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Genetic Improvement in Banana: A Breeding Approach

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ABSTRACT: Banana is a major fruit crop grown in the tropical and sub-tropical regions. The sustainable banana production is threatened by an ever increasing range of pests and diseases and adverse environmental conditions like drought and salinity. Enhancing the genetic makeup of bananas is essential for creating novel varieties that are both high-yielding and well-suited to various environmental circumstances. The combination of traditional breeding methods with advanced biotechnology, such as genomic research and transformation techniques, holds significant promise in driving the attainment of a sustainable banana production system. The breeding programs include introduction, selection, mutation breeding, hybridization and biotechnological approach to expedite the development of improved banana varieties with desired traits, including disease resistance, climate resilience, and nutritional value. Thus, developing banana varieties/ hybrids suitable for sustainable production under changing climatic conditions is the need of the hour.

Keywords: Conventional, biotechnological, introduction, selection, mutation, hybridization.

INTRODUCTION

Banana, a vital food crop grown in tropical and subtropical regions, serves as a staple food in many African countries and is a beloved fruit worldwide. The process of developing new banana varieties differs from seed crops because bananas are propagated vegetatively, without the use of seeds. Unlike seed crops, where desirable traits can be obtained through sexual reproduction, the lack of natural variation in vegetatively propagated crops like bananas makes the breeding process more demanding. However, dedicated breeders and researchers are actively engaged in creating improved banana varieties with desirable characteristics. Despite the challenges, various techniques are employed in banana improvement programs to introduce variability and enhance genetic diversity.

Breeding programme. In India, commercially significant banana cultivars include Robusta (AAA), 'Red banana' (AAA), 'Rasthali' (AAB), 'Poovan' (AAB), 'Virupakshi' (Pome, AAB), 'Nendran' (AAB), 'Karpooravalli' (ABB), 'Monthan' (ABB), and 'Ney poovan' (AB). Banana production in the country faces challenges such as sigatoka leaf spot diseases, Fusarium wilt (race 1 and race 2), viral diseases (BBTV, BBMV, BSV, and CMV), nematodes (*Pratylenchus coffeae*, *Radopholus similis*, and *Meloidogyne incognita*), as well as weevil and borer infestations (*Cosmpolites sordidus* and *Odoiporus longicollis*) (Ploetz *et al.*, 2015).

To improve banana varieties, conventional and new breeding techniques have been employed. Recurrent breeding conducted at the diploid stage has led to the creation of synthetic diploid strains possessing drought resistance and immunity against diseases like banana leaf spot and nematode *Radopholus similis*. Crosses between triploids and diploids have further led to the creation of tetraploids, which have been evaluated for their yield, fruit quality, and resistance to nematodes, fusarium wilt, and leaf spot diseases. Notably, two improved hybrids, namely BRS-1 and BRS-2, have been developed and assessed at Banana Research Station, Kannara, and NRCB.BRS-1 is a parthenocarpic and sterile tetraploid Pome hybrid (Agniswar × Pisang Lilin) shows immunity to *Mycosphaerella*, *M. eumusa*, and *M. musicola*, and it yields an average bunch weight of 25 kg. On the other hand, BRS-2 is another tetraploid (AAAB) hybrid, resulting from Vannan × Pisang Lilin, and it exhibits tolerance to *M. musicola* and *M. eumusae*. In summary, India's banana industry faces several challenges, but through conventional and new breeding techniques, notable progress has been made in developing improved hybrids with desirable traits such as disease resistance and higher yields (Blomme *et al.*, 2017).

Several banana cultivars have been introduced to combat biotic stresses, such as Lady Finger (EC 160160), which was brought in from Australia and is currently under evaluation at Indian Institute of Horticultural Research, Bangalore, and TNAU, Coimbatore, due to its resistance to bunchy top virus. Additionally, cultivars like Valery from West and Indies Naine MS from France were introduced to be used in breeding programs. Another exotic variety, Popoulu, was also introduced from Musa International

Transit Centre (ITC), Belgium. The preliminary evaluation- Banana Research Station (BRS), Kannara, potential terms of yield, quality and processing attributes (Backiyarani *et al*., 2019).

In the field of banana cultivation, efforts have been made to introduce certain cultivars with resistance to biotic stresses. For example, the 'Lady Finger' (EC 160160) cultivar from Australia is currently being evaluated at the Indian Institute of Horticultural Research (IIHR) and Tamil Nadu Agricultural University (TNAU) in India. This cultivar has shown resistance to the bunchy top virus, which is a significant concern in banana production.

In addition to disease resistance, cultivars like Naine MS (EC 27237) from France and 'Valery' from the West Indies have been introduced for improvement programs. These cultivars were selected based on specific traits that could contribute to enhancing banana varieties. Furthermore, another cultivar called 'Popoulu' was introduced from the Musa International Transit Centre (ITC) in Belgium. This exotic introduction underwent preliminary evaluation at the Banana Research Station (BRS) in Kannara, focusing on assessing its potential in terms of yield, quality, and processing attributes.

These introductions of different banana cultivars aim to increase the genetic diversity available for banana improvement programs. By introducing cultivars with desirable traits, such as disease resistance, improved yield, and quality, researchers and breeders can enhance the overall performance and characteristics of banana varieties.

MUTATION BREEDING

The natural mutants or sports are observed in many banana cultivars and that include High gate (AAA) which is a semi-dwarf mutant of Gross Michel (AAA), Motta Poovan (AAB) is a sport of Poovan (AAB), Ayiranka Rasthali is the sport of Rasthali (Silk), Barhari Malbhog is a sport of Malbhog, Krishna Vazhai is a natural mutant of Virupakshi, Sambrani Monthan (ABB) is the mutant of Monthan (ABB)., Moongli is mutant of nendran , Attu Nendran, Nana Nendran, Myndoli, Velathan are mutants of nendran selected for their desirable character, Ambalakadali, Erachi vazhai and pacha kappa are mutants of Red banana.

In banana breeding, induced mutation has been employed by treating *in vitro* material with physical or chemical mutagens like ethyl methane-sulphonate, diethylsulphate, or sodium azide (Kulkarni *et al*., 2007). This approach aims to compensate for weaknesses in existing banana cultivars and generate commercially interesting variants. Although the production of desirable mutants through induced mutation is possible, it has had limited success overall (Heslop-Harrison & Schwarzacher 2007).

Successfully released commercially are two banana cultivars, Novaria and Klue Hom Tong KU1, which were derived from gamma ray-induced mutation. Novaria exhibits early flowering, while Klue Hom Tong KU1 produces large bunches of fruit, making them agriculturally valuable (Roux, 2004). Many of the commercially released mutants of banana have been induced through gamma irradiation.

Inducing tetraploids from diploids through colchicine treatment of parental tissues is part of another breeding strategy known as the triploid approach. Improved tetraploid lines are then selected and hybridized with diploids to produce triploids that are suitable for final evaluation (Smith *et al*., 2006). Colchicine and oryzalin have been used as mutagens to induce tetraploids and auto tetraploids in banana (Duren *et al*., 1990). This manipulation of chromosome number by *in vitro* mutation technology has been used in several *Musa* breeding programs.

HYBRIDIZATION APPROACH

A. Development of primary tetraploids

Triploid varieties are plants with three sets of chromosomes (n = 3x = 33), while diploid clones have two sets of chromosomes (n = 2x). Triploid varieties are typically highly sterile, but some of them show residual fertility and produce a few seeds. In this breeding strategy, the few unreduced triploid egg cells in the triploid variety can be fertilized with normal haploid pollen from a diploid clone, resulting in tetraploid embryos. Tetraploid embryos have four sets of chromosomes (n = 4x), which leads to improved fertility and other desirable traits in the resulting plants. One advantage of this breeding strategy is that the genes from the mother plant, which is the triploid variety, do not segregate. As a result, the progeny retains the maternal characteristics without genetic variation. This can be beneficial when trying to maintain specific traits from the mother plant in the offspring.

The 3x/2x breeding strategy also allows for the creation of various types of AA diploid hybrids. This strategy has been successful in creating hybrids in subgroups such as Gross Michel (AAA), Mysore (AAB), Pome (AAB), Plantain (AAB), and Pisang Awak (ABB). Two improved tetraploid hybrids, BRS-1 and BRS-2, which were released by Kannara and evaluated at NRCB. These hybrids exhibit immunity to *Mycosphaerella musicola* and *M. eumusae*, which are fungal pathogens. This indicates that these tetraploid hybrids have enhanced resistance to these diseases.

B. Improvement of the diploid male parents

In many countries where bananas are grown, initial breeding efforts used wild diploid bananas (AA) as male parents. However, this approach led to the inheritance of undesirable traits in the resulting tetraploid offspring. As a result, banana breeders recognized the importance of creating a superior male parent. The ideal male parent should have strong resistance to Panama and Sigatoka diseases, as well as compact bunches with upright orientation. Additionally, it should produce large fruits within the limits of diploidy and exhibit parthenocarpy, while still providing enough pollen for use in breeding.

The National Research Centre for Banana and the TNAU have successfully developed numerous mono-specific and inter-specific diploid hybrids. More recently, they have focused on using fertile diploid AA hybrids resulting from 3x/2x crosses as parents for creating high-quality diploid varieties. Specifically, they have produced plantain hybrids that show resistance to Panama wilt and sigatoka leaf spot diseases (SLSDs), while maintaining the desirable fruit characteristics of plantains. This approach has been particularly adopted by organizations like CARBAP and IITA to develop diploid varieties tailored to specific sub-groups, including plantains and East African highland bananas.

C. Production of secondary triploids from tetraploid hybrids

Fertile tetraploids and diploid hybrids (F1 hybrids) are crossed to produce F2 hybrids. The genetic gain achieved through nuclear restitution in the earlier stage, where heterosis is transmitted from the triploid variety to the tetraploid hybrid through FRD (First Division restitution), will be negated due to the reshuffling and recombination of alleles at each locus that takes place during meiosis of the tetraploid hybrid.

D. Production of Triploid Hybrids from Diploid

To begin the agronomic evaluation and fertility testing phase, carefully selected mono- and inter-specific diploid banana genotypes were used. These genotypes were exposed to colchicine treatment in a controlled in vitro environment to induce chromosome doubling, leading to the formation of auto- or allotetraploids. The choice of genotypes for colchicine treatment was based on type of banana desired (cooking or dessert), agronomic characteristics, resistance to disease, and fertility of paternal and maternal parents. Additionally, improved diploids resulting from hybridization and showing high resistance to various diseases were also included in the treatment process. As a result of this approach, more than 25 AAAA auto tetraploids and AABB allotetraploids have already been obtained.

After obtaining tetraploids through colchicine treatment, reciprocal crosses were conducted between tetraploid and diploid clones to create both mono- and inter-specific triploid hybrids. The genetic combinations chosen during the diploid stage were largely preserved in the resultant triploids. These triploid hybrids possess desired sterility due to their triploid nature, which allows breeders to preserve the desired characteristics of the parent plants. By incorporating highly fertile wild parents in some cases, breeders could generate large triploid progenies, which facilitated effective selection. This breeding strategy taps into the wide diversity present in diploid banana varieties (Tenkouano *et al*., 1998).

Moreover, this approach offers flexibility in incorporating new selection criteria at any stage by introducing new parents. This adaptability enables breeders to respond promptly to the emergence of new pathogenic fungi races or to address other specific selection objectives.

BIOTECHNOLOGICAL APPROACH

Genetic modification is a powerful tool in crop improvement that bypasses traditional breeding barriers. This process entails changing an organism's genetic material by introducing particular genes or making alterations to those already present. This technique has been particularly useful in improving traits such as disease resistance and fruit quality in bananas**.** The significant progress in genetic engineering methods, combined with the release of the banana genome, has greatly eased the enhancement of traits using genetic transformation methods. Genetic modification techniques offer a means to introduce variability in plants by either increasing or decreasing the expression of specific traits, such as disease resistance or fruit quality (D'Hont *et al*., 2000; Maxmen, 2019; Hwang, 2001). Several studies have demonstrated successful outcomes in this regard. For example, Dale *et al*. (2017) effectively conferred resistance to Foc TR4 in the Grand Naine cultivar by overexpressing a potential nucleotide-binding and leucine-rich repeat (NB-LRR)-type resistance gene (RGA2) from the TR4 resistant line, *M. ac. ssp. Malaccensis* (Peraza-Echeverria, 2008). Ghag *et al.* (2014) targeted pathogenicity genes for inducing resistance, including Fusarium transcription factor 1 (ftf1) and Velvet genes of Foc race 1, through host-induced post-transcriptional hairpin RNA-mediated gene silencing. Another approach involved creating Foc TR4 resistant plants by employing gene silencing techniques to target the ergosterol biosynthesis genes ERG6 and ERG11 (Dou *et al*., 2019). These studies demonstrate the potential of genetic modification as a tool to enhance desirable traits in plants.

To overcome regulatory challenges and the introduction of foreign genes, scientists have developed techniques for gene modification that do not require insertion into the plant's chromosome. One such technique involves the transient expression of the C as protein and single-guide RNA (sgRNA) using protoplast transformation. This method allows for high transfection efficiency and is suitable for testing new CRISPR-Cas9 designs in various plant species (Tripathi *et al.,* 2019, Kaur *et al.,* 2017). Overall, genetic modification and genome editing techniques have demonstrated significant potential in bananas. They offer precise and rapid methods for introducing desirable traits while reducing the risks associated with traditional genetic modification approaches.

CONCLUSIONS

Integration of high-throughput technologies from various scientific fields further accelerates the breeding process. Molecular biology techniques such as Marker-Assisted Selection (MAS) and Genomic Selection (GS) enable breeders to rapidly identify and select plants with the desired traits based on their genetic makeup. Tissue culture methods, including the embryo culture, multiple shoot induction etc. aid in the production of huge numbers of plants with favorable traits, allowing for faster evaluation and selection.

Furthermore, information technology plays a crucial role in streamlining breeding programs. Tools like the banana tracker, which likely refers to software or systems designed for tracking and managing breeding data, facilitate data organization, analysis, and decision-making. These technological advancements collectively contribute to the acceleration of breeding programs and enable the development of desirable banana hybrids in a shorter time frame. In summary, the combination of genetic modification, genome editing, and advanced scientific technologies in banana breeding programs offers immense potential for creating varieties with desirable traits. The existing natural variation in bananas, coupled with high-throughput molecular biology, tissue culture, and information technology, paves the way for rapid and efficient crop improvement in the years to come.

FUTURE SCOPE

The future of banana breeding programs holds significant potential for addressing various challenges, including disease management, climate change, and nutritional needs, while also improving the overall sustainability and profitability of banana cultivation. Collaboration, innovation, and a focus on consumer and farmer needs will be crucial in shaping the future of banana breeding programs.

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Conflict of Interest. None.

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