Adv. Hort. Sci., 2024 38(3): 297-310

DOI: 10.36253/ahsc-15047
https://oaj.fupress.net/index.php/ahs

Review paper

AHS
Advances in Horticultural Science

Future prospects and challenges in developing saline-tolerant banana

E.F. Hasrianda (*), R.H.B. Setiarto, L.O.M.M. Davis, Y.S. Poerba
National Research, and Innovation Agency (BRIN), Jl. Raya Jakarta-Bogor Km 46, Cibinong Science Center, Bogor 16911, West Java, Indonesia.

OPEN ACCESS

(*) **Corresponding author:**
erwi011@brin.co.id
erwin.fraa@gmail.com

Citation:
HASRIANDA E.F., SETIARTO R.H.B., DAVIS L.O.M.M., POERBA Y.S., 2024 - *Future prospects and challenges in developing saline-tolerant banana.* - Adv. Hort. Sci., 38(3): 297-310.

ORCID:
EFH: 0000-0002-9072-0305
RHBS: 0000-0001-6894-7119
LOMMD: 0000-0002-5465-7177
YSP: 0000-0002-1388-8745

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no conflict of interests.

Received for publication 8 February 2024
Accepted for publication 27 June 2024

Key words: Banana, breeding, genetic diversity, saline-stress, transgenesis.

Abstract: Banana (*Musa* spp.) is an essential fruit with high economic value worldwide. One of the main environmental constraints in banana cultivation is abiotic stress on marginal land caused by salinity stress. This salinity stress can significantly reduce banana productivity and even cause plant death. Global climate change due to warming and reduced rainfall has increased the number of agricultural areas affected by salinity. This review article will describe current knowledge of biodiversity and various biological responses when banana plants are exposed to saline stress. This article also mentions the challenges in the banana breeding program with the current development of plant breeding and genetic engineering technology that could be used to create saline-tolerant banana accessions, its prospects, and the societal controversy underlying this technology. Thus, the information presented in this article could be a reference in the saline-tolerant banana breeding program.

1. Introduction

Bananas (*Musa* spp.) are ancient monocotyledonous plants grown in nearly 120 countries in tropical and subtropical regions worldwide. Moreover, banana is also a staple food and a major source of nutrition for more than 500 million people (Wang *et al.*, 2019). After rice, maize, and wheat, bananas are among the world's most important food crops and the second most popular fruit. The worldwide production of bananas (*Musa* spp.) totaled 113.9 million metric tons in 2017 (Evans *et al.*, 2020), highlighting its importance as a substantial crop in global diets and commerce. Banana has a significant market share globally due to their broad appeal (De Langhe *et al.*, 2009; Wang *et al.*, 2021). It is rich in protein, vitamins, and minerals and is a premium export commodity in some countries (Aquil *et al.*, 2012; Ji *et al.*, 2021). Banana consumption worldwide continues to increase year by year. Consequently, the area of banana cultivation will expand as well.

In order to support the growth of the increasing demand for bananas, marginal land has emerged as a viable option for agricultural land development for planting bananas (Kusdianti *et al.*, 2016). However, marginal land has relatively poor agricultural yield potential (Dikayani *et al.*, 2017). This land is widely accessible, but its agricultural potential remains largely untapped for agricultural activities. As an illustration, in Indonesia, coastal agricultural areas often face salinity issues attributed to rising sea levels resulting from climate change. Approximately 12.020 million hectares, or 6.20% of Indonesia's total land area, are estimated to be prone to salinity near the coast (Karolinoerita and Annisa, 2020).

Salinity is among the most critical abiotic stressors affecting plant development and productivity worldwide. Salinity is a prominent concern among the abiotic stressors affecting farming (Rao *et al.*, 2016). Salinity induces two types of stress: direct osmotic stress and delayed ion toxicity stress. They will affect plant development directly and indirectly. Osmotic stress occurs when excessive salt concentration disrupts osmotic balance, causing a physiological drought and thus inhibiting plant water uptake (Endris and Mohammad, 2007; Manojkumar *et al.*, 2022).

Salinity problems can arise in coastline areas, dry environments on saline soil and from using low-quality irrigation water. Furthermore, an area with higher water evaporation rates in agricultural lands than rainfall also has a significant amount of land at risk of salinity stress. Salinity stress can cause reduced growth, fruit yield, and quality, which can have significant economic impacts on banana producers. Excessive salinity also increases salt content in the roots, reducing potassium absorption, slowing development, delaying flowering, and reducing crop production (Rao *et al.*, 2016). Developing banana plants with improved salinity tolerance is crucial to ensure food security, especially in regions prone to stress.

Fortunately, some plant accessions possess natural tolerance to environmental stressors, enabling them to withstand adverse conditions. This adaptation relies on the activation of intricate molecular networks responsible for stress detection, signal transduction, metabolite production, and the expression of stress-related genes. Additionally, it is crucial to consider the interplay between signaling molecules to understand the synergistic or antagonistic interactions that influence plant responses to abiotic stressors. We can better understand plant performance under stress conditions by identifying the genes involved in stress tolerance, including those responsible for cellular protection and stress response regulation. This comprehensive understanding is vital for developing effective strategies to enhance plant tolerance to salinity (Nguyen *et al.*, 2018).

To our knowledge, no national or global-scale banana breeding efforts have yet been executed to generate salt-tolerant banana cultivars successfully deployed for utilization by general farmers and the banana cultivation sector. This review explores the importance of banana biodiversity, genetic engineering technology advancements, challenges, and opportunities to improve banana salinity tolerance. It also highlights crucial aspects of banana breeding, identifies the unknown physiological and molecular processes underlying saline stress adaptation, evaluates the current status of banana genetic transformation technology, and discusses the potential application of newly discovered genome editing technology in banana breeding in saline stress adaptation.

2. Saline stress response in plants and bananas

Soil water salinity generally restricts plant development by affecting the soil solution's osmotic potential. It hinders plant water uptake and retards plant growth, which is commonly referred to as water deficit due to salinity. Moreover, excess salt entering the plant system through transpiration force can produce ion toxicity and cell damage in plant organs, further reducing plant development (Greenway and Munns, 1980). At the cellular level, high salt concentration could also deactivate plants' main cellular activities (Cuartero *et al.*, 2006; Anusuya and Soorianathasundaram, 2014). Because the osmotic pressure in the soil is higher than in plant cells, excessive soil salt levels can also trigger cell plasmolysis in plants. In addition, plant growth caused by excessive salinity has the following characteristics: (1) plants with low water potential, (2) Na^+ and Cl^- ion toxicity, and (3) nutrient imbalance in plants (Dikayani *et al.*, 2017). Next, salinity will also affect plant physiology, including CO_2 uptake, protein synthesis, respiration, and phytohormone cycles (Mengel and Kirkby, 1987; Ravi *et al.*, 2013; Rao *et al.*, 2016).

Firstly, saline stress reduced water availability, higher respiration rates, mineral distribution changes, membrane instability, and inability to maintain turgor pressure are some factors that contribute to poor plant performance due to saline stress (Cuartero *et al.*, 2006; Anusuya and Soorianathasundaram, 2014). Secondly, ionic stress causes the accumulation of *sodium chloride* (*NaCl*) ions in plant cells and tissues. Ionic stress could result in toxicity and physiological disturbances. Thus, salinity has an inhibitory effect on plant development due to the excess of Na^+ and Cl^- ions toxicity towards water uptake as well (Cherian and Reddy, 2003; Sharif *et al.*, 2018; Verma *et al.*, 2019; Manojkumar *et al.*, 2022).

Generally, saline-tolerant plants can show one or a combination of processes, such as plant organ robustness, salt excretion, and osmotic adjustment, by producing solutes that can counteract ion accumulation in the tonoplast with more unsaturated fatty acids in the membrane composition (Mengel and Kirkby, 1987). Additionally, plants perform tolerance-related responses such as protein stabilization, osmotic protection, reactive oxygen species (*ROS*) detoxification, and ion homeostasis to adapt to a saline environment, enhancing plant durability (Jacob *et al.*, 2017; Nguyen *et al.*, 2018). Furthermore, protein stabilization compounds for osmotic adjustment, such as Proline (*Pro*), Trehalose (*Tre*), and other soluble sugars, are excellent osmoprotectants that can reduce osmotic damage caused by salt pressure (Türkan and Demiral, 2009; Deinlein *et al.*, 2014).

On the other hand, during saline stress, *peroxidase (APX), superoxide dismutase (SOD), peroxidase family protein (POD), thioredoxin family protein, and glutathione S-transferase family protein (GST)* were identified as differentially expressed genes (DEGs) in a plant. Transcription factors (TFs) can function as gene activators, repressors, or both (Ji *et al.*, 2021). On top of that, saline tolerance is a complex characteristic involving multiple genes and various physiological and biochemical adaptive pathways (Cuartero *et al.*, 2006; Anusuya and Soorianathasundaram, 2014). It also implies that there are genetic and metabolic mechanisms that plants can use to adapt to saline stress and enhance their ability to better adapt to these environmental conditions.

Bananas are especially vulnerable to saline stress, as high salinity levels can significantly reduce banana yields (Dikayani. *et al.*, 2017). Saline stress injuries manifest as leaf edge necrosis and then spread to the centre of the banana leaf. Moreover, salinity stress also causes yellowing and decreased chlorophyll content and stability, followed by leaf drying, significantly affecting plant development. The impact of salt is then significantly reducing stem thickness and prolonged flowering period of vulnerable cultivars. Due to lower photosynthesis activity in these leaves, harvest yields are reduced due to decreased banana finger weight which also produces low-quality commercial bunches and fails to develop the characteristic finger (fruit) physical characteristics of fruit, such as length, circumference, fruit flesh, skin weight, volume, and density (Israeli *et al.*, 1986; Ravi *et al.*, 2013; Ravi *et al.*, 2014; Rao *et al.*, 2016; Ravi and Vaganan, 2016). Thus, salinity reduces banana production significantly. Further research on the genetic mechanisms responsible for adaptation to saline environments is expected to facilitate breeding of high-yielding banana cultivars that can thrive in saline environments and meet market demand.

Fig. 1 - From left to right, pictures A to D show the conditions of the banana seedling phase with a plant height of ±40 cm exposed to salinity through hydroponic treatment. Pictures E to H show the conditions of banana leaves exposed to a saline environment. Starting from before the salt application treatment until the banana plant eventually dies. The plant wilts and dries; it starts from the older leaves to the younger ones, gradually from the leaf edges until eventually drying out completely in three months (author personal collection, unpublished).

3. Breeding for saline tolerant banana

Genetic diversity for salinity adaptation

The potential tolerance sources should be identified before breeding stress tolerance bananas. Thus, screening cultivars is the main approach to identifying genetic resources for plant breeding programs in the future (Anusuya and

Soorianathasundaram, 2014). Genetic resources for environmental saline stress tolerance could be derived at least from (1) the natural gene pool of banana accessions and their close relatives already present in the wild, (2) by promoting natural mutations in existing banana accessions using cis-genesis methods, and (3) by incorporating genes from entirely different species using genetic engineering approaches (transgenesis).

Several types of banana accessions are known to be more tolerant of environmental stress than other accessions. These accessions have several genes related to their useful agronomic traits, including height, leaf angle, root spread, and resistance to abiotic and biotic stresses (Heslop-Harrison and Schwarzacher, 2007). The B genome of bananas, for example, has long been the target of breeding programs because of the strong tolerance of bananas to abiotic stress (Tripathi *et al.*, 2019). Therefore, genotyping and phenotyping exploration of the B banana genome, both accessions of cultivars and their wild relatives, should be considered as a way to find novel metabolisms or genes that can play a significant role in increasing the tolerance of banana cultivars to saline environments.

Previously, research has been carried out on the tolerance level to saline stress on various genotypes of banana cultivars. Various banana cultivars such as cv. Tongat (AA), cv. Ney Poovan (AB), cv. H 212 (AB), cv. Grand Naine (AAA), cv. Karpooravalli (ABB), cv. H 96/7 (ABB), cv. Poovan (AAB), cv. Rasthali (AAB), cv. Chakkaiya (ABB), cv. FHIA-1 (AAAB), cv. FHIA-23 (AAAA), and cv. FHIA-17 (AAAA) which were tested with four salt concentrations, namely 0 mM NaCl NaCl, 150 mM NaCl, and 200 mM NaCl, in a research by Anusuya and Soorianathasundaram (2014). The experiment demonstrated significant variability in the tolerance response of bananas to salinity stress, whereas the banana accessions 'FHIA-1' and 'Grand Naine' are very sensitive to the saline treatment given. Meanwhile, the banana accessions 'Ney Poovan', 'Poovan' and 'Karpooravalli' appeared to be more tolerant compared to other banana cultivars in the study (Anusuya and Soorianathasundaram, 2014). This finding suggests that while bananas are generally susceptible to saline stress, unique molecular processes in the different banana accessions can be utilized in breeding programs to develop new, saline-tolerant banana cultivars. In short, genetic diversity is crucial for successful plant breeding (Nandariyah *et al.*, 2020) and in producing saline-tolerant bananas.

In addition, Rao *et al.* (2016) found that among the tested genotypes, Ney Poovan, Poovan, and Karpooravalli can be classified as tolerant bananas based on their capacity to develop and maintain tolerable physiological conditions even under concentrated saline stress levels of 200 mM. However, saline stress affected fruit growth in vulnerable banana cultivars, particularly in the Cavendish group but not in the tolerant Saba genotype. Mature Saba bananas grown in affected areas produced normal bunches (Rao *et al.*, 2016; Ravi and Vaganan, 2016).

On the other hand, Southeast Asia is known as the origin of bananas (Wang *et al.*, 2021). This location also has the highest diversity of wild *Musa* species *(Musa acuminata AA* and *Musa balbisiana BB)* found today, as well as the earliest domestication (Simmonds, 1962; Rao *et al.*, 2016). However, testing and recording the saline tolerance traits of various cultivars and endemic wild banana accessions in Southeast Asia are not widely reported. Research on the tolerance level of salinity stress in banana cultivars and wild species is expected to be used to identify banana accessions as donor genes for the salinity stress tolerance trait in bananas that have not been documented previously.

Essentially, the most significant cultivated banana type in the world is triploid, resulting from inter- or intraspecific hybridization of two wild diploid species, *Musa acuminate* (genome A) and *Musa balbisiana* (genome B) (Wang *et al.*, 2019). As a result, most banana cultivars are sterile, and their genetic diversity is limited, making traditional breeding schemes challenging to implement for this plant. Based on this, although candidate genes for saline tolerance in bananas have been identified, there will be further challenges in transferring these genes of interest through conventional breeding methods to existing industrial and consumer banana accessions. On the opposite, since the genetic engineering method avoids reproductive barriers and promotes the production of superior strains based on large cultivars, this may be a solid alternative to traditional banana breeding (Heslop-Harrison and Schwarzacher, 2007). Considering these conditions, the genetic engineering approach could be a reasonable option to produce saline-tolerant banana accessions for consumption and industry.

An additional issue with cultivated banana plants is that they are propagated vegetatively, leading to a decrease in genetic variation (López *et al.*, 2017; Hasim *et al*., 2021). However, improving plant traits can be achieved by increasing genetic diversity, including inducing mutations. Induced mutations can enhance the genetics of vegetatively propagated plants (Ghag and Ganapathi, 2017; Nandariyah *et al.*, 2020). Gamma radiation technology can be an option to increase the genetic diversity of bananas. Gamma radiation mutagen is frequently used as a mutagenic agent to induce mutations (Indrayanti *et al.*, 2012). Gamma rays are commonly used in mutation breeding because they can interact with atoms or molecules in cells with water to form free radicals. These radicals can damage or alter key components in plant cells, affecting plants' morphology, anatomy, biochemistry, and physiological differentiation (Datta *et al.*, 2018). Gamma radiation is high-energy electromagnetic radiation produced by radioactive isotopes or nuclear reactors. When applied to plant tissue, it can affect the gene structure, increasing genetic variability in irradiated plants (Astutik, 2009; Khumaida *et al.*, 2015; Maharani *et al.*, 2015).

In another research, Nandariyah *et al.* (2020) developed a gamma radiation protocol to generate genetic variability in bananas, which is one of the options that can be used. Observations of the number of fruits per bunch on each cv. Raja Bulu banana plant that received gamma radiation treatment from the first to the third bunch showed significant differences. The study showed that gamma radiation treatment had a more significant effect on the average number of fruits per bunch, bunch weight, and fruit weight than the control. According to Datta *et al.* (2018), gamma radiation with a dose of 10 Gy changed the appearance of morphological varieties in the generative phase of the M1V1 Raja Bulubanana (Nandariyah *et al.*, 2020). In their study, Nandariyah *et al.* (2020) successfully produced a new genotype of a banana with superior traits compared to its parent.

However, this method also poses challenges that need to be considered. One of them is that gamma radiation can cause random mutations. It means that this method will require laborious phenotyping selection which could take considerable time to separate the desired mutant plants from the undesired ones. The random nature of this method also means that there is a possibility that the survived mutants from the experiment may ultimately fail to produce a better cultivar than their parent.

Screening methods for salinity stress tolerance in bananas

Screening for salinity tolerance plants has been conducted using various methodological approaches, including controlled artificial conditions such as *in vitro* culture, pot planting conditions, hydroponic media, greenhouse cultivation, and direct field planting. However, the latter approach is relatively challenging to control due to high environmental variability and difficulty fully controlling field conditions.

Tissue culture techniques have rapidly assessed the saline tolerance traits of different tested banana accessions on an *in vitro* scale. It provides a controlled environment where the effects of salinity can be evaluated by exposing banana explants or tissue cultures to different salt concentrations. Currently, *in vitro* culture of bananas has been developed using various explants, such as shoot tips (Matheka *et al.*, 2019), immature male flowers (Jalil *et al.*, 2003), apical meristems (Novak *et al.*, 1989), bulbs (May *et al.*, 1995), flower tops (Liu *et al.*, 2017), and Embryogenic Cell Suspension (ECS) (Vuylsteke and De Langhe, 1985; Sági *et al.*, 1995; Côte *et al.*, 1996; Cote *et al.*, 2000). In addition, the development of *in vitro Musa acuminata* L. is likely influenced by salt. The treatment in cv. Barangan with $NaCl$ in 200 mM for plant height character was lower than the control. The height of banana plantlets decreased after the cultivation age in the 4th week. This study also showed that $NaCl$ treatment could simulate saline tolerance in banana plants *in vitro* (Dikayani *et al.*, 2017). This strategy can yield reliable data in a short period as salinity can significantly impact the growth of banana plantlets.

The *in vitro* test approach allows for evaluating various specific physiological and biochemical responses to salinity stress, including changes in ion accumulation, osmotic adjustment, and antioxidant enzyme activity. Morphologically, the impact of salinity is characterized by a brown coloration, particularly in the leaves, and dark brown coloration in the roots. This is attributed to the high concentration of Na^+ ions, which hinders the plant's uptake of K^+ ions. Normal plant cells typically contain a higher concentration of K^+ than Na^+. Potassium ions are crucial in maintaining osmotic pressure in cells, regulating stomatal opening and closing, protein synthesis, and functioning as enzymes such as pyruvate kinase. Therefore, low K^+ concentration in cells leads to chlorosis and necrosis (Munns and Termaat, 1986; Hasegawa *et al.*, 2000; Mahajan and Tuteja, 2005; Dikayani *et al.*, 2017).

In a different research study using the *in vitr*o assay technique, Kusdianti *et al.* (2016) found that the activity of banana plantlets induced proline accumulation after exposure to saline stress. This study also found that saline stress affected the gene expression pattern *of Heat Shock Protein 81-2 (HSP 81-2)* and *delta-1-pyrroline-5-carboxylate synthase (P5CS1)*. In this experiment, shoot meristem *Musa acuminata* cv. Barangan was cultured *in vitro* with 25, 50, 75, and 100 mM *NaCl* (Kusdianti *et al.*, 2016). Thus, *in vitro* testing of saline-tolerant banana traits can be considered one of the most powerful research methods for screening saline tolerance cultivars. This method enables testing many samples in a relatively short time.

On the other hand, hydroponic methods also offer another advantageous platform for studying saline stress in bananas. By growing banana plants in nutrient solutions with controlled salt concentrations, researchers can investigate the effects of different salinity levels on plant growth, physiological parameters, and gene expression profiles. Thus, hydroponic-based screening enables the identification of key genes and pathways involved in saline tolerance, providing valuable insights into the molecular mechanisms underlying plant responses to saline stress. Moreover, evaluating various banana genotypes under hydroponic conditions allows identifying potential candidates with more specific salinity tolerance levels.

However, while *in vitro* and hydroponic testing offers controlled conditions, it must be complemented by direct testing in saline-affected fields to ensure accuracy. This is because both tests are conducted in controlled environments, simplifying the real-world complexity of abiotic-biotic interactions in a field. Thus, field experiments with actual cultivation conditions are still essential to evaluate banana plants' performance under saline stress accurately. Nevertheless, the inherent environmental variability in field settings, including temperature fluctuations, rainfall patterns, and soil heterogeneity, poses challenges in obtaining consistent data. Factors such as temperature fluctuations, rainfall patterns, and soil heterogeneity contribute to the complexity of field trials, making it difficult to assess the true genetic potential and performance of banana genotypes under saline stress.

Based on the aforementioned points, screening banana genotypes for salinity tolerance necessitates a combination of controlled artificial conditions and realistic evaluations in field settings. *In vitro* culture and hydroponic systems provide controlled environments where physiological and molecular responses to saline stress can be studied in detail. On the other hand, field evaluations offer insights into the performance of banana plants under actual cultivation conditions. Ultimately, the breeding outcomes of bananas will be grown by farmers in agricultural fields. Therefore, in the end, banana accessions selected through rapid *in vitro* testing still need to be validated through multi-location field trials. Integrating these approaches, robust experimental design, and statistical analysis will contribute to developing saline-tolerant banana varieties through combined plant breeding programs and precise molecular analyses.

4. Saline-tolerant genes in Banana

Potential saline-tolerant genes in banana

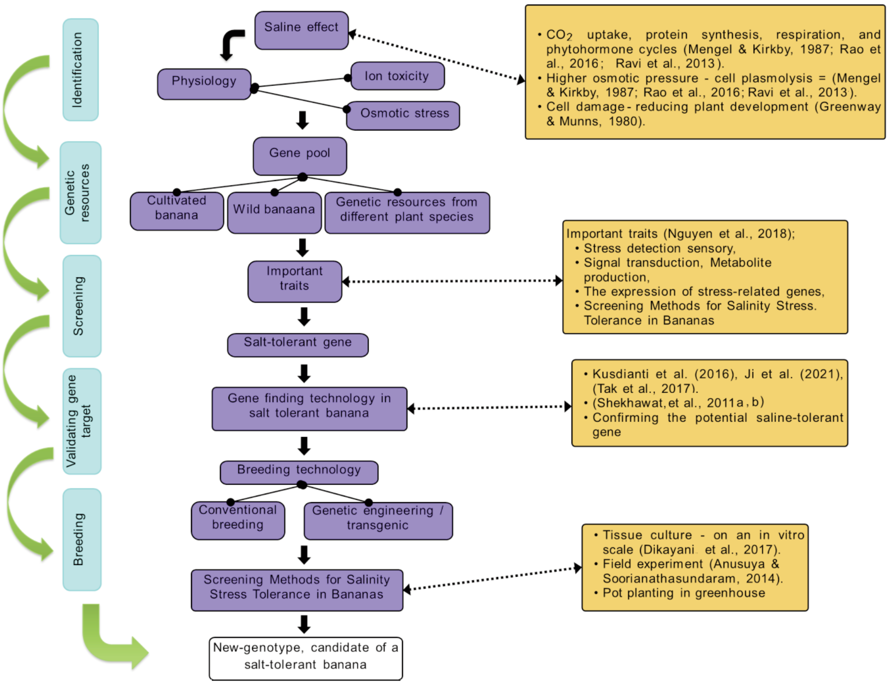
Various genes in multiple plant species have been discovered to enhance plant tolerance to abiotic stress. These include the over production of enzymes involved in metabolite synthesis, membrane lipid biosynthesis, antioxidant defense, protective proteins, and transporters (Yang *et al.*, 2018; Zheng *et al.*, 2018). Genes like *PROLINE DEHYDROGENASE (ProDH)* and *1-pyrroline-5-carboxylate synthetase (P5CS)* regulate proline synthesis and contribute to stress response (Villao *et al.*, 2021). Up-regulation of *OsbZIP23* (Dey *et al.*, 2016) and *OsNAC6* (Nakashima *et al.*, 2007) in transgenic rice increases salt and drought tolerance, while *SNAC1* and *SNAC2* enhance cold, salt, and drought tolerance (An *et al.*, 2018). The expression of protein kinases such as *SnRK2, CIPK*, and *CDPK* has also been shown to improve salt, cold, and drought tolerancein various crops (Saijo *et al.*, 2000; Boudsocq and Lauriere, 2005; Xiang *et al.*, 2007; Zhang *et al.*, 2016; Fedorowicz-Strońska *et al.*, 2017). The *Mitogen-Activated Protein Kinase (MAPK)* cascade (Boudsocq and Lauriere, 2005) and *Protein Phosphatase 1a (OsPP1a)* (Liao *et al.*, 2016) are involved in stress response pathways. Identifying key regulators and utilizing them for stress acclimation is an important research focus. Such findings can be valuable references for identifying potential gene donors in breeding programs to enhance abiotic stress tolerance in bananas.

Various attempts have also already been made to make bananas more tolerant of saline stress. Among them is to look for genes that play a significant role in making plants able to tolerate the effects of environmental salinity. The search for this target gene can be carried out using the RNA-seq method, as done by Ji *et al.* (2021). They use the RNA-seq approach to compare transcriptome changes in banana roots exposed to salt (60 mM *NaCl*) with those grown under normal conditions. After 12 and 24 hours of salt treatment, 1466 and 2089 DEGs were found, consisting of 542 up-regulated and 924 down-regulated genes, 507 up-regulated and 1582 down-regulated genes, respectively. Stress signalling transduction, reactive oxygen species (*ROS*) scavenging, osmoregulation, and TFs are all elements of the saline stress response. A total of 3,355 DEGs were classified into 40 functional groupings, with biological processes (15 subcategories), cellular components (10 subcategories), and molecular functions (7 subcategories) being the most common (Ji *et al.*, 2021).

In that research, *HSP 81-2* and *P5CS1* genes were expressed at varying levels in all banana plantlets. With 75 mMNaCl treatment, *HSP 81-2* was most abundant in the shoots and roots of the plants. Similarly, this treatment resulted in the most significant accumulation of proline. The expression of the *HSP 81-2* gene was greater than the *P5CS1* gene in all roots and shoots given NaCl treatment. They concluded that *Musa acuminata* cv.*Barangan* has a defense mechanism against salt (Kusdianti *et al.*, 2016). Proline accumulation by plantlets given NaCl and expression of the *HSP 81-2* and *P5CS1* genes was found to vary among all plantlets, and maximum *HSP 81-2* was expressed by the shoots and roots of plants given 75 mMNaCl treatment (Kusdianti *et al.*, 2016). In general, gene findings using genetic factors and molecular methods are rapidly growing research activity and widely used in the past decade. This method allows for discovering various genes that contribute to a specific trait. Thus, understanding genetic and molecular mechanisms underlying the trait can be more comprehensive.

Confirming the potential saline-tolerant gene

Our current understanding of saline stress perception, signaling pathways, and downstream



- CO_2 uptake, protein synthesis, respiration, and phytohormone cycles (Mengel & Kirkby, 1987; Rao et al., 2016; Ravi et al., 2013).
- Higher osmotic pressure - cell plasmolysis = (Mengel & Kirkby, 1987; Rao et al., 2016; Ravi et al., 2013).
- Cell damage - reducing plant development (Greenway & Munns, 1980).

Important traits (Nguyen et al., 2018);
- Stress detection sensory,
- Signal transduction, Metabolite production,
- The expression of stress-related genes,
- Screening Methods for Salinity Stress. Tolerance in Bananas

- Kusdianti et al. (2016), Ji et al. (2021), (Tak et al., 2017).
- (Shekhawat, et al., 2011a, b)
- Confirming the potential saline-tolerant gene

- Tissue culture - on an in vitro scale (Dikayani et al., 2017).
- Field experiment (Anusuya & Soorianathasundaram, 2014).
- Pot planting in greenhouse

Fig. 2 - Phases of breeding salt-tolerant banana plants. These phases initiates with identifying the negative effects of saline stress on banana plants. The next steps entail exploring genetic diversity for salt stress tolerance within the existing gene pool and developing an appropriate breeding scheme. These steps aim to produce new banana genotype as potential candidates for salt-resistant banana cultivar.

factors is primarily based on research on the model plant *Arabidopsis thaliana*. It has yet to be widely applied to other crops. To effectively enhance saline tolerance in different plant species, it is crucial to identify and understand the specific growth adaptations to salt that benefit each species. Even in *Arabidopsis*, saline stress's early cellular perception and signaling are still obscure. During saline stress, intracellular Ca_2^+ spikes and wave signals seem to be a convergent sensory mechanism through which the cell perceives high salinity. It triggers a signaling pathway involving the Salt Overly Sensitive (*SOS*) proteins, which regulate sodium concentration in the cytosol. The *SOS* pathway comprises *SOS1, SOS2*, and *SOS3* and is induced by saline stress. Upon binding with Ca_2^+, *SOS2* is activated and forms a complex with *SOS3*, which then phosphorylates the H^+/cation antiporter *SOS1*, excluding sodium from the cell. This mechanism has been reviewed in detail in 2020 (van Zelm *et al.,* 2020).

Functional genetic studies aimed at elucidating the function of banana genes in saline stress are even more scarce. Table 1 lists known validated factors in the body of current literature. Two transcription factors from the banana cv. Karibale Monthan (ABB), *Musa NAC04* (Tak *et al.*, 2017), and *Musa WRKY71* (Shekhawat *et al.*, 2011 a) have been identified to be involved in the control of the saline stress response. *WRKY71* expression is elevated by many stressors, such as cold, dehydration, salt, and other hormones, while *Musa NAC04* is activated by salt treatment. *NAC04* or *WRKY71* overexpression in transgenic bananas has been associated with increased saline tolerance. It is indicated by sustained chlorophyll content, lower oxidative damage, and a better Fv/Fm ratio than wild-type (Tak *et al.*, 2017). Moreover, the induction of putative gene expression related to abiotic stress responses in *WRKY71* overexpression lines upon salt treatment suggests that *WRKY71* is involved in the up-regulation of down stream stress response genes in bananas (Shekhawat *et al.*, 2011 a).

Aquaporins are among the other factors that have been validated to be involved in banana saline tolerance. Aquaporins are water channels that promote symplastic water movement. The banana aquaporin gene, *MaTIP1;2,* is known to be expressed in the root, stem, and leaves and is strongly upregulated by salt and drought conditions. Transgenic *Arabidopsis* overexpressing *MaPIP1;1* is more tolerant to saline stress. Similarly, over expression of *MusaPIP2;6* in transgenic bananas resulted in higher saline tolerance than that of the wild type. A protection mechanism from osmotic shock through up-regulation of Banana Dehydrin1 was suggested in a study by Shekhawat *et al.* (Shekhawat *et al.*, 2011 b). *Banana Dehydrin1*, a type of Late Embryo Abundance (LEA) protein encoded by the *MusaDHN-1* gene, was induced by drought, cold, and high salt (Shekhawat *et al.*, 2011 b). Over expression of *MusaDHN1* leads to enhanced banana tolerance via accumulation of proline and reduced malondialdehyde levels upon drought and saline stress (Shekhawat *et al.*, 2011 b).

Nevertheless, various genetic profiling studies recorded in bananas are generally held on cultivated bananas. Exploration and research of genetic profiling of saline-tolerant traits in wild banana relatives in their center of diversity in Southeast Asia can be expected to provide information and potential tolerance traits that are not yet known. This is partly due to wild bananas undergoing more complex adaptation processes in nature than those cultivated on agricultural land. This diversity of traits is expected to enrich the genetic and molecular mechanism toolbox that can be applied to cultivated bananas. Therefore, it can provide new possibilities in breeding programs to produce cultivated bananas tolerant to saline environments.

The prospect of genetic transformation technology in Banana breeding

Natural selection and conventional banana crossing have been the most commonly used breeding methods worldwide. This is based on considerations of its simplicity and not requiring sophisticated instruments in their implementation. However, conventional breeding is based on natural genetic variation, and an extensive backcross program is required to add desirable characteristics to elite plants. This has led to the use of this method in banana breeding being considered laborious and time-consuming. Moreover, the availability of favorable alleles or genetic variation in nature is limited to exploit this approach (Manshardt, 2004).

Meanwhile, breeding through random mutagenesis (physical, chemical, or biological mutations) can result in mutations of many traits and undesirable changes. Breeding these mutations should also be followed by screening huge and time-

Table 1 - List of genes with validated functions related to saline stress

Gene	Protein	Role	Origin oganism	Expression nduced by	Expression pattern	Functional genetic testing plant	Phenotypes	Molecular mode of action	Publication(s)
MaROP5g	Rho-like GTPases	Signaling	Fenjiao and Baxi Jiao banana	Mainly salt, but also cold and drought	Protein localized at the plasma membrane	Overexpressed in *A. thaliana*	Longer primary roots and increased survival rates in response to salt stress	The increased salt toleranceconferred by *MaROP5g*gene might be related to reduced membrane injury and the increased cytosolic *K+/Na+* ratio and *Ca2+* concentration in the transgenic plants	(Miao *et al.*, 2018)
MusaDHN-1	Novel Banana SK3-type dehydrin, highly hydrophilic proteins (LEA family protein)?	Protecting cells from osmotic shock	Banana cv. Karibale Monthan	Drought, salinity, cold, oxidative and heavy metal stress, as well as by treatment with signalling molecules like abscisic acid, ethylene and methyl jasmonate	Leaves	Overexpressed in banana cv. Rastali	Improved toleranceto drought and salt-stress treatments in both i*n vitro* and *ex-vitro* assays	Enhanced accumulation of proline and reduced malondialdehyde levels in drought and salt-stressed	(Shekhawat *et al.*, 2011 b)
MaTIP1;2 promoter	Aquaporin	Water channels in symplastic pathways	*Musa acuminata* L. AAA group cv. Brazilian	Drought and salt	Roots, stems, leaves, flowers and fruits. Highest in leaves	Tested in *A. thaliana*	Reporter responds to salt and drought stress	Reporter responds	(Song *et al.*, 2018)
MusaNAC042	Signaling	Transcription factor	cv. Karibale Monthan	Induced by salt	Leaves	Transgenic *Musa acuminate* L. cv. Rasthali	Transgenic plants retained higher levels of total chlorophyll and lower levels of MDA content in response to salt stress. Transgenic plants have higher proline content and better Fv/Fm ratio	Modulating the expression of *CBF/DREB, LEA,* and *WRKY*	(Tak *et al.*, 2017)
MusaPIP2;6	Aquaporin	Water channels in symplastic pathways	cv. Karibale Monthan	Induced by salt and drought	Leaves	Overexpressed and using inducible construct transgenic banana (cv. Rasthali)	Transgenic plant has higher salt tolerance	better Fv/Fm, lower MDA	(Sreedharan *et al.*, 2015)
MaPIP2;7	Aquaporin	Water channel protein function in symplastic pathways	*Musa acuminata* L. AAA group, cv. Brazilian	Up-regulated after osmotic (mannitol), cold, and salt treatments	No data	Gongjiao Banana (*Musa acuminata* L. AA group, cv. Mas)	Overexpression of *MaPIP2-7* in bananas enhanced toleranceto drought, cold, and salt	lower levels of MDA and ion leakage, but higher contents of chlorophyll, proline, soluble sugar, and abscisic acid (ABA) compared with wild-type (WT) plants under stress and recovery conditions	(Xu *et al.*, 2020)
MaPIP1;1	Aquaporin	Water channel in symplastic pathways	*Musa acuminata* L. AAA group, cv. Brazilian	Induced by salt and drought	Root. Localized in plasma membrane	*Arabidopsis thaliana*	Overexpression of *MaPIP1;1* in Arabidopsis resulted in increased primary root elongation, root hair numbers and survival rates compared to WT under salt or drought conditions	Reduced membrane injury and high cytosolic K^+/Na^+ ratio	(Xu *et al.*, 2014, 2021)
MusaWRKY71	Transcription factor		*Musa acuminata* L. cv. Karibale Monthan (ABB)	Up-regulated by cold, dehydration, salt, ABA, H_2O_2, ethylene, salicylic acid and methyl jasmonate	Localized in nucleus	*Musa acuminata* L. cv. Rasthali suspension culture cells	Overexpressed line exhibited upregulation of putative genes related to abiotic stress responses	Transcriptional upregulation of stress response genes	(Shekhawat *et al.*, 2011 a)

consuming populations to identify mutants with the desired traits (McCallum *et al.*, 2000). Breeding mutations usually have a shallow frequency (0.1% of total mutations). In contrast, crossbreeding using markers is often very expensive, and linking markers to the desired trait is sometimes very difficult and time-consuming. This method will also produce products requiring complex regulatory processes, time-consuming requirements, and expensive safety analyses (Lusser *et al.*, 2012). Moreover, most Banana consumption cultivars are triploid. Thus, the sterility and polyploidy of bananas greatly inhibit conventional or molecular breeding from providing a practical and effective way for genetic improvement (Tenkouano *et al.*, 2011). In order to overcome this problem, transgenic bananas achieved through genetic engineering techniques might be a reasonable way to produce bananas with saline tolerance traits. In addition, genetic transformation technology also allows the addition or insertion of genes from groups of living things that are phylogenetically distant or sexually incompatible.

In the modern genetic engineering approach using agrobacterium plasmid, tissue culture methods are generally needed in the implementation stage. Usually, *in vitro* bananas could be genetically modified using particle bombardment or *Agrobacterium*-mediated transformation (May *et al.*, 1995; Sági *et al.*, 1995; Wang *et al.*, 2021). It is also worth noting that the approach using *Agrobacterium tumefaciens* is one of the most widely used due to its ease of use and ability to be applied to various plant tissues (Villao *et al.*, 2021). In addition, regeneration in the genetic modification of bananas is often carried out by organogenesis based on meristematic tissue or somatic embryogenesis based on ECS (Novak *et al.*, 1989; May *et al.*, 1995). Because of its rapid regeneration rate, meristematic tissue from shoot tips and flower buds are usually used as explants for cloning and genetic transformation (May *et al.*, 1995; Liu *et al.*, 2017; Wang *et al.*, 2021). Additionally, scientists commonly prefer to use plant materials for the genetic transformation process using the Agrobacterium-mediated transformation technique in somatic embryos, callus, and protoplast cultures (Villao *et al.*, 2021).

Development of saline-tolerance banana through transgenesis and gene editing

Perhaps the most known successful transgenesis in bananas so far is transgenic Cavendish banana using *Agrobacterium tumefaciens* from Dale *et al.* (2017). Normally, Cavendish bananas are highly susceptible to TR4, a destructive fungal disease that poses a significant threat to plantations worldwide. Currently, there is no known effective resistant replacement for Cavendish banana. The researchers conducted a 3-year field trial using transgenic banana lines transformed with genes *RGA2* and *Ced9* in this experiment. Both lines remained disease-free, indicating resistance to TR4. This study demonstrates how transgenic and gene editing approaches can practically enhance Banana cultivars to overcome environmental challenges through single gene transformation (Dale *et al.*, 2017).

In addition, just recently, in 2021, one experiment by Villao *et al.* (2021) successfully developed a genetic transformation protocol from banana apical meristem for the cv. Williams (genotype AAA). This protocol was based on the co-cultivation of explants (whole *in vitro* plants or meristematic tissue divided in two from *in vitro* plants) with *Agrobacterium tumefaciens* separately. As an illustration of the timeline required to implement this protocol, Villao *et al*. also mentioned that after embryogenic cell suspension transformation mediated by Agrobacterium, it would take six to twelve months to obtain transgenic banana plants *in vitro* (Villao *et al.*, 2021). In addition, the maturation of embryogenic cell suspensions can take eight to twelve months (Tenkouano *et al.*, 2011). The technique designed in the Dale *et al*. and Villao *et al.* experiments, mentioned above, could aid in genetically transforming various banana cultivars. That approach could also be considered to produce transgenic banana tolerant.

Recently, genome editing in plant breeding has been on the rise due to the development of CRISPR/Cas9 technology. This is partly because the CRISPR/Cas9 system allows for various methods of genome editing, depending on the repair route and the availability of repair templates (Bortesi and Fischer, 2015), contributing to its precision. As a result, with the open-access publication of the banana genome and the development of viable genetic transformation methods, gene editing systems such as host-induced gene silencing (HIGS) and CRISPR have been increasingly used to mutate specific genes, resulting in the creation of mutant plants without the need for foreign gene insertion (Tripathi *et al.*, 2019; Wang and Chen, 2020; Wang *et al.*, 2021). Hence, CRISPR technology has emerged as a promising option in genetic transformation programs to produce banana cultivars that are tolerantto saline environments. By considering this factor, the development of genetic engineering technology for producing saline-tolerantbanana cultivars can progress more smoothly than before.

Although there have not been many massive genetic transformation programs to produce banana cultivars that can tolerate and thrive in saline environments, scientists have successfully created saline-tolerantcultivars of other crops using genetic transformation. The information from these studies is becoming more relevant as various molecular mechanisms involved in the process are also known to occur in banana tissues. This information can help study genetic transformation techniques and their potential application in banana cultivars in the near future.

5. Concluding remarks and future prospect

Bananas are popular worldwide, but abiotic stress factors often limit their production. One such stressor is salinity, exacerbated by global warming, particularly in tropical regions like Southeast Asia with extensive coastlines. As the center of banana diversity, Southeast Asia has abundant genetic variability in wild and local bananas. This biodiversity richness can be utilized further to address the various challenges in banana cultivation. Additionally, countries experiencing higher water evaporation rates in agricultural lands than rainfall face significant risks from salinity stress.

However, developing elite cultivars tolerant to salinity stress presents a unique challenge, mainly since elite cultivar bananas are typically seedless and triploid or parthenocarpic. Various breeding and genetic engineering techniques can be employed to overcome these challenges. By using these techniques, it is possible to produce elite banana cultivars that are more tolerant to saline stress, have high yields, and are preferred by consumers in a shorter period. Therefore, continued efforts towards developing improved banana cultivars for salinity tolerance are necessary for the sustainable production of this vital crop.

The following are some essential topics related to banana breeding for saline tolerance that may be of significant research interest in producing an elite cultivar of saline-tolerant banana. Firstly, utilizing wild banana genetic resources alone or combined with mutation can enhance genetic variability for screening saline tolerance. Additionally, translational studies in model plants and omics approaches like genomics, transcriptomics, and metabolomics can uncover biological response and tolerance genes in bananas. Investigating the physiological and biochemical mechanisms underlying saline tolerance, such as ion transporters, osmoprotectants, and antioxidant enzymes, provides insights into the molecular basis of saline tolerance in bananas. Furthermore, improving micropropagation, embryogeniccell induction, and regeneration techniques facilitate genetic transformation and gene editing in diverse elite banana cultivars. Additionally, marker-assisted selection strategies integrated with molecular markers linked to saline tolerance traits can expedite the screening and selection of saline-tolerant genotypes. Finally, integrating traditional breeding approaches with biotechnological tools like marker-assisted backcrossing and genome editing can accelerate breeding progress and the development of this field.

As for the safety concerns surrounding the use of genetically modified crops, including transgenic bananas, this will still be a controversial topic for the foreseeable future. It is crucial for all stakeholders, including scientists and governments, to apply the precautionary principle when dealing with GMO products and technology. The emergence of gene editing approaches such as CRISPR/Cas9 offers an alternative approach to conventional transgenesis, which may change the acceptance of genetically modified products in the coming years.

Acknowledgements

The authors would like to sincerely thank the Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN) Indonesia, for the financial support of this study.

References

AN J.-P., LI R., QU F.-J., YOU C.-X., WANG X.-F., HAO Y.-J., 2018 - *An apple NAC transcription factor negatively regulates cold tolerance via CBF-dependent pathway*. - J. Plant Physiol., 221: 74-80.

ANUSUYA P., SOORIANATHASUNDARAM K., 2014 -

Screening studies on banana genotypes for salt stress. - Biochemical Cellular Arch., 14(1): 51-58.

AQUIL B., JAN A.T., SARIN N.B., HAQ Q.M.R., 2012 - *Micropropagation and genetic transformation of banana for crop improvement and sustainable agriculture*. - J. Crop Sci., 3(2): 64.

ASTUTIK A., 2009 - *Peningkatan kualitas bibit pisang kepok melalui radiasi sinar gamma secara* in vitro. - Buana Sains, 9(1): 69-75.

BORTESI L., FISCHER R., 2015 - *The CRISPR/Cas9 system for plant genome editing and beyond*. - Biotechn. Adv., 33(1): 41-52.

BOUDSOCQ M., LAURIERE C., 2005 - *Osmotic signaling in plants. Multiple pathways mediated by emerging kinase families.* - Plant Physiol., 138(3): 1185-1194.

CHERIAN S., REDDY M.P., 2003 - *Evaluation of NaCl tolerance in the callus cultures of* Suaeda nudiflora Moq. Biologia Plantarum, 46: 193-198.

CÔTE F.-X., DOMERGUE R., MONMARSON S., SCHWENDIMAN J., TEISSON C., ESCALANT J.-V., 1996 - *Embryogenic cell suspensions from the male flower of Musa AAA cv. Grand nain*. - Physiologia Plantarum, 97(2): 285-290.

CÔTE F., GOUE O., DOMERGUE R., PANIS B., JENNY, C., 2000 - *In-field behaviour of banana plants (*Musa *AA sp) obtained after regeneration of cryopreserved embryogenic cell suspensions. - Cryo Letters*, 21(1): 19-24.

CUARTERO J., BOLARIN M.C., ASINS M.J., MORENO V., 2006 - *Increasing salt tolerance in the tomato*. - J. Exp. Bot., 57(5): 1045-1058.

DALE J., JAMES A., PAUL J.-Y., KHANNA H., SMITH M., PERAZA-ECHEVERRIA S., GARCIA-BASTIDAS F., KEMA G., WATERHOUSE P., MENGERSEN K., 2017 - *Transgenic Cavendish bananas with resistance to Fusarium wilt tropical race 4*. - Nature Commu., 8(1): 1496.

DATTA S., JANKOWICZ-CIESLAK J., NIELEN S., INGELBRECHT I., TILL B.J., 2018 - *Induction and recovery of copy number variation in banana through gamma irradiation and low-coverage whole-genome sequencing*. - Plant Biotech. J., 16(9): 1644-1653.

DE LANGHE E., VRYDAGHS L., DE MARET P., PERRIER X., DENHAM T., 2009 - *Why bananas matter: An introduction to the history of banana domestication*. - Ethnobot. Res. Appl., 7: 165-177.

DEINLEIN U., STEPHAN A.B., HORIE T., LUO W., XU G., SCHROEDER J.I., 2014 - *Plant salt-tolerance mechanisms*. - Trends Plant Sci., 19(6): 371-379.

DEY A., SAMANTA M.K., GAYEN S., SEN S.K., MAITI M.K., 2016 - *Enhanced gene expression rather than natural polymorphism in coding sequence of the OsbZIP23 determines drought tolerance and yield improvement in rice genotypes*. - PLoS One, 11(3): e0150763.

DIKAYAN I., ANAS N., QOSIM A.A., WARI D., 2017 - *Response of shoot and root* in vitro *cultures of banana plant* (Musa acuminata *L.) cv. Barangan to salinity stresses*. - Asian J. Agric. Res., 11(4): 103-107.

ENDRIS S., MOHAMMAD M.J., 2007 - *Nutrient acquisition and yield response of barley exposed to salt stress under different levels of potassium nutrition*. - Inter. J. Environ. Sci. Technol., 4: 323-330.

EVANS E.A., BALLEN F.H., SIDDIQ M., 2020 - *Banana production, global trade, consumption trends, postharvest handling, and processing*. - Handbook of Banana Production, Postharvest Science, Processing Technology, and Nutrition, pp. 1-18.

FEDOROWICZ-STROŃSKA O., KOCZYK G., KACZMAREK M., KRAJEWSKI P., SADOWSKI J., 2017 - *Genome-wide identification, characterisation and expression profiles of calcium-dependent protein kinase genes in barley (*Hordeum vulgare *L.)*. - J. Appl. Gen., 58: 11-22.

GHAG S.B., GANAPATHI T.R., 2017 - *Genetically modified bananas: To mitigate food security concerns*. - Scientia Hortic., 214: 91-98.

GREENWAY H., MUNNS R., 1980 - *Mechanisms of salt tolerance in nonhalophytes*. - Annual Rev. Plant Physiol., 31(1): 149-190.

HASEGAWA P.M., BRESSAN R.A., ZHU J.-K., BOHNERT H.J., 2000 - *Plant cellular and molecular responses to high salinity*. - Annual Rev. Plant Biol., 51(1): 463-499.

HASIM A.A., SHAMSIAH A., HUSSEIN S., 2021 - I*nduced mutations using gamma ray and multiplication of plantlet through micro cross section culture of banana (*Musa acuminata *cv. Berangan)*. - IOP Conference Series: Earth Environmental Science, 757(1).

HESLOP-HARRISON J.S., SCHWARZACHER T., 2007 - *Domestication, genomics and the future for banana*. - Annals Bot., 100(5): 1073-1084.

INDRAYANTI R., MATTJIK N.A., SETIAWANA., 2012 - *Evaluasi Keragaman Fenotipik Pisang Cv. Ampyang Hasil Iradiasi Sinar Gamma di Rumah Kaca*. - J. Hortikultura Indonesia, 3(1): 24-34.

ISRAELI Y., LAHAV E., NAMERI N., 1986 - *The effect of salinity and sodium adsorption ratio in the irrigation water, on growth and productivity of bananas under drip irrigation conditions*. - Fruits, 41(5): 297-302.

JACOB P., HIRT H., BENDAHMANE A., 2017 - *The heat-shock protein/chaperone network and multiple stress resistance*. - Plant Biotech. J., 15(4): 405-414.

JALIL M., KHALID N., YASMIN OTHMAN R., 2003 - *Plant regeneration from embryogenic suspension cultures of* Musa acuminata *cv. Mas (AA)*. - Plant Cell, Tissue Organ Cult., 75: 209-214.

JI F., TANG L., YANG Z., LI Y., WANG W., XU Y., LI S., LI X., 2021 - *Transcriptome sequencing and comparative analysis of differentially expressed genes in the roots of* Musa Paradisiaca *under salt stress*. - Plant Biotech. Rep., 15(3): 389-401.

KAROLINOERITA V., ANNISA W., 2020 - *Salinisasi Lahan dan Permasalahannya di Indonesia*. - J. Sumberdaya Lahan, 14(2): 91.

KHUMAIDA N., ARDIE S., DIANASARI M., SYUKUR M., 2015 - *Cassava (*Manihot esculenta *Crantz.) improvement through gamma irradiation*. - Procedia Food Science, 3: 27-34.

KUSDIANTI, DININGRAT D.S., VIAWATI, WIDIYANTO S.N., 2016 - *P5CS and HSP 81-2 gene expression profile of banana (*Musa acuminata*)* in vitro *culture under salt stress condition*. - J. Plant Sci., 11(4-5): 91-95.

LIAO Y.-D., LIN K.-H., CHEN C.-C., CHIANG C.-M., 2016 - *Oryza sativa protein phosphatase 1a (OsPP1a) involved in salt stress tolerance in transgenic rice*. - Molecular Breeding, 36: 1-19.

LIU J., GAO P., SUN X., ZHANG J., SUN P., WANG J., JIA C., ZHANG J., HU W., XU B., 2017 - *Efficient regeneration and genetic transformation platform applicable to five* Musa *varieties*. - Electronic J. Biotech., 25: 33-38.

LÓPEZ J., RAYAS A., SANTOS A., MEDERO V., BEOVIDES Y., BASAIL M., 2017 - *Mutation induction using gamma irradiation and embryogenic cell suspensions in plantain (*Musa *spp.).* - Biotech. Plant Mutation Breeding: Protocols, pp. 55-71.

LUSSER M., PARISI C., PLAN D., RODRÍGUEZ-CEREZO E., 2012 - *Deployment of new biotechnologies in plant breeding*. - Nature Biotech., 30(3): 231-239.

MAHAJAN S., TUTEJA N., 2005 -*Cold, salinity and drought stresses: an overview*. - Archives Biochem. Biophysics, 444(2): 139-158.

MAHARANI S., KHUMAIDA N., SYUKUR M., ARDIE S.W., 2015 - *Radiosensitivitas dan keragaman ubi kayu* (Manihot esculenta *Crantz) hasil iradiasi sinar gamma*. - Jurnal Agronomi Indonesia, 43(2): 111-117.

MANOJKUMAR P., DHEEPANCHAKKRAVARTHY A., SENTHIL KUMAR P., TAMILSEVI S., SRINIVASAN C., 2022 - *Effect of physiological and morphological response of* Musa acuminata *under stress condition with different salinity levels using IoT*. - Int. J. Envir. Sci. Techn., 19(6): 5227-5238.

MANSHARDT R., 2004 - *Crop improvement by conventional breeding or genetic engineering: how different are they?* - UH-CTAHR, Biotechnology, Jan., BIO-5, pp. 1-3.

MATHEKA J., TRIPATHI J.N., MERGA I., GEBRE E., TRIPATHI L., 2019 - *A simple and rapid protocol for the genetic transformation of* Ensete ventricosum. - Plant Methods, 15(1): 1-17.

MAY G.D., AFZA R., MASON H.S., WIECKO A., NOVAK F.J., ARNTZEN C.J., 1995 - *Generation of transgenic banana (*Musa acuminata*) plants via Agrobacterium-mediated transformation*. - Bio/Technology, 13(5): 486-492.

MCCALLUM C.M., COMAI L., GREENE E.A., HENIKOFF S., 2000 - *Targeting induced local lesions in genomes (TILLING) for plant functional genomics*. - Plant Physiol., 123(2): 439-442.

MENGEL K., KIRKBY E.A., 1987 - *Principles of plant nutrition.*- Int. Potash Institute, Bern, Switzerland, pp. 687-695.

MIAO H., SUN P., LIU J., WANG J., XU B., JIN Z., 2018 - *Overexpression of a novel ROP gene from the Banana (MaROP5g) confers increased salt stress tolerance*. - Int. J. Mol. Sci., 19(10): 3108.

MUNNS R., TERMAAT A., 1986 - *Whole-plant responses to salinity*. - Functional Plant Biol., 13(1): 143-160.

NAKASHIMA K., TRAN L.P., VAN NGUYEN D., FUJITA M., MARUYAMA K., TODAKA D., ITO Y., HAYASHI N., SHINOZAKI K., YAMAGUCHI-SHINOZAKI K., 2007 - *Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice*. - Plant J., 51(4): 617-630.

NANDARIYAH N., YUNIASTUTI E., SUKAYA S., YUDHITA S.I., 2020 - *Selection for growth traits on M1V1 generation of raja Bulu Banana (*Musa paradisiaca *Linn.) obtained by gamma rays irradiation*. - *Caraka* Tani: J. Sust. Agric., 36(1), 97.

NGUYEN H.C., LIN K.H., HO S.L., CHIANG C.M., YANG C.M., 2018 - *Enhancing the abiotic stress tolerance of plants: from chemical treatment to biotechnological approaches*. - Physiologia Plantarum, 164(4): 452-466.

NOVAK F.J., AFZA R., VAN DUREN M., PEREA-DALLOS M., CONGER B.V., XIAOLANG T., 1989 - *Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (*Musa *spp.).* - Bio/Technology, 7(2): 154-159.

RAO N.K.S., SHIVASHANKARA K.S., LAXMAN R.H., 2016 - *Abiotic stress physiology of horticultural crops.* - Springer New Delhi, pp. 368.

RAVI I., UMA S., VAGANAN M.M., MUSTAFFA M.M., 2013 - *Phenotyping bananas for drought resistance.* - Frontiers Physiol., 4(9): 1-15.

RAVI I., VAGANAN M.M., 2016 - *Abiotic stress tolerance in banana*, pp. 207-222. - In: RAO N.K.S., K.S. SHIVASHANKARA, and R.H. LAXMAN (eds.) *Abiotic stress physiology of horticultural crops*. Springer, New Delhi, pp. 368.

RAVI I., VAGANAN M.M., MUSTAFFA M.M., 2014 - *Management of drought and salt stresses in banana*. - ICAR-NRCB, Technical folder, 6, pp. 1-6.

SÁGI L., PANIS B., REMY S., SCHOOFS H., DE SMET K., SWENNEN R., CAMMUE B.P.A., 1995 - *Genetic transformation of banana and plantain (*Musa *spp.) via particle bombardment.* - Bio/Technology, 13(5): 481-485.

SAIJO Y., HATA S., KYOZUKA J., SHIMAMOTO K., IZUI K., 2000 - *Over-expression of a single Ca_{2}^{+}-dependent protein kinase confers both cold and salt/drought tolerance on rice plants*. - Plant J., 23(3): 319-327.

SHARIF P., SEYEDSALEHI M., PALADINO O., VAN DAMME P., SILLANPÄÄ M., SHARIFI A.A., 2018 - *Effect of drought and salinity stresses on morphological and physiological characteristics of canola*. - Int. J. Environ. Sci. Techn., 15: 1859-1866.

SHEKHAWAT U.K.S., GANAPATHI T.R., SRINIVAS L., 2011 a - *Cloning and characterization of a novel stress-responsive WRKY transcription factor gene (Musa*

WRKY71) from Musa spp. cv. Karibale Monthan (ABB group) using transformed banana cells. - Mol. Biol. Rep., 38(6): 4023-4035.

SHEKHAWAT U.K.S., SRINIVAS L., GANAPATHI T.R., 2011 b - *MusaDHN-1, a novel multiple stress-inducible SK 3-type dehydrin gene, contributes affirmatively to drought- and salt-stress tolerance in banana*. - Planta, 234(5): 915-932.

SIMMONDS N.W., 1962 - *The evolution of the bananas.* - Longmans, Green, London, UK, pp. 170.

SONG S., XU Y., HUANG D., MIAO H., LIU J., JIA C., HU W., VALAREZO A.V., XU B., JIN Z., 2018 - *Identification of a novel promoter from banana aquaporin family gene (MaTIP1;2) which responses to drought and salt-stress in transgenic* Arabidopsis thaliana. - Plant Physiol. Biochem., 128: 163-169.

SREEDHARAN S., SHEKHAWAT U.K.S., GANAPATHI T.R., 2015 - *Constitutive and stress-inducible overexpression of a native aquaporin gene (MusaPIP2; 6) in transgenic banana plants signals its pivotal role in salt tolerance*. - Plant Mol. Biol., 88: 41-52.

TAK H., NEGI S., GANAPATHI T.R., 2017 - *Banana NAC transcription factor MusaNAC042 is positively associated with drought and salinity tolerance*. - Protoplasma, 254(2): 803-816.

TENKOUANO A., PILLAY M., ORTIZ R., 2011 - *Breeding techniques*, pp. 181-202. - In: PILLAY M., and A. TENKOUANO (eds.) *Banana breeding progress and challenges.* CRC Press, Boca Raton, FL, USA, pp. 383.

TRIPATHI L., NTUI V.O., TRIPATHI J.N., 2019 - *Application of genetic modification and genome editing for developing climate-smart banana*. - Food Energy Sec., 8(4): e00168.

TÜRKAN I., DEMIRAL T., 2009 - *Recent developments in understanding salinity tolerance*. - Environ. Exp. Bot., 67(1): 2-9.

VERMA E., SINGH S., MISHRA A.K., 2019 - *Salinity-induced oxidative stress-mediated change in fatty acids composition of cyanobacterium Synechococcus sp. PCC7942*. - Int. J. Environ. Sci. Techn., 16: 875-886.

VILLAO L., FLORES J., EFRÉN S.O., 2021 - *Genetic transformation of apical meristematic shoots in the banana cultivar "Williams".* - BioNatura, 6(1): 1462-1465.

VUYLSTEKE D., DE LANGHE E.A.L., 1985 - *Feasibility of* in vitro *propagation of bananas and plantains*. - Tropical Agric., 62(4): 323-328.

WANG J., CHEN H., 2020 - *A novel CRISPR/Cas9 system for efficiently generating Cas9-free multiplex mutants in* Arabidopsis. - Abiotech, 1(1): 6-14.

WANG X., YU R., LI J., 2021 - *Using genetic engineering techniques to develop banana cultivars with Fusarium wilt resistance and ideal plant architecture*. - Front. Plant Sci., 11: 617528.

WANG Z., MIAO H., LIU J., XU B., YAO X., XU C., ZHAO S., FANG X., JIA C., WANG J., 2019 - Musa balbisiana *genome reveals subgenome evolution and functional divergence*. - Nat. Plants, 5(8): 810-821.

XIANG Y., HUANG Y., XIONG L., 2007 - *Characterization of stress-responsive CIPK genes in rice for stress tolerance improvement*. - Plant Physiol., 144(3): 1416-1428.

XU Y., HU W., LIU J., SONG S., HOU X., JIA C., LI J., MIAO H., WANG Z., TIE W., XU B., JIN Z., 2020 - *An aquaporin gene MaPIP2-7 is involved in tolerance to drought, cold and salt stresses in transgenic banana* (Musa acuminata L.). - Plant Physiol. Biochem., 147: 66-76.

XU Y., HU W., LIU J., ZHANG J., JIA C., MIAO H., XU B., JIN Z.,2014 - *A banana aquaporin gene, MaPIP1;1, is involved in tolerance to drought and salt stresses.* - BMC Plant Biology, 14(1): 59.

XU Y., LIU J., JIA C., HU W., SONG S., XU B., JIN Z., 2021 - *Overexpression of a Banana Aquaporin Gene MaPIP1;1 enhances tolerance to multiple abiotic stresses in transgenic banana and analysis of its interacting transcription factors*. - Front. Plant Sci., 12: 699230.

YANG L., WU L., CHANG W., LI Z., MIAO M., LI Y., YANG J., LIU Z., TAN J., 2018 - *Overexpression of the maize E3 ubiquitin ligase gene ZmAIRP4 enhances drought stress tolerance in* Arabidopsis. - Plant Physiol. Biochem., 123: 34-42.

ZHANG H., LI W., MAO X., JING R., JIA H., 2016 - *Differential activation of the wheat SnRK2 family by abiotic stresses*. - Front. Plant Sci., 7: 420.

ZHENG L., CHEN S., XIE L., LU Z., LIU M., HAN X., QIAO G., JIANG J., ZHUO R., QIU W., 2018 - *Overexpression of cysteine protease gene from Salix matsudana enhances salt tolerance in transgenic* Arabidopsis. - Environ. Exp. Bot., 147: 53-62.