

Review

Rice Breeding in Vietnam: Retrospects, Challenges and Prospects

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Abstract: Rice breeding was conducted for a long time during historical times and is an important job in Vietnam because rice is the major food for domestic consumption and export. In this review, we have provided a comprehensive insight into the importance of promising rice germplasm resources, breeding achievements, and breeding approaches as well as discussed challenges and perspectives of rice breeding in this country. With rice germplasm and wild rice relative resources with rich and various genetic diversity, their useful genes and traits have been exploited and integrated into commercial varieties as the final outputs of rice breeding programs. New achievements of the modern genetics era have been approached and effectively contributed to breeding activities in this country. Genome sequences, molecular breeding, and mutation are powerful tools and playing vital roles in developing new varieties with characteristics of interest that should be followed by the current market demands. In the last decades, there has been a plethora of newly generated varieties by Vietnamese scientists and rice breeders and approved by the state authorities. However, very few domestic mega varieties have prevailed over the imported varieties. Therefore, rice breeding in this country is faced with big challenges, including limitations of backgrounds, budgets, and even talents in basic research to compete with other rice-producing countries. The target goals and long-term approaches for rice breeding should be paid explicitly in priority to ensure national food security and the advantage and development of rice breeding in this country.

Keywords: rice; molecular breeding; mutation; germplasm; genome sequence; wild rice relatives

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important crops and provides for daily meals for over half of the worldwide population, and estimated areas have grown approximately 163 million ha in over 100 countries [1]. Climate change and global warming have led to increasing severe abiotic and biotic stresses, which have been narrowing both rice

production areas and global rice productivity in the past decades [2]. Like other Asian rice-producing countries, rice was long cultivated in Vietnam and estimated for over 4000 years [3,4]. However, approximately two-thirds of the total 96 million Vietnamese people are working in agriculture and cultivating on less than 4 million ha in total [5]. Rice is grown in many areas in this country, especially in two major rice bowls of flat deltas, including the Red River and Mekong deltas (Figure 1). Most farmers are cultivating rice on small scales and have met with numerous difficulties to get enough income for their daily living expenses. Besides that, a large number of rice varieties are sensitive to biotic or abiotic stresses, which are the consequences of rice yield decrease or complete loss of harvest [6].

Vietnam is an agricultural country and is considered the center of rice genetic diversity where it is rich in various rice germplasms. Hence, breeding was long conducted during historical times in Vietnam [4]. Nevertheless, only after the green revolution, with the release of newly improved rice varieties carrying the semi-dwarf gene imported from the International Rice Research Institute (IRRI), the breeding activity was much encouraged. Starting from a country with a lack of food facing widespread poverty and chronic hunger, Vietnam has ensured national food security and become one of the biggest rice-exporting countries by increasing rice production by more than triple [7].

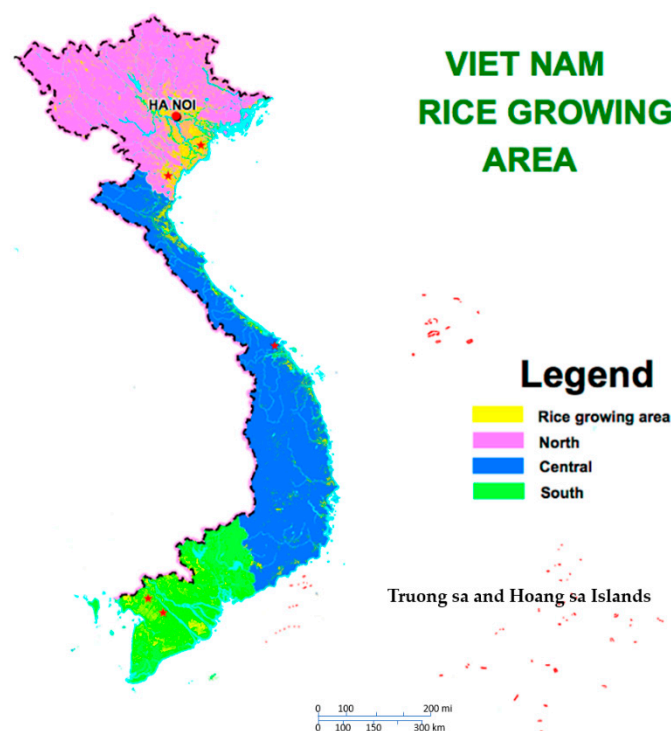


Figure 1. Ecological rice-growing map in Vietnam [8].

Famous imported rice brands such as NongNghiep 8 rice variety (Thannong 8 in the South) from IR8 were long cultivated [9], and CR203, adopted from IRRI, increased the average rice yield in Vietnam [9,10]. The imported Khang Dan 18, improved with higher quality and resistance to pests and diseases via the national breeding program [11], and Bac thom 7 (BT7), a high-quality variety with acceptable resistance to bacterial blight (*Xanthomonas oryzae*), has been cultivated [12]. Improved varieties such as Khangdan Dotbien (mutated rice variety), with BT7 resistance to bacterial blight and salinity tolerance, have been cultivated in many rice-growing areas in this country [12,13].

With the dramatic development of rice genetics and the advanced biotechnology applications in rice breeding as powerful tools, most obstacles in rice research have been gradually solved. The whole genomic sequence (WGS) of rice helps scientists accelerate and shorten time for breeding programs [14]. After full completion of the genomic sequence

(GS) from Nipponbare (RAP-DB 2008) and Kasalath [15], the 3000 rice genomes project was started (3000 rice genomes project, 2014) [16]. Variations of GS have revealed and effective marker systems such as SNPs [17], Indels, and SSRs [18] were established and sped up the rice breeding programs. By integration of the target genes, trait(s) of interests were introgressed into the elite varieties using molecular breeding such as marker-assisted selection (MAS) and marker-assisted backcrossing (MABC), which helps to minimize labor force and time needed compared with conventional breeding [19].

In Vietnam, several hundreds of native rice accessions have been re-sequenced to explore genetic diversity and trait association and a dozen newly novel QTLs involved in abiotic and biotic tolerance stresses have been identified [20,21]. Novel polymorphisms in two native Vietnamese aromatic japonica rice landraces were found that may facilitate the identification of the fragrance-related genes. GS information can provide a comprehensive background for MAS [22]. As the goal of rice breeding has been targeted, previously, Vietnam could not produce enough rice to serve the national population, but recently, is the second biggest rice-exporting country in the world [23]. However, as it is, rice production in this country is faced with the decline of rice-growing areas due to industrialization and severely adverse impacts from climate change such as high sea-level rise, salinity, and submergence inundation. It is anticipated that the rice yield of two rice bowl deltas (Red River and Cuu Long River deltas) will be reduced by at least 2.2% and 5.6% by 2030 [24]. In addition, the world population is rapidly increasing and climate change is causing adverse impacts on rice production. Breeding new varieties adapted to unfavorable conditions must be done to stabilize rice yield and ensure worldwide food security [25,26].

Rice breeding is an imperative job in Vietnam. In recent years, numerous studies on molecular breeding and exploiting genes of interest from rice germplasm integrated with improved rice varieties to develop abiotic and biotic tolerances of rice via the national rice breeding programs have been extensively carried out. Much information is available. However, the reviews on rice breeding have appeared sporadically. Buu and Lang [23] and Du and Loan [26] documented some achievements of conventional molecular breeding, and improved rice breeding in the intensive cropping system in Cuu Long Rice Research Institute, Mekong Delta. Hence, the objectives of this review are to (1) highlight the importance of promising rice germplasm resources and typical achievements of rice breeding over the decades, (2) address the current breeding approaches (conventional and molecular approaches) of the target rice breeding programs, and (3) discuss the challenges and perspectives and indicate further works needed about rice breeding in this country.

2. Rice Germplasm: An Important Input

Vietnam is considered the center of rice variation, with vast genetic diversity because of its geographical situation, latitudinal range, differences in habitats, and diversity of ecosystems, and has over 4000 years of rice cultivation experience [27–30]. Most rice germplasms in Vietnam are comprised of indica and japonica types and wild rice relatives and are considered the traditional, native rice landraces.

Most aromatic varieties in the north of Vietnam are considered the japonica type, with some unique aromatic indigenous rice landraces such as Tam Thom, Nang Thom Cho Dao, Seng Cu, Nep Cai Hoa Vang, etc. They possess various traits of interest such as aroma and tolerance to abiotic and biotic stresses [24]. Numerous wild rice relatives can be found throughout the country, including *O. rufipogon*, *O. nivara*, *O. officinalis*, and *O. granulata*, for example, *O. officinalis*, *O. fatua*, and *O. granulata* are found in the northern and western areas; *O. fatua* is found in the north-central coastal region (Thanh Hoa province); *O. latifolia* is found in the swamp areas; *O. nyriana* and *O. granulata* are found in the forest of the highland region (Tay Nguyen province); and *O. rufipogon*, *O. nivara*, *O. minata*, and *O. fatua spontanea* are found in the Mekong Delta (Figure 2). Generally, most wild rice and its relatives in Southeast Asia can be found in Vietnam [4], and are valuable materials and provide great potential for rice breeding programs to cope with abiotic and biotic stresses. Tan et al. [31] successfully selected a rice variety with short growth duration

by consecutive backcrossing between indica IR24 and *O. rufipogon* from BC4F5. Three groups of Vietnamese rice varieties were classified as A, B, and C using RFLP analysis. Of these, group A corresponded to the indica rice varieties, whereas, based on the variation of chloroplast DNA, groups B and C belonged to the japonica subspecies [32]. Genetic diversity analysis on the cultivated rice landraces by using enzymatic polymorphism of more than 1000 rice varieties of South Asia revealed that the germplasm derived from the northern mountainous areas in Vietnam have the highest index of genetic diversity [33].



Figure 2. Some wild species of *Oryza* available in Vietnam. The photos were collected by some scientists [34–37]. The photo of *O. granulata* was taken by Dr. Rod A. Wing (IRRI).

Naturally, these cultivars may possess potential genetic resources involving traits to adapt to specific environmental conditions [38,39]. One of the most famous native rice varieties, Tetep, is rich in a blast genetic resistance source and has been frequently used as the resistant donor plant in numerous worldwide studies [40–42]. The blast resistance gene of this variety, *Pi-kh*, was mapped and cloned by a map-based cloning aspect [42]. Other novel blast-resistance genes, *Pi-tp(t)* and *Pi-ta/Ptr*, were identified using near-isogenic lines [43] and have been introgressed into some US rice germplasms and cultivars [44,45]. Another QTL, *qSBR11-1*, which confers resistance to sheath blight (*Rhizoctonia solani*), was detected on chromosome 11 and named *LOC_Os11g47510* as a novel rice chitinase gene [46]. Like other genetic resource centers introduced by IRRI, the Genetic Resource Center, NARO (NGRC) in Japan, the rice germplasms of the different plants have been conserved in the Plant Resources Center (National Gene Bank of Vietnam). A total of 11,356 cereal samples, including rice, are stocked in this center [47], of which Vietnamese native rice germplasms constitute about 6000 accessions [48]. Meanwhile, the gene bank at CLRRRI (Cuu Long Rice Research Institute) and Vietnam National University of Agriculture has collected and recognized over 2000 rice accessions, which are being used for rice breeding programs [30]. Among them, it has been reported that 158 accessions showed good quality, 144 were resistant to blast, another 100 resisted bacterial blight, and 30 accessions revealed resistance to other insects/pests or diseases [49].

3. Commercial Rice Varieties, an Output of Breeding Activities

Over the last decades, Vietnam terminated the period of rice import and started exporting over 1 million tons of milled rice in 1986 [50]. During the innovation period (1986–1999) “Doi moi”, the growth rate, growing areas, yield, and production of rice were strikingly increased to 7.65 million ha and attained 31.5 million tons [50]. The leading rice varieties at this time could be listed as Moc Tuyen and Tran Chau Lun in the north; and Trang Chum, Nanh Chon, Than Nong 73-2, TN5, TN8, and Taichung 1 in the south. Some mutant varieties were released at the University of Agriculture and VASI to saline regions [51]. Owing to international collaboration (IRRI), newly improved rice varieties with a much higher yield compared to the leading local varieties were tested and released to farmers, such as IR8, NN75-2, NN75-6, NN75-10, Xuan No-2, and CR203 in the north and Than Nong 73-2, IR36, IR42, IR19660, IR48, IR4570, IR8423, and IR64 in the south. Some high-yield varieties were introduced from China, such as Q5 and Khang Dan. These varieties have helped to continuously enhance the average grain yield of 0.11 tons per ha for over 20 years [51].

Hybrid rice was first tested in Vietnam in 1991. It was rapidly expanded up to 11,000 ha in 1992 and continuously significantly increased by 73,000 ha in 1995, 437,000 ha in 2000, and 710,000 ha in 2009 [51], up to October 2013, when 516 rice varieties were released. Of those, 342 varieties are pure sticky and non-sticky and the remaining 174 are hybrid rice, including two-line hybrid and three-line hybrid. A total of 194 varieties were permitted to commercially produce [52], as shown in Figure 3.

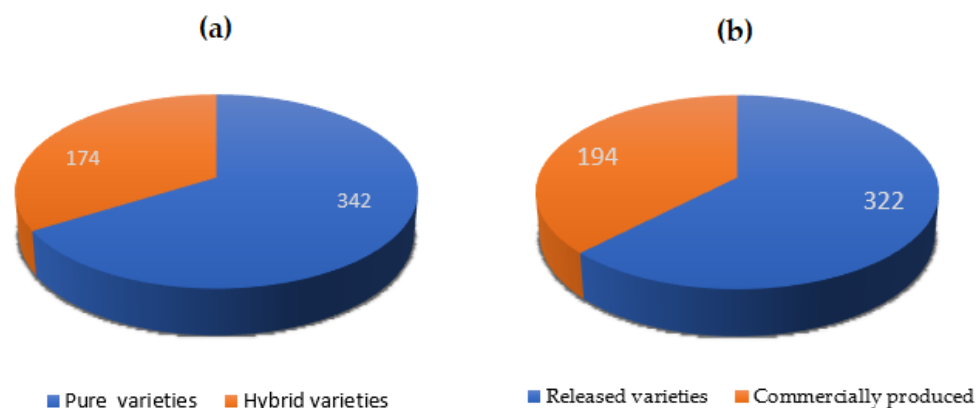


Figure 3. Number of released rice varieties in Vietnam up to October 2013. (a) Number of pure rice and hybrid varieties and (b) number of commercial rice varieties.

Among the released rice varieties, 12 are sticky rice, 14 are hybrids, and 168 varieties have been classified as “non-sticky rice” to differentiate from sticky rice, including both indica and japonica subspecies. Up to date, for three years from 2017 to 2019, a total of 197 varieties were accepted for the plant breeder’s rights [52] and assigned; 144 varieties were derived from companies; 37 varieties were released by institutions, universities, and breeding centers; and 16 varieties were introduced by persons or groups of people [52], as presented in Figure 4. Commercial varieties are normally derived in two ways, including conventional breeding and domesticated selection. Recently, some typical japonica rice varieties were imported from Japan, such as Koshihikari, Hinohikari, etc., with different brands and have been being cultivated and consumed in Vietnam. In the south, some varieties that are regularly planted can be seen, such as Fuji Sakura, sushi rice, Hitomebore, and Nigata Koshihikari [53]. In the north, another brand of japonica rice from Japan that is mostly planted are the J01, J02, and QJ4 varieties [53].

One of the most popular varieties that is widely cultivated in the north is BT7 and was imported from China over 30 years ago. Other quality varieties such as Jasmine 85 from IRRI and Dai Thom No. 8 from Taipei have been being exploited for both domestic consumption and exportation.

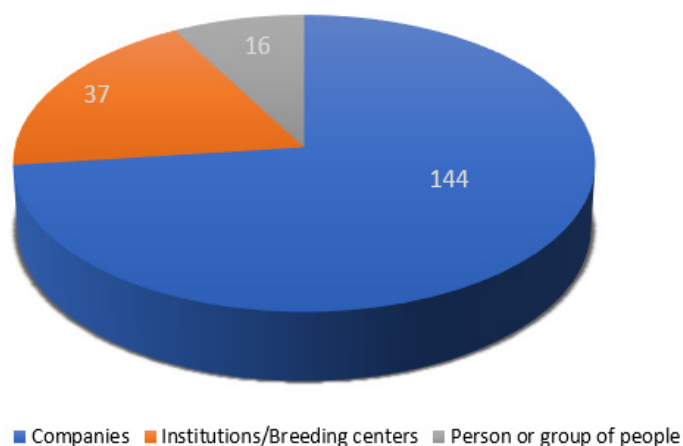


Figure 4. Number of rice varieties were assigned for plant breeder’s rights in 2017.

According to the report by the Department of Plant Production in 2015 [54,55], there were 255 rice varieties, including 155 inbred rice, 81/85 hybrids, and 9/22 sticky rice varieties, which was equivalent to 88%, 10%, and 2%, respectively. However, only 66 rice varieties were inbred, 5 were sticky rice, and 15 were hybrid varieties, which accounted for 91% of rice growing in this country (Figure 5). Among them, there were 12 main rice varieties, including inbred non-sticky rice and hybrid, being grown in most areas across

the country and reached 47% of total rice-growing areas (Figure 6). They included IR50404, OM5451, OM4900, Khang dan 18, OM6976, BC15, Jasmine 85, OM4218, Bac Thom 7, Nhi Uu 383 (hybrid), and TH3-3 (hybrid) (Figure 6).

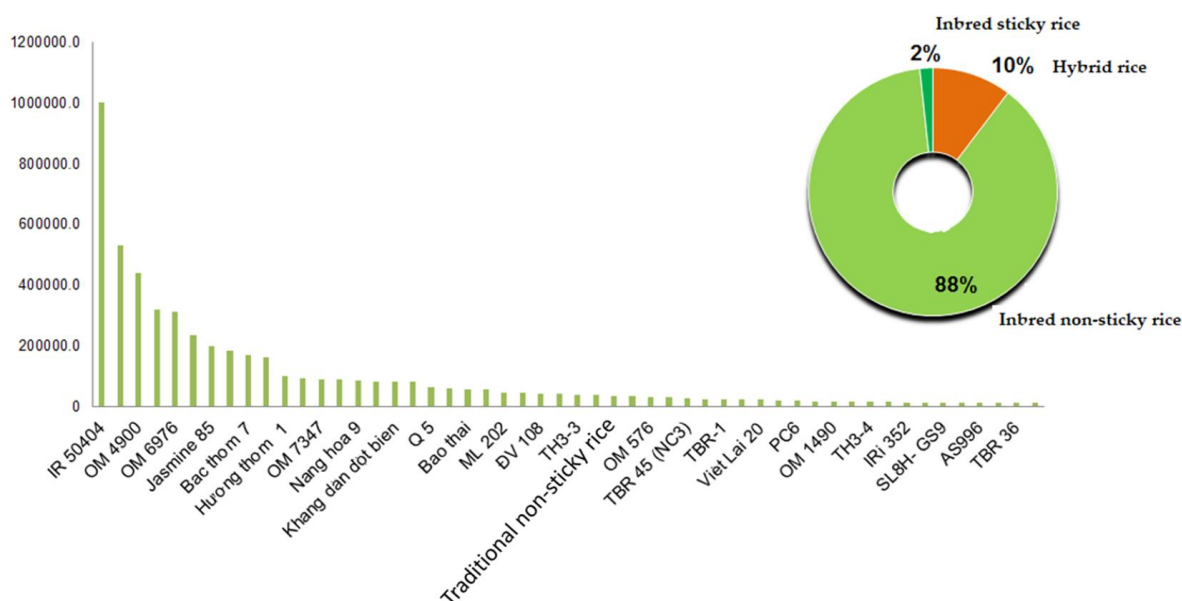


Figure 5. The structures of rice varieties grown in Vietnam.

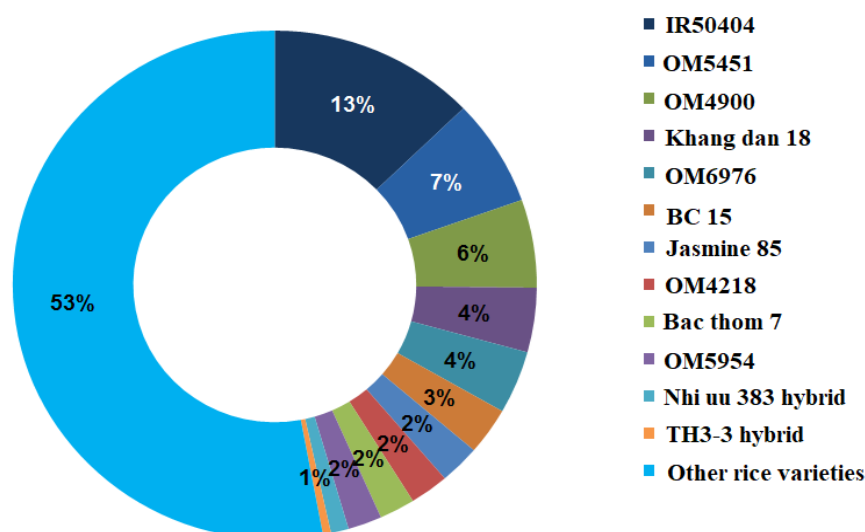


Figure 6. Major rice varieties are grown in Vietnam.

In recent years, numerous newly improved rice varieties have been developed by Vietnamese scientists and rice breeders. However, it has been estimated that still approximately 50–70% of the rice varieties being grown in the southern deltas are imported from IRRI, whereas the improved varieties imported from China have been planted in the northern deltas [56,57].

4. Breeding Goals by Applying Newly Improved Rice Varieties

In fact, the high yield of rice is a key trait that most released varieties must fulfill. The improved rice cultivars should reach an average yield at least higher than that of the standard varieties in the same group. Some newly imported varieties such as “new plant type variety” and “high supper rice varieties” have been released to the farmers. However, those varieties need special intensive farming techniques and are very sensitive to native

pests, insects, and diseases. Hence, it is difficult to develop them as a mega variety in this country. The newly improved ones need to be typically involved in a high-quality trait such as low amylose content, high protein, aroma, and/or resistance to biotic or abiotic stresses or possess advantageous characteristics. Initial screening to select materials is a prerequisite to initially launching any breeding program [58].

4.1. High-Yielding Rice Cultivars

The average rice yield in Vietnam in 2017 was 555 quintals per hectare, according to the published data from General Statistics Office of Vietnam [59]. Compared to the average yield produced in 1995 (36.9 quintals/ha), this was equal to a 50.4% increase. This made a dramatic change in the total amount of rice, from 24.9 million tons in 1995 to 42.8 million tons in 2017, which was increased by 71.5% of the total rice amount. Breeding and applying new rice varieties helped bridge the gap and promote self-supply, and have made Vietnam the leading rice-exporting country in the world [60]. Rice yield is dependent on multiple complexes of traits, such as plant vigor, heterosis, plant height, number of tillers per hill, panicle length, number of grains per panicle, grain weight, and filled seed percentage, etc. The most common way is using hybrid varieties that increase the heterosis of plants and therefore increase yield. By acquiring hybrid rice from China and IRRI, the change in yield was up to 81.1% compared with the check variety CR203 at that period [61].

4.2. Improvement of Biotic and Abiotic Stress Tolerances to Stabilize Rice Production

The exploitation of resistant varieties helps to prevent damage and infestation of diseases, and stabilize and increase grain yield of rice cultivars to cope with adverse impacts from climate change. By screening 500 varieties for blast disease resistance in the Mekong Delta, Luu et al. [62] reported that 23 varieties showed high resistance and 80 varieties were moderately resistant to blast. Inbred line PB10, derived from the cross between N46 and BT13, showed short growth duration, high yield, high grain quality, and relatively good resistance to blast and bacterial leaf blight compared with the check varieties [63]. Jasmine 85, a high-quality variety, has been introgressed with the *Pita* gene, the blast disease resistance gene *Pikp*, and *Bph14*, a brown planthopper resistance gene [64]. In case of bacterial leaf blight, using a resistant cultivar is one of the most effective ways to control this disease. The *Xa-4* gene had been used for a long time and became susceptible due to the appearance of new bacterial races [65]. Other genes resistant to bacterial leaf blight were screened from Vietnamese rice landraces to use for breeding programs [66,67]. *Xa-7* was successfully introgressed into the restorer line to develop a two-line hybrid resistant to bacterial blight [68]. Recently, the integration of the candidate genes *xa5*, *Xa7*, and *xa13* by exploited from the rice landraces was successfully pyramided in the elite rice variety [69]. Discrimination and classification of the races, virulence, and diversity of bacterial blight from northern Vietnam and an assessment of the interaction between resistance genes and virulence genes has been carried out and effectively exploited to stabilize rice production [70,71].

Abiotic stresses have recently been a hot topic because of the severely adverse impacts of climate change on worldwide crops. Vietnam is the most vulnerable nation to climate change impacts, particularly the Red River delta and Mekong delta, where the largest rice production occurs in this country. However, salinity intrusion and submergence are causing severely adverse impacts on rice production in these areas [72]. In Mekong delta, salinity has become the most serious problem, with sea-level rise, saline water intrusion, and freshwater exhausted by the presence of dams, reservoirs, and hydroelectric plants from upstream in China [73,74]. That fact requires anticipated strategies to improve and stabilize rice productivity and yield to maintain food security as well as stabilize farmers' daily life [75]. Three native rice landraces, Lua Soi, Mot Bui Hong, and Nang Quot Bien, were analyzed for salt tolerance compared with the check variety Doc Phung. These varieties showed high salt tolerance at level 5 with 10% to 12.5% salinity, respectively [76]. Similarly, three other native rice varieties, Chanh trui, Cuom dang 2, and Nep cuc, disclosed a strong

salinity tolerance (level 3) in both bioassays and field screening [77]. Rice variety BT7 was introduced with *Saltol* QTL to improve salt tolerance by crossing it with FL478-*Saltol*, a salt tolerance donor variety. Improved lines after several backcrossing generations and self-pollinating carried the *Saltol* allele of the donor parent FL478 and major agronomic traits were similar to genetic background recipient donor BT7 [72]. In other reports, the same QTL, *Saltol* from FL478-*Saltol* was introgressed into an AS996 recipient parent, a popular cultivar planted in the south of Vietnam. Information on genetic background was surveyed with approximately 500 SSR markers to attain the genome content of AS996 [78]. In the same way, *Saltol* was introgressed into Q5DB to improve the salinity tolerance lines carrying *Saltol* with the genetic background of the recipient parents [79].

Since salinity is the most serious problem of abiotic stress, submergence is also a big trouble in rice production in Vietnam, especially in the Red River delta. Experiments on the complete submergence conditions of some commercial rice varieties were conducted, and the results showed that the yield of the CR203 variety decreased severely after five days of full submergence treatment. At the early tillering stage, the Tap Giao 4 cultivar was dead after the experiment ended, and the Moc Tuyen variety showed severe damage due to the impact of submergence [80]. Lines carrying *Sub1* QTL derived from the cross between AS996 and IR64, a submergence tolerance variety, could be recovered after submergence stress lasted for 21 days with a similar level as that of IR64 [81]. BT7-*Sub1*, the improved BT7 line carrying *Sub1* QTL, obviously affirmed an equal tolerance level compared with the donor parent IR64-*Sub1* after 14 days in complete submergence and recovered rapidly after de-submergence [82]. In contrast to submergence, drought stress has seriously increased in recent years and is one of the main constraints of rice production that needs to be judiciously considered. Vietnam is situated downstream of rivers that originate in China. Drought frequently occurs due to the appearance of a lot of hydropower plants [83] and is one of the reasons causing irrigated water deficiency stress in the deltas [84]. In the early months of 2020, the Mekong River delta was devastated and suffered 33,000 hectares of rice field losses and had 362,000 severely affected hectares of rice [85]. By screening more than 3000 rice lines/cultivars in different conditions, including bioassays, greenhouse, and field trials, a set of varieties that showed drought tolerance was selected [86,87]. In another work, 75 rice lines were analyzed using SSR markers to detect polymorphisms and make potential crosses for drought tolerance. Of them, eight crossed combinations showed characteristics expected to develop drought tolerance cultivars that should be useful for breeding programs [88].

Wild rice relatives (*O. rufipogon*) and sub-species Japonica from introgression lines (ILs) and chromosomal segment substitution lines (CSSL) were tested to screen drought tolerance lines at the tillering stage. With different mechanisms such as an increase in root growth, water uptake, or reduction in leaf number and leaf area, these lines could be ideal materials for drought tolerance breeding [89]. Two QTLs related to root length and two other QTLs associated with dry root weight were identified from the cross between OM1490 and WAB880-1-38-18-20-P1-HB, which contributed useful information to the molecular breeding of drought tolerance in rice [90].

4.3. High-Quality Rice for Export

As mentioned before, rice production has strikingly increased from the “Doi Moi” period that brought Vietnam from a lack of food to self-supplication to one of the top three rice-exporting countries in the world. The quantity and value of rice exportation improve year by year due to the application of high-yield and -quality varieties, with new production technology [91]. The grain quality of 55 rice varieties collected from the coastal provinces in the south was evaluated and scored with various categories, including intermediate amylose and protein contents [92]. Milling quality, physical properties, and cooking quality from 148 varieties were analyzed to identify good grain quality [93]. Pham et al. [94] examined 11 lines/varieties selected from the landraces and developed a cross combination for high-quality characteristics. The quality of parental lines for hybrid breeding was

analyzed to improve the quality of hybrid rice. Of the results attained, eight lines were identified as carrying aromatic *fgt* gene including three TGMS lines, two restorer lines were low amylose contents [95]. Two promising combinations were selected for high yield, good quality, slight fragrance, growth duration, and plant type suitable for Red River delta cultivation conditions [96].

4.4. New Medicinal Rice Varieties for Health Care

The appearance of golden rice carrying β -carotene has made a great change in the supplementation of vitamin A in daily meals instead of using vitamin as a medicine or drug for targeting vitamin deficiencies [97]. However, golden rice is a genetically modified crop (GMC), hence causing national controversies and worldwide debates and not allowed to grow in this country. Diabetes patients will have reasons to be delighted by the inhibition of this disease by momilactones A and B extracted from rice bran, which was detected in high amounts in Vietnamese rice varieties [98]. They have exhibited potential inhibitory activities on pancreatic α -amylase and α -glucosidase and skin aging inhibitors, which are even higher than those of γ -oryzanol, which is commonly used [99,100]. In Vietnam, medicinal rice is now receiving much attention by people due to the dramatic change in the economy and health care demands that have led to a malnutrition situation. Rice varieties with high iron content were developed, namely, OM6976, OM5451, OM5472, and OM3995, which contain more than 6 mg/kg of iron in white rice, similar to the iron levels of the international high-iron variety IR68144 [101]. Although Vietnam is among the important worldwide rice producers and exporters, very few Vietnamese scientists work in the field of nutrition and medicinal rice compared to those of other traits because of the high pressure for studies on high yield and abiotic and biotic stress tolerance of rice, which are still a priority.

5. Rice Breeding in the Modern Genetics Era

Rice breeding has advanced dramatically through the development of new technologies of genetic and molecular breeding since the availability of full reference genome sequences of rice. These achievements have revealed the shadow of rice genetics, which is quite different from traditional genetics and conventional breeding. Mechanisms of gene expressions and gene interactions at molecular levels have been better understood. Presently, a large number of useful genes and QTLs and various genetic resources against abiotic and biotic stresses have been identified over the last two decades, which has increasingly facilitated genomic assisted breeding in nature to release acceptable rice varieties with prearranged genetic compositional traits of stress tolerance QTLs and genes [102]. However, what we need, in the case of Vietnam, is to effectively apply for great outputs of modern genetics such as riding the wave to reach rice breeding targets.

5.1. Genome-Wide Association Studies (GWAS)

Nowadays, information on rice genomes from the typically different rice varieties could be easy to perform by next-generation genome sequencing (NGS). With a large number of common SNPs, with genome-wide association studies, a full understanding of allelic variation in natural populations will be possible [103]. Many QTLs exist in wild rice species and rice landraces could be simultaneously identified and then promptly applied via advanced breeding programs. By using 3.6 million SNPs from sequence data of 517 rice landraces, 14 important agronomic traits have been analyzed with both the simple model and the compressed mixed linear model (MLM) and a total of 80 associations for these traits have been identified [104]. In another report, four new genes from 26 loci that were associated with agronomic traits were identified based on the estimated effects of nucleotide polymorphism from 176 Japonica rice varieties developed in breeding programs [105]. New QTL tolerance to water deficits during the vegetative phase was detected by comparing identified associations with the 442 QTLs for drought tolerance-related traits in 180 Vietnamese rice landraces [21]. Root traits (root length, root mass, root thickness,

and number of crown roots) in a panel of 182 varieties (115 Indica accessions, 64 japonica accessions, and 3 controls: Nipponbare, IR64, and Azucena) were investigated to identify the significant associations used in genome-wide association mapping. A set of QTLs was detected in all examined traits. In the attained results, 13 additional candidate genes related to these mentioned traits were revealed [106]. A recent report studied 155 Vietnamese rice accessions conferring jasmonate correspondence on the growth regulation of shoot and root by using GWAS, and found three QTLs and 232 regulated genes that were involved in stress response [107]. Simultaneously, Hoang et al. [108] examined a panel of 180 Vietnamese rice landraces using 21,623 SNPs markers and GWAS for some traits of rice leaves in the vegetative stage and identified dozens of QTLs that are significantly related to the phenotypic variation of leaf mass traits. Our recent study resequenced 672 Vietnamese rice landraces and compared their genomes, including population structures and genetic diversity, to 3000 genomes of Asian cultivated rice. We found 21 new QTLs involved in 12 traits involved in genome composition and trait associations among the adaptation diversity of rice landraces in this country [20].

5.2. Molecular Breeding

Marker-assisted selection (MAS) and marker-assisted backcrossing (MAB) are common terms in molecular breeding that have been widely applied in plant breeding programs. With the development of genome sequencing (GS), various systems of DNA markers (RFLP, RGA, AFLP, RAPD, STS, SSR, SNP, etc.) have been established, which made the big change and have widely become an approach for precision plant breeding in the 21st century. The vast number of QTL-mapping studies in crop species has contributed to a rich source of marker-trait associations [109]. Molecular breeding helps plant breeders expedite three major purposes: (i) to trace favorable alleles across generations to accumulate favorable alleles, (ii) to identify the most suitable individuals among segregating progenies, and (iii) to avoid linkage drag of undesirable loci [110]. Four bacterial blight resistance genes, *Xa-4*, *xa-5*, *xa-13*, and *Xa-21*, were incorporated into the IR24 variety and pyramided lines showed a wider spectrum and higher resistance level than that of lines with only a single gene [111]. Besides that, linkage drag with unfavorable traits could be controlled [112].

In Vietnam, molecular breeding (MAS and MABC) has been extensively used for rice breeding for the last 10 years. Salt tolerance lines were successfully developed from the cross between IR64 and OMCS2000 by applying SSR marker RM223, which significantly associated with the salt tolerance locus on chromosome 8 with an accuracy of more than 95% [113,114]. *Saltol* QTL derived from FL478 was introduced into the BT7 variety by MABC. The genetic background of the selected lines was significantly attained [115]. In order to generate heat tolerance in rice, 50 lines carrying heat QTLs from the donors N22 and Dular were selected and tested for heat tolerance, of which 12 lines harboring a homozygous allele to QTL at the RM3586 marker on chromosome 3 and the RM3735 marker on chromosome 4 were selected to further evaluate agronomic traits [116]. A QTL conferring a number of grains per panicle from KC25 was successfully introduced into KD18 by MABC [117,118]. Two SSR markers tightly linked to this QTL were used to screen progenies of BC1F1 populations and 84 individuals in a total of 312 individuals carrying this QTL were identified. Advanced generations BC2F1 and BC3F1 were developed for future selection of near-isogenic lines carrying grains/panicle QTL [118]. In another study, the target gene *fgr* involving aromatic traits and bacterial blight resistance genes *Xa4*, *xa5*, and *Xa7* were pyramided by MAS to improve bacterial leaf blight resistance in aromatic rice varieties HT1, HDT8, BT7, Nghi Huong, SH8, AC15, and N46. Advanced generation BC5F3 carrying *fgr* and/or *Xa4*, *xa5*, and *Xa7* were developed and evaluated for agronomic traits, aroma, and bacterial blight resistance [119]. In a new modern genetic era, molecular breeding is playing a vital role in breeding programs in this country.

5.3. Mutation Breeding

Conventional breeding can promote plant vigor in hybrids and introduce new traits from one individual to other individuals. Mutation breeding frequently uses mutant factors (chemical, radiant, physical, etc.) to generate huge changes in DNA molecules that can be inherited by the next generations. By rearranging DNA sequences, expected new traits may come out and unexpected ones could be replaced or permanently removed [120]. To the best of our knowledge, a total of 823 rice mutant events have been released from 30 countries in the world, of which Japan and China have been responsible for 26.8% and 35.6% of this total, respectively [121]. In Vietnam, mutation breeding was first reported in the early 1970s and was documented by Vinh et al. [122]. However, since 1992 rice mutation breeding has been intensively performed, leading to the successful selection of mutant rice cultivars with typical and distinct traits, which remarkably enhanced socio-economic impacts in this country [123]. Moreover, Vietnam was on the list of top 10 countries with the highest achievements in mutation breeding in crops [124]. In the case of rice, a total of 32 varieties had been released and DT10 rice variety had reached the cultivation area of 1 million ha (1990 to 2009). Anther mutant variety, VND95-20, which originated from IR64, extended up to 900,000 ha within three years [122]. Some promising mutant varieties, namely, TNDB-100, VND95-19, VND99-3, VN212, VN214, OM2717, and OM2718, have been released and grown for large-scale production in the Mekong River delta [123]. A set of local aromatic rice varieties was used to develop the improved aromatic varieties using a gamma-ray. From a total of 20 varieties, 10 improved ones were obtained after treatments. Agronomic traits and zinc and iron contents were analyzed for comparison and differences from the original varieties were found [125]. Low phytic acid mutant rice could prevent a lack of iron and zinc in the human body due to the anti-nutritional factor that reduces the bioavailability of iron and zinc [126]. The changes in phytic acid fluctuation levels were also investigated in the mutant lines [127]. The volatility of several genetic traits and characteristics of mutant lines after repeated irradiation treatment were surveyed to find the advantages of the mutation induction method [128]. Changes in DNA molecules were also characterized in the mutant lines to better understand the effects of irradiation on the rearrangement of genetic materials. By using RAPD and microsatellite markers, Hoang et al. [129] investigated genetic changes for salt tolerance from two Vietnamese rice mutants. A G-statistic demonstrated that comparisons between Am and TT, M2-2, and M2-4 revealed moderate genetic differentiation between TT and M2-2 or M2-4 exhibited low genetic differentiation. Ten percent of polymorphisms were detected in both RAPD-PCR and RAMP-PCR between TT, M2-2, M2-4, and Am [129].

5.4. Genetic Engineering and Genome Editing Studies

Vietnam is a leading rice-producing country and genetically modified rice is not allowed to be commercialized yet. However, studies on rice transformation have been conducted in limited and strict spaces over the years even with the ongoing national controversy taking place [130,131]. Transgenic rice lines resistance to insect/pests were developed using an *Agrobacterium tumefaciens*-mediated transformation. Two constructs carrying *CryIAb* and *CryIIAc* were transformed into IR64 and Mot Bui varieties, selected through a mannose selection system, and confirmed by using a Southern blot. The homozygous transgenic lines (F8) were selected and developed, and showed high resistance to yellow stemborer in a bioassay [131]. Another report on the *GUS* gene (β -glucuronidase), the most common use reporter gene, was expressed with a different promoter in Indica rice variety IR68144. The presence of *GUS* was further confirmed by PCR and a Southern blot contributed to the expression in transgenic plants [132]. In another study, the transformation protocol to transform *GUS* via *A. tumefaciens* into Taichung 65, a japonica variety, was optimized. *GUS* expressions were detected in several different tissues of transgenic plants and showed stability by examining through the next generation [133]. The effectiveness of the transformation system was also analyzed to investigate factors affecting the efficiency of transformation. Callus treatment, light regimes, and mannose were adjusted and modified

to find the most suitable condition. Transgenic Taipei 309 plants carrying the *PMI* gene (Phosphomannose isomerase) were selected and were confirmed by PCR [134]. By applying advanced biotechnologies, genome editing can be done using the CRISPR/CAS system in rice to mutate target genes and at specific genomic sites [135]. Plant breeding can be sped up using the CRISPR/Cas9 system and plant breeders can precisely modify the DNA of crop plants [136]. Accurate genome editing using a highly versatile, cost-effective, and highly superior CRISPR/Cas system opens the prospects for the research communities and can be widely applied in the improvement of plants in the foreseeable future [137]. So far, worldwide achievements with 109 modified genes in rice have been documented, which is the largest number of modified genes compared to other cereals [138]. As mentioned before, with great potential applications, the applications and studies of this achievement are still at the infant stage in this country. A recent study by Phuong and Hoi [139] successfully designed the gRNA sequence to edit the *OsP5CS* gene to improve the salt and drought tolerances of the BC15 rice variety by CRISPR/CAS9. However, in Vietnam, limitations in research budgets and scientific backgrounds are the main constraints to implement this technique in rice and other major crops.

6. Challenges and Perspectives

As indicated above, Vietnam is one of the richest rice germplasm countries with over 4000 years of rice-cultivating experience. However, most landraces and germplasms have been sporadically planted by ethnic minority people in the mountainous and outback areas due to their long growth duration and low yield. Since imported rice varieties were introduced, the rice yields have significantly increased for both national security and export. Nevertheless, those varieties have been imported from nearby countries such as Thailand, China, and IRRI, etc., and their genetic backgrounds have been therefore poorly understood [57]. Moreover, these varieties are neither tolerant and resistant nor sensitive to both abiotic and biotic stresses compared to the native landraces. For instance, Bachthom 7 (BT7) was imported from China for its good quality and stable yield; however, this variety is severely infected by bacterial leaf blight disease. Much effort to introgress the resistant genes into those varieties has been made by molecular breeding, whereas the resistant genes are neither inactivated nor provide unstable resistance in nature, which shows low resistance to pests and diseases, especially bacterial leaf blight disease. Indeed, dozens of newly developed varieties with high yield and quality potential, desirable traits, and specific abiotic and biotic tolerances are being released, which have gradually replaced the antiquated varieties that no longer conform to newly increasing demands. For example, the popular variety CR203, imported from IRRI, which had long been cultivated in Vietnam for the last few decades, is now envisaged as the history of rice breeding. Other varieties include KD18, with reasonably stable productivity. The new KD18 mutant has a rather high yield and was recently planted to harvest for noodle processing only. The taste of Vietnamese people shows a trend of moving to other higher-quality varieties even with the higher prices [140]. That is an opportunity for high-quality rice breeding, including varieties with low amylose content, aroma, and rich microelements. Besides breeding activities that exploit the local rice landraces, the wave of imported good rice brands from outside countries for domestication is common to shorten time-consuming breeding, which could take up to 10 years. Several hundred new rice varieties have been annually developed and released to the farmers; however, they are still cultivated on a small scale with the estimation of reaching only over 30% in total rice-growing areas. On the other hand, there has been a plethora of new varieties developed by Vietnamese scientists and rice breeders, but a lack of the mega variety that adapts to the changing environment and ecosystem. Hence, with the targeted goal in rice breeding, great efforts by scientists and breeders should generate multiple resistant rice varieties with high adaptability to changing environments in both abiotic and biotic stresses in the coming time to cope with climate change in this country.

Vietnam is located in a subtropical area where rice is cultivated year-round (in the south), where there is an abundant source of increasing diseases and is the ecological niche of insect pests. Resistance genes, such as the *Xa4* and *xa-5* gene for bacterial leaf blight and *Bph1* and *bph2* for brown planthopper were broken by the development of virulence and the occurrence of new races [29,141,142]. Increasingly, climate change has caused a lot of serious problems that require rice breeders to establish suitable breeding strategies and flexible reactions to new conditions and practices [73,143]. Changes in types and virulence of diseases and pests normally are faster than the speed that new resistant varieties can be released. Therefore, frequent findings of new resistance gene sources and germplasms to integrate into new improved varieties must be advanced. The application of molecular breeding helps breeders shorten the time to produce new cultivars. A single or a set of genes for trait(s) can be accumulated in the target cultivars to improve the resistance ability, resistance level, and durability of resistance by molecular breeding [144]. Expression mechanisms of resistance genes and the interaction between genes/QTLs have been revealed and better understood, which supports breeders in designing accurate breeding schemes and gene pyramids to develop higher-resistance rice varieties or resistance to different diseases or insect pests [145]. Frankly stated, rice is our life; however, scant Vietnamese rice brands and rice trademarks have been successfully established compared with other rice-growing countries. Furthermore, limitations in backgrounds, budgets, talents in basic research, and the lack of a core collection and long-term breeding strategies prevent promotion in this field. Not only research but also the application of new knowledge in studies, investigations, and practical aspects present many challenges. Vietnamese rice breeders are now making great efforts to overcome this situation with promising perspectives; however, there are highly competitive international markets where rice is the most important crop that secures food safety and farmer income. Nevertheless, breeding programs are generally faced with the difficulties of integrating conventional and advance biotechnology due to research infrastructure and scientists' capacity. It is true that numerous genomes of rice accessions were resequenced [20], but genomics and bioinformatics analyses are still a maiden approach, as is how to integrate and effectively utilize the profuse existing rice genome databases and actual experimental data in nature for breeding application. Even though rice breeding programs in this country are projected to meet the rising demands, high yield, good quality, and both abiotic and biotic stresses tolerances, the rice breeders should speed up their approach to up-to-date worldwide breeding methods such as rapid generation advance (RGA) [146] and speed-breeding methods [147], advanced genomic breeding to modernize, and harmoniously integrate conventional and advanced tools to simultaneously enhance the rate of targeted genetic gain and minimize the performance costs commensurate with the rapid growth of the population and trend of market demands. Another challenge is that rice-growing areas have reduced due to rapid industrialization and modernization in this country and farmers are uninterested in farming because of the low income. Moreover, in recent years, the reforms and restructures to agriculture by the state authorities have had cropping transfer to vegetables, fruits, and aquacultures with higher values. Doubtlessly, in flat earth with open markets, advantages are accompanied by numerous difficulties. International companies and corporations can throw away the attempts of local rice breeders, companies, and groups with economic and financial powers. These facts put Vietnamese rice breeders in a struggle to control themselves in the development of global markets that are supposed to be a survivor or death fight. Except for numerous challenges as mentioned above, rice breeding in Vietnam has some opportunities and should be focused as follows: (1) effectively exploiting and sustainably conserving rice landraces (germplasms), gene pools, and wild rice relatives integrated with improved rice varieties for long-term approaches and strategies with targeted goals such as promoting specialty rice and high grain quality; (2) generating multiple resistance against both abiotic and biotic stresses with various rice ecosystems; (3) identifying the geographical origin of the landraces to meet the demand of developing specialty rice; (4) applying advanced molecular, genomics, bioinformatics, etc., as an interdisciplinary

breeding strategy to enhance rice-yield potential and grain quality following the trend of market demands and exports as well as to reduce the yield gap at the national level; (5) improving irrigation modification, seed quality, fertilizer management, efficient plant protection, and mitigated post-harvest loss [51]; (6) extending international cooperation in order to set up key technology pipelines, together with exchanging rice materials for breeding and strengthening infrastructures and the capacity of scientists; and (7) changing government agriculture policies for rice flexibly and promptly following the demands of international markets and providing a chance to penetrate into potential markets and seeking potential export contracts in developed countries as special privileges under World Trade Organization (WTO) commitments. Despite being endowed with great opportunities, perspectives, and favorable conditions to exploit and develop rice, numerous challenges are being coped with in rice breeding. The prompt actions of government policies, together with the attempts of scientists, rice breeders, agricultural policymakers, economists, etc., should be met with cautious optimism, and impressive new reforms for rice breeding in this country should be discovered.

7. Conclusions

In summary, the approaches and strategies of rice breeding in this country have indisputably contributed to successful rice improvement for national food security and export. This review not only highlighted the achievements of rice breeding but also provided insight into retrospects, challenges, and perspectives in rice breeding in this country. Booming advanced biotechnology tools and full rice genome sequences have helped scientists and rice breeders to unravel the functions and mechanisms of QTLs and genes and improve rice yield potential, multiple tolerances of stresses, and high grain quality in this country. As an afterword for this review, we borrowed the remark of Charles Darwin: “If the misery of the poor be caused not by the laws of nature but by our institutions, great is our sin”. This wise advice can still be effectively applied nowadays.

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