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Review

The Possibility of Gene Flow Between Cultivated and Wild Rice in Ghana, A Review.

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Abstract: Several transgenic rice lines have been developed and are currently under field trials around the world. There are future plans for the commercial release of transgenic rice into the environment. Rice is an autogamous plant and therefore not perceived to be a very high candidate for pollen mediated gene flow to wild and weedy relatives. However, in a tropical environment like Ghana, where sexually compatible wild *Oryza* species which belongs to the AA genome are present within the ecology of cultivated rice, the possibility of gene flow to wild species cannot be overlooked. There is little evidence on gene flow and its consequences on the wild rice species should they acquire useful genes through gene flow. This review discusses the chances of cultivated to wild rice gene flow in Ghana and the biosafety considerations that should be put in place before the commercial release of genetically modified (GM) rice.

Keywords: Gene flow; *Oryza* species, Wild rice; GM crops; Biosafety

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1. Introduction

Half of the world's population depends on rice (*Oryza sativa*) as a staple food [1-3]. Rice contributes significantly to food security, particularly in the developing world such as Ghana, with demand increasing [4]. The global importance of rice has led to the development of several improved varieties using classical breeding [5], and in recent times using modern biotechnological tools [6]. Besides its economic importance, this annual crop is a model research crop because of its diversity and relatively small genome size. The rice genome was the first crop to be fully sequenced [3, 7]. The sequencing of the genome facilitated massive research into the genetics of rice, including the use of transgenic technology. A large number of transgenic rice lines have been developed; for yield enhancement, for higher quality, for disease and stress resistance, and water and soil nutrient use-efficiency [8-10]. These improved varieties are expected to be released into the environment for commercial production in the near future [11, 12]. Field trials of different types of transgenic rice are currently being conducted around the world, including in Ghana(Figure 1) [13, 14].

Figure 1. GM Field trial site in Nobewam (-1◦26'6", 6◦63'3"), Ashanti Region, Ghana

The development and commercial release of transgenic crops are always associated with biosafety concerns [15, 16]. A significant perceived ecological risk is gene flow from GM crops to their related wild species and the consequences on the wild relatives [17, 18]. The majority of important crops have been found to be cross compatible with their wild relatives, leading to gene flow from cultivated crops to wild relatives [19]. Pollen mediated gene flow from GM crops to wild species has been reported in crops including soybean, maize, wheat and canola [20]. Gene flow a natural ecological phenomenon has always been part of the evolutionary process [21]. Current concerns about gene flow is due to of the fear of useful genes escaping to wild and weedy relatives, which may lead to super weeds [15]. The presence of wild relatives of rice *Oryza barthii* and *Oryza longistaminata* in Ghana means that gene flow from cultivated to wild rice is plausible [22]. Considering the fact that *O. barthii* and *O. longistaminata* are closely related to the cultivated rice, *O. glaberrima* and *O. sativa,* belonging to the same genome (the AA genome), and can be found growing together with cultivated rice in farmers' fields (Figure 2) [23, 24]. *O. longistaminata* and *O. barthii* have been cited among the major weeds of lowland rice in Africa [25, 26]. We later discuss in detail the devastating effects of *O. barthii* and *O. longistaminata* in rice paddy fields. There is a concern of the possible flow of useful genes such as abiotic and biotic stress tolerance genes from transgenic rice to wild and weedy rice species like *O. longistaminata* and *O. barthii* may likely make them more aggressive and difficult to control, leading to severe consequences on the ecosystem [27].

However, these wild relatives of rice and the African cultivated rice, *O. glaberrima* possess very useful genes that has contributed greatly to rice crop improvement around the world [23, 28]. In order to ensure effective conservation and utilization of these resources, knowledge about how gene flow could affect the genetic diversity of such species is highly imperative [28]. In this review, we seek to understand the possibility of gene flow from transgenic rice to wild rice, and the potential consequences on the environment. Particularly, we discuss how gene flow from transgenic rice could affect the genetic constitution of local wild relatives.

Figure 2. Rice field infested with wild rice in Golinga (9◦21'0", 0◦57'0"), Northern region, Ghana. Such shared ecology of cultivated and wild taxa might lead to gene flow [12].

2. The wild relatives of rice present in Ghana are weeds

Wild and weedy relatives of rice are economically important weeds in the lowland rice cultivation in Africa [25]. The wild rice population found in Africa are either weedy rice species or non-cultivated rice species, and they share major genetic similarities with the cultivated rice species *O. sativa L.* and *O. glaberrima*, [27]. Weedy rice comprises a wide group of the genus *Oryza,* including red rice that is parasitic to crops via infestation and competition with cultivated crops, leading to yield reduction or poor grain quality [27].

Morphologically, wild rice appears like cultivated rice at the early stages of growth, when it is very competitive. The morphological appearance of wild rice can be distinguished from cultivated rice at later stages of growth [22]. This phenomenon makes wild rice a significant constraint in lowland rice production [25]. It is difficult to control wild rice in rice fields because wild rice grows tall, is vigorous, awned, and produces a large amount of seed and disperses them into the soil, which grows the next season [6, 27]. The most widespread wild rice species in Africa are *O. longistaminata*, a perennial, and *O. barthii* and *O. punctata* both which are annuals (Figure 3) [29]. *O. longistaminata* and *O. barthii* are of specific importance in our discussion due to their presence in Ghana [22]. *O. longistaminata* is a perennial weed with an advanced underground rhizome making it difficult to manage and control [25, 29]. The consequences of higher *O. longistaminata* infestation can be devastating. Close to 85% reduction of yield have been reported in Mali [29], and infestation is a severe problem in the floodplains in Tanzania [30]. Annual wild rice species in Africa *O. barthii* and *O. punctata* also pose a threat to cultivated rice by reducing yield [25]. *O. longistaminata* and *O. barthii* account for about 50% of weeds in rice fields in Senegal [31]. *O. barthii* infestation can also be highly destructive, (leaving farmers with huge economic loses). An estimated 97% reduction in rice yield has been reported in Senegal due to higher *O. barthii* infestation [25]. It is unclear if the problems posed by these wild relatives of rice will be accelerated, should there be an escape of transgenes that confer tolerance to abiotic stress into the wild rice through gene flow.

Another problem posed by wild rice (aside from competing with cultivated rice for nutrients, water and light) is that it serves as an alternate host to a major rice pest, the African rice gall midge, and the rice yellow mottle virus [29].

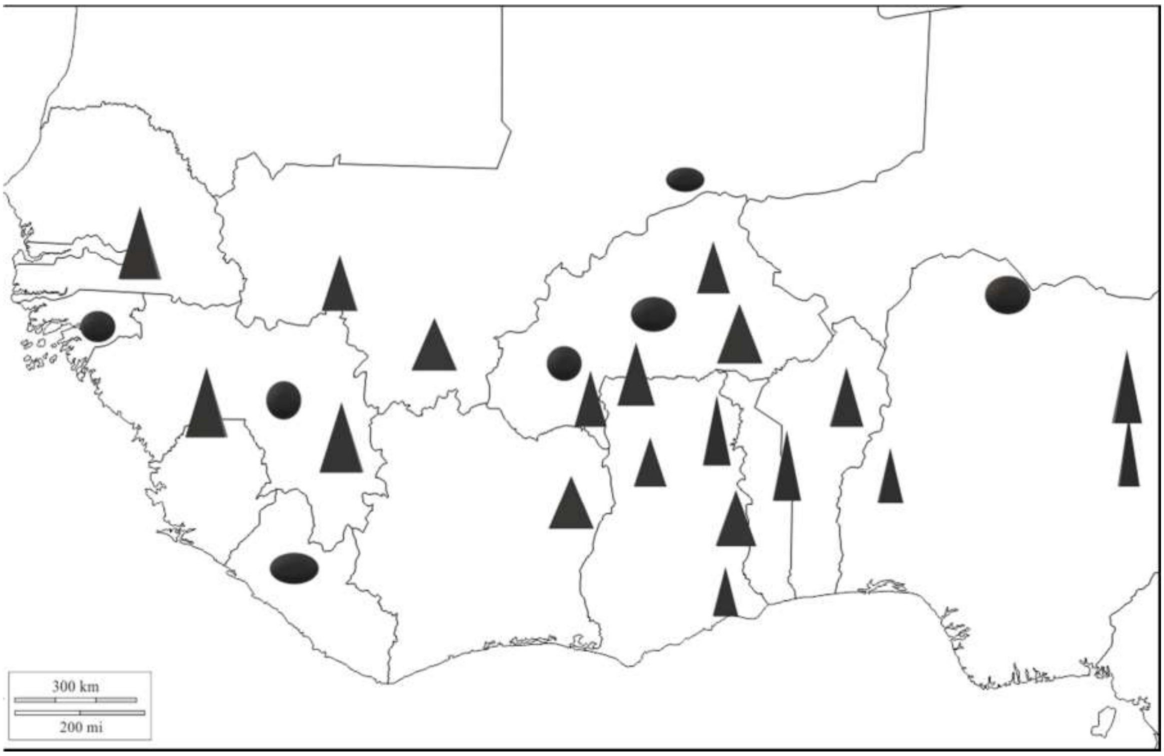

Figure 3. Distribution of *O. longistaminata* (▲) and *O. barthii* (●) species in West Africa**,** [32].

3. The gene pool of the genus *Oryza*

The genus Oryza can be grouped into three subdivisions *Padia*, *Brachyantha* and *Oryza* [33]. *Padia* is adapted to forest areas, *Brachyantha* is adapted to iron rocky areas, and Oryza to low and high lands (Table 1) [34]. For the purpose of this review our focus will be on the *Oryza* species. The species within the genus *Oryza* belong to nine different genomes, namely AA, BB, CC, BBCC, CCDD, EE, FF, GG and JJHH [22, 35]. Species of unknown genomes are assigned to genomes based on whether they are able to form hybrids with species of known genome or not [35]. Species belonging to different genome have reproductive barriers that prevent them from hybridizing, even artificially [6]. Species belonging to the same genome, specifically those belonging to the AA genome, can hybridize, because they are sexually compatible and they have complete chromosome pairing in meiosis of F1 interspecific hybrids (Figure 4) [36]. The possibility of hybridization of the species within the AA genome presents an important case for crop to wild gene flow consideration in Ghana before the release of a transgenic rice.

Species belonging to the A and C genome are widely distributed in the tropics [34]. Within the AA genome, there are six wild species; the perennials *O. rufipogon*, *O. longistaminata* and *O. glumaepatula* from Asia, Africa and Latin America respectively, as well as the annuals *O. nivara* from Asia, *O. barthii* from Africa and *O. meridionalis* from northern Australia and New Guinea [22]. These wild species, which normally grow together with cultivated rice, are capable of crossing and hence gene flow between cultivated rice and wild species belonging to the AA genome. This should be of significant interest in the era of GM rice production [6, 22].

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Table 1. Species in the genus *Oryza*, their chromosome number and Genome type Distribution

Species Complex	Chromosome number	Genome type
Oryza		
***Oryza sativa* complex**		
***Oryza sativa* L**	24	AA
***O. rufipogon* sensu lacto (syn: *O. nivara* for the annual form *O. rufipogon* sensu stricto for the perennial form)**	24	AA
***O. glaberrima* Steud**	24	AA
***O. barthii* A. Chev**	24	AA
***O. longistaminata* Chev. et Roehr**	24	AA
***O. meridionalis* Ng**	24	AA
***O. glumaepatula* Steud.z**	24	AA
***O. officinalis* complex**		
***O. officinalis* Wall ex Wat**	24	CC
***O. minuta* JS Presl. ex CB Presl**	48	BBCC
***O. rhizomatis* Vaughan**	24	CC
***O. eichingeri* Peter**	24	CC
***O. malapuzhaensis* Krishnaswamy and Chandrasakara**	48	BBCC
***O. punctata* Kotschy ex Steud**	24 48	BB, BBCC
***O. latifolia* Desv**	48	CCDD
***O. alta* Swallen**	48	CCDD
***O. grandiglumis* (Doell.) Prod**	48	CCDD
***O. australiensis* Domin**	24	EE
Ridleyanae Tateoka		
***O. schlechteri* Pilger**	48	Not known
***O. ridleyi* complex**		
O. ridleyi Hook	48	HHJJ
***O. longiglumis* Jansen**	48	HHJJ
***O. granulata* complex**		
***O. granulata* Nees et Arn ex Watt**	24	GG
***O. meyeriana* (Zoll. et Mor. ex Steud.)**	24	GG
***Brachyantha* B.R. Lu**		
***O. brachyantha* Chev. Et Roehr**	24	FF

Adapted from [37]

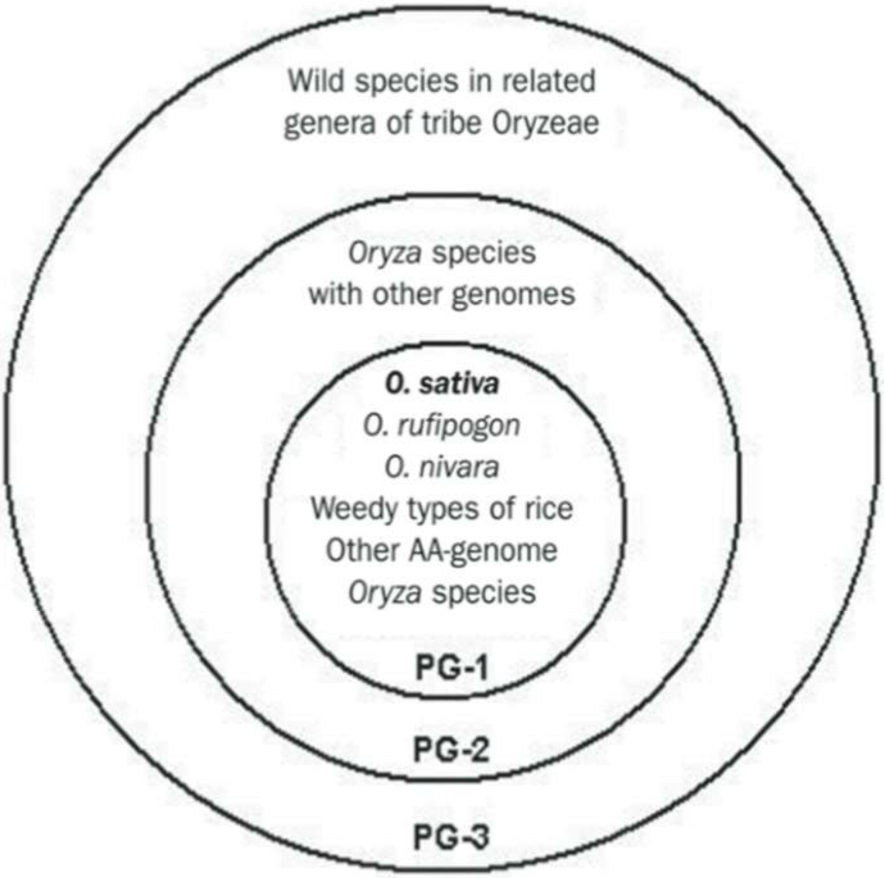

Figure 4. The gene pool of the species *O. sativa*, *O. longistaminata*, *O. barthii* and *O. glaberrima* are all within the primary gene pool. Species within the primary gene pool have a chance of forming hybrids. Modified from [6, 38]

3.1 Gene flow

Gene flow is the movement of genetic information between two organisms, whereby one organism serves as the donor, and the other serves as the recipient [39]. Gene flow can arise from three sources, pollen-mediated through outcrossing, seed-mediated through seed dispersal, and vegetative propagule-mediated gene flow via the dispersal of vegetative organs [39, 40]. In this review we examine seed-mediated and pollen mediated gene flow.

Seed-mediated gene flow is mainly brought about by means of international trade. In the case of rice, the hull is normally removed before shipping, and therefore most of the seeds will not be viable. However, if seeds are imported for the purpose of planting, then such seeds are viable and can contribute to gene flow. The extent to which seed admixtures contribute to gene flow in rice is also unknown [6].

Pollen-mediated gene flow has been found to be a high source of gene flow in cross pollinated plants, and while gene flow from self-pollinated plants, such as rice, is rare, it cannot be excluded [41]. Pollen-mediated gene flow normally occurs between individuals that are sexually compatible. Pollen-mediated gene flow occurs naturally and has been part of the plant evolutionary process. But it could be of concern due to the fear of possible movement of useful transgenes to wild species, which may impact negatively on the environment [39]. Understanding gene flow from crop to wild relatives will help to minimize the potential environmental risk posed by transgene escape [42]. Pollen-mediated gene flow, which will be the focus of this review, depends on several factors including:

- Proximity of the individual plants, which allows an agent to transfer pollen between them. This means that a gene flow will not occur if a crop and its wild relative are not in sympatric occurrence. We propose that when buffer zones and isolation distances are put in place before a transgenic rice is commercialized it will be able to prevent the possibility of gene flow. In this paper we will expand on how buffer zones and isolation distances can be used as a biosafety measure [39].
- The kind of pollinating agents (wind, water, insects and animals) that are present to carry pollen between the plants. The nature of pollinating agents should be taken into consideration when citing buffer zones and isolation distances [43, 44].
- The overlapping and/or synchronicity of flowering period of both plants. Knowledge about the flowering time of wild relatives can be used to stop fertilization between wild and transgenic rice, and such measures could be put in place to minimize the chances of crop to wild gene flow [44].
- The prevailing climatic conditions (direction and speed of wind, light, temperature and humidity) [45].
- The similarities in the physiological appearance of both plants such as plant height [13, 26, 40].

3.1.1 Gene flow in rice

Gene flow from GM and non-GM crops to wild and weedy species has been reported in a number of crops, including rice, in different parts of the world [13, 26, 41, 46]. Generally, cultivated rice is autogamous and therefore the rate of outcrossing with adjacent plants is low [41], ranging from 0% to 0.06% [12, 47]. However rates of 2.94% - 3% have also been reported in some studies [6, 42]. Spontaneous hybridization between cultivated rice species and their wild taxa happens frequently around rice fields, when the wild relatives are present [48]. Langevin [49] reported high natural outcrossing rates ranging from 1-52% using allozyme progeny analysis in Louisiana. When the wild relatives present are weedy species, such as in the case of Ghana, then hybridization with the wild relatives could be of a serious concern [26]. The wild relatives of rice in Ghana are considered as weeds and therefore it is important to study the rate of gene flow between cultivated and wild rice.

The frequency of gene flow recorded in rice varies, and is dependent on environmental and genetic factors [11, 41, 50]. Frequency of gene flow below 0.1 % between GM and red rice was reported by Messeguer [50]. A subsequent experiment using a circular experimental design even recorded lower frequencies of between 0.013% and 0.063%, due to differences in wind direction. Gene flow frequency tends to decrease as distance increases [6]. Sun [13] reported an outcrossing rate of 0.302% at 1m, which significantly decreased to 0.011% at 12m in a gene flow study involving transgenic rice and weedy rice in China. Similarly, Song [42] found that 95 % of rice crop-wild hybrids were located within 30m range. These research works affirm that our suggestion of using isolation distance to reduce or eliminate gene flow from transgenic to non-transgenic rice can be effective.

3.2 *Fitness of cultivated and wild rice hybrids*

Fitness is the ability of an organism to adapt and produce offspring in a particular environment [39]. To the best of our knowledge not much is known about the fitness effect of transgenes. High fitness is necessary for the survival of hybrids arising from natural crop-wild hybridization [26]. Crop alleles may become prevalent in wild and weedy relatives if they possess high fitness. Such alleles can be easily detected in crop-wild hybrids by using specific molecular markers [48]. The fitness of hybrids can be used to monitor and estimate the ecological risk of a transgene, as to whether it will pose a threat to the environment or not [40]. The ecological consequences of a transgene will depend on the success of its incorporation into the wild species [6]. For transgenes that code for traits that do not enhance reproduction it is unlikely they will form new genes, and therefore no environmental consequences can occur [51]. However if a transgene has a normal expression, or expresses higher in the wild rice, then the fitness of the wild population can be altered [40].

Hybrids between cultivated and wild rice in some cases have been found to exhibit high fitness. These hybrids tend to be stabilized and appear as a new species. [26]. Varieties of rice that belong to the AA genome possess a relatively high fitness, because they undergo a complete pairing of their chromosome in meiosis of F_1 interspecific hybrids. They also possess a relatively high fertile F1 hybrids pollen and panicle [52, 53]. Therefore, even with a low frequency of gene flow among species in the AA genome, there is a possibility for genes to escape into wild and weedy species, due to the high survivability of their intraspecific and interspecific hybrids [6]. Previous studies have shown that, under high insect prevalence, insect-resistance transgenes exhibit fitness advantage for crop-wild rice hybrids [48, 54-57]. However, this is unlikely to happen because when insect-resistant GM rice is planted insect pressure will be low under normal field conditions [54, 57, 58]. Conversely, the use of herbicide-resistant GM plants normally leads to high doses of herbicide application. Therefore, even a low fitness of hybrids of herbicide-resistant and wild rice could potentially affect the evolutionary process of the hybrids, which could cause weediness problems [59].

Future studies of gene flow between cultivated and wild rice in Ghana should take into consideration the fitness of cultivated and wild rice hybrids for biosafety assessment. Research should be conducted by measuring the fitness benefits of GM rice and local rice varieties hybrids.

3.3 Consequences of gene flow on genetic diversity of rice

Wild relatives of rice are a significant component of the diverse *Oryza* genetic community that are viewed as most essential for future breeding programs [39]. Wild rice species serve as a reservoir for quality traits for breeding quality cultivated rice. *O. barthii and O. punctata*, for instance, have been reported to possess useful traits against a number of abiotic stresses like drought, and biotic stresses such as bacterial blight, brown plant hopper, and green leafhopper [60, 61]. *O. longistaminata* is indicated to possess resistance to bacterial blight and nematodes, tolerance to drought and rhizomatous [61-65]. *O. brachyantha* has been found to be resistant to bacterial blight, yellow stem borer, leaf-folder, whorl maggot, and laterite soil [61, 66, 67]. *O. glaberrima* indigenous to West Africa is a precious genetic treasure [32, 68]. This species is reported to hold useful traits for several biotic and abiotic stresses. These include tolerance to drought, iron toxicity, waterlogging, resistance to nematodes, African gall Midge, stem borers, and Rice yellow mottle virus. It also adapts well in acidic and low phosphorous soils and weed competitiveness [61, 69-71]. The genetic importance of wild relatives of rice highlights why they should be protected from extinction [6].

Gene flow have the potential of increasing the genetic diversity of a population by adding valuable alleles to a population. An example is the introgression of aroma, a very desirable trait in NERICA 1, a variety developed by West Africa Rice Development Association (WARDA). NERICA 1 gained this gene through gene flow from aromatic rice varieties grown near the nursery of the crosses of NERICA 1 parents [72]. Gene flow has always been part of the evolutionary process which could even lead to speciation in some cases [21, 23].

However, crop to wild gene flow is capable of adding new genes into wild populations, which may cause genetic combinations, and thereafter lead to significant consequences, such as a decrease in the diversity of wild populations [42]. In addition, when genes from cultivated crops escape to, and persist in, populations of wild species, the crop-wild hybrid may spread faster and may dominate the original wild populations. If there is a better ecological fitness, crop-wild hybrids can lead to the extinction of endangered wild species populations in local ecosystems [73]. For instance, *O. rufipogon,* once endemic in Taiwan, is close to extinction due to natural hybridization with cultivated rice [46].

A decrease in the genetic diversity of rice will be a threat to local and world food security, and therefore wild species require protection from genetic erosion and extinction [40].

3.4 Measurement of gene flow

Gene flow can be measured using direct or indirect methods in both GM and non-GM crops. In the direct approach, the range of gene flow is measured based on observations of pollen flow and/or seed dispersal [42]. In the indirect methods, allele frequencies or paternity analysis are used to determine gene flow between adjacent plants. The latter approach employs the use of specific molecular markers [74]. Molecular markers have served as an indispensable tool for the genetic identification of cultivated and wild rice hybrids [75].

4. Biosafety risk assessment

The aim of risk assessment is to eliminate or reduce the risk from happening. Risk assessment can be done by using quantitative or qualitative methods. However, it is complex to quantify risk associated with transgenic crops. Therefore, in many cases, quantitative data is absent [40]. In the risk assessment process if the likelihood of a risk occurring is high but the impact of the risk is low, then there is low risk. However, steps must be taken to reduce the risk to a negligible level. Risk elimination and reduction is based on early detection and better evaluation of procedures [76]. Rice is considered a low risk crop when it comes to gene flow to wild and weedy species because of its autogamous nature [77]. However, if the transgenes involved have a high fitness advantage, as discussed earlier, then it may spread at a faster rate among the wild progenies, even with low gene flow frequency [39, 46]. Many studies have examined the best ways to control transgene flow to wild relatives [45, 55]. In China an isolation distance of more than100 m is recommended for field trials [78]. As we discussed above gene flow is dependent on the microclimate, pollinating agents as well as fitness potentials of the F_1 hybrids. Therefore, we recommend risk assessment and regulations should be based on the prevailing conditions present at the planting site. Jia [45] endorsed the use of threshold management, a system that predicts gene flow based on the purity of the GM seeds and calculates reasonable isolation distances on that basis. Biosafety risk assessment and regulation organizations can adopt this model to ensure the flow of transgenes is minimized.

5. Conclusions and information gap

Previous research on gene flow between transgenic and/or cultivated rice and wild rice both in the glass house and under field conditions, have focused on gene flow between wild rice and Asian rice *O. sativa* [12, 13, 41, 42]. Recent studies on gene flow in the West African region only focused on interspecific hybridization between *O. sativa* and *O. glaberrima* in farmers' fields, and how farmers influence gene flow [23, 24]. There is inadequate study on gene flow between wild rice and African rice *O. glaberrima* and its progenies. There is also insufficient information on the effects of gene flow on the genetic diversity and weediness of wild rice in Ghana.

Currently in Ghana there is a confined field trial of a nitrogen use efficient, water use efficient and salt tolerant transgenic rice. In the immediate future there are plans to introgress these genes into the local varieties and make them available to farmers [14]. However, there is insufficient knowledge about gene flow between the wild relatives of rice and cultivated rice in Ghana, and its consequences on the wild rice species and the environment. A better understanding of crop-to-wild gene flow, including its frequencies and directions under field conditions in Ghana, is therefore necessary. These will be important to access and analyze plausible biosafety concerns associated with gene flow, such as weediness, genetic drift and/or a decrease in genetic diversity [12]. However, if the current biosafety requirement of a minimum of 50m between transgenics and conventional rice is also applied for wild relatives, the possibility of gene flow will be largely contained.

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