Protocol for Management of Weedy Rice (Red Rice) in Biofortified Rice Production Systems

PROTOCOL FOR MANAGEMENT OF WEEDY RICE (RED RICE) IN BIOFORTIFIED RICE PRODUCTION SYSTEMS

Iván Mauricio Hurtado Rivas [1, 2]

Mahalingam Govindaraj [1, 2]

Maria Fernanda Alvarez [2]

Jerome Bartholome [2]

Cristian Camilo Herrera [2]

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International Food Policy Research Institute (IFPRI), Washington, D.C, USA [1]

Alliance of Bioversity International and International Center for Tropical Agriculture (CIAT), Cali, Colombia [2]

Foreword

The Alliance of Bioversity International and CIAT and HarvestPlus are committed to delivering nutrition-sensitive agricultural innovations that empower farmers and improve public health. Biofortified rice, enhanced with essential micronutrients like zinc and iron, is one of our flagship interventions to combat hidden hunger and support resilient food systems across Latin America. It is important to note that the increasing threat of weedy rice (commonly known as red rice)—a biologically similar yet agronomically disruptive plant—poses a major risk to the productivity, profitability, and seed integrity of rice systems. This threat is particularly pressing as seed multiplication and dissemination scale up. The inadvertent multiplication of seeds contaminated with weedy rice perpetuates a cycle of infestation, making field purification and quality seed production increasingly difficult. This issue affects not only biofortified rice but also conventional rice systems, where weedy rice similarly compromises seed quality and field performance. Therefore, maintaining the genetic purity of rice seeds in the field is essential to preserving the zinc integrity of biofortified rice.

Understanding and managing weedy rice in farmers' fields in Latin America and the Caribbean (LAC) is therefore crucial. This publication presents a comprehensive protocol for the evaluation and management of weedy rice in biofortified rice production systems, with an emphasis on practical, field-based solutions. It includes a simple yet effective method to estimate the percentage of weedy rice in the field, empowering farmers to take informed decisions on control measures.

The protocol outlines a diverse set of management strategies ranging from labour-intensive but effective manual removal to stale seedbed techniques and herbicide application. It also cautions against unsustainable practices like repeated herbicide use, which may cause resistance and lead to the emergence of "super weeds," and stubble burning, which harms soil microbiota and contributes to air pollution. Importantly, these guidelines are designed to maintain the genetic purity, yield potential, and nutritional value of biofortified rice.

By compiling best practices tailored to different rice ecosystems, this protocol aims to support a broad range of stakeholders, seed producers, farmers, agronomists, and researchers in reducing the burden of weedy rice and preserving the integrity of biofortified varietal systems. It reflects our shared commitment to scaling nutrition innovations in a sustainable and science-based manner.

We sincerely thank the national partners, technical teams, and field practitioners whose feedback, insights and experience have shaped this valuable resource. Together, we can ensure that biofortified rice continues to serve as a reliable solution to both malnutrition and livelihood enhancement.

Maria Fernanda Alvarez

Rice program leader

Alliance of Bioversity International and CIAT, Cali, Colombia

Erick Boy

Chief Nutritionist,

International Food Policy Research Institute (IFPRI), Washington, DC, USA

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We also extend our thanks to HarvestPlus for their support in refining and publishing this protocol for dissemination to farmers and seed producers around the world. We are grateful for the support for the designing process to James Decker, who with patience and wisdom was able to create the final version of this protocol.

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1. INTRODUCTION

Rice (*Oryza sativa*) is a basic staple food, which is widely consumed around the world, it is a source of food for at least half of the world's population (Makmuang *et al*., 2021). Rice is a crucial source of energy, as it contributes almost 20% of the daily caloric intake of an individual. It is a widely consumed food and is cultivated in numerous countries across different continents. According to FAO Stat, the total rice production for 2022 was led by the Asian continent with 698.767.821 tons, followed by Africa with 39.876.974 tons, and the Americas produced 34.052.692 tons.

The concept of weeds has been used for plants that are located in commercial fields, becoming unwanted due to negative interactions with cultivated plants (de Aguiar *et al*., 2022). Usually, this negative interactions occur for the uptake of environmental resources and conditions, resources are consumable factors such water availability, light, nutrients and CO_2 and conditions are factors not directly consumable, such as pH, soil density and temperature (da Silva *et al*., 2022).

One of the most prominent weeds generating negative impacts in rice productive systems is "red rice," which gets its name for the red pericarp encountered in many cases (Nadir, et al. 2017). Red rice has genetic and morphologic features that resemble commercial rice exactly. In fact, it is the same specie as commercial rice, and it is classified as (*Oryza sativa* f. *spontanea)* (Yang, 2019).

Weedy rice causes issues, mainly competing for light and nutrients (Durant-morat et al., 2018). It has reproductive advantages such as early and easy seed shattering, long periods of dormancy, and the ability to adapt to various environments. These characteristics contribute to the main reasons for crop loss and reduced yield and also encourage the formation of a "seed bank" in the soil. (Nadir, et al. 2017).

Weedy rice's skills to thrive in productive fields and its ability to impersonate other crops make its management difficult using a single method (Yang, 2019). As weedy rice presents these challenges in management, it is necessary to apply diverse management techniques, which will be presented in this protocol for evaluating and managing weedy plants in rice crops.

In Colombia, rice is established using different methods. One approach involves direct seeding, where seeds are sown after tilling the soil, another method entails using pre-germinated seeds, which are broadcast and sown, lastly, transplanting seedlings is utilized as a means to control weeds in plots.

2. EVALUATION OF PRESENCE OF WEEDY RICE

2.1 IN FIELDS

According to Scott *et al* (2018), it is necessary to have a complete understanding of how weeds and weedy rice affect and compete with cultivated rice, in order to establish an appropriate management program. In the next paragraph, we explain details of the process for evaluating the presence of weedy rice in the field.

First, use a quadrat to delimitate a fixed area in the field. It is recommended to use durable materials to construct a 1.0-meter square. See Figure 1 below for an example.

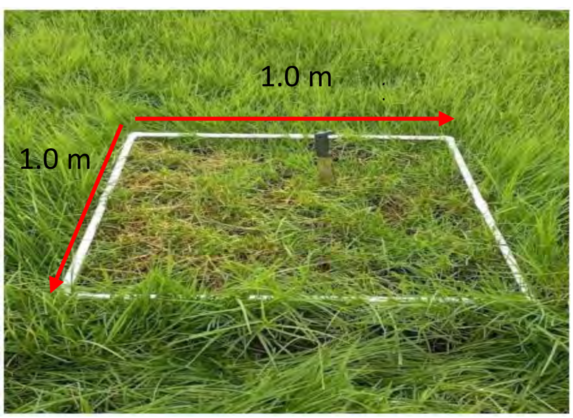

Fig.1. Quadrat used for measurements is built in PVC (polyvinyl chloride). Source: http://repository.unilasallista.edu.co/dspace/bitstream/10567/3496/1/1039471502.pdf

The next step is to conduct a representative spatial sampling of the field. It is recommended to have twenty measuring points distributed over the entire field. See Figure 2 for an example of how to distribute the measurement points with the quadrat over the plot.

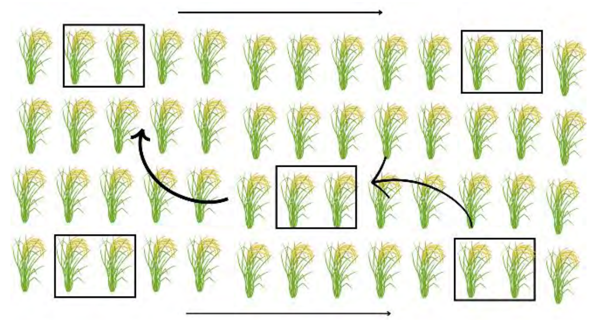

Fig.2. Example of the spatial distribution of the measurements in field.

At each measurement point, the quadrat is used to count the number of plants that exhibit phenotypic differences, like height, flowering precocity, or any other distinguishing characteristics. After this sampling process, the mean of the total number of plants is calculated using the following equation:

$$Mean\ (red\ rice) = \frac{sum\ of\ all\ weedy\ rice\ plants\ found}{total\ number\ of\ samples\ taken\ (20\ samples)}$$

Equation 1. Calculation of mean.

According to (Revista Arroz, 2015), the percentage of yield reduction can be related to the presence of weedy rice in farmer's fields. The following data was obtained for Colombia. Table 1 specifically focuses on the occurrence of weedy rice and can be used as a reference for predicting the yield losses that may occur in the presence of weedy rice.

Refer to Annex 2, which will guide you through the decision-making flowchart on how to act in each of the management steps presented in this manual.

INFESTATION		YIELD REDUCTION (%)
# OF PLANTS m²	PERCENTAGE (%)	
<10	20	1 to 20
11 to 20	40	21 to 40
21 to 30	60	41 to 60
31 to 40	80	61 to 80
>40	100	>80

Table. 1. Infestation and reduction of yield caused by weedy rice plants (source: Revista Arroz, 2015).

2.2 IN SEED STOCKS.

This measurement should be done after the harvest when the rice is stored for the next growing season. To conduct a thorough evaluation, the following steps are required to ensure an accurate evaluation:

- First, estimate the seed index by counting the number of individual grains found in a one-gram sample of rice grains.
- After this, 20 grams of rice are selected from the storage bags for each sample, for a total of 20 samples representing the planting area. The number of weedy rice grains in each sample is then counted. Subsequently, a simple mathematical equation (1), is used to calculate the total harvested rice grains and determine how many of them are classified as weedy rice.

$$seed\ index = \frac{Number\ of\ grains\ (individuals)}{Sample\ weight\ (grams)}$$

Equation 2. used for calculating seed index.

- Next, let us consider an example: Five individual grains of weedy rice were found in 20 samples, each weighing 20 grams, totaling 400 grams. As an estimate, the farmer harvested one ton or 1.000.000 grams.

400 grams → 5 weedy rice grains

1000000 grams → 12.500 weedy rice grains found in the total harvested by farmer.

In this example, we have an approximate estimate of the number of rice grains in one ton of harvested rice. It also highlights the reproductive abilities of weedy plants. Without effective and decisive management, this can directly affect rice yield, resulting in a decrease in the quantity of rice harvested.

Below is a reference table indicating percentage deductions from the agreed acquisition price at a grain mill, which is located in Magangué, Bolivar, (Colombia).

Predefined criteria for acceptance – Magangué (Colombia)		Deductions to be applied if the predefined criteria for acceptance are surpassed.
Humidity	22%	1.33%
Impurities	3%	1.03%
Broken	2%	0.50%
Red	______	0.50%
Diseases (fungi)	______	0.50%

Table. 2. Deductions in percentage according to agreed price: Source mill grain located in Colombia.

These deductions are enforced on green paddy rice that farmers sell to the grain mill, resulting in a reduction in the final acquisition price. While the deductions may not significantly impact on the overall price, they still signify funds lost by the farmer. Additionally, as outlined in this process, the presence of weedy rice led to decrease in rice yield, further affecting the farmer's output.

3. MANAGEMENT OF WEEDS INCLUDING WEEDY RICE

3.1 PREVENTIVE MEASURES

As an essential component of weedy management, prevention is a key strategy. According to Chauhan (2013), buying seeds from a reliable supplier with the equipment, infrastructure, and knowledge is essential to ensure the rice seed is free from contamination. It is important to highlight another preventive measure, which is tool cleaning. The main idea behind this technique is to remove the excess soil that sticks to the tools when a farmer moves from one plot to another. This can be achieved by using scraping tools and water. Below is a diagram of the process:

Fig.3. Illustration depicting the optimal procedure for cleaning tools.

Following this, another preventive measure involves phenotypic identification, which entails selecting plants, grains, and seeds based on their physical resemblance to Bio-Zn 035 rice. Annex 1 contains pictures of Bio-Zn 035 plants and rice grains, both in their paddy and polished stage. To support farmers, these images provide a quick and accurate reference for identifying Bio-Zn 035 rice. Furthermore, the annex includes pictures of weedy rice plants and peeled grains for identification purposes.

3.2 WATER MANAGEMENT

This approach entails establishing a water layer of about 5-10 centimeters. When employed, this method delays or halts the germination and growth of weed seeds. However, it proves ineffective if weedy rice has already germinated in the field. Maintaining an optimal water height is strongly advised, as a decrease in water depth can promote the germination and growth of weedy rice and other weed species (Chauhan, 2013).

Fig.4. An example of an appropriate water height in rice fields typically ranges from 5 to 10 centimeters.

Fig.5. Mismanagement of water levels can lead to soil cracking and the proliferation of weeds.

3.3 CROP ROTATION

The crop rotation technique has been practiced since ancient times, involving the cultivation of a sequence of crops from different plant families within the same plot (Reddy, 2017). Crop rotation can address issues such as soil erosion, water and nutrient deficiency, as well as infestation by weeds, pests, and diseases (Boincean and Dent, 2019). Utilizing crops with allelopathic properties can help to reduce weed populations in the plot. Another method of fighting weed infestation is through crop rotation, which allows for the alteration of crop types (broadleaf vs narrowleaf) and facilitates the use of herbicides that may otherwise harm the crop (Tariq et al., 2019).

Continuous cultivation of crops from the same botanical family in a plot should be avoided. When selecting crops for rotation, it is crucial to ensure, that the selected crops yield economically valuable produce, while also avoiding the attraction or introduction of new pests and diseases (Reddy, 2017). Implementing crop rotation leads to improved grain yields and soil nitrogen transformation, particularly when incorporating crop residues into the soil, especially legumes (Yu et al., 2013). According to T.R. Banjara et al. (2021), rice yields have been observed to increase in plots where crop rotation is applied, especially through intercropping rice with potatoes and rice with cabbage and cowpea fodder. This practice enhances nitrogen levels in the soil, and the inclusion of legumes and vegetables in the cropping sequence boosts productivity and profitability.

Fig.6. Crop rotation with diverse bean varieties is practiced at the CIAT campus.

Fig.7. Crop rotation with "Crotalaria" (Crotalaria juncea L.) is implemented at the CIAT campus.

3.4 PLANTING USING SEEDLINGS

This involves establishing nurseries plots using the technique of wet bed, which consists of broadcasting seeds to a field that has been previously puddled and leveled and has water availability and possess drainage canals (IRRI, 2008). According with IRRI (2008), appropriate time for transplanting is 15-21 days after establishment; CIAT’s Rice Program recommends to transplant seedlings in nurseries 22 days after establishment.

Utilizing well-developed, nourished, and well-adapted seedlings in the field results in stronger plants that can effectively compete against red rice plants.

Fig.8. Nurseries are established in plots using wet-bed technique.

Fig.9. Recently transplanted plots.

4. ADAPTED FIELD MANAGEMENT

4.1 STALE SEEDBED PRACTICE

As a common practice to mitigate the presence of the "weedy rice seed bank," a technique involves lightly irrigating the rice fields after harvesting. This boosts the germination of weedy plants, and remaining seeds of commercial rice in the soil after each harvest. Following the light irrigation, it is essential to discontinue irrigation for a period of 10-15 days. This allows weedy plants and red rice to emerge and begin development. It is crucial to ensure that weedy plants do not exceed the development stage of 3 to 4 leaves, If this stage is surpassed, herbicide application becomes less effective.

Fig.10. Automated Harvest.

Fig.11. Preparing herbicide doses.

Fig.12. Sprinkling herbicide in plot, using protective garment.

Fig.13. Weedy rice field after sprinkling herbicide.

4.2 STUBBLE BURNING

Stubble burning has been employed as a direct method to combat weedy rice and weedy plants due to its affordability and ease of implementation. However, it is not recommended due to its adverse effects on soil health, including the reduction of organic matter and beneficial microorganisms. According to Revista Arroz (2015), if the stubble is not burned against the wind direction and does not gather adequately, the heat's impact on seeds will vary. This variation could lead to the stimulation of seed germination rates and the preservation of other seeds. Therefore, proper land preparation is necessary before initiating the burning process.

4.3 TILLING PRACTICES

Tilling operations are not only a critical step in each sowing season but also help bury weedy rice seeds. The majority of these seeds are found within the first 5-8 centimeters of soil depth, suggesting that tilling should exceed this depth to effectively bury them (Chauhan, 2013). However, this practice may have negative consequences in subsequent seasons as shallow tilling in the future could bring weedy plant seeds to the surface, accelerating infestation rates (Chauhan, 2013). Furthermore, this method proves useful when farmers allow a fallow season, permitting the germination and development of weedy plants. Subsequent tilling eradicates these seedlings or plants. However, it is crucial to have a proper fallow period for effective physical elimination of red rice seedlings and plants. Unfortunately, due to farmers' eagerness to sow, this fallow period is often overlooked, hindering the physical elimination of weedy rice (Revista Arroz, 2015).

Fig.14. Mechanical tilling of soil.

Fig.15. Bringing weedy plants and weedy rice seeds to the soil surface.

4.4 ERADICATING LEAVES & CLEANSE TECHNIQUES IN COMMERCIAL FIELDS

Eradication of leaves is a method that involves manually removing leaves from plants that exhibit greater height, or any distinguishing feature compared to cultivated rice plants in the same plot. Implementing this method results in two outcomes: it accelerates the flowering stage, If the procedure is repeated it helps to reduce the yield of weedy rice. Subsequently, a process known as "cleansing" is applied, which entails manually removing panicles from early maturity rice. This helps distinguish between cultivated rice and weedy rice (Revista Arroz, 2015). It is important to note that both methods require significant manual labor. Additionally, the physical similarity between red rice and commercial rice makes it challenging to visually identify weedy rice.

4.5 APPLICATION OF HERBICIDE - RESISTANT PLANT TECHNOLOGIES

This technique makes use of various technologies which include developing a variety/line of rice resistant to herbicide molecules such as imidazoline. This variety/line is not considered a genetically modified organism (GMO) or a transgenic organism since it is developed using mutagens. Conversely, varieties resistant to glufosinate and glyphosate are indeed GMOs. In both cases, this resistance allows the usage of specific herbicides to control weedy rice and weedy plants (Sudianto et al., 2013; Shailani et al., 2021).
It is crucial to note that the development of herbicide-resistant plants comes with certain restrictions. The resistance only protects against specific herbicide molecules, and farmers must purchase additional commodities such as herbicides and certified seeds.

This technology ought to be implemented once the infestation rate surpasses threshold limits and after employing integrated management techniques. Moreover farmers need to have the necessary resources to prevent cross-contamination of the developed hybrid or variety.

5. CONCLUSIONS

In certain regions of Latin America and Colombia, managing weedy plants, particularly red rice, presents a significant challenge that demands the application of several preventive methods to reduce the germination of contaminated seeds. However, if weedy rice seeds are already in the field, it becomes necessary to implement measures to rapidly diminish its future effects. Therefore, it is anticipated that the techniques and management strategies outlined in this protocol can assist farmers dealing with such infestations in their fields.

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ANNEX 1.

Fig. 16. Plant of rice Bio Zn-035 in plot.

Fig. 17. Grain of rice Bio Zn-035 in paddy.

Fig. 18. Grain of rice Bio Zn-035 peeled and polished.

Fig. 19. Panicle of weedy rice in Colombia. Source: Camilo Velasquez.

Fig. 20. Harvested red rice grain.

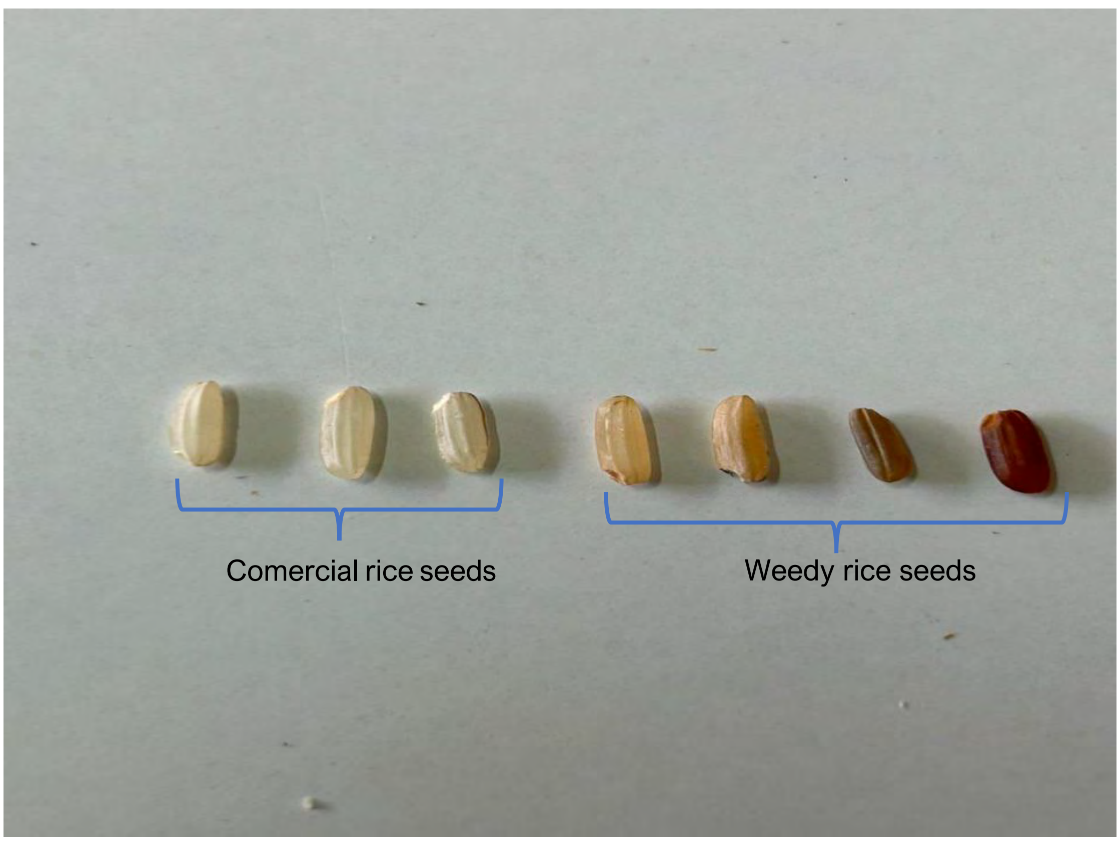

Fig. 21. Diverse phenotypes of weedy rice seeds. Source: FEDEARROZ FNA.

Fig. 22. Weedy rice expressing early flowering and seed development. Source: FEDEARROZ FNA.

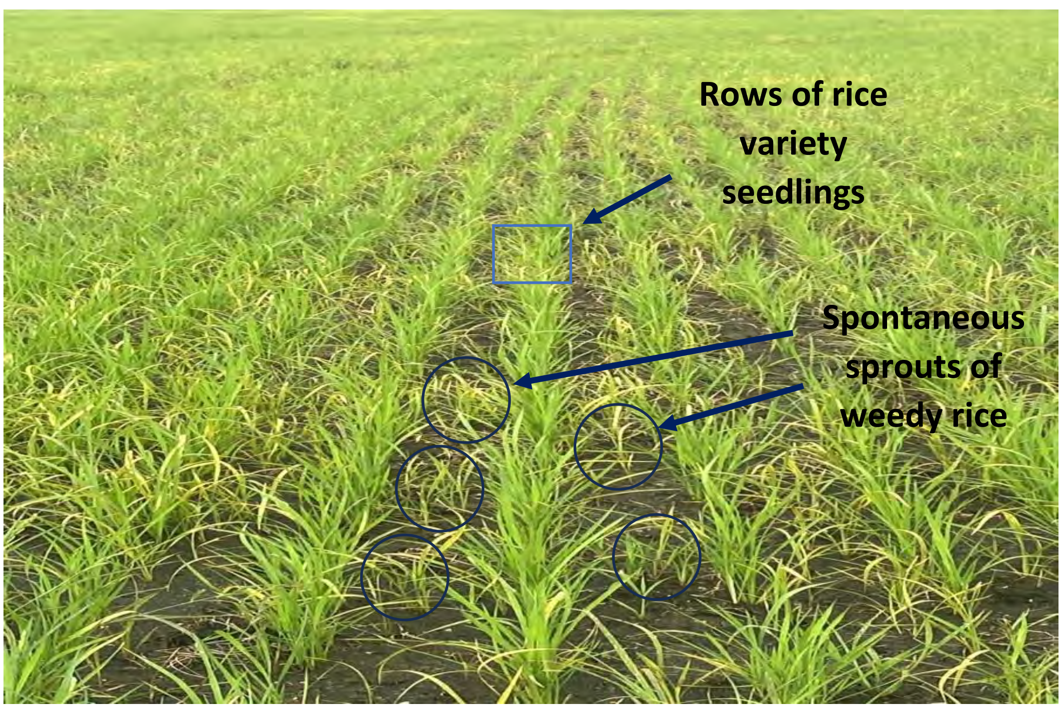

Fig. 23. Rows of rice variety and spontaneous sprouts (weedy rice) can be observed between rows, this indicating growing of weedy rice plants. Source: FEDEARROZ FNA.

Fig. 24. Seedling of weedy rice found in rice fields. Source: FEDEARROZ FNA.

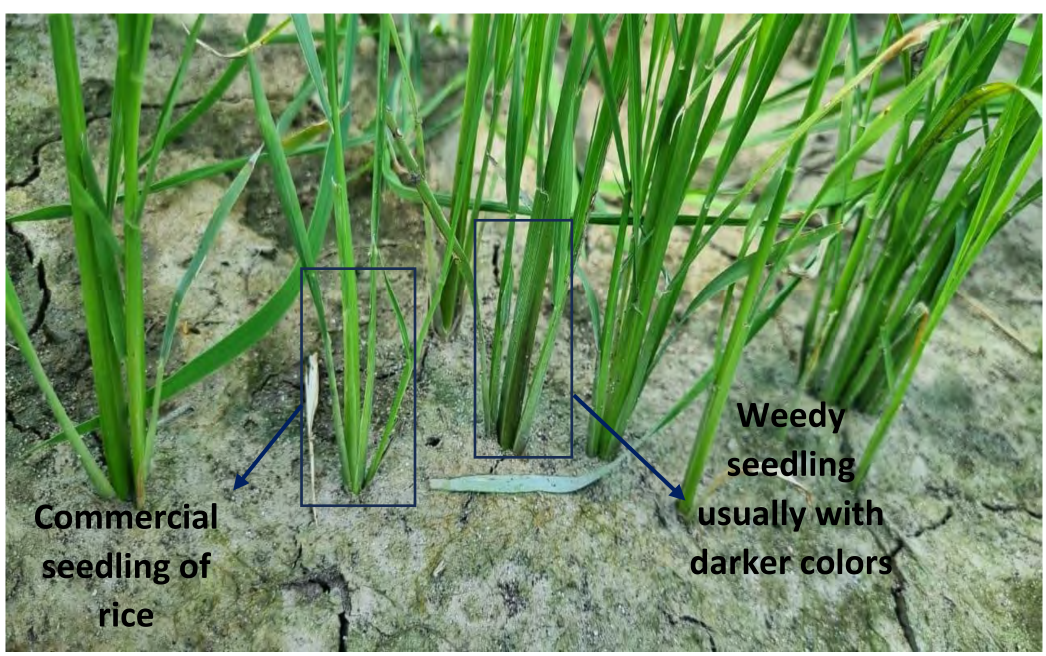

Fig. 25. Seedling of weedy rice it usually has darker colors compared to commercial rice.

ANNEX 2

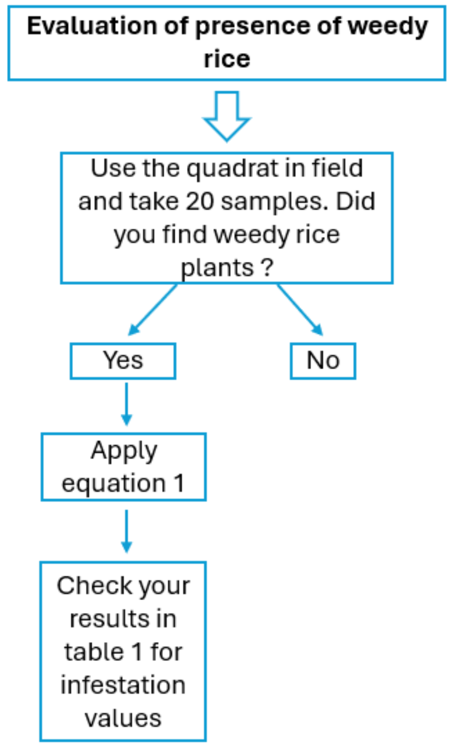

Fig. 26. Flow chart for decision making.

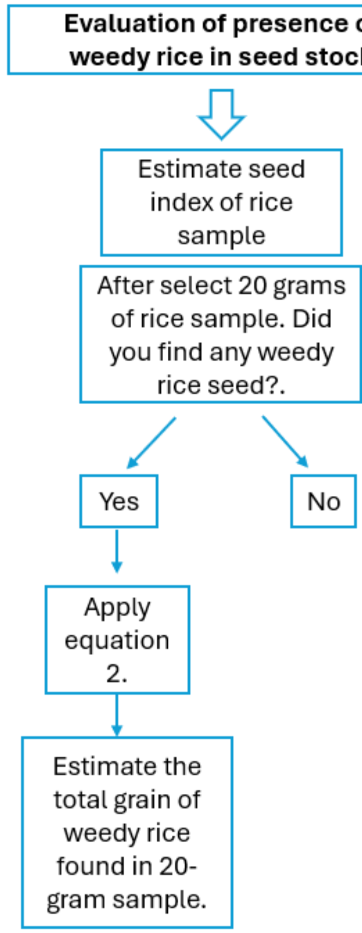

Fig. 27. Flow chart for decision making.

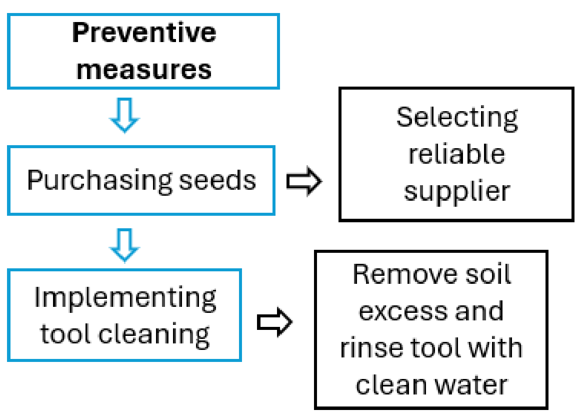

Fig. 28. Flow chart for decision making.

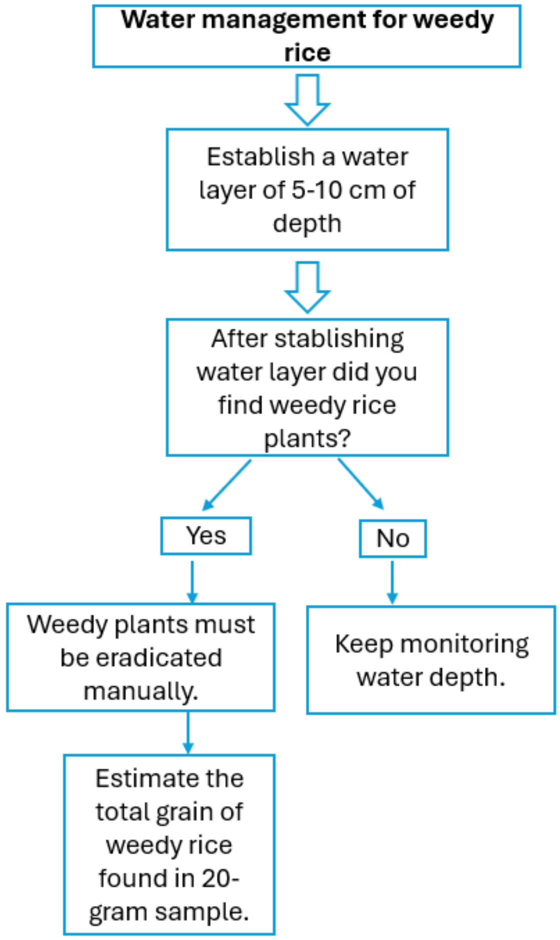

Fig. 29. Flow chart for decision making.

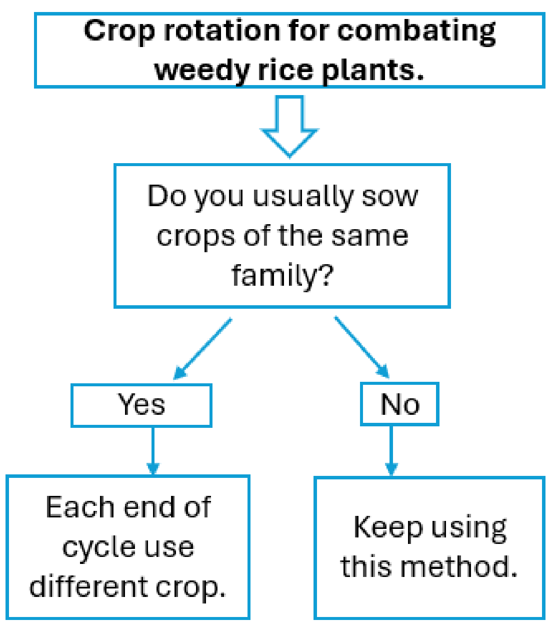

Fig. 30. Flow chart for decision making.

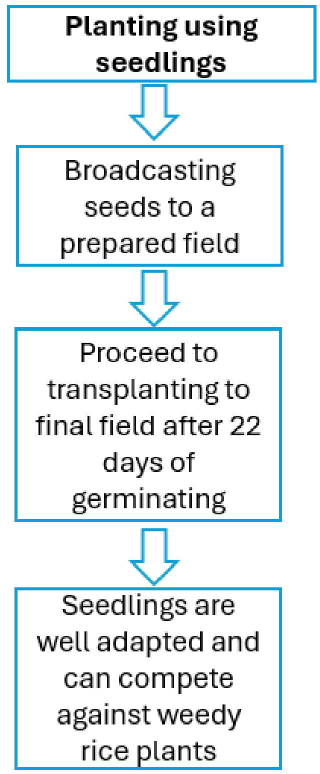

Fig. 31. Flow chart for decision making.

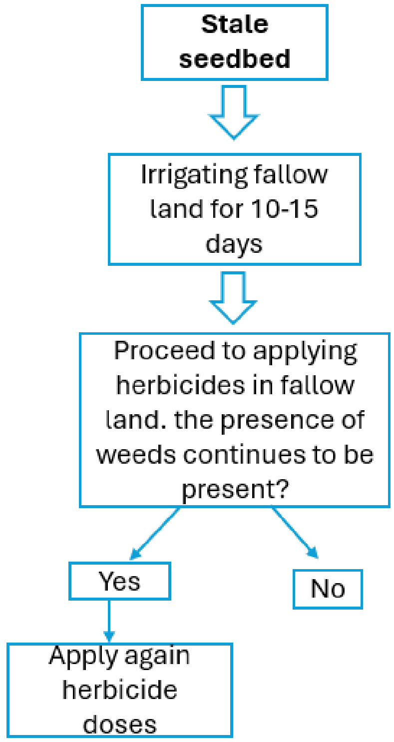

Fig. 32. Flow chart for decision making.

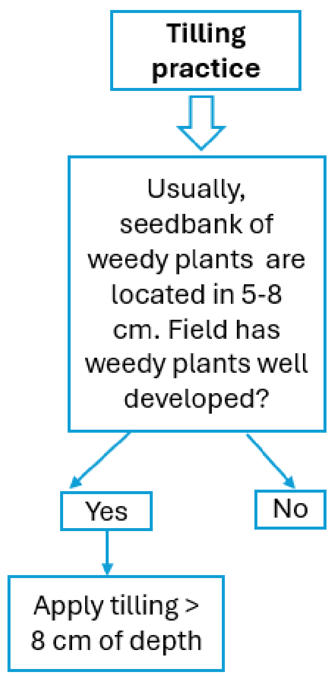

Fig. 33. Flow chart for decision making.

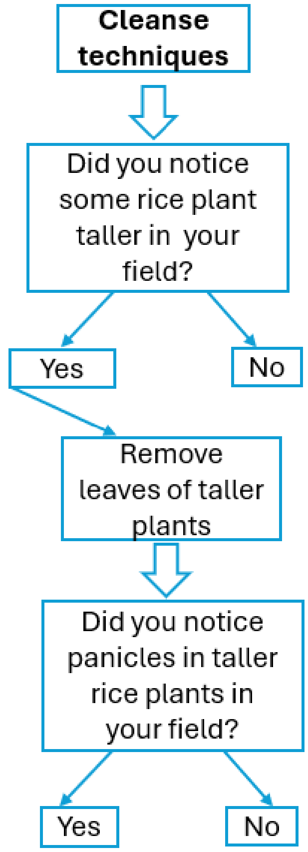

Fig. 34. Flow chart for decision making.