HORIZON 2020

Herbicide Resistant Rice Development For European Continental Project

Reporting

Project Information

HerbaRice

Grant agreement ID: 897192

DOI
10.3030/897192

Closed project

EC signature date
23 April 2020

Start date
16 November 2020

End date
15 November 2023

Funded under
EXCELLENT SCIENCE - Marie Skłodowska-Curie Actions

Total cost
€ 255 943,68

EU contribution
€ 255 943,68

Coordinated by
MINISTRY OF AGRICULTURE AND FORESTRY
Türkiye

Periodic Reporting for period 1 - HerbaRice (Herbicide Resistant Rice Development For European Continental Project)

Reporting period: 2020-11-16 to 2022-11-15

Summary of the context and overall objectives of the project

Rice is a staple food for more than half of the world's population. The rice farming system has problems such as weed management, disease control, abiotic stress tolerance, etc. The issue of weed and weedy rice (red rice) is one of the most important problems in terms of reducing both yield and quality. Chemical application as herbicide is a common technique to control weeds, but herbicides are losing their effectiveness due to various reasons day by day. Chemical weed control is a laborious and costly business, in some cases it is repeated several times, increasing the negative effects on the environment as well as costly. On the other hand, since weedy rice is a close relative of cultivated rice, its chemical control is very difficult. Two herbicide resistance systems, Clearfield and Provisia, were developed so far for the use of rice farmers. After Clearfield was used, it started to lose its effectiveness due to reasons such as gene flow and misuse. The second one, Provisia, entered the farming system in 2018 but is not yet available in Europe. With the refrain from these systems will be damaged over time, it is important to make new resilience resources ready for use by European countries. We aim to develop a new herbicide resistance source in rice and put it at the disposal of European farmers. The developed resistance source will not only be used directly but also EU countries will be able to improve their breeding programs according to their needs via this base material.

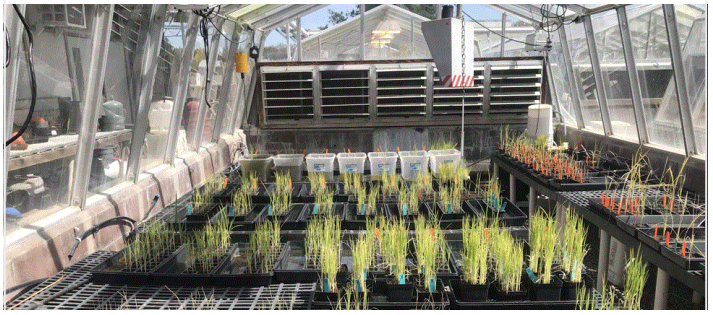
Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far

In order to develop a rice base material resistant to the clethodim herbicide, firstly, mutation application was carried out. Then low dose resistance study, high-dose resistance study, and determination of mutation points study were carried out. In the mutation generation study, the standard protocol for EMS mutagenesis application in rice was developed. It is useful to obtain a sufficient number of mutant plants instead of working with a severe mutation in a few plants. This application protocol contains presoaking for 12 hours, 0.5% dose EMS application for six hours, final washing for six hours, and dry for 72 hours. According to the created protocol, the chemical mutation was applied to 5 rice varieties and a low-dose clethodim resistance study was started. Chemical mutation applied seeds 10,000 seeds each of 5 rice varieties, were planted in the plant growth chamber. It was known that clethodim herbicide can be killed the rice, however, the doses needed to be standardized. Therefore, a dose-response study was carried out with clethodim in rice. As a result of the study, the dose that killed 90% (ED90) of the rice was determined in the range of 70-80 g ai ha-1. Clethodim 1x was utilized 150 gr ai ha-1 in the experiments. In the low-dose experiment, herbicide was applied at 1/2x doses on the m1 plants. Clethodim was applied when the plants were at the 3-4 leaf stage in the spray chamber. Visual injury ratings were conducted 28 days after treatment (DAT) based on 0=not injury and 100=plant killed. A total of 30 plants survived. The frequency of survival was found as 0.06%. Then, the mutation determination process started. Clethodim herbicide binding point was on the ACCase carboxtransferase region on the 5th chromosome in rice. Five primers were designed to cut this region. With this primer, the target region was cut and amplified by PCR and sequenced by the Sanger method. Although the plants were found to be resistant, it was observed that the original sequence was not changed in the target region. In the high dose resistance study, the mutation was applied to 10000 seeds of 5 varieties according to the determined protocol. First-year mutant rice was planted and generation advancement was achieved from M1 to M2 plant. The M2 generation was grown in the field and harvested for herbicide scanning. The second-year M2 mutant rice plant was

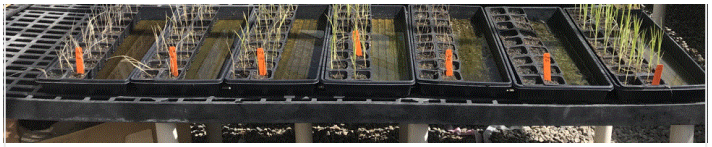
grown up 3-4 leaf stage on the field. Double doses of clethodim were sprayed on mutant rice varieties two times when first at the 3-4 leaf stage and second 14 days later the first application, and 750 surviving plants were selected. The clethodim resistance was tested in the greenhouse at fully controlled conditions, such as an automatic herbicide sprayer and arranged climatic conditions, and 3 out of 750 M3 plant genotypes survived high-dose clethodim treatment. At the next stage observed that these 3 genotypes had no resistance exceeding ½ × dose detected in the herbicide dose-response study and they had no mutation in the ACCase gene region according to the DNA sequence study. As a result, 30 low-dose resistant genotypes were developed, but no material was determined at high-dose resistance for commercial resistance. The dynamic structure of the study allows us to test the newly produced material again until the study is terminated. High-dose resistance study will be re-implemented to achieve success.

Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)

The state-of-the-art of project is to develop Non-GMO resistant clethodim herbicide for rice. Up to now, 30 low-dose clethodim-resistant plants selected but no one high-dose resistant plant was developed. A dynamic structure has been created in the study, that is, the mutant material used in the experiment is constantly supported. This gives us the opportunity to test the material again until the study is terminated. Although high-dose resistance could not be developed from the data obtained at the end of the two-year study, this work package will be re-implemented and the possibility of achieving success will be increased. The project aimed to develop a patentable technology. The patent application requires the confidentiality of some information, therefore, no scientific or nonscientific article has been published directly on the subject. On the other hand to make a standardization use of clethodim herbicide some results were published in scientific journals and congresses. The publications included a series of studies such as clethodim and dose-response experiments on rice, red rice, and some weeds, application time, and application method. The website was released and updates were made to increase the visibility of the project. The effects of the project are to obtain scientific literature, standardize the use of the chemical, and raise awareness subject so far. The main effects of the project will be seen in the years after the project is completed. Releasing the patented product is closely related to rice seed companies, herbicide companies, and end-user farmers. This product, which is clethodim herbicide-resistant rice seed, can be used directly by sowing the base material, or it can be utilized by the different countries' own seeds by gene transfer via classical breeding. The product was developed primarily for the use of the European rice farmer. Starting from here, it can be predicted to spread to other countries and continents.



Weed Science Information Day in UC Davis



Greenhouse experient

RESEARCH ARTICLE

REVISED **Protocol for ethyl methanesulphonate (EMS) mutagenesis application in rice [version 2; peer review: 1 approved, 1 approved with reservations]**

Rasim Unan[1], Ilyas Deligoz[1], Kassim Al-Khatib[2], Husrev Mennan[3]

[1]Black Sea Agricultural Research Institute, Samsun, Turkey
[2]University of California, Davis, CA, USA
[3]Ondokuz Mayis University, Samsun, Turkey

v2 **First published:** 24 Mar 2021, 1:19
https://doi.org/10.12688/openreseurope.13317.1
Latest published: 08 Oct 2021, 1:19
https://doi.org/10.12688/openreseurope.13317.2

Abstract

Background: Non-transgenic chemical mutagen application, particularly ethyl methanesulfonate (EMS), is an important tool to create mutations and gain a new genetic makeup for plants. It is useful to obtain a sufficient number of mutant plants instead of working with a severe mutation in a few plants. EMS dose and exposure period have been previously studied in several crops; however, EMS used to create point mutations in presoaked rice seeds has not been sufficiently studied and there is no standard protocol for such treatment. The aim of this study is to establish a standard protocol for EMS mutagenesis application in rice.
Methods: Two studies were conducted to evaluate the effect of four durations of rice seed presoaking (0, 6, 12, and 24 hours), four EMS concentration doses (0.0%, 0.5%, 1.0%, and 2.0%), and four EMS exposure periods (6, 12, 24, and 48 hours). Germination rate, plumula and radicle length, seedling survival, LD_{50} (Lethal Dose) determination, shoot length, root length and fresh seedling weight were evaluated.
Results: Results showed that a 12-hour presoaking duration, 0.5% EMS dose, and six hours of EMS exposure were the best practices for the optimum number of mutant plants.
Conclusions: In light of both this study and the literature, a standard application protocol was established. This application protocol, detailed in this article, contains the following guidelines: (1) Presoaking: 12 hours, (2) EMS application: 0.5% dose EMS and six hours, (3) Final washing: six hours, (4) Drying: 72 hours at 38°C. A user-friendly protocol has been presented for utilization by researchers.

Open Peer Review

Reviewer Status ✓ ?

	Invited Reviewers	
	1	**2**
version 2 (revision) 08 Oct 2021	✓ report	? report
version 1 24 Mar 2021	? report	

1. **R. Kleynhans**, Tshwane University of Technology, Pretoria, South Africa

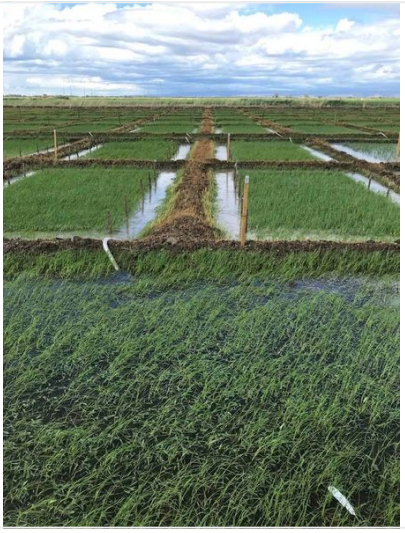
2. **Ashok Somalraju**, AAFC, Charlottetown, Canada

Any reports and responses or comments on the article can be found at the end of the article.

Research article

UC Davis Universty

Herbicide application in field



Experiment plots in field

Last update: 4 July 2023

Permalink: https://cordis.europa.eu/project/id/897192/reporting