GENERICE

Generation & Deployment of Genome-Edited, Nitrogen-use-Efficient Rice Varieties

ABSTRACT

The GENERICE project aims to provide rice research and development community with tailored technical methods to develop improved rice varieties using genome editing technology and with approaches to accompany such technical innovation throughout its journey to the small farmers' fields and local market in developing countries.
The proposed strategy calls for in-depth dialogue between biologists, social scientists and professionals using established methods with demonstrated efficiency, exploratory research to prepare the next generation of knowledge and methods, and in-the-job training of professionals, scientists, postdoc, PhD and MS fellows.
Initial background
Improving nitrogen use efficiency (NUE) in cereal crops is an important avenue for agroecological intensification of their production. It allows small farmers without o with limited access to chemical fertilizers to make the best use of the natural nitrogen cycle in the soil. Genome Editing (GE) technology is a new powerful tool allowing targeted genome modifications quickly, precisely and predictably. It is emerging as a potential new instrument to strengthen the adaptive capacity of plant improvement programs to face changing societal demand and climate change.
The project was complementary to GENIUS/PIA3, ImpresS and Asirpa national research projects and initiatives, international CGIAR Rice research programs, and debates within the European Biotech Councils.

Keywords : Madagascar, Rice, Rice, Impact pathways, Innovation system, Nitrogen-use-efficiency, CRISPR/Cas9 gene editing

Year : 2016
Project number : 1605-019
Type of funding : AAP OS
Project type : AAP
Research units in the network : BPMP INNOVATION
Start date : 2017-07-01
End date : 2019-12-31
Flagship project : no

Project leader : Gilles Trouche
Project leader's institution : CIRAD
Project leader's RU : AGAP

Budget allocated : 237000 €
Total budget allocated (including co-financing) : 470361 €
Funding : Labex

GOAL

Project objectives:
1. Proof of concept for GE-assisted variety development for a complex trait of agronomic interest such as NUE
2. Analyze the societal and institutional feasibility (regulations, environmental and social risks, impacts) of the deployment of GE-assisted varieties for a food crop such as rice, in the context of the emergence of regulatory structures for biosecurity in a developing country, Madagascar
3. Strengthen the collective capacities through information exchange and training of Malagasy biosafety

regulation bodies and other stakeholders to assess the risks and opportunities associated with the deployment of new crop varieties improved through GE technology and to make decision
Research questions
1. Optimize CRISPR/Cas9 technology in rice for simultaneous KO and base substitution, including the investigation of a non-integrative approaches,
2. Establish relationships between our main target gene BT2/OsBT, plant N status, nitrate transporter genes such as NRT1.1/OsNRT1.1Bs, OsCCA1& OsELF4 clock genes, and TGA transcription factors between Arabidopsis and rice plant models,
3. Decipher the eco-physiological components associated with NUE for improved rice varieties developed with GE,
4. Determine the conditions for the social acceptability of the proposed GE-new varieties with regards to potential socio-economic risks and impacts for farmers and other stakeholders of the local rice value chain,
5. Address the needs for capacity building or institutional adaptations to implement national biosafety legislation.

ACTION

Development of rice lines improved for NUE using genome editing CRISPR / Cas9 of rice varieties with better NUE
- Preparation of the next generation of methods and target genes for GE-assisted improvement of rice for NUE.
- Participatory analysis of institutional impacts to characterize, assess and manage the social and environmental risks linked to the dissemination of genome-edited rice varieties in Madagascar
- Agronomic evaluation and ecophysiological characterization of the performance of GE-assisted rice lines
-I377Identification and implementation of training actions (students' thesis, professional and academic training, participatory workshops) to strengthen the research and decision-making capacities of stakeholders

RESULTS

Main results
On the biological level
1. Demonstration of the feasibility to use genome editing technology for developing rice varieties with improved NUE
- Feasibility of the evaluation of CRISPR-CAS9 constructs in rice by transformation of protoplasts and regeneration of fertile plants, which could be multiplied and tested under laboratory and field controlled conditions for their NUE potential;
- Development of a system able to produce mutations by deletions in rice;
- Feasibility of target insertion/deletion of few bases in rice genome leading to target gene knock-out (KO) by frameshift introduction or single base substitutions (base edited);
- Demonstration at field level in Colombia (CIAT) that some of the base edited lines obtained from an upland rice cultivar Chhomrong Dhan (CHD) have a positive effect on NUE and yield: increases in yield were of about 15% in both low N and high N fertilization treatments (Figure 1).
2. New knowledge on the regulation pathways for NO3 transport and uptake:
- In Arabidopsis thaliana, BTs genes are not involved in the NRT1.1 signaling pathway for the regulation of NRT2 by nitrates, but results obtained in 2019 and 2020 show that HRS1 and HHO1 genes are part of this signaling pathway and involved in the repression by nitrate of NRT2s and root nitrate uptake;
- preliminary results indicate that, unlike what we observed in Arabidopsis, the repression by nitrate of NRT2s does not exist in rice and thus the mutation of NRT1.1B has no impact;
- Overexpression of BT reduces plant growth and NUE under limiting N while overexpression of CCA1 reduces growth and NUE under non-limiting N (need confirmation with on-going experiment).
On the socio-economic level :
- The sociotechnical transition model (Greels and Schot, 2007) constitutes a relevant theoretical framework for the analysis of the determinants of varietal innovation based on genome editing;
- the project actions generated a collective improvement in the capacity of the different stakeholders to

understand the conditions of use of GE varieties;
- the project has allowed to structure interactions between civil society, public authorities and research in the joint development of a methodological framework including the participation of stakeholders in the context of improving public regulatory systems;
- A main recommendation concerns the need to involve stakeholders in the risk assessment methods that must compare the benefits expected from GE varieties with other possible variety and agronomic options usable by farmers;
- These methods must also integrate, with regard to societal demand, the assessment of socioeconomic risks associated with the possible privatization of property rights on newly created varieties.
On the capacity building and communication levels:
- Academic education: 3 master students, 5 PhD students (including two from Madagascar and one from Cameroun), 1 postdoc;
- 5 training workshops or academic modules on new plant breeding technologies, genome-editing technique and its issues, participatory risk evaluation for disseminating GE rice varieties;
- 6 research articles in peer-review journals with IF (2 published, 4 on-going), one policy brief, 6 communications in conferences or events, 3 posters, 4 PhD and master student thesis monographies (+ 2 on-going) and 3 working documents

PERSPECTIVES

Propsects
-Results acquired and new questions arising from the activities of WP3 will feed into the discussions underway within the UMR Innovation on risk assessment methods linked to the governance of biotechnology innovations, which will help to orientate and structure future project calls to come within the framework of the CTS Cirad “one health” and the Ecophyto 2 plan;
-An application for a MOGBA post-doctoral fellowship has just been submitted for a project Malagasy partner to continue work on risk assessment approaches on the one Health theme;
-Co-supervision of Fanilo Ny Aina RAMANITRINIZAKA's PhD thesis between the University of Antananarivo and CIRAD will strengthen relationships between the two institutions;
-Involved teams of PBMP and AGAP research units have initiated discussions to set up a new project based on the achievements and new research questions resulting from the project, probably in 2022;
-Technical mastery of the technology of the "base editor" allowing to modify DNA sequences to the base, which was acquired within the framework of this project, is certainly a technology that will be reused in future projects or activities within the AGAP unit