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NGT rice with reduced glutelin content

The NGT rice could enter the European agriculture and food market without risk assessment

Summary
The first rice obtained from new genomic techniques (NGTs) could soon be commercially available in Japan, according to a recent publication (Wakasa et al., 2024). The rice is meant to be used as functional food and supposed to have a positive effects on the health of consumers who suffer from conditions, such as diabetes or renal insufficiency. The rice was engineered to have a reduced content of a group of proteins known as glutelins. Several attempts have been made in the past to achieve comparable results with conventional breeding. However, CRISPR/Cas gene scissors have now made it possible to achieve results beyond any prior breeding attempts.

Glutelins are major seed storage proteins in rice and a major protein source for humans. The reduction of glutelins in rice is associated with trade-offs: it means that the content of other proteins is increased and therefore, e.g. specific attention has to be paid to allergenic factors in plant composition. There are some further indications that the texture of the grains may be altered in a way that will reduce their food value. Therefore, the plants should definitely be required to undergo health risk assessment prior to their commercialisation.

In addition, environmental risk assessment is also necessary and measures to avoid uncontrolled gene flow have to be taken. It is known that gene flow from cultivated rice to weedy rice actually occurs also beyond the fields. Therefore, unwanted genetic contamination and the resulting adverse effects can have a wide-ranging and long-term impact on rice-growing regions, which may also affect regional and traditional varieties. In the present case, the fitness of the NGT rice was enhanced by subsequent crossbreeding with a conventionally-bred variety showing improved lodging resistance, thus enabling a greater potential to persist and spread in the environment.

Figure 1: Conventional rice grown in Spain, near Valencia.

The researchers announced that they intend to make the rice commercially available in Japan, where it will be subject to Japanese regulations for genome-edited crops. There also may be some interest in European markets, especially for import or cultivation in rice growing regions, such as Italy, Spain, France and Hungary. According to current European Commission plans for the deregulation of NGT plants, the rice could be imported and grown without mandatory GMO risk assessment.

Research in Japan and China

To our knowledge, there are at least two NGT projects attempting to produce rice with reduced glutelin content.

- In a Chinese study, Chen et al. (2022) knocked out up to seven relevant genes. The glutelin content of two NGT lines was lower than that of the low-glutelin cultivar used for comparison and supposed to be without major pay-offs in agronomic performance, but unintended effects in grain composition.

- In a Japanese project (Wakasa et al., 2024), CRISPR/Cas was used to knock out five glutelin genes. In this case, the starting point for introducing the genetic changes was a rice variety derived from conventional breeding that already lacked four glutelin genes. Wakasa et al. reported that the NGT rice showed “a drastically reduced content of glutelin proteins” compared to conventionally bred rice plants.

In both cases, the first step towards producing the transgenic rice plants involved transformation with *Agrobacterium tumefaciens*. After subsequent introduction of the (desired) genetic changes via the gene scissors, the transgenes were removed via segregation breeding. Both studies used japonica rice (*Oryza sativa* L. ssp. japonica) varieties, but with different genetic backgrounds. The Japanese study used a Koshihikari variety with a brown phenotype. Koshihikari has been the most widely grown cultivar in Japan for more than 35 years, making it the most important rice for Japanese consumers. The variety is well known for its excellent taste and texture. It shows good adaptation to different environments, tolerance to pre-harvest sprouting, and cold tolerance during the booting stage, but low lodging resistance (Kobayashi et al., 2018). In China, the researchers used the "Wuyunjing 7" (WYJ7) variety, a high-yield and high-quality japonica rice that is widely cultivated in Jiangsu Province, China (Chen et al., 2022).

Differences to conventional breeding

One problem facing scientists are facing using NGTs is the chromosomal arrangement of the glutelin genes, as there is functional redundancy and chromosomal linkage in the relevant genomic regions, with at least twelve genes involved, nine of them located on the same chromosome (chromosome 2), most of them in close proximity (see Figure 2). The genetic redundancy requires the knock-out of multiple genes to obtain discernable mutant phenotypes carrying the intended characteristics. Furthermore, the close linkage of those genes hinders efficient generation of high-order knock-out mutants by crossing single mutants of individual genes (Liu et al., 2023).

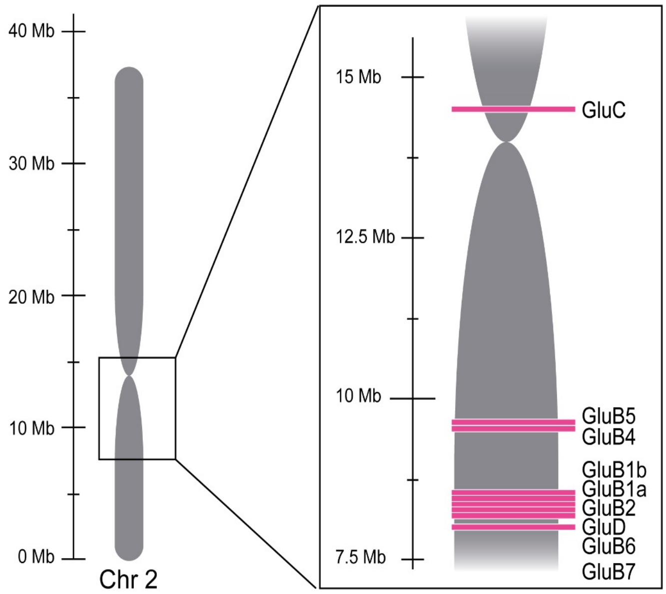

Figure 2: At least twelve genes are involved in the production of glutelins, nine of them located on chromosome 2, most of them in close proximity.

Unintended genetic changes
Chen et al. (2022) investigated unintended genetic changes in predictable off-target regions (regions with some sequence similarity to the target regions) and for transgenes possibly remaining after the transformation with *Agrobacterium tumefaciens*. Wakasa et al. (2024) only checked for transgenes. Neither of the research groups reported any significant findings. However, the insufficient methodology may have led researchers to overlook other unintended genetic changes resulting in major deletions, insertions, or so-called delinvers (Liu et al., 2023), or chromothripsis (Samach et al., 2023) which is described as being caused by NGTs (see also Koller & Cieslak, 2023).

Trade-offs & potential side effects
The NGT rice produced in China (Chen et al., 2022) showed phenotypical changes in the grains, e.g. chalkiness, which is indicative of deformation in the starch granules. Grain chalkiness may cause grain breakage during milling, and have inferior cooking and eating qualities (Hori et al., 2017). Wakasa et al. (2024) reported deficiency in major glutelins and accumulated higher levels of prolamins (also found by Chen et al., 2022), but did not report notable morphological changes. Chen et al. (2022) also identified significant changes in albumin and globulin protein fractions. Wakasa et al. (2024) investigated some of the major rice allergens and detected slight differences in some of the NGT lines compared to the grains of Koshihikari a123 (which is already deficient in four glutelins) that was used as starting material. They suggest that allergenicity in the NGT rice should not be expected to lead to strong allergic reactions compared to the conventionally-bred Koshihikari a123.

Health risks
The trade-offs and other uncertainties identified above would require an in-depth health risk assessment before the NGT rice is introduced into the food market. Some examples of risks include: (i) it is known that reduction in glutelin can cause various other proteins to be increased (see, for example, Kim et al., 2016). This can also apply to proteins that have an allergenic potential or can trigger other immune reactions; (ii) furthermore, since the NGT applications are reported to result in frameshift mutations, it cannot be excluded that new fragmented peptides or other biologically active molecules are produced at the target sites (Kapahnke et al., 2016; Lalonde et al., 2017; Mou et al., 2017; Tuladhar et al., 2019; Jia et al., 2022); (iii) in addition, the (over) consumption of rice with major changes in the composition of the grains may also result in malnutrition effects or fail to provide the expected benefits.

Environmental risks
In addition to health risks for consumers, there are also environmental risks and potential hazards in breeding this kind of rice, which may be caused by or associated with unintended gene flow. It is known that gene flow from rice to weedy rice can occur also beyond the fields (Chen et al., 2004; Lu & Yan, 2009; Shivrain et al., 2009). In the past, this has been the likely cause of substantial commercial damage due to contamination of rice with transgenes (see, for example, GAO, 2008).

As a result, unwanted genetic contamination or adverse effects caused by the cultivation of NGT rice may, over longer periods of time and at large scale, impact rice growing regions, and also affect regional and traditional varieties. In the present case, the fitness of the NGT rice was enhanced by crossbreeding with a conventionally-bred variety showing improved lodging resistance. Therefore, the plants may have a greater potential to persist and spread in the environment. In addition, spontaneous hybrids resulting from crosses with weedy rice may show higher fitness compared to other varieties due to next generation effects (see overview in Bauer-Panskus et al., 2020).

It should also be noted that in order to explain differences in comparison to Chen et al. (2022), Wakasa et al. refer to the different genetic backgrounds and growth conditions to explain unintended effects. From the heterogeneous background of weedy rice as well as various environmental stress factors, it can be assumed that the spontaneous offspring of NGT plants may also exhibit a broad range of unintended effects.

The intended and unintended effects caused by the NGT processes may also negatively impact yield and/or further breeding. If unintended effects are overlooked, they may, for example, accumulate in rice used for further breeding.

So far, no empirical data on environmental risks are available, thus increasing the need for more detailed elaborated risk scenarios to guide further research in this area.

EU: regulatory issues

Rice production has a tradition in at least eight EU Member States (Bulgaria, Greece, France, Hungary, Italy, Portugal, Romania and Spain[1]). The current proposals made by the EU Commission and the EU Parliament in regard to the future regulation of NGT plants would not require GMO risk assessment, as currently foreseen under Directive 2001/18 or Regulation 1879/2003. Despite the geno- and phenotype described by Wakasa et al. (2024) being strikingly different to plants obtained from conventional breeding, the NGT rice would have the same legal status as conventionally-bred plants.

Under these circumstances, NGT rice might only be assessed as a ‘novel food’ in regard to the known characteristics of the final product. However, this approach is setting aside unintended effects and health risks caused by the NGT processes. In addition, there would be no requirement for the assessment of environmental risks. Consequently, the NGT rice could be cultivated in Europe without environmental risk assessment, potentially causing gene flow to weedy rice and other rice fields. If damage occurs, it is doubtful whether the plants could be removed from the environment.

Without more detailed data, no conclusions can be drawn on the assessment of short, mid- or long term risks resulting from cultivation or uncontrolled gene flow. Therefore, traditional rice breeding and rice farming systems in countries like e.g. Italy, Spain, France and Hungary, would be exposed to hazards and risks without being able to protect specific regions and productions systems.

Furthermore, as mentioned, Wakasa et al. (2024) used a randomly mutated Koshihikari rice line, a123, which is already lacking four glutelins, as their starting point, and subsequently used NGTs to further knock out five major glutelin genes. In other words, Wakasa et al. (2024) combined existing genetic material (already present in the breeders’ gene pool) with additional genetic changes introduced by NGTs.

The possibility of using plants already present in the breeders’ gene pool for further NGT applications is explicitly allowed in the current proposals made by the European Commission. This approach may also be applied to further genetic engineering of plants obtained from (i) previous NGT applications or (ii) the use of transgenic plants obtained from previous methods of genetic engineering, as currently carried out by the US company Inari. The company is known for its practice of applying NGT processes in plants produced by other companies, which inherit transgenes for herbicide resistance or insecticidal proteins, and subsequently attempting to re-

1 https://agriculture.ec.europa.eu/system/files/2019-02/factsheet-eu-rice-market_en_0.pdf

introduce them into the market as their own 'new' varieties.[2] It appears that, under the conditions proposed by the EU Commission, plants with a new combination of genotypes or phenotypes would not have to undergo mandatory risk assessment if the parental plants, which may also inherit transgenes, already have marketing approval.

Conclusions

The data provided by Wakasa et al. (2024) show that the NGT rice with low glutelin content is unlikely to be achieved with conventional breeding techniques. It thus requires in-depth assessment of health and environmental risks. If cultivated in the fields, rice growing regions would be exposed to spontaneous gene flow, with potentially long term and significant consequences for breeding, rice production and biodiversity. Additional regulatory issues are related to potential further crossing of the rice with transgenic plants that have already been granted marketing approval.

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