**DEVELOPMENT**

# Transgenic Biofortified Crops

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Biofortification can be achieved through conventional plant breeding, where parent lines with high vitamin or mineral levels are crossed over several generations to produce plants that have the desired nutrient and agronomic traits. Transgenic approaches are advantageous when the nutrient does not naturally exist in a crop (for example, provitamin A in rice) or when sufficient amounts of bioavailable micronutrients cannot be effectively bred into the crop. However, once a transgenic line is obtained, several years of conventional breeding are needed to ensure that the transgenes are stably inherited and to incorporate the transgenic line into varieties that farmers prefer. While transgenic breeding can sometimes offer micronutrient gains beyond those available to conventional breeders, many countries lack legal frameworks to allow release and commercialization of these varieties.

To attain higher levels of provitamin A, zinc, and iron content in crops where genetic variation for these traits has not been identified, HarvestPlus, its partners, and other organizations have explored transgenic approaches, discussed below.

**Golden and High-Iron Rice:** Golden Rice was first developed at the Swiss Federal Institute of Technology and the University of Freiburg, Germany. The inventors donated the technology for public sector research and development and farmers' use, free of charge, in developing countries. This effort was assisted by Syngenta who arranged, for humanitarian purposes, royalty-free access to intellectual property for a number of key technologies used in Golden Rice held by several biotechnology companies. These arrangements allow the International Rice Research Institute (IRRI) and others to develop Golden Rice on a non-for-profit basis. In parallel, Golden Rice product development was furthered by Syngenta as part of their then-commercial pipeline. Transgenic events with higher levels of provitamin A, up to 37 ppm in a U.S. variety (GR2 events), were produced and were then donated for use by the Golden Rice Network when Syngenta decided not to pursue the trait as a commercial product (1). The development of Golden Rice is currently coordinated by IRRI in collaboration with national rice research institutes, such as PhilRice (Philippines), Bangladesh Rice Research Institute (BRRI), and Indonesian Centre for Rice Research (ICRR). Starting in 2006, the GR2 events were backcrossed into varieties for these countries. Field testing is currently ongoing.

Bioavailability testing has confirmed that Golden Rice is an effective source of vitamin A in humans, with an estimated conversion rate of beta-carotene to retinol of 3.8:1 and 2:1 (2,3). Golden Rice will be required to pass biosafety tests prior to release. An efficacy trial, evaluated by Helen Keller International, is planned in the Philippines after biosafety approval is granted. For additional information, see www.goldenrice.org and http://irri.org/golden-rice.

Additionally, a transgenic high-iron rice variety has been developed by the University of Melbourne and IRRI that contains 14 ppm iron in the white rice grain and translocates iron to accumulate in the endosperm, where it is unlikely to be bound by phytic acid and therefore likely to be bioavailable (4). The University of Melbourne has produced a number of transformants of Nipponbare carrying the rice nicotianamine synthase (NAS2) over-expression genetic constructs, suggesting, at screen-house level, the ability to reach target levels for iron and zinc. Teams at IRRI have produced several thousand transformants of IR64 and IR69428 that carry the soybean or rice ferritin and rice nicotianamine synthase (NAS2) over expression genetic constructs and, in the field, demonstrate the target level for iron and surpass that for zinc. Achieving the iron and now higher zinc levels in the field requires both a ferritin and NAS gene to be expressed correctly. The project at IRRI is now moving beyond proof of concept to product development for high-iron and high-zinc, highly adapted rice genotypes. Bioavailability trials are expected to begin next year, and release is projected for about 2022 in Bangladesh.

**BioCassava Plus:** The BioCassava Plus (BC+) program genetically engineers cassava with increased levels of iron and provitamin A. Additional traits addressed by BC+ include increased shelf life, reduced cyanide levels, and improved disease resistance. The first field trials for a provitamin A biofortified cassava began in 2009, followed by trials for high-iron cassava (5). Delivery of the biofortified crops is expected in 2017. Retention and bioavailability of transgenic cassava are similar to the findings of HarvestPlus on conventional biofortification research (6). For additional information, see BioCassava Plus at http://www.danforthcenter.org.

**Vitamin A and Iron Bananas:** Queensland University of Technology and the National Agricultural Research Organization of Uganda are developing transgenic provitamin A and iron bananas for Uganda. Bananas with up to 20 ppm provitamin A have been developed and trials have commenced in Uganda (7). Provitamin A bananas are expected to be released in 2019. A human bioavailability study using transgenic provitamin A banana began in late 2013. High-iron bananas are not yet ready for use in human trials. For additional information, see Banana21 at http://www.banana21.org/index.html.

**Iron Wheat:** Efforts to increase iron concentrations in wheat by conventional breeding have not been successful, and there are currently no iron-biofortified wheat varieties available for farmers. Whole wheat grain contains approximately 30 ppm iron, of which only 5% is estimated to be bioavailable. It is estimated that wheat requires an additional 22 ppm iron in the whole wheat grain, for a total concentration of 52 ppm iron, to adequately biofortify a wheat-based diet with iron.

The University of Melbourne is employing the approach that has proven highly effective in rice, using NAS to increase iron concentrations in wheat and produce biofortified wheat varieties with 52 ppm iron in whole grain. The project places strong emphasis on multi-location field trials of wheat plants transformed with a rice nicotianamine synthase gene (OsNAS2) under regulatory control of the maize ubiquitin promoter, Ubi1, to provide proof of concept of the transgenics approach in wheat. Additionally, selectable marker-free transgenic populations will be developed and evaluated in commercially important wheat backgrounds.

The John Innes Center is investigating several independent strategies to increase iron concentration and bioavailability in wheat grains through transgenic means. The approaches will target distinct stages and tissues including uptake from the soil, remobilization from vegetative tissue during grain filling, and accumulation within grains to enhance total iron in the grain (8).

### Challenges:

- Regulatory concerns are often the biggest sticking point for the roll-out and adoption of transgenic crops and continue to be a significant barrier (9).

### Recommendations:

- Use conventional breeding where the genetic variability for the nutritional trait is sufficiently large and breeding is feasible. Apply recombinant transgenic technologies when this is not available.

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