



February 2015

CONSUMER RESEARCH

## CONTENTS

Genetically Engineered Crops with Enhanced Micronutrient Levels: Present Status and Market Potential	1
Enhancing Photosynthesis in Crops with a Faster Rubisco	7
Impact of Soil Water Content and Temperature on GE Crop Cry1Ac Protein Degradation	8
USDA Announces Deregulation of Non-Browning Apples	11

## Genetically Engineered Crops with Enhanced Micronutrient Levels: Present Status and Market Potential

*Hans De Steur, Dieter Blancquaert, Simon Strobbe, Willy Lambert, Xavier Gellynck & Dominique Van Der Straeten*

Genetically engineered (GE) or transgenic biofortified crops with an increased vitamin and/or mineral content have large potential to improve public health, but their availability for consumers is still hampered. Over the last years, various GM crops with health benefits have been developed in which genes, mostly originating from other organisms, have been added. Notable examples include rice enriched with pro-vitamin A (also known as 'Golden Rice') and folate-enriched rice, developed at Ghent University. Nevertheless, fifteen years after the development of 'Golden Rice', i.e., the first GMO with health benefits, the developers of such GE biofortified crops have little reason to celebrate. To date, none of these GMOs are approved for cultivation, unlike GMOs with agronomic traits or conventionally bred biofortified crops.

A multidisciplinary team from Ghent University has summarized the current state of research in the field of GE biofortification by focusing on biotechnology research as well as socio-economic research at micro (acceptance, willingness-to-pay) and macro level (health impacts, cost-effectiveness). Their findings, a compilation of three review studies (35 R&D studies; 25 market studies), have been recently published in *Nature Biotechnology*<sup>1</sup>. This novel study aims to inform various stakeholders among which are biotech developers and agricultural economists, policy makers, regulators, and health planners in the field of micronutrient malnutrition prevention.

### Research & Development

Staple crops, such as rice, wheat, and potato, are widely consumed as the main source of daily caloric intake; therefore enhancing their micronutrient content is highly desirable in the battle against so-called 'hidden hunger'. In theory, this can be achieved via two approaches: conventional breeding and metabolic engineering. The former relies on the variability of micronutrient levels in sexually compatible organisms, hence its limitation. Furthermore, conventional breeding is very time-consuming because it requires selfing or back-crossing over several generations. Conventional breeding has been successfully applied in the creation of pro-vitamin A biofortified yellow corn, but is of little use in other crops, where the natural variation of a micronutrient between cultivars, subspecies, or species is low. Moreover, in rice, most of the micronutrients are concentrated in the outer layers of the kernel (the aleurone layer), which is often removed to enhance its shelf-life. For these crops, metabolic engineering is regarded as a valuable alternative. In addition, through the use of tissue-specific promoters controlling (trans)gene expression, biofortification can be directed to tissues, such as rice endosperm, where the micronutrient does not accumulate naturally. Metabolic engineering requires a profound understanding of the metabolism of the micronutrient. Luckily, this

PUBLISHED BY

**Information Systems  
for Biotechnology**

Virginia Tech  
1900 Kraft Drive, Suite 103  
Blacksburg, VA 24061

Tel. 540-231-3747  
Fax 540-231-4434

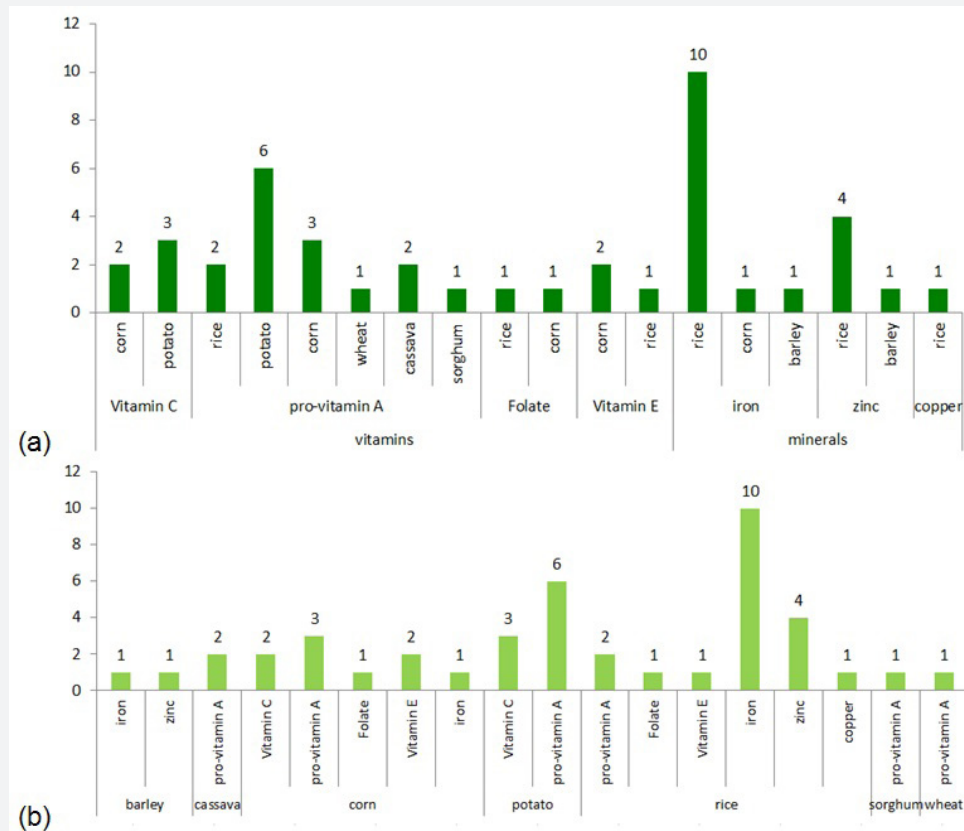
Subscribe to and access  
archived issues of the ISB  
News Report online:

[www.isb.vt.edu](http://www.isb.vt.edu)

Editor:  
Ruth Irwin  
[rirwin@vt.edu](mailto:rirwin@vt.edu)

ISB welcomes your comments  
and encourages article  
submissions. If you have a  
suitable article relevant to our  
coverage of agricultural and  
environmental applications of  
genetic engineering, please  
email it to the Editor for  
consideration.

The material in this News Report is  
compiled by Information Systems  
for Biotechnology, Virginia  
Tech. Any opinions, findings,  
conclusions, or recommendations  
expressed in this publication are  
those of the author(s) and do not  
necessarily reflect the view of  
the US Department of Agriculture  
or of Virginia Tech. The News  
Report may be freely photocopied  
or otherwise distributed for non-  
commercial purposes only, with  
attribution.



**Figure 1.** Research & development of GE biofortified products. Number of studies, organized by (a) micronutrient and (b) crop.

Note: 6 R&D studies deal with multi-biofortification: corn (1 study: pro-vitamin A, folate, vitamin C), rice (3 studies: iron, zinc; 1 study: iron, zinc, copper) and barley (1 study: iron, zinc). Source: De Steur et al.<sup>1</sup>

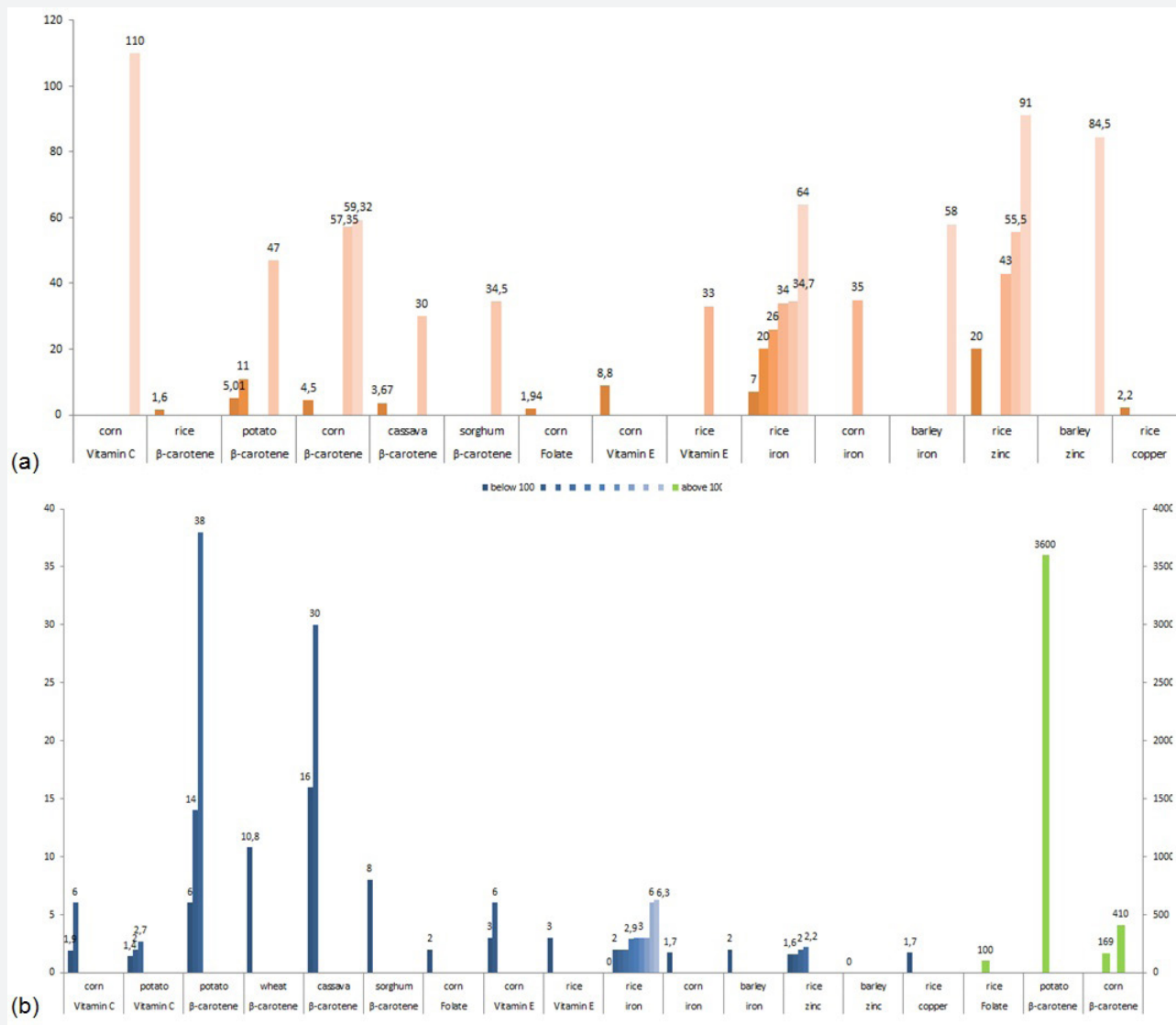
knowledge is steadily growing, resulting in an increasing number of reports describing a successful enhancement of vitamin or mineral content in staple crops (see Figure 1).

Vitamin biofortification by metabolic engineering can be achieved by increasing its bioavailability and/or by the enhancement of its content. Thus far, most studies have focused on the latter. Since metabolic engineers are not restricted to a sexually compatible gene pool, they often make use of genes originating from non-related organisms, such as bacteria, mammals, or other plant species. This way, endogenous regulatory mechanisms, when present, can be dodged, often resulting in a higher accumulation of the target compound as compared to engineered crops where endogenous genes were used. Golden Rice 1 is a prime example of this technology. Here, genes from daffodil and *Pantoea* (formerly known as *Erwinia*) were overexpressed in rice endosperm. Genes from other organisms further increased pro-vitamin A content, leading to the creation of Golden Rice 2. Golden Rice opened the door for the creation of a series of ‘Golden’ (= pro-vitamin A biofortified) crops, such as wheat, corn, potato, and cassava (Figure 2). Another well-known example is folate (vitamin B9) biofortified rice, in which the endosperm-specific overexpression of two *Arabidopsis* genes, encoding the first steps in the biosynthesis of folate biosynthesis intermediates para-aminobenzoate and pterin, resulted in a 100-fold increase of folate levels in white rice (Figure 2).

As many disorders, such as anemia, are often caused by insufficient mineral intake, there is growing interest in improving the mineral content in staple crops, mainly focusing on iron and zinc. However, unlike vitamins, minerals cannot be synthesized by plants, and their content in crops is directly correlated with their availability in the soil. Again, both mineral availability and content can be targeted for engineering. Phytic acid, for instance, binds iron and zinc, which limits the absorptions of these minerals by the human intestine. Here, both conventional breeding and metabolic engineering can be used to lower phytic acid levels. To increase mineral content in edible tissues, several steps need to be engineered, from mineral

uptake by the roots, to transport to and accumulation in the target tissues. These steps need to be correctly orchestrated to maximally increase mineral levels in staple crops, which requires the overexpression of multiple genes.

With the current knowledge on single micronutrient biofortification in staple crops, a multi-biofortification approach is highly desirable to tackle a broad range of deficiencies. Thus far, only a handful of studies report on multi-biofortification. Moreover, there is a growing awareness of the importance of a general nutritional enhancement of staple crops, focusing on both essential and non-essential nutrients. In this respect, a combination of conventional breeding and metabolic engineering



**Figure 2.** Successful biofortification reports through metabolic engineering in staple crops. **(a)** compound level (µg/g DW) and **(b)** fold increase. DW, dry weight. Note: not all values of the reviewed studies are presented. Source: De Steur et al.<sup>1</sup>.

would be the best way to reach this goal.

Despite the fact that progressively more reports on the successful biofortification of staple crops become available, none of these engineered crops are currently accessible for consumers. Scientists often provide a proof-of-principle in laboratory varieties of a staple crop. This knowledge needs to be transferred to commercially and locally adapted varieties, which then need to be regulated for market release. This regulatory pipeline is well defined and includes a molecular characterization to detect a regulatory ‘clean’ transgenic event, a biosafety assessment, and a transgene expression analysis over a number of generations. Afterwards, field trials are conducted to reveal yield performances, as well as a compositional analysis and an environmental risk assessment.

Unfortunately, although the impact that these crops could have on human health is convincingly proven, a negative public opinion, often based on non-scientific arguments, prevents them from saving thousands of lives worldwide.

### Market potential

Because GE biofortified foods are not only considered agricultural biotechnology innovations but also as potential micronutrient strategies, researchers gradually started to estimate ex-ante the market potential of these new product developments. Thereby, the focus was on consumer research (acceptance, willingness-to-pay) and intervention analysis (health impacts, cost-effectiveness)(**Figure 3**).

While consumers often demand a discount for GE foods with improved agronomic traits, the premiums they are prepared to pay for GE biofortified crops are all positive and relatively high (**Figure 4a**). These premiums, which range from 19.5% to 70.0%, support decision making and resource allocation by policy makers (together with optimistic purchase intentions, preference rates, and acceptance levels; see De Steur *et al.*<sup>1</sup>). In regions with high prevalence of micronutrient deficiencies as in China and Brazil, which are considered key target markets, willingness-to-pay levels are as high as and often exceed those in developed regions such as Europe, the US, and New Zealand. However, it is important to note that high premiums are not intended for future price setting of GE biofortified crops, but should be interpreted as an

indication of a potentially high market demand.

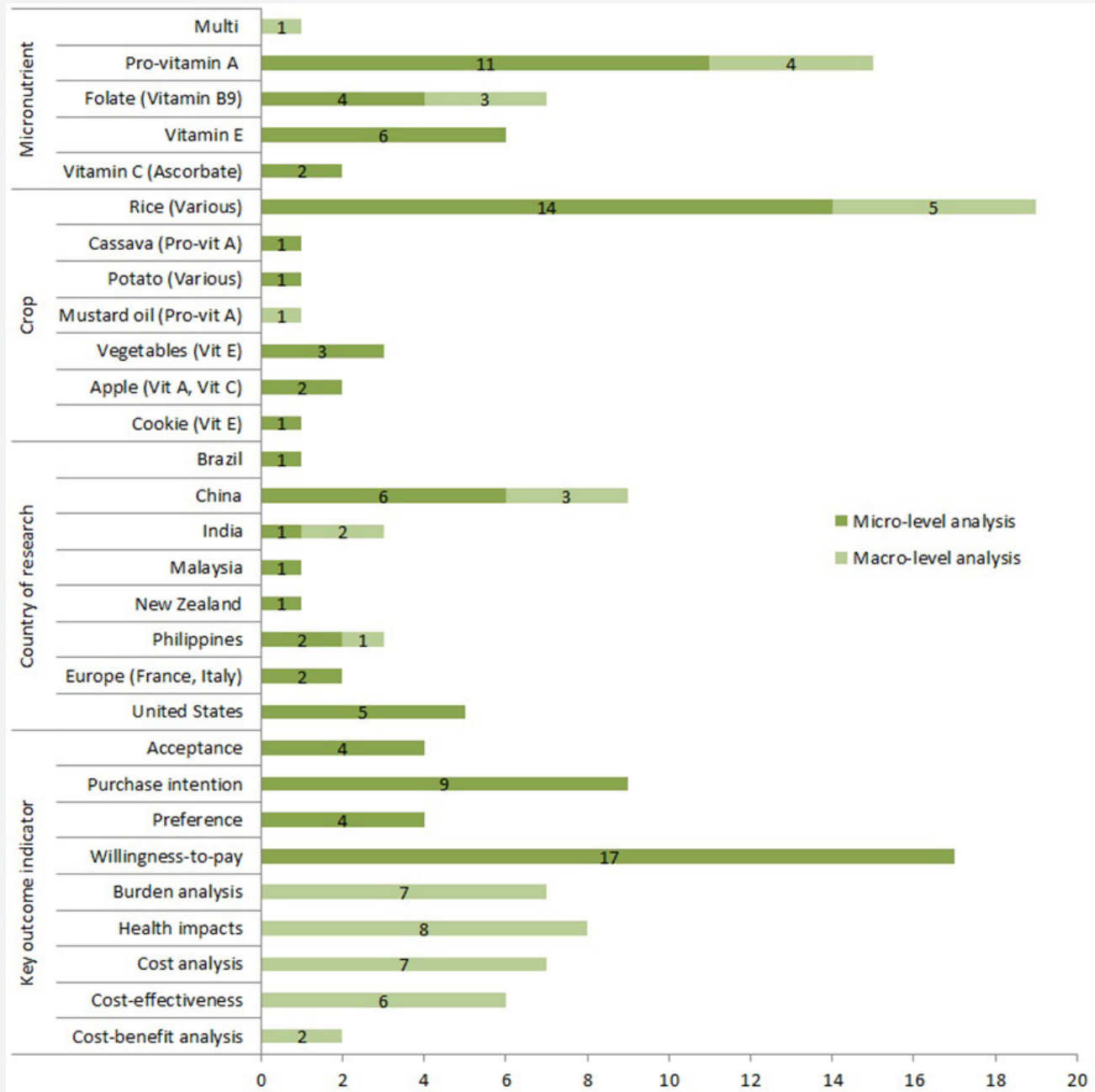
By applying the Disability-Adjusted Life Year (DALY) framework, researchers have also determined the potential health benefits of GE biofortified crops (in DALYs gained per year) by measuring the difference in the burden of the targeted micronutrient deficiency before (current burden) and after (health impacts) the release of biofortified crops (**Figure 4b**). Golden Rice, for example, has the potential to lower the burden of vitamin A deficiency by 9–59%, 6–32%, and 17–60% in, respectively, India, the Philippines, and China. Regarding the latter, folate deficiency and micronutrient malnutrition can be reduced by, respectively, 20–82% and 11–46% when folate biofortified or multi-biofortified rice would be put on the market. Although caution is needed when interpreting and comparing these findings, they can be used as input for benchmarking the effectiveness of various micronutrient interventions. Building upon these impacts, cost-effectiveness analyses were conducted to evaluate whether the costs of the implementation of GE biofortified crops can be justified (in US\$ per DALY gained). Our study reveals that multi-biofortification is by far the most promising option, followed by Golden Rice and folate biofortified rice. This is because the former generates aggregated health benefits at a relatively low additional cost. Nevertheless, all GE biofortified rice varieties are considered to be highly cost-effective interventions.

### Conclusions – the bottom line

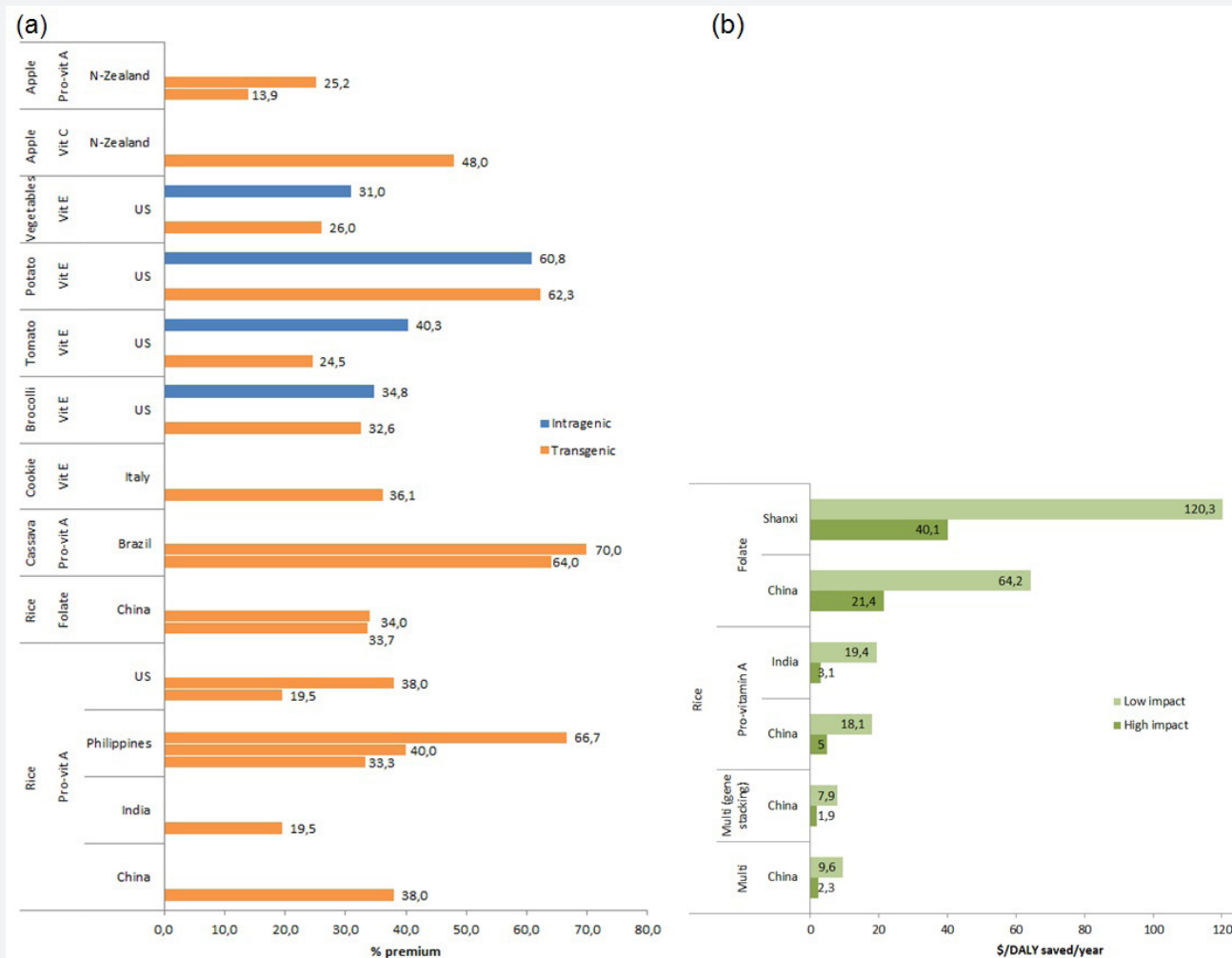
Our study shows that biotech developers are clearly advancing, extending and fine-tuning GE biofortification research, with currently six major staple crops that were successfully biofortified with one or more vitamins or minerals. Moreover, not only the impact of these GE crops on human health, but also their market potential was convincingly demonstrated.

Even though GE crops with health benefits are not a panacea for eradicating malnutrition, they offer a cost-effective alternative when other strategies are less successful or feasible. Unfortunately, GE biofortified crops are expected to face similar commercialization barriers as GE crops with improved agronomic traits, as the ever-repeating delays in Golden Rice commercialization illustrate. In this respect, our review hopes to bring parties together, stimulate the current

scientific and public debate on GE crops, and improve the understanding of biofortification as a means to alleviate the burden of micronutrient malnutrition.



**Figure 3.** Market research on GE biofortified Crops. Number of studies, organized by micronutrient, crop, country and outcome indicator. Source: De Steur et al.<sup>1</sup>



**Figure 4.** Findings of market studies on biofortified crops, per target crop, micronutrient and country. **(a)** Willingness to pay (percentage premium). Premium levels are compared with a conventional, non-GE crop, except for pro-vitamin A apples in New Zealand, which were compared with apples treated with vacuum-infiltration and irradiation, respectively. All values are based on a sample of adults, except for the studies in the Philippines (students) and one study in China (33.7%, women of childbearing age). **(b)** Cost effectiveness (US\$/DALY saved/year). Impact scenarios differ in terms of efficacy (e.g., improved micronutrient content, post-harvest losses and, for pro-vitamin A, bioavailability) and estimated market coverage (i.e., consumption levels), by which low-impact scenarios are rather pessimistic compared with the more optimistic high-impact scenarios. Shanxi Province is a high-risk region of folate deficiency in China.  
 Note: For detailed information and findings on market shares (acceptance, purchase intention and preference) and the cost-benefits (internal rate of return), see De Steur et al.1. Source: De Steur et al.1.

**References**

De Steur H, Blancquaert D, Strobbe S, et al. (2015) Present Status and Market Potential of Transgenic Biofortified Crops. *Nat Biotechnol* **33**, 25-9.

Hans De Steur\*, Dieter Blancquaert, Simon Strobbe, Willy Lambert, Xavier Gellynck & Dominique Van Der Straeten  
 Department of Agricultural Economics – Department of Physiology – Department of Bioanalysis  
 Ghent University, B-9000 Ghent, Belgium  
 \* Email for correspondence: hans.desteur@ugent.be

## Enhancing Photosynthesis in Crops with a Faster Rubisco

P S Janaki Krishna

The enzyme Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) catalyzes the primary photosynthetic CO<sub>2</sub> reduction reaction that fixes atmospheric CO<sub>2</sub> to ribulose-1,5-bisphosphate (RuBP) to form two molecules of 3-phosphoglycerate (3PGA). The two PGAs subsequently are the foundation of all organic molecules. It is difficult to exaggerate the significance of the Rubisco enzyme due to its key role in photosynthetic carbon fixation. Rubisco is found in most autotrophic organisms including photosynthetic bacteria, cyanobacteria, algae, and plants. Carbon fixation resulting from Rubisco's activity amounts to more than 10<sup>11</sup> tons of atmospheric CO<sub>2</sub> annually. However, Rubisco is an inefficient enzyme, and its carboxylation activity is compromised by numerous side-reactions, and the slow turnover of Rubisco hinders the photosynthetic efficiency of vascular plants, especially the C3 plants. Improving Rubisco efficiency has been a research goal for many years. The introduction of the CO<sub>2</sub> concentrating mechanism (CCM) from cyanobacteria into plants, which allows the utilization of a form of Rubisco that has a higher catalytic rate, has been proposed as a means to enhance crop yields. However, the complex nature and structure of Rubisco enzyme challenges researchers who attempt to replace it with enzymes from cyanobacteria.

A team of researchers from the Department of Molecular Biology and Genetics, Cornell University, USA, and Plant Biology and Crop Sciences, Rothamsted Research, Hertfordshire, UK, has reported the development of two transplastomic tobacco lines that have functional Rubisco taken from cyanobacterium *Synechococcus elongates* (Se7942). The researchers developed these transplastomic tobacco lines by knocking out the *rbcl* gene encoding the large subunit of Rubisco by inserting both the large and small subunit genes of the Se7942 enzyme in combination with either the corresponding Se7942 assembly chaperone, Rcbx, or an internal carboxysomal protein, CcmM35, which incorporates three small subunit-like domains. The researchers examined whether cyanobacterial large subunits (LSU) and small subunits (SSU) could assemble into a functional enzyme in higher plant chloroplasts. The

presence of the transgenes was confirmed through RFLP and RT-PCR tests. The expression of the cyanobacterial proteins was tested by SDS-PAGE and through immunoblots.

Transformants showed higher protein molecular mass of cyanobacterial LSU Rubisco compared to wild type tobacco plants. Tobacco SSU was absent in the transformants indicating that it could not form a stable complex with the cyanobacterial LSU. The configuration of the cyanobacterial Rubisco in the transgenic lines was examined through Transmission Electron Microscopy. It was observed that though the enzyme was localized in the chloroplast stroma, the pattern of molecular organization was different in the transformant SeLSX and SeLSM35 lines. In the leaves of SeLSX line, the cyanobacterial Rubisco showed a similar pattern to wild type tobacco, in contrast to the SeLSM35 line in which the Rubisco protein is coexpressed with CcmM35 and both were aggregated as a giant complex in all chloroplasts. Also, the structures observed in chloroplasts almost appeared like procarboxysomes, which are found in early stage carboxysome assembly. The researchers further tested and found that these transgenic plants are completely dependent on the cyanobacterial Rubisco for carbon fixation. As the two transgenic lines could grow autotrophically, it was demonstrated that active cyanobacterial Rubisco was assembled in the chloroplast.

The team opined that if the oxygenation reaction of local Rubisco is suppressed and the CO<sub>2</sub> concentration near the enzyme is increased by further engineering, assimilation of CO<sub>2</sub> may be increased. The researchers also demonstrated in another experiment that the shell proteins of  $\beta$ -carboxysomes also form similar empty compartments in the chloroplast stroma. Hence, the introduction of shell proteins and cyanobacterial Rubisco might enhance photosynthetic performance significantly in vascular plants. In summary, the present study shows that cyanobacterial Rubisco can assemble into active enzyme in C3 vascular plants that support autotrophic photosynthesis, which is a significant step towards introduction of complex CCM that is functional in the chloroplasts of vascular plants, thereby enhancing photosynthetic efficiency and crop yields.

## RISK ASSESSMENT RESEARCH

### References

- Lin Myat T, Occhialini Alessandro, Andralojc P John, Parry Martin A J, Hanson Maureen R. (2014) A faster Rubisco with potential to increase photosynthesis in crops, *Nature*, Vol. 313, 25, S 47-S50
- McGrath J M and Long S P. (2014) Can the Cyanobacterial carbon-concentrating mechanism increase photosynthesis in crop species? A theoretical analysis, *Plant Physiol.* 164, 2247-2261.
- Whitney SM, Houltz RL and Laonso H. (2011) Advancing our understanding and capacity to engineer nature's CO<sub>2</sub> sequestering enzyme, Rubisco. *Plant Physiol.* 155, 27-35,

P S Janaki Krishna  
Associate Professor & Coordinator, PGDM-Biotechnology  
Institute of Public Enterprise, Hyderabad, India  
jankrisp@gmail.com

## Impact of Soil Water Content and Temperature on GE Crop Cry1Ac Protein Degradation

Mei-jun Zhang, Mei-chen Feng, Lu-jie Xiao, Xiao-yan Song, Wu-de Yang, and Guang-Wei Ding

### Background and scope

The rapid adoption of genetically engineered (GE) crops around the world has aroused public controversy. On one hand a number of scientists, regulatory organizations, and governmental agencies have demonstrated that GE crops can generate a high yield cost-effectively in agricultural production. In addition, a few reports indicate that GE crops may be associated with safety issues for human consumption and the environment. Previous researchers have investigated the degradation of purified Cry protein and Cry protein released by transgenic Bt crops such as rice and corn. There are relatively fewer reports on the degradation of Cry protein in cotton. However, based on the report by the International Service for the Acquisition of Agri-biotech Applications (ISAAA), the global planting area of transgenic cotton reached 24.7 million ha in 2011, which accounted for 68.6% of the total cotton production. The insect-resistant transgenic Bt cotton is a major cotton variety used around the world. Therefore, we have initiated an investigation on the degradation of Cry1Ac protein in leaves and buds of Bt cotton in the soil.

Research literature indicates that Cry protein expressed exogenously in transgenic Bt (*Bacillus thuringiensis*) crops can enter the soil ecosystem after secretion by crop roots, through aboveground crop and post-harvest residues returning to soil, and through pollen dissemination. If it is in the soil environment, it is possible for Cry protein to be rapidly absorbed and bound on surface-active particles (e.g., clay minerals, humic

acids, and complexes of montmorillonite-humic acids-Al hydroxypolymers). Obviously, the binding of Cry protein diminishes its bioavailability to microbes, which is likely responsible for the persistence of Cry protein in soil. Therefore, the degradation dynamic characteristics of Cry protein residues in soil is an essential issue for assessing the ecological risk of transgenic Bt crops in environment.

Previous researchers have showed that the Cry protein degradation rate varies significantly, probably due to the diversity of materials, soil type, and experimental methods used. As an illustration, enzyme-linked immunosorbent assay (ELISA) test was used to monitor the purified protein from *Bacillus thuringiensis* subsp. *kurstaki* and the Cry1Ac protein from Bt cotton, which persisted at detectable levels for up to 28 and 56 d in soil, respectively. A further report indicates that the DT<sub>50</sub> (50% degradation time) and DT<sub>90</sub> (90% degradation time) of Cry1Ab protein released from Bt corn tissue in soil are 1.6 d and 15 d, respectively. However, different cultivation techniques (such as thinning of seedlings, final singling, topping, and pruning during cotton growing period), the aboveground residues resulting from these procedures, as well as residual stubble returned to the field by tillage are also closely associated with the degradation rate of Cry protein. We were particularly interested in the soil water content and temperature as the major factors.

### Experimental approach and result

The purpose of our study was to determine the correlation

between degradation of Cry1Ac protein from transgenic Bt cotton (expressing the Cry1Ac protein) and soil water content and temperature. The degradation of Cry1Ac protein in leaves at the thinning stage and in buds in soil under different soil water content and temperature was evaluated by ELISA. The degradation dynamics of Cry1Ac protein in soil was tentatively fit to an exponential model and a shift-log model. In addition,  $DT_{50}$  and  $DT_{90}$  values were also documented. The statistical approach (ANOVA) was adopted to analyze the impact of soil temperature and moisture on the Cry1Ac degradation rate. The results of this work may be useful in assessing the ecological risks of Bt cotton planting.

Soil samples were collected from the top 0 to 15 cm layer in an agricultural experimental cotton field of Shanxi Agriculture University (China). The field had never been planted with transgenic Bt crops before our study. The transgenic Bt cotton variety used was Jinmian 26 (expressing the Cry1Ac protein), which was derived from a commercial cotton variety Jinmian 7 and transformed by *Agrobacterium* infection. Our experimental design consisted of two soil factors (*i.e.*, soil water content and temperature). Three levels of soil water content (50%, 70%, and 100% of water-holding capacity (WHC)) and three levels of soil temperatures (15 °C, 25 °C, and 35 °C) were applied to the experiment. A two-factor completely randomized design was adopted. Nine treatments were initiated for each leaf and bud sample. Each treatment was prepared with 18 replicates of which three replicates were destructively-sampled at each of the six time intervals (*i.e.*, collected on 16, 32, 48, 64, 80, and 96 d). For each treatment, three tubes (triplicate) were removed and stored at -80 °C for analyzing Cry1Ac protein content by ELISA. The Bt protein content was determined by using a commercial ELISA kit according to the protocol described in the literature. Two steps were used in preparing Cry1Ac protein (extraction and quantification). A six-point calibration curve of purified Cry1Ac (supplied with the kit) was used for analysis and comparison.

The first-order kinetics model (exponential model)  $Y(t) = Ae^{-kt}$  was fit to the data set. Where  $Y(t)$  was the amount of residues of Cry1Ac protein in soil at time  $t$ ,  $A$  was the amount of Cry1Ac protein in soil at the initial time,  $k$  was the Cry1Ac protein degradation rate constant,

$t$  was the degradation time (d). The expressions for shift-log model was  $Y(t) = B \times (t+k)^m$ .  $Y(t)$  and  $t$  were the same as that of the exponential model.  $B$ ,  $k$ , and  $m$  were the Cry1Ac protein degradation rate constants. The  $DT_{50}$  and  $DT_{90}$  values under different settings of soil water content and temperature were calculated by using the model in Excel (Microsoft, USA). The statistical analysis of the amount of residue and degradation rate of Cry1Ac protein at each sampling time in the soil as well as the fitting of the degradation equation was carried out using Excel and DPS (data processing system) software. A level of  $P < 0.05$  was considered statistically significant. The analysis of variance (ANOVA, two-way) statistical method was implemented to compare the different impacts of soil temperature and moisture on the Cry1Ac degradation rate ( $P < 0.01$ ).

Generally, the Cry1Ac protein degradation rate demonstrated the same dynamic trend with the same soil water content at various temperatures within the sampling period. As an illustration, on day 48, 56.18 to 93.26% Cry1Ac protein was degraded. After day 48 for each treatment, there was no significant difference in the percentage of residual Cry1Ac protein compared to adjacent sampling period, indicating a declining rate of Cry1Ac degradation. At the end of 48 days, a significant difference ( $P < 0.05$ ) of the amount of Cry1Ac protein remaining in the leaf-soil mixture was observed between the different incubation temperatures if a single water holding capacity was fixed. A similar trend was also obtained in the bud-soil mixture if 70% water holding capacity was used. Moreover, after 32 days there was a significant difference ( $P < 0.05$ ) of Cry1Ac protein in the bud-soil mixture recorded with varying temperature if 50% and 100% water holding capacities were maintained. We observed that relative higher temperature facilitates the degradation of Cry1Ac protein in the soil. The findings of this study imply that under appropriate soil temperature and water moisture, Cry1Ac protein would not persist and accumulate in soil.

Our data indicate that a significant interaction between soil water content and soil temperature impacted the degradation rate of Cry1Ac protein in leaf-soil and bud-soil mixtures, especially before day 32. Under a constant temperature, the degradation rates of Cry1Ac protein in leaf-soil and bud-soil mixture at 100% water holding capacity was significantly lower ( $P < 0.05$ ) than those at

50% and 70% water holding capacity throughout the entire sampling period. Furthermore, the degradation rates of Cry1Ac protein in leaf-soil and bud-soil mixtures with 70% soil water holding capacity and soil temperature of 25 °C and 35 °C either before day 32 or day 48 were significantly higher ( $P < 0.05$ ) than those under 50% water holding capacity. These results further emphasized that excessive soil water content (*e.g.*, 100% WHC) would retard the degradation of Cry1Ac protein in the soil. On the other hand, moderate soil water content (*e.g.*, 70% WHC) was conducive to a rapid degradation of Cry1Ac protein in the early stage. The degradation rates of Cry1Ac protein under 25 °C and 35 °C conditions with different soil water contents were significantly higher ( $P < 0.05$ ) than those at 15 °C with different soil water content over the entire sampling period. Obviously, this result demonstrated that temperature was a major factor affecting the degradation of Cry1Ac protein in the soil. Overall, the degradation rates of Cry1Ac protein in leaf-soil and bud-soil mixtures at 35 °C with 70% water holding capacity were the highest before day 32 and day 48, respectively. The lowest degradation rate of Cry1Ac protein was recorded at 15 °C with 100% water holding capacity over the entire sampling period.

Mathematically, the precision of the shift-log model fit was less accurate under 50% water holding capacity and 35 °C in bud-soil mixture conditions. Then, the exponential model was chosen to estimate the DT values of Cry1Ac protein degradation. If the soil water content was kept constant, the  $DT_{50}$  and  $DT_{90}$  values were least at 35 °C. Alternatively, if the temperature was constant,

a least  $DT_{50}$  and  $DT_{90}$  value was documented under 70% water holding capacity conditions.

### Implications

In summary, the degradation of Cry1Ac protein in both the leaves and buds of Bt cotton in the soil was fastest during the early period. In other words, a slow degradation rate was observed in the later stage. Our results support the observation that temperature is a major factor affecting the degradation rate of Cry1Ac protein in the ecosystem. In addition, a relative higher temperature (25 °C, 35 °C) favors Cry1Ac protein degradation in soil. The highest water content – 100% water holding capacity – is unfavorable to Cry1Ac protein degradation. Furthermore, an exponential model was a better fit of Cry1Ac protein degradation dynamics than that of a shift-log model under different soil water content and temperature conditions. The largest Cry1Ac protein degradation rate was observed at 70% water holding capacity and 35 °C. The  $DT_{50}$  values were 12.29 d and 10.17 d; while the  $DT_{90}$  values were 41.06 d and 33.96 d in the leaf-soil and bud-soil mixture, respectively. Our experimental data indicate that under an appropriate soil temperature and water content, Cry1Ac protein may not accumulate and/or be recalcitrant in soil. The current study also emphasized the significance of interaction effect between soil matrix and the climate factors on Cry1Ac degradation of Bt cotton residues in soil. It is advocated that under natural field conditions, the impact of environment factors on Cry1Ac protein degradation may be more realistic and practical. Additional field investigation is required to further validate the effect of these factors on the degradation of Cry1Ac protein.

### Reference

Mei-jun ZHANG, Mei-chen FENG, Lu-jie XIAO, Xiao-yan Song, Wu-de YANG, Guang-Wei DING. 2015. Impacts of water content and temperature on the degradation of Cry1Ac protein in leaves and buds of Bt cotton in the soil. Published: PLoS ONE. DOI: 10.1371/journal.pone.0115240

*Mei-jun Zhang<sup>1</sup>, Mei-chen Feng<sup>1</sup>, Lu-jie Xiao<sup>1</sup>, Xiao-yan Song<sup>1</sup>, Wu-de Yang<sup>1,\*</sup>, and Guang-Wei Ding<sup>2,\*</sup>*

<sup>1</sup>*College of Agriculture, Shanxi Agricultural University, Taigu, People's Republic of China*

<sup>2</sup>*Department of Chemistry, Northern State University, Aberdeen, South Dakota, United States of America*

*\*sxaywd@126.com; \*guangwei.ding@northern.edu*

## USDA Announces Deregulation of Non-Browning Apples

The U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS) is announcing its decision to deregulate two apple varieties genetically engineered (GE) to resist browning. APHIS is taking this action based on a final plant pest risk assessment (PPRA) that finds the GE apples are unlikely to pose a plant pest risk to agriculture and other plants in the United States. APHIS also completed an environmental assessment (EA) to comply with the National Environmental Policy Act (NEPA) that finds deregulation is not likely to have a significant impact on the human environment. Under APHIS' regulations, pursuant to the Plant Protection Act (PPA), APHIS is specifically required to evaluate if the apple varieties are a plant pest risk to agricultural crops or other plants or plant products. The Act defines a plant pest as organisms, such as bacteria, fungi, or insects that can cause harm to agricultural crops or other plants or plant products. If APHIS finds through its rigorous scientific review that a new GE plant is unlikely to pose a plant pest risk, then under the law and its regulations, it is required to deregulate the GE plant. These varieties, developed by Okanagan Specialty Fruits Inc. (OSF), will be marketed as the Arctic® Granny and Arctic® Golden. OSF is also currently engaging in a voluntary food safety assessment consultation with the Food and Drug Administration regarding its Arctic® Apples. APHIS' final PPRA and final environmental assessment can be found at <http://www.aphis.usda.gov/biotechnology/news>

**Source:**

[http://www.aphis.usda.gov/stakeholders/downloads/2015/SA\\_arctic\\_apples.pdf](http://www.aphis.usda.gov/stakeholders/downloads/2015/SA_arctic_apples.pdf)

