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RISK ASSESSMENT RESEARCH

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Trends in GE Food/Feed Safety Research: Analysis of Potential Conflict of Interests

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Background and scope

Genetically Engineered (GE) crops have been the most rapidly adopted agricultural technology in mankind history, as evidenced by a 100-fold increase from 1.7 to 181.5 million hectares globally cultivated between 1996 and 2014¹. Their environmental and economic benefits for farmers, farm workers, and society have been extensively reported elsewhere^{2,3}.

Although GE crops are not inherently riskier than non-GE crops⁴, the safety of these plants is a key aspect of the rigorous regulatory processes leading to their approval for environmental release and/or food/feed purposes. It is also one of the main topics addressed in the public debate associated with GE crops. Consequently, the safety of GE crops has been an object of intense research and study over the past 20 years⁵.

In terms of food/feed safety, GE crops are thoroughly assessed for potential toxicity, allergenicity potential, and possible unintended effects among other factors that might result from the expression of foreign proteins driven by genes inserted into a host plant. Detailed compositional analyses of nutrients and antinutrients specific to individual plant transformation events in different crops and the nutrition and health of animals fed on GE crops or feedstuffs are also evaluated extensively on a regular basis⁶.

The scientific literature produced over the years on GE crop safety is extensive, and it has reported the existence of more than 31,848 reports up to 2006⁶. Nevertheless, the vast majority of these reports do not represent original research papers published in scientific journals, but rather abstracts, notes, opinions, and commentaries not subjected to peer review, and scientific reviews. Further, only 237 of those reports dealt with GE food/feed safety matters, including detection, compositional, toxicological, and nutritional analyses⁷.

Recently, a list of scientific papers on GE crop safety has been compiled, analyzing the distribution and composition of the literature published from 2002 to October 2012. A total of 1,783 reports, including original research papers, reviews, relevant opinions, commentaries, and reports addressing all the major issues that emerged in the debate on GE crops, were classified, and 770 out of the total were related to GE food/feed safety issues. The main conclusion was that scientific research conducted thus far has not detected any significant hazards directly associated with the use of GE crops⁵.

In this context, GE crop safety scientific literature is sometimes questioned or even plainly disregarded in the public debate, either arguing the limited number of studies, or the possibility of conflicts of interests (COIs) that might bias and/or cast doubt on their findings^{8,9}. This has raised concerns over the need for more research in this field and the possible influence of financial and professional conflict of interests in the design and outcomes of scientific studies addressing GE food/feed safety issues. Financial COIs arise when research is fully or partially

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funded by a party with a stake in the development of GE crops, whereas professional COIs arise when at least one author is affiliated with a company developing GE crops even though the research is supported through public funding. I was particularly interested to determine how many original research papers related to GE food/feed safety have been published in peer review journals up to present. A further investigation of COIs would certainly indicate the independence or bias of the researchers.

Methodological approach and results

My work presented an assessment of original research scientific papers addressing food/feed safety aspects of GE crops published in scientific journals with peer review (hereafter referred to as reports)¹⁰. Variables such as composition, evolution, COIs, and the scientific journal of publication of GE crops or products derived thereof were analyzed. In order to retrieve those reports, the databases PubMed and Web of Science were scanned and search terms were derived from previous work^{5,7}. Additionally, bibliographical references cited in the identified reports were further analyzed to identify additional reports not recovered from database searches. The full list of identified reports is provided as Supplementary Material in the original publication in Nature Biotechnology¹⁰. Abstracts, opinions, and commentaries were excluded from this analysis. In order to avoid redundancy, scientific reviews were excluded as well. Furthermore, to gain insight about COIs, individual reports were manually checked, enabling us to assess information regarding authors' affiliation and funding sources.

A total of 707 original reports were identified. In order to understand the dynamics of GE food/feed safety scientific publications over a 21 years period (1993-2014), only those 698 that had full text access (digital or physical) and a peer review process before publication were inspected and considered for further analyses. Two kinds of papers were considered: those suggesting a lack of GE food/feed adverse effects; and also reports suggesting potential hazards. It is worth noting that less than 5% of all reports analyzed fell in the latter category.

Each of those publications was classified according to the main objective of the study, namely allergenicity potential, animal health, animal nutrition, equivalence, mycotoxins, processing (effects on protein functionality during food manufacturing), traceability/digestion (DNA or proteins), and unintended effects.

The category with the most reports was animal health, with 204 reports representing 29.2% of reports analyzed. Allergenicity potential had 46 reports (6.6%), whereas mycotoxins and processing were less represented with 18 papers each (2.6%). Animal nutrition had 111 reports (15.9%), equivalence 106 (15.2%), unintended effects 104 (14.9%), and traceability and/or digestion (DNA or proteins) 91 (13%).

In terms of presence/absence of COIs, 407 out of 698 reports, or 58.3%, lack declared financial and professional COIs. Overall, 180 out of 698 articles, or 25.8%, had declared COIs either in terms of the author affiliation or funding source. The distribution of COIs across functional categories is uneven. A considerable number of reports related to animal health (67.2%), traceability/digestion (69.2%), allergenicity potential (71.7%), processing (77.8%) and unintended effects (77.9%), lack of any kind of COIs (financial or professional). Meanwhile, categories related to equivalence (43.4%), animal nutrition (27.9%), and mycotoxins (11.1%) showed lower rates of lack of COIs. Reports where the authors were not related to companies that develop

GE crops, but they did not provide funding information, represented a remaining 15.9% (111 articles).

From another perspective, categories related to equivalence (43.4%), animal nutrition (53.2%), and mycotoxins (66.7%) showed the highest rates of presence of COIs. The other five categories had less than 20% of financial or professional COI: unintended effects (8.7%); traceability/digestion (11%); processing (11.1%); animal health (16.2%); and allergenicity potential (19.6%).

Implications

According to my results, GE food/feed safety issues have been and continue to be extensively studied. The cumulative number of original research reports has dramatically increased over the last years and publication levels remain high. Different areas of GE food/feed safety have been addressed from a scientific perspective where animal health is the most frequently studied topic. My analysis indicates that only approximately one-quarter of all reports investigated here have COIs related to author

affiliation and/or declared funding source, with 15.9% not reporting funding information. Even though sometimes the public argues that the presence of COIs in scientific research related to GE food/feed safety might raise concerns about the outcome of the studies, I confirmed that the majority of the reports have no conflict from author affiliation and funding source. In other words, at least 58.3% have no COI.

Overall, the analysis of all 698 reports collected indicates that GE crops are extensively evaluated for potential hazards and also that genetic modification technologies based on recombinant DNA do not carry a risk *per se*. Considering all reports analyzed, and taking into account the pros and cons of the experimental models used in each investigation, their findings are consistent with the principles underpinning food/feed regulatory approval of GE crops, that they must be as safe as their non-GE counterparts. Finally, claims either that there is not sufficient peer-reviewed literature evaluating GE food/feed safety issues or that COIs prevail in the published literature are not supported by this analysis.

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“GE Food” Labels: Polls and Politics

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Genetically engineered (GE) crops are more popular than ever – at least among farmers. According to the International Service for the Acquisition of Agri-Biotech Applications, farmers in 28 countries cultivated a record 181.5 million hectares of GE crops during 2014. This represents an increase of more than six million hectares from the previous year. Once again, the United States led the pack with 73.1 million hectares, an increase of three million hectares from 2013. Some public opinion polls indicate that the increasing cultivation of GE crops parallels a rise in the American public’s distrust of food made from GE crops. Many polls focus on one aspect of this distrust: The notion that the government should establish mandatory labeling of food made from GE crops. In the United States, decisions about food labeling start with the Food and Drug Administration (FDA).

Clear Facts About GE Food Labels

The FDA ensures the safety and wholesomeness of most foods, an authority conferred by the Federal Food, Drug, and Cosmetic Act (FD&C Act). The FD&C Act authorizes the FDA to require information on a label only if the information is accurate and material.

More than two decades ago, the FDA considered if food produced from GE crops should be labeled to reveal that fact to consumers. In 1992, the FDA announced that the agency does not consider the method of development of a new plant variety to be generally material information. The FDA refused to require GE food labels. This decision reflects the FDA’s science-based approach to evaluate the safety of foods produced from new plants: Focus on the characteristics of food and its components, rather than the fact that a new method was used at some point to produce the food.

Advocates of mandatory GE food labeling promote the popular – albeit illusory – “consumers’ right to know.” Yet the FDA has stated that the FD&C Act does not provide the authority to mandate labeling based solely upon a consumer’s right to know the method of food production if the agency considers the final food product to be safe. The FDA has offered guidelines

for companies to voluntarily label their food products to inform consumers that they do not use ingredients produced with biotechnology.

Murky Measures of Public Opinion

At least one poll indicates that Americans applaud FDA policy on labeling. During May 2014, the International Food Information Council Foundation announced the results of its consumer survey performed in March and April with 1,000 US participants. This poll indicated that 63 percent of Americans approve the FDA’s current voluntary policy for labeling GE foods. Only four percent named “biotech” as something they want information about on their food labels. About 70 percent said that they would be likely to buy “GE foods” that provide benefits lacking in conventional foods.

Other surveys paint a different picture of the public’s attitude about labeling. For example, an Associated Press-GfK poll revealed that 66% of Americans favor GE food labels. The survey of 1,010 US adults was performed online during early December 2014. GE food label advocates would require food manufacturers to put labels on products to indicate that they contain GE ingredients. Twenty-four percent of those surveyed said that they did not care, whereas 7% opposed labels. Although 66% voted in favor of labels, only 42% said that the presence of GE ingredients in food is extremely or very important in determining “whether a food item is a healthy choice or not.” Apparently, many people just like to have as much information as possible on their food labels.

Despite polls indicating that Americans want GE food labels, voters in recent years defeated ballot initiatives to require labeling in California, Washington, and Oregon. What do polls really say about GE food label preferences? Perhaps, very little.

Many factors can affect the results of a survey. “Respondents tend to want to please the pollster by answering in ways that they perceive are sought, even though they might not be aware of it,” says *Los Angeles Times*’ Karin Klein.

Klein suggests that polls also can prime respondents to answer in a certain way. This may explain why a recent

Oklahoma State University survey revealed that eighty percent of respondents support mandatory labels on any food that contains DNA. The DNA question appeared within a long list of questions about government policies regarding the safety of food. “So people who favor protective policies were in a way primed or lulled into saying yes,” Klein says, “unless they looked and questioned closely.”

Another factor that may explain the failure of GE food ballot initiatives, Klein suggests, is that respondents “didn’t think about any of the counter-arguments until there was an actual vote.” Some survey respondents support labeling even though a GE food label would not affect their decision to buy the product. They might feel differently about labeling if the survey included information about the increased cost of food under a labeling scheme.

Vermont’s GE Food Labeling Law: Model or Muddle?

Legislators from several states forged ahead with bills that require labels on food products that contain ingredients from GE crops. Maine and Connecticut passed GE label laws that will not take effect until neighboring states pass similar legislation. The Vermont General Assembly passed a GE label bill in 2014 that lacks a neighboring state proviso. Governor Peter Shumlin signed the bill – known as Act 120 – into law, while encouraging label advocates to support a fund to help pay legal expenses for the expected lawsuit.

The Grocery Manufacturers Association (GMA), the Snack Food Association, International Dairy Foods Association, and the National Association of Manufacturers filed a complaint at the US district court in Burlington during June 2014. Citing various

Constitutional grounds for declaring Act 120 invalid, the plaintiffs asked the court to issue a permanent injunction that would prevent the defendants from enforcing or implementing any aspect of the Act.

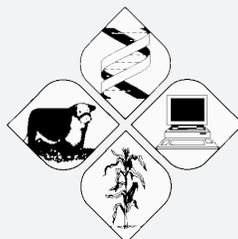
“Vermont’s mandatory GMO labeling law,” the GMA asserted in a press release, “is a costly and misguided measure that will set the nation on a path toward a 50-state patchwork of GMO labeling policies that do nothing to advance the health and safety of consumers.”

Unless it is struck down, Act 120 will become effective on July 1, 2016. Meanwhile, Vermont attorney general William Sorrell issued proposed regulations designed to implement Act 120, and held a public meeting to discuss the rules in January 2015. The proposed regulations raised new potential problems.

In comments filed with the Vermont attorney general, the Washington Legal Foundation, a national public interest law firm and policy center, warned that the rules could create massive tort liability for food manufacturers. “Vermont’s law requiring labeling of food containing genetically modified ingredients is an unworkable ‘Frankenstatute’ that could lead to massive litigation every time food intended for shipment elsewhere accidentally finds its way into the state,” the foundation’s chief counsel stated in an issued release. “The labeling requirement is highly questionable to begin with, given the absence of any material difference between food that contains GM ingredients and food that does not. There is no reason to compound Act 120’s folly by permitting the plaintiffs’ bar to pile on.”

The problem highlighted by the Washington Legal Foundation shows the impracticality of relying on state-based laws to create a GE food labeling system. A practical system must originate from the FDA.

“The labeling requirement is highly questionable to begin with, given the absence of any material difference between food that contains GM ingredients and food that does not.”



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Improving the Salinity Tolerance of Soybean

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Introduction

Soil salinity reduces crop yield. The extent and severity of salt-affected agricultural land around the globe is predicted to worsen due to the use of poor quality irrigation water, rising water tables, and climate change. The growth and yield of the majority of crop species is adversely affected by soil salinity, but varied adaptations can allow some crop cultivars to continue to grow and produce a harvestable yield under moderate soil salinity. Soybean, which is ranked as the fourth largest crop in terms of global yield, is a major food, fuel and feed crop, and has been classified as sensitive to salinity. Identification of genes that improve soybean salt tolerance would be useful for extending the agricultural range of soybean into soils with moderate salinity and therefore would be of use for meeting the challenging global food security targets set for 2050. Here, we summarize our recent research finding that *GmSALT3* (*Glycine max* Salt Tolerance-associated gene on chromosome 3) is a major gene involved in tolerance of soil salinity by soybean.

***GmSALT3*, a dominant gene responsible for salt tolerance in soybean**

Soybean germplasm displays a spectrum of salt-tolerance phenotypes. We used this natural variation in

salt tolerance to identify genes that increase soybean production under saline conditions. In 1969, a major quantitative trait locus (QTL) for salt tolerance was discovered in soybean that was predicted to be conferred by a single gene. Many research groups have since mapped this QTL to the same region on soybean linkage group N (chromosome 3), despite using distinct soybean parents and crosses between cultivated soybeans or crosses between wild and cultivated soybeans. These consistent results for the presence of a salt tolerance QTL indicate that this region on chromosome 3 might play an essential role in salt tolerance of soybean. Furthermore, the same region has been highlighted using different genetic backgrounds, which has led some to hypothesize that a conserved gene or several genes control salt tolerance in diverse soybean germplasm. We used map-based cloning within a soybean population derived from the salt-tolerant variety Tiefeng 8 and the salt-sensitive variety 85-140 to identify the salt-tolerance associated gene *GmSALT3* underlying this major QTL. RNA sequencing (RNA-seq) indicated that the *GmSALT3* coding sequence obtained from Tiefeng 8 was over twice the length of that from 85-140, with the sequence from 85-140 likely to result in a truncated and non-functional protein.

To understand the function of a candidate gene, it is useful to explore where it is expressed in the plant. Expression analysis demonstrated *GmSALT3* was in greater abundance in roots compared to shoots of Tiefeng 8 (the salt tolerant parent), and although the transcript decreased transiently when initially challenged with 200 mM NaCl, it gradually recovered within three days. Using *in situ* PCR, a technique that allows the localization of transcripts within tissue, we detected the expression of *GmSALT3* predominantly within endodermal cells and cells associated with phloem and xylem of salt-tolerant Tiefeng 8 soybean roots; the localization was unchanged under salinity treatment. We used a genetic engineering technique (using the fusion of a fluorescent marker protein with our protein of interest and its expression in cells isolated from tobacco and *Arabidopsis*) to detect to which membrane the *GmSALT3* protein localized. We observed that *GmSALT3* localized to an endomembrane, the endoplasmic reticulum (ER), and so shares this localization with the nearest characterized homolog to *GmSALT3*, CHX20 from the brassica *Arabidopsis thaliana*.

To investigate the role of *GmSALT3*, sodium ion (Na^+) accumulation within the two soybean parents was compared. The Na^+ content in roots of the parents was similar, but following NaCl treatment (200 mM) Tiefeng 8 accumulated significantly less Na^+ than 85-140 in both stems (after 5 days) and leaves (after 7 days). To compare the function of the two *GmSALT3/Gmsalt3* alleles, we developed a pair of near isogenic lines (NILs) NIL-T (*GmSALT3* from Tiefeng 8) and NIL-S (*Gmsalt3* from 85-140). Under control conditions, the NILs had no significant difference in agronomic traits, such as 100-seed weight, and protein and oil content, but had differential salt tolerance. The Na^+ content in stems and leaves of self-grafted NIL-S was much higher than that in NIL-T when the NIL-S scion was grafted on the NIL-T rootstock. The Na^+ content in the NIL-S scion was 49% lower in stems and 71% lower in leaves when grafted to NIL-T roots. In contrast, the Na^+ content in stems and leaves of the NIL-T scion grafted to the NIL-S root increased by 79 and 139%, respectively, compared with self-grafted NIL-T.

Collectively, these results suggest that *GmSALT3* is likely to function in the root (and stem), which is consistent with the predominant expression pattern of *GmSALT3* in Tiefeng 8 in roots and stem, and that this ER-localized protein constrains Na^+ translocation to the leaves.

The salt tolerant haplotype (H1) of *GmSALT3* is likely to be the ancestral allele and a target of natural and artificial selection

By screening 172 landraces and 57 wild soybean, we identified a total of nine haplotypes (i.e., versions of this gene, H1-H9), five from landraces and eight from wild soybean. Two of these alleles were associated with conferring salt tolerance and seven resulted in salt sensitivity. Furthermore, our study identified minimal genetic variation between the two salt-tolerant alleles (H1/H7) compared with the more extensive variation in the seven salt-sensitive alleles (H2-H6, H8, H9), with H6-H9 only found in wild soybean. The salt-tolerant H1 (which is the version of the gene found in Tiefeng 8) is the most frequently found haplotype in both wild soybean and landraces, and it has the widest geographical range. By integrating genotype and phenotype information, we found a clear relationship between H1 and salt tolerance. The high frequency of H1, its co-occurrence with salt-affected soils, the low genetic diversity of salt-tolerant alleles, and the high genetic diversity in the salt-sensitive alleles are all hallmarks that the H1 allele is likely to be the ancestral allele and has been strongly favored during natural and/or artificial selection around salinity affected areas.

Hypothesized model of salt tolerance in soybean related to *GmSALT3*

Based on the data we have gathered so far, we hypothesize that once the salinity selection pressure was released on soybean (i.e., when the plants encountered low concentrations of salt in the soil) *GmSALT3* was no longer sensitive to acquiring genetic change, including insertions and deletions, as it resulted in no detrimental effect on the plant phenotype in the low-salt environment. However, the resulting mutations have led to a loss, or reduced function, of the gene product, resulting in a loss of salt tolerance. This greater mutation rate in the salt-sensitive alleles may indicate that the loss of function of *GmSALT3* confers a growth advantage for these plants on non-saline soils. However, we find no evidence that this is the case, as we have not detected any growth or yield advantage in the NIL-S lines compared with the NIL-T line when grown under control conditions for the agronomic traits we tested. Interestingly, there is a small subset of soybean cultivars, such as Peking and Baipihuangdou, that contains H1 but is salt sensitive. They are likely to contain recent mutations

in other key salt-tolerance genes such as *SOS1* or *HKT1*, or contain genetic elements that are yet to be identified that may be a useful source for discovering novel genes involved in salt tolerance in soybean.

GmSALT3 is expressed in root stelar cells, which are cell types already known to have a role in limiting salt transport to the shoot. For instance, the Na⁺ transporters (*HKT1*;5-like proteins) expressed in these cells directly retrieve Na⁺ from the root xylem. In plants expressing the correct *HKT1*;5-like allele, this is associated with reduced Na⁺ content in shoots and superior salt tolerance in rice, wheat, and *Arabidopsis*. A similar lower Na⁺ content in the shoots of salt stressed H1-containing plants compared with H2 suggests that this gene may also affect transport of Na⁺ from root to shoot, and the grafting of NIL-T and NIL-S lines showed that the root and stems were sufficient to limit the accumulation of Na⁺ in the shoot. However, the *GmSALT3* transcript level was first down-regulated and then gradually recovered in roots; this is different from the pattern of other salt-tolerance genes, indicating a distinct salt response in soybean or a different role for this gene. Consistent with this is the ER localization we observed for *GmSALT3* compared with the plasma membrane localization of *HKT* or *SOS1*

proteins. Therefore, *GmSALT3* is unlikely to play a direct role in the retrieval of salt from the xylem but instead may have a role in sensing or responding to salt.

Conclusions and implications for agri-biotechnology

The identification of the gene underlying this important soybean salt-tolerance allele has provided an insight into the molecular basis of both natural and human selection of salt tolerance in soybean. In light of the predicted 70–110% increase in food production that will be needed by 2050 to feed the global population over the same period, and with no current option for expanding the area of agricultural land, an increase in the salt tolerance of conventional crops will be required to assist in improving crop productivity and food security. To achieve this goal, both traditional plant breeding programs using marker-assisted selection and genetic engineering (GE) technologies can contribute. We have previously bred durum wheat to contain an ancestral *HKT1*;5-like gene and this led to a 25% increase in grain yield under saline field conditions. We expect *GmSALT3* to confer an even larger increase in yield in soybean—these experiments

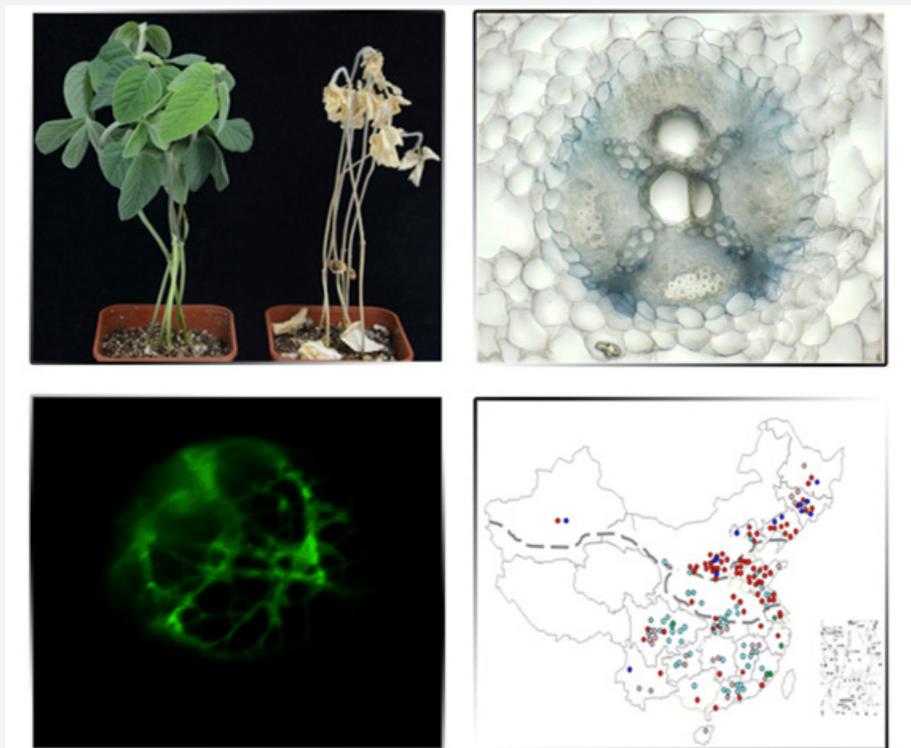


Figure 1. Identification of genes that improve crop salt tolerance is essential for the effective utilization of saline soils by agriculture. Here, we use fine mapping in a soybean [*Glycine max* (L.) Merr.] population derived from the commercial cultivars Tiefeng 8 and 85-140, to identify *GmSALT3* (*Glycine max* Salt Tolerance-associated gene on chromosome 3), a dominant gene associated with sodium (Na⁺) exclusion from the shoot and soybean salt tolerance. The above montage describes the phenotypic variation between Tiefeng 8 and 85-140 treated with 200 mM NaCl for 18 d (top left); the tissue localization of *GmSALT3* within root phloem- and xylem-associated cells in the salt-tolerant parent Tiefeng 8, as indicated by blue colour (top right); the subcellular localization of *GmSALT3* to the endoplasmic reticulum (ER) in *Nicotiana benthamiana* protoplasts (bottom left); distribution of different haplotypes of *GmSALT3* gene in China, indicated by different color dots (bottom right). Source: Guan et al. 2014.

are underway. Our most recent study in soybean can now provide markers to breeders for targeting the salt resistance traits into new soybean germplasm, which should facilitate the rapid development of new elite salt-tolerant soybean cultivars by marker-assistant selection. Alternatively this gene can also be introduced via GE technology. Whilst the

exact mechanism of how GmSALT3 imparts salt tolerance is still unknown, this protein also offers the opportunity for improving salt tolerance in other crop species. To this end, the role of homologs of *GmSALT3* is being currently investigated—as is the exact mechanism by which GmSALT3 impart salt tolerance.

More detailed information about GmSALT3 can be found in the open access publication on the Plant Journal: "Salinity tolerance in soybean is modulated by natural variation in GmSALT3." Markers and additional information are available on request.

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