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PLANT RESEARCH NEWS

CONTENTS

How Do Plants Deal with Flood and Drought? Biotechnological Strategies to Enhance the Natural Response and Improve Yield

Karina F. Ribichich and Raquel L. Chan

Plants cope with drought and flood triggering different strategies

Water deficit and water excess are major factors affecting plants yield worldwide. To cope with such factors, plants trigger physiological and biochemical changes allowing them to survive for a limited period of time.

Plants deal with **water deficit** by modifying some of their morphological and physiological characteristics. Examples of these characteristics are a cuticle that reduces transpiration, the ability to close stomata and reduce leaf surface area, and the ability to accelerate senescence. However, these adaptive modifications usually have detrimental consequences, such as a decrease in biomass and yield until plant death, depending on the strength and duration of the stress.

To cope with **water excess**, plants trigger one of two strategies; quiescence and escape. Quiescence involves a reduction in carbohydrate consumption for a few days, to recommence after the water drains. This occurs in some species that tolerate complete flooding. On the other hand, the escape strategy consists of the fast elongation of internodes, maintaining a low metabolic activity for months to continue growth. This strategy is triggered by cultivars partially covered with water that overgrow the water level. Both strategies exhibit a few common characteristics such as the emergence of adventitious roots, the formation of aerenchyma, and hyponastic growth.

When plants are flooded, both submerged or waterlogged, respiration and photosynthesis are drastically reduced with a concomitant loss in biomass and yield. Notably, after complete desubmergence and re-oxygenation, plants must overcome dehydration—an objective that is not always reached, and hence they die from drought.

Water deficit and excess seem to be opposites; however, the stresses caused by these factors, as well as the responses triggered by the plants to deal with them, share several common features.

Transcription factors are crucial to plant adaptation

Transcription factors (TFs) are regulatory proteins with a DNA binding domain that recognizes specific sequences in target genes and other domains that are able to interact with the basal transcriptional machinery. These regulatory proteins can induce or repress entire transduction signal pathways. In plants, TFs are more abundant than in other kingdoms. They have been classified into families and subfamilies according to the conserved binding domains and also to other structural characteristics and functions. Using functional studies, some of these families are associated with responses to abiotic stresses. As examples of

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these families, the WRKY (after the WRKY domain composed by these four amino acids), the bHlH (basic-Helix-loop-Helix), the b-Zip (basic domain associated to a Leucine Zipper), the AP2/ERF (after APETALA2 and Ethylene Responsive Factor), the HD-Zip (Homeodomain-Leucine Zipper), and the NAC (after NAM, ATAF1/2 and CUC2) families have members well characterized as negative or positive regulators of the responses to drought and flooding stresses, mostly to only one of such responses, in several species including crops. The response pathways to drought, mediated by such TFs, are described in some cases as abscisic acid (ABA)-dependent and in others as ABA-independent. ABA is called the "stress hormone" because it has been broadly involved in abiotic stress signaling. However, not all the TFs participating in abiotic stress responses and plant adaptation are regulated by ABA.

Crosstalk between drought and flood stress response mediated by transcription factors

As mentioned above, the responses triggered by plants to deal with flooding and drought stresses share common features, among which TFs play key roles. Most studies of common responses have been conducted in the model species *Arabidopsis thaliana* (Arabidopsis) and *Oryza sativa* (rice). Proteins belonging to the AP2/ERF family are especially important in such response crosstalk. For example in rice, *SUB1A*, a gene belonging to the AP2/ERF family, acts in both responses: drought and submergence. Also in the dicotyledonous Arabidopsis, several ERFs from group VII have different roles, sensing anoxia and thereafter inducing hypoxia-responsive genes.

The responses to drought and flood in non-model species are less explored, although among these species, several have adapted during evolution to live in different water-regime environments. In particular, Asteraceae species have adapted to diverse environments, climates, and topographies and are characterized by their plasticity. This could be the case of *Helianthus annuus* or common sunflower, a crop able to grow in very different agronomic situations, in particular on soils with variable water levels (**Fig.1**).

Transcription factors from Asteraceae species involved in abiotic stress responses

The scientific literature shows that several TFs of Asteraceae species involved in abiotic stress tolerance have been characterized. Some examples of Asteraceae TFs characterized during the last five years are: sunflower HaWRKY6, involved in temperature response and regulated by an miRNA; chrysanthemum DgWRKY3, involved in salt tolerance; sunflower HD-Zip I members HaHB4, HaHB1 and HaHB11, conferring drought, freezing, and submergence tolerances, respectively; chrysanthemum DREB subfamily member of the AP2/ERF family CgDREBa and the bHLH member CdICE1, both involved in freezing, salt, and drought tolerance; chrysanthemum MYB TF CmMYB2, conferring salinity and drought tolerance; chrysanthemum NAC DgNAC1, enhancing salt tolerance; and chrysanthemum zinc finger protein DgZFP, conferring salt tolerance (**Table 1**). Because of the unavailability of mutant libraries of Asteraceae species, most of these functions were

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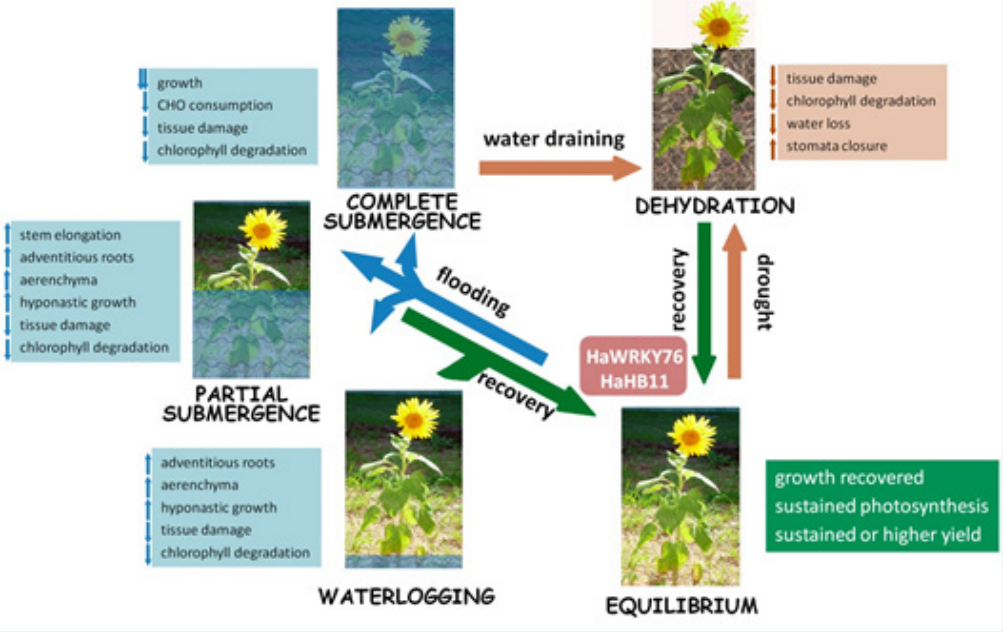

Figure 1. Probable responses of sunflower plants subjected to different water regimes
Schematic representation of different stressful situations suffered by sunflower plants because of water scarcity or excess, and their probable tolerant responses. Boxes: main effects caused by these situations. Arrows indicate the adaptive response in sunflower which would be different than in other species subjected to the same stresses. HaWRKY76 and HaHB11 are signaled as positive regulators of recovery.

assigned after transformation of heterologous species like Arabidopsis, tobacco, or rice, and a few after transforming the Asteraceae chrysanthemum. Some of these TFs have homologues in model species presenting high sequence similarities and functions, whereas others are unique to Asteraceae species, named divergent TFs.

Are Asteraceae species better adapted to stress because of their divergent transcription factors?

Considering the outstanding adaptation of Asteraceae species to different environments and having TFs key regulatory proteins, divergent TFs are particularly good candidates to study such adaptation. Among Asteraceae species, sunflower is the most important from an economic point of view. Although its genome is not completely known, because of its great size and complexity and that there are not mutant resources or routine protocols to transform it, sunflower remains especially interesting to research adaptation to abiotic stresses such drought and flood.

Our research group is interested in understanding how sunflowers are so well adapted and, for this reason, sunflower TFs were chosen as research subject. Most sunflower TFs function remains elusive, but some knowledge has been acquired. Our initial hypothesis was that the differential adaptation of this species could be due, at least in part, to divergent genes encoding TFs that are not conserved in model plants.

Because of difficulties in studying sunflower TFs, a general experimental strategy was applied. Using sequences published in public databases and bioinformatic analyses, we selected TFs that diverge from typical TFs in

Table 1. Examples of Asteraceae genes encoding Transcription Factors involved in abiotic stress responses

Gene name and source	Function	Reference
Sunflower *HaWRKY6*	High temperatures	Giacomelli et al., 2012
Chrysanthemum *DgWRKY3*	Salt tolerance	Liu et al., 2013
Sunflower *HaHB4*	Water deficit	Dezar et al., 2005
Chrysanthemum *CgDREBa*	Freezing temperatures, salinity and drought	Chen et al., 2012
Sunflower *HaHB1*	Freezing temperatures and drought	Cabello et al., 2012
Chrysanthemum *CdICE1*	Freezing, salt and drought tolerance	Song et al., 2014
Chrysanthemum *CmMYB2*	Salinity and drought tolerance	Shan et al., 2012
Chrysanthemum *DgNAC1*	Salinity and drought tolerance	Liu et al., 2011

model species for further analysis. The first step was to determine the expression pattern of the encoding genes in plants grown in standard conditions or subjected to different stress treatments, particularly flood and drought. Genes responsive to such stresses were selected as they are likely to have an adaptive role. These genes or their corresponding cDNAs were cloned in vectors suitable to transform plants and used to stably or transiently transform Arabidopsis or sunflower leaf-disks, respecitvely. Once transgenic Arabidopsis plants were obtained, phenotypical characteristics were determined in both standard and stressful conditions. Transcriptome analyses in transiently transformed sunflower leaves or in stable transgenic Arabidopsis were conducted in order to determine which pathways were regulated by each TF. In this way, functions of sunflower divergent TFs were determined, and additionally, a few of were used for crop improvement.

Sunflower transcription factors role in drought and flood responses

Using the strategy described above, two sunflower TF families were studied: the HD-Zip I and the WRKY families. HD-Zip proteins are characterized by the presence of a homeodomain (HD) and a leucine zipper (LZ); the HD is able to bind DNA and the LZ conforms dimers, a prerequisite for DNA binding. Besides these conserved domains, HD-Zip proteins exhibit different conserved motifs, mostly of unknown function, in their carboxy- and amino-termini, whereas WRKY proteins have the WRKY domain, responsible for DNA binding, a zinc finger domain, and other motifs, also mostly of unknown function. Because the sunflower genome is not completely known, it is not possible to state how many members of each of these families exist in this species. However, analyses of public EST databases and the available literature allow an estimation of 97 sunflower WRKYs and 19 HD-Zip I proteins. These estimations are imprecise, and divergent members were identified in both families. For both TF members, divergence is defined as the presence of conserved motifs that are absent in similar proteins of model plants.

Expression analyses of transcripts of HaWRKY76 from the WRKY family and HaHB11 from the HD-Zip I family showed an induction caused by both water deficit and water excess treatments. In both cases the induction by flooding was detected during desubmergence, indicating a role in drought stress responses. Transcriptomic studies of transformed sunflower leaf-disks indicated that genes encoding enzymes from fermentative pathways were regulated by HaHB11. Moreover, transgenic Arabidopsis plants ectopically expressing these genes driven by the constitutive *35S CaMV* promoter exhibited enhanced tolerance to flooding and drought to different degrees, depending on the transgene expression level.

Both types of transgenic plants displayed the quiescent strategy when flooded, whereas the mechanisms by which drought tolerance was achieved is less clear; however, experimental evidence indicated that such mechanisms are ABA-independent.

Biotechnological applications

Adaptive responses to flooding and drought stresses usually cause unfavorable consequences, which can vary depending on the stress strength and duration. When transgenic plants are evaluated for their ability to better respond to such stresses, survival rate of the plants is the most frequently reported metric, whereas biomass production or seeds yield are not usually assessed. In crop production, the latter traits are more important, and survival rates are not good indicators of the viability of a given technology. In crop improvement programs, the yield or biomass, depending on the crop, should be correlated with stress tolerance under varying conditions.

Arabidopsis transgenic plants ectopically expressing *HaWRKY76* or *HaWRKY11* showed enhanced tolerance to both drought and flooding and, more importantly, these plants exhibited enhanced yields under standard growth conditions, and fewer penalties after stress treatments, than the controls, leading us to propose the transcripts as potential biotechnological tools to improve crops. Because these studies were conducted in Arabidopsis, further work is needed demonstrate that these technologies are suitable for crops.

Concluding remarks

Plant responses to abiotic stresses are complex and use different mechanisms depending on the type of stress, developmental stage, growth conditions, and other internal and external factors. Such responses vary between species, and some species are better adapted

than others to survive and grow in adverse environments. Asteraceae species are particularly adapted to such adverse growth conditions; therefore, the study of differential responses in Asteraceae constitutes a strategy not only also to understand plant adaptive mechanisms, but also to identify the genes involved in these responses use them to confer stress tolerance. Transcription factors, as key molecules participating in adaptive mechanisms, are good candidates to use as biotechnological tools for less-well adapted crops. Our work demonstrates that the sunflower divergent transcription factors confered tolerance to water deficit and water excess conditions and increased yield to varying degrees. Although using current techniques it is not possible to affirm that these TFs are unique and responsible for the sunflower adaptation to stress, we can propose that both play significant roles in this species adaptation and both are potential biotechnological tools to improve crops.

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USDA Slowly Adapts to New Technology in the Regulation of Biotech Products

Phillip Jones

On Oct. 9, 2008, the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) published a proposal to amend regulations for the importation, interstate movement, and environmental release of certain genetically engineered (GE) organisms. The agency explained that the proposal was prompted by advancements in technology and by experience in implementing the current regulations.

APHIS is part of the federal government's Coordinated Framework for the Regulation of Biotechnology, a system first devised three decades ago.

Federal Regulation of Biotech Products

The Coordinated Framework (1986; revised 1992) established the federal government's risk-based system intended to ensure that new products of biotechnology would be safe for the environment, human health, and animal health. The system is based on several guiding principles. One principle is that federal agencies should define which transgenic organisms are subject to review to the extent permitted by an agency's statutory authorities. Federal agencies also must focus on the characteristics and risks of the biotechnology product, not the process used to create the product. Finally, federal agencies are directed to exercise oversight of GE organisms only if there is evidence of "unreasonable" risk.

Based upon existing laws, the Coordinated Framework provides regulatory roles for three agencies: the Food and Drug Administration (FDA), the Environmental Protection Agency (EPA), and APHIS. A biotech product may be subject to the jurisdiction of one or more of these agencies.

Under the authority of the Federal Food, Drug, and Cosmetic Act, the FDA must ensure the safety and proper labeling of all plant-derived foods and animal feeds, including GE foods and feeds. Food ingredients produced from GE plants must adhere to the same safety requirements under the Act that apply to food ingredients produced from traditionally bred plants.

The EPA regulates the sale, distribution and use of pesticides, including pesticides produced by biotechnology. The Federal Insecticide, Fungicide, and Rodenticide Act gives the EPA authority to regulate plant-incorporated protectants, such as anti-insect toxins produced by GE plants. The EPA also sets tolerance limits for residues of pesticides associated with food and feed, or the agency establishes an exemption from the tolerance requirement under the Federal Food, Drug and Cosmetic Act.

APHIS is responsible for protecting agriculture from pests and diseases. The agency has regulatory oversight over biotech products that could pose such a risk under the authority of the Plant Protection Act. Organisms and products known or suspected to be plant pests or to pose a plant pest risk are classified as regulated articles. APHIS regulates the import, handling, interstate movement, and release into the environment of regulated organisms, including those undergoing confined experimental use or field trials. These include organisms and products altered or produced by genetic engineering.

USDA's Branch of the Coordinated Framework Gets Creaky

The USDA's duty to protect agriculture from pests and diseases encompasses GE plants in several ways. During the 1980s, a popular method for producing transgenic plants required segments of the tumor-inducing plasmid of *Agrobacterium tumefaciens*, a bacterial parasite that can inflict crown gall disease in plants. Transgenes were introduced into plants by infection with modified *Agrobacterium* cells. Moreover, transgene expression was often controlled by regulatory elements from plant virus genomes.

Advances in technology change the way that researchers alter plants. These new methods create plants that can fall outside APHIS regulations.

About 12 years ago, the Scotts Miracle-Gro Company was developing GE grass until the plants spread beyond test plots. The escape ended government approval for commercial use of the grass and almost

led to the closing of the company's biotech program. Today, Scotts has new types of grasses. Characteristics, such as Roundup® resistance, have been introduced with plant genes and microprojectile bombardment. The novel plants do not need federal approval before they can be field-tested and marketed.

Scotts chief executive Jim Hagedorn explained the company's new strategy to analysts in December 2013, as reported by Andrew Pollack of *The New York Times*. "If you take genetic material from a plant and it's not considered a pest," Hagedorn said, "and you don't use a transformation technology that would sort of violate the rules, there's a bunch of stuff you can do that at least technically is unregulated."

Gene editing enables a researcher to alter a plant's genes without the need of introducing a transgene. Gene editing can be achieved using site-specific endonucleases to target a double-stranded break in a gene. The gene is then disrupted or otherwise modified. Scientists argue that gene editing introduces modifications in a manner that is more precise than traditional – and unregulated – techniques of treating plants with chemicals or radiation to induce random mutations in the hope that the exposure will produce a desirable change in the genome.

Cibus, a San Diego-based company, produces plants with new traits using a gene editing platform. For instance, the company's SU Canola™ is a non-transgenic sulfonylurea herbicide tolerant canola available in the United States. The USDA regards Cibus' technology to be a modern form of mutagenesis, which should not be regulated by US agencies.

More generally, APHIS has decided that agricultural plants produced with gene editing using meganucleases, zinc finger nucleases, and transcription activator-like effector nucleases do not fall within its regulatory authority. The financial benefit of avoiding APHIS regulation is clear. According to one study, companies spend about $35 million in regulatory costs to develop a GE agricultural plant. The USDA can require more than two years to review an application. The costs in money and time have shaped the development of agricultural biotechnology: Large companies dominate the industry.

Alan B. Bennett and colleagues (Department of Plant Sciences, University of California, Davis) found an increase in requests to the USDA for nonregulated status of GE crops produced with new technology. They suggest that small companies and public institutions may be altering plant genomes with gene editing and other newer technologies to avoid the burdens of the US regulatory system. It is time to update that system, they say.

> The fact that the US Coordinated Framework is on the one hand failing to oversee these new product types and on the other overregulating GE crops and technologies with proven track records of safety should be a cause for concern. We conclude that it is time to reevaluate the US regulatory framework for GE crops and build a system that is based on science, with enough flexibility to evolve with accumulating scientific knowledge and technologies and, importantly, that allows the participation of small companies and public sector institutions.

Reinforcing the Coordinated Framework

During February 2015, APHIS announced that the agency withdrew its 2008 proposed rule that would have amended regulations covering GE crops. This, after receiving more than 88,000 comments. Three months later, APHIS asked for comments on whether GE crops should be regulated as noxious weeds and about situations that justify biotech regulations. In June, dozens of businesses and consumer groups filed their recommendations. The White House soon overshadowed this effort.

On July 2, White House science adviser John P. Holdren and three other senior White House officials announced a plan to revise the Coordinated Framework, noting that advances in science and technology since the 1992 revision have altered the product landscape. Echoing Alan Bennett and his colleagues, the White House officials said that "the complexity of the array of regulations and guidance documents developed by the three Federal agencies with jurisdiction over biotechnology products can make it difficult for the public to understand how the safety of biotechnology products is evaluated, and navigating the regulatory process for these products can be unduly challenging, especially for small companies."

The goal of this project is to "ensure public confidence in the regulatory system and improve the transparency, predictability, coordination, and, ultimately, efficiency of the biotechnology regulatory system." To achieve this goal, the Coordinated Framework update has

three objectives.

First, following public comments, the Administration will update the Coordinated Framework by clarifying the responsibilities of the EPA, USDA, and FDA in the biotech regulatory process. The update should spell out which biotech product areas are within the authority of each agency and outline how the agencies should work together to regulate products that fall under the authorities of multiple agencies.

Second (again, following public input), the Administration will develop a long-term strategy to ensure that the regulatory system can efficiently assess any risks associated with future products of biotechnology. In addition, the updated Coordinated Framework should support innovation, protect health and the environment, increase transparency and predictability, and reduce unnecessary costs and burdens.

Third, the EPA, FDA, and USDA will commission a series of independent analyses of future biotech products. The reports will identify potential new risks and methods for risk assessment. An analysis should be completed at least every five years.

Starting with a Washington, DC, meeting next fall, the Administration will sponsor three public sessions to discuss the Coordinated Framework revision. Revisions also will undergo public notice and comment before the new Coordinated Framework is finalized.

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EU's GRACE CONFERENCE

GMO Risk Assessment and Communication of Evidence

9th-10th November 2015

Potsdam, Germany

Registration deadline: 30th September 2015
Website: http://www.grace-fp7.eu/de/content/invitation
Press meeting on 10th November 2015 at 2.30 pm

This conference will mark the end of GRACE, a research project funded by the European Commission's 7th framework programme from 2012-2015. 18 research institutions from 13 countries are involved in the project.

GRACE pursues two key research objectives:

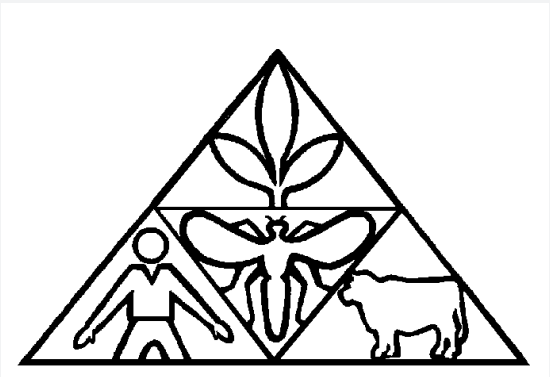
Firstly, it aims to provide comprehensive reviews of the existing evidence on the health, environmental and socio-economic impacts of GM plants – considering both risks and possible benefits. GRACE reviews go beyond what has been done so far and are conducted in a highly systematic, transparent and inclusive way, based on concepts of systematic reviews and evidence maps. Both types of evidence synthesis tasks have been proposed or used as a valuable tool to support policy making in many areas including medicine, environmental studies, social studies, and - more recently - food safety. In the context of GMO risk research and risk assessment systematic evidence synthesis methods are fairly new and their potentials were investigated in the frame of the GRACE project.

Secondly, GRACE evaluates animal feeding trials (90-day, 1-year) and alternative methods for use in GMO risk assessment. This is a particular topical issue as the need for mandatory animal feeding studies in GMO risk assessment in the European Union will be evaluated in 2016.

The conference will not only provide an overview of conclusions and recommendations drawn from the GRACE studies in the course of a multistep stakeholder engagement process (day 1), but will also offer opportunities to discuss the broader perspectives and future implications of GRACE and related research projects for the refinement of GMO impact assessment and policy making (day 2). In this context, we will also address the role of society in fostering the design of research and innovation in order to better align them with the values, needs and expectations of society.

This conference is open to all stakeholders interested in GMO impact assessment. This includes but is not limited to GMO risk assessors, risk managers, policy makers as well as representatives of all relevant sectors (competent authorities, industry, professional organisations, civil society organisations and academia).

Prof. Joachim Schiemann
GRACE project coordinator



DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. APHIS-2014-0076]

J.R. Simplot Co.; Determination of Nonregulated Status of Potato Genetically Engineered for Late Blight Resistance, Low Acrylamide Potential, Reduced Black Spot Bruising, and Lowered Reducing Sugars

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that InnateTM Potato designated as Russet Burbank event W8, which has been genetically engineered for late blight resistance, low acrylamide potential, reduced black spot bruising, and lowered reducing sugars, is no longer considered a regulated article under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by J.R. Simplot Company, in its petition for a determination of nonregulated status, our analysis of available scientific data, and comments received from the public in response to our previous notices announcing the availability of the petition for nonregulated status and its associated environmental assessment and plant pest risk assessment. This notice also announces the availability of our written determination and finding of no significant impact.

DATES: Effective September 2, 2015.

ADDRESSES: You may read the documents referenced in this notice and the comments we received at http://www.regulations.gov/#!docketDetail;D=APHIS-2014-0076 or in our reading room, which is located in room 1141 of the USDA South Building, 14th Street and Independence Avenue SW., Washington, DC. Normal reading room hours are 8 a.m. to 4:30 p.m., Monday through Friday, except holidays. To be sure someone is there to help you, please call (202) 7997039 before coming.

Supporting documents are also available on the APHIS Web site at http://www.aphis.usda.gov/biotechnology/petitions_table_pending.shtml under APHIS Petition Number 14-093-01p.

FOR FURTHER INFORMATION CONTACT: Dr. John Turner, Director, Environmental Risk Analysis Programs, Biotechnology Regulatory Services, APHIS, 4700 River Road Unit 147, Riverdale, MD 20737-1236; (301) 851-3954, email: john.t.turner@aphis.usda.gov. To obtain copies of the supporting documents for this petition, contact Ms. Cindy Eck at (301) 851-3892, email: cynthia.a.eck@aphis.usda.gov.