Biofortified sorghum in Africa: using problem formulation to inform risk assessment

To the Editor:

Most of the genetically modified (GM) crops approved to date (e.g., corn, cotton and soybean improved for insect resistance or herbicide tolerance) do not have compatible wild relatives near their intended area of cultivation, and those that do are not being cultivated in the center of diversity of the species. However, many GM crops being developed to solve agronomic or nutritional problems in developing countries may be grown near centers of origin and diversity of the crop, where these plants were first domesticated and remain major crops[1]. Furthermore, they are often being developed by publicly funded, nonprofit institutions[2]. Such developers, and the regulatory authorities that oversee them, often have relatively limited experience and resources for risk assessment and are faced with some of the first decisions regarding risks associated with gene flow in centers of diversity.

Although the potential for negative effects of gene flow from GM crops in centers of diversity must be considered, some would argue that another kind of risk will be increased if the benefit offered by these products is delayed[3,4]. It is essential, therefore, that data required for risk assessment, including those related to gene flow, are limited to information necessary to allow sound regulatory decisions. Numerous studies related to gene flow from GM crops have been conducted or proposed to address interesting research questions, including evaluations of distance and rates of gene flow, fitness of hybrids, ecosystem dynamics and other parameters[5]. Although some of these studies are useful for decision making, many lack a clear identification of the harm and how the study relates to a causal pathway from the GM crop to that harm. This accumulation of data under the name of 'risk assessment' can lead to considerable confusion about what is necessary for a regulatory decision[6].

The use of appropriate problem formulation to identify data needs has gained attention recently in discussions on risk assessment of GM crops[7–12]. Problem formulation begins with the identification of the protection goals of the law or other instrument that triggered the risk assessment (e.g., protection of biodiversity). A proper problem formulation then derives adverse effects (harm) as operational assessment endpoints (e.g., the abundance of a valued species) based on the protection goals. This is followed by the development of possible scenarios of harm (that is, how there may be adverse change to the assessment endpoints given what is known about the crop plant, the introduced traits and the environment; a risk scenario or conceptual model). Testable hypotheses can then be formulated and an experimental plan to test them can be determined.

The advantage of following the steps of problem formulation is that it focuses data acquisition on clear questions to help decision makers, rather than on attempts to exhaustively characterize all possible outcomes following cultivation of GM crops. It is important to recognize that for risk assessment to be effective, harm must be defined before data acquisition. Definitions of harm are necessarily subjective, and subjectivity in risk assessment cannot be eliminated by doing more scientific research. Thus, extensive collection of data cannot substitute for clear decision-making criteria[6,8,10].

In the following article, we present a case study that shows how these concepts can be applied to risk assessment for GM nutritionally enhanced sorghum intended for cultivation in the center of diversity of the crop and provides a model to help focus the criteria for risk assessments of other GM crops in their centers of diversity. The case is based on a discussion among a panel of individuals (including the authors of this correspondence) with expertise and experience in risk assessment, gene flow, sorghum biology and sorghum as a crop in Africa. This was assembled at the Donald Danforth Plant Science Center in St. Louis in October 2008 by the Program for Biosafety Systems, an organization involved in capacity building for regulation of biotech, to discuss the environmental risks associated with gene flow to wild relatives in the case of African biofortified sorghum (ABS). This panel was not convened to make a determination of the level of risk, but to discuss how it is possible to assess the risk. The steps of problem formulation were used to guide this discussion.

Sorghum is a major crop and staple food in sub-Saharan Africa. ABS is being developed with funding from the public sector in a humanitarian effort to bring better nutrition to the people of Africa (see http://www.grandchallenges.org/ImproveNutrition). Biotech is being used to introduce genes into sorghum for increased lysine and threonine, increased protein digestibility, reduced phytic acid to enhance the availability of iron and zinc, as well as increased levels of the vitamin A precursor beta carotene. The specific genes inserted into ABS and their modes of action were considered during our discussion. The genes are being combined in a single unit that will behave as a locus, to be expressed in the seed endosperm only. These sorghum lines will soon be ready for field trials and for breeding to introduce the genes into suitable local varieties.

The center of origin and diversity for sorghum is in the Ethiopia-Sudan region of Africa[13]. Existing data suggest that gene flow does occur readily between the crop and nearby or sympatric weedy populations, although very rarely to distant, more-or-less truly 'wild' populations[13–15]. According to theory, even neutral genes from cultivated sorghum, which are not expected to have a selective advantage or disadvantage by definition, may persist in the wild populations, even if gene flow should be rare[16,17]. The discussion panel agreed that when GM sorghum is grown in standard conditions for the cultivation of sorghum, transgenes are likely to be transferred to and persist in the wild populations, as with other genes from cultivated sorghum. For the purposes of a risk assessment, in this case, it should not be necessary to carry out any additional studies to test for the likelihood or frequency of gene flow to wild sorghum.

The important question the panel identified for environmental risk assessment of gene flow from ABS in Africa is whether there may be harmful consequences when the transgenes enter the wild populations through gene flow. To answer this question using problem formulation, the first steps are to determine the protection goals and identify assessment endpoints that fit those goals. In many countries, protection goals are defined by law. If no legal definition exists, it may be necessary to define the goals in the risk assessment, perhaps using precedent from similar assessments elsewhere.

Identification of the harm presents one of the greatest challenges for risk assessors. As noted before, 'harm' is subjective and cannot be deduced scientifically; science can help us predict whether there will be consequences of actions, but it cannot determine whether those consequences are acceptable[18]. In this case study, harm is defined as adverse changes to ecological assessment endpoints. We recognize that assessment endpoints could also be cultural, political or economic but did not consider those endpoints in our discussion.

In this case, we considered specific adverse changes to valued entities (that is, harms) and scenarios by which they could result from gene flow from ABS to wild sorghum (**Table 1**). The harms we identified include loss of valuable genetic diversity in the crop, loss in abundance or diversity of valued flora or fauna, and loss of crop yield. More than one scenario could lead to each of the identified harms, and each scenario is based on our knowledge of the biology of sorghum, the introduced traits, the environment where it will be grown and population genetics theory. Some of these scenarios are those typically associated with gene flow, such as loss of diversity due to a selective sweep or genetic swamping. Other scenarios are more specifically related to knowledge about the biology of the crop and the introduced traits. For example, the panel recognized that bird feeding is a serious problem already in sorghum but did not agree about whether this would have an impact in wild relatives of sorghum, or that there was a reason to expect the traits being introduced into ABS would make the seeds more attractive to birds; however, birds are known to prefer seeds with low-tannins[19], and the panel agreed that an unintended reduction in the level of tannins should be considered (**Table 1**; harm 1, scenario 4). During the problem formulation phase of risk assessment, it must be decided which scenarios are plausible, warranting further investigation, and which scenarios are so unlikely that they do not need to be considered[7–12]. We included most of the scenarios that we discussed, although there was some disagreement about which were plausible.

Having clearly outlined the harms and possible ways that ABS might lead to them, we developed a testable hypothesis of 'no harm' for each scenario identified, which can then be corroborated or refuted with existing or new observations. A testable hypothesis for each of these potential scenarios for harm is shown in **Table 1**.

Various other hypotheses could have been formulated related to each scenario. It is not necessary to test every possible hypothesis, but ideally the hypothesis to test is one that will give most confidence that the scenario leading to harm is not likely[8]. In the case of ABS, the panel (including the authors of this correspondence) agreed that the scenarios by which the identified harms could occur are only likely if there are unintended changes associated with the transformation. All of these hypotheses of no difference can be tested by conducting a thorough comparison of the GM and non-GM sorghum for the specific characteristics in the hypotheses, to evaluate the likelihood that the identified harms will not occur from ABS.

Such a thorough characterization of a GM crop, which includes characteristics related to agronomic performance, survival and reproduction, disease and insect susceptibility, nutritional composition and known toxicants, is standard practice during GM crop development. Comparative assessment to detect differences between the GM crop and a comparator, usually its non-GM counterpart, forms the foundation of risk assessment for GM crops currently[12,16]. This is generally conducted in the laboratory and in field trials, which may be carried out over multiple seasons and in multiple locations. Field trials are conducted with appropriate measures for confinement of plant material, including the restriction of gene flow. If any potentially harmful unanticipated changes are detected during this characterization, further assessment or risk management options would be considered. It should be noted that certain unanticipated changes such as disease or pest susceptibility could have a significant effect on the comparative yield of the GM crop, in which case the product may not be deployed owing to poor agronomic performance not related to biosafety.

The panel also determined that an additional study to compare characteristics related to survival and reproduction in 'ABS × wild' hybrids and 'non-ABS × wild' hybrids could be conducted to test the hypothesis that transgene interaction with wild genetic backgrounds will not significantly increase the survival and reproduction of hybrids. Each of the harms identified is possible if there is an increase in survival and reproduction due to such an interaction (**Table 1**). Interactions between transgenes and 'wild' genes are not expected to increase hybrid survival

Table 1 A plan to assess the potential environmental risks of gene flow ABS to wild sorghums in Africa

Harm	Risk scenarios	Hypotheses	Experimental plan
Harm 1. Loss of valuable genetic diversity in the crop or compatible species	Scenario 1. Loss of allelic diversity in the wild sorghum due to a 'selective sweep'. A selective sweep following the movement of transgenes into the wild populations would likely leave the populations more genetically uniform in parts of the genome closely linked to the transgenes under strong selection[17,20]. This requires a substantial selective advantage for plants with the ABS transgene.	Hypothesis. ABS traits will not increase survival or reproduction of sorghum.	A thorough comparison of ABS and non-GM sorghum for characteristics related to survival and reproduction, disease and insect susceptibility, nutritional composition, and known toxicants.
	Scenario 2. Loss of allelic diversity due to 'genetic swamping'. 'Genetic swamping', whereby the wild species becomes genetically inextinguishable from the crop plant ('extinction by assimilation') is often cited as a risk from gene flow, but circumstances that would lead to such swamping are likely to be rare[17]. Genetic swamping from crop sorghum to wild sorghum does not occur currently; therefore, harm via this route would require a substantial increase in the hybridization frequency associated with ABS.	Hypothesis. ABS traits will not change the hybridization frequency of sorghum.	
	Scenario 3. Loss of abundance of wild sorghum due to 'outbreeding depression'. In certain circumstances, populations may decline if there is a reduction in the ability of hybrids to survive and reproduce following hybridization[17]. If the ABS transgenes reduce survival and reproduction, populations of wild sorghum could decline following hybridization with ABS.	Hypothesis. ABS traits will not reduce the survival or reproduction of sorghum.	
	Scenario 4. Loss in abundance of wild sorghum due to increased bird preference. Higher levels of tannins in sorghum seeds can make them less palatable to birds[18]. If the level of tannins decreases in ABS compared with those in other cultivated sorghums, birds may preferentially feed on the wild sorghum with the ABS traits over other nonsorghum seed sources. It is difficult to predict how this change in bird behavior could affect the dynamics of wild sorghum populations. It could potentially decrease the abundance of wild sorghums.	Hypothesis. ABS traits will not decrease tannin levels (increase bird preference) in sorghum.	
Harm 2. Loss in abundance or diversity of valued flora (native)	Scenario. Loss of native plants due to competition with wild sorghum. Loss of abundance or diversity of flora is possible if the wild sorghums that carry the transgene become invasive in unmanaged ecosystems (outside of agriculture) and outcompete other native plants.	Hypothesis. ABS traits will not increase the survival and reproduction of sorghum.	
Harm 3. Reduction in abundance or diversity of valued fauna (wildlife or domestic animals)	Scenario 1. Reduction of a critical food source for native fauna due to competition with wild sorghum. The loss of plant species abundance or diversity (flora, as in harm 2) could also have a detrimental impact on the abundance or diversity of native fauna that co-habit with wild sorghum.	Hypothesis. ABS traits will not increase the survival and reproduction of sorghum.	
	Scenario 2. Increased toxicity to native fauna. Native fauna, as well as domestic animals, that feed on wild sorghum could be affected if the GM traits introduced into sorghum should lead indirectly to an increase in the toxicants in sorghum. Endogenous toxins known to occur in sorghum include cyanide, tannins and nitrate.	Hypothesis. ABS traits will not increase the endogenous toxicity of sorghum.	
	Scenario 3. Decreased nutritional value for native fauna. Should the introduced ABS traits affect an unintended change that decreases the value of the existing nutritional composition of sorghum, there may be a detrimental effect on animals that feed regularly on wild sorghum.	Hypothesis. ABS traits will not decrease the existing nutritional value of sorghum.	
Harm 4. Significant decrease in yield of crops	Scenario 1. Increased abundance or persistence of wild sorghum in cultivated plantings. If the ABS traits lead to an increase in the weediness that renders wild sorghum, which can already be a problematic weed, more difficult to control in cultivated plantings or more competitive with crop plants, the result could be a loss of crop yields.	Hypothesis. ABS traits will not increase the survival and reproduction of sorghum.	
	Scenario 2. Increased reservoirs for crop pests. Crop yield could also be affected if the ABS traits are associated with an increase of disease or pest infestation in the wild sorghums, as these plants could serve as a reservoir for the pests and contribute to an increase in pest incidence in crop plantings. Changes in amino acid composition using mutation breeding in the past have been associated with decreased seed hardness and increased fungal and insect susceptibility[21].	Hypothesis. ABS traits will not increase disease or insect infestation in sorghum.	
Harms 1–4	Scenario. Increased selective advantage, invasiveness or weediness due to interactions between the transgene and wild genes. If there is an interaction between the transgene and the genes in the wild sorghum which results in an increase in survival and reproduction, the 'harms' that have been identified above might be possible, where an increase in survival and reproduction is part of the risk scenario/hypothesis.	Hypothesis. ABS transgene-interaction with wild genetic backgrounds will not increase the survival and reproduction of the hybrids.	A thorough comparison of characteristics related to survival and reproduction in 'ABS x wild' hybrids and 'non-ABS x wild' hybrids.

and reproduction relative to non-GM hybrids, but few studies have been done specifically to address this question. A carefully designed set of experiments would test this hypothesis. Although it would not be necessary to repeat a study like this for every GM crop, especially if collective evidence or prior experience with the transgene demonstrates no potentially harmful gene interaction effect, most panel members agreed that these hybrid studies would be useful for a regulatory decision concerning ABS.

In this case of ABS, we considered environmental impacts associated with gene flow that are commonly of regulatory concern, including loss of diversity in flora or fauna due to invasiveness or toxicity and yield loss in crops due to increases in weediness, and we considered case-specific scenarios by which these harms could occur. We determined that experimentation to test whether the identified harms are likely to occur only requires a thorough characterization of the GM plants and GM plant × wild plant hybrids for specific characteristics compared with non-GM plants. If a hypothesis is falsified, then additional experiments would be necessary.

Although the harms that we identified in this case of nutritionally enhanced sorghum in Africa may be typical of those to consider for any transgenic crop cultivated in its center of diversity, it is conceivable that other harms might be identified, based on the protection goals within a particular regulatory framework, or on the specific details of a different case (that is, crop, trait or environment). Even when the harms are similar, a different set of hypotheses and experiments may be developed depending on the case. For example, if the introduced trait were one that might be expected to confer a fitness advantage (e.g., insect, disease or stress tolerance) in sorghum, some harms may be more likely or other scenarios more plausible, and therefore a different set of hypotheses and experiments might be developed[7,8,12]. This might also be true if the same nutritionally enhanced traits were introduced into a different crop or environment. In a similar manner, problem formulation can be applied for risk assessment related to concerns other than from gene flow, such as impacts on nontarget organisms[11,12].

By focusing on the initial problem formulation phase of a risk assessment, it is possible for developers and regulators to gain a clear indication of the important questions to answer, and the data required to address them. By clearly identifying what are the harms, considering scenarios that might lead to them and developing testable hypothesis when necessary, risk assessments can be conducted in a manner that is open and transparent for all parties. This will allow developers and regulators, especially those with relatively limited experience in risk assessment, to move forward with confidence in their efforts to develop products and assess the risks, and to safely provide these technologies that hold such promise.

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The authors declare no competing financial interests.

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