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CONTENTS

Did Transgenic Cotton Cause Varietal Diversity Erosion in India?

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Introduction

Several studies have examined the on-farm impacts of transgenic crops. A consensus has been reached on the technology's agronomic and economic potentials to contribute to farmers' income and welfare[1]. However, there are still widespread concerns that the introduction of transgenic crops could exacerbate the loss of agrobiodiversity[2]. Alongside the possibilities of 'gene flow' occurring through outcrossing of transgenes into wild relatives of domesticated crops, critiques are apprehensive about farmers replacing a diverse portfolio of indigenous crop varieties and landraces by a few genetically uniform high-yielding transgenic varieties. A counterargument is that if transgenic technology were introduced in many existing (local) crop varieties simultaneously, the rate of adoption of the technology and the associated welfare impacts would be higher, while biodiversity loss could be prevented[3]. However, to the best of our knowledge, the relationship between the adoption of transgenic crops and the diversity of crop varieties on-farm has never been empirically tested, especially not in developing countries.

In a recent paper published in the *European Review of Agricultural Economics*[4], we postulated that whether or not varietal diversity erosion occurs through the introduction of transgenic crops will depend on both demand- and supply-side factors in the local seed market. We examined this hypothesis using the case of Bt cotton in India. When Bt technology was introduced in India in 2002, only three Bt hybrids were officially released, even though the cotton-growing regions are highly heterogeneous with respect to soil and agro-climatic conditions. In subsequent years, the number of companies and varieties with Bt technology has grown rapidly owing to an active proprietary seed network system that already existed in the country. By 2012, over 1000 different Bt varieties were supplied in India[5]. Using four rounds of panel data collected between 2002 and 2008, we tested the hypothesis that the supply of only a few transgenic varieties would reduce varietal diversity, whereas varietal diversity could be preserved when more transgenic varieties are supplied.

2. Concept

Smallholders often maintain a relatively high level of on-farm varietal diversity in the traditional farming systems. However, they also constantly experiment with new varieties under a dynamic management regime. Farmers' demand for varieties depends on the relative importance of two functions of agrobiodiversity — *insurance* function and *productivity-enhancement* function. There is a trade-off between these functions, potentially determining the farmers' demand for on-farm varietal diversity. Farmers who seek to avoid downside risk

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may choose to include lower-productive but more adaptable (less vulnerable) local varieties in the portfolio, even though the full adoption of higher-yielding varieties could be more profitable in a normal season. Here, maintenance of diversity is associated with an opportunity cost; a relative reduction of production risk would allow farmers to lower on-farm diversity. Many risk-reducing inputs, such as irrigation and pesticides, could thus have a negative diversity impact, as the farmer can achieve a given level of production with more certainty using these inputs.

The productivity-enhancement function of varietal diversity implies that growing several varieties on the same farm may also increase mean yield levels, which may be due to complementarity or scale effects[6]. Complementarity effects occur in a production system when particular varieties perform better in the presence of others, for instance through lower infestation levels of certain pests or diseases. Scale effects arise when the functioning of the system is affected by its degree of fragmentation. Different plots on the same farm may differ in terms of soil type, slope, and other characteristics, so that crop performance may be increased when varieties that are optimally suited for each plot are cultivated. Again, a new high-yielding variety that is not optimally suited for all plots may be adopted only partially, especially when plot heterogeneity is significant.

How and to what extent will a farmers' demand for on-farm varietal diversity change with the adoption of a new transgenic variety? The answer depends on the relative importance of the insurance and productivity-enhancement functions of diversity. Most transgenic crop technologies available so far involve insect resistance, virus resistance, or herbicide tolerance[5], hence, introduction of such transgenic crops is likely to reduce production risk. At the same time, it is likely to increase yield, not necessarily through higher yield potential but through more effective damage control[1].

3. Background and data

In India, cotton is typically cultivated by smallholders in farms with less than 5 ha of land[7]. Before the introduction of Bt cotton in 2002, pest management was highly chemical intensive. Despite this, cotton bollworms (mainly *Helicoverpa armigera*) caused significant economic loss. Aiming to manage the bollworm complex, genes from the soil bacterium *Bacillus thuringiensis* (Bt) were transferred to the cotton genome, making it resistant to borer pests, thus reducing the dependency on chemical measures of pest control. The insect resistance trait was introgressed from a US Bt cotton variety (event MON531) developed by Monsanto. Within a decade of introduction of Bt cotton in India, the adoption rate has increased to over 90%. India is now the country with the largest area under transgenic crops in Asia[5].

Apart from the significant direct economic benefits of Bt technology, the specific impacts of regulatory policies associated with technology diffusion as well as indirect yield impacts through affecting on-farm varietal diversity have hardly been examined. When Bt cotton technology was officially introduced in India in 2002, there were only 3 Bt hybrids approved for sale. All of them were released under the joint venture of Monsanto with the Indian firm Maharashtra Hybrid Seed Company (MAHYCO). The Bt hybrids were marketed under the trade name Bollgard™. The slow increase in the number of transgenic varieties until 2004 could be attributed

to the fact that every single Bt variety needs approval by the national Genetic Engineering Approval Committee (GEAC). However, in 2005 and 2006 GEAC approved more than 60 new Bt cotton hybrids developed by 13 seed companies. By 2012, over 1000 different Bt cotton varieties were planted in India (**Figure 1**). In terms of varietal diversity, this is a significant improvement over the initial phase of transgenic technology diffusion.

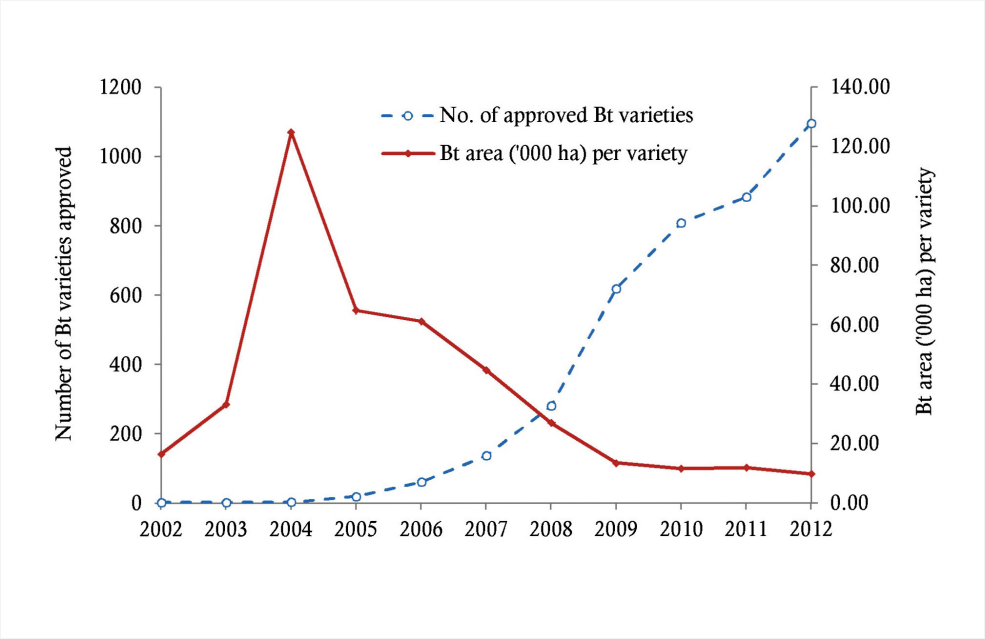

Figure 1: Diffusion of Bt cotton varieties in India

We used survey data from Indian cotton farmers, collected in four rounds between 2002 and 2008. In a multistage sampling framework, four states in central and southern India were purposely selected, namely Maharashtra, Karnataka, Andhra Pradesh, and Tamil Nadu. In these states, we randomly selected 10 cotton-growing districts and 58 villages, using a combination of census data and agricultural production statistics. In total, 341 farmers were sampled in 2002. In 2004, a second round of the survey was performed with the same farmer participants, whereby the overall sample size was slightly increased. A third and a fourth round of data collection with these same farmers took place in 2006 and 2008, respectively. In total, we have an unbalanced panel of 1431 household observations over the four survey rounds. The sample is representative for cotton growers in central and southern India.

4. Impact of Bt technology and varietal diversity on cotton yield

The analysis, following a moment-based stochastic production function approach, shows that Bt adoption has a positive and significant effect on mean production levels. Controlling for other factors and estimating at the sample mean of varietal diversity, Bt adoption has increased cotton yield by about 20%. This effect has not changed significantly over time. The number of cotton varieties grown on-farm also has a positive impact on mean production levels, confirming the productivity-enhancing function of varietal diversity. One additional variety grown on the farm increases cotton yield by about 5%. The interaction between Bt adoption and varietal diversity is negative and significant, indicating that Bt adoption reduces the productivity-enhancing function of varietal diversity. Comparing percentage effects, the yield impact of varietal diversity is lower than that of Bt adoption. For instance, an average farmer with five conventional cotton varieties could switch to one single Bt variety, without suffering any significant decline in mean yield. This confirms the hypothesis that there may be incentives to reduce on-farm varietal diversity when only a small number of Bt varieties is supplied.

Estimates of the production variance function suggest that both Bt and varietal diversity reduce production variance. Full adoption of Bt (100% cotton area under Bt) reduces production variance by 2% over non-adoption. In comparison, the variance effect of varietal diversity is much smaller. Neither Bt adoption nor varietal diversity seem to have a significant impact on skewness or downside risk. However, both variables significantly reduce kurtosis, implying that Bt adoption and varietal diversity contribute to reducing rare events in the tails of the yield distribution. The effect is again stronger for Bt than for varietal diversity.

5. Impact of Bt adoption on varietal diversity

We found that Bt technology increases mean yield and reduces production risk, and the effect is stronger than that of varietal diversity. Hence, Bt-adopting farmers have an incentive to reduce on-farm varietal diversity when only a small number of Bt varieties is available in the seed market. Transgenic technology may contribute to agrobiodiversity erosion in such situations. We further investigated whether such erosion actually occurred due to Bt cotton adoption in India. We began by comparing different diversity indicators between the early Bt diffusion phase with a low number of approved Bt varieties (phase I; 2002 – 2004) and the later diffusion phase with a much larger number of approved Bt varieties (phase II; 2006 – 2008). Bt technology adoption, measured as the share of Bt cotton in the total cotton area, had increased from 25% in phase I to 92% in phase II.

We estimated different regression models, showing that Bt adoption had a positive and significant effect on varietal diversity. This effect was primarily driven by partial adoption. With full adoption, Bt has decreased varietal diversity. However, this negative impact of Bt technology on varietal diversity only holds when the supply of different Bt varieties is small. With more Bt varieties supplied, the loss of diversity is reduced. Model predictions suggest that varietal diversity in phase II, with much higher Bt adoption intensity, was in the same magnitude as with zero adoption in phase I. To analyze diversity at higher geographic levels, we used our data and counted the number of different cotton varieties that sample farmers reported at the village, district, and state levels. We did not observe a decline in varietal diversity over time at any geographic level.

6. Conclusion and policy implications

During the Green Revolution it was observed that many local landraces were replaced with a much smaller number of high-yielding varieties in large parts of the developing world. There are widespread concerns that such agrobiodiversity erosion may continue and be accelerated through transgenic crop technologies. However, transgenic crops differ from high-yielding varieties of the Green Revolution and so warrant a closer look. From the private perspective of farmers, varietal diversity can have productivity-enhancing and risk-reducing effects. Transgenic crops can also increase productivity and reduce production risk and may therefore substitute for on-farm varietal diversity. Yet, transgenic technology need not be introduced in a new variety; the same genes coding for desirable traits can be introgressed into many varieties that are well-adapted to various soil and climate conditions. If many transgenic varieties with the same traits are developed and adopted, agrobiodiversity can be preserved. The empirical analysis showed that cotton varietal diversity in India with a Bt adoption rate of over 90% is now at the same level or even higher than it was before the introduction of this transgenic technology. We derive some policy implications.

First, the biosafety regulatory framework matters. In India, the regulatory authorities were slow in the beginning to approve additional transgenic varieties, mainly due to the public debate about possible risks associated with transgenic technology. However, once a transgenic event has been tested and deregulated, introgressing that same event into other varieties cannot reasonably be expected to lead to new risks[8]. Hence, a complex regulatory process for each new transgenic variety jeopardizes agrobiodiversity without increasing safety levels.

Second, local breeding capacities in a country play an important role. India has a strong public and private breeding sector for cotton. Hence, many companies were technically able to introgress a transgenic trait into their varieties and breeding lines. Such introgression of an available transgenic trait is less complicated than identifying the trait and developing the transformation event, but it still requires some R&D capacity that may not be available in many poorer countries.

Third, intellectual property rights (IPRs) may play affect agrobiodiversity erosion. Many of the transgenic technologies available so far are not patented in developing countries, so that local organizations can use these technologies for free or with relatively simple licensing agreements for introgression into their own varieties and breeding lines. Stronger IPRs may involve more complex licensing agreements. Restricting licenses to only one or a few organizations could contribute to agrobiodiversity erosion.

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Bt Crops Prove Harmless for Non-Target Insects and Soil Organisms: A Risk Assessment Approach

Ahmad Ali Shahid and Amina Yaqoob

The commercial utilization of genetically modified (GM) plants containing *Bacillus thuringiensis* (Bt) genes invokes biosafety concerns worldwide. Therefore, assessing the risks of Bt crops prior to their cultivation is a critical issue. In agrobiotechnology, one goal of safety assessment is not just to identify the food and feed safety of a GM plant, but also to demonstrate its impact on an ecosystem. Many experimental studies conducted worldwide over the last 20 years have studied Bt-crop risks to non-target organisms (NTO's). NTO's include beneficial insects, natural pest controllers, plant growth promoting rhizobacteria (PGPR), pollinators, soil dwellers, aquatic and terrestrial vertebrates, mammals, and even human beings. Although no adverse effects have been reported relative to approved GM events, critical risk assessments continue to be needed before commercialization of these crops.

GM crops with *Bacillus thuringiensis* (Bt) gene

Insecticidal Bt Cry proteins, derived from the spore-forming bacterium *Bacillus thuringiensis* (Bt), specifically cause lesions in the epithelial layer of the susceptible insect midgut. Bt crops are grown globally over 35 million hectares in 13 different countries and carry either *Cry1Ab* or *Cry3Bb1* proteins to control pest groups, e.g., *Lepidopteran* and *Coleopteran* species. Plants such as tobacco, potato, rice, tomato, maize, eggplant, and cotton have been successfully transformed with Bt genes. Although Bt crops have proved successful in reducing or eliminating certain plant pests, the global agriculture community continues to debate their use. Opponents raise questions of environmental safety, changes in biodiversity in Bt crop fields, safety of consumption of Cry proteins residues by humans, possible effects of Bt crops on NTOs, and effects on financial gain or loss in developing

countries. Over the last 20 years, risk assessment studies of Cry proteins have been extensively conducted. The present report reviews the results from multiple experimental trials regarding Bt crop biosafety and its consequences for plant biotechnology.

Effect of Bt crops on non-target insects

One major aspect in risk assessment of Bt crops is the identification of the lethal effects of Cry proteins on beneficial or non-target arthropods. Most experimental studies have fed insects with Bt grain directly, and the outcomes have generally supported the safety of Bt crops. Negative effects of Bt corn were indicated by Losey[1] (1999) when feeding trials of Bt pollens were performed with the larvae of monarch butterfly *Danaus plexippus*; however, the feeding strategy was considered non-applicable to larvae in nature because high levels of Bt pollen were artificially used. Later experimental studies using levels of Bt-supplemented feed similar to that found around crops indicated harmless effects of this Cry protein on rice grasshopper (*Acrida exaltata*), green leaf hoppers (*Nephotittix cincticeps*), white-backed plant-hoppers (*Sogatella furcifera*), paddy grasshopper (*Oxya hyla*), Rice bug (*Leptocorisa acuta*), rice thrips (*Stenchaetothrips biformis*), etc. Because Cry genes have specific targets, the protein encoded by these genes also has a narrow range of activity against particular target organisms and does not harm other non-target insects or herbivores.

Honey bee (*Apismellifera* L.) — the most efficient and important pollinator among all pollinators. Babendreier et al.[2], studied the effect of Bt toxin on honey bee by feeding it Bt pollen directly, and they observed a very small amount of Bt toxin in the hypo-pharyngeal glands of young bees after 10 days. However he reported no negative effect on insect survival and development. Other experimental trials also rendered no effect of Bt pollen to the survival, developmental stages, learning behavior, diversity, and abundance of honey bees.

Ladybird (*Adalia bipunctata*) — Schmidt and coworkers[3] performed an ecotoxicity test on *Adalia bipunctata* by feeding *Cry1Ab* toxin directly to immature larvae, and a high mortality was observed in tested larvae as compared to the control group. These results might be due to poor study designs or low nutritional quality, because subsequent studies revealed no fatal effect of *Cry1Ab* and *Cry3Bb1*on the weight, development, and differentiation of *A. bipunctata* larvae.

Green lacewing (*Chrysoperla carnea*) — a common and predominant pollen-consumer in maize fields. The insects showed non-significant change in fitness parameters such as survival, pre-oviposition period, fertility, and dry weight when fed Bt pollen (supplemented with *Cry3Bb1* or *Cry1Ab*) from transgenic maize and rice for ≥ 28 days.

Flower bug (*Orius majusculus*) — natural predators found in cotton and maize fields. A study by Lumbierres et al., (2012) indicated reduced nymph development of *Orius majusculus* fed Bt-containing spider mites. But later experimental trials revealed that ingestion of Bt proteins either through plant leaves or via the food web was not lethal for these beneficial predatory insects.

Butterfly (*Rhopalocera spp.*) —one of the most important Phytophilous or sap sucking insects. A 2-year study on the Monarch butterfly suggested Bt corn pollen was harmless. Another field study on a swallowtail butterfly population further confirmed this result. However another research group showed that Bt proteins somehow negatively affect the development, survival, body weight, wing size, and larval behavior of this natural pollinator. Similarly, the larvae of the Peackock butterfly (*Inachis io spp.* L.) fed Bt maize pollen showed a reduction in larval body weight, and it was assumed that this reduction is directly proportional to the Bt concentration in maize pollen. Although Bt pollen was somehow adversely affecting the health of this butterfly species, no lethal effect was claimed by any researcher. Therefore more studies are needed.

Aphid (*spp.*) — an important position in food web, and therefore considered a significant species in biosafety studies. Traces of *Cry1-Ab* protein have been noted after quantified exposure of aphids to Bt-containing tissues. Since the Cry protein does not translocate into the phloem, no toxin can be transferred to sap sucking insects such as aphids. However a trace of Bt found in some aphids could be the result of intracellular sap

uptake by the insect while piercing the plant tissue. No negative effects on developmental stages, survival, or other biological parameters have been observed in tested species, i.e., *Rhopalosiphum maidis* F., *R. padi* L., and *Sitobion avenae*.

Effect of Bt crops on soil organisms

The Bt toxin rapidly adheres to soil particles and is quickly degraded; therefore soil organisms are considered to be at greater risk of contact with these toxins.

Plant growth promoting rhizobacteria (PGPR) — promote plant growth by nitrogen fixation, salt tolerance, nutrient solubilization, pathogen control, and plant hormone production. Very few risk assessment studies have been done using PGPR strains with regard to Bt crops. An evaluation made by Sun, Chen, & Wu[5] showed a decreased rizhospheric phosphatase activity of bacteria in Bt soil as compared to bacteria in non-Bt soil. Another study also reported decreased efficiency of soil bacteria in a medium supplemented with Bt-maize residue. However, the experimental design was questioned, as the direct addition of Bt-maize straw greatly increased the CO_2 concentration of the substrate and eventually resulted in high mortality rates of soil bacteria. Later studies suggest that the cultivation of transgenic Bt plants has a minor or no effect at all on rhizobacteria. A four-year experimental study by Barriuso et al.[6], on the risk assessment of rhizobacteria in Bt-maize fields found no change in the structure and ecology of Bt-maize rhizobacterial communities when compared to those in the non-Bt maize fields. Successive studies comparing the soil of Bt crops with control soil showed that neither the soil microbial biomass nor the microbial enzymes are adversely affected by incorporation of Cry proteins into the rhizosphere. Conversely, microbial colonies are also co-affected by other biotic and abiotic environmental factors of the rhizosphere.

Mycorrhizae — beneficial symbionts of plants. Cheeke et al.[7], discovered reduced mycorrhizal colonization in the roots of Bt maize lines and showed unexpected harmful effects of Bt crop cultivation on non-target soil fungi. However, successive studies using molecular fingerprinting and nucleic acid-based pyrosequencing methodologies indicated no risk for mycorrhizal association in Bt crops. The results indicate a need for more critical experimentation than previous trials to accurately evaluate the possible impacts of Cry proteins.

Soil protozoa — important agents for soil mineralization and feed upon bacteria or fungi. Unfortunately, very few studies have been performed to assess the risks or benefits associated with these microscopic organisms in regards to GM events. Lack of experimental setup under laboratory conditions is one of the major problems in studying soil protozoa.

Earthworms (*Lumbricina spp.*) — the most active soil macroorganism that enhances water and nutrient transport through soil layers and is termed a natural plow. Earthworms ingest Bt toxins along with bulk of soil particles and are thought to be affected by Bt toxin directly. But in light of the results from different experimental studies on earthworms, it is evident that Bt proteins neither harm the adult or juvenile earthworm in soil nor its characteristics like reproduction, growth rate, abundance, biomass, and mortality rate.

Nematode (*Caenorhabditis elegans*) — commonly known as round worms and found by the millions in the top soil layer. Effects of Bt crops have been assessed for free living nematodes, and it is estimated that *C. elegans* is not affected significantly by Cry proteins; their abundance and diversity are essentially the same among different Bt maize cultivars. Other studies indicated that Cry proteins somehow negatively affect the reproduction rate of *C. elegans* because of the presence of similar receptors to nematicidal Cry proteins. However, it must be kept in mind that *C. elegans* can defend itself against the *Cry6Aa2* toxin through upregulation of defense initiating genes as well as behavioral responses, i.e., by reduced oral uptake and physical avoidance. More trials are needed to analyze the ecological effect of Bt toxins on soil nematodes.

Concluding remarks

Bt crops have a narrow spectrum of activity so do not adversely affect non-target organisms. The comparative results of numerous experimental trials regarding the risk assessments of Bt crops in non-target organisms favor their use and show no lethal impact of Bt protein to biodiversity.

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A Novel Approach for Engineering Durable Disease Resistance in Crops

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Genetically determined disease resistance is the most cost effective and environmentally safe approach to protecting crops from disease. Unfortunately, many classically defined disease resistance (*R*) genes are rapidly overcome in field settings due to evolution of the pathogen. The rate at which *R* genes are overcome is defined as their durability. Most *R* genes characterized to date encode intracellular receptors that mediate detection of pathogen virulence factors, often referred to as 'effector' proteins, that are injected or translocated into the cytoplasm of host cells. The durability of *R* genes is thus determined by how easily a pathogen can modify its effector repertoire to evade detection. To assure durability, *R* proteins should detect effectors that are essential to pathogen virulence, and which are highly conserved among pathogen strains. Identification of such conserved effectors is thus a priority of current research in the molecular plant pathology field.

Once conserved effectors are identified, the next step toward engineering durable resistance is the identification of *R* proteins capable of detecting such effectors. Ideally, such *R* proteins can be identified in available germplasm of the crop species of interest, or alternatively, in sexually compatible wild species. Unfortunately, this is not always feasible, and may account for the ability of conserved effectors to persist in the pathogen population in the first place. My laboratory has been developing novel methods for modifying the recognition specificity of existing *R* proteins so that they can be used to detect new effectors, including conserved effectors.

Our approach is based on over two decades of investigations into the molecular mechanisms underlying pathogen recognition. This work led to the identification of *R* genes in both Arabidopsis and soybean that mediate recognition of specific effector proteins from the bacterial pathogen *Pseudomonas syringae*[1-3]. These effector proteins are injected into host cells where they target components of the host immune system to suppress immunity[4]. Work in my lab and others revealed that recognition of these effector

proteins by *R* proteins occurs indirectly, via detection of effector-induced modifications of host proteins[5]. Arguably the best understood example of such indirect recognition is that of AvrPphB, a cysteine protease from *P. syringae* pv. *Phaseolicola*[6]. AvrPphB targets a family of plant protein kinases involved in regulating basal immunity[7,8]. Specifically, AvrPphB catalyzes cleavage of these kinases at the apex of their activation loops, which likely renders them inactive[7,9]. In Arabidopsis, cleavage of one of these kinases, PBS1, activates the *R* protein RPS5, which thus confers resistance to *P. syringae* strains expressing AvrPphB[7,10,11]. In other words, AvrPphB is recognized by RPS5 via its proteolytic activity on PBS1 (**Figure 1**).

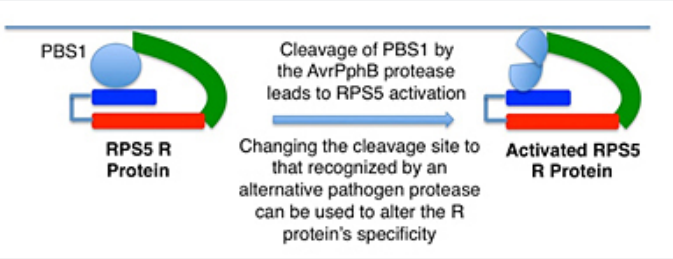

Figure 1. Protease-mediated activation of the plant disease resistance protein RPS5. RPS5 and PBS1 form a pre-activation complex that maintains RPS5 in the off state. Cleavage of PBS1 by a protease induces a conformational change in PBS1 that activates RPS5. Modifying PBS1 to contain cleavage sites for proteases from various pathogens enables RPS5 to confer resistance to those pathogens.

We have been investigating precisely how RPS5 senses cleavage of PBS1 and have shown that RPS5 does not recognize the free ends generated by cleavage, but rather senses an overall conformational change in PBS1 that results from cleavage[9]. This was an important discovery as it indicated that the amino acid sequence at the cleavage site might be irrelevant to RPS5 function, as long as cleavage could occur. To test this hypothesis, we replaced seven amino acids flanking the AvrPphB cleavage site with a seven amino acid sequence known to be cleaved by a different protease from *P. syringae*, AvrRpt2. Transformation of an Arabidopsis line normally unable to recognize AvrRpt2 with this modified *PBS1* gene (designated $PBS1^{RCS2}$) resulted in AvrRpt2 recognition and resistance to infection by *P. syringae* strain DC3000(AvrRpt2)[12]. Importantly, these lines retained their ability to recognize AvrPphB, indicating that both the modified $PBS1^{RCS2}$ and wild-type PBS1 proteins were being monitored by RPS5. We are thus able to expand the recognition specificity of RPS5 by adding additional PBS1 'decoys' within the plant cell. Collectively, these data demonstrate that the specificity of RPS5 can be altered simply by modifying the protease recognition sequence in PBS1. This finding suggests that we can engineer resistance to any pathogen that employs a protease as part of its effector repertoire, provided that the protease is targeted to the cytoplasm of the host cell. Fortunately, many of the most important viral, bacterial, oomycete, fungal, and nematode pathogens express proteases during host infection[13-17]. We must now determine where these proteases localize in the host.

As a proof-of-principle for the above protease detection system, we engineered recognition of a protease from Turnip mosaic virus (TuMV). TuMV is a member of the potyvirus family, which are plus-stranded RNA viruses. Upon entry into a host cell, potyviruses initiate replication by translating the entire length of their RNA genomes (~10 kb) as a single large polyprotein that is then self-processed into 10 separate proteins via three embedded proteases, NIa-Pro, P1 and HC-Pro. All three proteases are essential to viral replication; thus an R protein that could detect one or more of these proteases should be quite durable. NIa-Pro is noteworthy for its ability to cleave substrate proteins *in trans* (i.e., substrates do not need to be part of the original polyprotein), and for their sequence specificity in terms of what sites they will cleave. For most characterized NIa proteases, a seven amino acid sequence is sufficient to specify cleavage and confer 'cleavability' upon a protein of interest. We reasoned that replacing the AvrPphB cleavage sequence within PBS1 with a cleavage sequence for TuMV NIa-Pro (GGCSHQS) would render PBS1 cleavable by NIa-Pro, which should then activate RPS5. Transient assays in *Nicotiana benthamiana* confirmed this hypothesis; thus we generated transgenic Arabidopsis expressing the modified PBS1. When these transgenic Arabidopsis were infected with TuMV, they developed a systemic necrosis phenotype[12], indicating that the modified PBS1 enabled recognition of TuMV and the activation of programmed cell death. Ideally, cell death would be activated in time to contain the virus within the initially infected leaf. In the case of systemic necrosis, however, the virus spreads from the originally infected leaf, leaving a trail of dying cells in its wake.

Although systemic necrosis is not an optimum

resistance phenotype, it is a frequently observed outcome in plant-virus interactions. For example, the *I* gene of common bean has been used widely by bean breeders to confer resistance to Bean common mosaic virus (BCMV), a potyvirus[18]; however, this same gene confers a systemic necrosis phenotype in response to the closely related virus, Bean common mosaic necrosis virus, leading to death of the plant upon infection[18]. As a second example, a derivative of the Rx protein of potato confers full resistance to Potato virus X, but triggers a systemic necrosis when infected with Poplar mosaic virus[19]. Importantly, this systemic necrosis phenotype can be switched to full resistance by four different single amino acid substitutions in Rx[20]. Interestingly, these amino acid substitutions are not in the domain of Rx believed to interact with viral proteins, but instead, are in the nucleotide binding domain of Rx, which likely enhances the ability of Rx to switch from the off state to the on state[20]. These observations suggest that we may be able to engineer RPS5 to confer full resistance to TuMV infection by making similar substitutions. We are also investigating whether increasing the concentration of RPS5 in the plant cell would have a similar effect, as this should increase the number of activated R proteins upon virus infection, and thus decrease the time required to trigger cell death.

Thus far the RPS5-PBS1 protease detection platform has only been tested in model plant systems (Arabidopsis and *Nicotiana benthamiana*). We are currently working toward deploying it in soybean. Specifically, we are engineering recognition of Soybean mosaic virus (SMV), which can cause significant losses, especially when plants are co-infected with Bean pod mottle virus[21,22]. SMV incidence appears to be spreading northward, likely as a consequence of warmer winters and the introduction of soybean aphid (*Aphis glycines*) to the US[23]. Like TuMV, SMV is a potyvirus and thus relies on an NIa protease to process its polyprotein. We have already identified orthologs of Arabidopsis *PBS1* in soybean and have modified them for cleavage by SMV NIa. Transient expression assays in *N. benthamiana* have confirmed that the modified soybean PBS1 protein is cleaved by SMV NIa protease. Importantly, we have also shown that AvrPphB induces a resistance response in most soybean varieties, which indicates that soybean contains one or more endogenous *R* genes that can detect AvrPphB protease activity[24]. We thus predict that expression of the modified soybean PBS1 protein should enable soybean to recognize SMV. If correct, it should be feasible to modify an endogenous soybean *PBS1* gene using a genome editing tool such as CRISPR/Cas9. Although regulations for genome-edited plants are still being developed, it is hoped that such plants will not be considered 'GMOs' since they will not contain any foreign genes. In this example, only seven amino acids of one gene will have been changed.

In addition to SMV resistance, we have initiated a project aimed at engineering resistance to Asian soybean rust (ASR; *Phakopsora pachyrhizi*), one of the most costly diseases of soybean worldwide in terms of fungicide costs and yield losses[25]. RNA-seq analyses of infected soybean leaves indicate that *P. pachyrhizi* expresses at least one secreted protease during infection. Importantly, this protease is conserved among all *P. pachyrhizi* isolates examined, with orthologs present in many other rust species. It is not yet known whether this protease has a defined cleavage site specificity that will be amenable to the PBS1 decoy approach, but if it does, the PBS1 protease detection platform would represent a very attractive strategy for conferring genetic-based resistance to this pathogen, something that is urgently needed. Given the conservation of this protease, such resistance should be quite durable.

In summary, we have developed a novel system for modifying the recognition specificity of a plant R protein via modification of the effector target guarded by the R protein. We expect that this general approach may be applicable to other R proteins that detect pathogen effectors indirectly via detection of their enzymatic activity. Recently it has been shown that many plant R proteins contain integrated decoy domains that activate their R proteins following modification by a pathogen effector[26-30]. It will be most interesting to test whether such integrated decoys can be engineered to be substrates for multiple different effectors. If so, we may be on the brink of engineering resistance to many different pathogens for which we currently lack robust and durable genetic resistance.

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