

The Good, the Bad, and the Ugly: What the Future Could Hold for *Bs2* Tomatoes¹

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Over the past several years there has been considerable discussion within the Florida tomato industry about *Bs2* tomatoes. Previous and ongoing trials conducted by University of Florida researchers have consistently and repeatedly demonstrated the benefits of these cultivars for bacterial spot disease management, while growers and industry members who have visited these trials likewise recognize the potential for *Bs2* tomatoes to make Florida tomato production a much more sustainable operation. But what does the future really hold for this technology? What benefits might be realized by the adoption of *Bs2* tomato varieties, and what challenges stand in the way of their commercial production?

What Are *Bs2* Tomatoes?

Bs2 tomatoes are transgenic tomatoes that have been engineered to contain the *Bs2* gene from pepper. As such, they are considered a genetically modified (GM) food, or a genetically modified organism (GMO). (For more information about GMOs see Schneider, Schneider, and Richardson 2002). *Bs2* transgenic tomatoes were developed by the Two Blades Foundation, a charitable scientific organization, which holds an exclusive license to the *Bs2* gene, in collaboration with scientists at the University of California and the University of Florida.

Bacterial spot is a major disease of both pepper and tomato, especially in Florida and other warm, humid production regions of the world. Plant resistance is desired because chemical control is costly and sometimes ineffective when conditions are favorable for development of the disease. In pepper, conventional (non-GMO) breeding efforts have been very successful due to the discovery and use of several individual resistance genes. Seven of these resistance genes have been reported (Potnis et al. 2012; Stall, Jones, and Minsavage 2009), four of which have been exploited in commercial varieties (*Bs1*, *Bs2*, *Bs3*, and *bs5*). The majority of these genes behave in a manner consistent with the gene-for-gene hypothesis devised by Henry Flor (1955). By this model, a resistance gene in the plant must recognize a corresponding gene, commonly referred to as an avirulence gene, in the bacterium in order for the plant to be resistant; thus both genes are necessary for resistance to bacterial spot. In most cases where single resistance genes are deployed, crops remain disease-free for a period of time (usually a few years), until a mutation occurs in the pathogen's avirulence gene, rendering the corresponding resistance gene ineffective. This was the case with pepper varieties containing *Bs2* alone, which became available in 1984 (Cook 1984) and were widely grown in the 1990s. But after only several years, bacteria containing mutations in the *Bs2* avirulence gene became prevalent in the field

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(Pernezny and Collins 1999), and when deployed alone, *Bs2* resistance was no longer effective against such strains. Fortunately, not all pathogen strains carry a mutant *Bs2* avirulence gene (Wichmann 2005), and pepper breeders have enjoyed some success against bacterial spot by pyramiding *Bs2* with other resistance genes (for example, *Bs2* combined with *Bs3*). This strategy also helps prolong the “life” of the resistance genes, since the pathogen can only survive/spread if mutations occur in all avirulence genes at the same time.

In contrast to pepper, tomato breeders have been unsuccessful in developing bacterial spot resistant varieties by conventional approaches. Although the UF/IFAS tomato breeding program has maintained an active breeding project for resistance since the early 1980s, no resistant varieties have been developed. There are several reasons for this, including limited sources of resistance, resistance that is conferred by multiple genes rather than a single gene (which makes the breeding process much more complicated), mutations in pathogen avirulence genes resulting in ineffective resistance genes, and introduction of exotic pathogen strains which overcome the resistance (Hutton et al. 2010). In short, tremendous efforts on the breeding front have been unable to combine horticultural acceptability with high levels of resistance in tomato.

The *Bs2* gene from pepper was cloned in the late 1990s when the gene conferring resistance was identified (Tai et al. 1999). The researchers also determined that transgenic tomatoes containing the pepper *Bs2* gene were resistant to bacterial spot. Because of the difficulty in developing resistant tomato varieties by conventional means, many have considered *Bs2* tomatoes an important tool to manage this devastating disease.

The Good

There are several reasons why *Bs2* tomatoes are an attractive strategy for management of bacterial spot:

- *The Bs2 gene occurs naturally in plants.* What is more, it occurs naturally in a major food crop, pepper. The protein product of the *Bs2* gene is safe for consumption, attested to by more than two decades of the public’s consumption of bell peppers containing *Bs2*.
- *The Bs2 gene provides excellent disease control in tomatoes* (Figure 1). This was demonstrated in a multi-year experiment where *Bs2* tomatoes maintained extremely low levels of disease compared to susceptible controls, while inbred lines with conventionally-bred resistance had intermediate levels of infection (Horvath et al. 2012).



Figure 1. Bacterial spot resistance in tomato conferred by the pepper *Bs2* gene. On the left are symptomless *Bs2* transgenic plants of the hybrid, Fla. 8314; on the right are severely infected non-transgenic plants of the cultivar VF36. The picture was taken from a trial conducted in Florida in spring 2012, for which all plants in the trial were inoculated with the bacterial spot pathogen.

- *Bs2 is effective against all field strains of the tomato bacterial spot pathogen.* This was determined by surveying bacteria samples from the production regions of Florida and parts of Georgia; all strains contained a recognizable *Bs2* avirulence gene, meaning that *Bs2* would provide effective resistance throughout the southeast production region (Horvath et al. 2012). Additionally, many of the mutations which have occurred in the *Bs2* avirulence gene and which provide the pathogen a means to escape detection by the resistance gene also result in a loss of fitness of the bacterium (Kearny and Staskawicz 1990), meaning that the mutant strains are often weaker and less likely to survive and/or cause severe infections.
- *Higher yields are obtained with Bs2 tomatoes.* In repeated trials, *Bs2* inbreds and hybrids maintain a 1.5-fold or greater yield increase over the non-transgenic versions of these inbreds and hybrids; when conditions were favorable for disease, these increases were often 2-fold or greater (Horvath et al., 2012, 2014).
- *Bs2 tomatoes are a green technology.* Because these tomatoes have a significant yield advantage over traditional varieties, increased production can be realized without increasing fertilizer, pesticide, plastic, or other inputs. Thus the carbon footprint per unit of production can be reduced. In addition, because *Bs2* tomatoes provide good control for bacterial spot, copper and other chemical sprays for management of this disease can be reduced or eliminated, which can further reduce environmental impact.
- *The Bs2 gene is a simple, highly effective tool for tomato breeders to utilize.* As described above, most of the conventionally bred resistance is controlled by multiple

genes, meaning that breeders have to sift through many more plants to identify those that are most resistant. Furthermore, unlike the resistance provided by the *Bs2* gene, current conventionally bred resistance genes only provide tolerance or partial resistance; thus breeders spend a great deal more time and effort trying to distinguish between “shades of gray,” vs. presence or absence of disease.

The Bad

Although years of repeated trials have demonstrated the ability of the *Bs2* gene to effectively eliminate bacterial spot in tomatoes, the success of this gene in tomatoes hinges on its ability to recognize the pathogen’s *Bs2* avirulence gene. So if mutations occur in the *Bs2* avirulence gene (which they will) that prevent recognition, *Bs2* could be rendered ineffective at controlling bacterial spot of tomato. As was discussed earlier, this is what occurred in non-transgenic *Bs2* pepper varieties in only a matter of years. In fact, such mutations already have been observed on a limited scale in *Bs2* tomato trials (Horvath et al. 2014).

In order to prolong the “life” of *Bs2* resistant tomatoes, care will need to be taken to limit the emergence and spread of resistance-breaking strains. Several strategies are available, any number of which might be employed.

- *Deployment of *Bs2* exclusively in varieties that contain conventionally bred tolerance or partial resistance to bacterial spot.* This is a good strategy because a pathogen must simultaneously overcome multiple mechanisms of resistance. But this is easier said than done because of the challenges of conventional breeding for tolerance or partial resistance conferred by multiple genes, as already discussed.
- *Employment of cultural practices to minimize the emergence and spread of mutant strains of the pathogen.* Further research is needed to identify helpful strategies that are not already practiced. Ultimately, those cultural practices that are based on good sanitation all the way from seed production to the growers’ fields will go a long way to minimize bacterial spot outbreaks and the introduction of resistance-breaking strains.
- *Deployment of *Bs2* in combination with other novel resistance genes.* As scientists expand their understanding of plant disease resistance, there will no doubt be additional resistance genes discovered—whether in relatives of tomato, in other Solanaceous species, or in entirely different plant families—and some of these genes may provide useful levels and alternative mechanisms of resistance. As long as these genes do not rely on recognition of the same avirulence gene in the pathogen, the pyramiding of *Bs2*

with one or more of these likely would prove extremely long-lasting. This strategy of pyramiding resistance genes to promote their durability is not novel; examples include combining multiple conventionally bred resistance genes to *Striga* in sorghum (Ejeta 2007), as well as pyramiding multiple transgenic insect resistance genes in cotton (Li et al. 2014).

The Ugly

There currently are no *Bs2* tomatoes being produced for sale or consumption, and this will not change until two hurdles are passed. The first is the de-regulation process. It takes years for a transgenic crop to be de-regulated, and the process is costly. The Two Blades Foundation has invested significant resources toward the development and testing of this GMO. However, additional funds are needed in order to complete the de-regulation process, and many potential investors are wary due to concerns about public acceptance—which is the second hurdle.

Even though this gene has the potential to increase yields while decreasing pesticide applications, and even though it is naturally present in peppers, which are very closely related to tomatoes, the *Bs2* resistance gene does not naturally occur in tomatoes. Since peppers and tomatoes cannot be intercrossed, the only way to utilize this gene in tomatoes is through the use of transgenic technology. Ultimately, because growers will only produce what they can sell, the future of *Bs2* tomatoes relies on whether or not the public will accept and buy their product.

Going Forward

If deployed carefully, *Bs2* tomatoes have the potential to significantly advance the sustainability of tomato production in bacterial spot-prone environments by increasing yields while reducing pesticide inputs. The *Bs2* protein product is known to be safe based on decades of its consumption in pepper. But before *Bs2* tomatoes can be grown, the de-regulation process must be completed; and before *Bs2* tomatoes *will* be grown, producers must be satisfied that they can sell their product. Thus public controversy over GMO technology has everything to do with the future of *Bs2* tomatoes.

Although there is considerable opposition to and great skepticism over GM technology, it is evident that much of this is based on the public’s perception of the science. A recent Intelligence Squared U.S. debate illustrated this: after hearing concerns over GMOs addressed by both GMO-skeptics and by supporters of GMO technology, an audience changed from 32% supportive of GM technology

before the debate, to 60% afterward (Fraley et al. 2014). The opinion shift after that debate suggests that increasing acceptance of Bs2 tomatoes (and other GMOs) will depend on transparency and open discussion between scientists and the general public to show these crops' benefits to consumers, growers, and the environment.

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