The Good, the Bad, and the Ugly: What the Future Could Hold for *Bs2* Tomatoes[1]

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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 advantages of these cultivars for bacterial spot disease management. Likewise, growers and industry members who have visited these trials 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 new techniques and genes are in the pipeline which may address some of these challenges?

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 et al. [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 University of California and University of Florida.

What is the source of the gene *Bs2* and how does it work?

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 largely 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 et al. 2009), and at least four of these 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 in the bacterium (commonly referred to as an avirulence gene) in order for the plant to be resistant. Thus, both the resistance gene in the plant and the avirulence gene in the pathogen are necessary for resistance to work against bacterial spot. In most cases where single resistance genes are deployed, crops remain disease-free for a period

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of time (usually several years), until a mutation occurs in the pathogen's avirulence gene that renders it undetectable by the corresponding resistance gene in the plant. 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 (Pernezny and Collins 1999), and when deployed alone, *Bs2* resistance was no longer effective against such bacterial strains. Fortunately, not all bacterial 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 to prolong the "life" of the resistance genes, since the pathogen can only survive/spread if mutations simultaneously occur in all avirulence genes.

Why do we need a pepper gene in tomato?

In contrast to pepper, tomato breeders have been unsuccessful at developing bacterial-spot-resistant varieties by conventional approaches. Although the UF/IFAS tomato breeding program has maintained an active breeding project for bacterial spot resistance since the early 1980s, no varieties with appreciable levels of resistance have yet been developed. There are several reasons for this, including limited sources of resistance, resistance that is conferred by multiple genes with small effects 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.

One strategy to address these challenges has been to leverage in tomato some of the available resistance gene resources found naturally in pepper. The *Bs2* gene, which confers strong resistance to bacterial spot, was cloned from pepper in the late 1990s (Tai et al. 1999). The researchers also determined that transgenic tomatoes containing the pepper *Bs2* gene were likewise resistant to bacterial spot. Because of the difficulty in developing resistant tomato varieties by conventional means, many have considered *Bs2* tomatoes an important tool for management of this devastating disease.

The Future of *Bs2* Tomatoes

The Good

The use of *Bs2* tomatoes is an attractive strategy for the management of bacterial spot, and here are several reasons in support of this approach:

- *The Bs2 gene occurs naturally in plants*: What's more, it occurs naturally in a major food crop, pepper. The protein product of the *Bs2* gene is safe for consumption, attested to by nearly three 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 multi-year experiments 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; Kunwar et al. 2018).
- *The Bs2 gene 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 tested 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-times 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, 2015; Kunwar et al. 2018).
- *Bs2 tomatoes are a green technology*: Because these tomatoes have a significant yield advantage over traditional varieties, increased production can be realized without corresponding increases in water, fertilizer, pesticide, plastic, or other inputs. Thus, the carbon footprint per unit of production may be reduced. In addition, because *Bs2* tomatoes provide good control for bacterial spot, copper and other chemical sprays for management of this disease may 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 in tomatoes is controlled by multiple genes, meaning that breeders have to sift through many more plants to identify those that are most resistant. Further, 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. a simple black-or-white.

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.

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. Since the generation time of bacterial pathogens is much faster than plants, mutations in the *Bs2* avirulence gene that can escape its recognition by the *Bs2* resistance gene are forthcoming. When such mutations occur, the *Bs2* transgene may 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 have already been observed on occasion in *Bs2* tomato trials (Horvath et al. 2015).

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.
- *Exercise 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. Pyramiding of *Bs2* with additional resistance genes from related plant species will improve the durability of resistance. Recently, transgenic tomato containing another resistance gene, *EFR* from the cabbage family was developed. *EFR* alone or in combination with *Bs2* was also effective against bacterial spot under multi-year field trials (Kunwar et al. 2018). Researchers are also investigating several new resistance genes which have shown effective control of bacterial spot. 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 would likely 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 are currently no *Bs2* tomatoes being produced for sale or consumption, and this will not change until two hurdles are passed. The first is the deregulation process. It takes years for a transgenic crop to be deregulated, and the process is costly. The Two Blades Foundation has invested significant resources towards the development and testing of this GMO. However, additional funds are needed in order to complete the deregulation 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, a functional *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 this product.

Going Forward

If deployed carefully, *Bs2* tomatoes have the potential to significantly improve 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 deregulation process must be completed, and before *Bs2* tomatoes *will* be grown, producers must be confident that they can sell their product. Thus, public controversy over GMO technology has everything to do with the future of *Bs2* tomatoes. It is clear that the path towards acceptance of *Bs2* tomatoes (and other GMOs) will no doubt involve a great deal of transparency and many open discussions as scientists seek to make known the potential benefits to consumers, growers, and the environment.

Looking ahead, it is likely that additional resources for bacterial spot disease management will become available, especially as our knowledge of plant disease resistance grows and as new scientific tools are developed. For example, the deployment of multiple bacterial resistance genes, together, should not only enhance the level of disease control, but should simultaneously prolong the time for which these genes are effective. Mention has already been made of the resistance achieved by combining the *Bs2* and *EFR* genes for disease management. Similarly, ongoing research trials are evaluating the effects of additional genes, such as *Roq1* from tobacco and *bs5* from pepper (Schultink et al. 2017). Positively, the deployment of such genes—in combination with, or instead of *Bs2*—may contribute greatly to sustainable disease management. Negatively, the introduction of some of these genes into tomato may only be possible by transgenic technology, which presents the same challenges as those currently faced by *Bs2*. Alternatively, in recent years, scientists have developed genome editing technology that can be used to modify or repair native genes. This technique can be particularly useful for introducing specific modifications to a gene that is already present. In contrast to transgenic (GMO) approaches, the United States Department of Agriculture (USDA) currently does not regulate most uses of gene editing, and it may thus be a great resource in plant breeders' toolkits for rapidly introducing new traits.

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