

# Utilizing Genetic Resources and Precision Agriculture to Enhance Resistance to Biotic and Abiotic Stress in Watermelon

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## Abstract

Originally from Africa, watermelon is a staple crop in South Carolina and rich source of important phytochemicals that promote human health. As a result of many years of domestication and selection for desired fruit quality, modern watermelon cultivars are susceptible to biotic and abiotic stress. The present review discusses how genetic selection and breeding combined with geospatial technologies (precision agriculture) may help enhance watermelon varieties for resistance to biotic and abiotic stress. Gene loci identified and selected in undomesticated watermelon accessions are responsible for resistance to diseases, pests and abiotic stress. Vegetable breeding programs use traditional breeding methodologies and genomic tools to introduce gene loci conferring biotic or abiotic resistance into the genome background of elite watermelon cultivars. This continuous approach of collecting, evaluating and identifying useful genetic material is valuable for enhancing genetic diversity and tolerance and combined with precision agriculture could increase food security in the Southeast.

**Keywords:** *Citrullus*; next generation sequencing (NGS) technologies; food security; precision agriculture; resistance

## Introduction

Watermelon is a vegetable crop known for its beneficial phytochemicals for human health. It is also one of the most widely cultivated crops in the world (Tannin-Spitz *et al.*, 2007; Guner and Wehner, 2008; Wechter *et al.*, 2008; Liu, 2013). Climate change however has been impacting watermelon yields, especially in areas affected by drought (Cooley *et al.*, 2015). Consequently, in efforts to circumvent future food insecurity, scientists all over the world have focused on the development of varieties that manifest resistance to different diseases, pests, drought and salt as well as on harvesting novel technology for reducing crop placements such as nutrients, pesticides and water through the utilization of precision agriculture.

In Somalia for example, where drought has led to some of the worst recorded famines, development of drought resistance plants is crucial (USAID, 2017). Watermelon is one of the vegetables that was used in demonstrations farms to educate local farmers as well as to test and identify best local seed varieties (USAID, 2017). To address these problems researches use different methods to incorporate resistance into plants (traditional breeding, plant transformation, CRISPR-CAS9).

The *Citrullus* germplasm collected in Africa (the center of origin of this genus), represents a wide genetic diversity and contains a large number of novel gene sequences (among them, a considerable number of genes associated with disease resistance) that have been lost or diverged during the many years of evolution or domestication and selection for sweet-red watermelon with desirable fruit qualities (Levi *et al.*, 2012). Watermelon cultivars (*Citrullus lanatus* var. *lanatus* Cll) lost their disease resistance allele and they also share a narrow genetic base which makes them susceptible to a large number of diseases and pests (Levi *et al.*, 2001, 2017; Nimmakayala *et al.*, 2010, 2014; Reddy *et al.*, 2014 a, b). The loss of gene sequences associated with disease resistance was further reinforced with the sequencing and assembly of the watermelon genome (Guo *et al.*, 2012; Levi, 2013). Dessert watermelon is susceptible to the soil born pathogen *Fusarium oxysporum* and nematodes, potyviruses and other bacterial and fungal diseases. Since the ban of the chemicals used for soil disinfection, researchers and breeders focused on finding rootstocks resistant to the pathogen (Levi *et al.*, 2014) and have been releasing new watermelon lines that have resistance to different pathogens (Levi *et al.*, 2014, 2016 a,b; Kousik *et al.*, 2014). The present review will discuss the major watermelon diseases and pests that are impacting the crop yield as well as applications of precision agriculture in watermelon research.

### Major viral diseases

Potviruses such as zucchini yellow mosaic virus (ZYMV) and papaya ringspot virus (PRSV) are the most common and most economically important watermelon diseases throughout the world and there is a continuous need to enhance resistance to these viruses (Webb *et al.*, 2003). Gunner *et al.* (2004) and Ling *et al.* (2009) reported that resistance to PRSV and ZYMV is controlled by a single homozygous recessive gene.

To date, a few Plant Introductions (PIs) have been reported to have resistance to PRSV-W; among them, the United States PI 595203 (*Cl*), that also contains resistance to ZYMV and WMV, and the *Clc* PI 244019 and PI 244017 (Guner, 2004). Also, a study performed by Araujo and Souza (1988) reported the watermelon accession (Ouricuri) and PI 595201 to have resistance to PRSV (Nascimento *et al.*, 2011; Levi *et al.*, 2016a). Scientists recently identified resistance genes in desert watermelon (*C. colocynthis-Clo*) (Levi *et al.*, 2016a). After testing thirty-one *Clo* accessions, 4 PIs showed high resistance to PRSV which were self-pollinated and also crossed and backcrossed with watermelon cultivars obtaining valuable genetic populations with resistance to PRSV which could be used for watermelon breeding programs (Levi *et al.*, 2016a).

Breeding efforts using the three ZYMV-resistance molecular markers developed by Ling *et al.* (2009) and Harris *et al.* (2010) have yielded advanced watermelon lines BC<sub>3</sub>F<sub>5</sub> and BC<sub>4</sub>F<sub>4</sub> that contain resistance to ZYMV. These lines are derived from crossing the ZYMV resistant PI 595203 (donor parent) with the heirloom watermelon cultivars Charleston Gray or Crimson Sweet (recurrent parents) to have ZYMV-resistant lines with desirable fruit quality (including red flesh), high sugar content, and appealing flavor. Recently, a new ZYMV resistant line was released by Levi *et al.* (2016) after 7 years of breeding process, which included testing each generation using molecular markers, enzyme-linked immunosorbent selection (ELISA) as well as stringent phenotypic ratings.

### Major bacterial and fungal diseases

Since there is very little variation within all the sweet watermelon varieties they are vulnerable to a large number of diseases and pests (Levi *et al.*, 2001). Breeders and scientists have researched genebank accessions of *Citrullus lanatus*, *C. mucospermus* and *C. amarus* (previously known as *Citrullus lanatus* var. *citroides*) which were found to be a valuable source of resistance to powdery mildew (PM), a major disease of watermelon (Davis *et al.*, 2007; Tetteh *et al.*, 2010; Kousik *et al.*, 2012, 2014; Levi *et al.*, 2017).

Fusarium wilt caused by the pathogen *Fusarium oxysporum* is one of the most important diseases in watermelon, causing important economic losses (sometimes even total yield losses) and limiting the production (Ling *et al.*, 2013). Different methods were tested to find viable solutions to overcome fusarium, such crop rotation, grafting onto resistant rootstocks, using resistant rootstocks,

different chemical fungicides, cover crops, using host resistant cultivars and solarization (Ling *et al.*, 2013; Everts and Himmelstein, 2015). The most effective way to fight the pathogen is by development of host resistance watermelons lines, a difficult task to accomplish since watermelon lacks resistance to all races of the fungus (Meyer *et al.*, 2016). Breeders worked on developing resistant watermelon lines since the early 1900's (Martyn, 2014; Everts and Himmelstein, 2015). Seed companies and research stations developed many diploid and triploid lines with different levels of resistance to fusarium; still, to date none of the lines has high levels of resistance to FON race 1 and 2 (Everts and Himmelstein, 2015).

It was demonstrated that adding strains of beneficial bacteria *Pseudomonas fluorescens* could lead to suppression of fusarium wilt in watermelon up to 60 percent when applied as seed coat treatment (Meyer *et al.*, 2016). The inhibition of fusarium by these beneficial bacteria could be the effect of the production of secondary metabolites such as 2,4 diacetylphloroglucinol (DAPG) which proved to affect plant vigor (De Leij *et al.*, 2002; Brazelton *et al.*, 2008; Meyer *et al.*, 2016) and to inhibit bacterial, fungal, nematodes and viruses (Delany *et al.*, 2001; De Souza *et al.*, 2003; Weller *et al.*, 2007; Zhou *et al.*, 2014; Meyer *et al.*, 2016).

Powdery mildew is a major disease in watermelon caused by *Podosphaera xanthii*. Once it infects the plants it can be controlled using different fungicides (Ben-Naim and Cohen, 2015). Various *Citrullus* PIs were reported to have resistance to race 1 or 2W of powdery mildew and the resistance mechanism was determined in several PIs (Davis *et al.*, 2007; Tetteh *et al.*, 2013; Ben-Naim and Cohen, 2015; Levi *et al.*, 2017). Resistance to powdery mildew is different depending on the plant age. At cotyledonary stage, resistance is controlled by a single gene; when the plant is at the 4<sup>th</sup> -leaf stage and 15<sup>th</sup> -leaf stage it is controlled by three complimentary partially dominant genes (Ben-Naim and Cohen, 2015). Valuable genetic material that can be used as sources of resistance to powdery mildew and phytophthora fruit rot was developed by the USDA/ARS/USVL and it is available to be used in breeding programs (Levi *et al.*, 2017).

Various *C. amarus* PIs genotypes manifest resistance to the major watermelon diseases and pests. Several *Ca* PIs were reported to manifest resistance to: root-knot nematodes (Thies and Levi, 2003, 2007; Levi *et al.*, 2017), Fusarium wilt race 2 (Martyn, 1989; Dane *et al.*, 1998; Netzer and Wechter *et al.*, 2012; Levi *et al.*, 2017) and potyviruses (Strange *et al.*, 2002; Guner, 2004; Levi *et al.*, 2017). Because multiple resistance genes were identified in the *Ca* PIs it was suggested that the PIs could represent valuable genetic material to be used as rootstock (Thies *et al.*, 2010, 2015; Levi *et al.*, 2013, 2017).

### Major insects

One of the pests that largely affect watermelon is the whitefly (*Bemisia tabaci*). The most common control strategy for whiteflies involves the application of insecticides; unfortunately, whiteflies develop resistance to

insecticides quickly, so the most desirable way to control them is to develop new watermelon lines with resistance to whiteflies (Simmons and Levi, 2002; Coffey *et al.*, 2015). Recent research focused on sequencing the whitefly genome and managed to identify 15,664 genes from which 5,330 (3,417 single-copy genes) were found unique to *B. tabaci* (Chen *et al.*, 2016). The recent technical advancement in gene discovery has the potential to lead to identification of the ones that could be involved in virus transmission and insecticide resistance (Chen *et al.*, 2016). Scientists also tested different cultivars, namely one triploid line, 10 *C. amarus* PIs and 8 *C. colocynthis* PIs while aiming to identify useful sources of germplasm that could be used for watermelon breeding programs to enhance watermelon resistance to whiteflies (Simmons and Levi, 2002). Their findings indicated that *C. colocynthis* (desert watermelon) had the highest resistance to the whiteflies and could be used as a source of genetic material for the breeding programs. In 2015, Coffey *et al.* also evaluated 30 different *Ca* PIs to identify sources of resistance to *B. tabaci* and recommended PI 346082 and PI 537277 to be used for future breeding projects.

The best method to identify if a line has resistance or is susceptible to whiteflies is by performing an olfactometer bioassay (Togni *et al.*, 2010). By performing this type of bioassay it can be determined whether whitefly has a nonpreference for volatiles produced by the host plant (Togni *et al.*, 2010; Coffey *et al.*, 2015). Combining resistant watermelon lines with reflective mulch proved to be the best method to control whiteflies (Simmons *et al.*, 2010).

Since 2012, when the U.S. Environmental Protection Agency banned the utilization of methyl bromide initially used to control nematodes infestations, researchers have been continually working on finding solutions to the nematodes problem. Other chemicals are available for preplant fumigation, but there are very expensive and hard to apply (Morris *et al.*, 2015; Thies *et al.*, 2016). Scientists searched and evaluated different watermelon germplasms and managed to identify several accessions of *Ca* which manifested moderate resistance to rootknot nematodes (Thies *et al.*, 2010, 2015a, 2015b, 2016). In a comparative study that included 19 accessions of *Ca* as well as reference entries of *Clo* and sweet watermelon showed that *Ca* had lesser degree of nematode reproduction and plants had more root mass (Thies *et al.*, 2016). Recently, a new watermelon line was released as a root-knot resistant line and recommended to be used as a rootstock for grafting watermelon (Thies *et al.*, 2015c). The line released by Thies *et al.* (2015c) proved to be a valuable rootstock in field testing, conferring nematodes resistance to southern root-knot, peanut and Javanese nematodes.

### Genetic enhancement

After the draft sweet watermelon genome was sequenced by Guo *et al.* (2013) it reinforced results from previous reports (Levi *et al.*, 2001; Levi *et al.*, 2011a) that the watermelon cultivars share a narrow genetic base due years of domestication and selection for fruit quality.

Because of the narrow genetic base that the sweet cultivars share researches had to find *Citrullus* species which have resistance. In a study using DNA markers it was determined that the desert watermelon *Citrullus colocynthis*, is closely related to the heirloom watermelon cultivars such as 'Charleston Gray' or 'Black Diamond' (Levi *et al.*, 2013). Also, in a recent study (Levi *et al.*, 2016) it was determined that the desert watermelon accessions collected in northern Africa, the Middle East and Asia contain wide genetic diversity and exotic gene sequences that could be useful in enhancing medicinal and nutritional values of watermelon cultivars. However, to date, there is no sufficient information about the genome of this important watermelon species (Levi *et al.*, 2016).

Although the watermelon cultivars are easily crossed with *Clo*, wide genetic diversity exists between these two groups (Jarret *et al.*, 1997; Levi *et al.*, 2001, 2012). Genetic mapping studies using F<sub>2</sub>, BC<sub>1</sub> and test cross populations derived from a cross between *Citrullus amarus* (*Ca*), *Cll*, and *Clo* genotypes resulted in a strong preferential (non-Mendelian) segregation for most DNA markers, indicating that differences in their genome structure prevent normal Mendelian segregation and may produce quasi-linkage for many gene loci (Levi *et al.*, 2001, 2002, 2006).

To date, there are several SNP-based genetic maps for watermelon derived from crossing *Cll* and *Ca* genotypes (Cecilia) the *Cll* PI 595203 and the watermelon cultivar Calhoun Gray (Levi *et al.*, 2012). Although *Clo* is considered an important source of resistance genes and includes genes conferring resistance to fusarium wilt, powdery mildew, anthracnose, or whiteflies (Simmons and Levi, 2002), no genetic map has been constructed and gene loci associated with ZYMV (Guner, 2004); similarly, PRSV resistance has not been mapped in this *Citrullus* species. Breeding efforts using the three ZYMV-resistance molecular markers developed by Ling *et al.* (2009) and Harris *et al.* (2010) have yielded advanced watermelon lines BC<sub>3</sub>F<sub>5</sub> and BC<sub>4</sub>F<sub>4</sub> that contain resistance to ZYMV.

### Watermelon and resistance to abiotic factors

The two major abiotic factors that breeders try to improve in watermelon are drought resistance and salt tolerance (Kotuby-Amacher *et al.*, 2000; Cheng *et al.*, 2015). The most common methods to induce resistance to abiotic factors are using resistant rootstocks or developing resistant lines through traditional breeding methods. Salt level in soil is directly dependent on climate conditions (Kotuby-Amacher *et al.*, 2000); as the soil dries, salt concentrations become higher resulting in a increased level of salt stress on the plant. Fortunately, controlling and managing soil salinity is not difficult: with proper drainage it can be controlled very easily (Kotuby-Amacher *et al.*, 2000). Dessert watermelon is known to have a very low tolerance to salt; thus, research has shown that salt tolerance in watermelon can be improved by application of foliar treatment using 24-epibrassinolide (Cheng *et al.*, 2015). Also, foliar applications using 5mmol/L of salicylic acid was found to be very effective in alleviation of salinity (Ayyub *et*

*al.*, 2015). Using salt tolerant rootstock could represent a viable solution to the problem. In 2015, a study compared four different watermelon rootstocks on which mini watermelons were grafted at three different salinity levels ranging from 0 to 3dS·m<sup>-1</sup> (Simpson *et al.*, 2015). The best results were obtained using 'Strong Tosa' rootstock (Simpson *et al.*, 2015).

Drought is another important and limiting abiotic factor to which most of the desert watermelon are susceptible to. A study performed by Zhang *et al.* (2011) analyzed the USDA watermelon germplasm aiming identification of drought tolerance watermelon varieties. After screening more than 1000 accessions and breeding lines they were rated for drought tolerance and grouped in four classes based on their drought tolerance levels. The study identified twenty five watermelons (12 *Ca* accessions and 13 cultivars), all of which with African origins (Zhang *et al.*, 2011).

### Precision agriculture

Precision agriculture involves the application of the right treatment in the right place at the right time (Srinivasan, 1999; Robert, 2002; Stafford, 2006; Gebbers and Adamchuck, 2010). Since 2007, the Natural Resources Conservation Service (NRCS) has been highlighting the benefits of precision agriculture. Its two major advantages (economical and environmental) are the result of targeted placement of crop inputs - fertilizers, pesticides and water (McLoud *et al.*, 2007). Precision agriculture technologies typology systems could be divided into three main categories such guidance systems (automatic steering/guidance for tractors and self-propelled agricultural machinery); recording technologies (soil mapping, canopy mapping, yield mapping); and reacting technologies (variable rate irrigation and weeding, variable rate application of pesticides and fertilizers) (Schwarz *et al.*, 2011; Balafoutis *et al.*, 2017).

The information collected through a variety of methods (grid soil sampling, yield monitoring, detailed soil information, remote sensing and environmentally sensitive areas) is combined and compiled into detailed application maps to determine the appropriate fertilizer rates. By using Differential GPS systems such as Wide Area Augmentation Systems (WAAS), the application equipment can use the variable rate fertilizer application (McLoud *et al.*, 2007). Precision agricultural systems require the use of Global Navigation Satellite Systems irrespective of the typology (Balafoutis *et al.*, 2017). Based on existent forecasts, the fertilizer consumption may increase from 166 million tons in 2005/2007 to 263 million tons by 2050, fueling already existent concerns over the negative impact of agriculture on the environment (Alexandratos and Bruinsma, 2012). Precision agriculture technologies are needed to reduce the amount of mineral fertilizer per unit of crop output while increasing the productivity (Alexandratos and Bruinsma, 2012). The important role that precision agriculture may have on reducing the use of fertilizer is described in a study performed by Khosla *et al.* (2002). The study suggested that nitrogen management using site-specific management zones provides the N amounts needed to obtain higher yields as

well as to maximize the use of applied N.

Variable rates for pesticide application may be developed using control maps or sensor inputs similar to the ones described for nutrients. However, additional planning information and equipment would be needed for implementation (McLoud *et al.*, 2007; Ess *et al.*, 2001). Guidance technologies play an important role in improving the pass-to-pass efficiency while also reducing the overlapping and application gaps (Balafoutis *et al.*, 2017). Variable rate technologies can lead to a lower pesticide application that does not exceed the application rate indicated for the diagnosed disease (Balafoutis *et al.*, 2017).

Irrigation accounts for about 40-60 percent of the total cereal production in developing countries with approximately half of the irrigated area being found in India and China (Alexandratos and Bruinsma, 2012). The need for irrigation increases with a rising global population while renewable water resources have already become extremely scarce in several countries. Currently, the water usage threshold of imminent water scarcity established by FAO is at 20 percent of a country's renewable water resources for irrigation. Twenty-two countries already pass the threshold, with four of them (Libya, Saudi Arabia, Yemen and Egypt) using more water through irrigation than their renewable resources (Alexandratos and Bruinsma, 2012). Since the soil conditions vary within the field, a way to address the irrigation induced water scarcity problem is by using precision agriculture techniques that could help identify these variations (Tomasiewicz *et al.*, 2013). The application of precision agriculture to irrigation is known as variable rate irrigation (VRI) and refers to applying different irrigation amounts to match crop water demands (Tomasiewicz *et al.*, 2013). The VRI system has an important economic advantage by increasing the yield while reducing the water and energy use (Tomasiewicz *et al.*, 2013; Balafoutis *et al.*, 2017).

One of the easiest ways to collect different data about soil measurements is by using soil electrical conductivity (EC) defined as a measurement that correlates with soil properties that affect crop production. The measurement includes parameters such as soil texture, cation exchange capacity (CEC), drainage conditions, organic matter level, salinity, and subsoil characteristics (Grisso *et al.*, 2009). Electrical conductivity is one of the most useful and frequently used measurements to characterize field variability for application to precision agriculture (Corwin and Lesch, 2003).

The only study related to the influence of precision agriculture on watermelon production was conducted by Fountas *et al.* (2015). The researchers looked for correlations between soil electrical conductivity and yields as well as fruit quality. The study was conducted for two consecutive years. While results from year one of the study showed a correlation between EC, yield and fruit quality, no correlation was found during year two (Fountas *et al.*, 2015). Lack of more data suggests the need for more in depth research in this field.

Precision agriculture is believed to have great potential to improve the efficiency of farm operations and to help increase farmers' net returns (Shockley *et al.*, 2012; Smith *et al.*, 2013; Castle *et al.*, 2016), while also lowering water usage (Evans and Sadler, 2008).

## Conclusions

With a global population rising very rapidly and predicted to reach 9.2 billion in 2050 and 11.2 billion by 2100 (UN, 2015) scientists in the agricultural and horticultural field need to also consider and implement geospatial technologies that may contribute to higher yields and thus insure the production of necessary food supplies. The utilization of high-yielding cultivars with disease and insect resistant packages produced through different genetic enhancement procedures and of resistant rootstocks combined with precision-farming systems, coupled with increased education of the growers and their awareness of emergent technologies may lead to increased yields to sustain a rapidly growing human population. Watermelon along potato, tomato, garlic and onion is one of the most cultivated vegetables worldwide (Camargo and Camargo, 2017). A multidisciplinary effort to improve the process of its production is needed to ensure that the future market demands are being met.

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