ABSTRACT

Fahey, Kyle Stephen. Phenotypic Characterization and Marker Development for Non-Race 1 Verticillium Wilt Resistance in Two Tomato Recombinant Inbred Line Populations

(Under the direction of Drs. Reza Shekasteband and Gina Fernandez).

Host resistance has been a core strategy for managing verticillium wilt caused by *Verticillium dahliae* in tomato production for over 70 years. While resistance to race one has been thoroughly introgressed into most round-type commercial tomato cultivars, non-race one *V. dahliae* (Vdn) has no known resistance in any cultivated varieties to date. Vdn remains a major challenge for growers in western North Carolina and numerous other tomato-growing regions globally. *V. dahliae* remains one of the most difficult diseases to control in fresh market tomato production, due in part to dormancy structures (microsclerotia) that can survive within the soil for decades. Further complicating this, a lengthy list of alternative host species including many weedy species, solanaceous vegetables, non-solanaceous vegetables, fruit crops, cotton, alfalfa, and many others have made crop rotation strategies ineffective. In the past, the fumigant methyl bromide (CH_3Br) was shown to be highly effective, however, it was phased out due to environmental concerns. Vdn has been an issue in tomato production for over 50 years, but the loss of methyl bromide as a control measure has elevated this issue to one of the most important breeding objectives in western NC. These two challenges, the loss of methyl bromide and the development of *V. dahliae* isolates with no known host resistance have necessitated new breeding strategies to identify and map new sources of resistance and use this resistance to develop new hybrids or cultivars.

The current project had two main goals, first, we aimed to identify variation in resistance to Vdn of two advanced generation (F_5 and F_6) recombinant inbred line (RIL) populations in the

field with potential resistance to Vdn, and second to identify linkage-based markers for resistance to Vdn.

In the first study, we phenotyped two RIL mapping populations using natural inoculum across two on-farm and two research station trials spread across 2 years and found ample diversity for resistance to Vdn. A statistically significant and biologically relevant association was found between an acute angle between the compound leaves and the main stem to Vdn resistance within the population. The acute angle in combination with superior Vdn resistance is unique to one of the parental lines, 'NC 7GEM', and the resulting population. Earliness was not found to be associated with an improvement in Vdn resistance, indicating a biological basis for resistance. One finding was a change in Vdn disease severity phenotypes in one location in 2023. One possible cause could be a change in the pathogen population, Vdn was sampled and a morphologically unique isolate to NC of *V. dahliae* was found. This isolate was shown to be more aggressive in phenotypic extremes in one population in a greenhouse pathology assay than an isolate with the expected NC morphology found in the on-farm trial in 2023. In the second study, a marker trait analysis was performed. The morphological and genetic markers identified (association between a growth habit and an improvement of resistance scores) serve to advance the efforts of tomato breeding for *V. dahliae* resistance in this material.

Phenotypic Characterization and Marker Development for Non-Race 1 Verticillium Wilt Resistance in Two Tomato Recombinant Inbred Line Populations

by
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DEDICATION

I dedicate this thesis to my family, and my partner Emma.

BIOGRAPHY

Kyle was born in Halifax County, Virginia. He grew up in Apex NC, a short drive from where his great grandparents grew tobacco. In his early years, he spent a lot of time exploring the land there and finding various rusting farm equipment and curing setups in the overgrown woods. Part of the acreage and the original farmhouse still stands to this day on Kelly Road, just past Fahey Drive. Kyle's passion for the outdoors and the environment came from his dad, Tim Fahey, and being a boy scout. Kyle's love for plants came from his mom, Amy Fahey, who is the most enthusiastic horticulturist he knows, growing lots of veggies in her garden.

For hobbies, Kyle enjoys blacksmithing, camping, gardening, fishing at the beach, and any particularly challenging DIY repair.

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I would like to thank Dr. Gabriel Giestera, he was a major net positive on this project and on my development and learning. I would also like to thank Dr. Tom Ingram for teaching me about the root dipping assay. I would also like to thank Dr. Randy Gardner for his support, and the creation of these parental lines, the world of tomato breeding and plant breeding is so much better for his past and continued participation. I really appreciate his support for developing traditional plant breeding practices in me. I think without his voice and guidance I would not be where I am today.

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CHAPTER 1:LITERATURE REVIEW

INTRODUCTION

Modern tomato breeding has played a critical role in the commercialization and improvement of fresh market tomato production. Outcomes that can be attributed to tomato breeders include increasing marketable yields, improving fruit quality traits, increasing shelf life, and providing host resistance to pests. Plant pests shape the availability and quality of food supplies globally, with plant pests accounting for 35% of crop losses in agricultural output (Oerke 2006).

Tomato Production Overview

Tomato production is often split into two broad categories by end use, fresh market, and processing production. These two production systems contrast in many ways, one major difference is the technology employed, Fresh market has no current mechanized harvest available, while processing tomatoes do have mechanization employed. Fresh market tomatoes are produced under plasticulture, including drip irrigation tape that is also used to deliver fertilizer directly, also known as fertigation. Fresh market tomatoes are supported by interweaving strings by hand. As one of the most valuable global crops, tomatoes gross $102 billion US dollars and $10.52 billion within the United States (FAO, 2022). Tomatoes are also a critical supply of essential nutrients to people, including carotenoids, lycopene, and other essential micronutrients (Perveen, 2015). There are a broad range of skin and flesh colors, but historically the industry has focused on red skinned, red fleshed tomatoes. In the US, fresh market tomatoes are produced primarily in Florida, California, and North Carolina, with some production in Georgia, Tennessee, and Virginia (Zhu et al., 2013).

The Tomato Industry in North Carolina

The primary large round-type cultivars grown are primarily of the ‘Mountain’ Series from Randy Gardner, commonly ‘Mountain Fresh Plus’, ‘Mountain Spring’, ‘Mountain Gem’ ‘Mountain Glory’, ‘Mountain Merit’ ‘Mountain Rogue’ ‘Mountain Spring’ ‘Red Defender’, and ‘Fletcher'. Some cultivars recommended for NC by the Southeastern US 2023 vegetable crop handbook for the southeastern United States are: ‘Amelia VR’, ‘BHN 602’, ‘Celebrity’, ‘Defiant PhR’, ‘Florida 47R’, ‘Jolene’, ‘Pheonix’, ‘Primo Red’, ’Red Snapper’ and ‘Rocky Top’. A selection of divergent (non-red fruited, large round fruited) market classes are grown, some of the cultivars recommended are ‘Mountain Magic’(Campari, unique market class, see Gardner and Panthee, 2012 for details), ‘Sun Gold’(yellow cherry), ’Sun Sugar’(yellow cherry),’Elfin’, ‘Mountain Honey’(grape type), ‘Mountain Vinyard’(grape type), and ‘Smarty’(grape type) (Southeastern US Vegetable crop handbook, 2023). Fresh market tomatoes grown in North Carolina come in various shapes, the most common are large round, grape, cherry, and plum types.

Diversity of Market Classes in Tomato

Fresh market tomatoes as a horticultural crop have a wide diversity of market classes, wider than most horticultural crops. In the eighties, large red tomatoes dominated the tomato market, with few other colors and shapes to select from. Since then, diverse market classes have come through from breeders to home gardeners or grocery stores. While the determinant, large round red tomato is not as common in-home gardens, it is worth acknowledging the impact of the home garden on the demands of new commercial cultivars. Some cultivars and fruit shapes are considered distinctly heirloom, which has a definition subject to some interpretation and could be used to describe cultivars and fruit shapes that are not intended or employed in mass production. These are commonly cultivars that diverge significantly from large round, cherry or grape, cultivars that diverge significantly from unpatterned red coloration, or a combination of both. A heirloom fruit shape is defined as having extreme ‘shoulders’ that protrude above the pedicel connection point. It could be said that all heirloom fruit-shaped cultivars fall under “heirloom cultivars” but not all heirloom cultivars are heirloom fruit-shaped. These shoulders are bad for tomato packers so are mostly avoided or demand a high price on the market as a specialty product. In general, these groups are not grown at scale commercially but represent an important influence on tomato breeding.

Verticillium Wilt in Tomatoes and Impacts of the Disease in North Carolina

Verticillium wilt (*Verticillium dahlia* Kleb.) is one of the most important fungal diseases impacting tomato production in North Carolina. Verticillium wilt is a vascular wilting fungus in tomatoes and commonly occurs in temperate, high-precipitation regions. *V. dahliae* is a highly challenging pathogen to manage due to dormancy structures that ensure pathogen survival for 10-15 years in the soil, plant tissue, and seed (Wilhelm, 1955). Additionally, numerous weedy and crop hosts including *Solanaceous* vegetable crops, cotton, ragweed, and over two hundred more hosts complicate cultural and chemical management of *V. dahliae.* In tomatoes, host resistance to race one *V. dahliae* is widely adopted in all modern, commercially produced cultivars (Schaible et al., 1951; Ingram et al., 2020). In older literature, *V. dahliae* was often considered a subgroup of *V. albo-atrum and* was highly controversial throughout the 60s and 70s. It is widely accepted that the primary delineator of *V. dahliae* and *V. Albo-atrum* is the presence or absence of microsclerotia (see Pegg and Brady, 2002 for a review of the literature surrounding this topic). *V. Albo-atrum*, which is similar to *V. dahliae* is defined as described in Reinke and Berthold's initial work on the pathogen- an isolate of Verticillium wilt that lacks structures described as microsclerotia (Reinke and Berthold, 1879; Fradin and Thomma, 2006).

Race 1 and 2 *Verticillium dahliae* in NC was first reported by Bender and Shoemaker, along with *Verticillium albo-atrum* (Bender and Shoemaker, 1984). In Bender and Shoemakers initial survey for verticillium wilt pathogens, 56% of the 108 fields surveyed (56.5% of 65.6 hectares) had isolates that were confirmed to be some species of Verticillium wilt and identified to be *V. dahliae*. Isolates that are/are not pathogenic on lines containing resistance descended from the work of Dr. Loran Blood and Schaible et al. are considered "race one" and "race two" respectively (Schaible et al., 1951). In recent years, isolates from NC as well as isolates sourced from Japan, Florida and California have been sequenced and identified as non-race one.

The NC tomato industry is valued at $30 million dollars. (USDA NASS, 2023). *V. dahliae* can have severe economic impacts on tomatoes, while a contemporary economic analysis for NC is needed, a past study in South Africa on the impact of Verticillium on fresh market tomatoes indicates marketable yield losses between 8% and 21% by the end of the season (Visser, 1977). Invasive plant pathogens in the US alone cost twenty-one billion dollars across various agricultural systems (Rossman, 2009).

Due to the phaseout of methyl bromide, growers have come to rely heavily on alternatives, most prominently chloropicrin. The reduction of yield attributable to chloropicrin was found to reduce yields by 7116 pounds per acre in comparison to methyl bromide, the next best alternative (Louws et al., 2004). In the study, this loss totaled 11% of total yield. Additional fumigants including 1,3-dichloropropene (1,3-D), trichloronitromethane (chloropicrin), dimethyl disulfide (DMDS), sodium-N-methyldithiocarbanate (metam sodium) and potassium-N-methyldithiocarbanate (metam potassium) 3,5-dimethyl-(2H)-tetrahydro-1,3,5-thiadiazine-2-thione(dazomet) have been evaluated for effectiveness to verticillium wilt with varying degrees of success(Pecchia et al., 2017; Zhao et al., 2018; Acharya et al., 2020). A completely effective chemical control of *V. dahliae* remains elusive, as many of the fumigants listed are only effective in moderate effects, whether it is delaying onset, reducing incidence, or slowing progression.

Tracing the Roots: A Brief History of the Pursuit of Verticillium Wilt Resistance in Tomatoes

The development of Verticillium wilt resistant cultivars in tomatoes has been an objective with “deep roots”- figuratively speaking- in the history of tomato breeding that often is overlooked. The story that is commonly mentioned in the literature starts with the release of ‘Loran Blood’ and ‘VR Moscow’ by Schaible et al. split verticillium into two races releasing cultivars by the single dominant gene *Ve* (Schaible et al., 1951). What is often difficult to convey is the lifetime of effort that Dr. Loran Blood, the namesake of the release, put into the verticillium wilt resistance in addition to the work Schaible et al. did. The resistance was sourced from two crosses between a small fruited wild tomato accession known as Peru Wild and the cultivars ‘Stone’ and ‘Century’, by Blood, who bred for a race one *Verticillium dahliae* resistant cultivar over the course of 20 years following its receipt in 1932 (Blood, 1932). Blood mentions that he had identified single plant selections with marketable fruit size from these crosses (Blood, 1942). Records indicate that between 1930 and 1940, 1444 single plant selections for disease resistance were made, 1250 varieties of wild, novelty, and commercial tomatoes, and over three hundred crosses were tested for resistance on 15 acres in Hurricane, Utah (Blood, 1948). Dr. H Loran Blood passed away in 1948, three years before another group of scientists were able to develop a novel root dipping assay to screen and identify the most resistant lines out of Bloods population to release (Schaible et al., 1951). In the decades following this achievement, almost all commercialized, round type tomato cultivars now have introgressed this resistance. Unfortunately, 6 years after this achievement, isolates of verticillium were found to break the resistance in ‘Loran Blood’ were uncovered (Robinson et al., 1957).

In NC, almost all NC material, the *Ve* gene, is fixed thanks to the persistent efforts of Dr. Randy Gardner, who started with Florida material (Okie and Gardner, 1982a). Okie and Gardner did manage to identify moderate resistance to non-race one resistant material under root dipping conditions and pursued that resistance in a follow up greenhouse assays and field trials (Okie and Gardner, 1982b). They found that the resistance trait from ‘Morden MEL2668107G’ had relatively low heritability but could be attributed to a small number of genes based on the number of F_3 progeny with resistance comparable to the parental line. This led to further progeny advancement, the release of ‘Mountain Fresh’ (Gardner, 1999).

In this paper, another step towards the identification of a non-race one resistant, large round fruited tomato cultivar will be taken, with some notable parallels to the work described above, performed 73 and 42 years ago (Schaible et al., 1951; Okie and Gardner, 1982a). As a field of study, published scientific efforts directly breeding for Verticillium wilt in tomatoes are rare between Okie and Gardner’s publications in 1982, and the recent phase-out of methyl bromide. Dr. Gardner shared a race-2 resistant line, 8V’ which was crossed with ‘Ohio CR-6’ which was used to generate 2 F_4 breeding lines ‘Ohio 11’ and ‘Ohio 12’, reported to have resistance to non-race 1 verticillium wilt, Fusarium Crown Rot resistance and Tobacco mosaic virus (homozygous, and heterozygous TMV resistance respectively) (Berry and Oakes, 1987). It is unknown what the present status of this material is and requires further investigation, as no contemporary assessments have been performed.

Emergence of Additional Races of Verticillium and the Need for a New Breeding Focus

As described above, the phaseout of methyl bromide and less than optimal performance of chloropicrin, a need for new sources of resistance to forms of Verticillium wilt are needed. Further complicating the matter is that in recent years, non-race one *V. dahliae* has been split into 2 races using differential wild-type rootstocks under root dipping conditions, with ‘race two’ being isolates that overcome the *Ve* gene, but do not overcome the resistance conferred by the rootstock ‘Aibou’,’Back attack’, and ‘Ganbarune-Karis’. Race three is composed of isolates that overcome the rootstock-based resistance and *Ve* (Usami et al., 2017). It appears that all isolates obtained from western NC have been defined as race three thus far (Ingram et al., 2020). Further, ‘Aibou’, ‘Back attack’ and ‘Ganbarune-Karis’ are wild accessions of *Solanum neorickii* (syn. *Lycopersicon parviflorum*), which would require a focused breeding project to make available in an environmentally adapted, elite, large round-fruited cultivar. In this manuscript, the resistant material described has no known shared background with this material and is resulting from different wild material. Marker-assisted selection is one of the most important innovations in plant breeding technologies and has resulted in numerous advances in breeding populations worldwide. Numerous tomato breeding markers have been developed and remain in use by tomato breeding programs employing marker-assisted selection (Lee et al., 2015). While numerous markers have been generated for various disease resistance traits, markers are available only available for race one and race two resistance along with the corresponding avirulence genes, the *Ve1* and *V2* loci (De Jonge et al., 2012, Chavarro-Carrero et al., 2020). Ingram et al. (2020) hypothesizes race 3 may encompass many races that are undefined as of yet.

Single Seed Descent as a Breeding Strategy

Single seed descent (SSD) is a strategy where plants are advanced through a single plant each generation during the inbreeding phase to allow for the advancement of multiple generations per year by growing generations within the greenhouse. SSD was first described in the seminal paper Problems in Plant Selection (Goulden, 1939). In this initial paper, Goulden does point out the strategy was primarily intended to assist statisticians at the time to assist plant breeders with theoretical and practical studies of specific problems. In cultivar development scenarios, a pure interpretation of SSD would prevent the implementation of selections, so Brim (1966) adapted the SSD method to include selections while maintaining the SSD nature-enabling the breeder to combine the benefits of off-season generation advancement with the benefits of selection. Brim (1966) refers to this method as the "Modified pedigree method" but it is important to make clear this method is far more similar to SSD than pedigree method.

Application of Association Studies to Improve Diploid Crops

While there are many approaches to marker development, a few key considerations are common among all approaches in diploids. These considerations are one. availability of sufficient genetic diversity for a trait of interest within either a structured population or a panel of diverse individuals two. cost of obtaining genotypic information three. cost and ease of phenotyping the trait of interest (Collard et al., 2005). The first step to any marker development project is to obtain phenotypic and genotypic information from a subset of a population, or a diverse panel with sufficient genetic diversity. Ideally, this subset will be representative of the distribution of the desired phenotype in the population to optimally detect highly relevant SNPs. Generally speaking, larger datasets with finer coverage are more desirable, but they come with higher costs. One of the easiest places to begin the process of selecting a sample size/coverage pairing for diploids is to consider the recombination rate. Tomatoes tend to have an exceedingly low recombination rate compared to other crop species (de Haas et al., 2017). For this reason, it is optimal to prioritize larger populations over finer coverage, as much of the tomato genome is made up of large linkage blocks. After selecting a genotyping design, DNA can be extracted, and samples can be prepared to send for sequencing. By analyzing the phenotypic associations and the sequence data, potential molecular markers can be revealed.

DNA Sequencing

To identify molecular markers, DNA sequence data must be obtained. The advent of DNA sequencing, and its development has made tremendous strides since its development in the 1970s. Initially, the two methods were the chain terminator procedure developed by Sanger and Coulson, and the chemical cleavage procedure developed by Maxam and Gilbert (Shendure et al., 2017). Both methods relied on distances from a radioactive label along a DNA molecule. Specifically, Sanger's method involved taking a labeled primer through extension phase using DNA polymerase four times, with chain-terminating nucleotides to generate fragments of different lengths. The fragments underwent gel electrophoresis to separate by size using a DNA ladder. In the 80s and 90s, second-generation sequencing methods began to emerge, characterized by ‘massively parallel’ sequencing using multiplexing with fluorescently labeled nucleotides. By allowing for a single sample volume to have access to all DNA templates, copies of each sample to be sequenced could be generated and base information extracted using various strategies like imaging to obtain genotypic data simultaneously, rather than base pair by base pair. Increasingly, methods based on template amplification like massively parallel (second-generation sequencing) and Sanger sequencing remains the most popular sequencing method to date, albeit with less dangerous chemicals involved (Eren and Pirim, 2022). There are many reasons for this, including the time and complexity along with possible errors in the strands amplified. The first approach to be developed for this was to image the synthesis mediated by polymerase in real time by Webb, Craighead, Korlach, Turner and Pacific Biosciences (PacBio) (Eid et al., 2009; Levene et al., 2003). This allowed for the synthesis of ultra-high-quality reads with minimal bias compared to earlier methods. Another method developed concurrently, nanopore sequencing, was based on electronic signals as DNA passes through a channel.

Justification for Research

V. dahliae remains a major threat to tomato production due in part to the resistance-breaking strains that have been identified and remain prevalent in western North Carolina (NC) (Ingram et al, 2020). The ability to persist in soil for years on end within microsclerotia during the winter is one of the driving factors in creating such a difficult pest. As the *V. dahliae* pathogen population has adapted to the pressure of resistant plant material in agronomic systems, and the pest management systems themselves have faced regulatory pressure, the need to identify host resistance is greater than ever before (Usami et al., 2017, Castroverde et. al., 2017). Past work has shown that breeding for Verticillium wilt resistance is a complex, multifaceted challenge. In this project, tools will be identified to increase selection intensity for Verticillium wilt resistance in tomatoes.

REFERENCES

Acharya, B., Ingram, T. W., Oh, Y., Adhikari, T. B., Dean, R. A., & Louws, F. J. (2020). Opportunities and Challenges in Studies of Host-Pathogen Interactions and Management of Verticillium dahliae in Tomatoes. *Plants*, *9*(11), 1662. https://doi.org/10.3390/plants9111622

Bender, C. G. (1984). Prevalence of Verticillium Wilt of Tomato and Virulence of Verticillium dahlia Race 1 and Race 2 Isolates in Western North Carolina. *Plant Disease, 68*(1), 305. https://doi.org/10.1094/pd-68-305.

Bertucci, M., Bilbo, T., Jennings, K., Meadows, I., Rodrigues, C., Walgenbach, J., & Wszelaki, A. (2023). Southeastern U.S. 2023 Vegetable Crop handbook. Southeastern Vegetable Extension Workshop Group, *1*, 110–115.

Blood, H., Loran. (1942). Results of Station Research [Lead to] Practical Applications to War Emergency Agricultural Production Problems. *Farm & Home Science, 3*(4), 8.

Brim, C. (1966). A modified pedigree method of selection in soyabeans. *Crop Science 6*(2), 220. Portico. https://doi.org/10.2135/cropsci1966.0011183x000600020041x

Castroverde, C. D., Xu, X., Blaya Fernández, J., Nazar, R. N., & Robb, J. (2017). Epistatic influence in Tomato *Ve1*-mediated resistance. *Plant Biology, 19*(5), 843–847. Portico. https://doi.org/10.1111/plb.12582

Chavarro-Carrero, E. A., Vermeulen, J. P., E. Torres, D., Usami, T., Schouten, H. J., Bai, Y., Seidl, M. F., & Thomma, B. P. H. J. (2020). Comparative genomics reveals the in planta-secreted Verticillium dahliae Av2 effector protein recognized in tomato plants that carry the *V2* resistance locus. *Environmental Microbiology, 23*(4), 1941–1958. Portico. https://doi.org/10.1111/1462-2920.15288

Collard, B. C. Y., M. Z. Z. Jahufer, J. B. Brouwer, and E. C. K. Pang. (2005) An Introduction to Markers, Quantitative Trait Loci (QTL) Mapping and Marker-Assisted Selection for Crop Improvement: The Basic Concepts. *Euphytica 142*(1–2), 169–196. https://doi.org/10.1007/s10681-005-1681-5

de Haas, L. S., Koopmans, R., Lelivelt, C. L. C., Ursem, R., Dirks, R., & Velikkakam James, G. (2017). Low-coverage resequencing detects meiotic recombination pattern and features in tomato RILs. *DNA Research*, *24*(6), 549–558. https://doi.org/10.1093/dnares/dsx024

Eren, K., Taktakoğlu, N. and Pirim, I., (2022). DNA sequencing methods: from past to present. *The Eurasian journal of medicine*, *54*(1), 47. https://doi.org/ 10.5152/eurasianjmed.2022.22280

Blood, H. L. (1948), *Farm & Home Science 9*(2), 16.

Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., Peluso, P., Rank, D., Baybayan, P., Bettman, B., Bibillo, A., Bjornson, K., Chaudhuri, B., Christians, F., Cicero, R., Clark, S., Dalal, R., deWinter, A., Dixon, J., … Turner, S. (2009). Real-Time DNA Sequencing from Single Polymerase Molecules. *Science*, *323*(5910), 133–138. https://doi.org/10.1126/science.1162986

Fradin, E. F., & Thomma, B. P. H. J. (2006). Physiology and molecular aspects of Verticillium wilt diseases caused by *V. dahliae* and *V. albo-atrum*. *Molecular Plant Pathology*, *7*(2), 71–86. https://doi.org/10.1111/j.1364-3703.2006.00323.x

Gardner, R. G. (1999). NC 109 Tomato Breeding Line: Mountain Fresh'F1 Hybrid. *HortScience*, *34*(5), 941–942. https://doi.org/10.21273/hortsci.34.5.941

Gardner, Randy G., and Dilip R. Panthee. (2012) 'Mountain Magic': An Early Blight and Late Blight-Resistant Specialty Type F1 Hybrid Tomato. *HortScience, 47*(2), 299–300. https://doi.org/10.21273/hortsci.47.2.299

Goulden, C. (1939). "Problems in plant selection. Proceedings of the Seventh International Genetics Congress, *Cambridge University Press*, 132-33.

Hamer, H., McDaniel, J., Dorn, T., Edwards, M., Troxler, S., Smith, N. D., Teachey, S., Galiano, M., & Woodard, T. (2023) *United States Department of Agriculture National Agricultural Statistics Service*.

Ingram, T. W., Oh, Y., Adhikari, T. B., Louws, F. J., & Dean, R. A. (2020). Comparative Genome Analyses of 18 Verticillium dahliae Tomato Isolates Reveals Phylogenetic and Race Specific Signatures. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.573755

Levene, M. J., Korlach, J., Turner, S. W., Foquet, M., Craighead, H. G., & Webb, W. W. (2003). Zero-Mode Waveguides for Single-Molecule Analysis at High Concentrations. *Science, 299*(5607), 682–686. https://doi.org/10.1126/science.1079700

Louws, F. J., Ferguson, L. M., Ivors, K., Driver, J., Jennings, K., Milks, D., … & Monks, D. W. (2004). Efficacy of methyl bromide alternatives for Verticillium and weed management in tomatoes. *North Carolina State University.*

Okie, W. R., & Gardner, R. G. (1982a). Breeding for Resistance to Verticillium dahliae Race 2 of Tomato in North Carolina. *J Am Soc Hortic Sci*, *107*(4), 552–555. https://doi.org/10.21273/JASHS.107.4.552

Okie, W. R., & Gardner, R. G. (1982b). *Screening Tomato Seedlings for Resistance to Verticillium Dahliae Races 1 and 2*. *Plant Disease*, *66*(1), 34. https://doi.org/10.1094/pd-66-34

Pecchia, S., Franceschini, A., Santori, A., Vannacci, G., & Myrta, A. (2017). Efficacy of dimethyl disulfide (DMDS) for the control of chrysanthemum Verticillium wilt in Italy. *Crop Protection*, *93*, 28–32. https://doi.org/10.1016/j.cropro.2016.11.019

Pegg, G. , & Brady, B. (Eds.). (2002). Verticillium wilts. https://doi.org/10.1079/9780851995298.0000

Reinke, J., & Berthold, G. (1879). Die Zersetzung der Kartoffel durch Pielze. *Ullersuch. Bot. Lab. Univ. Gottingen 1*. 1-100.

Robinson, D., Larson, R., Walker, J. (1957). Verticillium Wilt of Potato in Relation to Symptoms, Epidemiology and Variability of the Pathogen. *Uni. Wisc. Agric. Exp. Stn. Res. Bull. 202,* 46.

Rossman, A. Y. (2009). The impact of invasive fungi on agricultural ecosystems in the United States. *Biological Invasions*, *11*(1), 97–107. https://doi.org/10.1007/s10530-008-9322-2

Schaible, L., Cannon, O., & Waddoups, V. (1951). Inheritance of resistance to Verticillium Wilt in A tomato cross 1951. *Phytopathol*, *41*(11), 986–990.

Shendure, J., Balasubramanian, S., Church, G. M., Gilbert, W., Rogers, J., Schloss, J. A., & Waterston, R. H. (2017). DNA sequencing at 40: Past, present and future. *Nature*, *550*(7676), 345–353. https://doi.org/10.1038/nature24286

Sim, S.-C., Deynze, A. V., Stoffel, K., Douches, D. S., Zarka, D., Ganal, M. W., Chetelat, R. T., Hutton, S. F., Scott, J. W., Gardner, R. G., Panthee, D. R., Mutschler, M., Myers, J. R., & Francis, D. M. (2012). High-Density SNP Genotyping of Tomato (Solanum lycopersicum L.) Reveals Patterns of Genetic Variation Due to Breeding. *PLOS ONE*, *7*(9), e45520. https://doi.org/10.1371/journal.pone.0045520

Usami, T., Momma, N., Kikuchi, S., Watanabe, H., Hayashi, A., Mizukawa, M., Yoshino, K., & Ohmori, Y. (2017). Race 2 of Verticillium dahliae infecting tomato in Japan can be split into two races with differential pathogenicity on resistant rootstocks. *Plant Pathol*, *66*(2), 230–238. https://doi.org/10.1111/ppa.12576

Visser, S. (1977). The effect of Verticillium dahliae on the yield of tomato plants. *Phytophylactica*, *9*(3), 65–70.

Wilhelm, S. (1955). Longevity of the Verticillium wilt fungus in the laboratory and field. *Phytopathol*, *45*(3). https://www.cabdirect.org/cabdirect/abstract/19551102133

Zhao, J., Zhou, X., Jiang, A., Fan, J., Lan, T., Zhang, J., & Cai, Z. (2018). Distinct impacts of reductive soil disinfestation and chemical soil disinfestation on soil fungal communities and memberships. *Appl Microbiol Biotechnol*, *102*(17), 7623–7634. https://doi.org/10.1007/s00253-018-9107-1

Zhu, M., Zhengfei G., and Feng W. (2013). An Overview of the US Tomato Industry. *The Florida Tomato Proceedings 1*(1) 28-30.

CHAPTER 2: PHENOTYPING RESISTANCE TO NON-RACE 1 VERTICILLIUM WILT IN TWO ADVANCED TOMATO BREEDING RIL POPULATIONS AND SELECTIONS WITHIN THE FIELD AND GREENHOUSE

SUMMARY

The fresh market Tomato (*Solanum lycopersicum)* industry in NC is valued at $30 million dollars (US Department of Agriculture, National Agricultural Statistics Service 2023). Total land use ranges between 2800 and 3000 acres (US Department of Agriculture, National Agricultural Statistics Service 2023). The majority of the fruit is grown in Henderson, Buncombe, Haywood, Macon, Polk, Waynesville, Rowan, and Rutherford counties of the state (US Department of Agriculture, National Agricultural Statistics Service 2023). Verticillium wilt caused by *Verticillium dahliae* has been a challenging disease for the tomato industry in NC. In this work, disease severity data was gathered across four locations and seasons, a morphological marker was identified, and an irregular strain of non-race one verticillium wilt (for North Carolina isolates at least) was uncovered.

INTRODUCTION

Verticillium Wilt Symptomatology

Verticillium is a late-season vascular disease that presents a characteristic “checkmark” on the lower leaves. (Fig. 2.1) This checkmark can also be associated with extreme drought stress, but given fields are irrigated in NC as well as high rainfall numbers, extreme drought stress is rarely if ever a concern. Verticillium is also associated with harsh yellowing starting from the “check” of browning and rapidly encompassing most or all of the leaf at symptom onset. It is also possible to cut the main growing stem at the base of the plant and observe browning of the vasculature. This method is destructive, disease severity information can also be obtained from disease severity index ratings based on foliage.

The development of a marketable, large, round-fruited tomato with Verticillium wilt resistance has been a challenge to breeding programs (Vermeulen et al., 2022). There could be a plethora of reasons for this. Vermeulen et al. (2022) proposed (although states that it is highly speculative) that the main reason for the scarcity of major resistance genes to *V. dahliae* could be due to *V. dahliae* being partially endophytic under natural conditions and sometimes in agronomic conditions. Within the field, Verticillium which is impacting contemporary tomato production does not appear to be fully endophytic and does show symptoms at the end of the season. Further, mono-cultured tomato fields can create optimal conditions for rapid pathogen evolution in response to control measures.

Verticillium dahliae **- The Pathogen**

Verticillium wilt is caused by numerous species of *Verticillium* but *Verticillium dahliae* is the primary species of concern for NC tomato growers. Contemporary isolates from NC tomato fields have all turned out to be non-race one *V. dahliae (*Vdn) (Ingram et al., 2020). Another factor impacting the focus on Vdn is that only vintage cultivars (pre-1980s or earlier) lack the *Ve1* gene that confers resistance to non-race one, meaning that non-race one is the most relevant to modern tomato production (personal communication, Reza Shekasteband and Randy Gardner). Resistance to non-race one verticillium has proven to be a difficult trait to discover and develop host resistance for because it seems to be a highly quantitative trait influenced significantly by environmental factors (Vermeulen et al., 2022, Usami et al., 2017). While some progress for possible diversity for resistance was made by Usami et al. (2017) in identifying a rootstock ‘Aibou’ which was shown to be resistant under greenhouse conditions, the resistance was not maintained under field conditions in Japan, and subsets of the non-race one verticillium population could overcome this resistance. Ingram et al. (2020) found that of the isolates collected in NC, all were not only non-race one, but all could break the rootstock's resistance. Oftentimes "race three" has been adopted as a delimiter for these resistance-breaking isolates, but within this manuscript, non-race one is used because one. Previous work indicates that "race three" likely comprises many races because the resistance-breaking component is due to a deletion rather than an insertion. two. While previous work indicates isolates obtained were likely of the "race three" character, it is possible there could be additional delineations in the near future, which would rapidly nullify this terminology, further increasing the complexity of the taxonomy of an already taxonomically complex genus and species.

The use of cultural practices including rootstocks like 'Aibou' have seen some success conferring partial resistance- or delay of onset of non-race one verticillium but the demand for additional tools such as a resistant large round-type tomato cultivar remains (Kubota et al., 2010). The options for non-field-based verticillium assays are generally limited to dipping seedlings in homogenized verticillium inoculum or injecting the inoculum into the soil. While these two options have continued to fill a large gap for alternatives to field-based pathology, and has been irreplaceable for gene dissection, as with many non-field-based approaches in plant breeding, can be a contentious topic when applied to pursue plant breeding objectives (see discussion section of chapter 2). Interestingly, the first application of root dipping was the basis for the original race one resistance, which was of a single dominant character (Schaible et al., 1951).

OBJECTIVES

1. *Characterize non-race one verticillium resistance within two replicated RIL mapping populations at F_5 and F_6 generations in field conditions.*

2. *Test for associations between Verticillium wilt resistance and an upright growth habit characteristic of individual parents*
3. *Characterize natural inoculum of verticillium wilt as it relates to applied plant breeding objectives through a combination of confirming the presence of known resistance genes to Race 1 in the population and employing a root dipping assay.*

MATERIAL AND METHODS

Origin of Populations

In 2014, a cross was made between two determinant large round fruited breeding lines ‘NC 6GEM’ and ‘NC 7GEM’, along with ‘NC 6GEM’ and ‘NC 9BS’. ‘NC 6GEM’ and ‘NC 7GEM’ both carry resistance to race one and non-race one Verticillium wilt and Bacterial spot. ‘NC 6GEM’ and ‘NC 7GEM’ both show symptoms of non-race one verticillium wilt at the end of the season. The resistances originate from two distinct sources (Gardner, Personal communication). ‘NC 9BS’ is susceptible to both bacterial spot, and non-race one verticillium wilt, but carries resistance to race one verticillium wilt. The ‘NC 6GEM’ breeding line is derived from selfing the ‘Mountain Fresh Plus’ hybrid (Gardner, 1999). The source of the resistance ‘NC 6GEM’ was confirmed based on continued field trials to be associated with ‘NC 109’, a parent of the original ‘Mountain Fresh’ Release (Gardner, 1999). ‘NC 109’ was the result of a cross between the University of California-Davis breeding line ‘T5’ and North Carolina State University breeding line ‘NC 50-7’ (Gardner, 1999, Gardner, 1982). While the resistance status of ‘T5’ is unknown, ‘NC 50-7’ did not appear to carry resistance to Vdn, leading to ‘T5’ being the likely source for ‘NC 7GEM’ resistance (Gardner, 1999, personal Communication, Randy Gardner). Shekasteband identified ‘Hawaii 7998’ as the likely wild source of Verticillium wilt resistance in ‘NC 7GEM’ (personal communication, Reza Shekasteband) In order to generate a RIL population, the lines within these populations were randomly selected in the F_2 generation and advanced to F_5 through a single seed descent scheme with selections within the field, as described in Brim (1966). Lines were advanced in greenhouses during the off-season and fields surrounding the Mountain Horticultural Crops Research and Extension Center (MHCREC) in Mills River, NC. All three parents of the population are known to carry the *Ve1* gene, which ensures that the symptoms observed are due to non-race one verticillium wilt. The presence of the Ve*1* locus was also confirmed by whole genome sequencing. ‘NC 6GEM’ is a determinant “large-round type” red tomato with plant height that is similar to most commercially employed determinant cultivars, falling in the range for ideal for staked cultural practices as defined by the Southeastern US Vegetable Crop Handbook (2023), standing at approximately 100 centimeters under good growing practices at the end of the season. ‘NC 6GEM’ has a typical growth habit for commercialized fresh market cultivars. ‘NC 7GEM’ is also determinant with large round red tomatoes, but ‘NC 7GEM’ has a particular plant structure that can best be described as upright. ‘NC 7GEM’ has slightly paler, yellow leaves than a typical tomato plant under all conditions (including optimal fertility), which can be observed under both field and greenhouse conditions. The upright structure of ‘NC 7GEM’ is clearly distinct from stress conditions, as has been observed for over a decade alongside plants under the same condition that do not show this phenotype. ‘NC 7GEM’ also tends to grow taller than typical tomato vertical growth rates but is determinant. In addition to carrying both race one and non-race one *V. dahliae* resistance, ‘NC 7GEM’ carries resistance to bacterial canker and bacterial spot. ‘NC 9BS’ is a high-yielding, determinant Vdn susceptible plant (although it carries resistance to race one *V. dahliae* through the *Ve* gene). ‘NC 9BS’ is also known to be susceptible to bacterial spot. (Randy Gardner, personal communication).

Plant Care and Maintenance

A recombinant inbred line population numbering two hundred and fifteen RILs and seventy-eight plant selections (increasing to ninety-three in the F_6 generation) resulting from a cross between ‘NC 6GEM’ x ‘NC 7GEM’ were planted. A recombinant inbred line population numbering two hundred and fourteen RILs and ten selections (increasing to twenty-four in the F_6 generation resulting from a cross between ‘NC 6GEM’ x ‘NC 9BS’ were planted as well. Both populations were grown in randomized complete block design trials in the four locations described in Table 2.1. Seeds were sown in soilless media with nutrients. Seedlings were grown in a heated, bilayered plastic hoop house in preparation for transplanting in late May. Seedlings were watered, fertilized, and transplanted following conventional grower practices. Per industry standards for the western NC region, Plants were grown under plasticulture, and fertigated through drip tape irrigation below the plastic. Plants were strung between wooden stakes to support the plants as they grew. Industry-standard practices for fertigation and foliar pesticide applications were followed for both locations in both years. The on-farm trials were fumigated with a broad-spectrum fumigant while the research station trials did not fumigate. Roots were sampled and inspected visually for root-knot nematodes by pulling up the plants at the end of the season in 2023.

Rating Non-Race 1 Verticillium Wilt

Best phytopathometric practices in estimating disease severity visually, as described in Bock (2022) were employed. Upon the first observed incidence of Verticillium symptoms, plants were rated from 0-12 on a categorical disease severity scale. Disease severity was rated as the proportion of the sampling unit (the plot) displaying yellowing and browning characteristics of

Verticillium. Where differences in plant height were observed, the proportion of the plot was maintained, for example, in taller plots there were greater distances between each rating. Because of the nature of Verticillium as a soil-borne vascular disease, categorical ratings must be taken indirectly based on the percent of the plot displaying the characteristic yellowing and browning of the leaves. Yellowing was treated as part of the symptomology and was seen to radiate from the browning symptoms at a similar distance in both locations. Where there were differences in plant height, proportional ratings were maintained. See (Fig. 2.3)

Rating Foliage Color and Growth Habit

The yellow-green phenotype attributable to `NC 7GEM` was scored visually in the field in the 2023 growing season. Scoring was taken as a binary boolean as middling observations were not found. The yellow-green phenotype tends to be subtle when viewed under full sun but can be clearly observed on cloudy days. The yellow-green phenotype can also be clearly seen in artificial white light. All foliage color data was gathered under cloudy conditions. Growth habits were collected under greenhouse conditions in the 2022 spring greenhouse season, in the 2022 summer field season, and in the 2023 summer growing season visually. Upright growth habit was characterized as plants with an acute growing angle that is less than 45°∠. Upright growth habit was also rated on a binary boolean scale because the trait also appeared to be binary with no middling observations.

Rating Earliness

Earliness data was gathered around 60 days after field transplanting. This was done visually by identifying the quantity of fruits on the plant, then of those, how many fruits were mature. The proportion of the fruits that were underripe, ripe, and overripe rated on a 0-5 scale with a rating of three being most fruits were ready to harvest but not overripe at 60 days. If between 50% and 25% were underripe at 60 days, it was scored as a two, if more than 50% but less than 75% were underripe it was scored a one, and if 75% or more were underripe at 60 days after planting it was scored a zero. If more than 25% but less than 50% were scored to be overripe that was considered to be a four, and if more than 50% were overripe that was considered a five.

Obtaining and Verifying Isolates from the Field

In the later stage of the 2023 season, numerous sections of plant main stem material were obtained randomly from both locations. Segments ~3.8 centimeters were cut from the main stem of mature plants and kept on ice. In the lab, a fresh cut was made to both ends of the stem under sterile conditions, and small pieces were then cut for further sterilization. The pieces were then washed with a 90% EtOH solution for 60 seconds. The fragments were transferred to one milliliter(mL) of 1% sodium hypochlorite (NaOCl) using sterilized forceps. After 60 seconds, the samples were rinsed with sterile distilled water three times, allowing the water to soak into the samples for 60 seconds each rinse. These samples were then dried on sterile filter paper and transferred to acidified potato dextrose agar plates (prepared using lactic acid, APDA).

After one-week, fungal cultures that had the appearance of *V. dahliae* were selected for further isolation. Samples were verified to be *Verticillium dahliae* through markers targeting housekeeping genes as described by Usami et al. (2007) and then confirmed to be non-race one by employing available markers described in De Jonge, et al. (2012) that ensured both samples were non-race one (Personal communication, Yeonyee Oh).

Root Dipping Assessment of Resistant and Susceptible Selections

From both the 'NC 6GEM' x 'NC 7GEM' population and 'NC 6GEM' x 'NC 9BS' population, Ten F_6 lines resulting appearing to be resistant in the field and ten F_6 lines appearing to be susceptible in the field across all four locations and two seasons were planted. Plants were grown in the winter of 2023 in a heated glasshouse, seedlings were prepared for inoculation in a heated hoop house with bilayered plastic. Parents 'NC 6GEM', 'NC 7GEM' and 'NC 9BS' were planted alongside them. After three weeks of growth, healthy plants were split into four replicates of four of each genotype. One replicate was left uninoculated and transplanted, while the other three replicates were inoculated as described in the fungal inoculation section of Oh et al. (2023). Fungal cultures were obtained from samples taken from both the on-farm trials and the research station trials, as described in the previous section. Plant height was taken three weeks after inoculation by measuring between the bottom of the topmost terminal bud and the soil line. The average of the four measurements were taken for each replicate and the uninoculated controls. Effect of inoculation on plant height was then obtained using the following formula:

$$\%\ change\ in\ plant\ height = \frac{uninoculated\ plant\ height\ \ -\ inoculated\ plant\ height}{uninoculated\ plant\ height}$$

Statistical Analyses

Statistical analyses and plotting were performed within an R environment, version 4.3.1 (R core team, 2023). To identify morphological markers as described by Chesnokov (2020) a one-way analysis of variance (ANOVA) was employed. Traits were modeled in the following fixed effects model:

$$Y_{ij} = \mu + \tau_i + e_{ij}$$

Where Y_{ij} is the observed Verticillium wilt score, μ is the mean value, τ_i is the effect of the phenotype being tested for association with verticillium (earliness for example) and e_{ij} is the random error term. Effects were estimated using linear regression, and Cohen's d was extracted:

$$d = \frac{difference\ between\ means}{pooled\ standard\ deviation}$$

Data tidying for analysis was completed using the R package 'Tidyverse' version 2.0.0 (Wickham et al. 2019). Plotting was completed by ggplot2(Wickham, 2016)

RESULTS

Phenotypic Assessment of Verticillium Wilt Disease Severity in the Field

Across two years and two locations, symptoms characteristic of a verticillium wilt infestation were scored on a disease severity index (DSI) and summary statistics were obtained for each population (Fig. 2.3, Tables 2.1-2.3). Symptoms were found extensively on plant material (Figs. 2.1-2.2). A rating scale from 0-12 where plants were rated on proportion of the plant height that was showing *V. dahliae* symptoms (Fig. 2.3). Varying levels of discoloration was observed in the vasculature of material when cut at the end of the season (Fig. 2.4). In the 215 F_5 and F_6 RILs descended from a cross between 'NC 6GEM' x 'NC 7GEM' material, a gradient of resistance/susceptibility approximating a normal distribution was observed in the on-farm and research station trials in the first season, and the on-farm trial in the second season (Fig. 2.5A-C). An approximate normal distribution of DSI scores was also observed in the on-farm and research station trials in the first season, and in one of the on-farm trials (2023) within the 215 F_5 and F_6 RILs descended from a cross between 'NC 6GEM' x 'NC 9BS' (Fig. 2.6A-C). A departure from the approximately normal distribution of DSI scores were observed in the second season on the research station trial in both populations, in the F_6 generation RILs (Figs. 2.5D and 2.6D).

Association Between Verticillium Resistance and Population-Specific Phenotypes

Variation in growth habit and leaf coloration unique to the RILs resulting from ‘NC 6GEM’ x ‘NC 7GEM’ were gathered to identify valuable breeding tools (Fig. 2.7). Both phenotypes appeared to be segregating within the material. In particular, upright growth habit could be observed starting from the first two cotyledons in the greenhouse to final observations late in the field season, across both locations and years (Fig. 2.7). Through a one-way analysis of variance, the upright growth habit was found to be significantly associated with a reduction in DSI scoring (P value of 0.02402). Cohen’s d was calculated to be 0.64, indicating somewhere between 38.2% and 43.0% non-overlap of verticillium wilt scores between groups of individuals with upright growth habit and those without. The effect the upright growth habit had on Vdn scoring was found to be a reduction of 1.151 in verticillium scoring on the categorical disease severity index through the same fixed linear model. Another one-way analysis of variance found a significant association (P value of 0.05) between pale yellow-green leaf types specific to ‘NC 7GEM’ and a fixed linear model identified a decrease in Verticillium wilt DSI scoring by 0.95 categorical disease ratings.

Earliness was also gathered as well as elucidate if the nature of the resistance to non-race one *V. dahliae* was independent of this critical trait. It was found that earliness was not significantly associated with a change in Vdn DSI scoring (P value 0.45).

Characterizing Impact of a Morphologically Unique Non-Race One *Verticillium dahliae* on RIL Populations

A morphologically unique isolate of non-race one *V. dahliae* was obtained in the second season, from the research station trial (2.5D, 2.6D). A sample from the second on-farm trial was also obtained, which was consistent with isolates previously obtained from NC. There was no significant difference (P value = 0.117) between the percentage reduction in plant height between the uninoculated and inoculated ‘NC 6GEM’ x ‘NC 7GEM’ resistant and susceptible extremes and those inoculated with the isolate of common NC appearance (isolate 1) and the irregular NC isolate (isolate 2) (Fig. 2.9), indicating that the differences between the inoculum did not alter the distributions. In the ‘NC 6GEM’ x ‘NC 7GEM’ susceptible and resistant extremes, there were significant (P value=.004) differences among the 20 taxa tested, but these differences did not appear to be associated with the extreme resistant and susceptible expectation found in the field. In the 20 lines selected from ‘NC 6GEM’ x ‘NC 9BS’ There was a significant (P value=0.04) increase in the difference in percent reduction in plant height between the uninoculated and inoculated ‘NC 6GEM’ x ‘NC 9BS’ selected lines when inoculated with the pigmented isolate (isolate 2) compared to the common appearance isolate (isolate 1) (Fig. 2.9). There was also a mildly significant association between the differences in the taxa and a percent reduction in plant height, but similarly to the previous population, phenotypic extremes were not consistent with what was observed in the field.

DISCUSSION

Replicated disease data identified highly resistant and susceptible lines within two RIL mapping populations in the first replicated assessment of this material (Fig. 2.1). This work fits into a larger effort to breed for resistance to non-race one Verticillium wilt, which has been a challenging pursuit that has recently increased in relevance because of the changing landscape of control measures available (Vermeulen et al. 2022, Usami et al. 2017, Okie and Gardner 1982a, Okie and Gardner 1982b). The phenotypic distributions indicate this trait is quantitative in character. The most likely scenario is that this trait is attributed to multiple genes, as observations have shown a gradient of resistance distributed across taxa which has been replicated in multiple locations and years. It also appears to be a trait of moderate to low heritability, although this could be better supported with replications at the same location. Repeated observations have found a number of highly resistant lines as well as overall distributions that lay the foundation for further assessments of these populations through association to genotypic data and in better understanding the genetic basis for this trait.

Darkening of the vasculature was confirmed to be present in the parental lines and a subset of resistant and susceptible lines, with resistant parents ‘NC 6GEM’ and ‘NC 7GEM’ showing less darkening of the vasculature(Fig. 2.4), In three of the four locations tested, the distribution of the two RIL populations Vdn observations appeared approximately normal(Figs. 2.5-2.6). This distribution indicates a single gene-for-gene interaction as Flor described is not the dominant force driving resistance in either population(Figs. 2.5-2.6). A polygenic basis is more likely given the repeated observations of resistant and susceptible material across years.

Environmental conditions for each of the four locations were identified (Table 2.1). Western NC is a temperate rainforest with semi-mountainous terrain. Soil appeared to be a mix of organic matter and primarily clay, with a smooth feel (indicating low sand content) in all four locations. Soil was transplanted into beds covered by plastic. The four locations selected represent two scenarios for growers, a scenario where fumigation is employed (the two on-farm locations) and a scenario where fumigation is not employed (the two research trial locations). All four fields are located in what is now USDA zone 7b as of 2023, changed from zone 7a in 2012. The plastic was white in 2023 and 2022 on-farm and 2023 research station trial locations, black in 2022 on-farm trial locations. The switch from black plastic to white plastic was not likely to make a large difference in the phenotype given the soil-borne nature of the disease. Among the two years and four locations, severe flooding did not occur but has historically occurred in the research farm. Late observations are taken in verticillium because the visual symptoms are typically very close to the end of the season. Ratings encompassed most of the DSI in all cases and were approximately centered (Table 2.2). A disease diagram was generated for Vdn (Fig. 2.3)

It is critical to confirm whether any resistance trait is associated with earliness. Earliness is a critical consideration for growers when selecting a cultivar to employ, so it follows it is important to consider when introgressing disease traits. Given the nature of verticillium as a disease based in robbing the shoot tissue of water (vascular wilting disease) it is necessary to consider factors impacting the water needs of shoot tissue. Plants that develop fruit early will likely have increased water needs in the entire season, whereas later plants are likely to have comparatively less water stress. A similar statement can be said of both marketable and unmarketable yield, which while not assessed formally in this work due to general similarity in parental yield and labor/financial limitations is an important consideration for future work on these populations. By establishing that Vdn resistance is not associated with differences in fruiting timing, one part of this complex water stress relationship can be removed as a variable in verticillium scoring, which also establishes no part of the resistance is based in mechanisms outside of simply putting fruit on later than its counterparts, which would detract from its application if true. Morphological markers have continued to be a valuable strategy to identify resistant material sooner than the resistance can be observed. Two morphological markers were identified in this work. A significant association was identified between non-race one verticillium wilt resistance and yellow-green leaves, with a p value of .05. Because the probability(*P*) value is so low ($p<.001$), it can be concluded that within population 1, upright growth habit can be confidently associated with improved resistance for non-race one verticillium wilt Fig. 2.7). Morphological markers continue to be employed to great effect in breeding programs globally, in spite of the availability of molecular markers(Chesnokov, 2020) This type of marker is even more effective in transplanted crops, as growth habits like this one can be observed without cost by growing the plant to cotyledon stage, with materials as ubiquitous as soil, sunlight, and a tray that could have hundreds of wells if desired. For a DNA marker, the breeder needs to grow the plants out anyway, sample, extract DNA with reagents, create a master mix using additional costly reagents and run on an expensive PCR machine. While most modern breeding labs have some access to these materials, it is far easier and cheaper to select phenotypically at the seedling stage. Both morphological markers could be used in combination with pedigree information from 'NC 7GEM' to identify at least one of the wild resistance sources if desired, although that information is not publicly available.

In the research station trial in 2023, more lines seemed to overperform (reduced DSI scores) in the 'NC 6GEM' x 'NC 7GEM' population, and more lines seemed to overperform in the 'NC 6GEM' x 'NC 9BS', which could be attributable to many factors including environmental considerations, differences in the natural *V. dahliae* inoculum response, and differences in the distribution of the natural Vdn inoculum. No major weather events, severe flooding of rows, or blatant deviations from normal weather conditions occurred in the 2023 season in either location, the same can be said for the 2022 season as well. A random sampling of twenty plots in the research station trial did not uncover any root galls characteristic of *Meloidogyne incognita* (Root-knot nematode), which is known to interact with Vdn infection (Çürük et al., 2009, Hasan and Khan., 1985). An overall summary of the observed natural inoculum for that environment was that the verticillium was far earlier than previous seasons. The Verticillium scores in the research station trial in 2023 seemed to generally be divergent with some resistant material being far better than the mean, and other resistant material performing much worse. A much greater variation in plant height was observed in this season, which could be a contributing factor to this even with proportional ratings. *V. dahliae* was observed much earlier in this season as well. In some cases,

A deviation from the disease distributions observed in previous trials occurred in 2023 at the research station trial when the disease severity index distribution collapsed, particularly in the case of 'NC 6GEM' x 'NC 7GEM' (Fig. 2.5). a small number of 'NC GEM' x 'NC 7GEM' lines overperformed while others performed worse than the previous year leading to a wider spread (Fig. 2.5). There also was a noticeable effect on the parents within these two plots. It is difficult to pinpoint with certainty the singular reason, but the pigmented *V. dahliae* isolate that was gathered in that location seems to be a strong possibility. given the isolate was significantly different and more aggressive in the pathology assay, it is postulated that this isolate is a more aggressive version of verticillium (Figs. 2.6,2.9). These factors led to a root dipping assay that identified a significant(P value 0.04) worsening of stunting symptoms in the included 'NC 6GEM' x 'NC 9BS' lines which were selected from the most resistant and susceptible lines(Fig. 2.9). Interestingly, this significant (P value= 0.12) worsening was not observed in 'NC 6GEM' x 'NC 7GEM' selected lines. Association between different taxa performance in the field and taxa performance in the inoculation study did not align well, leading to testing the samples as a group.

Further, this field also showed symptoms remarkably early, with disease progression continuing from the early onset faster. Within the on-farm trials, normality was maintained, and when isolates were obtained from the same location at the same time, no visual differences between the isolate found and the isolates originally obtained in NC were observed on plates (Fig. 2.8). While there are a great many unknowns that remain about this *V. dahliae* isolate, a few things have been observed. It should be made clear that this isolate is not *V. albo-atrum*, as confirmed by Dr. Yeonyee Oh using a PCR marker for *V. dahliae* and taxonomically- The darkness is not because of a dark mycelia that is characteristic of *V. albo-atrum* (Personal communication, Yeonyee Oh, Pegg and Brady, 2002). Another is that while pigmented *Verticillium* isolates have been found in various locations, no *V. dahliae* with this type of pigmentation has been found in NC.

The primary alternatives to phenotyping in the field have been colloquially known as ‘Root dipping’ and ‘Root Drenching’ referring to the delivery method of the inoculum. The originators of Root dipping, as well as a major contributor to the release of the original race one VW resistant plants, executed this assay in sandy loam soil under greenhouse conditions, on a monogenic based resistance (Shaible et al., 1951). This can be observed in the extreme phenotypic differences observed. The phenotypic differences observed do not appear to match a simple mendelian gene-for-gene interaction as Flor described(Figs. 2.5-2.6). Across the selected lines, a statistically significant negative % reduction on plant height was observed in those lines that were inoculated with the irregular pigmented isolate (Figs. 2.9). It should be noted, one outlier taxa performed slightly better than the uninoculated control which appeared to be because of a short water control, but all others performed worse as expected. Over one thousand plants were measured for change in plant height compared to the uninoculated control. In summary, the root dipping assay achieved the objective of informing on how the new isolate impacts the core objectives of gathering Vdn resistance disease severity data.

Significance

The identification of morphological markers represents an important step among other works towards a non-race one Vdn resistant tomato cultivar (Vermeulen et al 2022, Okie and Gardner 1982a, Okie and Gardner 1982b). Further, the emergence of an irregular isolate that was more aggressive on the plants certainly is a critical matter to be investigated further, there is definitely a great deal of more focused study remaining on that topic. Some of the breeding materials were identified as promising for the isolate if it becomes a larger issue. In summary, the contributions of the morphological markers, confirmation of resistance and susceptibility across two mapping populations, and assessments against a possible emerging problem fit into an expanding body of work on the topic of non-race one verticillium wilt resistance.

REFERENCES

Bertucci, M., Bilbo, T., Jennings, K., Meadows, I., Rodrigues, C., Walgenbach, J., & Wszelaki, A. (2023). Tomatoes (*Solanum lycopersicum*). *Southeastern U.S. 2023 Vegetable Crop handbook 1*. 110–115.

Brim, C. (1966). A modified pedigree method of selection in soyabeans. *Crop Science*. *6*, 220. Portico. https://doi.org/10.2135/cropsci1966.0011183x000600020041x

Bock, Clive H., Kuo-Szu Chiang, and Emerson M. Del Ponte. (2022). Plant Disease Severity Estimated Visually: A Century of Research, Best Practices, and Opportunities for Improving Methods and Practices to Maximize Accuracy. *Tropical Plant Pathology 47*(1) 25–42. https://doi.org/10.1007/s40858-021-00439-z.

Chesnokov, Y. V., Kosolapov, V. M., & Savchenko, I. V. (2020). Morphological genetic markers in plants. *Russian Journal of Genetics, 56*, 1406-1415.

Çürük, S., Dasgan, H. Y., Mansuroğlu, S., Kurt, Ş., Mazmanoğlu, M., Antaklı, Ö., & Tarla, G. (2009). Grafted eggplant yield, quality, and growth in infested soil with *Verticillium dahliae* and *Meloidogyne incognita*. *Pesquisa Agropecuária Brasileira, 44* (12), 1673–1681. https://doi.org/10.1590/s0100-204x2009001200017

de Jonge, R., van Esse, H. P., Maruthachalam, K., Bolton, M. D., Santhanam, P., Saber, M. K., Zhang, Z., Usami, T., Lievens, B., Subbarao, K. V., and Thomma, B. P. H. J. (2012). Tomato immune receptor *Ve1* recognizes effector of multiple fungal pathogens uncovered by genome and RNA sequencing. *Proceedings of the National Academy of Sciences*, *109*(13), 5110–5115. https://doi.org/10.1073/pnas.1119623109

Gardner, R.G. (1982) NC50-7 breeding line, 'Cherokee', and 'Mountain Pride' tomato. *HortScience 17*(1), 92–93. https://doi.org/10.21273/hortsci.17.1.92

Gardner, R. (1999). NC 109 Tomato Breeding Line: Mountain Fresh'F1 Hybrid." *HortScience 34*(5), 941–942. https://doi.org/10.21273/hortsci.34.5.941

Gardner, R. G., & Panthee, D. R. (2010). NC 1 CELBR and NC 2 CELBR: Early Blight and Late Blight-resistant Fresh Market Tomato Breeding Lines. *HortScience*, *45*(6), 975–976. https://doi.org/10.21273/hortsci.45.6.975

Hamer, H., McDaniel, J., Dorn, T., Edwards, M., Troxler, S., Smith, N. D., Teachey, S., Galiano, M., & Woodard, T. (2023) United States Department of Agriculture National Agricultural Statistics Service. *USDA.*

Hasan, A., & Khan, M. N. (1985). The effect of *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Verticillium dahliae* on the resistance of tomato to *Meloidogyne incognita*. *Nematologia Mediterranea 13*(2) 133-136.

Okie, W. R., & Gardner, R. G. (1982a). Breeding for Resistance to Verticillium dahliae Race 2 of Tomato in North Carolina. *J Am Soc Hortic Sci*, *107*(4), 552–555. https://doi.org/10.21273/JASHS.107.4.552

Okie, W. R., & Gardner, R. G. (1982b). *Screening Tomato Seedlings for Resistance to Verticillium Dahliae Races 1 and 2*. *Plant Disease, 66*(1), 34. https://doi.org/10.1094/pd-66-34

Pegg, G. F., & Brady, B. L. (Eds.). (2002). *Verticillium wilts*. https://doi.org/10.1079/9780851995298.0000

R Core Team 2023. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing,* Vienna, Austria. <https://www.R-project.org/>.

Usami, T., Ishigaki, S., Takashina, H., Matsubara, Y., and Amemiya, Y. 2007 Cloning of DNA fragments specific to the pathotype and race of Verticillium dahliae. *J. Gen. Plant Pathol. 73*(2), 89–95. https://doi.org/10.1007/s10327-006-0334-4

Vermeulen, J. P., Hanika, K., Thomma, B. P. H. J., Bai, Y., & Schouten, H. J. (2022). Scarcity of major resistance genes against Verticillium wilt caused by Verticillium dahliae. *Plant Breeding, 141*(5), 681–694. Portico. https://doi.org/10.1111/pbr.13046

Wickham H. (2016) ggplot2: Elegant Graphics for Data Analysis. *Springer cham 1*(2) 1-260.

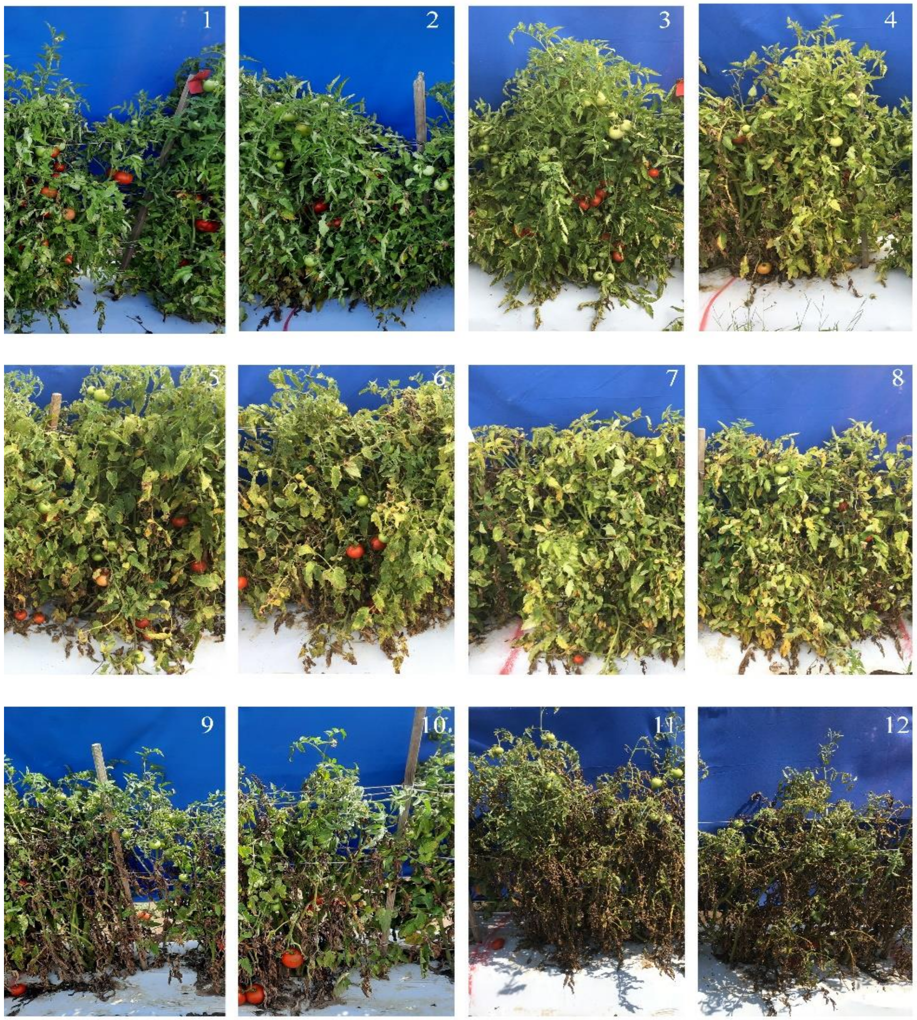
Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019). Welcome to the tidyverse. *Journal of Open-Source Software, 4*(43), 1686. doi:10.21105/joss.01686

Yigit, S., & Mendes, M. (2018). Which effect size measure is appropriate for one-way and two-way ANOVA models? : A Monte Carlo simulation study. *REVSTAT-Statistical Journal, 16*(3), 295-313.

FIGURES

Figure 2.1: Typical Verticillium symptoms are a "V" or checkmark shape of necrosis (browning) surrounded by chlorosis which precedes and surrounds necrosis symptoms (yellowing) on leaves (left) and leaflet (Right).

Figure 2.2: Typical early Verticillium symptoms *in vivo.*



Verticillium Disease Severity Index (DSI) scoring

Figure 2.3: Representation of Verticillium disease severity index (DSI) used to rate Verticillium wilt from a lack of visible symptoms (DSI=1) to plant death (DSI=12).

Figure 2.4: Discoloration and browning of vasculature attributable to Verticillium wilt in the main stem at the end of the season.

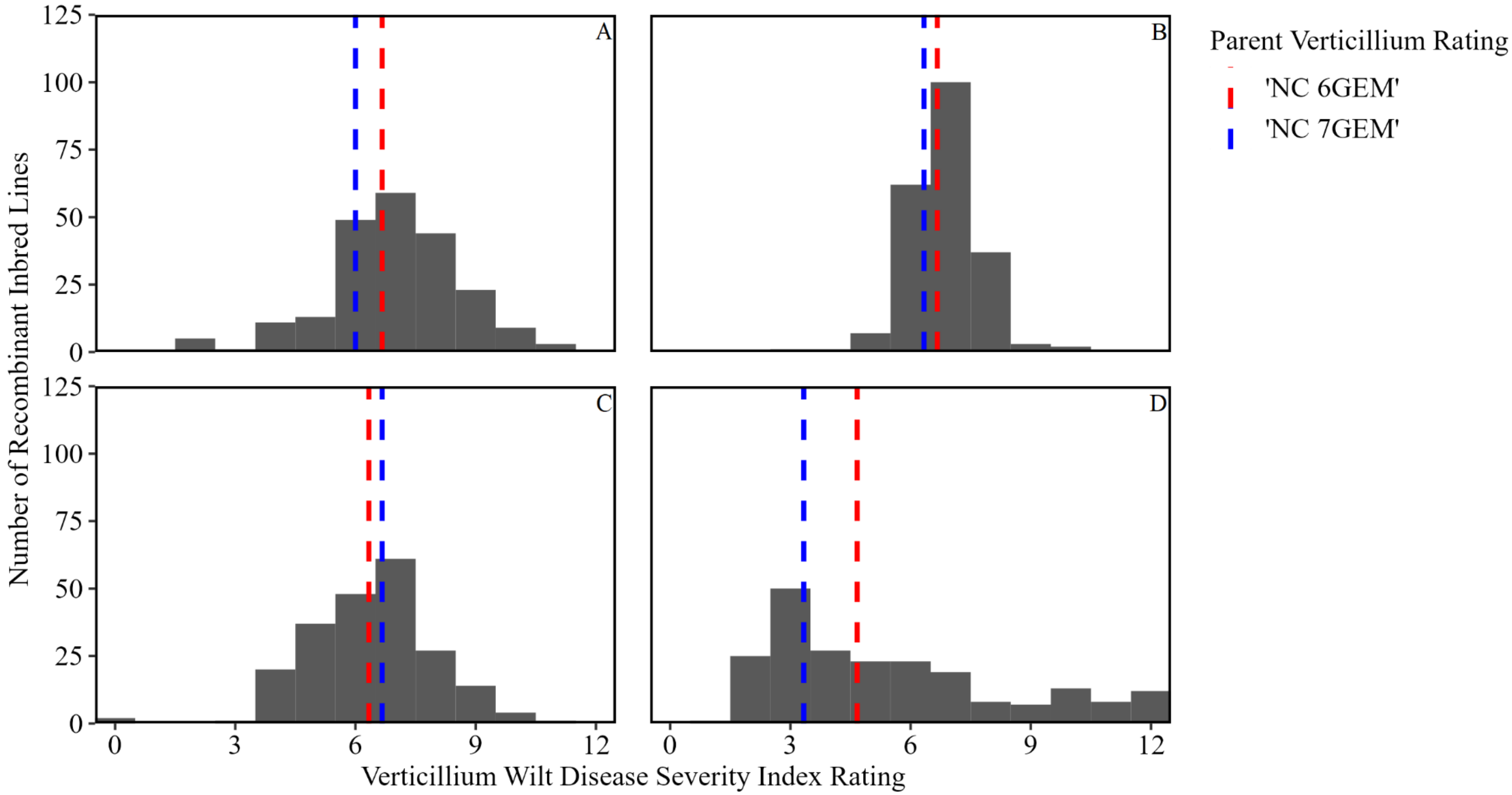


Figure 2.5: Final Verticillium wilt (VW) disease rating Distribution for ‘NC 6GEM’ x ‘NC 7GEM’ RIL population across two years in two locations. Figure A represents the distribution from the on-farm trial in 2022, figure B represents the distribution from the on-farm trial in 2023 figure C represents the distribution from the research station trial in 2022, and figure D represents the distribution from the research station trial in 2023. Red dashed lines indicate mean ‘NC6GEM’ VW score in the environment (Year x Location), Blue dashed lines indicate Mean ‘NC7GEM’ VW score in the environment.

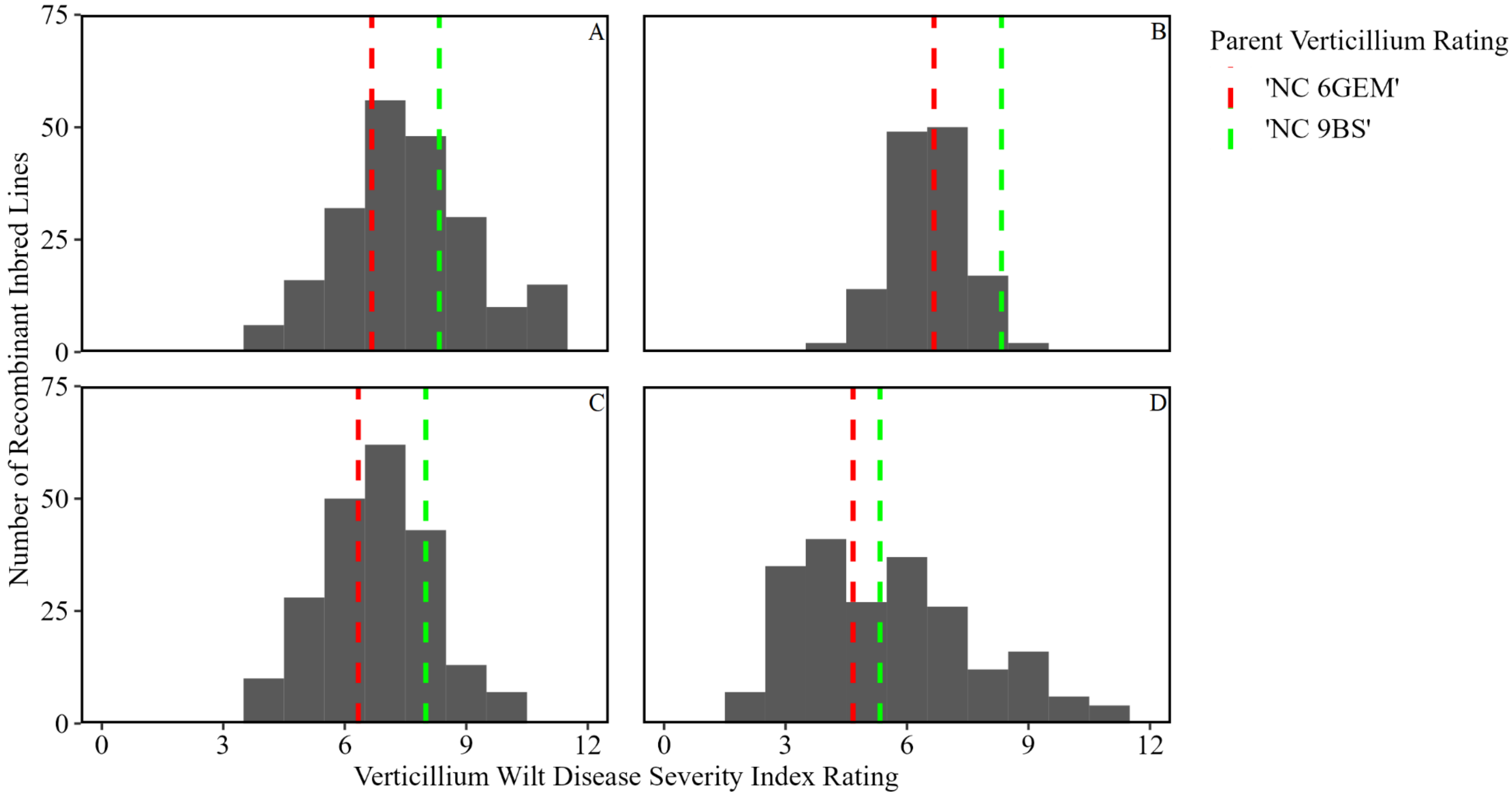


Figure 2.6: Final Verticillium disease rating Distribution for ‘NC 6GEM’ x ‘NC 9BS’ RIL population across two years in two locations. Figure A represents the distribution from the on-farm trial in 2022, figure B represents the distribution from the on-farm trial in 2023, figure C represents the distribution from the research station trial in 2022, and figure D represents the distribution from the research station trial in 2023. Red dashed lines indicate mean ‘NC6GEM’ score in the environment (Year x Location), green dashed lines indicate Mean ‘NC9BS’ score in the environment.

Figure 2.7: Examples of "upright" growth habit significantly associated with improvement of verticillium scores as well as one example of "yellow-green" leaf coloration. Prostrate (Figs. A, C, E, and G) and Upright (Figs. B, D, F, and H) growth habit across growth stages. Fig. G is an example of typical green leaf coloration before the onset of disease, and Fig H is an example of yellow-green phenotype before the onset of disease.

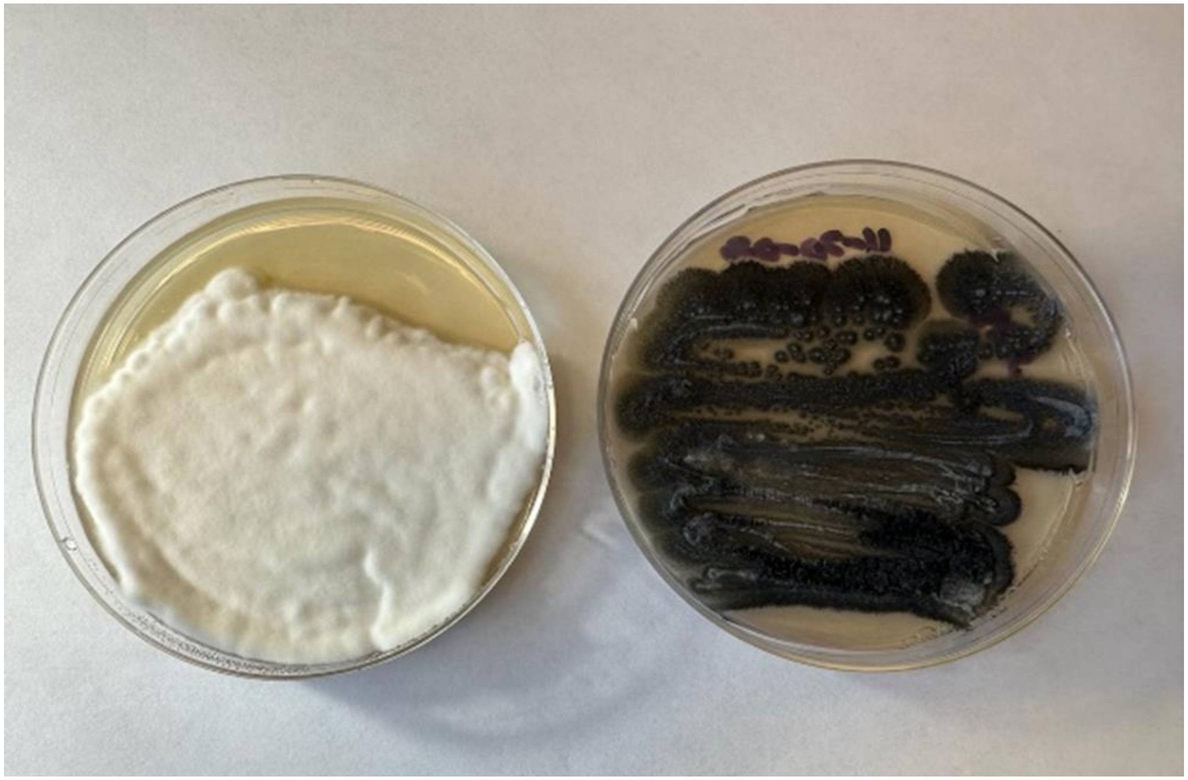


Figure 2.8: V. dahliae isolates obtained from the on-farm trial (left) Research station trial (right). V. dahliae species classification was confirmed by sequencing housekeeping genes (Personal communication Yeonyee Oh), and taxonomic assessment (Dark portion is not due to a dark mycelium as is described in *V. albo-atrum*). image credit: Dr. Yeonyee Oh

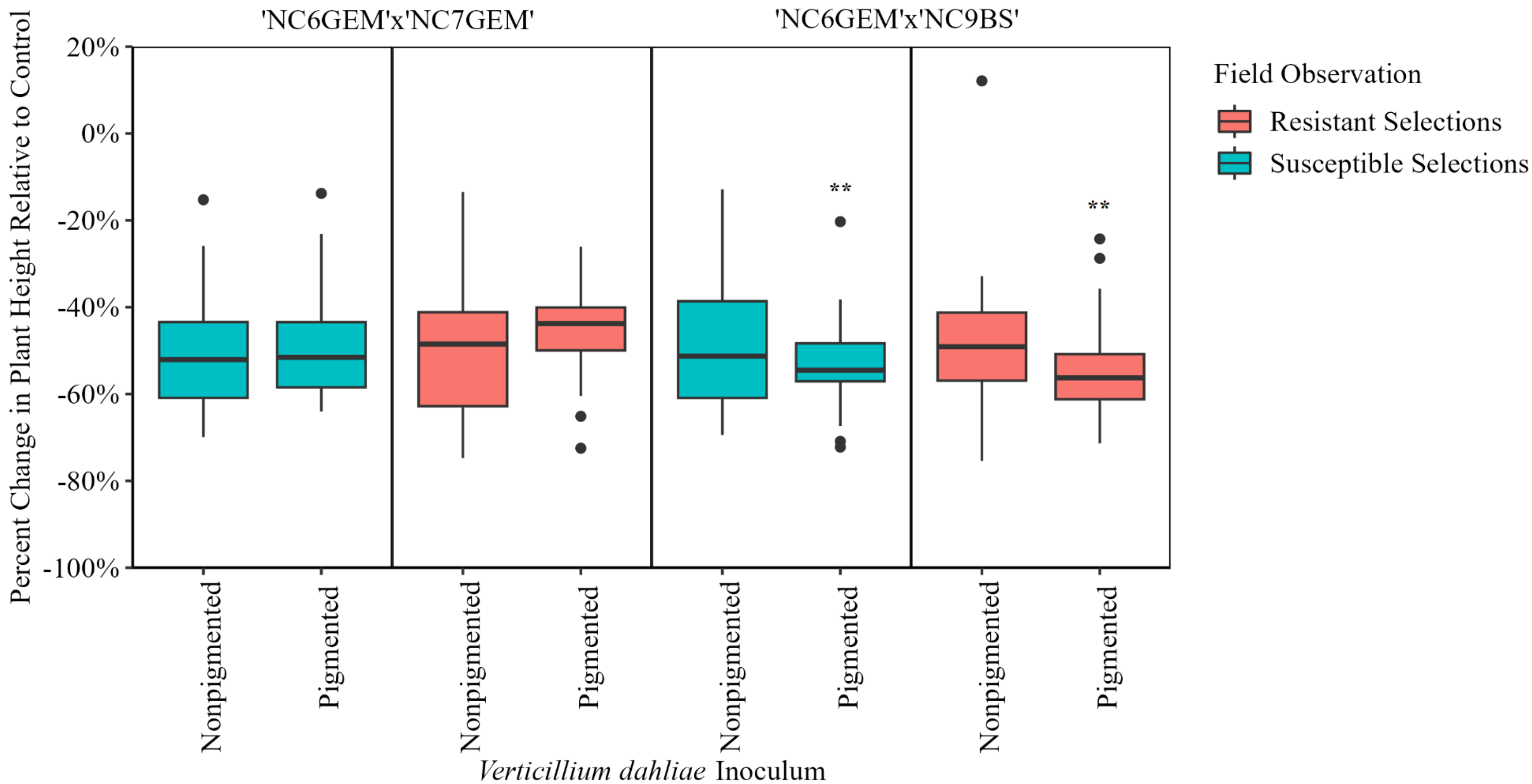


Figure 2.9: The x axis represents the percent change in plant height across 2 RIL mapping populations. The percent change in plant height is relative to uninoculated controls within 40 F_6 RILs in a greenhouse root dipping assessment. The ten susceptible(blue) and ten resistant(red) RILs were selected out of each population. Plants were inoculated with non-race one *V. dahliae* isolates without pigmentation, and a non-race one *V. dahliae* isolate with pigmentation found in the research station trial, previously unseen in North Carolina fields.

TABLES

Table 2.1: Locations of V. dahliae resistance trials.

Loc.[i]	Coord.[ii]	Elev.[iii]	Year	Notes
On-farm trial (2022)	35°23'38"N 82°33'32"W	630 m	2022	Fumigated with trichloronitromethane. Noted presence of root-knot nematode.
On-farm trial (2023)	35°23'55"N 82°34'15"W	637 m	2023	Fumigated with trichloronitromethane
Research Station trial (2022)	35°25'05"N 82°33'19"W	629 m	2022	Area historically known to flood. Field is unfumigated.
Research Station trial (2023)	35°25'06"N 82°33'20"W	628 m	2023	Field is unfumigated. ~10 meters distance from 2022 research station trial, no part or portion overlaps.

[i] Loc=location
[ii] Coord= coordinates
[iii] Elev= elevation

Table 2.2: Summary statistics for NC6GEM x NC 7GEM RIL population *V. dahliae* DSI scoring across 2 years and 2 locations in the F_5 and F_6 Generation.

Year	Loc.[i]	Min.[ii]	Q1[iii]	Mdn[iv]	Mn[v]	Q3[vi]	Max.[vii]
2022	On-farm trial	2	6	7	6.753	8	11
2022	Research station trial	0	5	6	6.263	7	11
2023	On-farm trial	3	6	7	6.840	7	10
2023	Research station trial	1	3	5	5.481	7	12

[i]Loc=location
[ii]Min=minimum
[iii] Q1=1st quartile
[iv]Mdn=median
[v]Mn=Mean
[vi]Q3=3rd quartile
[vii]Max=maximum

Table 2.3: Summary statistics for NC6GEM x NC 9BS RIL population *V. dahliae* DSI scoring across 2 years and 2 locations in the F_5 and F_6 Generation.

Year	Loc.[t]	Min.[u]	Q1[v]	Mdn[w]	Mn[x]	Q3[y]	Max.[z]
2022	On-farm trial	4	6.750	7	7.558	9	11
2022	Research station trial	4	6	7	6.763	8	10
2023	On-farm trial	4	6	7	6.537	7	9
2023	Research station trial	2	4	5	5.519	7	11

[i]Loc=location
[ii]Min=minimum
[iii] Q1=1st quartile
[iv]Mdn=median
[v]Mn=Mean
[vi]Q3=3rd quartile
[vii]Max=maximum

CHAPTER 3:MARKER-TRAIT ASSOCIATION ANALYSIS OF NON-RACE 1 VERTICILLIUM WILT RESISTANCE IN TOMATOES USING A COLLECTION OF THE MOST RESISTANT AND SUSCEPTIBLE RILS UNDER SINGLE PRIMER ENRICHMENT TECHNOLOGY (SPET)

INTRODUCTION

Tomatoes (*Solanum lycopersicum)* represent the world's most valuable vegetable crop grossing $102 billion US dollars globally and $10.52 billion US dollars nationally (FAO, 2022). North Carolina alone accounts for $30 million US dollars of that $10.52 billion. North Carolina is entirely based on the fresh market, so oftentimes the profit margin is much higher, costs are higher, and scale is smaller than processing tomatoes. Growers land use in NC fluctuates between 2800 and 3000 acres concentrated in the western part of the state. Large-fruited cultivars are the primary industry in NC, with grape and cherry-type tomatoes being the second most common and a small proportion of tomatoes are heirloom-type shapes. For commercial tomatoes, it is rare to see fruit colors other than red in commercial growing operations. Field tomatoes are grown strung between stakes in NC. The vast majority of growers use plastic mulch and drip irrigation. The majority of tomato growing operations are conventionally grown, with a small number of organic producers in the state. Host based disease resistance represents one of the most important control measures available to growers in NC.

Verticillium wilt presents itself as a characteristic “V” or checkmark on the margins of the leaves. Radiating out from this check mark, are yellowing symptoms that advance first before browning occurs. The browning slowly consumes the lower leaves first and progresses up the plant. The symptoms can be analogous to drought stress, but the symptoms will present even under irrigated conditions with no water stress. Within thick enough stem material late enough in infection, browning of vascular tissue can be observed. As a disease, Verticillium has three races, but delimiting race two and race three in the field is not possible. The *V. dalhiae* currently present in NC, is highly likely to be race three based on testing done by Ingram et al. which indicated all isolates gathered from NC could break the resistance of the 'Aibou' rootstock as of 2020(Ingram et al., 2020). Further evidence of this could be attributed to unpublished data showing the rootstock in NC was not able to maintain its resistance in two locations (both on-farm and research station trial locations in 2023) described in this manuscript. The delimiter between race two and three was found using the rootstock 'Aibou' under greenhouse inoculation conditions and did not hold its resistance in the fields they tested (Usami et al., 2017). Similarly, all races found in NC tomato fields have been *V. dahliae*. Race one resistance developed in 1952 is conferred by the *Ve* gene, which is present within all modern commercially grown varieties and breeding lines (Schaible et al., 1951, Kawchuk et al., 2001). Within this manuscript, "non-race one" will be used to denote these two races although it is very likely race 3 based on what has been obtained in NC thus far. As an area of study, Verticillium wilt assessment can be done in three ways- field-based strategies using native inoculum (due to limitations and preferences not to inoculate fields with VW given its long dormancy period), inoculation in the greenhouse in root dipping/drenching assays, inoculation in the lab using root dipping/drenching assays. This limitation on the available approaches has proven difficult to identify non-race one *V. dahliae* resistance genes (Vermeulen et al., 2022).

OBJECTIVES

1. *Utilize previously obtained Allegro targeted genotyping information (Shekasteband, unpublished data) to identify QTLs with significant associations with resistance to non-race one V. dahliae.*
2. *Perform a single marker analysis on significant QTLs identified.*

3. *Generate effect sizes for each associated loci to establish the biological relevance.*

MATERIAL AND METHODS

Genotypic Data

The genotyping by sequencing dataset of a collection of RILs in the F_4 generation was generated prior to the beginning of this work (Shekasteband, unpublished data, Personal communication, Reza Shekasteband). The whole-genome sequencing data of the three parental lines associated with the two mapping populations in this study was also generated previously (unpublished data, Reza Shekasteband, Personal communication, Reza Shekasteband). The genotyping by sequencing dataset was generated utilizing single primer enrichment technology (SPET) and the allegro Targeted Genotyping by Sequencing (TGBS) platform (Scaglione, 2019, Tecan Group, 2021). VW disease evaluation data of a non-replicated field trial at MHCREC, Mills River, NC in 2021 were used to select the collection of 43 RILs with two extreme VW disease phenotypes for the targeted GBS approach (Personal communication, Reza Shekasteband). Five samples were filtered out from the dataset due to low sequencing coverage. The SNP calls from 38 RILs (19 samples from each mapping population) were kept and filtered by removing SNP calls not supported by parental WGS data or a minimum of 10x sequencing coverage per sample (unpublished data, Reza Shekasteband, Personal communication, Reza Shekasteband). After the filtering process, GBS dataset contained 71,780 and 49,094 SNPs from (NC 6GEM x NC 7GEM) and (NC 6GEM x NC 9BS) mapping populations, respectively. Phenotypic data from four replicated field trials were generated during this study in the summer of 2022 and 2023 and were used for the marker-trait association study.

The Targeted GBS datasets were generated using a single plant per line from F_4 progeny (Personal communication, Reza Shekasteband). Phenotypic data, however, were generated using replicated trials of F_5 and F_6 progenies in the following years. Furthermore, additional F_4- and F_5-derived RILs were selected and included in the replicated field trials. To account for possible residual heterozygosity of the selected F_4 plants, the disease severity index of 10-12 plants measured in three replicates as well as F_4- and F_5-derived sister lines was averaged into a single disease severity score and used for the marker-trait association study through a general linear model approach (Personal communication, Reza Shekasteband). Linked markers were filtered using WGS data obtained from the parents.

Marker Trait Association Analysis

A general linear model (GLM) through TASSEL v.5.0 (Bradbury et al, 2007) was employed to study marker trait associations in this study.

The model used was a fixed effects linear model:

$$Y \sim X_s \beta_s + e$$

where Y is a vector of phenotypic estimates, X_s is a vector of genotypic values for the SNP read at locus s, β_s is the fixed effect (also known as SNP effect) size at locus s, and e is a random effect of residual errors, which were assumed to be normally distributed with a mean of zero and a constant variance, represented as $e = N(0,\sigma^2,\boldsymbol{I})$. Here, σ^2 denotes the residual variance, and **I** is the identity matrix, indicating that the residuals are uncorrelated and homoscedastic (Uffelmann et al., 2021). It is important to note, from Uffelmann et al., (2021), the kinship or a Q matrix was not used due to the pre-defined kinship structure of the dataset (Uffelmann et al., 2021).

Transformation of Phenotypic Data

Given the selection of phenotypic extremes from the F_4 generation, lines that performed better than the mean for that line (IE a lower DSI score than the rest of the plants in that plot) were selected, which were then averaged into the randomly selected RIL for those lines (Personal communication, Reza Shekasteband). The average was taken from two reps as well as advancement material.

Height Data

Height data was gathered by pulling the plant to tension and using a meter stick to take the height of the plant from the soil level. Plants were measured by lightly placing a meter stick on ground level next to the main stem. The plant was gently pulled so the full plant height unsupported by strings could be measured. Measurements were taken from ground level at the main stem to the bottom of the terminal bud. Each of the four plants within each plot were measured individually then an average was taken for the four plants. Plant height was taken forty-five days after planting in 2022. A second measurement was taken in 2023 seventy-six days after planting, with the latter including replicated and across both seasons, Plant height was only collected for the ‘NC 6GEM’ x ‘NC 7GEM’ population each year in the on-farm Trial locations.

Estimating Effects of Associated Loci

After completing an analysis, it is critical to estimate the effect on the Verticillium wilt phenotype that the SNPs identified as significantly associated have. The reasons for this are twofold: for one, it establishes biological relevance of those SNPs, and it can inform breeding decisions. The model used to assess the impact of a single marker using a reduced best linear unbiased predictor (BLUP) is:

$$Y_{\mathrm{j}} \sim \mu + \beta M_{\mathrm{j}} + e$$

where Y_j is the observed verticillium wilt score for the j-th individual, μ is the overall mean, β is the additive SNP effect, M_j is the genotypes of the significant SNP genotypes at locus j, and e is the residual error. This model allows us to quantify the effect of the significant marker on the trait by obtaining the Best Linear Unbiased Estimates (BLUEs) β provides an estimate of how much the trait value changes with each additional copy of the minor allele at the marker locus (Balding, 2006).

Statistical Analyses

Statistical analyses and plotting were performed within an R environment, version 4.3.1 (R core team, 2023). Duncan new multiple range test was completed using Agricolae version 1.3-6(De Mendiburu, 2009). Tidying of data for the phenotypic analyses was completed using Tidyverse version 2.0.0 (Wickham et al., 2019). Bar and boxplots were plotted by ggplot2 (Wickham, 2016). Spearman's rank correlation coefficient was employed using the "cor" command in Tidyverse to calculate the correlation between the four locations and was plotted with "corrR" package and command (Kuhn and Jackson, 2016).

RESULTS

The objective of this work was to identify single nucleotide polymorphisms (SNPs) significantly associated with the observed resistance to *V. dahliae* by harnessing genotypic data gathered from two tomato RIL populations and selections. The key finding of this section is a number of SNPs were identified to have P values less than 0.001, which has been shown to be biologically useful in previous work completed by tomato breeding programs, including work predating the completion of the tomato genome (See QTL scan in Genetic data analysis Sierra-Orzoco et al., 2021, Agrama and Scott, 2006). The lines descended from the ‘NC 6GEM’ x ‘NC 7GEM’ (population 1) F_4 selections were included in a broader replicated assessment under randomized complete block design applied to the entire population. For the subset of lines included in the marker trait association, the DSI scores of the RILs and direct sibling material was averaged across replicates (Figs. 3.1-3.4). The same was completed for ‘NC 6GEM’ x ‘NC 9BS’ (population 2) selections (Figs. 3.5, 3.8). Four analyses of variance for the DSI scoring and the differences between the 19 lines indicated differences in taxa had a significant effect in ‘NC 6GEM’ x ‘NC 7GEM’ in the on-farm trial in 2022 and research station trial in 23, all others were not significant. Analyses of variance between Vdn DSI scoring against genotypes found that all locations and years were significant apart from the research station trial in 2022 for population 2. Duncan’s multiple range test was completed to assess the significant breakpoints of groups within the genotypes resulting from population one, and a number of groups emerged. Two individuals were significantly different from part of the broader group, RIL 210 and 202 in the research station trial in 2022 were identified in population 1, but this observation was not repeated in other years(Figs.3.1). For population 1 at the on-farm trial in 2023, some statistically significant minor differences were observed in these lines (Fig. 3.2). No significant differences were found in population one at the research station trial in 2022 (Fig. 3.3). For population 1 at the research station trial in 2023, some grouping occurred but with much higher variability within the lines (Fig. 3.4). Duncan’s new multiple range test was completed for progeny resulting from population 2 and no lines had significant differences from the majority of lines, with being grouped together and significant differences found between large groups encompassing the small number of lines (Figs. 3.5-3.8)

Spearman's rank coefficients were calculated, and a similarity matrix was generated for population 1 (Fig. 3.9). Based on the interpretation as described in Schober et al. (2018), the on-farm trial was moderately correlated across years, but all other possible combinations for population one was weakly correlated. The research station trial in 2023 was inversely weakly correlated to both on-farm trial years (Fig. 3.9). In the second population, moderate positive correlations were found between the on-farm trial years, the research station years, and the research station in 2022 and the on-farm trial in 2023 (Fig. 3.10). All other correlations were positive but weak in population two. Height observations were taken for lines descended from the targeted genotyping by sequencing material and RIL lines were averaged with sibling lines as part of a larger assessment. Replicated height measurements of the entire population were taken later in season 2 on the on-farm trial, those descended from the F_4 selections were averaged with the siblings.

The distribution of the previously obtained Targeted Genotyping data was plotted for both F4 populations, indicating large fluctuations in SNP reads between chromosomes (Figs. 3.11 and 3.12). A general linear model (GLM) approach resulted in four marker trait associations with p values less than 0.001 in both populations (Figs. 3.13 and 3.15, See QTL scan in genetic data analysis Sierra-Orzoco et al., 2021, Agrama and Scott, 2006, Personal communication, Reza Shekasteband). For the first population, a quantile-quantile plot showed how P values were expected to perform under a null model and how the observed P values were distributed in relation to the null model (Fig. 3.14A-D). Observed P values tended to depart from the null model N(0,1) beginning at the origin of the plot (0,0), with a number p values showing a slight negative (particularly in the case of the research station trial in 2023), sometimes meandering trend in association signal that indicates P values are systemically mildly enriched or unenriched compared to the null model (Fig. 3.14A-D). Some stratification was observed in population 1 p values, causing p values to be repeated forming short stretches of loci carrying the same information as can be seen in the horizontal lines represented in each quantile-quantile plot (Fig. 3.15A-D). While some stratification can be observed in the population 2 quantile-quantile plots, the stratification was not considered severe enough to invalidate the analysis(Fig. 3.16, Personal communication, Reza Shekasteband)

In assessment of the F_4 genotype against the progeny resulting in 2022, a clear peak emerged with a top SNP on the short arm of chromosome 6, with the additive effect being 1.091 (Fig. 3.13A and

Table **3.1**). The SNP appeared to show the greatest reduction in the year and location it was identified from when lines carried the allele from ‘NC 7GEM’ but showed a mild reduction in 2023 and 2024 in the median. In population 2 during the same year, the top SNP had an additive effect of 1.203, but was located in the centromeric region of chromosome 6 (Fig. 3.4 and Table 3.3). Several of the most significant SNPs had moderate effects ranging from 0.550-0.937 that account for a small proportion of the variance (Tables 3.1-3.4) Population 1 and population 2 on the research station trial in season 2, which notably observed deviated from the expected phenotypic distribution indicated high effects for the most significant SNPs-1.291 and 1.768 respectively (Tables 3.2 and 3.4). Top loci identified in population 1 in the on-farm trial in 2023 (8.36 Megabasepairs on chromosome 4) was consistent with repeated improvement of disease scoring by biologically relevant quantities, indicating the loci is likely the most promising for associated loci coming from this assessment (Fig. 3.18). Loci identified in the on-farm trial in

2023 showed DSI scoring consistent with an improvement of DSI scores across locations and years (Fig. 3.19) similarly, markers identified from the research station trial appeared to be reducing DSI scores across locations, although only slightly in the on-farm trial in 2023 (Figs. 3.20-3.21)

DISCUSSION

The non-race one Verticillium wilt (Vdn) disease severity index(DSI) scores replicated F_5 and F_6 resulting from the F_4 selection of ‘NC 6GEM’ x ‘NC 7GEM’ (population 1) maintained some consistency for the selections when accounting for environmental variance through averaging replications (Figs. 3.1-3.4). Population 1 Vdn DSI appeared to have more variability in the research station trial than the on-farm trial. Both were true for F_4 selections from ‘NC 6GEM’ x ‘NC 9BS’ (population 2) (Figs. 3.5-3.8). Given the breakdown in normality in the 2023 research station, it is possible that the basis for Vdn scoring could be different in the form of a different race or strain of Vdn- which is reported to be encompassing multiple races beyond race one and race two by Ingram et al in western NC (2020). An inverse weak correlation found between the research station trial and both on m trial years in the first population aligns with the observation that all lines including susceptible lines tended to perform better in population 1 in terms of proportional ratings, but plant height appeared unusually high in variability among both populations in 2023 (Fig. 3.9) the same combinations were weakly positively correlated in the second season. It is critical to note that both plots represent a subset of the population which, while originally resistant and susceptible selections in 2021 based on initial data, the replicated disease trials have found a gradient of Vdn DSI scores rarely strayed far from DSI= 6 (Figs. 3.1-3.8)

Plant height data was gathered for the individuals within the marker trait association study in both seasons on the on-farm trial for population 1 only (Fig. 2.9). Population one segregates for height, as ‘NC 7GEM’ is taller (but still determinant) than ‘NC 6GEM’. One symptom of Vdn infection is stunting, which, coupled with the above segregation, is why ratings were taken as a proportion of the plant (Pegg and Brady, 2002). This combination of symptoms and phenotype in a natural inoculum scenario with environmental considerations means that plant height is difficult to use as a direct phenotypic value but was carefully considered in the gathering of DSI scores.

In population 1, few of the individual DSI scores had significant differences from the main group, which could be explained because the two parents both had moderate resistance to Vdn, (Figs. 3.1-3.4). A number of individuals were significantly different from the main group of F_5 and F_6 lines(Figs. 3.5-3.8) The increased number of significant differences between the two was logical because the population included a moderately resistant parent and a susceptible parent, allowing for a wider range of phenotypes that are more easily selected for and representative of susceptibility.

Correlation matrices of Vdn scoring found moderate to weak association between year x location combinations, indicating a major environmental component that is a critical consideration(Figs. 3.9 and 3.10). Wherever possible, environmental variance was combated with experimental design decisions, such as rating proportional to plant height. While variability in plant height was observed in population one, given the proportional basis the verticillium scoring, this should not impact scoring downstream. In all locations except for the research trial in 2023, plant height in population two was generally similar across lines, but plant height data was not gathered for these locations because their status as unfumigated fields raised concerns about lurking factors like root knot nematode infestations, although not observed after the final season was completed. The gathering of plant height for the research station trial location in the future, while labor intensive could yield dividends given the knowledge that something is causing stunting there, although determining if it was simple environmental factors or a difference in *V. dahliae* is yet unknown.

A dense genotypic dataset obtained in years prior was obtained using WGS data of parental lines to identify polymorphic sites as targets for targeted genotyping by sequencing(Figs. 3.11 and 3.12). A number of chromosomes contained a few polymorphic SNPs while others were densely packed in both targeted genotyping by sequencing (TGBS) datasets. The reason for this is likely due to the polymorphic markers being unevenly distributed on the parental lines- which single primers were designed around as targets (personal communication, Reza Shekasteband, Figs. 3.11 and 3.12). This aligns with findings of low genetic diversity in breeding lines in a crop with low recombination like tomato, leading to large regions of identical loci, especially in the same market class (Sim et al., 2012). The shared parent 'NC 6GEM' allows for some interesting interpolation, for example it appears that chromosome six on 'NC 6GEM' is unique compared to the other two parents. 'NC 7GEM' differs from 'NC 6GEM' in a large part of chromosomes 2 and 11. This must be assessed in context with the phenotypic values, just because a large portion of SNPs are present within a chromosome does not mean that those loci are necessarily associated with any meaningful phenotype on its own.

A multitude of statistically significant, biologically relevant SNPs were found in this study for a much needed host plant resistance within the tomato industry (Figs. 3.13-3.16).In this work, SNPs with probability values below 0.001 were considered to be significant, of which a multitude were, with effects ranging from 0.49-1.77 DSI points (Tables 3.1-3.4).

Effects found are relative to the 12-point disease severity index. A major peak emerged when assessing population 1 against the DSI scores at chromosome 6 (Fig. 3.13A) The top SNP was located on the short arm of chromosome six, but not close to where resistance to *Meloidogyne incognita* (root knot nematode, or RKN) is segregating in the populations (Messenguer et al. 1991).If the locus was closer, This would be an important consideration, as RKN has known interaction with Vdn infection, and was not rated in this season. Interpersonal communication with the grower later on indicated that there were suspected nematode problems with the field (Çürük et al 2009, Hasan and Khan 1985). Repeated identification of significant loci on chromosomes two and four indicate those may be highly relevant in population 1 in relation to reduced DSI scoring (Fig 3.13). The SNP p values were deflated (lower observed values than expectation of normality), which is likely due to small sample sizes that may be insufficient for QTL identification (Collard et al. 2005, Fig. 3.14). Further, this constraint also limits the possible combinations of SNPs, which leads to stratification as can be observed in both the Manhattan plots and QQ plots for population 2 (Figs. 3.15 and 3.16). The SNPs with the lowest p values but the highest effects were prioritized as valuable loci to assess, or SNPs of interest hereafter denoted SNPoi. The SNPoi identified from ‘NC 6GEM’ x ‘NC 7GEM’ in the on-farm trial appeared to be consistent with the expectation that the addition of a relevant locus would reduce DSI scoring in all cases but in the research station trial in 2023(Fig. 3.17). While this could be promising, the difference did not appear to cause large departures in any trial but the trial which the SNP was identified in. SNPoi identified from population 1 in the on-farm trial during 2023 appeared to be consistent across all locations and years indicating this may be a valuable candidate marker, albeit for a reduction of ~1 on the DSI scale(Fig. 3.18). The SNPoi identified in the on-farm trial in 2023, and both years of the research station trial all show the best promise as candidate markers, with a strong trend of consistent biologically relevant changes to Vdn DSI scoring (Figs. 3.19-3.21). This consistency even carried over into the research station trial in 2023 which carried the aforementioned uncertainty. It is likely that the inclusion of a susceptible parent enabled far better resolution and better loci to identify large differences in Vdn scoring. While effects could be larger, if the right combination of these three markers are employed, a large effect could be attained.

Significance

Given the importance of this disease, and the complete lack of tools available to plant breeders to select for resistance to this disease, these loci coupled with the morphological markers (see chapter 2) and gathering of Vdn DSI values for this population could represent a crucial step in breeding for verticillium wilt resistance. Breeding for new resistance has always been hard, breeding for resistance to soil borne pathogens is notoriously even harder, and -for tomatoes- breeding for verticillium wilt resistance has been next to impossible for the past 73 years. One other important finding to consider in tandem with this data was the emergence of an irregular strain of *V. dahliae* that was initially observed last season on the research station trial to be earlier and more aggressive, which has never been seen in NC isolates. This was highly unusual but could be valuable in a number of possible scenarios. Given the new pigmented isolate in NC was confirmed to be more aggressive in a controlled experiment, and an expectation of multiple races of unknown character encompassing non-race one, a scenario where this more aggressive isolate becomes more problematic appears to be somewhat likely but far from confirmed. It is possible that this is an isolated incident, and perhaps this isolate remains rare in horticultural cropping systems, limited by transmission or by inferior fitness compared to other races. In the former, this work could serve as a head start on that, and a glimmer of hope because of the resistant material that scored at or better than previous years. The amplification of markers on chromosome 11 in 2023 at the research station trial in population 1 contrasted with the other years, these loci might be worth investigating if there comes a day when resistance to this more aggressive isolate is needed(Fig. 3.13D) and fortunately, advanced material is available in the form of this population. If the more common isolate continues to dominate as expected, the identified SNPs in both populations in the other three environments would serve to fill this need for tools to breed for resistance within this population.

REFERENCES

Agrama, H. A., & Scott, J. W. (2006). Quantitative trait loci for tomato yellow leaf curl virus and tomato mottle virus resistance in tomato. *Journal of the American Society for Horticultural Science, 131*(2), 267-272. https://doi.org/10.21273/jashs.131.2.267

Balding, D. J. (2006). A tutorial on statistical methods for population association studies. *Nature Reviews Genetics*, *7*(10), 781-791. https://doi.org/10.1038/nrg1916

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*(19), 2633–2635. https://doi.org/10.1093/bioinformatics/btm308

Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., & Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping, and marker-assisted selection for crop improvement: The basic concepts. *Euphytica*, *142*(1), 169–196. https://doi.org/10.1007/s10681-005-1681-5

Çürük, S., Dasgan, H. Y., Mansuroğlu, S., Kurt, Ş., Mazmanoğlu, M., Antaklı, Ö., & Tarla, G. (2009). Grafted eggplant yield, quality, and growth in infested soil with *Verticillium dahliae* and *Meloidogyne incognita*. *Pesquisa Agropecuária Brasileira, 44* (12), 1673–1681. https://doi.org/10.1590/s0100-204x2009001200017

De Mendiburu, F. (2009). *Una herramienta de análisis estadístico para la investigación agrícola* [Thesis]. Universidad Nacional de Ingeniería (UNI-PERU), Universidad Nacional Agraria La Molina, Lima-PERU.

Hasan, A., & Khan, M. N. (1985). The effect of *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Verticillium dahliae* on the resistance of tomato to *Meloidogyne incognita*. *Nematologia Mediterranea 13*(2) 133-136.

Ingram, T. W., Oh, Y., Adhikari, T. B., Louws, F. J., & Dean, R. A. 2020. Comparative Genome Analyses of 18 Verticillium dahliae Tomato Isolates Reveals Phylogenetic and Race Specific Signatures. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.573755

Kuhn, M., Jackson, S., & Cimentada, J. (2016). *corrr: Correlations in R* [dataset]. In CRAN: Contributed Packages. The R Foundation. https://doi.org/10.32614/cran.package.corrr

Messeguer, R., Ganal, M., De Vicente, M. C., Young, N. D., Bolkan, H., & Tanksley, S. D. (1991). High resolution RFLP map around the root knot nematode resistance gene (Mi) in tomato. *Theoretical and Applied Genetics*, *82*(5), 529-536. https://doi.org/10.1007/bf00226787

Pegg, G. F., & Brady, B. L. (Eds.). (2002). *Verticillium wilts*. https://doi.org/10.1079/9780851995298.0000

R Core Team 2023. R: A Language and Environment for Statistical Computing. *R Foundation for Statistical Computing,* Vienna, Austria. <https://www.R-project.org/>.

Schaible, L., Cannon, O., & Waddoups, V. (1951). Inheritance of resistance to Verticillium Wilt in A tomato cross. *Phytopathology*, *41*(11), 986–990.

Schober, P., Boer, C., & Schwarte, L. A. (2018). Correlation coefficients: appropriate use and interpretation. *Anesthesia & analgesia*, *126*(5), 1763-1768. https://doi.org/10.1213/ane.0000000000002864

Sim, S. C., Van Deynze, A., Stoffel, K., Douches, D. S., Zarka, D., Ganal, M. W., ... & Francis, D. M. (2012). High-density SNP genotyping of tomato (Solanum lycopersicum L.) reveals patterns of genetic variation due to breeding. *PLoS ONE*, *7*(9), e45520. https://doi.org/10.1371/journal.pone.0045520

Uffelmann, E., Huang, Q. Q., Munung, N. S., de Vries, J., Okada, Y., Martin, A. R., Martin, H. C., Lappalainen, T., & Posthuma, D. (2021). Genome-wide association studies. *Nature Reviews Methods Primers*, *1*(1), 1–21. https://doi.org/10.1038/s43586-021-00056-9

Vermeulen, J. P., Hanika, K., Thomma, B. P. H. J., Bai, Y., & Schouten, H. J. (2022). Scarcity of major resistance genes against Verticillium wilt caused by Verticillium dahliae. *Plant Breeding*, *141*(5), 681–694. Portico. https://doi.org/10.1111/pbr.13046

FIGURES

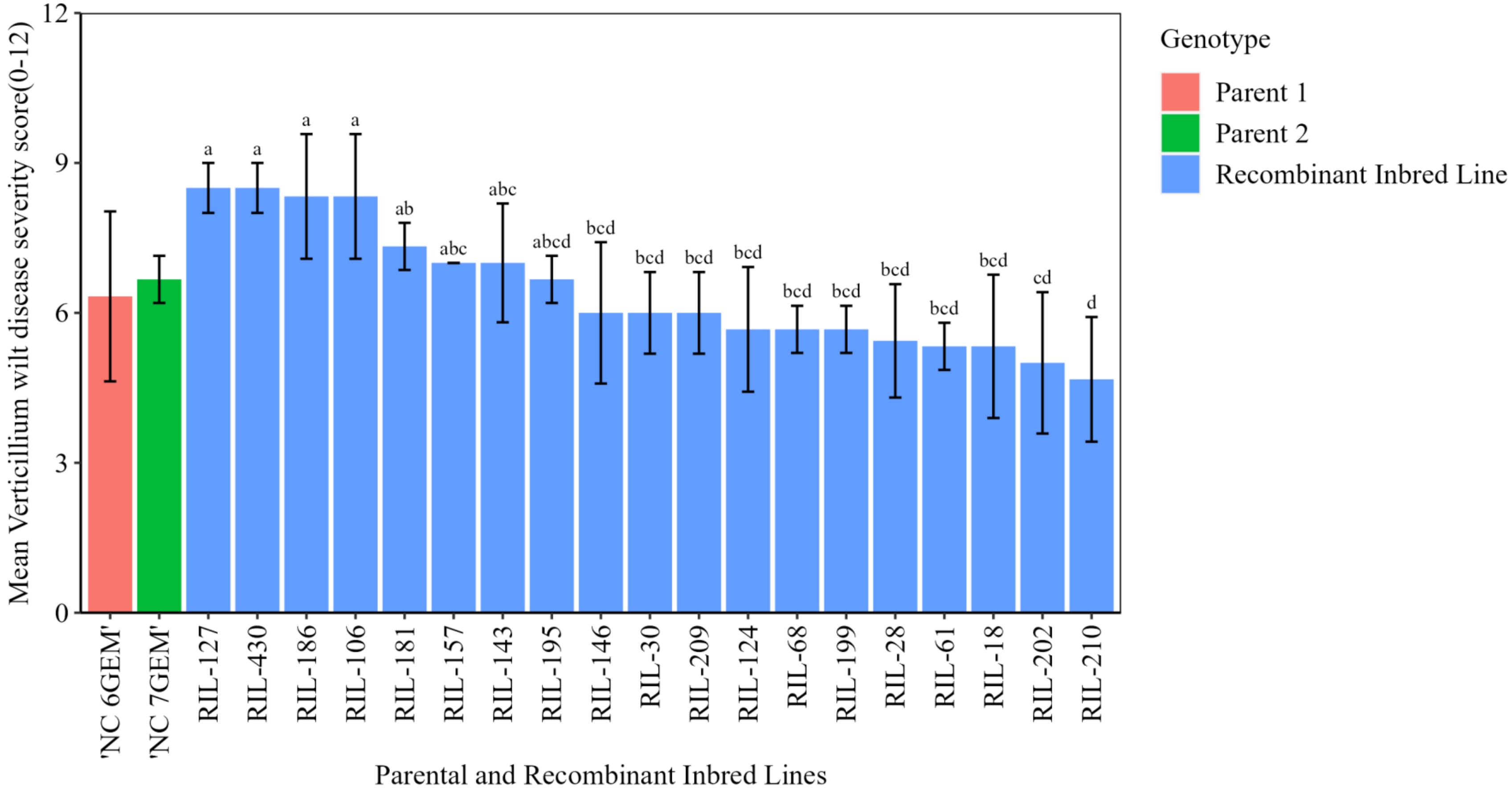


Figure 3.1. Average Verticillium wilt disease severity index scores in a randomized replicated on-farm trial (2022) of F_5 'NC 6GEM' x 'NC 7GEM' RILs and related material descended from 19 F_4 genotyped lines from RILs. Lines were resistant and susceptible extreme selections in the F_4 generation. Parental line Verticillium disease severity scoring was highlighted in Red and Green ('NC 6GEM' and 'NC 7GEM') Values sharing a letter were not significantly different by Duncan's multiple range test to α=05

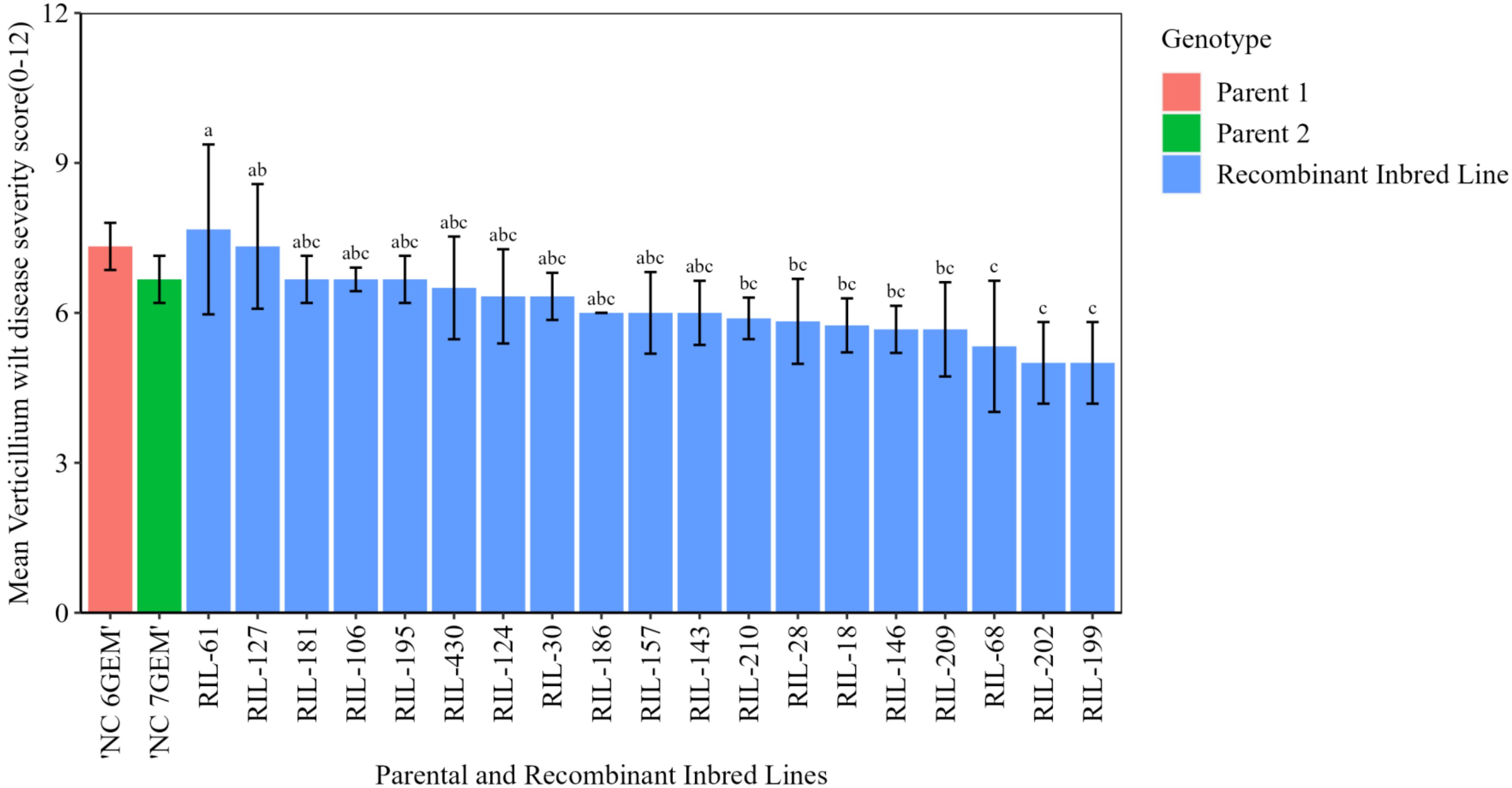


Figure 3.2. Average Verticillium wilt disease severity index scores in a randomized replicated trial on-farm trial (2023) of F_6 ‘NC 6GEM’ x ‘NC 7GEM’ RILs and related material descended from 19 F_4 genotyped lines from RILs. Lines were resistant and susceptible extreme selections in the F_4 generation. Parental line Verticillium disease severity scoring was highlighted in Red and Green (‘NC 6GEM’ and ‘NC 7GEM’) Values sharing a letter were not significantly different by Duncan’s multiple range test to α=05

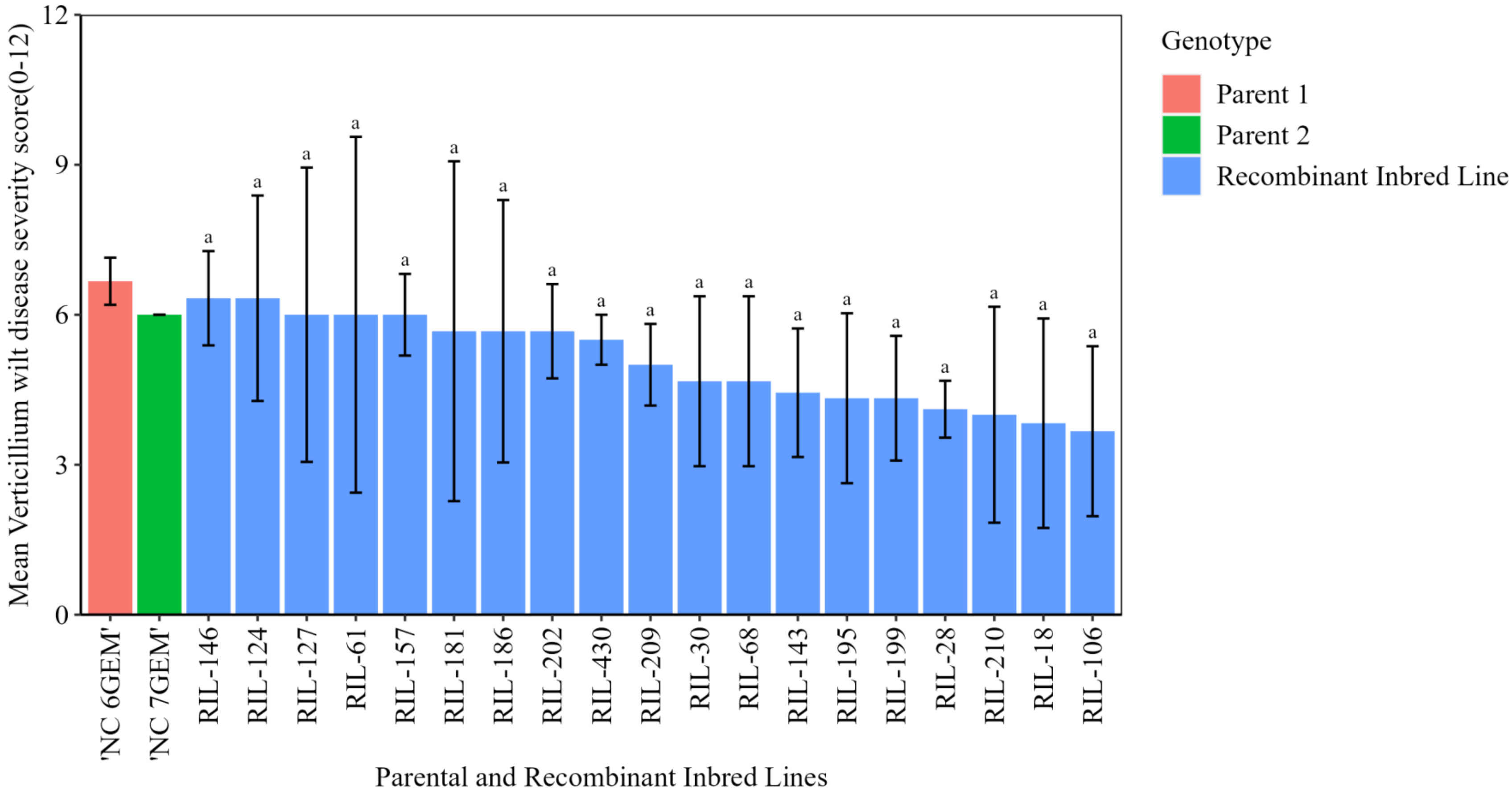


Figure 3.3. Average Verticillium wilt disease severity index scores in a randomized replicated research station trial (2022) of F_5 'NC 6GEM' x 'NC 7GEM' RILs and related material descended from 19 F4 genotyped lines from RILs. Lines were resistant and susceptible extreme selections in the F_4 generation. Parental line Verticillium disease severity scoring was highlighted in Red and Green ('NC 6GEM' and 'NC 7GEM') No significant differences were detected by Duncan's multiple range test in these lines among these lines.

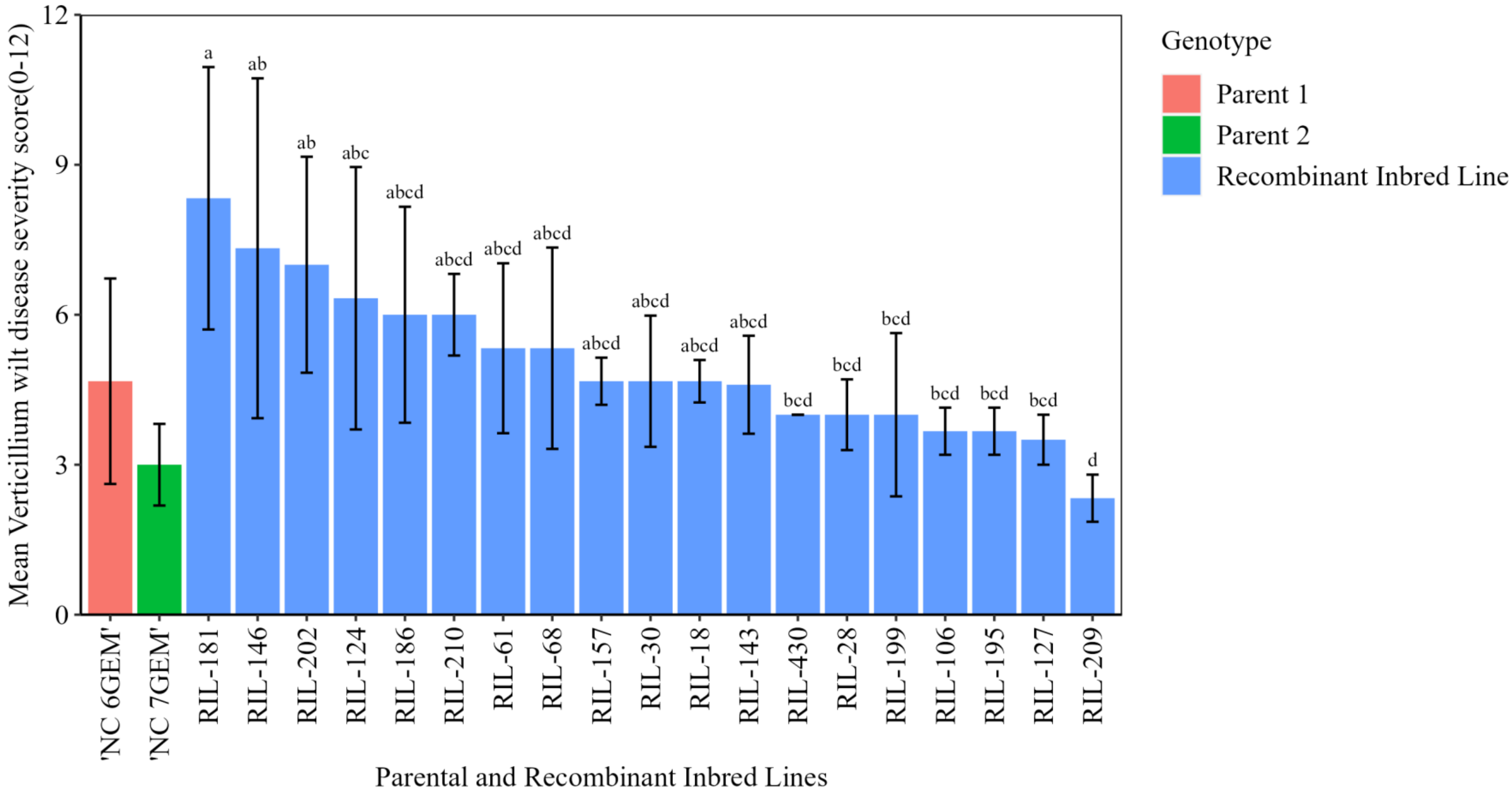


Figure 3.4. Average Verticillium wilt disease severity index scores in a randomized replicated research station trial (2023) of F_6 'NC 6GEM' x 'NC 7GEM' RILs and related material descended from 19 F_4 genotyped lines from RILs. Lines were resistant and susceptible extreme selections in the F_4 generation. Parental line Verticillium disease severity scoring was highlighted in Red and Green ('NC 6GEM' and 'NC 7GEM') Values sharing a letter were not significantly different by Duncan's multiple range test to α=05.

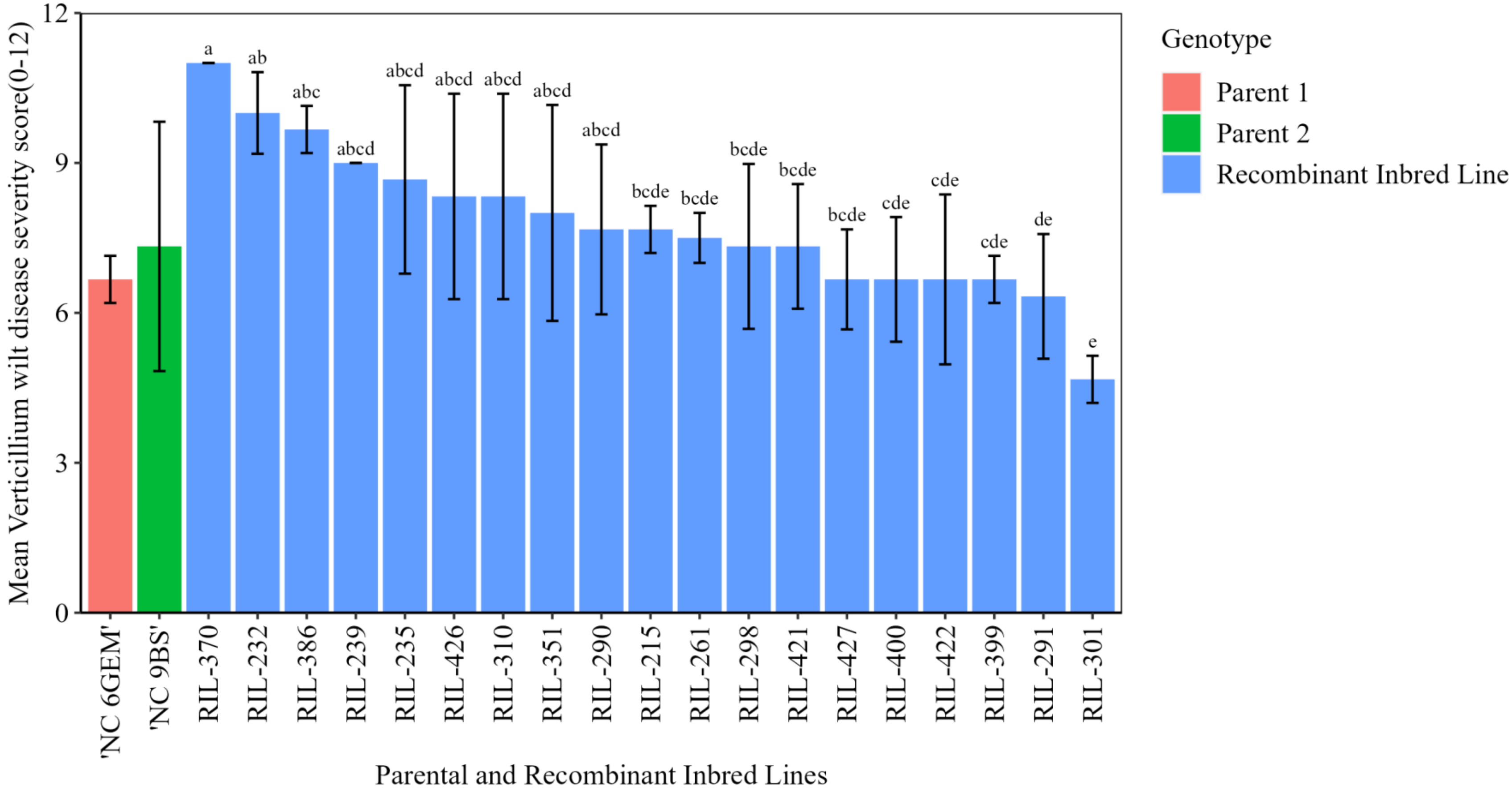


Figure 3.5. Average Verticillium wilt disease severity index scores in a randomized replicated on-farm trial (2022) of F_5 ‘NC 6GEM’ x ‘NC 9BS’ RILs and related material descended from 19 F_4 genotyped lines from RILs. Lines were resistant and susceptible extreme selections in the F_4 generation. Parental line Verticillium disease severity scoring was highlighted in Red and Green (‘NC 6GEM’ and ‘NC 9BS) Values sharing a letter were not significantly different by Duncan’s multiple range test to α=05.

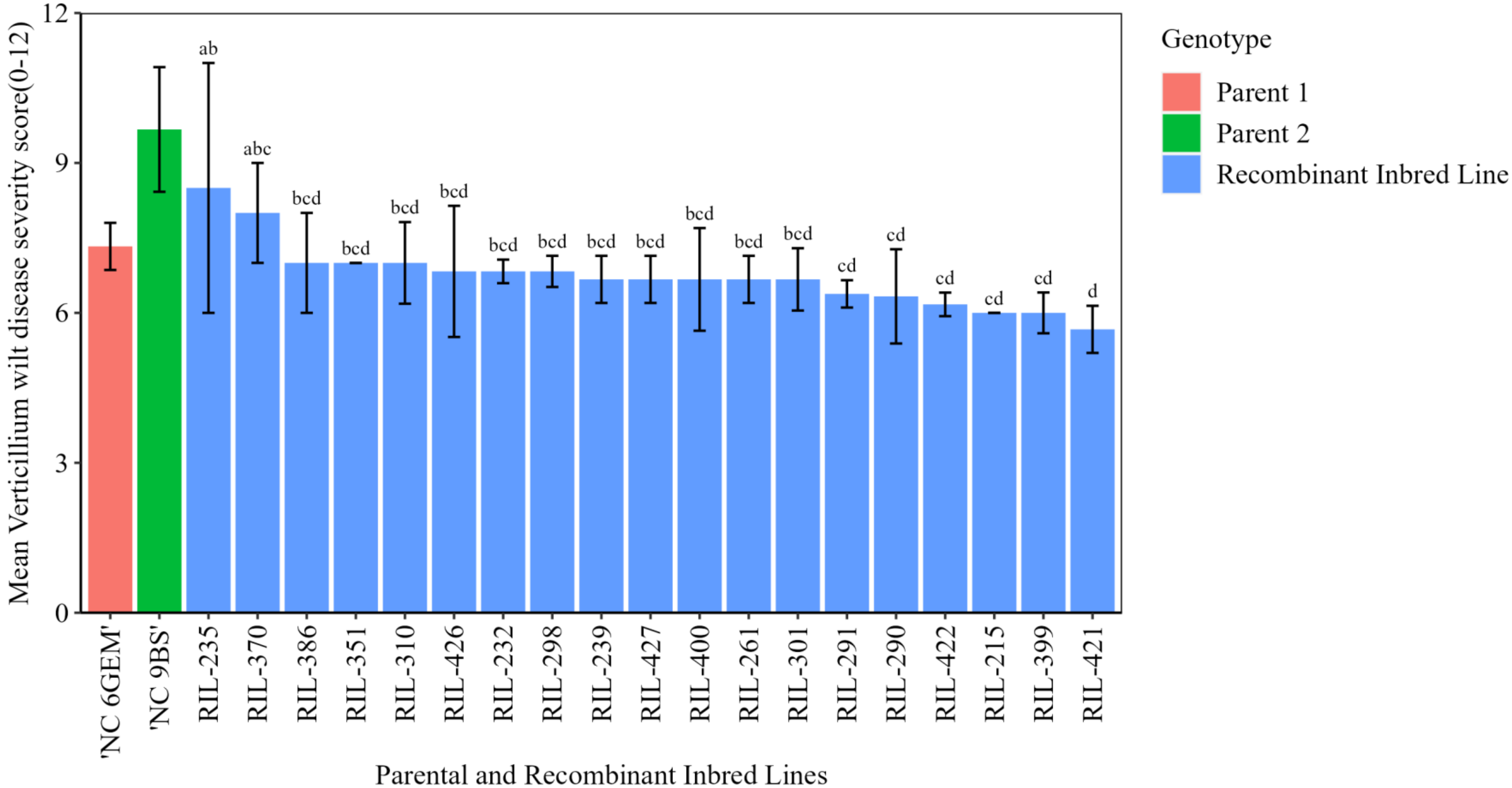


Figure 3.6. Average Verticillium wilt disease severity index scores in a randomized replicated on-farm trial (2023) of F_6 'NC 6GEM' x 'NC 9BS' RILs and related material descended from 19 F_4 genotyped lines from RILs. Lines were resistant and susceptible extreme selections in the F_4 generation. Parental line Verticillium disease severity scoring was highlighted in Red and Green ('NC 6GEM' and 'NC 9BS) Values sharing a letter were not significantly different by Duncan's multiple range test to α=05.

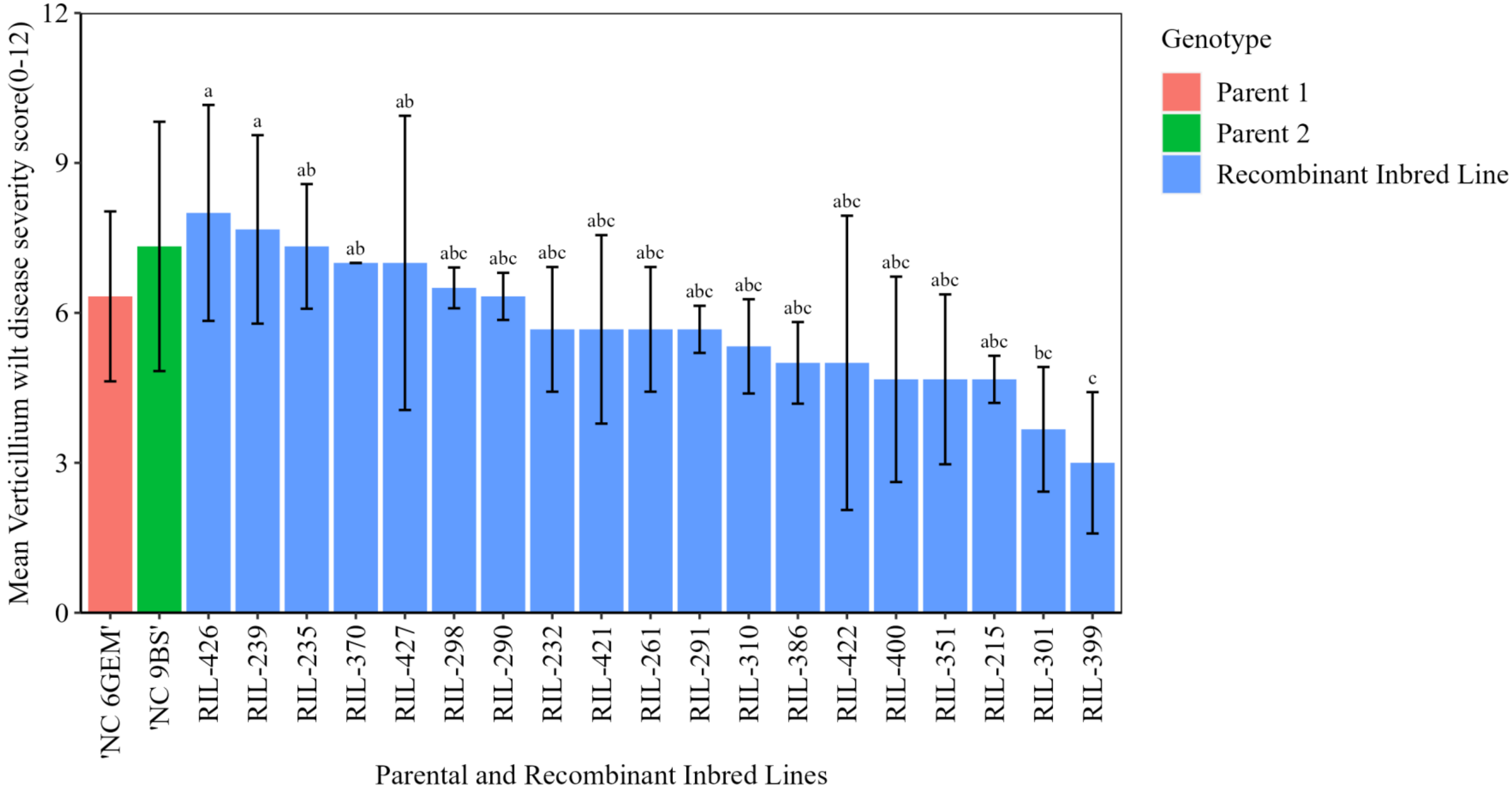


Figure 3.7. Average Verticillium wilt disease severity index scores in a randomized replicated research station trial (2022) of F_5 ‘NC 6GEM’ x ‘NC 9BS’ RILs and related material descended from 19 F_4 genotyped lines from RILs. Lines were resistant and susceptible extreme selections in the F_4 generation. Parental line Verticillium disease severity scoring was highlighted in Red and Green (‘NC 6GEM’ and ‘NC 9BS) Values sharing a letter were not significantly different by Duncan’s multiple range test to α=05.

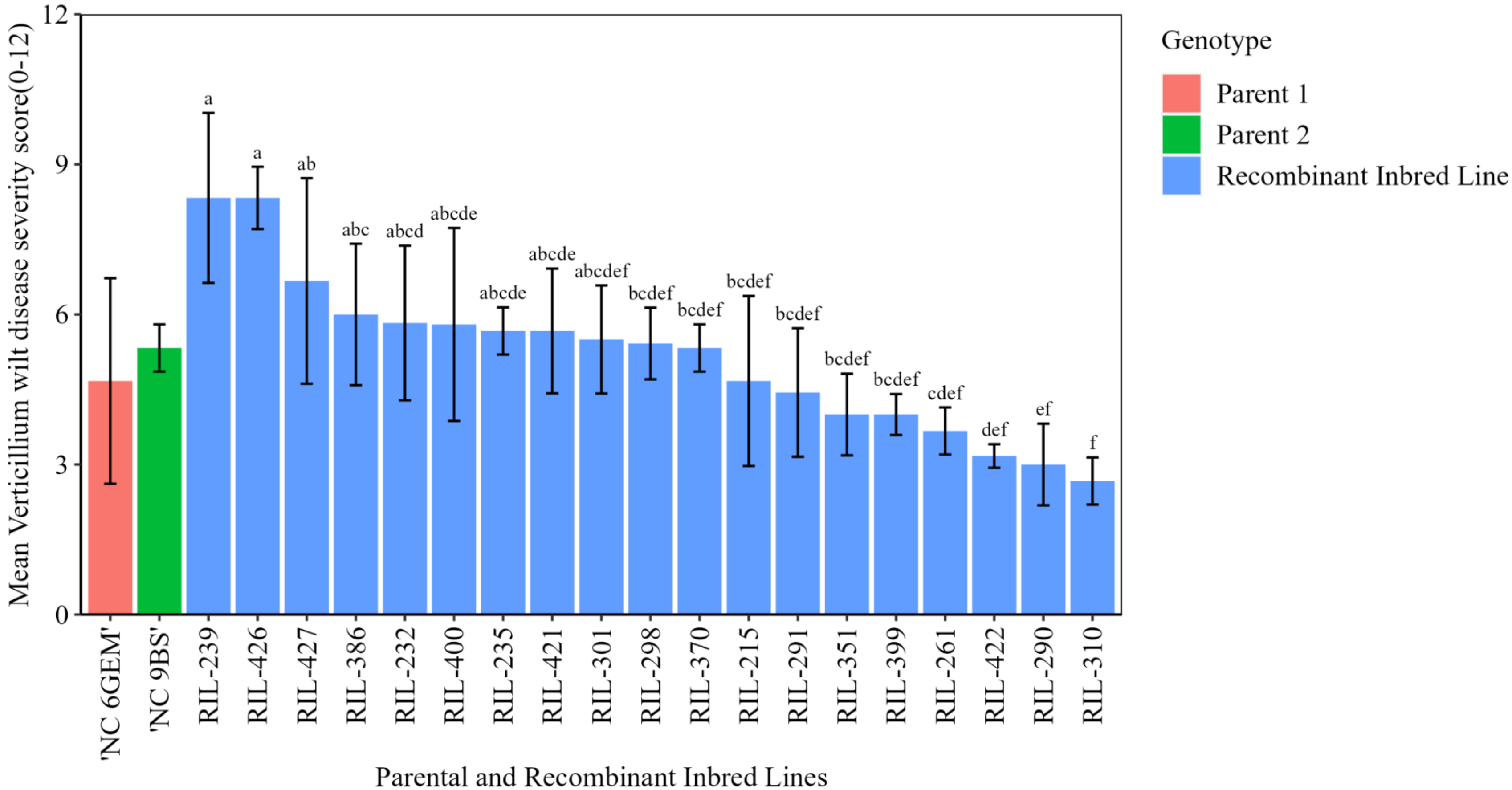


Figure 3.8. Average Verticillium wilt disease severity index scores in a randomized replicated research station trial (2023) of F_6 ‘NC 6GEM’ x ‘NC 9BS’ RILs and related material descended from 19 F_4 genotyped lines from RILs. Lines were resistant and susceptible extreme selections in the F_4 generation. Parental line Verticillium disease severity scoring was highlighted in Red and Green (‘NC 6GEM’ and ‘NC 9BS) Values sharing a letter were not significantly different by Duncan’s multiple range test to α=05.

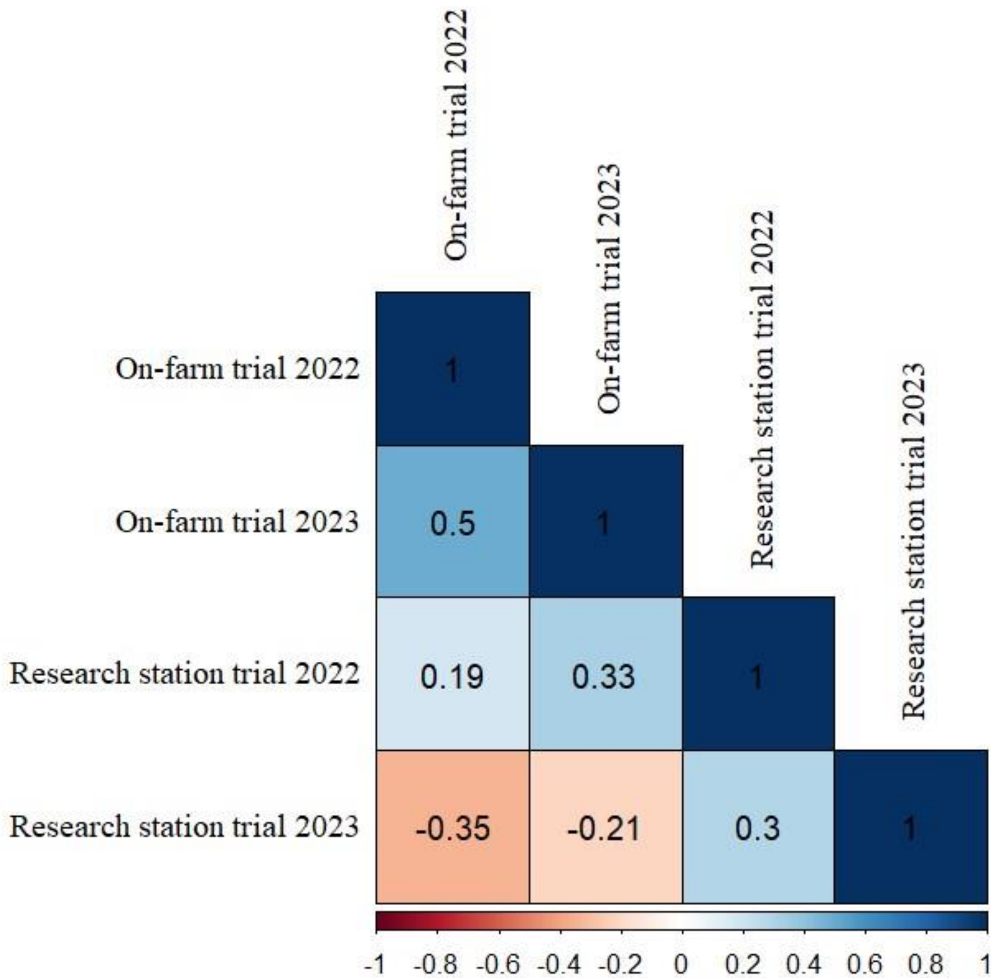


Figure 3.9. Pairwise correlation matrix depicting Spearman's rank correlation coefficients of Verticillium wilt disease severity index scoring for the 19 F5 and F6 RILs descended from 'NC 6GEM' x 'NC 7GEM' selections for resistant and susceptible extremes used in marker trait association across years and locations.

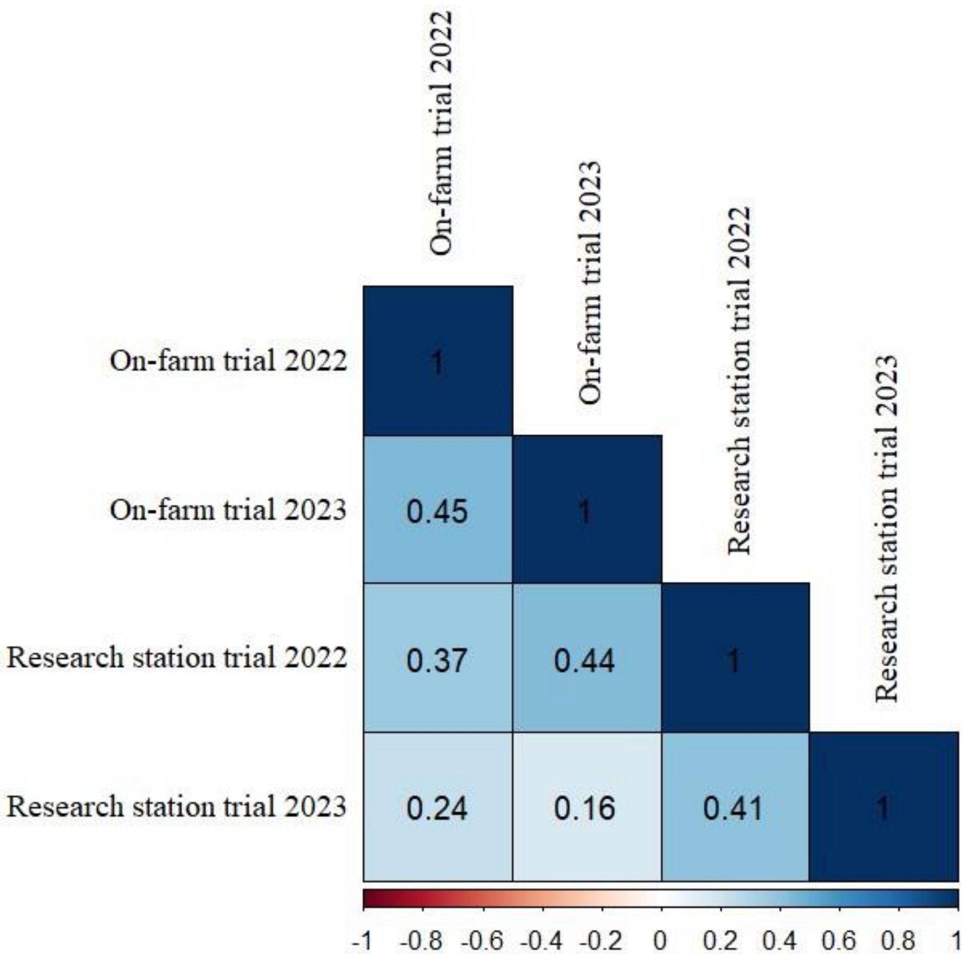


Figure 3.10. Pairwise correlation matrix depicting Spearman's rank correlation coefficients of Verticillium wilt disease severity index scoring for the 19 F5 and F6 RILs descended from 'NC 6GEM' x 'NC 9BS' selections for resistant and susceptible extremes used in marker trait association across years and locations.

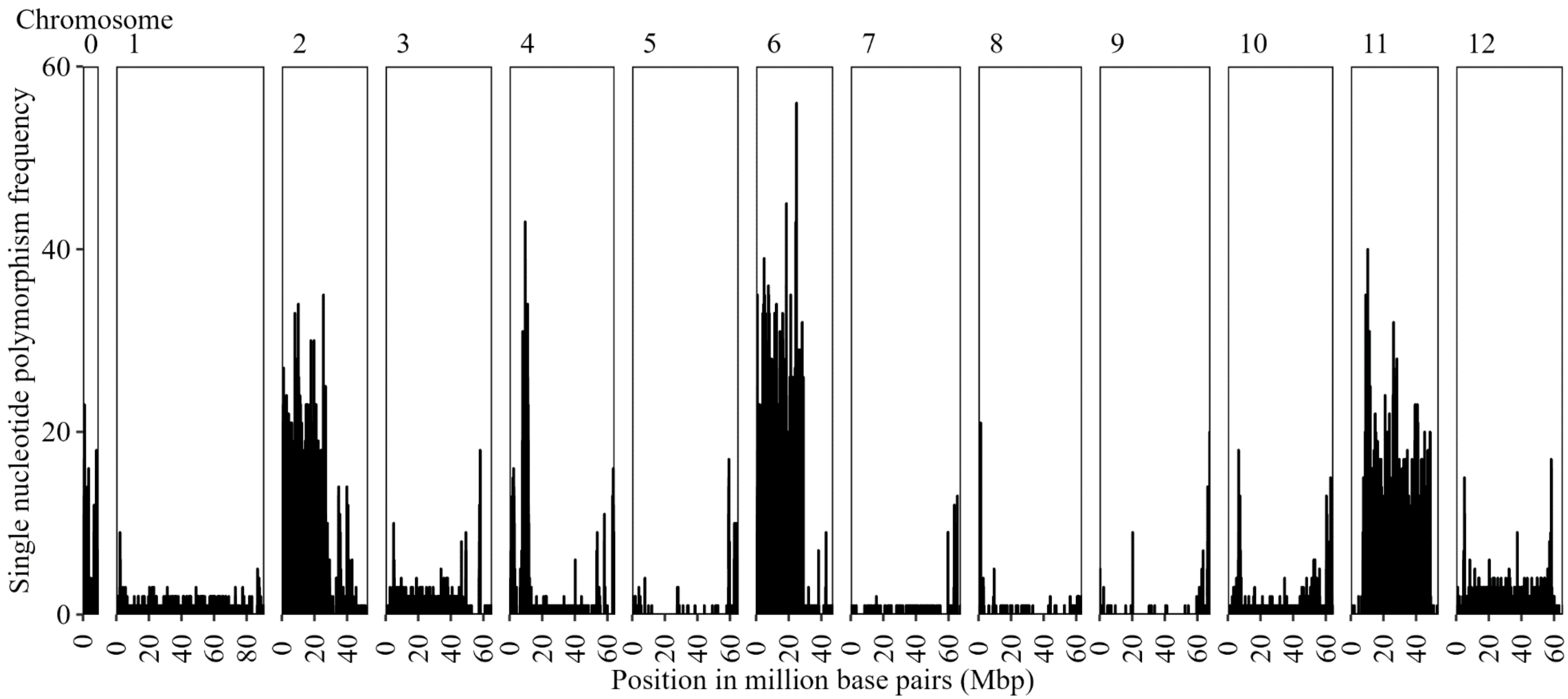


Figure 3.11: Distribution of single nucleotide polymorphisms within an F_4 (NC 6GEM x NC 7GEM) targeted genotyping by sequencing dataset on tomato chromosomes.

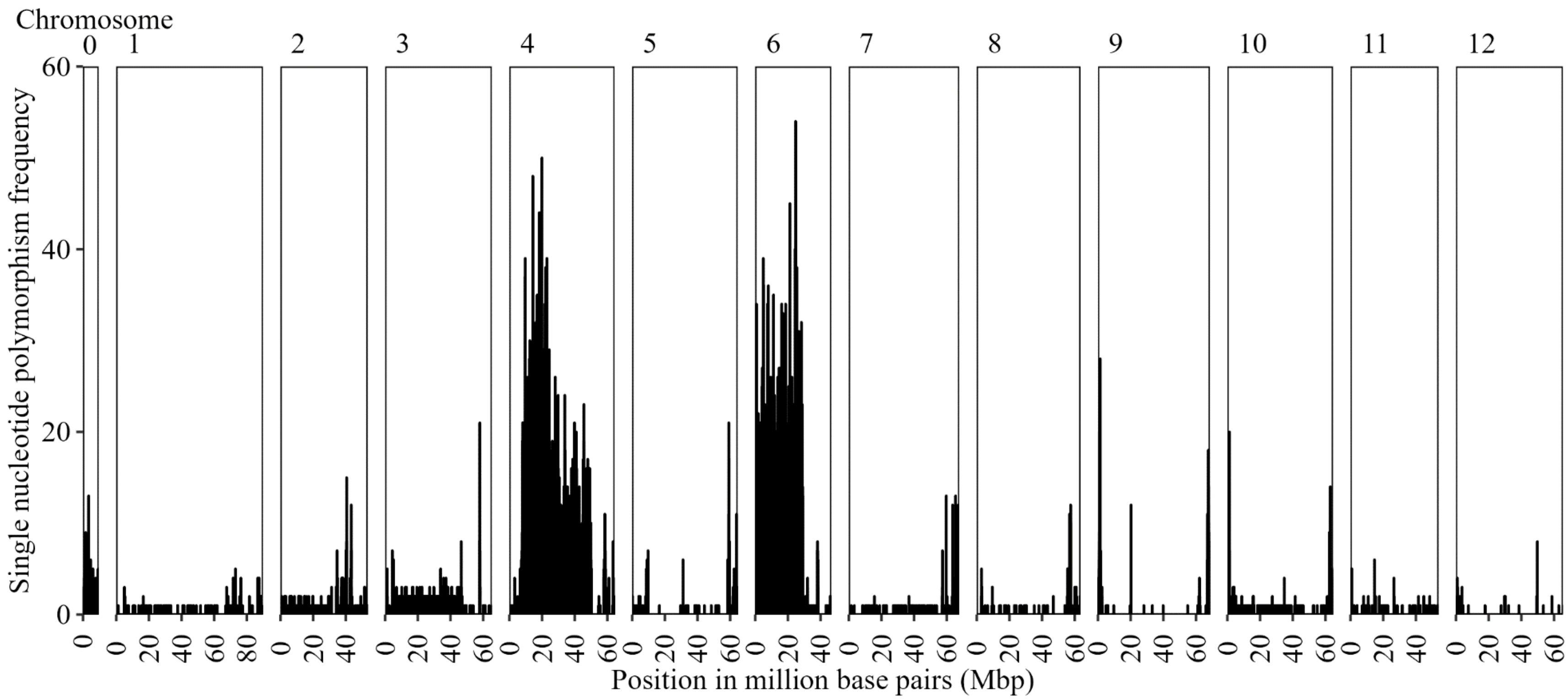


Figure 3.12: Distribution of single nucleotide polymorphisms within an F_4 (NC 6GEM x NC 9BS) targeted genotyping by sequencing dataset on tomato's chromosomes.

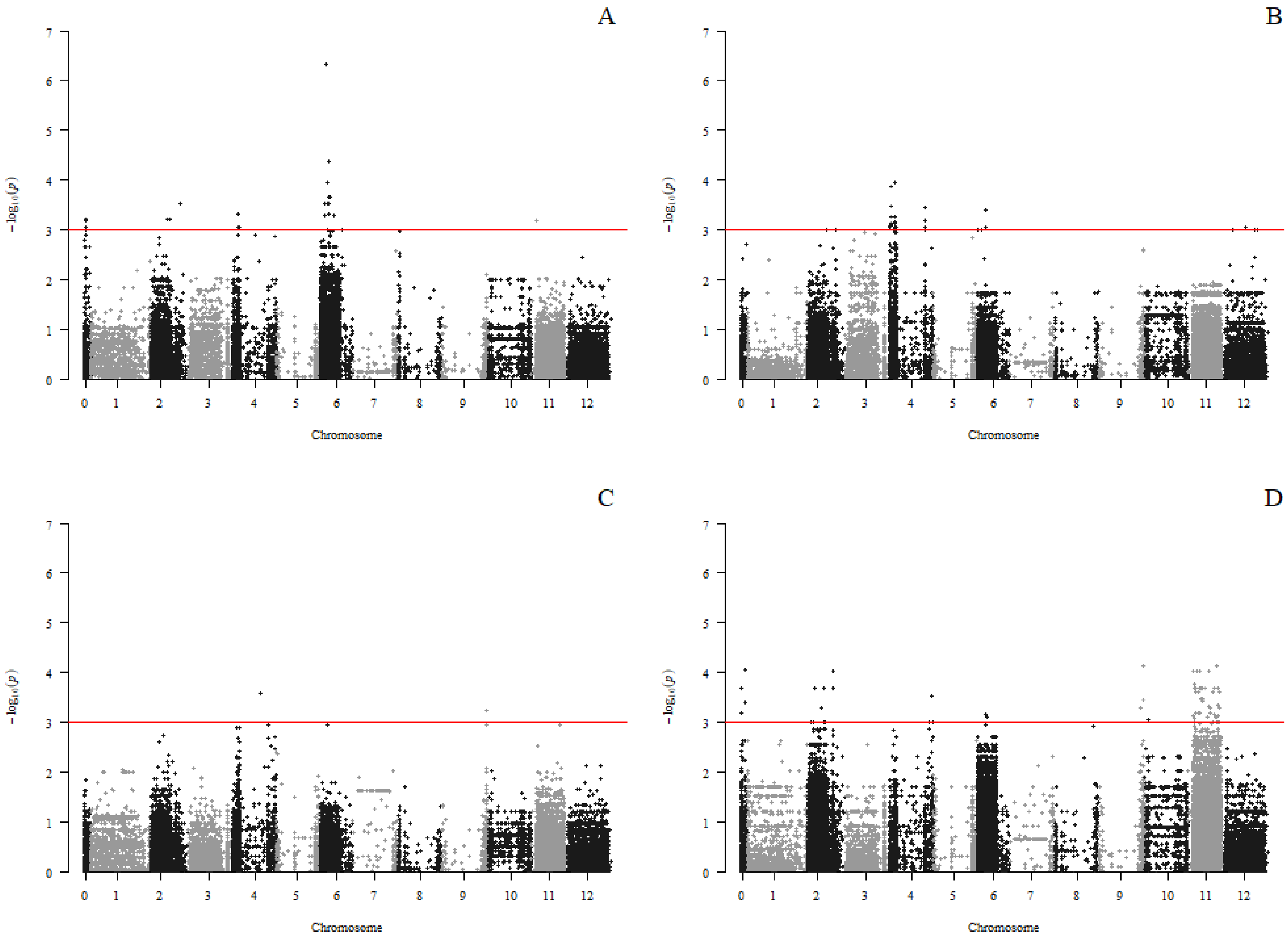


Figure 3.13: Associations resulting from a general linear model approach applied to selection of F_5 and F_6 'NC6GEM' x 'NC7GEM' RILs. Subfigures are labeled as follows: the phenotypic information from A. 2022 on-farm trial, B. 2023 on-farm trial C. 2022 research station trial D. 2023 research station trial. A -log10(p) score of three was marked in red for reference.

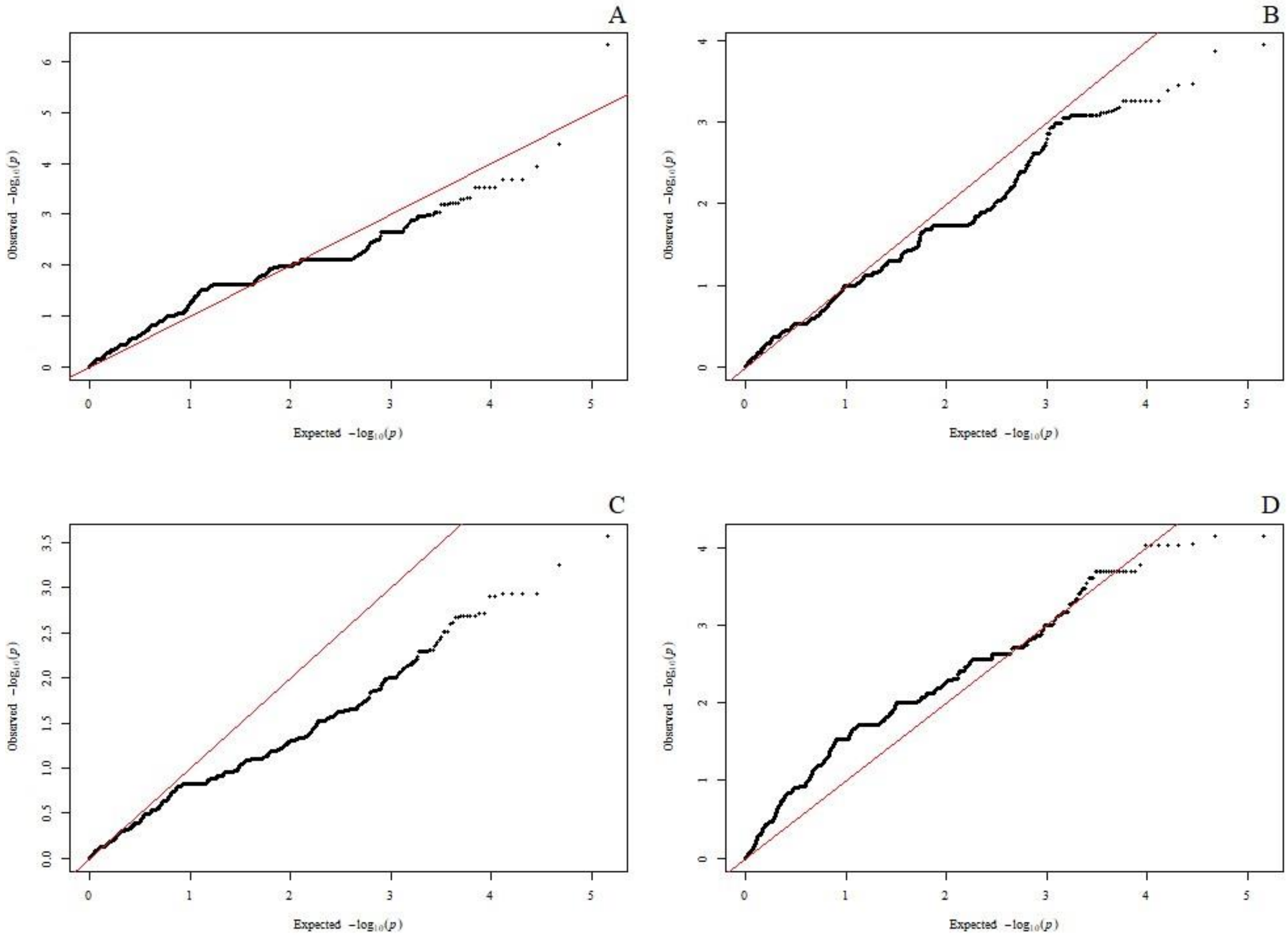


Figure 3.14: Quantile-Quantile plots for the SNP P values for associations between A. 2022 on-farm trial, B. 2023 on-farm trial C. 2022 research station trial D. 2023 research station trial.

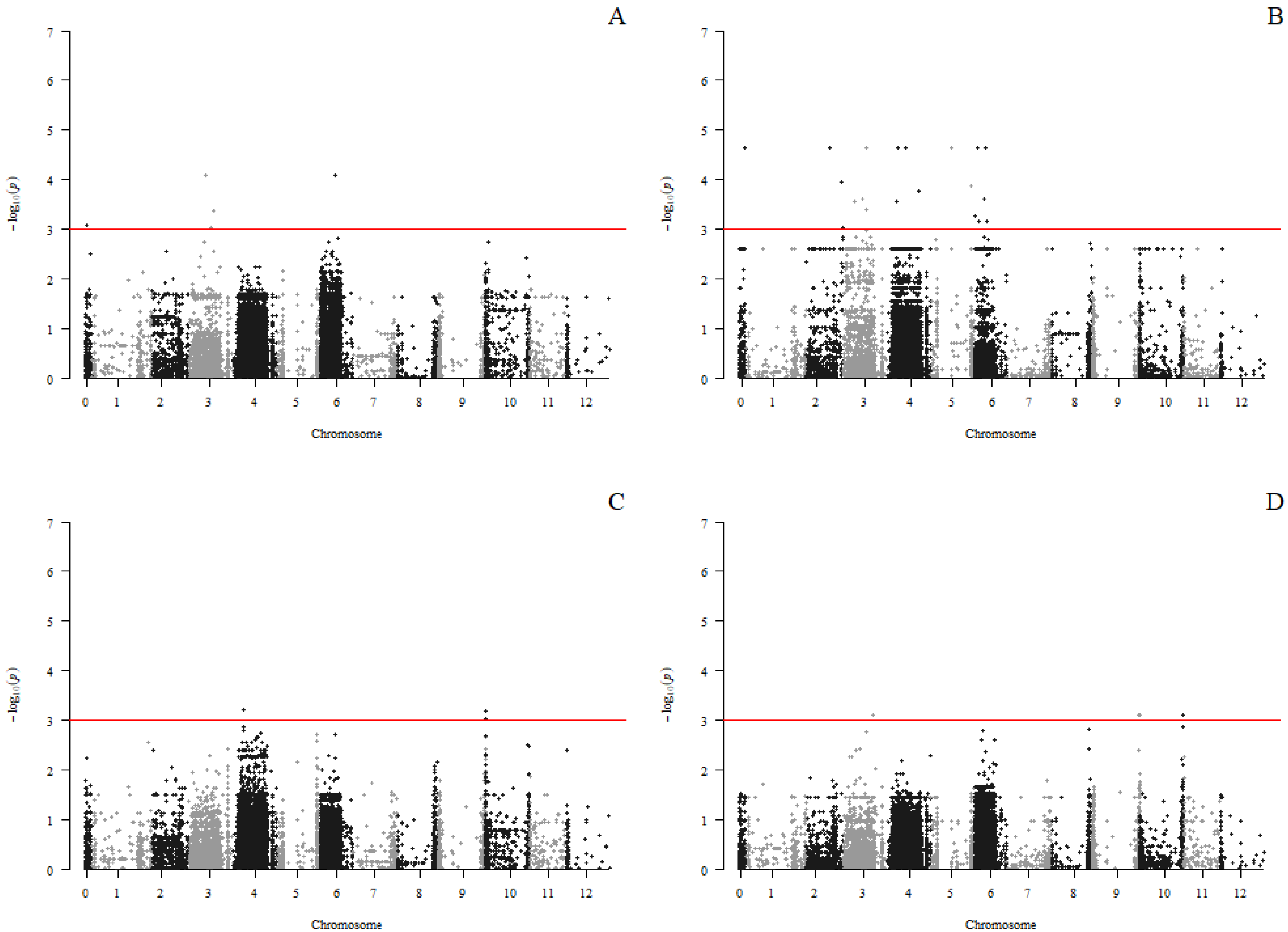


Figure 3.15: Associations resulting from a general linear model approach applied to a selection of F5 and F6 'NC6GEM' x 'NC9BS' RILs. Subfigures are labeled as follows: the phenotypic information from A. 2022 on-farm trial, B. 2023 on-farm trial C. 2022 research station trial D. 2023 research station trial. A -log10(p) score of three was marked in red for reference.

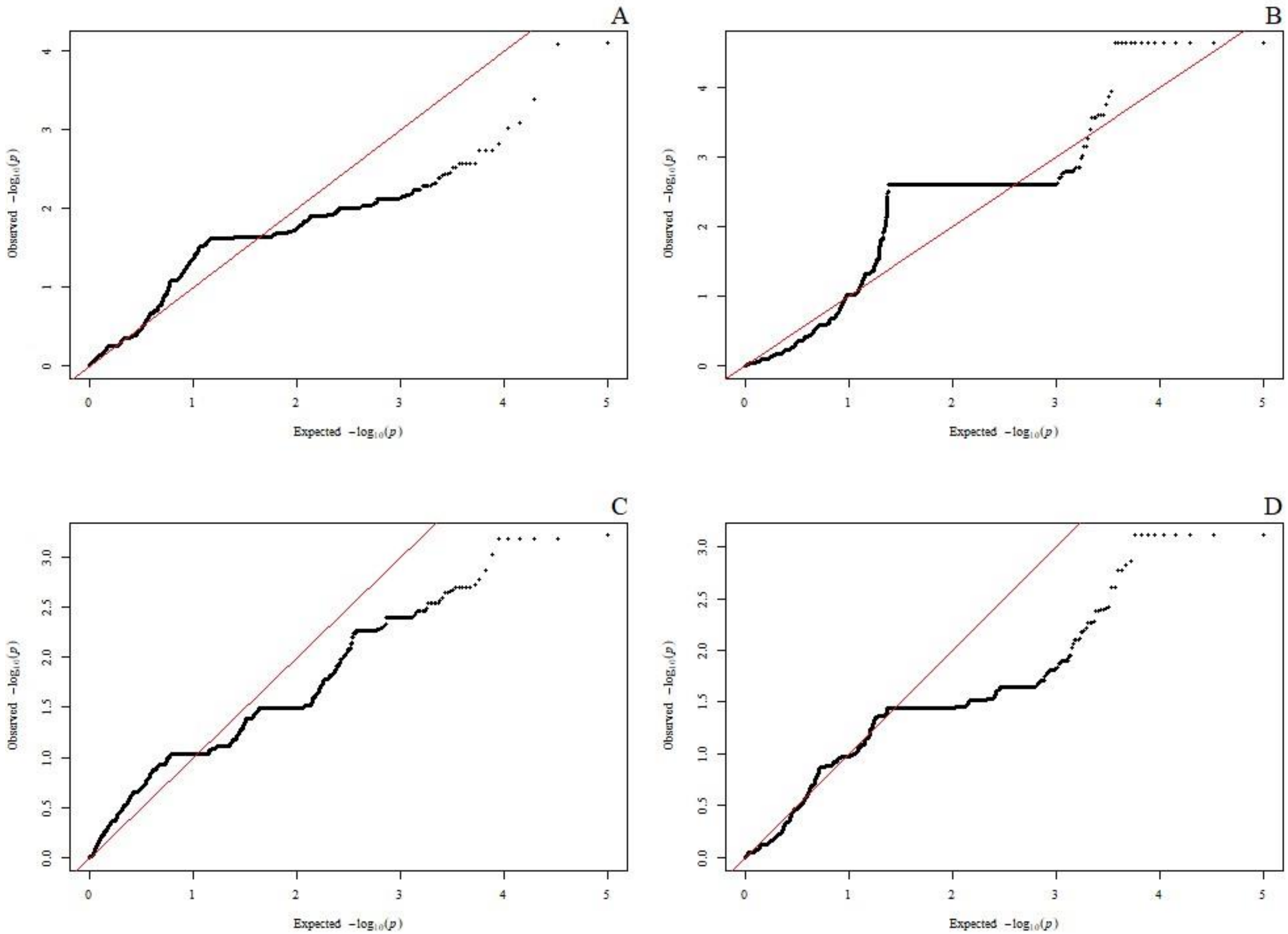


Figure 3.16: Quantile-Quantile plots for the SNP P values for associations F_5 and F_6 'NC6GEM' x 'NC9BS' RILs between A. 2022 on-farm trial, B. 2023 on-farm trial C. 2022 research station trial D. 2023 research station trial.

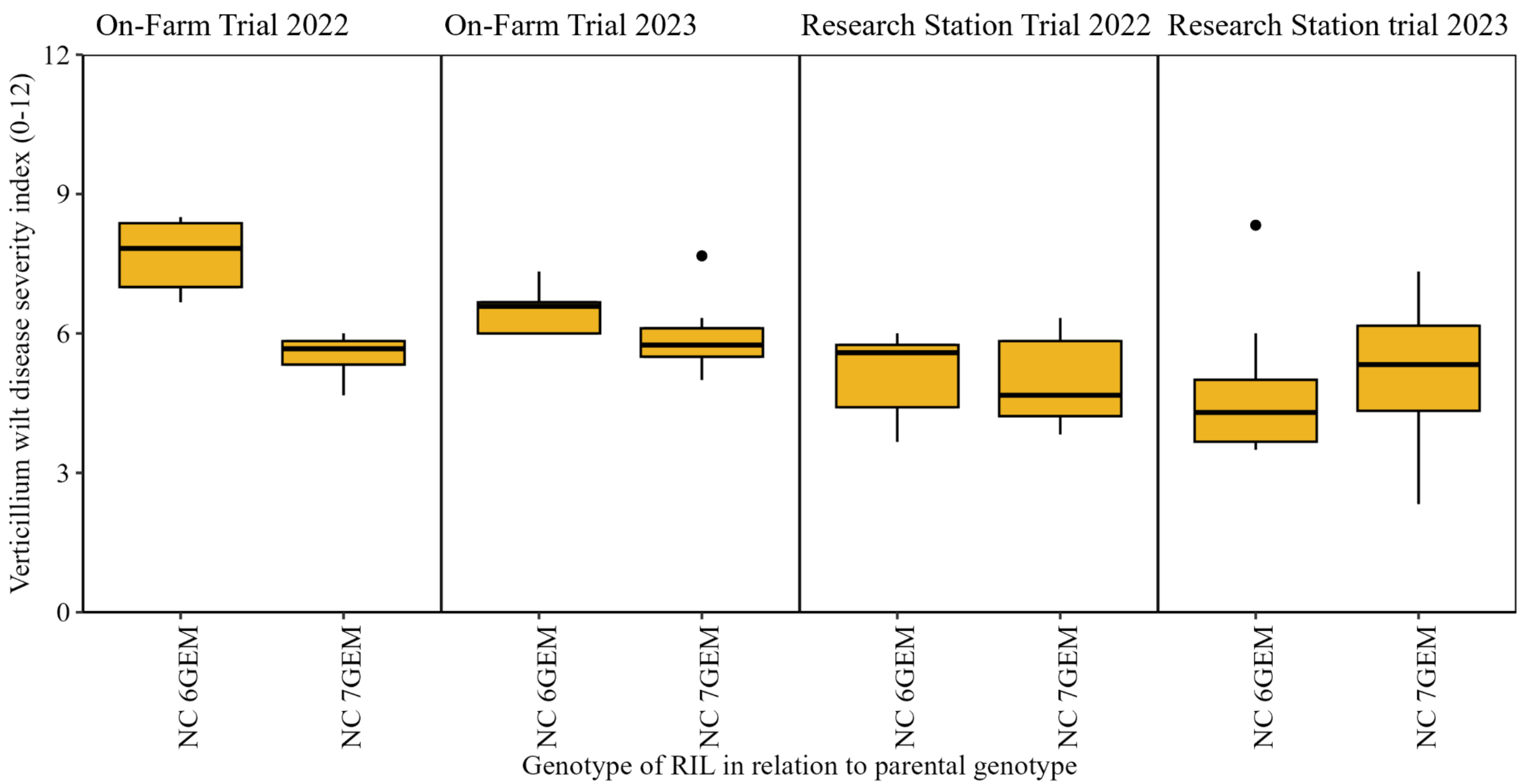


Figure 3.17: Phenotypic delineation by parental representation of biologically relevant marker with lowest P value in the on-farm trial in 2022 of the 'NC 6GEM' x 'NC 7GEM' genotyped RILs (9.60 Mbp on chromosome 6). Figure A is Vdn DSI at the on-farm trial in 2022, figure B is Vdn DSI at the on-farm trial 2023 figure C is Vdn DSI at the research station trial in 2022, figure D is Vdn DSI at the research station trial in 2023.

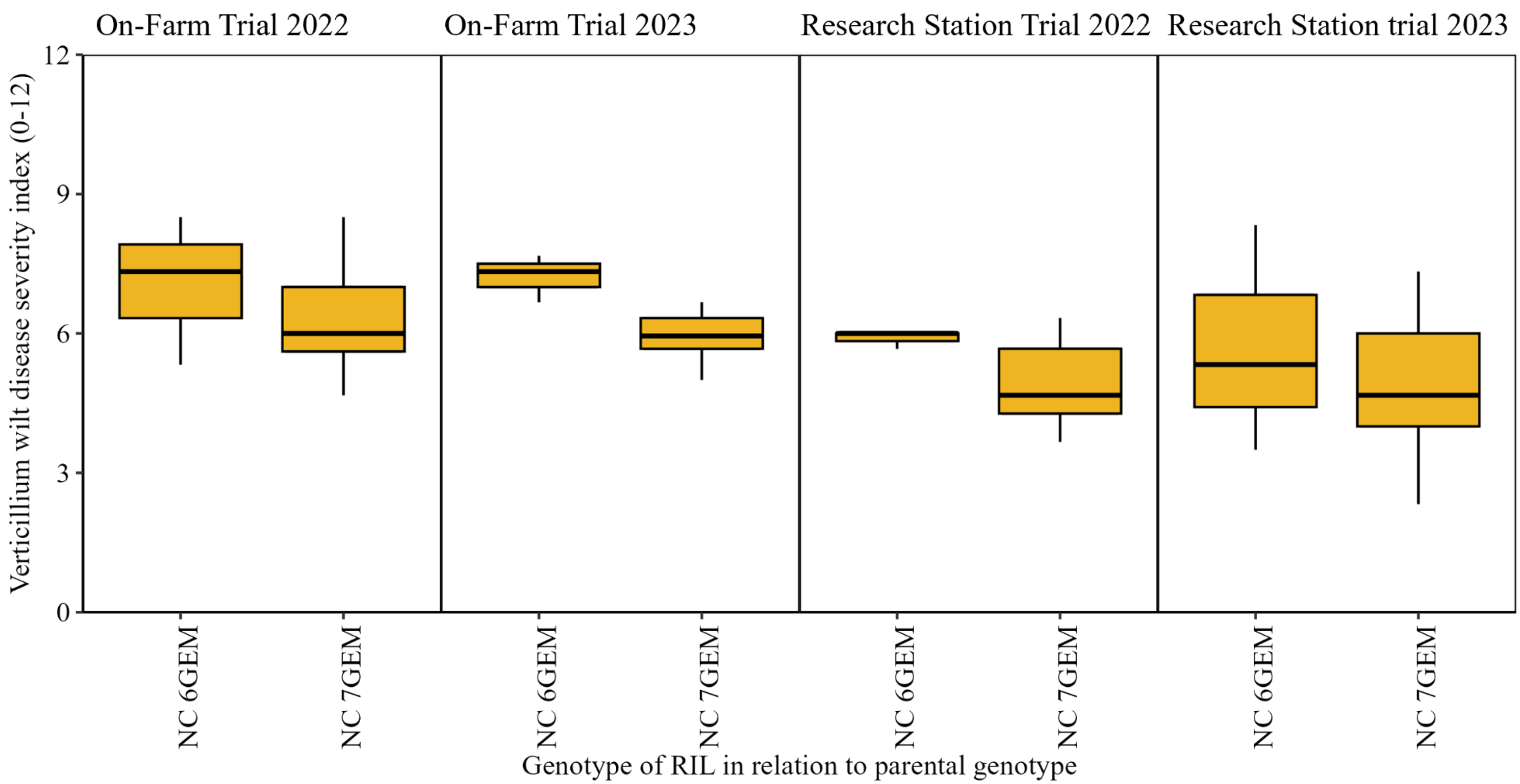


Figure 3.18: Phenotypic delineation by parental representation of biologically relevant marker with lowest P value in the on-farm trial in 2023 of the 'NC 6GEM' x 'NC 7GEM' genotyped RILs (8.36 Mbp on chromosome 4). Figure A is Vdn DSI at the on-farm trial in 2022, figure B is Vdn DSI at the on-farm trial 2023 figure C is Vdn DSI at the research station trial in 2022, figure D is Vdn DSI at the research station trial in 2023.figure D is Vdn DSI at the research station trial in 2023.

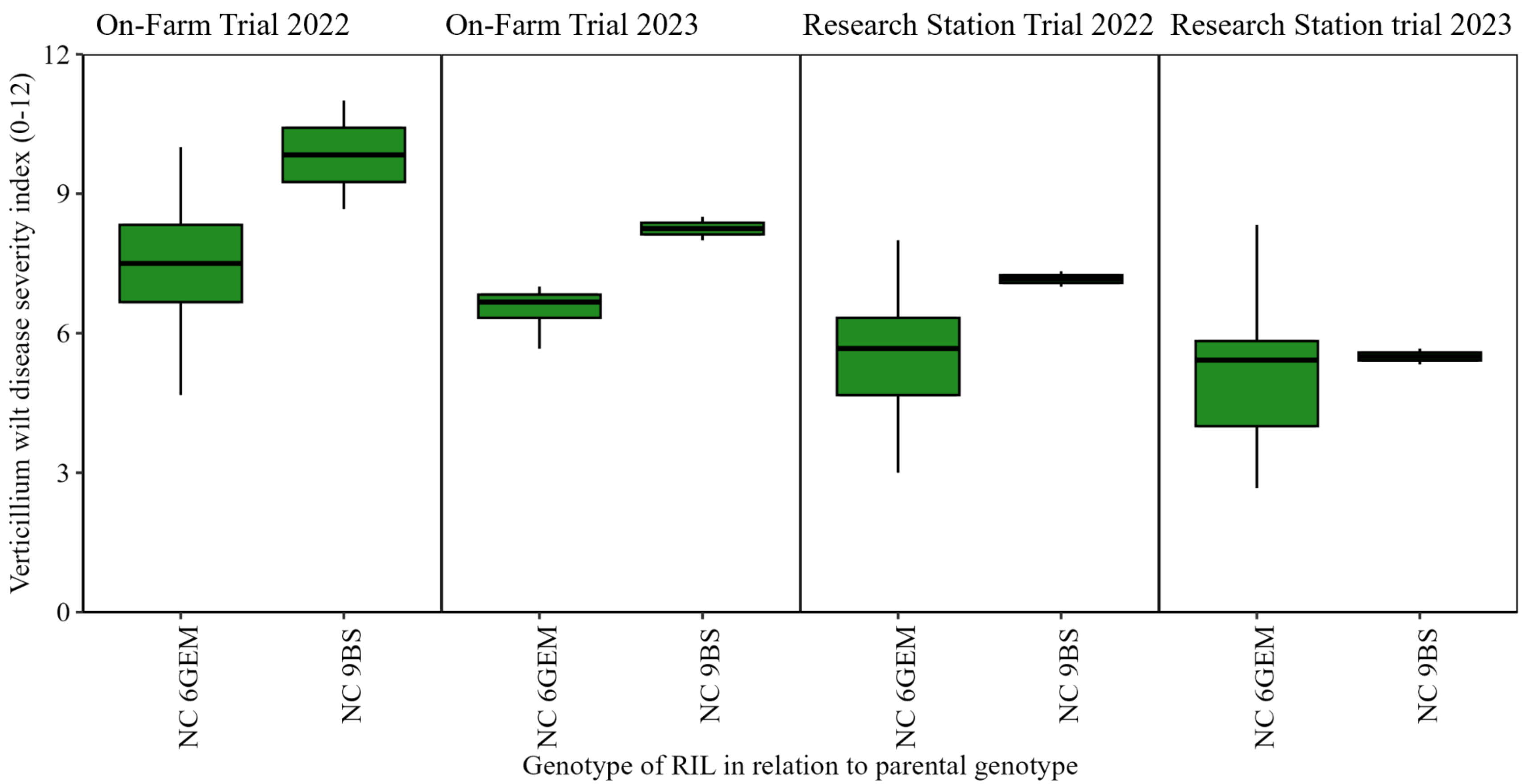


Figure 3.19: Phenotypic delineation by parental representation of biologically relevant marker with lowest P value in the on-farm trial in 2023 of the ‘NC 6GEM’ x ‘NC 9BS’ genotyped RILs (8.74 Mbp on chromosome 0). Figure A is Vdn DSI at the on-farm trial in 2022, figure B is Vdn DSI at the on-farm trial 2023 figure C is Vdn DSI at the research station trial in 2022, figure D is Vdn DSI at the research station trial in 2023.

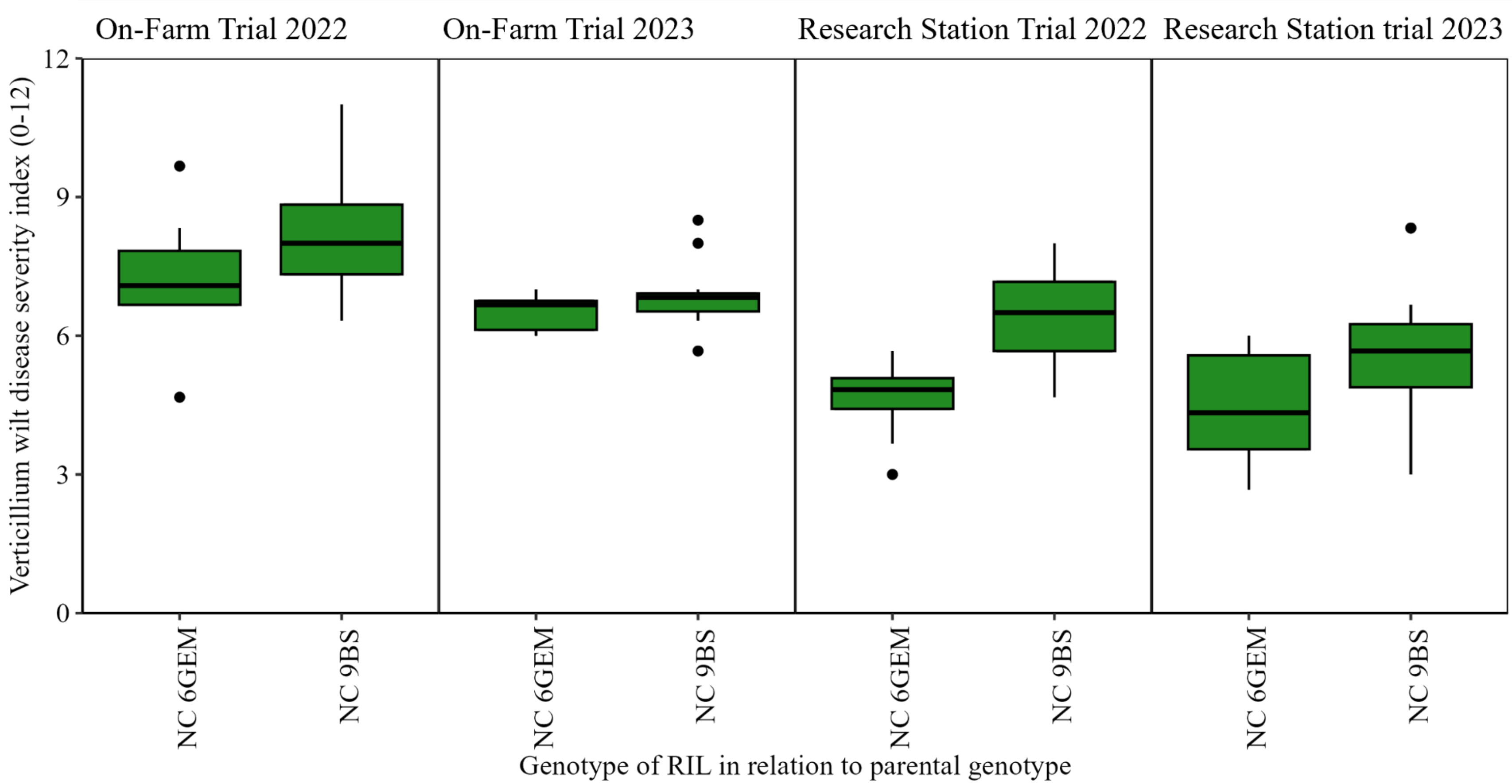


Figure 3.20: Phenotypic delineation by parental representation of biologically relevant marker with lowest P value in the research station trial in 2022 of the 'NC 6GEM' x 'NC 9BS' genotyped RILs (16.56 Mbp on chromosome 4). Figure A is Vdn DSI at the on-farm trial in 2022, figure B is Vdn DSI at the on-farm trial 2023 figure C is Vdn DSI at the research station trial in 2022, figure D is Vdn DSI at the research station trial in 2023.

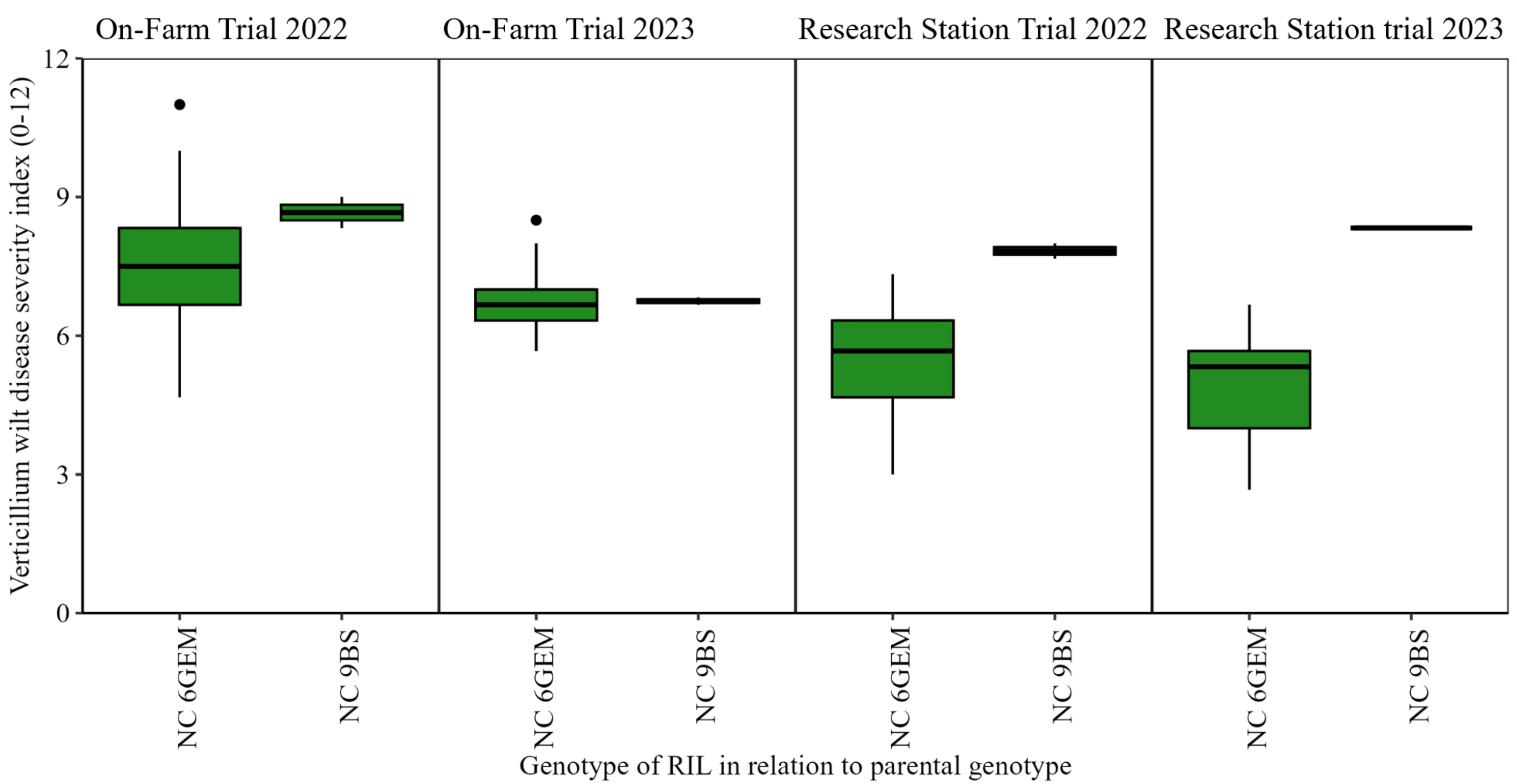


Figure 3.21: Phenotypic delineation by parental representation of biologically relevant marker with lowest P value in the research station trial in 2023 on the 'NC 6GEM' x 'NC 9BS' genotyped RILs(67.91 Mbp on chromosome 9). Figure A is Vdn DSI at the on-farm trial in season 1, figure B is Vdn DSI at the on-farm trial season 2 figure C is Vdn DSI at the research station trial in season 1, figure D is Vdn DSI at the research station trial in season 2.

TABLES

Table 3.1: Identified SNPs with an unadjusted p value less than 0.001 within NC6GEM x NC7GEM population in the on-farm trial in 2022 and 2023. Table extends to page 89.

CH[i]	P (Mbp)[ii]	R[iii]	A[iv]	AE[v]	Year
CH00	3.48	T	-	1.063	2022
CH00	3.48	T	-	0.875	2022
CH00	3.52	G	A	0.913	2022
CH00	3.52	G	T	0.913	2022
CH02	22.26	C	A	1.063	2022
CH02	22.26	A	C	1.063	2022
CH02	26.61	T	C	1.063	2022
CH02	42.78	T	-	0.975	2022
CH04	0.48	C	G	0.654	2023
CH04	1.65	T	C	0.536	2023
CH04	2.87	G	A	0.536	2023
CH04	3.03	T	A	0.520	2023
CH04	8.36	C	G	0.654	2023
CH04	9.21	C	T	0.508	2023
CH04	9.69	C	G	0.913	2022
CH04	9.73	A	T	0.910	2022
CH04	9.76	G	C	0.483	2023
CH04	11.03	A	T	0.654	2023
CH04	11.23	C	A	0.913	2022
CH04	53.74	G	A	0.521	2023
CH06	6.99	T	G	1.096	2022
CH06	7.68	T	C	0.912	2022
CH06	9.60	G	C	1.091	2022
CH06	10.86	A	C	0.911	2022
CH06	11.14	G	T	0.938	2022
CH06	12.66	A	-	0.879	2022
CH06	12.89	T	-	0.983	2022
CH06	12.89	-	C	0.914	2022
CH06	12.92	T	-	0.912	2022
CH06	13.30	-	T	0.914	2022
CH06	13.31	A	C	0.610	2023
CH06	14.13	T	-	0.914	2022
CH06	19.90	A	-	0.887	2022
CH11	8.11	C	T	1.187	2022
CH11	8.11	A	G	1.187	2022
CH11	8.11	A	G	1.187	2022
CH12	32.39	C	-	0.654	2023

[i] Chromosome
[ii] Reference allele A = Adenine, T = Thymine, G = Guanine, C = Cytosine, - = deletion
[iii] Alternative allele A=Adenine, T=Thymine, G=Guanine, C=Cytosine, - = deletion
[iv] Additive effect of the marker under a fixed effect model, on 0-12 disease severity index.

Table 3.2: Identified SNPs with an unadjusted p value less than 0.001 within 'NC6GEM' x 'NC7GEM' population on the research station trial in 2022 and 2023.

CH[i]	P (Mbp)[ii]	R[iii]	A[iv]	AE[v]	Year
0	0.45	A	G	1.353	2023
2	2.83	C	T	1.247	2023
2	6.50	T	A	1.247	2023
2	22.65	G	A	1.353	2023
2	35.82	G	A	1.291	2023
4	41.11	A	G	0.648	2022
4	58.19	A	G	1.247	2023
4	64.43	G	A	1.247	2023
6	13.92	T	C	1.067	2023
9	67.01	T	C	0.700	2022
9	67.01	A	G	1.205	2023
11	7.38	T	-	1.291	2023
11	9.11	A	G	1.353	2023
11	10.19	T	C	1.196	2023
11	11.71	G	T	1.499	2023
11	15.82	A	G	1.499	2023
11	17.46	A	G	1.353	2023
11	20.18	T	A	1.353	2023
11	25.38	C	T	1.353	2023
11	30.08	A	C	1.070	2023
11	33.91	A	T	1.053	2023
11	36.32	T	A	1.499	2023
11	45.60	T	G	1.247	2023

[i] Chromosome
[ii] Reference allele A = Adenine, T = Thymine, G = Guanine, C = Cytosine, - = deletion
[iii] Alternative allele A=Adenine, T=Thymine, G=Guanine, C=Cytosine, - = deletion
[iv] Additive effect of the marker under a fixed effect model, on 0-12 disease severity index.

Table 3.3: Identified SNPs with an unadjusted p value less than 0.001 within 'NC6GEM' x 'NC9BS' population on the on-farm trial in 2022 and 2023

CH[i]	P (Mbp)[ii]	R[iii]	A[iv]	AE[v]	Year
0	2.88	G	-	1.005	2022
0	8.74	-	C	0.849	2023
2	34.67	A	G	0.849	2023
3	25.02	T	A	1.268	2022
3	27.89	G	C	0.654	2023
3	32.51	A	G	1.131	2022
3	34.59	T	A	0.849	2023
3	36.24	A	G	1.121	2022
4	16.62	T	C	0.849	2023
4	27.79	G	A	0.849	2023
5	31.17	T	C	0.849	2023
5	59.66	A	-	0.857	2023
6	1.37	T	A	0.522	2023
6	17.36	C	T	0.621	2023
6	20.64	A	T	1.203	2022

[i] Chromosome
[ii] Reference allele A = Adenine, T = Thymine, G = Guanine, C = Cytosine, - = deletion
[iii] Alternative allele A=Adenine, T=Thymine, G=Guanine, C=Cytosine, - = deletion
[iv] Additive effect of the marker under a fixed effect model, on 0-12 disease severity index.

Table 3.4. Identified SNPs with an unadjusted p value less than 0.001 within 'NC6GEM' x 'NC9BS' population on the research station trial in 2022 and 2023.

CH[i]	P (Mbp)[ii]	R[iii]	A[iv]	AE[v]	Year
3	44219687	A	G	1.768	2023
4	16566292	T	A	0.937	2022
9	67909913	A	G	1.768	2023
10	1279292	A	T	1.024	2022
10	62984027	A	G	1.768	2023

[i] Chromosome
[ii] Reference allele. A=Adenine, T=Thymine, G=Guanine, C=Cytosine
[iii] Alternative allele. A=Adenine, T=Thymine, G=Guanine, C=Cytosine
[iv] Additive effect of the marker under a fixed effect model, on 0-12 disease severity index.

APPENDIX

SUPPLEMENTAL FIGURES

Figure S3.1: Examples of irregular stunting in the research station trial in 2023. A B and C were all taken at the same time point, representing atypical stunting for 74 days after transplanting.