CHAPTER 5

Impact of Soil Moisture Regimes on Wilt Disease in Tomatoes: Current Understanding

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1. INTRODUCTION

Ralstonia solanacearum is a causal agent of vascular wilt disease in more than 200 crop species, including the tomato. *R. solanacearum* is a strict soil-borne pathogen and thrives in moist soils (Van der Wolf et al., 1998). The bacterium can live for years in an infected field, and has been reported to persist for 12 months in potato fields (van Elsas et al., 2000). The sources of inoculum for agricultural fields are irrigation and surface water, weeds, infested soil, latently infected propagative plant material, and contaminated farm tools and equipment. The bacteria exhibit subterranean movement and spread from the infected plants' roots to the healthy ones (Hayward, 1991). *R. solanacearum*-caused wilt in tomato amounts to a 35%–90% yield loss under high temperatures and high moisture conditions (Singh et al., 2015). *R. solanacearum* colonizes the nutrient-poor xylem vessels, which are characterized by dead tracheary elements that have a relatively low osmotic pressure, which makes the pathogen penetration easy (Yadeta and Thomma, 2013). Vasse et al. (1995) observed that in tomatoes, the bacteria are attracted to the root wounds through an unknown mechanism, and stick to the epidermal cells' surface. The bacteria

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then colonize the intercellular spaces in the root epidermis, followed by successive invasion of the intercellular spaces of the inner cortex, forming biofilm structures and residing there as intercellular micro-colonies (Vasse et al., 1995; Mori et al., 2016). Through the root endodermis, the *R. solanacearum* in tomatoes subsequently colonizes the vascular parenchyma. Xylem-dwelling *R. solanacearum* releases a virulence factor, extracellular polysaccharides (EPS), that increase the viscosity of the xylem fluid, block the xylem vessels, and hinder the water transport; making the plant succumb to bacterial wilt (McGarvey et al., 1999). Interestingly, under field conditions, the *R. solanacearum* incidence was drastically reduced with the decrease in soil moisture content (van Elsas et al., 2000; www.data.gov.in; Mondal et al., 2014), thus highlighting the importance of soil and plant water levels in determining the disease severity and spread. However, the interaction between the two stressors and the impact of their concurrence on the plant, specifically in tomatoes, has not been studied. In this chapter, we have reviewed the potential interaction between the *R. solanacearum* and different soil moisture regimes, and the consequent impact of the combined stress on the plant. We also present different instances of the interaction between low soil moisture and other major xylem-dwelling pathogens, such as *Xanthomonas campestris* (causes black rot and bacterial wilt), *Xylella fastidiosa* (causes bacterial leaf scorch and Pierce's disease), and *Tomato spotted wilt virus* (causes tomato spotted wilt). We have also reviewed the anatomical and physiological changes that are adopted by the tomato plants that are resistant to drought or wilt.

2. STRESS INTERACTION: AT THE JUNCTURE OF THE RHIZOSPHERE AND THE ROOTS

The soil moisture influences and interacts with the wilt pathogen in different ways. It (i) manipulates the survival of the *R. solanacearum* in soil and regulating its inoculum density, (ii) alters the pathogen infection, and (iii) modulates the pathogen progression after infection. The first two points of interaction occur in soil and at the plant's root surface in the rhizospere. The interaction described in the third point occurs *in planta* and is dependent on the soil moisture level.

2.1 Impact of Soil Moisture on Pathogen Multiplication

In a study conducted in potato fields, the *R. solanacearum* inoculum was negatively impacted by severe drought (van Elsas et al., 2000). It caused severe wilt under high moisture in the early part of the potato growing season. The coincidence of average rainfall and *R. solanacearum*-caused disease prevalence data from West Bengal, India, further supports the earlier study and reflected a negative correlation between the two (Fig. 1; Mondal et al., 2014). However, other wilt-causing pathogens, for example, *Xanthomonas campestris* pv. *musacearum* (*Xcm*, causes wilt in bananas) caused severe disease in banana plants maintained under low soil moisture as compared with normal soil moisture levels

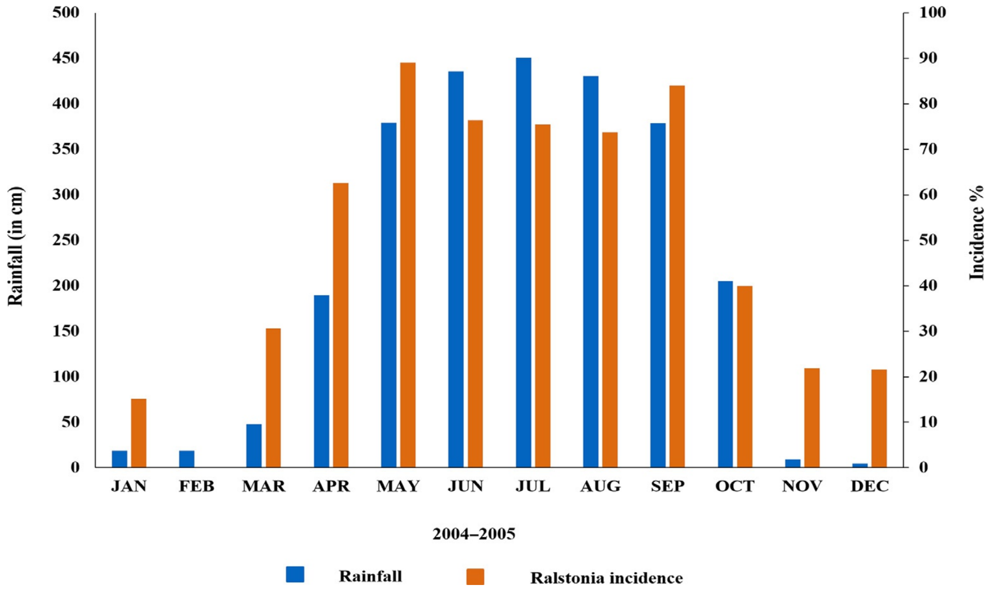

Fig. 1 Co-occurrence pattern of average rainfall and *Ralstonia* bacterial wilt incidence during 2004–2005 in West Bengal: figure displays the relationship between monthly precipitation (www.data.gov.in/) and *Ralstonia solanacearum* wilt incidence in tomatoes during 2004–2005 in West Bengal (Mondal et al., 2014). During periods of low rainfall (low soil moisture conditions), there is reduced *R. solanacearum* wilt incidence when compared with high rainfall (high soil moisture conditions) periods.

(Ochola et al., 2015). Drought stress aggravated the disease severity and advancement of *X. fastidiosa* in *Parthenocissus quinquefolia* vine (McElrone et al., 2001). Under low soil moisture conditions, *Streptomyces scabies* (causes common scabs in potatoes) multiplied to high numbers in the rhizosphere, which increased the chances for disease in potato plants (Goto, 1985). In the presented reports, soil moisture deficit effects were significant for the disease incidence and severity, whereas the resultant stress interaction between low soil moisture levels and the rhizospheric wilt pathogens varies depending upon the nature of the pathogen and the plant species.

2.2 Impact of Soil Moisture on Pathogen Infection

Despite the low soil moisture-inflicted reduction in the pathogen inoculum in the rhizosphere, the residual amount of inoculum potentially causes infection in a drought-stressed plant. *R. solanacearum* enters plant roots through small entry points, such as those generated during lateral root emergence (Vasse et al., 1995). During drought stress, the plant tends to increase the root biomass (to absorb maximum available water) by developing lateral roots. This, as such, provides an opportunity for a proximal pathogen to infect and make the plant more prone to *R. solanacearum* infection. Moreover, the concentration, composition, and the diffusion of host plant root exudates (involved in chemotaxis) may be altered under low soil moisture conditions such as to attract the bacterium to plant roots and cause infection. Thus, the low soil moisture provides the bacteria with an opportunity to infect. Moreover, the resulting stress weakens the defense systems of the tomato, allowing *R. solanacearum* to proliferate *in planta* and cause disease.

2.3 Drought and Wilt: Interactions at the Plant Interface

By influencing the host plant anatomy, the low soil moisture level can restrict the pathogen movement and the resultant disease symptoms. Under drought stress, the tomato plants develop reduced vessel size, diameter, and the number of pits leading to a compact xylem. *R. solanacearum* moves from vessel to vessel, and during its course of movement, it degenerates pit membranes (Nakaho et al., 2000). The anatomy of the xylem under such situations is one of the deciding factors of plant resistance. In a wilt-resistant tomato cultivar, the thickened pit membranes halted pathogen movement in xylem vessels, which was not observed in cultivars susceptible to *R. solanacearum* (Nakaho et al., 2000; Kim et al., 2016). Unlike the resistant tomato cultivar, a large number of the *R. solanacearum* population was observed in the vessels of both the primary and secondary xylem of a wilting-susceptible tomato cultivar. The resistant trait (to vascular wilt) was therefore associated with the thickened pits of the plant that restrained *R. solanacearum* colonization in the stem, rather than by resistance during entry through the roots (Grimault and Prior, 1993). Thus the reduced pits under low soil moisture imply reduced movement, and spread of the pathogen across the plant.

The drought stress leads to the stomatal closure, which in turn reduces the transpiration pull. Transpiration pull in the xylem vessels is one of the possible means of longitudinal movement of pathogens *in planta*. This is akin to the instance where the longitudinal spread of *R. solanacearum* in the aerial parts is inhibited in resistant cultivars (to vascular wilt) than in susceptible tomato cultivars (Grimault et al., 1994; Prior et al., 1997). Under a scenario where the low water availability restricts *R. solanacearum* movement across plant tissues, drought confers plant tolerance to wilt pathogens by restricting the systemic spread of the bacterium.

Reportedly, a low soil moisture level has been implicated in reducing the *in planta* multiplication and spread of other wilt-causing pathogens. It has been shown that the banana wilt (caused by *Xcm*) development was hastened by continued water stress, as compared with instances in which the plants were maintained under control conditions or were stressed only before inoculation. The observations in these plants suggested that both timing of infection (before or after water stress), and duration of exposure to water stress (continued after infection) are important in determining the multiplication and spread of *Xcm* in the vascular tissues of the banana (Ochola et al., 2015). Drought stress ameliorated the viral wilt disease symptoms in tomato plants by limiting the *in planta* movement of *Tomato spotted wilt virus* (Córdoba et al., 1991).

The xylem sap is a nutrient-poor medium with extremely low levels of organic and inorganic compounds (Siebrecht et al., 2003). Reportedly, the composition of xylem sap is drastically influenced by drought stress, where the nitrate, ammonium, potassium, and phosphate concentrations are reduced by about 50% (Bahrun et al., 2002; Jia and Davies, 2007). Importantly, the *R. solanacearum* wilt-resistant tomato exhibited reduced nitrate levels in comparison with the wilt-susceptible ones (Hacisalihoglu et al., 2008). This suggests an inherent dependency of the pathogen on the inorganic compounds for its pathogenicity, and may explain the negative effect of drought stress on pathogen infection.

3. PHYSIOLOGICAL CHANGES DURING STRESS INTERACTION

Based on the positive or negative interaction between the two stresses, the plant can exhibit a response additive (aggravated response), or canceled (reduced effect). In a Plant—*R. solanacearum*—drought interaction, wilting can be considered as one of the parameters to assess the net impact of the stress interaction. When wilt caused by vascular-limited pathogens occurs in drought-stressed plants, the damage may be much greater than in plants in a high soil moisture situation. Mostly, due to the xylem occlusion in the wilt infection, the plant is predisposed to drought stress (Yadeta and Thomma, 2013). Additionally, tyloses that are formed by the parenchyma cells in response to wilt pathogens, can further contribute to xylem occlusion (Fradin and Thomma, 2006; Klosterman et al., 2009; Beattie, 2011). Plants develop tyloses in the xylem vessels to inhibit pathogen movement and spread to adjoining vessels (Wallis and Truter, 1978; Rahman et al., 1999). These tyloses also block vessels and thereby inhibit water transport. In wilt-resistant tomato cultivars, tyloses blocked the colonized vessels, and although they restricted the bacterial spread, they predisposed the plant to water deficit. On the other hand in *R. solanacearum*-susceptible tomato cultivars, no tyloses were observed in the infected vessels, which failed to restrict the bacterial spread, and eventually the plant succumbed to wilt. Thus, in a wilt-resistant plant, tylose formation can make a plant more prone to drought stress (Fradin and Thomma, 2006).

Other vascular wilt pathogens also inflict dehydration in host plants. As a result of the dehydration response, the plant closes its stomata, which leads to reduced photosynthesis and decreased partitioning of photo-assimilates to the roots. The eventual reduced root growth in infected plants further predisposes a plant to low soil moisture stress (Yadeta and Thomma, 2013). *X. fastidiosa* causes an infection that prompts drought stress in alfalfa plants (Daugherty et al., 2010). Tomato plants infected with *Verticillium dahliae* (which causes Verticillium wilt) exhibited decreased leaf water potential (Ayres, 1978). The reduced shoot hydraulic conductance caused by *X. fastidiosa* infection acts additively when imposed upon by drought stress (McElrone et al., 2003). *X. fastidiosa*-infected grape plants displayed the early occurrence of embolism (the formation of air bubbles), which correlated with decreased xylem conductivity and aggravated drought stress (Pérez-Donoso et al., 2007).

These examples explain that the xylem inhabitation by wilt pathogens exposes the plant to more water-limiting conditions, such that plants become susceptible to drought stress. However, in a contrasting instance, the *Arabidopsis thaliana* infected with the xylem occluding pathogen *Verticillium longisporum* (the causal agent of vascular wilt) was tolerant to drought stress. *V. longispourum* induced the expression of the vascular-related NAC domain (*VND7*) gene in host plants and activated de novo xylem formation, which led to enhanced water storage or conductance capacity under drought stress conditions (Reusche et al., 2012).

4. CONCLUSIONS AND FUTURE PERSPECTIVES

The emergence of water limitations and increased wilt disease pressure are of serious concern to agriculture in a changing climate. In some cases of stress interaction, the plant responses under combined stressors are canceled, and some of the responses during stress interaction are neutralized by one of the dominant stressors. In the case of drought and wilt pathogens, plants display a negative interaction where the *R. solanacearum* wilt is favored by high soil moisture, and the incidence of wilt under low soil moisture is reportedly reduced. As one of the measures to control *R. solanacearum* infection in fields, the use of well drained and leveled fields is recommended. The low moisture content in the soil reduces bacterial rhizospheric inoculum, but induces lateral root development, which in turn increases the potential entry points for *R. solanacearum*. At the same time, drought stress-inflicted structural alterations, such as reduced cortical cell size, hinder *R. solanacearum* entry in the host plant. The host plant exhibits inhibited transpiration pull under low soil moisture regimes, which restricts the longitudinal movement of *R. solanacearum* in the plant. Evidence shows that both drought stress and *R. solanacearum* infection reduce the xylem vessels (Fig. 2). The production of EPS, a key virulence factor of *R. solanacearum*, is enhanced under drought conditions. Additionally, the induced tylose production in response to pathogen defense will further clog the xylem vessel and make the host plant more prone to drought stress. The low soil moisture may also intensify the wilt symptoms caused by the pathogen in drought-stressed plants. The wilt caused by *R. solanacearum* involves irreversible damage to the vasculature, but drought recovery is common. The timing and concentration of each stress, in this case, would be the determining factor in shaping the net impact in the tomato plant, which divergently affects plant fitness under combined drought stress and *R. solanacearum* infection. Under low soil moisture conditions, tomato plants reduce the vessel's size and increase thickening of pit walls, as compared with the ones under high soil moisture conditions. The more compact vasculature under low soil moisture conditions potentially restricts the *R. solanacearum* movement. Thus, as a means of predicting the outcome of plant responses to combined low soil moisture and *R. solanacearum* stress, one would need to focus on the common anatomical and physiological features modulated by these two stressors during their individual occurrences.

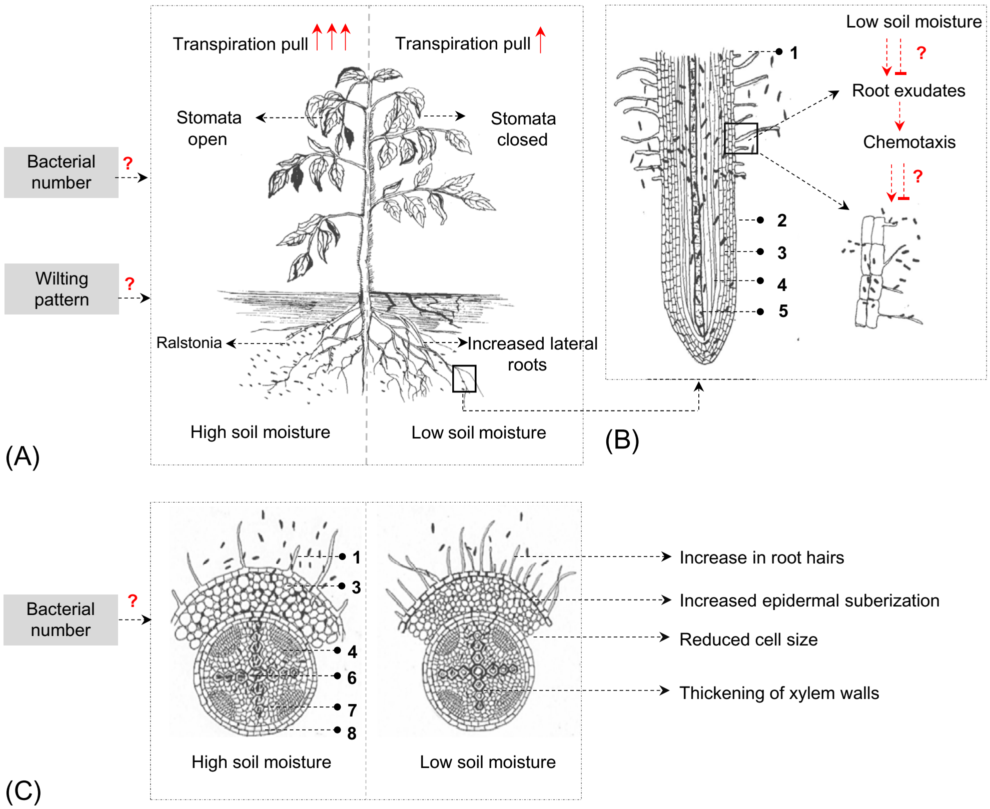

Fig. 2 Hypothetical model on effect of soil moisture on *Ralstonia solanacearum* infection in the tomato: (A) an illustration of the morpho-physiological responses of tomatoes to *R. Solanocearum* (Rs) at the above-ground and below-ground levels under high soil moisture (left side) and low soil moisture (right side) conditions. The low moisture content in the soil reduces the bacterial (Rs) population, but induces enhanced lateral root development in plants, which may increase the chances of Rs infection (through the emerging lateral root hairs). Moreover, the plants tend to close their stomata under low soil moisture, which may inhibit the transpiration pull, and hence the movement of Rs in an aerial part of the plant. (B) shows critical steps involved in Rs—root—rhizosphere interaction with a specific focus on the role of tomato root exudates and the impact of soil moisture on it. Under low soil moisture, the concentration and composition of the root exudates may change, which may regulate chemotaxis. (C) compares a transverse section of the root displaying the potential variations under different soil moisture regimes impacting Rs infection in the tomato. A plant growing under low moisture conditions has been shown to display increased root hair development, epidermal suberization, and reduced cell size, all of these posing hindrance to the Rs entry.

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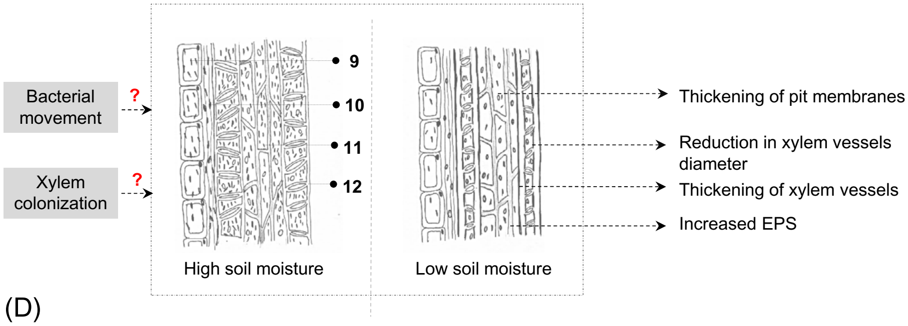

Fig. 2, cont'd (D) shows changes in the xylem tissue due to soil moisture variation, impacting Rs colonization and movement. Under low soil moisture conditions, tomato plants tend to reduce the vessel size, and increased thickening of pit walls as compared with those under high soil moisture conditions. The more compact vasculature under low soil moisture conditions potentially restricts the Rs movement. As a means of virulence, Rs tend to release exopolysaccharides (EPS) and clog the xylem vessels. Evidence from other related species has shown enhanced EPS production under drought stress. At present, the conflicting variations in the plant and the pathogen interactions under low soil moisture makes it difficult to draw any inference in the intensity of infection under low soil moisture as compared with high soil moisture. Key: 1, root hair; 2, epidermis; 3, cortex; 4, phloem; 5, xylem; 6, meta-xylem; 7, proto-xylem; 8, endodermis; 9, xylem parenchyma; 10, fibers; 11, tracheids; 12, xylem vessels. *Red upward arrows*: the intensity and direction of transpiration pull; *blunt arrows*: inhibition; *red downward arrows*: positive regulation of the process. The boxes with a question mark represent the scenarios that need to be unraveled upon study of actual combined stressors. *Rs*, *Ralstonia solanacearum*; *EPS*, exopolysaccharide.

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