A Comparative Study of the Pathogen Load in Wilted and Healthy looking Tomato Seedlings Inoculated by the Leaf Clip Method

3.1 Abstract

Ralstonia solanacearum causes a lethal bacterial wilt disease in numerous plants distributed globally. While studying its pathogenicity in the model host plant tomato, it is often observed that several of the inoculated susceptible plants escape the disease, referred to as escapees. The reason for this mysterious behavior of the pathogen is yet to be explored. Here, we have studied *R. solanacearum* association and load in escapees in comparison with the wilted plants. The experiment was carried out on two leaves cotyledon stage tomato seedlings inoculated by the leaf clip method. Bacterial load in the inoculated seedlings was measured at different days post inoculation (DPI) starting from the inoculation day. Comparison of bacterial load between the wilted and healthy looking inoculated leaves of the seedlings on any particular DPI suggested that bacterial loads in both the seedlings were not significantly different. Bacterial load in a healthy looking seedling on specific DPI was higher than that of a wilted seedling of previous DPI. Seedling that wilted during early DPI had lesser bacterial load than seedlings that wilted during later DPI. Our observations suggest that bacterial load within a seedling cannot be a sole determining factor for the disease appearance in tomato seedlings. As disease is a plant phenotype, we believe that tomato plants susceptibility for disease to occur by *R. solanacearum* is a complex process.

3.2 Introduction

How does a pathogen cause disease in its host plant is a major interest in host pathogen interaction research. In case of some pathogenic organisms, their mere presence in host may lead to the disease occurrence. These types of pathogens are likely to have limited host infection ability as well as within a host they might have limited niche to survive. There is another type of pathogens that live within the host for several days and colonizes different regions of the host before causing the disease. The disease caused by this pathogen is likely to be lethal for the organism. Therefore host response to all pathogens may not be the same and all pathogens' offense to host

may not be the same in all aspects. This is important for having different bacterial pathogen and host interaction models in plant pathogen research.

R. solanacearum, the devastating wilt pathogen, infects many host plants. It is a systemic pathogen. During pathogenesis, *R. solanacearum* rapidly and effectively colonizes entire host vascular system and then kills the plant [1]. The bacterium has been evolved with well equipped genetic means that facilitates it to adapt to different environmental niches and cause disease in vast diversity of plants covering herbs to trees. The pathogen expresses different genes for its adaptation to conditions encountered during the entire infection cycle. Interestingly, it can colonize some hosts (distant hosts) asymptomatically as latent infections maintaining high bacterial populations for example in weeds [1,2,3,4]. The disease causing mechanism of this mysterious pathogen is quite complex and may vary from host to host. Very less has been understood about the colonization as well as pathogenicity phase of the bacterium as very few plants have been considered as model host plants to address this exciting behavior of this complex pathogen.

In tomato plant, one of the model hosts of *R. solanacearum*, stem pricking and soil drenching methods are widely used to study its pathogenicity [5]. It is quite intriguing to notice that among all the tomato plants inoculated with the pathogen through any inoculation mode, about 10-20% plants don't exhibit disease symptoms or escapes the disease, which can be called as escapees for that study period (we are also using the term "healthy looking seedlings" to refer escapees). Although several exciting insights into disease causing mechanisms have been uncovered [6,7,8,9,10,11], this enigmatic behavior in its pathogenicity has not yet been explained.

Recently, a simple leaf clip inoculation method has been developed in our laboratory to study pathogenicity in seedlings stages of tomato host which is very consistent and reproducible [12]. This method resembles in several ways the pathogenicity study done in grown up tomato plants by soil drenching method as *hrpB, hrpG, phcA* and *gspD* mutants of *R. solanacearum* were found to be deficient in virulence. In this method also, 10-20% pathogen inoculated tomato seedlings escaped the disease (Fig.3.1). However, the leaf clip method one way is different from the soil drenching as that disease appeared from the site of inoculation and spread gradually

downwards to the root region. Moreover, this method avoids any influence of xylem residing endophytes as well as soil microflora which plays important role in the pathogenicity of this bacterium. Therefore, the escapees found in case of tomato seedlings inoculated by the leaf clip method provided us a better opportunity to understand the enigmatic behavior of *R. solanacearum* in its pathogenicity.

Fig.3.1: Picture showing escapee tomato seedlings inoculated with *R. solanacearum* after ten days of inoculation. Tomato seedlings were inoculated with F1C1. Some of the inoculated tomato seedlings were looking healthy; they escaped the disease after ten days post inoculation.

In this study, we have tried to address this question by doing inoculation study in one leaf *vs* two leaves employing 6-7 days old tomato seedlings. We observed disease progression in terms of escapees number under different bacterial concentrations in the inoculum and counting bacterial population in wilted as well as in escapee seedlings. We observed higher number of escapees in case of one leaf inoculation than two leaves inoculation with same pathogen concentration and escapee number increases with decreasing concentration of pathogen in the inoculums. Most importantly, our study revealed the presence of high bacterial population in escapees tomato seedlings which suggest that bacterial presence may not be the only factor in causing disease in susceptible hosts.

3.3 Material and Methods

3.3.1 Bacterial strains and growth conditions

Wild type *R. solanacearum* F1C1 and mCherry tagged F1C1; TRS1016 were grown in BG medium [13] supplemented with 0.5 % glucose at 28°C for 48 h. For

bacterial suspension culture, F1C1 cells were grown in 10 ml BG broth kept in a shaking incubator (Orbitek, Scigenics, India) maintained at 28°C and 200 rpm. Gentamycin antibiotic was used to grow TRS1016 at a concentration of 50μg/ml. For the pathogenicity assay, inoculums were prepared following the steps as described in section 2.3.4 of the previous chapter.

All bacterial culture media components, chemicals and antibiotic used were procured from Hi-Media, Mumbai, India. Tomato seeds were bought from Sonitpur Nursery, Tezpur, Assam. Plastic wares were purchased from Tarsons, Kolkata, India; glasswares were bought from Borosil, Kolkata, India.

3.3.2 *R. solanacearum* pathogenicity study in tomato seedlings by the leaf clip method

Pathogenicity assay of *R. solanacearum* F1C1 in tomato seedlings was performed by the leaf clip method as described in the previous chapter. We used disease susceptible Durga (Ruby) cultivar of tomato seeds for this study. Tomato seeds procured from the nursery were first washed vigorously with sterile distilled water and transferred to wet cotton and tissue paper bed allowing them to germinate. The germination tray was kept at growth chamber (Orbitek) maintained at 28°C temperature, 75% relative humidity and 12 hr photoperiod. After 7 days, two cotyledon leaves staged germinated seedlings were gently transferred to 1.5 ml microfuge tube to which 1 ml of sterile distilled water was added. Then a pair of sterile scissors were dipped in the bacterial suspension of F1C1 (~10^9 CFU/ml or of required CFU) and a portion of the cotyledon leaves from the tip were clipped off in each tomato seedling kept in microfuge tube.

For one leaf inoculation study, one cotyledon leaf of each seedling was inoculated with bacterial inoculum and the other cotyledon leaf was inoculated with sterile distilled water. For two leaves inoculation, both the cotyledon leaves were inoculated with bacterial inoculum of required concentrations. The schematic representation of the strategies used has been shown in Fig.3.2.

We investigated disease appearance and progression in tomato seedlings in relation with pathogen concentration in the inoculums. For that study, we used ~10^9 CFU/ml, 10^8 CFU/ml, 10^7 CFU/ml and 10^6 CFU/ml concentrations of *R.*

solanacearum TRS1016 in the inoculums. The saturated inoculum (~10^9 CFU/ml) was serially diluted with sterile distilled water to obtain respective concentrations. Then we inoculated both cotyledon leaves of tomato seedlings and followed the disease progression.

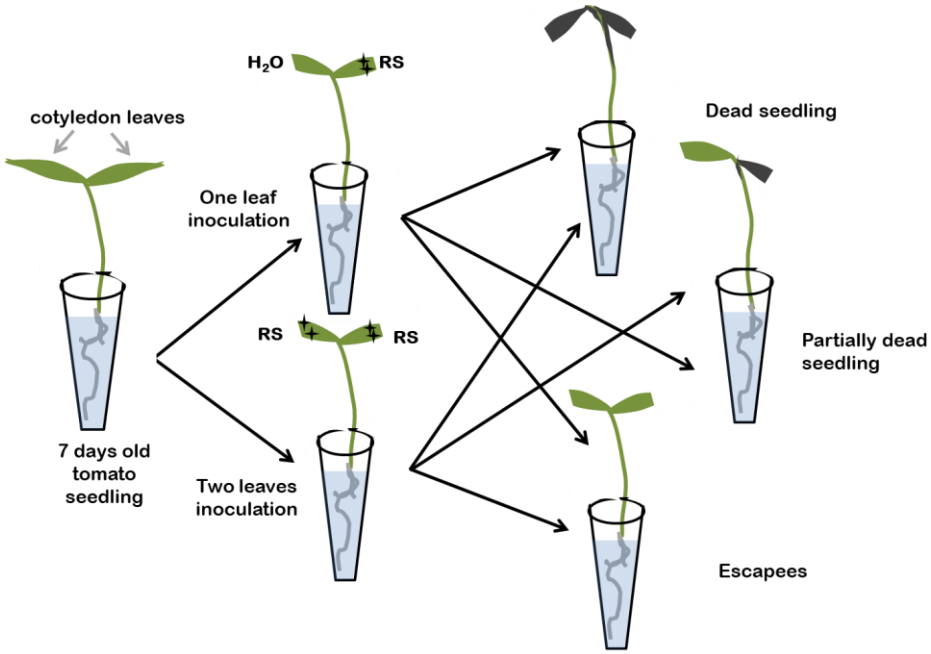


Fig.3.2: Schematic representation depicting pathogenicity assay set up in tomato seedlings by the leaf clip method. 7 days old tomato seedlings were inoculated with *R. solanacearum* (RS) by one leaf inoculation as well as by two leaves inoculations. Dead, partially dead and escapee were the expected outcomes of inoculated seedlings.

In all the pathogenicity assays, 40 seedlings were inoculated in a set and three independent experiments were performed with two replicates. Same number of seedlings mock inoculated with sterile distilled water was kept as control. After inoculation, seedlings were transferred to a growth chamber (Orbitek) maintained at 28°C temperature, 75% relative humidity and 12 h light and 12 h dark cycle. Sterile distilled water was added everyday to sustain the inoculated seedlings in the microfuge tubes and seedlings were carefully observed for disease progression till 7^{th} DPI.

3.3.3 *R. solanacearum* colonization study in tomato seedlings

To check the colonization of *R. solanacearum* in tomato seedlings, we used mCherry marked F1C1 strain, TRS1016 and inoculated in both the cotyledon leaves by the clip inoculation as described above. Starting from the day of inoculation till 6^{th}

day, we checked the pathogen growth and colonization. Each day, we took four seedlings, washed twice with sterile distilled water and then kept submersed in 70% ethanol for 1-2 min for surface sterilization. Further, we washed seedlings with sterile distilled and were observed for red fluorescence under the fluorescence microscope (EVOS FL, Life technologies) equipped with 4X magnification.

3.3.4 Bacterial population count in inoculated tomato seedlings

To investigate the bacterial load in infected seedlings, 7 days old tomato seedlings were inoculated with TRS1016 at a concentration of ~10^9 CFU/ml following the method as described above. Bacterial load in the inoculated seedlings were counted at 0, 1, 2, 3, 4, 5, 6, 7 days post inoculation (DPI). We counted bacteria in seedlings that exhibited the wilting disease and also in seedlings that appeared healthy at different DPI. For counting bacterial load, only the leaf part of the seedlings was considered (Fig.3.3). A total of 3 seedlings i.e. six inoculated leaves were taken per set for studying the bacterial load. The average bacterial load per one inoculated leaf was calculated by dividing the value by six. Considering six leaves and taking average of it might minimizes the error due to size differences of leaves in seedlings, the size of the inoculated leaf left over after the leaf clip inoculation, number of bacteria deposited initially at the time of inoculation. Leaf is only considered because the disease symptom first appears in the leaf in leaf clip inoculation method. The inoculated seedlings were dipped in 70% ethanol for 1 min followed by washing twice with sterile distilled water by keeping submerged for 1-2 minute each. The two cotyledon leaves at the stem junction were cut out as shown above (Fig.3.3). At 0, 1 and 2 DPI, all seedlings were looking healthy. From 3 DPI onwards, the escapees set were selected from healthy seedling and the disease seedling set was selected from the wilted seedlings. Samples from both the sets were homogenised separately using sterile micro pestle (Abdos, India) in microfuge tubes and then 1 ml sterile distilled water was added. Each homogenate was serially diluted with sterile distilled water and plated on BG medium supplemented with gentamycin antibiotic. In case of 0 and 1 DPI, dilutions were made 10^3 and 10^4 fold; on 2 DPI dilutions were made 10^4 and 10^5 fold; on 3 and 4 DPI, dilutions were made 10^4, 10^5 and 10^6 fold; on 5 DPI, dilution was made 10^5 and 10^6 fold and on 6 and 7 DPI, dilutions were made 10^5, 10^6 and 10^7

fold. The plates were incubated at 28°C for 48 hr. Colonies recovered in both the cases were counted. The experiment was performed in three sets for each day.

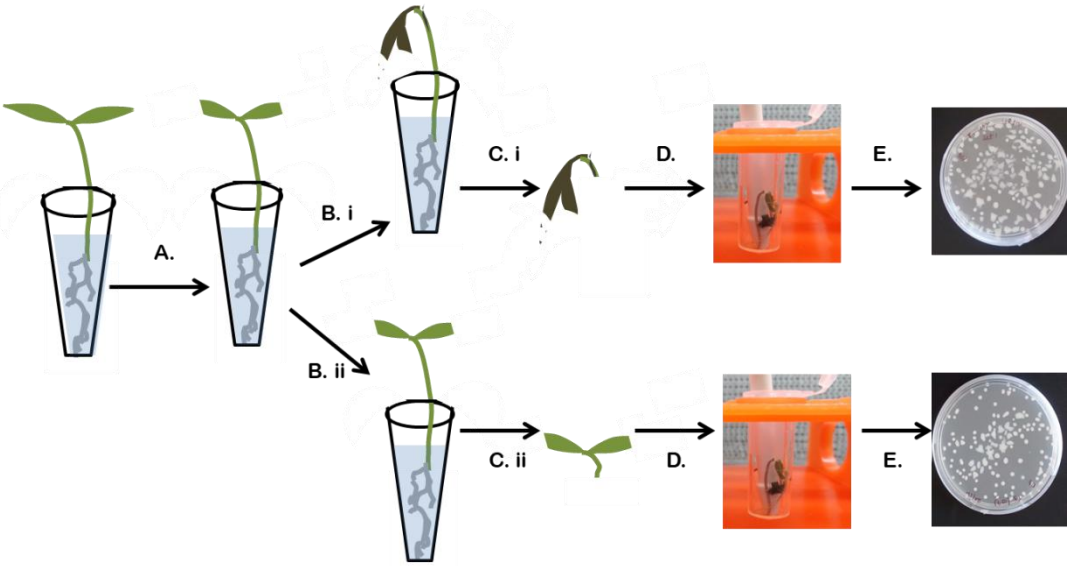


Fig.3.3: Schematic representation depicting steps followed to count bacterial load in infected seedlings. A. *R. solanacearum* inoculation in tomato seedlings via both the leaves, B. Outcomes of inoculation; i. Infected seedling and ii. Healthy looking seedling, C. Sampled out leaf portion of i. Infected seedling and ii. Healthy looking seedling after surface sterilization, D. Crushing and E. Dilution plating.

3.4 Results

3.4.1 Inoculation of *R. solanacearum* in two cotyledon leaves has a higher chance of wilting occurrence than inoculation in one cotyledon leaf of a tomato seedling

Upon inoculation in root of seedlings or grown up plants, *R. solanacearum* colonizes different part of the host before killing it. Therefore, it was believed that inoculation of the pathogen at any point in host will be sufficient to cause disease in a susceptible host plant and inoculation at different points may not cause a significant difference in disease outcome. Since each seedling has two cotyledon leaves, we decided to compare wilting in seedlings by inoculating in one leaf *vs* inoculation in two leaves.

It was consistently observed that number of seedlings killed by two leaves inoculation was more than that by one leaf inoculation. In both the cases death of seedlings started appearing from 3 DPI onwards. On 4 DPI, less than 25% seedlings were killed means more than 75% seedlings were healthy in case of one leaf

inoculation while in case of two leaves inoculation, 50% seedlings were killed on that day. The study was carried out till 7 DPI. Consistently total number of seedlings killed in case of two leaves inoculation was more than one leaf inoculation. By 7 DPI, ~ 90% seedlings were killed in two leaves inoculation whereas ~75% seedlings were killed in one leaf inoculation (Fig.3.4a). The observations were also analyzed by Kaplan-Meier survival curve [14] as shown in Fig.3.4b.

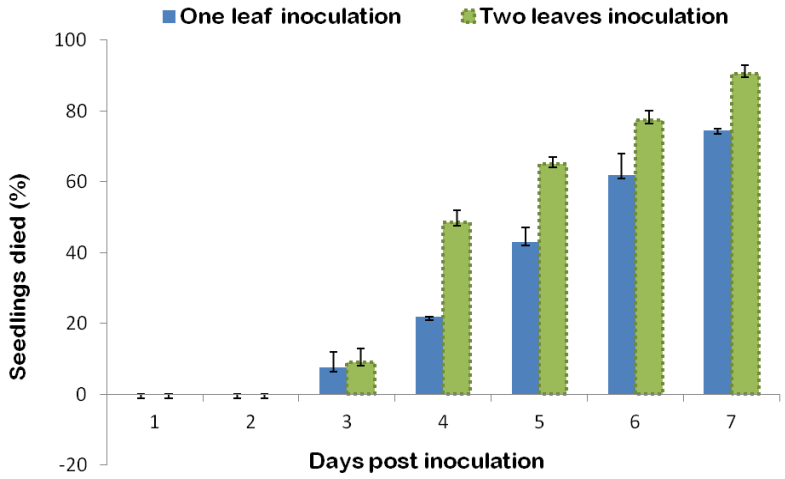


Fig.3.4a: Pathogenicity of *R. solanacearum* F1C1 in tomato seedlings inoculated in one leaf and two leaves by clip inoculation method. Tomato seedlings were inoculated with saturated concentrations (10^9 CFU/ml) of F1C1 wild type. X-axis presents DPI and Y-axis presents percentage of seedlings killed. From 3 DPI onwards, inoculated seedlings through one way and two ways started dying and distinct difference was observed in percentage of seedlings killed in both the cases in subsequent days post inoculation ($p<0.001$; log-rank test). The result is an average of three independent experiments with two replicates. Error bars are depicting standard errors.

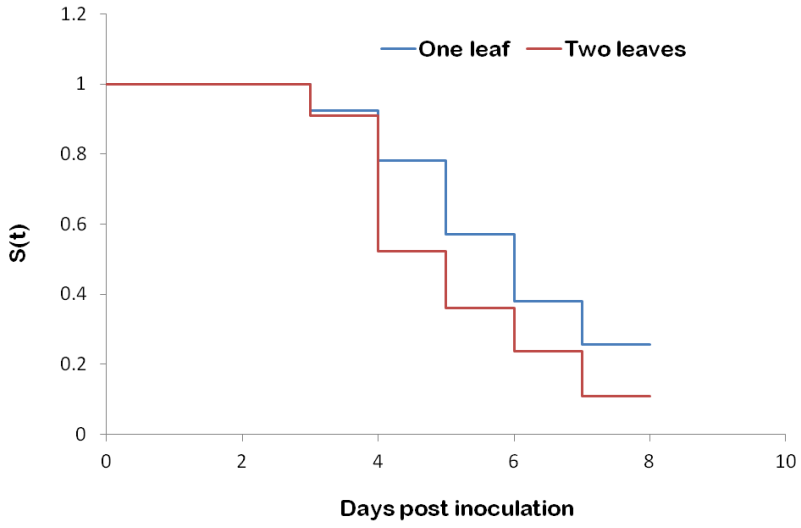


Fig.3.4b: Kaplan–Meier survival probability [S(t)] curve of tomato seedlings inoculated in one leaf and two leaves by clip inoculation method. Seedlings inoculated through one leaf showed significantly more survival probability than two leaves inoculations.

To find out if this difference in disease magnitude will be followed in lower titer of the pathogen in the inoculum, we inoculated tomato seedlings with ~10^8, 10^7 and 10^6 CFU/ml concentration of *R. solanacearum* through both one leaf and two leaves inoculations. In all concentrations, the pathogen started killing tomato seedlings from 3 DPI onwards though the number of wilted seedlings was different. In 10^8 CFU/ml, by 7 DPI, ~ 63% seedlings were killed in case of one leaf whereas ~ 83% were killed in case of two leaves inoculations. In case of 10^7 CFU/ml, ~ 53% and ~ 59% seedlings were killed in one leaf and two leaves inoculations, respectively. In case of 10^6 CFU/ml, almost similar number, ~35% seedlings were killed in both one and two leaves inoculations. Result is presented in Fig.3.5.

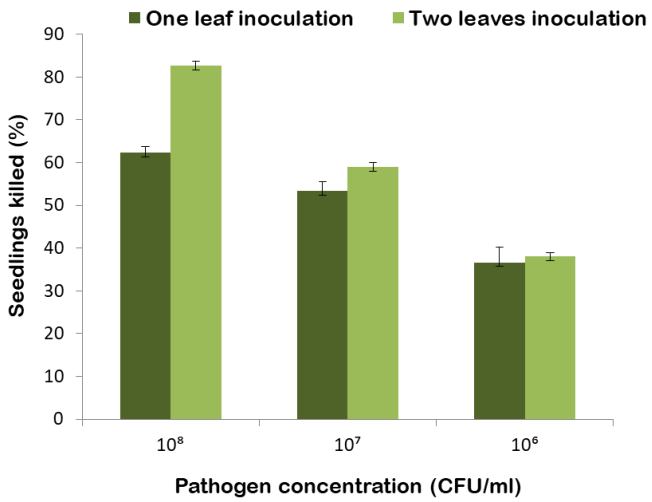


Fig.3.5: Pathogenicity of *R. solanacearum* F1C1 at lower concentrations inoculated in one leaf *vs* two leaves of tomato seedlings. Wild type F1C1 of concentrations 10^8, 10^7 and 10^6 CFU/ml were inoculated in tomato seedlings through one leaf and two leaves. We observed percentage of seedlings killed was more in two leaves than one leaf inoculation. Interestingly, the magnitude difference between one leaf and two leaves inoculated seedlings decreases from higher concentration to lower concentration of the pathogen. The result is an average of three independent experiments with two replicates. Error bars are depicting standard errors.

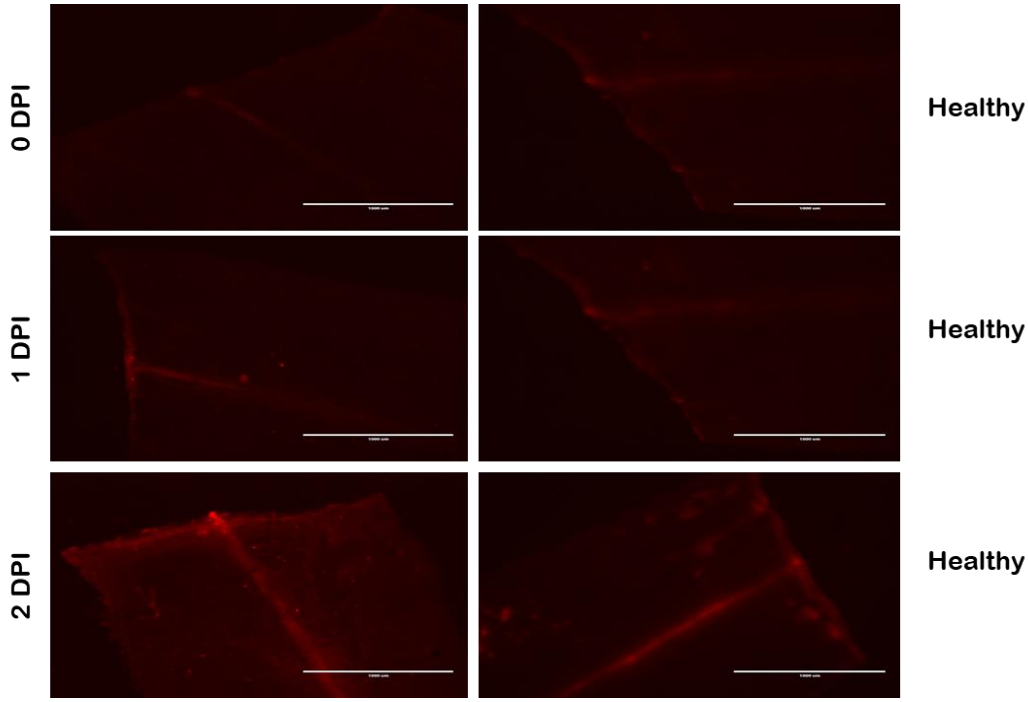
Observations from the above experiments with tomato seedlings suggested as follows: (i) escapees number is more in case of one leaf inoculation than two leaves inoculation at a given concentration; (ii) escapes number are more in case of low pathogen load in the inoculum than that in the high pathogen load in the inoculum;

and (iii) escapees number difference between two leaves and one leaf inoculation is dependent upon the pathogen load in the inoculum.

From all the experiments above, escapees were observed till 7 DPI. It is pertinent to note that all seedlings are not visually alike with regard to the size of their cotyledon leaves and size of the shoot. But there was no such observation regarding seedlings looking bigger being more susceptible or resistant to *R. solanacearum* pathogenicity than smaller looking seedlings. However, the two cotyledon leaves of a seedling were alike with regard to their size. But when inoculated in the two cotyledon leaves, the DPI by which disease appeared in the two leaves was not always the same for both the leaves. Moreover, sometimes disease appeared only in one of two leaves inoculated via both leaves. Therefore it was intriguing for us to know the reason behind the healthy looking seedlings.

3.4.2 Pathogen occurrence in the escapees like the wilted plants

We were interested to check the bacterial presence or colonization if any in escapee seedlings. For that we used mCherry marked *R. solanacearum* strain which caused disease like the wild type. It was easy for us to track the bacterial presence in seedlings under fluorescence microscope. We infected the 6-7 days old seedlings in both the leaves and observed pathogen colonization for six days post inoculations starting from the day of inoculation both in infected as well as in healthy looking tomato seedlings. At 0, 1 and 2 DPI, all the inoculated seedlings were healthy looking. From 3, 4, 5 and 6 DPI, we took seedlings from healthy looking as well as from wilted seedlings for the observation. In 0 and 1 DPI, red fluorescence was not detectable in the seedlings, may be due to low bacterial load. On 3 DPI, we observed detectable fluorescence in both healthy looking and infected seedlings suggesting the bacterial presence in both the types of the seedlings. Similarly, in 4, 5 and 6 DPI, we could observe pathogen colonization both in healthy looking and infected seedlings (Fig 3.6).


0 DPI
Healthy
1 DPI
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2 DPI
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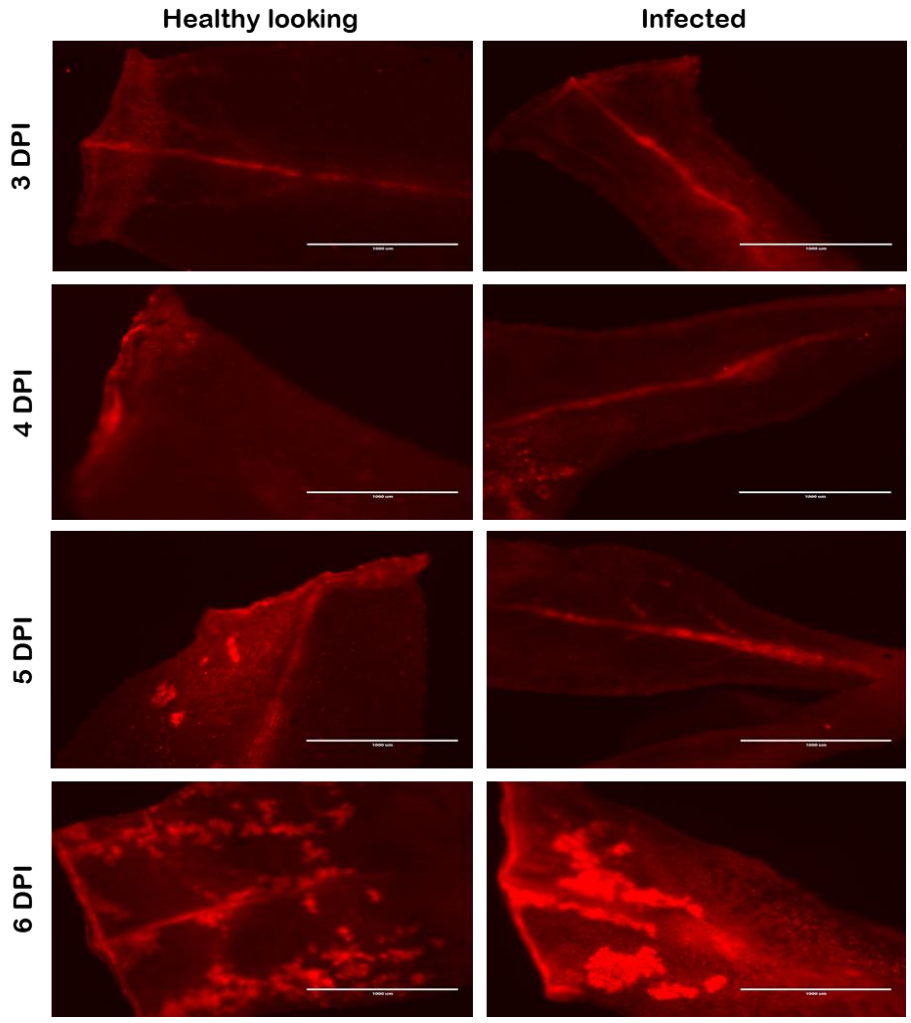
Healthy looking
Infected
3 DPI
4 DPI
5 DPI
6 DPI

Fig.3.6: Fluorescence staining of healthy looking and infected tomato seedlings. Tomato seedlings were inoculated with TRS1016 in both the leaves. On 0, 1, 2, 3, 4, 5 and 6 DPI, infected seedlings were observed under fluorescence microscope. At 0, 1 and 2 DPI, all seedlings were healthy. From 3 DPI onwards, detection of red fluorescence in inoculated leaves confirmed the bacterial colonization in both healthy looking and infected seedlings.

3.4.3 Similar bacterial load in wilted and healthy looking seedlings

After our observation of pathogen colonization in escapees like the wilted seedlings, we were then interested to quantify the bacterial load in healthy looking and wilted seedlings at different DPI. It was easy to specifically select the *R. solanacearum* colonies on gentamycin containing BG-agar plate to avoid contamination.

At each DPI, we sampled out both the inoculated leaves along with the leaf junction from the healthy looking and infected/wilted seedlings, homogenized and dilution plated the homogenates prepared in distilled water. For each day, we kept three replicates for both escapees and wilted seedlings and experiment was performed from 0 to 7 days post inoculations (Fig. 3.7). The general statistics observed regarding the pathogen growth inside the tomato seedlings was as follows: (i) per leaf the pathogen deposited was ~ 50,000 CFU to 1,00,000 CFU. At 1 DPI, 2 DPI and 3 DPI, the pathogen load increased as 10, 100 and 1000 times with regard to the load at 0 DPI. Considering the bacterium generation time is ~4.0 h in BG medium, the growth rate inside the tomato leaves was ~ 7.2 h, which was slower than the BG medium. This is expected because inside the host the pathogen has to adapt to the host defense response and will grow in a way to least provoke the host. When disease appeared in a leaf, pathogen population is around 10^8 CFU. The maximum pathogen load attained inside a leaf was observed as ~10^9 CFU.

It was interesting to observe that pathogen load was increasing gradually from 0 DPI to 6 DPI both in case of healthy looking and wilted seedlings. On 7 DPI, pathogen load decreased both in healthy looking as well as in wilted seedlings. Looking at the pathogen load in both types of leaves, it was evident that pathogen load in healthy looking leaves was similar like the wilted leaves (Fig.3.7 and Fig.3.8). Comparison among the wilted seedlings with regard to pathogen load indicates that pathogen load was not the only deciding for the disease because pathogen load was different in different DPI though both were wilted. Therefore, the leaf that got wilted

on 4 DPI might had similar bacterial load on 3 DPI like the leaf that got wilted on 3 DPI, but did not wilt on 3 DPI and wilted only on 4 DPI with higher pathogen load. This further supports why escapees having similar bacterial loads like the wilted but did not wilt. These observations suggested that tomato escapee seedlings carry high pathogen load in the inoculated areas after two leaves inoculation and was indeed sufficient to give a clue that colonization of pathogen may not be the only factor to cause disease in the hosts.

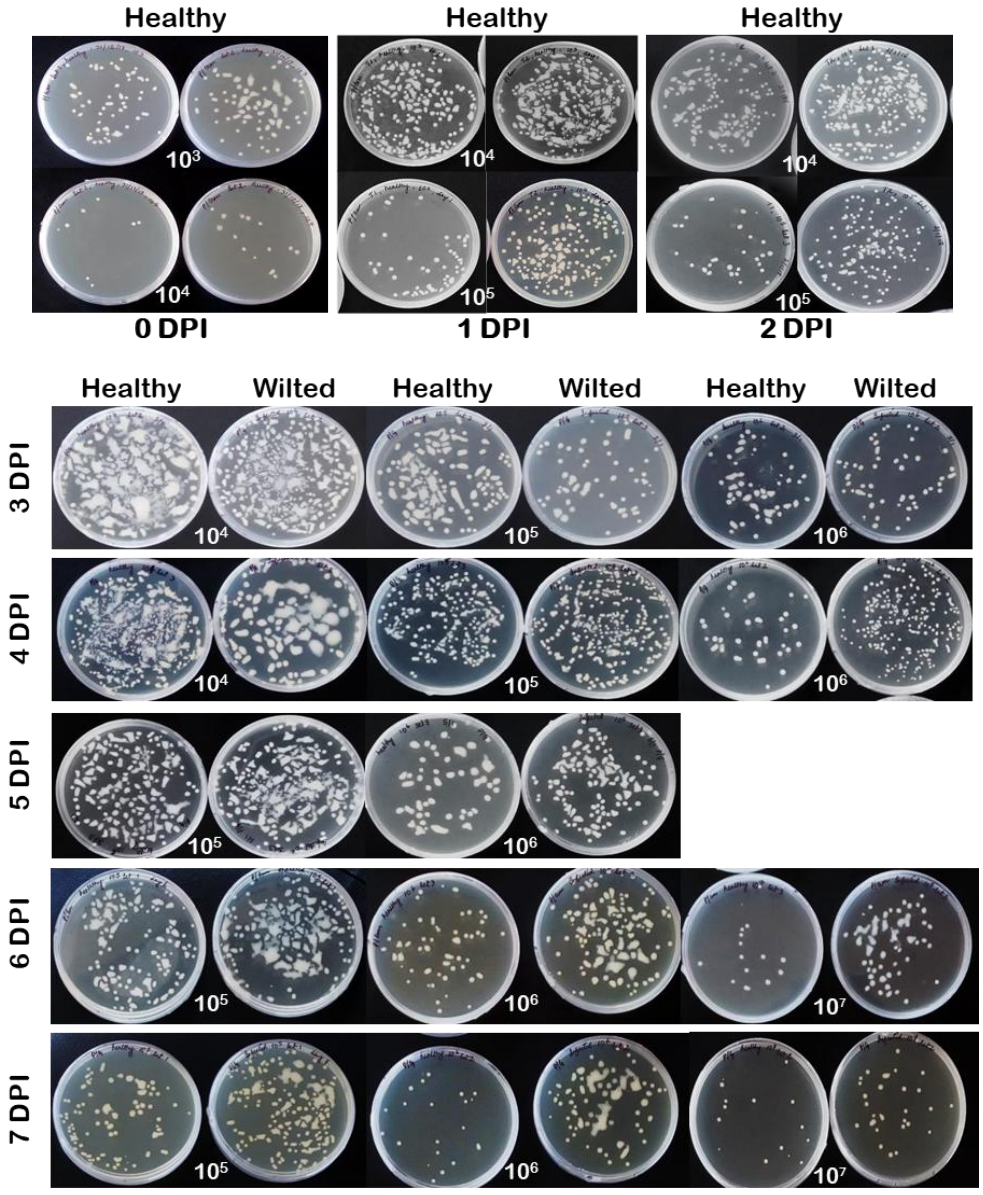

Fig. 3.7: Representative picture showing bacterial colonies isolated from leaves of healthy looking and infected tomato seedlings inoculated with *R. solanacearum*. Tomato seedlings were inoculated with saturated concentration (10^9 CFU/ml) of mCherry tagged F1C1 strain (TRS1016) in both the leaves. At 0, 1, 2, 3, 4, 5, 6 and 7 days post inoculations,

leaves of inoculated seedlings were samples out, crushed and dilution plated. Colonies were observed after 48 hr of incubation.

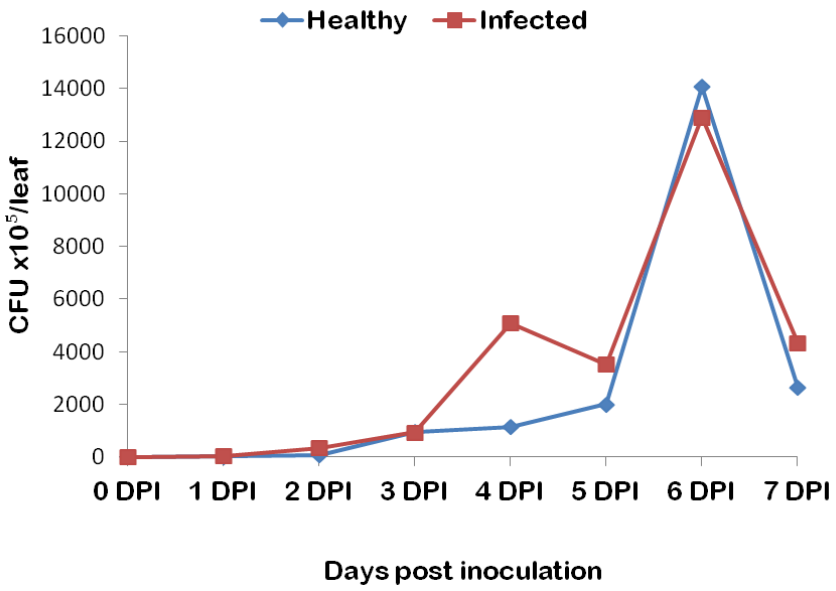

Fig.3.8: Comparative bacterial population in leaves of infected and healthy looking tomato seedlings inoculated with *R. solanacearum*. Tomato seedlings were inoculated with TRS1016 of saturated concentration (10^9 CFU/ml) in both the leaves. At each day starting from 0 DPI till 7 DPI, the healthy looking and infected seedlings were surface sterilized, sampled out leaves, homogenized and dilution plated. X-axis presents DPI and Y-axis presents CFU/ml. Colonies (CFU x10^5/ml) observed were increasing with days up to 6 DPI while in 7 DPI it dropped in both types of seedlings. All data point in this graph is an average of three independent experimental sets.

3.5 Discussion

Our study demonstrated that healthy looking inoculated seedlings have pathogen colonization and growth. We observed that pathogen load was increasing from initial day of inoculation in both healthy looking seedlings as well as in wilted seedlings and pathogen loads were not drastically different in both the cases. So it was reasoned out from bacterial load count that it was not the sole determinant of the wilting in plants. This was very important with regard to our earlier observation that two leaves inoculation results higher disease frequency than one leaf inoculation in seedlings.

One of the interesting question emerged from our two leaves inoculation *vs* one leaf inoculation study is that whether a plant fight against its pathogen independently at the site of inoculation or it fights as a whole. The observation of

more number of escapees in case of one leaf inoculation than two leaves inoculation can be explained as follows supporting both the models: (i). if we consider both the cotyledon leaves as independent entities, each leaf will fight against the pathogen independently and will have chance to escape from the pathogen attack. Hence, in one leaf inoculated seedling, the chance that the seedling will escape from pathogen will be always more than the seedling inoculated through both the leaves. Moreover, the pathogen load at the cut end of a leaf is not always the same but can be variable between two to three folds. So in case of two leaves inoculation, the chance of minimum one leaf having a higher pathogen load is higher than that in case of single leave inoculation. In addition, the amount of pathogen inoculated in case of two leaves inoculations per seedling as a whole is higher than that in case of one leaf inoculation. So, the magnitude of plant defense response required to fight against the bacterial population in case of two leaves inoculations is more than that of one leaf inoculation hence less chance to escape the disease. Further, in lower pathogen concentration also we found similar pattern of less escapee seedlings in two leaves inoculations in comparison to one leaf inoculation. Again the magnitude difference in escapee seedling between one leaf and two leaves inoculation was decreasing with decreasing concentration of the pathogen. This observation suggests the role of critical bacterial inoculum to cause the disease in susceptible hosts.

R. solanacearum is a systemic pathogen. Under natural conditions, the pathogen enters through the root, colonizes the whole plant and then cause disease. Unlike pathogens that restrict their association to specific parts of their hosts, this systemic pathogen develops intimate association with the host plant. There are colonization phase and pathogenicity phase of this bacterium through root inoculation and the genes involved in both the phases are under tight and complex regulation [15]. This gene regulation favors the bacterium to manipulate or alter the host environment better suited for the bacterium and makes it systemic pathogen [16]. The experimental evolution has demonstrated the role of transcription regulators in different host adaptation, to change the niche inside the infected hosts [4,17,18]. Therefore, we believe that in *R. solanacearum*, the disease outcome is a stochastic process.

We hypothesize that pathogenicity results when seedling fails to adapt to the pathogen. Mere presence of the pathogen not necessarily will result in the disease.

The pathogen and the host interaction can attain an equilibrium which will not result into disease but survival of both the host as well as of the pathogen, which is a state like in case of endophytes. But when the plant cannot adapt to the pathogen, it results into disease and plant death and escape of the pathogen to the soil. Adaptation to the pathogen is a gradual process for which it is dependent upon the initial concentration. Therefore, higher the initial concentration, less are the escapees and *vice versa*. Plants that are escapees are not of resistant nature as disease can appear when inoculated again.

Two leaves inoculation methods have advantages such as pathogen is deposited at both the cut ends, so the chance of adaptation at both the leaves may not occur so disease should be more. The plant adaptation model also explains why sometimes disease is more and sometimes disease is less. We believe that the disease caused by this bacterium is a manifestation of its high population inside the plant where the plant had failed to adapt to its demand, result in change in the plant physiology and metabolism leading to the breakdown of the plant system.

We believe that pathogens are of two types: one type attacks the plant cells soon after it enters into the host plant. If they survive and grows, then disease occurs. These pathogens may not be influenced too much by plant physiology and whenever they come across the host they try to infect and cause disease. These are likely to be narrow host range pathogens. One example can be *Xanthomonas oryzae* pv *oryzae* and rice interaction; the second type likely to go to a colonization stage first after invasion. In these colonization phase inside the plant the pathogen, disease is less likely to occur. The pathogen remains associated with the host for a long duration. Infection by *R. solanacearum* may fall into this category. This indicates that though host-pathogen relation results into same phenotype i.e. disease, different interactions make every host-pathogen model unique.

It is pertinent to note that *R. solanacearum* exhibits latent infection in several host plants, known as tolerant hosts. In latent infection bacterial load *in planta* is very high like susceptible hosts but disease symptom is not developed. This is a mysterious behavior of *R. solanacearum* pathogenicity which is less understood. In contrast to latent infection observed in tolerant hosts, latent infection is also observed in susceptible hosts, which are referred to as escapees. Unlike the tolerant hosts, the

escapees are temporary latent infection and the disease may occur at any point of time during the development of the host. Moreover, escapees develop disease upon re-inoculation (unpublished result), unlike the tolerant or resistant hosts. We observed high bacterial population in escapee seedlings also without any visible disease symptom and most importantly, almost similar population like in infected seedlings. Moreover, we observed cases where we had found higher bacterial population in escapees on a particular day than in infected seedlings on the previous day. These observations indicate that the escapees and pathogen interaction might involve a more dynamic as well as complex mechanism in compared to latent infections in resistant plants.

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