New Symptoms Identified in Phytoplasma-Infected Plants Reveal Extra Stages of Pathogen-Induced Meristem Fate-Derailment

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In flowering plants, the transition of a shoot apical meristem from vegetative to reproductive destiny is a graduated, multi-stage process that involves sequential conversion of the vegetative meristem to an inflorescence meristem, initiation of floral meristems, emergence of flower organ primordia, and formation of floral organs. This orderly process can be derailed by phytoplasma, a bacterium that parasitizes phloem sieve cells. In a previous study, we showed that phytoplasma-induced malformation of flowers reflects stage-specific derailment of shoot apical meristems from their genetically preprogrammed reproductive destiny. Our current study unveiled new symptoms of abnormal morphogenesis, pointing to derailment of meristem transition at additional stages previously unidentified. We also found that the fate of developing meristems may be derailed even after normal termination of the floral meristem and onset of seed production. Although previous reports by others have indicated that different symptoms may be induced by different phytoplasmal effectors, the phenomenon observed in our experiment raises interesting questions as to (i) whether effectors can act at specific stages of meristem transition and (ii) whether specific floral abnormalities are attributable to meristem fate-derailment events triggered by different effectors that each act at a specific stage in meristem transition. Research addressing such questions may lead to discoveries of an array of phytoplasmal effectors.

Keywords: disease symptom, meristem fate, phytoplasma, stem cells, virulence factor

Sexual reproduction is a pivotal process in the life cycle of seed plants, and the process often proceeds in three phases: flowering, fruit setting, and seed dispersal and germination (Lord and Russell 2002). Flowering marks the transition from vegetative growth to reproduction. During floral transition, the shoot apical meristem undergoes a series of fate changes, including

conversion of the vegetative meristem (VM) to an inflorescence meristem (IM), initiation of floral meristems (FMs) from the flask of the IM, emergence of flower organ primordia from FMs, and formation of floral organs such as sepals, petals, stamens (male reproductive organ), and carpels (female reproductive organ, including ovaries and ovules) (Kater et al. 2006). FMs stop producing meristematic cells once carpel primordia emerge. Successful pollination and fertilization marks the beginning of a second phase, during which the ovary develops into fruit and the ovule develops into seed (Seymour et al. 2013). The seed undergoes a period of dormancy until conditions are favorable for germination. Upon germination, the nascent shoot and root apical meristems become active and start a new life cycle (Koorneef et al. 2002). The sequential events of plant growth and reproduction are controlled by both intrinsic signals and environmental cues (Barthélémy and Caraglio 2007; de Kroon et al. 2005).

Phytoplasmas are phloem-inhabiting, cell-wall-less bacteria responsible for numerous diseases in diverse plant species. Plants with phytoplasma infection often exhibit symptoms that manifest developmental and morphological alterations such as stunting, shoot proliferation, witches'-broom growth, and flower malformation. Common floral symptoms arising from phytoplasmal infection include phyllody (development of leaf-like structures in place of normal flowers), virescence (VIR) (green pigmentation of floral organs, especially petals), and proliferous flower (formation of a shoot or a new flower from the midst of a flower). Such flower malformations directly affect sexual reproduction.

Mechanisms of phytoplasma pathogenesis remain to be fully elucidated. Thus far, four putative virulence factors (namely, TENGU, SAP54, SAP11, and phyllogen) have been functionally identified from two '*Candidatus* Phytoplasma asteris'-related strains (Hoshi et al. 2009; Maejima et al. 2014; MacLean et al. 2011; Sugio et al. 2014). While TENGU and SAP11 were implicated in development of vegetative symptoms of witches'-broom growth, SAP54 and phyllogen were suspected to play a key role in floral malformation. On the host side, several studies revealed that misregulation of floral meristem and flower organ identity genes occurred in malformed flowers of phytoplasma-infected plants (Cettul and Firrao 2011; Himeno et al. 2011; Pracros et al. 2006; Su et al. 2011; Wei et al. 2013). Proteins of the RAD23 family, an important component of a plant's ubiquitin/proteasome system, were found to interact with phytoplasma virulence factor SAP54, leading to subsequent degradation of MADS-box transcription factors and eventual

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flower malformation (MacLean et al. 2014; Maejima et al. 2014). A recent study demonstrated that phytoplasma effector SAP11 was able to interact with and destabilize class II TCP transcription factor TB/CYC, resulting in proliferation symptoms (Chang et al. 2018). Based on analyses of a series of sequentially developed symptoms induced by Columbia Basin potato purple top (PPT) phytoplasma infection in tomato, a working model was constructed, depicting phytoplasmal disease symptoms as pathogen-induced alterations of growth pattern (PIAGP) and linking each symptom to a stage-specific event of apical meristem destiny derailment or reprogramming (Wei et al. 2013).

PPT phytoplasma is a '*Ca. P. trifolii*'-related strain belonging to the lineage previously termed as beet leafhopper-transmitted VIR agent (BLTVA). The phytoplasma has a wide host range and has been attributed to diseases of diverse agriculturally important crops, including radish, dry bean, potato, and tomato (Munyaneza 2005; Munyaneza et al. 2007). Symptoms of PPT phytoplasma infection vary, depending on the host plant species and developmental stages of the plant being affected. In a previous study, using a normal-flowering tomato cultivar (*Solanum lycopersicum* Mill. 'Moneymaker') as host, we identified four mutually distinct, sequentially developed symptoms: big bud (BB), cauliflower-like inflorescence (CLI), disrupted sympodial growth pattern (DSGP), and witches'-broom growth (WB). According to our PIAGP working model, these four disease symptoms were results of premature termination of FM, arrested transition from IM to FM,

delayed conversion of VM to IM, and repeated initiation and outgrowth of lateral VM, respectively (Wei et al. 2013). There was an apparent inverse relationship between the order of symptom appearance and the developmental stage of the fate-derailed apical meristem when it became affected (i.e., an early-appearing symptom corresponded to a late meristem fate-derailment event).

In the present article, we report the identification of four additional symptoms or morphotypes induced by PPT phytoplasma infection. These additional symptoms included vivipary (VP), parthenocarpy (PC), floral organ duplication (FOD), and VIR, all of which occurred before the appearance of BB, revealing additional phases of meristem fate reprogramming after the initiation of FM. Findings from this study extend our initial PIAGP model and provide fresh insights into the impact of phytoplasma infection on sexual reproduction in plants, from flower malformation to abnormal fruit setting and premature seed germination.

RESULTS

Identification of new symptoms.

Our previous study revealed that, using normal-flowering tomato cultivar Moneymaker as the host of the PPT phytoplasma, BB was the earliest appearing symptom and was attributed to premature termination of FM (Wei et al. 2013). In the present study, we hypothesized that, after FM initiation, the

Fig. 1. Diagrammatic representation of normal and early-flowering tomato plants after the onset of floral transition. We hypothesized that, over the time course of reproductive development, compared with normal-flowering plants, early-flowering plants possess apical meristems representing a broader array of stages in developmental commitment to reproduction. These, in turn, offer wider opportunities for interactions with phytoplasmal effectors, with consequent manifestation of a broader array of abnormal morphogenesis. Blue circles indicate the point of phytoplasma graft-inoculation. Pink ovals indicate critical stages at which the fate of a developing meristem may be derailed or modified. Horizontal orange lines labeled with first, second, third, and so on represent sequentially developed inflorescences. IM = inflorescence meristem, FM = floral meristem, FOP = flower organ primordia, FOD = floral organ duplication, PC = parthenocarpy, VIR = virescence, BB = big bud, DSGP = disrupted sympodial growth pattern, and CLI = cauliflower-like inflorescence.

fate of the meristem may still be derailed and modified at multiple stages in addition to premature FM termination. We reasoned that, over the time course of reproductive development, compared with normal-flowering plants, early-flowering plants possess apical meristems representing a broader array of stages in developmental commitment to reproduction. These, in turn, offer wider opportunities for interactions with phytoplasmal effectors, with consequent manifestation of additional abnormal morphogenesis (Fig. 1). Therefore, using PPT phytoplasma and an early-flowering tomato line, additional symptoms may be observed before BB, and such additional symptoms may lead to identification of extra meristem fate-derailment events post FM initiation.

To test our hypotheses, a transgenic tomato line that constitutively expresses the *single flower truss* (*SFT*) gene was used as the host of the PPT phytoplasma. *SFT*, the tomato ortholog of

Arabidopsis flower locus t, encodes florigen, the mobile signal that induces flowering. The 35S:*SFT* transgenic tomato line (Moneymaker) possesses an early-flowering trait (Lifschitz et al. 2006; Shalit et al. 2009); it develops the first inflorescence after producing 3 to 5 leaves (Fig. 2A) in contrast to 10 to 12 leaves in normal-flowering Moneymaker tomato plants.

In our experiment, phytoplasma infection was established once and at a single site. At the time of phytoplasma inoculation, the apexes (meristems) of different shoots, including emerging and yet-to-emerge ones, were at different developmental stages. During systemic infection, whereas phytoplasma cells couldn't move into developing meristems, phytoplasmal effector molecules could (Hoshi et al. 2009). The fate of a given shoot apex and the final morphology (symptom) of the corresponding shoot varied, depending on the developmental stage of the apex when it became affected by the

Fig. 2. Symptoms of potato purple top (PPT) phytoplasma infection in early-flowering tomato transgenic line 35S:*SFT*. **A**, Healthy (mock-inoculated) 35S:*SFT* tomato seedlings, showing the first inflorescence. **B**, PPT phytoplasma-induced carpel enlargement at stage 6. **C**, PPT phytoplasma-induced uplifted and enlarged precocious ovary development. **D**, Longitudinal view of **C**. **E**, PPT phytoplasma-induced parthenocarp in a 35S:*SFT* plant. **F**, Longitudinal view of **E**. **G**, Flower of a mock-inoculated 35S:*SFT* tomato plant. **H**, Twin flowers produced by a PPT phytoplasma-infected 35S:*SFT* tomato plant. **I**, PPT phytoplasma-induced floral organ duplication, showing excessive number of stamens and carpels. **J**, PPT phytoplasma-induced virescence in a 35S:*SFT* tomato plant. **K**, PPT phytoplasma-induced big buds on a 35S:*SFT* tomato plant. **L**, Dome shape meristem at stage 6 in a mock-inoculated 35S:*SFT* plant. **M**, Flat floral meristem at stage 6 in PPT-induced 35S:*SFT* plants. **N**, Longitudinal section of a stage 15 flower bud from a mock-inoculated 35S:*SFT* plant, showing sepals, petals, stamens, and carpels. **O**, PPT phytoplasma-induced virescence at stage 15 in 35S:*SFT* plants, showing smaller petals, stamens, carpels, and enlarged sepals. **P**, PPT phytoplasma-induced big bud symptom in 35S:*SFT* plants, showing fused and enlarged sepals and aborted inner whorls. **Q**, Fruit produced by a mock-inoculated 35S:*SFT* plant. **R**, Fruit produced by a PPT phytoplasma-infected 35S:*SFT* plant. **S**, Close-up image of the red rectangle area of **Q**, showing dormant seed covered with seed coats. **T**, Close-up image of the red rectangle area of **R**, showing viviparous shoots germinated from seed inside of a fruit. **U**, Cultivation of viviparous shoots on Murashige-Skoog medium. Scale bars indicate 0.5 mm.

phytoplasmal effector molecules. The early-flowering 35S:SFT transgenic tomato line expressed the following new symptoms.

PC. At appropriately 20 days postinoculation (dpi), in the second and third inflorescences of PPT phytoplasma-infected 35S:SFT tomato plants, the size of floral meristem increased by 1.46 ± 0.08 -fold ($P < 0.01$) (Fig. 3B and C). Enlarged carpel primordia and, subsequently, multiple fused carpels formed (Fig. 2B). The carpels continued to overgrow beyond the length of other flower organs, leading to an exposure of the carpels without coverage of sepals and petals (Fig. 2C), a sign of precocious ovary formation. Such overdeveloped carpels tend to be vascularized, and ovule-like structure could still be found inside the carpels (Fig. 2C and D). Although carpels were overdeveloped, the stamens were grossly underdeveloped, lacking a tight cone (Fig. 2B and C). Eventually, without successful pollination and fertilization, vascularized ovaries developed into parthenocarpic (seedless) fruit (Fig. 2E and F). Compared with normal fruit, the parthenocarpic fruit produced by PPT phytoplasma-infected tomato plants underwent a much longer ripening process. They were triangular in shape, smaller in size, and orangish in color (Fig. 2E and F).

FOD. In flowers in the second and third inflorescences of PPT phytoplasma-infected 35S:SFT tomato plants, in addition to precocious ovary development, the numbers of flower organs, especially stamens and carpels, were increased. Occasionally, within a given flower, all organs doubled their number, forming twin flowers (Fig. 2H). Microscopic investigations of floral meristem tissues revealed an exceptionally large floral meristem indistinguishable from that of PC (Fig. 3B and C) and revealed an

excessive number of flower organ primordia (Figs. 2I and 3D). Apparent splitting of floral organ primordia (e.g., splitting of stamen primordia) was observed under a scanning electron microscope (Fig. 3E). Conceivably, such floral organ primordia split was responsible for the increase of flower organs and formation of twin flowers. Flowers with FOD symptoms rarely set fruit.

VIR. Another symptom observed in PPT phytoplasma-infected 35S:SFT plants was floral VIR in the fifth and sixth inflorescences. Instead of having normal yellow petals and stamens, affected flowers developed pale-green and smaller-sized petals and stamens. In addition to abnormal pigmentation and size, the stamens failed to form a tight cone and were unable to produce pollen. The carpels were also much smaller when compared with those of healthy (mock-inoculated) flowers. Sepals were enlarged (Fig. 2J). A microscopic analysis visualized a flat floral meristem (Fig. 2M), indicating an early termination of floral meristem comparable with the one that led to the BB symptom, as described previously (Wei et al. 2013). Fruit setting was completely abolished in flowers with such VIR symptoms.

In the present study, we noticed that two floral symptoms, VIR and BB (the latter is a form of phyllody in tomato), developed sequentially and sometimes both could be observed in the same inflorescence. VIR symptoms appeared earlier than BB. When the symptoms first emerged, the floral organ identities of petal, stamen, and carpel still existed. However, as symptoms progressed, the development of petals, stamens, and carpels was completely arrested, and sepals started to enlarge and became fused: BB, the most characteristic symptom of PPT phytoplasma infection in tomato, began taking its shape (Fig. 2K).

Fig. 3. Electron microscopic analysis of floral buds from healthy (mock-inoculated) and potato purple top (PPT) phytoplasma-infected 35S:SFT tomato plants. **A**, Floral meristem in a floral bud of a mock-inoculated 35S:SFT tomato plant. **B** and **C**, Floral meristems in floral buds of a PPT phytoplasma-infected 35S:SFT tomato plant; such buds later develop into parthenocarp or floral organ duplication (FOD) symptoms. **D**, floral bud at stage 8 of a mock-inoculated 35S:SFT tomato plant. **E**, PPT phytoplasma-induced excessive numbers of stamens and carpels at stage 8; such bud leads to FOD symptoms. Scale bars indicate 100 μm.

VP. In addition to PC, FOD, and VIR, we observed another PPT phytoplasma infection-induced abnormality in 35S:SFT tomato plants during sexual reproduction: VP. Appropriately 14 days after PPT phytoplasma graft inoculation, the first inflorescence appeared and developed into normal flowers, indicating that FM was not affected by the phytoplasma. However, fruit setting was significantly reduced. In all, 20 PPT phytoplasma-infected 35S:SFT tomato plants produced only 13 fruit with normal size, color, and shape (Fig. 2R); in contrast, each of the mock-inoculated 35S:SFT tomato plants (Fig. 2Q) produced more than 20 fruit. In addition to low yield, PPT phytoplasma infection affected seed development. The seed produced by PPT phytoplasma-infected 35S:SFT tomato plants lacked a protective seed coat (Fig. 2T) and started germinating without dormancy, bypassing a physiological condition important for offspring survival and dispersal (Koornneef et al. 2002). Both green cotyledons and viviparous shoots were observed inside the fruit (Fig. 2R and T).

Viviparous shoots were transferred to Murashige-Skoog (MS) medium for cultivation (Fig. 2U) for 3 days. After they showed signs of survival and growth, they were potted in the greenhouse. The seedlings grown from these viviparous shoots showed no difference from healthy 35S:SFT tomato plants. No phytoplasma was detected in the VP seedlings by nested PCR amplification, which is consistent with the general belief that phytoplasmas are not vertically transmitted by seed (Jović et al. 2007).

Because phytoplasma infection often induces malformed flowers that lead to sterility, incidence of VP in phytoplasma-infected plants is rare (Cagiran et al. 2013; Hamed et al. 2014; Limberk and Ulrychová 1972; Singh et al. 2013). Our results indicate that, if a phytoplasma infection signal reaches a given apex destined to become an FM too late, the FM will develop into normal flowers and proceed with fruit setting as well as seed development. However, the phytoplasma may still be able to change the physiology of the mother plant and induce precocious seed germination, altering the activities of the embryonic apical meristems of the next generation.

Expression profiles of genes involved in symptom development.

In PPT phytoplasma-infected 35S:SFT plants, floral meristem enlargement was observed in early stages of PC and FOD symptom development. In searching for genes responsible for such floral meristem enlargement, we turned our attention to

tomato orthologs of *Arabidopsis CLAVATA 1 (clv1)* and *CLAVATA 3 (clv3)* because, in *Arabidopsis*, loss-of-function mutation in *clv1* and *clv3* leads to accumulation of stem cells and, in turn, enlarged floral meristems and supernumerary reproductive organs (Schoof et al. 2000). We examined the expression levels of tomato *clv1* and *clv3* orthologs in infected plants versus mock-inoculated control plants at flower developmental stages 3 and 6. Results revealed that, while at stage 3, both *clv1* and *clv3* genes were significantly downregulated; only slight upregulations of *clv1* and *clv3* genes were detected at stage 6 (Fig. 4A). This result suggested that *clv1* and *clv3* genes may play a role in PPT phytoplasma induced enlargement of floral meristem at early developmental stages of flower bud formation.

The expression levels of five classes (from class A to class E) of flower organ identity genes were also investigated. Because PC and FOD cannot be distinguished until developmental stage 8, RNA samples from stage 8 PC and FOD buds were used for quantitative real-time (qRT)-PCR analysis. Likewise, RNA samples from stage 15 VIR buds were used for qRT-PCR analysis because, at this developmental stage, VIR and BB can be easily distinguished (Fig. 2O and P).

In PPT phytoplasma-induced PC samples, class C genes (*TAG* and *TAGL1*) and class D genes (*SLMBP3* and *TAGL11*) were significantly upregulated (Fig. 4B). *TM6*, a class B gene that functions as a class C gene in tomato (de Martino et al. 2006), was also significantly upregulated (Fig. 4B). Such upregulations of class C and class D genes were likely responsible for the precocious ovary development and ovule formation. Downregulation of class B gene *TAP3* could be responsible for underdeveloped petals. In addition, an array of class E genes was also differentially regulated. Consistent with findings from a previously reported study in tomato (Ampomah-Dwamena et al. 2002), downregulation of *TM29* likely played a role in PC formation. Downregulations of *MADS1* and *RIN* may confer delayed ripening (Dong et al. 2013; Vrebalov et al. 2002). Class E gene *SLMBP21* is known to promote abscission of unfertilized flowers, ripe fruit, and senescent organs (Roldan et al. 2017); an upregulation of the gene in PC symptom development remains to be elucidated.

The PPT phytoplasma-induced FOD symptom is unique; no known plant mutant has exhibited such a phenotype. In PPT phytoplasma-induced FOD samples, although all 14 flower organ identity genes examined were differentially regulated,

Fig. 4. Expression profiles of meristem maintenance and floral organ identity genes in potato purple top (PPT) phytoplasma-infected 35S:SFT tomato plants. **A**, Expression of *clv1*, *clv3*, *AN*, and *WUS* in PPT phytoplasma-induced enlarged floral meristems at stages 3 and 6, respectively. **B**, Expression of floral organ identity genes in PPT phytoplasma-induced parthenocarpy (PC, at stage 8), floral organ duplication (FOD, at stage 8), and virescence (VIR, at stage 15). *TM6*, while being classified as a class B gene, functions as a class C gene in tomato. Gene expression fold changes are relative to healthy (mock-inoculated) controls to which a value of 1.00 was assigned. The β -actin gene was used as an internal reference. Vertical bars indicate two times the value of the standard deviation for the corresponding dataset ($n = 9$). Up- or downregulations of all differentially expressed genes presented in this figure are statistically significant ($P < 0.05$). Abbreviations st3, st6, st8, and st15 stand for flower developmental stages 3, 6, 8, and 15, respectively.

the magnitudes of expression changes of 11 genes were rather low (Fig. 4B). Such results seemed to agree with the fact that, in flowers with FOD symptoms, there was no change in identities of individual flower organs, although flower organ number was increased. Class B genes *TAP3* and *TM6* as well as class C gene *TAG* were significantly upregulated; however, the roles of these three genes in PPT phytoplasma-induced FOD symptom development remain unknown.

In PPT phytoplasma-affected floral buds destined to form VIR symptoms, the expressions of class A (*MC*), class B (*LePI*, *TAP3*, *TM6*, and *TPI*), class C (*TAG*), class D (*SLMBP3* and *TAGL1*), and class E (*MADS1*, *TM29*, and *TM5*) floral organ identity genes were all downregulated to various degrees (Fig. 4B). The downregulations of these genes could be responsible for arrested development of petals, stamens, carpels, and ovules. The expression profiles of the 11 floral organ identity genes that we observed from the present investigation into VIR symptoms (Fig. 4B) were comparable with the data obtained from our previous study of BB symptoms (Wei et al. 2013), indicating that VIR and BB shared similar transcriptional reprogramming of most flower organ identity genes. In floral buds destined to form VIR symptoms, the expression profiles of floral meristem-switching genes *AN* and *WUS* were also comparable with that of respective genes in floral buds destined to form BB symptoms (Fig. 4A) (Wei et al. 2013). One class C gene (*TAGL1*) and two class E genes (*SLMBP21* and *RIN*) were upregulated in floral buds destined to form VIR symptoms (Fig. 4B); this contrasts with the expression profiles of the same three genes in floral buds destined to form BB symptoms, in which all three genes were downregulated (Wei et al. 2013). It would be interesting to learn whether the upregulations of *TAGL1*, *SLMBP21*, and *RIN* genes in the VIR-destined floral buds contributed to VIR symptom formation or simply reflected different developmental stages of the VIR-destined floral buds (stage 15) versus BB-destined floral buds (stages 3 and 6).

Abscisic acid content in seed from infected plants.

To our knowledge, no VP-related plant gene has been identified thus far. Results from studies of various flowering plants have suggested that abscisic acid (ABA) and gibberellic acid (GA) play important roles in regulating seed dormancy. Although ABA promotes seed dormancy and inhibits germination, GA exerts the opposite effect (Karssen et al. 1983; Skubacz and Daszkowska-Golec 2017). Because our previous study suggested that, in PPT phytoplasma-infected tomato plants, levels of bioactive GAs were low and there was a diminished sensitivity of GA feedback regulation (Ding et al. 2013), we only examined possible effects of ABA in PPT phytoplasma-induced VP. ABA contents in seed from mock-inoculated and PPT phytoplasma-infected 35S:SFT tomato plants were measured by enzyme-linked immunosorbent assay (see Material and Methods). Results showed that the ABA level was 2.59-fold lower in seed from PPT phytoplasma-infected plants compared with the ABA level in seed from mock-inoculated plants (Fig. 5). It would be interesting to learn whether this decrease in endogenous ABA level played a role in inducing precocious germination or VP in fruit of PPT phytoplasma-infected plants. Additional studies will be required to unveil genes, including hormone biosynthesis and signaling genes, that are involved in regulating properties and, therefore, the fate of embryonic apical meristems.

DISCUSSION

In our previous study, using normal-flowering tomato cultivar Moneymaker as the host of the PPT phytoplasma, we identified BB as the earliest appearing symptom and attributed

the symptom to premature termination of FM (Wei et al. 2013). Because no earlier symptom could be detected before the appearance of BB, premature termination of FM became the only identifiable fate-derailment event for an FM (and the last identifiable fate-derailment event for a shoot apex) in such host–pathogen pairs. In the present study, we further explored the relationship between PPT phytoplasma infection symptoms and corresponding meristem fate derailment events, using an early-flowering tomato as the host. Results from the present study revealed that, after FM initiation, the fate of the meristem can still be derailed and modified at multiple stages in addition to premature FM termination; and, therefore, cause additional abnormal morphogenesis or disease symptoms.

In the present study, after the appearance of PC, FOD, VIR, and BB, two previously reported symptoms (CLI and WB) also appeared subsequently. However, the previously reported DSGP symptom never occurred in PPT phytoplasma-infected 35S:SFT tomato plants. According to Wei et al. (2013), downregulation of the *SFT* gene is responsible for DSGP symptoms in PPT phytoplasma-infected normal-flowering tomato cultivar Moneymaker. Because, in the 35S:SFT transgenic tomato line, expression of the *SFT* gene is under the control of a constitutively active promoter, a relatively stable expression level of the gene may be maintained even under phytoplasma infection. This may explain why our PPT phytoplasma-infected 35S:SFT tomato plants upheld a normal “three leaves and one inflorescence” sympodial growth pattern throughout the course of the experiment.

Because phytoplasma infection often induces malformed flowers that lead to sterility, incidence of VP in phytoplasma-infected plants is rare (Cagiran et al. 2013; Hamed et al. 2014; Limberk and Ulrychová 1972; Singh et al. 2013). Our results indicate that, if a phytoplasma infection signal reaches a given apex destined to become an FM too late, the FM will develop into normal flowers and proceed with fruit setting as well as seed development. However, the phytoplasma may still be able to change the physiology of the mother plant and induce precocious seed germination, altering the activities of the embryonic apical meristems of the next generation.

Extension to the initial PIAGP model.

The initial PIAGP working model summarizes our previous findings that phytoplasma infection can divert the host plant's apical meristems from their genetically preprogrammed destiny, thereby changing the morphology and fertility of the plant (Wei et al. 2013). The working model depicts each phytoplasma disease symptom as a pathogen-induced growth pattern

Fig. 5. Comparison of abscisic acid (ABA) concentrations in seed of healthy (mock-inoculated) and potato purple top (PPT) phytoplasma-infected 35S:SFT tomato plants. **A**, Standard curve generated from a series of ABA dilutions. **B**, ABA levels in mock-inoculated and PPT phytoplasma-infected plants. *SFT* = single flower truss gene.

Fig. 6. Extended working model of pathogen-induced alteration of meristem fates. Potato purple top (PPT) phytoplasma-induced floral reversion and vegetative proliferation takes place through transcriptional suppression of key meristem switching genes, altering meristem fate. The meristem fate modification types include release of apical dominance, causing witches'-broom growth (WB) symptoms; delayed transition from apical meristem to inflorescence meristem (IM), causing disrupted sympodial growth pattern (DSGP) symptoms; arrested IM to floral meristem (FM) transition, causing cauliflower-like inflorescence (CLI) symptoms; premature termination of FM, causing big bud (BB) and virescence (VIR) symptoms; FM enlargement and floral organ primordia doubling, causing floral organ duplication (FOD) symptoms; and FM enlargement and carpel primordia over growth, causing parthenocarpy (PC) symptoms. In addition, if an apex becomes affected by the phytoplasma after normal FM termination and during fruit setting, apical meristems of the next generation (seed) will bypass dormancy, resulting in vivipary (VP) symptoms. *WUS* = *Wuschel* gene, *SFT* = *single flower truss* gene, *AN* = *anantha* gene, *CLV1* = *CLAVATA 1* gene, *CLV3* = *CLAVATA 3* gene, and ABA = abscisic acid. Downward arrowheads indicate downregulations of respective genes. Pink crosses indicate points of meristem fate derailment.

modification that arises from a phase-specific fate derailment of apical meristem cells. An important aspect of the working model was a pairwise inverse relationship between phytoplasmal disease symptoms and meristem fate-derailment events, pairing an early-appearing symptom to a late-stage meristem fate derailment, and vice versa. The purpose of the present study was to further examine such a relationship and to explore the extensibility of our initial PIAGP working model.

The initial PIAGP model was constructed based on identification and analyses of the symptoms exhibited by PPT phytoplasma-diseased normal-flowering tomato cultivar Money-maker. The initial model covered four symptom types (BB, CLI, DSGP, and WB), with BB being the earliest appearing symptom and representing the latest meristem fate-derailment event, premature FM termination. In the present study, using an early-flowering tomato line, 35S:SFT, as the host, four additional symptoms (VP, PC, FOD, and VIR) were identified (Fig. 2E, H, J, and T). All four symptoms appeared before BB, and each symptom represented a meristem fate-derailment event after FM initiation. Our extended PIAGP working model, adding the four new symptom types and the corresponding meristem fate-derailment events identified in the present study, is illustrated in Figure 6. The extended working model can be summarized as follows.

A single phytoplasma infection may induce multiple mutually distinct symptoms in a single host plant. At the time of initial infection, shoot apices (meristems) of different shoots differ in their developmental stages. During the course of systemic infection, whereas phytoplasma cells are always restricted to phloem elements, phytoplasmal effector molecules can reach meristematic cells. The fate of a given shoot apex and the final morphology or symptom of the corresponding shoot vary, depending on the developmental stage of the apex when it becomes affected by the phytoplasmal effector molecules. If an apex becomes affected at early vegetative development stages before floral transition, apical dominance will be released and lateral VM will be initiated and outgrow repetitively, resulting in WB symptoms. If an apex is affected at or right before IM initiation, the conversion of VM to IM will be delayed, resulting in DSGP symptoms in the branch. If an apex becomes affected before FM initiation, the initiation of FM from IM will be arrested for that apex, resulting in CLI symptoms. If an apex originally destined to be an FM becomes affected by the phytoplasma shortly after its initiation as an FM, the further development of the FM will be terminated prematurely, resulting in BB and VIR symptoms. If the apex becomes affected by the phytoplasma after FM initiation and at the point of flower organ primordia emergence, the FM will enlarge abnormally and excessive numbers of flower organ primordia will form, resulting in FOD symptoms. If the apex becomes affected by the phytoplasma after FM initiation and flower organ primordia emergence, the FM will enlarge abnormally and produce imbalanced reproductive organs (overdeveloped carpels and underdeveloped stamens), leading to PC symptoms. If a phytoplasma infection signal reaches a given apex after the meristem had already fully committed to reproductive destiny, the activities of the embryonic apical meristems of the next generation may be altered through the infected mother plant, resulting in precocious seed germination.

Conclusion and further hypothesis.

Findings from our previous and present studies indicate that phytoplasma infection and symptom induction in host plants are a multifaceted process, involving complex interactions between the invading pathogen and its plant host. On the plant side, such interactions may derail the fate of a developing meristem at every possible stage during vegetative growth, floral transition, and

flower development. Even an apex becomes affected by a phytoplasma after normal FM termination and during fruit setting, the properties and activities of the apical meristems of the next generation (seed) may change. On the pathogen side, previous reports have suggested that different symptoms may be induced by different virulence factors: although OY phytoplasma TENGU and AYWB phytoplasma SAP11 were attributed to witches'-broom growth, SAP54 and OY phytoplasma phyllogen and AYWB phytoplasma SAP54 were implicated in floral malformation (Hoshi et al. 2009; MacLean et al. 2011; Maejima et al. 2014; Sugio et al. 2014). Taking findings from others and our present study together, we hypothesize that additional phytoplasma virulence factors may be present, and each such yet-to-be-identified virulence factor interacts with host components in a stage-specific manner, derailing the fate of developing meristems at multiple points and causing distinct symptoms. We envision that such virulence factors will eventually be identified, and that some may become exploited as biotechnological tools to mitigate unwanted phytoplasma infection symptoms in agricultural crops and induce favorable phenotypes for ornamental plants.

MATERIALS AND METHODS

Phytoplasma strain and plant material.

The Columbia Basin PPT phytoplasma, also known as BLTVA, was identified from diseased potato plants growing in a production field located in the Pacific Northwest of the United States (Munyanza 2005; Munyanza et al. 2007). Prior to the present study, the PPT phytoplasma had been maintained in its alternate host, normal-flowering Money-maker tomato, in a greenhouse. In this study, an early-flowering tomato transgenic line, 35S:SFT (a gift from Dr. Eliezer Lifschitz, Technion-Israel Institute of Technology, Haifa, Israel), was used as the host of the PPT phytoplasma. The transgenic tomato line bears an *SFT* gene under the control of the 35S promoter of *Cauliflower mosaic virus*.

Phytoplasma graft inoculation.

PPT phytoplasma infection in early-flowering 35S:SFT tomato plants was established by a single grafting inoculation. Symptomatic shoots from previously infected tomato plants were used as PPT phytoplasma inocula (scions). The infectious scions were grafted onto the primary stem of healthy 35S:SFT tomato seedlings (rootstocks) at right above the second leaf at the four-leaf stage. The grafted plants were maintained in a greenhouse under a photoperiod of 16 h of light and 8 h of darkness.

Symptom recording and stereomicroscopic imaging.

Post-PPT phytoplasma graft inoculation, disease symptom development in the infected plants was monitored and recorded daily with respect to flowering timing, flower and leaf morphology and color, inflorescence formation, and sympodial growth habit. To trace interior morphological changes, inflorescences and shoot apex were dissected and examined under a stereomicroscope (Stereo Discovery V20; Zeiss, Jena, Germany). The images were captured by a digital camera (Axio-Cam; Zeiss) attached to the microscope.

Scanning-electron microscopy.

Floral meristems and early-stage floral buds from greenhouse-grown, PPT phytoplasma-infected and mock-inoculated 35S:SFT tomato plants were dissected from the floral buds and placed onto 25-mm scanning-electron microscopy specimen stubs (Ted Pella, Redding, PA, U.S.A.) with ultrasmooth, round (25 mm in diameter) carbon adhesive tabs (Electron Microscopy Sciences, Inc., Hatfield, PA, U.S.A.) affixed to the surface

of the stub. The plant materials were not pretreated with a fixative, coated with a metal, or critical point dried; they were simply removed from live plants. Images of the meristems and floral buds were obtained using a Hitachi TM-3030 Table Top Scanning Electron Microscope (Hitachi High Technologies America, Inc., Dallas, TX, U.S.A.) with a Deben TM-3000 Coolstage (Deben UK Ltd., Suffolk, U.K.) set at -25°C . Images were viewed using the charge-reduced mode (variable-pressure mode) with 15 kV of accelerating voltage, a working distance of between 8 and 12 mm, magnification of between $\times 40$ and $\times 1,000$, and backscatter detectors.

DNA extraction from viviparous plants and phytoplasma detection.

Viviparous shoots from PPT phytoplasma-infected 35S:SFT tomato fruit were collected and cultured on MS medium for 3 days, then potted in soil and maintained in greenhouse at 25°C under a photoperiod of 16 h of light and 8 h of darkness. Leaf tissues (0.1 g) were collected from 2-month-old PPT phytoplasma-induced viviparous plants, and total DNA was extracted using a modified cetyltrimethylammonium bromide procedure, as described previously (Wei et al. 2011). Presence or absence of PPT phytoplasma in viviparous plants was investigated by nested PCR assays using primer pair P1/P7 followed by P1A/16S-SR (Deng and Hiruki 1991). PCRs were carried out for 35 cycles in 50 μl of PCR mixture that contained 1 μl of DNA template; 25 pmol each forward and a reverse primer; 200 μM each dATP, dCTP, dGTP, and dTTP; 5 μl of GeneAmp High Fidelity 10 $\times$ PCR buffer (with 25 mM MgCl_2); and 2.5 U of GeneAmp High Fidelity enzyme mix (Applied Biosystems, Foster City, CA, U.S.A.). The thermocycling parameters were denaturation at 95°C for 60 s, annealing at 56°C for 60 s, and extension at 72°C for 75 s. Amplification results were analyzed using agarose gel electrophoresis.

RNA extraction and qRT-PCR analysis of gene expression.

Floral meristematic tissues and floral buds were dissected from PPT phytoplasma-affected, abnormally developed floral buds destined to VIR, FOD, or PC separately at stages 3, 6, 8, and 15. Tissue samples representing the same symptom type or floral development stage were pooled (approximately 5 mg). Total RNAs were extracted from the above collected tissue samples using the Trizol (Invitrogen, San Diego, CA, U.S.A.) or RNeasy (Qiagen, Valencia, CA, U.S.A.) hybrid protocol, as described previously (Lai et al. 2006). Total RNAs were also extracted from floral meristematic tissues from floral buds of mock-inoculated control plants at the comparable developmental stages.

First-strand cDNA was synthesized from 1 μg of the above extracted total RNA using a AffinityScript Multi Temperature cDNA synthesis kit, per the manufacturer's instructions (Agilent Technologies, Santa Clara, CA, U.S.A.). qPCR was carried out using Brilliant SYBR Green QPCR Master Mix (Agilent Technologies) and run on the STRATAGENE Mx3000P QPCR system (Stratagene, La Jolla, CA, U.S.A.). Primer pairs for tomato *clv1* and *clv3* orthologs SIClv1 (SIClv1F: 5'-TTCAATTGCGCTTTGTTTCAG-3' and SIClv1R: 5'-GCCATTCATCACTCCGACTT-3') and SIClv3 (SIClv3F: 5'-AAAGGAAGTTGCTCCTGTGAA-3' and SIClv3R: 5'-ATGCAATGGATCAGGACCAG-3') were designed using online software Primer3. The primers for tomato flower organ identity genes, floral meristem switching genes, and the internal control β -actin gene were designed previously (Wei et al. 2013). Each qPCR was performed in a total volume 25 μl , containing 12.5 μl of 2 $\times$ Brilliant SYBR Green QPCR Master Mix, 2 μl of the cDNA, and 1.25 μl of 5 μM forward and reverse primers. The thermocycling parameters were 10 min at

95°C for initial template denaturation, followed by 40 cycles of 95°C for 30 s, 55°C for 1 min, and 72°C for 30 s. Quantitation of the target gene expression was carried out using the comparative cycle threshold (C_t) method ($\Delta\Delta C_t$ method) (Schmittgen and Livak 2008). Gene expression fold changes are relative to mock-inoculated control samples with the β -actin gene as an internal reference. For each sample type, three independent RNA samples from three plants (biological replicates) were subjected to qRT-PCR analysis. Each experiment was repeated three times (technical replicates). Data were analyzed statistically using Student's *t* test imbedded in Microsoft Excel (Microsoft Corporation, Seattle, WA, U.S.A.).

ABA extraction and concentration measurement.

ABA was extracted from seed produced by PPT phytoplasma-infected 35S:SFT plants and mock-inoculated control plants according to a modified protocol described by Ali et al. (2011). First, seed were dried on paper towels. Dry seed (1 g) in triplicate were homogenized to powder with a mortar and pestle in liquid nitrogen. The homogenate was then immersed in 5 ml of 80% methanol at 4°C for 30 min. The mixture was filtered using Whatman filter paper number 1, and the filtrate was dried by evaporation in a Speed Vac. The completely dried residue was redissolved in 100 μl of 1 $\times$ Tris-buffered saline solution. ABA concentration was measured using an ABA immunoassay detection kit (Sigma-Aldrich, St. Louis, MO, U.S.A.).

AUTHOR-RECOMMENDED INTERNET RESOURCE

Primer3: <http://bioinfo.ut.ee/primer3-0.4.0/>

LITERATURE CITED

- Ali, K., Gujjar, R. S., Niwas, R., Gopal, M., and Tyagi, A. 2011. A rapid method for estimation of abscisic acid and characterization of ABA regulated gene in response to water deficit stress from rice. *Am. J. Plant Physiol.* 6:144-156.
- Ampomah-Dwamena, C., Morris, B. A., Sutherland, P., Veit, B., and Yao, J. L. 2002. Down-regulation of TM29, a tomato SEPALLATA homolog, causes parthenocarpic fruit development and floral reversion. *Plant Physiol.* 130:605-617.
- Barthélémy, D., and Caraglio, Y. 2007. Plant architecture: A dynamic, multilevel and comprehensive approach to plant form, structure and ontogeny. *Ann. Bot.* 99:375-407.
- Cagiran, M. I., Mbaye, N., Silme, R. S., Ouedraogo, N., and Topuz, H. 2013. The impact of climate variability on occurrence of sesame phyllody and symptomatology of the disease in a Mediterranean environment. *Turk. J. Field Crops* 18:101-108.
- Cettul, E., and Firrao, G. 2011. Development of phytoplasma-induced flower symptoms in *Arabidopsis thaliana*. *Physiol. Mol. Plant Pathol.* 76:204-211.
- Chang, S. H., Tan, C. M., Wu, C. T., Lin, T. H., Jiang, S. Y., Liu, R. C., Tsai, M. C., Su, L. W., and Yang, J. Y. 2018. Alterations of plant architecture and phase transition by the phytoplasma virulence factor SAP11. *J. Exp. Bot.* 69:5389-5401.
- de Kroon, H., Huber, H., Stuefer, J. F., and van Groenendael, J. M. 2005. A modular concept of phenotypic plasticity in plants. *New Phytol.* 166:73-82.
- de Martino, G., Pan, I., Emmanuel, E., Levy, A., and Irish, V. F. 2006. Functional analyses of two tomato APETALA3 genes demonstrate diversification in their roles in regulating floral development. *Plant Cell* 18:1833-1845.
- Deng, S., and Hiruki, C. 1991. Amplification of 16S rRNA genes from culturable and nonculturable Mollicutes. *J. Microbiol. Methods* 14: 53-61.
- Ding, Y., Wei, W., Wu, W., Davis, R. E., Jinag, Y., Lee, I.-M., Hammond, R. W., Shen, L., Sheng, J., and Zhao, Y. 2013. Role of gibberellic acid in tomato defense against potato purple top phytoplasma infection. *Ann. Appl. Biol.* 162:191-199.
- Dong, T., Hu, Z., Deng, L., Wang, Y., Zhu, M., Zhang, J., and Chen, G. 2013. A tomato MADS-box transcription factor, SIMADS1, acts as a negative regulator of fruit ripening. *Plant Physiol.* 163:1026-1036.

- Hamed, A. H., El Attar, A. K., and El-Banna, O.-H. M. 2014. First record of a phytoplasma associated with faba bean (*Vicia faba* L.) witches'-broom in Egypt. *Int. J. Virol.* 10:129-135.
- Himeno, M., Neriya, Y., Minato, N., Miura, C., Sugawara, K., Ishii, Y., Yamaji, Y., Kakizawa, S., Oshima, K., and Namba, S. 2011. Unique morphological changes in plant pathogenic phytoplasma-infected petunia flowers are related to transcriptional regulation of floral homeotic genes in an organ-specific manner. *Plant J.* 67:971-979.
- Hoshi, A., Oshima, K., Kakizawa, S., Ishii, Y., Ozeki, J., Hashimoto, M., Komatsu, K., Kagiwada, S., Yamaji, Y., and Namba, S. 2009. A unique virulence factor for proliferation and dwarfism in plants identified from a phytopathogenic bacterium. *Proc. Natl. Acad. Sci. U.S.A.* 106:6416-6421.
- Jović, J., Cvrković, T., Mitrović, M., Krnjajić, S., Redinbaugh, M. G., Pratt, R. C., Gingery, R. E., Hogenhout, S. A., and Toševski, I. 2007. Roles of stolbur phytoplasma and *Reptalus panzeri* (Cixiidae, Auchenorrhyncha) in the epidemiology of maize redness in Serbia. *Eur. J. Plant Pathol.* 118:85-89.
- Karssen, C. M., Brinkhorst-van der Swan, D. L. C., Breckland, A. E., and Koornneef, M. 1983. Induction of dormancy during seed development by endogenous abscisic acid: Studies on abscisic acid deficient genotypes of *Arabidopsis thaliana* (L.) Heynh. *Planta* 157:158-165.
- Kater, M. M., Dreni, L., and Colombo, L. 2006. Functional conservation of MADS-box factors controlling floral organ identity in rice and *Arabidopsis*. *J. Exp. Bot.* 57:3433-3444.
- Koornneef, M., Bentsink, L., and Hilhorst, H. 2002. Seed dormancy and germination. *Curr. Opin. Plant Biol.* 5:33-36.
- Lai, Z., Gross, B. L., Zou, Y., Andrews, J., and Rieseberg, L. H. 2006. Microarray analysis reveals differential gene expression in hybrid sunflower species. *Mol. Ecol.* 15:1213-1227.
- Lifschitz, E., Eviatar, T., Rozman, A., Shalit, A., Goldshmidt, A., Amsellem, Z., Alvarez, J. P., and Eshed, Y. 2006. The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. *Proc. Natl. Acad. Sci. U.S.A.* 103:6398-6403.
- Limberk, J., and Ulrychová, M. 1972. Vivipary in fruits of tomato plants infected with a mycoplasma disease—Potato witches' broom. *J. Phytopathol.* 73:227-234.
- Lord, E. M., and Russell, S. D. 2002. The mechanisms of pollination and fertilization in plants. *Annu. Rev. Cell Dev. Biol.* 18:81-105.
- MacLean, A. M., Orlovskis, Z., Kowitzanich, K., Zdziarska, A. M., Angenent, G. C., Immink, R. G., and Hogenhout, S. A. 2014. Phytoplasma effector SAP54 hijacks plant reproduction by degrading MADS-box proteins and promotes insect colonization in a RAD23-dependent manner. *PLoS Biol.* 12:e1001835.
- MacLean, A. M., Sugio, A., Makarova, O. V., Findlay, K. C., Grieve, V. M., Tóth, R., Nicolaisen, M., and Hogenhout, S. A. 2011. Phytoplasma effector SAP54 induces indeterminate leaf-like flower development in *Arabidopsis* plants. *Plant Physiol.* 157:831-841.
- Maejima, K., Iwai, R., Himeno, M., Komatsu, K., Kitazawa, Y., Fujita, N., Ishikawa, K., Fukuoka, M., Minato, N., Yamaji, Y., Oshima, K., and Namba, S. 2014. Recognition of floral homeotic MADS domain transcription factors by a phytoplasmal effector, phyllogen, induces phyllody. *Plant J.* 78:541-554.
- Munyanze, J. E. 2005. Purple top disease and beet leafhopper-transmitted virescence agent (BLTVA) phytoplasma in potatoes of the Pacific Northwest of the United States. Pages 211-220 in: *Potato in Progress: Science Meets Practice*. A. J. Haverkort and P.C. Struik, eds. Wageningen Academic Publishers, Wageningen, The Netherlands.
- Munyanze, J. E., Crosslin, J. M., and Lee, I.-M. 2007. Phytoplasmas diseases and insect vectors in potatoes of the Pacific Northwest of the United States. *Bull. Insectol.* 60:181-182.
- Pracros, P., Renaudin, J., Eveillard, S., Mouras, A., and Hernould, M. 2006. Tomato flower abnormalities induced by stolbur phytoplasma infection are associated with changes of expression of floral development genes. *Mol. Plant-Microbe Interact.* 19:62-68.
- Roldan, M. V. G., Périlleux, C., Morin, H., Huerga-Fernandez, S., Latrasse, D., Benhamed, M., and Bendahmane, A. 2017. Natural and induced loss of function mutations in SIMBP21 MADS-box gene led to jointless-2 phenotype in tomato. *Sci. Rep.* 7: Article 4402.
- Schmittgen, T. D., and Livak, K. J. 2008. Analyzing real-time PCR data by the comparative C(T) method. *Nat. Protoc.* 3:1101-1108.
- Schoof, H., Lenhard, M., Haecker, A., Mayer, K. F. X., Jürgens, G., and Laux, T. 2000. The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes. *Cell* 100:635-644.
- Seymour, G. B., Østergaard, L., Chapman, N. H., Knapp, S., and Martin, C. 2013. Fruit development and ripening. *Annu. Rev. Plant Biol.* 64: 219-241.
- Shalit, A., Rozman, A., Goldshmidt, A., Alvarez, J. P., Bowman, J. L., Eshed, Y., and Lifschitz, E. 2009. The flowering hormone florigen functions as a general systemic regulator of growth and termination. *Proc. Natl. Acad. Sci. U.S.A.* 106:8392-8397.
- Singh, A. K., Bhatt, B. P., and Manibhushan. 2013. Occurrence of phytoplasma phyllody and witches' broom disease of faba bean in Bihar. *J. Environ. Biol.* 34:837-840.
- Skubacz, A., and Daszkowska-Golec, A. 2017. Seed dormancy: The complex process regulated by abscisic acid, gibberellins, and other phytohormones that makes seed germination work. Pages 77-100 in: *Phytohormones—Signaling Mechanisms and Crosstalk in Plant Development and Stress Responses*. M. El-Esawi, ed. InTech, London.
- Su, Y. T., Chen, J. C., and Lin, C. P. 2011. Phytoplasma-induced floral abnormalities in *Catharanthus roseus* are associated with phytoplasma accumulation and transcript repression of floral organ identity genes. *Mol. Plant-Microbe Interact.* 24:1502-1512.
- Sugio, A., MacLean, A. M., and Hogenhout, S. A. 2014. The small phytoplasma virulence effector SAP11 contains distinct domains required for nuclear targeting and CIN-TCP binding and destabilization. *New Phytol.* 202:838-848.
- Vrebalov, J., Ruezinsky, D., Padmanabhan, V., White, R., Medrano, D., Drake, R., Schuch, W., and Giovannoni, J. 2002. A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (rin) locus. *Science* 296:343-346.
- Wei, W., Cai, H., Jiang, Y., Lee, I.-M., Davis, R. E., Ding, Y., Yuan, E., Chen, H., and Zhao, Y. 2011. A new phytoplasma associated with little leaf disease in azalea: Multilocus sequence characterization reveals a distinct lineage within the aster yellows phytoplasma group. *Ann. Appl. Biol.* 158:318-330.
- Wei, W., Davis, R. E., Nuss, D. L., and Zhao, Y. 2013. Phytoplasmal infection derails genetically preprogrammed meristem fate and alters plant architecture. *Proc. Natl. Acad. Sci. U.S.A.* 110:19149-19154.