Stomate-based defense and environmental cues

Shweta Panchal¹ and Maeli Melotto²,*

Molecular Mycology Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India

Department of Plant Sciences; University of California, Davis; CA USA

*Correspondence to: Maeli Melotto; Email: melotto@ucdavis.edu

Addendum to:

Panchal S, Chitrakar R, Thompson B, Obulareddy N, Roy D, Hambright WS, Melotto M. 2016. Regulation of stomatal defense by air relative humidity. Plant Physiol. 172:2021-2032.

Panchal S, Roy D, Chitrakar R, Price L, Breitbach ZS, Armstrong DW, Melotto M. 2016. Coronatine facilitates Pseudomonas syringae infection of Arabidopsis leaves at night. Front. Plant Sci. 7:880.

Submitted: 19 May 2017

Keywords: air relative humidity, abiotic stress, biotic stress, hormone balance, salicylic acid, jasmonate, abscisic acid, darkness, bacterial diseases, Arabidopsis
Abstract

Environmental conditions play crucial roles in modulating immunity and disease in plants. For instance, many bacterial disease outbreaks occur after periods of high humidity and rain. A critical step in bacterial infection is entry into the plant interior through wounds or natural openings, such as stomata. Bacterial-triggered stomatal closure is an integral part of the plant immune response to reduce pathogen invasion. Recently, we found that high humidity compromises stomatal defense, which is accompanied by regulation of the salicylic acid and jasmonic acid pathways in guard cells. Periods of darkness, when most stomata are closed, are effective in decreasing pathogen penetration into leaves. However, coronatine produced by Pseudomonas syringae pv. tomato (Pst) DC3000 cells can open dark-closed stomata facilitating infection. Thus, a well-known disease-promoting environmental condition (high humidity) acts in part by suppressing stomatal defense, whereas an anti-stomatal defense factor such as coronatine, may provide epidemiological advantages to ensure bacterial infection when environmental conditions (darkness and insufficient humidity) favor stomatal defense.

Plant disease is a successful culmination of three important factors viz. high pathogen virulence, ineffective plant immunity, and favorable environmental conditions. This central dogma of plant pathology is a 50-year-old concept of the disease triangle (Stevens, 1960) and is relevant in all aspects of plant-pathogen interactions (Scholthof 2007). Environmental abiotic factors such as relative humidity (RH) and light conditions have a drastic effect on prevalence of disease in different geographical regions. Plants need to adapt to simultaneous exposure to variable biotic and abiotic stresses, sometimes with opposing effects, for maintenance of healthy whole plant
physiology. For instance, high disease incidence can be explained by the occurrence of climatic
conditions that favor pathogen growth and weaken the plant immune system\cite{Panchal2016} \cite{Panchal2016a}. It is well known that the outbreak of late blight of potato caused by \textit{Phytophthora infestans} that lead to the unfortunate Irish potato famine of 1845 was initiated and spread rapidly\cite{Scholthof2007}. Still, current knowledge on the molecular basis of environment-mediated regulation of plant responses to pathogens is still in its infancy. Moreover, we have gathered evidence that different cell types (\textit{e.g.}, guard cell and mesophyll cell) may have variable molecular responses to the same environmental condition\cite{Panchal2016} (\textit{Panchal et al. 2016}) adding additional levels of complexity in plant immune responses.

Plant immune system consists of a complex network of signals tuned to respond to specific types of biotic stresses. One of the first outputs of pattern-triggered immunity (PTI) consists of stomatal defense\cite{Melotto2006}. The microscopic stomatal pores in the leaves are important not only for transpiration and exchange of gases, but also as entry points for some pathogenic microbes, which otherwise could not transit from the phylloplane to the leaf apoplast. However, recognition of microbe-associated molecular patterns (MAMPs) by plant pattern-recognition receptors (PRRs) is a signal to close stomata that serve as guarding gates against microbe invasion\cite{Arnaud2015}. A rapid (<2h) bacterium-triggered stomatal closure is also observed when the plant perceives non-pathogens such as \textit{Escherichia coli}, \textit{Salmonella enterica}, and \textit{Bacillus subtilis}\cite{Melotto2006; Kroupitski2009; Roy2013; Kumar2012}. Molecular mechanisms underlying stomatal defense have been studied mostly in the \textit{Arabidopsis-Pst} pathosystem. This well-studied system has been very useful to decipher both
stomatal defense and counter-defense mainly due to the initial PTI response and subsequent induction of coronatine production in the bacterium that overrides PTI\(^9,10\) (Melotto et al. 2017; Xin et al. 2013). This temporal response in the Arabidopsis guard cell is mediated by phytohormones\(^5\) (Arnaud and Hwang 2015). For instance, abscisic acid (ABA), salicylic acid (SA), and jasmonic acid (JA) play important roles in guard cell signaling during Arabidopsis/P. syringae interaction.

Endogenous ABA and SA are important for stomatal closure in response to bacteria or purified MAMPs\(^4,11,12,14,15,16,17\) (Melotto et al. 2006, Zhang et al. 2008, Zeng and He, 2010; Zeng et al. 2011; Montillet et al. 2013, Du et al. 2014; Lim et al. 2014, Derger et al. 2015). By contrast, strong evidence suggests that, similar to its structural and functional mimic coronatine, jasmonoyl-L-isoleucine (JA-Ile) mediates stomatal opening\(^3,18\) (Panchal et al. 2016; Okada et al. 2009).

Intriguingly, control of stomatal movement by air RH also seems to operate through hormone signaling. As an example, low RH induced-stomatal closure is associated with ABA biosynthesis\(^19\) (Bauer et al. 2013), whereas activation of stomatal opening by high RH is associated with ABA catabolism\(^20\) (Okamoto et al. 2009). However, we have found that exogenous treatment of ABA does not close stomata to the full extent under high RH as compared to plants at moderate RH\(^3\) (Panchal et al. 2016). This finding indicates that while ABA has a prominent role in RH-mediated stomatal movement, it does not seem to be the only target of high RH in guard cells.

Previously, SA-dependent phenotypes have also been shown to be suppressed under high RH\(^21\) (Yoshioka et al. 2001), including the suppression of SA-dependent activation of PR genes in Arabidopsis leaves at 24 h after shifting plants to high RH\(^22\) (Zhou et al. 2004). As SA signaling is required for stomatal closure\(^4,13\) (Melotto et al., 2006; Zeng et al., 2011), we performed guard
cell-specific analysis and determined that high RH also repressed the expression of PR1 gene in this cell type (Fig. 1; Panchal et al. 2016). On the other hand, JA-responsive genes are upregulated in guard cells within 1h of plant exposure to high RH (Panchal et al; 2016). However, this regulation is independent of the JA-Ile receptor, COI1. COI1-independent and JA-dependent signaling pathway has been previously proposed and induction of some JAZ genes in coi1 plants has been reported when Arabidopsis leaves are infected with Sclerotinia sclerotiorum (Stotz et al. 2011). In addition, P. syringae pv. maculicola ES4326 infection in coi1-1 plants also leads to induction of JA-regulated genes, indicating that JA response can be activated downstream or independent of COI1 (Chen et al. 2001). Moreover, an effector from Pst DC3000, HopX1 triggers degradation of JAZ proteins in a COI1-independent manner and promotes stomatal opening (Gimenez-Ibanez et al. 2014). Consistent with this, we observed that the JA biosynthesis genes, LOX3 and OPR3 are repressed within 1h of exposure to high RH (Panchal et al. 2016). This finding suggests that JA-Ile replenishment may not be required as the signaling occurs independent of COI1 in guard cells. Specific branches of the SA and JA signaling pathways regulated by RH are yet to be determined.

In several circumstances, JA and SA act antagonistically and some key regulators in this crosstalk have been identified. SA inhibits JA signaling through the regulatory protein, NONEXPRESSOR OF PR GENES 1 (NPR1) (Spoel et al. 2003). By contrast, JA and coronatine inhibit SA biosynthesis genes (isochorismate synthase, ICS1) and activate SA degradation genes (benzoic acid/SA carboxyl methyltransferase 1, BSMT1) through three NAC transcription factors, ANAC019, ANAC055, and ANAC072 (Zheng et al. 2012). However, we observed that both activation of JA and suppression of SA occur simultaneously in guard cells of plants exposed to high RH (Panchal et al. 2016) and hence these pathways are likely to be
regulated independently by RH. Guard cell response to RH is much quicker (<1h) than that of whole leaves (>8h) suggesting the existence of an independent regulation of guard cell signaling by RH. However, it is possible that JA/SA antagonism exist in guard cell under high RH at a step downstream of the signaling components tested so far, which still needs further investigation. Based on current evidence, we propose that the shift of balance between SA and JA signaling leads to repression of bacterium-triggered stomatal closure and consequently bacteria that are otherwise unable to overcome PTI can still penetrate leaf tissue under high RH (Fig. 1).

High humidity also promotes rapid proliferation of bacteria in the epiphytic phase (Hirano and Upper 2000). However, in general, phyllosphere is a water-limiting environment (Beattie 2011) that imposes a challenge for epiphytic survival of pathogens in this niche. To counter this challenge, bacteria produce extracellular polymeric substances (EPS) to maintain hydration and form aggregates on the leaf surface (Monier and Lindow 2003; Yu et al 1999). High humidity positively affects such aggregate formation of P. syringae pv. syringae B728a on bean leaf surface and aids in rapid proliferation of the bacteria and subsequent entry into the endophytic phase (Monier and Lindow 2003). To maintain epiphytic fitness, virulent bacteria can physically alter the wettability of the leaf surface by producing biosurfactants (Bunster et al. 1989; Schreiber et al. 2005). Furthermore, bacterial-dependency on high RH to establish apoplastic infection while suppressing host immunity has also been demonstrated recently (Xin et al. 2016). These observations emphasize that RH participates in multiple steps of molecular plant-pathogen interaction and influences its outcome.

In contrast to high RH that aids plant susceptibility and counteracts stomatal defense, several other abiotic factors may favor a robust stomatal defense. In particular, absence of light may lead to stomatal closure; indeed, most stomata of C3 and C4 plants are closed at night. This suggests
that bacterial penetration of leaves through stomata would be minimal at night. Interestingly, the clock proteins CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) not only control the circadian stomatal movement, but they are also required for flagellin-mediated immune response \(^{35}\) (Zhang et al. 2013). Disruption of the clock activity through CCA1 and LHY resulted in stomata that are less responsive to dark and \(P. syringae\ pv. maculicola\), thus rendering Arabidopsis plants more susceptible to infection at night.

Furthermore, surface-inoculated plants, but not syringe-infiltrated plants, are more resistant to bacterium infection at dusk than at dawn \(^{35}\) (Zhang et al. 2013). These findings mechanistically link stomatal defense and the circadian clock.

Interestingly, the levels of the two most well-known hormones associated with biotic stress, JA and SA, naturally oscillate throughout a 24 h cycle. While the JA level peaks in the daytime, the SA level is highest during the night in whole leaves \(^{36,37}\) (Goodspeed et al. 2012; Grundy et al. 2015). These oscillations are under the control of the clock and several clock-associated proteins \(^{37}\) (Grundy et al. 2015). If the JA/SA hormone balance determines the opening and closing of stomata (Fig. 1), then one would assume that inducing JA signaling at night could promote stomatal opening. Previously, others and we have determined that coronatine, a molecular mimic of JA-Ile, overcomes bacterium-triggered stomatal closure by upregulating JA signaling and repressing SA signaling \(^{4,38}\) (Melotto et al. 2006; Zhang et al. 2015). Consistently, \(Pst\ DC3000\) senses the leaf surface, produces coronatine, and opens dark-closed stomata \(^{39}\) (Panchal et al. 2016). It remains to be determined whether coronatine disrupts the natural guard cell circadian movement by actively suppressing CCA and LHY1 mediated signaling.

Nonetheless, it is evident that a stomatal defense-favoring environmental condition such as
darkness can be overcome by a virulent pathogen that shifts the hormone balance in guard cell towards $\text{JA}_{39}$ (Fig. 2).

Acknowledgments

This research topic in the M. Melotto Lab is supported by grants from the U.S. National Institute of Allergy and Infectious Disease (5R01AI068718), the U.S. Department of Agriculture – National Institute of Food and Agriculture (2015-67017-23360 and 2017-67017-26180), Center for Produce Safety (CPF43206), and UC Davis-FAPESP SPRINT (Award 40747474).

References

1. Stevens RB. Plant Pathology, an Advanced Treatise. Vol. 3, 357–429 (Academic, 1960).
2. Scholthof KB. The disease triangle: pathogens, the environment and society. Nat Rev Microbiol 2007; 5(2):152-6; PMID: 17191075; DOI: 10.1038/nrmicro1596
3. Panchal S, Chitrakar R, Thompson B, Obulareddy N, Roy D, Hambright WS, Melotto M. Regulation of stomatal defense by air relative humidity. Plant Physiol 2016; 172:2021-2032; PMID: 27702841; DOI: 10.1104/pp.16.00696
4. Melotto M, Underwood W, Koczanski J, Nomura K, He SY. Plant stomata function in innate immunity against bacterial invasion. Cell 2006; 126: 969-980; PMID:16959575; DOI: 10.1016/j.cell.2006.06.054
5. Arnaud D, Hwang I. A sophisticated network of signaling pathways regulates stomatal defenses to bacterial pathogens. Mol Plant 2015; 8(4):566-81; PMID: 25366179; DOI: 10.1016/j.molp.2014.10.012
6. Kroupitski Y, Golberg D, Belausov E, Pinto R, Swartzberg D, Granot D, Sela S. Internalization of *Salmonella enterica* in leaves is induced by light and involves chemotaxis and penetration through open stomata. Appl Environ Microbiol 2009; 75:6076-86; PMID: 19648358; DOI: 10.1128/AEM.01084-09
7. Roy D, Panchal S, Rosa BA, Melotto M. *Escherichia coli* O157:H7 induces stronger plant immunity than *Salmonella enterica* Typhimurium SL1344. Phytopathology 2013; 103: 326-332; PMID: 23301812; DOI: 10.1094PHYTO-09-12-0230-FI

8. Kumar AS, Lakshmanan V, Caplan JL, Powell D, Czymmek KJ, Levia DF, et al. Rhizobacteria *Bacillus subtilis* restricts foliar pathogen entry through stomata. Plant J 2012; 72:694–706; PMID: 22862801; DOI: 10.1111/j.1365-313X.2012.05116

9. Melotto M, Zhang L, Oblessuc PR, He SY. Stomatal defense a decade later. Plant Physiol 2017; 174: 561-571; PMID: 28341769; DOI: 10.1104/pp.16.01853

10. Xin XF, He SY. *Pseudomonas syringae* pv. *tomato* DC3000: a model pathogen for probing disease susceptibility and hormone signaling in plants. Annu Rev Phytopathol 2013; 51:473-98; PMID: 23725467; DOI: 10.1146/annurev-phyto-082712-102321

11. Zhang W, He SY, Assmann SM. The plant innate immunity response in stomatal guard cells invokes G-protein-dependent ion channel regulation. Plant J 2008; 56: 984-996; PMID: 18702674; DOI: 10.1111/j.1365-313X.2008.03657.x

12. Zeng W, He SY. A Prominent role of the flagellin receptor FLAGELLIN-SENSING2 in mediating stomatal response to *Pseudomonas syringae* pv. *tomato* DC3000 in *Arabidopsis*. Plant Physiol 2010; 153: 1188-1198; PMID: 20457804; DOI: 10.1104/pp.110.157016

13. Zeng W, Brutus A, Kremer JM, Withers JC, Gao X, Jones AD, He SY. A genetic screen reveals *Arabidopsis* stomatal and/or apoplastic defenses against *Pseudomonas syringae* pv. *tomato* DC3000. PLoS Pathog 2011; 7: e1002291; PMID: 21998587; DOI: 10.1371/journal.ppat.1002291

14. Montillet JL, Hirt H. New checkpoints in stomatal defense. Trends Plant Sci 2013; 18: 295-297; PMID: 23582764; DOI: 10.1016/j.tplants.2013.03.007

15. Du M, Zhai Q, Deng L, Li S, Li H, Yan L, Huang Z, Wang B, Jiang H, Huang T, Li CB. Closely related NAC transcription factors of tomato differentially regulate stomatal closure and reopening during pathogen attack. Plant Cell 2014; 26: 3167-3184; PMID: 25005917; DOI: 10.1105/tpc.114.128272

16. Lim CW, Luan S, Lee SC. A prominent role for RCAR3-mediated ABA signaling in response to *Pseudomonas syringae* pv. *tomato* DC3000 infection in *Arabidopsis*. Plant Cell Physiol 2014; 55: 1691-1703; PMID: 25063782 DOI: 10.1093/pcp/pcu100
17. Deger AG, Scherzer S, Nuhkat M, Kedzierska J, Kollist H, Brosché M, et al. Guard cell SLAC1-type anion channels mediate flagellin-induced stomatal closure. New Phytologist 2015; 208: 162-173; PMID: 25932909; DOI: 10.1111/nph.13435

18. Okada M, Ito S, Marsubara A, Iwakura I, Egoshi S, Ueda M. Total syntheses of coronatine by exo-selective Diels-Alder reaction and their biological activities on stomatal opening. Org Biomol Chem 2009; 7: 3065-3073; DOI:10.1039/B905159G

19. Bauer H, Ache P, Lautner S, Fromm J, Hartung W, Al-Rasheid KA, et al. The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis. Curr Biol 2013; 23: 53-57; PMID: 23219726; DOI: 10.1016/j.cub.2012.11.022

20. Okamoto M, Tanaka Y, Abrams SR, Kamiya Y, Seki M, Nambara E. High humidity induces abscisic acid 8'-hydroxylase in stomata and vasculature to regulate local and systemic abscisic acid responses in Arabidopsis. Plant Physiol 2009; 149: 825-834; PMID: 19036833; DOI: 10.1104/pp.108.130823

21. Yoshioka K, Kachroo P, Tsiu F, Sharma SB, Shah J, Klessig D. Environmentally sensitive, SA-dependent defense responses in the cpr22 mutant of Arabidopsis. Plant J 2001; 26: 227-259; PMID: 11439131; DOI: 10.1046/j.1365-313X.2001.2641039.x

22. Zhou F, Menke FL, Yoshioka K, Moder W, Shirano Y, Klessig DF. High humidity suppresses ssi4-mediated cell death and disease resistance upstream of MAP kinase activation, H2O2 production and defense gene expression. Plant J 2004; 39: 920-932; PMID: 15341634; DOI: 10.1111/j.1365-313X.2004.02180.x

23. Stotz HU, Jikumaru Y, Shimada Y, Sasaki E, Stingl N, Mueller MJ, Kamiya Y. Jasmonate-dependent and COI1-independent defense responses against Sclerotinia sclerotiorum in Arabidopsis thaliana: auxin is part of COI1-independent defense signaling. Plant Cell Physiol. 2011; 52(11): 1941-56; PMID: 21937677; DOI: 10.1093/pcp/pcr127

24. Chen W, Provart NJ, Glazebrook J, Katagiri F, Chang HS, Eulgem T. Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 2002; 14(3): 559-74; PMID: 11910004; DOI: 10.1105/tpc.010410

25. Gimenez-Ibanez S, Boter M, Fernández-Barbero G, Chini A, Rathjen JP, Solano R. The bacterial effector Hopx1 targets JAZ transcriptional repressors to activate jasmonate
26. Spoel SH, Koornneef A, Claessens SM, Korzelius JP, Van Pelt JA, et al. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. The Plant Cell 2003; 15: 760–770; PMID: 12615947; DOI: 10.1105/tpc.009159

27. Zheng X-Y, Spivey NW, Zeng W, Liu P-P, Fu ZQ, Klessig DF, et al. Coronatine promotes Pseudomonas syringae virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. Cell Host Microbe 2012; 11: 587-596; PMID: 22704619; DOI: 10.1016/j.chom.2012.04.014

28. Hirano SS, Upper CD. Bacteria in the leaf ecosystem with emphasis on Pseudomonas syringae - a pathogen, ice nucleus, and epiphyte. Microbiol Mol Biol Rev 2000; 64: 624–653; PMID: 10974129; DOI: 10.1128/MMBR.64.3.624-653.2000

29. Beattie GA. Water relations in the interaction of foliar bacterial pathogens with plants. Annu Rev Phytopathol 2011; 49: 533–555; PMID: 21438680; DOI: 10.1146/annurev-phyto-073009-114436

30. Monier JM, Lindow SE. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. Proc Natl Acad Sci USA 2003; 100: 15977–15982; DOI: 10.1073/pnas.2436560100

31. Yu J, Penaloza-Vazquez A, Chakrabarty AM, Bender CL. Involvement of the exopolysaccharide alginate in the virulence and epiphytic fitness of Pseudomonas syringae pv. syringae. Mol Microbiol 1999; 33: 712–720; PMID: 10447881; DOI: 10.1046/j.1365-2958.1999.01516.x

32. Bunster L, Fokkema NJ, Schippers B. Effect of surface-active Pseudomonas spp. on leaf wettability. Appl Environ Microbiol 1989; 55: 1340–1345; PMID: 16347926

33. Schreiber L, Krimm U, Knoll D, Sayed M, Auling G, Kroppenstedt RM. Plant–microbe interactions: identification of epiphytic bacteria and their ability to alter leaf surface permeability. New Phytol 2005; 166: 589–594; PMID: 15819920 DOI: 10.1111/j.1469-8137.2005.01343.x
34. Xin XF, Nomura K, Aung K, Velásquez AC, Yao J, Boutrot F, et al. Bacteria establish an aqueous living space in plants crucial for virulence. Nature 2016; 539(7630):524-529; PMID: 27882964; DOI: 10.1038/nature20166

35. Zhang C, Xie Q, Anderson RG. Ng G, Seitz NC, Peterson T, et al. Crosstalk between the circadian clock and innate immunity in Arabidopsis. PLoS Pathogens 2013; 9(6): e1003370; PMID: 23754942; DOI: 10.1371/journal.ppat.1003370

36. Goodspeed D, Chehab EW, Min-Venditti A, Braam J, Covington MF. Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. Proc Natl Acad Sci USA 2012; 109:4674-7; PMID: 22331878; DOI: 10.1073/pnas.1116368109

37. Grundy J, Stoker C, Carré IA. Circadian regulation of abiotic stress tolerance in plants. Front Plant Sci 2015; 6: 648; PMID: 26379680; DOI: 10.3389/fpls.2015.00648

38. Zhang L, Yao J, Withers J, Xin XF, Banerjee R, Fariduddin Q, et al. Host target modification as a strategy to counter pathogen hijacking of the jasmonate hormone receptor. Proc Natl Acad Sci USA 2015; 112: 14354–14359; PMID: 26578782; DOI: 10.1073/pnas.1510745112

39. Panchal S, Roy D, Chitrakar R, Price L, Breitbach ZS, Armstrong DW, Melotto M. Coronatine facilitates Pseudomonas syringae infection of Arabidopsis leaves at night. Front. Plant Sci 2016; 7:880; PMID: 27446113 DOI: 10.3389/fpls.2016.00880