

Interactive Effects of Intraspecific Competition and Drought on Stomatal Conductance and Hormone Concentrations in Different Tomato Genotypes

Yang Gao (✉ gaoyang@caas.cn)

Chinese Academy of Agricultural Sciences

Yueping Liang

Chinese Academy of Agricultural Sciences

Shuang Li

Chinese Academy of Agricultural Sciences

Zhuanyun Si

Chinese Academy of Agricultural Sciences

Abdoul.Kader.Mounkaila Hamani

Graduate School of Chinese Academy of Agricultural Sciences (GSCAAS)

Research Article

Keywords: Intraspecific competition, Tomato, Plant hormones, Above- and below-ground competition

Posted Date: October 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-942000/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Horticulturae on January 4th, 2022. See the published version at <https://doi.org/10.3390/horticulturae8010045>.

Abstract

We elucidated the effects of intraspecific competition on plant growth, stomatal opening and hormone concentrations in different tomato genotypes under different water regimes. Intraspecific competition reduced plant leaf area and stomatal conductance (g_s) of wild-type tomato (Ailsa Craig), which was accompanied by abscisic acid (ABA) accumulation and ethylene evolution. Intraspecific competition-induced decrease in g_s was absent in *flacca*, an ABA-deficient mutant, and in never-ripe, a partially ethylene-insensitive genotype, indicating ABA and ethylene involved in plant response to intraspecific competition. As soil water becomes dry, the competition decreased g_s by elevating ABA and ethylene accumulations. Under severe drought, the competition-induced decline in g_s was covered by the severe drought-induced decrease in g_s , as hydraulic signals most probably dominate. Absence of canopy competition had no significant influence on plant stomatal opening of well-watered tomato, due to canopy separation minimized the plant neighbor sensing by ethylene and other signals. Whereas under water deficit condition, absence of canopy competition significantly reduced ABA accumulation in roots and then stomatal conductance, indicating the belowground neighbour detection signals maybe enhanced by soil drought. Absence of root competition increased ethylene evolution, confirming the importance of ethylene in neighbor detection and plant response to environmental stress.

1. Introduction

Both biotic and abiotic stresses affect plants normal growth and development, and significantly decrease their productivity [1]. As plants responses to these stresses are complex, more attentions are focused on plants responses to particular abiotic or biotic stress [2]. Phytohormones play central roles in sensing biotic and abiotic stresses [3–8]. Moreover, physiological effects of signal response to various stresses are characterized by interaction and coupling, while the intrinsic mechanism is still unclear.

Inter-/intra-specific competition for growing space and limited resources are the important biotic and abiotic factors inhibiting plant growth [7–8]. Plants can detect neighbors by multiple hormones and respond to them in multiple ways [3, 9–11]. Over-representation of phytohormone-responsive genes was observed in competing Arabidopsis plants, con-forming that competing-induced involvement of plant hormones [12]. Ethylene is an important hormone in determining plant responses to neighbors, such as shoot elongation and leaf and stomatal movements [7, 13–14]. Pierik et al. [9] found that the ethylene-insensitive transgenic tobacco could reduce shade avoidance responses to the neighbors. Ethylene generally maintains stomata at sub-maximum apertures despite the relatively non-stressful conditions [7]. Vysotskaya et al. [7] explained that the decline in stomatal conductance induced by the neighborhood was due to the increased ethylene production in competing plants. Abscisic acid (ABA), an essential hormone adjusting stomatal opening, also involved in plant's response to neighborhood [7]. Apart from the effect of ABA on stomatal closure, little attention was paid to the interaction between hormones [15–16]. As ethylene has an opposing effect on the stomata by inhibiting ABA-induced stomatal closure [17], more experiments are necessary to investigate the co-regulation of ABA and ethylene in stomatal responses to the presence of neighbors. Moreover, recent researches indicated that cytokinin and auxin participated in plant adaptation to competitions [3, 18]. As response of hormones to stressful environments is a complex signaling network, the mechanism of phytohormone's regulation in plant adaptations to stresses was not well elucidated.

Under the condition of low population density, the inter-/intra-specific competition was mainly observed belowground [19]. However, limitation in aboveground space or belowground resources in high population environments may favor different suites of plant traits [18]. Despite the interdependence of above- and below-ground competition [20], no studies have addressed the effects of below- and above-ground interaction between different species on plant's response to environmental stresses, when grown in mixture under both abiotic and biotic stresses conditions. Stomatal opening is a fundamental response of plants to environment, regulating carbon gain and water loss [7, 21–22]. Signaling network of hormones controlling stomatal movement has been well-established [23–26], while little information was available on stomatal response to the presence of neighbors, more specifically, the above- and below-ground completion. Therefore, the objectives of this study were to investigate the interactive effects of intraspecific competition and drought on plant growth and stomatal response in different tomato genotypes, and to analyze the influence of aboveground and belowground competition on plant hormones accumulation (ABA and ethylene) and stomatal opening.

2. Results

2.1. Effects of intraspecific competition on plant growth and stomatal opening of WT tomato

At 4 days after competition, there was no significant difference in leaf area between the single and competing plants of WT tomato (Fig. 1a and b). Subsequently, compared with the single plant, the competing averagely decreased leaf area by 21% under full irrigation and by 26% under deficit irrigation, respectively. Transpiration was calculated per-unit leaf area basis [5]. During the sampling period, the intraspecific competition significantly decreased transpiration, on average 33% and 28% lower than the single plant under full irrigation and deficit irrigation, respectively (Fig. 1c and d).

Under full irrigation, the leaf water potential (LWP) of single WT plant (-0.58 ± 0.03 MPa) was similar to that of the competing plants (-0.60 ± 0.03 MPa). Furthermore, there was no significant difference in LWP between the single plant and competing plants under deficit irrigation (Fig. 2a). Abaxial stomatal conductance (g_s) of fully expanding leaves are shown in Fig. 2b and c. Under the well-watered condition, the intraspecific competition significantly reduced the g_s of WT tomato. As soil moisture depleted gradually, the difference in g_s between the single tomato and the competing plants was markedly decreased from 26% at 4 days after competition to 16% at 10 days after competition. Although the LWP was similar between the single plant and competing plants, the difference in g_s between the two treatments was measured in our experiment. The result indicated that the non-hydraulic signals contributed for regulating stomatal opening of competing plants. However, under severe drought (at 12 days after competition), the g_s of single tomato was not significantly higher than that of competing plants, as the LWP in competing plants was lower than that in single plant.

2.2. Involvement of ABA and ethylene in plant response to intraspecific competition

As a mutant of ABA-deficient, in the WT/FL competing pot, the g_s of well-watered FL plant (686 ± 24 mmol m⁻² s⁻¹) was similar to the value (681 ± 18 mmol m⁻² s⁻¹) of water-stressed FL tomato (Fig. 3). Like the WT/WT competing pot, the intraspecific competition from the WT/FL competing pot significantly reduced the g_s of well-watered WT. When soil moisture reduced from 36–26%, the difference in g_s between the single WT tomato and the competing WT in the WT/FL competing pot was averagely 16% ($P < 0.01$); this difference was not significant as soil moisture depleted below 20%. The difference response of stomatal opening to intraspecific competing between WT and FL indicated that ABA involved in tomato respond to competition. ABA concentration in the single and competing WT tomatoes under full and deficit irrigations is shown in Table 1. Whether under well-watered condition or water deficit condition, the intraspecific competition markedly increased the foliar ABA accumulation in WT tomato (Table 1). Under full irrigation, the intraspecific competition significantly decreased the root ABA concentration, whereas increased ABA accumulation in roots under soil drought.

Table 1
ABA concentration (ng g⁻¹ DW) in leaf and root of the single and competing WT tomato under full and deficit irrigation.

Treatment	Deficit irrigation		Full irrigation	
	Leaf	Root	Leaf	Root
WT	961.5±52b	115.4±4.5c	332.6±23c	65.4±3.4b
WT/WT-WT	1080.4±75a	155.3±3.2b	505.2±17a	43.5±2.1a
WT/FL-WT	1136.6±61a	174.7±4.1a	458.6±21ab	47.7±3.1a

When growing WT and NR tomato in a pot, whether under full or deficit irrigation, the intraspecific competition did not reduce leaf area, transpiration and abaxial g_s of the NR plant (Fig. 4). In the WT/NR competing pot, the intraspecific competition had more significant influence on the g_s of WT tomato (Fig. 5a). Under full irrigation, the g_s of WT plant was averaging lower than that of NR plant by 22%. The difference in g_s between the WT and NR tomato gradually decreased from 25–7% with decreasing in soil moisture. Moreover, this difference was not significant as soil moisture was lower than 28%. Overall, the distinct response to intraspecific competition between WT and NR tomato indicated that ethylene contributed to tomato response to intraspecific competition.

In the WT/NR competing pot under full irrigation, the foliar accumulation of ABA in competing WT plants (WT-C) was significantly greater than that in single WT plant by 39% and 44%, respectively. In contrast, the single WT plant had higher ABA concentration in roots, 35% and 23% greater than that in the competing plants (WT-C), respectively (Fig. 5b). Under water deficit condition, the foliar accumulation of ABA in the competing plants was significantly greater than that in the single WT plant, and the value in the competing NR tomato was significantly higher (35%) than that in the competing WT plant. However, there was no significant difference in root ABA accumulation between the competing plants and the single plant under water deficit condition. The effects of intraspecific competition on foliar ethylene evolution between the single WT and competing WT and NR tomato are shown in Fig. 5c. Under the well-watered condition, the foliar ethylene evolution in the competing NR and WT tomato was 73% and 28% higher than that in the single WT plant, and the value in the competing WT tomato was significantly greater than the competing NR plant by 26%. No significant difference in foliar ethylene was detected between the competing plants and the single WT plant under deficit irrigation.

2.3. Effects of above- and below-ground competition on plant response to competition

With sufficient water supply, the pot size had insignificant influences on plant growth, stomatal opening, and hormone synthesis of the competing tomato (data not shown). From the first day to 10 days after deficit irrigation, soil moisture in the small pot with a volume of 0.94 L was slightly

lower than that in the big pot with a volume of 1.86 L, the difference was not significant. While at 12 days after deficit irrigation, the value in the big pot was 17.3%, significantly greater than 13.0% in the small pot. At 10 days after deficit irrigation, there were insignificant differences in leaf area, transpiration and foliar ABA concentration of tomato between the small pot and big pot, whereas the difference in foliar ethylene evolution was significant (Table 2). At 12 days after deficit irrigation, the significant difference in plant growth and hormone synthesis was detected between the small and big pot with severe drought.

Table 2
Effects of pot size on leaf area, transpiration and foliar ABA content.

Days after deficit irrigation	Leaf area		Transpiration		Foliar ABA		Ethylene evolution	
	(cm ²)		(g m ⁻² hr ⁻¹)		(ng g ⁻¹ DW)		(nl g ⁻¹ FW)	
	1.86L	0.94L	1.86L	0.94L	1.86L	0.94L	1.86L	0.94L
10	53.95±4.6a	48.54±3.6a	261.83±21.3a	243.52±23.5a	872.45±67.5a	983.12±56.3a	1.37±0.04b	2.02±0.05a
12	92.39±6.3a	78.56±8.1b	170.78±15.2a	136.43±10.5b	1474.32±112.3b	2043.23±151.2a	2.56±0.62b	3.42±0.81a

Effects of absence of canopy competition or root competition on g_s , ABA content and ethylene evolution in the WT tomato under different treatments are presented in Table 3. The value of g_s in the well-watered WT plants without canopy competition was significantly higher than that in the WT plant with above-and below-ground competition by 25%. The influence of belowground competition on stomatal opening was insignificant under well-watered condition, as the g_s between the WT tomato with and without root competition was comparable. The belowground competition, i.e., absence of canopy competition, significantly decreased the foliar ABA concentration of the competing plants, while the aboveground competition did not affect the foliar accumulation of ABA. Whereas, both the root separation and canopy separation had no remarkable effects on the concentration of ABA in the well-watered WT tomato roots. For foliar ethylene evolution of tomato, absence of canopy competition reduced the foliar ethylene evolution distinctly, but root separation had insignificant influence on foliar ethylene evolution.

Under the condition of water deficit, the absence of aboveground competition had in-distinctive influence on the g_s of the competing WT tomato, which was in contrast with the result under full irrigation. The result indicating water deficit strengthened the effects of root competition on stomatal opening. Both root separation and canopy separation did not influence the foliar concentration of ABA. In contrast to the result under full irrigation, the belowground competition increased ABA accumulation in the roots of the competing WT tomato. The absence of belowground competition significantly increased the foliar ethylene evolution in the WT tomato, while the absence of aboveground competition had insignificant effects on ethylene evolution.

Table 3
Abaxial stomatal conductance (mmol m⁻² s⁻¹), ABA content (ng g⁻¹ DW) and ethylene evolution (nl g⁻¹ FW) in competing WT plants under full irrigation and deficit irrigation. NC-without canopy competition, NR-without root competition.

Treatment	Full irrigation				Deficit irrigation			
	g_s	Foliar ABA	Root ABA	Ethylene	g_s	Foliar ABA	Root ABA	Ethylene
WT/WT	238.62 ±21.34b	601.34 ±49.32a	49.34 ±4.39a	1.23 ±0.07a	144.17 ±11.14a	1643.14 ±137.92a	145.44 ±14.33b	2.11 ±0.15b
WT/WT-NC	298.83 ±22.31a	561.83 ±38.51b	48.43 ±3.67a	0.91 ±0.04b	143.36 ±11.31a	1701.36 ±132.47a	178.37 ±13.07a	1.91 ±0.14b
WT/WT-NR	247.31 ±19.45b	611.23 ±51.11a	51.23 ±5.34a	1.27 ±0.06a	157.31 ±14.15a	1681.35 ±138.13a	131.31 ±15.43b	2.47 ±0.16a

3. Discussion

Intra-/inter-specific competition is the key external factor influencing plant growth, physiologies and functions [3, 27]. The few studies that have explored links plant growth traits and competition have shown the relationships were complex, as a few of plant hormones involved in plant response to competition [3, 7, 9, 11, 14, 18]. Growing space for shoot and roots plays an important role in interception of radiation and absorption of water and nutrients, respectively. These authors usually planted the single plant and competing plants in pots with same volume, the difference in growing space was overlooked. Unlike to the competition literatures, the effect of pot size on plant response was explicitly accounted for in our experiment. Our results shown that the effects of pot size on plant growth and hormone synthesis were dependent on soil water availability. Soil drying stimulates ABA formation in roots, translocation to leaves, then reduction in stomatal opening and plant growth [24]. Moreover, soil drying

promotes soil compaction and increase in plant ethylene production [28]. Our results also demonstrated that soil drying-induced compaction increased foliar ethylene evolution in both the big and small pot. The severe drought (12 days after deficit irrigation) significantly decreased ABA and ethylene synthesis in the small pot compared with the big pot. Moreover, as the physical properties were different between the compost we used in this experiment and soil, soil drying-induced compaction in soil maybe more significant. Therefore, to compare soil drying-induced influences on plant physiology and phenotype in different volumes, soil compaction and soil water availability need to be considered.

Plant hormone ABA and ethylene are known to regulate stomatal opening in response to competition from a neighbor [3, 7, 18]. With growing WT tomato and NR or FL tomato in one pot, we also concluded that ABA and ethylene involved in plant response to intraspecific competition. The decreased in stomatal conductance, leaf area and transpiration, which was induced by intraspecific competition, was accompanied by an increased in plant hormone concentrations. Vysotskaya et al. [7] found no significant difference in ABA concentration in xylem sap between single tomato and competing tomato. Whereas the elevated foliar ABA concentration in competing plants was measured in our experiment, which was in accord with earlier conclusions [18, 29]. Vysotskaya et al. [18] indicated that the competing from neighbors increased ABA concentration in lettuce shoot. Kurepin et al. [29] attributed the increased ABA concentration in *Helianthus annuus* leaves to shade light-reduced R/FR ratio. Intraspecific competition increased ABA concentration in tomato in our experiment, which contrasted with the lettuce data of Vysotskaya et al. [18]. This may be explained by different species, soil and environments.

Both ABA and ethylene are known to regulate stomatal opening in response to reduced water availability [28, 30–31]. As soil dries, the competition decreased g_s by elevating ABA and ethylene accumulations, whereas, under severe drought, the competition cannot reduce g_s even increasing in these plant hormones accumulations. During the early stages of soil drought before hydraulic signals were produced, plant hormones dominated plant in response to stress [32]. Under severe drought, chemical signals became less important when LWP declines and leaves wilt [30]. The results indicated that the competition-induced decline in g_s may be covered by the severe soil-drying-induced decrease in g_s , as hydraulic signals most probably dominate (Fig. 2a).

Except for severe, competition regulated plant stomatal closure mainly through non-hydraulic signals [11–12]. Ethylene is an important phytohormone of sensing competing neighbors and determining plant responses to neighbors [7, 13–14]. Under well-watered condition, the absence of canopy competition cannot significantly reduce the stomatal opening (Table 3). Ethylene and the red: far-red light ratio (R:FR) are most important above-ground signals of plant neighbor detection [13, 33]. Canopy separation minimized the plant neighbor sensing by ethylene and R:FR. Although root exudates can serve as a belowground neighbor detection signal [34], belowground neighbor detection most probably occur through reduction of local soil water and nutrients [35]. As presence of above-ground sensing signals in the competing pot with root separation, the competition-induced influences on plant growth occurred observably.

Neighbors can be detected by several sensing signals in aboveground and below-ground through neighbor-induced changes in resource availability. The absence of canopy competition significantly decreased ABA accumulation in roots and then stomatal conductance, which contrasted with the result under full irrigation, indicating the below-ground neighbor detection signals were enhanced by soil drought. Although ethylene evolution was increased by drought, elevated ethylene synthesis did not significantly affect stomatal opening. Under some circumstances, ethylene can modulate stomatal responses to a given ABA concentration [17, 36–37].

Vysotskaya et al. [7, 18] suggested that several plant hormones, such as ABA, ethylene, auxin and cytokinins, involved in plant growth response to competition from neighbors, while the interactive mechanism of multi-hormones regulating plant response to competition still was unclear. Further experiments are necessary to learn more about interaction between competition and defense responses. Moreover, only a few of competition experiments were conducted under natural field conditions. Therefore, better understanding the multi-hormones mediated plant-plant interactions could help to optimize plant density and to understand plant behaviors in natural environment.

4. Materials And Methods

4.1. Plant materials

Tomato (the wild-type, Never-ripe mutant, and *flacca* mutant) was used as a model species. The wild-type (WT) of tomato was Ailsa Craig. The Never-ripe mutant (NR) was the partially ethylene-insensitive genotype, and the *flacca* mutant (FL) was the abscisic acid (ABA) -deficient tomato mutant. Seeds from the three genotypes, which were obtained from the Tomato Genetics Resource Center (University of California, Davis, USA), were germinated in compost (John Innes No.2) and covered with black plastic. After 6-7 days, the plastic was removed to prevent etiolation of the seedlings. After a further 8-days, seedlings were transferred to pots, which were filled with the same substrate, and grown in a walk-in controlled environment room with a day/night temperature of 32/16°C and a 12h photo-period (06:00-18:00). Light intensity at plant height was between 400 and 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD (Photosynthetic Photon Flux Density).

4.2. Experimental design

Two irrigation levels were designed as full irrigation (irrigation amount=daily transpiration) and deficit irrigation (60% of irrigation amount in full irrigation). Three kinds of competition were designed, i.e., with root and canopy competition, without root competition and without canopy competition, respectively. There were three sub-treatments (two plants in one pot) in each competition, i.e., WT/WT, WT/NR and WT/FL, respectively. CK was single plant of the three species in one pot. Two different size pots were used for the treatment of single plant, i.e., 1.86 L and 0.94 L, respectively. The pot size for the competing plants was 1.86 L. Therefore, 30 treatments with 10 replications were carried out in this experiment (Table 4). Each treatment was replicated twice. The pot in the treatment with-out root competition was completely separated into two equal parts using an acrylic divider, which was glued to the inner wall and bottom of pot. For the treatment without canopy competition, a transparent glass barrier was placed between the aerial portions of two plants to totally separate the shoot components.

Table 4
Experimental design in this study. WT-wild type, NR-never ripe mutant, FL-*flacca* mutant.

Irrigation factor	Competition factor		
Full Irrigation	Single Plant	^a 1.86L	WT, NR, FL
		^a 0.94L	WT, NR, FL
	Competing Plants	With root and canopy competition	WT/WT, WT/NR, WT/FL
		Without root competition	WT/WT, WT/NR, WT/FL
		Without canopy competition	WT/WT, WT/NR, WT/FL
	Deficit Irrigation	Single Plant	^a 1.86L
^a 0.94L			WT, NR, FL
Competing Plants		With root and canopy competition	WT/WT, WT/NR, WT/FL
		Without root competition	WT/WT, WT/NR, WT/FL
		Without canopy competition	WT/WT, WT/NR, WT/FL
^a The volume of pots.			

4.3. Plant measurements

At 10 days after transferring tomato plants into pots, plants were harvested to measure leaf area using a leaf area meter (Licor Model 3100 Area Meter, Cambridge, UK). The dry weight of leaf, stem, and root was also measured. Leaf water potential (LWP) of tissue discs from mature leaves of lettuce plants was measured with Wescor 5100 thermocouple psychrometers (Logan, UT, USA). The stomatal conductance (g_s) was measured between 10:00 and 10:30 using a porometer (AP4, Delta-T Devices Ltd, Cambridge, UK). In some experiments, the soil surface was covered with aluminum foil to prevent water evaporation, and then the plant transpiration was determined.

4.4. Plant hormone analysis

Bulk leaf ABA concentration as well as root ABA concentration was measured with a radioimmunoassay (RIA) using the monoclonal antibody AFRC MAC 252 [38]. The youngest and fully expanded leaflet was harvested for ABA measurement. Plants were sampled at the same time (10:00-10:30) on each harvesting day to avoid diurnal effects on foliar ABA concentration. Leaflets and roots (on the same plant) were sampled and snap frozen in liquid nitrogen, freeze-dried for 48h, then finely ground. A small number of samples (10-15 mg dry weight for leaflets, and 30-40 mg dry weight for roots) was needed for ABA analysis. Then, the samples were diluted with deionized, distilled water (1:70 for leaflets, and 1:25 for roots). Samples were then placed on a shaker in a cold room (4°C) overnight to extract ABA. A standard curve was determined with standards in a serial dilution of synthetic unlabeled (\pm)-cis, trans-ABA (Sigma Let., Dorset, UK). ABA concentration was calculated by reference to the standard curve after linearization using the 'logit' transformation.

For the determination of ethylene evolution rates, leaflets and roots on the same plant sampled for ABA determination were weighed and placed in 28 ml glass vials containing saturated filter paper, which was then sealed with a rubber puncture cap. The leaflets and roots samples were incubated for 60 min under a lamp ($200 \mu \text{mol m}^{-2} \text{s}^{-1}$) and in a dark chamber, respectively. A 1 ml headspace sample was withdrawn using a gas-tight syringe, then manually injected into a gas chromatograph (6890N, Agilent Technologies UK Ltd, Wokingham, UK; Networked GC system, method: Ethylenesplit. M, software: Enhanced Chemstation Online GC) equipped with a J&W HP-AL/S (50 m \times 0.537 mm \times 15.0 mm) column (HiChrom Ltd, Reading, UK). This was maintained for the first 5 min at 100°C to resolve ethylene, and then ramped at 15°C min⁻¹ to 150°C and held for 1.5 min to drive off any water vapor introduced onto the column by sample injection. The carrier gas was helium at a flow rate of 5.7 mL

min⁻¹, and detection was by flame ionization. The rate of ethylene evolution was determined with reference to peak areas of known ethylene standards (99.995% minimum purity, BOC Special Gases, Manchester, UK), and corrected for tissue FW and time in incubation.

4.5. Statistical analysis

All data were analyzed by one-way analysis of variance (ANOVA). Less significant difference (LSD) and Student's t-test were carried out with SPSS21.

Declarations

Author Contributions:

Conceptualization, Y.G.; methodology, Y.G.; software, Y.L. and S.L.; formal analysis, Y.G. and Z.S.; investigation, Y.G. and S.L.; data curation, Y.L.; writing—original draft preparation, Y.G. and Z.S.; writing—review and editing, A.K.M.H.; project administration, Y.G.; funding acquisition, Y.G. All authors have read and agreed to the published version of the manuscript.

Funding:

This research was funded by the National Natural Science Foundation of China (Grant No. 51879267) and the Agricultural Science and Technology Innovation Program (ASTIP).

Data Availability Statement:

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments:

We thank Dr. Ian Dodd in Lancaster University for his invaluable guidance and assistance.

Conflicts of Interest:

The authors declare no conflict of interest.

References

1. Rejeb IB, Pastor V, Mauch-Mani B. **Plant responses to simultaneous biotic and abiotic stress: molecular mechanisms.** *Plants* 2014, **3**: 458–475.
2. Qin F, Shinozaki K, Yamaguchi-Shinozaki K. **Achievements and challenges in understanding plant abiotic stress responses and tolerance.** *Plant Cell Physiol* 2011, **52**: 1569–1582.
3. Arkhipova T, Vysotskaya L, Martinenko E, Ivanov I, Kudoyarova G. **Participation of cytokinins in plant response to competitors.** *Russ J Plant Physiol* 2015, **62**: 524–533.
4. Kazan K. **Diverse roles of jasmonates and ethylene in abiotic stress tolerance.** *Trends Plant Sci* 2015, **20**: 219–229.
5. Peleg Z, Blumwald E. **Hormone balance and abiotic stress tolerance in crop plants.** *Curr Opin Plant Biol* 2011, **14**: 290–295.
6. Verma V, Ravindran P, Kumar PP. **Plant hormone-mediated regulation of stress responses.** *BMC Plant Biol* 2016, **16**: 86.
7. Vysotskaya L, Wilkinson S, Davies WJ, Arkhipova T, Kudoyarova G. **The effect of competition from neighbors on stomatal conductance in lettuce and tomato plants.** *Plant Cell Environ* 2011, **34**: 729–737.
8. Zhu JK. **Salt and drought stress signal transduction in plants.** *Annu Rev Plant Biol* 2002, **53**: 247–273.
9. Pierik R, Whitelam GC, Voesenek LACJ, De Kroon H, Visser EJW. **Canopy studies on ethylene-insensitive tobacco identify ethylene as a novel element in blue light and plant–plant signalling.** *Plant J* 2004, **38**: 310–319.
10. Semchenko M, Hutchings MJ, John EA. **Challenging the tragedy of the commons in root competition: confounding effects of neighbour presence and substrate volume.** *J Ecol* 2007, **95**: 252–260.
11. Vysotskaya L, Arkhipova TN, Kudoyarova GR, Veselov SY. **Dependence of growth inhibiting action of increased planting density on capacity of lettuce plants to synthesize ABA.** *J Plant Physiol* 2018, **220**: 69–73.

12. Masclaux FG, Bruessow F, Schweizer F, Gouhier-Darimont C, Keller L, Reymond P. **Transcriptome analysis of intra-specific competition in *Arabidopsis thaliana* reveals organ-specific signatures related to nutrient acquisition and general stress response pathways.** *BMC Plant Biol* 2012, **12**: 227.
13. Kegge W, Pierik R. **Biogenic volatile organic compounds and plant competition.** *Trends Plant Sci* 2010, **15**: 126–132.
14. Pierik R, Sasidharan R, Voeselek LA. **Growth control by ethylene: adjusting phenotypes to the environment.** *J Plant Growth Regul* 2007, **26**: 188–200.
15. Tholen D, Pons TL, Voeselek LA, Poorter H. **Ethylene insensitivity results in down-regulation of Rubisco expression and photosynthetic capacity in tobacco.** *Plant Physiol* 2007, **144**: 1305–1315.
16. Nazareno AL, Hernandez BS. **A mathematical model of the interaction of abscisic acid, ethylene and methyl jasmonate on stomatal closure in plants.** *PLoS One* 2017, **12**: e0171065.
17. Wilkinson S, Davies WJ. **Ozone suppresses soil drying-and abscisic acid (ABA)-induced stomatal closure via an ethylene-dependent mechanism.** *Plant Cell Environ* 2009, **32**: 949–959.
18. Vysotskaya L, Veselov SY, Kudoyarova GR. **Effect of competition and treatment with inhibitor of ethylene perception on growth and hormone content of lettuce plants.** *J Plant Growth Regul* 2017, **36**: 450–459.
19. di Iorio A, Montagnoli A, Terzaghi M, Scippa GS, Chiatante D. **Effect of tree density on root distribution in *Fagus sylvatica* stands: a semi-automatic digitising device approach to trench wall method.** *Trees* 2013, **27**: 1503–1513.
20. Contador ML, Comas LH, Metcalf SG, Stewart WL, Porris Gomez I, Negron C, Lampinen BD. **Root growth dynamics linked to above-ground growth in walnut (*Juglans regia*).** *Ann Bot* 2015, **116**: 49–60
21. Buckley TN. **Stomatal responses to humidity: has the 'black box' finally been opened?** *Plant Cell Environ* 2016, **39**: 482–484.
22. Wolz KJ, Wertin TM, Abordo M, Wang D, Leakey ADB. **Diversity in stomatal function is integral to modelling plant carbon and water fluxes.** *Nat Ecol Evol* 2017, **1**: 1292–1298.
23. Albert R, Acharya BR, Jeon BW, Zañudo JG, Zhu M, Osman K, Assmann SM. **A new discrete dynamic model of ABA-induced stomatal closure predicts key feedback loops.** *PLoS Biol* 2017, **15**: e2003451.
24. Davies WJ, Kudoyarova G, Hartung W. **Long-distance ABA signaling and its relation to other signaling pathways in the detection of soil drying and the mediation of the plant's response to drought.** *J Plant Growth Regul* 2005, **24**: 285.
25. Munemasa S, Hauser F, Park J, Waadt R, Brandt B, Schroeder JI. **Mechanisms of abscisic acid-mediated control of stomatal aperture.** *Curr Opin Plant Biol* 2015, **28**: 154–162.
26. Sarwat M, Tuteja N. **Hormonal signaling to control stomatal movement during drought stress.** *Plant Gene* 2017, **11**: 143–153.
27. Kunstler G, Falster D, Coomes DA, Hui F, Kooyman RM, Laughlin DC, Poorter L, Vanderwel M, Vieilledent G, Wright SJ, Aiba M, Baraloto C, Caspersen J, Cornelissen JHC, Gourlet-Fleury S, Hanewinkel M, Herault B, Kattge J, Kurokawa H, Onoda Y, Peñuelas J, Poorter H, Uriarte M, Richardson S, Ruiz-Benito P, Sun IF, Ståhl G, Swenson NG, Thompson J, Westerlund B, Wirth C, Zavala MA, Zeng H, Zimmerman JK, Zimmermann NE, Westoby M. **Plant functional traits have globally consistent effects on competition.** *Nature* 2015, **529**: 204.
28. Wilkinson S, Davies WJ. **Drought, ozone, ABA and ethylene: new insights from cell to plant to community.** *Plant Cell Environ* 2010, **33**: 510–525.
29. Kurepin LV, Emery RN, Pharis RP, Reid DM. **Uncoupling light quality from light irradiance effects in *Helianthus annuus* shoots: putative roles for plant hormones in leaf and internode growth.** *J Exp Bot* 2007, **58**: 2145–2157.
30. Schachtman DP, Goodger JQD. **Chemical root to shoot signaling under drought.** *Trends in Plant Sci* 2008, **13**: 281–287.
31. Boyle RKA, McAinsh M, Dodd IC. **Stomatal closure of *Pelargonium × hortorum* in response to soil water deficit is associated with decreased leaf water potential only under rapid soil drying.** *Physiol Plantarum* 2016, **156**: 84–96.
32. Goodger JQ, Sharp RE, Marsh EL, Schachtman DP. **Relationships between xylem sap constituents and leaf conductance of well-watered and water-stressed maize across three xylem sap sampling techniques.** *J Exp Bot* 2005, **56**: 2389–2400.
33. Ballaré CL. **Phytochrome Responses: Think Globally, Act Locally.** *Trends in Plant Sci* 2017, **22**: 909–911.
34. de Kroon H. **How do roots interact?** *Science* 2007, **318**: 1562.
35. Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. **The role of root exudates in rhizosphere interactions with plants and other organisms.** *Ann Rev Plant Biol* 2006, **57**: 233–266.
36. Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S. **Ethylene inhibits abscisic acid-induced stomatal closure in *Arabidopsis*.** *Plant Physiol* 2005, **138**: 2337–2343.
37. She X, Song X. **Ethylene inhibits abscisic acid-induced stomatal closure in *Vicia faba* via reducing nitric oxide levels in guard cells.** *New Zeal J Bot* 2012, **50**: 203–216.
38. Quarrie S, Whitford P, Appleford N, Wang T, Cook S, Henson I, Loveys B. **A monoclonal antibody to (S)-abscisic acid: its characterisation and use in a radioimmunoassay for measuring abscisic acid in crude extracts of cereal and lupin leaves.** *Planta* 1988, **173**: 330–339.

Figures

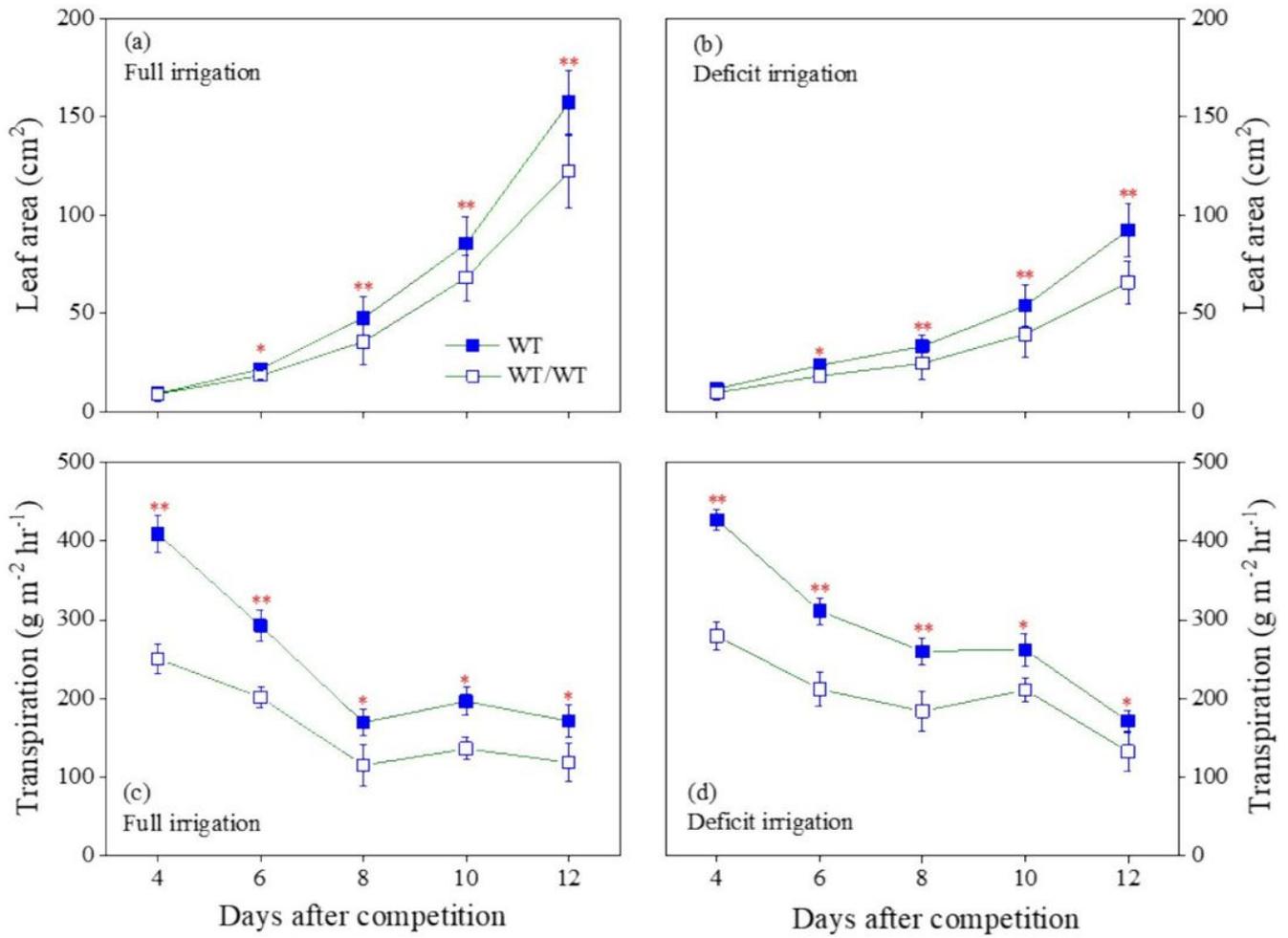


Figure 1
 Variations of leaf area and transpiration in CK and the treatment of competition under full irrigation and deficit irrigation. * indicates significant difference between treatments (* $P < 0.05$, ** $P < 0.01$).

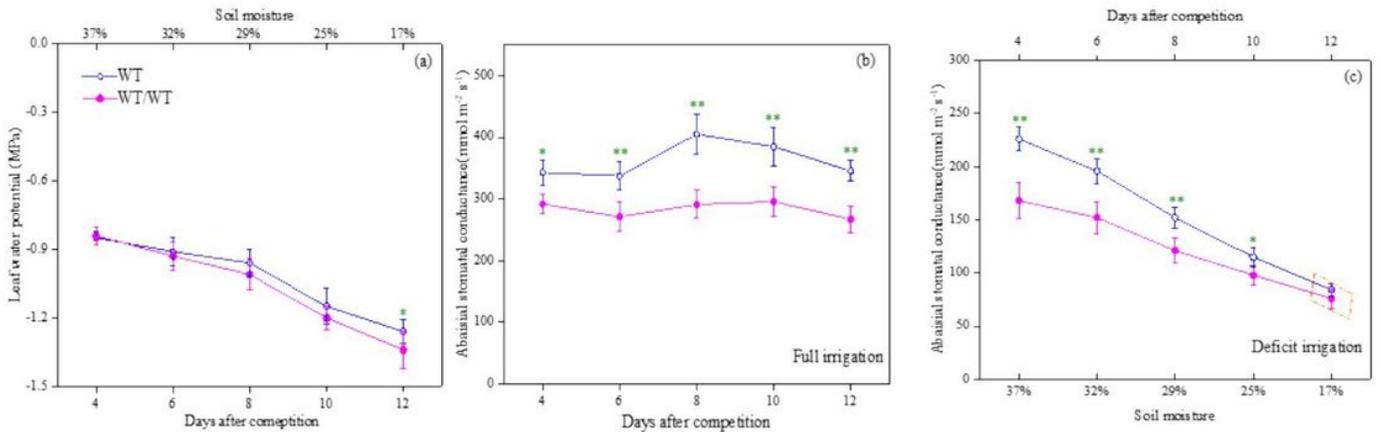


Figure 2

Variation of leaf water potential (a-deficit irrigation), abaxial stomatal conductance (b-full irrigation, c-deficit irrigation) in the single plant and competing plants of wild type tomato (*P<0.05, **P<0.01).

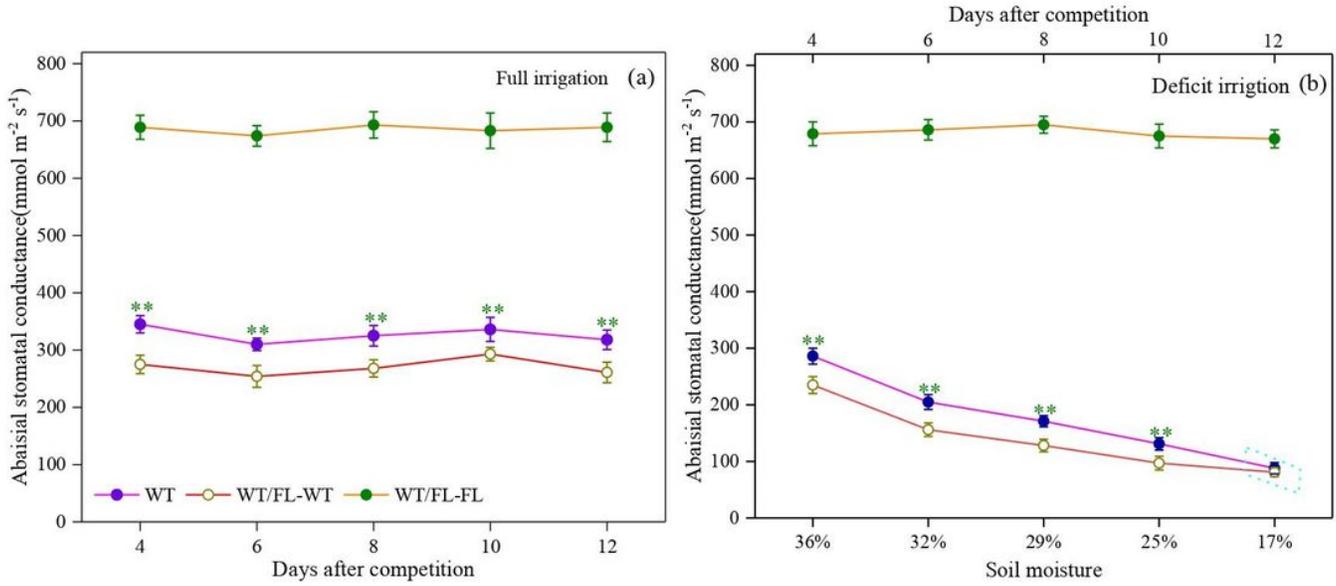


Figure 3

Variation of abaxial stomatal conductance (a-full irrigation, b-deficit irrigation) of FL and WT in competing pot. The wild-type tomato and flacca mutant was planted in one pot (WT/FL) (*P<0.05, **P<0.01).

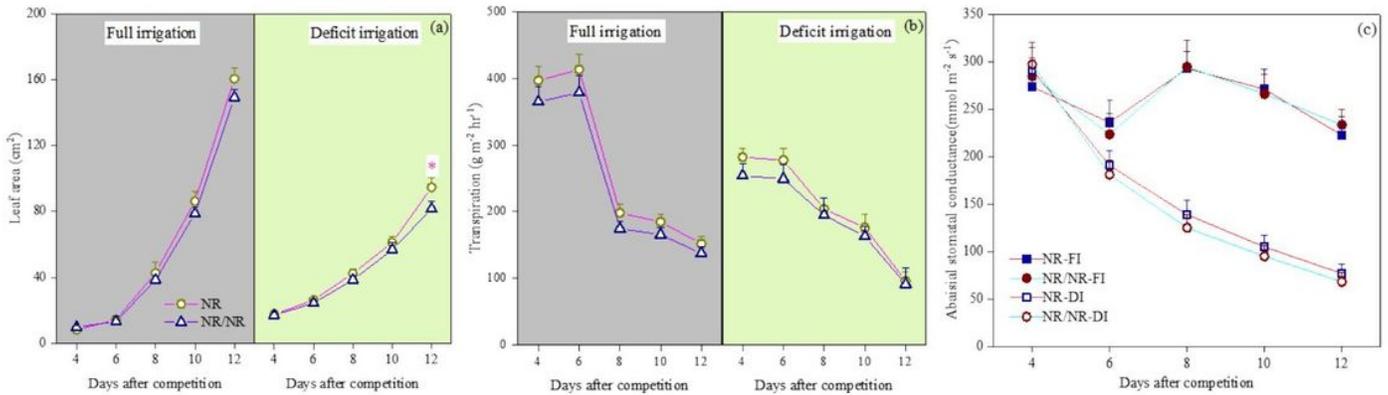


Figure 4

Variation of leaf area (a), transpiration (b) and abaxial stomatal conductance (c) of the single and competing plant of NR tomato under full irrigation and deficit irrigation.

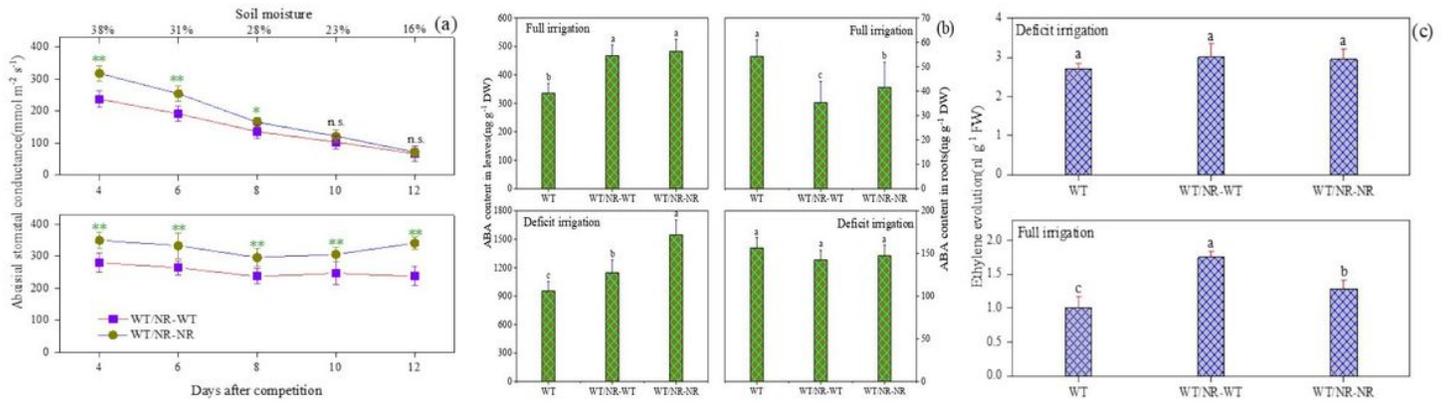


Figure 5

Effects of intraspecific competition on stomatal conductance (a), ABA content in leaves and roots (b), and ethylene evolution (c) of the single WT tomato and the competing plants from pot with WT and NR tomato under full irrigation and deficit irrigation.