Title: United we stand: Plants that physically touch each other are more resilient to stress

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Abstract:

Plants use sophisticated signaling networks to communicate with each other. This process is thought to support the overall health and resilience of plant communities, but could also reflect eavesdropping between plants, used for competition. Here we reveal that plants that physically touch each other aboveground are more resilient to stress, and that this phenomenon is dependent on the ability of plants to exchange electric, calcium, and reactive oxygen species (ROS) signals with each other. We further separate electric- from calcium/ROS-dependent plant-to-plant signals and transcriptional landscapes and show that the function of several calcium/ROS-dependent transcripts is required for stress acclimation. Our study reveals that plants that live together and physically touch each other establish an aboveground community-wide signaling network that enhances their collective resilience to stress.

Main Text:

Plants are vital to most ecosystems on Earth. They establish biological networks with other organisms, as well as communicate with each other in multiple ways (*1–3*). Some of the well-studied plant-to-plant (P-T-P) communication pathways include belowground routes involving direct physical touch between roots, interactions between root-associated arbuscular mycorrhiza networks and other microorganisms, and exchange of different compounds/RNA molecules between roots of different plants (*4–7*). In addition, aboveground pathways involving volatiles, acoustic sounds, light reflection/filtering, and physical touch between leaves, are also thought to play a role in P-T-P communication (*8–11*). Although communication between plants was proposed to support the overall health and resilience of different plant communities and ecosystems (*8*, *12–14*), direct evidence supporting such a role for this process is scarce. Moreover, eavesdropping between plants, used for competition instead of cooperation, was also proposed as a possible role for P-T-P communication (*15*, *16*). We recently reported that two plants that physically touch each other aboveground, via leaf-to-leaf contacts or through a parasitic plant that connects them, can exchange systemic electric and reactive oxygen species (ROS) signals, or waves, between each other (*9*, *17*). However, whether this P-T-P communication process plays a key role in enhancing the resilience of a community of plants to stress is unknown. As plants growing within many ecosystems in nature physically touch each other, and this physical touch can mediate electric and/or ROS P-T-P communication between them (*9*, *17*), we hypothesized that plants living in a group or a community, touching each other, will exchange systemic signals with each other, and that this communication process will allow the entire community to become more resilient to stress.

To test this hypothesis, we compared the transcriptome and stress resilience of individual *Arabidopsis thaliana* (*A. thaliana*) plants to that of a group of *A. thaliana* plants that physically touch each other (Fig. 1A; fig. S1). We found that, compared to individual plants, the transcriptome of plants that touch each other for 1 hour or 1 week contained a significantly higher number of stress and hormone response transcripts (2,248 and 1,780 transcripts significantly altered in response to a 1 hour or 1 week of touching, respectively, with 595 transcripts common to these two responses; Fig. 1B,C; tables S1-S2). These were especially enriched in touch, cold, waterlogging, ROS, and salicylic acid (SA) response transcripts, as well as included transcripts involved in responses to excess light (EL), salt, wounding, and brassinosteroids (Fig. 1D; table S3). In agreement with these findings, compared to plants living in a group and touching each other, individual plants accumulated more anthocyanins (a common sign of stress in *A. thaliana*), as well as experienced more cellular damage (measured by ion leakage from injured cells), when challenged by growth under EL stress for 24 hours (Fig. 1E, fig. S1). The findings presented in Fig. 1 reveal that *A. thaliana* plants that physically touch each other are primed to resist stress and can withstand EL stress better than individual plants that do not touch each other.

Two or more plants that touch each other aboveground, via leaf-to-leaf contacts, were found to transfer electric and ROS signals between each other (*9*). However, whether these signals are required to induce resilience in a group of plants that live together (Fig. 1) remains to be answered. To address this question, we studied the transfer of systemic membrane potential (electric), calcium (Ca^{2+}), and ROS signals between 3 different plants that touch each other in a successive arrangement (*i.e.,* transmitter - T, mediator - M, and receiver - R) in response to EL stress applied using a fiber optic to a single leaf of the transmitter plant (a leaf not involved in the P-T-P connection; termed the local leaf; L; Fig. 2A-C, fig. S2). To separate the systemic electric wave from the systemic Ca^{2+} and ROS signals we either used wild type (WT) or a mutant that transmits

the electric, but not the Ca^{2+} and ROS waves [HYDROGEN PEROXIDE INDUCED Ca^{2+} INCREASES 1; *hpca1* (*18*)], as the mediator plant (Fig. 2A-C, fig. S2). The application of EL to a local leaf of the transmitter plant in the WT-WT-WT arrangement resulted in the transfer of systemic electric, Ca^{2+}, and ROS signals from the transmitter plant, through the mediator plant, to the receiver plant (Fig. 2A-C; no systemic ROS signals were detected in similarly arranged plants, not subjected to the EL stress; fig. S3). In contrast, in the WT-*hpca1*-WT setting, only the electric signal was transferred all the way from the transmitter to the receiver plant, while the systemic Ca^{2+} and ROS signals were restricted to the transmitter plant (Fig. 2A-C). The two arrangements shown in Fig. 2A-C (*i.e,* WT-WT-WT and WT-*hpca1*-WT) allowed us therefore to separate the electric from the Ca^{2+}/ROS systemic signals during P-T-P communication. Using these two setups (Fig. 2A-C), we then asked what signals are required to induce acclimation to EL in the receiver plant in response to an EL stress applied to the transmitting plant? In agreement with previous studies of individual plant acclimation (*19–21*), when EL was applied to the local (L) leaf of the transmitting plant, it induced acclimation to EL in the systemic leaf of the same plant (S1; Fig. 2D). Remarkably, in the WT-WT-WT setup, it also induced acclimation in a systemic leaf of the receiver plant (S3; Fig. 2D, fig S4A; in agreement with Fig. 1E). In contrast, while application of EL to the local leaf of the transmitter plant in the WT-*hpca1*-WT setup induced acclimation in the systemic leaf of this plant (S1), it was unable to induce acclimation in the systemic leaf of the receiver plant (S3; Fig. 2D). This finding reveals that the transfer of the electric wave between all 3 plants in the WT-*hpca1*-WT setup is insufficient to induce acclimation to EL in the receiver plant (Fig. 2). The transfer of all 3 signals between different plants is likely therefore required for the acclimation of a community or group of plants to EL stress (Figs. 1, 2, figs. S2, S3).

To determine what systemic transcriptomic responses are associated with the electric, or Ca^{2+}/ROS waves, during P-T-P communication (Fig. 2A-C), we conducted an RNA-Seq analysis of the local (L) and systemic (S1-S3) leaves of plants arranged in the WT-WT-WT and WT-*hpca1*-WT setups in response to an EL stress applied to a single leaf of the transmitter plant (not involved in the P-T-P interaction; Fig. 3A, figs. S2 and S5; table S4). While the steady-state level of over 6000 transcripts was significantly altered in the local leaf of plants from both setups in response to EL stress (with an overlap of over 4500 transcripts; Fig. 3A-C), except for S1 in the WT-*hpca1*-WT setup, fewer transcripts (ranging from 1400 to 3400) were altered in their expression in the systemic leaves of each setup in response to EL stress applied to the local leaf (S1-S3; Fig. 3A-C; tables S5-S6; figs. S6-S7). Notably, transcripts present in S2 and S3 in both setups had a considerable overlap with their corresponding local leaf (1500-2100 in the WT-WT-WT setup and 1700-1800 in the WT-*hpca1*-WT setup), as well as an overlap of about 1000-1200 transcripts with each other (Fig. 3A-C; tables S5-S6). The findings presented in Fig. 3A-C reveal therefore that P-T-P communication (Fig. 2) can result in the altered expression of thousands of transcripts in the systemic leaves of mediator and receiver plants (S2 and S3) that are connected to the transmitter (L, S1) plant through P-T-P communication, but are not directly exposed to the EL stress (only applied using a fiber optic to leaf L of the transmitter plant; Fig. 3A-C; fig. S2). As many of the transcripts altered in the systemic leaves of the mediator and receiver plants (S2 and S3 in both setups) had a considerable overlap with the local (L) leaf of the transmitter plant that was exposed to the EL stress (Fig. 3A-C), it is likely that the transfer of some of the different systemic signals between the successively arranged plants that touched each other had an important biological context and could induce acclimation to EL stress (Fig. 2D). The exceptionally large number of transcripts altered in the systemic leaf of the transmitter plant (6037; S1) in the WT-*hpca1*-WT setup, compared to the low number of overall transcripts in the S1 leaf in the WT-WT-WT setup (1428), could suggest that the disruption in P-T-P communication caused by the inclusion of the

hpca1 mutant as the mediator plant in the WT-*hpca1*-WT setup (Fig. 2) caused some type of a feedback response in the transmitter plant of this setup (Fig. 3A-C). These transcripts were enriched in SA, H_2O_2, ozone and ROS response transcripts (table S7), suggesting that the inability of the transmitter plant to induce the Ca^{2+}/ROS waves in the mediator (*hpca1*) plant in the WT-*hpca1*-WT setup resulted in a ROS associated feedback response.

Compared to the WT-WT-WT setup, the systemic Ca^{2+}/ROS waves were suppressed in the mediator and receiver plants in the WT-*hpca1*-WT setup (Figs. 2A-C, 3D), in which the systemic leaf of the receiver plants (S3) was unable to acclimate to EL stress (Fig. 2D). In contrast, the electric wave was present in all plants belonging to both setups (Figs. 2A-C, 3D). This difference allowed us to separate systemic P-T-P transcriptomics responses to the electric wave from those to the Ca^{2+}/ROS waves during P-T-P communication. Thus, we identified transcripts absent from S2 and S3 in the WT-*hpca1*-WT setup (compared to the WT-WT-WT setup; marked with an 'X' in Fig. 3D; 37 transcripts; table S8) and termed them 'Ca^{2+}/ROS dependent transcripts', as well as identified transcripts present in S2 and S3 in the WT-*hpca1*-WT setup (compared to the WT-WT-WT setup; marked with an '√' in Fig. 3D; 589 transcripts; table S9) and termed them 'electric wave dependent transcripts' (Fig. 3D). While wounding and touch response transcripts were equally represented in both groups of transcripts, electric wave dependent transcripts were especially enriched in jasmonic acid (JA) response transcripts, while Ca^{2+} and ROS dependent transcripts were especially enriched in EL, drought, salt, abscisic acid (ABA), and H_2O_2 response transcripts (Fig. 3D; table S10). As systemic Ca^{2+}/ROS signaling is required for plant acclimation to EL stress (Fig. 2D), we hypothesized that some of the systemically induced Ca^{2+}/ROS response transcripts present in the WT-WT-WT setup in S2 and S3, but absent in the WT-*hpca1*-WT setup (37 transcripts; Fig. 3D; table S8; fig. S8), are required for this acclimation process. To test this hypothesis, we obtained mutants deficient in the expression of several Ca^{2+}/ROS dependent transcripts and tested their ability to acclimate to EL stress (Fig. 4A-C).

Altogether, we studied the role of 6 different genes (using two independent mutant alleles per locus; table S11), identified as encoding Ca^{2+}/ROS response transcripts to EL stress (Fig. 3D; table S8) and identified 5 that had altered ability to acclimate to EL stress (Fig. 4A-C; fig. S9). While WT plants were able to locally and systemically acclimate to an extended (45 min) EL stress following a short (10 min) EL stress applied to a single leaf [leaf L; Fig. 4A, fig. S4B; previously reported by (*19*, *20*, *21*)], two independent mutant alleles per locus of *WRKY DNA-BINDING PROTEIN 33* (*WRKY33*; AT2G38470; *22*), a transcriptional regulator involved in responses to various biotic and abiotic stresses, and *ETHYLENE RESPONSE FACTOR 22* (*ERF22*; AT1G33760; *23*), a transcriptional regulator involved in ethylene responses, were able to acclimate to EL stress, as well as displayed enhanced resilience to this stress in the absence of a pretreatment, suggesting that they could function as suppressors of the EL acclimation response (Fig. 4B). In contrast, two independent mutant alleles per locus for *BERBERINE BRIDGE ENZYME 1* (*BBE1*; AT1G30730; *24*), an oligogalacturonide oxidase involved in damage-associated molecular patterns (DAMPs) signaling as suppressor of immune responses, *IMPAIRED OOMYCETE SUSCEPTIBILITY 1* (*IOS1*; AT1G51800; *25*), an LRR-RLK protein family member involved in Pattern-Triggered Immunity activation, and *SALT-UP A 1* (*SUPA1*; AT5G65300; *26*), a peroxisomal protein of unknown function, rapidly expressed in response to different biotic and abiotic stresses, were unable to systemically or locally acclimate to an extended EL stress following a short local application of EL, suggesting that they are required for plant acclimation to EL stress (Fig. 4C). The findings presented in Figs. 3 and 4A-C reveal therefore that several different transcripts associated with the systemic Ca^{2+} and ROS signals, mediated from plant to plant during P-T-P communication, are required for plant acclimation to EL stress. In addition,

they demonstrate that acclimation to EL stress and responses to biotic challenges may share similar regulators and/or pathways.

As plants touching each other express many stress-response transcripts, even in the absence of stress (compared to plants growing individually; Fig. 1), we hypothesized that they would also have a higher basal level of ROS that causes them to be more resilient. Testing this hypothesis, we found that plants growing individually had a lower basal level of ROS, compared to plants growing in a group, touching each other (fig. S10). In agreement with our findings for systemic ROS signals in the WT-WT-WT setup (Fig. 2), applying EL stress to several members of a plant group, comprised of multiple *A. thaliana* seedlings growing in soil and touching each other above- and below-ground, resulted in further enhancement in ROS levels in the entire group (Fig. 4D). Thus, much like interactions with a beneficial microbe that caused elevated basal levels of ROS in plants, that could be elevated even further following stress (*27*), plants living together and touching each other displayed a higher basal level of ROS that could be enhanced even further during P-T-P responses to stress (Fig. 4D; fig. S10).

Although the transfer of systemic Ca^{2+} and ROS signals between different plants was shown to be required for the enhanced acclimation of receiver plants to EL (Fig. 2D), we cannot rule out the possibility that electric signals are also required; especially since mutations in the GLUTAMATE-LIKE RECEPTORs GLR 3.3 and GLR3.6, that block the electric wave, were found to block the Ca^{2+}/ROS waves within a plant (*28*), and the inclusion of a *glr3.3glr3.6* double mutant as a mediator plant, blocked the transfer of the ROS wave from transmitter to receiver plants (*9*). It is therefore possible that the transfer of the electric signal from a transmitting plant to a receiving plant is required for the activation of Ca^{2+}/ROS signals/responses in the receiver plant and that, in turn, the activation of the Ca^{2+}/ROS responses in the receiving plant are required for acclimation (Fig. 4E). However, why the activation of Ca^{2+}/ROS signals in a transmitting plant are required for the activation of Ca^{2+}/ROS signals/responses in a receiving plant (revealed by the WT-*hpca1*-WT setup; Fig. 2) is currently unknown. As H_2O_2 can easily diffuse in and out of cells (*29, 30, 31*), it is possible that H_2O_2 generated by a transmitting leaf at the leaf-to-leaf contact site between the transmitting and receiving plants enters the leaf cells of the receiver plant and triggers the Ca^{2+}/ROS waves in the receiver plant (*18*; as long as the electric wave was transferred between plants, and humidity is high enough to allow such a transfer). More studies are needed to address this point.

Although in our experimental setup the roots of plants that touched each other via leaf-to-leaf contacts did not touch each other (fig. S2), we cannot rule out the possibility that volatiles and/or acoustic signals were exchanged between plants. Further studies are needed to uncover the role of these communication pathways in inducing resilience to EL stress in a plant community. In this respect it should be noted that two independent alleles of a trichomeless mutant [*glabra1*; trichomes are major producers and storage sites for volatiles; (*32*)] that touched each other via leaf-to-leaf connections were able to exchange systemic ROS signals between each other (fig. S11), suggesting that the exchange of aboveground systemic signals reported here might not involve volatiles.

Taken together, our findings suggest that plants growing in a community, touching each other in nature may have a better P-T-P communication ability and display a higher level of resilience to stress, compared to plants that do not physically touch or communicate with each other (Fig. 4F). This advantage would of course come at the cost of less resources for growth, due to competition for water, nutrients, light, and/or space (*33*, *34*), but may offset such cost when the entire community experiences harsh environmental conditions (*35*). A tradeoff between acclimation

stemming from living together and overall plant growth and reproduction may also occur within different agricultural settings, such as crop fields or plantations, in which crowding of plants could result in yield penalty (*36*, *37*). As environmental conditions on our planet are worsening due to climate change and increased pollution levels (*38*, *39*), that generate conditions of multifactorial stress combination (*40*, *41*), our findings may offer a promise for future agriculture and ecosystem engineering, highlighting the potential of using plant communities with enhanced P-T-P communication for heightened resilience. In this respect, it should be noted that a key acclimation mechanism of the unicellular green algae *Chlamydomonas reinhardtii* to conditions of multifactorial stress combination, identified recently, is the formation of cell aggregates (*42*). At least one possible answer to the growing complexity of environmental stress conditions on our planet could therefore be living together. The principle of 'United We Stand' may hence also apply to plants living on Earth.

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Supplementary Materials

Materials and Methods

Figs. S1 to S11

Tables S1 to S11

References (*43-51*)

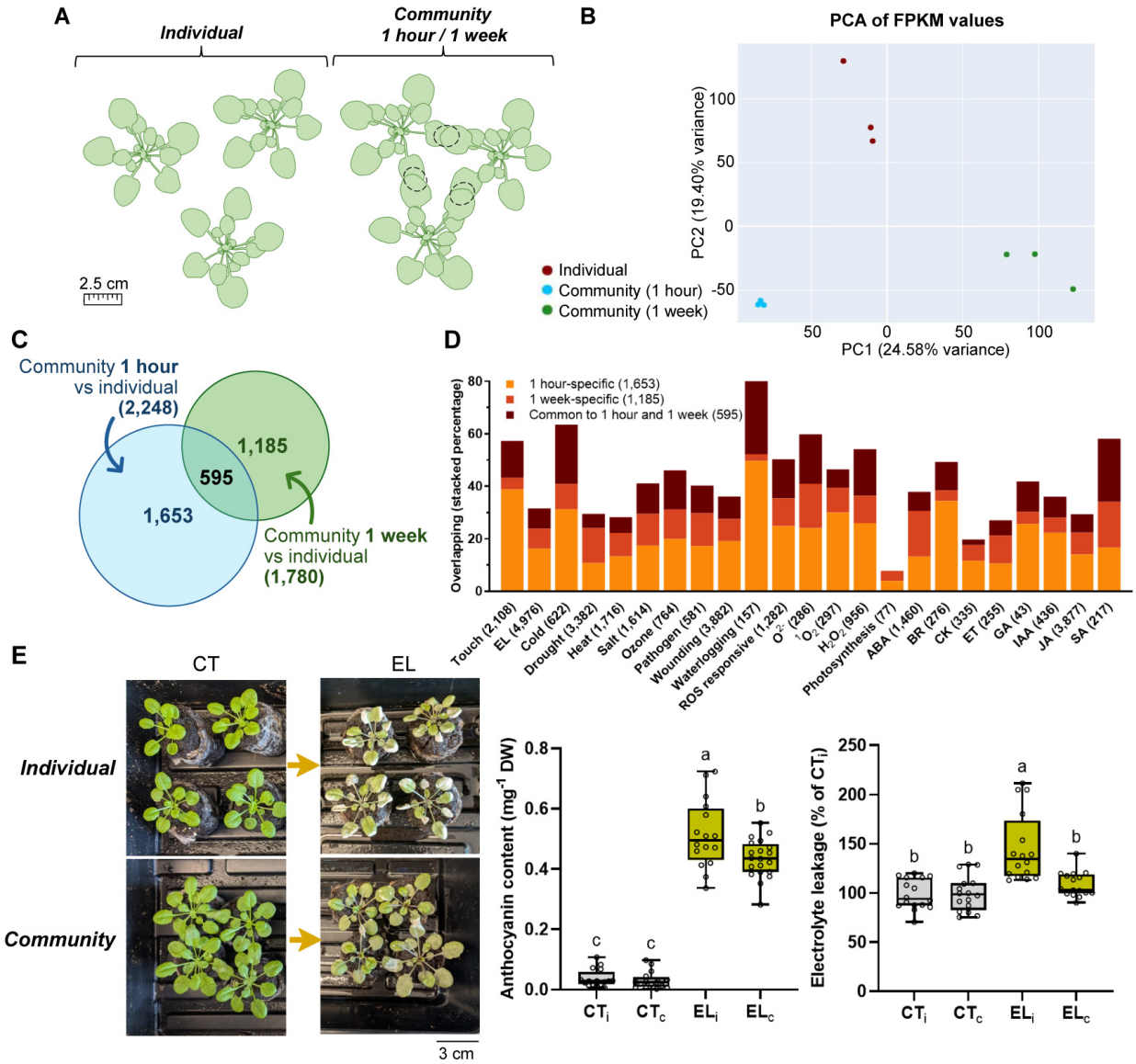


Fig. 1. Plants that touch each other aboveground are more resilient to excess light stress. (A) The experimental setup used to study plants (*Arabidopsis thaliana*) growing individually or in a community touching each other aboveground (fig. S1). **(B)** A PCA plot showing the distinct transcriptomics grouping of plants growing individually or in a community touching each other for 1 hour or 1 week (*N*=3). **(C)** A Venn diagram showing the overlap in transcripts significantly altered in their expression in plants growing as a community and touching each other for 1 hour or 1 week, compared to plants growing individually. **(D)** Representation of different stress-, hormone-, and reactive oxygen species (ROS)-response transcripts significantly altered in their expression in plants living as a community and touching each other compared to individual plants (from C). **(E)** Decreased levels of anthocyanins and decreased cellular damage (measured as ion leakage from cells) in plants living as a community touching each other, compared to individual plants, subjected to excess light stress for 24 hours. A representative image of the experimental setup is shown on left, decreased accumulation of anthocyanins (*N*=16) is shown in the middle, and decrease cell death (*N*=16) is shown on right. Significance for (B-C) was determined by negative binomial Wald test followed by Benjamini–Hochberg correction (*N*=3), and significance for (E) was determined by one-way ANOVA followed by a Tukey post hoc test (different letters denote statistical significance at $p \leq 0.05$). Abbreviations: ABA, abscisic acid; BR, brassinolide; CK, cytokinins; CT_i, individual plants under control conditions; CT_c, plants growing as a community under control conditions; EL, excess light; EL_i, individual plants subjected to excess light; EL_c, plants growing as a community subjected to excess light; ET, ethylene; GA, gibberellic acid; IAA, indole-3-acetic acid; JA, jasmonic acid; SA, salicylic acid.

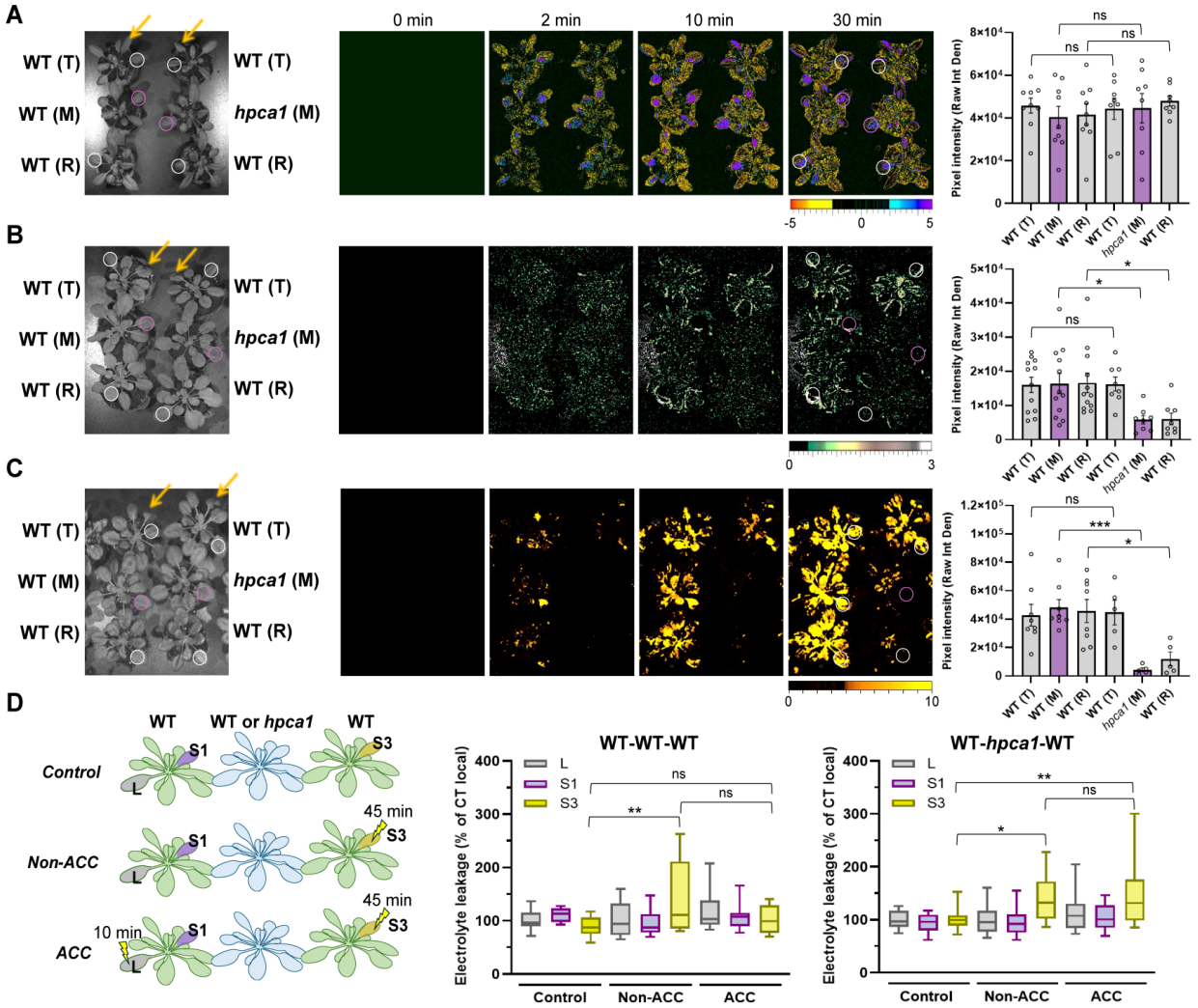


Fig. 2. Activation of systemic Ca^{2+} and reactive oxygen species waves in receiver plants is required for plant acclimation to excess light stress during plant-to-plant communication. (A) An image showing the experimental setups used to study the transfer of the systemic membrane potential (electric) wave between three successively arranged plants touching each other (transmitter – T, mediator – M, and receiver – R; Left; fig. S2), time lapse images of membrane potential change in plants touching each other in response to an excess light (EL) stress applied to one leaf of the transmitter plant (Middle), and a bar graph showing statistical analysis of membrane potential change intensity at 30 min (Right; *N*=8). **(B)** Same as (A), but for the systemic calcium (Ca^{2+}) wave (*N*=8). **(C)** Same as (A), but for the systemic reactive oxygen species (ROS) wave (*N*=5). See fig. S3 for imaging of plants touching each other but not subjected to EL stress. Local leaf subjected to EL stress is indicated with an arrow. Significance was determined by two-tailed Student's t-test ($p \leq 0.05$) comparing the values of T, M and receiver plants in both experimental setups. **(D)** The experimental setup used to study local (L) and systemic (S1, S3) leaf acclimation to EL stress in three successively arranged plants touching each other (Left; fig. S4A), and bar graphs showing cellular damage, measured as ion leakage from cells, in leaves L, S1 and S3 following an extended EL stress (45 min) in the presence or absence of a short (10 min) EL stress applied to leaf L of the transmitter plant (Right; *N*=7). Significance was determined by two-tailed Student's t-test ($p \leq 0.05$) comparing L, S1 and S3 of non-acclimated (ACC) and ACC plants versus control plants. Abbreviations: ACC, acclimated; HPCA1, HYDROGEN PEROXIDE INDUCED Ca^{2+} INCREASES 1; ns, non-significant; WT, wild type.

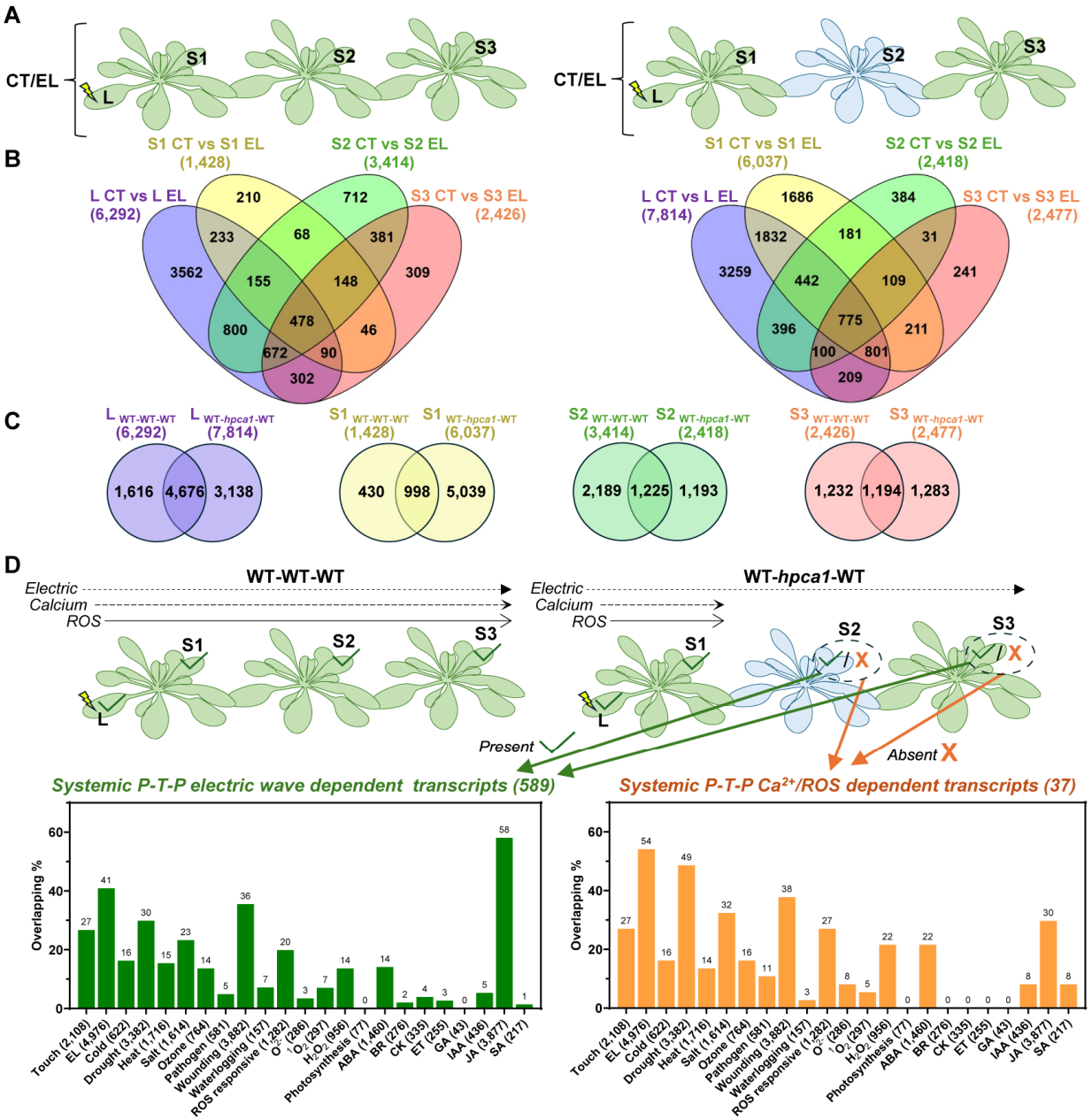


Fig. 3. Identification of electric- and Ca^{2+}/reactive oxygen species (ROS)- associated transcripts significantly altered in their expression in plants during plant-to-plant communication. (A) The experimental setups used for the transcriptomics study of plant-to-plant (P-T-P) communication between three successively arranged plants touching each other (transmitter – T, mediator – M, and receiver – R). **(B)** Venn diagrams showing the overlap between transcripts significantly altered in their expression in local (L) and systemic (S1-S3) leaves of plants from the two setups shown in (A), in response to excess light (EL) stress applied to leaf L (see fig. S2). **(C)** Venn diagrams showing the overlap between L and S1-S3 from the two different setups shown in (A) and (B). **(D)** The scheme used to identify electric- and Ca^{2+}/ROS- associated transcripts significantly altered in their expression in plants during P-T-P communication (Top), and representation of different stress-, hormone-, or ROS- response transcripts in the two different groups (electric – 589 transcripts, and Ca^{2+}/ROS – 37 transcripts; Bottom). Significance was determined by negative binomial Wald test followed by Benjamini–Hochberg correction (*N*=3). Abbreviations: ABA, abscisic acid; BR, brassinolide; CK, cytokinins; CT, control; EL, excess light; ET, ethylene; GA, gibberellic acid; HPCA1, HYDROGEN PEROXIDE INDUCED Ca^{2+} INCREASES 1; IAA, indole-3-acetic acid; JA, jasmonic acid; L, local; P-T-P, plant-to-plant; S1, systemic leaf 1; S2, systemic leaf 2; S3, systemic leaf 3; SA, salicylic acid.

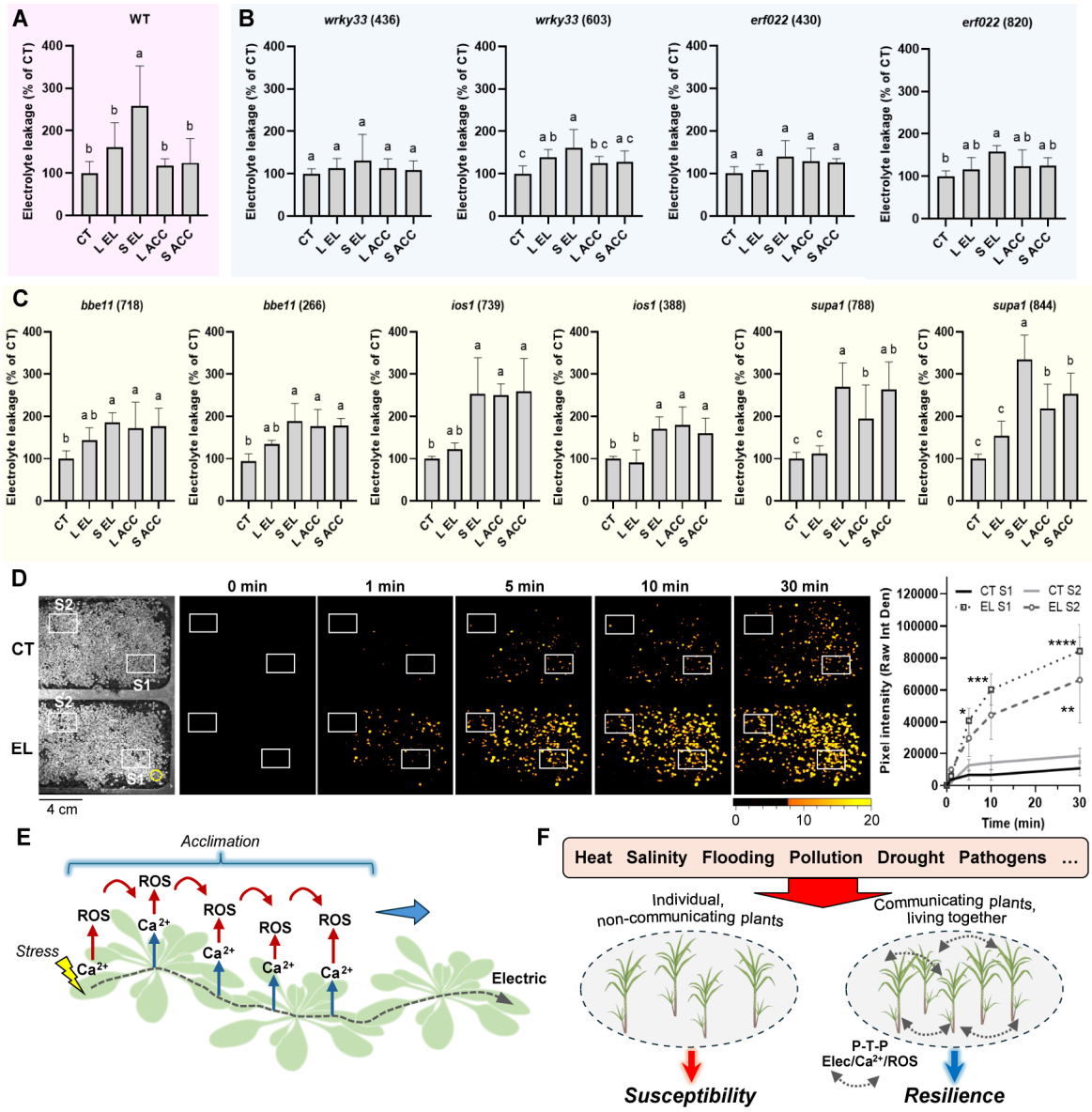


Fig. 4. Genes encoding Ca^{2+}/reactive oxygen species (ROS)-associated plant-to-plant communication transcripts are essential for plant acclimation to excess light stress. (A) Decrease in cellular damage (measured as ion leakage) in local (L) or systemic (S) leaves from wild type plants subjected to a prolonged (45 min) excess light (EL) stress following a short (10 min) pretreatment of EL stress applied to leaf L (*i.e.,* acclimation to EL stress; fig. S4; *N*=5). **(B)** Enhanced basal acclimation to EL stress in mutants deficient in the expression of different Ca^{2+}/ROS-associated P-T-P communication transcripts (*N*=5). **(C)** Failure of mutants deficient in the expression of different Ca^{2+}/ROS-associated P-T-P communication transcripts to acclimate to EL stress following a short (10 min) pretreatment of EL stress applied to leaf L (*N*=5). **(D)** Time lapse images of ROS accumulation in a dense group of seedlings touching each other in response to an EL stress applied to a few plants (circle) and a line graph showing quantification of ROS accumulation in two groups of systemic seedlings (white boxes) growing 1 (S1) or 8 (S2) cm away from the treated seedlings, respectively (Right; *N*=8). **(E)** A hypothetical model showing that the electric wave mobilized between plants triggers the Ca^{2+} and ROS waves in each of the plants that touch each other, and that the Ca^{2+} and ROS waves are required for plant acclimation as well as for their own propagation. **(F)** A hypothetical model proposing that plants living in a community, touching each other and exchanging aboveground systemic signals (electric and Ca^{2+}/ROS), are more resilient than individual plants to different stresses. Significance was determined by one (letters)- or two (*)- way ANOVA followed by a Tukey post hoc test ($p \leq 0.05$). Abbreviations: BBE, berberine bridge enzyme; CT, control; EL, excess light; Elec, electric; ERF, ethylene response factor; IOS, impaired oomycete susceptibility; L, local; ROS, reactive oxygen species; S, systemic; SUPA, salt-up a; WRKY, WRKY DNA-binding; WT, wild type.

1. Materials and Methods

Plant material, growth conditions, and stress treatments. *Arabidopsis thaliana* wild-type Col-0 (WT) and mutant (table S11) plants were germinated and grown on peat pellets (Jiffy 7; Jiffy International) under controlled conditions of 10-hour/14-hour light/dark regime, 50 µmol photons s^{-1} m^{-2}, and 21°C for 4 to 5 weeks. Leaf-to-leaf connections between different plants were established by adding a drop of a solution containing 0.05 M phosphate buffer, pH 7.4, 0.01% (v/v) Silwet L-77 and 0.05% (w/v) agarose, containing the different dyes used for imaging, to ensure continuous contact between touching leaves (*9*). Leaf-to-leaf connections between different plants were also established in the absence of any agar or buffer solution, however, following treatment of plants with the imaging buffer (*9*). One leaf from each plant was connected to a similar shape and size leaf from a different plant. Connections were maintained for different periods of time depending on the specific experiment. To induce a local excess light (EL) stress treatment, either in individual plants or plants connected to others in a community, a single leaf (local, L) of the transmitter plant was illuminated with 2000 µmol photons s^{-1} m^{-2} using a ColdVision fiber optic LED light source (Schott) for 10 min (fig. S2). For each experiment, plants of the same age and developmental stage were used, and all imaging or sampling were conducted between 9AM to 1PM.

RNA-Seq analysis. For RNA-seq analysis comparing individual plants to a group of plants that touch each other aboveground, the experiment was designed as shown in Fig. 1A (similar to control plants shown in fig. S1 but in triplets). WT plants were grown as described for 4 weeks. Plants were then divided into three groups: (i) For individual plants, triplets of individual plants were maintained at a distance of 2.5 cm from each other (measured from leaf edges) with no leaves connected; (ii) and (iii) for communities, two leaves of each plant were connected with other two plants (forming a triangle) as explained above (Fig. 1A; fig. S1). Triplets of connected plants as well as triplets of individual plants were maintained under control conditions for 1 hour (ii) or 1 week (iii). Three non-connected leaves per plant were sampled and flash frozen in liquid nitrogen in three replicates and a total of 10 plant triplets per condition were collected. All plants used for the experiments were of the same age and developmental stage and sampled on the same day (between 10-11AM).

For the RNA-seq analysis involving excess light (EL) stress treatment applied to a single leaf (local leaf; L), WT and *hpca1* plants were grown as described above for 4 weeks. Plants were then organized in two different setups as shown in Fig. 3A (fig. S2), allowing a single leaf from each plant to touch the other: (i) a chain of three WT (Col-0) plants touching each other consisting of a transmitter (T), mediator (M), and receiver (R) plants (WT-WT-WT), and (ii) a chain of three plants (T, M, R) in which the T and R plants were WT (Col-0), and the M plant an *hpca1* mutant (WT-*hpca1*-WT). Leaves of plants were connected as described above for 0.5 hour before the EL stress treatment. One leaf (local, L) of the T plant was subjected to excess light stress (1700 µmol photons s^{-1} m^{-1} for 30 min). Local and systemic leaves (L, S1, S3; Fig. 3A) from control and treated plants were sampled. Leaves from 60 different plants were pooled into three replicates, and every replica included 20 leaves/plants for each sample (L, S1, S2 and S3).

Samples from the two experiments described above were processed similarly. Leaves were ground to a fine powder and total RNA was isolated using RNAeasy Plant Mini Kit (Qiagen, MD, USA; 74904) according to manufacturer instructions. RNA libraries were prepared using standard Illumina protocols, and RNA sequencing was performed using NovaSeq 6000 PE150 by Novogene Co. Ltd (https://en.novogene.com/; Sacramento, CA, USA). Quality control was conducted using

FastQC v0.11.9 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), and summary reports were generated with MultiQC v1.12 (https://github.com/MultiQC/MultiQC/). Read trimming was performed to remove low-quality bases and adapter sequences using Trim Galore v0.6.4 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/). Alignment of sequence reads to the Arabidopsis reference genome TAIR10, release 57 (https://ftp.ensemblgenomes.ebi.ac.uk/pub/plants/release-57/fasta/arabidopsis_thaliana/) was performed with Hisat2 v2.2.1 (*43*). Aligned sequences were sorted with SAMtools v1.9 (*44*) to facilitate downstream analyses. Transcript abundance expressed as fragments per kilobase million (FPKM) was generated using the Cufflinks tool v.2.2.1 from the TUXEDO suite (*45*) guided by genome annotation files (downloaded from https://ftp.ensemblgenomes.ebi.ac.uk/pub/plants/release-54/gff3/arabidopsis_thaliana/). Differential gene expression analysis was performed using the Cuffdiff 2 method (*46*), also from the same TUXEDO suite. Differentially expressed transcripts were defined as those that had a fold-change with an adjusted $p \leq 0.05$ (negative binomial Wald test followed by Benjamini–Hochberg correction). For the analysis and visualization of the data, R was used with methods and packages available through CRAN (R Core Team 2022, https://www.R-project.org/) or Bioconductor (*47*). Principal Component Analysis (PCA) was performed to assess the clustering of biological replicates, confirming the reproducibility and consistency of the experimental conditions. RNA-Seq data files from the two different experiments (no stress and excess light stress) are available at GEO (GSE301070).

Transcript datasets of stress, hormone, and reactive oxygen species (ROS) responses used in Figs. 1D and 3D were obtained from (*48*) with some updates as follows: transcripts associated with a 'touch' response were obtained from (*49*); transcripts related to 'excess light' (EL) response were obtained from (*20*) and transcripts from 'waterlogging' list were filter by fold change ≤ -1 and ≥ 1 (*48*). Venn diagrams were created in VENNY 2.1 (BioinfoGP, CNB-CSIC; https://bioinfogp.cnb.csic.es/tools/venny/index.html). UpSet plots were generated using the UpSetR (*50*) package.

Anthocyanin content quantification. Whole individual plants or communities composed of four plants (touching each other for 1 hour) were exposed to excess light conditions (2000 µmol photons s^{-1} m^{-2}) using a full spectrum led grow light (BESTVA, CA, USA) positioned 22 cm above plants as shown in fig. S1 for 24 hours. Control plants were grown in parallel and sampled at the same time. Whole rosettes were collected independently (flash frozen in liquid nitrogen), and each plant was treated as a technical replicate. Anthocyanin content was determined as described in (*51*) with some modifications. Briefly, ~100 mg of ground tissue was incubated in 1 ml of 1% HCl in methanol (v/v) and then incubated overnight in the dark at 4°C with gentle shaking. After extraction, samples were centrifuged at 12,000 rpm for 2 min and the pellet was dried at 36°C overnight. The absorbance of the supernatant was measured at 530 and 657 nm using a spectrophotometer (GENESYS 10 UV-Vis Thermo Fisher Scientific), and the concentration of anthocyanin was calculated using $A_{530} - 0.25 \times A_{657}$ and expressed as g^{-1} dry weight (DW). Acidified methanol was used as blank. Results include anthocyanin content from at least 16 different plants for each treatment and from two independent experiments.

Acclimation assays. Acclimation was measured in individual plants as well as in plants that touched each other connected by one leaf (fig. S4). For individual plants, systemic acclimation to excess light stress was tested by exposing a local leaf to excess light (2000 µmol photons s^{-1} m^{-2})

for 10 min, incubating the plant under controlled conditions for 50 min, and then exposing the same leaf (local) or another younger leaf (systemic) to excess stress (2000 µmol photons s^{-1} m^{-2}) for 45 min (fig. S4B), as previously described (*18, 20*). Results are presented as a percentage relative to control (electrolyte leakage from leaves not exposed to the light stress treatment) and include measurements from at least two independent experiments with three plants for each treatment. For measuring acclimation in a chain of plants, as shown in fig. S4A, plants were misted with water for 10 min and leaves were connected as described above for 30-60 min. Chains of plants composed of WT(T)-WT(M)-WT(R), as well as chains of plants composed of WT(T)-*hpca*(M)-WT(R) were treated side by side. For non-acclimated chains, excess light was applied to a systemic leaf (S3) of the receiver plant (WT in both setups) for 45 min with no stress pre-treatment (*i.e.,* no EL-pretreatment was applied). For acclimated chain of plants, a short pre-treatment (10 min) of excess light (2000 µmol photons s^{-1} m^{-2}) was applied to a local (L) leaf of the transmitter plant (WT in both setups). Following the pre-treatment, plants were allowed to rest for 2 hours to induce acclimation. Excess light (2000 µmol photons s^{-1} m^{-2}) was then applied to a systemic leaf (S3) of the receiver plant (WT) for 45 min. Control plants were maintained under the same control conditions and sampled at the same time. For the three groups, the local (L) leaf and a systemic leaf (S1) of the transmitter (T) plant, together with the systemic (S3) leaf of the receiver (R) plant were sampled for conductivity measurement (described below). Results are presented as percentage of control (electrolyte leakage from leaves not exposed to the light stress treatment) and include measurements from at least three independent experiments.

Electrolyte leakage assay. Leaf injury after excess light stress was measured using the electrolyte leakage assay, as described previously (*18, 20*). For whole plants exposed to excess light, either individually or in a community, stress was applied for 24 hours following the setup shown in fig. S1 as explained above. Following excess light treatment, one non-connected leaf per plant was sampled and placed in a tube containing 30 mL of ddH_20 and moved to a gentle shaker for one hour. After one hour, the electrolyte leakage was measured for each sample (treated, untreated, individual, and community) using a conductivity meter Oakton CON 700 (Thermo Fisher Scientific). The samples were then boiled in a water bath at 95°C for 20 minutes, cooled down to room temperature while shaking and the electrolytic leakage was measured for a second time. The percentage of electrolyte leakage in each sample was calculated as the ratio of the conductivity before boiling to that after boiling.

Whole-plant imaging of ROS, calcium and membrane potential. As previously described (*18*), plants were treated with fluorescent dyes via fumigation (misting with fine droplets) in a glass container using a nebulizer (Punasi Direct, Hong Kong, China) for 30 minutes with: 50 µM H_2DCFDA (Millipore-Sigma, St. Louis, MO, USA) for ROS imaging; 4.5 µM Fluo-4-AM (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for calcium imaging; 20 µM $DiBAC_4(3)$ (Biotium, Fermont, CA, USA) for imaging changes in membrane potential. Following fumigation, a chain of physically connected plants was established, consisting of a transmitter (T) and a receiver (R) WT (Col-0) plants that were separated by a mediator plant (M), that was either WT, *hpca1,* or *gl1* mutant (table S11). Transmitter plants were then treated with a local excess light (EL; 2000 µmol photons s^{-1} m^{-2}) for 10 min and imaged using the IVIS Lumina S5 fluorescence imaging system (PerkinElmer, Waltham, MA, USA) as previously described (*18*). Fluorescence images (excitation/emission 480/520 nm) were acquired every minute for 30-60 min. Accumulation of ROS, calcium and electric (membrane potential) signals, in treated and untreated

plants, were compared to the 0-min time point and determined by subtracting the fluorescent signal of the initial time point (0-min) from the time point of interest. The 0-min time point was the initial image, which served as a baseline, resulting in no visible signal after subtraction using the Living Image 4.7.2 software (PerkinElmer). The total radiant efficiency of regions of interest (ROI; indicated with white circles in the different figures) of only non-plant-connected leaves were quantified as pixel intensity (Raw Int Den) using ImageJ. Each experiment was repeated at least three times, *N* for each experiment is indicated in the Figure legends.

For imaging ROS accumulation in large plant communities, Col-0 seeds (5 for individual and >100 for communities), were sown on 8.5 cm x 12.5 cm pots filled with Promix BX growing medium (Premier Tech Horticulture, Quakertown, PA, USA). Plants were grown under controlled conditions, as previously described, for 3 to 4 weeks. Leaves and roots were permitted to grow without spatial restriction. Plant communities were fumigated with the fluorescent dye H_2DCFDA for 30 minutes, as described above. After fumigation, plants were maintained under the same controlled conditions for an additional hour before stress application. Excess light stress (2000 μmol photons s^{-1} m^{-2}) was applied to a local region (indicated with a yellow arrow pointing to a circle in Fig. 4D) for 10 min and plant communities were imaged using the IVIS Lumina S5 fluorescence imaging system. Fluorescence images (excitation/emission 480/520 nm) were acquired every minute for 30 min. Image processing was conducted as described above, and two distinct regions of interest (S1 and S2, marked with white rectangles in Fig. 4) were quantified using ImageJ. Fluorescence intensity was measured as pixel intensity (Raw Int Den).

Statistics and data analysis. Data analysis and statistics were performed in GraphPad Prism 10. Different letters indicate significant differences ($p \leq 0.05$) as determined by a one-way ANOVA followed by a Tukey's HSD or uncorrected Fisher's LSD post hoc tests, and results are presented as means ± standard errors (SE). Asterisks denote statistically significant differences based on two-sided Student's *t* test with significance levels: *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$. Asterisks in Fig. 4D indicate significant differences ($p \leq 0.05$) as determined by a two-way ANOVA followed by Tukey's HSD post hoc test. Differentially expressed transcripts were defined as those that had a fold-change with an adjusted $p \leq 0.05$ (negative binomial Wald test followed by Benjamini–Hochberg correction).

2. Supplementary Figure Legends

Fig. S1. Images showing the experimental setup used to compare the resilience of individual *A. thaliana* plants to that of groups of *A. thaliana* plants that physically touch each other, to excess light stress. Individual plants were maintained at a distance of 2.5 cm from each other and groups of plants (touching each other) were connected for 1 hour by a drop of a solution containing water, or 0.05 M phosphate buffer, pH 7.4, 0.01% (v/v) Silwet L-77 and 0.05% (w/v) agarose, to ensure continuous contact between touching leaves. Quadruplets of individual plants were maintained under control or stress conditions for 24 hours and then sampled for quantification of anthocyanin content and leaf injury.

Fig. S2. Images showing the experimental setup used to study the transfer of systemic waves (electric, calcium and reactive oxygen species) between three successively arranged plants touching each other, in response to excess light. Leaf-to-leaf connections between different

plants were established by adding a drop of water or a solution containing 0.05 M phosphate buffer, pH 7.4, 0.01% (v/v) Silwet L-77 and 0.05% (w/v) agarose, containing the different dyes used for imaging, to ensure continuous contact between touching leaves. Following 30-60 min incubation, excess light (EL) stress was applied to one leaf of the transmitter plant. Transmitter (T), mediator (M), and receiver (R).

Fig. S3. The systemic reactive oxygen species (ROS) wave is not transmitted between plants under non-stress conditions. An image showing the experimental setups used to study the transfer of the systemic waves between three successively arranged plants touching each other (transmitter – T, mediator – M, and receiver – R) and time lapse images of ROS accumulation in plants touching each in the absence of an excess light (EL) stress treatment (Left), and a bar graph showing statistical analysis of ROS accumulation at 30 min (Right; N=3). Significance was determined by one-way ANOVA followed by a Tukey post hoc test ($p \leq 0.05$). In support of Fig. 2. Control (CT), Transmitter (T), mediator (M), and receiver (R).

Fig. S4. Experimental setup used to study local (L) and systemic (S) leaf acclimation to excess light stress in three successively arranged plants touching each other (A) or in individual plants (B). In support of Figs. 2D and 4.

Fig. S5. A PCA plot showing the distinct transcriptomics grouping of plants arranged in the WT-WT-WT and WT-*hpca1*-WT setups in response to an EL/high light (HL) stress applied to a single leaf of the transmitter plant. In support of Fig. 3. Abbreviations in the legend: 'WT' for WT-WT-WT setup and 'WhW' for WT-*hpca1*-WT setup; 'L' is local leaf, 'S1' is systemic leaf 1 of the transmitter plant, 'S2' is systemic leaf 2 of the mediator plant, 'S3' is systemic leaf 3 of the receiver plant; 'CT' is control and 'HL' is excess light.

Fig. S6. An UpSet plot showing the unique and common transcripts significantly altered in their expression under control conditions (CT), in the systemic leaves 1 (S1), 2 (S2) and 3 (S3) compared to local leaf (L), in the WT-WT-WT (WT) and WT-*hpca1*-WT (WhW) setups. In support of Fig. 3.

Fig. S7. An UpSet plot showing the unique and common transcripts significantly altered in their expression in the systemic leaves 1 (S1), 2 (S2) and 3 (S3) compared to local leaf (L) in the WT-WT-WT (WT) and WT-*hpca1*-WT (WhW) setups in response to excess light/high light (HL) stress applied to the local leaf of the transmitter plant. In support of Fig. 3.

Fig. S8. A heat map showing the expression pattern (fold change) of all systemic calcium/reactive oxygen species-dependent transcripts significantly altered during plant-to-plant communication in response to a local application of excess light (EL) stress applied to the local leaf of the transmitter plant. Abbreviations: 'WT' for WT-WT-WT setup, and 'WhW' for WT-*hpca1*-WT setup; 'L' is local leaf, 'S1' is systemic leaf 1 of the transmitter plant, 'S2' is systemic leaf 2 of the mediator plant, 'S3' is systemic leaf 3 of the receiver plant. In support of Fig. 3.

Fig. S9. Cellular damage (measured as ion leakage) in local (L) and systemic (S) leaves of two knockout mutants of the nudix hydrolase 4 gene (*NUDT4)* subjected to an extended EL stress

following a short (10 min) pretreatment of EL stress. Significance was determined by one-way ANOVA followed by a Tukey post hoc test (letters denote statistical significance; $p \leq 0.05$; *N*=5). In support of Fig. 4. Abbreviations: acclimated (ACC), control (CT).

Fig. S10. Reactive oxygen species (ROS) accumulation in a dense group of seedlings touching each other (community, top) and individual plants (bottom) under control conditions. Bright field, fluorescence image for ROS accumulation, and overlay of both images, at 30 min are shown on left. A box plot graph showing statistical analysis of ROS accumulation (pixel intensity measured as Raw Int Den) at 30 min from 2 independent experiments (*N*=10, 30 ROIs measured) is shown on left. Significance was determined by two-tailed Student's t-test ($p \leq 0.05$).

Fig. S11. The systemic reactive oxygen species (ROS) wave can be transmitted between wild type and trichomeless mutants, or from one trichomeless mutant to another, in response to excess light (EL) applied to a local leaf of a transmitter plant (wild type or a trichomeless mutant). Images showing the experimental setups used to study the transfer of the systemic waves between three successively arranged plants touching each other (transmitter – T, mediator – M, and receiver – R; Left), time lapse images of ROS accumulation in plants touching each in response to an excess light (EL) stress treatment (0-30 min; middle), and bar graphs showing statistical analysis of ROS accumulation at 30 min (Right; *N*=3). The different setups used included two independent alleles of a trichomeless mutant (*glabra1; gl1-1;* Top; *and gl1-2*; Bottom*).* Significance was determined by one-way ANOVA followed by a Tukey post hoc test ($p \leq 0.05$). In support of Fig. 2. Transmitter (T), mediator (M), and receiver (R).

3. List of Supplementary Tables

Table S1. Transcripts significantly altered in their expression in wild type *Arabidopsis thaliana* plants growing individually (individual) or in a community touching each other (community) for 1 hour or 1 week. In support of Fig. 1.

Table S2. Overlap between transcripts significantly altered in their expression in plants growing as a community and touching each other for 1 hour or 1 week, compared to plants growing individually. In support of Fig. 1C.

Table S3. Percent representation of transcripts related to different stress-, hormone-, and reactive oxygen species (ROS)-response (left column) among transcripts significantly altered in their expression in plants living as a community and touching each other, compared to individual plants. The number of transcripts is shown in parentheses. In support of data presented in Fig. 1C-D.

Table S4. Transcripts significantly altered in wild type and the mutant *hpca1* in response to excess light stress applied to a local leaf of the transmitter plant in the two setups WT-WT-WT and WT-*hpca1*-WT. WT-WT-WT setup is abbreviated as 'WT' and WT-*hpca1*-WT setup as 'WhW'; 'L' is local leaf, 'S1' is systemic leaf 1 of the transmitter plant, 'S2' is systemic leaf 2 of

the mediator plant, ‘S3’ is systemic leaf 3 of the receiver plant, ‘CT’ is control and ‘EL’ is excess light.

Table S5. Overlap between transcripts significantly altered in their expression in local (L) and systemic (S1-S3) leaves of plants from two setups (WT-WT-WT, abbreviated as 'WT'; WT-*hpca1*-WT, abbreviated as 'WhW') in response to excess light (EL) stress applied to the local leaf of the transmitter plant. In support of Fig. 3B. Control, CT.

Table S6. Overlap between transcripts significantly altered in their expression in local (L) and systemic (S1-S3) leaves of plants from two setups (WT-WT-WT, abbreviated as 'WT'; WT-*hpca1*-WT, abbreviated as 'WhW') in response to excess light (EL) stress applied to the local leaf of the transmitter plant. In support of Fig. 3C.

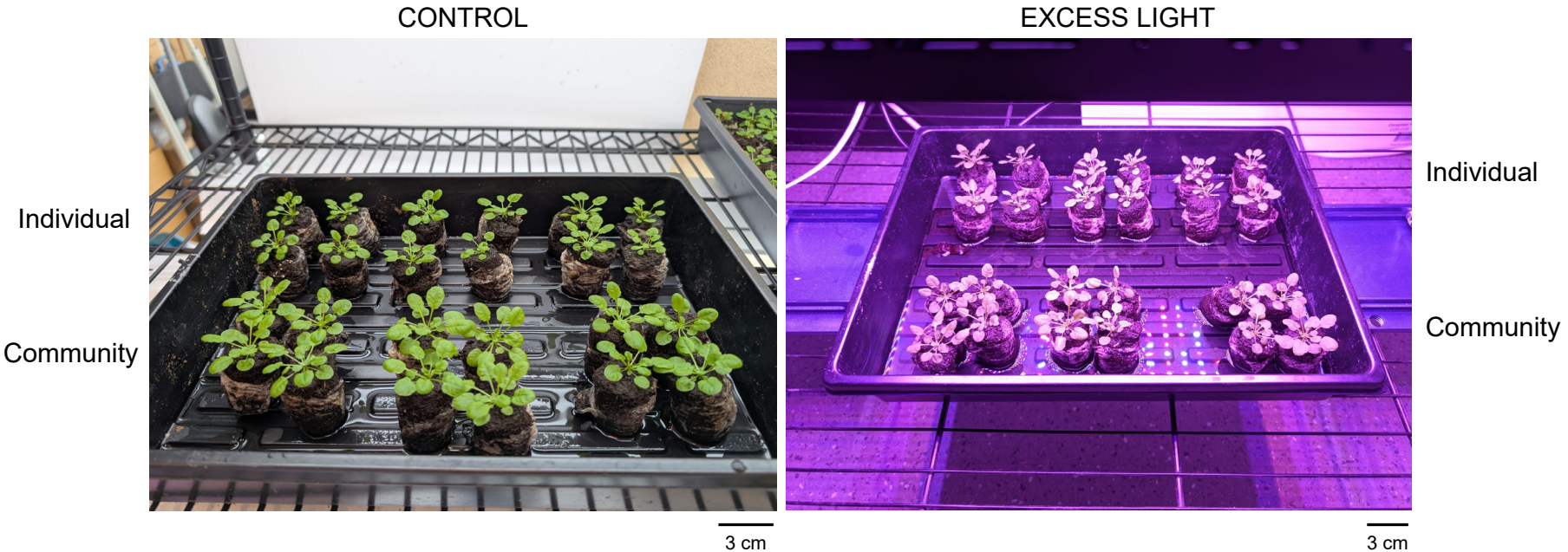
Table S7. Percent representation of transcripts related to different stress-, hormone-, and reactive oxygen species (ROS)-response (left column) among transcripts significantly altered in their expression in systemic leaf 1 (S1) from the transmitter plant in the setup WT-*hpca1*-WT in response to excess light stress applied to the local leaf of the transmitter plant. The number of transcripts is shown in parentheses. In support of data presented in Fig. 3.

Table S8. List of calcium/reactive oxygen species (ROS)-associated transcripts significantly altered in their expression in plants during P-T-P communication. In support of data presented in Fig. 3.

Table S9. List of electric (membrane potential)-associated transcripts significantly altered in their expression in plants during P-T-P communication. In support of data presented in Fig. 3.

Table S10. Percent representation of transcripts related to different stress-, hormone-, and reactive oxygen species (ROS)-response (left column) among electric and calcium/reactive oxygen species associated transcripts significantly altered in their expression in plants during plant-to-plant communication. The number of transcripts is shown in parentheses. In support of data presented in Fig. 3.

Table S11. The different mutants used in this work.



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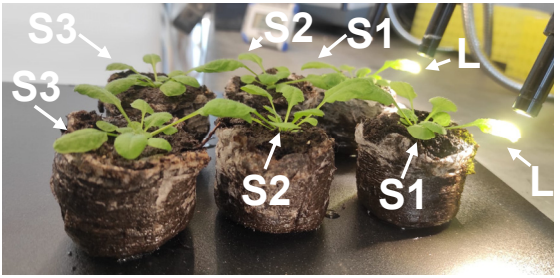

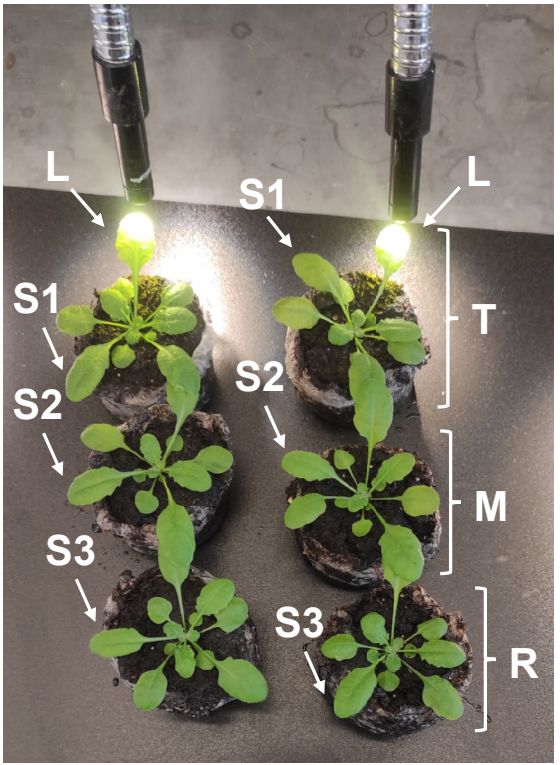

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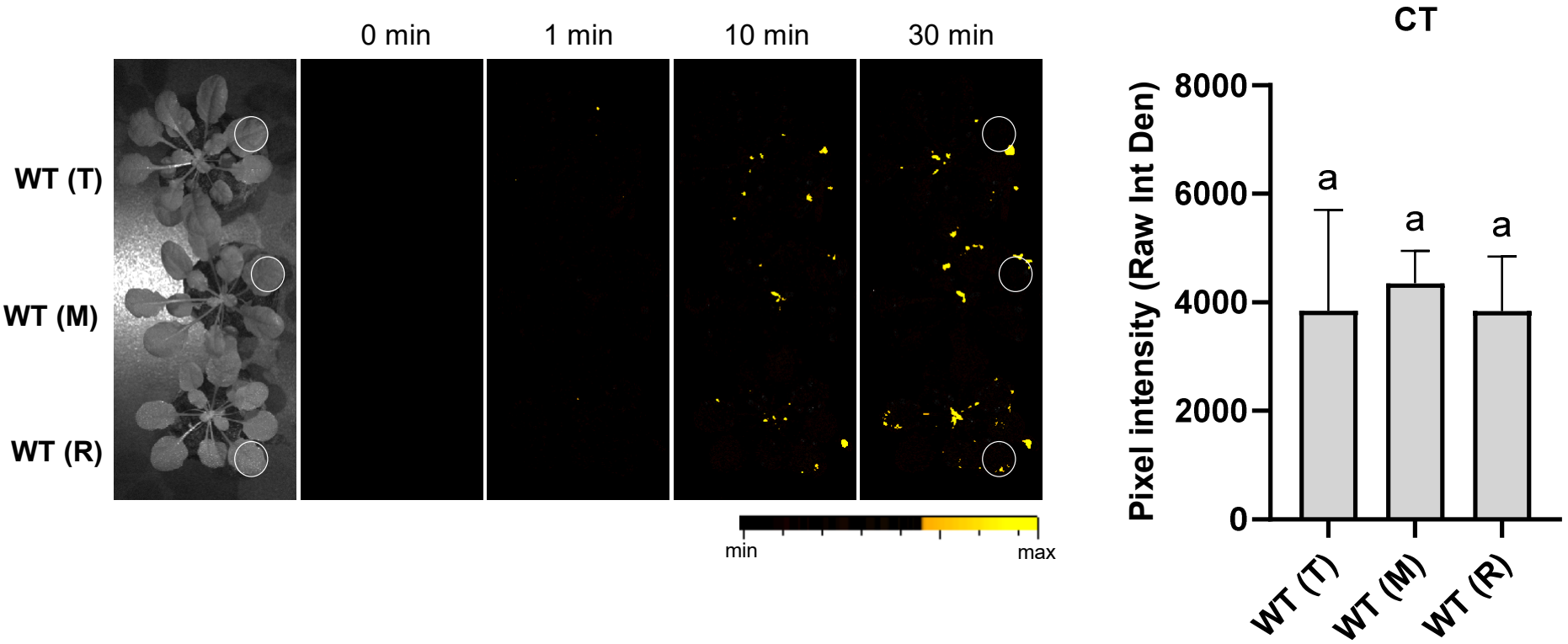

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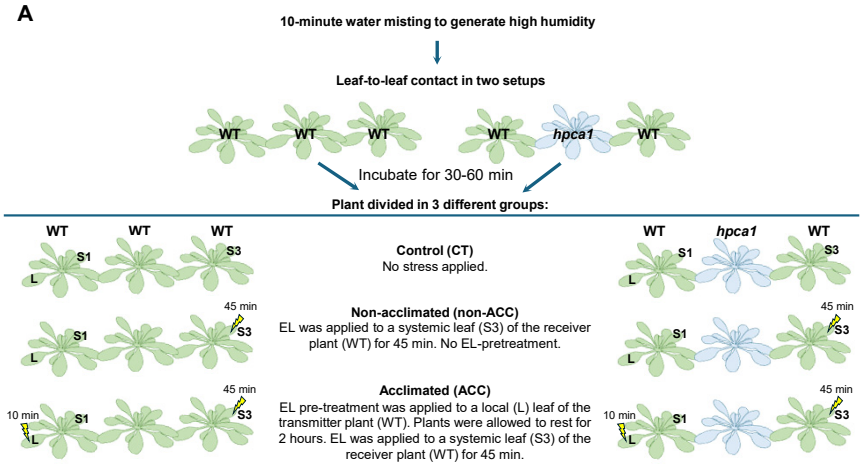

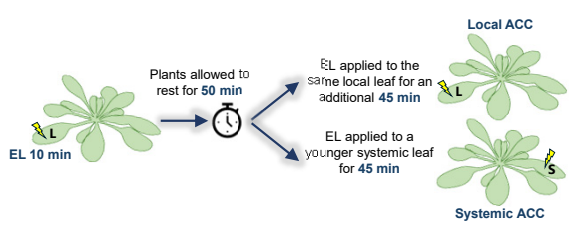

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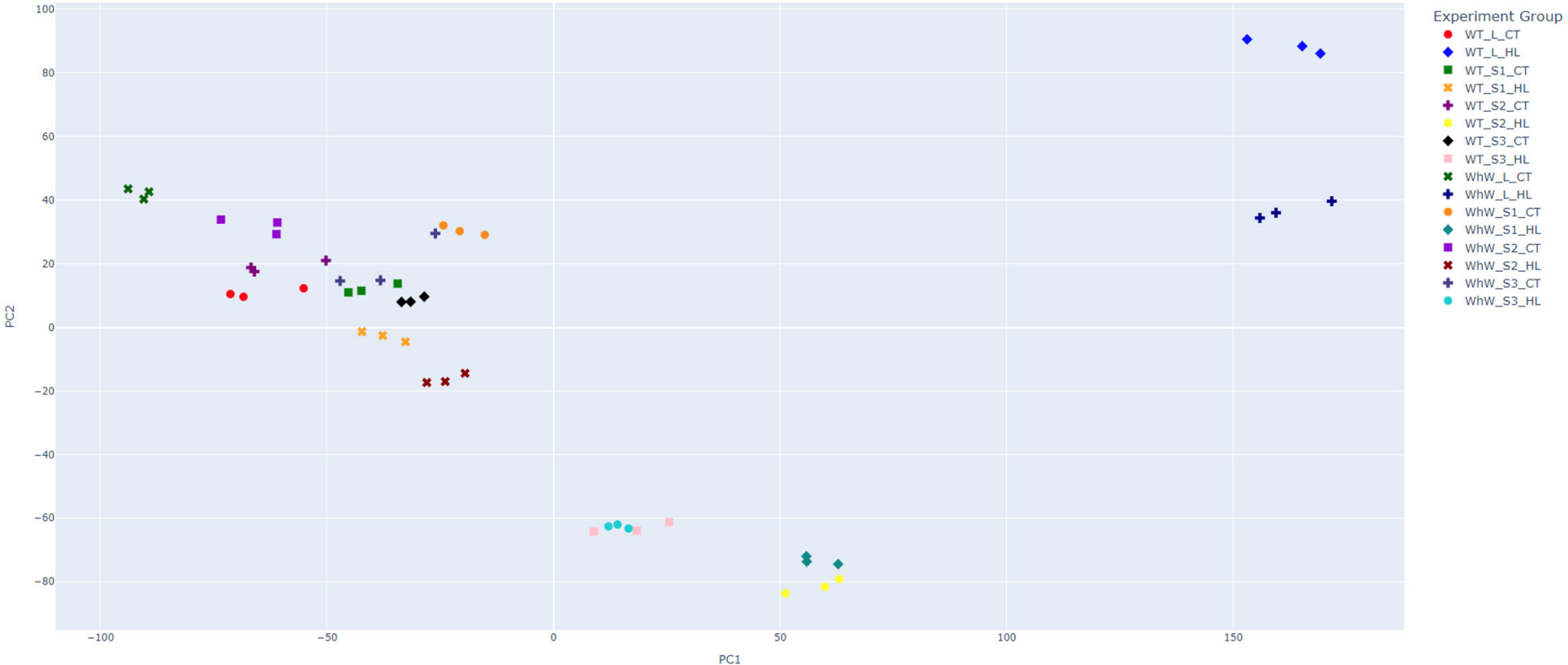

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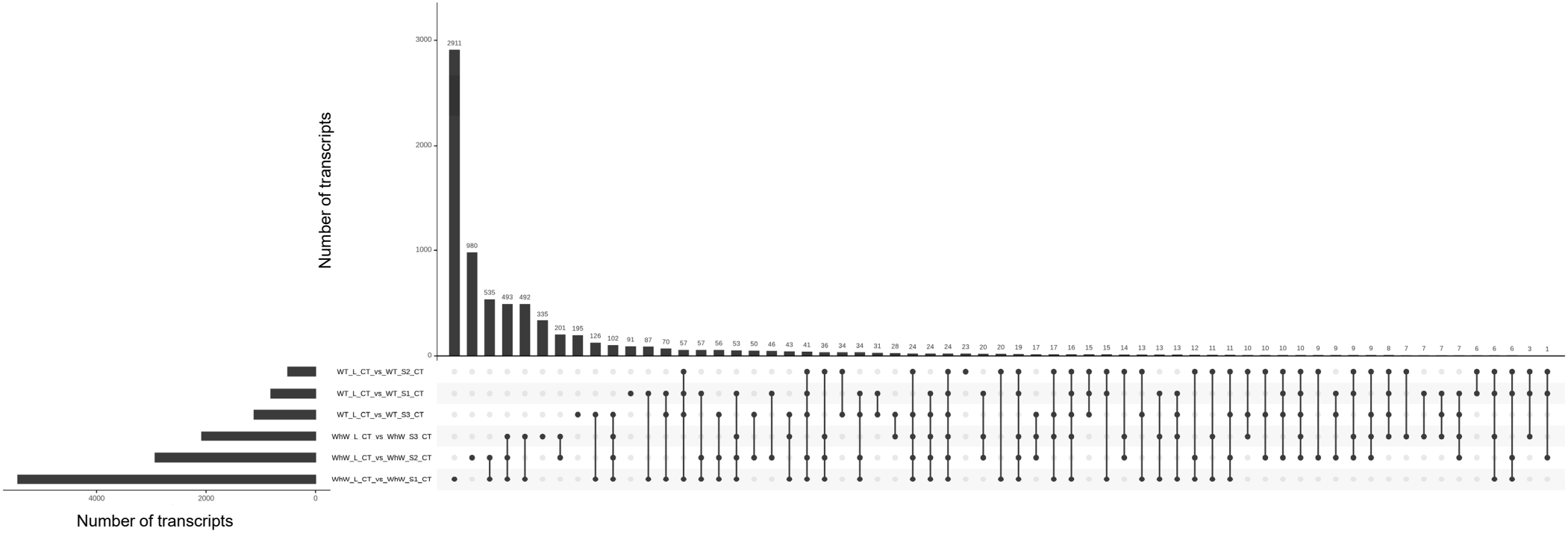

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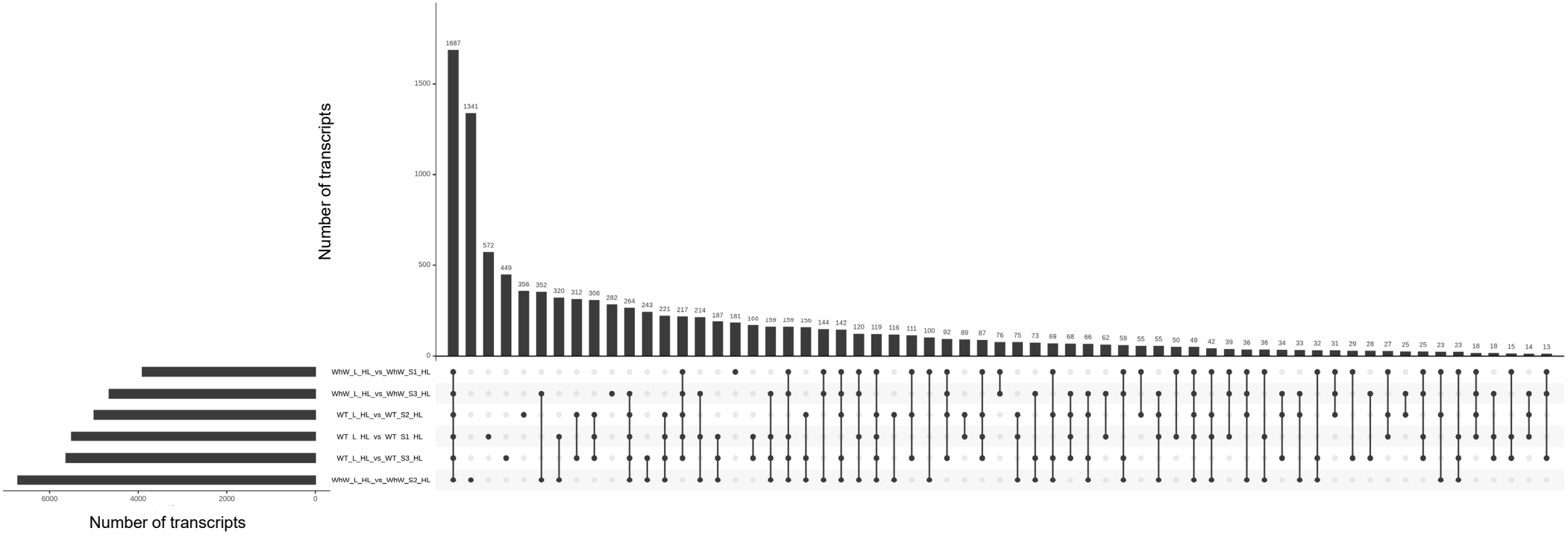

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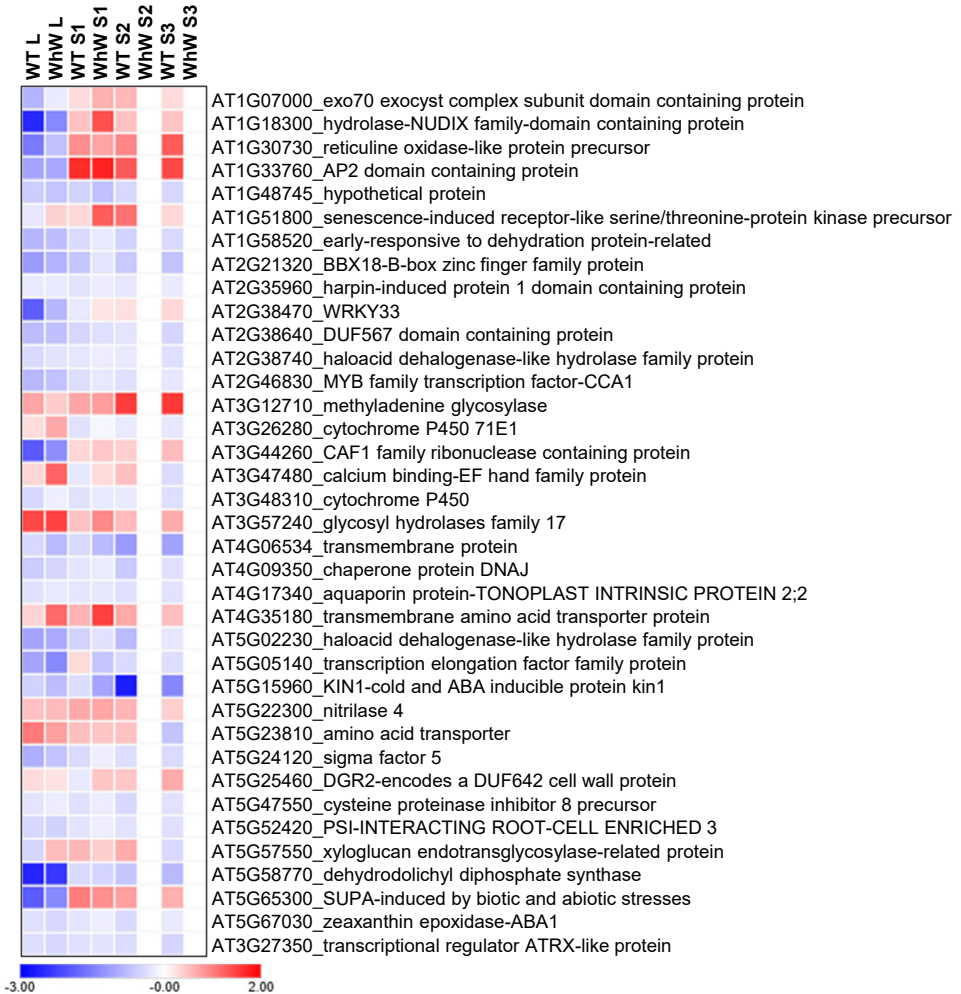

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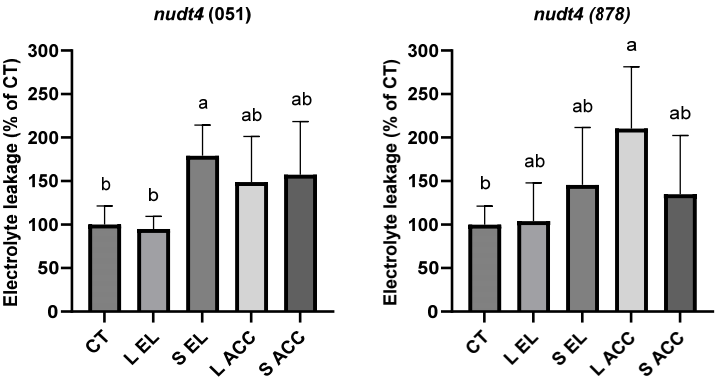

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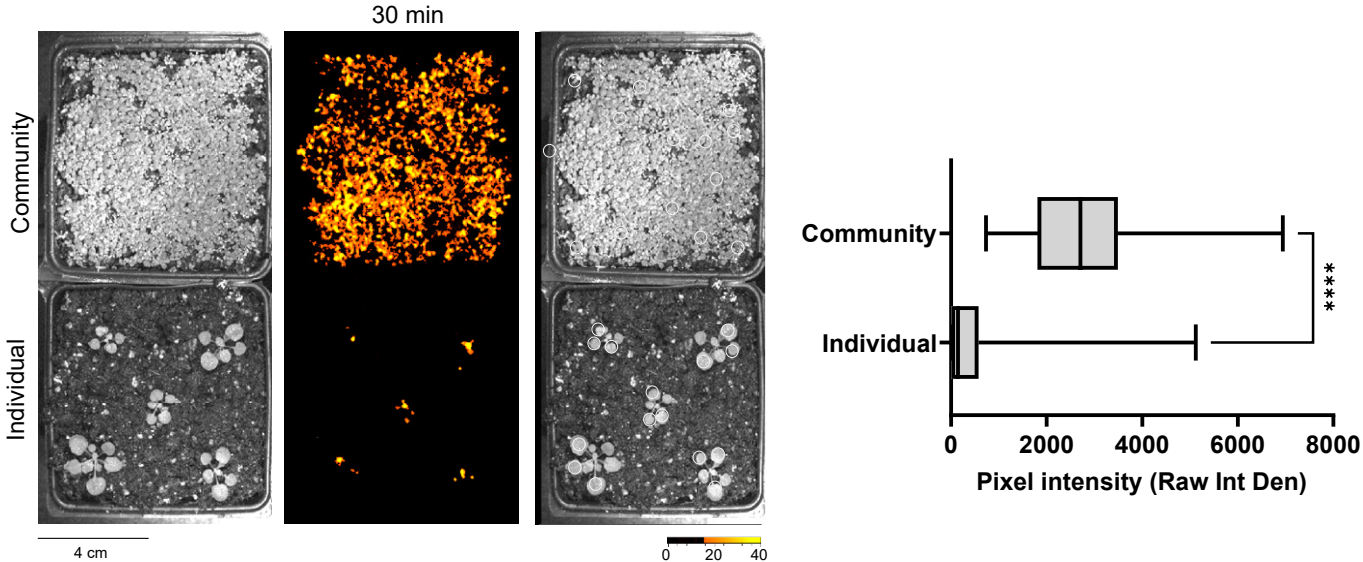

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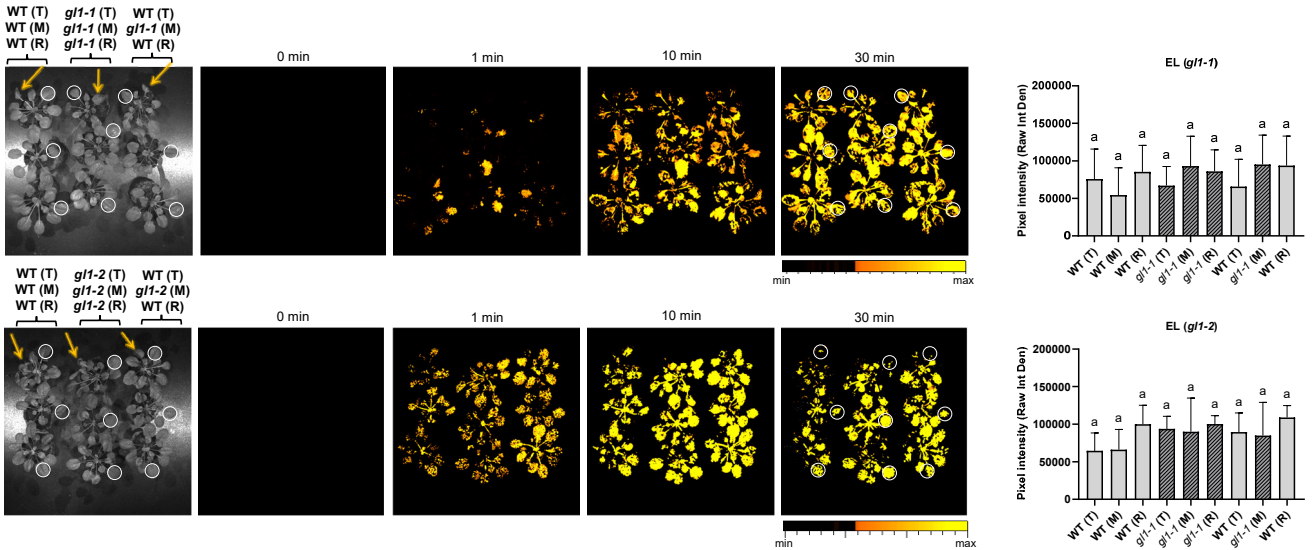

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