

The ecological implications of interplant drought cuing

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10

11 **Abstract**

12 Plants are able to perceive, integrate and respond to multiple signals and cues informative
13 of imminent threats and opportunities. Here we studied the ecological implications of
14 interplant drought cuing by subjecting unstressed target plants to short periods of root
15 cuing from neighbors that were either stressed or unstressed by drought. Our findings show
16 for the first time that cues emitted from the roots of drought-inflicted plants could elicit
17 greater survival in neighboring plants under a subsequent drought, yet at the expense of
18 lowered performance under benign conditions. Interestingly, these adaptations and costs
19 could be similarly elicited in immediate neighbors via direct cuing and in more distant
20 neighbors via relayed-cuing. Further investigation into the mechanism of interplant drought
21 cuing implicated abscisic acid (ABA) as the exogenous vector based on three lines of
22 evidence- a) drought cuing was greatly reduced in ABA-deficient plants such as *Wilty* mutant
23 plants or fluridone-treated plants, b) metabolomic analysis demonstrated that the roots of
24 both drought-stressed plants and their unstressed neighbors exuded significantly more ABA
25 than their uncued controls, c) Both direct and relayed cuing from ABA-treated neighbors
26 increased the survival and performance of unstressed target plants under a subsequent
27 drought, and inflicted performance costs on target plants that grew under benign
28 conditions. were not subjected to a subsequent drought. Our findings suggest that
29 interplant root communication of drought cues could have novel implications for plant
30 interactions, survival and performance under both natural and agricultural settings, calling
31 for its further investigation under field conditions.

32

33 **Introduction**

34 Biological adaptations invariably rely on the integration of functionally-relevant
35 information from the environment. At the population level, such information can be
36 genetically incorporated via Darwinian evolution regardless of the awareness or the
37 responsiveness of the involved organisms. In contrast, adaptive responses of individuals
38 require that organisms perceive and integrate fitness-relevant information during their
39 lifetimes¹. While most animals are able to sense and interpret their environments by
40 sophisticated Central Nervous Systems (CNS), intricate environmental perception, decision
41 making and behavior can be observed in most CNS-less organisms, including sponges²,
42 archaea³, fungi⁴, bacteria⁵ and viruses⁶. Due to their sessile nature, plants experience high
43 levels of environmental heterogeneity, which selects for remarkable levels of phenotypic
44 plasticity fostered by structural simplicity, morphological modularity, and continuous
45 meristematic activity⁷. The adaptive value of plastic modifications depends on a tight
46 correlation between the plastically-altered phenotype and the conditions under which it
47 functions. As plastic modifications require (sometimes considerable) time, relevant
48 environmental information should pertain to forthcoming rather than to present
49 conditions^{8,9}. Plants are able to utilize early cues and signals to increase their readiness (AKA
50 priming) to anticipated conditions such as salinity¹⁰, drought¹¹, nutrient availability^{12,13},
51 competition^{8,14} and neighbour proximity^{15,16,17}. In some cases, relevant information is

52 perceived from neighboring plants and can greatly increase anticipative adaptations to e.g.
53 impending damage by herbivory¹⁸ or pathogens¹⁹.

54 In response to stress cues emitted from the roots of their drought-inflicted neighbours,
55 unstressed plants readily close their stomata, and via relayed cuing induce stomatal closure
56 in additional unstressed plants^{20,21}. Nevertheless, while drought-stressed plants show
57 persistent stomatal closure, their unstressed neighbors fully reopen their stomata 3 to 24 h
58 following the onset of drought cuing²¹. These findings raise important questions regarding
59 the possible ecological implications of interplant drought cuing. What are the adaptive
60 implications of drought cuing? Are such adaptations only expressed in directly-cued plants
61 or also in their relayed neighbors? What is the mechanism of interplant drought cuing?

62 Here, we studied the ecological implications of interplant drought cuing by subjecting
63 unstressed *S. secundatum* target plants to short periods of root cuing from neighbors that
64 were either stressed or unstressed by drought. The survival and performance of the target
65 plants were monitored during a subsequent period, during which they were subjected to
66 either benign or drought conditions. We predicted that both direct and relayed interplant
67 cuing would increase the survival and performance of unstressed plants under a subsequent
68 drought. In addition, we expected that drought cuing would inflict performance costs on
69 target plants that would not be subjected to a subsequent drought.

70 The mechanism of interplant drought cuing was studied in additional experiments. Previous
71 findings demonstrated that interplant drought cuing was attained amongst neighboring
72 roots, suggesting that the involved vectors are emitted by the roots of drought-inflicted
73 plants. Abscisic acid (ABA) is a promising candidate that satisfies these specific criteria. ABA
74 is produced in most plant tissues and is involved in the induction of resistance and tolerance
75 to drought and other stresses²². Previous studies have demonstrated that in some legumes
76 and grasses, drought may cause ABA leakage from the roots^{23,24}. Other studies showed that
77 exogenous ABA can be taken up by roots and elicit stress responses in receiving plants^{25,26}.
78 Accordingly, we hypothesized that ABA is the exogenous vector of interplant drought cuing.
79 We studied the possible involvement of ABA in four complimentary experiments in which
80 plant responses to interplant drought cuing was monitored while ABA levels or presence
81 were manipulated. Specifically, we compared stomatal closure in unstressed *Pisum sativum*
82 plants subjected to drought root cuing from neighboring plants deficient in their ability to
83 produce ABA (ABA-deficient *wilty* mutant plants²⁷ or plants treated with fluridone that
84 interferes with ABA biosynthesis²⁸). Next, we analyzed ABA content in the rhizosphere of
85 drought-stressed plants and their unstressed neighbors. Based on previous studies²⁷, we
86 expected to find higher concentrations of ABA in the rhizosphere of drought-inflicted plants
87 and their unstressed neighbors compared to uncued controls. Finally, we compared the
88 survival and performance of *P. sativum* plants growing under either drought or benign
89 conditions after being subjected to a short period of direct or relayed root cuing from
90 neighboring plants that were either treated or untreated with ABA. We predicted that both
91 direct and relayed cuing from ABA-treated neighbors would increase the survival and
92 performance of unstressed target plants under a subsequent drought and that ABA cuing

93 would inflict performance costs on target plants that have not been subjected to a
94 subsequent drought.

95

96 **Results**

97 Ecological implications of interplant drought cuing

98 Early interplant drought cuing significantly affected plant survival and performance under a
99 subsequent drought. As expected, all plants growing under benign conditions survived to
100 the end of the experiment (not shown). Both direct and relayed interplant drought cuing
101 affected the survival time of plants under drought. While one day of drought cuing only
102 slightly and non-significantly affected the survival of the target plants (Fig. 2a-c), five days of
103 drought cuing significantly increased survival time of both directly-cued (T1) and relayed-
104 cued (T2) plants by 14% and 28%, respectively (Fig. 2d-f). Shoot size was 2.05 times greater
105 under benign conditions than under drought (one-way ANOVA, $F(1,158) = 44.83$, $p <$
106 0.0001). Early drought cuing differentially affected the size of plants growing at different
107 positions and under different drought-cuing conditions (Fig. 3). Target plants experiencing
108 one day of relayed drought cuing (T2) had 34% greater shoot biomass, 88% more branches
109 and 73% greater branch length than their uncued controls (Fig. 3c, g, j); however, no such
110 effects were found in directly-cued (T1) plants (significant cuing treatment X target
111 interaction for shoot biomass ($F(1, 72) = 4.22$, $P = 0.043$), Branch number ($F(1, 72) = 6.27$, $P =$
112 0.015) and branch length ($F(1, 72) = 5.17$, $P = 0.026$), or when drought cuing was
113 implemented for 5 days (Fig. 3d, h, l). T2 plants that experienced five days of relayed
114 drought cuing and grew under benign conditions showed 40%, 36% and 42% lower shoot
115 biomass, branch number and branch length, compared to their uncued controls (Fig. 3b, f,
116 k), but no such effects were observed in directly-cued T1 plants or when drought cuing was
117 implemented for one day (Figs. 3a, e, i).

118 *Soil water*: soil water content during the cuing period was non-significantly different in
119 drought-cued treatments and in their uncued controls (Supplementary Fig. 2). Incidental
120 differences in soil water content were observed during the drought period, but in all these
121 cases values were higher in the drought treatment than in the benign controls
122 (Supplementary Fig. 2).

123

124 The involvement of ABA in interplant drought cuing

125 a. Interplant root cuing by ABA-deficient plants

126 As expected, 60 min following the onset of drought treatment to one of the roots of the IND
127 plant (pot 1, Fig 4a, c), similar stomatal closure was observed in stressed IND plants and in
128 their unstressed neighbors (nonsignificant difference in stomatal width between IND, T1 -
129 T4: *wilty*- $F(4,50) = 0.83$, $p = 0.51$; *fluridone*- $F(4,25) = 1.61$, $p = 0.20$) (Fig. 4b, d). The presence of
130 an ABA-deficient plant significantly interfered with drought cuing. While stomatal aperture

131 was similarly low in IND, T1 and T2 plants of the ABA-deficient and in their control plants
132 (*wilty*: IND- $F_{(1,18)} = 3.26$, $p = 0.087$; T1- $F_{(1,18)} = 0.40$, $p = 0.53$; T2- $F_{(1,18)} = 0.15$, $p = 0.70$;
133 fluridone: IND- $F_{(1,11)} = 1.86$, $p = 0.20$; T1- $F_{(1,11)} = 4.64$, $p = 0.06$; T2- $F_{(1,11)} = 0.33$, $p = 0.58$),
134 *wilty* and fluridone-treated plants exhibited 79% and 45% greater stomatal aperture than
135 their WT and untreated controls, respectively (*wilty*: $F_{(1,18)} = 8.91$, $p = 0.00795$; fluridone:
136 $F_{(1,11)} = 24.91$, $p = 0.00041$), and their T4 neighbors showed 29% and 39% greater stomatal
137 aperture than their WT and untreated controls, respectively (*wilty*: $F_{(1,18)} = 13.89$, $p =$
138 0.00179 ; fluridone: $F_{(1,11)} = 27.24$, $p = 0.00029$) (Fig 4b, d).

139

140 b. ABA in the rhizosphere

141 Drought cuing significantly affected ABA concentration in the rhizosphere of both drought-
142 stressed plants and their unstressed neighbors. Sixty minutes following the onset of a
143 drought treatment to one of the roots of the IND plant (receptacle 1, Fig. 5a), ABA
144 concentrations were 106% and 145% higher in pots 2 and 3 of the drought treatments than
145 in their respective controls, with a more pronounced difference in receptacle 3 than in
146 receptacle 2 (Fig. 5b).

147

148 c. Ecological implications of interplant ABA cuing

149 *Survival*: early interplant ABA cuing significantly affected the survival of cued *P. sativum*
150 plants under a subsequent period of drought. Regardless of drought cuing, all plants
151 growing under benign conditions survived to the end of the experiment (not shown).
152 Treating one of the roots of the IND plant (pot 1, Fig. 1) with ABA for three days significantly
153 increased leaf survival in both directly-cued (T1) and in relayed-cued (T2) neighbors,
154 compared to their uncued controls (Fig. 6). At day 52 (following 27 d of drought), leaf
155 survival was greater than 98% in both ABA-cued plants, but only 88% and 87% in their
156 uncued T1 and T2 controls, respectively (Fig. 6). At day 82, at the end of a 30-day rewatering
157 'revival treatment', during which all plants were grown under benign conditions, leaf
158 survival was 92.4% and 89.4% in the T1 and T2 ABA-cued plants, respectively, but none of
159 their uncued controls survived (Fig. 6). Leaf greenness increased in all treatments through
160 the initial part of the drought period but it started to decline in uncued T1 and T2 plants
161 after day 36 (Fig. 7). At day 82, leaf greenness of ABA-cued plants was 16% and 12% lower
162 than in plants that grew under benign conditions throughout the entire experiment (-ABA →
163 benign) in T1 and T2, respectively, and 59% and 58% higher than in their uncued (-ABA →
164 drought) controls (Fig. 7). Nineteen days following the onset of the revival treatment, leaf
165 resprouting was observed in 32% and 44% of the T1 and T2 ABA-cued plants, respectively,
166 and by day 82, leaf resprouting was observed in 48% and 52% of the ABA-cued T1 and T2
167 plants, respectively (Fig. 8). By contrast, merely 4% and 12% of the uncued T1 and T2
168 controls resprouted, but by day 82 none of them survived (Figs. 6, 8).

169 *Performance*: as expected, the largest and the smallest shoots belonged to uncued plants
170 that grew under benign conditions (-ABA → benign) and to uncued plants that grew under

171 drought (-ABA → drought), respectively (Figs. 9, 10). Early ABA cuing significantly increased
172 plant size under a subsequent drought. At the end of the experiment and following the
173 rewatering revival treatment, ABA-cued T1 and T2 plants had 25% and 29% more leaves,
174 15% and 21% longer seminal shoots, and 38 and 35% greater shoot biomass than their
175 uncued controls, respectively (Figs. 9, 10). Under benign conditions, ABA-cued plants
176 incurred significant size reductions. ABA-cued T1 and T2 plants that grew under benign
177 conditions had 18% and 21% less leaves, 27% and 20% shorter shoots, and 13% and 20%
178 lower shoot biomass than their uncued controls, respectively (Figs. 9, 10). No flowers were
179 developed by uncued plants under drought and the low flower production in other
180 treatments mirrored shoot growth (Fig. 10b).

181 *Soil water*: soil water content during the cuing period was non-significantly different in ABA-
182 cued treatments and in their uncued controls (Supplementary Fig. 3).

183

184 **Discussion**

185 Plants are able to perceive and integrate intricate signals and cues that enable them to
186 plastically adapt to imminent risks and opportunities⁹. It was previously demonstrated that
187 unstressed plants rapidly close their stomata in response to stress cues emitted by the roots
188 of their drought-stressed neighbors, and that via ‘relay cuing’ can elicit stomatal closure in
189 additional unstressed plants^{20,21}. In the following, we discuss the ecological implications of
190 interplant drought cuing and its underlying mechanism as reflected from the results.

191 In *S. secundatum*, both direct and relayed drought cuing can significantly increase plant
192 survival time and performance under a subsequent drought (Figs. 2, 3). The criticality of
193 water limitation to plant survival, growth and functioning cannot be overemphasized and
194 predictive information regarding forthcoming droughts could be crucial for plant survival
195 and fitness. Plants are known to respond to mild soil drying before any changes in leaf water
196 potential are detectable^{11,29} and competitive depletion of soil water have been shown to
197 elicit large plastic modifications in resource allocation that facilitate increased survival under
198 drought¹⁴. Our results show that ‘eavesdropping’ on neighbors allows plants to exploit even
199 earlier indications of an ensuing drought, before they experience any decreases in water
200 availability (Supplementary Fig. 2). In arid environments, such predictive cues may help
201 plants increase their survival and performance during dry spells within and between
202 growing seasons^{30,31,32,33} and prompt annual plants to more timely and less traumatically
203 modulate their resource allocation and phenology in preparation for the end of the growing
204 season^{34,35}.

205 The findings demonstrate that drought cuing can be relayed via a chain response of multiple
206 neighboring plants (Figs. 2, 3). As the perception of drought cues elicits further cuing,
207 relayed drought cuing is expected to be rapidly amplified by a feedforward positive-
208 feedback loop (see Wenig et al. 2019 for a similar phenomenon related to volatile defense
209 cuing³⁶). Nevertheless, for such a self-enhancing system to be ecologically-relevant and
210 reliable, it is essential that plants are not engaged in an inappropriate and costly permanent
211 overly-escalated state of alert³⁷. It is thus expected that both drought responses and cuing

212 rapidly subside if drought conditions do not materialize following cue perception or where
213 there is a consistent contrast between the perceived drought cues and the water status of
214 the cued plants.

215 The duration of drought cuing significantly affected plant survival ($F_{(1,212)} = 4.98$), $p = 0.027$).
216 While one-day cuing only slightly and non-significantly affected plant survival, five-day cuing
217 had a significant effect on canopy survival (Fig. 2). This finding demonstrates the importance
218 of the integration of environmental information over longer periods, which could
219 considerably increase the quality of the perceived information and response
220 appropriateness, and reduce the costs of erroneous responses to false alarms. Similar
221 integration of environmental cues has been demonstrated in other environmentally-cued
222 responses such as seed germination³⁸ and shade avoidance³⁹, where inappropriate
223 responses could be critically detrimental for plant survival and fitness⁴⁰.

224 As expected, the drastic limitation in water availability only allowed limited effects of
225 drought cuing on plant performance under a subsequent drought (Fig. 3) but the results
226 show that induced stress tolerance and increased survival may incur performance costs
227 under both drought and benign conditions. While one day of drought cuing increased plant
228 size under drought and inflicted no costs under benign conditions, five days of drought cuing
229 did not improve plant growth under drought yet caused some cost under benign conditions
230 (Figs. 2, 3). Indeed, functional tradeoffs between stress tolerance and growth rates under
231 benign conditions have been commonly demonstrated in comparative studies^{e.g. 41,42,43}. A
232 dramatic manifestation of adaptive plastic responsiveness that alleviates some of the costs
233 of stress adaptations is demonstrated by plants such as *Portulaca oleracea* and
234 *Mesembryanthemum crystallinum* that are able to facultatively switch from fast-growing C4
235 or C3 photosynthesis under benign conditions to a slow growing stress-tolerant
236 Crassulacean acid metabolism (CAM) mode under drought or saline conditions, without
237 incurring reduced growth rates under favorable conditions⁴⁴.

238 Interestingly and perhaps counterintuitively, both survival and growth effects of drought
239 cuing tended to be consistently greater in relayed target plants (T2) than in directly-cued
240 plants (T1) (Figs. 2, 3). While we cannot provide a conclusive explanation for this trend, it
241 can be speculated that it might have been caused by the slightly faster soil drying of pots 3
242 and 4 (T2) compared to pots 2 and 3 (T1), 10-20 d following the onset of drought
243 (Supplementary Fig. 2). Although this trend was indistinguishably similar in all cuing
244 treatments, it is conceivable that the match between the experienced water status and the
245 perceived drought cues was tighter and thus potentially more reliable for the relayed T2
246 than for the directly-cued T1 plants. An alternative though not mutually-exclusively
247 hypothesis could be that T2 was experiencing stronger drought cuing than T1 plants, which
248 in turn increased hydraulic conductance into the plant⁴⁵ and accelerated soil dehydration.
249 Such a scenario could have increased plant survival under drought and decreased plant
250 growth under benign conditions (Figs. 2, 3).

251 While it is relatively easy to understand the adaptive benefits of plant responsiveness to
252 sufficiently-reliable drought cues, the selective advantage of cue emission is much less
253 obvious. Selection is only expected to prefer cue 'leakiness' from stressed plants where the

254 average fitness benefits of stress cuing outweigh their production and competitive costs^{21,46}.
255 Accordingly, leakiness of honest drought cues is expected to benefit emitters a) if the
256 presence of drought cues (e.g. ABA) in the rhizosphere could increase drought resistance⁴⁷,
257 b) in large plants or plants with strict anatomical sectoriality⁴⁸, where exogenous root
258 signaling could be more rapid and efficient than endogenous signaling⁴⁹, c) in clonal plants
259 or in plants where kin or clone mates are spatially clumped^{50,51}, where the probability of
260 benefiting genetically-alien competitors is low.

261 Roots exchange with the rhizosphere myriad substances, some of which carry vital adaptive
262 information⁵². For example, under nutrient deficiency some plants increase production and
263 root exudation of strigolactones that promote mycorrhizal development and
264 establishment⁵³. ABA is a plant hormone involved in growth and responses to abiotic
265 stresses such as drought, salinity and nutrient deficiencies⁵⁴. In drying soils, long-distance
266 endogenous ABA signaling regulates multiple pathways related to stomata aperture, root
267 development and hydraulic conductivity⁵⁵. In some plants drought not only increases ABA
268 production and accumulation but also elicits ABA exudation to the rhizosphere^{23,25,56}, Fig. 5).
269 As roots can absorb ABA from the soil solution and transfer it to the shoots²⁵, the combined
270 effects of ABA exudation and uptake may be the mechanism underlying interplant drought
271 cuing^{20,21} (Figs. 2, 3).

272 The involvement of ABA in interplant drought cuing is implicated here by a few lines of
273 evidence. Drought cuing was greatly reduced in *P. sativum* plants with diminished ABA
274 synthesis (Fig. 4). Although this finding demonstrates the essentiality of ABA for interplant
275 cuing, it does not prove that ABA is the exogenous cuing agent. Both drought-stressed *P.*
276 *sativum* plants and their unstressed neighbors exuded significantly more ABA than their
277 unstressed controls (Fig. 5), more directly implying that ABA could be the exogenous vector
278 of drought cuing. ABA concentrations in the rhizosphere are known to increase following
279 soil drying, which in turn elicit adaptive drought responses in plants^{25,57}. Various soil
280 bacteria and fungi⁸⁰ have been shown to produce ABA under developing drought conditions,
281 which could further augment plant responses to forthcoming drought⁵⁸. On the other hand,
282 soil bacteria, such as *Rhodococcus* and *Novosphingobium*, were shown to metabolize soil
283 ABA⁵⁹, which could potentially alleviate performance costs in previously stressed or
284 communicated plants under benign conditions²⁰ (Figs. 9, 10), and increase the reliability of
285 ABA cuing by rapidly reducing soil ABA concentrations following the cessation of its
286 exudation. Finally, the effects of early ABA cuing in *P. sativum* largely emulated the effects
287 of drought cuing in *S. secundatum* by eliciting significant increases in plant survival and
288 performance under a subsequent drought in both directly-cued and relayed plants, and by
289 inflicting performance costs in cued plants under benign conditions (Fig. 6-10). Although the
290 similarities between the drought-cuing experiment with *S. secundatum* and the ABA-cuing
291 experiment with *P. sativum* are admittedly limited, the resemblance between the results of
292 the two experiments strongly suggests that ABA is the exogenous vector of interplant
293 drought cuing. These findings, the ubiquity of ABA as a stress hormone⁶⁰ and the existence
294 of similar drought cuing in a variety of plant species²³ imply that drought cuing could exist
295 between neighboring plants belonging to different taxa. Testing this hypothesis, we found

296 that *S. secundatum* and *Cynodon dactylon* closed their stomata when neighboring each
297 other's drought-stressed plants (Omer Falik and Ariel Novoplansky, *in preparation*).

298 Natural selection is expected to favor plastic responsiveness to reliable cues and signals that
299 help organisms track environmental changes while reducing costs of constitutive
300 adaptations⁶¹. Our findings show for the first time that the perception of early interplant
301 root cues could help unstressed plants to become better adapted to forthcoming drought,
302 yet at the expense of reduced performance under benign conditions. Interestingly, these
303 adaptations and costs could be similarly elicited in both directly-cued and relayed plants,
304 implying that besides direct communication of warning cues, stress cuing and priming may
305 be elicited by unstressed plants. The findings raise interesting questions as to the
306 circumstances under which plants are expected to respond to stress cues in spite of their
307 growing under benign conditions. For example, it is expected that due to their potential high
308 costs, drought adaptations will not be triggered in plants growing under water-abundant
309 conditions or where the temporal horizon of plastic responses spans beyond the time plants
310 are expected to be able to adaptively respond, e.g. in annual plants nearing the end of the
311 growing season. Cost-benefit considerations further imply that responsiveness to early
312 stress cues is expected to diminish over time if the expected stress does not materialize.
313 Previous studies have provided impressive evidence for the effects of plant communication
314 on population- and community-level interactions^{e.g.18,62,63} and thus the findings call for
315 further investigation into the implications of interplant stress cuing under realistic field
316 conditions. For example, how and to what extent can direct and longer-distance relayed
317 interplant stress cuing affect competitive and facilitative interactions between plants? It can
318 be speculated that under some circumstances interplant stress cuing could ameliorate
319 competition beyond the direct effects of abiotic stresses though evolutionary game
320 considerations related to the variable abilities of particular taxa to plastically modify their
321 responsiveness to stress cuing could give rise to rather intricate effects. The results also call
322 for the investigation of potential applied implications of both direct and relayed ABA root
323 cuing⁶⁴. For example, what are the implications of exogenous ABA treatments for interplant
324 cuing in agricultural settings or what are the effects of ABA root exudation on soil bacteria,
325 and the spatial and temporal ranges of local ABA application on increasingly-distant
326 neighboring plants and rhizosphere microbiota?

327

328 **Methods**

329 **Ecological implications of interplant drought cuing**

330 *Experimental design and setup.* The goal of this experiment was to study the survival and
331 performance implications of interplant drought cuing. The experiment was conducted on
332 *Stenotaphrum secundatum* (buffalo grass), a perennial stoloniferous grass native to North
333 America, West Indies and Australia. *S. secundatum* is a strong competitor typical to
334 anthropomorphically-disturbed habitats and is commonly used as a lawn grass⁶⁵. Testing for
335 the effects of drought cuing required that specific induced plants (IND) would experience a
336 drought event or benign conditions while their neighboring target plants (T1, T2) would only

337 experience cuing from the IND plants (Fig. 1). This was achieved by using triplets of two-root
338 *S. secundatum* plants planted in rows of four pots (Fig 1). One of the roots of the IND plant
339 was subjected to either drought or benign conditions while its other root shared a pot with
340 one of the roots of its nearest unstressed neighbor (T1). The other root of T1 shared its pot
341 with one of the roots of an additional unstressed target plant (T2). This configuration
342 permitted T1 to exchange stress cues with both IND and T2, while preventing direct root
343 cuing between IND and T2 and thus allowing to separately study the effects of direct and
344 relayed drought cuing on T1 and T2, respectively^{20,21} (Fig 1). The experiment comprised
345 three distinct periods as follows:

346 a) Establishment period, during which the plants were grown in the triplet experimental
347 systems so their roots became well intermingled. During this period all plants (IND, T1, T2)
348 were grown under benign conditions.

349 b) Cuing period, during which one of the roots of the IND plant (pot 1) was subjected to
350 either drought or benign conditions for one or five days, while the target plants (T1, T2)
351 experienced benign conditions. The IND plant was removed at the end of this period.

352 c) Survival period, during which the survival and growth of the target plants (T1, T2) were
353 assessed while experiencing either drought or benign conditions.

354 *Growth conditions and plant preparation.* The experiment was conducted in a greenhouse
355 under 30% full sunlight on the Sede Boqer campus, Israel (30 852'N, 34 847'E), partially
356 controlled by an automated pad-and-fan evaporative cooling system (Termotecnica Pericoli,
357 Albenga, Italy), keeping day temperatures at 25-35° C during the summer. Commercial lawn
358 mats of *S. secundatum* (Etz Hasade, Sede Yaakov, Israel) were planted in 15 cm deep flats
359 for 21 d for root regeneration and acclimation, during which they were irrigated to field
360 capacity every 3-4 d. The establishment period started by planting triplets of 6 cm long
361 rhizome cuttings in 200 mL, 65 mm-diameter plastic pots (Miniplast, Ein Shemer, Israel);
362 (Fig. 1). The pots were wrapped with aluminium foil to protect the soil and roots from light
363 exposure. To allow rapid and non-destructive drought conditions, the induction pot (pot 1,
364 Fig. 1) was filled with tap water and the other pots were filled with a commercial soil
365 mixture (Deshanit, Be'er Yaakov, Israel). In clonal plants such as *S. secundatum*, resource
366 translocation is predominantly acropetal⁶⁶ and in response to herbivory, endogenous
367 warning signals are more efficiently transferred acropetally than basipetally⁶⁷. To avoid
368 potential variability due to axis polarity, all plants were planted so their proximal ramets
369 were planted nearest to the IND pot (Fig 1). In order to increase root intermingling and plant
370 uniformity, plant cuttings were grown in the experimental systems for 30 d, after which
371 their tillers were clipped and they were allowed to regenerate for additional seven days
372 before starting the cuing period.

373 The cuing period started by evacuating the water from pot 1 and filling it with either dry
374 (drought treatment) or wet (benign control) 9:1 mixture of no. 2 vermiculite (Agregal,
375 Habonim, Israel) and bentonite (Agat, Be'er Sheva, Israel), (VB mixture)²¹, for either one or
376 five days. The IND plants were removed at the end of the cuing period to ensure that
377 drought cuing was restricted to the designated periods and that the performance of the

378 target plants during the survival period would not be affected by the presence of the IND
379 plant (Fig. 1).

380 *Plant performance.* During the survival period, pots assigned to drought treatment were not
381 irrigated and pots assigned to benign conditions were irrigated every 3-4 d. Plant
382 aboveground survival (AGS) was estimated every 7 d from digital photographs by the
383 percentage of living aboveground canopy. Plants shoots were individually harvested at day
384 60, upon the death of the last surviving drought-treated plant. Harvested plants were
385 surveyed for branch number and length and shoot dry biomass was estimated after drying
386 the samples in a ventilated oven at 60 °C for 72 h. Soil water content was measured using a
387 portable Time-Domain Reflectometer (TDR100, Campbell Scientific)⁶⁸. Daily TDR surveys
388 were conducted in pots 2-4 during the first 20 d of the experiment at which time all the
389 drought-treated pots reached a stable soil water content. TDR measurements were
390 conducted between 21:00 and 00:00, when greenhouse temperatures were the most stable
391 and thus introduced the least temperature-induced variability⁶⁹. Each TDR probe consisted
392 of a pair of parallel 2 mm-diameter, 100 mm-long stainless-steel rods (Zahavi Steels, Tel
393 Aviv), fastened 20 mm apart by an isolated terminal connector block. Probes were
394 permanently installed at a vertical position, 70 mm below the soil surface, in the center of
395 each pot. Soil water content was derived from paired gravimetric-TDR measurements.

396

397 The involvement of ABA in interplant drought cuing

398 *Pisum sativum* was chosen as a model plant because of the comprehensive knowledge on
399 the involvement of ABA in its responses to various stresses and the ease of its cultivation
400 under both laboratory and greenhouse conditions.

401 a. Interplant root cuing in ABA-deficient plants

402 Interplant root cuing of drought cues was studied in *P. sativum* with diminished ABA
403 synthesis. Groups of five split-root plants were planted in rows of 50 mL receptacles (Fig 4a,
404 c). Split-root plants were prepared as described in Falik et al. (2011)²⁰. Stomatal aperture
405 was estimated after subjecting one of the roots (pot 1) of the first plant (IND) to either
406 drought or benign conditions for 1 h using the above protocol. The effects of diminished
407 ABA synthesis were studied in two separate experiments. In the first experiment (Fig. 4a),
408 the third target plant (T3) was either a JI 1194 wild-type *P. sativum* (WT control) or a *wilty*
409 (line JI 1069) *P. sativum* mutant in which ABA biosynthesis is reduced by ca. 90% compared
410 to WT²⁷. In a second experiment (Fig. 4c), the T3 plant was either untreated (control) or
411 treated with fluridone, known to drastically interfere with ABA biosynthesis²⁸. In both
412 experiments we predicted that diminished ABA production in T3 plants would significantly
413 reduce their abilities to perceive and relay drought cues. Accordingly, we expected that
414 when subjecting the IND plant to drought, both T3 and T4 plants would demonstrate greater
415 stomatal aperture than their IND, T1 and T2 neighbors. Stomatal aperture measurements
416 were conducted using epidermal imprints as described in Falik et al. (2011)²⁰.

417

418 b. ABA in the rhizosphere

419 ABA concentrations were estimated in root leachates separately collected from drought-
420 stressed plants and their unstressed neighbors. Triplets of split-root *P. sativum* cv. Dunn
421 plants were grown in rows as described above (Fig 5a). One of the roots of the IND plant
422 (pot 1) was subjected to either drought (dry VB) or benign conditions (wet VB) for one hour,
423 after which the contents of the rooting receptacles of stressed plants (receptacle 2) and of
424 their cued unstressed neighbors (receptacle 3) were analyzed. The experiment was
425 conducted with 12 biological replications and leachates were pooled into six 100 mL
426 technical replicates to increase metabolite concentrations in the analyzed samples. The
427 leachate samples were lyophilized and the dry pellets were analyzed for ABA content at the
428 Instituto de Biología Molecular y Celular de Plantas, CSIC, Universidad Politécnica de
429 Valencia, Spain. Individual samples were macerated in liquid nitrogen with the addition of
430 1.8 mL of the extraction solution (80% methanol, 1% acetic acid and 19% distilled water),
431 following the addition of the deuterated analogue of ABA (Oilchemim Ltd, Olomuc, Czech
432 Republic) to be quantified (30 μ L of a solution containing ABA). Following, the samples were
433 shaken for 1 h at 4 °C and then centrifuged at 10000g at 4 °C for 4 min. The supernatant was
434 removed and conditioned in a 2 mL tube for 24 h at -20 °C for precipitation of proteins, and
435 the samples were centrifuged again at 10000g at 4 °C for 4 min; the supernatant was
436 transferred to 5 mL glass tubes, and the samples were concentrated in a rotovapor
437 (Thermos Scientific®) for 3 h. The concentrated samples were finalized with 1 mL of 1%
438 acetic acid, and after a rapid shaking, filtered in Oasis HLB® columns (reverse phase). ABA
439 was recovered by applying 1 mL of 95% methanol and the samples were dried in the
440 rotovapor and subsequently dissolved with 150 μ L of 5% acetyl nitrile (ACN) + 1% acetic
441 acid. Readings were retrieved from a spectrometer coupled to a UHPLC and an autosampler
442 (Accucore RP-MS column 2.6 μ m, 50 \times 2.1 mm; ThermoFisher Scientific)⁷⁰.

443

444 c. Implications of interplant ABA cuing

445 We tested the hypothesis that interplant ABA cuing could increase the survival and
446 performance of cued plants under drought conditions. Triplets of split-root *P. sativum*
447 cv. Dunn plants were grown in the greenhouse using the same setup used in the *S.*
448 *secundatum* experiment (Fig. 1). One of the roots of the IND plant was grown in water (pot
449 1) while its other root and the roots of the target plants were grown in a commercial soil
450 mixture. Cuing treatments started by evacuating the water from pot 1 and filling it with
451 either 100 μ M ABA solution (ABA cuing; +ABA) or water (-ABA controls). In all treatments,
452 the proximal part of the treated root of the IND plant and the rims of pots 1 and 2 were
453 coated with a thick layer of petroleum jelly to prevent capillary flow of ABA solution from
454 pot 1 to pot 2. The content of pot 1 was replaced daily for 3 d after which pot 1 was
455 emptied, the IND plant was removed and the target plants were subjected to either 27 d of
456 drought treatment (no irrigation) or benign conditions (irrigation to field capacity every 3-4
457 d). The ability of plants to recover following drought was tested by a rewatering 'revival
458 treatment'. Starting at day 52, when significant differences in leaf survival were apparent

459 between ABA-treated plants and their untreated controls (Fig. 6), and throughout the
460 remainder of the experiment, all plants were irrigated to field capacity every 3-4 d. Leaf
461 number and survival, and shoot length were periodically censused. Plant state was further
462 estimated from 3216 X 2136 pixels white-balanced JPEG photographs. The area of all leaf
463 blades of each plant was digitally separated from its background and red (R), green (G), and
464 blue (B) reflectance data were used to calculate average excess green index (2G-R-B)⁷¹ using
465 Adobe Photoshop CC. Soil-water content and final shoot biomass were measured as
466 described for the *S. secundatum* experiment.

467

468 Data analyses

469 The effects of drought cuing on the survival of *S. secundatum* under drought were analyzed
470 using log-rank analyses⁷². The effects of drought cuing on growth of *S. secundatum* under
471 drought, the effect of ABA deficiency on interplant drought cuing in *P. sativum*, the effects
472 of drought cuing on rhizosphere ABA concentration and the effects of ABA cuing on survival
473 and growth of *P. sativum* under drought were analyzed using one-way ANOVAs where data
474 met the assumptions of parametric analysis or Kruskal–Wallis one-way ANOVAs where data
475 did not meet the assumptions of parametric analysis. As the rates of false positive results
476 have been demonstrated to be only marginally affected by lack of normality⁷³, analyses of
477 experiments with large replication numbers, e.g., the effects of drought cuing on *S.*
478 *secundatum* (Fig. 3) were performed using ANOVAs on untransformed data. The statistical
479 analyses were conducted using SYSTAT 13 (SPSS).

480

481 Data availability

482 All data will be made accessible at publication.

483

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648

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655

656 **Author information**

657 **Contributions**

658 O.F. coordinated all technical aspects of the project and conducted the ABA deficiency, ABA
659 exudation and ABA cuing experiments. S.M. conducted and analyzed the results of some of
660 the drought cuing experiments with *S. secundatum*, L.K. conducted the greenhouse ABA
661 cuing experiment with *P. sativum*, A.N. conceived the project, designed the experiments,
662 analyzed the results and wrote the paper.

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665

666 **Ethics declarations**

667 **Competing interests**

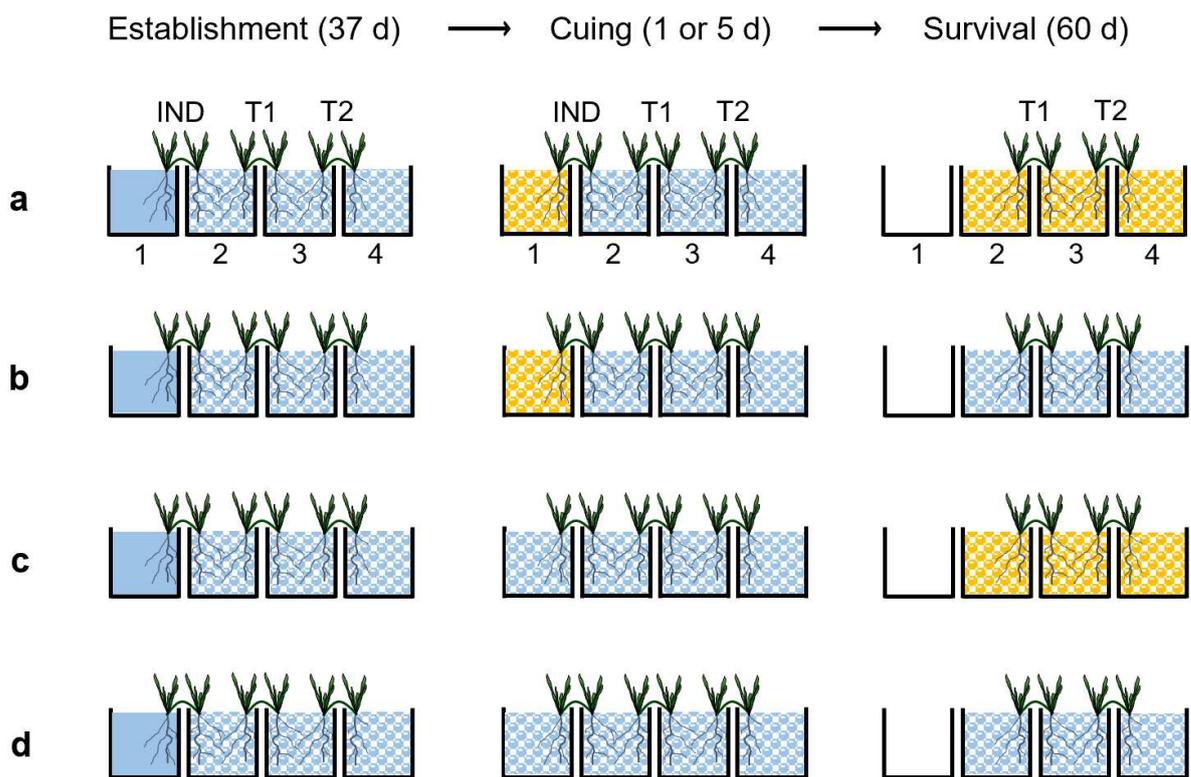
668 The authors declare no competing interests.

669

670

671 Figure 1: **Testing for the ecological implications of drought cuing in *S. secundatum* –**
 672 **experimental setup.** Triplets of two-ramet plants were planted in rows of four pots. One of
 673 the roots of the IND plant (pot 1) was subjected to either drought (yellow) or benign (blue)
 674 conditions while its other root shared pot 2 with one of the roots of its nearest unstressed
 675 neighbor (T1). The other root of T1 shared pot 3 with one of the roots of an additional
 676 unstressed target plant (T2). This configuration permitted T1 to exchange stress cues with
 677 both IND and T2, while preventing direct root cuing between IND and T2, thus allowing to
 678 separately study the effects of direct and relayed drought cuing on T1 and T2, respectively.
 679 Following an establishment period, one of the roots of the IND plant (pot 1) was subjected to
 680 either drought (**a, b**) or benign (**c, d**) conditions for one or five days after which the IND
 681 plant was removed (white) and the target plants were subjected to either benign (**b, d**; blue)
 682 or drought (**a, c**; yellow) conditions.

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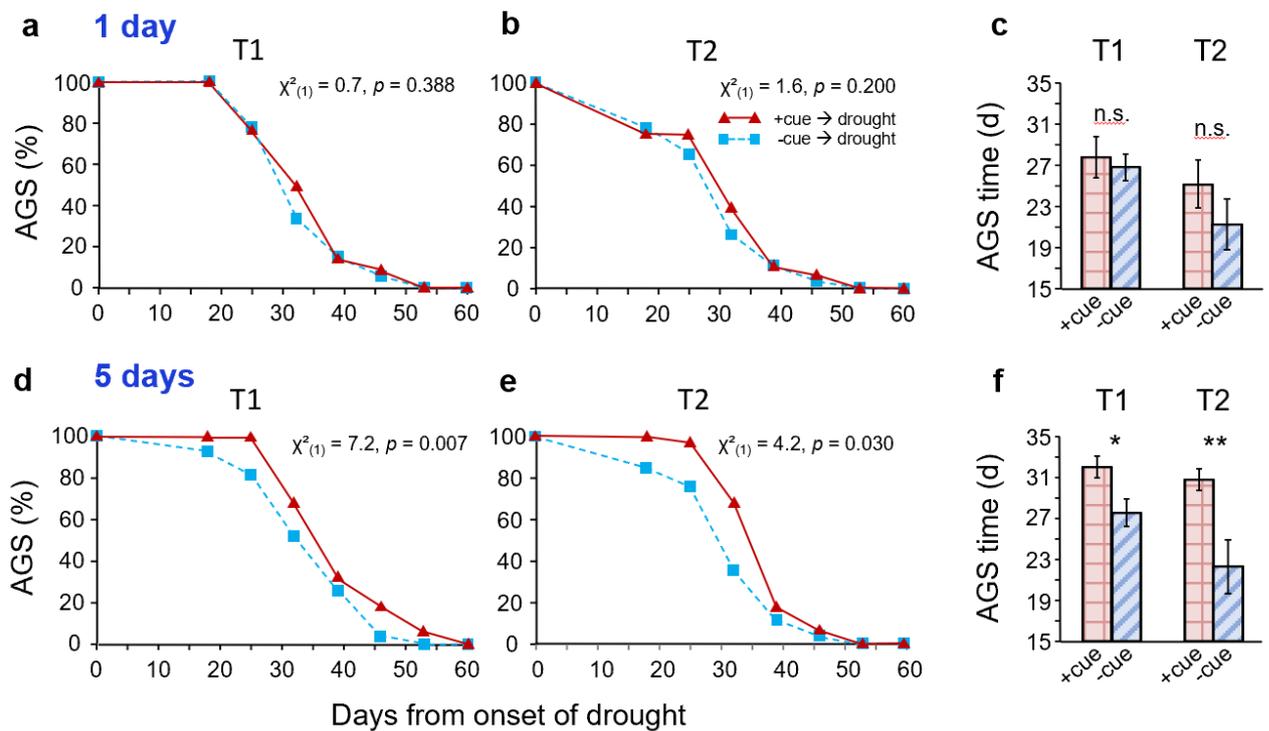


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686 Figure 2: **the effects of drought cuing on survival of *S. secundatum* under drought.** Values
 687 are for aboveground survival (AGS) kinetics (**a, b, d, e**) and for means \pm SEM of AGS survival
 688 time (**c, f**) of directly-cued (T1) (**a, b**) and relayed-cued (T2) (**d, e**) plants. Drought-cuing and
 689 control treatments are indicated in cyan and red, respectively. Analyses are for Kaplan-
 690 Meier log rank tests (**a, b, d, e**) and one-way ANOVAs (**c, f**), between target plants that
 691 either received or not received drought cues ($n = 25$).

692



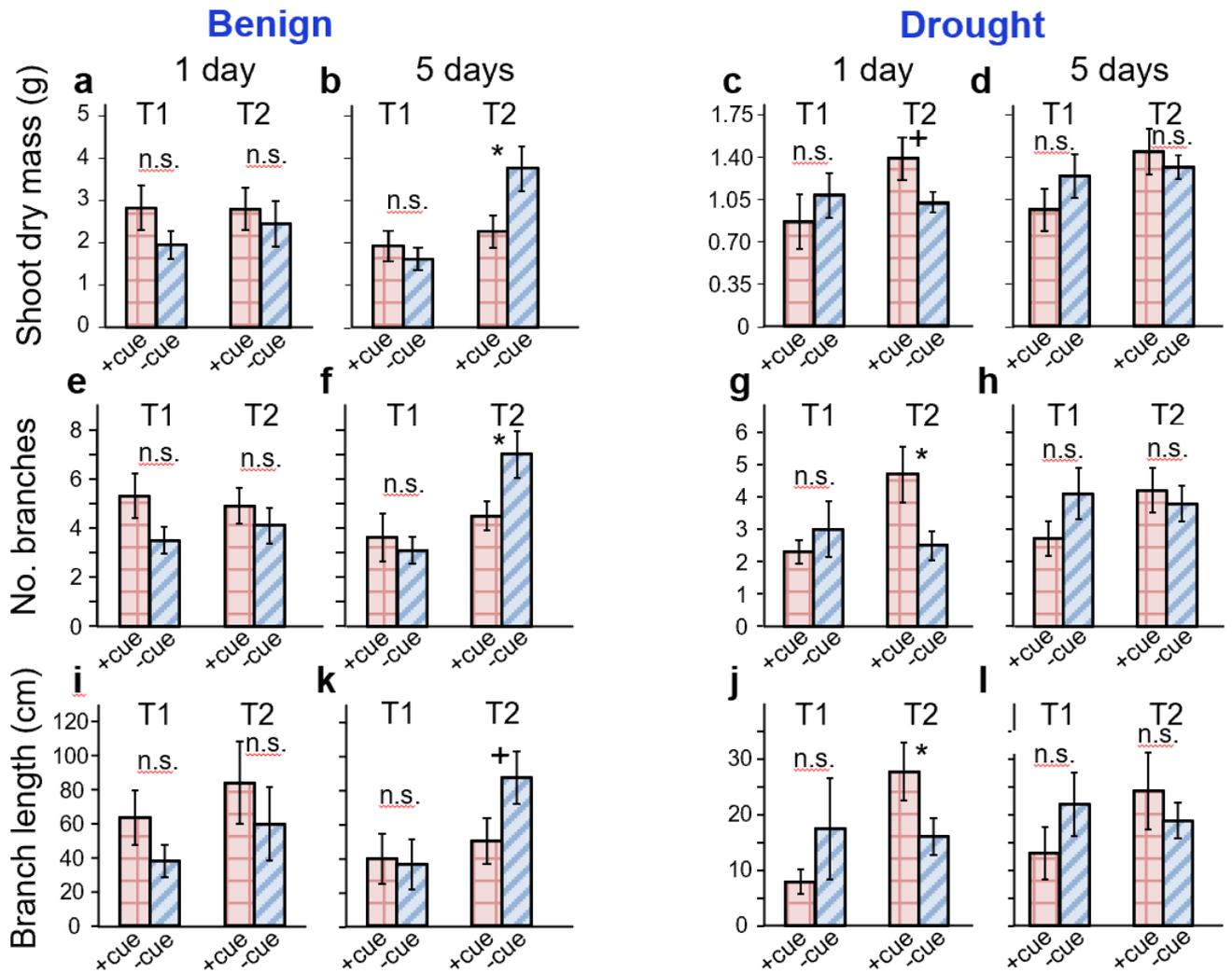
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696 Figure 3: **the effects of drought cuing on growth of *S. secundatum* under drought.** Values
 697 are means \pm SEM of shoot dry biomass (**a-d**), Number of branches (**e-h**) and branch length (**i-**
 698 **l**) of directly cued (T1) or relayed-cued (T2) plants. Significance values are for one-way
 699 ANOVA comparisons between target plants that either received (red) or not received (blue)
 700 drought cues, $+0.1 < P < 0.05$, * $P < 0.05$, (n = 25).

701

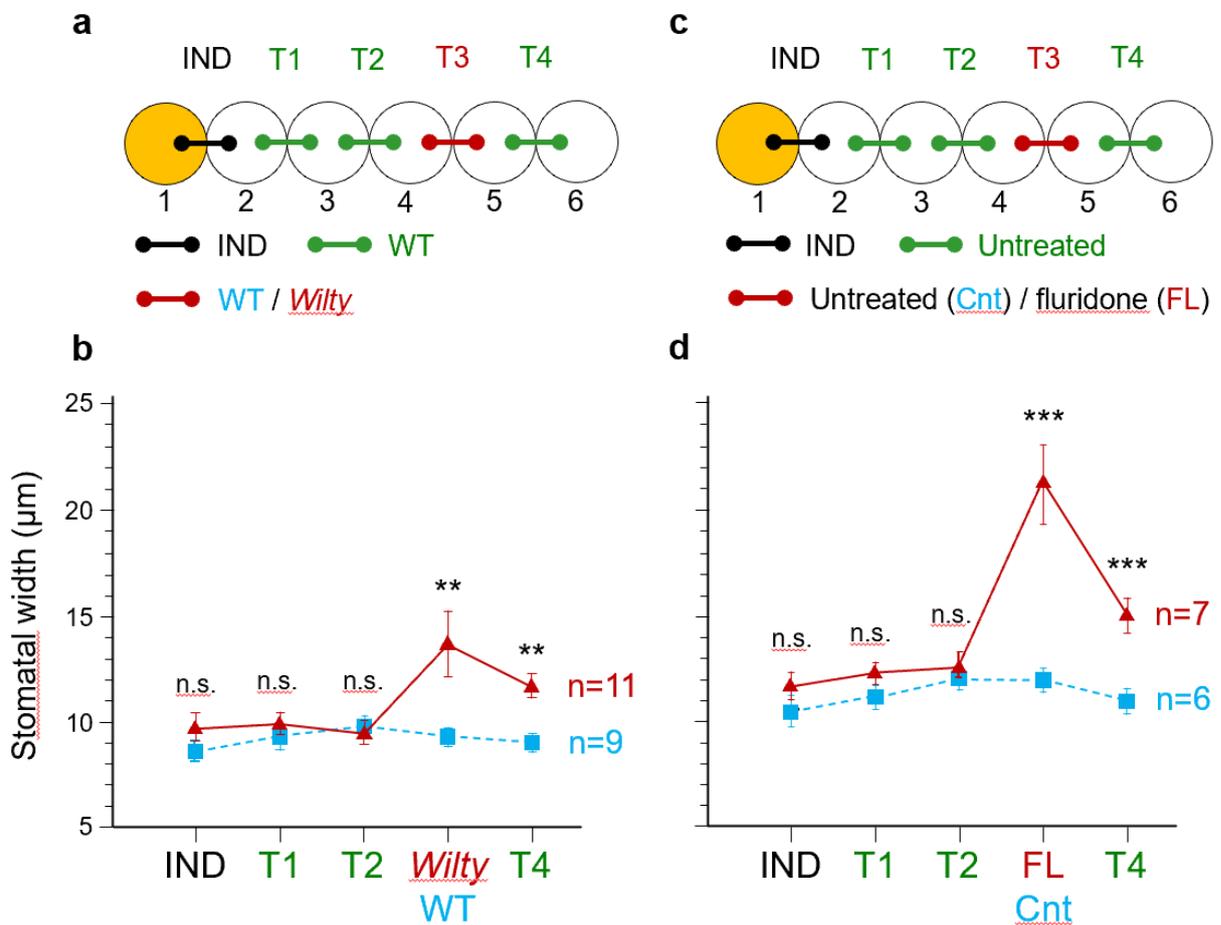


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703

704 Figure 4: **the effect of ABA deficiency on interplant drought cuing**. Sets of five split-root *P.*
 705 *sativum* plants were planted in a row. One of the roots of the IND plant (pot 1) was
 706 subjected to drought conditions for one hour while its other root shared pot 2 with one of
 707 the roots of its nearest unstressed neighbor (T1). The other root of T1 shared pot 3 with one
 708 of the roots of an additional unstressed target plant (T2) etc. In one experiment (a, b), the
 709 third target plant (T3) was either a wild-type *Pisum sativum* (WT) or a *Wilty* mutant plant. In
 710 another experiment (c, d), the T3 plant was either untreated (Cnt) or treated with fluridone
 711 (FL). Values (b, d) are means \pm SEM of stomatal width in plant rows including either WT (b) /
 712 control (d) plants (cyan) or *Wilty* (b) / fluridone-treated (d) plants (red). Significance values
 713 are for one-way ANOVA comparisons between the *Wilty* (b) and fluridone (d) treatments
 714 and their controls, ** $P < 0.01$, *** $P < 0.001$.

715

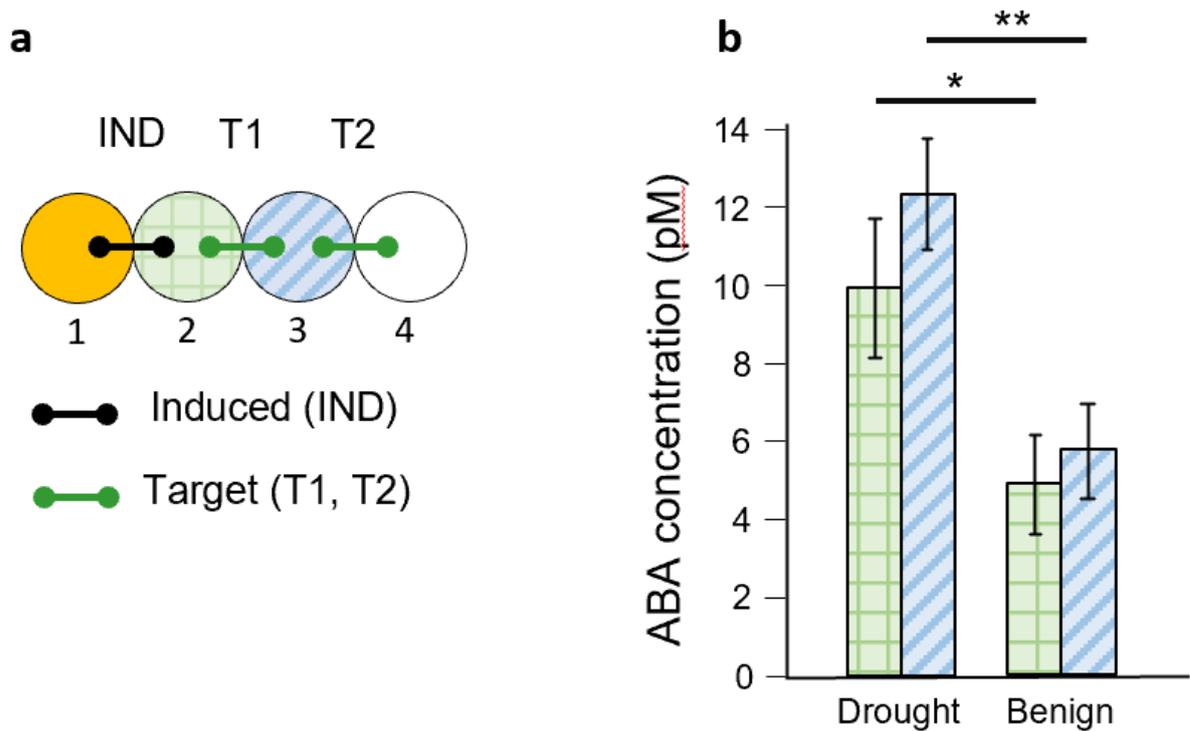


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718 Figure 5: **the effects of drought cuing on rhizosphere ABA concentration.** Triplets of split-
 719 root *P. sativum* plants were grown in rows as described in Fig. 1 (a). One of the roots of the
 720 IND plant (pot 1, orange) was subjected to either drought or benign conditions for one hour.
 721 ABA concentration was analyzed in the rhizospheric solution of pot 2 (shared by drought-
 722 treated IND and its unstressed T1 neighbor, green) and pot 3 (shared by unstressed T1 and
 723 T2 plants, blue). Values are means \pm SEM of ABA concentrations in pot 2 (green) and pot 3
 724 (blue). Significance values are for Kruskal–Wallis one-way comparisons between drought-
 725 cued plants and their controls, * $P < 0.05$, *** $P < 0.01$, $n = 6$.

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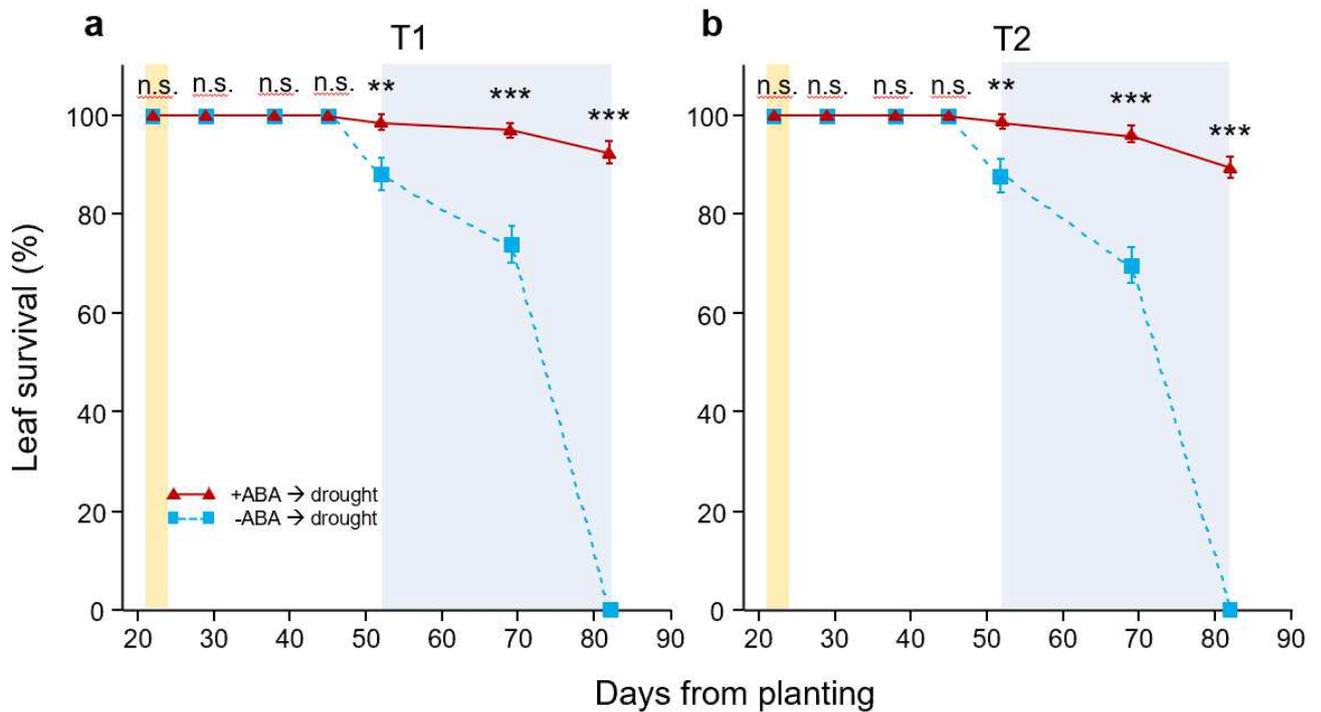


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728

729 Figure 6: **the effect of ABA cuing on leaf survival of *P. sativum* under drought.** Triplets of
 730 split-root *P. sativum* plants were grown in rows as described in Fig. 1. One of the roots of
 731 the IND plant (pot 1) was either treated or untreated (control) with ABA for three days
 732 (yellow background) after which the IND plant was removed and the target plants were
 733 subjected to a drought period (no background) and to a subsequent wet 'revival' period
 734 (blue background). Results are for percentage leaf survival of directly-cued (T1; **a**) and
 735 relayed-cued (T2; **b**) target plants. Significance values are for Kruskal–Wallis one-way
 736 comparisons between ABA-cued plants and their uncued controls, ** $P < 0.01$, ***
 737 $P < 0.001$, $n = 25$.

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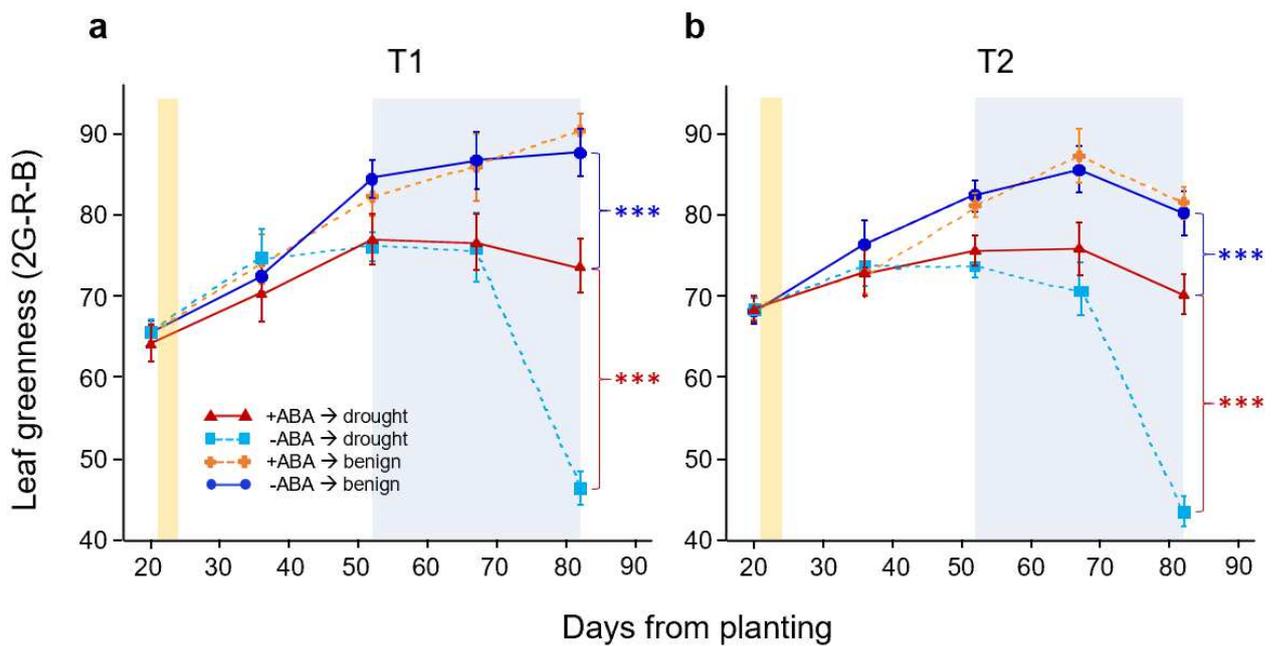


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741 Figure 7: **the effect of ABA cuing on leaf color of *P. sativum* under drought.** Triplets of split-
 742 root *P. sativum* plants were grown in rows as described in Fig. 1. One of the roots of the IND
 743 plant (pot 1) was either treated or untreated (control) with ABA for three days (yellow
 744 background) after which the IND plant was removed and the target plants were subjected to
 745 benign conditions or to drought (no background) and to a subsequent wet 'revival' period
 746 (blue background). Values are for leaf greenness (2G-R-B) of directly-cued (T1; **a**) and
 747 relayed-cued (T2; **b**) target plants. Significance values are for one-way ANOVA comparisons
 748 at day 82, *** $P < 0.001$, $n = 25$.

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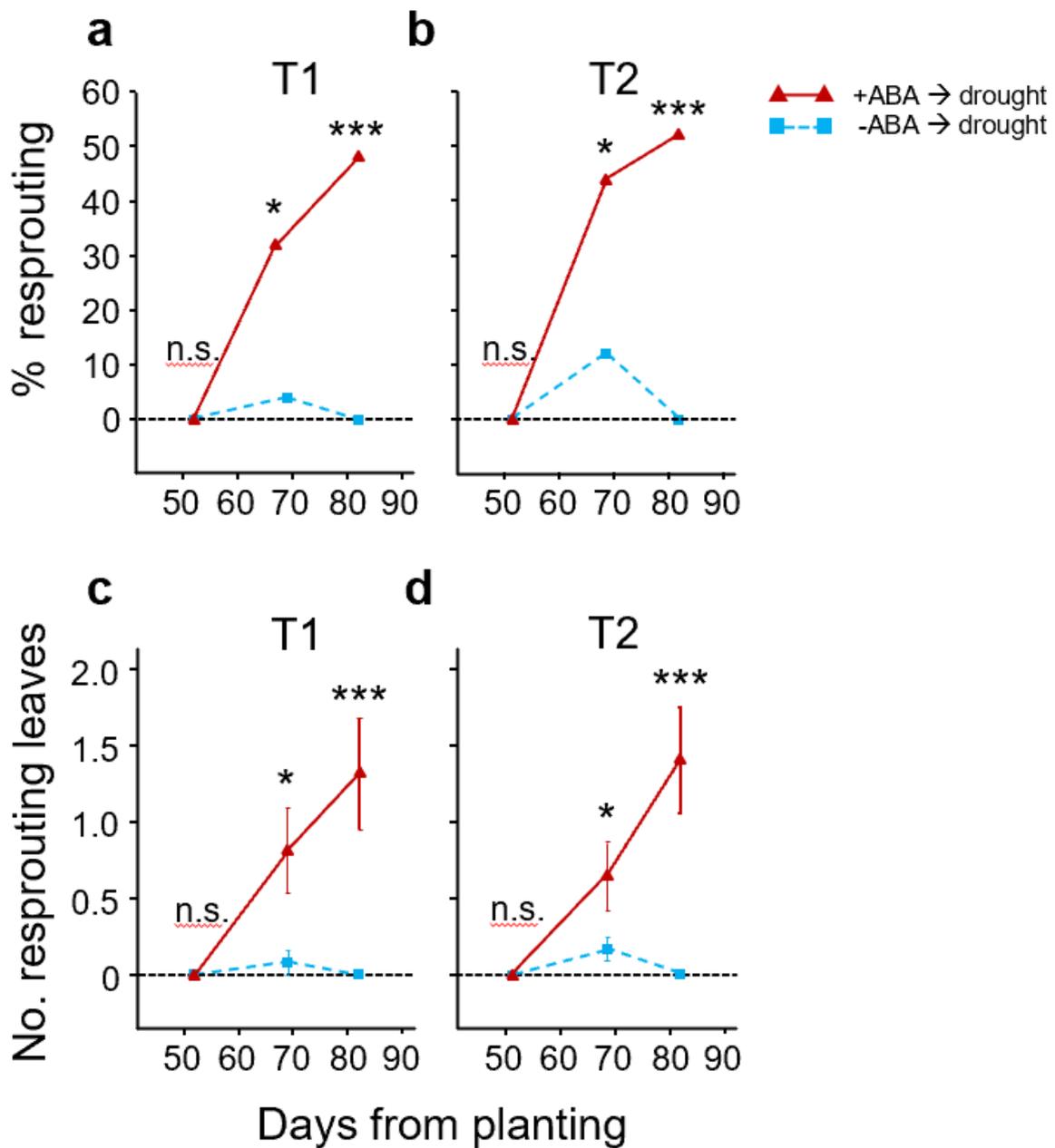


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751

752 Figure 8: **the effect of ABA cuing on post-drought revival in *P. sativum***. Triplets of split-root
 753 *P. sativum* plants were grown in rows as described in Fig. 1. One of the roots of the IND
 754 plant (pot 1) was either treated or untreated (control) with ABA for three days after which
 755 the IND plant was removed and the target plants were subjected to a drought period and to
 756 a subsequent wet 'revival' period. Values are for percentage of resprouting (**a, b**) and for
 757 means \pm SEM of number of sprouting leaves per plant (**c, d**) of directly-cued (T1- **a, c**) and
 758 relayed-cued (T2- **b, d**) plants. Significance values are for G-test (**a, b**) and Kruskal–Wallis
 759 one-way comparisons (**c, d**) between ABA-cued plants and their uncued controls, ***
 760 $P < 0.001$, $n = 25$.

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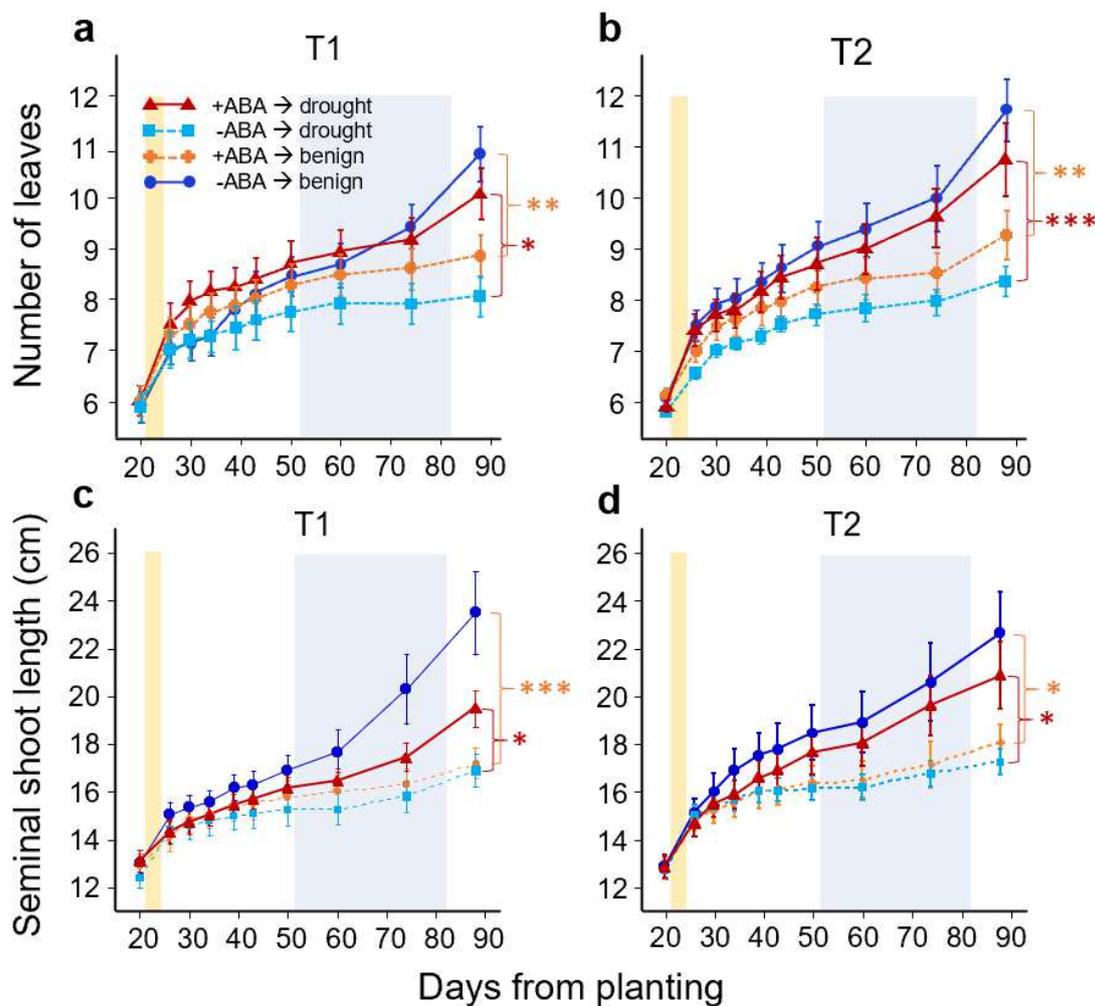


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763

764 Figure 9: **the effect of ABA cuing on number of leaves and shoot length of *P. sativum***
 765 **under drought.** Triplets of split-root *P. sativum* plants were grown in rows as described in
 766 Fig. 1. One of the roots of the IND plant (pot 1) was either treated or untreated (control)
 767 with ABA for three days (yellow background) after which the IND plant was removed and
 768 the target plants were subjected to benign conditions or to a drought period (no
 769 background) and to a subsequent wet 'revival' period (blue background). Values are for
 770 means \pm SEM of number of leaves (**a, b**) and length of seminal shoot (**c, d**) of directly-cued
 771 (T1- **a, c**) and relayed-cued (T2- **b, d**) target plants. Significance values are for one-way
 772 ANOVA comparisons at harvest, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n = 25$.

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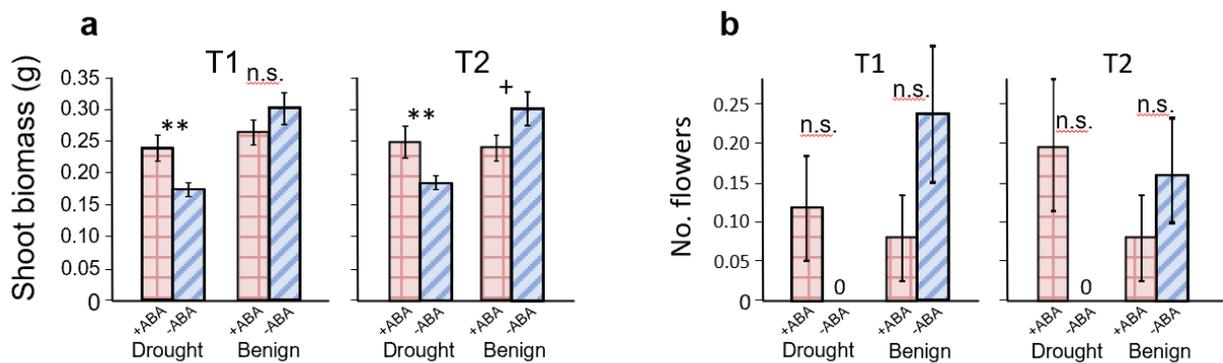
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777 Figure 10: **the effect of ABA cuing on shoot biomass and number of flowers of *P. sativum***
 778 **under drought.** Triplets of split-root *P. sativum* plants were grown in rows as described in
 779 Fig. 1. One of the roots of the IND plant (pot 1) was either treated or untreated (control)
 780 with ABA for three days after which the IND plant was removed and the target plants were
 781 subjected to benign conditions or to a drought period and to a subsequent wet 'revival'
 782 period. Values are for means \pm SEM of shoot biomass (a) and number of flowers (b) of
 783 directly-cued (T1) and relayed-cued (T2) target plants at harvest. Significance values are for
 784 one-way ANOVA (a) and Kruskal–Wallis one-way comparisons (b) at harvest between ABA-
 785 cued plants and their uncued controls, + 0.1 < *P* < 0.05, ** *P* < 0.01, *n* = 25.

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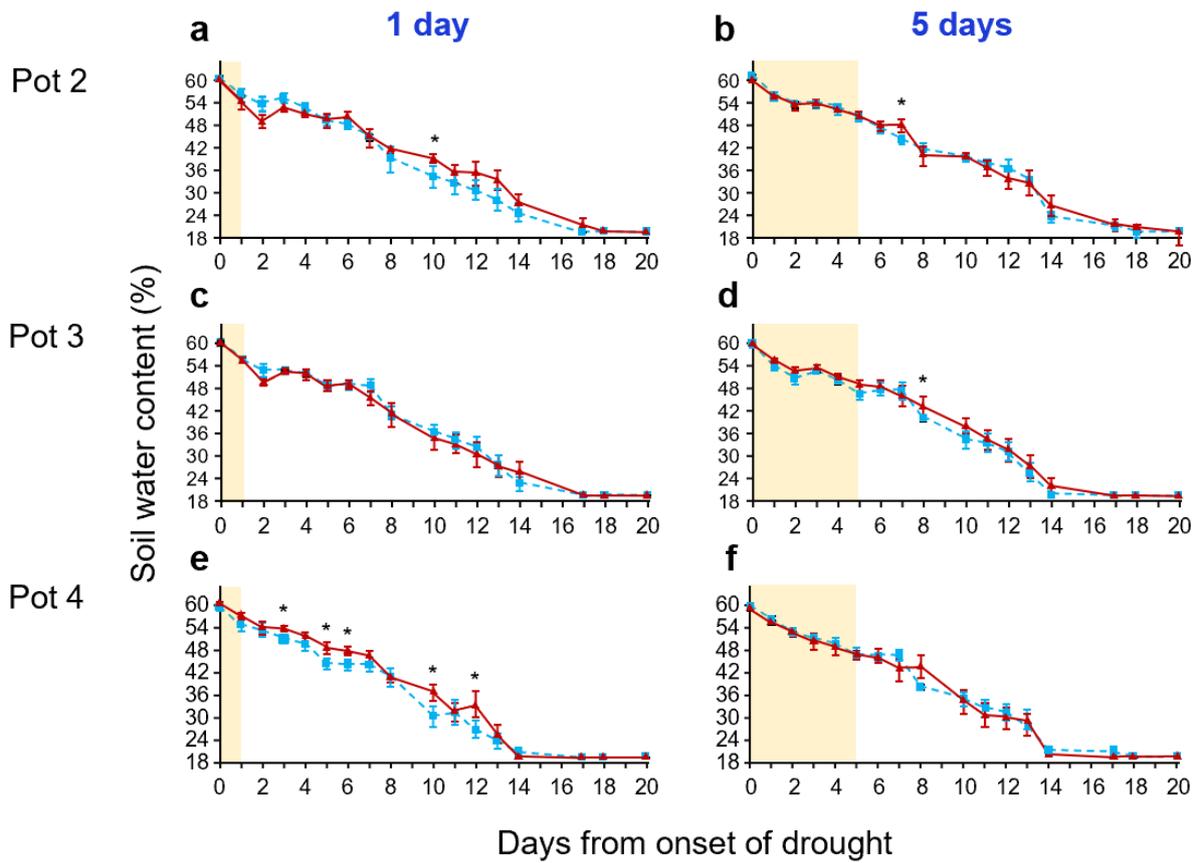


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789 Supplementary figure 1: **testing for the ecological implications of drought cuing in *S.***
 790 ***secundatum* – soil water content.** Soil water content was measured using time domain
 791 reflectometry (see methods). Plants were subjected to one or five days (yellow
 792 background) of either drought or benign conditions. Significance indications are for paired t-
 793 tests for the differences between drought (red) and benign (blue) conditions in each day, *
 794 $P < 0.05$, (n = 10).

795

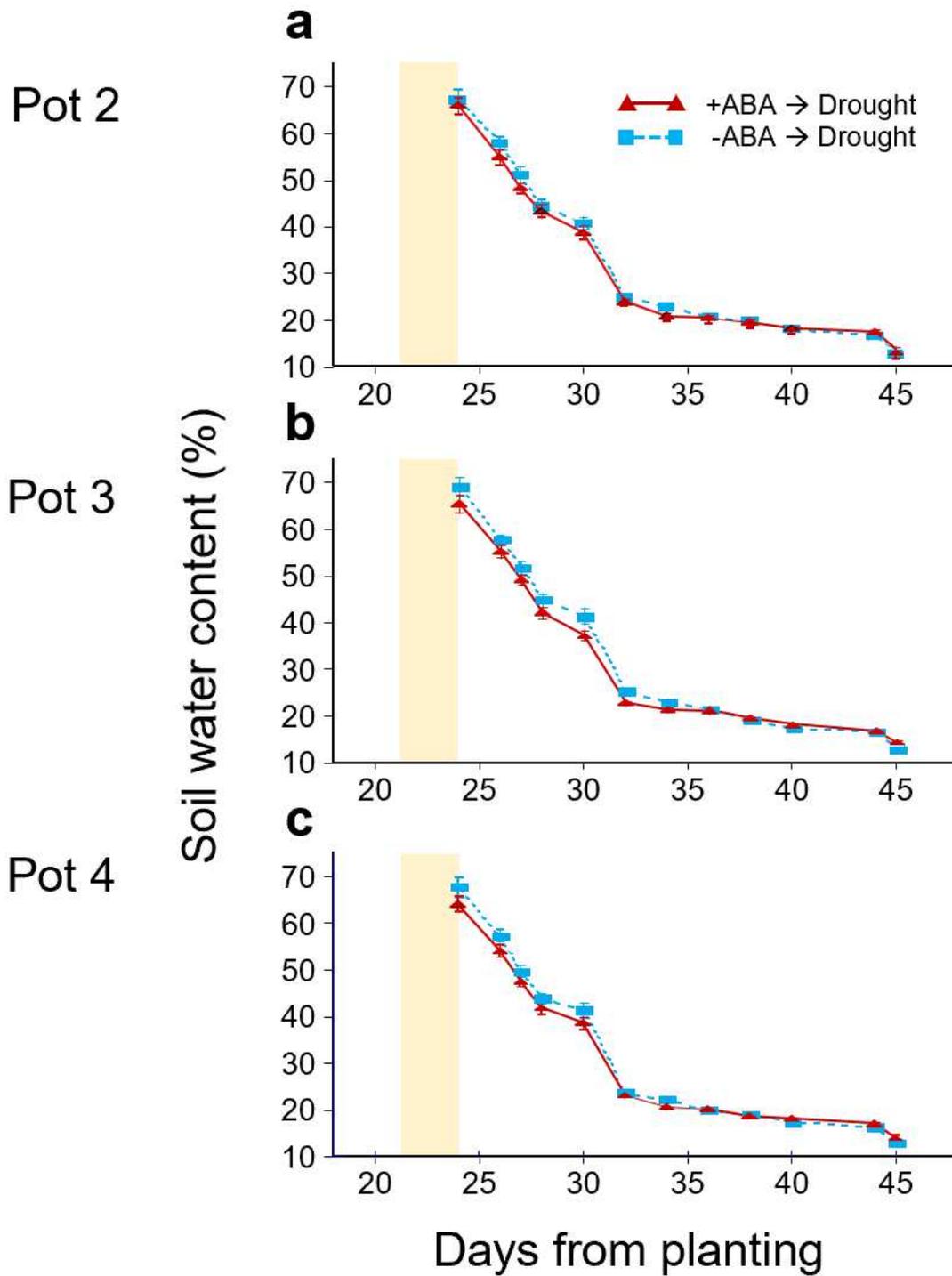


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798 Supplementary figure 2: **testing for the effects of ABA cuing on the survival and**
799 **performance of *P. sativum* under drought – soil water content.** Soil water content was
800 measured using time domain reflectometry (see methods). Plants were subjected to three
801 days (yellow background) of ABA cuing (red) or control treatments (blue), (n = 10).

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Figures

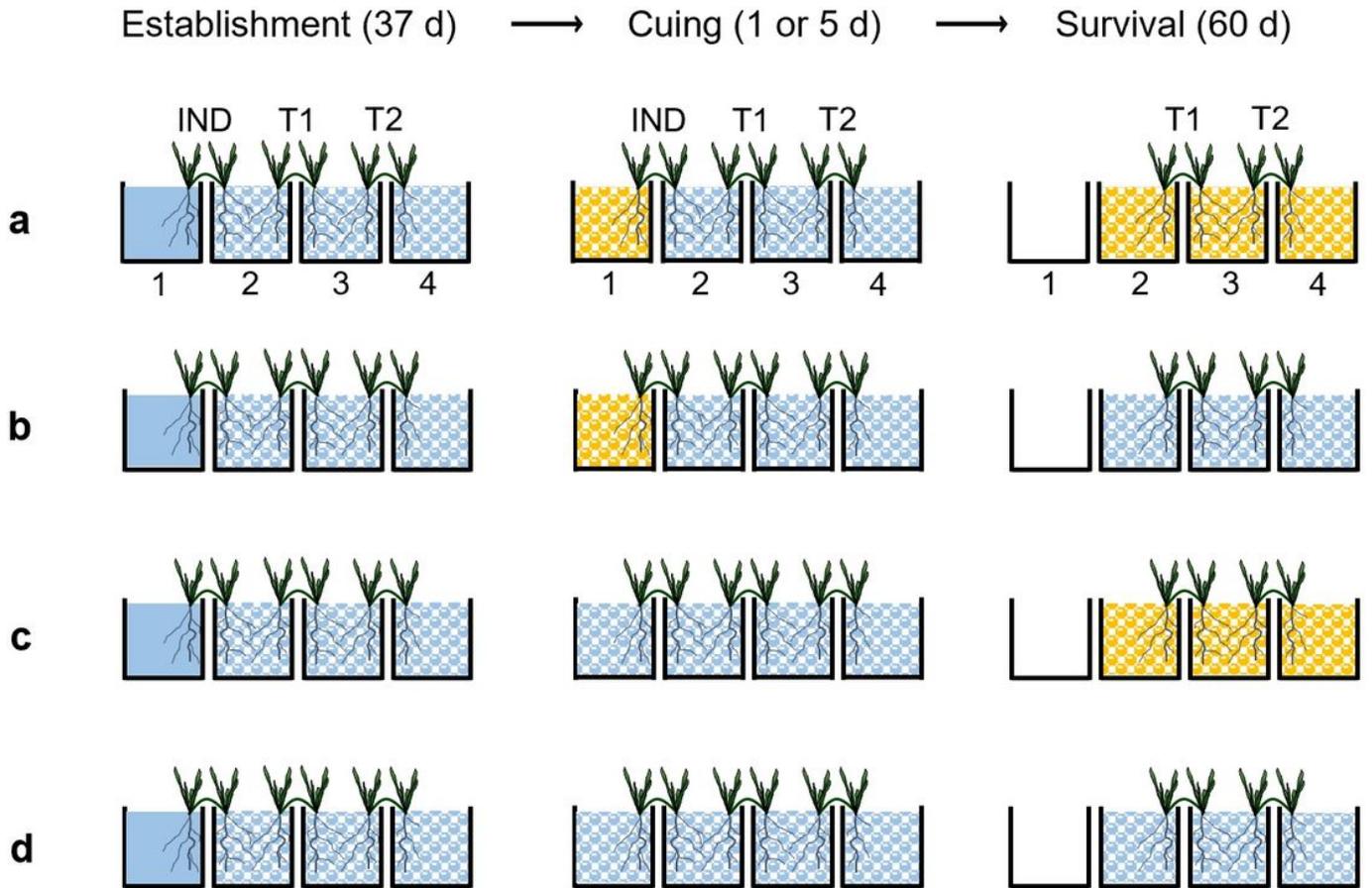


Figure 1

Testing for the ecological implications of drought cuing in *S. secundatum* – experimental setup. Triplets of two-ramet plants were planted in rows of four pots. One of the roots of the IND plant (pot 1) was subjected to either drought (yellow) or benign (blue) conditions while its other root shared pot 2 with one of the roots of its nearest unstressed neighbor (T1). The other root of T1 shared pot 3 with one of the roots of an additional unstressed target plant (T2). This configuration permitted T1 to exchange stress cues with both IND and T2, while preventing direct root cuing between IND and T2, thus allowing to separately study the effects of direct and relayed drought cuing on T1 and T2, respectively. Following an establishment period, one of the roots of the IND plant (pot 1) was subjected to either drought (a, b) or benign (c, d) conditions for one or five days after which the IND plant was removed (white) and the target plants were subjected to either benign (b, d; blue) or drought (a, c; yellow) conditions.

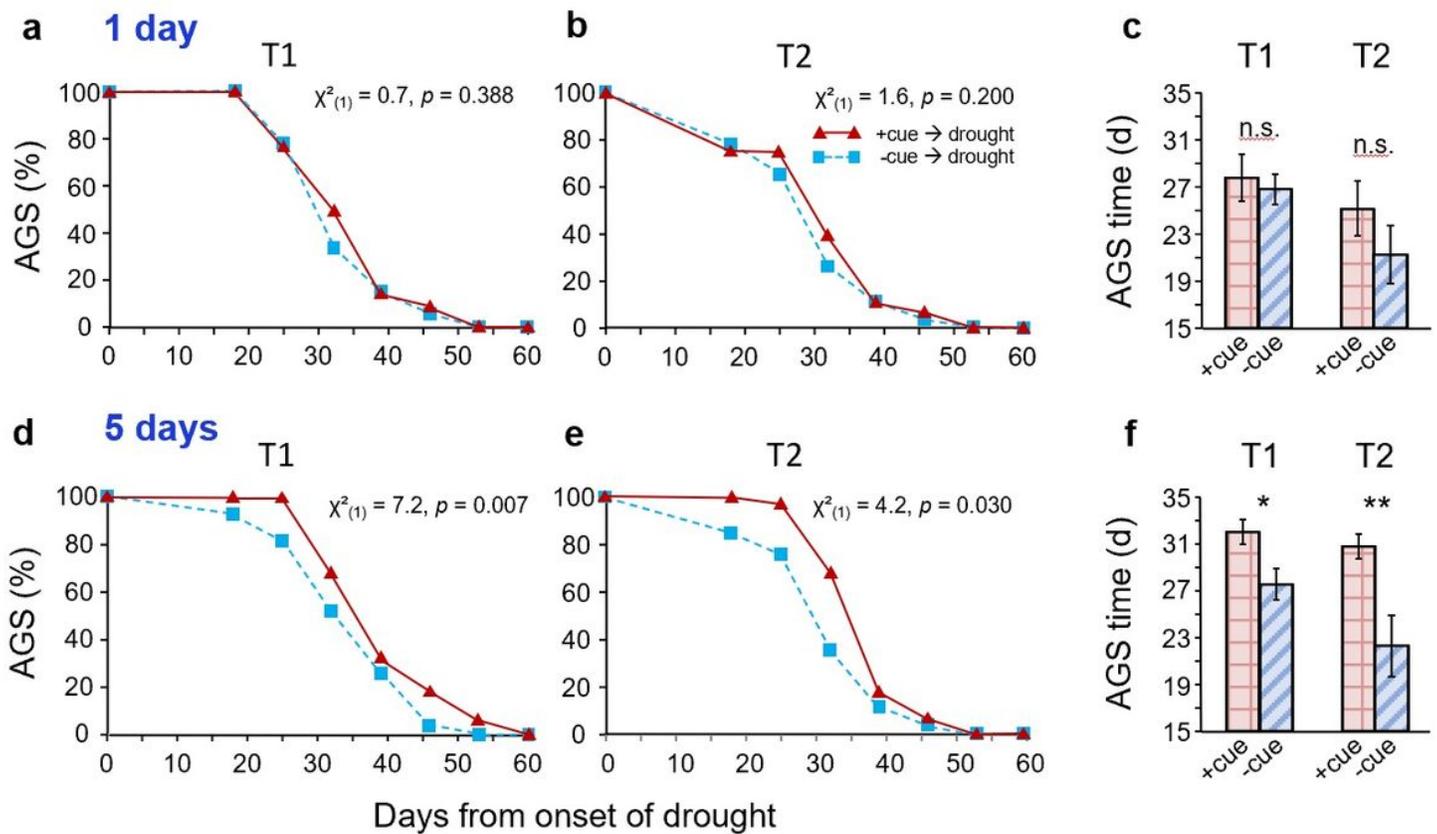


Figure 2

the effects of drought cuing on survival of *S. secundatum* under drought. Values are for aboveground survival (AGS) kinetics (a, b, d, e) and for means \pm SEM of AGS survival time (c, f) of directly-cued (T1) (a, b) and relayed-cued (T2) (d, e) plants. Drought-cuing and control treatments are indicated in cyan and red, respectively. Analyses are for Kaplan-Meier log rank tests (a, b, d, e) and one-way ANOVAs (c, f), between target plants that either received or not received drought cues ($n = 25$).

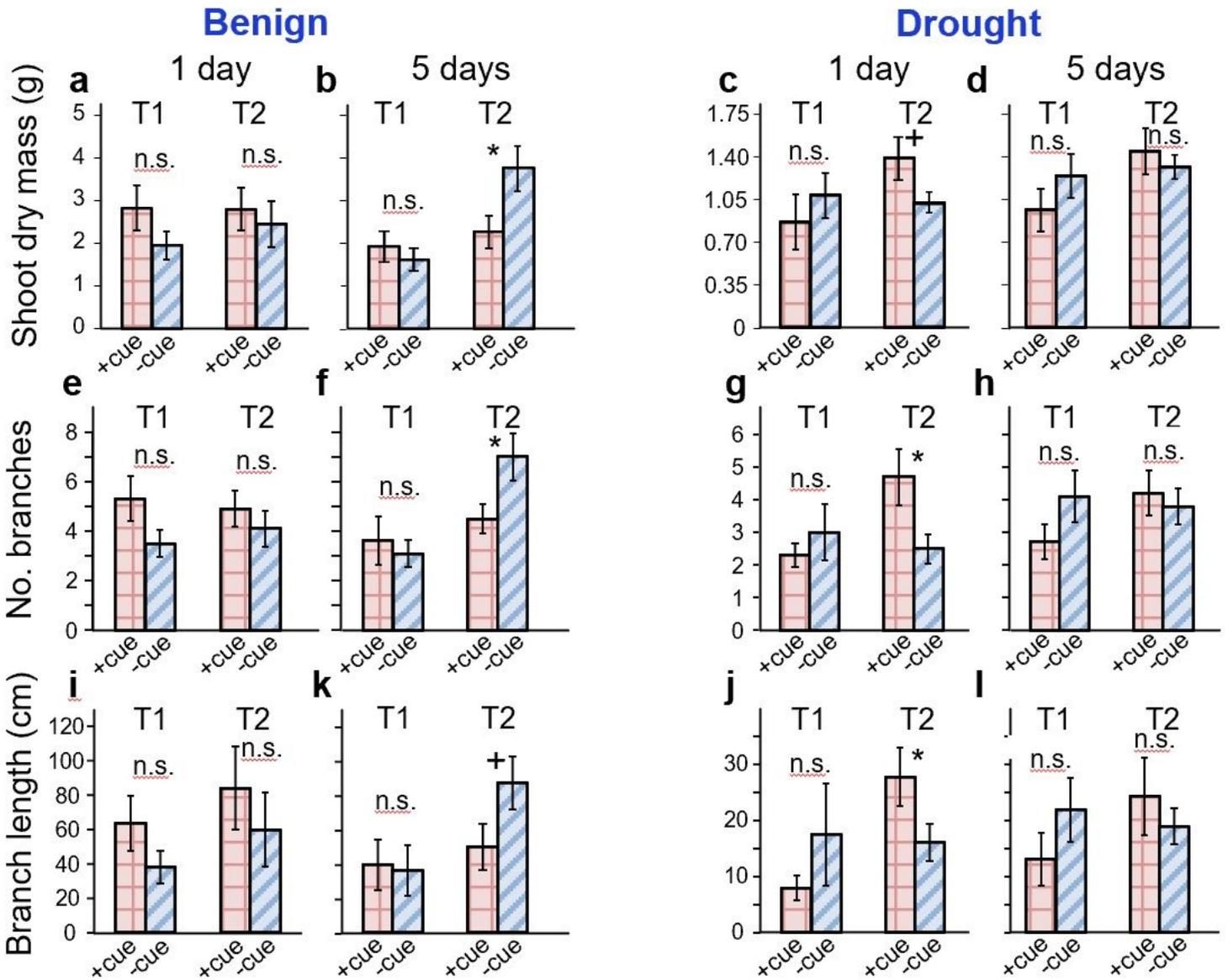


Figure 3

the effects of drought cuing on growth of *S. secundatum* under drought. Values are means \pm SEM of shoot dry biomass (a-d), Number of branches (e-h) and branch length (i-l) of directly cued (T1) or relayed-cued (T2) plants. Significance values are for one-way ANOVA comparisons between target plants that either received (red) or not received (blue) drought cues, $0.1 < P < 0.05$, $* P < 0.05$, (n = 25).

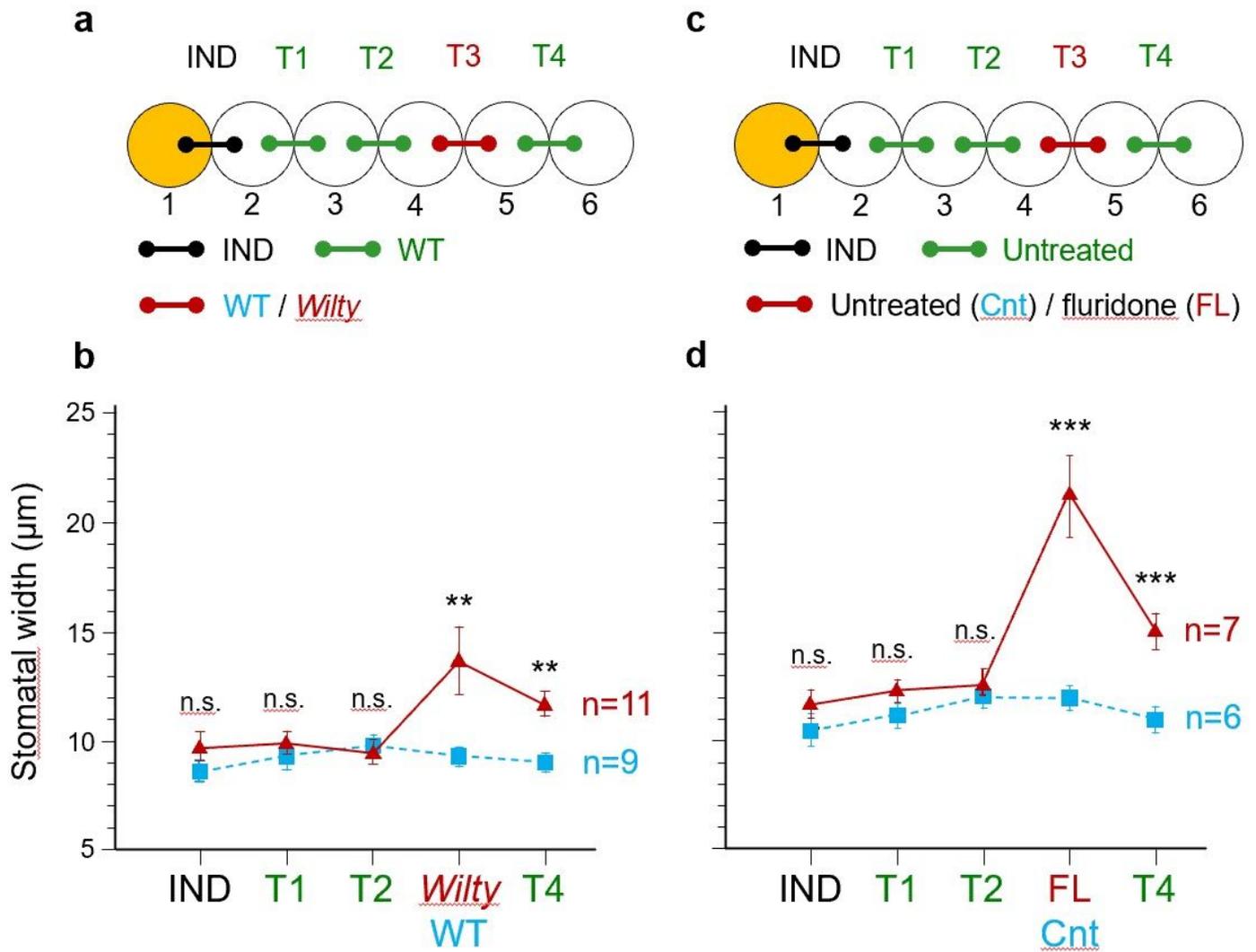


Figure 4

the effect of ABA deficiency on interplant drought cuing. Sets of five split-root *P. sativum* plants were planted in a row. One of the roots of the IND plant (pot 1) was subjected to drought conditions for one hour while its other root shared pot 2 with one of the roots of its nearest unstressed neighbor (T1). The other root of T1 shared pot 3 with one of the roots of an additional unstressed target plant (T2) etc. In one experiment (a, b), the third target plant (T3) was either a wild-type *Pisum sativum* (WT) or a *Wilty* mutant plant. In another experiment (c, d), the T3 plant was either untreated (Cnt) or treated with fluridone (FL). Values (b, d) are means \pm SEM of stomatal width in plant rows including either WT (b) / control (d) plants (cyan) or *Wilty* (b) / fluridone-treated (d) plants (red). Significance values are for one-way ANOVA comparisons between the *Wilty* (b) and fluridone (d) treatments and their controls, ** $P < 0.01$, *** $P < 0.001$.

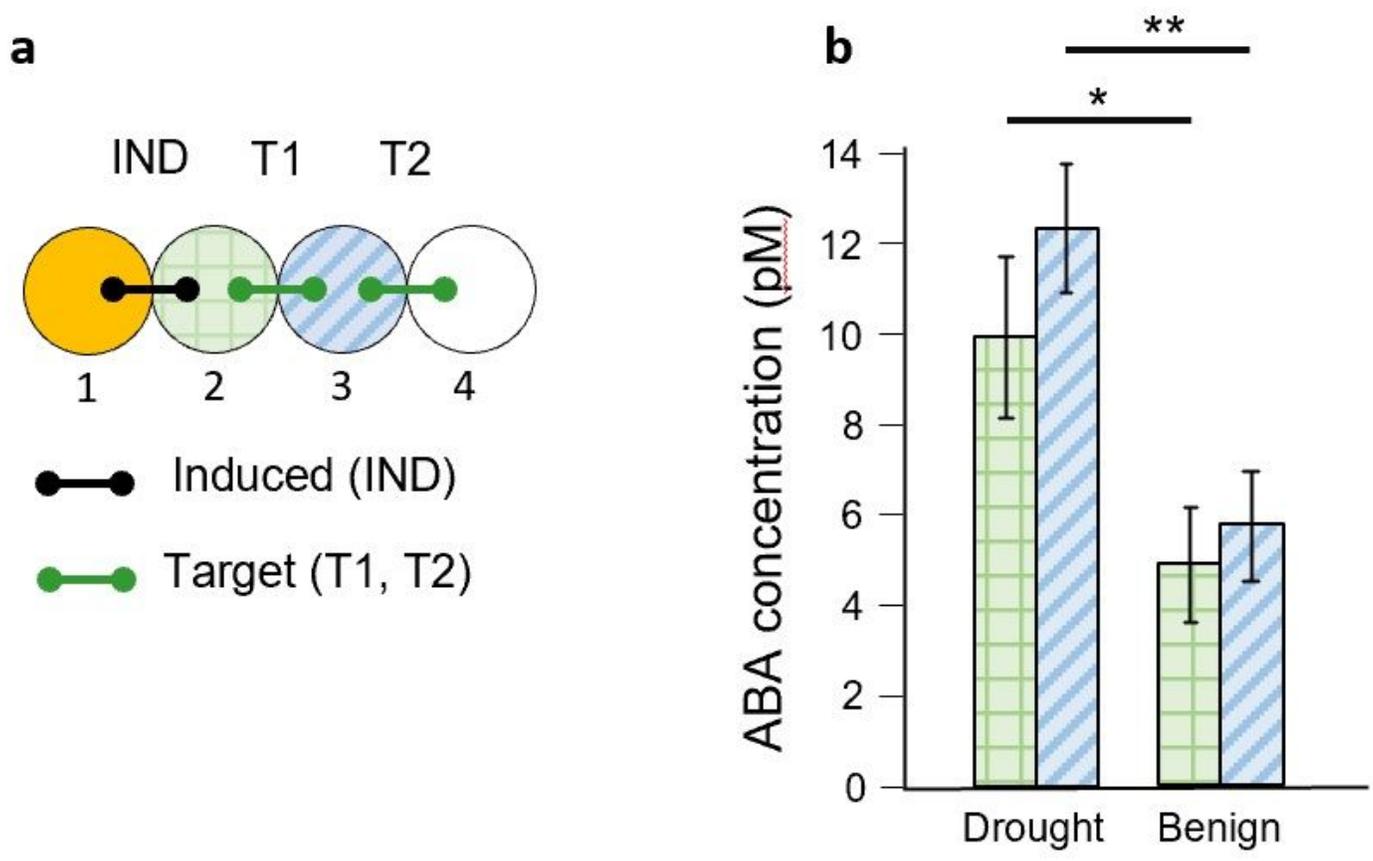


Figure 5

the effects of drought cuing on rhizosphere ABA concentration. Triplets of split-root *P. sativum* plants were grown in rows as described in Fig. 1 (a). One of the roots of the IND plant (pot 1, orange) was subjected to either drought or benign conditions for one hour. ABA concentration was analyzed in the rhizospheric solution of pot 2 (shared by drought-treated IND and its unstressed T1 neighbor, green) and pot 3 (shared by unstressed T1 and T2 plants, blue). Values are means \pm SEM of ABA concentrations in pot 2 (green) and pot 3 (blue). Significance values are for Kruskal–Wallis one-way comparisons between drought-cued plants and their controls, * $P < 0.05$, *** $P < 0.01$, $n = 6$.

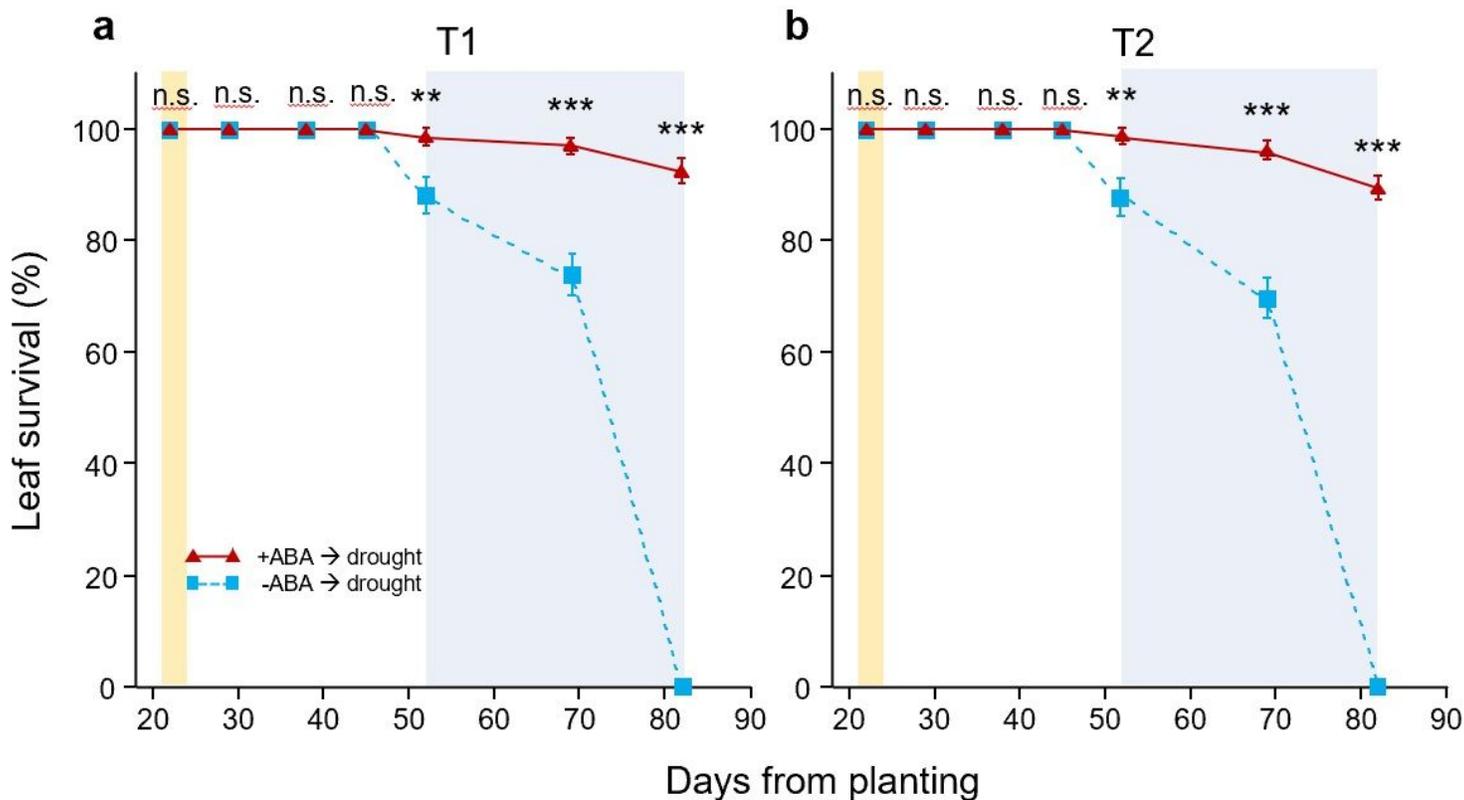


Figure 6

the effect of ABA cuing on leaf survival of *P. sativum* under drought. Triplets of split-root *P. sativum* plants were grown in rows as described in Fig. 1. One of the roots of 7th IND plant (pot 1) was either treated or untreated (control) with ABA for three days (yellow background) after which the IND plant was removed and the target plants were subjected to a drought period (no background) and to a subsequent wet 'revival' period (blue background). Results are for percentage leaf survival of directly-cued (T1; a) and relayed-cued (T2; b) target plants. Significance values are for Kruskal–Wallis one-way comparisons between ABA-cued plants and their uncued controls, ** $P < 0.01$, *** $P < 0.001$, $n = 25$.

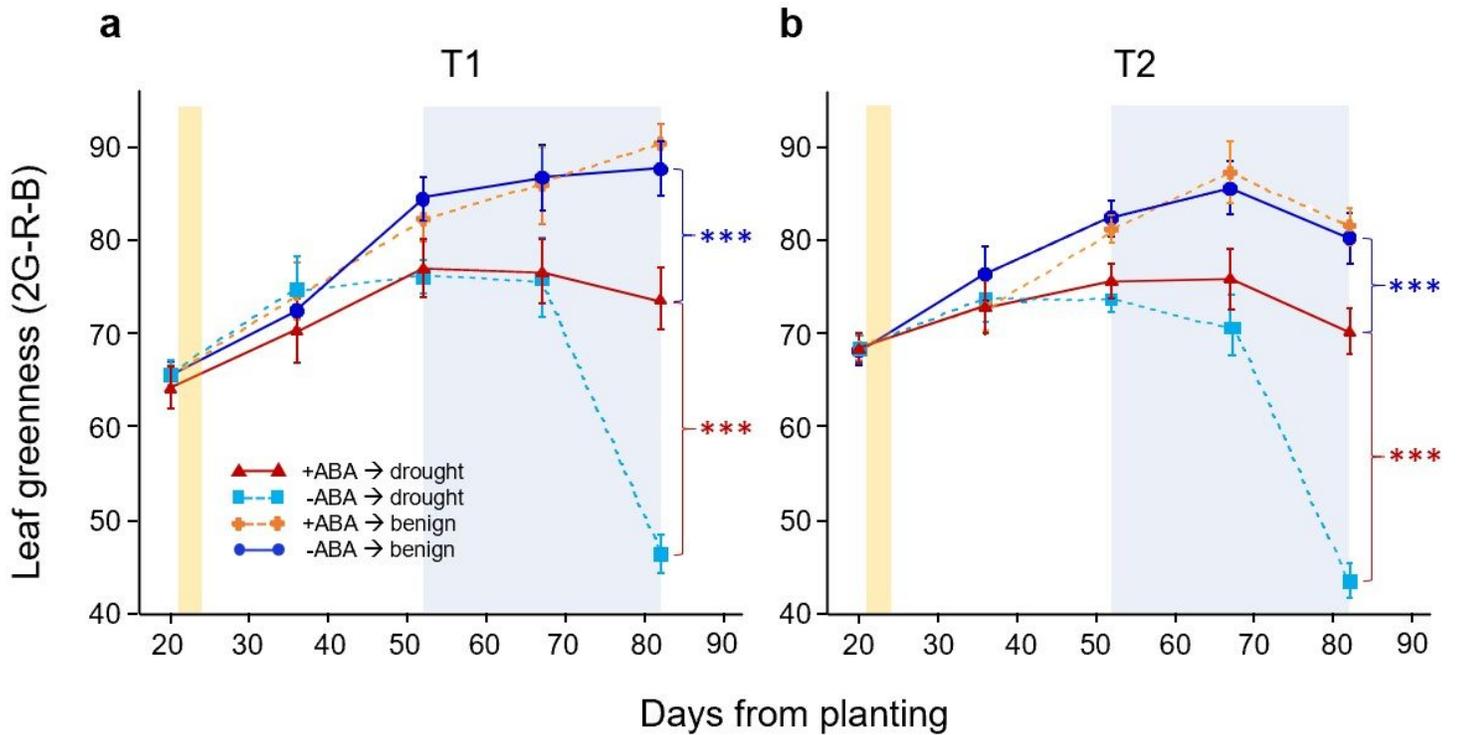


Figure 7

the effect of ABA cuing on leaf color of *P. sativum* under drought. Triplets of split-root *P. sativum* plants were grown in rows as described in Fig. 1. One of the roots of the IND plant (pot 1) was either treated or untreated (control) with ABA for three days (yellow background) after which the IND plant was removed and the target plants were subjected to benign conditions or to drought (no background) and to a subsequent wet 'revival' period (blue background). Values are for leaf greenness (2G-R-B) of directly-cued (T1; a) and relayed-cued (T2; b) target plants. Significance values are for one-way ANOVA comparisons at day 82, *** $P < 0.001$, $n = 25$.

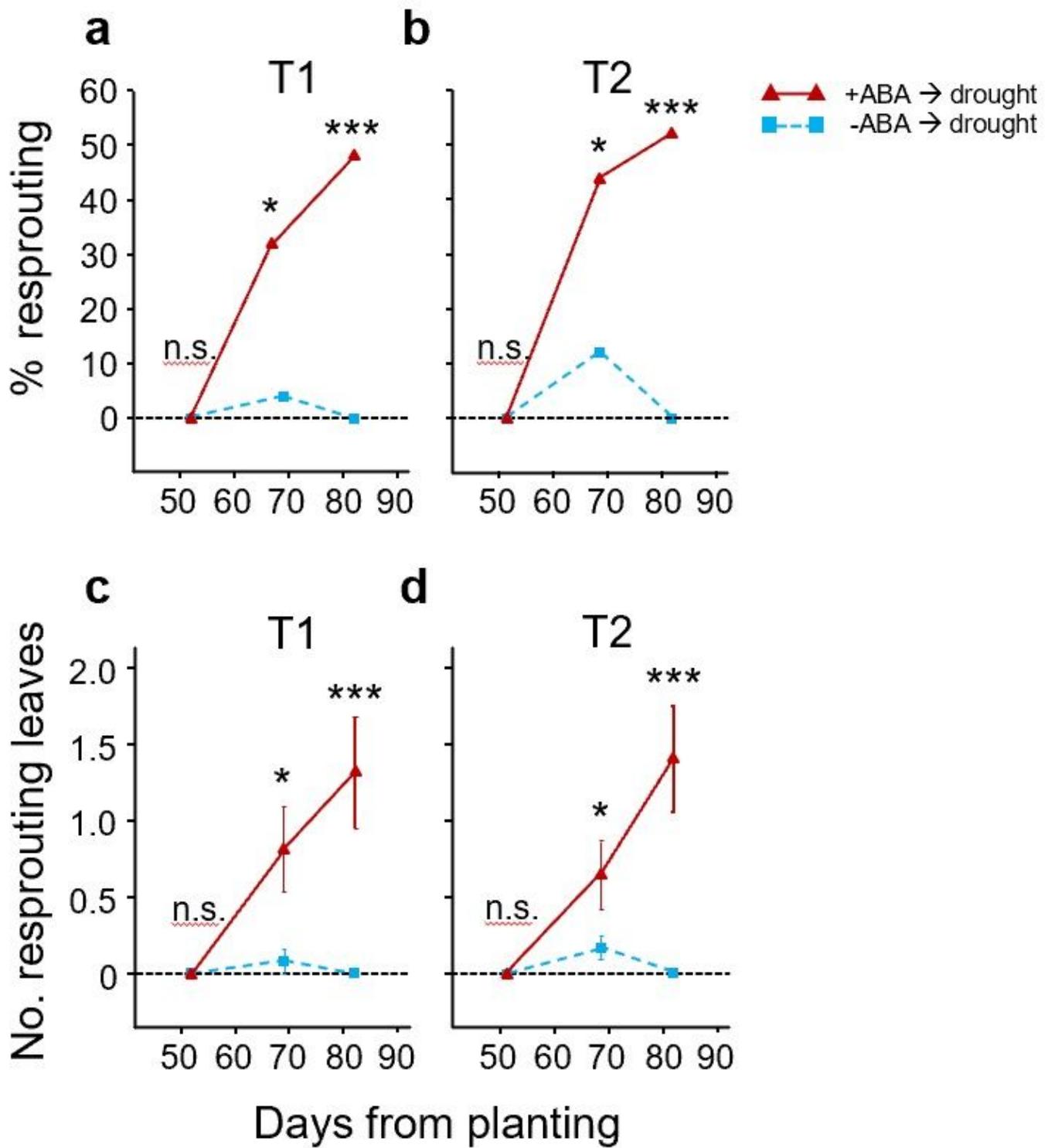


Figure 8

the effect of ABA cuing on post-drought revival in *P. sativum*. Triplets of split-root *P. sativum* plants were grown in rows as described in Fig. 1. One of the roots of the IND plant (pot 1) was either treated or untreated (control) with ABA for three days after which the IND plant was removed and the target plants were subjected to a drought period and to a subsequent wet 'revival' period. Values are for percentage of resprouting (a, b) and for means \pm SEM of number of sprouting leaves per plant (c, d) of directly-cued (T1

- a, c) and relayed-cued (T2 - b, d) plants. Significance values are for G-test (a, b) and Kruskal–Wallis one-way comparisons (c, d) between ABA-cued plants and their uncued controls, *** P < 0.001, n = 25.

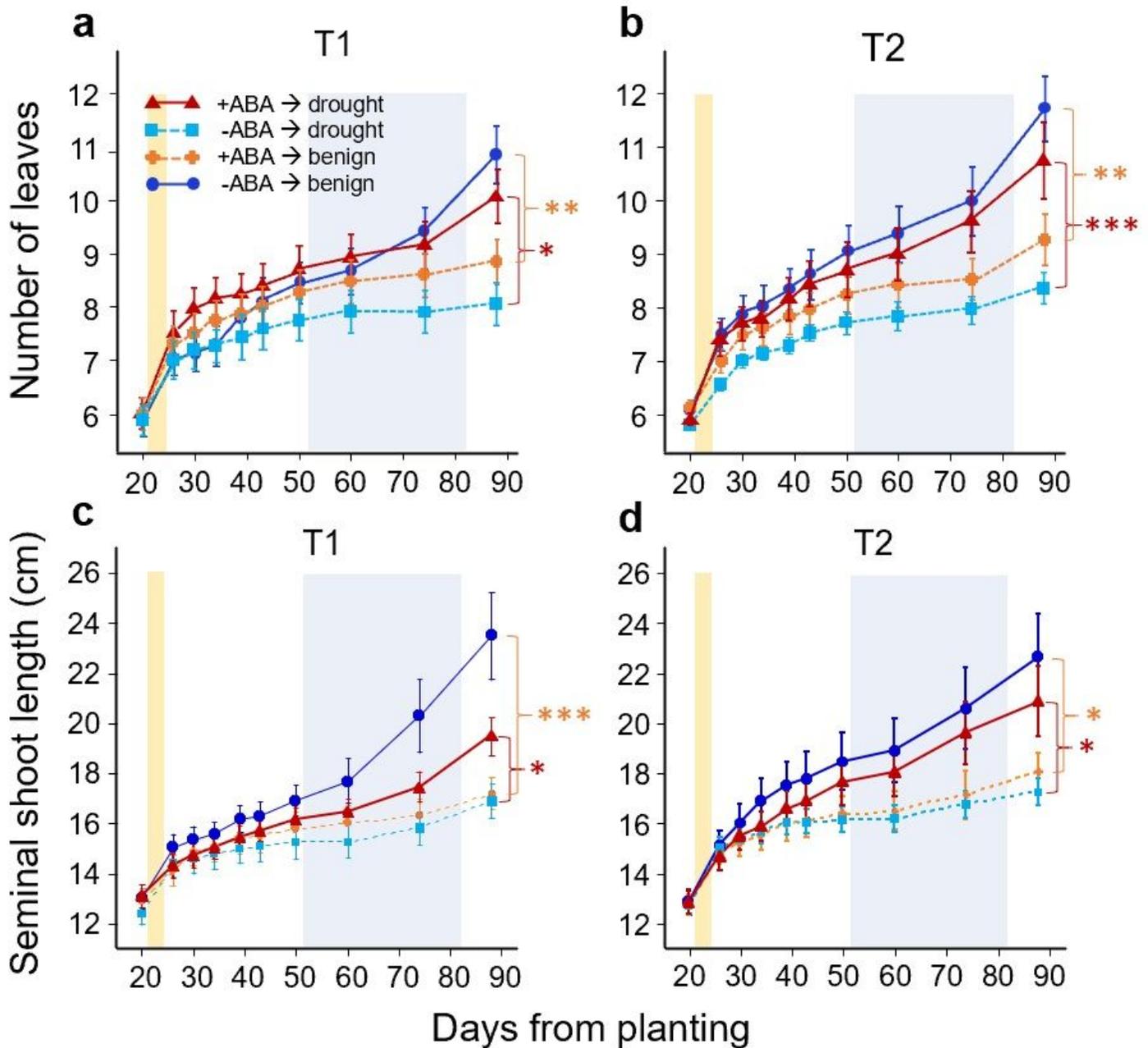


Figure 9

the effect of ABA cuing on number of leaves and shoot length of *P. sativum* under drought. Triplets of split-root *P. sativum* plants were grown in rows as described in Fig. 1. One of the roots of the IND plant (pot 1) was either treated or untreated (control) with ABA for three days (yellow background) after which the IND plant was removed and the target plants were subjected to benign conditions or to a drought period (no background) and to a subsequent wet 'revival' period (blue background). Values are for means \pm SEM of number of leaves (a, b) and length of seminal shoot (c, d) of directly-cued (T1- a, c) and relayed-

cued (T2- b, d) target plants. Significance values are for one-way ANOVA comparisons at harvest, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n = 25$.

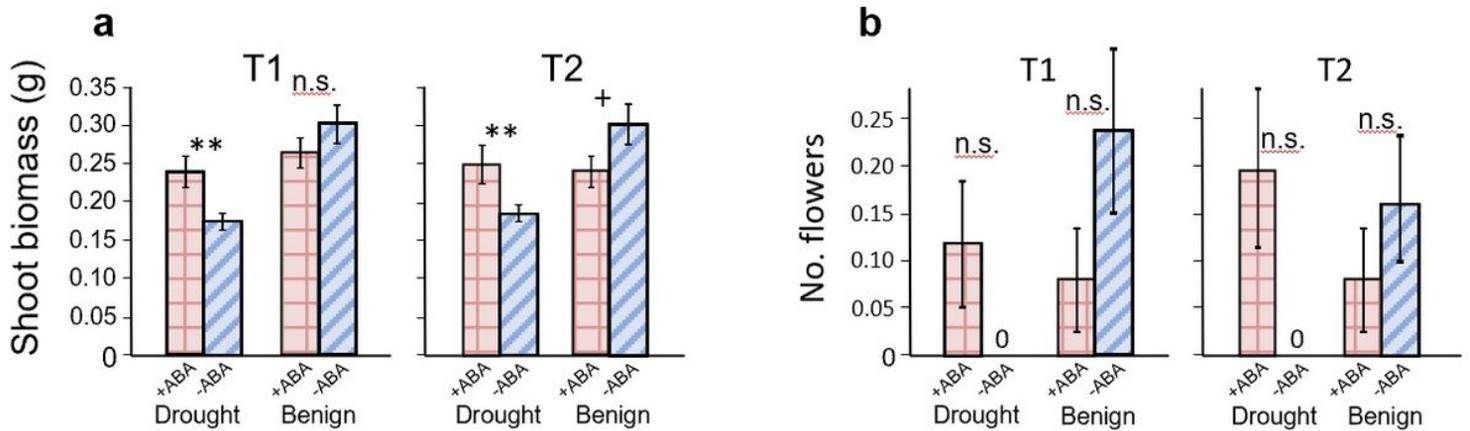


Figure 10

the effect of ABA cuing on shoot biomass and number of flowers of *P. sativum* under drought. Triplets of split-root *P. sativum* plants were grown in rows as described in Fig. 1. One of the roots of the IND plant (pot 1) was either treated or untreated (control) with ABA for three days after which the IND plant was removed and the target plants were subjected to benign conditions or to a drought period and to a subsequent wet 'revival' period. Values are for means \pm SEM of shoot biomass (a) and number of flowers (b) of directly-cued (T1) and relayed-cued (T2) target plants at harvest. Significance values are for one-way ANOVA (a) and Kruskal–Wallis one-way comparisons (b) at harvest between ABA- cued plants and their uncued controls, + $0.1 < P < 0.05$, ** $P < 0.01$, $n = 25$.