



Short communication

Is the zone of influence colonized by roots of neighboring species? Field tests in a Patagonian steppe



M. Fernanda Reyes*, Martín R. Aguiar

IFEVA, Facultad de Agronomía, Universidad de Buenos Aires, CONICET, San Martín 4453, Buenos Aires C1417DSE, Argentina

ARTICLE INFO

Article history:

Received 15 January 2016

Received in revised form

22 July 2016

Accepted 28 October 2016

Keywords:

Belowground biomass

Belowground relative growth rate

Plant territoriality

Root segregation

Spatial distribution of roots

ABSTRACT

In general, there is more knowledge about aboveground structure and processes than about those belowground. It has been proposed that plants are belowground territorial and root segregation explains species coexistence. We explored this idea by studying root intermingling of perennial graminoid plants in an arid Patagonian steppe. We extracted soil samples under target species. Additionally, we buried root traps filled with sieved soil to quantify root colonization close to plants (zone of high influence), of five dominant graminoid species. We sorted root biomass by species from samples and traps using a root-trait taxonomic key with 95% accuracy. Both studies indicated that the zone of high influence is occupied by roots of target and neighbor species (1–4 species) but showed differences in relative biomass of species. After 4 months, species with high root RGR show dominance in traps. This soil pre-emption seems transitory because in the descriptive study dominant species predominated independently of target species. We propose that coexistence among active roots of several species could be explained by different soil occupation rates in the zone of high influence. More studies are needed in relation with resource use in order to comprehend relationship between above and belowground biomass distributions.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Results from field studies with tracer or molecular methods support the notion that in grasslands, root systems from different herbaceous species overlap in the same soil volume (de Kroon and Mommer, 2006; Frank et al., 2010; de Kroon et al., 2012). Here we further explore this pattern of root overlapping by studying how root biomass of several species is able to invade the soil directly below or close to a plant under natural conditions. This volume of soil has been defined as the zone of influence (Casper et al., 2003).

We tested if there are specific differences in the ability to maintain the exclusive use of the soil volume. A high relative growth rate (hereafter RGR) could generate a high effect over resource availability. Then its neighbor could be more affected on their development, providing evidence of the competitive advantage of the species with the high RGR (Poorter, 1989). We proposed the hypothesis that species have territorial behavior (*sensu* Schenk et al., 1999) and therefore there is root segregation among neighbor

plants. Alternatively, because in the steppe there are no absolute dominant species, we expected that all species have the capacity to invade the zone of high influence according to their RGR. We proposed that target species with high RGR are dominant in the soil volume under themselves. Additionally, roots of species with high RGR (functioning as neighbors) are able to invade the soil volume under species with low RGR. To study these hypotheses we measured specific root biomass of grasses under target plants and we also set up a root traps experiment that lasted 4 months to estimate RGR and measure fine alive roots.

2. Materials and methods

2.1. Study site and grass species

The study was conducted in the semi-arid Patagonian steppe ecosystem (45° 25'S, 70° 20'W). The climate is cold arid, with an intense summer drought. Mean annual precipitation is 131 ± 40 mm (mean \pm SD) and most precipitation occurs during winter and early spring (May–September) (INTA, 2015). The study site is characterized by low vegetation density (48% plant cover) and species richness (26 species) (Golluscio et al., 1982). It is

* Corresponding author. Tel.: +54 011 4524800(8114), fax: +54 011 4524800(8120)

E-mail address: freyes@agro.uba.ar (M.F. Reyes).

dominated by perennial grasses, since shrubs only cover 18% of total surface. The grass community includes eight perennial-graminoid species, the five of them are common in domestic grazing exclusion (Oñatibia 2009): *Pappostipa speciosa* (Trin. & Rupr.) Romasch ($2950 \pm 50 \text{ kg ha}^{-1}$); *Pappostipa humilis* (Cav.) Romasch ($600 \pm 150 \text{ kg ha}^{-1}$); *Poa ligularis* Nees ex Steud ($2050 \pm 30 \text{ kg ha}^{-1}$); *Bromus pictus* Hook ($200 \pm 30 \text{ kg ha}^{-1}$), all Poaceae species, and *Carex* sp. ($58 \pm 12 \text{ kg ha}^{-1}$), Cyperaceae species, and other three with less than 3% frequency in the field: *Bromus setifolius* J. Presl *setifolius* (Hook. f.) Skottsb; *Festuca argentina* (Speg.) Parodi and *Hordeum comosum* J. Presl, all Poaceae species.

2.2. Test of taxonomic key

Leva et al. (2009) built a taxonomic key using the main features of root harvested in the field, such as: diameter, color and hairiness, including all the graminoid species (Supplementary material, FigA). We tested the accuracy of this taxonomic key. We composed root samples with known composition and specific biomass. In the study site, we collected whole plants of the eight graminoid species mentioned in the previous section. In the lab, plants were taxonomically identified and grouped by species. Only root attached to plants were used, to be sure about the identity of them. Not attached roots were discarded. Using identified roots, 20 samples were prepared with 2–5 species. Samples were prepared following aboveground species field cover. The total biomass per sample was around 0.1 g. A member of our team (MFR) processed blind samples using the taxonomic key to sort species biomass. After being sorted by species, roots were weighed. We calculated the relative difference between specific root biomass in the sample and biomass recovered after sorting. Differences were expressed as %. We performed simple regression analysis for specific biomass.

2.3. Field experimental designs

We conducted two field studies. The first one was descriptive to define the relation between target species and root biomass under their canopy and the second one was a manipulative study with root traps.

2.3.1. Descriptive study

We took soil samples from four different locations (grazing enclosures) spread over a 150 km² area. The samples were taken every 2 m along a 50 m transect, with a soil corer (6 cm of diameter and 20 cm of depth). Here we only presented specific root biomass of samples that fall under individual plants, not associated with shrubs. ANOVA tests were used to examine the effects of target and neighboring species on specific and total root biomass ($n = 4$). A post-hoc test (Tukey) was used to identify homogeneous groups when a variable showed significant differences.

2.3.2. Root trap study

We set root traps in the proximity of individual plants of five species commonly present in grazing enclosure: *Bromus pictus*, *Poa ligularis*, *Carex* sp., *Pappostipa speciosa* and *Pappostipa humilis* (according to decreasing belowground RGR- Leva et al., 2009). We selected individual plants of the five species (target plants) growing isolated from neighbor plant (the nearest was >15 cm away). One root trap filled with sieved soil was buried 4 cm from the base of each target plant (following Graff et al., 2007), to generate a soil free of roots in a zone of high influence of the plant. We defined the distance from target plant based on the findings of Casper et al. (2003). We set root traps at two depths: 5–11 cm and 15–21 cm. Each target plant had either one or the other depth. The experiment

had a factorial design with two factors: target species (5 levels) and depth (2 levels), with 10 replicates for each combination.

The root trap consisted of a plastic tube with 10 mm² perforations all along and around, 4 cm of diameter and 6 cm of high (commercially a hair curler, Supplementary material, FigB). Traps allowed colonization by roots of different diameters such as the species studied (Leva et al., 2009). After 4 months, we dug up to the border of the trap, passed a sharp knife around the trap to cut the roots, and carefully removed the trap with a small gardening shovel. Target species and depth effects on richness and RGR were characterized from grasses roots found in each trap. We estimated RGR as $(\ln W_2 - \ln W_1) \times T^{-1}$, where W_2 : root biomass weight at the end of the study (g m^{-2}) + 0.5; W_1 : initial root biomass weight (0 g) + 0.5; T: 120 days. We transformed W_1 and W_2 by adding 0.5 to both weights in order to avoid zero values in W_1 and be able to calculate $\ln W_1$, because the sieved soil contained in traps was initially free of roots. Root traps as other method, such a rhizotrons, may sever roots upon establishment. Therefore RGR from excised roots may differ from that of intact roots. ANOVA tests were used to examine the effects of target and neighboring species between depths on richness and RGR under the aboveground portion of target species ($n = 10$). A post-hoc test (Tukey) was used to identify homogeneous groups when a variable showed significant differences. We also performed simple regression among target and neighboring RGR to analyze the effect of target growth over the neighbors' in the grass community ($n = 5$).

For both studies, after field collection, roots were sorted by species in the laboratory following the procedure described in the Test of taxonomic key. Even though roots obtained corresponding to detached roots from plants, we managed to recognize them color mainly and also for diameter and hairiness (Supplementary material, FigA). Roots were oven-dried at 70 °C for 48 h and weighed.

3. Results

3.1. Taxonomic key test

Root traits included in the taxonomic key were reliable to identify species. After sorting, we found that differences in specific root biomass varied between 1.4% and 0.3%. Sorting was accurate in all species ($r^2 > 0.90$, $P < 0.001$), except for *Bromus pictus* and *B. setifolius*, whose roots could not be distinguished ($r^2 < 0.5$, $P > 0.05$). For this reason we pooled both species together (*Bromus*, hereafter). *Festuca argentina* did not show a good fit ($P = 0.09$). Regression could not be performed for *Hordeum comosum* because the frequency in samples was too low, but a chi-square test comparing original and sorted biomass supported a good identification ($\chi^2 < 0.01$, $P = 0.95$). Pooling all species together in a single analysis indicated an overall high degree of accuracy ($r^2 = 0.95$, $P < 0.001$).

3.2. Field experiments

3.2.1. Descriptive study

There were no differences in total graminoid biomass among target species ($P = 0.51$, Fig. 1). Several samples had some root fragments that could not be identified, which represented $17.1 \pm 0.34\%$ of total root biomass. *Bromus*, *Poa*, *Pappostipa speciosa* and *Pappostipa humilis* represented the target species in this study. We did not find plants of *Carex* sp., *Festuca argentina* and *Hordeum comosum* in the four enclosure studied. Roots of *Bromus*, *Poa*, *Pappostipa speciosa*, *Pappostipa humilis* and *Carex* sp. were frequently found, roots of *Festuca argentina* only appeared in 20% of samples of one transect and roots of *Hordeum comosum* did not appear. In

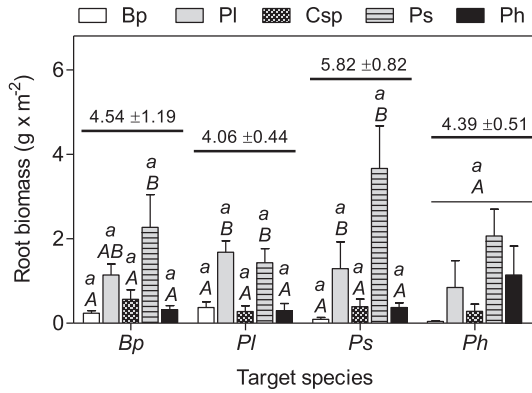


Fig. 1. Root biomass of neighbor grass species below individual plants of target species: Bp, *Bromus pictus*; Pl, *Poa ligularis*; Ps, *Pappostipa speciosa*; Ph, *Pappostipa humilis*. The bars represent the mean (\pm SE) root biomass of each species averaged over the four different sites. Lower case letters (a and b) indicate comparisons among the root biomass of one species under the different target species zone of influences. Upper case letters (A and B) indicate difference among species under one under one particular target species ($P < 0.05$). Total biomass of root under each target species is indicated by the numbers (mean \pm SE) over each group of bars.

general, the belowground portion of target plants was colonized by up to four neighbor species. Each species showed similar root biomass under the four target species ($P > 0.05$; lower case letters). Under *Bromus*, *Poa*, and *Pappostipa speciosa* plants, *Poa* and *Pappostipa speciosa* had the highest root biomass (all $P < 0.001$). This dominance disappeared under *Pappostipa humilis* ($P = 0.18$; upper case letters, Fig. 1).

3.2.2. Root trap study

After 4 months, fine roots colonized 99% of traps. Few samples (4%) had some roots that could not be identified. Among the eight perennial-graminoid species of the study site, only *Festuca argentina* and *Hordeum comosum* did not appear. In general, the belowground portion of target plants was colonized by up to four neighbor species. The mean species richness was 1.6 ± 0.1 species per trap and there were no differences either among the five target species ($P = 0.33$), the two depths ($P = 0.29$) or interaction between factors ($P = 0.33$, Table A, Supplementary material).

Relative growth rates varied according to target species zone of influence but were similar between depths (Table A, Supplementary material). Close to *Bromus*, *Poa*, *Carex* sp. and *Pappostipa humilis* RGR of target and neighboring species were different ($P < 0.01$, $P < 0.01$, $P < 0.05$, $P < 0.01$, respectively) and similar close to *Pappostipa speciosa* ($P = 0.09$). Only *Carex* sp. showed an interaction between RGR of target and neighboring species and depth ($P = 0.03$). In shallow traps, except for *Carex* sp. ($P = 0.59$), roots of target species grew at higher rates than all or some of the neighboring species ($P = 0.03$, $P < 0.01$, $P = 0.04$ and $P = 0.04$, for *Bromus*, *Poa*, *Pappostipa speciosa* and *P. humilis*, respectively; Fig. 2a). In deep traps, *Bromus* and *Poa* had the highest RGR under their aboveground portion (both species $P < 0.01$, Fig. 2b). *Carex* sp., *Pappostipa speciosa* and *P. humilis* roots grew at the same rate than neighboring species under their aboveground portion ($P = 0.18$, $P = 0.29$ and $P = 0.45$, respectively). The simple regression between mean values of RGR of neighboring and target species showed a strong negative relationship between them ($r^2 = -0.86$, $P = 0.02$, Fig. 3). Soil under *Poa* species presented the highest RGR of the target species and the lowest RGR of neighboring species; meanwhile *Carex* presented the opposite pattern.

4. Discussion

Occupation of soil space and degree of overlapping of root systems are critical determinants of belowground competition among plants (Casper and Jackson, 1997). Our studies indicate that roots of different species occupied the same soil volume in a zone of high influence (Figs. 1 and 2). Because more than one species may grow under a target plant, we found no evidence of territoriality as we proposed in the first hypothesis. Our alternative hypothesis was partially supported by field studies. We found evidence that in this steppe species differ in their capacity to invade and accumulate root biomass (under or close to the aboveground portion). Our studies show interesting differences between the short-time response of species to unoccupied soil and the root patterns in non-disturbed soil. Soil pre-emption by high RGR species was transitory as was shown by the root biomass found under plants of perennial grasses in the descriptive study.

In the root trap study, species that showed minimum invasibility of their zone of high influence (*Bromus* and *Poa*) were also able to invade traps close to other species. This pattern was present at the two studied depths. Reinforcing the idea that the lack of territoriality in this steppe is not transitory, the descriptive study also showed that there was root biomass of neighbor species under the different species found along transects. But, interestingly, the species that dominated the zone of high influence were *Poa* and *P. speciosa* (Fig. 1). In other words, *Bromus* was able to rapidly dominate unoccupied soil in the traps but over time this predominance decreased. On the contrary, *P. speciosa*, which was a minor component as neighbor in the trap study, became dominant in its own zone of high influence as well as in other species. Root trap results may be affected by differences in root phenology (Burke and Raynal, 1994) or in RGR of roots after its severing (Schmidt and Walter, 2010), when traps were buried. The descriptive study indicates that the lack of territoriality that we found in the zone of high influence was not the result of these factors. Frank et al. (2010) suggested that root segregation probably plays a comparatively minor role in maintaining species coexistence in grasslands. Coexistence of target and neighbor species could be explained also by species differences in soil colonization rates, associated with RGR, rather than only by segregation.

To the best of our knowledge this is the first time that root intermingling is tested in a zone of high influence of individual plants (i.e. under or close to the plant). Because we worked with root biomass instead of root frequency (the usual response variable of molecular methods of root identification) we are able to estimate with more precision the degree of intermingling. Biomass and RGR are variables more robust for ecological inferences (Chapin et al., 1987). At the same time we can formulate hypotheses about mechanisms that involve species biomass dynamics such as root growth and decomposition. For example, we found that grass species in this Patagonian steppe can be organized in a gradient of colonization ability of unoccupied soil measured as biomass accumulation ($Poa \geq Bromus > Carex$ sp., *P. speciosa*, *P. humilis*).

Relative growth rates of target species in the first months of occupation of vacant soil was negatively related to RGR of neighboring species found under the targets (Fig. 3). We propose RGR as a good index of territoriality degree in this first stage. Conversely, it can be proposed that the overlapping pattern of root biomass, in part, may derive from the accumulation of dead roots and only one species maintains the exclusive use. This hypothesis may be supported by the fact that decomposition rate of root in the steppe is rather low (Austin et al., 2009). Given our traps captured roots only during four months, at the start of the growing season, and we harvested them before the start of the drought period (summer), we claim that most of the roots we recovered were active. In other

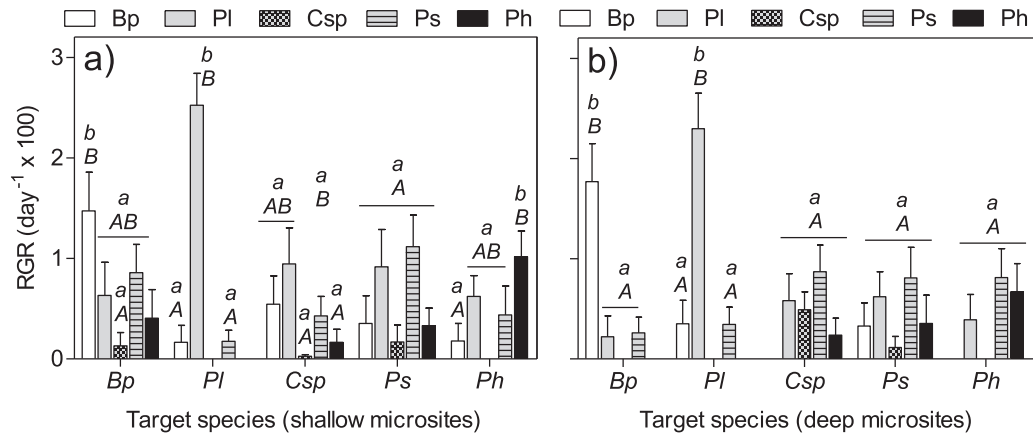


Fig. 2. Relative growth rate of grass roots according to target species. Roots in traps and target species are: *Bp*, *Bromus pictus*; *Pl*, *Poa ligularis*; *Csp*, *Carex* sp.; *Ps*, *Pappostipa speciosa*; *Ph*, *Pappostipa humilis*. The columns represent the mean RGR of each species and error bars represent SE ($n = 10$). Differences among the RGR of target and neighboring species under the aboveground portion of a target species in shallow traps (5 cm–11 cm depth) are shown in a) and in deep traps (15 cm–21 cm depth) are shown in b). Letters *a* and *b* indicate different homogeneous groups among the RGR of one species in the different target species zone of influences. Letters *A*, and *B* indicate comparisons among the same species under one target species ($P < 0.05$).

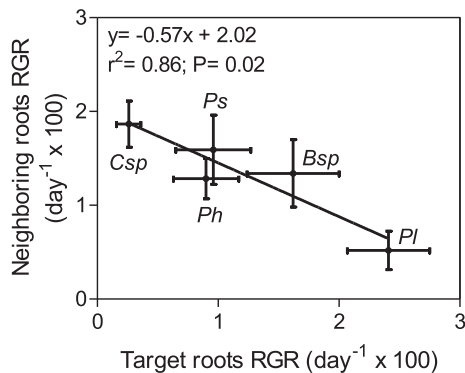


Fig. 3. Relationship between neighboring roots relative growth rate (RGR) in relation with target roots RGR, mean values \pm SE, $n = 20$. All root biomass of neighboring species was pooled before RGR calculation. Species are: *Bp*, *Bromus pictus*; *Pl*, *Poa ligularis*; *Csp*, *Carex* sp.; *Ps*, *Pappostipa speciosa*; *Ph*, *Pappostipa humilis*.

words, the same soil microsite is exploited by active roots of different species with different intensities according to RGR.

The difficulty of identifying roots by species is perhaps the cause that most experiments studying root distribution and root colonization were performed in pots or in field monocultures. However, experiments developed in the field and in natural conditions allow us to reach a better understanding of how root colonization really works (Hodge, 2004). Our study complements others that provided insights about intermingling root systems (Berger et al., 2006). Currently molecular techniques have been used to identifying species. We are confident in the trait-based technique (Leva et al., 2009) because of the precision obtained in the Test of taxonomic key with blind samples (Fig. 1). We acknowledge our study is only a first step in understanding of belowground plant structure and dynamics, nutrient foraging and root functionality. Under a scenario of root intermingling, tracer studies are a perfect complement of specific root biomass distribution. Relating these results with the response of roots to nutrient availability and dominance of species in natural communities will allow us to advance in understanding multi-species coexistence.

Author contributions

MFR developed methodology, conducted field and laboratory work, performed statistical analyses and wrote the manuscript and

MRA originally formulated the idea and contributed with the experimental design, analysis, discussion and writing.

Acknowledgements

We thank Celeste Silvano and Sofia Campana for helping in the root separation and accuracy test of root identification. Paula Leva for sharing her knowledge about grass roots, Beatriz Santos for her English corrections. Funding was provided by grants from ANPCyT (PICT 00462) and UBA (G0032) to MRA. MFR was sponsored by PhD fellowships from ANPCyT and CONICET. MRA is a member of CONICET. INTA granted the access to the facilities and experimental field.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jaridenv.2016.10.012>.

References

- Austin, A.T., Araujo, P.I., Leva, P.E., 2009. Interaction of position, litter type, and water pulses on decomposition of grasses from the semiarid Patagonian steppe. *Ecology* 90, 2642–2647.
- Berger, A.G., McDonald, A.J., Riha, S.J., 2006. Scaling plant size to below-ground zone of influence in annuals under contrasting competitive environments. *Funct. Ecol.* 20, 770–777.
- Burke, M.K., Raynal, D.J., 1994. Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. *Plant soil* 162, 135–146.
- Casper, B.B., Jackson, R.B., 1997. Plant competition underground. *Annu. Rev. Ecol. Syst.* 28, 545–570.
- Casper, B.B., Schenk, H.J., Jackson, R.B., 2003. Defining a plant's belowground zone of influence. *Ecology* 84, 2313–2321.
- Chapin, F.S., Bloom, A.J., Field, C.B., Waring, R.H., 1987. Plant responses to multiple environmental factors. *Bioscience* 37, 49–57.
- Frank, D.A., Pontes, A.W., Maine, E.M., Caruana, J., Raina, R., Raina, S., Fridley, J.D., 2010. Grassland root communities: species distributions and how they are linked to aboveground abundance. *Ecology* 91, 3201–3209.
- Golluscio, R., León, R., Perelman, S., 1982. Caracterización fitosociológica de la estepa del oeste del Chubut: su relación con el gradiente ambiental. *Boletín de la Sociedad Argentina de Botánica* 21, 299–324.
- Graff, P., Aguiar, M.R., Chaneton, E.J., 2007. Shifts in positive and negative plant interactions along a grazing intensity gradient. *Ecology* 88, 188–199.
- Hodge, A., 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol.* 162, 9–24.
- INTA, 2015. Sistema de Información de la Patagonia Sur. <http://anterior.inta.gov.ar/region/pas/sipas2/cmp/agromet/index.html#>.
- de Kroon, H., Mommer, L., 2006. Root foraging theory put to the test. *Trends Ecol. Evol.* 21, 113–116.
- de Kroon, H., Hendriks, M., van Ruijven, J., Ravenek, J., Padilla, F.M., Jongejans, E.,

- Visser, E.J.W., Mommer, L., 2012. Root responses to nutrients and soil biota: drivers of species coexistence and ecosystem productivity. *J. Ecol.* 100, 6–15.
- Leva, P.E., Aguiar, M.R., Oesterheld, M., 2009. Underground ecology in a Patagonian steppe: root traits permit identification of graminoid species and classification into functional types. *J. Arid. Environ.* 73, 428–434.
- Oñatibia, G.R., 2009. Régimen de pastoreo ovino y cambios en la biomasa y los reservorios aéreos de C y N en estepas arbustivo-graminosas patagónicas. Degree Thesis. Facultad de Agronomía, Universidad de Buenos Aires, Buenos Aires.
- Poorter, H., 1989. Interspecific variation in relative growth rate: on ecological causes and physiological consequences. *Causes consequences Var. growth rate Prod. High. plants* 24, 45–68.
- Schenk, H.J., Callaway, R.M., Mahall, B.E., 1999. Spatial root segregation: are plants territorial? *Adv. Ecol. Res.* 28, 145–180.
- Schmidt, L., Walter, A., 2010. Root growth is affected differently by mechanical wounding in seedlings of the ecological model species *Nicotiana attenuata* and the molecular model species *Arabidopsis thaliana*. *Plant Signal Behav.* 5, 290–292.