

MIZ1-regulated hydrotropism functions in the growth and survival of *Arabidopsis thaliana* under natural conditions

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- **Background and Aims** Root hydrotropism is a response to water-potential gradients that makes roots bend towards areas of higher water potential. The gene *MIZU-KUSSEI1* (*MIZ1*) that is essential for hydrotropism in *Arabidopsis* roots has previously been identified. However, the role of root hydrotropism in plant growth and survival under natural conditions has not yet been proven. This study assessed how hydrotropic response contributes to drought avoidance in nature.
- **Methods** An experimental system was established for the study of *Arabidopsis* hydrotropism in soil. Characteristics of hydrotropism were analysed by comparing the responses of the *miz1* mutant, transgenic plants overexpressing *MIZ1* (*MIZ1OE*) and wild-type plants.
- **Key Results** Wild-type plants developed root systems in regions with higher water potential, whereas the roots of *miz1* mutant plants did not show a similar response. This pattern of root distribution induced by hydrotropism was more pronounced in *MIZ1OE* plants than in wild-type plants. In addition, shoot biomass and the number of plants that survived under drought conditions were much greater in *MIZ1OE* plants.
- **Conclusions** These results show that hydrotropism plays an important role in root system development in soil and contributes to drought avoidance, which results in a greater yield and plant survival under water-limited conditions. The results also show that *MIZ1* overexpression can be used for improving plant productivity in arid areas.

Key words: *Arabidopsis thaliana*, drought avoidance, hydrotropism, root system, *MIZU-KUSSEI1* (*MIZ1*).

INTRODUCTION

The sessile nature of land plants does not allow them to move away from harsh conditions. To increase survival, plants have evolved numerous mechanisms for controlling directional growth in response to environmental cues. An example is tropism, which orients plant organs towards or away from an external stimulus. Tropism is thought to be a mechanism to adapt to unfavourable environmental conditions (Darwin and Darwin, 1880; Firm and Digby, 1980; Hart, 1990; Hangarter, 1997; Correll and Kiss, 2002; Kiss *et al.*, 2003; Gilroy and Masson, 2008). A crucial environmental cue for plants is soil water status. Drought is one of the major factors limiting crop productivity (Boyer, 1982), and the growth and development of lateral roots are greatly influenced by environmental factors such as water deficiency (Deak and Malamy, 2005). Plants utilize hydrotropism to bend their roots towards moistened areas of soil in the presence of moisture gradients (Takahashi *et al.*, 2009; Moriwaki *et al.*, 2013). Because roots play an important role in water uptake, hydrotropism may help plants to obtain water efficiently under drought conditions. To date, physiological studies of hydrotropism reveal its interaction with gravitropism, and the involvement of abscisic acid (ABA) and auxin in hydrotropic responses (Takahashi and Suge, 1991; Takahashi, 1997; Takahashi *et al.*, 2002, 2009; Kaneyasu *et al.*, 2007; Moriwaki *et al.*, 2013). An apparatus for sensing moisture gradients appears to reside in the root cap, and differential growth for the bending response takes place in the elongation zone, as observed

in root gravitropism (Jaffe *et al.*, 1985; Takahashi and Suge, 1991; Hirasawa *et al.*, 1997; Miyazawa *et al.*, 2008).

Hydrotropism studies with *Arabidopsis thaliana* have led to the isolation of ahydrotropic mutants such as *no hydrotropic response 1* (*nhr1*), *mizu-kusseil* (*miz1*) and *miz2* (Eapen *et al.*, 2003; Kobayashi *et al.*, 2007; Miyazawa *et al.*, 2009). Saucedo *et al.* (2012) reported an *altered hydrotropic response 1* (*ahr1*) mutant with an enhanced hydrotropic response, in which the growth of primary roots was directed towards the source of higher water availability. Two genes involved in the hydrotropic response, *MIZ1* and *MIZ2*, have been isolated (Kobayashi *et al.*, 2007; Miyazawa *et al.*, 2009). *MIZ1* is composed of 297 amino acids and contains an uncharacterized domain of unknown function (DUF617), which is termed the MIZ domain (Kobayashi *et al.*, 2007). The MIZ domain is conserved in land plants including mosses, but not in algae or animals (Kobayashi *et al.*, 2007). The *MIZ1* promoter is activated in root cap cells and the mature region of the roots, whereas *MIZ1* protein localizes in the lateral root cap and the cortical cells of the root proper (Kobayashi *et al.*, 2007; Moriwaki *et al.*, 2012, 2013; Yamazaki *et al.*, 2012). Light and ABA signalling participate in the hydrotropic response by up-regulating *MIZ1* expression (Moriwaki *et al.*, 2012, 2013). Transgenic plants overexpressing *MIZ1* (*MIZ1OE*) exhibit significantly enhanced hydrotropic responses (Miyazawa *et al.*, 2012).

The study of hydrotropism in *Arabidopsis* roots and the isolation of mutants defective in hydrotropism has enabled a molecular dissection of the hydrotropic response. However, these

studies focused on primary roots, although the root system responsible for water acquisition consists primarily of lateral roots (Dittmer, 1937; Yamauchi *et al.*, 1987). Iwata *et al.* (2012) established an experimental system to study hydrotropism of lateral roots in *Arabidopsis* seedlings, and showed that lateral roots display a hydrotropic response similar to that observed in primary roots. On the other hand, the responses to gravity differ between primary and lateral roots. Namely, primary roots display orthogravitropism, whereas lateral roots display diagravitropism, plagiogravitropism or orthogravitropism, depending upon the age of plants (Kiss *et al.*, 2002; Baldwin *et al.*, 2013). These results indicate that the mechanisms underlying root hydrotropism are probably common to the two types of roots, although different mechanisms are involved in their gravitropism.

A question arises as to whether hydrotropism of lateral roots plays a role in the development of the root system, because soil water status substantially influences root system development (Lynch, 1995). However, the potential role of hydrotropism in mediating adaptive plasticity of roots under natural conditions remains unknown. That is because most studies on hydrotropism have been done using its assay systems with agar media or aeroponic culture (Takahashi, 1997; Takahashi *et al.*, 2009). Currently, contradictory results are reported on the role of hydrotropism in nature (Tsuda *et al.*, 2003; Tsutsumi *et al.*, 2003; Cole and Mahall, 2006). Tsuda *et al.* (2003) observed positive hydrotropism when a steep water-potential gradient was applied across roots of an agravitropic pea mutant grown in vermiculite. A model simulation based on growth experiments in soybean predicts that hydrotropism plays a major role in orienting plant roots in a suitable direction (Tsutsumi *et al.*, 2003). By contrast, Cole and Mahall (2006) reported that a simulation experiment using coastal dune shrubs failed to find an ecological significance for hydrotropism.

This study aimed to clarify how the hydrotropic response contributes to the development of the root system in soil. We established

moisture gradients in soil and analysed root system development of the *miz1-1* mutant, *MIZ1* overexpressor (*MIZ1OE*) and wild-type plants of *Arabidopsis thaliana*. Furthermore, we assessed the relationship between the hydrotropism-influenced root architecture and drought avoidance by examining the growth and survival rates of these plants.

MATERIALS AND METHODS

Plant materials

Arabidopsis thaliana ecotype ‘Columbia’ was used in this study. The ahydrotropic mutant *miz1-1* and a transgenic plant overexpressing *MIZ1* (*MIZ1OE*) (OE7) were described previously (Kobayashi *et al.*, 2007; Moriwaki *et al.*, 2011; Miyazawa *et al.*, 2012).

Plant growth conditions

Seeds were surface sterilized with a solution of 5% (v/v) sodium hypochlorite, washed with distilled water, and germinated on half-strength Murashige and Skoog medium (Sigma-Aldrich, St Louis, MO, USA) with 0.8% (w/v) agar (Wako Pure Chemical Industries, Osaka, Japan). Then, 14-d-old plants were transplanted in the soil (Fig. 1). In brief, seedlings were placed in transparent polystyrene rectangular dishes (14 × 10 × 1.5 cm) and covered with commercially available soil (Metro-Mix 360; 0.07 g N, 0.07 g P, 0.1 g K kg⁻¹; Hyponex, Osaka, Japan) that was moistened at 65% (v/v) water content. A block of water-absorbable plastic foam, PVA sponge (type D; 1 × 1 × 11 cm) (AION, Osaka, Japan) containing 10 mL distilled water was placed on the two longest sides of the soil, parallel to the root system. To maintain the soil at higher water content, the dish was entirely covered with a lid. This set-up was the control chamber (Fig. 1B). To establish experimental moisture gradients, the dish had the block of

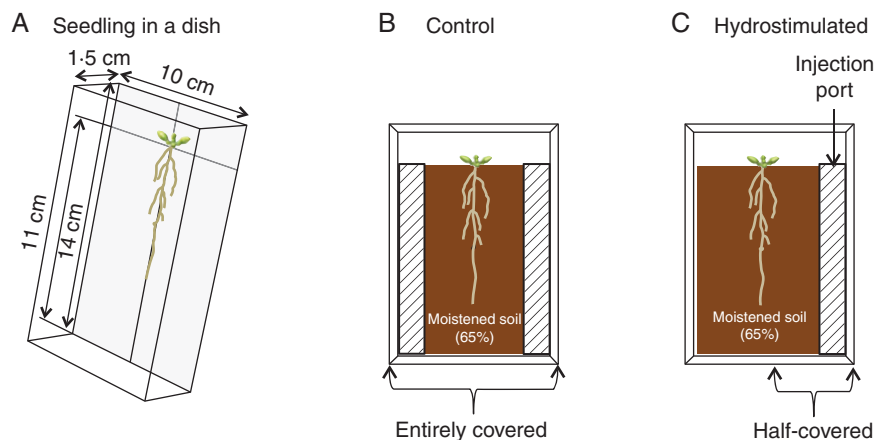


FIG. 1. Experimental system for the study of hydrotropism-influenced root system development in soil. (A) Fourteen-day-old plants were placed in the bottom of a transparent rectangular dish (14 × 10 × 1.5 cm). (B, C) The seedling roots were covered with soil (1.5 cm depth and 11 cm length). To maintain uniformly higher water content (Control), a block of water-absorbent plastic foam (1 × 1 × 11 cm; indicated by hatched bars) containing 10 mL distilled water was placed on each side of the soil, and the dish was entirely covered with a lid (B). To establish moisture gradients (Hydrostimulated), the water-absorbent plastic foam containing 10 mL distilled water was placed only on one side of the soil, and the half of the container with the plastic foam was covered with a lid (C). To maintain the moisture gradient in the hydrostimulated chamber for a longer time, 10 mL water was supplied to the plastic foam through the injection port every 5 d during the experiment. The chambers were held at an angle of 5° from the vertical to promote root growth along the transparent bottom side of the dish. Plants were grown under these conditions for 30 d.

water-absorbable plastic foam only on one side of the soil, and the side containing the plastic foam was half-covered with a lid. This set-up was the hydrostimulated chamber (Fig. 1C). To maintain the moisture gradient in the hydrostimulated chamber for a relatively long period of time, 10 mL water was supplied to the plastic foam through an injection port every 5 d during the experiment. All chambers were placed at an angle of 5° from the vertical to promote root growth along the bottom surface of the chamber. Plants were grown for 30 d at 23°C under continuous fluorescent light ($50\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$) with a relative humidity of 60 %.

Measurement of water distribution in soil

The entire soil media in the control and hydrostimulated chambers were divided into 24 and 28 zones, respectively (superficially, 1.25-cm width \times 2.75-cm height for each zone). Then, the soil water content of the zone was measured with a moisture meter (M-291; AS ONE, Tokyo, Japan) based on the change in permittivity at time zero; measurements were taken at 0, 2, 4, 6, 8, and 10 d after transplanting seedlings. A measurable range by this moisture meter is $<50\%$ (v/v) according to the manufacturer. Data are shown as means \pm s.e. ($n = 4$).

Observation of root system development

Images of root architecture were obtained by scanning the roots observed on the bottom surface of the transparent plastic container with an image scanner (CanoScan 8800F; Canon, Tokyo, Japan). Scans were performed at time zero and after exposing plants to moisture gradients for 2, 4, 6, 8, 10 and 30 d. The scanned images of roots were traced by visual discrimination of roots from the soil background using PowerPoint 2008 (Fig. 2). Then, the root system areas were measured using ImageJ (ver. 1.42) software.

Analysis of biomass production

The above-ground shoots were harvested 30 d after transplanting the seedlings into the soil for both control and hydrostimulated chambers. Shoots were dried at 28°C for 2 weeks in a constant-temperature oven (DK63; Yamato, Tokyo, Japan). Then, dried shoots were weighed using an analytical balance

(AB265-S/FACT; Mettler Toledo, Switzerland) for the measurement of shoot biomass. The experiment was repeated three times.

Assessment of plant survival rate under water-stressed conditions

To assess survival under drought conditions, 14-d-old plants were transplanted into soil in transparent polystyrene rectangular dishes. For these experiments, plant roots were covered with soil moistened to 65 % (v/v) water content, and moisture gradients were developed by covering half of the chamber with a lid. The formation of water distribution was determined by the moisture meter. The control chamber was fully covered with a lid to maintain a uniform and higher water content. The control and experimental chambers were kept at 23°C under continuous light for 12 d without any additionally supplied water. Then, plants were re-watered until soil water content reached $>50\%$, and the survival rate was calculated 2 d after re-watering. The experiment was repeated three times.

RESULTS AND DISCUSSION

We established an experimental system to induce and measure the hydrotropic response in soil to investigate its role in root system development (Figs 1 and 2). Figure 3 shows the soil water distribution for the control and hydrostimulated chambers. In the control, the soil kept a constantly high water content in all zones for 10 d (Fig. 3A). Water can move to the lower zone of the inclined chamber due to gravity, but $>50\%$ water content was maintained even in the uppermost zone of the soil. Accordingly, the results show that there were no detectable gradients in water content in the control chamber. By contrast, in the hydrostimulated chamber, the uncovered side lost water content much sooner than the covered side, which resulted in the formation of water-content gradients (Fig. 3B). Water gradient formation was observed 4 d after the start of the treatment. The water gradient was apparent in the upper zones (soil water contents were approx. 20 %), and probably formed in co-operation with gravity-forced water movement in the inclined chambers. On day 6, the soil water-content gradients were clear both longitudinally and laterally. The uncovered side was relatively dry (approx. 12 %), whereas the covered side ranged from 15 to 45 % water content towards the plastic foam, values that were maintained until the end of experiment. Thus, the results show

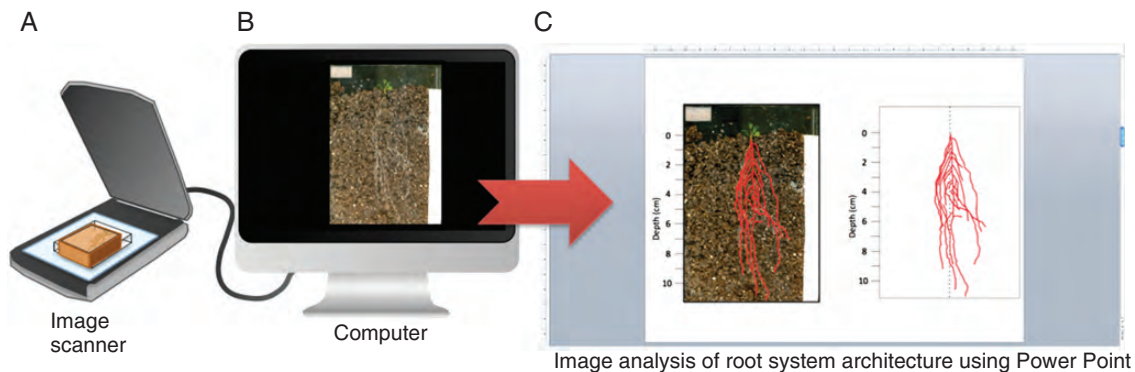


FIG. 2. Diagram of the experimental set-up for viewing the root system architecture. Root architecture was scanned two-dimensionally (A), and the image was subjected to computer-assisted analysis (B). PowerPoint2012 software enabled discrimination of the root system from the background (C).

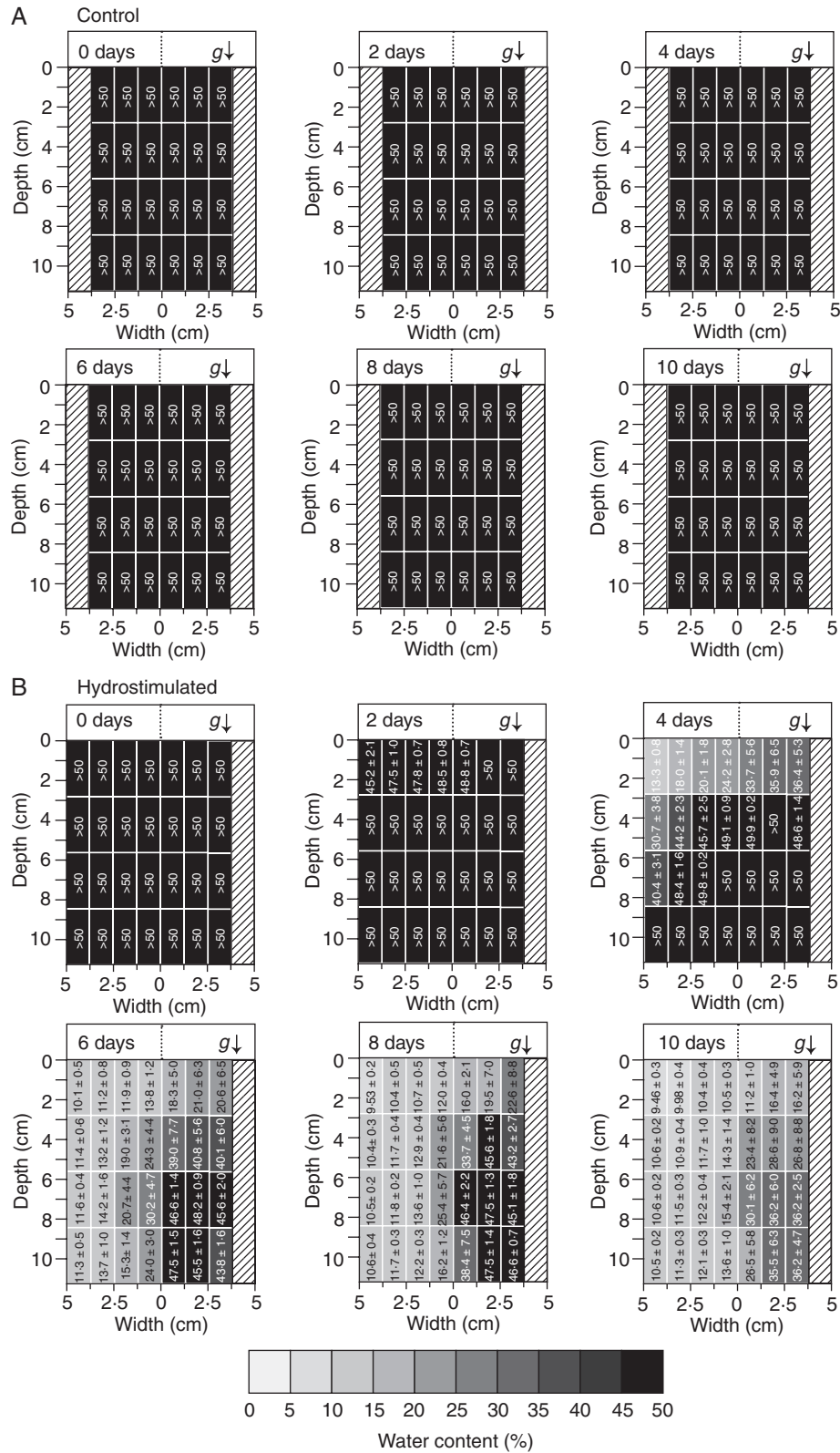


FIG. 3. Soil water distribution in control and hydrostimulated chambers. (A) Water distribution in the absence of hydrostimulation (Control) and (B) in the presence of hydrostimulation (Hydrostimulated) are shown. The soil media in control and hydrostimulated chambers were divided into 24 and 28 zones, respectively, and the water content in each zone was measured at time zero and 2, 4, 6, 8, and 10 days after hydrotropic stimulation. The bottom bar shows water contents as grey scales, which correspond to those of the water distribution map shown above. The arrow (g) indicates the direction of the gravitational force. Data show the means \pm s.e. ($n = 4$).

that these experimental techniques successfully induced gradients in soil water contents. Therefore, we grew *Arabidopsis* plants under these conditions to examine the role of hydrotropic responses in the formation of root architecture, biomass production and survival under drought conditions.

Contribution of hydrotropic response to root system development

We obtained images of root architecture at 0, 2, 4, 6, 8 and 10 d after the start of hydrostimulation, and merged the images with the data for soil water distribution in the chambers (Fig. 4). When wild-type plants were incubated under water-saturated conditions in the control chambers, they developed lateral roots almost symmetrically, within a 1.25-cm-wide zone from the primary roots (Fig. 4A). By contrast, in the presence of water-content gradients, lateral roots developed to approx. 8.0 cm deep in the moist side and did not exceed the 1.25-cm wide growth on the dry side (Fig. 4B). This asymmetrical distribution of lateral roots was attributed to the hydrotropic response to the presence of water-content gradients in the soil. Previous work demonstrated that *Arabidopsis* lateral roots exhibit hydrotropism in response to moisture gradients, similar to the hydrotropic response of primary roots (Iwata *et al.*, 2012). Thus, it is probable that differences in the root system architecture between control and hydrostimulated chambers are caused by the absence or presence of hydrotropic responses, respectively. That is, the hydrotropism could result in an increased development of the root system towards the moist side of the chamber. This result supports previous work that shows that the site of water supply affects the morphology of root system development and architecture (Tsutsumi *et al.*, 2003). The asymmetric root architecture was observed on day 4 when soil water contents were apparently still uniform. This suggests that plants can perceive water-content gradients that induce a hydrotropic response by day 4, but these gradients are below the detection limit of the moisture meter used in this study.

We previously showed that *MIZ1* is essential for hydrotropism in both primary and lateral roots of *Arabidopsis* (Iwata *et al.*, 2012). To verify that hydrotropism induced the observed root system architecture, we observed root system development of the ahydrotropic mutant *miz1-1* grown in control and hydrostimulated chambers (Fig. 5). In comparison with the root architecture of wild-type plants in hydrostimulated chambers, that of the *miz1-1* mutant did not show such asymmetrical development. In the soil water-content gradients of the hydrostimulated chambers, the *miz1-1* mutant developed a more symmetrical root system extending <1.25 cm in width from the primary root (Fig. 5). These results suggest that the hydrotropic response mediated by *MIZ1* functions to modify the root system architecture.

To elucidate further the effect of hydrotropism on root system architecture, transgenic plants overexpressing *MIZ1* (*MIZ1OE*) were examined. Overexpression of *MIZ1* substantially enhanced hydrotropism in *Arabidopsis* primary roots (Miyazawa *et al.*, 2012). *MIZ1* overexpression also resulted in an inhibition of lateral roots (Moriwaki *et al.*, 2011). The inhibition of lateral root formation was observed at an early age of seedling growth in soil, but it became indistinguishable by 4–6 d after transplanting seedlings into soil. In the hydrostimulated chambers, a number of the wild-type roots grew towards the moist side with a width range greater than 1.25 cm, which became obvious on

days 2–6 (Fig. 6A). This asymmetrical root growth was increased in *MIZ1OE* plants, and the lateral roots that were hydrotropically bending reached the plastic foam used as the water source (Fig. 6B). In *MIZ1OE* plants, several lateral roots grew far from the 1.25-cm distance even on the dry side of the chamber. This implies that the hydrotropic response enhanced by *MIZ1* overexpression causes an increase in the size of the whole root system. However, the extent of root growth was similar between wild-type and *MIZ1OE* plants when they were grown in control chambers (data not shown). These results indicate that root hydrotropism occurs in the soil and plays an important role in the formation of root architecture.

We examined root system architecture at 30 d after the start of hydrostimulation, and the percentage of plants that grew roots into individual zones in the rectangular dish was calculated ($n = 12–25$). In the control chamber, more than 80 % of wild-type plants developed symmetrical lateral roots within the 1.25-cm-wide zone from the primary root (Fig. 7A). More lateral roots grew into the moist zones in hydrostimulated chambers; approx. 80 % of wild-type plants developed roots that extended beyond the 1.25-cm-wide zone in the moist side of the chamber (Fig. 7B). By contrast, even in the presence of soil water-content gradients, *miz1-1* mutant roots developed symmetrical lateral roots within a 1.25-cm-wide zone, similar to that of wild-type plants in control chambers (Fig. 7C). Contrary to the ahydrotropic mutant, *MIZ1OE* transgenic plants showed a much greater distribution of lateral roots in the moist side of the chamber; more than 80 % of *MIZ1OE* plants developed roots that extended beyond the 2.5-cm zone (Fig. 7D).

We determined the total root area for each individual seedling (Table 1). In hydrostimulated chambers, wild-type and *MIZ1OE* transgenic plants showed greater root growth in the moist side of the chamber (13.2 and 21.1 cm², respectively) than in the dry side of the chamber (8.72 and 10.8 cm², respectively). By contrast, *miz1-1* plants did not show such asymmetry in root growth between moist and dry sides of the chamber (approx. 10 cm² for each) (Table 1). *MIZ1OE* plants developed a greater root system towards the moist side of the chamber than wild-type plants ($P < 0.001$). By contrast, root system development was similar among wild-type, *miz1-1* mutant and *MIZ1OE* transgenic plants in control chambers (Table 1). These results suggest that the strength of influence on hydrotropism-mediated development of root architecture occurs in the order *miz1* mutant < wild type < *MIZ1OE*. This implies that plant root hydrotropism could function in normal soil environments and play an important role in the development of root system architecture.

Effects of root hydrotropism on biomass production and survival under water-limited conditions

Plant productivity depends on environmental variables, especially during water-stress conditions (Chalmers *et al.*, 1981; Lynch, 1995). Root hydrotropism is considered to play a role in drought avoidance, but the relationship between the hydrotropic response and plant production under water-limited conditions remains unknown. Therefore, we examined whether the hydrotropism-influenced architecture of the root system ultimately affects biomass production.

Figure 8 shows the shoot dry weight at the end of the 30-d experiment. In control chambers, no differences in shoot biomass

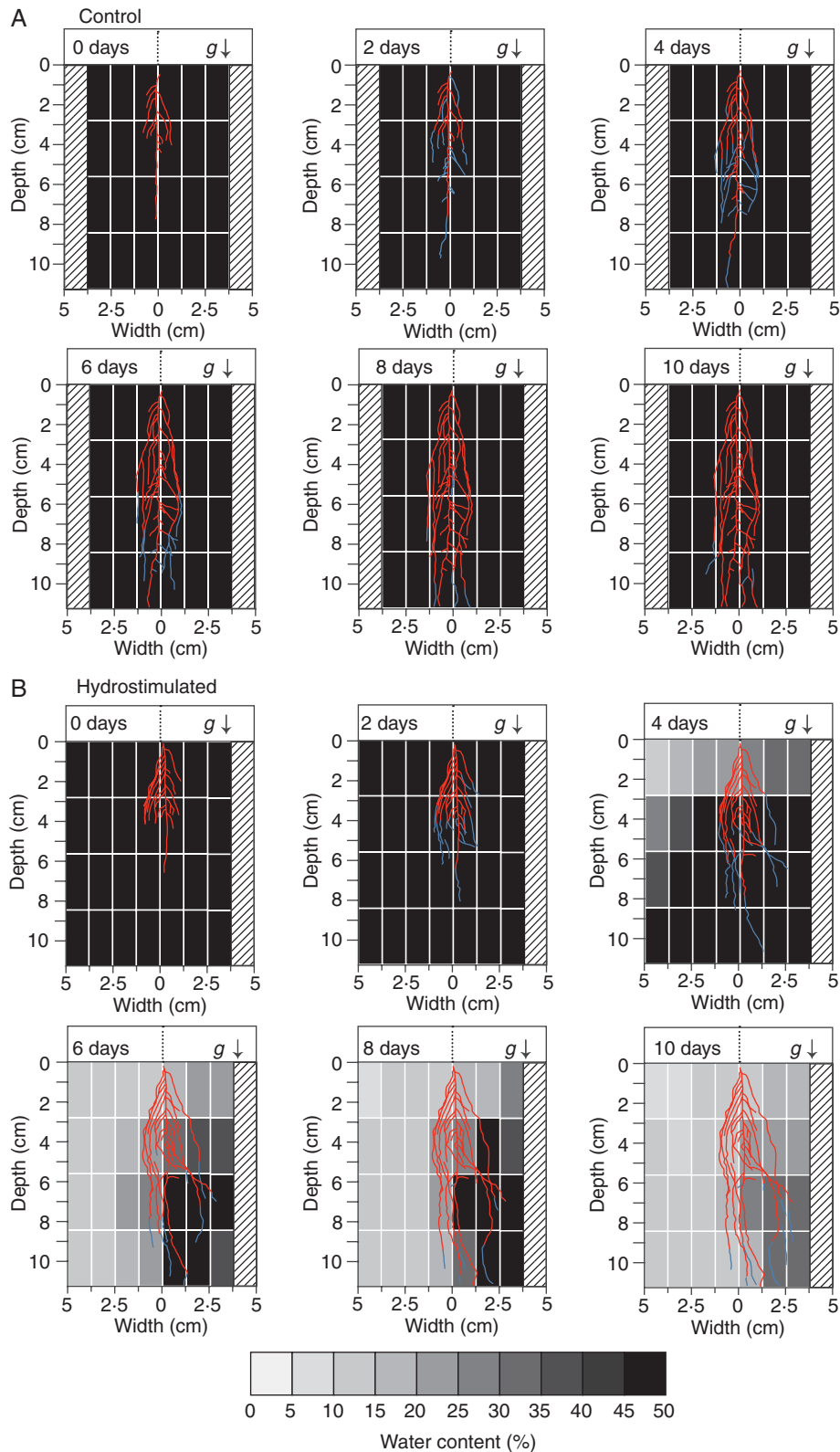


FIG. 4. Effects of moisture gradients on root system development of wild-type plants grown in soil. (A) Root systems in the absence of hydrostimulation (Control) and (B) in the presence of hydrostimulation (Hydrostimulated) are shown. The images of root architecture were obtained at time zero and 2, 4, 6, 8, and 10 d after hydrotropic stimulation. These images were merged with images of the water distribution shown in Fig. 2. Blue lines indicate the root regions that elongated during the preceding 2 d of growth (when the previous image was captured). The bottom bar shows water contents as grey scales, which correspond to those of the water distribution map shown above. The root system of a representative seedling is shown for each time point of imaging. Numbers of seedlings used and experiments repeated correspond to those of Table 1. The arrow (g) indicates the direction of the gravitational force.

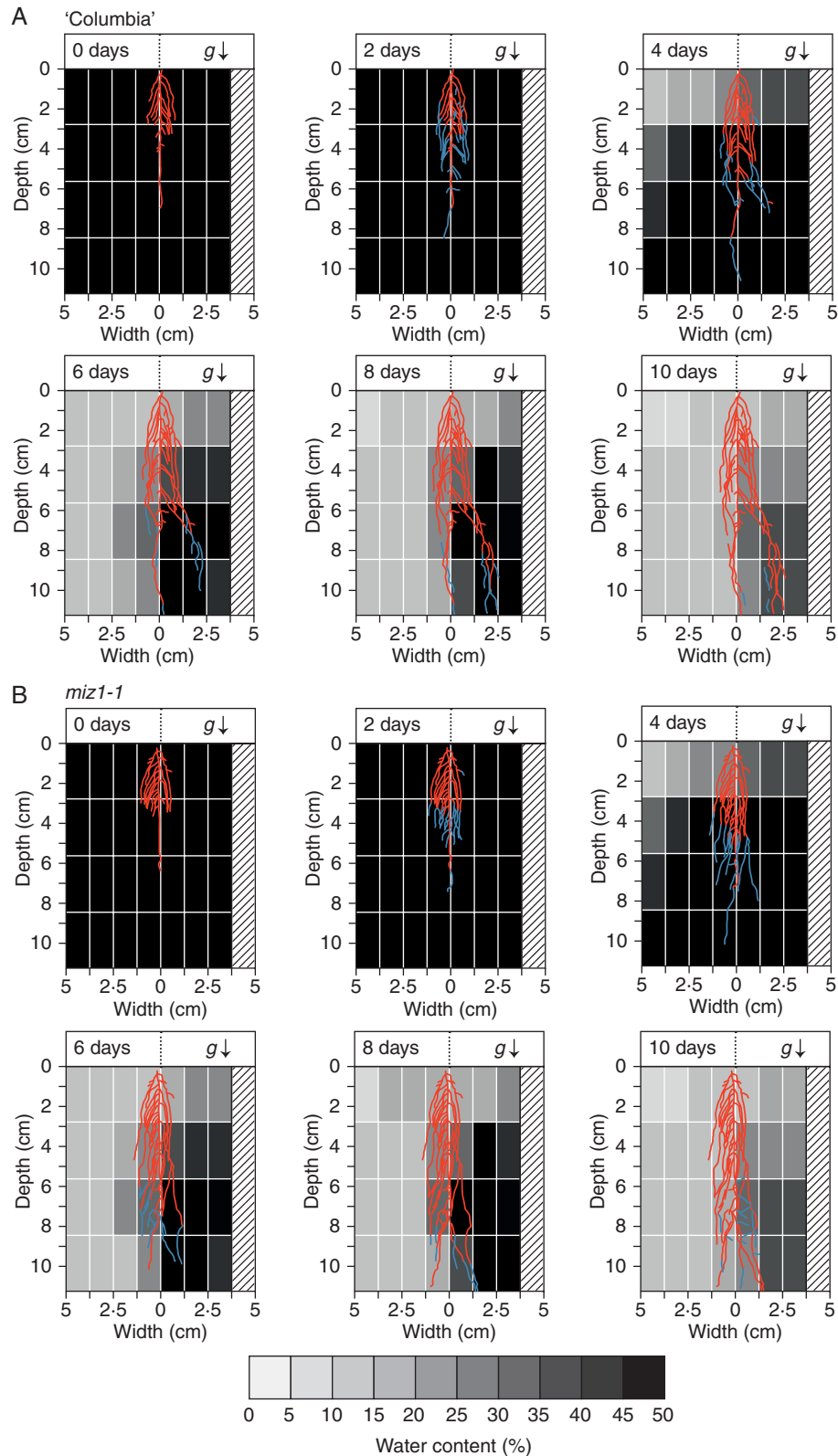


FIG. 5. Effects of the *miz1-1* mutation on root system development of plants grown in the hydrostimulation chambers. The root architecture images of wild-type (A) and *miz1-1* (B) plants were obtained at time zero and 2, 4, 6, 8 and 10 d after hydrotropic stimulation, and these images were merged with images of the water distribution shown in Fig. 2. Blue lines indicate the root regions that elongated during the preceding 2 d of growth (when the previous image was captured). The bottom bar shows water contents as grey scales, which correspond to those of the water distribution map shown above. The root system of a representative seedling is shown for each time point of imaging. Numbers of seedlings used and experiments repeated correspond to those of Table 1. The arrow (g) indicates the direction of the gravitational force.

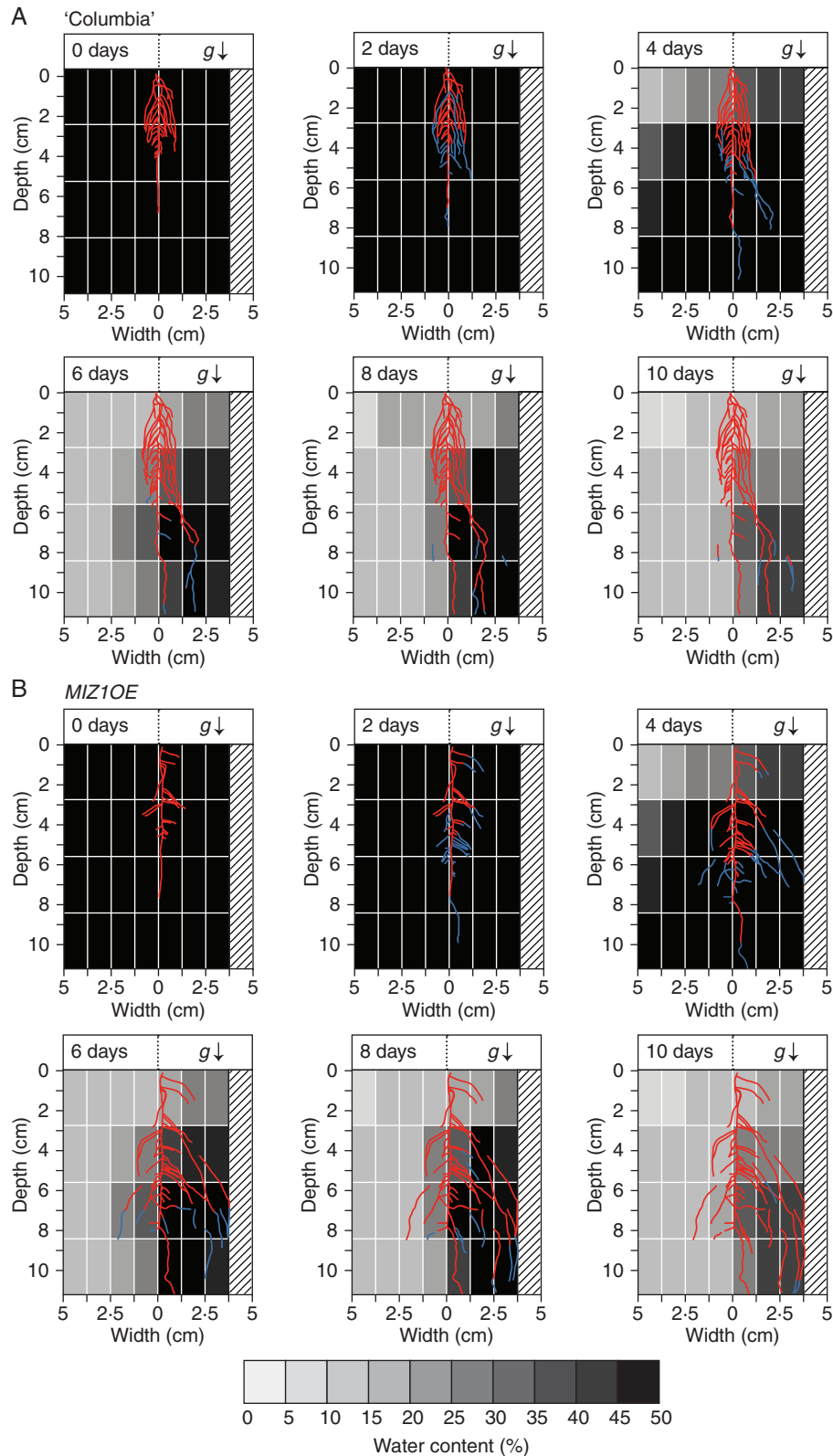


FIG. 6. Effects of *MIZ1* overexpression on root system development of plants grown in the hydrostimulation chambers. The root system images of (A) wild-type and (B) *MIZ1OE* plants were obtained at time zero and 2, 4, 6, 8 and 10 d after hydrotropic stimulation, and these images were merged with images of the water distribution shown in Fig. 2. Blue lines indicate the root regions that elongated during the preceding 2 d of growth (when the previous image was captured). The bottom bar shows water contents as grey scales, which correspond to those of the water distribution map shown above. The root system of a representative seedling is shown for each time point of imaging. Numbers of seedlings used and experiments repeated correspond to those of Table 1. The arrow (g) indicates the direction of gravitational force.

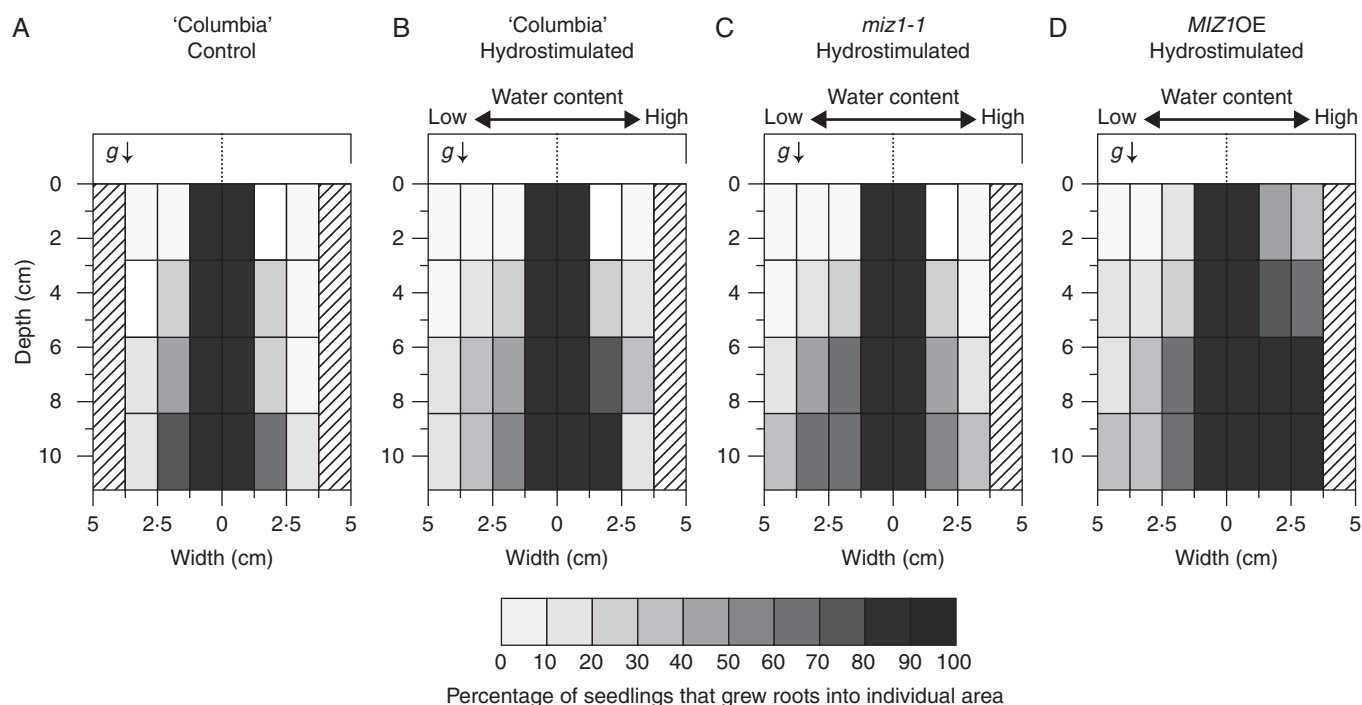


FIG. 7. The root system development of wild-type ('Columbia'), *miz1-1* and *MIZ1OE* plants after hydrotropic stimulation for 30 d. The root system images of (A, B) wild-type ('Columbia'), (C) *miz1-1* and (D) *MIZ1OE* plants were obtained after growing in the absence (A) or presence of hydrostimulation (B–D) for 30 d. The percentage of plants that grew roots into each zone of a rectangular dish was calculated, and is represented by grey scales according to the classification indicated in the bottom bar. Numbers of seedlings used and experiments repeated correspond to those of Table 1. The arrow (*g*) indicates the direction of gravitational force.

TABLE 1. Root areas of wild-type ('Columbia'), *miz1-1* mutant and *MIZ1OE* transgenic plants grown in control and hydrostimulated chambers for 30 d

	Control (cm ²)			Hydrostimulated (cm ²)		
	Left side	Right side	<i>n</i>	Dry side	Moist side	<i>n</i>
Columbia	10.5 ± 0.9	9.7 ± 0.7	12	8.7 ± 0.5	13.2 ± 0.9***	20
<i>miz1-1</i>	11.4 ± 1.1	9.8 ± 0.6	14	11.1 ± 0.7	10.1 ± 0.8	20
<i>MIZ1OE</i>	12.7 ± 1.4	11.8 ± 1.4	14	10.1 ± 1.3	21.2 ± 1.9****	20

Data are the mean root area per plant ± s.e. No statistically significant differences between the left and right sides of the control chambers were observed for all plants tested. In hydrostimulated chambers, 'Columbia' and *MIZ1OE* plants grew more roots in the moist side of the chamber, but *miz1-1* did not show such asymmetrical root growth. The experiments were repeated three times. Statistical analyses were performed using Student's *t*-test. Asterisks represent highly significant values (***P* < 0.001, *****P* < 0.0001).

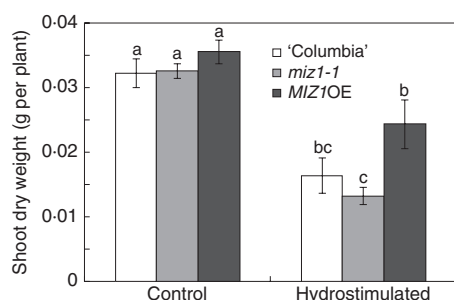


FIG. 8. Effect of hydrotropic response on biomass production of wild-type ('Columbia'), *miz1-1* and *MIZ1OE* plants. Seedlings were grown in the absence (Control) and presence (Hydrostimulated) of hydrotropic stimulation for 30 d, and the dry weights of the shoots were measured. Data are means ± s.e. (*n* = 11–20). Different letters above the bars indicate statistically significant differences at *P* < 0.05 by Tukey's HSD.

were observed among wild-type, *miz1-1* mutant and *MIZ1OE* transgenic plants. In hydrostimulated chambers, shoot dry weights decreased compared with those grown in control chambers. However, the decrease in shoot dry weight was significantly less for *MIZ1OE* plants than for *miz1-1* plants. Shoot biomass weight for the *miz1-1* mutant did not differ statistically from that of wild-type plants (Fig. 8). These results suggest that root hydrotropism does influence the growth of plant biomass under conditions of soil water-content gradients. Water deficit is a common environmental stress experienced by plants, and it strongly impairs their production (Boyer, 1982; Passioura, 1996). Root architecture is considered to be linked to the acquisition of water (Lynch, 1995). Our results show that hydrotropism is a factor that is involved in root system development, which results in better yield under water-limited conditions.

We next examined the survival rate of wild-type, *miz1-1* mutant and *MIZ1OE* plants when they were grown in control

and hydrostimulated chambers. For these experiments, 12-d-old plants were transplanted to control (covered) and hydrostimulated (partially uncovered) chambers (Fig. 1), and then the hydrostimulated chambers were gradually subjected to drought stress by withholding water for 12 d. Wet conditions were maintained in control chambers, whereas drought stress gradually became severe in the hydrostimulated chamber (Supplementary data

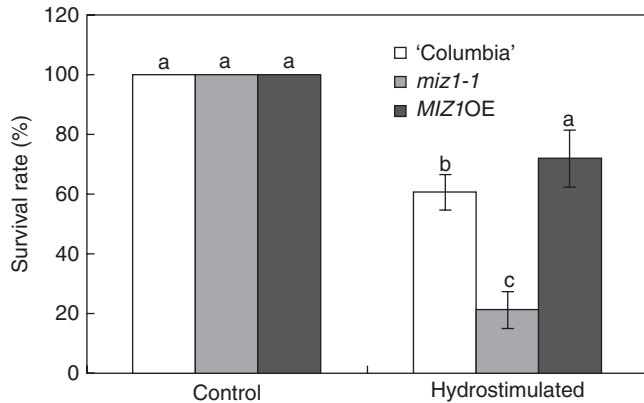


FIG. 9. Survival rates of wild-type ('Columbia'), *miz1-1* and *MIZ1OE* plants after treatment with 12-d drought stress. Fourteen-day-old plants were transplanted into moistened soil, and chambers were either entirely covered (Control) or were half-covered (Hydrostimulated) as shown in Fig. 1. Then, plants were grown without further water supply for 12 d. The plants were re-watered until soil water content reached $>50\%$, and the survival rate was counted 2 d after re-watering. Data are means \pm s.e. ($n = 21-29$). Different letters above the bars indicate statistically significant differences at $P < 0.05$ by Tukey's HSD.

Fig. S1). In the hydrostimulated chamber, plants were exposed to both soil water-content gradients and water stress. After 12 d of drought stress, plants were re-watered until soil water content reached $>50\%$ and allowed to grow for 2 d before the survival rates were determined.

Under the control condition, no water-stressed plants were observed, and 100 % of wild-type, *miz1-1* and *MIZ1OE* plants survived (Fig. 9). When plants were grown in the hydrostimulated chambers and subjected to drought stress for 12 d, the survival rate of *miz1-1* mutant plants significantly decreased, compared with that of wild-type plants (Fig. 9). By contrast, *MIZ1OE* plants showed an enhanced tolerance to drought stress and had a significantly greater survival rate than wild-type plants (Fig. 9).

In these experiments, we also assessed the development of the root system as shown in Fig. 10. More than 80 % of wild-type plants did not develop lateral roots that extended beyond the 1.25-cm-wide zone in the dry side, whereas more than 60 % of the plants developed lateral roots that extended beyond the 1.25-cm-wide zone in the moist side (Fig. 10A). The root system distribution of *miz1-1* mutant plants was symmetrical, with more than 60 % of plants developing lateral roots within the 1.25-cm-wide zone (Fig. 10B). By contrast, more than 80 % of *MIZ1OE* plants developed root systems that extended beyond the 1.25-cm-wide zone, and more than 50 % of *MIZ1OE* plants developed root systems that extended beyond the 2.5-cm-wide zone (Fig. 10C). These results indicate that the hydrotropic response occurred during drought stress treatment, and suggest that the drought tolerance shown in Fig. 9 correlates with the hydrotropism-influenced root system architecture shown in Fig. 10.

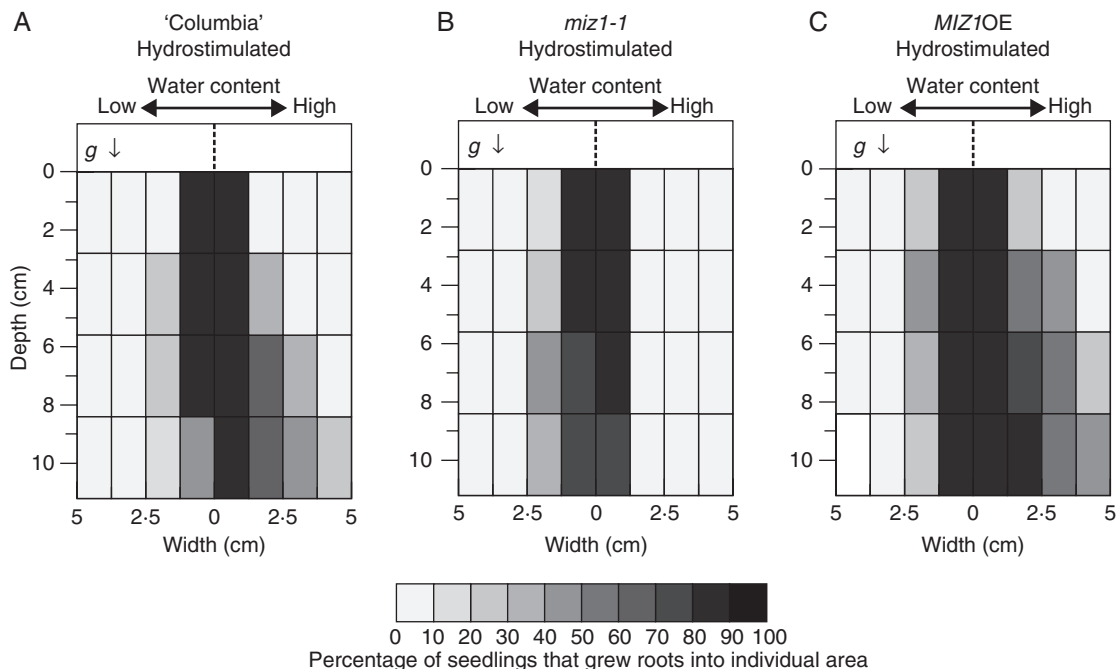


FIG. 10. Effects of hydrotropic response and drought stress on the development of root system of wild-type ('Columbia'), *miz1-1* and *MIZ1OE* plants. Fourteen-day-old plants were transplanted into moistened soil, and the chambers were half-covered (Hydrostimulated) as shown in Fig. 1. They were kept without further water supply for 10 d. The percentage of plants that grew roots into each zone of soil medium was calculated and represented by grey scales according to the classification indicated in the bottom bar. The arrow (g) indicates the direction of gravitational force.

Furthermore, the results suggest strongly that the hydrotropic response contributes to drought avoidance by promoting root growth towards the moistened region. Saucedo *et al.* (2012) discuss the potential of a crop fitness increase by altering pathways that regulate the response of roots to water-potential gradients. However, Cole and Mahall (2006) could find no evidence for the ecological significance of root hydrotropism in seedlings of two species of dune shrubs. In their report, it is not clear whether the roots of the observed dune shrubs are able to display hydrotropic responses by overcoming gravitropic responses. The ability of roots to respond to moisture gradients should be experimentally assessed in dune shrubs to examine the ecological significance of hydrotropism in these plant species.

Conclusions

This study has demonstrated that root hydrotropism plays a role in root system development in soil. In addition, the hydrotropism-influenced root system architecture appears to affect plant growth and survival under water-limited conditions. Root hydrotropism could function during plant responses to water-limited conditions by modifying root architecture for increased acquisition of water, which ultimately determines plant productivity. Our study suggests that constitutive expression of *MIZ1* could potentially increase crop productivity in arid areas. This technology could be applied to plant breeding programmes to improve drought tolerance.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of Figure S1: water distribution measured in the soil after withholding water supply for the evaluation of shoot biomass and plant survival rates.

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