Factors inducing cavity formation in the vascular cylinders of pea roots (Pisum sativum L., cv. Alaska)

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Abstract

When grown at relatively high temperatures pea primary roots (Pisum sativum L. cv. Alaska) often form long lysigenous cavities in the centers of their vascular cylinders. Factors other than temperature may be involved, however. Pea seedlings were grown at 10 and 25°C in vermiculite in a water-availability series (750–2200 ml water/2 l vermiculite) and hydroponically at various levels of aeration (0, 400, 800 ml air/min). Pea seedlings were also grown at 25°C in vermiculite moistened with 750 ml water/2 l along an oxygen gradient (2–21% O2). Growth was much slower and vascular cavities never formed at 10°C. At 25°C in vermiculite the rate of cavity formation (22–100%) was positively correlated with water-availability, so water-availability was a significant factor. In the hydroponic system, aeration had little effect on growth at 10°C but increased growth at 25°C. All primary roots grown hydroponically at 25°C contained cavities. As ambient oxygen level was increased so did growth rate, but the reverse was true for cavity formation. Growth rate, therefore, was not a factor in cavity formation but oxygen availability was. Primary roots that did not develop cavities at 25°C had intercellular spaces in parenchymatous tissues of their vascular cylinders, whereas those with cavities did not. These results support the hypothesis that at high temperature elevated respiratory demand exceeds the rate of oxygen diffusion to the center of the mature portions of pea roots that form cavities, and that this situation is aggravated by wet conditions. Therefore, vascular cavities may be functioning as a type of aerenchyma. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Root cavity formation; Root growth; Water-availability; Flooding response; Aerenchyma

1. Introduction

The observations of Popham (1955) regarding the formation of lysigenous cavities in the centers of vascular cylinders of primary roots of pea were largely disregarded until Rost et al. (1988) reported vascular cavities beginning near the tips of Alaska pea primary roots grown under standard conditions in vermiculite. Lu et al. (1991) showed good evidence of routine cavity formation in primary roots (cavities do not form in lateral roots) extending continuously from ca. 2 cm from the...
tips to within 2–3 cm of the bases. They described the phenomenon as temperature-dependent; i.e. vascular cavities form in pea primary roots at temperatures above 15°C when grown in a wide range of media. Cavities formed in up to 30 cm of the root axis. They also described specialized parenchyma cells (SP-cells), which later acropetally fill the cavities. In an examination of the vascular anatomies of the primary roots of 20 legume species, this phenomenon was shown to be general in cool season legumes (Rost et al., 1991). Niki et al. (1995) and Niki et al. (1998) have since described the fine details of the process of cavity formation by lysigeny and the later intrusion of SP-cells into the cavities in vascular cylinders of pea primary roots using thin-sectioning methods for light microscopy, transmission electron microscopy and scanning electron microscopy.

In a population of pea seedlings growing at an inductive temperature the frequency of occurrence of root cavities was usually not 100% (Lu et al., 1991). Even with seeds of the same lot, germinated and grown together under the same growing conditions, usually a portion of these previously described populations did not produce root cavities at inductive temperatures. This suggests that other factors in addition to temperature may be involved in inducing vascular cavity formation. In the present study we manipulated water conditions and the gaseous environment around developing seedlings as modulators of root development in order to examine the relationship of water status and oxygen availability with root growth and cavity formation. We hypothesized that at elevated temperature high water availability would be associated with hypoxia in the central vascular tissue and that this would contribute to cavity formation.

2. Materials and methods

2.1. Vermiculite method

Distilled water (DW) was added to vermiculite-filled, foil-covered 2 l beakers to fill them half-way (after Gladish and Rost, 1993), or according to the following specific series: 750, 1225, 1500, 1900 and 2200 ml (n = 3 beakers each). Water levels of 1900 and 2200 ml displaced nearly all free air in the vermiculite or filled the available airspace and covered the surface of the vermiculite, respectively. Garden pea seeds (Pisum sativum L. cv. Alaska) were surface sterilized in 10% household bleach. 7.5 g of seeds (ca. 40) were sown in each beaker under axenic conditions. The seeds were grown in the covered beakers at constant 10 or 25°C in a continuously dark growth chamber. After 18 days at 10°C or 8 days at 25°C, primary roots were collected.

For evaluating the frequency of occurrence of vascular cavities in the roots, freehand sections were cut transversely from 20 roots chosen at random from each beaker. These were stained with 0.025% toluidine blue O. For more detailed histological assessment, 2 mm segments of roots with and without cavities were immediately immersed in 2.4% glutaraldehyde/0.3% paraformaldehyde in 0.02 mol/l phosphate buffer (pH 7.2), and gently shaken overnight at room temperature. Following fixation the segments were rinsed in buffer, dehydrated by ethanol series, embedded in Jung HistoResin (Leica Instruments GmbH, Heidelberg), and sectioned at 2 µm. These were stained with 0.025% toluidine blue O. Sections were viewed with a Leica DMLB light microscope, and photographs were taken with Kodak Technical Pan film.

2.2. Hydroponics method

Seeds were germinated at 25°C in sterile, moist vermiculite for 3 days, and transferred to plastic tanks (1600 ml capacity) when primary roots were ca. 20 mm long. A sheet of gauze supported by a wire network was suspended at the water’s surface in the plastic tanks. The gauze layer held the seedlings in the proper orientation after the primary roots were inserted through it. Tanks were filled with tap water and aerated at the following rates: unaerated; 400 ml air/min; 800 ml air/min (n = 20 each for three trials). The plants were grown at 10°C for 17 days or 25°C for 5 days in continuous darkness. After these treatments, the lengths of the primary roots were measured. Roots 70–140 mm long were collected. As de-
scribed above, sections were cut 60–90 mm from the root tips. A minimum of ten primary roots per trial were scored for each treatment.

2.3. Root zone gas modification method

Two litre beakers filled with vermiculite moistened with 750 ml of water were prepared as described in Section 2.1 above, except that tubing was installed leading into the beakers at their tops, down one side, and around the bottoms of the beakers. The end of the tubing that looped inside around the bottom of each beaker was perforated so that a continuous stream of gas could be uniformly introduced at the bottom of the beaker. Two litre beakers filled with vermiculite moistened with 750 ml of water with no tubing (as described in Section 2.1 above) were used as negative controls (n = 3 beakers). Each beaker was sown under axenic conditions with 7.5 g of sterilized pea seed. Certified gas mixtures (Weiler, Moraine, OH) were prepared as follows: 2, 4, 8, and 14% O₂. Each of these mixtures contained 400 ppm CO₂ and balance N₂. These mixtures or compressed air (positive control) were used to infuse the root zones of developing pea seedlings at the rate of 40–50 ml/min (n = 3 beakers each).

Primary roots were harvested when they were ca. 15 cm in length, which was 7 days after imbibition for both controls, the 14% O₂, and the 8% O₂ trials, 11 days for the 4% O₂ trial, and 17 days for the 2% O₂ trial. To evaluate the frequency of occurrence of vascular cavities, freehand sections were cut transversely from 20 primary roots chosen at random from each beaker. These were stained with 0.025% toluidine blue O. Sections were viewed with a Nikon FLX light microscope, and photographs were taken with Kodak Technical Pan film. Negatives were digitized at 1200 dpi using a Polaroid Sprintscan 35/LE slide scanner, and the images printed.

2.4. Statistical analysis

Growth series data were evaluated using ANOVA and linear regression analysis. Because they are categorical (a cavity is either present or it is not), cavity series data were evaluated using the Chi-square test of independence.

3. Results

3.1. Cavity formation in roots grown in vermiculite

Cavity formation did not occur in the vascular cylinders of pea primary roots at 10°C (Fig. 1A), but they occurred variously (see below) at 25°C when grown in vermiculite (Fig. 1B–C). These cavities extended through the centers of vascular cylinders from ca. 15 to 90 mm from the tips of roots ca. 150 mm long. SP-cells were observed at the basal end of the cavities (data not shown).

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![Fig. 1. Freehand transverse sections of pea primary roots. A. Anatomy typical of roots grown at 10°C. B. Anatomy typical of roots grown at 25°C in 2 l vermiculite moistened with 750 ml of water. C. Anatomy typical of roots grown at 25°C in 2 l vermiculite moistened with 2200 ml of water. Arrow indicates vascular cavity. Bar = 350 μm.](image)
3.2. Effects of water volume on root growth and cavity formation in vermiculite medium

At 25°C cavities often did not form at low water volume (<1225 ml/beaker; Fig. 1B), but were significantly more frequent ($\chi^2 \geq 31.8$) at high water volume (Fig. 1C, Fig. 2). The relative growth response to varying water amounts was the same at both temperatures. The growth of primary roots appeared to increase only slightly with increasing water volume from 750 to 1500 ml and was, respectively less at 1900 and 2200 ml of water (Fig. 2 shows 25°C data), though these differences were not statistically significant (10°C: $F = 0.56$, $P = 0.70$; 25°C: $F = 0.78$, $P = 0.56$).

3.3. Growth and cavity formation of pea roots in hydroponic conditions

Root growth was clearly influenced by temperature (Table 1). Aeration treatments enhanced the growth of roots grown at 25°C ($F = 35$, $P \approx 0$), but the growth of roots at 10°C was not significantly enhanced by aeration ($F = 2.1$, $P = 0.14$). Cavities were not observed in the vascular cylinders of roots grown at 10°C, with or without aeration. On the other hand, at 25°C cavities were observed in all roots, despite aeration treatments.

3.4. Effects of oxygen availability on growth and cavity formation

Mean growth rate was positively and linearly correlated ($R^2 = 0.99$, $P \leq 0.01$) to the percentage of oxygen in the gas mixture to which the roots were exposed (Fig. 3). Vascular cavities were negatively and linearly correlated ($R^2 = 0.92$, $P \leq 0.01$) to oxygen level, and the differences were significant ($\chi^2 \geq 34.2$, Fig. 3). Vascular cavities were found from within 2 cm of the tip to the first node of the epicotyls. This included the basal portion of the roots where cavities were previously never found (Fig. 4A). The vasculature of the first internode of the epicotyl was in transition between a typical root protostele and a typical dicotyledonous stem eustele. Where a protostele persisted, its central xylem region was interrupted by a vascular cavity. In addition, the first internode often contained cortical aerenchyma (Fig. 4B). Cortical aerenchyma was never observed in the roots.

3.5. Morphology of roots grown under various conditions

The morphology of roots grown at 10 and 25°C with 750 ml of water available appeared normal.
Table 1
Mean growth and cavity formation in pea roots grown hydroponically at 10 or 25°C under different levels of aeration a

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Aeration b</th>
<th>Growth (mm) ± SD</th>
<th>Cavity formation c</th>
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<tr>
<td>10°C (after 20 days)</td>
<td>–</td>
<td>78 ± 19</td>
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<td>+</td>
<td>80 ± 16</td>
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<td>++</td>
<td>93 ± 18</td>
<td>–</td>
</tr>
<tr>
<td>25°C (after 8 days)</td>
<td>–</td>
<td>55 ± 12</td>
<td>+</td>
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<td>+</td>
<td>118 ± 26</td>
<td>+</td>
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<td></td>
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<td>136 ± 27</td>
<td>+</td>
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a Three trials, n = 10–20 each.
b (-): unaerated; (+): 400 ml air/min; (++): 800 ml air/min.
c Cavity formation was determined by freehand sections taken ca. 45 mm from the root tips.

(Fig. 5A–B). The morphology of roots grown at 25°C in vermiculite with 2200 ml water volume and those grown under hydroponic conditions at 25°C, even with high aeration, was often very different from that of roots grown at 25°C in vermiculite at lower levels of water availability. The former were often coiled into a helix (Fig. 5C).

3.6. Histological differences in roots with and without cavities

At 25°C, phloem sectors in roots with cavities had no or very few intercellular spaces (Fig. 6A), while parenchymatous cells in phloem sectors of roots without cavities invariably had adjacent intercellular spaces (Fig. 6B). Ground tissue of the root cortex always had intercellular spaces irrespective of the presence or absence of a vascular cavity (data not shown).

4. Discussion

The results support our hypothesis, as they show that vascular cavities occurred most frequently when temperature was high (25°C) and so was water availability (Figs. 1 and 2), and there was a negative correlation between O₂ availability and the frequency of cavity formation in a relatively dry vermiculite medium (Fig. 3). Aeration in a hydroponic system was insufficient, however, to minimize cavities at high temperature (Table 1), which indicates the importance of water in restricting the diffusion of O₂ through a growing medium (Taiz and Zeiger, 1991).

The process of vascular cavity formation in roots has been observed previously (Popham, 1955; Lu et al., 1991; Rost et al., 1991; Fukuoka and Kano, 1992; Niki et al., 1995, 1998). In these cases, the cavities were derived from the lysis of parenchymatous cells usually located axially along the center of the roots. However, factors inducing cavity formation have not been thoroughly studied. Lu et al. (1991) argued that vascular cavity formation in pea primary roots is a temperature-induced phenomenon. Fukuoka and Kano (1992)
made a similar claim for daikon radish. Results from the current study show that, while it is clear that relatively high temperature is necessary, it is not sufficient by itself to induce vascular cavities in pea roots.

Root growth rates in pea are relatively high at 25°C and low at 10°C (Lu et al., 1991, Table 1), and it is known from the current and previous studies that pea roots do not produce cavities when grown at low temperatures. In fact, Lu et al. (1991) showed that changing the temperature from 10 to 25°C after a primary root has been developing for awhile will result in an increase in root growth rate and the initiation of a vascular cavity. Therefore, it is tempting to conclude that there is a correlation between rapid root growth and cavity formation, but rapid root growth is not always concomitant with cavity formation. Pea primary roots achieve their highest growth rates at 15°C (Gladish and Rost, 1993), but only rarely form cavities at this temperature (Lu et al., 1991). Furthermore, the current study shows that increasing water volume in vermiculite promotes root growth insignificantly and only to a point (1500 ml water/2 l beaker). Addition of more water than this to the vermiculite produced waterlogged conditions, resulting in negligible repression of root growth but 80–100% cavity

Fig. 4. Free-hand transverse sections taken from a pea seedling grown at 4% oxygen at 25°C in vermiculite. A. Primary root 1 cm below the cotyledonary node. A cavity has formed in the center of the vascular cylinder (large arrow) and become nearly filled with SP-cells (small arrows). Bar = 100 μm. B. Epicotyl 2 cm above the cotyledonary node. A cavity (C) has formed in the vascular cylinder and SP-cells have begun to grow into the lumen (arrow). Cortical aerenchyma also can be seen (Ae). Bar = 200 μm.

Fig. 5. Morphology of typical pea primary roots grown at: (A) 10°C regardless of moisture; (B) at 25°C in relatively dry conditions (750 ml water in 2 l vermiculite) and (C) at 25°C in flooded conditions. Bar = 5.0 cm.
all of the roots regardless of aeration level. Given that the frequency of root vascular cavity formation in our populations of pea seedlings was negatively correlated with the availability of oxygen under conditions that otherwise would result in low rates of cavity formation (Fig. 3), we are led to conclude that at high temperatures high respiratory demand can be met by aeration in the narrow meristematic tip of the roots but not in mature parts of the root where the diameter is > 0.7 mm (Niki et al., 1995) and where the endodermis has Casparian strips (Fahn, 1982).

Peas are cool season plants with tolerance to flooding at cool temperatures, as our results confirm (Table 1), so they seem not to have evolved the typical histological response to flooding found in other species: autolytic aerenchyma in the cortex (Drew et al., 1979; Koning, 1982; Campbell and Drew, 1983; Webb and Jackson, 1986; Schussler and Longstreth, 1996; Uchimura et al., 1996). We conclude that respiration in cells of pea primary roots consume oxygen rapidly enough at 25°C, which is a supra-optimal temperature for pea root growth (Gladish and Rost, 1993), that the rate of oxygen diffusion radially through the medium and epidermis and longitudinally through intercellular spaces of the cortex, then past the endodermis to the center of the root was insufficient to satisfy the respiratory demands of developing cells of the xylem. This is consistent with the results and model of Armstrong et al. (1994), and this may explain why the cavities form in the center of pea roots rather than in the cortices, as is the case for roots of other species exposed to flooding or hypoxia. Although pea primary roots do have a Casparian strip system in the endodermis (Karahara and Shibaoka, 1992), it is not as well defined and sclerified as in maize (pea root: Niki et al., 1995, cf. Figs. 1–7; maize root: He et al., 1996, cf. Fig. 2) so it is unlikely that the endodermis in pea is a greater barrier to oxygen than in other plants. On the other hand, it may not be a coincidence that cavities form after the Casparian strip begins to form in the endodermis (Fahn, 1982; Niki et al., 1995). The presence or absence of intercellular spaces in the parenchymatous tissues of the vascular cylinders of roots with cavities seems to be the critical factor (Fig.

Fig. 6. Transverse thin sections through the phloem sectors of vascular cylinders in roots grown at 25°C in vermiculite. Bar = 35 μm. A. Roots with a cavity (C) had no intercellular spaces in this sector. B. Roots without a vascular cavity had prominent intercellular spaces (arrows) in this sector.

occurrence (Fig. 2). Consistent with previous results (Lu et al., 1991), growth rate is not a factor in vascular cavity formation, but our data show that water availability is.

Since aeration did not have an effect on the growth of the roots under hydroponic conditions at 10°C, the respiratory demand for oxygen in pea roots at low temperature was apparently low enough that simple diffusion was sufficient to satisfy the demand without aeration. On the other hand, under hydroponic conditions at high temperature, aeration treatments enhanced root growth. This suggests that high temperature increased the respiratory demand for oxygen beyond the rate which diffusion alone could supply it, and that oxygen availability limited growth under these experimental conditions (Taiz and Zeiger, 1991). But at high temperature in hydroponic conditions cavity formation was observed in
rather than limitations of diffusion caused by the endodermis. The cortical tissue in pea primary roots apparently is sufficiently aerated to prevent cortical aerenchyma formation even at 2% oxygen, although the cortex of the epicotyl is not (Fig. 4B). This shows that pea stems respond somewhat differently to hypoxia than pea roots.

Stress-induced cortical aerenchyma is thought to reduce hypoxia by allowing an internal pathway for oxygen to the root zone much larger than intercellular spaces to aid in respiration and the oxidation of toxic compounds (Crawford, 1982). Since pea primary roots that do not develop vascular cavities have a well developed system of intercellular spaces in the parenchymatous tissues of the vascular cylinder and those that do have a cavity lack these spaces (Fig. 6), we suggest that vascular cavities are a type of aerenchyma for increasing longitudinal oxygen transport in root tissue under wet to flooded conditions. This is consistent with previous results that showed that these cavities are empty except for cell remnants and gases (Niki et al., 1998). Lu et al. (1991) reported that cavities did not form in the basal 2–3 cm of the primary root, but we found that they could consistently be caused to form there and in the epicotyl by hypoxic conditions (Fig. 4). This is consistent with our assertion that vascular cavities are a type of aerenchyma. In the vermiculite systems used by Lu et al. (1991), Rost et al. (1991), and Niki et al. (1995) oxygen availability would necessarily be higher near the surface of the medium where the base of the roots and epicotyls were located.

Drew et al. (1979) and Koning (1982) showed that hypoxia causes an increase in ethylene concentration. They showed that induced ethylene produces cell lysis of parenchyma cells in the cortices of flooded roots. A similar process may be involved in the formation of pea root vascular cavities. It may be that under hypoxic conditions transduction of an ethylene signal leads to lysis of parenchymous cells of the vascular cylinder akin to the process of cortical aerenchyma formation in maize (He et al., 1996). Since epinasty is known to be caused by ethylene (Jackson and Campbell, 1976; Jackson, 1985), the helicoid growth pattern of the flooded roots in the current study (Fig. 5C) is perhaps also an ethylene response. Further studies are needed to determine if ethylene is involved in the process of vascular cavity formation.

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References


