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Potassium phosphate induces systemic protection in barley to powdery mildew infection†

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Abstract: In laboratory tests, treatment of the first leaves of barley (*Hordeum vulgare* L cv Golden Promise) with potassium phosphate led to significant reduction in infection of the second leaves with the powdery mildew fungus *Blumeria graminis* f sp *hordei* Marchal, with a 25 mM treatment giving 89% reduction in infection. Although the optimal interval between phosphate treatment of the first leaves and mildew inoculation of the second leaves was 2 days, significant protection was still obtained if the interval was increased to 12 days. Protection against powdery mildew infection was not as effective when the potassium phosphate was applied as a seed treatment or root drench. Phosphate treatment of the first leaves led to significant increases in activities of phenylalanine ammonia lyase (PAL), peroxidase and lipoxygenase in second leaves. Enzyme activities, especially PAL and peroxidase, were increased further when second leaves of phosphate-treated plants were inoculated with powdery mildew. Phosphate treatment of the first leaves did not adversely affect plant growth and, in a field trial, 25 mM potassium phosphate provided 70% control of mildew and gave a small increase in grain yield.

Keywords: barley; barley powdery mildew; systemic protection; potassium phosphate

1 INTRODUCTION

Pre-treatment of susceptible plants with virulent or avirulent pathogens, resulting in formation of necrotic lesions, or with various non-pathogens, can lead to the local or systemic induction of disease resistance to subsequent pathogen attack.[1] This induced resistance is characterized by a reduction in the number and severity of lesions caused following challenge inoculation with a normally virulent pathogen.[1] Induced resistance can be divided into the two broad groups: systemic acquired resistance (SAR), where resistance develops either locally or systemically following infection by a pathogen causing formation of a necrotic lesion, and induced systemic resistance (ISR), which develops systemically in response to colonization of plant roots by certain rhizosphere bacteria.[1]

However, resistance can also be induced by treating susceptible plants with abiotic chemicals,[1] for example, oxalates and phosphates have been shown to induce systemic resistance in cucumber, broad bean, grapevine, maize, pepper and rice.[2–8] It has been suggested[3] that phosphates might induce systemic resistance by sequestration of calcium within the target organism, thereby affecting membranes and disrupting cell compartmentation, and causing the release or synthesis of hydrolytic enzymes. According to this hypothesis, the action of hydrolytic enzymes in the cell walls would release pectic cell-wall fragments, thus triggering the production of an 'alarm signal'. Subsequent work[8] showed that systemic resistance in broad bean could be induced by treatment with the calcium sequestrator EDTA and, furthermore, that this could be partially reversed by treatment with calcium nitrate. Very recent work has shown that resistance induction by potassium phosphate against *Colletotrichum lagenarium* (Paserini) Ell + Halst in cucumber was associated with localized cell death. Such cell death was preceded by a rapid generation of superoxide and hydrogen peroxide, and a local and systemic increase in free and conjugated salicylic acid.[9]

Information on the induction of systemic protection in plants using phosphates is scant and, apart from the recent work of Orober *et al*,[9] little information exists on the mechanisms responsible for such protection. In this paper, we report on the induction of systemic protection against powdery mildew (*Blumeria graminis* f sp *hordei* Marchal) in barley (*Hordeum*

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vulgare L) using potassium phosphate, and discuss the activities of enzymes [phenylalanine ammonia lyase (PAL), peroxidase and lipoxygenase (LOX)] associated with plant resistance to pathogens, and effects on plant growth under controlled and field conditions. PAL is involved in phenylpropanoid synthesis and peroxidases are responsible for cross-linking of cell wall components, while LOX catalyzes the oxygenation of polyunsaturated fatty acids and all three enzymes have been implicated in defence against pathogens.[10,11]

2 MATERIALS AND METHODS

2.1 Growth of plant material

Seeds of barley cv Golden Promise were grown in Fison's Levington M3 compost in 9-cm pots, and, after germination, they were thinned to five seedlings per pot. Plants were grown in a glasshouse under natural daylight supplemented for 16 h daily by 400-W mercury vapour lamps. The maximum temperature was 22 °C during the day and fell to a minimum of 9 °C at night. Plants were used for experiments when they had two fully developed leaves (GS 12).[12]

2.2 Treatment of seedlings and inoculation with powdery mildew

In most experiments, the first leaves of barley seedlings were painted with a solution of potassium phosphate (K_3PO_4) using a camel-hair brush. Potassium phosphate was made up in distilled water containing Tween 20 (0.1 ml litre^{-1}) and, in initial experiments, was applied at 10, 25, 35 and 50 mM. Second leaves of treated seedlings were inoculated with powdery mildew conidia (25 conidia mm^{-2}) using a spore settling tower[13] 1, 2, 3, 4, 7 and 12 days after treatment of the first leaf, depending on the experiment (see figure legends for details). Infection intensity was assessed 10 days after inoculation by estimating the percentage of leaf area infected, using a standard area diagram. Sporulation usually occurred 6–7 days after inoculation.

Seeds were soaked in 25 mM phosphate for 5, 15, 30, 60, 120, 180 or 360 min and sown as described above. Once the resulting seedlings had reached growth stage (GS) 12, the second leaf was challenge-inoculated with powdery mildew conidia as described above, and the inoculated plants were then left for a further 10 days before mildew infection was assessed. For root drench experiments, plants at GS 12 were given a 100 ml root drench of 25 mM potassium phosphate 1, 2, 3, 4 or 5 days pre- and post-inoculation of the second leaf with powdery mildew. Infection intensity was assessed 10 days after inoculation.

2.3 Enzyme activity in plants treated with potassium phosphate

The first leaves of plants at GS 12 were treated with 25 mM potassium phosphate as described above or with distilled water containing Tween 20 (0.1 ml litre^{-1}). The second leaves of half of the plants were inoculated with powdery mildew 2 days later. Thereafter, second leaves of plants treated with potassium phosphate but not inoculated were harvested 1, 2, 3, 4, 7 or 12 days later, while second leaves of plants treated with potassium phosphate and inoculated with powdery mildew 2 days later were harvested starting at 1 day following inoculation, ie 3, 4, 7 or 12 days after treatment with potassium phosphate. All harvested leaves were frozen in liquid nitrogen for subsequent analysis.

PAL was assayed as described by Southerton and Deverall.[10] Briefly, a sample of leaf tissue (250 mg) was ground in a cold mortar and pestle containing sodium borate buffer (0.1 M, pH 8.8; 2.5 ml). The homogenate (1.5 ml) was transferred to a centrifuge tube and supplemented borate buffer (0.75 ml) containing β-mercaptoethanol (3 mM) and EDTA (3 mM) was added to the homogenate and mixed thoroughly. The tube was then centrifuged at 20 000*g* for 15 min at 4 °C. The supernatant was used as the enzyme extract and samples were kept on ice at all times.

Leaf extract (300 µl) was incubated for 2 h at 40 °C with borate buffer (0.6 ml) containing L-phenylalanine (0.6 µM). A blank with no L-phenylalanine was treated similarly. The reaction was then stopped by adding hydrochloric acid (6 M; 100 µl) and the mixture extracted by adding chloroform (1.0 ml) and mixing thoroughly on a vortex mixer. This was then centrifuged at 1300*g* for 5 min at 4 °C. A sample (0.5 ml) was then taken from the lower (chloroform) phase, the solvent evaporated by blowing nitrogen over the sample and the residue was then redissolved in borate buffer (0.1 M, pH 8.8; 0.1 ml). Absorbance was measured at 270 nm to estimate the amount of cinnamic acid produced, the result being compared with a standard curve produced using 0.1–10 µg ml^{-1} cinnamic acid. Enzyme activity was expressed as µg cinnamic acid (mg protein)$^{-1}$ h^{-1}.

Peroxidase activity was assayed as described by Southerton and Deverall.[10] Leaf tissue (250 mg) was ground in a cold mortar and pestle with sodium borate buffer (0.1 M, pH 8.8; 2.5 ml). A further 0.75 ml of buffer was then added and thoroughly mixed with the leaf preparation. A sample (1.5 ml) of this was transferred to a centrifuge tube and centrifuged at 20 000*g* for 15 min at 4 °C. The supernatant was used as the enzyme extract and samples were kept on ice until required in the assay. Enzyme extract (10 µl) was added to supplemented phosphate buffer (0.1 M, pH 7.0; 2.5 ml) containing guaiacol (0.9 µM) and hydrogen peroxide (0.36 µM). Absorbance at 470 nm was then recorded over 2 min at 25 °C in order to follow the conversion of guaicol to tetraguaiacol and enzyme activity expressed as the increase in absorbance (mg protein)$^{-1}$.

Lipoxygenase (LOX) was assayed as described by Ocampo *et al*.[14] Briefly, enzyme extracts were prepared by grinding leaf tissue (250 mg) with pre-cooled potassium phosphate buffer (0.1 M, pH 7.5;

2.5 ml) containing EDTA (1 mM), in a pre-cooled mortar and pestle. Samples were then centrifuged at 28 000*g* for 15 min at 2 °C and the supernatant used as the enzyme extract. The reaction mixture consisted of citrate phosphate buffer (0.1 M, pH 6.2; 1 ml) and substrate solution (100 µl), prepared using linoleic acid (2.5 mM) in phosphate buffer, (0.05 M, pH 9.0) containing Tween 20 (0.25 ml litre^{-1}). The reaction was initiated by the addition of enzyme extract (20 µl) and the formation of lipid hydroperoxide was followed for 15 min at 234 nm. Enzyme activity was expressed as increase in absorbance (mg protein)$^{-1}$.

2.4 Growth analysis

Plants were grown as described above, and at GS 13 the lower three leaves were painted with potassium phosphate (25 mM) or with distilled water containing Tween 20 (0.1 ml litre^{-1}) (controls). The second leaves of half of the plants were inoculated with powdery mildew 2 days later. Thereafter, second leaves of plants treated with potassium phosphate but not inoculated were harvested 1, 2, 3, 4, 7 or 12 days later, while second leaves of plants treated with potassium phosphate and inoculated with powdery mildew 2 days later were harvested starting at 1 day following inoculation, ie 3, 4, 7 or 12 days after treatment with potassium phosphate. The fourth leaves of a half of the plants were inoculated with powdery mildew 2 days later. Ten plants per treatment were harvested one week after treatment with potassium phosphate and at weekly intervals thereafter for a period of 8 weeks. Plants were separated into shoots and roots and root material was washed to remove extraneous matter. Roots were then blotted dry with absorbent tissue and root and shoot fresh weights (*FW*) determined immediately. Quickly thereafter, the total leaf area of each plant (*La*) was measured using a leaf area meter (Delta T Devices, UK). Plant material was then put into separate paper bags, labelled and oven dried at 95 °C for 2 days. The plant material was then re-weighed and the dry weights (*DW*) recorded. From these measurements it was possible to calculate the following:

(1) *FW*/*DW* ratio
(2) Root/shoot ratio (ratio of root *DW* to shoot *DW*; R:S)
(3) Relative growth rate (RGR; mg mg^{-1} day^{-1}) = $\ln[DW]_2 - \ln[DW]_1/T_2 - T_1$
when T_2 and T_1 refer to harvest times
(4) Net assimilation rate (NAR; mg cm^{-2} day^{-1})
$= [DW]_2 - [DW]_1/La_2 - La_1$
$\times \ln[La]_2 - \ln[La]_1/T_2 - T_1$
(5) Leaf area ratio (LAR; cm^{-2} mg^{-1})
$= La_1 + La_2/DW_1 + DW_2$
(6) Specific leaf area (SLA) = La/DW(leaf)
(7) Leaf weight ratio (LWR)
= DW(leaf)/DW(whole plant)

2.5 Field trial

Spring barley (cv Golf) was sown at 12-cm row spacing with a seed rate of 190 kg ha^{-1}. Seed was sown in plots (4.0 × 2.0 m) in a randomised block design with four replicates. Plots were sprayed with potassium phosphate (25 mM) or tebuconazole 250 g litre^{-1} EW (Bayer) applied at rate of 1 litre ha^{-1} at the first sign of mildew or at GS 31 (first node detectable), and again at GS45 (boots swollen). Control plots were left unsprayed. Potassium phosphate was applied using an A20 compressed air sprayer with a delivery rate of 50 ml s^{-1} and a spray boom width of 1.8 m. Ten plants per plot were assessed for the percentage powdery mildew infection 7 and 14 days after each spray (although only the data for infection of the flag leaf at 14 days after spraying are shown in Table 1). At the end of the trial, plants were harvested and measurements made of grain weight, plant height and plant dry weight. Data were analyzed by analysis of variance.

3 RESULTS

3.1 Induction of systemic protection by potassium phosphate

In glasshouse tests, potassium phosphate used on first leaves at concentrations ranging from 10–50 mM significantly reduced powdery mildew infection of second leaves (Fig 1). The greatest reductions in mildew infection were obtained with 10 and 25 mM potassium phosphate, which led to reductions of 84 and 89% respectively. These reductions, although statistically different from the control ($P < 0.01$), were not significantly different from each other (Fig 1). In contrast, increasing potassium phosphate concentration to 35 mM resulted in only a 31% reduction in powdery mildew infection (Fig 1). Although the greatest reduction in mildew infection was obtained when the second leaves were inoculated 2 days following treatment of the first leaves with phosphate, powdery mildew infection was still reduced by 42% if the interval between treatment and inoculation was increased to 12 days (Fig 2). Altering day-length also had a substantial effect on the induction of systemic protection by potassium phosphate. Thus, when plants were grown under a 16-h photoperiod, treatment of the first leaves with

Table 1. Effect of treatment with potassium phosphate on powdery mildew infection, plant height, plant dry weight and grain yield of spring barley in a field trial

Treatment	% mildew infection (flag leaf)	Plant height[a] (cm)	Plant dry weight[a] (g)	Grain yield (kg la plot)
Untreated	21.2	75.9	20.8	8.7
Potassium phosphate	6.4	72.4	30.2	9.8
Tebuconazole[b]	3.1	73.6	28.1	9.6
LSD ($P < 0.05$)	4.1	9.2	3.5	1.2

[a] Means of data for 10 plants per plot and four plots.
[b] Applied at 250 g AI litre^{-1} ha^{-1}.

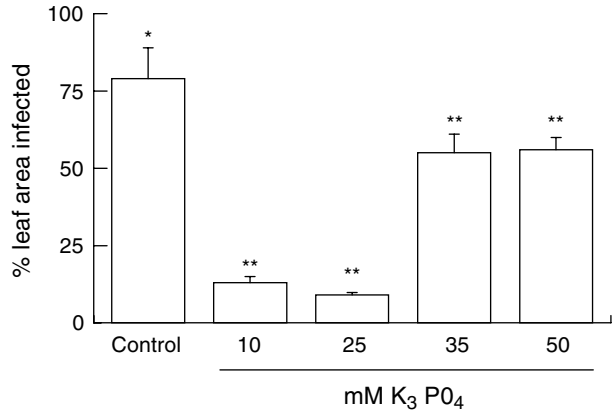

Figure 1. Effect of treating the first leaf of barley with various concentrations of potassium phosphate (K_3PO_4) on the infection of second leaves with powdery mildew. Second leaves were challenge-inoculated with powdery mildew 24 h after treatment of the first leaves with potassium phosphate. Values are the means of 10 replicates (±SE) and significant differences from the control are shown at $^{**}P < 0.01$.

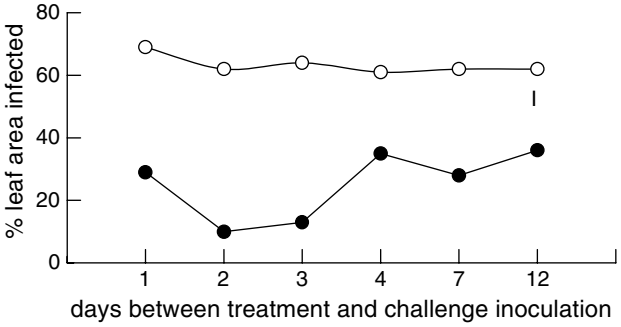

Figure 2. Effect of varying the time interval between treatment of the first leaf of barley with 25 mM potassium phosphate and challenge inoculation of the second leaf, on infection of the second leaves with powdery mildew. (○) control, (●) inoculated with powdery mildew. Values are the means of 10 replicates. SED = 1.3 (36 *df*).

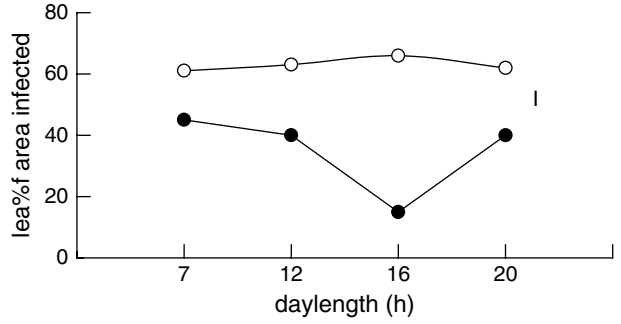

Figure 3. Effect of varying the photoperiod on infection of second leaves of barley with powdery mildew following prior treatment of the first leaves with 25 mM potassium phosphate. (○) control, (●) inoculated with powdery mildew. Values are the means of 10 replicates. SED = 1.5 (9 *df*).

25 mM potassium phosphate led to a 78% reduction in powdery mildew infection on the second leaves, while reducing the photoperiod to 12 h or increasing it to 20 h resulted in only a 36–37% reduction in mildew infection (Fig 3).

Soaking seeds for as little as 5 min in 25 mM potassium phosphate led to a 28% reduction in powdery mildew infection on second leaves of barley, compared with controls (Fig 4). This reduction in mildew infection was increased to 39% following a 30-min seed soak, but paradoxically, increasing the seed soak to 60 min and longer eliminated this effect and no reduction in mildew infection was observed (Fig 4). Application of 25 mM potassium phosphate to roots of barley seedlings at various times before or after inoculation of second leaves also reduced powdery mildew infection, although in most cases this was not significant (Fig 5). The exception to this was application of phosphate to roots 3 days following inoculation of second leaves, which led to a 30% reduction in powdery mildew infection (Fig 5).

3.2 Effect of phosphate treatment on enzyme activities

Treatment of the first leaves of barley seedlings with 25 mM potassium phosphate led to significant increases in the activities of PAL and peroxidase in second leaves, up to 12 days following treatment (Fig 6a,b). The largest increases in enzyme activity

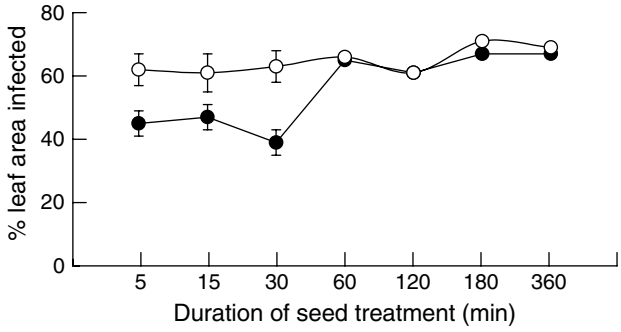

Figure 4. Effect of soaking barley seeds for different times in 25 mM potassium phosphate on powdery mildew infection of the second leaves. (○) control, (●) inoculated with powdery mildew. Values are the means of 10 replicates (±SE).

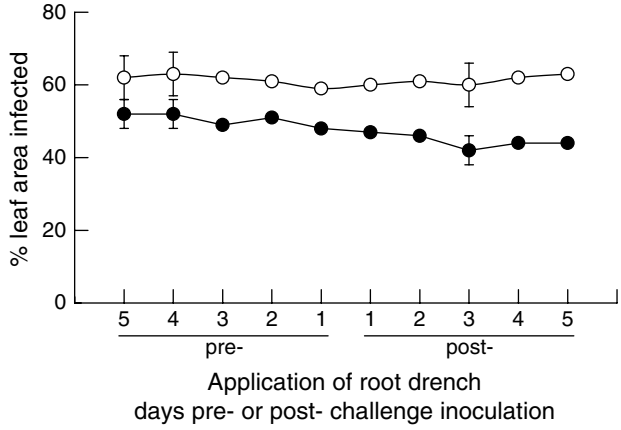

Figure 5. Effect of applying 25 mM potassium phosphate as a root drench on infection of second leaves of barley with powdery mildew. Root drenches were applied at different times prior to or post inoculation. (○) control, (●) inoculated with powdery mildew. Values are the means of 10 replicates (±SE).

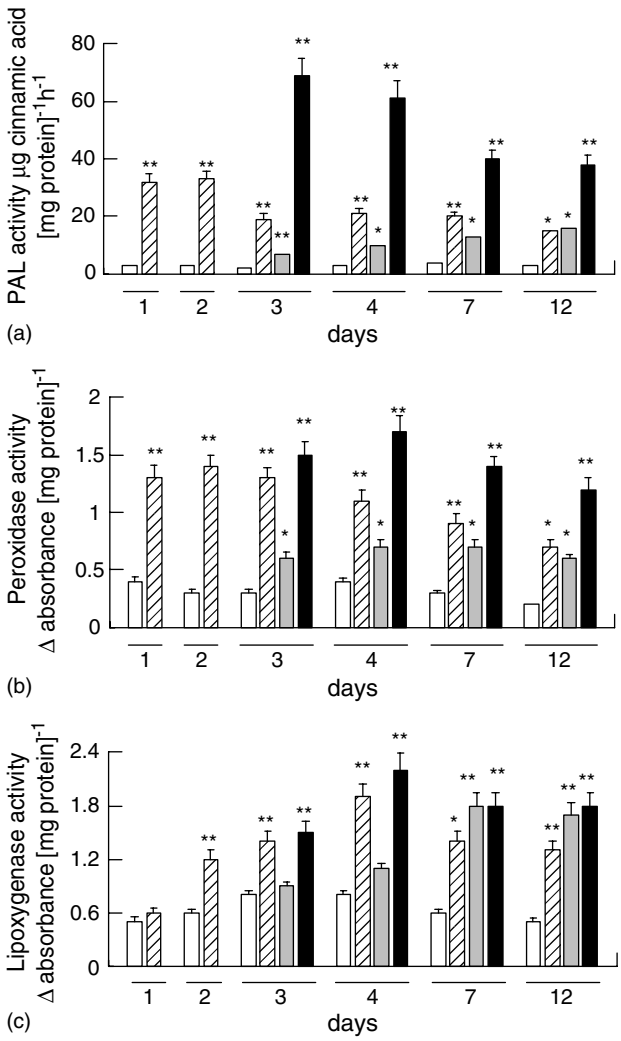

Figure 6. Effect of applying 25 mM potassium phosphate to first leaves on (a) phenylalanine ammonia lyase, (b) peroxidase and (c) lipoxygenase (c) activities in second leaves of barley. □ control; ▨ 25 mM potassium phosphate; ▩ second leaves inoculated with powdery mildew, but no potassium phosphate treatment; ■ second leaves inoculated with powdery mildew 2 days after treatment of first leaves with potassium phosphate. Values are the means of four replicates (±SE) and significant differences from the untreated control are shown at $^{*}P < 0.01$ and $^{**}P < 0.001$.

were observed 1 and 2 days following treatment, with, for example, 11-fold and 4.6-fold increases in PAL and peroxidase activities detected 2 days after treatment (Fig 6a,b). When second leaves were inoculated with mildew, small but significant increases in PAL and peroxidase activities, compared with untreated controls, were observed the following day (day 3 in Fig 6ab), with the greatest increases occurring 9 days later (day 12 in Fig 6a,b). The highest activities of PAL and peroxidase were observed in mildew-infected second leaves on plants where the first leaves had been treated with 25 mM potassium phosphate (Fig 6a,b). When second leaves were inoculated with powdery mildew 2 days after treatment of the first leaves with phosphate, PAL activity 1 day later (ie. day 3 in Fig 6a) had increased 3.6-fold compared to non-inoculated second leaves on phosphate treated plants. Activities of PAL and peroxidase in mildew-inoculated second leaves of phosphate-treated plants were still greater than for any other treatment at the end of the experiment (12 days following treatment of the first leaves with potassium phosphate; Fig 6a,b).

Lipoxygenase activity was also increased in second leaves of barley following treatment of the first leaves with 25 mM potassium phosphate (Fig 6c). The increase in LOX activity started 2 days after phosphate application to the first leaves and was still detectable 12 days following treatment. Inoculation of second leaves on plants where the first leaves had not been treated with phosphate also increased LOX activity, but significant increases were not observed until day 7 (Fig 6c). When second leaves on phosphate-treated plants were inoculated with mildew, the only further increase in LOX activity was detected on day 4 (Fig 6c).

3.3 Effect of phosphate treatment on plant growth

Application of 25 mM potassium phosphate to first leaves of barley led to significant increases in shoot dry weight, starting 2 weeks after treatment and continuing until the end of the experiment at 6 weeks following treatment (Fig 7a). Mildew inoculation of second leaves of plants that had received no phosphate treatment led to reductions in shoot dry weight compared to untreated controls. When second leaves of phosphate-treated plants were inoculated with powdery mildew, shoot dry weight was significantly greater than with inoculated plants that had not received phosphate treatment for most of the experiment. Six weeks after the start of the experiment, shoot dry weight was 33% greater in second leaves on phosphate-treated and inoculated plants than in inoculated plants receiving no phosphate treatment (Fig 7a).

Treatment of the first leaves of barley seedlings with 25 mM potassium phosphate had no significant effect on root dry weight over the 6-week experimental period (Fig 7b). When second leaves of non-phosphate-treated plants were inoculated with mildew, significant reductions in root dry weight were observed in weeks 5 and 6 compared with untreated controls. Root dry weight was not altered significantly in phosphate-treated, mildew-inoculated plants (Fig 7b).

Potassium phosphate application to first leaves had no significant effect on leaf area over the experimental period (Fig 7c). Powdery mildew inoculation of second leaves on non-phosphate-treated plants led to a reduction in leaf area in week 2 and increases in leaf area in weeks 3 and 4. The effects were very similar for plants when the first leaves had been treated with phosphate and the second leaves inoculated with mildew (Fig 7c).

Growth analyses based on the data described above showed that leaf area ratio (LAR) was reduced in phosphate treated plants compared to untreated controls over the entire experimental period, although

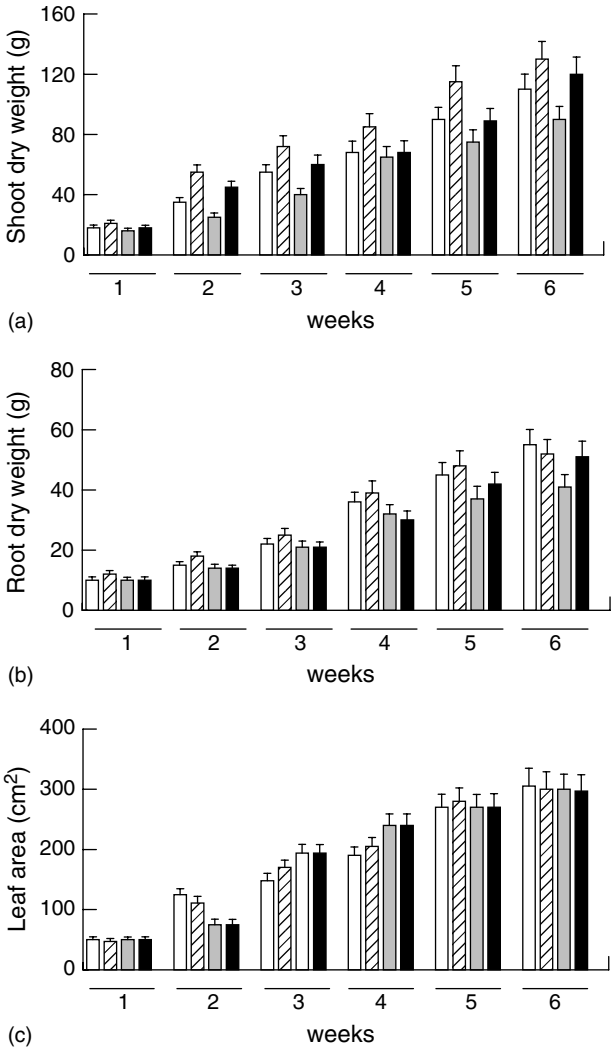

Figure 7. Effect of applying 25 mM potassium phosphate to the first leaves of barley on (a) shoot dry weight, (b) root dry weight and (c) leaf area of barley plants over the subsequent 6 weeks. □ control; ▨ 25 mM potassium phosphate; ▩ second leaves inoculated with powdery mildew, but no potassium phosphate treatment; ■ second leaves inoculated with powdery mildew 2 days after treatment of first leaves with potassium phosphate. Values are the means of 10 replicates (±SE).

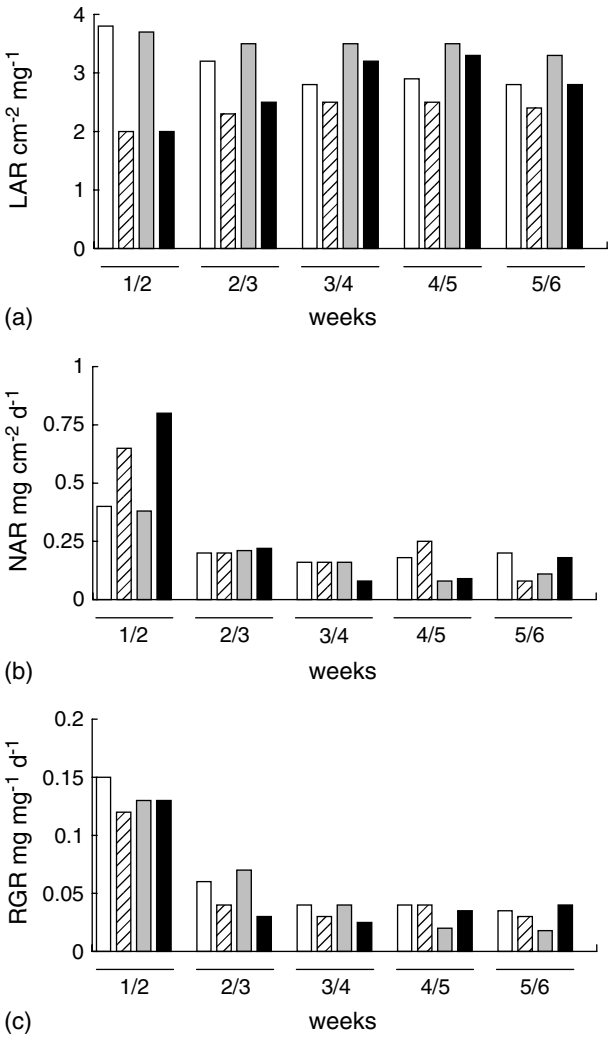

Figure 8. Growth analysis of plants where the first leaf was treated with 25 mM potassium phosphate. (a) leaf area ratio (b) net assimilation rate (c) relative growth rate □ control; ▨ 25 mM potassium phosphate; ▩ second leaves inoculated with powdery mildew, but no potassium phosphate treatment; ■ second leaves inoculated with powdery mildew 2 days after treatment of first leaves with potassium phosphate. Values are the means of 10 replicates.

the magnitude of this effect decreased as the experiment progressed (Fig 8a). Mildew inoculation of non-phosphate-treated plants resulted in a small increase in LAR compared to untreated controls over most of the experimental period. Powdery mildew inoculation of phosphate-treated plants led to reduced LAR ratio during the first three weeks compared with untreated controls, but this effect disappeared later in the experiment (Fig 8a).

During the first 2 weeks of the experiment net assimilation rate (NAR) was increased in phosphate-treated barley seedlings compared to untreated controls (Fig 8b). However, this effect was not observed subsequently and indeed at the end of the experiment, NAR in these plants was substantially lower than in untreated plants (Fig 8b). Mildew inoculation of second leaves of plants that had not received phosphate treatment produced no effect on NAR until week 5, when NAR was reduced substantially compared with untreated controls (Fig 8b). In phosphate-treated, mildew-inoculated plants, NAR was greater than for any other treatment during the first 2 weeks of the experiment, but by week 4 it was lower than for any other treatment, and during weeks 5 and 6 NAR in these plants was reduced compared with untreated controls.

The net effect of these changes in LAR and NAR was that the relative growth rate (RGR) was slightly reduced in phosphate-treated plants compared to untreated controls for the first 4 weeks of the experiment, but recovered thereafter (Fig 8c). Mildew infection of non-phosphate-treated plants had little effect on RGR until weeks 5 and 6, when it was a half of that calculated for untreated controls (Fig 8c). RGR in phosphate-treated, mildew-inoculated plants was lower than values calculated for untreated controls for the first 4 weeks of the experiment, but during the

final 2 weeks of the experiment, RGR in these plants was similar to that in untreated controls (Fig 8c).

3.4 Field trial

Application of 25 mM potassium phosphate to spring barley in the field at GS 31 and again at GS 45 led to 70% reduction in powdery mildew infection on the flag leaf. Tebuconazole applied at the same times led to 86% reduction in mildew infection on the flag leaf. Treatment with potassium phosphate did not produce a significant effect on plant height or plant DW compared with the control, and, although the effect on grain yield was not statistically significant, it was increased by 12% compared with the untreated control (Table 1). Treatment with tebuconazole increased grain yield by 10% compared with the untreated control.

4 DISCUSSION

The data reported here show that powdery mildew infection of the second leaves of barley seedlings can be reduced by prior treatment of the first leaves with potassium phosphate. This agrees with previous work on cucumber and barley which showed that treatment of the lower leaves with phosphate induced systemic protection in the upper leaves against anthracnose and powdery mildew respectively.[3,13] It also agrees with work which showed that a single application of 0.1 M phosphate induced systemic protection against powdery mildew in cucumber caused by *Sphaerotheca fuliginea* Poll and against common rust in maize caused by *Puccinia sorghi* Schw and northern leaf blight caused by *Exserohilum turcicum* Leonard + Suggs.[5] The results presented here also show, for the first time, that treating seeds with potassium phosphate, even for 5 min, leads to a reduction in powdery mildew infection in second leaves. The nature of the signal induced by phosphate treatment is not known. However, as indicated above, it has been suggested[3] that the effect of phosphates might be due to sequestration of calcium, thereby disrupting cell membranes and eliciting production of an 'alarm signal'. In the present work it is also not known whether the same signal(s) are responsible for the protection induced following treatment of lower leaves, seeds or roots.

Phenylalanine ammonia lyase catalyses the first committed step in phenylpropanoid synthesis and provides phenolics for, among other activities, plant defence. PAL activity is known to increase in interactions between barley and powdery mildew and the phenolic compounds that are synthesized as a result accumulate in papillae and cell wall haloes, and throughout the cytoplasm and walls of dead or dying cells.[15] It has been suggested[15] that synthesis of phenols is important in many forms of resistance to *B graminis*. Lignin biosynthesis includes the polymerisation of three cinnamoyl alcohols and is mediated by the peroxidase–hydrogen peroxide system. Cell-wall bound peroxidases are probably involved not only in the oxidative polymerization of hydroxylated cinnamoyl alcohols but also in the generation of hydrogen pereoxide necessary for lignification.[16] In the present work, treatment of the first leaves of barley seedlings with phosphate led to significant increases in the activities of PAL and peroxidase in second leaves. The activities of PAL and peroxidase were increased further when second leaves on phosphate-treated plants were inoculated with powdery mildew. Significant increases in activities of PAL and peroxidase were still detectable 12 days following treatment of the first leaves with phosphate. Similar results were reported by Irving and Kuc[17] who found that activities of peroxidase and chitinase were increased in second leaves of cucumber plants when the first leaves had been sprayed with 50 mM potassium hydrogen phosphate. They also showed that, when second leaves of cucumber were challenge-inoculated with *C lagenarium*, there was a further increase in peroxidase and chitinase activities. It was suggested[17] that, in the cucumber/*C lagenarium* system, systemic induced resistance resembled passive resistance, since it relies upon increased levels of enzyme activity associated with plant defence in a manner similar to physical barriers. It was also suggested[17] that since activities of peroxidase and chitinase continued to increase after challenge inoculation, an active response was also involved. In the present work, activities both PAL and peroxidase had increased by one day after phosphate treatment and increased further upon challenge inoculation, which is similar to the events described in cucumber/*C lagenarium*.[17] These results for the induction of systemic protection using phosphate appear to be different from systemic resistance induced by, for example, the synthetic compound *S*-methyl benzo[1,2,3]thiadiazole-7-carbothioate (acibenzolar-S-methyl; BTH; Bion®). Thus, although the latter induces systemic protection in wheat against powdery mildew infection, increases in activities of PAL, peroxidase and cinnamoyl alcohol dehydrogenase appeared to be increased only following challenge inoculation.[18,19] A similar situation was found in cowpea, where treatment of seeds with BTH led to increased resistance to *Colletotrichum destructivum* O'Gara in shoots, with increased in activities in PAL and chalcone isomerase observed only in challenged hypocotyls and not in induced but unchallenged tissues.[20] Results presented in this paper suggest that treatment of barley with potassium phosphate triggers activities of enzymes associated with plant defence responses (ie alters constitutive resistance) rather than potentiating an early defence response. The trigger for these changes in barley is not known, but it is interesting to note the recent work of Orober *et al*.[9] They found that systemic protection in cucumber, induced by treatment with potassium phosphate, led to rapid cell death, and was accompanied by an oxidative burst, indicative of a hypersensitive-like response.

Moreover, the phosphate-induced systemic protection was accompanied by local and systemic increases in salicylic acid content.[9]

The present study also shows that LOX activity is increased in second leaves of barley following treatment of the first leaves with potassium phosphate. However, unlike PAL and peroxidase activities, which had increased by 1 day following treatment, LOX activity was increased after 2 days following treatment. Furthermore, at only one point in the experiment did LOX activity increase further upon challenge inoculation with mildew (Fig 6c). These data agree with work[11] which demonstrated an increase in LOX activity in second leaves of cucumber plants following treatment of the first leaves with 50 mM potassium phosphate. LOX catalyzes the direct oxygenation of polyunsaturated fatty acids and products of LOX activity may contribute to defence reaction in various ways eg by their direct inhibition of pathogen growth and development,[21] by induction of phytoalexin accumulation,[22] or as a result of further catabolism into compounds with potential to participate in signal transduction eg jasmonic acid.[23] Whatever the role of the increased LOX activity in second leaves of phosphate-treated barley, the data provide further support for the suggestion stated above that phosphate treatment alters constitutive resistance and does not potentiate an early defence response.

Treatment of the first leaves of barley with 25 mM potassium phosphate resulted in increased shoot and root dry weights over most of the experimental period, but had little effect on leaf areas. When second leaves were subsequently inoculated with powdery mildew, although shoot dry weights were never as large as those obtained from non-inoculated, phosphate-treated plants, they were consistently greater than those obtained from mildew-inoculated plants that had not been treated with phosphate. The calculated values for LAR, LWR and SLA indicate that leaves in phosphate-treated plants may not be larger in terms of area, but they are heavier, ie they contain more dry weight than plants not treated with phosphate (eg 9% increase in LWR and 18% increase in SLA in weeks 3 and 4 in phosphate-treated plants; data not shown). This suggests that rates of photosynthesis are increased in such plants, although changes in NAR in phosphate-treated plants were not consistent over the experimental period. However, it is important to remember that NAR is a calculated indicator of the rates of net photosynthesis and dark respiration for all the leaves on the plant,[24] and that powdery mildew infection leads to increased rates of dark respiration.[25] What is required, therefore, is measurement of the rate of net photosynthesis in second leaves on phosphate-treated plants. The end result of the changes in LAR and NAR is that, although RGR of phosphate-treated, mildew-inoculated plants was less than in controls during the first four weeks of the experiment, RGR in such plants had recovered towards the end of the study, was little different from that in controls and was greater than RGR of mildew-inoculated plants that had not received phosphate treatment. When 25 mM potassium phosphate was applied to spring barley in a small field trial, it not only induced control of mildew infection on the flag leaf (70% reduction in mildew infection compared to 86% reduction obtained with a commercial fungicide) but also gave a small increase in grain yield compared to plants receiving no phosphate sprays. These data agree with work[26] which showed that not only did 100 mM potassium phosphate provide systemic protection in cucumber against the powdery mildew fungus *Sphaerotheca fuliginea* but also resulted in increased plant growth. It is interesting to compare this with data obtained by Heil *et al*[27] on the effect of the benzothiadiazole inducer of systemic acquired resistance, acibenzolar-S-methyl. These workers showed that treatment of wheat with acibenzolar-S-methyl led to reduced growth and seed set in wheat, especially under conditions of nitrogen shortage. Whether these differences between control and treated plants could be interpreted as a consequence of allocation costs, resulting from metabolic competition between processes involved in plant growth and the synthesis of defence-related compounds, is not known.

In conclusion, treatment of the first leaves of barley with 25 mM potassium phosphate led to systemic protection against powdery mildew infection in the second leaves, and this was associated with increased activities of PAL, peroxidase and LOX. It also induced significant control in the flag leaf in field trials. The fact that phosphate treatment alone led to increased enzyme activities suggests that it alters constitutive resistance and does not potentiate an early defence response. However, the precise mechanism of the induction process still remains unknown. Finally, induction of systemic protection with potassium phosphate did not have a detrimental effect on plant growth or yield in glasshouse or field experiments.

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