

# N–P–K ratio affects exudation of germination stimulants and resistance of tobacco seedlings to broomrapes

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**Abstract** Nutrient deficiency is known to aggravate the infestation of broomrape in a number of plant species. This effect is hypothesised to result from the promotion of the release of germination stimulants from the roots of the host plant. In the present study, three experiments (hydroponic culture, Petri dish culture and soil culture) were conducted to investigate the effects of macro-nutrient ratios on the production and release of germination stimulants and the resistance of host tobacco cultivars to broomrapes. The hydroponic culture study showed that nitrogen/phosphate deficiency and potassium excess increased the germination-inducing activity of root exudates on *Phelipanche aegyptiaca* and *Orobanche cumana* seeds, with two tobacco cultivars showing different responses to nutrient ratios. The production of germination stimulants was significantly increased under nitrogen- and phosphate-deficient soil conditions. In the Petri dish study, increased vulnerability to *P. aegyptiaca* infection was observed in RG tobacco seedlings under nitrogen- and potassium-deficient conditions and in WF tobacco seedlings under nitrogen- and phosphate-deficient conditions. The results showed that macro-nutrient ratios can affect both the exudation of germination stimulants and the resistance of

tobacco seedlings. Finally, we suggest that the classification of the resistance or tolerance of host species should fully consider the nutritional conditions of cultivation and the strategy of fertilisation in terms of the potential control of parasitic weeds prior to the identification of the key mechanism of parasite resistance.

**Keywords** Germination stimulants · Macro-nutrition · *Orobanche* · Resistance · Weed control

## Introduction

Broomrapes are members of a genus of over 200 species of root-parasitic herbaceous plants *Orobanchaceae* (Musselman and Press 1995). Several broomrape species are weedy, causing severe yield and quality losses in crops. These parasites damage crops by extracting water and nutrients, impairing photosynthesis and causing a phytotoxic effect within days of attachment to the host (Westwood 2013). A number of methods, such as hand-weeding, adjustment of the sowing dates, application of herbicides, selection and breed of resistant crop cultivars, inoculation with arbuscular mycorrhizal fungi and use of traps or catch crops have been proposed in broomrape-infested fields to control the parasite (Parker and Riches 1993; Dhanapal et al. 1996; Louarn et al. 2012; Ma et al. 2013). As the broomrape problem occurs most commonly in areas of poor soil fertility (Parker 2013), the application of fertiliser is considered as a direct and an easy operating strategy to improve the control of parasitic weeds in the field.

At the first phase of host-broomrape interaction, the host root releases chemical signals that stimulate the germination of the broomrape seed. Strigolactones (SLs) are the representative germination stimulants of the root-parasitic

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weeds *Orobancha* and *Striga* (Xie et al. 2010). It has been reported that nitrogen (N) and phosphorus (P) deficiency promote the production and exudation of SLs in plants. In sorghum, N-deficiency was found to increase the exudation of 5-deoxystrigol and sorgomol (Yoneyama et al. 2007). In rice, phosphate starvation dramatically increased the exudation of orobanchol and 2'-epi-5-deoxystrigol from roots to rhizosphere (Jamil et al. 2011). In tomato, phosphate starvation has been found to enhance SL content not only in the exudates but also, to a comparable extent, in root tissues (Lopez-Raez et al. 2008). These findings explain, in part, why fertiliser application can reduce the parasitic weed problem in some types of barren fields. However, nutrient status is also an essential factor that influences plant metabolism and the resistance to pathogens. It has been reported that excessive accumulation of N in the roots increases N content and asparagine formation, resulting in death in *Striga hermonthica* (Simier et al. 2006). The absorption of mineral nutrients by plants is not only related to dose response effects but also to the balance among nutrients, as toxic actions of single ions often occur in hydro-cultures and fields. Therefore, whether different nutrient application ratios affect either the exudation of germination stimulants or the resistance of the host to broomrape needs to be clarified.

In the present work, we studied the mechanism responsible for the reduction of *Orobancha* in relationship to the application ratio of N, P and potassium (K) fertilisers to host seedlings. Our hypothesis was that the application of different macro-nutrients ratios affects *Orobancha* infection either indirectly, by influencing the exudation of the germination stimulants into the rhizosphere, or directly, by altering the resistance of the host to *Orobancha*. To test this hypothesis, experiments were conducted under controlled conditions in a pot and hydro-culture system to analyse the relationship between the germination-inducing activity of host root exudates and the resistance of host seedlings under various application ratios of N, P and K, using tobacco as a model host crop.

## Materials and methods

### Experiment 1: hydroponic culture

#### *Plant materials and chemicals*

Two tobacco cultivars were used in experiment: RG11 (RG, an American cultivar) and Majiang Baihua (WF, a Chinese native cultivar). Seeds of the cultivars were kindly provided by the Chinese Central Southern Test Station of Tobacco Agriculture, Changsha, Hunan, China. Seeds of *Phelipanche aegyptiaca* and *Orobancha cumana* were

collected from tomato and sunflower fields, respectively, in the Xinjiang Uygur Autonomous Region of China in 2009. The field study did not involve endangered or protected species. Seeds of *P. aegyptiaca* and *O. cumana* were stored in a refrigerator at 4 °C before use. Synthetic strigolactone, GR24, was kindly supplied by Professor B. Zwanenburg, Radboud University, The Netherlands.

#### *Collection of tobacco root exudates*

The seeds used in the experiment were uniformly surface-sterilised by immersion in 1 % NaClO for 3 min, then in 75 % (V/V) ethanol for 3 min. The seeds were then rinsed five times with autoclaved distilled water and air-dried on a clean bench. After germination (25 °C in the dark for 5 days in Petri dishes), 54 tobacco seedlings were transferred to a strainer (36 cm × 26 cm × 8 cm) lined with a sheet of gauze that had been moistened by placing it in a slightly larger container (42 cm × 30 cm × 11 cm) containing 4 L of half-strength Tadano and Tanaka (TT) medium. The seedlings were placed in a growth chamber with a 12 h photoperiod at 120 μmol photons m<sup>-2</sup> s<sup>-1</sup> at 25 °C. The pH of the culture media was adjusted to 6.0 with H<sub>2</sub>SO<sub>4</sub> and NaOH. The TT medium was replaced every 3 days. On day 19, the medium was replaced with nutrient-deficient media (−N, −P and −K, 1/20 the strength of the elements in the TT medium, Table 1) or nutrient-excessive media (+N, +P and +K, 2 times the strength of the elements in the TT medium, Table 1). After 2 weeks of growth, the medium was circulated through an activated charcoal filter with an aquarium pump to adsorb root exudates. The medium and the charcoal filters were replaced every 3 days. At the same time, a root-exudate trapping system without seedlings was used as a system control. The root exudates were collected twice weekly.

The root exudates adsorbed onto the charcoal filters were eluted with acetone. The acetone was removed by vacuum evaporation in a rotary evaporator at 40 °C. The residue was dissolved in 50 mL distilled water and then extracted three times with 50 mL ethyl acetate (EtOAc). The EtOAc extracts were combined, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, and then evaporated to dryness under a vacuum at 35 °C using a rotary evaporator. The residues were dissolved in 5 mL acetone and then stored in sealed glass vials at 4 °C. The stored residues were diluted with distilled water to final concentrations of 100, 10, 1 and 0.1 mg L<sup>-1</sup> before use.

#### *Broomrape seed germination assays*

*P. aegyptiaca* and *O. cumana* seeds need to be conditioned before they become receptive to germination stimulants. Seed conditioning was performed using 4 mL of distilled

**Table 1** Macro-nutrient rates in the hydroponic/Petri dish culture and the soil culture media

Treatment	Hydroponic/Petri dish culture			Soil culture		
	N (mmol L <sup>-1</sup> )	P (mmol L <sup>-1</sup> )	K (mmol L <sup>-1</sup> )	N (g kg <sup>-1</sup> )	P <sub>2</sub> O <sub>5</sub> (g kg <sup>-1</sup> )	K <sub>2</sub> O (g kg <sup>-1</sup> )
CK	2.43	0.16	1.00	0.40	0.40	1.00
+N	4.86	0.16	1.00	0.80	0.40	1.00
+P	2.43	0.32	1.00	0.40	0.80	1.00
+K	2.43	0.16	2.00	0.40	0.40	2.00
-N	0.12	0.16	1.00	0.02	0.40	1.00
-P	2.43	0.01	1.00	0.40	0.02	1.00
-K	2.43	0.16	0.05	0.40	0.40	0.05

water applied to Petri dishes (9 cm diam) lined with double filter papers. Glass fiber filter discs (GFFP, 8 mm diam, Whatman GF/A) were placed on the filter paper, and approximately 30–50 broomrape seeds were then sown on each glass fiber disc. The Petri dishes with broomrape seeds were sealed with parafilm and incubated at 25 °C in the dark for 5 days.

Aliquots (20 µL) of tobacco root exudates were applied to the glass fiber discs with conditioned broomrape seeds in Petri dishes. Individual treatments were replicated five times. The Petri dishes were sealed and incubated at 25 °C. Germination rates were determined with a microscope after 10 days incubation. Broomrape seeds treated with GR24 (10<sup>-7</sup> M) and distilled water were used as positive and negative controls, respectively.

#### Experiment 2: Petri dish culture

To evaluate the effect of the macro-nutrient application ratio on the resistance of tobacco seedlings to broomrape infestation, Petri dish culture assays were conducted after the collection of the root exudates. A tobacco seedling was weighed and transplanted from the hydroculture system into a Petri dish lined with a layer of filter paper. The stem was placed through a cutout (1 cm<sup>2</sup>) on the ring of the base. Broomrape seeds, which had been induced to germinate with GR24, were sown (50–80 per cm<sup>2</sup>) on the roots of tobacco seedlings and the filter paper. Autoclaved vermiculite (30 g) was gently placed on the base. The lid was then placed on the Petri dish to cover it and fastened to the dish with waterproof adhesive tape. The Petri dishes planted with tobacco seedlings were returned to the nutrition treatment media in a vertical position at a depth of 1 cm (Fig. 1). The individual treatments were replicated 10 times. After 2 weeks, the Petri dishes were opened, and the tobacco seedlings were put into 200-mL flasks containing 150 mL distilled water and ultrasonicated for 20 min to wash off the vermiculite and the broomrape

seeds that had not infected the root. The number of parasites on each plant was determined with a microscope.

#### Experiment 3: soil culture

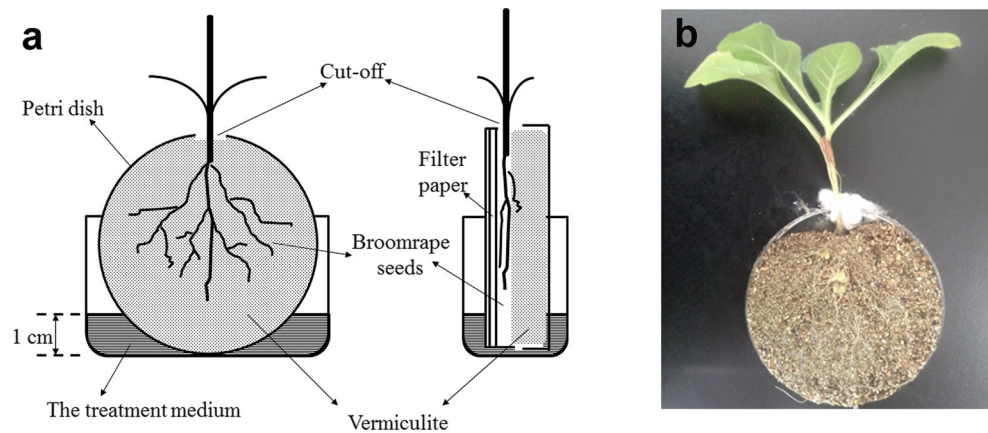
##### *Soil character and experimental setup*

Ten seeds of a given cultivar were sown in a pot containing 1 kg of soil. A total of 42 pots per cultivar were used in the macro-nutrition ratio treatments (Table 1) with NH<sub>4</sub>NO<sub>3</sub>, Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub> · H<sub>2</sub>O and K<sub>2</sub>SO<sub>4</sub> (AR, Xilong Chemical Co., Ltd, Shantou, Guangdong, China) as mineral fertilisers. The soil was a Lou soil (Typ-Eum-Orthic Anthrosol), collected from a field with a previous crop of wheat at the Institute of Soil and Water Conservation, Chinese Academy of Science, Yangling, China. The soil had the following chemical properties: pH 7.68, 13.8 g kg<sup>-1</sup> organic matter, 70.1 mg kg<sup>-1</sup> available N, 23.1 mg kg<sup>-1</sup> available P and 166 mg kg<sup>-1</sup> available K. The pots were placed in a greenhouse and watered as necessary.

##### *Sample collection and germination assays*

Plant and rhizosphere soil samples (Riley and Barber 1969, 1970) were collected 50 days after planting, when the tobacco in the control condition (CK) was at the six-leaf stage. Tissues were separated, freeze-dried, milled, and passed through a 0.35-mm sieve. The rhizosphere soil samples were analysed within a day after being collected. For each treatment, 5 g rhizosphere soil was put into a Petri dish (3.5 cm diam) and wetted with 1.5 mL distilled water. Five 8-mm discs of GFFP were placed on the soil surface. Conditioned broomrape seeds (50–70) were placed on the upper surface of each disc. The Petri dishes were sealed and incubated in the dark at 25 °C. The seeds were examined with a microscope after 10 days to determine the germination rate. Root tissues were extracted with methanol and used to test *P. aegyptiaca* and *O. cumana* seed germination. A sample (100 mg) of milled root tissue was

**Fig. 1** Schematic diagrams (a) and photograph (b) of Petri dish culture method



added to 1.5 mL centrifuge tubes, sonicated for 30 min in 1 mL of analytical grade methanol, and then centrifuged at 6,400 rpm for 2 min. The supernatants are hereafter referred to as the undiluted extracts. Aliquots (15  $\mu$ L) of the test solution (undiluted, 20-fold diluted and 400-fold diluted) were applied to 8-mm discs of GFFP, and the discs were allowed to dry. A disc with conditioned *Orobanchae* seeds was placed on top of the dried treated disc and moistened with 30  $\mu$ L deionised water. The Petri dishes were sealed and incubated at 25 °C. Germination rates were observed as described above.

#### Statistical analysis

The data were processed using Excel 2010 and DPS 9.50 software (DPS Soft Inc., Tang, Hangzhou, China). A Tukey's honest significant difference (HSD) test was used to compare the means. The germination data were arcsin transformed before analysis.

## Results

Distilled water stimulated negligible germination of *P. aegyptiaca* and *O. cumana* seeds (<8 %), whereas GR24 induced high germination rates in *P. aegyptiaca* (95.3 %) and *O. cumana* (67.2 %).

#### Germination-inducing activity of root exudates on broomrape seeds

Root exudates of RG and WF induced both *P. aegyptiaca* and *O. cumana* seeds to germinate. The germination rates of *P. aegyptiaca* seeds ranged from 9.7 to 94.9 %, decreasing with decreases in the concentration of root exudates (Table 2). At a concentration of 100 mg L<sup>-1</sup>, root exudates of RG induced a higher germination rate (>90 %) than those of WF with the exception of those in the N- or

P-deficiency treatments (-N and -P in Table 2). For WF, +N or +P significantly reduced the germination-inducing activity of root exudates on *P. aegyptiaca* seeds, whereas -N and -P increased the inducing activity compared with the control. At 1 and 10 mg L<sup>-1</sup>, the +K, -N and -P treatments resulted in the highest germination rate of *P. aegyptiaca* seeds found in the treatments. When the root exudates were diluted to 0.1 mg L<sup>-1</sup>, the germination rate of *P. aegyptiaca* seeds was halved or more than halved, but the N- and P-deficiency treatments still induced the highest germination among all treatments. It was evident that the +P treatment significantly reduced the inducing activity of WF root exudates on *P. aegyptiaca* seeds at a concentration of 0.1 mg L<sup>-1</sup>.

The germination rates of *O. cumana* seeds ranged from 0 to 51.2 % (Table 3). These values were much less than the corresponding rates for *P. aegyptiaca*. At concentrations of 10 and 100 mg L<sup>-1</sup>, the +K treatment induced the highest germination of *O. cumana* seeds among the treatments, with WF showing a higher inducing activity than RG. After the root exudates were diluted to 1 mg L<sup>-1</sup>, the difference between the two cultivars increased. The germination rates of *O. cumana* seeds induced by RG were below 10 %, and the difference between treatments disappeared. However, the germination-inducing activity of WF root exudates under the +K, -N and -P treatments was still >40 %, significantly higher than the control and the other treatments (Table 3). At 0.1 mg L<sup>-1</sup>, the root exudates of RG did not induce the *O. cumana* seeds to germinate, whereas WF under the -N and -P treatments still induced significantly higher germination (>30 %) than the other treatments (Table 3).

#### Germination-inducing activity of root tissue extracts on broomrape seeds

The germination rate of *P. aegyptiaca* and *O. cumana* seeds applied with 20-fold dilutions of the root tissue

**Table 2** Germination-inducing activity of root exudates of tobacco seedlings cultivars under various ratios of macro-nutrient to *P. aegyptiaca* seeds (%)

Cultivar	Nutrient level	Concentration of root exudates (mg L <sup>-1</sup> )			
		100	10	1	0.1
RG	CK	91.9 (76.0) a	85.1 (67.4) abc	63.2 (52.7) cde	30.4 (33.3) cde
	+N	90.5 (72.2) ab	81.2 (64.7) bcd	61.2 (51.5) de	31.6 (34.2) cde
	+P	92.0 (74.3) a	85.5 (67.7) abc	60.9 (51.3) de	29.5 (32.7) def
	+K	90.4 (72.0) ab	91.0 (73.1) a	89.3 (71.0) a	42.2 (40.5) abc
	-N	92.2 (74.3) a	87.6 (72.4) ab	71.1 (57.5) bcd	32.7 (34.9) cde
	-P	91.5 (73.2) a	93.6 (75.5) a	76.2 (60.8) bc	36.9 (37.4) bcde
	-K	94.9 (77.1) a	88.0 (70.5) ab	62.5 (52.3) de	26.3 (30.8) ef
WF	CK	72.1 (58.3) ef	77.7 (61.9) cd	41.0 (39.8) g	24.2 (29.4) ef
	+N	83.9 (66.4) bc	75.1 (60.2) d	44.2 (41.5) fg	21.8 (27.8) f
	+P	67.5 (55.3) f	65.6 (54.1) f	36.4 (37.1) g	9.7 (17.8) g
	+K	79.7 (63.4) cd	85.5 (67.7) abc	56.3 (48.6) ef	50.0 (45.0) ab
	-N	94.2 (76.2) a	90.4 (72.1) a	61.0 (51.3) de	43.6 (41.3) abc
	-P	96.4 (80.7) a	92.7 (74.5) a	82.0 (65.0) ab	51.3 (45.8) a
	-K	76.2 (60.9) de	81.3 (64.4) bcd	64.6 (53.5) cde	21.7 (27.7) f

Different letters indicate significant differences between means (arcsin) of treatments at  $P < 0.05$  (Tukey's HSD)

**Table 3** Germination-inducing activity of root exudates of tobacco seedlings cultivars under various ratios of macro-nutrient to *O. cumana* seeds (%)

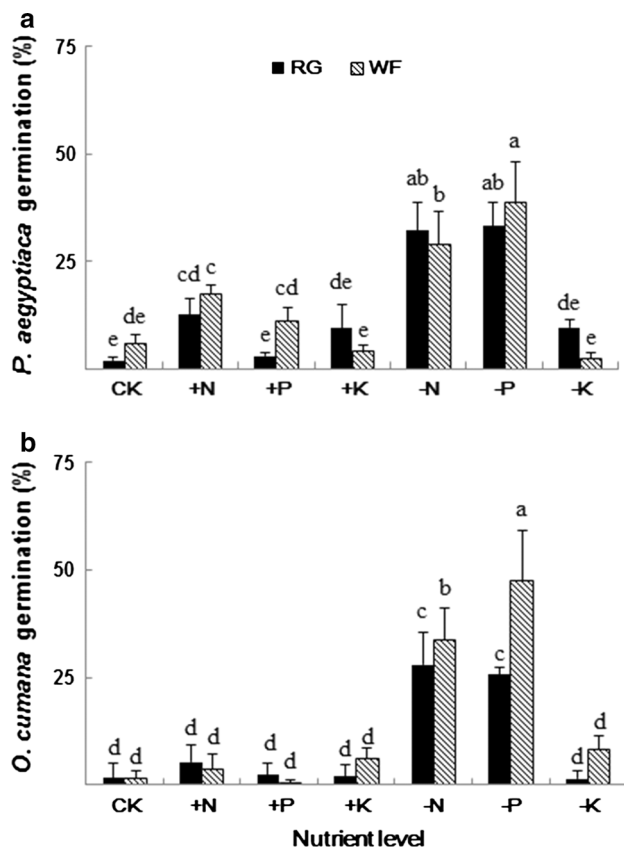
Cultivar	Nutrient level	Concentration of root exudates (mg L <sup>-1</sup> )			
		100	10	1	0.1
RG	CK	36.8 (37.3) b	33.1 (35.1) cd	7.5 (15.8) c	0
	+N	35.2 (36.4) b	29.5 (32.9) d	6.2 (14.3) c	0
	+P	38.5 (38.3) ab	35.1 (36.3) cd	7.2 (14.8) c	0
	+K	49.0 (44.4) ab	41.9 (40.3) bc	9.2 (17.5) bc	0
	-N	42.3 (40.6) ab	36.1 (36.9) bcd	6.7 (14.9) c	0
	-P	44.9 (42.1) ab	42.6 (40.8) bc	7.5 (15.5) c	0
	-K	46.1 (42.8) ab	22.5 (22.8) e	4.6 (11.8) c	0
WF	CK	37.0 (37.4) b	27.3 (31.4) d	6.0 (14.1) c	4.3 (4.2) c
	+N	42.4 (40.6) ab	28.3 (32.1) d	15.1 (22.8) b	7.0 (6.7) c
	+P	40.7 (39.6) ab	28.5 (32.2) d	11.8 (19.8) bc	5.6 (5.3) c
	+K	51.2 (45.7) a	54.4 (47.5) a	48.0 (43.8) a	15.9 (15.4) b
	-N	47.7 (43.7) ab	44.7 (41.9) b	42.8 (40.8) a	31.4 (31.1) a
	-P	46.6 (43.1) ab	45.4 (42.2) b	41.2 (39.9) a	33.4 (33.3) a
	-K	45.5 (42.4) b	34.4 (35.8) cd	11.2 (18.7) bc	4.2 (4.2) c

Different letters indicate significant differences between means (arcsin) of treatments at  $P < 0.05$  (Tukey's HSD)

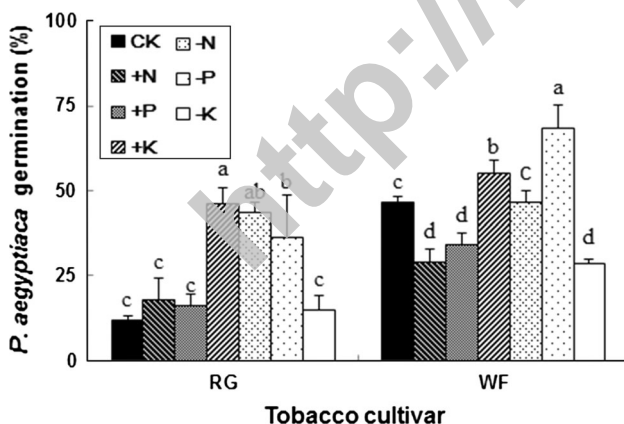
extracts ranged between 1 and 47 % (Fig. 2), whereas the undiluted and 400-fold diluted root extracts were unable to induce germination in either the *P. aegyptiaca* seeds or the *O. cumana* seeds. Root extracts of the seedlings under the -N and -P treatments induced significantly higher germination rates of *P. aegyptiaca* and *O. cumana* seeds than those resulting from the other treatments. However, the germination rates of both *P. aegyptiaca* and *O. cumana* seeds did not differ significantly between the +K treatment and the nutrient balance control in either cultivar (Fig. 2a, b), or between the +N/+P treatments and the nutrient balance control for WF.

Germination-inducing activity of the rhizosphere soil on broomrape seeds

The germination rates of *P. aegyptiaca* seeds in the rhizosphere soil bioassays ranged between 11.7 and 68.4 % depending on the cultivars and nutrient levels. WF generally induced a higher germination rate of *P. aegyptiaca* seeds than RG (Fig. 3). Rhizosphere soils of RG under the -N and -P treatments induced higher germination rates than those under the +N, +P treatments and control, whereas the rhizosphere soils of WF under the +K and -P treatments induced significantly higher germination rates



**Fig. 2** Germination of *P. aegyptiaca* (a) and *O. cumana* (b) seeds induced by the root tissue extracts of tobacco seedlings cultivars RG and WF. The different letters indicate significant differences between treatments at  $P < 0.05$  (Tukey's HSD)



**Fig. 3** Germination of *P. aegyptiaca* induced by the rhizosphere soils from two cultivars. Different letters indicate significant differences between treatments at  $P < 0.05$  (Tukey's HSD)

of *P. aegyptiaca* seeds than those under the control. It was interesting that N and P excess (+N and +P in Fig. 3) significantly reduced the inducing activity of rhizosphere soil of WF on *P. aegyptiaca* seeds. For RG, however, the N

and P excess treatments did not reduce the inducing activity of the rhizosphere soil of RG. However, the rhizosphere soil from RG seedlings and that from WF seedlings did not induce the germination of *O. cumana* seeds.

#### Biomass of tobacco seedlings and broomrape attachment numbers

Both the cultivar and the nutrient application ratio affected the biomass of the seedlings. Nitrogen or phosphorus excess (+N and +P in Table 4) significantly increased the fresh weight of RG, whereas seedlings of WF under the potassium deficiency or phosphorus excess conditions had the highest biomass. In contrast, nitrogen or phosphorus deficiency significantly reduced the biomass of the RG and WF seedlings compared with the control. The RG and WF seedlings responded differently to potassium levels. Potassium excess significantly decreased the fresh weight of the WF seedlings, whereas RG seedling growth was not suppressed. It was hypothesised that the nutrient requirements of the two tobacco cultivars differed.

As the rhizosphere soils of two tobacco cultivars did not induce the germination of *O. cumana* seeds, the infection test was only conducted on *P. aegyptiaca*. Nitrogen and phosphorus deficiency significantly increased the levels of infection of *P. aegyptiaca* on the tobacco seedlings (Table 4). However, an unexpected result was that neither nitrogen nor phosphorus excess clearly decreased the infection of *P. aegyptiaca*. Furthermore, the ratio of

**Table 4** Biomass and broomrape attachment numbers of tobacco seedlings

Cultivar	Nutrient level	Biomass (M) [g FW (5 plants) <sup>-1</sup> ]	Attachment number (AN) (No. plant <sup>-1</sup> )	AN:M (No. g <sup>-1</sup> FW plant <sup>-1</sup> )
RG	CK	3.5 c	8 b	11 d
	+N	4.4 b	13 b	15 cd
	+P	7.4 a	11 b	8 d
	+K	3.7 bc	10 b	14 cd
	-N	1.9 d	29 a	78 a
	-P	2.5 d	11 b	22 c
	-K	2.0 d	14 b	36 b
WF	CK	20.5 bc	17 c	4 c
	+N	14.6 cd	13 c	5 c
	+P	30.2 ab	18 c	3 c
	+K	6.3 d	10 c	8 c
	-N	12.6 cd	50 a	20 b
	-P	4.7 d	40 a	45 a
	-K	35.9 a	23 bc	3 c

Different letters indicate significant differences between treatments at  $P < 0.05$  (Tukey's HSD)

infection numbers to the seedling biomass showed that the seedlings of RG under the  $-N$  and  $-K$  treatments and the seedlings of WF under the  $-N$  and  $-P$  treatments were highly vulnerable to *P. aegyptiaca* infection.

## Discussion

Excess of nitrogen and phosphorous did not decrease the infestation of broomrape on tobacco seedlings

Mineral fertiliser deficiency is a key factor limiting crop production in Africa and has also been suggested as a reason for severe parasitic weed problems (Parker and Riches 1993). In our study, N or P deficiency increased the infestation of *P. aegyptiaca* on the tobacco seedlings, as previously shown in *Striga* (Jamil et al. 2011, 2012). However, N and P excess treatments did not significantly decrease the infestation of the broomrape on tobacco seedlings. These results suggested that the role of fertiliser application in the control of parasitic weeds is primarily to balance the nutritional status of the field (Foo et al. 2013; Jamil et al. 2012) or to produce an inhibitory effect on the germination of *Orobanchae* seeds (Abuirmaileh 1994; Haider et al. 2003; Jain and Foy 1992). Javed et al. (2013) reported that root exudates of *Eriophorum angustifolium* cause the rhizospheric basification which in turn limits the mobility of toxic elements in the soil plant systems. It is believed that the problem of weedy root parasites is associated with decreases in soil fertility. Accordingly, fertiliser application represents a strategy used to improve the control of parasitic weeds in infertile fields. However, in certain developing countries, especially China, excess application of mineral fertiliser has often occurred, and this practice has resulted in a number of eco-problems, such as water and soil pollution, algal blooms and an increased deposition of nitrogen. Thus, the nutrient requirements of individual crop species need to be fully understood before parasitic weeds are controlled by applying fertiliser.

Nutrient ratio influenced the germination-inducing activity of host root exudates

It has been estimated that 80 % of terrestrial plants excrete SLs under conditions of P deficiency to facilitate the establishment of plant-AMF (arbuscular mycorrhizal fungi) symbionts (Akiyama et al. 2005; Ruyter-Spira et al. 2013), which increase the plant's uptake of P from the soil. However, parasitic weeds have evolved adaptive strategies to take advantage of this plant-AMF signalling pathway. The production and exudation of SLs respond to nutrient deficiencies. These responses depend on the type of nutrient and degree of deficiency (Yoneyama et al. 2013). In our

study, the N and P deficiency treatment increased the inducing activities of root exudates on the germination of *P. aegyptiaca* and *O. cumana* seeds, indicating that N and P deficiency resulted in the increased exudation of germination stimulants (Table 2). Interestingly, the potassium excess treatment also resulted in an increased inducing activity of the root exudates, and this result was further confirmed by the rhizosphere soil test (Fig. 3). Furthermore, the K excess led to the secretion of the germination stimulants but did not significantly increase the accumulation of the stimulants in the roots (Fig. 2). Potassium is considered a symporter, participating in the transport of anions and photosynthates between plant tissues (Havlin et al. 2005). Excessive potassium transport from roots to leaves may have decreased the content of phosphorus or nitrogen in the roots and stems and resulted in increased secretion of SLs (Yoneyama et al. 2012). Another possible mechanism is that excessive K absorption by the seedling roots changed the permeability of the cells related to SL secretion, but this hypothesis needs further investigation.

Different resistance responses of cultivars under fertiliser application ratios

Within the host species range of a given parasite, individual host genotypes may display varied levels of resistance, tolerance or susceptibility to parasite attack (Timko and Scholes 2013). Many studies have focused on the genes involved in host resistance to parasitic weeds, and several genes that produce decreases in parasite infestation have been identified (Fernández-Martínez et al. 2000; Velasco et al. 2007). Unfortunately, it appears that a resistant variety or the resistance genes, once identified, would be overcome by the evolution of the parasitic weed. In our study, the two tobacco cultivars were susceptible to *P. aegyptiaca*, but the cultivars showed different germination-inducing activities and resistance to broomrape depending on the fertiliser application ratios. The Chinese native tobacco cultivar WF exhibited a higher sensitivity to the nutrient application ratios than RG. WF showed higher germination-inducing activities under N-/P-deficient conditions than RG. Moreover, the seedlings of RG under the  $-N$  and  $-K$  treatments and WF under the  $-N$  and  $-P$  treatments were highly vulnerable to *P. aegyptiaca* infection. Accordingly, we suggest that the classification of the resistance or tolerance of host species should fully consider the nutritional conditions of the cultivation and the fertiliser strategy in terms of the potential for the control of parasitic weeds before the key mechanism of parasite resistance can be identified.

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