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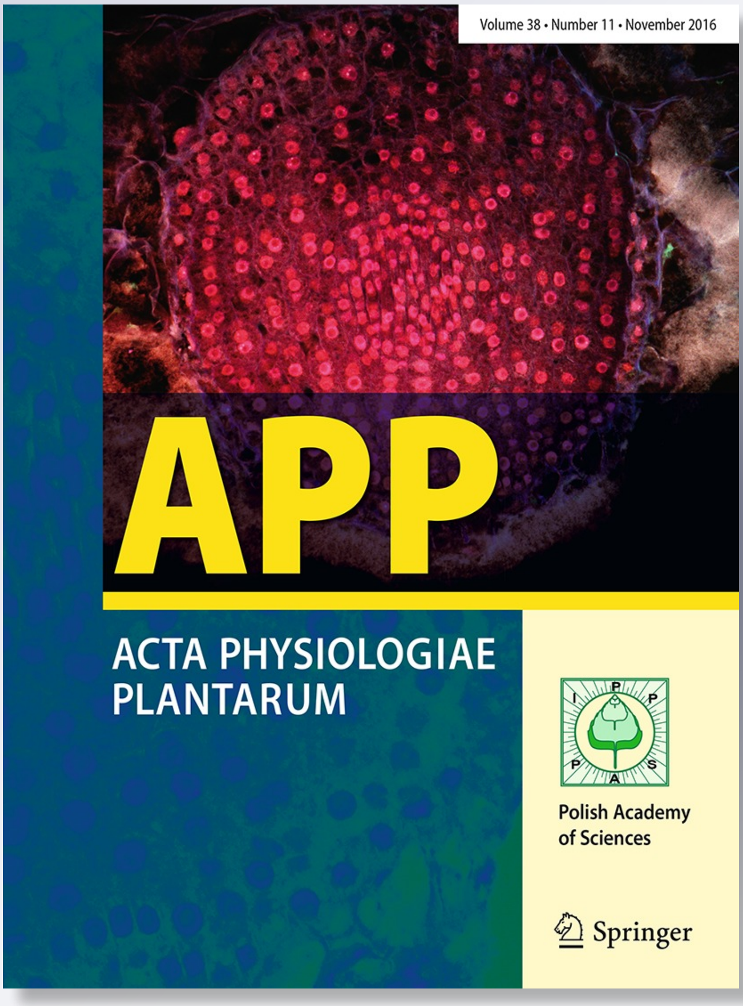

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ORIGINAL ARTICLE

The potential of foxtail millet as a trap crop for sunflower broomrape

Xiaoxin Ye[1] · Jie Chen[2] · Christopher S. P. McErlean[3] · Meng Zhang[4] · Rui Yu[1] · Yongqing Ma[1,5]

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Abstract Heavy infestation of large areas in China by the root parasitic sunflower broomrape (*Orobanche cumana* Wallr.) constitutes a major threat to sunflower production. This study was conducted to investigate the potential of foxtail millet as a trap crop for the management of sunflower broomrape (SB). To assay the ability of foxtail millet to stimulate germination of SB seeds, the rhizosphere soil of six foxtail millet cultivars cultured in various sand/soil ratios and fertilizer levels were employed to directly induce SB germination. Among all sand/soil treatments, Hong Guzi grown in a 1:1 sand/soil ratio induced the highest germination of SB (30.1%). Phosphorus resulted in a small but significantly higher germination of SB compared with the control without any fertilizer, while in contrast, nitrogen greatly suppressed the germination inducing capacity of foxtail millet even at concentrations as low as 28 mg kg^{-1} soil (based on N). Cropping rotation experiment was undertaken to quantify the effect of foxtail millet on SB infection in a subsequent sunflower crop. All foxtail millet rotation treatments reduced broomrape infection and gave higher shoot and flower disk dry mass of sunflowers than the control grown without a fore-rotating crop. These results suggest that efficacious cultivars of foxtail millet reduce SB parasitism and increase sunflower yields and that using foxtail millet as trap crop is probably an effective way to reduce the broomrape weed problem in China, especially in the dry, infertile, and sandy soils of the Loess Plateau.

Keywords Foxtail millet · Rhizosphere soil · Sunflower broomrape (SB) · Trap crop · Germination

Introduction

Species of broomrape (*Orobanche* spp. and *Phelipanche* spp.) are among the most devastating parasitic weed species worldwide (Parker 2009). Broomrape takes up water and/or nutrients from their host plants, often resulting in a reduction in host plant growth. The species of sunflower broomrape (SB) (*Orobanche cumana* Wallr.) which we focused on in this study exhibits a restricted host range and essentially only infests sunflower (*Helianthus annuus* L.) (Pujadas-Salva and Velasco 2000). The distribution of SB is centered on central Asia and south-eastern Europe, but it has been recently observed in Spain (Joel et al. 2013). In recent years, SB infestation has rapidly expanded in China and has become an increasingly serious problem (Guan 2007; Shi et al. 2015). For example, in Bayan Nur City, Inner Mongolia, 40% of sunflower fields are infested by

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✉ Yongqing Ma
mayongqing@ms.iswc.ac.cn

[1] College of Forestry, Northwest A&F University, Yangling 712100, Shaanxi, China

[2] College of Natural Resources and Environment, Northwest A&F University, Yangling 712100, Shaanxi, China

[3] School of Chemistry, University of Sydney, Sydney, NSW 2006, Australia

[4] Institute of Plant Nutrition and Environmental Resources, Henan Academy of Agriculture Sciences, Zhengzhou 450002, Henan, China

[5] The State Key Laboratory of Soil Erosion and Dryland Farming, Institute of Soil and Water Conservation, Northwest A&F University, Yangling 712100, Shaanxi, China

SB, resulting in 25–40% sunflower yield reductions (Jing et al. 2015). Sunflower has become the second most important oil crop next to soybean in the world (FAO 2011). The sunflower cultivation area in China was 9.23 × 10^5 hm^2, with a total yield of approx. 2.42 × 10^6 t (China Agriculture Statistical Yearbook 2013). Due to its adaptability to a wide range of soil types and climates, sunflower is currently one of the most popular crops cultivated by local farmers in Northwest of China (Wan et al. 2013).

Broomrapes are difficult to control because of their complex life cycle. The majority of yield losses caused by broomrape occur before it emerges from the soil, having already sequestered large amounts of water and nutrient from the hosts while underground (Ross et al. 2009). Another problematic aspect is that each mature broomrape plant can produce 500,000 seeds, which are capable of surviving for as long as 20 years in the soil (Kebreab and Murdoch 1999; Rubiales et al. 2009). Broomrape seeds require both certain environmental conditions and chemical germination stimulants to germinate. Thus, the effectiveness of some conventional control methods, such as hand weeding and herbicides, are limited (Hearne 2009). The reduction of these seed banks plays an essential role in better controlling the broomrape species and limiting their distribution (Rubiales et al. 2009). The use of false hosts (trap crops), which offer the advantage of stimulating broomrape germination without being parasitized, can be effective to some extent in reducing the broomrape seed bank (Kannan and Zwanenburg 2014). For example, maize is only infected by *Striga hermonthica*, but maize root exudates stimulate germination of SB (Ma et al. 2013), *Orobanche ramosa* (Zehhar et al. 2003), and small broomrape (*Orobanche minor* Sm.) that are known to infect other crops (Sunderland 1960). Thus, maize could be efficiently used as trap crop for these species by inducing suicidal seed germination of broomrapes (Abebe et al. 2005). Many researchers have assessed the potential of different cereal grain plants as trap crops (Kureh et al. 2006; Tarfa et al. 2006), because cereals are very important for human consumption and are cultivated in high frequency. For example, wheat (*Triticum aestivum* L.) can be utilized to reduce the amount of small broomrape seed in infested soil (Lins et al. 2006; Dong et al. 2012). The case of pearl millet (*Pennisetum glaucum* L.) that stimulated high SB germination (32.5%) is also remarkable (Fernández-Aparicio et al. 2009). Despite being one of the main food crops in China, foxtail millet (*Setaria italica* L.) has received relatively little attention, and its ability to induce parasitic weed germination is under-investigated.

It is generally believed that plants grown under nutrient-deficient conditions would face increasing incidence and severity of weedy parasitic plant damage (Emechebe et al. 2004). Thus, the nitrogen or phosphorus is considered as a direct and easy measure for controlling parasitic weeds (Ayongwa et al. 2006; Lopez-Raez et al. 2009). The inhibitory effect of high soil fertility on broomrape infection might be linked to the production and exudation of strigolactones (SLs). The latter, a class of carotenoid-derived molecules, has been identified as germination stimulants of root parasitic plants (Yoneyama et al. 2007). Several reports suggest that nutrient deficiencies markedly enhanced the secretion of SLs (López-Ráez et al. 2008; Yoneyama et al. 2007). However, other plant-produced compounds, such as dehydrocostus lactones (DCL) (Joel et al. 2011), dihydrosorgoleone, sesquiterpene lactones (Fischer et al. 1989), kinetin, coumarin, jasmonate, and fungal metabolites (fusicoccins and cotylenin), can also result in the germination of some broomrape species (Xie et al. 2010). Thus, for the crops whose main germination stimulant is not SLs, such as sunflower, the germination inducing activity is not regulated by phosphate availability (Joel et al. 2011). On the other hand, manure fertilization could also affect soil micro-biota, subsequently influencing the emergence of parasitic weeds (Fernández-Aparicio et al. 2011) or parasitic weed defense mechanisms (Mabrouk et al. 2007; Zhang et al. 2015). Thus, the effects of soil nutrients on the ability of plants to induce broomrape seed germination are still an ambiguous issue, especially with respect to the interaction of root exudates and the development of micro-biota communities.

The distribution of SB, coinciding with the distribution of sunflower fields in China, is north and northwest of China, where soils are mostly dry, infertile, and contain more than 50% fine sand (Shi et al. 2015). Foxtail millet's early maturity, tolerance to low soil fertility, and efficient use of available water make it suitable for cultivating in the dry areas of China which are suffering large-scale broomrape infestation (Li and Wu 1996). Thus, it is of interest to know whether foxtail millet is suitable for being used as a trap crop for SB in China, especially in sandy soil of Loess Plateau. Moreover, it is critical to know if SB germination stimulation varies with foxtail millet cultivar and fertilizer level, to optimize its effectiveness as a trap crop. The rhizosphere, a narrow zone of soil immediately surrounding the root system, is a highly complex ecosystem and is important in terms of root growth, exudate production, and community development of macro- and micro-biota (Bertin et al. 2003; Venturi and Keel 2016). The communication and interaction between host plants and parasites mainly happened in this area (Bouwmeester et al. 2007). It has been observed that *Striga* seeds germinate in high percentages within 5 mm of sorghum (*Sorghum bicolor* (L.) Moench) host root surface, but that no germination was observed at distances greater than 1 cm in agar (Fate et al. 1990). Thus, the rhizosphere soil has the potential to indicate the actual effects of plants on broomrape. Considering this, the rhizosphere soils of foxtail millet grown under different conditions were employed for direct SB seed germination. In addition to that, the actual effectiveness of foxtail millet rotation for SB management was studied. Pot trials were conducted to crop sunflower in rotation with foxtail millet (Cao Guzi and Jingu 29) for 1 or 2 years. Broomrape attachment and emergence on subsequent sunflower were quantified. The results will be of value for formulating integrated strategies to limit SB damage in China.

Materials and methods

Materials and plant culture conditions

Six foxtail millet cultivars (Jingu 21, Dingbian Huang guzi, Dingbian Hong guzi, Dingbian Xiaoxiang guzi, Jingu 29) were provided by Dingbian Bureau of Agriculture, Shaanxi Province of China. Seeds of SB were collected from infested sunflower fields in Xinjiang Uygur Autonomous Region of China in 2012. A susceptible sunflower to SB, T33, was provided by Associate Professor Enshi Xiao, from the College of Agronomy, Northwest A&F University, Yangling, Shaanxi Province of China. GR24, a germination inducing stimulant and a synthetic analogue of strigol, was kindly provided by Professor Binne Zwanenburg (the University of Nijmegen, The Netherlands). The pot experiments were conducted in Guyuan Ecological Station of the Institute of Soil and Water Conservation, Chinese Academy of Sciences located in Hechuan Village (35°99′N, 106°44′E), Guyuan City, Ningxia Hui Autonomous Region of China. The natural soil, which was collected from a cultivated field near the research institute, is common dark loessial soil with a pH of 7.41, a soil organic matter of 9.41 g/kg, a nitrogen total of 280 mg/kg, available phosphorus (P) of 2.28 mg/kg, and available potassium (K) of 206 mg/kg. The sand, brought from Guyuan, was sieved medium-sized natural river sand.

Experiment 1: foxtail millet germination inducing capacity assay

To evaluate the effect of different cultivars, sand/soil ratios, and fertilizer levels on the capacity of foxtail millet to induce SB germination, pot experiments were conducted to collect the rhizosphere soil. The different mixtures of sand and soil examined in the study were as follows (sand: soil, v: v): 0:1 (native soil), 1:1, 2:1, 3:1, 4:1, 5:1, and 1:0 (sand only). The natural soil used in this study was dried, pounded, and passed through 2 mm sieve and thoroughly mixed with sand in seven different proportions. The soil used in fertilizer trails was all mixed with the sand (1:1) thoroughly according to above protocol. The detailed rates of different fertilizer treatments are showed in Table 1. Nitrogen was used as urea at rates of 28, 56, 84, 112, and 140 mg kg^{-1} soil (based on N). Phosphorus was used as super phosphate at 31, 62, 93, 124, and 155 mg kg^{-1} soil (based on P_2O_5). All experiments were conducted in a randomized complete-block design. Treatments were replicated four times and this experiment was conducted twice. All treatments were carried out in 7.8-L pots (20 cm diameter × 25 cm height) filled with 8-kg soil prepared according to above protocol. In each pot, the seedlings were thinned to about 20 plants. The pots were placed in a sunny outdoor area and the plants were watered every other day. Foxtail millet was sampled at 30 days after emergence when the plants were in the five- to six-leaf stage. The foxtail millet's root plus adhering soil was carefully picked out of the non-rhizosphere soil which was more than 5 mm away from foxtail millet roots. The loosely held soil was gently shaken off the roots, which was referred to as rhizosphere soil (Riley and Barber 1969, 1970). The rhizosphere soil collected as samples was directly employed to induce SB seeds germination.

Before use in germination assays, the SB seeds were disinfected first with 1% (w/w) NaClO solution for 3 min and then with 75% (V/V) ethanol for 3 min (Parker et al. 1977). Then, the seeds were repeatedly rinsed with water and air-dried. Two pieces of filter paper were spread on the bottoms of petri dishes (9 cm) and 8-mm glass fiber filter disks (GFFP) (8-mm Whatman GF/A) were uniformly placed on the filter paper. Seeds of SB, 50–70, were sprinkled on each disk. The Petri dishes were sealed with

Table 1 Fertilizer treatments

Nitrogen	Nitrogen rate (mg kg^{-1} soil)	Phosphorus	Phosphorus rate (mg kg^{-1} soil)	Combined treatments	N/P rate (mg kg^{-1} soil)
N1	28	P1	142	N1P1	28/142
N2	56	P2	284	N1P5	28/710
N3	84	P3	426	N3P3	84/426
N4	112	P4	568	N5P1	140/142
N5	140	P5	710	N5P5	140/710

parafilm and then placed in growth chamber at 25 °C for 4 days (Ye et al. 2016). For each treatment, 5 g rhizosphere soil was placed into a Petri dish (3.5 cm diam.) and wetted with 3 mL distilled water. Five 8-mm disks of GFFP with conditioned broomrape seeds were placed on the soil surface. The Petri dishes were sealed and incubated in the dark at 25 °C for 2 weeks and subsequently examined for germination using a stereomicroscope. Conditioned seeds on disks, placed on the blank soil, or treated with water were used as negative control. Seeds treated with GR24 (1 mg L^{-1}) were used as positive control.

Experiment 2: foxtail millet-sunflower rotation assay

Cao Guzi and Jingu 21 were employed in this trial to investigate the effect of foxtail millet on SB parasitism in a subsequent sunflower. The soil used in this experiment was thoroughly mixed with sand in a 1:1 ratio. Experiments were conducted in 7.8-L plastic pots containing 8 kg of dry soil mix that was artificially infested with 32 mg of SB seeds (about 8000 seeds per pot). Pots were placed in a shed and watered as necessary. In each of the pots, the seedlings were thinned to about 20 plants. The rotation cycle treatments included: Cao Guzi-sunflower (CS), Cao Guzi-Cao Guzi-sunflower (CCS), Jingu 21-sunflower (JS), Jingu 21-Jingu 21-sunflower (JJS), sunflower (S), and blank-sunflower (BS), and blank-blank-sunflower (BBS). For the ‘blank’ treatment, pots contained SB seeds but without grown any plants were placed in a shed and watered as other treatments. The experimental details about the rotation system are shown in Table 2. At the end of the sunflower rotation in each cycle, sunflower roots were washed and separated from attached broomrape. Broomrape emergence and attachment were quantified. The flower disk, shoot, and root mass of sunflowers from harvest times were determined after drying for 48 h at 70 °C. Treatments were arranged in a completely randomized design with five replications for each treatment.

Statistical analysis

Data processing was done with Excel 2003 and DPS 9.5 (Tang and Zhang 2013). To satisfy the assumptions of ANOVA, the germination data of SB were arcsin transformed before analysis and proportions of 0/n were replaced with 1/4n to improve the arcsine transformation (Bartlett 1937). The back-transformed data are presented in figures. The arcsin transformed and the back-transformed data are presented in tables. Turkey’s honest significant difference (HSD) test was used to compare the means.

Results

Effect of sand–soil ratios on the ability of foxtail millet to induce SB seed germination

The germination of SB seeds treated with GR24 (1 mg L^{-1}) ranged between 60 and 70% (data not shown). The blank soil and distilled water did not induce germination. The rhizosphere soil collected at various sand/soil ratios induced 0–30.1% germination of SB. Germination of SB was significantly affected by the sand/soil ratio and the rhizosphere soil collected at the sand/soil ratio of 1:1 resulted in the highest SB germination. At the sand/soil ratio of 1:1, the highest germination was induced by the rhizosphere soil of Hong Guzi (30.1%) and the lowest was induced by the rhizosphere soil of Huang Guzi (14.5%). The next most effective sand–soil mixtures on inducing SB seeds germination were the native soil and mixed soil at the sand/soil ratio of 2:1, which formed a homogeneous group. If the ratio of sand in the mixture soil increased further, from 2:1 to 5:1, the germination of SB decreased gradually. Where foxtail millet was planted with the soil prepared with a sand/soil ratio of 4:1, 5:1, or only with sand, the rhizosphere soil simulated negligible SB germination (Table 3).

Table 2 Cropping rotation system management of Experiment 2

	Rotation crop							Sowing date	Harvesting date
	CS	CCS	JS	JJS	S	BS	BBS		
1st year	Cao Guzi	Cao Guzi	Jingu 21	Jingu 21	Sunflower	Blank	Blank	May 5	Sept.14
2nd year	Sunflower	Cao Guzi	Sunflower	Jingu 21		Sunflower	Blank	April 24	Sept. 29
3rd year		Sunflower		Sunflower			Sunflower	May 10	Sept. 26

CS Cao Guzi-sunflower, *CCS* Cao Guzi-Cao Guzi-sunflower, *JS* Jingu21-sunflower, *JJS* Jingu 21-Jingu 21-sunflower, *S* sunflower, *BS* blank-sunflower, *BBS* blank-blank-sunflower

Table 3 Effects of sand/soil ratio on induction of SB germination by foxtail millet rhizosphere soil

Sand-soil ratio	Cao Guzi	Huang Guzi	Jingu 21	Hong Guzi	Xiaoxiang Guzi	Jingu 29
1:1	25.8 (18.9) a	22.0 (14.0) ab	27.6 (21.4) a	32.4 (28.8) a	23.0 (15.3) a	23.1 (15.4) a
2:1	20.0 (11.7) ab	27.1 (20.7) a	17.3 (8.9) a	20.8 (12.6) b	19.8 (11.5) a	18.9 (10.5) a
3:1	11.1 (3.7) bc	14.7 (6.5) b	16.4 (8.0) a	12.2 (4.5) bc	7.4 (1.7) bc	15.1 (6.8) ab
4:1	3.4 (0.3) c	11.7 (4.1) c	4.6 (0.6) b	4.5 (0.6) c	6.0 (1.1) c	5.2 (0.8) bc
5:1	5.7 (1.0) c	3.7 (0.4) c	3.7 (0.4) b	4.9 (0.7) c	5.0 (0.8) c	7.3 (1.6) bc
Sand	3.4 (0.3) c	3.7 (0.4) ab	3.7 (0.4) b	3.7 (0.4) c	3.7 (0.4) c	3.7 (0.4) c
Soil	21.5 (13.5) ab	21.9 (13.9) bc	26.7 (20.1) a	17.4 (9.0) b	18.4 (10.0) ab	22.5 (14.7) a

Only the means in the same column were compared. Different letters indicate significant differences between arcsin transformed means (back-transformed means) of treatments at

$p < 0.05$ (Tukey's HSD)

Effect of fertilizer level on foxtail millet germination inducing activity

For experiments in which nitrogen was the only fertilizer, the level of nitrogen, the identity of the foxtail millet cultivars, and the interaction of the two, all significantly affected ($p < 0.01$) the SB germination (Table 4). The germination inducing activity of Xiaoxiang Guzi increased with the nitrogen up to 112 mg kg^{-1}, which was completely different from the other cultivars where nitrogen significantly decreased the germination inducing activity. The mean germination of SB (13.2%), induced by the rhizosphere soil with as little as 14 mg kg^{-1} nitrogen, was significantly lower than control soil (21.6%) (Fig. 1). In addition, the germination response of SB seeds to rhizosphere soil of all foxtail millet cultivars (except Xiaoxiang Guzi) decreased progressively with the increase in nitrogen ($p < 0.01$, data not shown). Nitrogen at 140 mg kg^{-1} effectively suppressed the stimulant activity of all foxtail millet cultivars on SB seeds (all lower than 6%).

Table 4 ANOVA results of effects of fertilizers and foxtail millet cultivars on the germination of SB

Source	*df*	*F* value		
		Nitrogen	Phosphorus	Combined
Fertilizer level	5	64.81**	31.94**	184.73**
Foxtail millet cultivars	5	4.69**	23.42**	9.19**
Interaction effects	25	4.23**	1.47	1.73*

The *F* value are expressed in the table

df degrees of freedom

*,** Difference at $p < 0.05$ and $p < 0.01$

For experiments in which phosphate was the only fertilizer, the inducing capacity of foxtail millet was significantly affected by the phosphorus level, the identity of the foxtail millet cultivars ($p < 0.01$), but not by their interaction. In general, Hong Guzi induced the highest mean germination

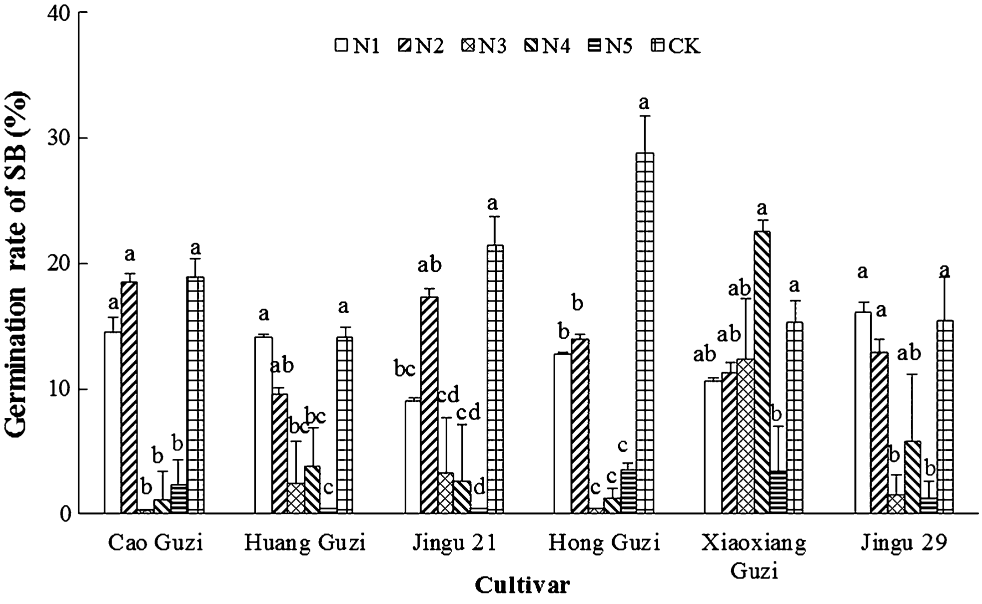

Fig. 1 Influence of nitrogen fertilization on induction of sunflower broomrape (SB) germination by foxtail millet rhizosphere soil. The back-transformed data ± SD are shown. Means followed by *different letters* are significantly different at $p < 0.05$ according to Tukey's test

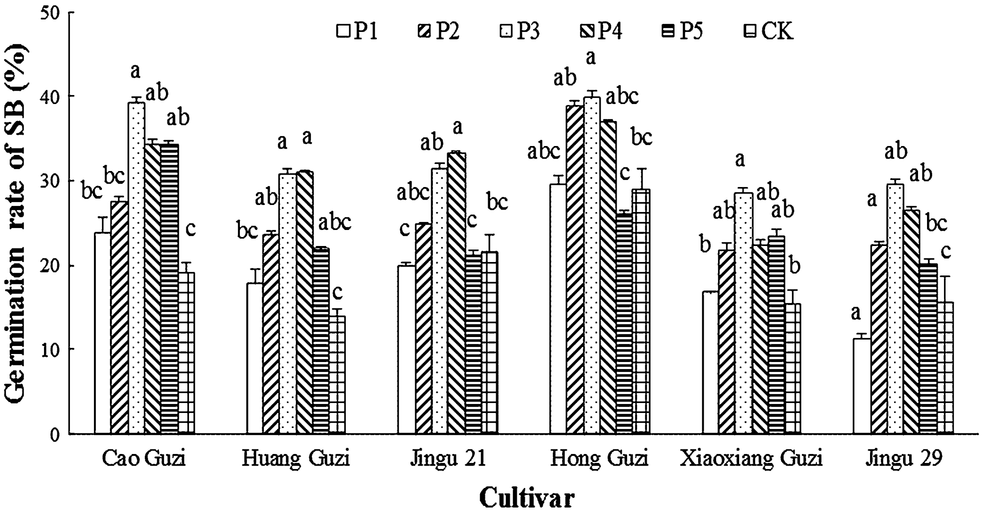

Fig. 2 Influence of phosphorus fertilization on induction of SB germination by foxtail millet rhizosphere soil. The back-transformed data ± SD are shown. Means followed by *different letters* are significantly different at $p < 0.05$ according to Tukey's test

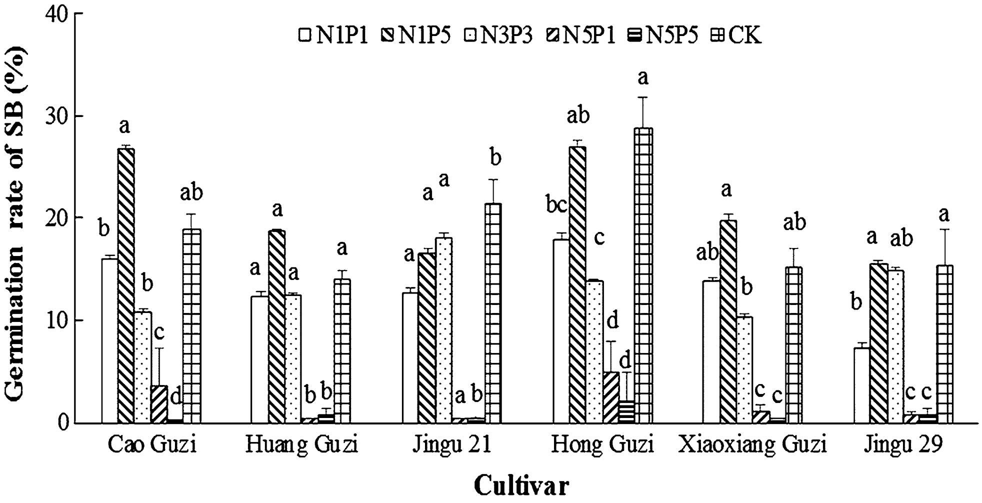

Fig. 3 Influence of nitrogen and phosphorus combinations on induction of SB germination by foxtail millet rhizosphere soil. The back-transformed data ± SD are shown. Means followed by *different letters* are significantly different at $p < 0.05$ according to Tukey's test

(33.7%) of SB among six foxtail millet cultivars (Fig. 2). The next most effective foxtail millet cultivar on inducing SB seeds germination was Cao Guzi, which exhibited a mean germination of 30.1% under different fertilizer treatments. The germination activity of Jingu 29 was significantly lower than other cultivars. In addition, increased germination inducing activities of different foxtail millet cultivars were observed in the treatments with phosphorus. Phosphorus at 426 mg kg^{-1} induced the highest mean germination of SB and the germination inducing activity of foxtail millet was reduced when treated with phosphorus at the highest concentration (710 mg kg^{-1}).

For experiments with combined of nitrogen and phosphorus, the germination of SB induced by different foxtail millet cultivars and fertilizer levels was significantly different. In general, Hong Guzi induced the highest germination of SB among the six foxtail millet cultivars. SB germination could reach a maximum rate (11.7%) when treated with 28 mg kg^{-1} nitrogen and 710 mg kg^{-1} phosphorus. In addition, the germination inducing activity of foxtail millet was completely suppressed when treated with 140 mg kg^{-1} nitrogen, even in combination with phosphorus (Fig. 3). Moreover, it was interesting to note that the fertilizer has a significantly interactive effect on the germination inducing capacity of foxtail millet (Table S1). To give an example, at low nitrogen, the germination inducing capacity of Cao Guzi increased when phosphorous increased, and at high nitrogen level, it decreased with phosphorus. For Cao Guzi and Xiaoxiang Guzi, the combined of both nitrogen and phosphorus gave a significantly lower germination inducing capacity than in the case of just nitrogen or phosphorus. In addition, it was detected that nitrogen plays the dominant role in the germination inducing capacity of foxtail millet (Table S1).

Effects of foxtail millet rotation on SB parasitism on subsequent sunflower

The number of emerged SB plants and the number of attachments per sunflower differed significantly among treatments. Sunflowers grown in soil without a fore-rotating crop had more than three parasitic emergences per plant in the first year and second year. All the other trap crop treatments significantly reduced the emergence of SB, generally to an average of less than one SB emergence. In the third year, all treatments, with or without fore-rotating crop, did not have any SB emergence (Fig. 4). For SB attachments, sunflowers grown in soil with a fore-rotating crop significantly reduced SB attachment up to 50–70% compared with the control. The sunflower grown after 2 years of Jingu 21 trap cropping induced the lowest SB attachment (4.4/plant). The sunflower grown in the third year without any rotation crop resulted in maximum SB attachment (19.4/plant) (Fig. 5). Moreover, the dry mass of sunflower was significantly affected by different treatments.

Sunflowers grown after 2 years of Cao Guzi trap cropping resulted in a significantly higher root dry mass than other treatments. The root dry mass of sunflower was similar in all other treatments. The mean shoot dry mass of sunflower grown in soil with a fore-rotating crop (16.0 g) was significantly higher than that of the control (4.7 g). The highest shoot dry mass was obtained from the sunflower grown after 2 years of Cao Guzi (19.3 g). The flower disk dry weight of sunflowers without a fore-rotating crop was lower, generally less than 1 g. However, sunflowers grown after foxtail millet resulted in significantly higher flower disk dry weight (mean weight of 8.1 g), which was an eightfold increase compared with the control (Table 5).

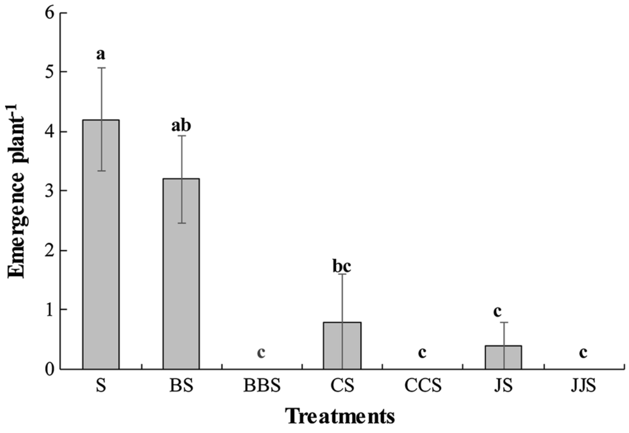

Fig. 4 Influence of crop rotation on SB emergence on sunflower. The back-transformed data ± SD are shown. Means followed by *different letters* are significantly different at $p < 0.05$ according to Tukey's test

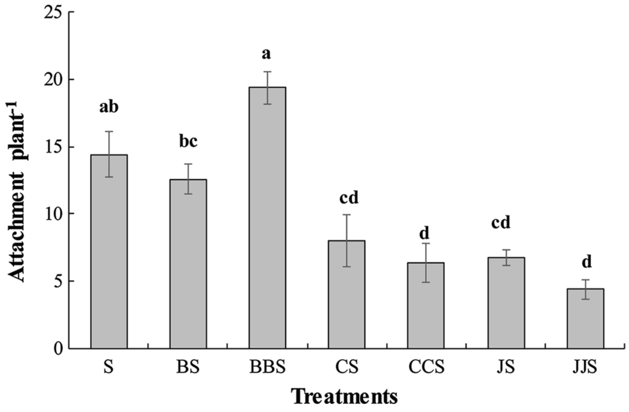

Fig. 5 Influence of crop rotation on SB emergence on sunflower. The back-transformed data ± SD are shown. Means followed by *different letters* are significantly different at $p < 0.05$ according to Tukey's test

Discussion

The rhizosphere soil is the narrow zone of soil surrounding the root system that is directly influenced by root secretions and associated soil microorganisms (Venturi and Keel 2016). The ability of rhizosphere soil to induce broomrape germination determines the capacity of foxtail millet to be used to control broomrape. Rhizosphere soil from six different foxtail millet cultivars all induced SB seed germination directly. This suggests that foxtail millet has the ability to directly induce SB germination to some extent. The germination response of SB seeds to different foxtail millet cultivars was significantly different. This conclusion was similar to previously reports that the germination inducing capacity varied among different soybean cultivars and among cotton genotypes (Ma et al. 2012; Zhang et al. 2013). The report of Jamil et al. (2011) suggests that the existence of large genetic variation for strigolactones production may lead to the difference in germination stimulatory activity among rice cultivars. The genetic variation in germination stimulatory activity indicates that selection of cultivars producing high levels of germination stimulants may be an important consideration when choosing a suitable trap crop. In this study, Hong Guzi induced significantly higher broomrape germination than other cultivars, followed by Cao Guzi and Jingu 21.

Environmental factors, including soil type, moisture, and nutrient availability, may directly and indirectly affect the production of germination stimulants (Sato et al. 2003). In the case of SB distributed in China, soil textures are an important influencing factor, since the infested fields occur mostly on the Loess Plateau which contains soil with more than 50% fine sand. This study shows for the first time that the soil texture dramatically influenced the capacity of plants to induce SB germination. The sand/soil ratio was found to significantly affect the ability of inducing SB germination by foxtail millet, with a sand/soil ratio of 1:1 resulting in the highest SB germination in Hong Guzi. The next most effective sand–soil mixtures for inducing SB

Table 5 Effects of foxtail millet rotation on shoot, root, and flower disk dry weight of sunflower grown under SB infestation

Cropping rotation system	Dry weight (g)		
	Shoot	Root	Flower disk
S	3.8 ± 0.4 b	2.3 ± 0.1 b	1.0 ± 0.4 c
BS	5.3 ± 0.6 b	2.4 ± 0.2 b	1.0 ± 0.6 c
BBS	5.1 ± 0.2 b	2.5 ± 0.1 b	0.5 ± 0.1 c
CS	11.0 ± 1.3 ab	2.0 ± 0.2 b	6.7 ± 1.8 abc
CCS	19.3 ± 4.4 a	5.4 ± 0.8 a	4.1 ± 0.1 bc
JS	18.9 ± 2.9 a	3.2 ± 0.4 b	12.6 ± 3.2 a
JJS	14.8 ± 0.3 a	2.6 ± 0.1 b	8.8 ± 1.0 ab

Only the means in the same column were compared. The means marked with different letters differed significantly at the 5% confidence level, according to a one-way ANOVA. Data represent the mean ± SE ($n = 5$)

seed germination were the native soil and mixed soil at a sand/soil ratio of 2:1. Accordingly, rhizosphere soil of foxtail millet cultured with less than 66% sand was still effective for inducing SB germination. As the sand proportion continuously increased, the germination inducing capacity of foxtail millet gradually decreased (Table 3).

It is now widely accepted that the parasitic plants generally prevail on nutrient-deficient soils (Jamil et al. 2011; Parker 2013). Data obtained in this study also suggest that the nitrogen significantly reduced the germination inducing activity of foxtail millet. However, phosphorus had a negligible effect on germination inducing capacity of foxtail millet, even promoting the germination of SB in general. The observed inactivity of phosphorus was not consistent with the previous research about the response of strigolactone secretion to phosphorus starvation in *Lotus japonicus* (Liu et al. 2015). All plants [Chinese milk vetch (*Astragalus sinicus* L), alfalfa (*Medicago sativa* L.), lettuce (*Lactuca sativa* L.), marigold (*Tagetes patula* L.), wheat (*Triticum aestivum* L.), and tomato (*Solanum lycopersicum* L.)] examined in the report of Yoneyama et al. (2012) exude higher levels of SLs under phosphorus deficiency. However, our results were consistent with the results obtained in sorghum (Raju et al. 1990). It was also recently reported that phosphorus starvation markedly decreased the stimulatory activity of sunflower root exudates (Joel et al. 2011). Moreover, the results discussed above revealed that the interaction of nitrogen and phosphorus significantly influenced the germination inducing capacity of foxtail millet. For Cao Guzi and Xiaoxiang Guzi, the combined of both nitrogen and phosphorus gave a significantly lower germination inducing capacity than in the case of just nitrogen or phosphorus (Table S1).

These cropping rotation trails demonstrated the potential of appropriate foxtail millet cultivars to reduce SB parasitism in subsequent sunflower. All tested cultivars greatly reduced SB attachment and emergence. In addition, the extent of reduction in SB infestation was dependent on the cultivars of foxtail millet and the number of year foxtail millet was cultivated before sunflower cultivation. For example, SB attachment was 37% lower after 1 year of Cao Guzi and 46% lower after 1 year of Jingu 21. However, the reduction of SB attachment was higher when the foxtail millet was cultivated for 2 years before sunflower was cultivated. SB attachment was 67% lower after 2 years of Cao Guzi and 77% lower after 2 years of Jingu 21 (compared with sunflower grown in the same year without fore-rotating crop). Moreover, it is interesting to note that all treatments, with or without a trap crop, did not have any SB emergence in the third year (Fig. 4). However, significantly higher SB attachments were displayed in the third year than in the second year. It was reported that delayed sowing date of a susceptible crop leads to a lower broomrape incidence compared to early sowing (Lopez-Granados and Garcia-Torres 1996).

Reduction of *Striga* infection due to lower temperatures has also been reported in the previous studies (Aflakpui et al. 1998). Thus, the occurrence of considerable variation in SB infestation between the 3 years of experiments, even though artificial infestation was used, may be due to differences in sowing time and weather conditions between the years. In addition to a reduction of SB incidence, cropping rotation also increased the dry mass of sunflower. The shoot dry mass and flower disk dry mass were both significantly higher. The flower disk dry mass of sunflower was 5.7 times higher after 1 year of Cao Guzi and 12.6 times higher after 1 year of Jingu 21 relative to that of sunflower grown in the same year without a fore-rotating crop. However, the root dry mass of sunflower did not differ significantly among all treatments, except for sunflower grown after 2 years of Cao Guzi. The previous reports have revealed that there was a large increase in root dry weight in the *Striga* infected plants relative to regular plants (Taylor et al. 1996).

Non-significant difference of the root dry weight was also observed between *Orobanche foetida* poir. infested and non-infested chickpea (*Cicer arietinum* L.) (Nefzi et al. 2016). In our study, the overall result of these alterations in biomass allocation was a significant increase in the shoot:root ratio in sunflower grown after foxtail millet. However, different responses have been reported in other broomrape host associations (Dale and Press 1998; Hibberd et al. 1998). Our result was consistent with the association between *Orobanche aegyptian* and tomato observed by Barker et al. (1996) that the parasite depressed shoot:root ratio of tomato. Even after growing foxtail millet for 2 years, the infection of SB still remained. This was similar to the previous results of Al-thahabi (2006) who observed that after 4 years of continuously cropping with wheat, small broomrape (*Orobanche minor* Sm.) attachment still remained. Al-Menoufi (1989) reported a 3 and 1% *Orobanche crenata* Forsk. infestation in faba bean following 3 and 4 crops of Egyptian clover (*Trifolium alexandrinum* L.). Kebreab and Murdoch (2001) predicted that sustainable control of the parasite can only be achieved by reducing the soil seed bank to levels of 1000–2000 seeds m^{-2}. In addition, it was predicted that it would take 3–4 years to reduce the infestation from 13,000 to 2000 seeds m^{-2}. Thus, further study is needed to investigate the impact of foxtail millet on the SB soil seed bank and to explore the number of foxtail millet cycles required to reduce parasitism of subsequent sunflower.

This set of trials demonstrated the potential of an appropriate foxtail millet cultivar to reduce SB parasitism in subsequent sunflower. Foxtail millet cultured with mediums contained less than 66% soil induced high germination of SB which demonstrated the effectiveness of using foxtail millet as trap crop in certain infected Chinese soils. The capacity of foxtail millet to induce suicidal germination of SB strongly depends on the levels of nutrients and their interaction with the culture media. Foxtail millet with 426 mg/kg phosphorus induced the highest germination of SB. The ability of induce SB germination can vary significantly among foxtail millet cultivars. Rotation of foxtail millet can reduce SB infestation and increase in sunflower biomass. The reduction of SB infestation and sunflower biomass increases will be higher if the foxtail millet is cultivated for more than 1 year before sunflower is introduced. Accordingly, foxtail millet can be used as a false host crop as part of SB management and this may lead to an integrated, biologically based strategy for SB control in China.

Author contribution statement Xiaoxin Ye, Jie Chen, and Rui Yu conducted out experiments. Yongqing Ma planned and supervised the experiments. Meng Zhang helped in statistical analysis. Xiaoxin Ye wrote the manuscript, and Christopher S. P. McErlean made the discussion and English language editing of the manuscript.

Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

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