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Original scientific paper

Effects of plant growth promoting rhizobacteria (PGPR) and cover crops on seed germination and early establishment of field dodder (*Cuscuta campestris* Yunk.)

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SUMMARY

Several bacterial cultures: *Bacillus licheniformis* (MO_1), *B. pumilus* (MO_2), and *B. amyloliquefaciens* (MO_3), isolated from manure; *B. megatherium* ZP6 (MO_4) isolated from maize rhizosphere; *Azotobacter chroococcum* Ps1 (MO_5) and *Pseudomonas fluorescens* (MO_6), were used to test the influence of plant growth promoting rhizobacteria (PGPR) on seed germination and germination rate of field dodder (*Cuscuta campestris* Yunk.). Also, to examine the effect of host seeds on germination and initial growth of seedlings of field dodder plants in the dark and under white light, the seeds of four host plants were used (watermelon, red clover, alfalfa and sugar beet). Germinated seeds were counted daily over a ten-day period and the length of seedlings was measured on the final day.

The results show that treatments MO_3, MO_4 and MO_6 had inhibitory effects (15%, 65% and 52%, respectively), while treatments MO_1, MO_2 and MO_5 had stimulating effects (3%, 3% and 19%, respectively) on seed germination of field dodder. The data for host seeds show that light was a significant initial factor (83-95%, control 95%) for stimulating seed germination of field dodder plants, apart from host presence (73-79%, control 80%).

Keywords: PGPR; Seed germination; Cover crops; Field dodder

INTRODUCTION

Plants belonging to the genus *Cuscuta* (common name: dodder) are obligate holoparasitic plants. Merely a few *Cuscuta* species still show residual photosynthesis (García et al., 2014) and have thus been designated as cryptically photosynthetic (Funk et al., 2007; McNeal et al., 2007a,b). However, all *Cuscuta* species depend absolutely on their hosts to complete their life cycle, and *Cuscuta* can be considered an obligate holoparasite.

Like other angiosperms, the life cycle of *Cuscuta* begins with seed germination. Germinating *Cuscuta*

seedlings depend on limited seed reserves, they are unable to survive alone for a longer time and must find an appropriate host plant stem within a few days after germination. To find and catch potential hosts, *Cuscuta* plants must recognize plant volatiles as chemo-attractants, which guide seedling growth and increase the chances of successful establishment of a connection (Runyon et al., 2006). However, expert opinions vary as to what is the necessary impulse for germination of field dodder seeds. Some researchers (Vail et al., 1990; Benvenuti et al., 2002) believe that *Cuscuta spp*. do not require host-root exudates to stimulate germination, similar to some important holoparasitic weeds of the genus *Orobanche* and some hemiparasitic weeds in the genus *Striga*. Field dodder as a stem parasite is strongly impacted by light signals, which stimulate germination of its seeds (Orr et al., 1996; Tada et al., 1996; Haidar, 2003). Field dodder seedlings tend to grow towards a light source, primarily red/far red light, which helps them find hosts, while far red and blue light have a significant role in prehaustoria formation. Recognition of a host occurs through phototropic mechanisms, and some authors claim that chemotropism (movement induced by chemical stimulus) and thigmotropism (movement induced by mechanical stimulus, i.e. by touch) have equally important roles in host recognition process (Haidar et al., 1997). Runyon et al. (2006) found that volatile chemical substances were also important for movement of *Cuscuta campestris* seedlings in the dark. *Cuscuta* can also change from one host to another and back. If the plant needs special volatile chemicals to search for a host, it is difficult to explain why it can parasitize so many different plants except that there is a wide overlap of volatile compositions of the various plants.

The effects of microorganisms (PGPR- Plant Growth Promoting Rhizobacteria) on seed germination and seedling growth have been studied by various authors (Bhat & Alagawadi, 1998; Gutiérrez-Mañero et al., 2001; Rodríguez & Fraga, 1999; Sturz & Nowak, 2000; Egamberdiyeva, 2007), while the effects of microorganisms on seed germination of weed species (particularly weed parasites) have been studied only sporadically (Miché et al., 2000; Vrbničanin et al., 2008a,b, 2011; Saric et al., 2009). Soils are inhabited by various microorganisms, mostly bacteria, which are specifically active in the root rhizosphere where their abundance is generally high (Gutiérrez-Mañero et al., 2001). Beneficial bacteria are mostly the plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1989). The group of bacterial cultures that promote plant growth includes different species and strains in the genera *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Rodríguez & Fraga, 1999; Sturz & Nowak, 2000). These bacteria colonize seedlings or their root systems and stimulate plant growth by improving the availability of nutrients, stimulating metabolic activities with phytohormones and similar substances or by reducing the number of phytopathogenic microorganisms (Ping & Boland, 2004). Today, PGPRs are often used as inoculants for biocontrol, biofertilization or phytostimulation (Ping & Boland, 2004).

Therefore, the objectives of this research were to: 1) examine the interaction between rhizobacteria (PGPR) and seed germination of field dodder; 2) to check whether certain crops as the most frequent hosts of field dodder exert an allelopathic effect on seed germination and early growth of field dodder seedlings.

MATERIAL AND METHODS

Petri dish germination bioassay with PGRP

The effects of rhizobacteria (PGPR) on seed germination of field dodder were examined under controlled conditions. Bacterial cultures (Pro Growth Promoting Rhizobacteria - PGPR) of *Bacillus licheniformis* (MO_1), *B. pumilus* (MO_2) and *B. amyloliquefaciens* (MO_3) were isolated from manure; *B. megatherium* ZP6 (MO_4) were isolated from maize rhizosphere; *Azotobacter chroococcum* Ps1 (MO_5) and *Pseudomonas fluorescens* (MO_6) were isolated from wheat rhizosphere and kept refrigirated at 4°C until use. Before planting, dodder seeds were disinfected with sodium hypochlorite (NaOCl) and distilled water at 1:1 ratio for 10 minutes and then rinced three times with distilled water to remove other microorganisms. Twenty disinfected and prepared seeds were placed into each petri dish and 5 ml of inoculum at a concentration of 10^8 ml^{-1} bacterial cells was added to each dish and kept in darkness in an incubator (Binder CE) at 25°C. Germinated seeds were counted over a period of ten days on a daily basis. Distilled water was used for control seeds. Seed germination rate was calculated using the formula of Maguire (1962). All treatments had four replicates and the bioassay was repeated twice.

Petri dish germination bioassay with cover crops

Seeds of *C. campestris,* collected in the fields around Jakovo during July 2012, were purified and kept in the laboratory at room temperature 22-25°C. The dodder host seeds used in the bioassay were: alfalfa, red clover, sugar beet and watermelon. Seeds of the cover crops and field dodder were germinated together in petri dishes (14 cm). Treatments consisted of 25 seeds of each cover crop and 25 seeds of field dodder uniformly interspersed per dish. Each petri dish received 5 ml of distilled water. Germination took place in an incubator (Binder CE) in the dark at 25°C temperature.

The same procedure was used for testing the germination of field dodder seeds under white light illumination in the climate room. The conditions included: 14/10 h photoperiod, 26/21°C (day/night) temperature and 300 $\mu E/m^2s$ light intensity. A Data Logger device was used for temperature and humidity measurements in the climate room.

Germinated seeds were counted daily over ten days and the length of seedlings was measured on the final day. All trial variants were performed in four replications and the trial was repeated twice.

Data analysis

Data were analyzed by a two-factorial analysis of variance (ANOVA) using STATISTICA 8.0. When F values were statistically significant ($p<0.05$) treatments were compared using the LSD test.

RESULTS AND DISCUSSION

Effects of PGPRs on field dodder seed germination

Germination of field dodder seeds *in vitro* depended on treatment, i.e. on the PGPRs (rhizobacteria) applied, and it ranged from 27 to 77 % (Figure 1). Data indicate that the treatments MO_3, MO_4 and MO_6 caused inhibitory effects (15%, 65% and 52%, respectively), while MO_1, MO_2 and MO_5 stimulated (3%, 3% and 19%, respectively) germination of field dodder seeds (Figure 1). The highest percentage of germinated seeds was found under MO_5 treatment (78%), and the lowest under MO_4 (27%), while 72% of control seeds germinated. Vrbničanin et al. (2008a) revealed positive effects of several rhizosphere bacteria (*A. chroococcum* Ps1, *B. megatherium* ZP6 and *B. circulans*), and their combinations, on seed germination of the weed species *Iva xanthifolia* Nutt., *Amaranthus retroflexus* L. and *Sorghum halepense* L. (Pers.). Other authors have also reported stimulating effects of the same bacterial cultures on seed germination and seedling growth of various cultivated, as well as weed species (Gutiérrez-Mañero et al., 2001; Carrillo-Castañeda et al., 2002; Ryu et al., 2003). Shishido et al. (1996) showed a positive effect of *Bacillus* bacterial culture on seedling growth of pine and spruce. Egamberdiyeva (2007) confirmed a stimulating PGPR activity on plant growth and nitrogen uptake in maize growing on two types of soils. However, whether their influence will be stimulating, neutral or inhibitory often depends on other factors too, such as the conditions in which seeds are stored prior to exposure to the activity of microorganisms. Thus, two isolates of *Bacillus licheniformis* had positive effect on seed germination of *Onopordon acanthium*, *Datura stramonium* and *Abutilon theophrasti* after seed storage at room temperature in the laboratory, while the effect was negative in case of *Datura stramonium* and *Abutilon theophrasti* seeds that had been previously exposed to 4°C temperature (Vrbničanin et al., 2008b). Data reported by Martinez-Toledo et al. (1988), Ahmad et al. (2008) and Vrbničanin et al. (2008a, 2011) reported stimulating activity of *A. chroococcum* on seed germination and seedling growth of various plant species. Consistently with those earlier data, the present study also showed that the bacterial culture *A. chroococcum* Ps1 (MO_5 treatment) had a stimulating effect on seed germination of field dodder. Statistical data analysis revealed significant ($p<0.05$) differences in seed germination between control seeds and treatments MO_3, MO_4 and MO_6.

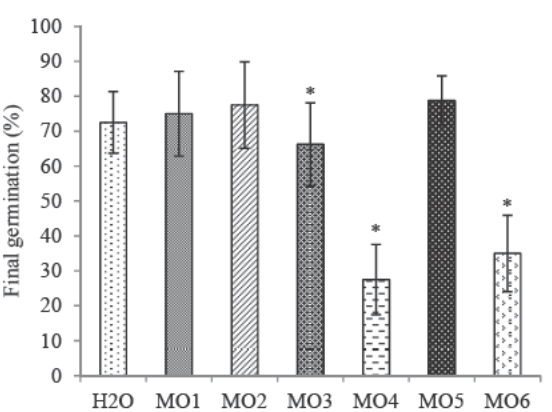

Figure 1. Effects of PGPRs on germination of field dodder seeds. H_2O-distilled water (control), MO_1-*Bacillus licheniformis*; MO_2-*B. pumilus*; MO_3-*B. amyloliquefaciens*; MO_4-*B. megatherium* ZP6; MO_5-*Azotobacter chroococcum* Ps1; MO_6-*Pseudomonas fluorescens*; LSD test * ($p<0.05$).

Germination rates of dodder seeds exposed to different PGPRs ranged from 11.73 to 27.38 (Table 1). Rueda-Puente et al. (2007) discussed that PGPR effects on seed germination could also depend on factors such as salinity and soil temperature as those factors significantly affected the germination rates of *Salicornia bigelovii* seeds in their trials. Statistically significant differences ($p<0.05$) in germination rates for field dodder were found between the control and treatments MO_5 and MO_6.

Petri dish germination bioassay with cover crops

The experimental data showed that cover crop (host) seeds had no stimulating effect on germination of field dodder seeds. The germination of host seeds ranged between 76% (sugar beet) and 97% (alfalfa), while field dodder germination under the same treatments was 73% and 79%, and 80% of control seeds. The percentage of germinated dodder seeds shows that there was virtually no difference bewteen treatments and control (Figure 2a).

Table 1. Effects of PGPRs on germination rates (no. day^{-1}) of field dodder seeds

Plant	H_2O	MO_1	MO_2	MO_3	MO_4	MO_5	MO_6
Cuscuta campestris	25.75	26.44	27.38	20.54	27.15	11.73*	12.87*

H_2O- distilled water (control), MO_1-*Bacillus licheniformis*; MO_2-*B. pumilus*; MO_3-*B. amyloliquefaciens*; MO_4-*B. megatherium* ZP6; MO_5-*Azotobacter chroococcum* Ps1; MO_6-*Pseudomonasfluorescens*; LSD * ($p<0.05$).

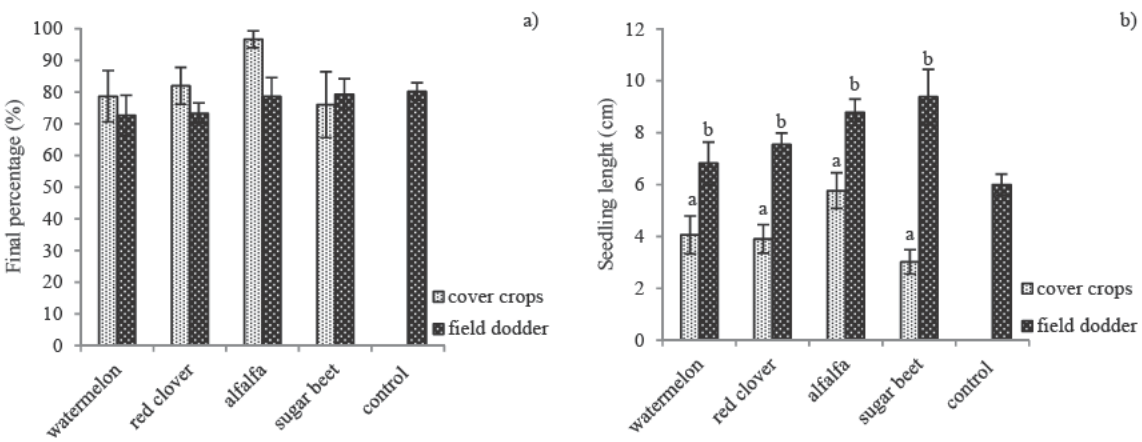

Figure 2. Final percentage (%) of seed germination - (a) and seedling length (cm) - (b) of cover crops and field dodder seeds in the dark; LSD test, a, b ($p<0.05$).

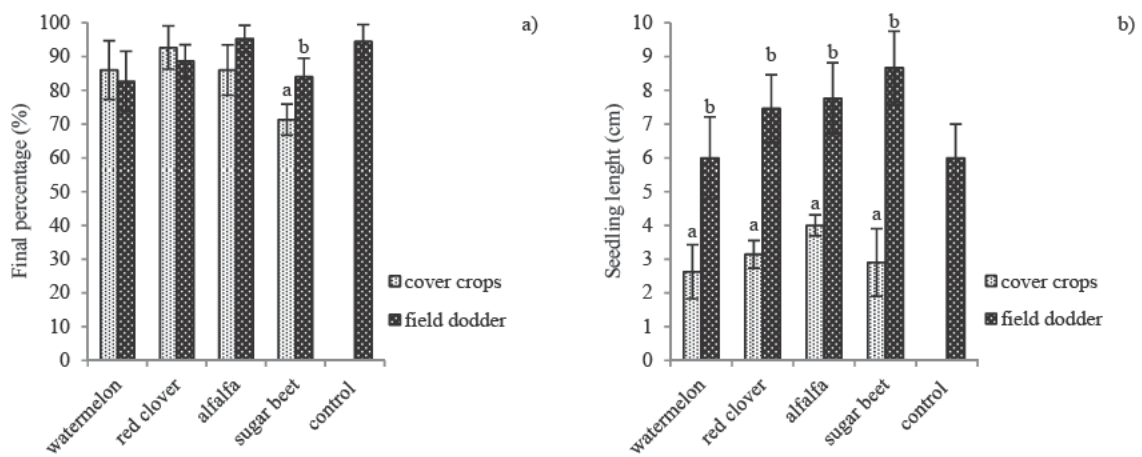

Figure 3. Final percentage (%) of seed germination - (a) and seedling length (cm) - (b) of cover crops and field dodder seeds under white light illumination; LSD test, a, b ($p<0.05$).

Conversely, a stimulating allelopathic effect was revealed on the initial growth of seedlings in the incubator in darkness at 25°C temperature. The length of control dodder seedlings (6 cm) was significantly ($p<0.05$) smaller than lengths under treatments with host seeds [from 7 cm (watermelon) to 9 cm (alfalfa and sugar beet)] (Figure 2b). Also, the lengths of host seedlings [from 3 cm (sugar beet) to 6 cm (alfalfa)] were significantly ($p<0.05$) smaller compared to dodder seedlings in corresponding treatments (Figure 2b).

Germination percentage of illuminated host seeds (71-93%) was similar to those germinating in the dark (79-97%) (Figure 3a). Conversely, germination percentage of control dodder seeds exposed to light (95%) was significantly higher than treatment in the dark (80%). Treatments with illuminated host seeds also showed higher percentages of germinated dodder seeds (83-95%) (Figure 3a). The data show that light is another important initial factor besides the host for stimulation of dodder seed germination. The length of treated dodder seedlings under illumination was between 6 cm and 9 cm, while control seedlings were 6 cm long on the average (Figure 3b). The greatest allelopathic effect on initial dodder seedling growth under illumination was detected for sugar beet (9 cm) and alfalfa (8 cm) seeds, then red clover (7 cm) and watermelon (6 cm) (Figure 3b).

The presented data show: 1) that development of a management strategy for field dodder growth and expansion in crops requires research into its life cycle, primarily a study of the biological character of its seeds and conditions of their germination since parasitic flowering plants germinate only in the presence of a potential host and under illumination, which was confirmed in our study, while initial seedling growth also requires adequate allelochemicals; 2) that knowing the interactions between rhizobacteria (PGPR) and seed germination and seedling growth of weed plants may be valuable for further developing the concept of biological control of weeds, i.e. systems of integrated plant protection. Therefore, by applying rhizobacteria that stimulate seed germination of weed plants it is possible to provoke uniform germination and emergence of weeds, which would then enable various agricultural practices or chemical control in a next step and so reduce potential weediness (seed bank) of agricultural plots. On the other hand, PGPRs that act inhibitory on seed germination and seedling growth of weeds, field dodder in this case, can also be used as a method of direct weed control in cover crops.

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Uticaj rizobakterija i useva na klijanje i rani porast viline kosice (*Cuscuta campestris* Yunk.)

REZIME

Za ispitivanje uticaja rizobakterija (Planth Growth Promoting Rhizobacteria - PGPR) na klijanje semena i stope klijanja viline kosice (*Cuscuta campestris* Yunk.), korišćene su bakterijske kulture: *Bacillus licheniformis* (MO_1), *B. pumilus* (MO_2) i *B. amyloliquefaciens* (MO_3) izolovane iz stajnjaka; *B. megatherium* ZP6 (MO_4) iz rizosfere kukuruza; *Azotobacter chroococcum* Ps1 (MO_5) i *Pseudomonas fluorescens* (MO_6). Svakodnevno u periodu od deset dana brojana su proklijala semena. Takođe, za praćenje uticaja semena biljaka domaćina na klijanje semena i početni porast klijanaca viline kosice u mraku i pri tretmanu belom svetlošću korišćena su semena četiri biljke domaćina (lubenica, crvena detelina, lucerka i šećerna repa). Proklijala semena su prebrojavana svakodnevno u periodu od deset dana, a poslednjeg dana su izmerene dužine klijanaca.

Dobijeni rezultati ukazuju da su tretmani MO_3, MO_4 i MO_6 ispoljili inhibitorni (15%, 65% i 52%), a tretmani MO_1 (*Bacillus licheniformis*), MO_2 (*B. amyloliquefaciens*) i MO_5 (*Azotobacter chroococcum* Ps1) stimulativni efekat (3%, 3% i 19%) na klijanje semena viline kosice. Takođe, rezultati dobijeni sa semenima biljaka domaćina ukazuju da je za podsticanje klijanja semena viline kosice, osim prisustva domaćina (73-79%, kontrola 80%), kao inicijalni faktor značajna i svetlost (83-95%, kontrola 95%).

Ključne reči: Rizobakterije; Klijanje semena; Usevi; Vilina kosica