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INFLUENCE OF NUTRITIVE MEDIA AND LOW TEMPERATURES ON BROOMRAPE SEED GERMINATION

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Abstract

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The germination of broomrape seeds on different nutritive media and at low temperatures was tested. Broomrape seed was collected from three localities in Northern part of Serbia. Nutritive media which were used are: water agar, water agar with gibberelic acid and water agar with biological agent Trifender. Temperature regimes were: without cooling, and in the fridge for 7, 14 and 21 days at + 4°C. The highest number of germinated seeds was observed on the agar medium with gibberelic acid in presence of sunflower roots. Cooling of broomrape seeds on 4°C during 21 day leads to stimulation of their germination and average radicle length. The effect of bio-agent Trifender should be further investigated.

Key words: broomrape, seed germination, sunflower, nutritive media

Abbreviations: water agar (WA); water agar with giberellic acid (GA); water agar medium with Trifender (T); WA-B, GA-B nutritive media (WA or GA) without cooling seeds (4°C); WA-7, GA-7 nutritive media (WA or GA) with cooling seeds (4°C) for 7 days; WA-14, GA-14 nutritive media (WA or GA) with cooling seeds (4°C) for 14 days; WA-21, GA-21– nutritive media (WA or GA) with cooling seeds (4°C) for 21 days

Introduction

Broomrape (*Orobanche cumana*) is one of the most important devastating parasites of sunflower. In the agroecological conditions of Serbia broomrape (*O. cumana*) as a parasite of sunflower has been appearing with varying intensity almost every year and can cause significant damage in sunflower production (Gulya et al., 1997; Masirevic, 2001). Resistant or tolerant sunflower hybrids are the most efficient and the most economical measures in the suppression of this parasitic plant (Skoric et al., 2012). In the tests of sunflower hybrids susceptibility to broomrape under artificial infestation, high germinativity of broomrape seeds used for infestation is an obligatory condition. The aim of this paper is to evaluate influence of different nutritive media and low temperature (4°C) on broomrape seed germination.

Materials and Methods

Seeds of *Orobanche cumana* were collected in the sunflower fields in Vojvodina, Northen part of Serbia (Senta, Vrsac and Backa Topola) during 2009 and 2010. Seed samples from Senta and Vrsac were kept in the fridge at + 4°C during ten months in order to break dormancy. Seed sample from Backa Topola were kept in different temperature regimes: without cooling, and in the fridge for 7, 14 and 21 days at + 4°C.

Surface sterilized seeds were placed in Petri dishes with nutritive media incubated at 25°C in the dark. The media which were used are: water agar (WA) with giberellic acid (GA_3, Sigma-Aldrich, USA) in concentration of 25 ppm, water agar medium with Trifender (T) at concentration of 1% and the check was water agar medium. Trifender is a biological pesticide from *Trichoderma asperellum* acting as plant growth promoter with beneficial side effect of controlling soil-born pathogens.

In order to test the influence of the sunflower roots on broomrape seed germination, five to eight days old roots of the susceptible sunflower hybrid (NS-H-111) were added on the above mentioned media. The same media without sunflower roots were used for comparison.

Germination rate and distance of the germinated seeds from sunflower root was determined every three to four days

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under dissecting microscope. For the seeds from Backa Topola the lengths of five radicles were measured for every combination of media and temperature. Data were analyzed by ANOVA and Duncan test.

Results

The influence of different nutritive media on broomrape seed germination was examined. Broomrape seeds from Senta locality were germinated on each nutritive media containing sunflower roots. After seven days the significant difference between used media were observed. The highest initial germination rates were determined on GA (23%), while rates were much lower on WA (3%) and T (0%) (Table 1).

Seeds from Senta locality germinated after 39 days on each used media (Figures 1 and 2). Germination rates did not have a high increase and reached 30% on GA, 6% on WA, and 5% on T. Such low percent of germinated broomrape seed on the media with Trifender could be the consequence of inhibition of germination by this biological agent. Germination rates of seeds from Senta locality on the same media without sunflower roots were under 5%. Broomrape seeds from locality Vrsac shared poor germinativity on all tested nutritive media. Germination of the sample form Vrsac was under 4%, even on the media with sunflower roots. There is no significant difference in the average number of germinated seed (from both localities) on the media without sunflower roots (Table 1).

Germination dynamics of broomrape seed on examined nutritive media WA, GA and T are shown on Figures 3, 4 and 5. The number of germinated seeds on WA and GA increased after the first evaluation and on medium with Trifender after second evaluation. The slowest germination rates were determined on T medium where seeds started to germinate after 12 days The number of germinated seeds on WA medium, after second, third and fourth assessment increased for 1% and in the further period germination stopped (Figure 3).

Germination of broomrape seeds on other media, ranged from 2-6% (Figure 4). Our experiments confirm that presence of roots of susceptible sunflower hybrids stimulated broom-

Fig. 1. Germinate broomrape seed

Fig. 2. Germinated broomrape seed and sunflower root

Table 1
Broomrape seed germination rate after on different nutritive media

Medium	Average number of geminated broomrape seed, %				
	Sample from Senta locality			Sample from Vrsac locality	
	Medium with sunflower roots		Medium without sunflower roots	Medium with sunflower roots	Medium without sunflower roots
	after 8 days	after 39 days	after 39 days	after 39 days	after 39 days
GA	23 a	30 a	2a	4a	2a
WA	3 b	6 b	3a	3a	3a
T	0 b	5 b	4a	1a	1a
*P < 0,05	0.0398*	0.0370*	0.6141ns	0.236ns	0.7118ns

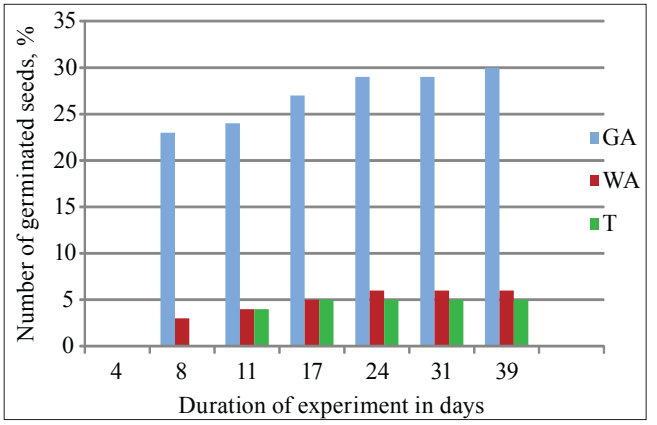

Fig. 3. Germination dynamics of broomrape seeds from Senta locality on different nutritive media with sunflower roots

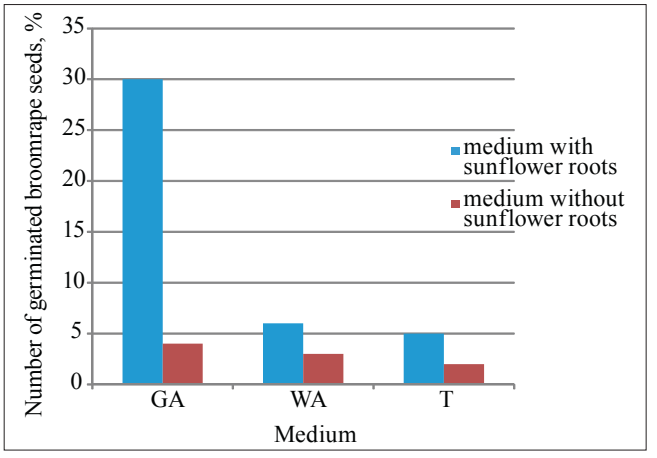

Fig. 4. Average number of germinated broomrape seeds from Senta locality on different nutritive media 39 days after experiment was set

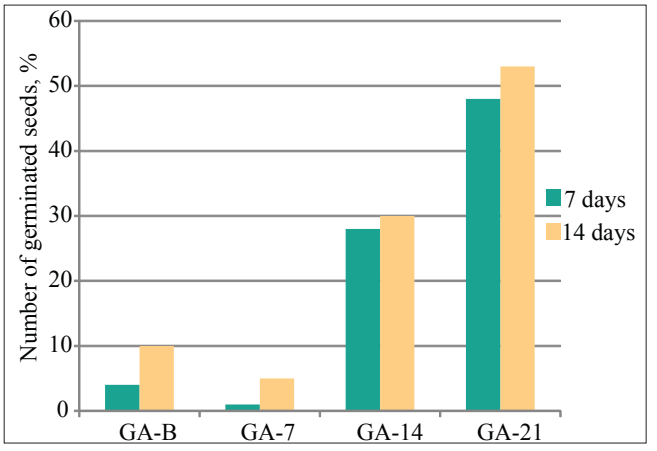

Fig. 5. Germination dynamics of broomrape seeds on GA medium depending on cooling period

rape seed germination from Senta locality *in vitro*. Influence of close vicinity of sunflower roots to germinated broomrape seed were evaluated (Table 2).

Obtained results indicate that average distance of broomrape germinated seeds from sunflower roots was the shortest on water agar medium, including both tested samples. Data for average distance of germinated broomrape seeds from sunflower roots on Trifender were similar for both samples while data for GA differ significantly between samples. However, on the GA medium seeds from Senta locality germinated at the longest distance from sunflower root (31 mm).

Influence of low temperature (4°C) on broomrape seed germination from Backa Topola, locality is shown in Table 3 and Figure 5. According to ANOVA and Duncan test, there are significant differences in average number of germinated broomrape seeds. Germination of broomrape seeds on WA medium in all temperature regimes and on GA medium with-

Table 2
Average distance of broomrape germinated seeds from sunflower roots

Nutritive media with sunflower root	Average distance of broomrape germinated seeds from sunflower roots, mm	
	Senta	Vrsac
WA	5.00 (1-18)	8.00 (2-19)
GA	8.98 (1-31)	19.25 (14-23)
T	13.50 (6-26)	14.00 (14)

Table 3
Influence of low temperatures on broomrape seed germination from locality Bačka Topola on WA and GA nutritive media 14 days after experiment was set

Nutritive media with sunflower roots	Number of germinated broomrape seeds, %
GA-21	53 a
GA-14	30 b
GA-B	10 c
GA-7	6 c
WA-21	4 c
WA-7	2 c
WA-14	2 c
WA-B	2 c
**P < 0,01	0,000**

out cooling and with 7 days on 4°C were low (up to 10%). The highest germination was observed on GA-21 (53%), while on GA-14 the average number of germinated seeds was 30%. This phenomenon indicates that exposure of broomrape seeds to low temperatures in duration of 21 and 14 days had positive influence on their germination.

Germination of broomrape seed which was cooled 21 day on GA medium was the highest 48% after 7 days and 53% after 14 days. It is interesting that on GA medium seed which was not cooled had doubled germination than seed cooled for 7 days.

Influence of close vicinity of sunflower roots to germinated broomrape seeds were evaluated (Table 4). Seeds on GA with different temperature regimes, had germinated on longer distances from sunflower roots than seeds on WA. It could be explained by positive joint effect of gibberelic acid on seed germination.

Average radicle length was shown in Table 5. The results showed that the highest average radicle length was on GA medium with the longest period of cooling. Decreasing of cooling period leads to decreasing of radicle length.

Table 4
Average distance of broomrape germinated seeds (locality Bačka Topola) from sunflower roots

Nutritive media with sunflower root	Average distance of broomrape germinated seeds from sunflower roots, mm
WA-B	10.00 (8-12)
GA-B	9.00 (3-18)
WA-7	4.50 (3-6)
GA-7	9.33 (2-23)
WA-14	2.50 (1-4)
GA-14	10.40 (1-29)
WA-21	9.20 (1-23)
GA-21	13.51 (2- 30)

Table 5
Average radicles length of broomrape seeds (locality Bačka Topola), 14 days after experiment was set

Nutritive media with sunflower root	Averge radicles length, mm
GA-B	0.30 (0.1-0.8)
GA-7	0.32 (0.1-0.95)
GA-14	1.08 (0.4-1.8)
GA-21	1.48 (0.7-2.3)

Discussion

The seed of parasitic weeds germinate only if they are exposed to stimulant molecules which are present in the root exudate of a suitable host plant (Musselman, 1987; Parker and Riches, 1993). Many authors (Pieterse, 1979; Press et al., 1990; Chae et al., 2003) reported that, *Orobanchaceae* seeds for germination under chemical stimulation, required conditioning special conditioning, for example to be kept for several days in a wet environment and suitable temperatures. However recent results (Plakhine et al., 2009; 2010), showed that non-conditioned seeds of both *Orobanche cumana* Wallr. and *O. aegyptiaca* Pers. were able to germinate in response to chemical stimulation by GR24 even without prior conditioning.

According to our preliminary investigations, medium with gibberellic acid and roots of susceptible sunflower hybrid is a good medium for provoking broomrape seed germination (Masirevic et al., 2011). In our experiment the most favorable medium for broomrape germination was the medium with sunflower roots and GA_3. Gibberellic acid in agar at the concentration rate of 1-20 ppm stimulated germination of *Orobanche ludoviciana* var. *cooperi* and *O. ramosa* seeds (Nash and Wilhelms, 1960). The same authors suggested that in nature gibberellins may be secreted in small amounts into the rhizosphere of young plants and may thus play a part in the germination of *Orobanche* spp. These plant hormones are involved in regulation of seed germination in *Orobanche* species. GA biosynthesis takes place during conditioning (Joel et al., 1989). The same authors reported that exogenous added GA to the medium during conditioning shortens the time for the response to germination stimulants. *O cernua* germinated in Petri dishes on agar medium and in the presence of isolated sunflower roots (Serghini et al., 2001). These authors also reported that germination became visible after 4 days and maximum values were reached on the eight day. In our experiment the germinated seeds were visible after 4 days and a major number of seeds germinated in that period, but seeds continued to germinate up to 39 days. The obtained germination on media without sunflower roots was very low.

Seeds of *O. cumana* - *in vitro* conditions germinate only in presence of sunflower roots and the highest germination was recorded in the most virulent race (Glijin et al., 2011). This phenomenon could be further investigated in the laboratory and field experiments of resistance testing. In a distance of 1-2 cm from the sunflower roots, germination of broomrape seeds is normally lower than between roots (Joel et al., 2011).

Trichoderma species are well known as bio-agents in control of phytopathogenic microorganisms (on seed, in the soil and postharvest) (Lo, 1998). The applied bioagent on the basis

of *T. asperellum* showed some kind of slow-down effect to the germination of broomrape seed. Bio-agent based on *T. asperellum* inhibited germination of broomrape *in vitro* and such seeds had shorter radicle lengths (Masirevic et al., 2012).

Conclusion

The highest number of germinated seeds was observed on the agar medium with gibberelic acid in presence of sunflower roots. Other tested media with or without sunflower roots weakly stimulated the germination of broomrape seeds. According to these results it can be concluded that gibberelic acid combined with sunflower roots had positive influence on broomrape seed germination. The effect of bio-pesticide to broomrape seed germination should be further investigated. Cooling of broomrape seeds on 4°C during 21 day leads to stimulation of their germination and average germ length. Further tests should be conducted with different broomrape populations and sunflower genotypes with different level of resistance to broomrape.

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