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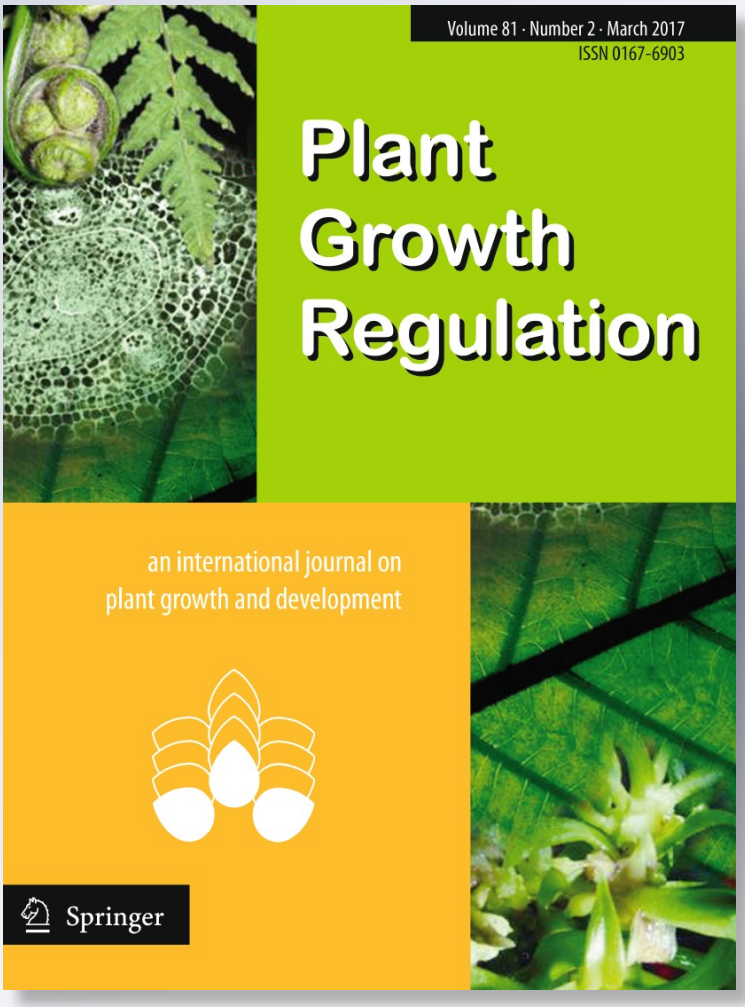


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ORIGINAL PAPER

# Conditioning duration and agents involved in broomrape seeds responding to germination stimulants

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**Abstract** Weedy broomrape species, such as sunflower broomrape (*Orobanche cumana* Wallr.) and Egyptian broomrape [*Phelipanche aegyptiaca* Pers. (syn. *O. aegyptiaca*)], require a period of pre-conditioning before they can respond to germination stimulants. Thus, the sensitivity of weedy broomrape seeds to germination stimulants could be an important factor for broomrape control. In this study, the influence of conditioning agents, conditioning period (0–21 days) and germination stimulants on the germination of sunflower broomrape and Egyptian broomrape seeds was analyzed. Without conditioning, the sunflower and Egyptian broomrape seeds exhibited negligible germination responses to the stimulants. The germination rate of the broomrape seeds increased rapidly with conditioning period and reached a maximum under a conditioning period of 4–10 days; further prolonged conditioning resulted in a decrease in the germination rate. Gibberellic acid ($GA_3$) could not only break the dormancy of the sunflower and Egyptian broomrape seeds but also maintained the high sensitivity of these seeds even after 21 days of conditioning. Furthermore, 100 μM of $GA_3$ induced the germination of the Egyptian broomrape seeds. The stimulants that induced Egyptian broomrape germination were ranked in decreasing order as GR24 (76.8%), strigol (76.1%), tobacco root exudates (49.5%), dehydrocostus lactones (DCL, 39.2%), and maize root exudates (18%). In contrast, $GA_3$ did not directly induce sunflower broomrape seed germination, which responded to strigol (62.8%) > maize root exudates (58.2%) > GR24 (57.9%) > tobacco root exudates (41.6%) > DCL (41.3%). These results indicate specialized recognition of germination stimulants by sunflower and Egyptian broomrape. This study may contribute to a better understanding of parasitic weed germination and may lead to improved control strategies.

**Keywords** Conditioning Gibberellic acid ($GA_3$) · Germination stimulants · Sunflower broomrape · Egyptian broomrape

## Introduction

Weedy broomrape (*Orobanche* spp. and *Phelipanche* spp.) species have parasitized a wide range of crops in the Mediterranean region, Western Asia and Eastern Europe (Parker 2009), causing substantial losses. Management strategies have been largely unsuccessful mainly because of the complex life cycle of broomrape (Goldwasser et al. 1997; Lins et al. 2009). The germination of broomrape seeds requires chemical signals from the host plants (Parker and Riches 1993). However, once a broomrape seed has germinated, it will die unless a direct connection is established with the

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host (Bouwmeester et al. 2003). In this respect, the germination of broomrape species can be targeted to develop new and practical control measures to combat the weedy broomrape problem (Wegmann 2006). A better understanding of the germination of sunflower broomrape (*Orobanche cumana* Wallr.) and Egyptian broomrape (*Phelipanche aegyptiaca* Pers. (syn. *O. aegyptiaca*)) seeds could facilitate the development of effective broomrape control programs.

For decades, it was believed that broomrape seeds require pre-incubation in a warm and moist environment for several days before becoming sensitive to germination stimulants; this is termed "conditioning" (Matusova et al. 2004). During conditioning, a series of physiological processes occur, such as decreases in the abscisic acid (ABA) content (Lechat et al. 2012), protein synthesis (Nun and Mayer 1993), changes in oxidase activity (Bar Nun et al. 2003) and DNA methylation (Lechat et al. 2015). All of these processes are potentially involved in the mechanisms of perception to germination stimulants. However, Plakhine et al. (2009) reported that seeds of some broomrape species do not require this conditioning phase. These authors reported that the time required for sunflower and Egyptian broomrape seeds to reach the maximum germination rate was 2 weeks after the application of GR24 (synthetic strigolactones), with or without conditioning. Prolonged conditioning of broomrape seeds could result in a reduction in germination (van Hezewijk 1994; Gibot-Leclerc et al. 2004). Thus, conditioning remains an ambiguous issue in parasitic plant studies (Parker and Riches 1993), especially when considering the effects of conditioning agents and the conditioning period.

Plant hormones and germination stimulants are two important chemical compounds that influence the germination of parasitic plants. Gibberellic acid ($GA_3$) has been reported to stimulate *O. crenata* seed germination and was thought to be a main stimulant in faba bean (*Vicia faba* L.) roots (El-Ghamrawy et al. 1990). However, subsequent research has revealed that $GA_3$ only results in limited germination of broomrape seeds, but enhances the germination induced by germination stimulants and significantly shortens the conditioning period needed of seeds (Takeuchi et al. 1995). It has been hypothesized that the biosynthesis of GA during conditioning is an important step in the germination of *Orobanche* seeds (Zehhar et al. 2002). On the other hand, most germination stimulants of broomrape seeds identified thus far belong to the strigolactones (Yoneyama et al. 2010, 2011). However, other plant-produced compounds, such as dehydrocostus lactones (DCL) (Joel et al. 2011), dihydrosorgoleone, sesquiterpene lactones (Fischer et al. 1989), kinetin, coumarin, jasmonate, peagol, peagoldione (Evidente et al. 2009) and fungal metabolites (fusicoccins, cotylenin), can also result in the germination of some broomrape species (Xie et al. 2010). It is generally believed that root parasites have significantly different responses to different stimulants (Bouwmeester et al. 2003; Yoneyama et al. 2009). However, whether interactions exist between the conditioning period, conditioning agent and germination stimulants, and their potential effects on the sensitivity and germination of broomrape seeds have not been systematically analyzed.

In this paper, we tested the germination responses of sunflower and Egyptian broomrape seeds to conditioning period (0–21 days), conditioning agents (distilled water or $GA_3$), and germination stimulants (strigol, GR24, DCL, maize root exudates and tobacco root exudates). A cubic curve was applied to determine the effect of the conditioning period on the germination of the sunflower and Egyptian broomrape seeds in the presence of different conditioning agents and germination stimulants. These results will be of value for developing new strategies to limit the damage caused by broomrape.

## Materials and methods

### Seed materials

Sunflower broomrape seeds were collected from sunflower (*Helianthus annuus* L.) fields in Dingbian County, Shaanxi Province, China, in 2010. Egyptian broomrape seeds were collected from tomato (*Lycopersicon esculentum* Mill.) fields in the Xinjiang Uygur Autonomous Region in 2010. Dry seeds were stored in a cloth bag in the dark at room temperature until use. Before use, all seeds were surface-sterilized in 1 % (w/w) sodium hypochlorite (NaClO) for 3 min and then soaked in 75 % (V/V) ethanol for 3 min. The surface-sterilized seeds were thoroughly rinsed with autoclaved distilled water and then air dried on a clean bench.

### Chemicals and root exudates

GR24 (synthetic strigolactones) was supplied by Professor Binne Zwanenburg from Radboud University, The Netherlands. Strigol was provided by Professor Koichi Yoneyama of Utsunomiya University, Japan, and DCL was purchased from the Baoji Biochemical Company (Baoji City, Shaanxi Province, China). Aqueous solutions of GR24, strigol and DCL (0.1 or 1 mg $L^{-1}$) were prepared by first dissolving the compounds in acetone and then diluting them with distilled water.

The maize seeds (Zhengdan 958) used to collect the root exudates were obtained from Professor Jianchang Mao in 2009 (College of Agricultural Science, Northwest A&F University, China). Tobacco seeds (Majiang Baihua, a Chinese native cultivar) were kindly provided by the Chinese Central Southern Test Station of Tobacco Agriculture, Changsha,

Hunan, China. Surface-sterilized maize and tobacco seeds were placed in sterile Petri dishes lined with moistened filter paper. The Petri dishes were wrapped in aluminum foil and incubated at 25 °C for 72 h. Then, the seedlings were removed from the Petri dishes and placed in culture medium in a growth chamber. The root exudates from the seedlings, which were released into water, were collected for 2 weeks, and the exudates were adsorbed using activated charcoal. The water containing 1 mM of Calcium chloride (CaCl2) and activated charcoal was replaced every 2 days. The activated charcoal containing the absorbed exudates was eluted with acetone. The acetone was removed by vacuum evaporation in a rotary evaporator at 40 °C. Then, the residue was transferred into a 50-mL volumetric flask and brought to volume using distilled water, and partitioned five times with 50 mL of ethyl acetate (EtOAc) in a separating funnel. The EtOAc phases were combined and concentrated by vacuum evaporation to dryness. The dried residues were suspended in 5 mL of acetone and stored in sealed glass vials at 4 °C. Before the germination tests were conducted, the residues were diluted with distilled water to final concentrations.

### Seed conditioning

The following procedure was used to condition the sunflower and Egyptian broomrape seeds. Approximately 40–80 broomrape seeds of each species were sown onto 8-mm glass fiber filter disks (Whatman GF/A). Then, 100 seed disks were placed in Petri dishes (9 cm diam.) lined with two layers of filter paper (9 cm diam.) that were wetted with aliquots (5 mL) of either distilled water or different conditioning media, i.e., $GA_3$ solutions at concentrations of 1, 10 and 100 µM. The Petri dishes were then sealed with parafilm and incubated in the dark at 25 °C (Parker et al. 1977) for conditioning. The conditioning period tests were conducted every day from 0 to 21 days. During conditioning, sterile water was added to each Petri dish every 3 days to maintain its initial weight and $GA_3$ concentration.

### Germination tests

After conditioning for the prescribed period, the seeds were blotted to remove excess water or conditioning media. The glass fiber filters disks that contained the conditioned seeds were then transferred to a new 9-cm Petri dish. Aliquots (20 µL) of the germination stimulants were applied to the glass fiber discs; GR24, strigol and DCL were applied at two concentrations: 0.1 and 1 mg $L^{-1}$, while the root exudates were applied at concentrations of 1 and 10 mg $L^{-1}$. In all experiments, distilled water was added to the conditioned seeds as a negative control. The Petri dishes were sealed and incubated at 25 °C in the dark as described above. The germination of the broomrape seeds in the Petri dishes was quantified directly under a binocular dissecting microscope at 20× magnification. Seeds were considered germinated when the length of the emerging radicle was equal to or longer than its width. The results of a pre-experiment (data not shown) suggested that maximum germination rates were achieved 10–14 days after the application of the germination stimulants, which is in agreement with Plakhine et al. (2009). Thus, the germination rates measured 14 days after the application of the germination stimulants were selected to compare the effects of the conditioning factors on the sensitivity of the broomrape seeds to various stimuli. The conditioning process and the germination tests are shown in the supplemental material (Supplementary Fig. S1).

### Statistical analysis

The experimental design was completely randomized. The treatments in each experiment were replicated five times; each experiment was conducted twice, and the data were combined for analyses. Analysis of variance (ANOVA) was performed on the germination rates depending on the response to the three factors. Tukey or Tamhanes T2 tests (depending on the homogeneity of variances) were used to separate the means. Cubic curve regression was used to evaluate the relationship between the duration of the conditioning period (days) and the germination rates. The data were processed using Excel 2003, DPS 9.5 software (Tang and Zhang 2013) and SPSS for Windows, version 22.0 (SPSS Inc., Chicago, Illinois, USA).

## Results and discussion

All three factors (conditioning agents, conditioning period and germination stimulants) tested in this experiment significantly influenced the germination of the sunflower and Egyptian broomrape seeds (Table 1). In addition, the interaction between the $GA_3$ concentration and the germination stimulants had a significant effect on the germination rates of the seeds. Detailed information on the germination rates is presented in a table format in the supplemental materials (Supplementary Table S1-S10).

### Conditioning released the dormancy of the sunflower and Egyptian broomrape seeds

It is generally believed that seeds of parasitic plants require a period of incubation in a warm and moist environment to release dormancy and become sensitive to germination stimulants (Matusova et al. 2004; Lechat et al. 2012). However, sunflower and Egyptian broomrape may not require this conditioning phase as their seeds have been found to be receptive without conditioning (Plakhine et al. 2009). In

**Table 1** ANOVA results of the effects of the conditioning agents, conditioning period and germination stimulants on the germination response of sunflower and Egyptian broomrape seeds

| F value | Sunflower broomrape | Egyptian broomrape |
|---|---|---|
| Conditioning agents (A) | 555.50** | 56.97** |
| Conditioning period (C) | 62.88** | 24.00** |
| Germination stimulants (S) | 164.49** | 485.04** |
| A × C | 4.16 | 0.88 |
| A × S | 25.14** | 12.11* |
| C × S | 1.96 | 1.79 |
| A × C × S | 0.99 | 0.52 |

*, **Indicate significant differences at the 0.05 and 0.01 level, respectively (Tukey's HSD)

the current study, the seeds of both sunflower and Egyptian broomrape were able to germinate in response to the germination stimulants without conditioning (Figs. 1, 2). However, significant increases in the germination rates of both types of broomrape seeds were observed in response to a conditioning period of 3 or 4 days. For instance, in the absence of conditioning, the sunflower broomrape seeds had germination rates that ranged from 0 to 26.9% in response to the various germination stimulants (Figs. 1, 3). After conditioning with either distilled water or $GA_3$ for 3 days, however, the germination rates of these seeds increased to 12% and 95%, respectively. Similar results were obtained for the Egyptian broomrape seeds treated with root exudates and DCL (Figs. 2, 4), where low germination rates (<20%) were observed. Unconditioned Egyptian broomrape seeds that responded to strigolactones exhibited a germination rate ranging between 45.6 and 58.2%; the maximal germination rate of conditioned Egyptian broomrape seeds (>90%) was significantly higher (Fig. 2).

The germination rates of sunflower broomrape and Egyptian broomrape generally declined when the seeds were conditioned with distilled water or $GA_3$ for >12 days. Under conditioning periods that were too long or too short, the sensitivity of sunflower and Egyptian broomrape to the germination stimulants generally decreased, which is in accordance with previous results on sunflower broomrape and *S. hermonthica* (Matusova 2004). This mechanism would prevent the germination of broomrape seeds in the absence of their host plant or at the end of its growing season. A cubic curve model was chosen to describe the relationship between the conditioning period and the germination rate of the broomrape seeds (Tables 2, 3, 4, 5 and Supplementary Table S11, S12, S13, S14). Many models, such as the hydrothermal time model (Kebreab and Murdoch 1999) and the Analysis β model (Moral et al. 2015), have been proposed to explain the effect of conditioning on broomrape seed germination. While numerous studies have focused on the effect of conditioning temperature and water stress,

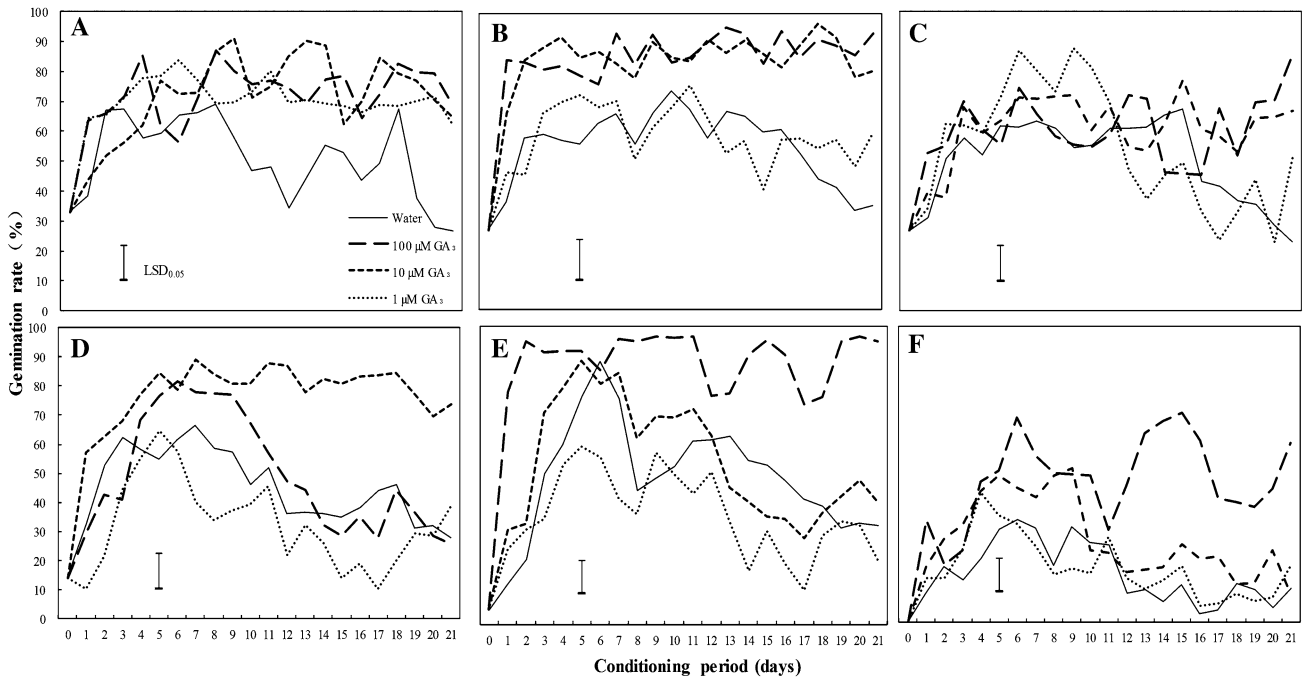


**Fig. 1** Germination rates of sunflower broomrape seeds in response to GR24 (**a, d**), strigol (**b, e**) and DCL (**c, f**) at concentrations of 1 mg $L^{-1}$ (**a–c**) and 0.1 mg $L^{-1}$ (**d–f**) over 21 days of conditioning. The *LSD bar* indicates the least significant difference ($p < 0.05$) between the mean germination rates in the presence of water and different concentrations of $GA_3$ treatments

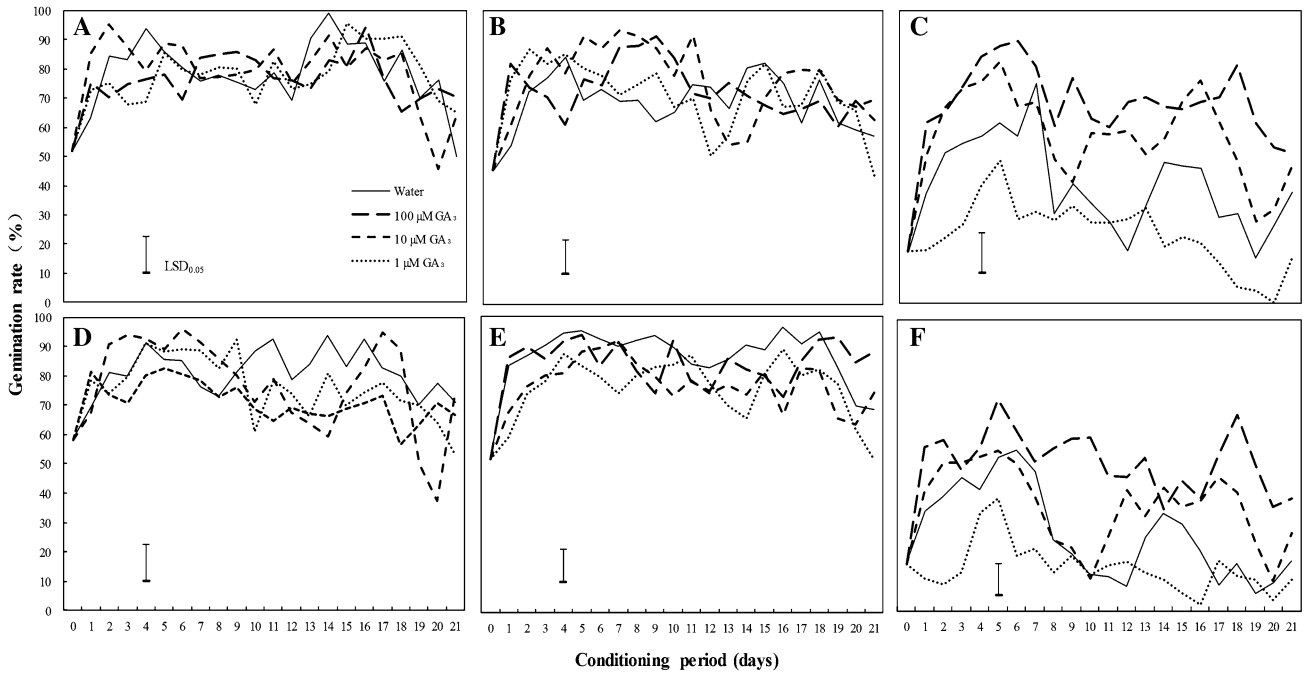


**Fig. 2** Germination rates of Egyptian broomrape seeds in response to GR24 (**a, d**), strigol (**b, e**) and DCL (**c, f**) at concentrations of 1 mg $L^{-1}$ (**a–c**) and 0.1 mg $L^{-1}$ (**d–f**) over 21 days of conditioning. The *LSD bar* indicates the least significant difference ($p < 0.05$) between the mean germination rates in the presence of water and different concentrations of $GA_3$ treatments

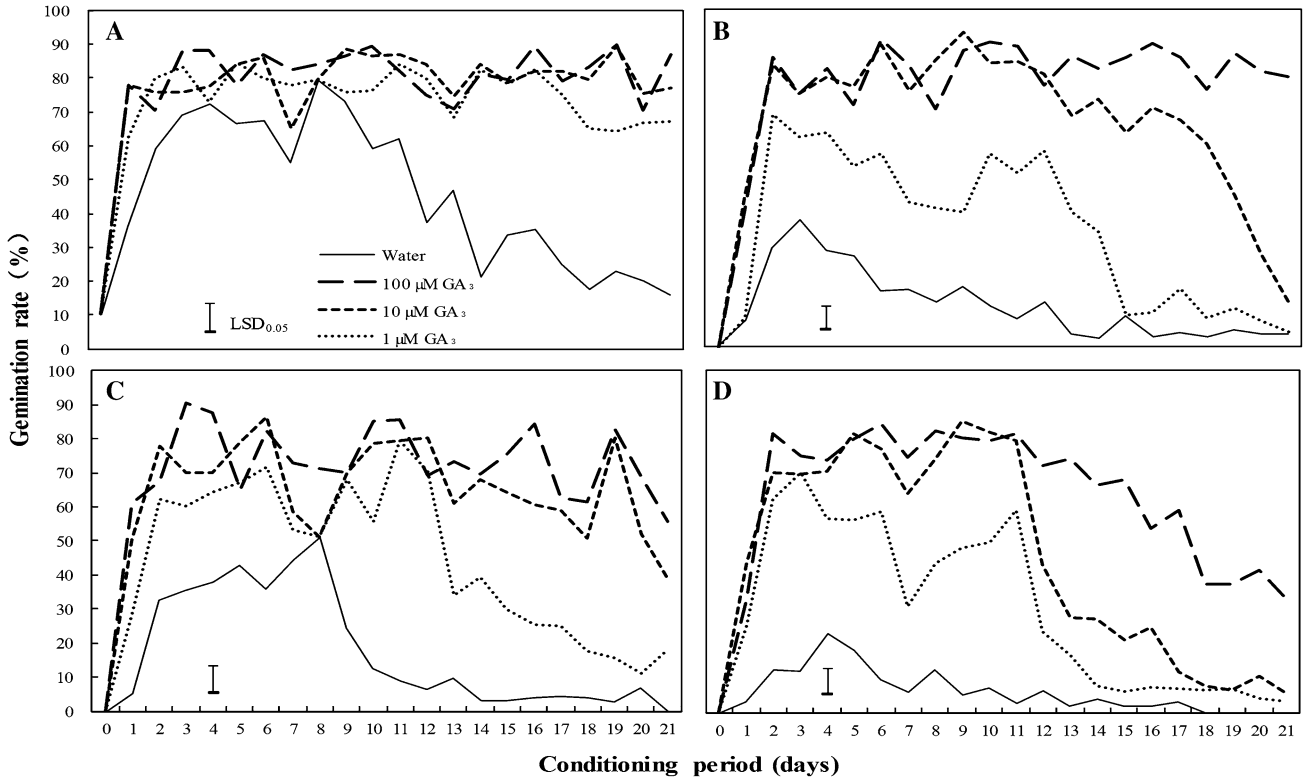


**Fig. 3** Germination rates of sunflower broomrape seeds in response to maize (**a, c**) and tobacco (**b, d**) root exudates at concentrations of 10 mg $L^{-1}$ (**a, b**) and 1 mg $L^{-1}$ (**c, d**). The *LSD bar* indicates the least significant difference ($p < 0.05$) between the mean germination rates in the presence of water and different concentrations of $GA_3$ treatments

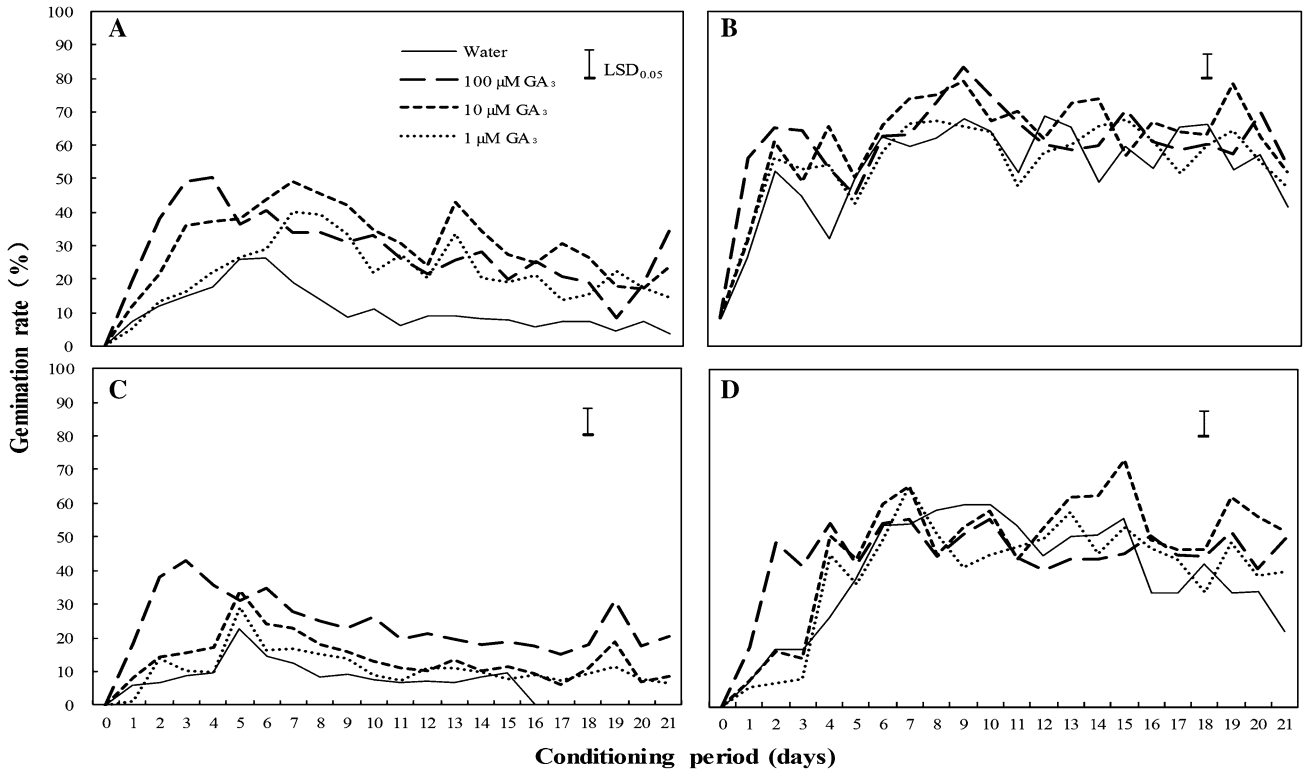


**Fig. 4** Germination rates of Egyptian broomrape seeds in response to maize (**a, c**) and tobacco (**b, d**) root exudates at concentrations of 10 mg $L^{-1}$ (**a, b**) and 1 mg $L^{-1}$ (**c, d**). The *LSD bar* indicates the least significant difference ($p < 0.05$) between the mean germination rates in the presence of water and different concentrations of $GA_3$ treatments

**Table 2** Effect of conditioning period on the germination of sunflower broomrape seeds conditioned with 100 μM $GA_3$ under various germination stimulants

| Germination stimulant | Concentration (mg $L^{-1}$) | Regression model | $R^2$ | F | Inflection point |
|---|---|---|---|---|---|
| GR24 | 1 | $y=47.17+7.96x-0.67x^2+0.017x^3$ | 0.46 | 5.15* | 9.2/17.0 |
| | 0.1 | $y=31.46+14.61x-1.17x^2+0.028x^3$ | 0.82 | 28.07** | 9.4/18.4 |
| Strigol | 1 | $y=53.28+9.72x-0.81x^2+0.021x^3$ | 0.54 | 6.90** | 9.7/16.0 |
| | 0.1 | $y=38.65+19.85x-1.97x^2+0.056x^3$ | 0.59 | 8.59** | 7.3/16.2 |
| DCL | 1 | $y=35.23+11.70x-1.33x^2+0.042x^3$ | 0.58 | 8.22** | 6.2/15.0 |
| | 0.1 | $y=7.56+11.93x-0.92x^2+0.022x^3$ | 0.55 | 7.29** | 10.1/17.8 |
| Maize root extracts | 10 | $y=38.91+16.24x-1.60x^2+0.045x^3$ | 0.55 | 7.31** | 7.4/16.3 |
| | 1 | $y=31.70+15.09x-1.35x^2+0.034x^3$ | 0.48 | 5.42** | 8.0/18.4 |
| Tobacco root extracts | 10 | $y=24.41+18.01x-1.54x^{22}+0.039x^3$ | 0.69 | 13.61** | 8.9/17.4 |
| | 1 | $y=17.50+21.02x-1.90x^2+0.045x^3$ | 0.81 | 24.67** | 7.6/20.5 |

The x value (number of conditioning days) of the inflection point derived from the model is presented

*, **Indicate significant correlations at the $P < 0.05$ and $P < 0.01$ level, respectively

only Song et al. (2005) proposed a negative linear model to explain the effect of the conditioning period. As the seed germination rate during conditioning was calculated every day (0–21 days) in our experiment, the cubic curve model provided a better fit for the germination of the broomrape seeds in contrast to a linear model. Moreover, the cubic curve model showed a good fit for the different broomrape species, conditioning mediums and germination stimulants, suggesting good potential for the general application of this model. Although this cubic curve model is essentially an empirical model, it may contribute to the development of mechanistic models of broomrape seed germination. The inflection points were derived from the cubic curve models, which indicated the optimal conditioning period

**Table 3** Effect of conditioning period on the germination of sunflower broomrape seeds conditioned with distilled water under various germination stimulants

| Germination stimulant | Concentration (mg $L^{-1}$) | Regression model | $R^2$ | F | Inflection point |
|---|---|---|---|---|---|
| GR24 | 1 | $y=42.00+6.46x-0.63x^2+0.015x^3$ | 0.41 | 4.17* | 6.8/21.0 |
| | 0.1 | $y=23.57+13.04x-1.37x^2+0.032x^3$ | 0.72 | 15.63** | 6.5/18.2 |
| Strigol | 1 | $y=32.82+7.73x-0.47x^2+0.005x^3$ | 0.84 | 31.28** | 9.6/53.6 |
| | 0.1 | $y=2.72+18.98x-1.65x^2+0.039x^3$ | 0.73 | 15.98** | 8.0/20.3 |
| DCL | 1 | $y=30.28+7.77x-0.49x^2+0.005x^3$ | 0.83 | 15.21** | 9.2/56.3 |
| | 0.1 | $y=-0.99+10.87x-1.17x^2+0.032x^3$ | 0.78 | 21.66** | 6.3/18.1 |
| Maize root extracts | 10 | $y=18.89+19.71x-2.14x^2+0.059x^3$ | 0.8 | 24.01** | 6.2/18.0 |
| | 1 | $y=3.33+13.41x-1.61x^2+0.047x^3$ | 0.77 | 19.49** | 5.5/17.3 |
| Tobacco root extracts | 10 | $y=5.31+3.32x-0.43x^2+0.013x^3$ | 0.48 | 5.58** | 5.0/16.9 |
| | 1 | $y=4.45+9.52x-1.14x^2+0.033x^3$ | 0.67 | 13.09** | 5.5/17.6 |

The x value (number of conditioning days) of the inflection point derived from the model is presented

*, **Indicate significant correlations at the $P<0.05$ and $P<0.01$ level, respectively

for inducing high germination rates of sunflower broomrape (Tables 2, 3 and Supplementary Table S11, S12) and Egyptian broomrape (Tables 4, 5 and Supplementary Table S13, S14). Though the maximal germinate rate differed significantly between the different types of broomrape seeds, the optimal number of conditioning days was similar and ranged between 4 and 10 days.

### $GA_3$ promotes/stimulates broomrape seed germination

$GA_3$ has been reported to play a key role in seed dormancy release and germination (Kucera et al. 2005). In this experiment, 100 µM of $GA_3$ induced the direct germination of the Egyptian broomrape seeds, with the highest germination rate of 62% (Supplementary Fig. S2). By contrast, $GA_3$ at a concentration ≤10 µM did not result in this type of phenomenon. Though El-Ghamrawy et al. (1990) noted that $GA_3$ could stimulate *O. crenata* seeds to germinate at rates up to 60%, other researchers indicated that GA alone did not directly induce broomrape seed germination (Takeuchi et al. 1995; Chae et al. 2004). In our research, the seeds of sunflower broomrape did not germinate under conditioning with $GA_3$ at any concentration. Thus, the germination-inducing effect of $GA_3$ on broomrape seeds appears to be species-specific.

Though $GA_3$ only induced the direct germination of the Egyptian broomrape seeds at a high concentration, $GA_3$ increased the sensitivity of the response of both species to subsequent germination stimulants. In our experiments, $GA_3$ did not significantly promote the germination of broomrape seeds at 1 µM, whereas concentrations of 10 and 100 µM significantly increased the germination of the seeds, which indicates that the $GA_3$ concentration played a critical role in broomrape seed germination (Figs. 1, 2, 3, 4). Notably, 10 µM of $GA_3$ had a significant promotive effect on the germination of the two types of seeds; this concentration was much higher than the level of this hormone in the seeds. Thus, whether endogenous and exogenous $GA_3$ exhibits a similar effect requires further study. Moreover, the promotive effect of $GA_3$ occurred mainly in the seeds without optimal conditioning. Sunflower broomrape seeds conditioned for only 1 d with $GA_3$ exhibited a higher mean germination rate (45%) than those conditioned with distilled water (21.4%). After 21 days of conditioning, a much higher germination rate (50.0%) was obtained for the sunflower broomrape seeds conditioned with $GA_3$ compared with those conditioned with distilled water (17.7%) (Figs. 1, 3). Similar results were observed for the Egyptian broomrape seeds (Figs. 2, 4). Our results provide additional proof that the application of $GA_3$ shortened the conditioning period requirement of the broomrape seeds, which resulted in a strong response to the germination stimulants and prevented the seeds from entering a stage of wet dormancy, as observed previously for different parasitic plants, such as small broomrape (*O. minor*) and Egyptian broomrape (Joel 2000; Song et al. 2006). In addition, $GA_3$ had a significantly greater promotive effect on the germination of the sunflower broomrape seeds compared with those of Egyptian broomrape.

### Specificity in the response of broomrape seeds to germination stimulants

The germination stimulants varied significantly ($p<0.01$) with respect to their ability to stimulate broomrape germination. The application of strigol had a significant effect on both the sunflower broomrape (mean germination rate of 62.8%) and the Egyptian broomrape (mean germination rate of 76.7%) germination. GR24 also had an efficient

**Table 4** Effect of conditioning period on the germination of Egyptian broomrape seeds conditioned with 100 µM of $GA_3$ under various germination stimulants

| Germination stimulant | Concentration (mg $L^{-1}$) | Regression model | $R^2$ | F | Inflection point |
|---|---|---|---|---|---|
| GR24 | 1 | $y=60.89+4.87x-0.33x^2+0.006x^3$ | 0.33 | 5.22 | 10.2/26.4 |
| | 0.1 | $y=66.74+4.63x-0.56x^2+0.017x^3$ | 0.47 | 5.41** | 5.4/16.7 |
| Strigol | 1 | $y=55.83+8.08x-0.76x^2+0.019x^3$ | 0.50 | 5.94** | 7.3/14.8 |
| | 0.1 | $y=69.31+7.26x-0.82x^2+0.026x^3$ | 0.34 | 3.09 | 6.3/14.8 |
| DCL | 1 | $y=40.17+12.45x-1.18x^2+0.03x^3$ | 45 | 4.94* | 7.4/18.8 |
| | 0.1 | $y=34.58+8.80x-0.91x^2+0.025x^3$ | 0.33 | 2.95 | 6.6/17.7 |
| Maize root extracts | 10 | $y=11.05+11.80x-1.35x^2+0.039x^3$ | 0.65 | 11.31** | 5.9/17.2 |
| | 1 | $y=13.12+8.10x-0.97x^2+0.029x^3$ | 0.52 | 6.41** | 5.6/16.6 |
| Tobacco root extracts | 10 | $y=29.17+11.19x-0.96x^2+0.024x^3$ | 0.50 | 6.03** | 8.7/17.8 |
| | 1 | $y=10.03+13.24x-1.25x^2+0.034x^3$ | 0.71 | 14.83** | 7.8/17.7 |

The x value (number of conditioning days) of the inflection point derived from the model is presented

*, **Indicate significant correlations at the $P<0.05$ and $P<0.01$ level, respectively

**Table 5** Effect of conditioning period on the germination of Egyptian broomrape seeds conditioned with distilled water under various germination stimulants

| Germination stimulant | Concentration (mg $L^{-1}$) | Regression model | $R^2$ | F | Inflection point |
|---|---|---|---|---|---|
| GR24 | 1 | $y=66.74+2.62x-0.03x^2-0.005x^3$ | 0.31 | 2.71 | –15.0/11.7 |
| | 0.1 | $y=66.99+3.97x-0.22x^2+0.002x^3$ | 0.45 | 5.41* | 10.7/61.6 |
| Strigol | 1 | $y=56.09+4.15x-0.27x^2+0.004x^3$ | 0.33 | 2.91 | 10.1/34.2 |
| | 0.1 | $y=69.64+6.19x-0.46x^2+0.008x^3$ | 0.47 | 5.28** | 8.7/29.5 |
| DCL | 1 | $y=29.63+9.57x-1.13x^2+0.033x^3$ | 0.38 | 3.70* | 5.7/17.1 |
| | 0.1 | $y=27.42+6.54x-0.90x^2+0.027x^3$ | 0.52 | 6.46** | 4.6/17.7 |
| Maize root extracts | 10 | $y=2.63+5.99x-0.67x^2+0.019x^3$ | 0.68 | 12.51** | 5.9/17.7 |
| | 1 | $y=1.21+4.16x-0.44x^2+0.011x^3$ | 0.70 | 14.25** | 6.2/20.3 |
| Tobacco root extracts | 10 | $y=16.72+10.27x-0.69x^2+0.013x^3$ | 0.71 | 14.67** | 10.6/24.9 |
| | 1 | $y=-7.20+14.75x-1.1x^2+0.023x^3$ | 0.78 | 20.92** | 9.5/22.4 |

The x value (number of conditioning days) of the inflection point derived from the model is presented

*, **Indicate significant correlations at the $P<0.05$ and $P<0.01$ level, respectively

and general inducing effect on broomrape seed germination as the mean germination rates of sunflower broomrape (57.9%) and Egyptian broomrape (76.8%) were relatively high. Though DCL is considered as the main germination stimulant from sunflower root exudates (Joel et al. 2011), the germination rates of the sunflower broomrape seeds induced by DCL were much lower than those induced by strigolactones. The Egyptian broomrape seeds also exhibited a low germination response to DCL, with a mean germination rate of 39.2%. The strigolactones (strigol and GR24) had a strong capacity to induce Egyptian broomrape germination (irrespective of concentration), and these compounds formed a homogeneous group (Fig. 2). By contrast, the germination of Egyptian broomrape was strongly dependent on the concentration of DCL and the root exudates, as the mean germination rates decreased markedly from 46.6 to 31.7%, from 22.5 to 13.5%, and from 57.3 to 41.6% when the respective concentrations of DCL were decreased from 1 to 0.1 mg $L^{-1}$ and the concentration of the maize and tobacco root exudates decreased from 10 to 1 mg $L^{-1}$ (Figs. 2, 4). Apart from the tobacco root exudates, the concentrations of the other germination stimulants, especially DCL, played a significant role in inducing sunflower broomrape seed germination. The germination rate of sunflower broomrape stimulated by 1 mg $L^{-1}$ DCL was 1.1-fold higher than that stimulated by 0.1 mg $L^{-1}$ DCL. In addition, it is interesting to note that while strigol was the major germination stimulant identified in maize root exudates (Awad et al. 2006), the germination response of Egyptian broomrape to the maize root exudates was very limited (13.6 and 22.4%), regardless of concentration. A possible explanation for this finding could be a unique combination of germination stimulators

or some germination inhibitors. Hence, the specialization of broomrape seeds may arise from a combination of many effects pertaining to germination stimulants with different levels of sensitivity, unique synergistic or antagonistic actions of these stimulants.

## Conclusions

In conclusion, our results provide additional proof that conditioning plays a key role in changing the sensitivity of sunflower and Egyptian broomrape seeds to germination stimulants, which has been underestimated or dismissed by some researchers. A cubic curve was selected to fit the relationship between the germination rate and the conditioning period, which indicated an optimized conditioning period of 4–10 days for both sunflower and Egyptian broomrape. Exogenous $GA_3$ application during conditioning may retain the sensitivity of these seeds to germination stimulants. The various chemical stimulants and root exudates had significantly different effects with respect to inducing broomrape seed germination. Currently, there are two main strategies for controlling broomrape, i.e., inducing suicide germination of the seeds to reduce the seed bank in the soil and inhibiting germination and parasitism. Thus, our results may contribute to a better understanding of parasitic weed germination and lead to the regulation of broomrape. For example, the role of $GA_3$ may help to retain the high sensitivity of the seeds and increase the effectiveness of suicide germination for broomrape control. Several publications report that a later crop-sowing date strongly reduces infection by parasitic weeds. It has been indicated that this is due to unfavorable conditioning (Sukno et al. 2001; Eizenberg et al. 2003). Thus, knowledge of the relationship between the conditioning period and the sensitivity of broomrape seeds to germination stimulants would help us to better understand the infection status affected by the sowing time of the host plants. In this case, a more thorough analysis of broomrape seed germination is needed to better control these parasitic plants.

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