A Two-Year Simulated Crop Rotation Confirmed the Differential Infestation of Broomrape Species in China Is Associated with Crop-Based Biostimulants

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Abstract: In Yanqi County of Xinjiang Uygur Autonomous Region, China, broomrape species (Orobanche cumana Wallr. and Phelipanche aegyptiaca Pers.) contribute to significant losses of processing tomato and sunflower. During the past decades, a significant infestation pattern was observed between these broomrape species with no scientific peer-reviewed explanation. A two-year pot experiment simulating the crop rotation and an independent hydroponic experiment were performed to address the problem and indicate the main reason behind the differential infestation pattern. Different varieties of three crops (sugar beet, pepper, and wheat) were grown in rotation with tomato and sunflower to identify a crop-rotation induced control mechanism on these two broomrape species. Germination bioassays were performed in vitro to identify stimulation of plant biochemicals collected as methanolic shoots/roots extracts and root exudates on the germination patterns of broomrape seeds. Results indicated that sunflower broomrape soil seed banks reduced during the two-year crop rotation; however, Egyptian broomrape seed banks did not alter and the resulting parasitism significantly reduced tomato growth. Seed germination bioassays confirmed that the methanolic shoot/root extracts successfully stimulate sunflower broomrape seeds germination but fail to stimulate Egyptian broomrape seeds germination. Root exudates collected from hydroponically grown crops also confirmed differential germination patterns in both broomrape species. Current results are of vital importance to explain the control effect of a crop rotation system and moreover, lay the foundation to study the genetic evolution of broomrape species that results in their differential germination responses to natural stimuli.

Keywords: Orobanche cumana; Phelipanche aegyptiaca; evolution; crop rotation; suicidal germination

1. Introduction

Broomrapes (Orobanche spp.) are holoparasitic weeds which significantly impair agricultural crops in many regions of the world [1]. In the Mediterranean and a number of European countries it is becoming a limiting factor to significantly reduce crop production [2]. In China, the prominent species are sunflower broomrape (Orobanche cumana Wallr) and Egyptian broomrape (Phelipanche aegyptiaca...
(Pers.) Pomel; synonym: *Orobanche aegyptiaca* Pers.) which attack and severely damage sunflower and processing tomato production in the Shaanxi Province and Xinjiang Uygur Autonomous Region [1,3,4]. These broomrape species are holoparasites and due to their lack of chlorophyll, entirely depend on the host plant for nutrition, thereby considerably reducing the growth and development of the host plants [5]. The mechanism of parasitism involves double parasitic action where the broomrape develops a haustorium to injure and bridge a xylem-oriented channel in order to obtain food from the host plant [6]. Due to this double action, the host plant not only has to bear the injury, but also has to provide its share of food with the parasite, which sometimes proves deadly to the life or life-cycle of the host plant. Additionally, seed germination of broomrape is a highly specialized process comprised of two prominent steps: the first is a conditioning period which is the provision of optimum temperature and humidity to the seeds and the second is the perception of a germination stimulant usually obtained from the host plant [7]. Hence all these processes occur underground; it is therefore extremely difficult to control these notorious weeds. Furthermore, these weeds have long dormancy spans and the seeds can remain viable in the soil for years [8]. Nonetheless, a significant behavior of these weeds is the selective germination capability that allows them to sustain the seed bank in the soil for extremely large time spans [5]. Due to their long dormancy span, achlorophyllous nature, and capability to survive harsh environmental conditions, these weeds arise as highly notorious to the crops and require considerable attention.

Due to the significance of broomrape infestation, an array of control methods has been proposed by various researchers to minimize its effects, such as using herbicides, hand weeding, solarization, and employing microbes such as bacteria and fungi [9–13]. These control measures may however be more or less effective due to the fact that most of them are only applicable once the broomrape seedlings develop above-ground [14], which in other words means that the major harm of parasitic processes have already started. Moreover, application of herbicide may lead to environmental pollution which risks the farming community and consumers’ health [15]. Hand weeding or herbicide control also fail due to the fact that weeds have unique style of seed dormancy and may sometimes remain dormant for a significant amount of time [4]. This dormancy protocol therefore makes it too difficult to monitor its germination and emergence as it takes months to develop from a seed to a complete plant above-ground, hence the utility of hand weeding or herbicide control remains largely impractical. Furthermore, soil solarization with plastic films has also been proposed to be effective against diminishing soil seed banks but this method also requires both an economical and labor burden upon the farmers and therefore cannot be extensively practiced. Apart from manual control methods, certain agronomic measures (crop rotation in particular) are suggested as significant to control broomrape infestation and improve host plants’ production and growth [16]. The mechanism of action is supposed to involve certain biochemicals or exudates that the host plant excretes to the rhizosphere stimulating the seed germination of various weeds. These crops are often termed as false crops or trap crops. Previous studies have shown that certain crops such as Egyptian clover, sesame, pepper, and cotton are able to successfully germinate broomrape seeds and thus help to reduce yield losses in the host crop such as tomato [17]. The biochemicals from these crops induce germination in the broomrape and hence the weeds are holoparasites; they can only live for a few days due to the absence of host plants. Thus, the seed banks of the soils are successfully eliminated. Earlier reports suggest foxtail millet and maize to induce germination of broomrape seeds and may therefore be used as trap crops to limit seed banks in the soil [4,18].

In Xinjiang Uygur Autonomous Region of China, farmers usually practice four crop-rotations: spring wheat, pepper, sugar beet rotated with processing tomato and sunflower. However, for the past decade, strange infestation behavior was noticed for broomrape species. For sunflower broomrape, the infestation is negligible, but the Egyptian broomrape infestation seemed to be ever-increasing causing considerable damage to the processing tomato produce. To help the farming community, it is inevitable to investigate the situation based on peer reviewed scientific evaluations to identify the root cause of ever-growing Egyptian broomrape infestation and furthermore, provide a satisfactory solution.
to the problem. We therefore conducted various experiments to investigate the problem involving hydroponic cultures and pot experiments. Our research work involves methods that clearly indicate the loopholes of the cropping system which leads to the expanding infestation of the notorious weeds. The outcomes of the study are therefore scientifically significant to develop understanding towards the infestation and control measures of broomrape species.

2. Materials and Methods

2.1. Pot Experiment

A two-year (2016–2017) pot experiment was designed to simulate the cropping system as practiced in Xinjiang Uygur Autonomous Region. On 20 March 2016, crops were grown in pots under a control shed at the Institute of Soil and Water Conservation, Northwest A&F University, China. The seeds were sown in pots (30 × 40 cm) at a depth of 2 cm. Each treatment had three repeats and a single repeat contained six pots per variety of the crop. For sugar beet, each pot contained 3 sugar beet plants; for wheat, 5 plants per pot were planted; and for pepper 4 plants were maintained in each pot. Lou soil was used in the experiment. The soil was prepared for the experiment by mixing organic fertilizer at a rate of 5%, urea (0.43 g kg\(^{-1}\)), and single super phosphate (SSP) (0.15 g kg\(^{-1}\)). Broomrape seeds at a rate of 0.017 g per pot were evenly mixed with this soil and the pots were regularly watered and administered for agronomic practices. In addition to the selected crop varieties as a particular treatment, two kinds of control treatments (pots having broomrape seeds only and pots having processing tomatoes without broomrape seeds) were used. On 19 July 2016, the plants of wheat, sugar beet, and pepper were harvested. Processing tomato and sunflower were grown in these pots on 20 July 2016 to simulate the rotation effects and were maintained for two months. Proper agronomic practices such as unwanted weeds removal and spring irrigation were performed to maintain the health of the plants. On 17 October 2016, the obtained tomato and sunflower plants were harvested, and data were collected for plant height, plant weight, and roots weight. The pots were subsequently opened, and data were recorded for broomrape attachment and total number of germinated vs. unearthed broomrape seedlings. In the year 2017, the whole experiment was repeated for a second time and data were collected in the same pattern.

2.2. Germination Stimulation of Sunflower and Egyptian Broomrape Seeds with Methanolic Shoot and Root Extracts from the Crops

The leaf (shoot) and root samples of pepper, wheat, and sugar beet varieties were collected from the pot experiment and their methanolic extracts were used to perform germination bioassays in the lab. For methanolic extracts preparation, 1.0 g of plant sample was crushed to homogenate in a sterile mortar and pestle. Next, 0.1 g from this homogenate was collected and added with 1 mL methanol in a 1.5 mL centrifuge tube. The sample was put in an ultrasonic cleaner bath for 30 min and centrifuged at 6400 rpm for 2 min. After the centrifugation, the supernatant was collected in a separate vial and stored for further use. In the current experiments, the obtained extracts were diluted in two different concentrations: 10-fold and 100-fold dilution. Briefly, the pre-conditioned seeds of sunflower and Egyptian broomrape were placed on a petri dish (9 cm) to exude the excess moisture from the seeds. The sterile glass fiber filter disks were used to germinate the seeds. For each disk, 25 µL of the solution was added and left for 20 min to volatilize the methanol. Then, 35 µL of sterile distilled water was added to the respective pieces and a wet/damp filter paper folded into a triangular shape was placed in the middle of each petri dish to retain the moisture inside. For the control treatment, the supernatant solution was replaced with distilled water and a positive control of GR24 (0.1 mg/L) was prepared to avoid experimental errors. Moreover, these prepared petri dishes were closed and sealed with paraffin film and finally placed in an incubator/growth chamber with a constant temperature and humidity while maintained in the dark. The germination rate of seeds was observed and counted after the
20 × 16-fold microscope. The germinating tube, which was observed as a seed under the microscope, was considered germination.

2.3. Hydroponic Culture and Root Exudates Collection

To understand the role of plant allelochemicals (root exudates) in the germination stimulation pattern of broomrape species, a hydroponic experiment was conducted using the same crops varieties as used in the pot experiment. Briefly, seeds of all the crops were surface sterilized with 75% (v/v) ethanol for 2 min and then with sodium hypochlorite (1% v/v) for 2 min. The seeds were further washed with sterile distilled water and dried. Plastic basins were prepared, and two layers of gauze were placed in these basins with care of avoiding contact of gauze with the water. Seeds of each crop were placed on the gauze and sprinkled evenly with water to provide moisture enough to germinate the seeds. When the seedlings grew roots, gauze wrapped with activated charcoal was placed in the water basins. The root exudates were collected, and the activated charcoal was periodically replaced to each basin every 2 days. The root exudates collected in the activated charcoal were eluted with acetone. By vacuum evaporation, the acetone was removed in a rotary evaporator at 40 °C. The residue was further transferred to a 50 mL volumetric flask and adjusted to final volume with distilled water. After this, 50 mL of ethyl acetate (EtOAc) was used to partition the obtained solution in a separate funnel. The EtOAc phases were further combined and concentrated using vacuum evaporation to dryness. Finally, the dried residues were added with 5 mL of acetone and stored in glass vials at 4 °C.

2.4. Data Processing

The obtained experimental data were subjected to two-way analysis of variance (ANOVA) in factorial arrangement. The obtained means were further determined for their significant differences using Tukey’s honest significant difference (HSD) test with 0.05 level of significance. Data analyses were performed using Statistix software V .8.0 and DPS software. Figures were drawn using Microsoft Excel 2016 and SigmaPlot V . 10 software.

2.5. Crops Used in Rotation and their Varietal Recognition

For data collection, the respective varieties were interpreted as Table 1.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Varieties</th>
<th>Label</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet</td>
<td>Ruima</td>
<td>TA</td>
</tr>
<tr>
<td></td>
<td>0143</td>
<td>TB</td>
</tr>
<tr>
<td></td>
<td>RG8001</td>
<td>TC</td>
</tr>
<tr>
<td>Pepper</td>
<td>Jinghong</td>
<td>LA</td>
</tr>
<tr>
<td></td>
<td>Qingdao Xinlilai</td>
<td>LB</td>
</tr>
<tr>
<td></td>
<td>Zi jinshan</td>
<td>LC</td>
</tr>
<tr>
<td>Wheat</td>
<td>Xinchun 6</td>
<td>MA</td>
</tr>
<tr>
<td></td>
<td>Yongliang 15</td>
<td>MB</td>
</tr>
<tr>
<td>Sunflower</td>
<td>Aidatou</td>
<td></td>
</tr>
<tr>
<td>Processing tomato</td>
<td>Shifan 33</td>
<td></td>
</tr>
</tbody>
</table>

3. Results

3.1. Effect of Crop Rotation on the Control of Egyptian Broomrape, and Plant Growth of Tomato Plants Grown in Pot Experiment

The results for plant growth parameters are presented in Figure 1. Statistical analysis of data indicated that the tomato plant growth under broomrape infestation was impaired in comparison to
the control (without broomrape). In general, the crop rotation could not improve the resulting plant growth and a tremendous decrease was noticed in the plant height when compared to those of control plants (74 and 69 cm for year 1 and year 2 respectively). The broomrape infestation obviously affected tomato growth by as much as 30% decrease to those of control plants. Similarly, a significant decrease in the dry weights of above-ground and roots was observed. Data obtained in both years showed a consistent decline in plant growth due to the broomrape infestation. When tomato plants were grown without broomrape, maximum dry weights for above-ground (more than 10 g) were observed, however, tomato plants grown in soils containing broomrape seeds exhibited fewer dry weights (below 7.00 g). The growth compared to the control was significantly lower particularly when pepper was used as a former crop followed by wheat. Obtained data suggested that the practiced crop rotation pattern lacks the ability to stimulate, and thereby limit, the broomrape infestation on the succeeding tomato crops as none of the trap crop could improve the tomato growth.

**Figure 1.** Tomato plant growth under crop rotation and Egyptian broomrape infestation. Bars represent the mean data for plant height (cm), above-ground (shoot), and root dry weight (g) collected at two years. CK represents control treatment (tomato plants grown in soil without broomrape seeds). All the data were subjected to two-way analysis of variance (ANOVA) and level of significance was determined using Tukey’s honest significance test (HSD) test with 0.05 level of significance. Difference in the letters indicate significant difference among means.

### 3.2. Effect of Crop Rotation on the Egyptian Broomrape Parasitism on Tomato Plants

The number of parasitic broomrapes were noticed both in the tomato plants rotated with beets, wheat, and pepper and processing tomato grown alone. The results are depicted in Figure 2.

As can be observed, no significant difference was found for parasitism of broomrape on the tomato plants with respect to the planting patterns. The tomatoes plants grown alone, and the tomato plants grown in rotation with the other crops had parasitic attachment of broomrape indicating that the crop rotation did not affect the soil seed banks of the weed species. On the other hand, data obtained for sunflower broomrape showed that compared to control plants, the number of sunflower attachments was significantly lowered (Figure 3).
Figure 2. Egyptian broomrape parasitic attachment on tomato plants effected by crop rotation. Bars represent the mean data of two years for broomrape attachment to the tomato plants. CK represents control (tomato plants without crop rotation). All the data were analyzed using analysis of variance and significant differences among the means were determined using Tukey’s HSD test with 0.05 level of significance. Means followed by same letter bear no significant differences.

Figure 3. Number of sunflower broomrape attachment under crop rotation. Bars represent means and standard deviation for the data collected in two years repetition. CK represent control plants grown without crop rotation pattern. The obtained data were analyzed using two-way analysis of variance (ANOVA). Difference in the letters indicate the level of significance among means according to Tukey’s HSD test at 0.05 significance level.

3.3. Effect of Methanolic Shoot Extracts of Various Crops on Broomrape Seed Germination

Broomrape seeds (O. cumana and P. aegyptiaca) were germinated under the influence of methanolic shoot extracts from the crops and are presented in Table 2.
Table 2. Effect of methanolic shoot extracts from different crops on broomrape seed germination.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sunflower Broomrape Germination %</th>
<th>Egyptian Broomrape Germination %</th>
</tr>
</thead>
</table>

**Rotation effect**
- Year 1: 65.59 a 16.18 a
- Year 2: 59.91 b 16.07 b

**Concentration effect**
- 10-fold: 68.48 a 17.00 a
- 100-fold: 57.02 b 15.25 b

**Treatment effect**
- CK: 8.18 e 6.98 bc
- CK GR24: 96.51 a 96.63 a
- LA: 68.83 bc 6.27 bc
- LB: 70.97 b 5.43 c
- LC: 71.19 b 8.11 bc
- MA: 62.44 bcd 6.26 bc
- MB: 60.21 cd 9.13 b
- TA: 64.88 bcd 7.60 bc
- TB: 59.11 d 6.00 bc
- TC: 65.15 bcd 5.53 c

**Interaction effect**
- CK
  - Year 1: 10-fold 8.24 m 8.17 bc
  - 100-fold 8.24 m 8.15 bc
- CK GR24
  - Year 1: 10-fold 96.24 ab 95.01 a
  - 100-fold 96.24 ab 96.10 a
- LA
  - Year 1: 10-fold 82.28 abcde 3.96 bc
  - 100-fold 59.12 fghijkl 9.09 bc
- LB
  - Year 1: 10-fold 84.59 abc 2.86 c
  - 100-fold 70.94 cdefghij 4.99 bc
- LC
  - Year 1: 10-fold 82.35 abcde 11.28 b
  - 100-fold 58.64 fghijkl 10.17 bc
- MA
  - Year 1: 10-fold 59.71 efghijkl 4.43 bc
  - 100-fold 74.07 abcdefgh 8.15 bc
- MB
  - Year 1: 10-fold 62.32 cdefghijkl 9.67 bc
  - 100-fold 80.49 abcdef 10.26 bc
- Year 2: 10-fold 55.03 hijkl 7.89 bc
  - 100-fold 43.00 1 8.71 bc

Note: Values followed by the same letter in the same column are not significantly different.
Table 2. Cont.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sunflower Broomrape Germination %</th>
<th>Egyptian Broomrape Germination %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>10-fold</td>
<td>72.79 cdefghi</td>
</tr>
<tr>
<td></td>
<td>100-fold</td>
<td>67.13 cdefghijkl</td>
</tr>
<tr>
<td>Year 2</td>
<td>10-fold</td>
<td>62.61 cdefghijklkl</td>
</tr>
<tr>
<td></td>
<td>100-fold</td>
<td>57.02 ghijkl</td>
</tr>
<tr>
<td>TB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>10-fold</td>
<td>66.63 cdefghijkl</td>
</tr>
<tr>
<td></td>
<td>100-fold</td>
<td>51.31 hijkl</td>
</tr>
<tr>
<td>Year 2</td>
<td>10-fold</td>
<td>65.32 cdefghijklkl</td>
</tr>
<tr>
<td></td>
<td>100-fold</td>
<td>53.19 hijkl</td>
</tr>
<tr>
<td>TC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year 1</td>
<td>10-fold</td>
<td>73.43 bcdefghi</td>
</tr>
<tr>
<td></td>
<td>100-fold</td>
<td>57.27 ghijkl</td>
</tr>
<tr>
<td>Year 2</td>
<td>10-fold</td>
<td>82.72 abcd</td>
</tr>
<tr>
<td></td>
<td>100-fold</td>
<td>47.22 kl</td>
</tr>
</tbody>
</table>

Note: All the data were statistically analyzed and subjected to two-way analysis of variance (ANOVA). Different letters in the same column indicate significant difference among treatments indicated through Tukey’s test \((p < 0.05)\).

Statistical analysis of the obtained data revealed that none of the crops extracts could successfully induce seed germination in Egyptian broomrape in comparison to the germination stimulant (GR24). Next to GR24, only pepper and wheat extracts resulted a slightly higher germination of 11.28% and 10.80%. It was noticed that dilution factor may interfere with the stimulation capability of the extracts because germination was slightly increased when the dilution was 100-fold. However, the overall germination was below 10%, far below the 96.5% germination result from GR24. No significant difference was noticed in the mean germination of broomrape seeds from year-wise comparison. As observed, sunflower broomrape responded significantly to almost all the crops extracts. Highest germination was observed in the seeds treated with GR24 (96.51%). Among the crops, pepper had the highest germination stimulation potential resulting in 71.19%. Similarly, sugar beet and wheat also successfully stimulated seed germination in sunflower broomrape. Lowest germination was observed in the water control where mean germination rate was 8.18%. Slight, but non-significant differences were observed from the year-wise repetition of the bioassays. The interaction of treatment \(\times\) concentration \(\times\) year also resulted considerable differences where the stimulatory effects were increased by increasing the dilution factor to 100-fold for most of the crops. Particularly pepper and sugar beet extracts had more stimulatory potential when the extracts were diluted to 100-fold. The mean data for all treatments showed that at 10-fold dilution, the germination percentage (68.48%) was significantly higher in comparison to that of 100-fold dilution (57.02%).

3.4. Effect of Methanolic Roots Extracts from the Crops on the Germination of Broomrape Seeds

Methanolic roots extracts from the crops were used to stimulate the germination process in the broomrape seeds and the analyzed data for the Egyptian broomrape seeds germination are depicted in Figure 4. Statistical analysis of the obtained data reveal that seed germination in methanolic root extracts was far below that of the germination stimulant GR24 (96.24%). The bioassay was repeated in the second year and similar results were noticed where the crops root extracts did not show significant stimulation of the seed germination. Compared to the water control (less than 10%), the methanolic root extracts of wheat cultivar, Yongliang, resulted in higher germination stimulation (14.6%) followed by 14.1% observed under the influence of sugar beet. Mean data analysis did not show any significant results for year-wise repetition or concentration of the extracts from roots.
Figure 4. Stimulation of Egyptian broomrape germination by the methanolic roots extracts of different varieties of sugar beet, pepper, and wheat. Columns represent means and bars represent standard deviation for percent germination of Egyptian broomrape seeds under methanolic root extracts of different crops. TA, TB, and TC represent sugar beet; MA and MB represent wheat; LA, LB, and LC represent pepper. CK water is the distilled water control and CK GR24 is the germination stimulant (GR24) as control treatment. Ten-fold and 100-fold dilution is used for each treatment. All the data were subjected to a two-way analysis of variance (ANOVA) and the significance of the results was confirmed using Tukey’s HSD with 0.05 level of significance. Difference in the letters indicates the level of significance among the means.

As can be observed, the extracts from roots had slightly higher germination stimulation potential for Egyptian broomrape, yet in comparison to the GR24, the overall germination rate was significantly lower and thus could not be regarded as successful germination.

The results obtained for the germination of sunflower broomrape under methanolic root extracts are summarized in Figure 5. Statistical analysis of the data confirmed that like the methanolic shoot extracts, the root extracts also possess potential to successfully germinate sunflower broomrape seeds. Germination stimulation was generally higher than 50% when the extracts were 10-fold diluted which was statistically different than germination under 100-fold dilution. The extracts collected at the second year bore no significant difference to the first year’s collections. Highest germination was recorded for pepper (88.41%) followed by 85.21% for sugar beet and 85.6% for wheat extracts. At 100-fold dilution, the effect decreased, and maximum germination was recorded for pepper (60.95%) followed by sugar beet extracts. Germination stimulation was different based on varietal differences according to the obtained data. Data suggested an overall successful germination in sunflower broomrape in response to all of the crops’ extracts suggesting the role of the crop rotation module in eliminating soil seed banks.
3.5. Broomrape Seeds Germination under the Influence of Roots Exudates Collected from Hydroponic Experiments

All the test crops were grown hydroponically, and their roots exudates were collected to study the germination of broomrape seeds. The results are summarized as the means of two repeated experiments in Table 3. Statistical analysis confirmed that there was no significant response of Egyptian broomrape to these extracts. In all the crops, only wheat roots exudates could slightly stimulate the germination of Egyptian broomrape seeds (3.2% and 2.1%, respectively). Also, the extraction of ethyl acetate phase showed significantly higher stimulation than the water phase extractions. However, there was no noticeable germination of Egyptian broomrape seeds in response to the crops roots exudates suggesting the inability of these crops to stimulate and thereby induce suicidal germination of these seeds. On the other hand, sunflower broomrape seeds significantly responded to these exudates and the germination percentage observed in response to the exudates collected in the ethyl acetate phase extraction was much higher than those collected in water phase extractions. Statistically significant and highest germination was observed in wheat root exudates (87.3%) followed by exudates collected from pepper (74.3% and 70.2%, respectively). Sugar beet roots exudates did not show higher stimulation and were below 40%. When the exudates were collected in water phase extraction, the stimulation potential seemed to decrease and was 77.0%, 60.3%, and 25.8%, respectively for wheat and pepper root exudates. These findings indicate that root exudates of these crops may be soluble in ethyl acetate and perhaps insoluble in aqueous phase extraction. Nonetheless, the obtained data also suggest that the content of germination stimulants in the first phase was higher in comparison to the second phase.
Table 3. Germination of broomrape seeds under hydroponically collected root exudates.

<table>
<thead>
<tr>
<th></th>
<th>Egyptian Broomrape</th>
<th>Sunflower Broomrape</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugar beet</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA</td>
<td>0.0 b</td>
<td>0.0</td>
</tr>
<tr>
<td>TB</td>
<td>0.0 b</td>
<td>0.0</td>
</tr>
<tr>
<td>TC</td>
<td>0.0 b</td>
<td>0.0</td>
</tr>
<tr>
<td>Pepper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LA</td>
<td>0.0 b</td>
<td>0.0</td>
</tr>
<tr>
<td>LB</td>
<td>0.0 b</td>
<td>0.0</td>
</tr>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MA</td>
<td>3.2 a</td>
<td>0.0</td>
</tr>
<tr>
<td>MB</td>
<td>2.1 a</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Note: Data represent the means of two years of experimental results. All the data were subjected to analysis of variance (ANOVA). Different letters in the same column indicate significant difference among treatments by Tukey’s HSD test (p < 0.05). EP: ethyl acetate (EtOAc) phase, WP: water phase.

4. Discussion

Based on the current experiment, insight into the control effect of the crop rotation system practiced in Xinjiang Province of China is anticipated. The findings are among the first to address the situation and therefore, hold important clues to understand stimulant-specific responses and genetic diversity of the broomrape species. Following is a two-dimensional explanation to understand the current findings.

4.1. Differential Response of Broomrape Species Indicates the Allelopathic Diversity of the Crops Biostimulants

Current findings revealed that in general, rotation planting patterns of sugar beet, wheat, and pepper had differential stimulatory effects on the germination of *Orobanche* seeds. Results obtained from the pot experiment indicated that the tested crops did not stimulate the germination of Egyptian broomrape seeds, therefore, the parasitism conceived on tomato plants and its respective effects on the growth could not be effectively relieved. The reduced tomato plant growth and yield suggest that the damage may directly be attributed to the parasitic sink activities. Studies on the host—*Orobanche* association have demonstrated yield losses in sunflower, tomato, and faba beans [11,12,18]. Similarly, trap crops such as Egyptian clover, sesame, mungbean, brown Indian hemp, common flex, cotton, pepper, and black-eyed pea were reported to successfully increase the yield of tomato in two-year crop rotation experiments [17]. These studies strongly support our results about reduced growth of the parasitized tomato plants in comparison to the non-parasitized (control) plants growth. Current findings demonstrate that the used crops in the rotation planting pattern may lack the allelopathic ability to successfully stimulate Egyptian broomrape seed germination. On the contrary, sunflower broomrape seeds showed a significant response to the crops root extracts and the effect was observed as a reduced parasitic attachment of the broomrape in the sunflower plants. As reported earlier, host—*Orobanche* association often involves enzymatic interactions and sometimes the non-host or trap crops may alter this enzymatic association, thereby depleting the chances of survival for the germinated broomrape seedlings [19]. The allelopathic potential of the observed crops seems to have stimulated seed germination of sunflower broomrape ahead of the host plant availability, thereby reducing the parasitic infestation in the later stages. Altering germinating conditions may considerably reduce the broomrape infection severity on sunflower, thereby effectively enhance its yield and biomass [20]. Allelopathic studies involving intercropping have shown it to reduce the infestation of *Orobanche crenata* on legumes [16], which agrees with the current idea that crop rotation exerts allelopathic influence to stimulate *Orobanche* seed germination. The parasitic strategy generally succeeds from coordinating early signals from the hosts termed as xenognosins [7]. Upon perception of xenognosins, these weeds initially activate seed germination which further leads to radicle development to bridge a parasitic tissue (haustorium) to the vascular system of host plants roots and extract nutrients [6,7]. The differential
responses of Egyptian and sunflower broomrape seeds to the crops’ allelochemicals indicate that these allelochemicals/biostimulants contain compounds which can stimulate the germination of these weeds. Root exudates obtained from the rhizosphere soils of foxtail millet were reported to induce seed germination of sunflower broomrape [4]. Based on current results, it can be anticipated that the germination-inducing effect of plant allelochemicals is associated with the reception and recognition by weeds seeds. When the seeds of broomrape species (O. cumana and P. aegyptiaca) were germinated under the methanolic extracts obtained from different crops, the differential germination pattern followed the pattern observed in the pot experiment. Almost every crop’s extract successfully induced germination of sunflower broomrape whereas, the Egyptian broomrape did not respond considerably to most of these extracts. The capricious germination behavior of broomrape seeds to these extracts indicates that these crops contain certain biochemicals that are either very specific in their biological activities or bioactive in a certain concentration. Plant extracts may comprise of numerous bioactive compounds and their activity often depends on the concentration as shown in previous studies based on pepper extracts and garlic allelochemicals [21–23]. It is possible that pepper plant extracts may contain a group of biochemicals that counteract with each other’s bioactivity and the concentration gradient may cause the bioactive potential to differentiate. Additionally, pepper extracts have been shown to induce biological responses in the receiver plants [21] and our findings therefore agree to the presence of highly bioactive chemicals in pepper extracts. Successful germination induction in the sunflower broomrape indicates that the crops used in rotation excrete certain chemical compounds that actively stimulate its germination. Previously, extracts from Chinese medicinal herbs have been reported to induce seed germination in numerous Orobanche species such as O. minor, O. cumana, and P. aegyptiaca [24], supporting our results that plant biochemicals induce germination of sunflower broomrape. Therefore, our findings lay the foundation for a research direction to identify bioactive compounds from wheat, pepper, and sugar beets using advanced technological protocols such as mass spectrophotometry. As an obligate parasite, broomrape seedlings can only survive for a few days before connecting to the host root [25]. In the current work, due to allelochemicals from non-host crops, the broomrape seeds germinated but could not survive due to the absence of host plants, hence reducing the soil seed banks. The succeeding sunflower crop therefore does not have to face the risk of parasitism in later stages due to the demise of the weed seed bank. On the other hand, no significant stimulatory effect was observed on the Egyptian broomrape seeds and therefore the seed bank in the soil remained largely unaffected. The lack of germination may therefore be the reason behind the indifference in the parasitism between the control and treated tomato plants. From the pot experiment, it is evident that the crop rotation pattern lacks the ability to germinate Egyptian broomrape and these crops may therefore be excluded when suggesting trap crops for this parasitic weed management.

Hydroponic culture and root exudates obtained from the studied crops further strengthen our speculations that the allelopathic influence is differentially perceived in the broomrape seeds. Results indicated that the root exudates of pepper had the highest potential to successfully induce or stimulate germination in the sunflower broomrape seeds. Our findings are in agreement with the reported allelopathic potential of wheat for germination stimulation of O. minor [26]. Previously, root exudates from rice have been shown to alter seed germination in O. cumana Wallr and O. minor [27], suggesting crop-based biostimulants for broomrape seed germination. Moreover, consistent effects were observed for the Egyptian broomrape seeds which showed no significant germination to these biochemicals. This strongly confirmed that all the test crops of the rotation system of Xinjiang Province of China lack the potential to stimulate germination and therefore, the problem of Egyptian broomrape is ever-increasing, affecting the yield potential of processing tomato. These crops may therefore be suggested as potential trap crops or false crops to control the seed bank of sunflower broomrape and help sustain the production of sunflower. It is therefore suggested that to minimize the hazards of Egyptian broomrape seeds banks in these soils, appropriate trap crops should be incorporated into the current planting system to allow the germination of these seeds prior to the host plant availability.
4.2. The Differential Germination Indicates a Genetic and Biochemical Evolution in the Broomrape Species

The differential responses of broomrape seeds to the biochemicals from the studied crops not only explain the observed problem of Xinjiang Uygur Autonomous Region of China, but also suggest significant contributions to the evolution of these species to assure their survival in the constantly challenging environment. To our understanding, seed germination is the imbibition of water that triggers certain biochemical responses leading to seed coat rupture and seedling emergence [28]. In certain weed species and broomrape in particular, seed germination is a specialized process that involves two steps: a conditioning phase and, upon reception of a certain stimulus, the germination phase [28–30]. The capricious germination response of sunflower and Egyptian broomrape was consistent throughout the experiments, where sunflower broomrape successfully germinated, while germination of the Egyptian broomrape proved otherwise. These findings clearly suggest significant differences in the chemo-detection that resulted in the differential germination. As described previously, germination potential is the combination of both the stimulatory capability of crop root exudates and the sensitivity of the parasitic receptors to recognize specific forms of germination-inducing factors [6]. Moreover, the qualitative and quantitative contents of germination-inducing factors may be summed up as the stimulatory potential of a particular crop’s root exudates and often varies across crops species or cultivars [5]. Current findings indicate a crosstalk of germination-inducing factors within the crop roots exudates and the receptors’ sensitivity of the broomrape seeds. These factors substantially explain the capricious germination behavior of broomrape to the studied crops root exudates. Previously, it was reported that Arabidopsis thaliana can differentially stimulate germination in numerous weed seeds [7] which strongly supports our findings. Furthermore, in a study, extracts from Chinese medicinal herbs have been shown to differentially stimulate seed germination in numerous Orobanche species [24] which is in agreement to our work. Current findings support the reported allelopathic influence of rice on the seed germination of sunflower broomrape and clover broomrape [27]. The sunflower and Egyptian broomrape seeds may contain receptors specifically designed to receive biochemical signals and respond to it depending on the perception [31,32]. Chemical interaction may not entirely be entitled to demonstrate this situation as the possible contribution of certain physical factors, such as seed coat structure, which can alter the reception and perception of a certain biochemical or stimulus. It is possible that structural differences may result in the differential permeability for a certain chemical and therefore result in a different response from the subject seeds [23]. The sunflower broomrape seeds’ structure seems to actively allow these crops’ allelochemicals to permeate and facilitate the chemo-detection, resulting in successful seed germination. Egyptian broomrape however, lacks this kind of structural ability which causes failure to germinate. However, this hypothesis requires further advanced investigation such as microscopic evaluation of the seed coat at various conditions. Based on the current findings, our future research directions include molecular and microscopic studies to unravel the biological barriers that result in the differential germination of sunflower and Egyptian broomrape seeds to different crop exudates.

5. Conclusions

At the outcome of the study, it is thoughtfully concluded that the cropping system practiced in Xinjiang Uygur Autonomous Region of China is responsible for the differential infestation of sunflower and Egyptian broomrape. The observed data revealed that wheat, pepper, and sugar beet plants contain certain biochemicals with germination-inducing capability for sunflower broomrape. The root exudates from these crops are able to successfully germinate the sunflower broomrape seeds and therefore reduce the seed bank considerably such that the area does not exhibit infestation of sunflower broomrape. On the other hand, the crop root exudates or the methanolic extracts do not induce the germination of Egyptian broomrape which causes an ever-increasing parasitic infestation in the tomato fields of Xinjiang Uygur Autonomous Region of China. These findings are among the very first to address the situation and provide significant scientific information to study these crops for their bioactive chemicals. Biochemicals from pepper exhibited capricious behavior closely related
to the concentration of a class of bioactive compounds whose activity may counteract this behavior depending on the concentration gradient. Moreover, it is suggested that the farming community in Xinjiang Uygur Autonomous Region of China may employ appropriate trap crops into the current cropping system to control the Egyptian broomrape seed banks in the soil. Therefore, our future research studies will include the evaluation of various trap crops such as maize and fennel to address this problem. Nonetheless, current findings provide research directions to study the germination behavior of sunflower and Egyptian broomrape based on molecular approaches and microscopic studies to identify genetic diversity among these species that control the differential responses to the studied botanicals of wheat, pepper, and sugar beet.

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References
16. Fernández-Aparicio, M.; Emeran, A.A.; Rubiales, D. Inter-cropping with berseem clover (*Trifolium alexandrinum*) reduces infection by *Orobanche crenata* in legumes. *Crop Prot.* 2010, 29, 867–871. [CrossRef]

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