

Response of Branched Broomrape (*Phelipanche ramosa* (L.) Pomel) to Amino Acid Treatments

Hilal Albayrak

Erciyes University

Doğan Isik

Erciyes University

Mehmet Arslan

mehmetarslan@erciyes.edu.tr

Erciyes University

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Abstract

Phelipanche ramosa (branched broomrape), a root holoparasitic weed causes great trouble in agriculture along Mediterranean, Europe, Asia, Africa, North and South America, cannot be easily controlled by the known management practices applied for non-parasitic weeds. Determination of novel compounds with higher herbicidal activity against *P. ramosa* can provide effective control for this parasitic weed. Certain amino acids have potential to inhibit early development of broomrape without any injury to host plants. A series of pot and in vitro studies were conducted to identify amino acids with higher inhibition efficacy on broomrape germination and growth. Germination inhibition rates of amino acids were significantly different. The highest inhibitory rates were obtained from alanine, lysine and tryptophan, while the lowest was obtained from theanine, leucine and isoleucine under the in vitro conditions. The inhibitory effect of amino acids on germination enhanced with the increasing concentrations. In the polyethylene bag studies, the number of germinated seed per plant varied between 46.00 and 205.50. The lowest and the highest germinated seeds were obtained from proline and theanine, respectively. Tubercle number per plant varied between 0.00 and 3.50. Glutamic acid had the highest tubercle number, while leucine, glycine, tryptophan and isoleucine had the lowest tubercle number per plant. In general, lysine, proline, phenylalanine, asparagine are thought to be the most effective inhibitors of branched broomrape germination. Further studies are needed to evaluate the usefulness of amino acids in *P. ramosa* control under field conditions.

Introduction

Broomrapes (*Orobanch* spp. and *Phelipanche* spp.) are chlorophyll-lacking root holoparasitic notorious weeds containing over 200 species that parasitize various host plants in Leguminosae, Solanaceae, Apiaceae, Brassicaceae and Asteraceae families (Joel 2000; Press et al. 2001; Nickrent and Musselman, 2004; Schneeweiss et al., 2004, Joel, 2009; Parker, 2009. Gevezova et al. 2012; Parker, 2013). Broomrapes grow primarily in arid and semi-arid Northern Hemisphere temperate continental regions in central and southern Europe, North and South America, Africa and Asia (Buschmann et al. 2005; Mohamed et al. 2006). A single broomrape can produce more than 500,000 extremely small dust like seeds that can stay dormant in the soil up to 20 years, until they stimulated to germinate by specific chemicals released the host plant roots (Gold et al. 1978; Škorić, 1988; Joel, 1987; Kebreab and Murdoch, 1999). Upon germination, the small seedlings attach to the host roots and differentiate in the attachment organ, called as haustoria that form a functional bridge into their hosts. Haustoria penetrates the host tissues and uptake water, nutrients and assimilates from the host plant (Joel et al., 2007). Broomrapes spend most of their life cycle below the soil surface. Crop damage and yield loss occur prior to the emergence and diagnosis of broomrape infection (Fernández-Aparicio et al., 2011). After emergence there is a short period between flowering and seed dispersal.

Phelipanche ramosa syn. *Orobanch ramosa*, native to the Mediterranean region of Eurasia, has a wide host range comprising Solanaceae, Brassicaceae and Fabaceae families. Yield losses in the infested fields can reach 5 to 100% (Joel et al., 2007, Tóth and Cagán, 2003; Schneeweiss, 2007; Mauromicale et al. 2008). Control of broomrapes can be classified into cultural, physical, chemical and biological methods (Joel et al., 2007; Rubiales et al., 2009; Fernández-Aparicio et al., 2016). However, none of the known methods alone is economically and practically effective against broomrapes (Perez-de-Luque et al., 2010, Goldwasser et al. 2008). Use of systemic herbicides and resistant cultivars are widely implemented control measures, but lack of resistance gene sources and host sensitivity to known herbicides limit these control measures (Rubiales et al., 2009). Therefore, a long-term integrated control measures are the only effective way to combat broomrapes.

Broomrape germination promoters or inhibitors can be used to reduce seed number in the seedbank. Application of synthetic strigolactones stimulates broomrape germination in the absence of a suitable host. Once germinated, broomrape seeds cannot return to dormancy and cannot survive longer than few days without supplied water, nutrients and assimilates by a host plant. Use of synthetic strigolactones seems to be an ideal strategy, but their usage is limited in the practices due to the instability of the compounds, especially at pH > 7.5 (Fernández Aparicio et al., 2011). The cultivation of trap crop and catch crop can reduce broomrape seed bank in the infested areas (Sauerborn, 1991; Acharya et al., 2002; Rubiales and Fernández-Aparicio 2012; Aksoy et al. 2016). Trap crops or false hosts stimulate germination of broomrape seeds without being infected themselves and consequently decrease the seed number in the soil. Each broomrape species responds only to certain trap crops, so it is required to determine potential trap crops for each species (Fernández-Aparicio et al. 2009). On the other hand, catch crops allow germination and development of the broomrape and they are destroyed before flowering and seed set. When resource sink established and tubercle started to develop after vascular connection, broomrape can be controlled by the application of glyphosate and imidazolinone herbicides (Hershenhorn et al., 2009). Broomrape parasitism can be controlled very efficiently by the application of systemic herbicides if the herbicide tolerant-cultivars are available. However, glyphosate and imidazolinone tolerant cultivars are not always available for many cultivated crop species that limits the application of this method.

Determinations of which amino acids and concentrations could be more suppressive on *P. ramosa* is very important for the implication of this approach to control this parasitic weed. Therefore, the laboratory and pot experiments were designed to determine which ones among the 20 proteinogenic amino acids and concentrations would strongly inhibit *P. ramosa* germination and growth and which amino acid would be indispensable to the inhibition of *P. ramosa* growth in the soil. These determinations were made by studying the germination inhibition and growth rate in the presence of single amino acids.

Materials and Methods

Experimental site

Experiments were conducted in Erciyes University Faculty of Agriculture Herbology laboratories of Plant Protection Department. Study area is located at 38°42' 33" N latitude and 35° 38' 34" E longitude and has an altitude of 1109 m. Experimental site (Kayseri province of Türkiye) has a semi-arid climate with cold and snowy winters and hot and dry summers.

Seed Preparation

The *P. ramosa* plants were collected from the broomrape infested tomato planting areas in Demirköprü village (36°15' N and 36° 20' E 90 m msl), Hatay, Turkey in July 2019. The broomrape plants were shade dried in the laboratory at ambient temperature (20–25 °C) for 20 days. The seeds were separated from the plant debris by gently rubbing and shaking. Broomrape seeds were surface sterilized by immersion in 1% sodium hypochlorite solution for 10 minutes then rinsed three times with sterile distilled water. After sterilization, the seeds were placed in the 1.5 mL sterile Eppendorf tubes and stored in darkness at 25–26°C for 3 weeks.

Germination bioassay in the petri dishes

The sterile 90 mm (90x15) petri dishes containing two layers of sterile filter paper were divided in 4 compartments. Each petri dish was wetted with 5 ml of distilled water then sterilized 20 broomrape seeds were placed in each

compartment. The petri dishes were sealed with Parafilm than stored in darkness at 26°C for 2 weeks. The GR 24 solution was prepared by dissolving 10 mg GR 24 in 10 ml of acetone and diluting 1,000-fold with a 0.3-mM buffer of 2-(N-morpholino) ethanesulfonic acid (MES)4, pH 6.1 (Johnson et al., 1981; Zwanenburg et al., 1986). After 15 days of incubation, 1 mg/L germination stimulant GR 24 solution, a synthetic analog of strigolactones, was added in each petri dish. The amino acids were purchased from Sigma-Aldrich. Each amino acid was diluted to 0.5, 1, 2, 3 and 4 µM concentrations. Following GR24 treatment, each amino acid concentration was added in the compartment of each petri dish. When the germ tube was at least 90 to 130 mm, seeds were considered to have germinated. The germinated seeds were counted under a stereo microscope after 7 and 21 days of incubation. Percent germination inhibition was calculated as:

$$GI = [(CG-TG)/CG] \times 100$$

where, GI is the germination inhibition (%); CG, germination rate in control treatment; TG, germination rate in amino acid treatment.

The experimental design used was entirely randomized in a factorial design with 20 amino acids and 5 concentrations (0, 0.5, 1, 2, 3, and 4 d µM). The experiment was composed of 3 replicates and 300 experimental units and the seed germination bioassay in the petri dishes was repeated twice. Analysis of variance was performed using a general linear model procedure SAS 9.2 (SAS Institute Inc.) and mean separations were determined by the least-squares difference.

Germination bioassay in the clear polyethylene bags

The seeds of tomato cultivar H-5803 were sown in plastic germination box containing peat and perlite mixture in 12 June 2020. Clear polyethylene zipper bags were used to monitor the host roots and the various stages of broomrape development during the parasitism period. Tomato seedlings at the four leaf stage were mounted onto 15 x 25 cm glass fibre filter papers (Whatman, GF/A, Whatman International Ltd., Maidstone, England) then the sterilized broomrape seeds were scattered around the roots. One tomato seedling was mounted in each glass fibre filter paper, and then the glass fibre sheets were inserted into a clear polyethylene bag (23 x 31). Twenty ml sterilized Hoagland nutrient solution (Hoagland and Arnon, 1950) was added in each root system on half of each GF/A sheet, together with the broomrape seeds. Twenty five ml Hoagland nutrient solution was added twice a week from the top of the polyethylene zipper bags. The polyethylene zipper bags were inserted in the aluminium foil bags to keep the roots in darkness. The root system zipped tomato seedlings were incubated in a growth chamber under a 12 h light/12 h dark photoperiod at 24°C with 55% relative humidity. After 3 weeks, 20 ml amino acid in 2 µM concentration was gently added to each aluminium foil covered zipper bags. Amino acid applications were repeated once a week for 21 days. No amino acid was added in the control treatment. Observations were carried out with a binocular microscope to detect and monitor the seed germination process.

The experimental design was completely randomized design with 4 replications and the seed germination bioassay in the clear polyethylene bags was reaped twice. The data was analysed using PROC GLM model in SAS 9.2 (SAS Institute Inc.), and the means were compared using least significant differences (LSD) at $P \leq 0.05$.

Germination and growth bioassay in the pots

Equal amounts of sterile soil, peat and perlite (1:1:1) were mixed to use for pod studies. The pH of the mixed growth media was 7.2. An amount of 1.5 kg of soil, peat and perlite mixture was mixed with a compound fertilizer (N:P:K 20:20:20) at a rate of 1 g/kg soil. The soil, peat and perlite mixture was mixed carefully with 30 mg/kg

broomrape seeds then the soil mixture was filled in the pots (26-cm diameter). The broomrape seeds were not mixed in the soil of the negative control group and same amount of broom rape seeds were mixed in the soil of positive control group. One tomato seedling at 2 leaf stage was planted in each pod. The pots were kept under full sunlight. Tomato plants were daily irrigated to keep soil water content at 80% of water holding capacity. The pots were kept 50 cm away from each other. After 20 days of transplanting, 100 ml amino acid in 2 μ M concentration were applied in each pot. Second amino acid treatment was done 20 days after the first amino acid treatments. No amino acid was applied to the broomrape inoculated and non-inoculated control groups. After 15 days of second amino acid treatments, the number of broomrape shoots was recorded. The tomato plants were harvested and the tubercle numbers were recorded.

All pots were arranged in a completely randomized design with 3 replicates and the seed germination bioassay in the clear polyethylene pots was reaped twice. The data was analysed using PROC GLM model in SAS9.2 (SAS Institute Inc.), means were compared using least significant difference (LSD) at $P \leq 0.05$.

Results

Germination bioassay in the petri dishes

In the in vitro method, the broomrape seed germinations after 7 and 21 days of the amino acid treatments were determined. The percent germination rate of the control seeds of broomrape was higher than that of the seeds treated with the different amino acids (Table 1).

Table 1

Effects of amino acid treatments on germination of *P. ramosa* seeds after 7 and 21 days and germination inhibition rate.

Amino acid	Germination rate after 7 days (%)	Germination rate after 21 days (%)	Germination inhibition, % of control
Control	19.12	95.14	--
Theanine	17.13	85.67	9.82
Leucine	16.48	82.33	13.33
Isoleucine	16.27	81.33	14.38
Valine	15.27	76.33	19.65
Cysteine	15.13	75.67	20.35
Arginine	15.00	75.00	21.05
Glutamine	13.53	67.67	28.77
Tyrosine	13.40	67.00	29.47
Methionine	13.27	66.33	30.18
Aspartic acid Serine	13.20	66.00	30.53
Proline	13.00	65.00	31.58
Phenylalanine	12.13	61.67	35.09
Asparagine	12.00	60.00	36.84
Glutamic acid	11.60	58.00	38.95
Glycine	11.60	58.00	38.95
Histidine	11.47	57.33	39.65
Tryptophan	10.87	54.33	42.81
Lysine	6.40	32.00	66.32
Alanine	3.80	19.00	80.29
	3.20	16.00	83.16
LSD 0.05	2.92	14.61	15.37

The germination rates after 7 and 21 days of treatments significantly varied among the tested amino acids. After 7 days of amino acid treatment, the highest germination rate was obtained from control treatment with 19.12%, while the lowest was obtained from alanine with 3.20%. When germination rate after 21 days of treatment was considered, germination rates varied between 16.00 and 95.00%. The highest and the lowest germination rates were obtained from the control and alanine treatments, respectively. Germination inhibition rates of amino acids were significantly different (Table-1) among the tested amino acids. Maximum inhibitory rate was obtained from alanine followed by lysine and tryptophan, while the minimum was obtained from theanine followed by leucine and

isoleucine. Among the twenty amino acids tested for germination inhibition, two amino acids, lysine and alanine, were identified that caused an inhibition rates greater than 80.00%.

Table 2
Effects of amino acid dose on the germination of *P. ramosa* seeds after 7 and 21 days and germination inhibition rate.

Amino acid treatment (µM)	Germination rate after 7 days (%)	Germination rate after 21 days (%)	Germination inhibition, % of control
0.5	16.00	80.00	15.79
1.0	14.38	71.90	24.31
2.0	12.82	64.12	32.49
3.0	10.88	54.44	42.69
4.0	8.74	43.73	53.96
LSD 0.05	1.42	7.12	7.50

Germination rates in the first and second count and germination inhibition rate of broomrape seeds incubated at different concentrations (0.5, 1.0, 2.0, 3.0, 4.0 µM) of amino acids has been shown in Table 2. When germination rates after 7 and 21 days of treatment were considered, there were significant germination rate differences among the amino acid concentrations (Table 2). A progressive decrease in the germination rate was recorded with the increasing amino acid concentration. Germination rate after 7 days of amino acid treatment varied between 8.74 and 16.00%. The lowest and the highest germination rates were obtained from 0.5 and 4.00 µM amino acid treatments, respectively. All of the applied concentrations of amino acids (0.5, 2, 3, and 4 µM) reduced the germination rate of the broomrape seed. Germination rates after 21 day of amino acid treatment showed similar germination pattern that germination rates reduced with the increased amino acid concentrations. The lowest and the highest germination rates were obtained from 0.5 µM amino acid concentration whit 80.00% and the lowest was obtained from 4.00 µM amino acid treatments with 43.73%.

The inhibitory effect on germination of each amino acid was evaluated in a bioassay against broomrape seed germination. The amino acid-mediated inhibition of broomrape seed germination exhibited a dose-dependency (Table 2). The germination inhibition rates gradually increased with the increasing amino acid concentrations. The range of germination inhibition was between 15.79 and 53.96%. The highest inhibition rate was obtained from 4 µM amino acid concentration while the lowest was obtained from 0.5 µM amino acid

Germination bioassay in the clear polyethylene bags

The effect of amino acid on the number of germinated *P. ramosa* seeds was tested under controlled conditions in the polyethylene bags.

Table 3
Effects of amino acid treatment on the number of *P. ramosa* seed germination and tubercle number after 7 and 21 days of incubation

Amino acid	Four weeks after amino acid treatment		Five weeks after amino acid treatment	
	Number of germinated seeds	Tubercle number	Number of germinated seeds	Tubercle number
Theanine	210.00	0.00	205.50	2.00
Asparagine	138.00	1.00	142.50	1.00
Glutamic acid	124.50	2.50	140.50	3.50
Glutamine	116.00	0.00	111.50	0.50
Alanine	114.50	2.00	109.00	2.50
Arginine	114.00	0.00	118.50	0.50
Leucine	109.50	0.00	117.50	0.00
Histidine	106.00	0.00	116.00	0.50
Glycine	104.50	0.00	112.00	0.00
Control	93.00	2.00	120.50	2.50
Valine	82.00	0.50	91.50	0.50
Aspartic acid	76.50	0.50	125.50	0.50
Tryptophan	71.50	0.00	74.00	0.00
Serine	62.00	0.50	91.00	0.50
Lysine	60.50	0.00	66.00	1.00
Methionine	54.00	1.00	69.50	1.00
Tyrosine	51.00	1.50	59.50	1.50
Cysteine	42.50	0.00	45.50	0.00
Phenylalanine	42.00	0.00	49.50	0.50
Isoleucine	40.00	0.00	45.50	0.00
Proline	37.50	0.00	46.00	0.50
LSD 0.05	61.77	2.17	57.93	2.79

As shown in Table 3, number of germinated seeds and tubercle number of broomrape seeds after 4 and 5 weeks after amino acid treatment varied significantly among the tested amino acids. Number of germinated seeds after 4 weeks of amino acid treatments varied between 37.50 and 210.00 seeds/plant. The highest and the lowest values were obtained from proline and theanine, respectively. The range of tubercle number per plant was between 0.00 and 2.50%. The lowest tubercle number was obtained from leucine, histidine, glycine, tryptophan, lysine, cysteine, phenylalanine, isoleucine and proline with 0.00 tubercle/plant, while the highest was obtained from glutamic acid with 2.5 tubercle/plant.

When five weeks after amino acid treatment was considered, the number of germinated seed/plant varied between 46.00 and 205.50 (Table 3). Similar to the number of germinated seed/plant after 4 weeks of amino acid treatment, the lowest and the highest values were obtained from proline and theanine, respectively. Tubercle number/plant varied between 0.00 and 3.50. Glutamic acid had the highest tubercle number while leucine, glycine, tryptophan and isoleucine had the lowest tubercle number.

Table 4

Effects of amino acid treatment on the branch number/plant, fresh weigh and dry weight of *P. ramosa* above and below soil surface, and fresh and dry weights of tomato plant.

Amin acid	Above-ground parameters of <i>P. ramosa</i>			Underground parameters of <i>P. ramosa</i>			Host crop parameters	
	Branch number/plant	Fresh weight (g)	Dry weight (g)	Branch number/plant	Fresh weight (g)	Dry weight (g)	Tomato fresh weight (g)	Tomato dry weight (g)
Tryptophan	13.50	27.21	11.54	28.00	30.11	12.88	216.50	43.00
Positive control	12.50	23.14	8.66	34.25	38.27	15.27	202.00	42.50
Histidine	11.50	30.17	12.76	52.00	26.33	10.43	148.50	42.25
Serine	11.00	14.12	4.44	49.00	27.54	10.16	142.00	20.75
Theanine	10.50	22.82	6.93	52.00	24.17	9.01	132.50	34.00
Alanine	9.25	15.06	5.04	48.25	46.97	21.34	214.00	43.50
Isoleucine	9.00	24.52	10.08	59.25	25.51	10.12	141.75	38.00
Valine	8.75	15.61	4.99	46.75	25.93	11.91	140.00	38.50
Arginine	7.50	8.41	2.72	72.00	31.79	10.70	150.50	33.25
Glycine	7.50	15.02	3.93	47.00	16.25	4.80	186.25	43.75
Methionine	7.00	24.41	11.75	51.75	18.78	8.50	132.25	26.50
Aspartic acid	5.75	10.10	3.01	50.50	29.71	9.20	137.75	36.00
Cysteine	5.25	12.22	5.38	46.00	28.31	11.30	162.25	34.75
Tyrosine	4.25	21.59	9.76	52.50	30.13	13.15	135.75	24.50
Proline	4.25	12.30	4.18	21.50	19.37	9.10	203.00	31.25
Lysine	4.00	10.36	3.41	22.25	21.73	10.56	161.25	28.75
Glutamic acid	2.75	8.29	2.67	20.00	28.04	13.37	144.50	25.75
Leucine	2.50	9.87	4.13	29.50	24.63	10.75	235.50	40.25
Phenylalanine	2.00	4.35	1.61	12.75	15.59	5.65	209.00	36.25
Glutamine	1.50	6.58	2.21	27.25	20.67	9.29	220.25	36.25
Asparagine	0.25	1.22	0.38	13.75	13.81	5.56	260.25	45.25
Negative control	0.00	0.00	0.00	0.00	0.00	0.00	274.00	62.00
LSD 0.05	11.28	27.96	12.18	30.52	22.73	10.66	80.20	19.67

Germination and growth bioassay in the pots

The effect of amino acid treatment on branch number/plant, fresh weight, dry weight of both underground and above ground parts of branched broomrape were determined in the pot studies. Compared with positive control

treatment, amino acid treatments significantly ($P < 0.01$) decreased both underground and above ground parts of broomrape, except for tryptophan (Table 4). Except for no GR 24 added negative control treatment, the lowest below ground brunch number per plant of broom rape was obtained from asparagine with 0.25, while the highest was obtained from tryptophan with 13.50. As shown in Table 4, the brunch number of positive control (12.50) was lower than that of tryptophan. Fresh weight of broomrape on the soil surface varied between 30.17 and 1.22 g among the tested amino acids, the highest and the lowest fresh weight values were obtained from histidine and asparagine, respectively. The positive control treatment had fresh weight that was significantly lower than that of the fresh weights obtained in histidine, tryptophan, izolosine and methionine. The effects of amino acids on above ground dry weight of broomrape matched the patterns recorded for above ground broomrape fresh weights. The highest broom rape dry weight was obtained from histidine with 12.76 g and the lowest was obtained from asparagine with 0.38 g. Five amino acids, tryptophan, histidine, izolosine, metionine and tyrosine, had greater above ground dry weights than that of the positive control treatment.

When underground plant parameters of *P. ramosa* were considered, brunch number per plant varied between 12.75 and 72.00. The lowest and the highest brunch number plant were obtained from phenylalanine and arginine, respectively. As seen from the Table 4 that brunch number per plant of broomrape below soil surface was higher than that of above soil surface. Compared with the positive control treatment twelve amino acids had higher brunch number per plant. Underground fresh weights of broomrape were greater than that of above ground fresh weights. The highest fresh weight was obtained from alanine with 46.97 g while the lowest was obtained from asparagine with 13.81 g. Compared with the positive control, only alanine had higher fresh weight. The range of underground broomrape dry weight was 4.65 and 21.34 g. The lowest and the highest underground broomrape dry weights were obtained from phenylalanine and alanine, respectively. Compared with the positive control, alanine had the higher underground broomrape dry weight than that of the positive control.

Tomato plants grown in broomrape infected and amino acid treated pots were harvested to determine how amino acid treatment effect fresh and dry weights of the host crop. There were significant tomato fresh and dry weights differences among amino acid treatments (Table 4). The tomato fresh weight varied between 132.25 and 274.00 g. Methionine had the lowest fresh weight, while the negative control treatment had the highest tomato fresh weight. Seven amino acids had higher fresh tomato weights than the positive control. When dry weight of tomato was consideration, it ranged between 20.75 and 62.00 g. The lowest and highest values were obtained from Serine and negative control treatments, respectively. Compared with the positive control, serine, alanine glycine and asparagine had the higher dry weight.

Discussion

As a bio-stimulant, amino acids play significant role on plant growth, nutrient availability, and plant development due to their functions as hormone precursor, injury repair caused by abiotic stresses factors, signal component in many physiological and biochemical processes, antioxidant metabolism, root development and regulation of nitrogen uptake (Ertani et al, 2013; Calvo et al., 2004; Miller et al., 2007; Kowalczyk et al., 2008; Forde, and Roberts, 2014; Maeda and Dudareva, 2012; Hildebrandt et al., 2014; Weiland et al. 2016; Teixeira et al., 2017; Roupheal and Colla, 2018; Roupheal et al., 2018).

Despite the above mentioned beneficial bio-stimulant effects, certain amino acids have inhibitory effects on plant growth and development due to feedback inhibition ((Vurro et al., 2006; Bertin et al., 2007; Nzioki et al., 2016; Fernández-Aparicio, 2017). Inhibitory effects of root exuded and pathogen over-excreted amino acids on plants

were previously reported (Bertin et al., 2007; Nzioki et al., 2016). Similarly exogenous supplied amino acids inhibited seed germination and tubercle formation of *O. ramosa* and *O. minor* (Vurro et al., 2006; Fernández-Aparicio, 2017).

The current study was we designed an in vitro and pot studies to investigate the inhibitory potential of amino acids on *P. ramosa* germination and growth. In the germination bioassay studies, 1 mg/ L concentration of GR 24, the strigolactone analogue, was used to excite the broomrape seed germination. After 21 days of incubation, 95% of the GR 24 treated and no amino acid added broomrape seeds were germinated. The higher germination percentage in the control group allowed us to accurately compare germination inhibition potential of each tested amino acid.

In the in vitro germination bioassay, *P. ramosa* seed germination was highly inhibited (> 80%) by lysine and alanine. On the other hand, the theanine had least inhibition rate (< 10%) among the tested amino acids. Similarly, Vurro et al., (2006) reported that lysine and alanine were found among the highest inhibitory amino acids on *P. ramosa* seed germination while lysine was found one of the strong inhibitory amino acids on *O. minor* seed germination under in vitro condition (Fernández-Aparicio et al. 2017). In contrast to our study, Vurro et al. (2006) reported strong germination inhibition from methionine and histidine treated *P. ramosa* verifying that these slight differences might be due to differences in the genetic make- up of *P. ramosa* populations. The present study confirmed that lysine and alanine had great potential on the germination inhibition of *P. ramosa*. This suggests that lysine and alanine possess strong inhibitory effects via specific mechanisms on *P. ramosa* seed germination. In the bacterial, valine and phenylalanine inhibited cell growth by repressing the enzymes involved in the synthesis of isoleucine and tyrosine, respectively (Bhatnagar et al., 1989).

All of the tested amino acid concentrations (0.5, 1.0, 2.0, 3.0 and 4.0 μ M) variously inhibited *P. ramosa* seed germination. A gradual increase in the inhibitory effect was observed with the increasing concentration of amino acids in the petri dishes. The findings about the inhibitory effects of different amino acid concentrations on broomrape germination showed that the negative impact increased with the increasing concentrations. At higher concentrations (3 and 4 μ M), the amino acids showed maximum inhibition on the germination of *P. ramosa*. It was observed that amino acid treatments with higher concentrations did not completely inhibit *P. ramosa* germination even up to 21 days of incubation.

Growth inhibitory effects of amino acids *P. ramosa* were investigated by using tomato seedling as a bioassay material. The treated (2 μ M) concentration of amino acids was inhibitory to the seedling growth of *P. ramosa*. Compared with the control, a significant decrease in the germination and tubercle number occurred when both the tomato seedling were grown in the clear polyethylene bags amended with 2 μ M isoleucine and proline. This difference among the amino acids in germination and tubercle number can be attributed to variability in efficient inhibition regulation of amino acids. Furthermore, the superiority of the isoleucine and proline might be due to better expression of inhibition attributes and efficient utilization of inhibition during the germination and tubercle development period of the *P. ramosa*. In the GR 24 excited germination lysine and alanine had the lowest germination rates while isoleucine and proline had the lowest germination rates in the tomato induced *P. ramosa* germination. We can speculate that inhibition potential of amino acid may vary due to source of germination stimuli.

In the pot growth bioassay studies, amino acids differently affected *P. ramosa* branch number. A great branch number variation was observed among the tested amino acids. Branch number is a good parameter as a growth indicator for *P. ramosa*. Asparagine and glutamine highly suppressed the above ground branch number of *P.*

ramosa in the pot studies. There was no *P. ramosa* germination and growth in the negative control group since this group was not infected with *P. ramosa* seeds. Fresh and dry weights *P. ramosa* were significantly affected from the amino acid treatments. Asparagine was the most inhibitory amino acid on fresh and dry weights of above and below ground parts of *P. ramosa*. These broomrape germination and growth inhibitions were attributed to mainly by feedback inhibition of an early enzyme in the amino acid biosynthesis pathway (Vurro et al., 2006, 2009; Sands and Pilgeram, 2009).

All of the tested amino acids did not suppress *P. ramosa* growth. In terms of above ground parts of *P. ramosa*, tryptophan, histidine and methionine had no inhibitory effect on *P. ramosa*. On the other hand, underground parts of *P. ramosa* were in consideration, alanine had no inhibitory effect on fresh and dry weights of *P. ramosa*. These results are in agreement with the study conducted by Bonner et al. (1992, 1996 and 1997) that not all of the tested proteinogenic amino acids inhibited *Nicotiana glauca* growth. Our study expanded on the work of Vurro et al. (2006) and Fernández-Aparicio et al. (2017) who showed that not all proteinogenic amino acids had strong growth inhibitory effect on broomrape species. These inhibitions of broomrape germination and growth were supposed to be caused mainly by feedback inhibition of an early enzyme in the synthetic pathway of the amino acid; this inhibition also suppresses the production of other amino acids that use the common enzyme for their syntheses.

In in vitro and clean polyethylene bag germination bioassays asparagine did not show a good performance as in growth bioassays studies. This could be attributed to physical and chemical properties of the soil, root exudates of the host crops and microbial metabolization of the amino acids; therefore, many factors might interfere to the inhibitory potential of amino acids in the soil. Our finding showed that in vitro and polyethylene bag studies did not reflect the inhibitory potential of amino acid under soil conditions. Therefore, determining the inhibition potential of amino acid under soil conditions was more realistic than that of in vitro and polyethylene bag studies. Broomrape infection reduced shoot and root dry weights of tomato compared with the no broomrape infected control group. Surprisingly, leucine phenylalanine, glutamine, asparagine, proline and tryptophan stimulated shoot and root dry weights of tomato compared with the positive control. Similarly, certain amino acids were reported to greatly inhibit the early development of broomrape without any phytotoxic effects on the host plants (Vurro et al., 2006). The inhibition potential of amino acids on parasitic weeds depends on parasitic plant species and their developmental stage (Fernández-Aparicio et al., 2017).

Conclusions

Amino acid sensitivity is an important phenomenon that should be considered in the management of *P. ramosa*. In this study we observed that the broomrape germination was significantly impacted by proteinogenic amino acid treatments. Among the twenty proteinogenic amino acids tested for germination inhibitions, four amino acids strongly inhibited the seed germination of broomrape, (42–83% inhibition) at a concentration of 2–4 μM . The inhibitory effect increased with the increasing amino acid concentrations and 4.0 μM concentrations had the highest level of germination inhibition. Amino acids imposed significant inhibitory effect on branch number, fresh and dry weights of branched broomrape. These results suggest that excesses of specific amino acids should be considered for more practical exhibitions of the inhibitory effect that amino acid is expected to show on broomrape growth in the soil. The main conclusion from the results presented above is that amino acids are clearly the superior candidate for germination inhibition agents to control broomrape. Further studies are needed to determine whether field applications of certain amino acids are effective to manage branched broomrape.

Declarations

Author Contribution

Hilal Albayrak and Doğan Işık designed and directed the project. Mehmet Arslan was involved in planning and supervised the work and processed the experimental data and assisted in interpreting the results and worked on the manuscript. All authors discussed the results and commented on the manuscript.

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Conflict of Interest

We declare no conflicts of interest.

Availability of data and materials: The datasets used and/or analyzed during the present work are available from the corresponding author on reasonable request.

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