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1 **Can amino acids be used to inhibit germination or deplete the soil seedbank of**

2 ***Phelipanche ramosa* (L.) Pomel?**

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Abstract

Phelipanche ramosa is a weed holoparasite which became a major agronomic problem for several crops in France. The yield and quality losses may be complete and lead to the abandonment of crops in the most infested fields. Chemical weed control using synthetic herbicides do not have a direct effect on this weed because the first half of its reproductive cycle occurs on the root system of its host. The aim of this study is to contribute to the definition of an integrated weed management program of *P. ramosa*, especially focusing on the use of the possible direct effect of amino acids on seed of broomrapes as a potential biocontrol solution. We studied a set of 14 amino acids in *in vitro* conditions to investigate whether some amino acids can have an inhibitory effect on the germination of the *P. ramosa* seeds, whether this effect may be permanent after the removal of the amino acid, and whether some amino acids can cause a high dormancy or seed mortality rate. Our main results showed (1) a strong inhibitory effect of four amino acids on the seed germination concerning arginine, lysine, phenylalanine and tryptophan, and (2) that this effect appeared temporary for arginine and lysine, but permanent for phenylalanine and tryptophan. Our study thus highlighted the interest of phenylalanine and tryptophan as they caused both high inhibition of germination and high seed mortality of *P. ramosa*. Based on these results, we suggest three options for the use of these amino acids that could provide concrete answers to farmers.

Keywords

Biocontrol · Weed management · Parasitic plant · Branched broomrape · Natural product

Introduction

Broomrapes constitute a large group of root holoparasites, including several major arable weeds damaging crop production and causing substantial economic damage. They remain a serious challenge in agriculture, especially in Mediterranean, Central and Eastern Europe (Parker 2009) because some of these weed broomrapes can infest a broad host range. The branched broomrape, *Phelipanche ramosa* (syn. *Orobancha ramosa* L., Joel 2009), can infest a wide set of major crops, but also many weeds among Brassicaceae, Cannabaceae, Cucurbitaceae, Fabaceae and Solanaceae (Brault et al. 2007; Gibot-Leclerc et al. 2003; Parker 2009; Gibot-Leclerc et al. 2016; Pointurier et al. 2019), thus increasing its harmfulness. In Europe, and especially in France, *P. ramosa* represents a major agronomic problem in tobacco fields, leading to yield and quality losses that may be complete, and then to the long-term abandonment of this crop production on highly infested arable fields (Brault et al. 2007; Julio et al. 2020).

Currently, crop protection against broomrapes has largely focused on chemical control using synthetic herbicides. This lever is usually combined with others, such as cropping practices preventing seed dispersal, trap crop, biological control and/or varietal resistance, as part of an integrated weed management program (Pérez-De-Luque et al. 2009; Rubiales and Fernández-Aparicio 2012; Fernández-Aparicio et al. 2016). However, agronomic, environmental, public-health and socio-economic issues related to the side effects of chemical control methods question the relevance of this lever to manage broomrapes (van der Werf 1996; Loyce et al. 2012; Bourguet and Guillemaud 2016; van Bruggen et al. 2018). The development of a more sustainable agriculture based on agro-ecological approaches is aimed at reducing the use of synthetic pesticides, while continuing to ensure a high level of production (Duru et al. 2015; Cordeau et al. 2016; Petit et al. 2018; Vila-Aiub 2019).

P. ramosa is characterized by a biological cycle consisting of two distinct phases (Gibot-Leclerc et al. 2012). The first phase is underground, including seed germination, attachment to host roots and penetration into host tissues with the development of an absorptive system called haustorium. The second phase is aboveground, with the emergence and growth of floral stems, flowering and fructification (Gibot-Leclerc et al. 2012). The long duration of the underground phase usually leads to a reduced effectiveness of the standard levers identified so far, making their use limited to the end of its biological cycle or/and very expensive (Gibot-Leclerc et al. 2012; Gaba et al. 2017). The end of the second phase leads to the release of several hundred thousand tiny seeds per floral spike, seeds characterized by a longevity in the soil of several decades (Gibot-Leclerc et al. 2012; Fernández-Aparicio et al. 2016). Moreover, the seed germination of *P. ramosa* is a two-step process, including first a conditioning period requiring suitable environmental conditions, and then a period during which seeds become sensitive to germination stimulants exuded by surrounding host roots, such as strigolactones and isothiocyanates (Fernández-Aparicio et al. 2011; Auger et al. 2012). All these traits explain why managing *P. ramosa* is particularly difficult.

Among several options based on bioherbicides, such as microbial herbicides, mycoherbicides, allelopathic plants producing herbicidal compounds and biochemical herbicides (Fernández-Aparicio et al. 2020; Gibot-Leclerc et al. 2022), an interesting one would be to use the potential herbicidal effect of natural products like amino acids, especially because this effect can potentially affect the germination rate of *P. ramosa* while depleting the soil seedbank by causing a high seed mortality rate (Vurro et al. 2006; Fernández-Aparicio et al. 2020). Amino acids are organic molecules that play an important role in cell metabolism. In particular, some amino acids can intervene in the regulation of biosynthesis pathways of other amino acids (Galili et al. 2016), or as precursors in pathways related to the storage of mineral elements such as nitrogen and sulphur. Some studies have assessed their effects on

91 parasitic plants (Vurro et al. 2006; Nzioki et al. 2016; Fernández-Aparicio et al. 2017; Kuruma
92 et al. 2021). In particular, Vurro et al. (2006) showed that some amino acids can decrease more
93 or less the germination rate of *P. ramosa* or even inhibit it. This is especially the case of
94 arginine, proline and histidine that appears to strongly inhibit *P. ramosa* germination.
95 Nevertheless, these studies did not assess the persistence of the inhibitory effect of germination
96 after the removal of the amino acid from the medium. Moreover, these studies do not indicate
97 the state of the seed in the absence of germination, even though the seed may be dormant or
98 dead.

99 The successful biocontrol with amino acid lies in distinguishing the different effects of
100 amino acids on seeds. As an annual plant, *P. ramosa* growth and survival depends on the seeds
101 occurring in the soil seedbank. Therefore, the management of the seeds by amino acid could
102 have a curative effect, by reducing or delaying the germination and emergence of *P. ramosa* or
103 a preventive effect, by depleting the soil seedbank. To our knowledge, no study has ever tested
104 these different effects: inhibiting, delaying or killing.

105 This study aims at identifying amino acids characterized by a high effect on the *P.*
106 *ramosa* seeds through in vitro experiments, especially by focusing on *P. ramosa* populations
107 parasitizing tobacco, so as to assess the relevance of using amino acids in field conditions. To
108 do this, fourteen amino acids were chosen and applied on *P. ramosa* seeds in order to assess
109 their potential effect and determine the various causes of this effect. Our main hypotheses are
110 (1) that at least some amino acids can reduce more or less strongly the germination rate of *P.*
111 *ramosa* seeds (inhibitory effect), (2) that this reduction of the germination rate may be
112 transitory and can disappear if the amino acids are removed from the medium (delaying effect),
113 (3) that this reduction effect may be permanent and related to seed death (killing effect).

Materials and methods

Seed material and conditioning

Seeds of *Phelipanche ramosa* were harvested from a natural population within a highly infested field of tobacco located in the Nouvelle-Aquitaine region, a region characterized by the highest levels of infestation of this parasitic weed in tobacco in 2017 (45° 53' 38.908" N, 0° 0' 36.803" E; Aigre). All seeds were kept in watertight glass containers at approximately 20°C until the beginning of the experiment.

Before any experiment, *P. ramosa* seeds were conditioned to make them susceptible to germination stimulants. Seeds were surface disinfected under a laminar flow hood by a 5-min immersion in 70% ethanol, followed by a 5-min immersion in a solution of Ca(OCl)₂ at 3% (p/v) and Tween 20 (0.1%) so as to limit fungal spread (Gibot-Leclerc et al. 2012). Seeds were rinsed five times with twice-distilled water and placed onto 1 × 1 cm paper squares (Whatman®, Glass microfiber filters GF/A) using a pipette. Pipetting resulted in a variable number of seeds per paper square, i.e. between 8 and 155 seeds. However, positioning a similar number of seeds onto the paper squares would have required using a stereoscopic microscope to transfer the seeds one by one because of their small size (350 × 250 µm, Gibot-Leclerc et al. 2012), which was not possible under sterile conditions under the laminar flow hood. The paper squares were placed on a Whatman® GF/A paper sheet (Ø 90 mm) at the bottom of a Petri dish (Ø 90 mm) and moistened with 3 mL of sterile distilled water. These Petri dishes were sealed with Parafilm® (American Can Company), wrapped in aluminum foil and placed in a dark growth chamber, at 20°C for 14 days, to condition *P. ramosa* seeds (Gibot-Leclerc et al. 2004).

Amino acids

Fourteen amino acids were tested in this study, including eight amino acids which had already been tested on *P. ramosa* populations harvested from infested fields of tobacco and tomato in Italy (Vurro et al. 2006): These chosen amino acids: alanine, arginine, glutamine, glycine, histidine, lysine, methionine and proline, reduced the germination rate by at least 60%, except for glutamine. Moreover, five amino acids had only been tested on other parasitic weeds, i.e. *Orobancha minor* (Fernández-Aparicio et al. 2017) and/or *Striga hermonthica* (Delile) Benth. (Nzioki et al. 2016): aspartate, isoleucine, phenylalanine, tryptophan and tyrosine, and one amino acid, asparagine, had never been tested on parasitic plants of the genera *Orobancha*, *Phelipanche* or *Striga*. All amino acids were supplied by the Sigma-Aldrich company (Sigma-Aldrich, St. Louis, MO, United States).

Seed germination assay

The germination stimulant strigol analogue GR24 was added to each Petri dish at 1 mg/L (Mangnus et al. 1992), a suitable concentration for this population of *P. ramosa* to achieve a maximum percentage of germinated seeds 10 days after application higher than 90%. The GR24 was provided by Dr. Binne Zwanenburg (Radboud University, Nijmegen, The Netherlands). The concentration of amino acids was set at 2 mM, based on the results of Vurro et al. (2006) on the application of amino acids to seeds of *P. ramosa* harvested from tobacco fields. We have grouped the 14 amino acids into four sessions, with each session including a positive control (only GR24, hypothesizing a high percentage of *P. ramosa* germinated seeds) and a negative control (only water, hypothesizing no germinated seeds). A seed was considered germinated when the radicle had pierced the seed coat (Gibot-Leclerc et al. 2004). In the following, viable seeds correspond to germinated seeds and seeds colored with TTC (see

section *Determination of seed viability*). In order to identify the optimal concentration of GR24 and the minimum duration of the exposure time before counting, two concentrations of GR24 were tested (1 mg/L and 10 mg/L) and the number of germinated seeds of *P. ramosa* was counted at two periods after application of the GR24 (5 and 10 days). The results obtained highlighted, for the concentrations 1 mg/L and 10 mg/L respectively, a germination rate of 67.2% and 86.5% 5 days after GR24 application, and of 99.2% and 94.7% 10 days after application, thus leading to retain a concentration of GR24 of 1mg/L and a minimum exposure time of 10 days after application of this concentration.

Potential inhibitory effect of amino acids on the germination of *P. ramosa* seeds

The amino acids were weighed and introduced into volumetric flasks with the GR24 solution at 1 mg/L, and then 3 mL of this solution were taken in order to moisten the paper at the bottom of the Petri dishes. Four paper squares with the conditioned seeds of *P. ramosa* were transferred into a Petri dish. Each modality (i.e. amino acids and the positive control) included three Petri dishes, for a total of 12 small paper squares, i.e. between 48 and 391 seeds per Petri dish, except for the negative control modality which had only one Petri dish with 4 paper squares, i.e. between 171 and 272 seeds. These Petri dishes were sealed with parafilm and wrapped in aluminum foil, and then placed at 20°C in the dark for 10 days. The number of germinated seeds of *P. ramosa* was counted 10 days after application of the treatment carried out in this first step using a stereoscopic microscope (1.95x–250x).

Potential reversibility effect of amino acids on the inhibition of germination of *P. ramosa* seeds

The aim of this second step was to assess whether the seeds of *P. ramosa* that did not germinate during the first step of the experiment can germinate after the removal of the amino acid from the medium. New Petri dishes were moistened with 3 mL of GR24 solution at 1 mg/L. Each paper square with the seeds of *P. ramosa* tested during the first step was transferred into these new Petri dishes. These dishes were sealed with parafilm and wrapped in aluminum foil, and then placed at 20°C in the dark for 10 additional days. The number of germinated seeds of *P. ramosa* was counted 10 days after application of the treatment carried out during the previous step, i.e. for 20 days after the beginning of the experiment, using a stereoscopic microscope (1.95x–250x). Given the small size and large number of *P. ramosa* seeds, it was technically impossible to wash the seeds during this step.

Determination of seed viability

The viability of the *P. ramosa* seeds that did not germinate after these two steps was assessed by adding 3 mL of 2,3,5-triphenyl tetrazolium chloride (TTC, Sigma-Aldrich) at 1% in each Petri dish. The Petri dishes were wrapped in aluminum foil, and placed in a drying oven at 40°C for 48 h. The seeds were then placed onto a new Whatman® GF/A paper sheet (Ø 90 mm) soaked with a saline solution (1% NaOCl). Viability was assessed after 20 min. Ungerminated viable and ungerminated non-viable seeds were counted under a stereoscopic microscope, seeds characterized by an embryo exhibiting no carmine red staining being considered as non-viable (Gibot-Leclerc et al. 2004).

Statistical analysis

For the subsequent statistical analyses and graphical representation, the number of seeds on the four squares per Petri dish and then on the three Petri dishes of a same modality was summed for each session, except for the negative control modality (only water), which had only one Petri dish for each session. In order to validate the absence of any session effect on the germination rates for the positive control between the four different sessions, a 4-sample test for equality of proportions without continuity correction was applied, the null hypothesis being that the proportions in these four sessions are the same. All analyses were made with R version 4.0.2 (R Core Team 2020) and the stats package. Raw data of this experiment are available in Supporting Information (Table S1).

Results

Control analysis

In each session, no seed germination was observed for the negative control (only water), but the seeds were still viable 20 days after the beginning of the experiment. The viability rate was always above 85% based on TTC test. In the absence of differences between sessions for the two controls, the raw results are presented for the entire experiment (refer to Supporting Information for details).

Inhibitory effect of amino acids on the germination of *P. ramosa* seeds

Considering the germination rate of *P. ramosa* seeds after the application of amino acids, we observed variable responses depending on the amino acid considered (Fig.1a). Based on the

confidence intervals (Table 1), four amino acids were characterized by a very strong inhibitory effect with germination rates below 10% (phenylalanine, arginine, lysine and tryptophan; Fig. 1a; Table 1). Six amino acids (aspartate, glycine, proline, histidine, methionine and isoleucine) had a low inhibitory effect with a germination rate above 70% (Fig. 1a; Table 1). Two amino acids (asparagine and glutamine) had no inhibitory effect with a germination rate comparable to that of the positive control (only GR24).

Permanent vs. temporary effect of amino acids on the inhibition of germination of *P. ramosa* seeds

For some of the four amino acids with a strong inhibitory effect, the removal of the amino acid from the medium highly increases this germination rate, thus suggesting a temporary effect of the amino acid on the inhibition of germination of *P. ramosa* seeds. This is the case for arginine and lysine, for which the germination rate after the removal of the amino acid was 85.0% and 56.9% respectively (Fig. 1b). For these two amino acids, the inhibitory effect would therefore be transient, whereas for phenylalanine and tryptophan, the germination rate remains very low, suggesting a permanent effect of the amino acid.

Determination of seed viability

Among the ungerminated seeds after the two previous steps of this experiment, the application of TTC showed that only a small number of seeds were still viable, from 0.0% at 2.2% with a mean of 0.9% similar to that of the positive control (only GR24). On the other hand, the mortality rate of ungerminated seeds varied very strongly depending on the amino acid, from about 7% to almost 100% (Fig. 2). For some amino acids, this rate was close to that observed

for the negative control (only water) but for most of the other amino acids, it appeared much higher. This result suggests that the amino acids for which the application decreased the overall germinated seed rate therefore induced the death of *P. ramosa* seeds (Fig. 2).

Discussion

Inhibitory effect of amino acids on the germination of *P. ramosa* seeds

Our study showed that *in vitro* application of several amino acids leads to an inhibitory effect on the germination of *P. ramosa* seeds. Among the 14 amino acids tested, only two, asparagine and glutamine, had no effect on the germination rate while only four, phenylalanine, arginine, lysine and tryptophan, had a strong effect on the germination rate of *P. ramosa* seeds. For these four amino acids, the germination rate is lower than 10%. When considering the results of the study by Vurro et al. (2006), carried out on *P. ramosa* seeds harvested from tobacco, i.e. the same pathosystem as in our study, we found that these results differed for most amino acids. As an example, Vurro et al. (2006) highlighted that arginine, histidine and proline showed the higher inhibitory effect of *P. ramosa* seeds, but only arginine had the same effect in our study (Table S2). We hypothesize that these differences may be due to differences in the experimental protocols concerning GR24 concentration and exposure time to amino acids before counting, or may be due to genetic differences between *P. ramosa* populations (Gibot-Leclerc et al. 2021).

For lysine and arginine, the inhibitory effect was transient, disappearing partially for lysine and completely for arginine when these amino acids are removed from the medium. This was not the case for the amino acids phenylalanine and tryptophan. Recently, L-tryptophan has been identified as a molecule characterized by a high inhibitory effect of the germination of

Orobancha minor seeds (Kuruma et al. 2021). Our study is the first to demonstrate the transient effect of some amino acids, while the inhibitory effect alone had been tested before (Vurro et al. 2006; Nzioki et al. 2016; Fernández-Aparicio et al. 2017).

Killing effect of amino acids on *P. ramosa* seeds

All the amino acids had a killing effect on the *P. ramosa* seeds, this effect varying from 7% to 99%. The lower the overall germinated seed rate, the higher the mortality rate for a given amino acid, suggesting especially that the low overall germinated seed rate (with and after removal of amino acid) of some amino acids is due to a high seed mortality rate. In any case, whatever the amino acid applied, the number of ungerminated but still viable seeds was low. For two amino acids, phenylalanine and tryptophan, the mortality rate was greater than 90%. Recent studies (Dor et al. 2017; Shilo et al. 2017) have shown that the effective control of *P. aegyptiaca* by glyphosate was due to a complex mechanism of action including inhibition of the aromatic amino acid biosynthetic pathway and, as a side effect, limitation of translocation of mobile solutes from the phloem to the parasite. According to Rubiales et al. (2018), some amino acids may have similar effects to such an herbicide due to feedback inhibition of metabolic pathways. Phenylalanine and tryptophan could then be used as alternatives to these synthetic herbicides through their direct effect on broomrape pre-attached stages, mainly during seed germination and development of the attachment organ (Vurro et al. 2006; Fernández-Aparicio et al. 2020; Kuruma et al. 2021).

Using phenylalanine and tryptophan for their biocide effect

Our study highlighted the interest of phenylalanine and tryptophan for their biocide effect on *P. ramosa* seeds. However, this effect has been observed only in *in vitro* conditions, and now its effectiveness remains to be demonstrated on the *P. ramosa*/tobacco pathosystem under

controlled conditions. Indeed, previous studies have shown that various factors must be taken into consideration to ensure appropriate use of this agronomic lever in field conditions (Vurro et al. 2006; Nzioki et al. 2016; Fernández-Aparicio et al. 2017).

The potential variability of the efficacy of amino acid application, as well as the associated costs, could be a major concern and would limit their use in field crops on a large scale. An optimization of the way amino acids are used could thus help improving their development in agriculture. Because of technical difficulties, it has so far been impossible to develop on a large scale the applications put forward by Vurro et al. (2006). However, we suggest proposals to deal with these difficulties for the coming years in the present study.

As a first option, we can use the allelochemical potential of some species and/or varieties to identify those most able to produce the amino acids of interest (Cimmino et al. 2018; Qasem and Issa 2018). As an example, *Avena sativa* L. seems to excrete L-tryptophan in these root exudates, which inhibits the growth of other plants (Kato-Noguchi et al. 1994). Then it might be possible to include this species in a crop mixture (Fernández-Aparicio et al. 2007; Fernández-Aparicio et al. 2010) or as a cover crop in a crop sequence (Rubiales and Fernández-Aparicio 2012). If this option proved effective, this lever could be used in cropping systems as a part of integrated strategy to reduce the seedbank of *P. ramosa*.

As a second option, the production of amino acid may be due to certain fungi that overproduce and excrete the relevant amino acid. For instance, Nzioki et al. (2016) developed amino acid overproducing lines of *F. oxysporum* f. sp. *strigae* and demonstrated their enhanced biocontrol action during management of *Striga hermonthica* on smallholder farms. Screening for fungi occurring in the soil rhizosphere (Vurro et al. 2018) or as endophytic community of *P. ramosa* and having deleterious effects of this weed is a promising approach. Currently, certain species have been isolated from *P. ramosa* and their pathogenic power has been characterized, as is the case for *Talaromyces trachyspermus* (Boari and Vurro 2004; Hemmati

and Gholizadeh 2019) and *Fusarium venenatum* (Gibot-Leclerc et al. 2022). At this stage, relying on these fungal species to create amino acid overproducing lines could be a relevant management option for *P. ramosa*.

As a last option, the biocide effect could be tested by encapsulating the amino acid in a matrix that would permit a targeted treatment and a prolonged release of the product onto the *P. ramosa* seeds. The encapsulation of traditional synthetic herbicides has already been proposed with this objective (Sopeña et al. 2009). Many formulations are based on the use of alginate gels (Ramos-Campos et al. 2015). This type of matrix provides a particulate form that appears suitable for use in the field during sowing or can be used as a seed coating material. The state of the art has also shown the interest of formulating these hydrogels by adding adsorbent agents of inorganic (silica/clays) or organic (cellulose fibers) nature to control the degree of release (Wang and Caruso 2005; Flores-Cespedes et al. 2007; Alila and Boufi 2009; Fernández-Pérez et al. 2011). Therefore, the approach would be to determine a formulation method combined with a determination of the lifetime of the amino acids in the environment. The successful application of phytotoxic amino acids on *P. ramosa* would depend mainly on how they are formulated. Among the new techniques that could be used, biopolymeric nanomaterials could make it possible to deliver active agro-compounds in a targeted, biocompatible, biodegradable and non-toxic way, while maintaining or even improving the efficacy of the product (Namasivayam et al. 2023).

This work in *in vitro* conditions constitutes the first step of use of amino acid to control seed germination and to deplete soil seedbank of *P. ramosa*. Future work will focus on the optimization of amino acid application in field conditions, including concentration, timing and formulation of this application.

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Statements and Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

Author contributions Conceived and designed the experiments: SGL, FD. Performed the experiments: SGL, MC. Analyzed the data: FD, RP. Wrote the paper: SGL, FD, MC, RP. Supervised the work: SGL, FD. Acquired funding: SGL.

Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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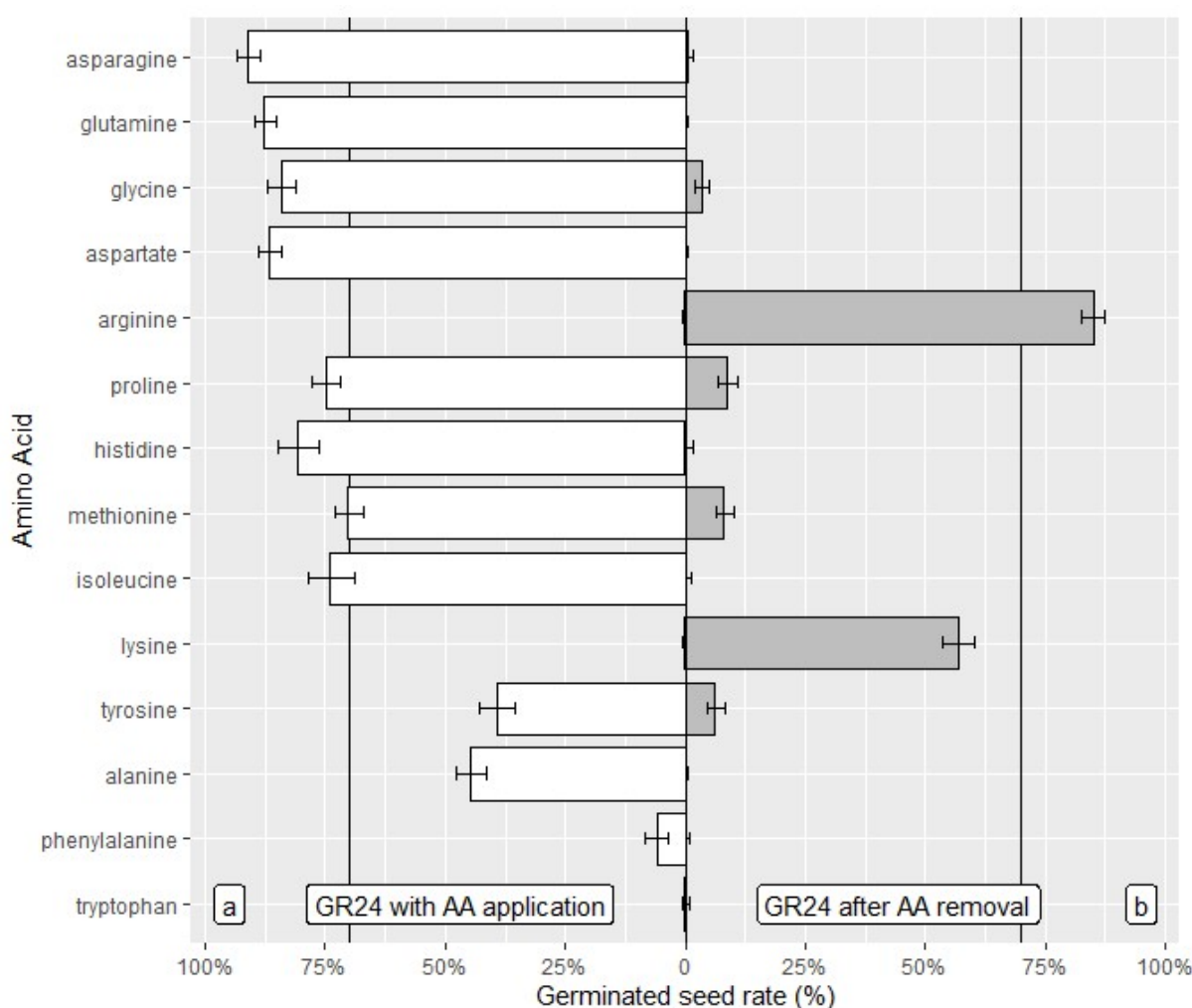
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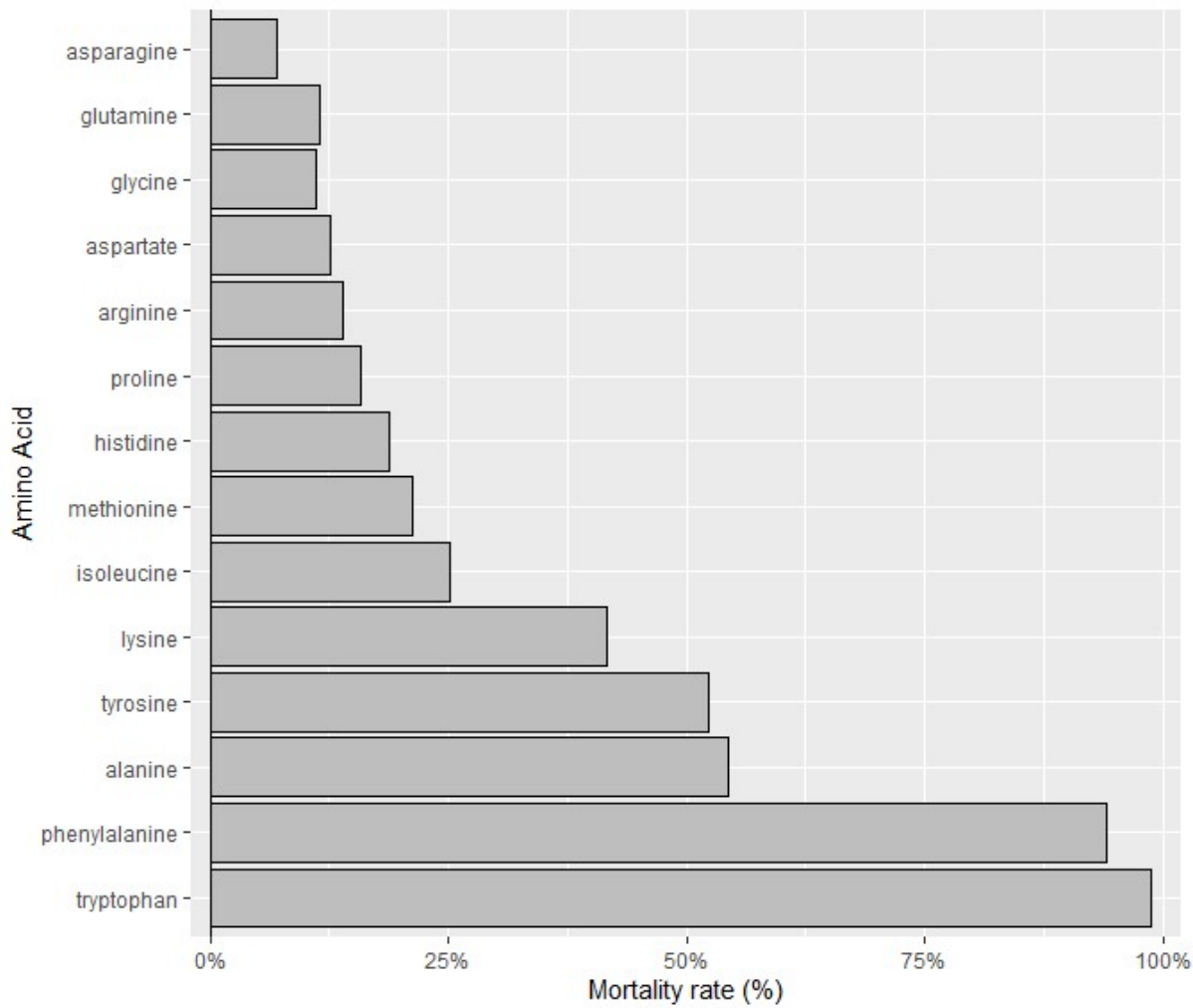
Figures

Fig. 1 Effect of amino acids on the germination rate of *P. ramosa* seeds: a) with GR24 and amino acid application; b) with GR24 after removal of amino acid. Amino acids are ordered according to their overall germinated seed rate (i.e. the sum of the germinated seeds with and after removal of amino acid). Error bars correspond to 95% confidence interval. See Material and methods for experimental details



557

558 **Fig. 2** Mortality rate of ungerminated seed of *P. ramosa*. Amino acids are ordered according
559 to their overall germinated seed rate, i.e. the same order as in Fig. 1. See Material and methods
560 for experimental details



561

562

Table 1 Germination rate (%) and confidence interval of *P. ramosa* with GR24 and amino acid (AA) application (step 1) and then with GR24 after removal of amino acid (step 2). Amino acids are ordered according to their overall germinated seed rate, i.e. the same order as in Fig. 1. * Amino acid with germination rate (step 1) not significantly different from GR24 only

Session	Amino acid	GR24 with AA application		GR24 after AA removal	
		Germination rate	CI 95%	Germination rate	CI 95%
S4	asparagine*	91.1%	[88.5; 93.3%]	0.7%	[0.2; 1.8%]
S3	glutamine*	87.7%	[85.3; 89.8%]	0.0%	[0.0; 0.4%]
S4	glycine	84.2%	[81.0; 87.0%]	3.4%	[2.1; 5.1%]
S3	aspartate	86.8%	[84.2; 89.1%]	0.0%	[0.0; 0.5%]
S2	arginine	0.1%	[0.0; 0.7%]	85.0%	[82.4; 87.3%]
S2	proline	74.9%	[71.7; 77.9%]	8.7%	[6.8; 10.9%]
S1	histidine	80.8%	[76.2; 84.9%]	0.3%	[0.0; 1.6%]
S2	methionine	70.2%	[67.1; 73.1%]	8.1%	[6.4; 10.1%]
S3	isoleucine	73.9%	[68.7; 78.6%]	0.0%	[0.0; 1.2%]
S2	lysine	0.1%	[0.0; 0.7%]	56.9%	[53.5; 60.3%]
S4	tyrosine	39.3%	[35.6; 43.1%]	6.2%	[4.5; 8.3%]
S1	alanine	44.6%	[41.4; 47.9%]	0.0%	[0.0; 0.4%]
S3	phenylalanine	5.7%	[3.5; 8.5%]	0.0%	[0.0; 1.0%]
S2	tryptophan	0.0%	[0.0; 0.5%]	0.3%	[0.0; 0.9%]