Solanum linnaeanum and Solanum sisymbriifolium as a sustainable strategy for the management of Meloidogyne chitwoodi

Laura Soraia Perpétuo1,2,3*, Maria J. M. da Cunha1,2, Maria Teresa Batista3 & Isabel Luci Conceição1

Root-knot nematodes (RKN), Meloidogyne spp., are important crop pests that cause severe losses in crop production worldwide, reducing both productivity and crop quality. Meloidogyne chitwoodi Golden, O’Bannon, Santo & Finley, 1980 is considered a quarantine organism by the European and Mediterranean Plant Protection Organization (EPPO) causing damage in tomato and potato crops. The development of nonchemical and sustainable management strategies to reduce nematode damage is crucial. The resistance of Solanum linnaeanum Hepper & P.-M.L. Jaeger and S. sisymbriifolium Lamarck cv. Sis 6001 to M. chitwoodi was evaluated based on gall index (GI), the Bridge & Page (1980) rating chart and reproduction factor (RF). Both plant species were resistant to M. chitwoodi. Solanum linnaeanum had an average of 519 small root swellings/plant, with 45% adult nematodes inside the roots, all males. Solanum sisymbriifolium had GI ≤ 2 and RF ≤ 1 with a high percentage (69%) of nematodes inside the roots that did not develop beyond the sexually undifferentiated second-stage. The use of S. linnaeanum as a new source of resistance is a good alternative for the control of RKN in the quest to develop nonchemical and sustainable management strategies to protect crops.

Plant-parasitic nematodes (PPN) are a focus of intense scientific research because they represent an important constraint for global food production, reducing yields and crop quality worldwide1-4. Most agricultural fields are infested with at least one PPN species and annual crop losses caused by them are estimated to be between 8.8 and 14.6% of total World crop production5,6. The nematodes with the greatest economic impact are the root-knot nematodes (RKN), Meloidogyne spp. Many species of this genus are quite aggressive, and they are widely distributed across a range of climatic conditions3,7,8. Some species are considered quarantine pests by the European and Mediterranean Plant Protection Organization (EPPO) and interfere with international trade9.

In Europe, RKN are increasingly important and they are responsible for significant yield losses, reaching up to 100% in tomato, S. lycopersicum (L.) H. Karst, one of the major vegetable crops cultivated and consumed internationally10,11. Potato, S. tuberosum L., is an important staple crop in Portugal and, according to “Instituto Nacional de Estatística” (INE), an average of 431,686 ha of potatoes are produced every year12. Meloidogyne chitwoodi Golden, O’Bannon, Santo & Finley, 1980 is considered a quarantine organism by EPPO (EPPO A2 list: No. 227) causing damage and making tubers unsuitable for consumption or processing. Even though M. chitwoodi is present in most major potato producing areas, its detection in tubers can lead to the rejection of entire shipments. Since the 1990s it has become prevalent in vegetable and potato production areas13,14.

The global distribution and wide range of hosts of RKN explains the high economic impact of these nematodes and the difficulties found in their management11. Although RKN are impossible to eradicate, it is crucial to reduce the amount of damage they cause if productivity is to be sustainable1. Their control is mainly achieved by the cultural practices of crop rotation and the use of resistant cultivars, combined with nematicide application15,16. Despite the fact that the use of chemical pesticides is an effective control strategy, this is an expensive method

---

1Centre for Functional Ecology-Science for People and the Planet (CFE), Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal. 2Polytechnic Institute of Coimbra, High School of Agriculture, Department of Agronomic Sciences and Research Centre for Natural Resources, Environment and Society (CERNAS), Bencanta, 3045-601 Coimbra, Portugal. 3Chemical Process Engineering and Forest Products Research Centre (CIEPQPF), Faculty of Pharmacy of the University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, University of Coimbra, 3000-548 Coimbra, Portugal. *email: soraiaperpetuo@gmail.com
and European legislation (Directive 69/465/CEE; Directive 2009/128/EC) is very strict regarding the use of nematicides in the field, focusing mainly on environmental safety issues and health risks. The increase of environmental concerns and regulatory restrictions on the application of chemical products in conventional systems have meant that the use of plant resistance to control PPN has increased. The most effective, environmentally friendly, and economical means of controlling Meloidogyne spp. is the use of resistant cultivars. However, up to now, there are no potato cultivars resistant to RKN.

Therefore, other control measures are being developed, including the use of plants as trap crops as an alternative to chemical pesticides. A trap crop is a plant species attractive to pests from another crop but in which the pest fails to survive or reproduce. Many wild Solanum species display resistance to Meloidogyne spp., although the resistance is often not complete, and the nematodes may form a few galls or eggs. A potentially useful source of resistance occurs in Solanum sisymbriifolium Lamarck.

Solanum sisymbriifolium, originates from warm, temperate South America, is an annual or perennial erect, rhizomatous, shrubby weed with an extensive root system and spiny leaves, currently distributed throughout the world and invasive in some countries. Interest in the study of this plant has increased since it was proved to be a good trap crop against potato cyst nematodes (PCN), Globodera spp. It was introduced in The Netherlands following research that identified the species as the most suitable candidate among a diversity of species tested as potential trap crops. Several studies have been done with this plant and it has proved to have effects on hatching, mortality, infectivity and/or reproduction in several nematode species, including PCN, RKN and root-lesion nematodes (RLN). The effects of S. sisymbriifolium on nematodes depend on the cultivars used, the genus and species of nematode present and on biotic and abiotic conditions.

In Portugal, S. sisymbriifolium is not part of the native flora, but other plants of the same genus, for instance Solanum lycopersicum cv. Coração de boi, are present in the South of Portugal. It is an invasive species of plant, spiny, with an extensive root system, and is probably native from Southern Africa although it is a common weed in North Africa and Southern Europe.

Solanum sisymbriifolium is also known to be a source of resistance, or partial resistance, to some diseases and plant pests, including fungi, bacteria, nematodes and insects. Solanum lycopersicum has been much less studied but has potential as a source of resistance to some fungi and viruses.
Index in the Bridge & Page (1980) rating chart was 0, because no egg masses developed on the roots. Observations of nematodes inside the roots were J2 (69%); of the remainder, 17% were adults, of which 63% were males (Fig. 3).

Some nematodes (total numbers values between 4 and 117 per plant) (Supplementary Table S2). The majority of the infestation in the Bridge & Page (1980) rating chart, obtained for S. lycopersicum cv. Coração de boi.

Table 1. Resistance degree (RD) of Solanum linnaeanum, S. sisybriifolium cv. Sis 6001 and respective controls (tomato and potato), 70 days after inoculation with 5000 eggs of Meloidogyne chitwoodi per plant (averages of ten replicates). GI (Gall Index) on a scale of 0–5; RF (Reproduction Factor) = Pf/Pi where Pi = initial population and Pf = final population; Resistance degree (RD): R = resistant (GI ≤ 2 and RF ≤ 1); S = Susceptible (GI > 2 and RF > 1); *Controls. Data from five plants per treatment of two independent experiments (n = 10) were submitted to ANOVA, and comparison of means by LSD test (P < 0.05) was carried out for GI and RF. Values with the same symbol are not significantly different.

The main goals of the present study were: to evaluate the potential value of two wild Solanum plants, S. linnaeanum and S. sisybriifolium, in controlling agricultural pests such as RKN; to assist in the development of sustainable and ecofriendly management methods of key enemies in agriculture, in order to reduce dependence on chemical pesticides; and to improve crop productivity. The resistance shown by S. linnaeanum and S. sisybriifolium cv. Sis 6001 was evaluated against M. chitwoodi. Although some studies have been made with S. sisybriifolium and PPN and it is already used as a trap crop in some places, not much is known about S. linnaeanum and its effects on nematodes. New sources of resistance may result in the development of sustainable and nonchemical management strategies to protect crops against PPN. This is the first report of S. linnaeanum being used in Portugal for the management of PPN.

Results

The environmental conditions used, and the period of the experiments, proved to be appropriate and sufficient for the development and reproduction of the nematodes. The results showed that there was no reproduction of M. chitwoodi in S. linnaeanum and that there was only a little reproduction in S. sisybriifolium cv. Sis 6001, when compared with the reproduction in the susceptible plants used as controls, tomato cv. Coração de boi and potato cv. Désirée (Fig. 1 and Supplementary Fig. S1).

Soil sterility was confirmed by the absence of galls and egg masses in uninoculated plants. The values of GI = 5 and RF > 1 (Table 1) obtained in the controls, tomato and potato, confirmed the viability of the inocula and that environmental conditions were favorable for penetration, development and reproduction of M. chitwoodi.

The same was verified by the index in the Bridge & Page (1980) rating chart, which varied between 4 in tomato plants, 40% roots infested with larger knots but main roots clean, and 5 in potato plants, 50% roots infested with knotting on parts of main roots. All inoculated plants presented symptoms of yellowing, wilting and leaf drop, related to the presence of nematodes.

Solanum linnaeanum was considered resistant to M. chitwoodi (Table 1 and Supplementary Table S1). The index in the Bridge & Page (1980) rating chart was 0, because no egg masses developed on the roots. Observation of the stained root systems showed the presence of many small root swellings (average of the 10 replicates was 519 small root swellings/plant) and nematodes inside the roots (total numbers values between 329 and 883 per plant) (Supplementary Table S2). Almost all the nematodes inside the roots were second-stage juveniles (J2) (44%) or adults (45%) and all the adults were males (100%) (Fig. 2).

The values of GI = 0 and RF = 0 (Table 1 and Supplementary Table S1), and the index 0 in the Bridge & Page (1980) rating chart, obtained for S. sisybriifolium cv. Sis 6001 confirmed its resistance to M. chitwoodi, and are consistent with the results obtained by Dias et al. (2012) for other S. sisybriifolium cultivars. However, when plants were analyzed separately some variability could be observed between them. Only two replicates (in a total of 10 replicates, 5 per assay) showed the presence of galls, one with one gall and without egg masses and another with 11 galls with 9 egg masses containing 187 eggs in total (Supplementary Table S1). Despite this, there are no statistically significant differences between numbers of galls, egg masses, eggs and RF between S. linnaeanum and S. sisybriifolium cv. Sis 6001. Observation of stained roots of S. sisybriifolium cv. Sis 6001 showed some nematodes (total numbers values between 4 and 117 per plant) (Supplementary Table S2). The majority of nematodes inside the roots were J1 (69%); of the remainder, 17% were adults, of which 63% were males (Fig. 3).

Discussion

The choice of the classification system of Sasser et al. (1984) and the rating chart of Bridge & Page (1980) for the resistance studies of S. linnaeanum and S. sisybriifolium cv. Sis 6001 to M. chitwoodi was because they can be applied to any crop and they also allow results from different studies to be compared without the need to include the susceptible controls.

The time that any Meloidogyne species needs to complete a generation depends on external factors, such as temperature, humidity, light and the quality and condition of the host plant in relation to its age and nutritional status. All of these factors are crucial as they determine how and when the effects of nematodes on the plant will be most visible. In our laboratory, it was observed that M. chitwoodi had a slower rate of development than other Meloidogyne isolates. Thus, the plants were uprooted 70 days after inoculation (DAI) instead of the usual
Figure 2. Number of nematodes in different development stages, J$_2$, J$_4$ and adults (all males), on *Solanum linnaeanum* root systems. The percentages are averages of the two assays (5 replicates/assay).

Figure 3. Number of nematodes in different development stages, J$_2$, J$_4$ and adults (males and females), on *Solanum sisymbriifolium* cv. Sis 6001 root systems. The percentages are averages of the two assays (5 replicates/assay).
60 DAI. Any differences found between species or replicates can only be attributed to characteristics inherent to the plants as the assay conditions were always the same.

The control of the population density of PPN in the soil is best achieved by resistance in the plant, characterized as the ability of a plant species to inhibit nematode development or reproduction38. So far, the capacity of J2 penetration into the roots has not been considered as a mechanism for characterization of the resistance. Generally, resistance to nematodes in plants becomes obvious after J2 penetration into the roots (i.e. post-infection)39. The J2 that penetrate the roots of resistant species may die or leave the roots, but they can also develop to the adult stage, as females without egg production (or the eggs produced are not viable) or as males40. Various procedures for determining the resistance of plants to nematodes are used. The most common procedures are the percentage or number of galls or egg masses formed as well as the RF (Pi/Pi) values37. The low numbers of nematodes that penetrated the roots of S. linnaeum and S. sisymbriifolium cv. Sis 6001 are probably due to substances produced by the roots of these plants, which prevented their penetration. Conceição and collaborators (2012) analyzed the effects of S. sisymbriifolium (cvs. Domino, Pion, Sharp and Sis 4004) exudates in five Meloidogyne isolates (M. arenaria, M. chitwoodi, M. hapla, M. hispanica and M. javanica) and showed the complexity and variability of the interactions between RKN and S. sisymbriifolium41. In this study, the cultivars studied demonstrated low or no inhibitory effect on M. chitwoodi hatching. The presence of resistance in some S. sisymbriifolium cultivars to PPN, has already been demonstrated for G. pallida, G. rostochiensis, M. chitwoodi and M. javanica17,24. This was confirmed by Hajihassani et al. (2020) for other major species of RKN (M. arenaria, M. haplanaria and M. incognita), although the effectiveness of resistance to M. arenaria varied a lot between S. sisymbriifolium cultivars41.

In S. linnaeum, in the present work, there was a higher nematode penetration of the roots than in S. sisymbriifolium cv. Sis 6001, but there was no reproduction in any of the plants, and all nematodes that developed into adults were males. This reaction is a form of active resistance (i.e. post-infection) in which, although the J2

The plant species we have considered are resistant, or partially resistant, to some diseases and plant pests. Solanum linnaeum exhibits resistance to Verticillium wilt and Liu and collaborators (2015) succeeded in the transference of this resistance to eggplants through the introgression of the disease resistance gene44. In the same way, if the S. linnaeum and S. sisymbriifolium resistance genes are isolated, they can be used to confer resistance to plants susceptible to M. chitwoodi, such as tomato and potato plants. They have a potential to be used as sources for resistance to M. chitwoodi in breeding programs.

In this study, it was demonstrated that S. linnaeum can reduce RKN densities in the soil. Its roots attract M. chitwoodi juveniles, removing them from the soil but at the same time reducing the reproduction of the nematodes. The fact that the nematodes developed into males, without females, avoids the risk of reproduction or leaving a dangerous, viable population of nematodes in the soil. Since the number of J2 that hatch and penetrate the roots is high, the population density of nematodes in the soil decreases and even those that hatch but do not enter the roots will die. In that way, the use of S. linnaeum as a trap crop, in a crop rotation system, or even as rootstocks, may be an appropriate component of Integrated Pest Management, keeping RKN populations at levels low enough not to cause economic losses, thereby increasing production and crop quality and avoiding or limiting the use of synthetic nematicides. Using a trap crop does not disturb the ecological balance in the soil and

www.nature.com/scientificreports/
the extensive and deep root systems of *S. linnaeanum* grow deeper into the soil than nematicides can penetrate. *Solanum linnaeanum* could be a good alternative to other PPN control measures but more studies are required, including field studies. The crop should be completely removed before flowering or incorporated into the soil as green manure before seeds are produced, preventing it from becoming invasive in areas where it does not yet exist. Also, different conditions should be tested to investigate whether the resistance of the plants is stable at different temperatures. There are no known uses for this plant in Portugal. In this way, it may be possible to find a use for a plant that, despite having existed in Portugal for many years, was only ever considered a weed. In this study, resistance to *M. chitwoodi* in another cultivar of *S. sisymbriifolium* (cv. Sis 6001) was also confirmed.

**Methods**

**Nematode isolates.** *Meloidogyne chitwoodi* was chosen because it is a problem for potato crops that has already been detected in Portugal and is considered an A2 quarantine pest by EPPO. An isolate of *M. chitwoodi* from the NEMATO-lab of the University of Coimbra, obtained from a potato field in Porto, Portugal, was maintained and multiplied on susceptible tomato plants, *S. lycopersicum* cv. Coração de boi, inoculated with 15 egg masses/plant. The plants were grown in pots filled with 500 g of steam-sterilized soil mix (sand:soil:peat 1:1:1 v/v) and kept in a glasshouse (20–25 °C, 70–75% relative humidity and 12 h photoperiod). Ninety DAI, the plants were uprooted and nematode eggs were extracted with 0.52% sodium hypochlorite (NaOCl) solution, according to Hussey & Barker (1973). This culture of *M. chitwoodi* is a pure isolate started initially from a single egg mass; its identification was confirmed by esterase phenotype analysis at the beginning and at the end of each assay.

**Plant materials.** *Solanum lycopersicum* cv. Coração de boi (tomato), *S. linnaeanum* and *S. sisymbriifolium* cv. Sis 6001 were grown from seeds. Our stock of *S. linnaeanum* is a wild isolate whose seeds were harvested from a plant growing on the roadside in the Algarve. The seeds were germinated at 25–27 °C on moist filter paper in Petri dishes and transplanted singly into 5 cm diameter plastic pots containing 60 cm³ of a steam-sterilized mixture of loam soil and sand (1:2 v/v). *Solanum sisymbriifolium* seeds were germinated in a glasshouse in polystyrene plates containing sterile peat. Fifteen days after germination the seedlings of this species were transplanted singly into pots filled with 300 g of steam-sterilized soil mix (sand:soil:peat 1:1:1 v/v). The potato plants, *S. tuberosum* sp. *tuberosum* L. (cv. Désirée), were obtained from pieces of potato tubers with sprouts in pots with the same mixture of soil (sand:soil:peat 1:1:1 v/v). All plants were kept in a glasshouse under the same conditions as described above.

**Pathogenicity tests.** Five four-weeks-old plants from each species were inoculated with 5000 *M. chitwoodi* eggs (initial population density, Pi). To confirm the soil sterility one plant of each species was potted in the sterilized soil mix and not inoculated. Five susceptible tomato plants cv. Désirée were also inoculated to confirm the viability of the inocula. Pots were kept in the conditions already mentioned and the plants were watered daily. Seventy DAI the plants were uprooted and the root systems washed. The root systems were stained with phloxine B (0.0015% solution) for 15 min, and galls and eggs masses were counted. Eggs were extracted as described above and counted to determine the final population (PF). Resistance rating of the species were based on Gall Index (GI) and Reproduction Factor (RF = Pf / Pi), according to the modified quantitative scheme of Canto-Sáenz (1985). The evaluation of the degree of resistance of the plants was based on GI and RF.

Roots that had less than 100 egg masses were stained with acid fuchsin, and the numbers of the different developmental stages of *M. chitwoodi* were recorded. Resistance ratings of these plants were based on the RKN rating chart of Bridge & Page (1980). This scale is based on the percentage and types of roots galled from 0 (0%, no galls) to 10 (100% galled). The roots were observed using a routine stereo microscope Leica M80, at a magnification of 60 x, and the nematodes found were transferred onto a glass slide and observed with an optical microscope Leica DM2500, at a magnification of 400 x. The identification of the different developmental stages was done by comparing their morphological characteristics with those described for *M. incognita* (Supplementary Fig. S2).

The assay was done twice, using the same conditions each time.

**Statistical analysis.** The data (values obtained for galls, egg masses and eggs counts and RF), were confirmed to meet the statistical assumptions of normality and homogeneity of variances (one way ANOVA), and were submitted to analysis of variance and the means compared by LSD (P < 0.05) using Statistic 10 software (Statsoft Inc.).
5. Baidya, S., Timila, R. D., Kc, R. B., Manandhar, H. K. & Manandhar, C. Management of root-knot nematode on tomato through

8. Hussey, R. S. & Janssen, G. J. W. Root-knot nematodes:

11. Wesemael, W. M. L., Viaene, N. & Moens, M. Root-knot nematodes (Meloidogyne spp.) in Europe. Nematology 13(1), 3–16. https://doi.org/10.1111/j.1744-7348.2011.01472.x (2011).

14. Ingham, R. E., Hamm, P. B., Williams, R. E. & Swanson, W. H. Control of

19. Sasaki-Crawley, A. et al. Characterization of resistance to major root-knot nematodes (Meloidogyne spp.) in Solanum sisymbriifolium. Phytopathology 110(3), 666–673. https://doi.org/10.1094/PHYTO-10-19-0393-R (2020).

15. Schomaker, C. H. & Been, T. H. A model for infestation foci of potato cyst nematodes (Globodera rostochiensis and Globodera pallida). Phytopathology 89(7), 583–590. https://doi.org/10.1094/PHYTO.1999.89.7.583 (1999).

19. Sasaki-Crawley, A. et al. Characterization of resistance to major root-knot nematodes (Meloidogyne spp.) in Solanum sisymbriifolium. Phytopathology 110(3), 666–673. https://doi.org/10.1094/PHYTO-10-19-0393-R (2020).

21. Ali, M., Matsuzoe, N., Okubo, H. & Fujieda, K. Resistance of non-tuberous Solanum to root-knot nematode. J. Jpn. Soc. Hortic. Sci. 60(4), 921–926 (1992).

24. Scholte, K. Screening of non-tuber bearing Solanaceae for resistance to and induction of juvenile hatch of potato cyst nematodes (Globodera rostochiensis and Globodera pallida). In Potato cyst nematodes, biology, distribution and control (eds Marks, R. J. & Brodie, B. J.), 135–152 (Wallingford, CAB International, 1998).

28. Roberts, P. A. & Stone, A. R. Comparisons of invasion and development of Globodera spp. and European potato cyst-nematode pathotypes in roots of resistant Solanum g. Leptostemumon spp. Nematologica 29, 95–108. https://doi.org/10.1163/187529283X00221 (1983).

35. Nefzi, A. et al. Characterization of resistance to major root-knot nematodes (Meloidogyne spp.) in Solanum sisymbriifolium. Phytopathology 110(3), 666–673. https://doi.org/10.1094/PHYTO-10-19-0393-R (2020).

38. Roberts, P. A. & Stone, A. R. Comparisons of invasion and development of Globodera spp. and European potato cyst-nematode pathotypes in roots of resistant Solanum g. Leptostemumon spp. Nematologica 29, 95–108. https://doi.org/10.1163/187529283X00221 (1983).

42. Conceição, I. L., Dias, A. M. C., Abrantes, I. & Cunha, M. J. M. Efeitos dos exsudatos radiculares de Solanum sisymbriifolium na resistência de solos à infestação de Pratylenchusgoodeyi. J. Nematol. 47(4), 238–253. https://doi.org/10.1111/j.1744-7348.2012.00035.x (2016).

43. Costa, M. V. C. & Fassuliotis, G. Efeito do exsudato radicular de Solanum sisymbriifolium sobre o nemátode-das-lesões-radiculares, Pratylenchusgoodeyi, parasita da bananeira. Rev. Ciênc. Agrar. 32(2), 173–181 (2008).

46. de Guiran, G. & Ritter, M. Life cycle of Meloidogyne species and factors influencing their development. In Root-knot nematodes (Meloidogyne species) systematic, biology and control (eds Lamberti, F. & Taylor, C. E.), 173–191 (Academic Press Inc, London, 1979).

50. Nelli, A. et al. Management of Fusarium crown and root rot of tomato by Solanum linnaeanum (Lam.) to Verticillium wilt. Plant Cell Tissue Organ Cult. 65(2), 123–129. https://doi.org/10.1007/s11240-004-0027-7 (2004).

52. Sasser, J. N. & Scott, J. E. Resistance of Solanum sisymbriifolium to and induction of juvenile hatch of potato cyst nematodes. Nematropica 36(4), 334–374. https://doi.org/10.1111/j.1744-7348.2012.00120.x (2013).

55. Evans, M. J., Janssen, G. J. W. & Rice, R. S. Root-knot nematodes: Management of root-knot nematode in organic horticulture in plastic greenhouse. Front. Plant Sci. 7, 1–15. https://doi.org/10.3389/fpls.2016.00164 (2016).

68. Minnis, S. et al. Potato cyst nematodes in England and Wales - occurrence and distribution. Ann. Appl. Biol. 140(2), 187–195. https://doi.org/10.1111/j.1744-7348.2002.tb01172.x (2002).

71. Campos, H. D., Campos, V. P. & Coimbra, J. L. Efeito do exsudato radicular de Brachiaria decumbens e do sorgoleone de Sorghum bicolor no desenvolvimento de Meloidogyne javanica. Nematol. Bras. 30(1), 59–65 (2006).

74. Baidya, S., Timila, R. D., Kc, R. B., Manandhar, H. K. & Manandhar, C. Management of root-knot nematode on tomato through grafting root stock of Solanum sisymbriifolium. J. Nepal. J. Sci. Technol. Ser. B. 3(2), 141–144. https://doi.org/10.3126/njstb.v3i2.5450 (2012).
Perpétuo was funded by FCT/ "Ministério da Ciência, Tecnologia e Ensino Superior" (MCTES) and the European Social Fund through the "Programa Operacional do Capital Humano" (POCH) of the National Strategic Reference Framework (SFRH/BD/129184/2017). Thanks, are also due to the Botanical Garden of the University of Trás-os-Montes and Alto Douro, Bragança, Portugal and to Vandinter Semo, Scheemda, The Netherlands, for supplying Solanum linnaceum and S. sisymbriifolium seeds, respectively, and to Prof. Óscar Machado and Dr. Filipe Melo, from High School of Agriculture of Coimbra, for multiplying S. linnaceum seeds and helping with plants maintenance. The authors would like to thank Dr. Kenneth Evans, retired researcher from Rothamsted Research, UK, for valuable comments and suggestions and his review of the final manuscript.

Acknowledgements
This work was supported by Centre for Functional Ecology—Science for People and the Planet (CFE), "Instituto do Ambiente, Tecnologia e Vida" (IATV), the European Regional Development Fund (FEDER) through the COMPETE 2020—“Programa Operacional Competitividade e Internacionalização” (POCI) and by National funds through "Fundaçao para a Ciência e Tecnologia" (FCT), under contracts UID/BI/04004/2020, POCI-01-0145-FEDER-029283 (PTDC/ASP-PLA/29283/2017) (Ref. HANDLER) and Project ReNATURE—Valorization of the Natural Endogenous Resources of the Centro Region (Centro2020, Centro-01-0145-FEDER-000007). Soraia Ferpêtu was funded by FCT/ “Ministério da Ciência, Tecnologia e Ensino Superior” (MCTES) and the European Social Fund through the “Programa Operacional do Capital Humano” (POCH) of the National Strategic Reference Framework (SFRH/BD/129184/2017). Thanks, are also due to the Botanical Garden of the University of Trás-os-Montes and Alto Douro, Bragança, Portugal and to Vandinter Semo, Scheemda, The Netherlands, for supplying Solanum linnaceum and S. sisymbriifolium seeds, respectively, and to Prof. Óscar Machado and Dr. Filipe Melo, from High School of Agriculture of Coimbra, for multiplying S. linnaceum seeds and helping with plants maintenance. The authors would like to thank Dr. Kenneth Evans, retired researcher from Rothamsted Research, UK, for valuable comments and suggestions and his review of the final manuscript.

Author contributions
Conceived and designed the experiments: L.S.P., M.J.M.C., M.T.B. and I.L.C. Analyzed the data: L.S.P. and I.L.C. Wrote the paper: L.S.P. All authors reviewed and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Additional information

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2021