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Perspective

Lotus spp.: a Mediterranean genus with high environment and economic impact in the Salado River Basin (Argentina)

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Abstract
This review has the principal objective in to hypothesise that the introduction of *Lotus* species could have significant benefits in constrained soils due their worldwide distribution. This idea is major based on results obtained in the lowlands of the most important livestock breeding area in Argentina denominated Salado River Basin (also called "Flooding Pampas"). Mostly of their land surface is dominated by salt-affected soils with severe constraints for traditional crop cultivation (i.e., maize, soybean, etc.). In order to increase their economic importance, farmers have utilized species such as non-native *L. tenuis* (ex- *Lotus glaber*), originating from European Mediterranean area, which shows a successfully and fast naturalization (in less than 60 years) in constrained areas improving forage performance. The increase in soil quality associated to this legume is achieved by an increment of the organic matter content and improvement of fertility and physicochemical parameters. Moreover, other studies have evidenced some genetic determinants associated with interesting agronomic traits such as plant tolerance to environmental stresses and the importance of leaves condensed tannins concentrations. This revision has many topics including a brief analysis of economic and environmental changes that occur under *Lotus* species implantation. In addition, we incorporate references recently published concerning the evaluation of the biochemical and physiological mechanisms involved in their adaptation to strong abiotic stresses characteristic of the region, the soil and plant microbiota diversity and soil physical and chemical characteristics associated to the presence of *Lotus* genotypes.

Keywords *Lotus* spp. · Global climate change · Marginal soils reclamation · Flooding lowlands · Sustainability of the livestock environment

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1 Introduction

The genus *Lotus* is included in the *Fabaceae* family, characterized for a high biological diversity (720 genera and more than 18,000 species) [21, 29, 31]. These legumes, are also recognized for stablishing benefic associations by root symbiosis with mycorrhizal fungi [33, 35, 41] and nitrogen-fixing bacteria [27, 34, 35]. These associations turn them more competitive and some of these species constitute "pioneer" plants in constrained soil environments. For these reasons, became an important "key-role" in the sustainable agricultural systems and in the improving of marginal soil ecosystems. Within the family, the tribe Loteae DC is a monophyletic group composed by four genera. Recently, modern molecular tools have significantly contributed to restrict the genus to 100–130 species. Most *Lotus* species are native to Mediterranean Europe, Asia, Africa and Australia and few ones from the Atlantic and Pacific Ocean Islands [23, 29, 31]. Mediterranean area is recognized for their richness in species and environments where the *Lotus* species constitute and important factor in the sustainability of the ecosystems [20]. In contrast, only few species were in 90's described originally in the Americas, but modern taxonomy determined that these species were not native. *Lotus* species have a worldwide distribution, except in very cold regions and certain tropical areas of Southeast Asia and Central America. In the Cone Sur of Americas there are species naturalized successfully specially in Argentina, Chile, Uruguay and Brazil [1, 21, 24, 29, 31]. Their advantages in adaptability and good implantation, determine that actually are important elements in the forage supply [36] and in soil environments remediation, including european soils [44]. Also, there are intends to contribute to the sustainability of them in South America through the development of better adapted genotypes of *Lotus corniculatus* L., *L. tenuis* Waldst. et Kit. (Syn. *L. glaber* Mill.), *L. uliginosus* Schkuhr (Syn. *L. pedunculatus* Cav.) and *L. subbiflorus* Lag. Otherwise, the autogamous specie *Lotus japonicus* (Regel) permit a model to design and understand tolerance mechanism useful for breeding of forage species [7, 29, 31]. Also, increasing the selection of microorganism to optimum N fixation improving legume nutrition and quality and soils sustainability [5, 13, 36].

These technological objectives are very important for the Salado River Basin in Argentina. This region is located in the center of the Flooding Pampa, a vast area located in the Buenos Aires province (Fig. 1).

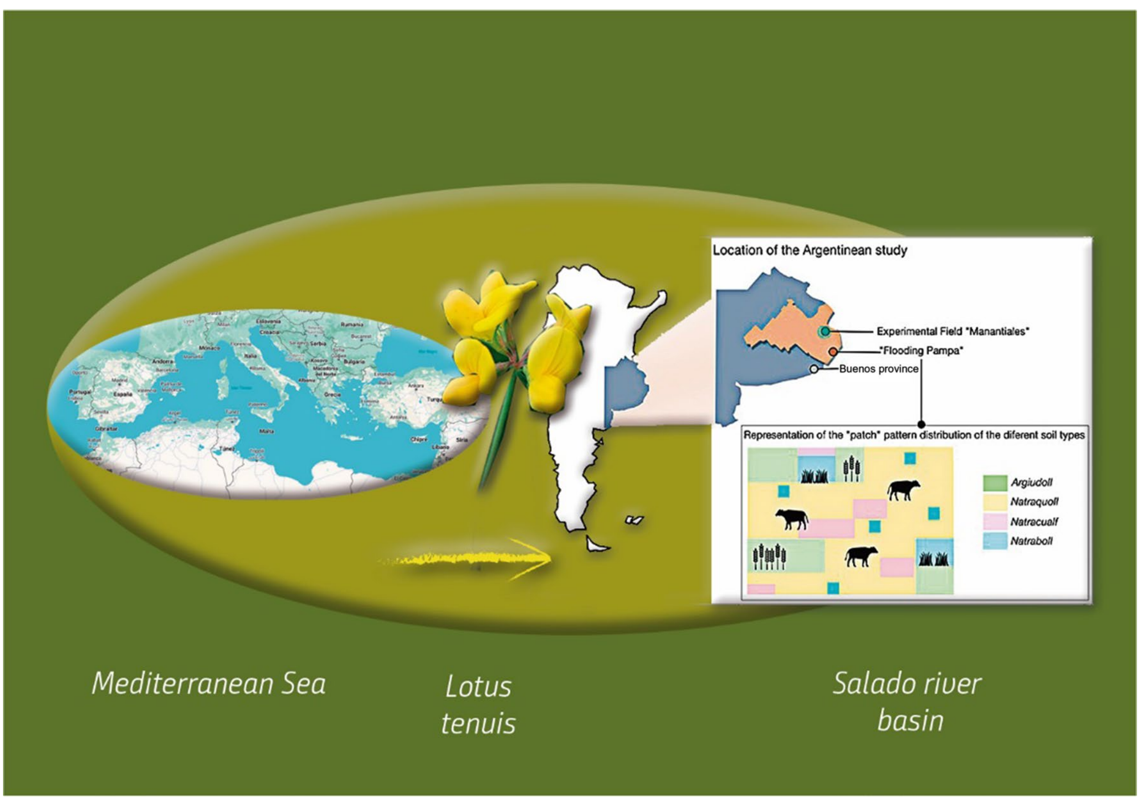

Fig. 1 Location of the *Lotus tenuis* naturalised in Argentina

It is a very flat region, constantly affected by periods of flood/drought. The economic development of this region has lagged due the existence of thousands of hectares of poor-quality salt-affected soils (approximately 60%). The distribution of natural grasslands in the Salado River Basin is also determined by alkalinity levels and flooding frequency. The grassland community growing on sodic soils is composed by *Distichlis scoparia*, *D. spicata*, *Paspalum vaginatum*, *Sporobolus pyramidatus* (all of them with low forage value) and *Nostoc* [1, 2].

The agricultural practices of the Salado River Basin have suffered significant changes [40]. The advance of agriculture over the grazing pastures, has limited the livestock to marginal areas on sodic and flooded soils with native species and low forage performance. Many attempts to replace grassland communities by exotic species have been made with relative success.

In this situation, the naturally implanted European legume *L. tenuis* increase significantly its abundance and became the only legume with forage supply significance [5, 13, 35, 36].

The tolerance of this species to alkalinity and flooding has allowed a wide and fast spread over in constrained soils in less than 60 years. *L. tenuis* can also grow under low extractable phosphorus (P) concentrations in association with mycorrhiza and Plant Growth Promoting Rizobacterias (PGPR´s) [11, 12, 14–16, 18, 19, 30].

In summary, the introduction of *L. tenuis* represents a substantial benefit for the regional soil environment due these areas have no abundant native legumes and generating not only an increase in forage supply [1, 5, 13, 34] but also a significant contribution to the C and N soil nutrition [6].

Another important aspect of this review, is the opportunity to disseminate a working methodology of scientific value oriented to solve problems and gaps of impact knowledge on big areas considered marginal or in degraded and contaminated soils where legumes of *Lotus* gender were native (Asia and Europe) or naturalised (America).

2 Materials and methods

The research on *Lotus* spp. in Argentina has implemented several approaches accordingly to the demands of the agricultural sector. The combination of basic and applied research has allowed the holistic description of the agro-ecosystem and the further development of technologies to improve the pasture systems and the conservation of natural resources [1, 36, 40]. The incorporation of *L. tenuis* in the Flooding Pampa has evidenced a clear event of naturalization of the legume. This fact has motivated the research by cooperation between research institutions and governmental agencies. The first stage of studies was focused on increase the knowledge of the promising forage source and its further development as a new key specie in the area [1, 5, 40]. With this objective, biochemical (levels of sugars, proteins and hormones in plants), physiological (photosynthetic parameters) and molecular (transcriptomes, metabolomes and proteomes) approaches were used [2, 4, 8–10, 23, 26, 29, 32–39]. In addition, numerous symbiotic microorganisms of forage and models' legumes of the gender *Lotus* were isolated, selected and identified and subsequently evaluated in their capacity to promote their growth or mitigate the situations of abiotic stresses that characterize the soils [9–12, 19, 30, 33, 41, 43, 45]. The use of a sort of *Lotus* species allowed the enhancement of exploratory studies and available tools and the use of the model legume *L. japonicus*, facilitating research into the stress tolerance attributes of the *Lotus* genus [4, 14–16, 18]. Additionally, the study of *L. tenuis* and *L. corniculatus* meant a challenge in the search for forage capable in restricted environment. The major evaluations on forage yields and efficiency of protocols for cow management avoiding overgrazing were performed in the experimental field of "Chacra Experimental Manantiales" (S35º 45` 01``, W 58º 02´ 22´´) located in Chascomús, Argentina [1, 5, 13, 17, 27, 29, 30, 33, 36, 40–42]. The information obtained from laboratory and field experiments was used to improve the agricultural performance of *L. tenuis* in restricted soil environments. Field experiments achieved during the 2010–2023 period were conducted based on fertilizers and promotion through herbicides [28, 34, 35]. The incorporation of *L. tenuis* as part of the agro-ecosystem requested the study of soil traits, including chemical and physical properties. The characterization of organic matter content, macro and micronutrients, pH, electric conductivity and infiltration rate, among others, were tested after a period of 7 years of implantation and promotion [1, 34, 35, 40].

The honey production in this improved ecosystem is a complementary and incremental economic activity (Fig. 2), and constitutes an extended criterion of agroecology and sustainability of the region, which is reflected by significantly impacting the presence and diversity of pollinators. Moreover, a significant reduction on seed harvest performance was evidenced when the interaction between bees and *Lotus* spp flowers was interrupted through an anti-aphid net. These results confirm the allogamy of the forage species of the gender *Lotus* (Unpublished results).

L. japonicus ecotype Gifu collected on a river bank in the Gifu prefecture on main-land Japan has been used for many experiments in our labs. Their behaviour on biotic [8] and abiotic stress conditions [2, 4, 9, 10, 12] were analysed. The

Fig. 2 Production of "*Lotus* honey" in experimental fields where *L. tenuis* becomes the predominant species

Gifu ecotype was the appropriated model legume for flooding, chilling, salinity and alkalinity stress as well as bacterial and fungal interactions assays by applying molecular biology approaches such as gene expression, microarray, RNASeq, proteomics, metabolomics and plant physiology indicators such as net photosynthesis, chlorophyll fluorescence, proline content and reactive oxygen species (ROS) [3, 4, 9, 10, 12, 29, 32, 36, 39, 46]. In some evaluations, also we utilised the *L. japonicus* MG-20 (Miyakojima) originates from one of the southern islands in Japan. [4, 9, 10, 32, 33, 46]. It is used for genome sequencing [29]. In total 91 ecotypes of *L. japonicus* can be obtained from LegumeBase (https://legumebase.nbrp.jp/legumebase/index.jsp?language=en). In addition, some labs used others *Lotus* models species as *L. filicaulis* (originated from Algeria), *L. burtii* (originated from Pakistan) to evaluated abiotic stresses [29, 32, 46] and to obtain inter-specific hybrids used to mapped genomic analysis in crops and models *Lotus* species [29].

The condensed tannins (CT) accumulation in leaves of *L. corniculatus* was evaluated as an interesting attribute to develop biotechnological tools [21–24]. The combination of the concentration of CT and the stress tolerance of *L. tenuis* has displayed the design of an interspecific hybrid to reinforce the quality of the naturalized legume in the Salado River lowlands. This new plant material was also evaluated under flooding and salinity conditions [3, 25] and its interaction with PGPR bacteria [11]. (Fig. 3).

As a legume, the interactions between *Lotus spp*. and *Rhizobiaceae* bacteria were evaluated [1, 13, 16, 18, 27, 30, 34, 40, 41, 44, 45]. In similar way, the beneficial fungal interactions with mycorrhizal fungi and P solubilizing bacteria were registered [11, 12, 14–19, 28, 29, 42, 43]. In both cases, the description of root microbial partners was accompanied of the holistic analysis of soil bacterial and fungal communities [34, 35].

3 Results

The introduction of *Lotus* in the constrained environments has involved significant modifications in the Flooding Pampa ecosystem. The implementation of agricultural technology, like fertilizers and herbicides-mediated promotion improved the implantation and yields of *L. tenuis* [1, 5, 34–36]. These practices have facilitated the naturalization of the legume in the area. Further evaluations have determined the improvement on the yield and quality of beef production, including cow management avoiding overgrazing, calf early weaning and meet composition [40]. This tendency has motivated the studies regarding the cattle component of the ecosystem [13]. The results obtained took a step forward into the analysis of nutritional effects on ruminal attributes [2]. This investigation imposes the focus on the ruminal gas production and microbiome composition [1, 2, 40].

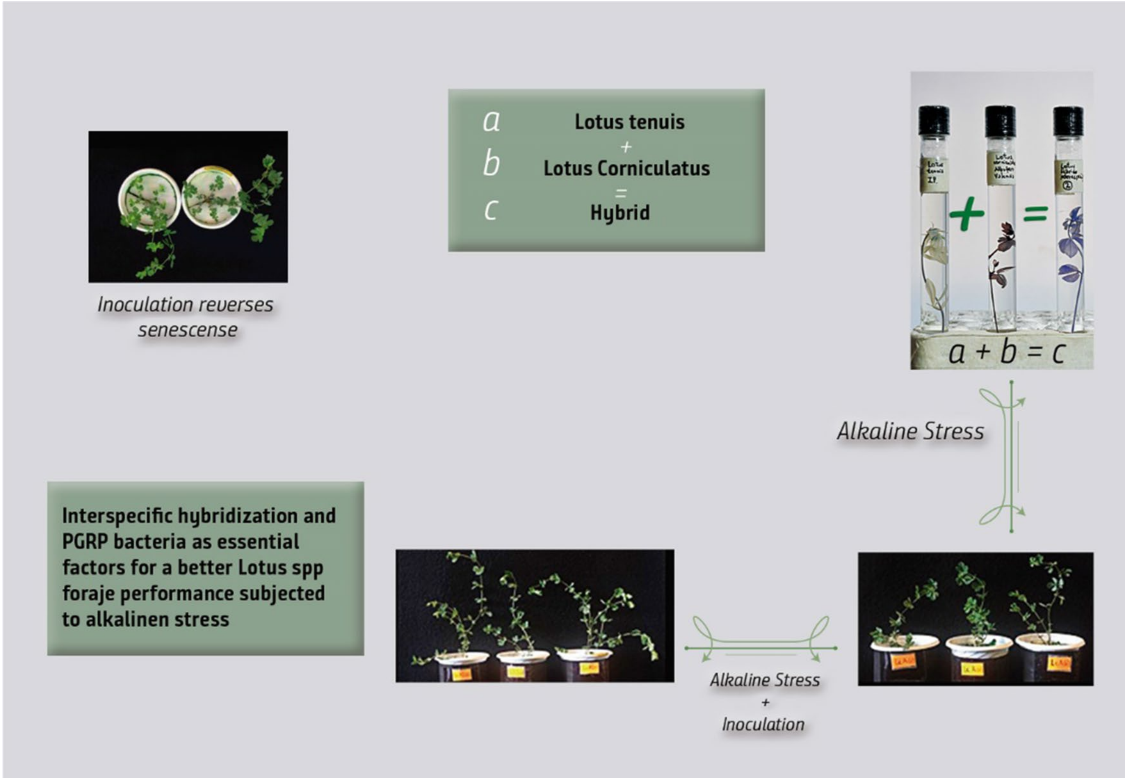

Fig. 3 Interspecific hybridization and the interaction with plant growth-promoting microorganisms (PGPRs) as tools to improve the forage performance of *Lotus* species (Adapted from a personal Graphical Abstract used for divulgation)

The research about condensed tannins (CT) of *Lotus* had a significant value for the animal health [22]. The hybrid (named "Albufera") meant a good example of transference of science to the technological and agricultural area [22]. The performance of this new source displayed significant tolerance to flooding and salinity stress, combining the benefits of tolerance of *L. tenuis* and the nutritional benefits of CT [3, 23, 25].

The introduction of *L. tenuis* has improved the quality of pasture sources as well as the characteristics of soil attributes. In this trend the research can be displayed into variables related to soil traits, such as physicochemical properties and soil microbial interactions. The conversion of the pasture systems from a native grass community mainly represented by limited depth roots, has improved the soil physical properties such as infiltration rate. This effect was also significant for some soil bacteria genera such as *Soilbacteria* and *Ktedonobacteria* [34]. The well know rhizobia and mycorrhizal interactions indicated the particular phylogeny, diversity and functional roles that are particular of the *L. tenuis* systems [1, 37–40]. The *Mesorhizobium* were the most frequent nitrogen-fixer bacteria in the "*Lotus* ecosystems" [13, 27, 41], while *Rhizophagus intraradices* (ex. *Glomus*) were the mycorrhiza most frequent detected in *Lotus* roots [35, 36, 42]. In spite of the presence of these symbiotic microorganisms, analysis of soil microbial ecology in the Salado River Basin indicated impairment of mycorrhiza spores [17] and culturable pseudomonads [28] as consequence of the herbicide-mediated promotion. Nevertheless, the metagenomics analysis revealed global soil bacterial community did not change its diversity, while the global fungal diversity increases with the *Lotus* presence [34–36, 40]. These studies including differential interactions between *Lotus* species and *Fusarium* sp. [33]. Its relevance in ecosystem plants biodiversity is actually under evaluation.

The study of the model *L. japonicus* allowed the deep investigation on specific issues regarding the biochemical and molecular mechanisms associated to the abiotic stress tolerance [3, 4, 9, 10, 12, 29, 40]. Other results obtained revealed the accumulation of polyamines, as key molecules involved in the response of *Lotus* spp. to the abiotic stress [26]. Additionally, photosynthetic traits revealed the reactive oxygen species (ROS) accumulation in chloroplast, PSII damage and alterations in the net gas exchange [9, 10]. Regarding biotic stress, the pathogenic strain *Pseudomonas* was analyzed by using molecular biology approaches *in L. japonicus*. This research was conducted on the model *L. japonicus* because was not reported yet a pathogen of *L. tenuis* isolated from the flooding pampa environment, with significant relevance to the health of naturalized *Lotus* crops species [8].

4 Discussion and conclusion

The concentration of research resources and scientific capacities in areas and countries with environments with productive limitations, is an imperative necessity for the next years looking to mitigate the disorders provoked by the global climatic change. The *Lotus* gender could be an opportunity to generate technological innovations to transform marginal areas into new productive and sustainable resources for the increasing global food demand. This review includes results of assays obtained in the project entitled *LOTASSA: bridging genomics and pastures in the XXI century* and subsequently research based on them. The nature of a project like this, executed by 14 institutions of Europe and the Southern Cone of America, deserves a sustained diffusion of its conclusions at all levels, both for users of different areas that can make the most of the advances generated, as well as for the interdisciplinary groups of researchers that can create new projects on the basis of the scientific and technological progress achieved by Lotassa project.

The experience in *L. tenuis* in flooding pampa environments has revealed a new perspective in the land use of agriculture restrictive soils. Further complementary studies with focus in economic and social impacts of the activities may complement a wide description of the productive ecosystem. Moreover, the new insights in the environmental issues and ecosystem conservation may include new perspectives on the research of grasslands and the ecosystems services. Twenty years of research on this genus and its phenomena as pioneer in the conversion from restricted to productive lands has experimented knowledge transitions in agreement with the technological and scientific innovation. Although more investigation must be conducted, this experience may constitute a model and the "start point" for the introduction of other *Lotus* sp key species in marginal lands. The results obtained look forward to increase the knowledge on soil microbial diversity in the ecosystems where *L. tenuis* in naturalized and evaluate potential relationship with soil C sequestration mitigating the climatic global change [6]. The topics under valuation are the physical, chemical and microbiological changes on soils caused by interseeding of *L. tenuis* in grasslands of the Salado River Basin with others forage species and the farm productivity when using this technological protocol and the legumes becomes the predominant species. Finally, new studies in international collaboration includes the better understanding of the molecular basis of condensed tannins biosynthesis in *Lotus* spp [23] and the role of these secondary metabolites on plant stress responses and GHG mitigation.

5 Future consideration

In summary, we are interested in collaborate with Mediterranean and others world-wide researching groups to analyze the technological transfer of the knowledge obtained in marginal environments of the Salado River Basin (Argentina) and the potential use of the species including in the genus *Lotus* in soil restoration and reclamation activities. The results obtained in the Salado River Basin (Argentina), a marginal ecosystem with sustainability very dependent on the naturalization of a species of the *Lotus* gender (*L. tenuis* in this case) can be added to the information previously obtained in Lotassa [29]. This project (at least to our knowledge) is the only one that integrated collaborations in molecular evaluations (mostly carried out by European Institutions) and its potential for application in pastures and grasslands of the Southern American Cone (all of them carried out in Argentina, Chile, Brazil and Uruguay). The *Lotus* Database, which includes a database on germplasms and specific nodulating strains, is available to facilitate academic or technological use in areas of marginal soils for agriculture or that require restoration and remediation and where Biological Nitrogen Fixation (BNF) plays an essential role in the recovery or improvement of eco-environmental services.

Author contributions M.G and J.P.E has developed evaluation tasks of plant materials and symbionts in the experimental field at extensive and plot levels; V.G.M and A.S.V.N have mainly developed and analyzed metagenomic studies; R.P.A. and M.A.B. have designed and supervised most of the studies involving Lotus spp. and grazing animals; M.P.C., C.J.A. and P.I.C. have designed and carried out most of the studies on abiotic stress in forage and models species; A.B.M. has been in charge of the biodiversity assessments included; F.J.E, P.M.C.S and F.P. have been in charge of most of the studies related to the metabolism and gene regulation of condensed tannins levels; A.G. has conducted the biotic stress studies and O.A.R has designed, wrote and corrected the review. This manuscript includes a brief summary of activities developed and reported by the authors in different journals and on different dates. Likewise, all authors agreed on their previous presentation at the 5th Mediterranean Conference for Environmental Integration (Presentation number 1339) held in Rende (Cosenza, Italy) October 2023. The general design of the Conference presentation was also carried out by the corresponding author.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate Because our brief review doesn't include human participants, we understand that is not applicable in our case.

Consent for publication Not applicable.

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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