

1. PROJECT DATA

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TÍTULO DEL PROYECTO (ACRÓNIMO): Mejora genética de guisantes y almortas para afrontar los retos de una agricultura sostenible (SUSTPEA)

TITLE OF THE PROJECT (ACRONYM): PEA AND GRASS PEA BREEDING FOR SUSTAINABLE AGRICULTURE (SUSTPEA)

1. JUSTIFICATION AND NOVELTY OF THE PROPOSAL

Grain legumes are multifunctional annual crops with extraordinary historical importance for the agriculture and the environment¹. However, in spite of the long tradition of their cultivation and consumption and their well-known environmental benefits by improving soil fertility legumes did not enter into modern European agriculture at the right speed and grain legumes have often been replaced in rotations by other crops that have adapted more quickly to technological progress and are more profitable for farmers in the short term². In parallel there is a continuously increasing demand of protein crops for animal feeding, with no domestic production to satisfy these needs. As result, the **EU countries import 70% of their requirements in vegetable protein**. Today the leader in feed industry is soybean, but peas have also huge potential for feed and manufacturers are ready to incorporate higher rates in their formulations provided there is a constant and homogeneous supply. **For food security and economic reasons, it makes strategic sense to set about improving its own capacity to grow legume crops**. Strategic plans to reintroduce legume crops have been considered a priority both at EU and global level. Producers, manufactures and consumers are demanding legumes, particularly in recent years, willing to grow more environmentally friendly crops, as soon as proper cultivars are available. As a result, in spite of the decline faced in past decades, cultivation is speedily recovering and we need to be ready with technical solutions to support this growth. Prospects are for further growth of grain legume production, with EU expectations of four-fold increase reaching >6% of the arable area, reducing the EU deficit in high protein commodities from about 70% to about 50%, with broad complementary agronomic, economic, environmental, and social impacts. For this, **European legumes must be competitive** for land at product prices that are competitive with imports. The issue with legumes is not a lack of demand, but rather the inability to produce them at a sufficient volume and at competitive price. Reintroducing legume crops into our rotations and market shelves will require an **integrative approach that involves adjusting cropping practices and the breeding of more adapted, attractive, and productive cultivars**³. The success histories of Canada and Australia with remarkable increases in legume cultivation show that legumes can thrive as cash crops with no subsidies on the global market.

Pea (*Pisum sativum*) is a cool season annual legume crop cultivated throughout the world⁴. Depending on its uses, three major types of peas are recognized, each with differing quality requirements. These types are dry or field peas, vegetable or green peas, and forage peas, each one with different uses, from dry seeds used for animal feed, dehulled/split seeds and meal for food industry, immature seeds or pods for food, to whole plant for silage or grazing⁶. Dry and forage peas are typically grown under low input conditions, unlike vegetable peas, which are grown under more intensive irrigation and fertilization. The dry pea is still the most grown temperate grain legume in Europe even

¹ Rubiales et al. 2020. Las leguminosas ante el cambio climático. Capítulo X, Serie Técn, CAAGP Murcia

² González & Rubiales 2016. Las leguminosas grano en la agricultura española. *Arbor* 192-779: a311

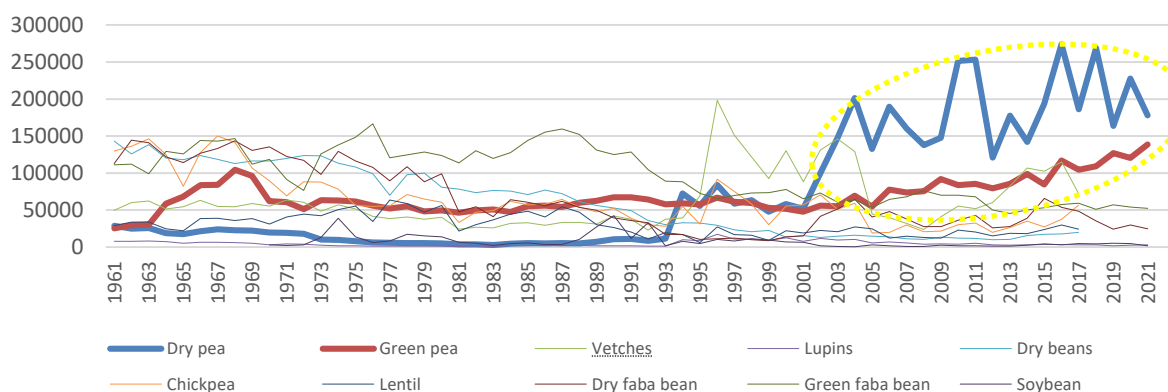
³ Rubiales, Vaz Patto et al. 2021. Legume breeding for the agroecological *Front Pl Sci* 12:782574

⁴ Rubiales, Vaz Patto et al. 2019. CH20 - Advances in breeding of peas. In: Burleigh Dodds Sci Publ. Ltd.

when cultivation decreased in past decades (now recovering). In Spain both dry and green peas are the legume crops more produced (Fig. 1), with clear expectative for a sustained growth.

However, even with this marked increase in pea cultivation in Spain pea cultivation is far below actual needs forcing to high imports to satisfy the demand of the food and feed industry. Therefore, **both to satisfy the domestic demand of pea seeds, and to satisfy the social demand of growing more legumes for their environmental benefits, pea acreage is likely to continue increasing in coming years. However, the breeding sector is not ready to satisfy this demand providing farmers with quality cultivars adapted to local needs.** Unfortunately, modern pea elite cultivars are little adapted to Mediterranean environments⁵, since breeding programs have concentrated their efforts to other environments (i.e Canada, Central and Northern Europe), not having targeted regions of the Mediterranean Basin. **Substantial breeding effort is needed in order to improve adaptation to Mediterranean rain fed farming systems and associated biotic and abiotic stresses.** There are therefore many Mediterranean specific constraints to be addressed locally.

Historic trend in legume production (Tons) in Spain (1961-2021 FAOSTAT data)



2. OBJETIVES, METHODOLOGY AND WORK PLAN

2.1. General and specific objectives.

The aim of the present project is to give continuity to our long-term pea (*Pisum sativum*) research program focused on resistance breeding, giving an insight into sanitation problems that are specific of Spain, and for extension, of the Mediterranean Basin. This program **started** with 1FD97-0393 project with a focus on resistance to broomrape (*Orobanche crenata*), and **became the major target of our group overt last two decades** under PID/AEI calls and its antecessors as indicated above. Taking advantage of our past experience we propose in this project to characterize deeper the stress resistance both in pea and grass pea at the molecular level to identify resistance genes and to complement conventional breeding with the efficient use of molecular tools. A range of pest and diseases and abiotic stresses will be covered at various degrees, characterizing plant/stress interactions, searching for resistance and studying its inheritance. This will provide a more comprehensive understanding of host molecular responses to the stresses making possible the elucidation of mechanisms and genes involved in resistance. Our final goal is to contribute to **developing more resilient cultivars, complementing classical breeding with molecular tools.** In addition to this contribution towards understanding plant/stress interaction, this project intent to provide applied solutions to farmers, delivering resistant germplasm together with alternative control strategies in integrated control packages. Even though our primary goal is to understand and exploit genetic resistance in breeding, we assume that existing resistance levels are still insufficient for adequate crop protection, and introduced resistance should be complemented by additional control approaches to increase the durability of resistance. Following our experience in other integrated control strategies, we will approach further studies on identifying pea metabolites with allelopathic activity and identifying phytotoxins produced by pea pathogens to understand their role in host-pathogen interaction. This will be approached by the 5 objectives, with sub-objectives and methodologies described later in section 2.2:

⁵ Rubiales, Barilli et al. 2023. Breeding for biotic stress resistance in pea. *Agriculture* 13:1825

1. Search for new sources of resistance to major biotic and abiotic stresses
2. Wide hybridization and breeding
3. Advanced QTL mapping and genome-enabled prediction of key traits
4. Establishment of the molecular bases of resistance
5. Alternative methods for disease management

2.2. Description of the methodology.

Task 1.- Search for new sources of resistance to major biotic and abiotic stresses

Our early efforts⁶ on pea and grass pea started with resistance to broomrape (*Orobanche crenata*) which was considered the major problem both for pea and grass pea in the region, not being covered by any other program^{7,8}. This was progressively expanded with activities on other stresses⁹ such as ascochyta blight (*Didymella pinodes*)^{10,11}, powdery mildew (*Erysiphe pisi*)^{12,13}, rust (*Uromyces pisi*)^{14,15}, bacterial blight (*Pseudomonas syringae*)¹⁶, fusarium wilt (*Fusarium oxysporum* f.sp. *psii*)^{17,18,19,20}, aphid (*Acyrtosiphon pisum*)^{21,22} and weevil (*Bruchus pisorum*)^{23,24} and more recently to root rot (*Aphanomyces euteiches*)²⁵ and to abiotic stresses²⁶ in close cooperation with world leading groups.

T1.1.- Identification and characterization of additional sources of resistance to airborne pests and pathogens:

T1.1.1. Airborn pathogens: in past projects we developed and refined methodologies for screening for resistance against ascochyta blight (*Dydymella pinodes*), rust (*Uromyces pisi*) and powdery mildew (*Erysiphe pisi*) and identified resistances in both pea⁷ and *Lathyrus*⁹. Some of these sources of resistance have been already used in the crossing program, succeeding in incorporating the broomrape and powdery mildew resistance by breeding in elite germplasm^{13,27}. However, we feel that in spite of the high scientific production generated on resistance to ascochyta blight in pea and grass pea, this has not been successfully incorporated into elite germplasm. We also realized that resistance to *E. pisi* might be broken down by *E. trifolii*. By focusing on a limited number of earlier identified sources of resistance, we neglected a number of valuable *Pisum* accessions (i.e. *P. abyssinicum* and *P. s. elatius*) that showed resistance in preliminary screenings, being insufficiently characterized and not exploited in the crossing

⁶ All across the proposal we will provide a number of references of the team describing recent achievements on the subject, with the only purpose to show familiarity of the team on such methodologies and therefore capability to perform the tasks committed. By no mean this should be understood as pretending to be the first or the only ones using them.

⁷ Rubiales 2018. Can we breed for durable resistance to broomrapes?. **Phytopath Med** 57:170-185

⁸ Rubiales 2023. Managing root parasitic weeds to facilitate legume reintroduction.... **Soil Systems** 7:99

⁹ Rubiales et al. 2015. Achievements and challenges in legume **Crit Rev Plant Sci** 34:195-236

¹⁰ Rubiales & Fondevilla 2012. Future prospects for ascochyta blight resistance ... **Front Pl Sci** 3:27

¹¹ Barilli, Rubiales et al. 2016. Clarification on host range of *D. pinodes* **Front Pl Sci** 7:59

¹² Fondevilla, Rubiales et al. 2022. Development of real-time PCR assays to quantify **Agronomy** 12:334

¹³ Santos, Rubiales, Vaz Patto et al. 2020. Resistance to *E. pisi* and *E. trifolii* ... **Plant Dis** 104:2875-2884

¹⁴ Osuna, Barilli, Rubiales et al. 2022. Identification of resistance to *U. pisi*... **Agronomy** 12:1582

¹⁵ Osuna, Rubiales et al. 2023. RGB image-based method for phenotyping rust ... **Plant Methods** 19:86

¹⁶ Fondevilla, Rubiales et al. 2012. Identification of QTL to *P. syringae* **Euphytica** 186:805-812

¹⁷ Bani, Rubiales et al. 2018. Physical and chemical barriers in root tissues.... **Front Pl Sci** 9:199

¹⁸ Rispaill & Rubiales 2015. Rapid and efficient estimation of pea resistance **Sensors** 15:3988-4000

¹⁹ Sampaio, Rubiales, Vaz Patto et al. 2021. A diversity of Fusarium resistance sources... **PI Soil** 463:19-38

²⁰ 5 PhDs co-supervised: Bani (FW-pea, UCO 2015), Pouralibaba (FW-lentil, UCO-2018), Leitao (FW-Phaseous, UNL-ITQB-2020), Sampaio (FW-grass pea UNL-ITQB, 2021), Wohor (FW-pea, UCO-2024)

²¹ Barilli, Rubiales et al. 2020. Candidate genes controlling pea aphid ... **Pest Man Sci** 76:1731-1742

²² Aznar & Rubiales 2018. Identification and characterization of antixenosis... **Ann App Biol** 3:268-281

²³ Aznar, Rubiales et al. 2017. Identification and multi-environment **J Pest Sci** 91:205-214

²⁴ Aznar & Rubiales 2019. Flower and pod source influence on pea weevil. **Front Pl Sci** 10:491

²⁵ Divyanth, Rubiales et al. 2022. Evaluation of effective ... assessment of *Aphanomyces*... **Sensors** 22:723

²⁶ Bagheri, Rubiales et al. 2023. Challenges in pea breeding for drought.... **Ann Appl Biol** 2023:1-13

²⁷ Cobos, Rubiales et al. 2018. *Er3* gene, conferring resistance to *Erysiphe pisi*... **Euphytica** 214:20

program at all. Therefore, we propose now to: i) look for higher levels of resistance to *D. pinodes* in *Pisum* and *Lathyrus* germplasm; ii) extend the screenings to *E. trifolii*; iii) go back to screenings of larger collections of *P. abyssinicum* and *P. s. elatius* to identify resistance to *E. pisi* and *E. trifolii* to be introduced in the breeding program (Obj. 2). Both *Pisum* and *Lathyrus* germplasm (>3000 accessions), screening protocols and facilities are in place at IAS-CSIC.

T1.1.2. Pea aphid (*Acyrtosiphon pisum*): with the tendency to reduce pesticide application pea aphid and weevil are emerging as major constraints reinforcing the need to develop resistant cultivars. We performed preliminary studies^{26,27} showing some levels of incomplete resistance, what need to be improved. Still, this was sufficient to attract the interest²⁸ of the French seed company RAGT-2N who is one of the major players in European seed industry. We propose to screen a starting collection of 300-400 accessions of pea and 150 of grass pea under field conditions using protocols already used by us^{26,27,29}. The 5-10 most resistant accessions will be studied in choice and no-choice experiments under controlled conditions following protocols already in place^{27,34}.

T1.2.- Identification of additional sources of resistance to soilborne pathogens:

Task 2.- Wide hybridization and breeding:

T2.1. Pea breeding: In the frame of past projects, in addition to the core scientific activities, we managed to run a small pea breeding program with a major focus on developing cultivars adapted to Mediterranean rain fed farming conditions. Our first priority was the resistance to broomrape, what was not easy since there was little resistance within pea germplasm, and we had to look for it in wild relatives with the associated problems of crossability and poor agronomic performance. After huge efforts in crossing and selection the first cultivars with resistance to broomrape were registered by CSIC³⁸. Similarly, we registered the first cultivars carrying the novel powdery resistance gene *Er3* discovered by us and transferred from *P. fulvum*^{32,37}. Still, being productive and highly resistant to broomrape and/or powdery mildew, those materials suffered from relatively weak standing ability with associated losses at harvest^{12,30}. To solve this, we paid big efforts during last 10 years to improve agronomic performance what successfully resulted in the development of novel cultivars combining broomrape resistance with excellent standing ability and productivity³⁷ that are submitted to OEVV (Oficina Española de Variedades Vegetales) for registration and provided to RAEA (Red Andaluza de Experimentación Agraria) for official comparative field testing during this season 2024. These activities were secured thanks to the recently terminated proof of concept projects PDC2021-120930-I00, CDTI-Batlle and the National Operative Group project GO-INPULSE. Fortunately, these applied breeding activities are having continuity thanks to the recently started public-private cooperation project CPP2022-009742 for the development of pea, faba bean and vetches cultivars with Semillas Batlle. The selections covered in those projects-contracts-MTA are to be made on demand addressing specific breeding targets agreed with each partner.

Still, we intend to allocate in the present proposal some activities to continue enlarging the pre-breeding program covering new crosses with new sources of resistances and new breeding targets looking for future developments in cooperation with other programs. Crossing will be extended with new crosses of selected accessions with desirable traits (identified in Task 1). Crosses will be performed at Córdoba. Selections will be made under field and controlled conditions for specific traits at Córdoba and Huelva. Selections of specific materials will also be made at Lebanon with ICARDA, Canada with Uni. Saskatchewan and USA with Univ. Clemson, ISEA-Italy, RAGT-France, KWS-Germany and Nordic Seeds-Denmark as we have already provided specific segregating populations or making new crosses on demand, covered in proper Material Transfer Agreements (MTA) to protect Intellectual Property. Selection criteria will include agronomic performance and disease resistance, according to regional priorities. To ease selection, Marker Assisted Selection (MAS) will be implemented for each trait in which efficient markers have been developed to replace resistance screening (T3.6). As soon as

²⁸ Contract 080109220001 with RAGT 2N SAS, France (2021-2024)

²⁹ PhD by T. Aznar 2018 and MsC by I. Lucato 2021

³⁰ Rubiales et al. 2021. Development of pea breeding lines resistance to *Orobanche* *Agronomy* 11:36



efficient Genomi Selection (GS) models are made available in T3.5, they will be also implemented in our selection program.

Task 3. Advanced QTL mapping and genome-enabled prediction of key traits

T3.1.- Linkage QTL mapping: In the frame of previous projects we established several genetic maps from RIL populations derived from inter and intraspecific crosses including *P. sativum* ssp. *syriacum* x *P. sativum* ssp. *sativum*, *P. fulvum* x *P. fulvum*, *P. sativum* ssp. *sativum* x *P. sativum* ssp. *sativum* and *P. fulvum* x *P. sativum* ssp. *sativum*. These populations were successfully used to identify QTLs controlling resistance to broomrape³¹, rust³², ascochyta³³, bacterial blight²¹, drought⁴⁴, aphid³⁴ and weevil³⁵. These maps were obtained by combining initially RAPDs with SSR markers and finally by DArT-Seq derived SNPs. We propose here to combine the genotypic data of the different pea RILs which allow the development of pea consensus maps with a much higher resolution and precision than individual bi-parental maps which will be useful to perform a meta-QTL analysis for multiple traits.

T3.2. Association mapping in pea: in the frame of past projects we gathered a core collection of around 340 pea accessions from worldwide origin. This was genotyped using DArT-Seq platform of Diversity array (Australia) generating more than 108.500 SNP markers which have been used to establish the Linkage disequilibrium decay distance, the population structure and genetic diversity of the collection confirming its suitability for GWAS³⁶. In parallel, the core collection was phenotyped for rust, broomrape and fusarium wilt resistance under controlled and/or field conditions. Genotypic and phenotypic data have been integrated to permit the genome-wide association analysis (GWAS) to deliver molecular markers closely linked to the traits of interest, to speed-up screening breeding steps through Marker Assisted Selection (MAS). The process has been slow as for quantitative traits, relevant journals rightly require a minimum of three environments and we are delivering this. Results from past PID2020 project are being published now^{37,38,39}. The collection was also phenotyped for aphid and weevil resistance in the field over 2 seasons, and for powdery mildews (*E. pisi* and *E. trifolii*) and ascochyta blight under controlled conditions. We intend now to complete the existing aphid and weevil phenotypings with two additional field seasons and to complement the existing powdery mildew and ascochyta blight phenotypings with adult plant screenings in the field.

T3.4 Development of multiparental pea populations (MAGIC and NAM): Although the use of bi-parental populations allows identification of QTLs for resistance to stresses, they often present low allelic variation that impede precise QTL localization and their usefulness are often limited to specific genetic backgrounds difficulting their application for MAS.

T3.4.1. Multi-parent Advanced Generation InterCrosses (MAGIC) populations may circumvent the low genetic diversity of bi-parental populations and increases the resolution of QTL localization. We have reached an international agreement protected with a proper MTA among public pea breeders in Australia, Canada, Italy and Spain so each of us have provided 2 elite accessions possessing different traits of particular value that are being used to cooperatively generate a pea MAGIC population through a joint effort that will extend to future phenotyping and genomic studies. The target is the development of 1600 inbred lines – to be all genotyped, while possibly restricting the initial phenotyping activities to a subset of them (e.g. 192). This initiative is led by Dr. P. Annicchiarico (CREA-Italy) with each country supporting its own activities. We already performed the first and second crossing stages, and, following the agreed plans, provided timely the resulting populations to Univ. Victoria-Australia where they would advance to F₆ by speed breeding. However, they faced a number

³¹ Fondevilla, Rubiales et al. 2010. Identification of QTL for specific ... **Mol Breed** 25:259-272

³² Barilli, Rubiales et al. 2018. A high-density DArTseq SNP-based map ... **Front Pl Sci** 9:167

³³ Carrillo, Rubiales et al. 2014. Identification of QTL *D. pinodes*... **Pl Cell Rep** 33:1133-1145

³⁴ Barilli, Rubiales et al. 2020. Candidate genes controlling pea aphid ... **Pest Man Sci** 76:1731-1742

³⁵ Aznar, Barilli, Rubiales et al. 2020. Identification of QTL for resistance to pea weevil **Sci Rep** 10:33

³⁶ Rispaill, Barilli, Rubiales et al. 2023 Genetic diversity and population structure... **Int J Mol Sci** 24:2470

³⁷ Osuna, Rubiales, Barilli et al. 2024. GWAS uncovers pea rust genes ... **Theor Appl Gen.** accepted

³⁸ Wohor, Rubiales et al. GWAS identifies *O. crenata* In prep

³⁹ Wohor, Rubiales et al. GWAS identifies *Fusarium osysporum*.... Ready for submission

of problems and this failed. The agreement now is that each partner should take care of 400 F_{2:3} lines (balanced across the crosses) to be advanced till F₆, that will then be shared to reconstruct the entire 1600 population. This is what we propose here, so by the end of the project the materials should be ready for joint genotyping and phenotyping.

T3.4.2. Nested association mapping (NAM) population: We have shown that value of pea wild relatives in pea breeding for broomrape resistance. However, estimating the performance of ‘wild’ alleles for crop improvement directly in wild germplasm is difficult. NAM populations are highly effective to estimate wild allele effects in adapted backgrounds. NAM represents a mixture of classical linkage mapping and association mapping and combines the advantages of both approaches. During last 2 years we crossed 25 highly diverse wild pea accessions (previously identified by us as resistant to different diseases) with a recurrent elite pea susceptible cultivar. We have now F₂s that we plan to submit to several cycles of selfing to generate recombinant inbred line (RIL). Like this, the NAM population will be finalized by the end of the project, available to be further characterized in future projects.

T3.5. Genomic Selection / Genome-enabled prediction of key traits: Genomic Selection (GS) is becoming a standard practice in contemporary plant breeding programs thanks to the decreasing costs of genomic tools and the acknowledgment that phenotyping has emerged as the bottleneck, being more expensive than genotyping. In the frame of PID2020 project we approached GS for rust resistance^{40,41}, selecting more favorable mathematical models, and applying covariates developing selection equations with better predictive ability than the ones currently available for other pea diseases. This enables optimal models that will be extrapolated to other traits assessed in the pea collection, establishing prediction models for agronomic traits and resistance to powdery mildew, ascochyta blight and broomrape. Analysis of adaptation through GxE evaluation of the pea core collection will determine whether different testing sites belong to a homogeneous region (with regard to genotype responses) and are, therefore, recipient of same GS models, or if individual sites (and the region represented by each of them) requires specific GS models. This will be used for breeding other lines (test population), by genotyping them and calculating their GEBVs using the prediction model, thus escaping the need for test trials of very large germplasm collection. Selection proceeds on the basis of these GEBVs leading to practice shifts since phenotypes will no longer be used to select, but to train prediction models.

T3.6. Implementation of Marker-Assisted Selection: Although we identified several QTLs controlling resistance/tolerance to numerous pea and grass pea constraints in previous projects, the large distance between the QTL and their closest markers hampered MAS implementation⁴². Thus, several objectives of this project aim to identify molecular markers more closely linked with resistance including the saturation of the existing maps by DArT-Seq approach (T3.1) and GWAS (T3.2). In addition, the sequence of resistance genes or their beneficial allelic variants that will be identified in T4.4 will be used to develop molecular markers linked to resistance. The usefulness of these markers to select for resistance will be tested on a selection of pea with differential responses to their corresponding diseases by conventional PCR approaches. The most efficient markers will be implemented in our pea breeding program for MAS.

Task 4. Establishment of the molecular bases of resistance

Considerable efforts have been paid to characterize resistance responses in the model legume *M. truncatula* and in pea and grass pea. The combination of transcriptomic approaches such as

⁴⁰ Osuna, Rubiales et al. 2022. Genomic Selection para resistencia a roya CMGP Pontevedra

⁴¹ Osuna, Rubiales et al. 2024. Genomic Prediction for Rust Resistance in Pea. Submitted

⁴² Pandey, Rubiales et al. 2021. Omics resources and omics-enabled ... pea. **Theor Appl Gen** 134:755-776



microarrays^{43,44}, transcription factors (TFs) analysis^{45,46,47}, deepSuperSAGE^{48,49}, RNASeq^{50,51} and MACE⁵² with different proteomic-based approaches^{53,54,55} provided us a global view of the genes and metabolic changes induced during compatible and incompatible responses to a number of stresses. Therefore, in this proposal we will take advantage of this experience to approach the study of the rather less studied pea / *Aphanomyces* pathosystem to identify candidate genes, and novel markers and allelic variants associated with resistance for their exploitation in breeding.

T4.4. Development of breeders' friendly molecular markers: Primer sequences will be developed for DNA screening of the co-dominant molecular markers identified by GWAS. For the resistance genes identified only at transcriptomic level (T4.1), a search for SNPs and SSRs in transcript expressed sequences will be performed using Geneious software. Molecular tools targeting SNP markers will be created by developing tetra-primers for unambiguously amplification of each allele using the ARMS (Amplification-refractory mutation system)-PCR technique. For SSR markers, primers will be designed on conserved flanking regions of the SSR. Amplification and specificity of each primer pair will be tested in the pea collection.

2.3. Previous results of the team in the theme of the proposal.

Results of the team in the theme of the proposal have been largely presented in previous sections. We opted to do that way as we understand that the proposal is very broad, covering many different aspects and we have a limited space (max 20 pages). We thought that as long as the proposal is designed as a follow up of a long-term research-breeding program updated with latest-state-of-the-art methodologies, each section would be better presented with a brief introduction of recent achievements to explain why and how. We also wanted to show that the team has sufficient familiarity on such methodologies and therefore capability to perform the tasks committed.

In brief, we have previous expertise in resistance breeding in cereals with above 75 major publications on the subject (that will not be listed here) as result of activities performed mainly in past projects AGF96-0463, AGF99-1036, PTR95-0635-OP and PET2007-0492-01. This experience helped us to start resistance breeding in legumes in the late 90's, focusing first on faba bean (*Vicia faba*, 60 JIF papers), and then also in lentil (*Lens culinaris*, 15 JIF papers), vetches (*Vicia* spp., 10 JIF papers), and to less extend chickpea (*Cicer arietinum*, 7 JIF papers), and common bean (*Phaseolus vulgaris*, 10 JIF papers). Our major focus was resistance to broomrape (*Orobanche crenata*) achievements that can be summarized in co-authorship in 80 major publications and co-direction of 6 PhD on *Orobanche* together with the registration of resistant cultivars⁵⁶. Broomrape focus was soon expanded to rusts on various crops (93 JIF publications and 10 PhD), powdery mildews (46 publications, 5 PhD), ascochyta (32 publications, 3 PhD), fusarium wilt (18 publications, 5 PhD), chocolate spot (8 publications, 1 PhD), drought (8 publications, 2

⁴³ Fondevilla, Rubiales et al. 2011. Identification of genes differentially **BMC Genomics** 12:28

⁴⁴ Curto, Rubiales et al. 2015. Plant defense responses in *M. truncatula* ... **PI Mol Biol Rep** 33:569-583

⁴⁵ Curto, Rubiales et al. 2015. Transcriptional profiling of *M. truncatula* during.... **Front Pl Sci** 6:517

⁴⁶ Madrid, Rubiales et al. 2010. Transcription factor profiling leading ... **Theor Appl Gen** 121:1311-132

⁴⁷ Villegas, Rubiales et al. 2014. Characterisation by TF profiling of **Plant Mol Biol Rep** 32:1030-1040

⁴⁸ Fondevilla, Rubiales et al. 2014. Identification of genes involved in **PI Mol Biol Rep** 32:258-269

⁴⁹ Almeida, Rubiales, Vaz Patto et al. 2014. Allelic diversity in the transcriptomes **BMC Plant Biol** 14:376

⁵⁰ Santos, Rubiales, Vaz Patto et al. 2018. First genetic linkage map ... based on RNAseq **Hort Res** 5:45

⁵¹ Jiménez, Rubiales et al. 2023. Pea resistance to *Erysiphe* ... via RNA-seq analysis. ILS4 Conf

⁵² Fondevilla, Rubiales et al. 2022. Transcriptomic analysis in a pea RIL ... **Scient Rep** 12:18159

⁵³ Castillejo, Rubiales et al. 2012. Proteomic analysis of response pea -*O. crenata*... **J Exp Bot** 63:107-119

⁵⁴ Catillejo, Rubiales et al. 2015. Understanding pea resistance mechanisms ... **Phytochem** 115:44-58

⁵⁵ Castillejo, Rubiales et al. 2016. Label-free quantitative proteomic drought ... **Proteomics** 16:2776-2782

⁵⁶ Broomrape faba bean resistant cvs. JOYA, OMEYA, HABANITA, BORJANA; pea resistant cvs. JARANA, FORANA, CHICANA, TIRANA, ANTOJO

PhD) and insect pests (7 publications, 1 PhD). This experience allowed us to consolidate a pea and grass pea programs at Córdoba, that became the major target of our group over the last two decades.

3. EXPECTED IMPACT OF THE RESULTS

3.1. *Expected impact on the generation of scientific-technical knowledge in the thematic area of the proposal.*

Returning legume crops to our rotations and market shelves can only be achieved through an integrative approach leading to the adjustment of cropping practices and the development of more adapted, attractive and productive cultivars. Pea is a versatile and inexpensive protein source for animal feeding but substantial breeding effort is needed in order to improve adaptation and resistance against major biotic and abiotic stresses. Unfortunately, modern pea elite cultivars are little adapted to Mediterranean environments as the big breeding programs have concentrated their efforts to other environments, not having targeted regions of the Mediterranean Basin. There are therefore many Mediterranean specific constraints to be addressed locally. The aim of this Project is to contribute to a pea program addressing problems that are specific of Spain, and for extension, of the Mediterranean Basin giving continuity to a long term research program started by us in 1996. Major limiting biotic and abiotic stresses will be covered at various degrees, searching for resistance and studying its inheritance. This will provide a more comprehensive understanding of host molecular responses during the infection process of these pathogens making possible the elucidation of mechanisms and genes involved in the resistance. Major limiting stresses will be covered at various degrees, searching for resistance and developing improved breeding materials to be further exploited by breeding/seeds sector. Latest State-of-the-Art techniques such as GBS and GWAS analyses will be used design markers to assist breeding and will be the basis for future Genomic-enabled predictive models. Adoption of these innovative technologies will speed the breeding process allowing new cultivar material reaching the farmer faster providing advantage for innovative breeding companies.

3.2. *Social and economic impact of the expected results.*

Besides relevant scientific contributions, the project will continue a number of applied tasks devoted to generate breeding material adapted to Mediterranean rain fed conditions. Our ambition is to promote the legume sector and strengthen its economic and social role in order to improve environmental balance, and improve field crop yields under climate change conditions. Scientific knowledge, protocols and pre-breeding material will be made accessible to the scientific community and to the end-users. This is ensured by the field testing in several countries and the strong interaction with stake holders. In the long run, developing legume cultivation in the Mediterranean Basin will contribute to the achievements of several critical points of utmost importance for crop management under anticipated climate-change, associated with a mandatory reduction of chemicals and pesticides inputs: i) Improve the profitability of cropping systems by integrating leguminous crops. This goes through reduced use of fertilizers, and possible increase of cereals crops cultivated using inter-, mixed- or rotation systems with legumes; ii) Valorize the marginalized dry areas, expected to increase in the near future, using adapted legume species; iii) Deliver varieties better adapted to less intensive cropping systems; iv) Preserve biodiversity and landscape through crops diversification via multiple legume species; v) Protect soils and improve fertilization.

3.3. *Plan for scientific communication and internationalization of the results.*

We commit to publish all project results preferably in reputed journals in Open Access what shall be facilitated by CSIC agreements with a number of major editorials (<https://bibliotecas.csic.es/publicacion-en-acceso-abierto>). In eventual cases in which this were not feasible due to budget limitations, we shall make preprints fully available via DIGITAL.CSIC platform (<https://digital.csic.es/>).

3.4. *Plan for dissemination, valorization and transfer of the results to the most relevant groups for the theme of the project and to society in general.*

We strongly commit with translating project results to society by making available to expedite commercialization and cultivation the advanced breeding lines validated in the proposal, accompanied with proper dissemination to the sector and with education and training.

As a result of final validations performed and seed multiplications selected cultivars will be submitted to the OEVV list of commercial cultivars (target number 5) by year 4. For this, formal procedures will first be shorted out with VICYT-CSIC patent office. As a result of our existing fluent communication with seed companies and the dissemination of the agronomic value of the cvs described above, we might be able to attract the interest of companies to license commercialization of the “future” cvs even before they are officially registered, as we successfully did in the past. Our target is to deliver excellent cultivars to be rapidly accessible to farmers. Terms and conditions will be negotiated by VICYT-CSIC and proper contracts licensing commercialization will likely be timely achieved, as seed companies are already interested in resulting cvs. as they have already seen the breeding lines.

We will actively interact with stake holders to promote pea and grass pea crops by showing agronomic and environmental benefits. We have experience on this with close collaboration with the feed industry, as we in fact participated in the operative group IMPULSE and interacted with farmers and manufacturers. We collaborated closely with RAEA (Red Andaluza de Experimentación Agraria), RedArax and SAT-Córdoba in cultivar comparison and will work on this to show farmers the benefits of the new cultivars even before they are registered and commercialized.

All this is further ensured by our:

- Strong involvement of the IPs in legume networking with IP1 being coordinator of RELEG (Spanish Legume Network, <https://releg.csic.es/>), past President of European Grain Legume Association, and Past President and current secretary of International Legume Society (<https://www.legumesociety.org/>).

- Strong involvement of relevant associations and industry in Spain (i.e. Semillas Batlle, COVAP, COAG, Cooperativas Aragón, LUGARTIS, Grupo AN, AGROSA) and with relevant pea breeding program in the world like ICARDA, Univ. Saskatchewan-Canada, Univ. Clemson-USA, CREA-Italy, RAGT-France, KWS-Germany, ISEA-Italy, GZPK-Switzerland, Nordic Seeds-Denmark.

- Proven commitment with outreach activities editing dissemination magazines such the past Gran Legumes Magazine and the current Legume Perspectives magazine and playing relevant editorial roles in major scientific journals (chief editor at Front Pl Sci, associate editor at Field Crops Res, Agron Sustain Dev, Agronomy, among others) as well as organization of 1st and 2nd Int. Legume Conferences.

4. TRAINING CAPACITY

4.1. *Training program planned in the context of the requested project*

Training capacity of the group is shown by the high number of PhDs supervised and major publications produced by the partners. University lectures, scientists and professors are members of the team, with wide national and international scientific recognition. The forecasted PhD student will enroll the Doctoral Program of the Univ. of Córdoba, in which the Institute for Sustainable Agriculture (CSIC) is very active offering its own courses as well as in the Training Network Courses of the CEIA3. Prof. Diego Rubiales, IP1 of the proposal, has wide international scientific recognition, as seen in the brief CV provided, that can be summarized by h-index 59 and 32 PhD theses successfully defended and 3 ones already submitted waiting for defense. Dr. Barilli, IP2 of the proposal, is a young scientist that has also a relevant scientific carrier (h-index 17). They together will co-supervise the PhD requested in this proposal, in close interaction with team members and with international collaborators.



The IAS-CSIC has a wide experience in research and training, both at the European and international level. Many national and international Post-doctorate scientists have been trained under national and international funding. The infrastructure for disease resistance studies and DNA analysis is available. The institute has considerable experience in studies on resistance to diseases in grain legumes, in the identification and characterization of sources of resistance, studies on inheritance and mapping and pathogen virulence determination. Training opportunities are ensured by the participation in this proposal of leading labs in legume breeding and marker development with whom we maintain a long-term fluent collaboration. National and international collaboration is also ensured by the strong leadership of IP1 in the legume community at national (coordinating RELEG, <https://releg.csic.es/>) and international level (<https://www.legumesociety.org/2019/11/27/executive-committee/>). The student requested will benefit from the long-term interaction of these international teams, and will pay regular training visits. He/she will perform his/her PhD in resistance to root diseases. He/she will identify and characterize sources of resistance, identify molecular markers linked to resistance and study plant disease interaction by using the transcriptomic technique RNASeq. We forecast a 3 month stay during year 1 at USDA-ARS for phenotyping under the guidance of Dr McGee. Then, we forecast a 3-4 months stay during year 2 at John Innes Center, Norwich, UK for VIGS studies under the guidance of Dr Sanu Arora. A third 3 months stay during year 3 at CREA, Italy for GS analysis under the guidance of Dr. P. Annicchiarico.

4.2. Thesis completed or in progress within the scope of the research team (last 10 years).

Out of 33 PhD thesis supervised, we list the 11 defended during the last 10 years (2014-2023) and 6 in process for defense during first trimester of 2024:

1. Montilla, 2014. Genetic and molecular approaches in oat breeding stress... Superv: Prats & Rubiales
2. Bani, 2015. Resistance to *Fusarium oxysporum* in pea and *M. truncatula*. Superv. Rubiales & Rispaill
3. Curto, 2015. Dissection of plant-disease interaction Superv: Rubiales, Jorrín & Maldonado.
4. Almeida, 2015. Comparative mapping of resistance in *Lathyrus* ... Superv: Vaz Patto & Rubiales
5. Pouralibaba, 2017. Resistance to Fusarium wilt in lentil. Superv: Rubiales & Fondevilla
6. Aznar, 2018. Resistance to aphid and weevil in pea. Superv: Rubiales
7. Leitao, 2020. Combined stress resistance in *Phaseolus* Superv. Vaz Patto, Araujo & Rubiales
8. Sampaio, 2021. Unravelling the genetic control of *Fusarium* Superv. Vaz Patto & Rubiales
9. Abou-Khaeter, 2022. Novel genes and tools for weeds management ... Superv Rubiales & Maalouf
10. Martins, 2022. Unravelling rust and powdery mildew resistance Sup. Vaz Patto & Rubiales
11. Amarna, 2023. Crop diversification to control biotic stresses legumes. Superv. Villegas & Rubiales
12. Balech, *submitted 27/11/23*. GWAS for herbicide tolerance in lentils. Sup. Maalouf & Rubiales
13. Agudo, *subm 07/12/23*. Alelopatía para el manejo de enfermedades. Sup. Rubiales & Barilli
14. Osuna, *subm 25/01/24*. Identification and characterization of rust Sup. Rubiales & Rispaill
15. Osman, *ready for subm*. Characterization of fusarium resistance in pea.... Sup. Rubiales & Rispaill
16. Gonçalves, *ready for subm*. Exploiting grass pea genetics as a tool for ... Sup. Vaz Patto & Rubiales