



Resilient, water- and energy-Efficient FORage and feed crops for Mediterranean Agricultural systems

- ❖ Crop-livestock and feed systems have a huge economic importance in Mediterranean regions, but are threatened by feed protein insufficiency, grassland overexploitation, decreasing irrigation water, and increasing drought, heat, and cost of fertilizers.
- ❖ REFORMA aims to alleviate these constraints by developing new breeding methods and varieties that enhance lucerne adaptation to drought, salinity, grazing, heat and mixed cropping and pea drought tolerance, and by optimizing lucerne- and pea-based crops.
- ❖ Extensive germplasm evaluation and innovative genotyping-by-sequencing molecular characterization were exploited to develop genomic selection models with good ability to predict lucerne or pea genotype breeding values in specific environments.



Objectives

Drought tolerance

Forage crops

Genomics

Grain legumes

Plant breeding

- ❑ Enhancing the forage yield and persistence of lucerne in Mediterranean environments prone to severe drought, salinity, grazing or heat stress, by selecting phenotypically stress-tolerant varieties and by defining innovative genome-enabled and ecologically-based selection procedures.
- ❑ Producing drought-tolerant pea varieties for grain or forage production, by phenotypic selection and definition of innovative genome-enabled and ecologically-based selection procedures.
- ❑ Optimizing the cultivation and use of lucerne-based and pea-based forage crops in different drought-prone Mediterranean environments, by assessing different pea-cereal and lucerne-grass mixtures, other mixtures and pure stand crops in terms of yielding ability, resilience, forage quality and acceptability by farmers.

Scientific results & innovation potential

- ✓ Large genetic variation emerged for breeding value of lucerne (alias alfalfa) and pea genotypes under various stress conditions, supporting the breeding of these crops for specific stress-prone environments.
- ✓ An evolutionary pea breeding scheme that exploits natural selection under drought stress proved valuable and cost-efficient.
- ✓ Genomic selection reached accuracy levels sufficient to potentially offset phenotypic selection for lucerne forage yield, and showed high predictive accuracy for pea grain yield under severe drought stress.
- ✓ Pea-cereal forage crops tended to out-yield vetch-based mixtures or cereal monocultures, and ranked high in farmer-participatory evaluations.
- ✓ Although most research work awaits completion in 2016, owing to delays in its implementation, our preliminary results confirm the high interest of pea and lucerne for more sustainable cropping systems and the scope for improving the resilience and breeding efficiency of these species via genomic selection and evolutionary breeding approaches. Various stress-tolerant varieties of lucerne and pea will be selected within the project.

Coordinator

Dr Paolo ANNICCHIARICO

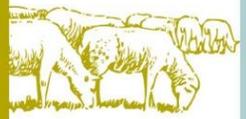
Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per le Produzioni Foraggere e Lattiero-Casearie [CREA-FLC], Lodi

ITALY

paolo.annicchiarico@crea.gov.it

Partners

- Institut National de la Recherche Agronomique, Unité de Recherche Pluridisciplinaire Prairies et Plantes Fourragères, Lusignan, **FRANCE** [INRA-URP3F]
- Institut National de la Recherche Agronomique, Unité de Recherche en Agroécologie, Dijon, **FRANCE** [INRA-URAgro]
- Institut National de la Recherche Agronomique (INRA), Centres Régionaux de Marrakech et de Rabat, **MOROCCO** [INRA-MOR]
- Institut National de Recherche Agronomique, Division des Agrosystèmes de l'Est, Sétif, **ALGERIA** [INRAA]
- Ecole Nationale Supérieure d'Agronomie, Alger, **ALGERIA** [ENSA]
- Consiglio Nazionale delle Ricerche, Istituto per il Sistema Produzione Animale in Ambiente Mediterraneo, Sassari, **ITALY** [CNR-ISPAAM]
- Institut des Régions Arides, Médenine, **TUNISIA** [IRA]
- The Samuel Roberts Noble Foundation, Forage Improvement Division, Ardmore, OK, **USA** [Noble F]



Lucerne and pea crop improvement

Ecological breeding and variety selection

Large breeding populations of lucerne genotypes and pea advanced lines underwent yield testing in different stress-prone agricultural environments for phenotyping and variety selection (Figure 1). Phenotyping platforms were used to assess their ability to reproduce genotype responses in drought-prone sites, for implementing evolutionary selection, and for heat tolerance studies.

Genome-enabled selection

Lucerne and pea reference populations were genotyped by genotyping-by-sequencing (GBS) methods, to define genomic selection models for yield in specific conditions and for genome-wide association (GWAS) studies.

Legume-based crop optimization

Innovative forage crops including pea (semi-dwarf or tall), common vetch or Narbon vetch in pure stand, binary and complex mixtures with oat and triticale, and lucerne (erect or semi-erect) in pure stand, binary and complex mixtures with tall fescue and cocksfoot, were evaluated for yield, quality and farmers' acceptance in rain-fed sites of Sardinia (Italy), Morocco and Algeria.

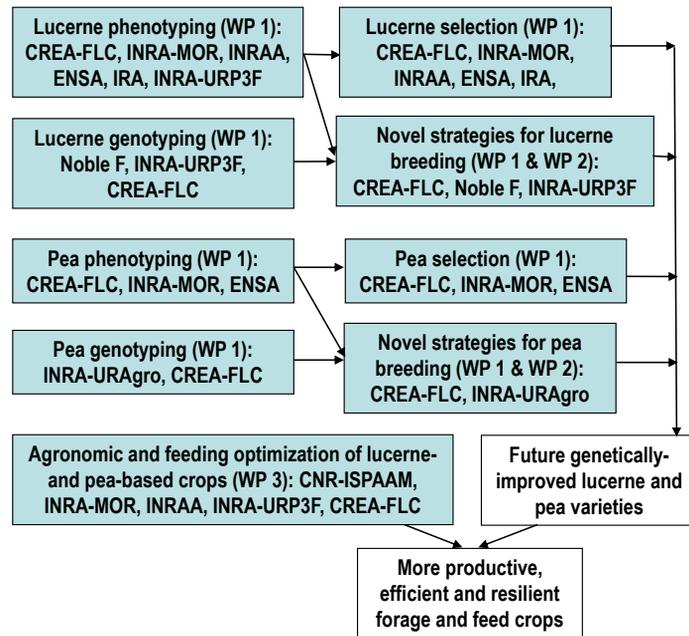


Figure 1. Partner tasks and interrelationships

Why collaborating?

Most activities implied close collaborative work and synergies among partners (Figures 1 and 2). Transnational cooperation provided remarkable added value, allowing for:

- Sharing the cost and effort of extensive phenotyping and the technical challenges required for the development of genome-enabled selection tools.
- Identifying opportunities for long-term cooperation between breeding programmes that share similar challenges and target environments.
- Improved understanding of adaptive responses of innovative crops and genotypes (through testing across climatically-contrasting sites).
- Exploiting plant responses in harsh environments of north Africa to improve the adaptation to climate change of crops and varieties in southern Europe.

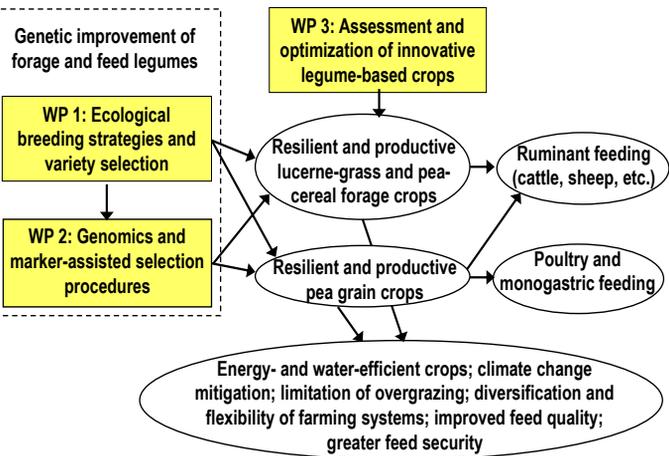
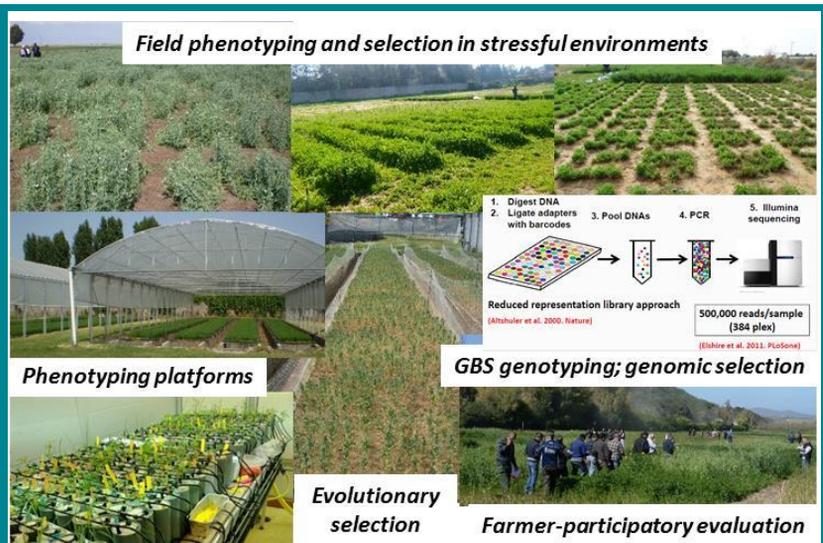


Figure 2. Work packages and their interrelationships

Figure 3. Some major research activities and study tools



ACHIEVEMENTS

Ecological breeding and variety selection

Lucerne

- Large numbers of half-sib families (over 150) tested in large phenotyping platforms showed modest correlation for forage yield or persistence across moderate and severe drought. Only moderate correlations emerged across pure stand and mixed stand with grasses.
- On-going trials in agricultural environments will allow to assess correlations across platform and field conditions, different stress types (drought, salinity), and different drought-prone sites. Synthetic varieties will be bred from each testing site from locally best plants.
- Growth chamber experiments highlighted fairly modest genetic variation for heat tolerance.

Pea

- Inbred lines issued by an inexpensive evolutionary breeding scheme implying natural selection under severe drought for three segregating generations were 18% higher yielding under severe drought than lines conventionally bred by a single-seed descent scheme.
- Varieties will be selected after completing the on-going phenotyping of 315 lines in various drought-prone sites.

Genome-enabled selection

Lucerne

- GBS provided many polymorphic SNP (Single Nucleotide Polymorphism) markers (10,000-15,000 for 20%-50% genotype missing data thresholds) for a reference population including 154 plants. Best genomic selection models for plant breeding value (based on yields of plants' half-sib families) in moisture-favourable conditions revealed accuracy (i.e. correlation between modelled and observed values) near 0.35 (Figure 4). This may imply definitely greater gains per year for genomic selection than phenotypic selection. GWAS revealed many QTL (Quantitative Trait Loci) with small effect, further reinforcing the interest of a genomic selection approach.
- Genomic selection for drought-prone, saline or grazed environments and forage quality, and GWAS, will be explored after completing the on-going phenotyping.

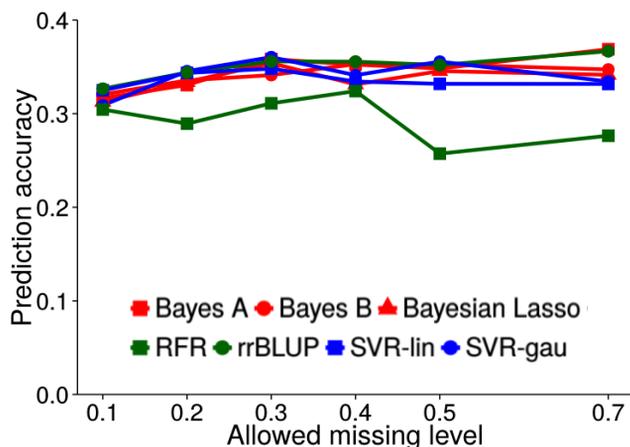


Figure 4. Accuracy of genomic selection models for lucerne breeding value (for varying genotype missing data thresholds). Genomic selection is accurate enough for implementation

Pea

- GBS provided at least 2,500-3,000 polymorphic SNP markers in three connected crosses between elite parent lines (each cross including 105 lines). Genomic selection displayed outstanding accuracy (> 0.70) in two crosses, and high accuracy (> 0.50) in one cross, for prediction of grain yield under severe terminal stress in phenotyping platforms. A preliminary GWAS study showed extensive co-localization of yield and early flowering (stress escape) QTL, and mostly small-effect QTL for drought resistance.
- A robust genomic selection model will be developed after completing the field phenotyping in drought-prone sites. More thorough GWAS studies are under way.

Legume-based crop optimization

- Results for 16 annual crops averaged across two years (2014-2015) and two rain-fed sites highlighted the high yield and farmers' acceptability of pea (in pure stand or mixed stand with oat) as a rain-fed forage crop (Table 1), confirming the interest assigned to pea in this project.

Table 1. Yield and farmers' global acceptability score of 16 forage crops, averaged across two years and two regions (Sardinia; inland Morocco). Pea in pure stand or mixed stand with oat combined high yield with high farmers' acceptability

Crop	Dry matter yield, t/ha	Farmers' score, 1-5 (5=highest)
Semi-dwarf pea	6.96	4.40
Tall pea	7.18	4.41
Common vetch	4.94	4.37
Narbon vetch	2.90	3.60
Oat	7.20	3.53
Triticale	5.91	2.82
Semi-dwarf pea - Oat	6.51	3.61
Tall pea - Oat	7.14	4.11
Semi-dwarf pea - Triticale	5.95	3.80
Tall pea - Triticale	6.39	3.91
Common vetch - Oat	6.77	4.07
Common vetch - Triticale	5.74	3.83
Narbon vetch - Oat	5.62	3.74
Narbon vetch - Triticale	4.81	3.38
Peas - Cereals complex mixt.	6.58	3.75
Vetches - Cereals complex mixt.	6.22	4.13

- These two-year results also revealed modest farmers' acceptability of cereal pure stands, a trend for mixtures to out-yield the mean yield of their components' pure stands (mainly in Morocco), and some advantage of complex mixtures over binary mixtures in Sardinia.
- Results from the same trials indicated good forage yield and persistence of lucerne in pure or mixed stand – even in Morocco, where lucerne is usually irrigated. This reinforces the project aim to breed drought-tolerant lucerne varieties for use in rain-fed systems.
- Final indications from 2014-2016 testing in these sites and one site in inland Algeria will allow to thoroughly assess perennial crops and compare them to annual crops. Forage quality data under generation will allow to consider crop utilization in crop recommendations.



Stakeholder engagement

- ❑ The extensive participation of farmers to crop evaluation allowed to verify project goals and to identify and promote the most promising legume-based crops.
- ❑ Relationships with scientists and extension officers were stimulated by two scientific workshops (Rabat and Médenine) and three seminars organized by the project - with two more workshops planned in 2016. Visits to the experiments were organized for seed companies in Italy.
- ❑ The project will produce a free electronic handbook of guidelines for cultivation and use of lucerne- and pea-based crops aimed to extension services and farmers.
- ❑ Results are disseminated via talks and posters in major conferences (EUCARPIA, FAO-CIHEAM, Int. Legume Soc., Plant & Animal Genome) and scientific articles.
- ❑ Experiment activities offered opportunities for training of students and young researchers, also through stages.
- ❑ REFORMA showed large scope for implementing more resilient, water- and energy-efficient, self-sufficient feed systems based on genetically improved legumes.
- ❑ Inter alia, REFORMA issued the first study of genome-enabled prediction of lucerne plants' breeding value, and unprecedented genomic selection results on pea.

Next steps

- ❖ Project activities and variety breeding (producing at least 3 candidate varieties for both lucerne and pea) will be completed using 2016 field data. We will also exploit data from two institutions (INTA, Argentina; IFC, Serbia) that joined our phenotyping work with own funds.
- ❖ The inclusion of pea or lucerne candidate varieties in variety registration trials (or other variety trials) will provide verification and promotion of this new material.



Figure 5. Project's relationships with stakeholders

- ❖ Our results on genome-enabled and ecologically-based selection procedures could modify in depth the strategies of lucerne and pea breeding for stressful environments.
- ❖ Their application in new regional breeding programmes could allow, inter alia, to compare our phenotypic selections with new material bred by genomic selection.
- ❖ Pea- and lucerne-based crops that showed highest yield and farmers' acceptance should be tested in on-farm trials, using lucerne and pea varieties bred by the project (presumably more adapted than those tested hitherto).
- ❖ Besides verification of innovative breeding techniques and most promising innovative crops, research and support actions will be needed for strengthening pea and lucerne seed production systems in Mediterranean areas.

Do you want to know more?

Contact: Dr Paolo ANNICCHIARICO

paolo.annicchiarico@crea.gov.it

Website: <http://reforma.entecra.it>

For more information on the ARIMNet2 Network

www.arimnet2.net

Contact: arimnet@arimnet.net

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