



**NAGREF**

NATIONAL  
AGRICULTURAL  
RESEARCH  
FOUNDATION

**Agricultural Research  
Center of Northern Greece  
(ARCNG)**

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## **SEELEGUMES**

**Research Activities 2011-2012**



## SEELEGUMES

### Legume Genetic Diversity of Balkan Peninsula

- ▶ the large legume biodiversity of the Balkan Peninsula remains still unexplored
- ▶ local landraces were evolved under the micro-climatic environmental conditions, harboring invaluable genetic traits
- ▶ assessment of genetic diversity is timely and indispensable



## SEELEGUMES

### Current trends in agriculture and nutrition

- ▶ well-adapted cultivars in view of the forthcoming climate change
- ▶ production of high added value products
- ▶ of all legumes, chickpea is an important source of nutrients for human diet, particularly for sensitive groups and an excellent alternative to soybean for livestock feed



## SEELEGUMES

**Assessment of legume genetic diversity provides means for:**

- ▶ **coupling conservation and biotechnology actions**
- ▶ **conservation of legume genetic resources**
- ▶ **utilization in breeding strategies**
- ▶ **exploitation in production of high added value food products**



## SEELEGUMES

♠ Large plant biodiversity of Greece

♠ Project timeframe - 24 months

♠ *Cicer arietinum*

### *Cicer arietinum*





## SEELEGUMES

### Goals

- ▶ collect chickpea varieties and local landraces
- ▶ morphological and genetic characterization
- ▶ assess genetic variability





**SEELEGUMES**

## Collection *Cicer arietinum* accessions

A/A	Cultivar Name	Location of origin
1	Amorgos	NAGREF
2	Andros	↓
3	Gavdos	
4	Gravia	
5	Evros	
6	Thiva	
7	Kerinia	
8	M14719	
9	Serifos	
10	Sifnos	
11	E210	
12	E216	NAGREF
	<b>Local landraces</b>	<b>all over Greece</b>
	52 accessions	

**A total of 64 chickpea accessions**





Map of Greece indicating origin of *Cicer arietinum* collection of local landraces

SEELEGUMES





## SEELEGUMES

### Experimental Plan

- ▶ **Morphological characterization of *C. arietinum* accessions**
- ▶ **Growth of plants in greenhouse**
- ▶ **Molecular characterization**
- ▶ **Assessment of genetic diversity of Greek accessions**



## Molecular characterization of *Cicer* accessions

### SEELEGUMES

#### Isolation of genomic DNA

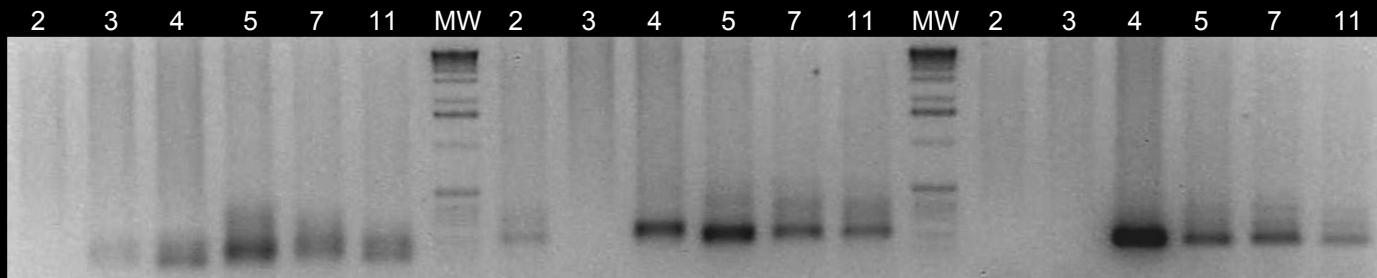
Extraction of gDNA has been performed from individual samples of selected accessions. Quality and quantity of isolated gDNA has been assessed spectrophotometrically.



#### Selection of molecular markers



#### PCR analysis of gDNA



Cultivars are as follows:  
2= Andros  
3= Gavdos  
4= Gravia  
5= Evros  
7= Kerinia  
11= E210



# Identification and calculation of MW of SSR bands using the GelAnalyser software.

SEELEGUMES

GelAnalyzer - gel\_9-5-2012\_TA2\_SSR.gap

File Analysis Windows Help

Image - gel-part1.jpg

Zoom: 75 %

Profile

Zoom: Fit profile

Intensity

Pixel

Analysis info

Show

Lane #	Band #	Rf	Raw volume	Cal. volume	MW
1.	1.	0.07	1297	-	398
1.	2.	0.264	1311	-	300
1.	3.	0.496	1158	-	200
1.	4.	0.775	1496	-	100
2.	1.	0.589	4020	-	164
3.	1.	0.527	4228	-	188
4.	1.	0.55	4600	-	179
5.	1.	0.527	4169	-	188
6.	1.	0.558	5730	-	176
7.	1.	0.527	3623	-	188
8.	1.	0.535	3934	-	185
9.	1.	0.589	1893	-	164
10.	1.	0.659	3261	-	139
11.	1.	0.589	4789	-	164
12.	1.	0.558	3042	-	176

Cal. curves

MW Cal Quantity Cal

Bps

Rf

$y = 782.595 * e^{(-0.7244486 * x)} - 346.34784$

$R^2 = 1.0$



## SEELEGUMES

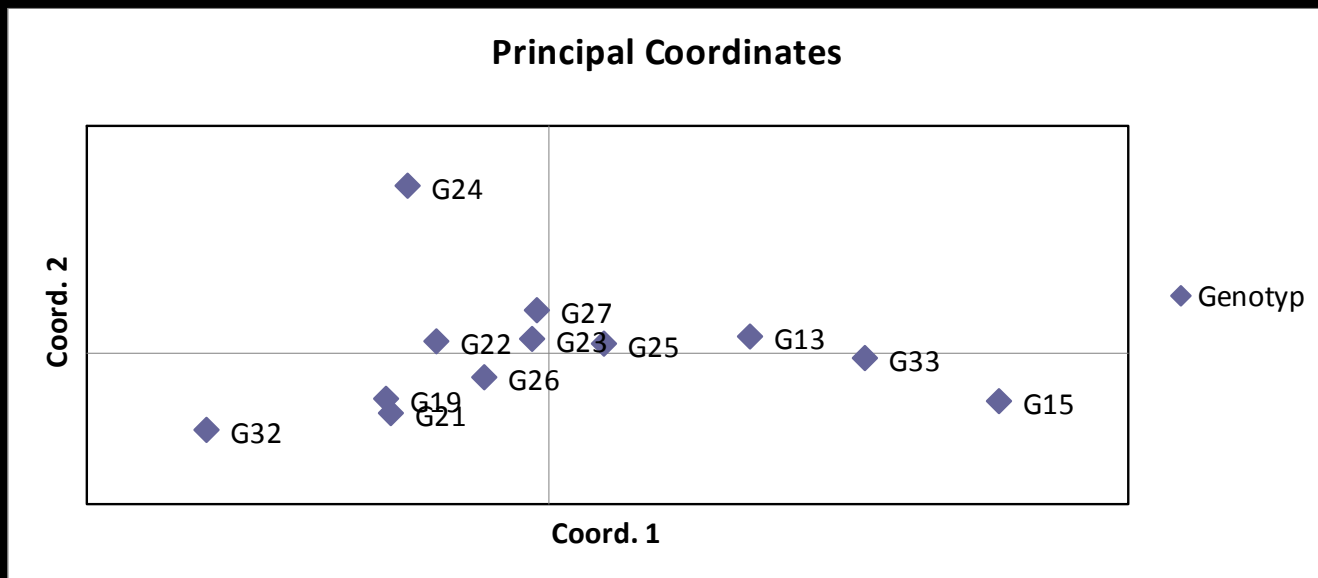
### Preliminary results of genetic analysis for

- ▶ 12 chickpea landraces
- ▶ Using a set of TA SSR markers
- ▶ Statistic analysis of genetic relationships



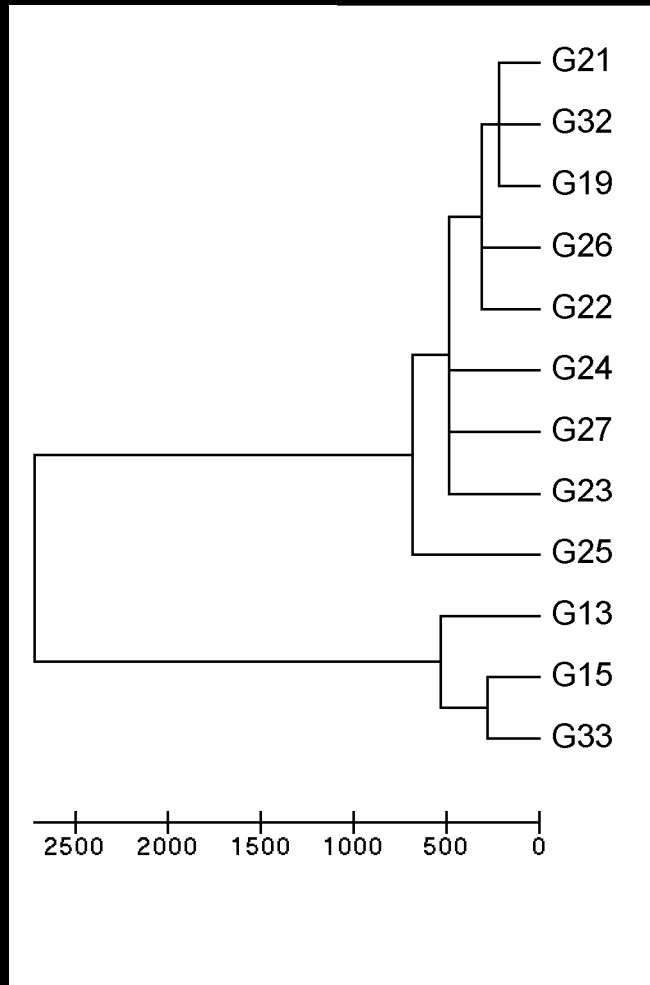
## SEELEGUMES

### A Principal Coordinate Analysis using Genetic Distance data





## SEELEGUMES



**Dendrogram for 12 chickpea landraces derived from UPGMA cluster analysis using simple matching dissimilarity index based on a set of SSR markers.**





## SEELEGUMES

### On going

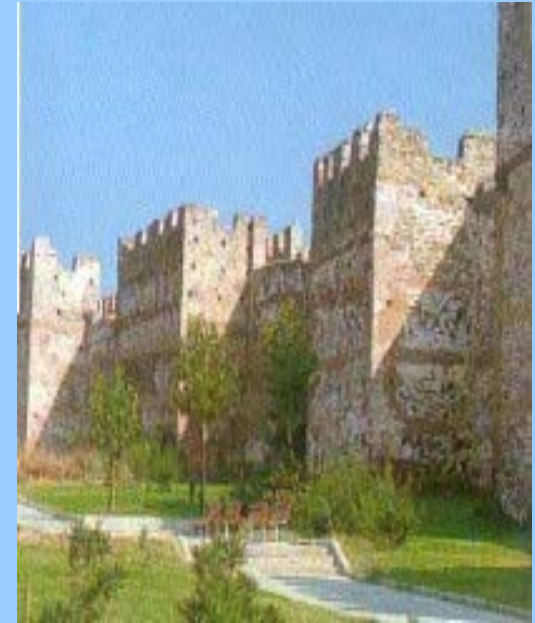
- ▶ Genetic analysis of genotypes using more SSR markers
- ▶ Estimation of correlation between genetic and geographic distance
- ▶ Determine population structure



## SEELEGUMES

### Also

- ▶ Collection of 48 accessions of *Cicer arietinum* from Balkan countries
- ▶ Isolation of gDNA from each accession
- ▶ Assessment of chickpea genetic diversity by Peter Winter's group



**Thank you !**





# Legume plant breeding

## Perspectives and Actions

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# Legume crops in Europe

Occupy 3-5% of the Europe's arable land

Supply only 30% of the protein crops consumed as animal feed in the EU

EU imports significant amounts of protein crops

Significant decrease of legume research projects in Europe

# Genetics and Plant Breeding

novel ways  
to  
manipulate  
genetic  
variability

stabilize  
genotype X  
environment  
interactions

# What are the Breeding targets for a modern legume breeding program?



## Breeding for:



Resistance to Biotic stresses  
diseases, pests, competitiveness to weeds



Resistance to Abiotic stresses  
drought, salinity, degraded soils, cold tolerance, climatic change etc.



Adaptation to Modern Culture Systems  
(sustainable culture systems as organic culture systems,  
low-input culture systems etc



# *What are the Breeding targets for a modern legume breeding program?*



**Breeding for:**

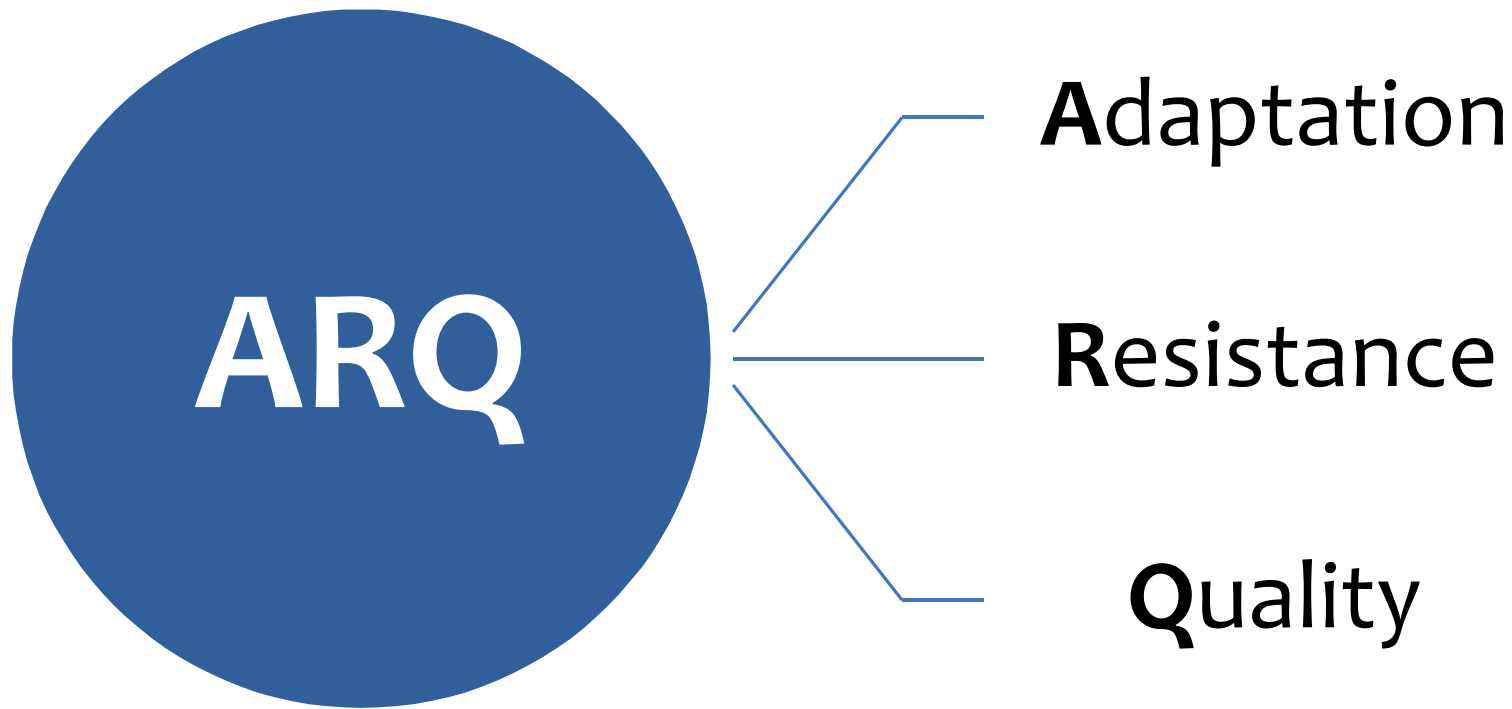


Resource use efficiency (WUE, PUE)



Quality for food and feed products (pulses: taste, color, shape, nutritional value and the ability to retain the quality characteristics, fodder crops: low level of anti-nutritional factors, high level of protein content)

*What is the legume ideotype that fit  
in the modern agricultural needs?*



# *What is the method for the development of varieties with ARQ?*

The crucial point during the breeding process is the need for a reliable and early indication about the genetic potential of the material evaluated in the field.

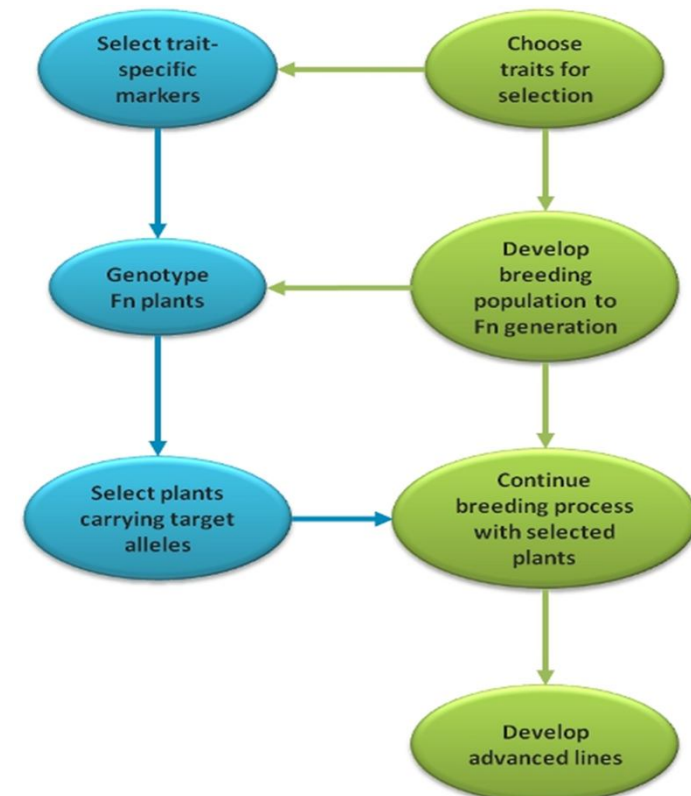
Classical breeding has proved to be effective, although it is often constrained by environmental fluctuations that make breeder's efforts more difficult and time-consuming.

New technologies could be exploited to make the procedure more efficient and precise

# New technology-New breeding tools

## Genomics

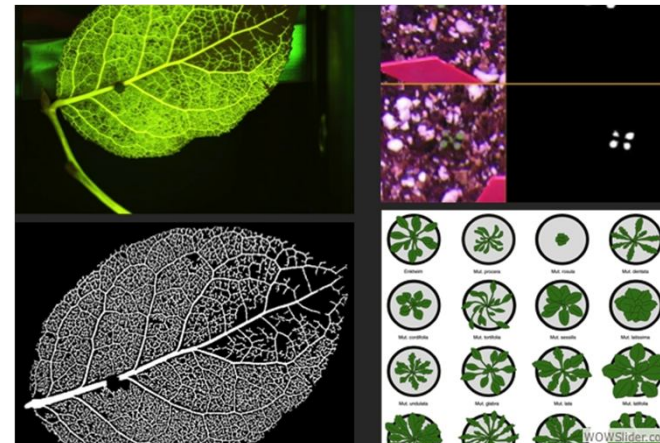
- Complete genome sequences allows a more efficient characterization of agronomically important genes and along with marker assisted selection may contribute efficiently in achievement of plant breeding targets



# New technology-New breeding tools

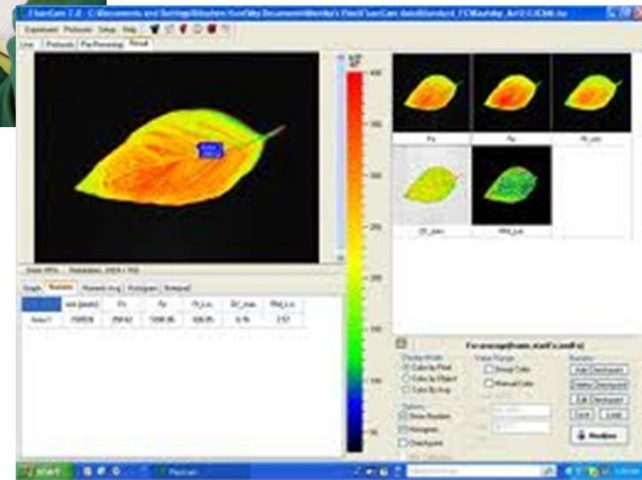
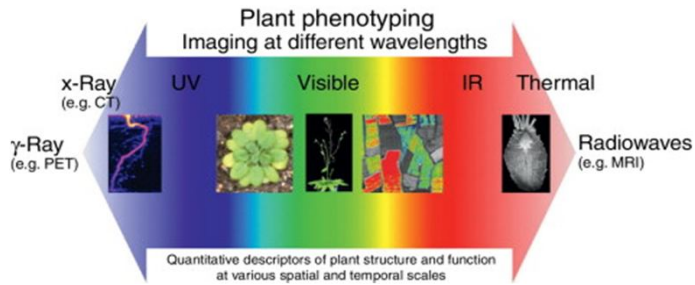
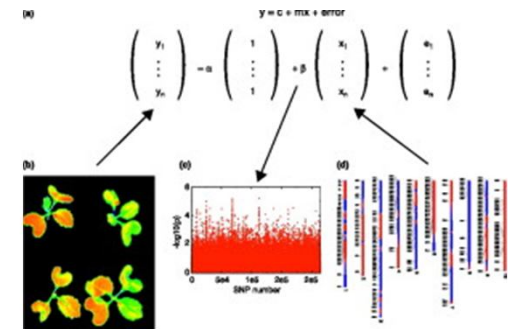
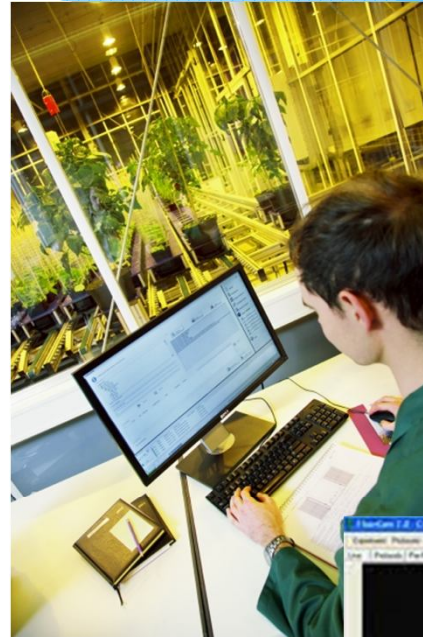
## Phenomics

- Non-destructive phenotyping provides a valuable new tool which allows the dissection of plant genotypes into a series of component traits in various developmental stages





# High through-put Phenotyping



## *In conclusion*



The skill and success of plant breeders will be determined by their ability to use their resources efficiently, retaining those proven methodologies and augmenting them with novel approaches to meet their breeding targets.



# Fodder Crops & Pastures Inst.



FCPI was  
founded in  
1933

Located in  
Central  
Greece in the  
city of  
Larissa

# Crops of research interest



Fodder Crops cultivated for grain production

*Vicia faba, Lathyrus cicera, Cicer arietinum, Soybean, Lupines*



Fodder Crops cultivated for silage or hay production

*Medicago sativa, Trifolium sp., Vicia sativa, Pisum sativum*



Pulses

*Lentil, Chickpea, Phaseolus sp. , Lathyrus sativus*

# Scientific personnel

Dr. C. Tsadilas – Senior Researcher (Director)

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J. Researcher  
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Dr. D. Baxevanos

J. Researcher  
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# Objectives of the Fodder Crops & Pastures Inst.



Applied breeding research for the development of new varieties in fodder crops, pastures and pulses with stability and high yield production (Long-term research programs)



Basic breeding research is conducted mainly in collaboration with other Institutes and Agricultural Universities.



Cultivation technique and culture systems (Intercropping, Low-input and organic culture systems)



Seed production for breeder's seed, pre-basic and basic seed of the cultivars developed in the FCPI and registered in Greek National Catalog.



# Funding resources





# Breeding methodology

## Honeycomb selection

- Basic principal: The unit of evaluation and selection is the individual plant grown at the critical distance, where the range of phenotypic expression is enlarged and the negative effect of the competition on selection efficiency is eliminated
- In honeycomb field layout each single plant holds the centre of a circular complete replicate.
- Honeycomb Selection is supported by a special statistical program





# Pre-breeding programs

Evaluation of  
different kinds of  
genetic material:  
varieties, breeding  
lines, genebank  
resources,  
populations, mixtures





# Weed tolerance & Weed suppression ability of common vetch varieties under low-inputs

**WT:** the ability of the variety to yield high despite the presence of weeds

**Indirect selection Indices:** Relationship among phenotypic growth traits, yield, WT and WSA

**WSA:** the ability of the crop to suppress weed development



# Selection for enhanced yield and tolerance to viral and vascular diseases within lentil landraces (Funding: FP7/2011-15)

Evaluation of Greek landraces in order to develop lentil varieties with resistance to Fusarium wilt and viral diseases

Identification of molecular markers that are tightly linked with the resistance genes in order to develop a protocol for Marker Assisted Selection



Viral infection



Infection of Fusarium wilt

