

ILS2 Session 4 overview: Genetic resources

Genetic Resources: From Mendel's peas to underutilized legume species

Chaired by Rodomiro Ortiz^{1*} and Hari D. Upadhyaya²

Abstract: Plant domestication is evolution in a human-made environment. A diversity “bottleneck” changed the sample of genes passing from one generation to another. Today's crops depend on humans for habitat and propagation because some of desired traits are often maladaptive in nature. Legume genetic resources (wild species, landraces, cultivars, breeding lines, segregating populations, genetic stocks and mutants) are most often used for studying genetic diversity, agro-morphological and nutritional quality traits, and host plant resistance to pathogens and insect pests. They also offer means for understanding plant domestication. Their diversity also shows a great potential for improving crops. Advances in omics are providing new knowledge for using this germplasm diversity in legume genetic enhancement.

Key words: chickpea, cowpea, DNA markers, lupin, mutants, pea, pulses

Legumes are the second most important family (*Fabaceae*) of crop plants after the grass family (*Poaceae*). As noted by the key lecture given by Prof. Noel Ellis (his presentation may be viewed on YouTube, <https://www.youtube.com/watch?v=kQNh-mEBktho>) in the Session on Genetics Resources at the Second International Legume Society Conference (ILS2, Tróia, Portugal, 2016.10.12) there are a variety of forms for various traits in legume germplasm (Figures 1a, 1b and 1c). They are broadly defined as either natural or bred populations. Segregating individuals or lines derived from crossing are included in the latter. Gregor Mendel (1866) was the first to show –using peas (*Pisum sativum*)– the value of genetic analysis to understand trait inheritance (6), while N.I. Vavilov (1920) began comparative genetics with the law of homologous series in variation (10), which established parallelism in the variability of organisms; i.e., a particular variation observed in a crop is also expected to be available in its related species.

Allelic diversity and genetic analysis

Herman Joseph Muller (1927) and C. Auerbach (1946) led the discovery of

mutations induced by X-rays (7) and ethyl methane sulfonate or EMS (1), respectively. Mutants are other source of useful allelic variation, and they also provide a powerful analytical tool for legume genetics. They are used to detect genes regulating a process (forward genetics) or to identify processes regulated by a gene (reverse genetics), while wild or bred populations allow finding genes that survived selection. Prof. Ellis provided three examples from research in which he was involved to illustrate their use. The characterization of mutants shows that novel filamentous leaf organs present in peas and their allies known as tendrils are modified leaflets inhibited from laminar development by the action of the *Tendrill-less* gene, which encodes a Class I homeodomain leucine zipper transcription factor (4). Mendel used flower color to study inheritance in pea. The genome sequence of model legumes along with their synteny to the pea genome facilitated the finding of candidate genes determining anthocyanin pigmentation in pea (2). Likewise, the DNA marker analysis of genetic diversity of a large pea germplasm collection provided a means for understanding variation and evolution of this species as well as for defining a core subset of *Pisum* considering both the major distinct gene pools within this genus and their

¹ Swedish University of Agricultural Sciences (SLU), Sweden

² International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India

*rodomiro.ortiz@slu.se



Figure 1a. Diversity for pod (left) and seed (center and right) traits in chickpea

geographical distribution (5).

Restriction site associated DNA (RAD) sequence data also enabled the study of the genetic determinant of the mutant *Stipules reduced* (St) (9) in peas, which was a transcription factor on chromosome 5 (3). These examples show both the different types of population that are available for further characterization of genetic determination of traits in legumes, and the role of genetic analysis in the high

throughput omics era.

Population bottlenecks in domestication

Eric von Wettberg (Florida International University) illustrated how he and co-workers used chickpea (*Cicer arietinum*) to elucidate loss of genetic diversity due to strong selection when domesticating annual crops. Their work included collecting wild relatives of chickpea in areas within the main

center of diversity (~ 60,000 km²) for about 2 months. This study shows that a thorough survey of source population for wild relatives along with their habitats may allow to infer the variation level that was available to early farmers as well as about the shifting environments resulting from the beginning of agriculture in the Fertile Crescent. Likewise, their research calls for having in depth collecting of crop wild relatives and landraces for making suitable conjectures about domestication.

A model for understanding plant domestication

Domestication offers an insightful model for understanding causes and consequences of evolution. Thousands of years took for taming a wild plant into a productive agricultural crop. Plants accumulated – through a gradual process– domestication traits that increase their usefulness to people. Fully documented, scientific age events are known for a 20th century domesticate such as narrow-leaved lupin, which was grown as a green manure and fodder crop in the Baltic States in the 19th Century and became a high-quality grain in the 20th century. Matthew N. Nelson (Royal Botanical Garden Kew, United Kingdom) told how he and research partners used 11690 DArTseq SNPs with unambiguous locations in the narrow-leaved lupin genome for genotyping 233 accessions (147 wild and 86 cultigens) and studying both its recent and rapid domestication. Their research confirms that modern era domestication in narrow-leaved lupin reduced diversity, generated a differentiated gene pool and increased linkage disequilibrium. It appears that the founder populations of narrow-leaved lupin were of Iberian origin. The dominant mutation in the *Ku* locus removed the vernalisation requirement for flowering, thus allowing the adaptation of narrow-leaved lupin to cropping in southern Australia and northern Europe. *Ku* has been widely used in lupin breeding to confer early flowering and maturity (8). Reduced seed indehiscence and alkaloid content, flower color and removal of physical seed dormancy are other domestication traits in narrow-leaved lupin. This research highlights the impact of domestication on genomic-wide diversity in a modern legume crop.

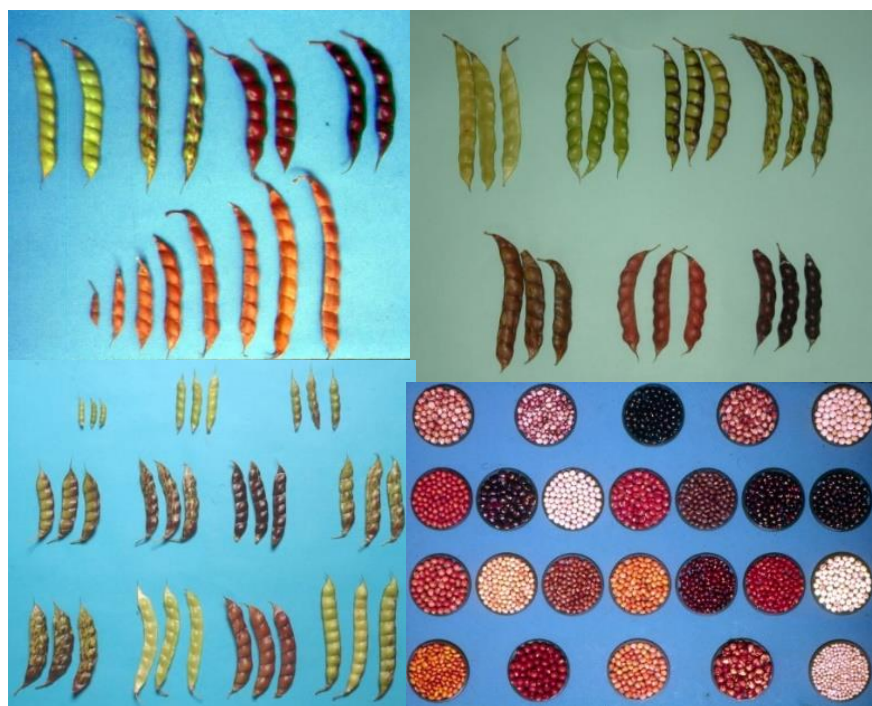


Figure 1b. Diversity for pod (top and bottom-left) and seed (bottom-right) traits in pigeonpea



Figure 1c. Diversity for pod (left) and seed (right) traits in groundnut (or peanut)

Characterizing cultigen pools with high density DNA markers

Single-nucleotide polymorphisms (SNPs) are utilized for studying genetic diversity in crop gene pools. Such an assessment allows an enhanced conservation genetic resources and their further use in plant breeding. Márcia Carvalho (Centro de Investigação e Tecnologias Agroambientais e Biológicas, Portugal) described her PhD research for characterizing the genetic diversity of 33 cowpea (*Vigna unguiculata*) landraces from Portugal and other 63 from elsewhere with the Illumina Cowpea iSelect Consortium Array that contains 51,128 SNPs. She found 44,054 high quality polymorphic SNPs useful for population genetics research using STRUCTURE v2.3.4 and principal component analysis with TASSEL v5.0. There were four subpopulations in these 99 cowpea accessions. The accessions belonging to the subspecies *sesquipedalis* were together in subpopulation 1, while the clusters of those belonging to subspecies *unguiculata* related to their geographical origin. There were two cowpea accessions from Portugal that were categorized as either admixed or belonging to other subpopulation (instead of being in the Mediterranean cluster). This study shows the value of using a high-density SNP array to characterize sources of genetic diversity, which is a requisite for the success of legume breeding.

Valorizing genetic variation

LupiBreed began in the Spring of 2015 with the goal of improving productivity and yield stability on both narrow-leaved sweet lupin (*Lupinus angustifolius*) and yellow sweet lupin (*L. luteus*). K. Fisher (Julius Kuhn Institute, Germany), on behalf of her public and private partners, provided an overview on how EMS-based mutagenesis allows broadening the genetic variability in advanced breeding materials of narrow-leaved lupin. Their research led to identifying and selecting novel growth types with high yield potential, which are undergoing multi-environmental testing. Preliminary results are very encouraging since some M lines had both higher yield and higher protein content than their “donor”. EMS mutagenesis provides a means for developing high yielding M lines showing great variability for growth type, maturity, pod number, seed number and weight, and protein yield.

Conference Session presentations:


Ellis N (2016) Where are we after 150 years of legume genetics? In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 35

von Wettberg E, Chang P, Singh V, Cordeiro M, Greenspan A, Alford B, Carrasquilla N, Dacosta-Calheiros E, Warschewsky E, Rouf Mir R, Bukun B, Kahraman A, Aydoğan A, Berger JD, Nuzhdin SV, Penmetsa RV, Cook DR (2016) Using expanded collections of wild relatives of chickpea to understand domestication and improve climate resilience. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 88

Carvalho M, Castro I, Matos M, Lino-Neto T, Close T, Munoz-Amatriain M, Carnide V (2016) Characterizing the genetic diversity of cowpea accessions using a high-density SNP array. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 89

Fischer K, Roux S, Jansen G, Ulrich H-J, Dieterich R, Wehling P, Ruge-Wehling B (2016) LupiBreed - Valorisation of novel genetic variability in narrow-leaved lupin. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 90

Nelson M, Mousavi M, Taylor C, Bayer P, Kamphuis L, Berger J, Clements J, Hane J, Edwards D, Erskine W, Cowling W (2016) Domestic bliss? Causes and consequences of a modern era domestication event in narrow-leaved lupin. In Second International Legume Society Conference ILS2, Tróia, Portugal, 11-14 October 2016. ITQB NOVA, Oeiras, Portugal. p. 91

There were other 20 posters related to the ILS2 Session on Genetics Resources including the model legume species barrel medic (*Medicago truncatula*) and pulses such as broad bean (*Vicia faba*), chickpea, common and climbing beans (*Phaseolus vulgaris*), cowpea, grass pea (*Lathyrus sativus*), lentil (*Lens culinaris*), pea, tepary bean (*Phaseolus acutifolius*) and white lupin (*Lupinus albus*), among others. The research subjects vary from germplasm conservation, characterization, evaluation and regeneration, as well as gene identification, genetic diversity analysis, and a participatory approach involving farmers, the local government and scientists to identify sources of interesting traits for further pre-breeding. 

References

- (1) Auerbach CC, Robson JM (1946) Chemical production of mutations. *Nature* 157:302
- (2) Hellens RP, Moreau C, Lin-Wang K, *et al.* (2010) Identification of Mendel's white flower character. *PLoS ONE* 5(10): e13230
- (3) Hofer JMI, Moreau C, Ambrose M, *et al.* (2014) Sequence analysis of a pea (*Pisum sativum*) fast neutron mutant population identifies the gene corresponding to *Stipules reduced*. In 6th International Food Legume Research Conference – 7th International Conference on Legume Genetics and Genomics; Saskatoon, Saskatchewan, Canada; 7–11 July 2014. University of Saskatchewan, Saskatoon, Canada, p 38
- (4) Hofer J, Turner L, Moreau C, *et al.* (2009) *Tendrill-less* regulates tendrill formation in pea leaves. *Plant Cell* 21:420–428
- (5) Jing R, Vershini A, Grzebyta J, *et al.* (2010) The genetic diversity and evolution of field pea (*Pisum*) studied by high throughput retrotransposon based insertion polymorphism (RBIP) marker analysis. *BMC Evol Biol* 10:44
- (6) Mendel G (1866) Versuche über Pflanzenhybriden. *Verhandlungen des naturforschenden Vereines in Brünn*, Bd. IV für das Jahr 1865, Abhandlungen, 3–47
- (7) Muller HJ (1927) The problem of genetic modification. *Zeit ind Abst-und Verer Suppl* 1:234–260
- (8) Nelson MN, Książkiewicz M, Rychel S, *et al.* (2016) The loss of vernalization requirement in narrow-leaved lupin is associated with a deletion in the promoter and de-repressed expression of a *Flowering Locus T* (FT) homologue. *New Phytol* doi: 10.1111/nph.14094
- (9) Pellew C, Sverdrup A (1923) New observations on the genetics of peas. *J Genet* 13:125–131
- (10) Vavilov NI (1922) The law of homologous series in variation. *J Genet* 12:47–69