

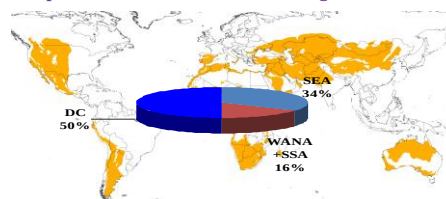
Genomics enabled Improvement of Lentil

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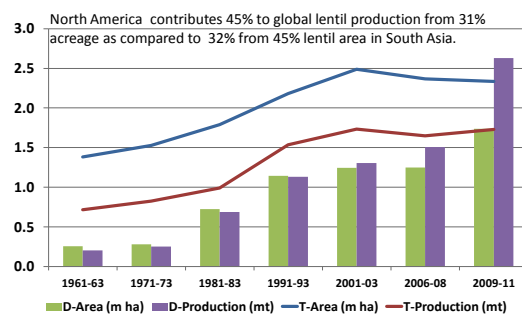


Outline of Presentation

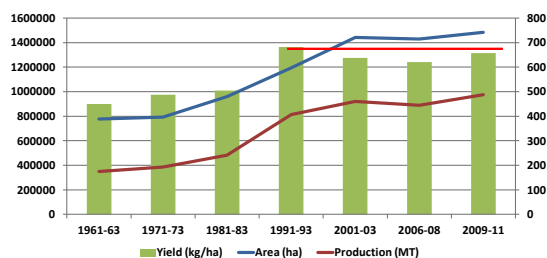
- Introduction
- Genetic Resources
- Genomic Resources
- Trait-Marker Association
- Prospect of Genomics enabled Breeding



But the growth is in the developed countries.....



Lentil yield in Indian Subcontinent is stagnant...



Enhancing economic competitiveness and stability in performance of lentil- a major challenge



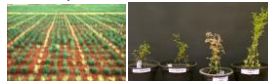


Precision phenotyping – a priority

FW and salinity screening in hydroponics



Wilt sick plots for Fusarium wilt



AB screening



Orobanche screening



Cold screening



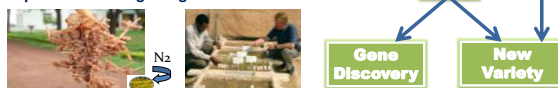
Drought and heat screening



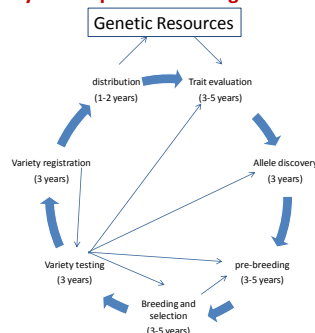
Gene Discovery & Deployment in Varieties

- Heat and drought tolerance
- Biological nitrogen fixation
- Extra short duration
- Herbicide tolerance
- Orobanche tolerance
- Machine harvestability
- Biofortification

In addition to key diseases and insect pests of the target region



Variety development is a long and time consuming process

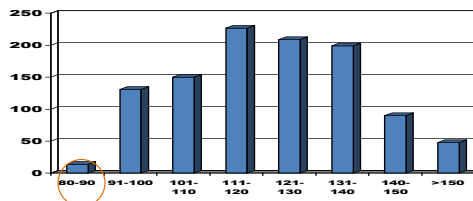


- From trait evaluation to gene discovery to its deployment into varieties is long drawn process taking as many as 12-15 years.
- Urgent need for enriched genomic and genetic resources



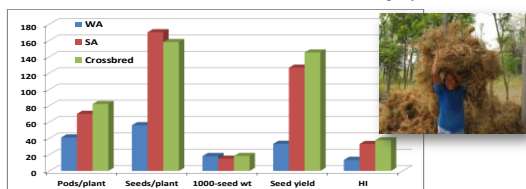
Pre-breeding efforts for earliness

- Wild species and land races: Source of useful genes
- Progenies of crosses between SA and WANA germplasm for South Asia
- New genes for earliness identified through diverse crosses (SA, WANA and S. America)
- Cross between late x late parents – early segregants
- Cross between early segregants and early parents from South Asia resulted in extra early
- Genes for earliness in West Asia germplasm different from South Asia and S. America
- Two decades of consistent efforts



Widening the genetic base of lentil in South Asia- Introgression of diverse genes for high yield

- Elites lines emanating from SA x SA, WA x WA, and SA x WA crosses
- Seeds /plant more in SA x SA and seed weight in WA x WA crosses
- Progenies emanating from SA x WA crosses showed high grain yield because of more pods per plants, higher HI and improved seed size
- Two decades of consistent efforts of more than 500 crosses per year

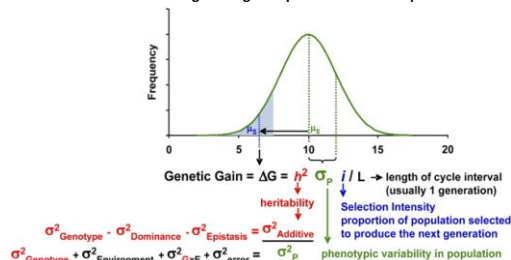


How to enhance breeding efficiency and genetic gains?



Integration of genomics tools enhances the genetic gain

The genetic gain equation and its component variables

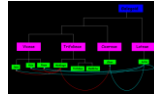


Moose S. P., Mumm R. H. Plant Physiol. 2010:147:969-977

Genomics Enabled Improvement

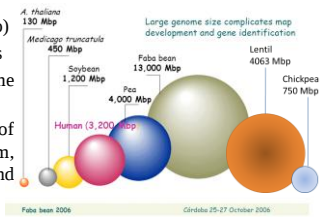
- **EST (expressed sequence tags) sequencing and gene annotation-**
 - Development of EST derived simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) markers
- **Construction of high-density genetic linkage maps**
- **Phenotyping for target traits**
 - Biotic stress tolerance (FW, Rust, AB)
 - Abiotic stress tolerance (Drought, heat, cold, salinity, boron toxicity)
 - Quality traits (Fe, Zn)
- **Identification of QTLs associated with key traits**
- **Marker-assisted selection for varietal development**

Model legumes: *M. truncatula* – 500 Mbp; *L. japonicus* – 470 Mbp



Major Constraints in Genomics assisted Lentil Breeding

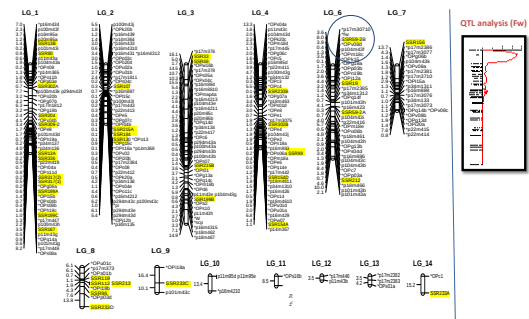
- Large genome size (>4 gbp)
- Limited molecular markers
- No high density genome wide maps
- Limited availability of appropriate germplasm, mapping populations and phenotyping data
- Non-availability of trait-allele association for marker assisted breeding



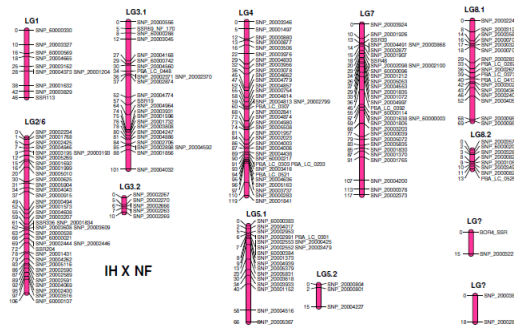
Genome-wide molecular maps in lentil is lacking

Population used for Mapping	No. of loci	Type of markers	Genetic map length (cM)	References
F2 (<i>Lens culinaris</i> ssp. <i>culinaris</i> X <i>L. c. ssp. orientalis</i>)	34	20 RFLPs, 8 isozymes, 6 Morphological	333	Havey and Muehlbauer 1989
RILs (ILL5588 X L692-16-1)	177	RAPD, AFLP, RFLP	1073	Eujayl et al., 1998a
F2 (ILL5588 X ILL7537)	114	RAPD, ISSR	784	Rubeena et al., 2003
<i>Lens culinaris</i> ssp. <i>culinaris</i> X <i>L. c. ssp. orientalis</i>	161	71 RAPD, 39 ISSR, 83 AFLP, 2 SSR	2172	Duman et al., 2004
RILs (ILL5588 X L692-16-1)	283	41 SSR, 45 AFLP	751	Hammieh et al., 2005
F2 (L830 X ILLW177)	199	28 SSRs, 9 ISSRs and 162 RAPDs	3843.4	Gupta et al. 2012b
RIL (ILL5588 X ILL5722)	196	RAPD, ISSR, 15 MEST-SSR, and SSR	1156.4	Gupta et al. 2012
RILs (CDC Robin X 9644-46)	543	6 SSRs and 537 SNPs	834.7	Sharpe et al. 2013
RILs (Cassab x ILL2024)	318	57 SSRs and 267 SNPs	1,178 cM	Kaur et al. 2013

ICARDA developed first SSR based linkage map in 2005



Current Linkage Maps in Lentil



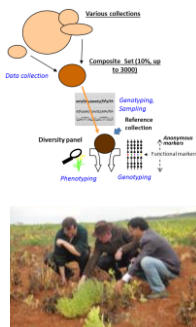
Source: Kaur et al. 2011

Genomic resources in lentil are still limited compared to its genome size

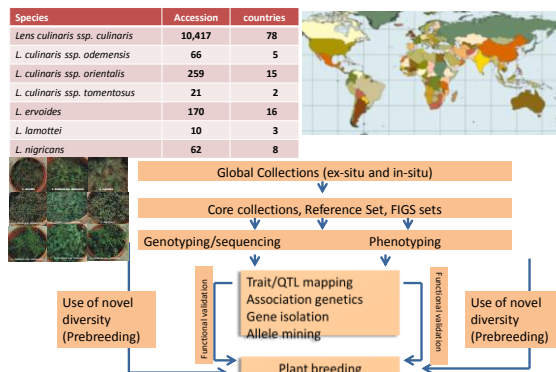
Resource	Number of markers developed
SSRs	2,393 EST-SSRs (Kaur et al. 2011); 219 EST-SSRs (Debijothi et al. unpublished); 5 673 EST-SSRs (Verma et al. 2012); 360 gSSRs (Andeden et al. 2013); 5409 EST-SSRs (Agarwal et al. 2012)
GoldenGate assays	1,536 SNP (Sharpe et al. 2013); 768 SNPs (Kaur et al. 2013)
KASPar assays	148 SNP (Sharpe et al. 2013)
Roche 454/FLX reads	4,918,154 reads (Sharpe et al. 2013);
Illumina reads	111,105,153 reads (Bahattin Tanyolac Persl communication); 119 855 798 reads (Verma et al. 2013)
Transcriptome assembly	11,050 contigs (Sharpe et al. 2013); 97,528 contigs (Bahattin Tanyolac Persl communication)

Genomics-assisted selection in lentil is still in a training phase

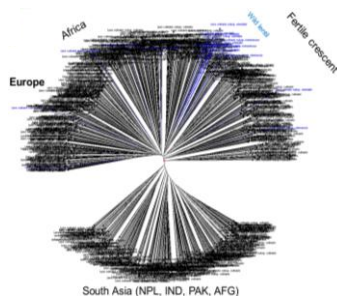
- **Augmentation of genetic resources**
 - Development of core collection
 - Development of reference set
 - Focused Identification of Germplasm Strategy (FIGS)
- **Genotyping and phenotyping**
 - Linkage mapping
 - Association mapping/LD mapping
 - Tagged markers



Genotyping a collection: Where to begin?



Development of Reference Set and Core Collection



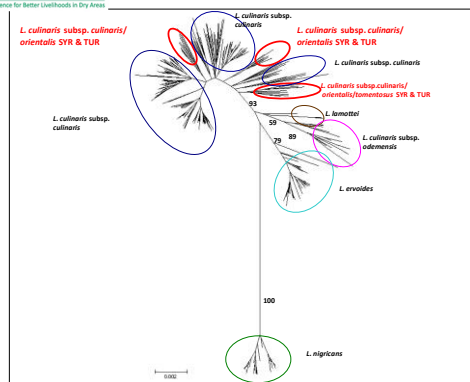
Core Set

- Consists of 960 accessions (10% of global collection)
- Harnessing maximum variability present in the global collection

Reference Set

- 136 accessions

MEGA Results



Lentil Genetic Resources at ICARDA

Genetic resources	Traits of interest
I. Reference set (136 genotypes) from 11500 accessions	All morphological and stress related traits – multi-environmental trials
II. Mapping populations at least 150 lines per population	
1. Morphological traits	Days to maturity, Seed size, Root length
2. Abiotic stresses	Drought, Cold, Boron toxicity
3. Biotic stresses	Rust, Wilt
4. Nutritional quality	Iron, Zinc

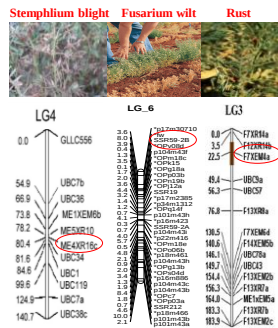
Molecular markers linked to desirable genes/QTLs

Traits	QTL/genes	Type of markers	References
Ascochyta blight resistance	QTL <i>Ra/2</i>	RAPD RAPD, SCAR	Ford et al. (1999) Chowdhury et al. (2001), Tar'an et al. (2003a) Rubeena et al. (2006)
Anthracnose resistance	QTLs <i>LC1-2</i>	AFLP AFLP, RAPD	Tullu et al. (2003), Tar'an et al. (2003a)
Fusarium wilt resistance	<i>Fw</i>	RAPD, SSR	Eujayl et al. (1998b), Hamviah et al. (2005)
Cold Winter hardiness	<i>Frt</i>	RAPD, SSR	Eujayl et al. (1999)
Earliness and plant height	QTL	RAPD, SSR, AFLP	Kahraman et al. (2004)s
Plant structure, growth, and yield	QTL	RAPD, SSR, AFLP	Tullu et al. 2008
Stemphylium blight resistance	QTLs	RAPDs, ISSRs, AFLPs, SSRs	Fratini et al. 2007
Rust resistance	QTLs	SSRs, SRAPs, RAPDs	Saha et al. (2010a)
Ascochyta lentis resistance	QTLs	STS, SSRs, RFPLs, RAPDs, CAPS, dCAPS	Saha et al. (2010b)
Boron tolerance QTL	QTL/Candidate gene	RAPD, ISSR, EST-SSR, SSR	Gupta et al. (2012a)
Seed Quality Characteristics	SNP	SNPs	Kaur et al. (2013)
	QTLs/genes	SNPs	Fedoruk et al. (2013)

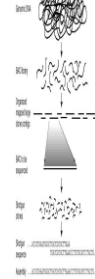


Molecular Markers for Disease Resistance

- Fusarium wilt, stemphylium blight and rust - three major diseases causing enormous yield losses in South Asia.
- Resistance genes/QTLs identified and linked with molecular markers.
- SSR marker (SRS59B) with wilt on LG6, SRAP marker (F7XEM4a) with rust on LG3 mapped.
- Three QTLs for stemphylium blight resistance detected. QTL QLGA480-81 accounted for 25.2-46.0% variation. SRAP marker (ME4XR16c) linked with QLGA480-81 mapped on LG4.



Lentil Genome Sequence (LenGen)



For establishing a comprehensive platform for molecular breeding

Activities

- **Establish a reference lentil genome sequence var. 'CDC Redberry'**
- **High Density Map Construction (Restriction site associated DNA-RAD)**
 - Genotyping by sequencing RILs segregating for high value agronomic traits → uncover candidate genes → provide genetic markers for marker-assisted breeding
- **Characterizing genomic diversity in lentil germplasm**
 - Sequence at lower depth 192 accessions; these accessions representing landraces, all high value breeding and pre-breeding material.

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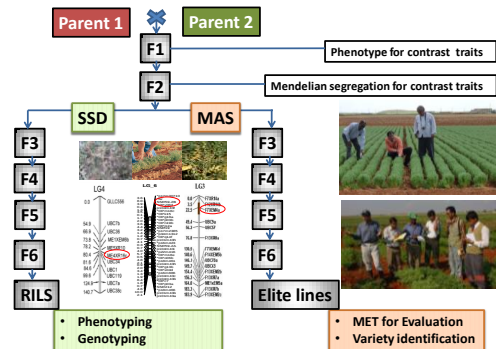
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Plan of Work

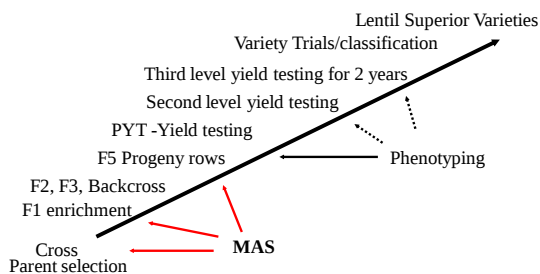
- Lentil reference genome sequence – soon to be available
- Re-sequencing of breeding lines, germplasm accessions, and wild relatives will identify SNPs
- Use of SNPs for detecting variations in lentil germplasm for marker-assisted selection and genomic selections
- Trait mapping and association genetics.
- Development of high-throughput cost-effective genotyping platforms is crucial for applications in lentil breeding and genetics



Molecular Marker assisted Breeding



Precision Breeding



Time to variety release is cut by 2-3 years



Thank you