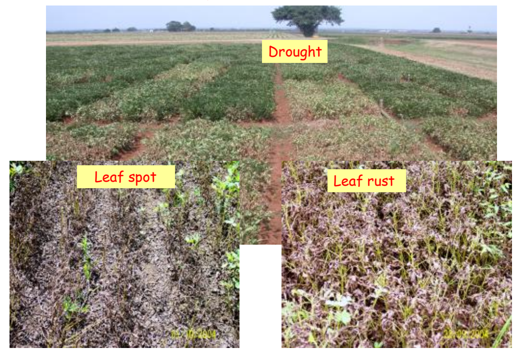
QTLs From Genome to Field Using Markers and Genetic Maps for Peanut Improvement

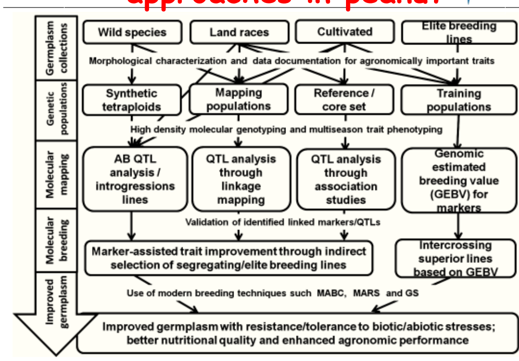
Manish Pandey

ICRISAT, INDIA

Constraints for low production



Integrated genomics approaches in peanut



Biotechnol Adv 2012, 30:639-651

Contents

- Markers repository
- Genetic/consensus maps
- QTLs and linked markers
- Molecular breeding
- Summary

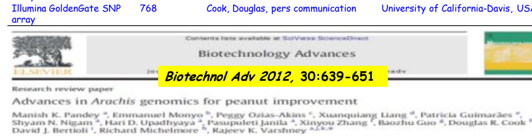
Arachis SSR markers

Marker series	Markers	References	Research Institute/University
Ah, Lec	26	Hopkins et al. 1999	USDA-ARS, USA
pPGPseq, pPGSseq	226	Ferguson et al. 2004	University of Georgia, USA/Cornell University, USA
Ah, Lec, Ap	32	Palmieri et al. 2002, 2005, Gimenes et al. 2007	Universidade Estadual Paulista (UNESP), Brazil
PM	103	He et al. 2003, Luo et al. 2005	USDA-ARS/Tuskegee University, USA
AC, Ah, gi, RN, TC, Seq	338	Moretzsohn et al. 2004, 2005	EMBRAPA, Brazil/USDA-ARS, USA
S	103	Nelson et al. 2006	University of Western Australia, Australia
LG, Lup	188	Proite et al. 2007	University of Brasília/EMBRAPA, Brazil
RN, RM	123	Wang et al. 2007	Shandong Peanut Research Institute, China
AhM	63	Naito et al. 2008	Ibaraki University/Mitsubishi Chemical Medience Co. , Japan
Lup, Dal, Stylo, Ades, Amor, Chaet, IPAHM, ICGM	178	Mace et al. 2007, Cuc et al. 2008, Gautami et al. 2009	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India
EM	290	Liang et al. 2009	Guangdong Academy of Agricultural Sciences, China/USDA-ARS, USA
ES	685	Hong et al. 2009	Guangdong Academy of Agricultural Sciences, China
PM	138	Yuan et al. 2010	Shandong Peanut Research Institute, China/USDA-ARS/Tuskegee University, USA
F, H, PD	94	Song et al. 2010	Shandong Academy of Agricultural Sciences, China
GM710-GM2847	2138	Guo et al. 2012	University of Georgia, USA
AHGS	6680	Shirasawa et al. 2012a	Kazusa DNA Research Institute (KDRI), Japan
Fl, Ahl	1152	Douglas R Cook, unpublished	University of California, USA
Transposon markers	1039	Shirasawa et al. 2012b, 2012c	Kazusa DNA Research Institute (KDRI), Japan

~13,000 SSR markers

Contd…………….

Marker series	Markers	References	Research Institute/University
Development of highly informative SSR markers (PIC >0.50)			
PGPSeq, TC, gi, IPAHM, PM, S, GM, GNB	199	Pandey et al. 2012b	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India
Ah, S, GNB, IPAHM, gi, PGPseq, AS, PGSseq	143	Zhao et al. 2012	Tuskegee University, USA
TC	66	Macedo et al. 2012	University of Brasilia, Brazil
Diversity arrays technology (DArT) markers			
DArT features	15,000	Killian 2008, Varshney et al. 2012	DArT Pty Ltd (Australia), ICRISAT (India), CIRAD (France), and Catholic University of Brasília & EMBRAPA (Brazil)
Single nucleotide polymorphism (SNP) markers			
Illumina GoldenGate SNP array	1,536	Ozias-Akins, Peggy, pers communication	University of Georgia, USA
Illumina GoldenGate SNP array	768	Cook, Douglas, pers communication	University of California-Davis, USA


Biotechnology Advances

Research review paper

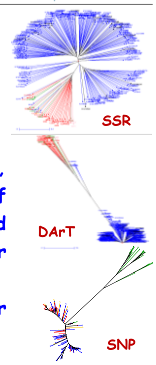
Advances in *Arachis* genomics for peanut improvement

Manish K. Pandey, Emmanuel Monyo, Peggy Ozias-Akins, Xuanquiang Liang, Patricia Guimarães, Shyam N. Nigam, Hari D. Upadhyaya, Pasupuleti Janila, Xinyou Zhang, Baozhu Guo, Douglas R. Cook, David J. Bertioli, Richard Michelmore, Rajeev K. Varshney

Biotechnol Adv 2012, 30:639-651

DArT and SNP markers

- DArT array showed low polymorphism
- Sequencing >200 genes in parents of mapping populations as well as screening of 1536 SNPs (from UG,USA) also yielded very few polymorphic SNPs
- As an collaborative effort with UGA, USA (Dr Peggy Ozias-Akins), a set of 96 highly informative SNPs in cultivated germplasm were converted into KASPar assays
- Validated KASPar assays available for 90 SNPs



Contents

- Markers repository
- Genetic/consensus maps
- QTLs and linked markers
- Molecular breeding
- Summary

Five genetic maps maps for 4x groundnut

Details of different linkage maps	TAG 24 x ICGV 86031 (RIL-1)	ICGS 76 x CSMG 84-1 (RIL-2)	ICGS 44 x ICGS 76 (RIL-3)	TAG 24 x GPBD 4 (RIL-4)	TG 26 x GPBD 4 (RIL-5)
Marker loci mapped	191	119	83	188	181
Linkage groups	22	18	16	20	20
Marker loci/LG	2-19	2-14	2-10	2-17	2-15
Avg. marker loci/LG	8	7	5	9	8
Total map distance (cM)	1785	888	2203	1922	1964
Avg. distance/LG (cM)	81.15	59.2	110.1	96.1	85.4
Avg. inter-locus distance (cM)	9.54	11.88	15.47	10.23	9.9

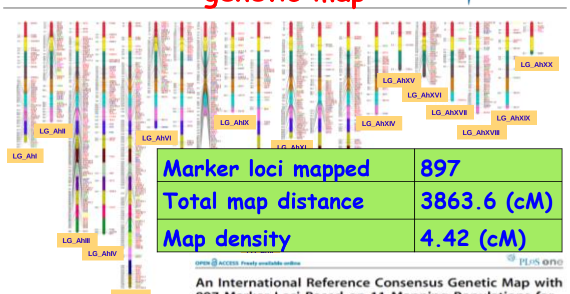
TAG 2009, 118:729-739; TAG 2010, 121:971-984; Field Crops Res 2011, 122:49-59; TAG 2011 122:1119-1132; Mol Breeding 2012, 32:757-772; Mol Breeding 2012, 32:773-788

Eleven 4X- genetic maps used for consensus map

Maps	Linkage groups	Polymorphic loci	Mapped loci	Map length (cM)	Map density (cM)	References
RIL-1	22	211	191	1785.4	9.35	Varshney et al. 2009b; Ravi et al. 2011
RIL-2	20	128	119	2208.2	18.56	Gautami et al. 2012a
RIL-3	15	87	82	831.4	10.14	Gautami et al. 2012a
RIL-4	20	209	188	1922.4	10.23	Khedikar et al. 2010; Sujay et al. 2012
RIL-5	21	209	181	1963	10.85	Sarvamangala et al. 2011; Sujay et al. 2012
RIL-6	19	146	132	793.1	6.01	Hong et al. 2010
RIL-7	21	124	109	503.1	4.62	Hong et al. 2010
RIL-8	13	64	46	357.4	7.76	Hong et al. 2010
RIL-9	26	261	233	1304.9	5.6	Qin et al. 2012
RIL-10	22	183	173	917.45	5.3	Qin et al. 2012
BC1F1-11	21	339	332	847.4	2.53	Foncéka et al. 2009
Consensus map	20	-	897	3,863.6	4.42	Gautami et al. 2012b

RIL-1: TAG 24 x ICGV 86031; RIL-2: ICGS 76 x CSMG 84-1; RIL-3: ICGS 44 x ICGS 76; RIL-4: TAG 24 x ICGV 86031; RIL-5: TG 26 x GPBD 4; RIL-6: Yueyou 13 × Zhenzhuhei; RIL-7: Yueyou 13 × Fu 95-5; RIL-8: Yueyou 13 × J11; RIL-8: Tifrunner × GT-C20; RIL-10: SunOleic 97R × NC94022; RIL-11: Fleur11 × AiAd (synthetic amphidiploid)

Reference consensus genetic map



Marker loci mapped	897
Total map distance	3863.6 (cM)
Map density	4.42 (cM)

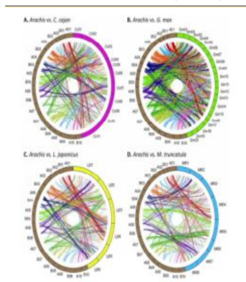
OPEN ACCESS Freely available online PLoS one

An International Reference Consensus Genetic Map with 897 Marker Loci Based on 11 Mapping Populations for Tetraploid Groundnut (*Arachis hypogaea* L.)

Bhimana Gautami, Daniel Fonceka, Manish K. Pandey, Márcio C. Moretzsohn, Venkataswamy Sujay, Hongde Qin, Yanbin Hong, Issa Faye, Xiaoping Chen, Amindala BhanuPrakash, Trushar M. Shah, Makanahally V. C. Gowda, Shyam N. Nigam, Xuanqiang Liang, Dave A. Hoisington, Baozhu Guo, David J. Bertioli, Jean-Francois Rami, Rajeev K. Varshney

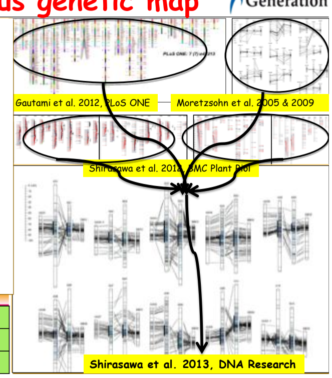
PLoS ONE 2012, 7 (7): e41213

High density reference consensus genetic map



SKF2 | A. hypogaea | KDRI, Japan

Marker loci mapped	3,693
Total map distance (cM)	2,651
Map density (loci/cM)	1.39



Contents

- Markers repository
- Genetic/consensus maps
- QTLs and linked markers
- Molecular breeding
- Summary

Phenotyping of mapping populations

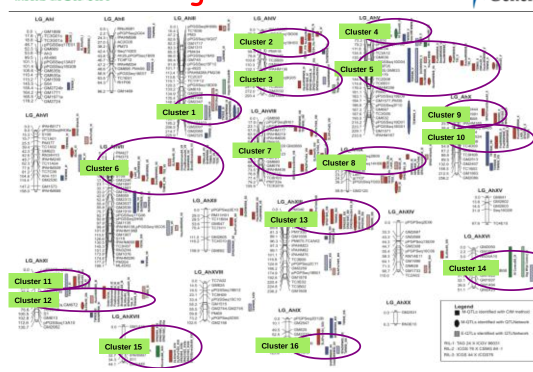
Phenotyping for WUE and surrogate traits in rain out shelters at ICRISAT, India

16 QTL clusters identified for drought related traits

LGs	QTLs	Traits	R^2 (%)
LG_AhIII	5	LDW, TW, ShDW, TDW,TE	3.64-22.39
LG_AhIV	6	SLA, ISC04, T, ShDW	3.91-22.24
LG_AhIV	7	HaulmWt, SCMR, TDW, Veg wt	5.06-33.36
LG_AhV	5	HI, T, Total DW	6.91-7.29
LG_AhV	18	T,TE, ShDW, Pod Wt, Seed Wt, HaulmWt, TDM, DWInc05,	1.7-13.44
LG_AhVII	16	LA, SeedWt, Pod Wt, TDM, T, SLAHar, Biomass, ShDW, DWInc, TE	2.93-9.85
LG_AhVIII	9	SLA, Haulm Wt, SCMR, ShDW, TE	3.90-9.87
LG_AhIX	5	SCMR , ISC05, LA	6.23-10.49
LG_AhX	4	SCMR	7.10-12.15
LG_AhX	7	SCMR, Pod Wt, Haulm Wt, LA, TE	4.67-7.74
LG_AhXI	16	Initial DW, SLA, T, TDM, Haulm Wt, Delta13C04, Biomass, SCMR, TEbis04	4.19-20.32
LG_AhXI	12	T, Haulm Wt, ISC05, Biomass, SLA, SCMR, TE, TDM	3.44-12.60
LG_AhXIII	9	SLA, SCMR, T, ShDW	3.11-13.96
LG_AhXVI	6	HI, Veg wt/pl, Total DW, Pod Wt, ShDW	6.62-40.10
LG_AhXVII	9	SCMR,HI, SLA	5.41-19.53
LG_AhXIX	3	Total DW, SCMR, T	2.51-9.87

Mol Breeding 2012, 32:757-772

Consensus QTL map for drought tolerance traits



Mol Breed 2012, 32:757-772

Phenotyping of populations for LLS & rust

Creation of artificial disease epiphytotics

RILs segregating for LLS and rust

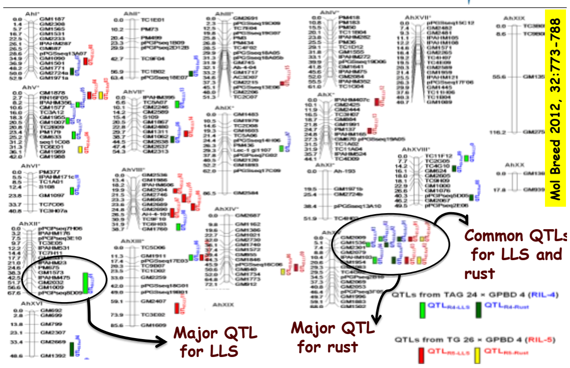
Stable QTLs identified for LLS and rust resistance

Major QTLs detected in two or more than two out of 6 environments

QTLs	Linkage group	Marker interval	No. of environments	R^2 (%)
Late leaf spot (LLS) resistance				
$QTL_{R4-LLS}01$	AhXII	GM1573-pPGPseq8D09	6	10.27-62.34
$QTL_{R4-LLS}02$	AhXV	GM2009-GM1536	2	12.49-67.98
$QTL_{R4-LLS}03$	AhXV	GM1536-GM2301/GM2079	2	10.83-17.37
$QTL_{R4-LLS}05$	AhV	IPAHM356-GM1577	4	10.81-15.34
$QTL_{R4-LLS}09$	AhXVIII	TC11F12-TC2G05	3	3.39-8.5
$QTL_{R5-LLS}01$	AhXV	GM2009-GM1536	2	7.58-49.64
$QTL_{R5-LLS}02$	AhVIII	GM2504-GM2746	2	9.79-22.46
Rust resistance				
$QTL_{R4-Rust}01$	AhXV	GM2009-GM1536	6	10.68-82.27
$QTL_{R4-Rust}02$	AhXV	GM1536-GM2301/GM2079	6	12.43-62.35
$QTL_{R4-Rust}03$	AhXV	IPAHM103-GM1954	6	23.12-82.96
$QTL_{R4-Rust}05$	AhII	TC1B02-pPGSseq18E07	2	2.54-3.29
$QTL_{R5-Rust}01$	AhXV	GM2009-GM1536	7	17.57-66.05
$QTL_{R5-Rust}02$	AhXV	IPAHM103-GM1954	6	17.42-78.96
$QTL_{R5-Rust}03$	AhV	RN16F05-GM1988	2	15.07-29.02

Mol Breed 2012, 32:773-788

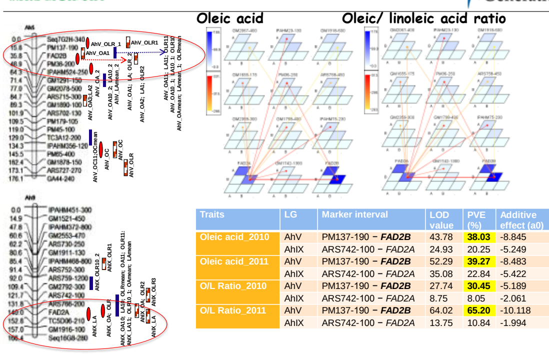
Consensus map with QTLs for LLS and rust resistance



Importance of high oleic acid in peanut oil

- Peanut seeds contain about 44–56% oil which comprised up to 12 fatty acids.
- Two major fatty acids oleic and linoleic acid account for approximately 80% of the oil composition (Ahmed and Young 1982).
- Peanut oil with high % of linoleic acid are prone to oxidation, leading to rancidity, off-flavors, and short shelf- life during seed storage.
- High levels of oleic acid is beneficial to human health by reducing low-density lipoproteins, maintaining high-density lipoprotein, slowing down atherosclerosis, and reversing the inhibitory effect of insulin production.

SunOleic 97R X NC94022



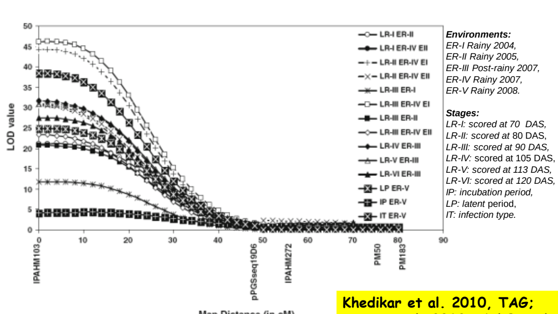
Traits	LG	Marker interval	LOD value	PVE (%)	Additive effect (a0)
Oleic acid_2010	AhV	PM137-190 – ***FAD2B***	43.78	**38.03**	-8.845
	AhIX	ARS742-100 – *FAD2A*	24.93	20.25	-5.249
Oleic acid_2011	AhV	PM137-190 – ***FAD2B***	52.29	**39.27**	-8.483
	AhIX	ARS742-100 – *FAD2A*	35.08	22.84	-5.422
O/L Ratio_2010	AhV	PM137-190 – ***FAD2B***	27.74	**30.45**	-5.189
	AhIX	ARS742-100 – *FAD2A*	8.75	8.05	-2.061
O/L Ratio_2011	AhV	PM137-190 – ***FAD2B***	64.02	**65.20**	-10.118
	AhIX	ARS742-100 – *FAD2A*	13.75	10.84	-1.994

Contents

- Markers repository
- Genetic/consensus maps
- QTLs and linked markers
- Molecular breeding
- Summary

QTLs/markers for molecular breeding

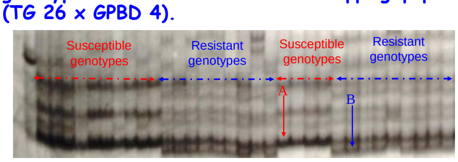
A major QTL for rust in peanut (PVE upto 82.96%)



Khedikar et al. 2010, TAG; Sujay et al. 2012, Mol Breed

A diagnostic marker for rust resistance

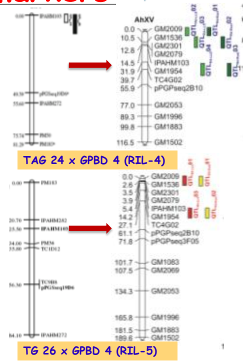
- A SSR marker (IPAHM103) was found associated with the major QTL (*QTLrust01*) contributing up to 82.60% PVE.
- Validated among a set of resistant/susceptible genotypes as well as in another mapping population (TG 26 x GPBD 4).



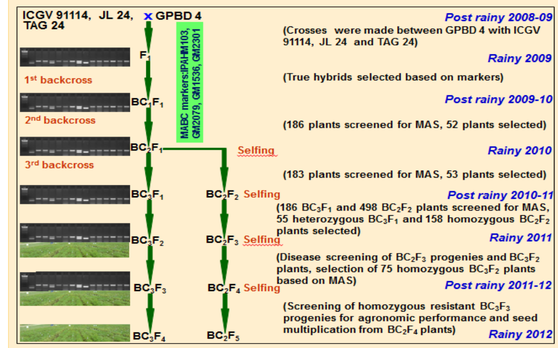
- The marker efficiently differentiated resistant/ susceptible genotypes and found suitable for marker-assisted breeding

Map saturation and new co-dominant markers

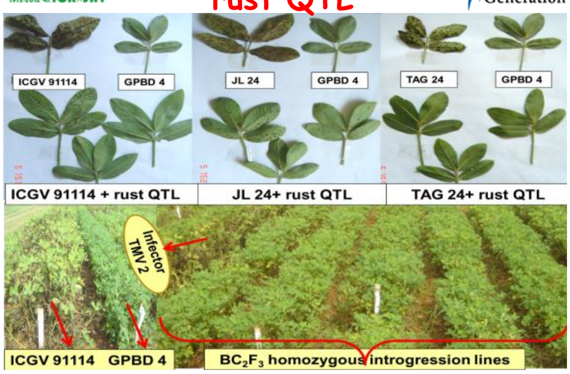
- Identification of four more markers (GM2009, GM2079, GM1536, GM2301), all four markers are co-dominant in nature as compared to dominant marker IPAHM103.
- All these markers are deployed to introgress QTL for rust resistance into the genetic background of three elite cultivars (TAG 24, JL 24 and ICGV 91114).
- This is the first effort towards moving QTL from one to another genotype through marker-assisted breeding to improve any trait in peanut.



Marker-assisted breeding for rust resistance

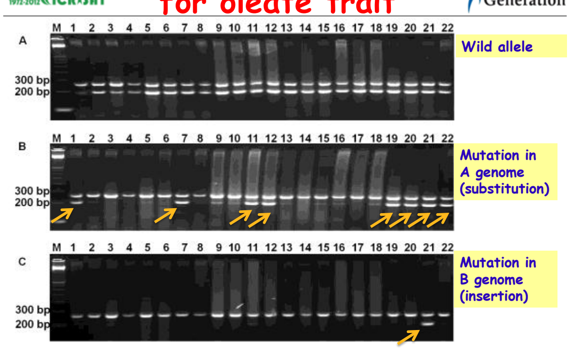


Phenotypic expression of rust QTL



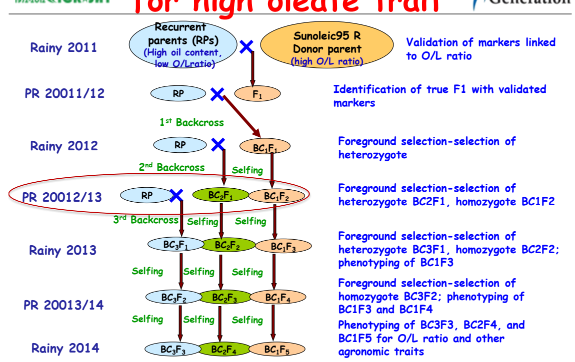
Yield assessment of promising resistant lines

Allele specific markers for oleate trait



1-20: Elite genotypes; 21:Sunoleic 95R; 22: TMV NLM mutant

Marker-assisted breeding for high oleate trait



Summary

- Development of large number of markers
- Construction of several SSR based genetic linkage maps for cultivated groundnut
- Development of highly dense consensus genetic maps
- Genome sequencing is in progress for diploid and tetraploids
- Involvement of few main effect (M-QTLs) and several epistatic (E-QTLs) QTLs for drought tolerance related traits
- Availability of linked markers for foliar diseases (rust and late leaf spot), tomato spotted wilt virus (TSWV) and high oleate trait
- Initiation of marker-assisted introgression of foliar diseases and high oleate trait for improvement of elite cultivars
- Promising introgression lines possessing rust QTL available with improved resistance

Acknowledgements

This work was carried out at CEG, ICRISAT under supervision of **Rajeev Varshney** in collaboration with several collaborators:

- **ICRISAT, Patancheru, India**: P Janila, Vincent Vadez, Shyam Nigam, Hari Upadhyaya, B Gautami, K Ravi, Bryan Moss, M Sriswathi, Pawan Khera, Manish Roorkiwal, Y Shasidhar
- **UAS-Dharwad, India:** MVC Gowda, R Bhat, V Sujay
- **DGR-Junagadh, India:** T Radhakrishnan
- **Univ Brasilia, Brazil:** David Bertioli
- **EMBRAPA, Brazil**: Soraya Bertioli, Marcio Moretzoshn
- **UGA, USA:** Steve Knapp, Peggy Ozias-Akins
- **UC-Davis, USA:** Doug Cook
- **Tuskegee Univ, USA**: Guahao He
- **USDA-ARS, USA:** Baozhu Guo
- **KDRI, Japan:** Sachiko Isobe
- **GAAS, China:** Xuanquiang Liang, Yanbin Hong Xiaoping Chen

Thanks for your patience

Center of Excellence in Genomics @ ICRISAT