

A photograph of ricebean plants with green leaves and yellow flowers. The text is overlaid on the image.

Molecular marker diversity of ricebean germplasm and its relationships

FOSRIN – WP3

**Final Workshop, Country Villa, Nagarkot
(01- 04 February, 2010)**

NARC/CAZS-NR

Biosystematics of Ricebean

- Ricebean – [*Vigna umbellata* Ohwi and Ohashi], an important but under-utilised minor grain legume
- Cultivated in hill areas of Nepal often as an intercrop with maize for local consumption, no modern plant breeding
- One of the eight *Vigna* cultigen species domesticated in Asia
- Belongs to *Ceratotropis, angulares* and closely related to Adzuki bean (*Vigna angularis*)
- Domesticated from a wild form *V. umbellata* var *gracilis* - a cross fertile type reported to occur in natural and disturbed habitats
- Indeterminate, photoperiod-sensitive, freely-branching, twining plant types with small seeds.
- Most ricebean landraces cultivated in Nepal are similar to these wild types

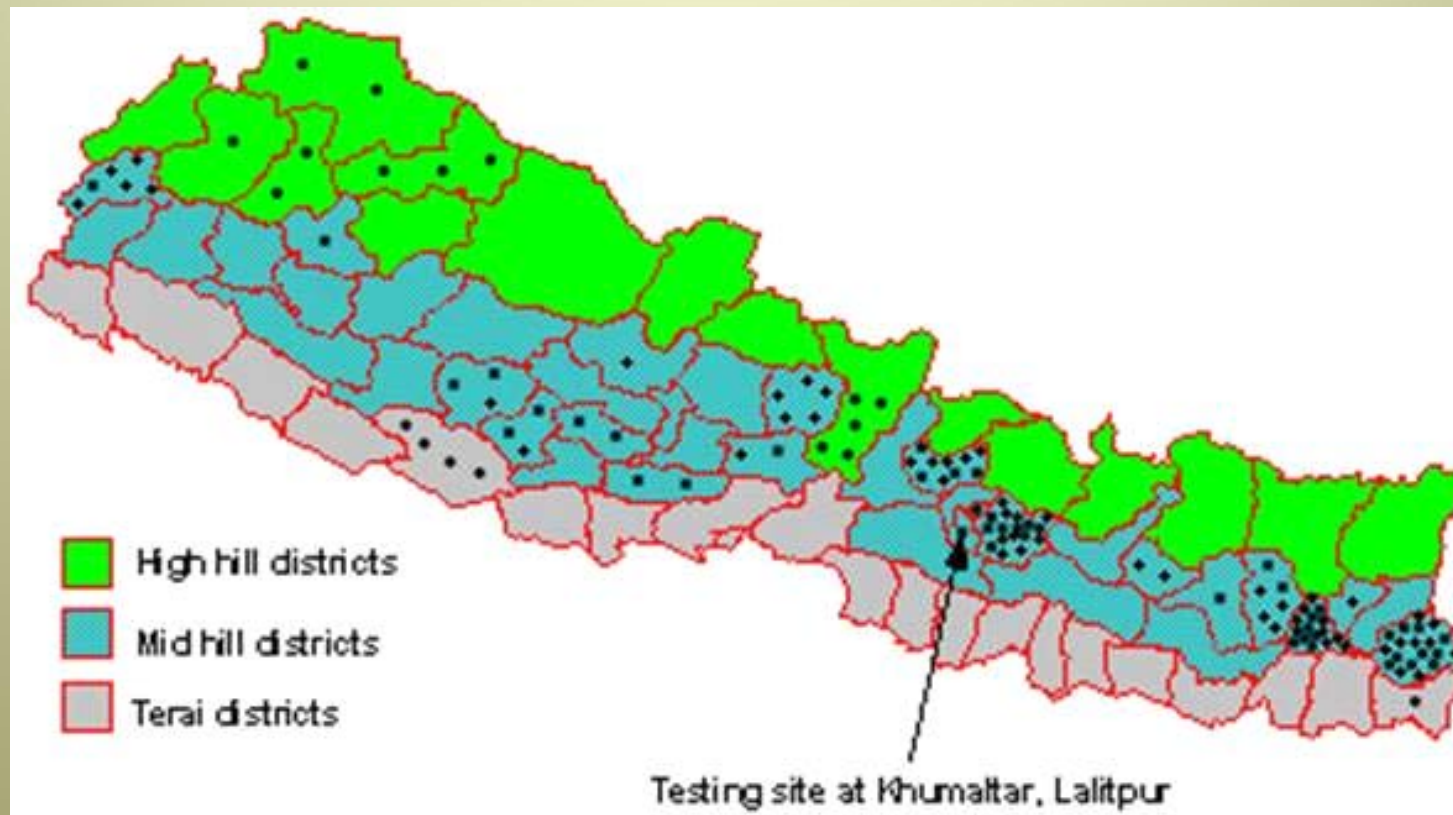
Eight wild and cultigens of Asian *vigna* under *Ceratotropis* subgenus

Table 1: The eight cultivated species of the Asian *Vigna* of subgenus *Ceratotropis*.

Species	Common name /local name	use	Taxa of wild form	Distribution of wild form
<i>V. radiata</i>	mungbean green gram	food	var. sublobata	E. Africa, Madagascar, Asia, New Guinea, Australia
<i>V. mungo</i>	black gram black matpe urd	food	var. silvestris	India, Myanmar
<i>V. angularis</i>	adzuki bean red bean	food	var. nipponensis	Himalayas, N. Myanmar, China, Korea, Japan
<i>V. umbellata</i>	rice bean	food	as for cultigen	E. India, Myanmar, Thailand, Indo-China, China
<i>V. aconitifolia</i>	moth bean mat bean	food cover crop	as for cultigen	Pakistan, India
<i>V. trilobata</i> or	pillipsesara bean jungali bean	forage food	as for cultigen	India, Sri Lanka, Myanmar
<i>V. stipulacea</i>		cover crop		
<i>V. reflexo- pilosa</i> var. <i>glabra</i> = <i>V. glabrescens</i>	lentille de creole	forage food	var. reflexo- pilosa	S.E. Asia, S Japan
<i>V. trinervia</i>	tua pee	cover crop food	as for cultigen	Madagascar, India, Sri Lanka, Thailand, Malaysia, Indonesia, Papua New Guinea

Adapted from Tomooka et al 2003

Ricebean germplasm collection

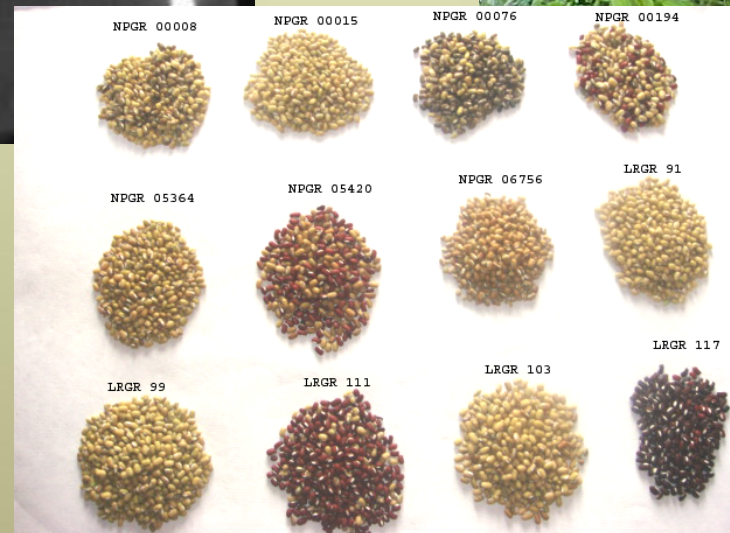
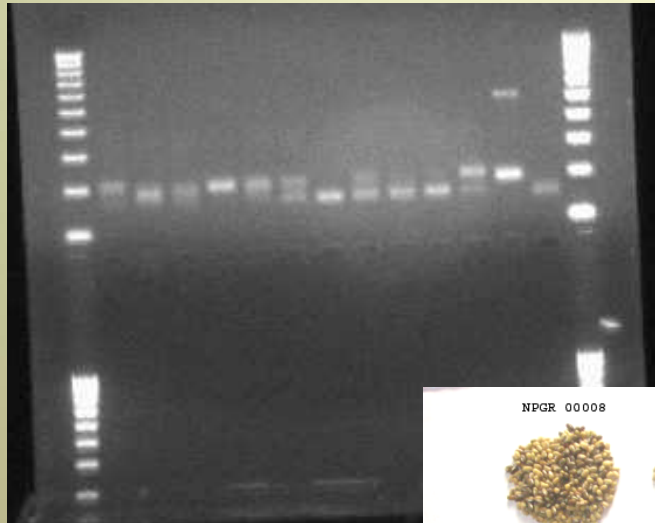


Molecular Marker Diversity

Objectives

- To identify the set of useful polymorphic markers
- To analyse a comprehensive collection of ricebean landraces diversity using molecular marker techniques
- To determine the estimates of genetic diversity and relate with phenotypic diversity

Molecular marker diversity



- Agro-morphological traits - 22
- SSR marker traits - 109

Identification of polymorphic loci

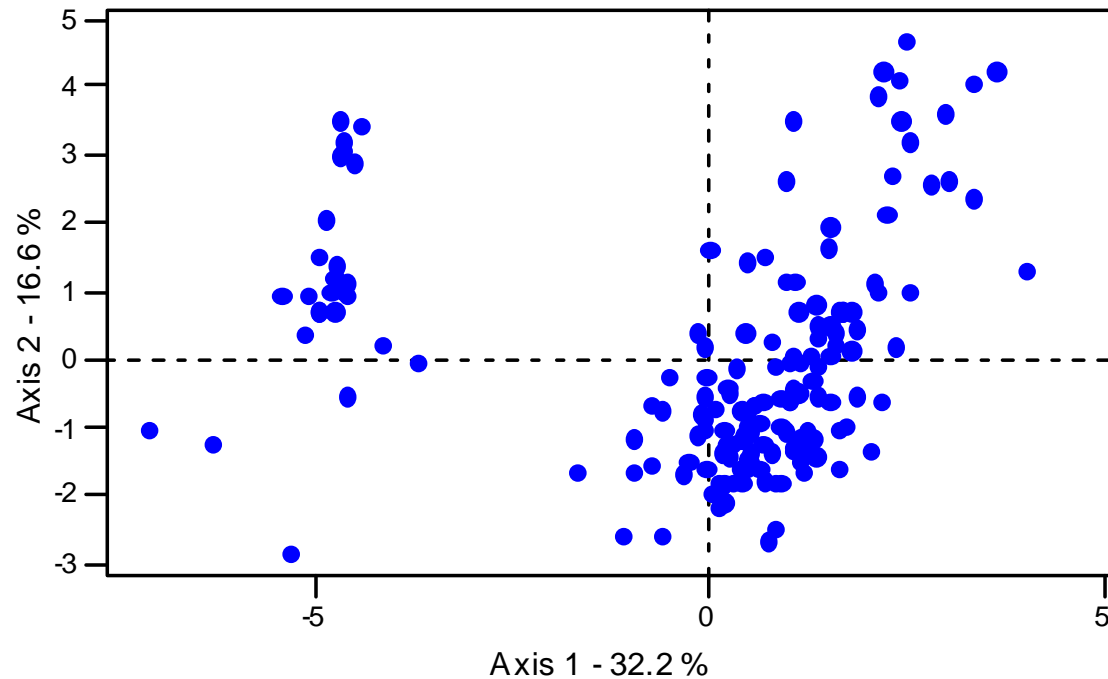
- 109 Adzuki bean SSR markers of different linkage groups screened on 27 accessions (NPGR/LRGR collections) and 2 checks (Ricebean, adzuki bean, Gurans)
- thermal cycling programme of 35 cycles of denaturation (94oC, 30 sec), annealing (50oC, 30 sec) and elongation (68oC, 30 sec)
- products run to electrophoresis in 2.5% SFR agarose and estimated the approximate product size estimated and compared with checks
- 49 (45%) amplified with 1-4 alleles
- 35 (71%) found polymorphic with 2-4 alleles

Molecular Marker Diversity

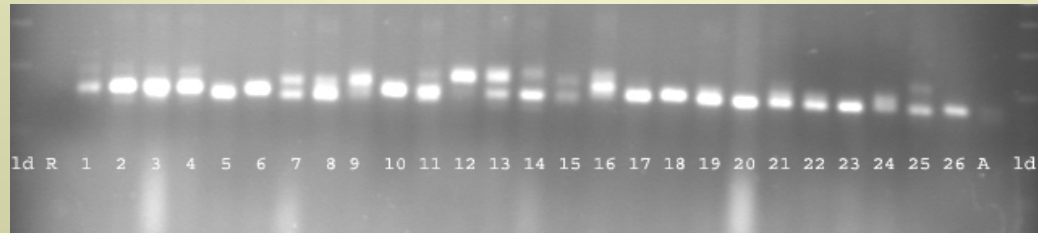
- 112 accessions (91 from Nepal, 21 from India)
- Selected randomly from samples under agromorphological diversity study
- Include the collections from 35 different districts
- Bulk DNA (15 individuals) for each sample isolated
- 35 polymorphic loci tested (derived from D3.1 on identification of polymorphic loci)
- Diversity indices, PCA and cluster analysis carried on molecular and agromorphological data

Random samples selected for SSR diversity

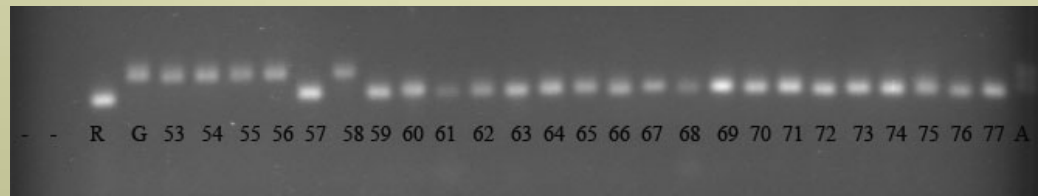
Scatter plot of ricebean accessions (non core) based on qualitative and quantitative traits



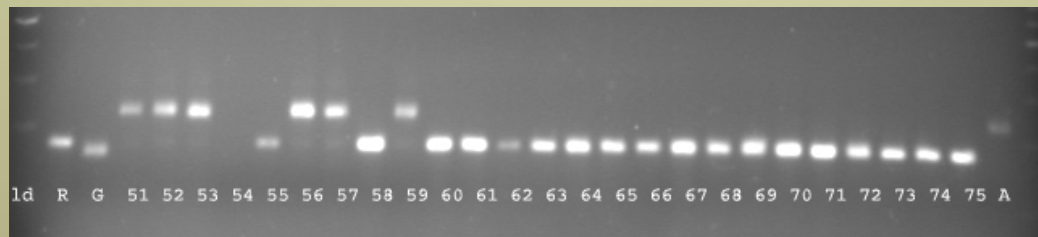
Polymorphism in Ricebean



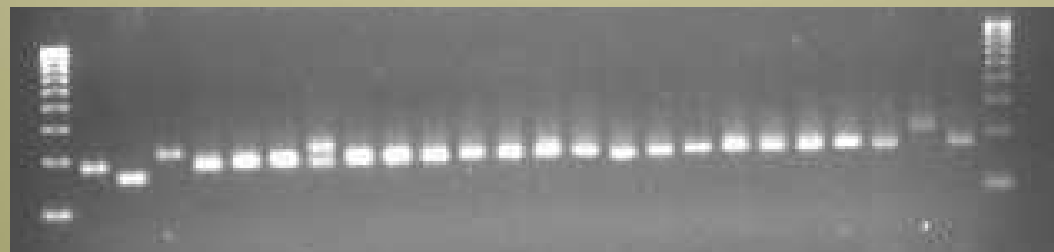
CEDG073



CEDG021



CEDG286



CEDG015

Polymorphic loci useful for diversity

Loci	Linkage	Loci	Linkage
CEDGAAG002	2	CEDG178	1
CEDG015	1	CEDG195	6
CEDG037	6	CEDG232	9
CEDG044	11	CEDG253	8
CEDG050	2	CEDG286	3
CEDG073	8	CEDG294	2
CEDG134	10		

Diversity values observed

Diversity parameters	Nepal	India
Total sample analysed	91	21
Total SSR loci assayed	35	27
Total polymorphic loci	30	16
% of polymorphic loci	86	56
Total number of alleles	80	46
Alleles/locus	2.2	1.7
Total polymorphic alleles	75	35
Alleles/poly locus	2.5	2.2
% of polymorphic alleles	94	76
H' (diversity index)	0.25	0.34

Molecular diversity indices observed in Ricebean (Nepal)

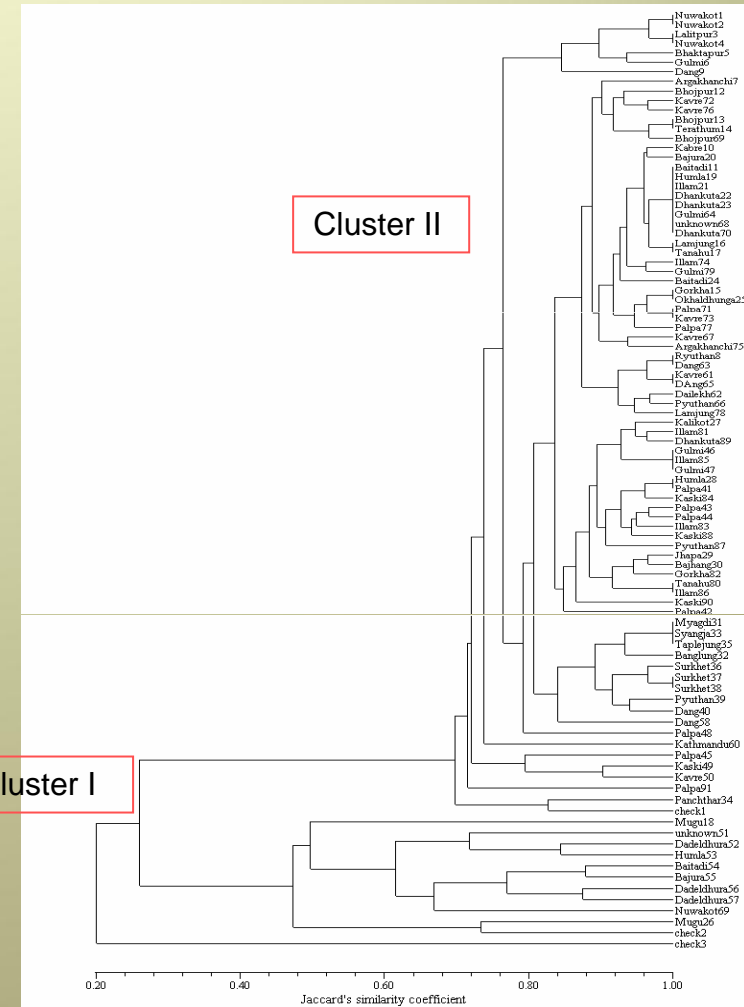
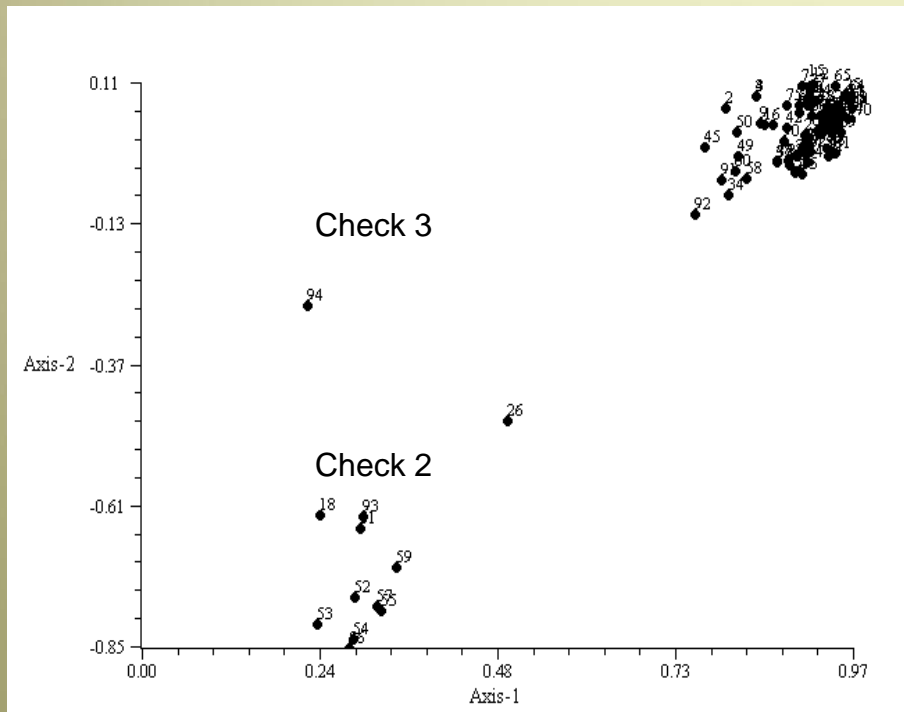
SN	Primers	Total sample	alleles	SW index	PIC
1	CEDG073	91	2	0.84	0.51
2	CEDG015	84	3	0.84	0.45
3	CEDG033	87	4	1.06	0.50
4	CEDG090	90	3	0.94	0.55
5	CEDG037	90	2	0.37	0.22
6	CEDG043	85	2	0.22	0.11
7	CEDG029	87	4	0.54	0.23
8	CEDG232	84	2	0.39	0.23
9	CEDG253	90	2	0.18	0.08
10	CEDG286	89	3	0.36	0.18
11	CEDG292	89	3	0.47	0.24
12	CEDG294	77	2	0.12	0.05
13	CEDG071	66	2	0.08	0.03
14	CEDG050	93	3	0.38	0.19
15	CEDG044	93	3	1.15	0.63
16	CEDG084	79	2	0.35	0.20

SN	Primers	Total sample	alleles	SW index	PIC
17	CEDG021	81	2	0.37	0.22
18	CEDG118	91	2	0.37	0.21
19	CEDG007	87	2	0.37	0.20
20	CEDG141	86	2	0.33	0.15
21	CEDG195	94	2	0.47	0.23
22	CEDG204	85	3	0.49	0.28
23	CEDG026	86	3	0.43	0.21
24	CEDG154	89	3	0.17	0.07
25	CEDG178	91	2	0.65	0.46
26	CEDG214	92	3	0.44	0.22
27	CEDAAG002	81	2	0.55	0.31
28	CEDG134	82	2	0.32	0.18
29	CEDG087	85	2	0.15	0.07
30	CEDG305	83	3	0.70	0.30
	Total		75	14.1	7.5
	mean		3	0.47	0.25

Molecular diversity indices observed in Ricebean (India)

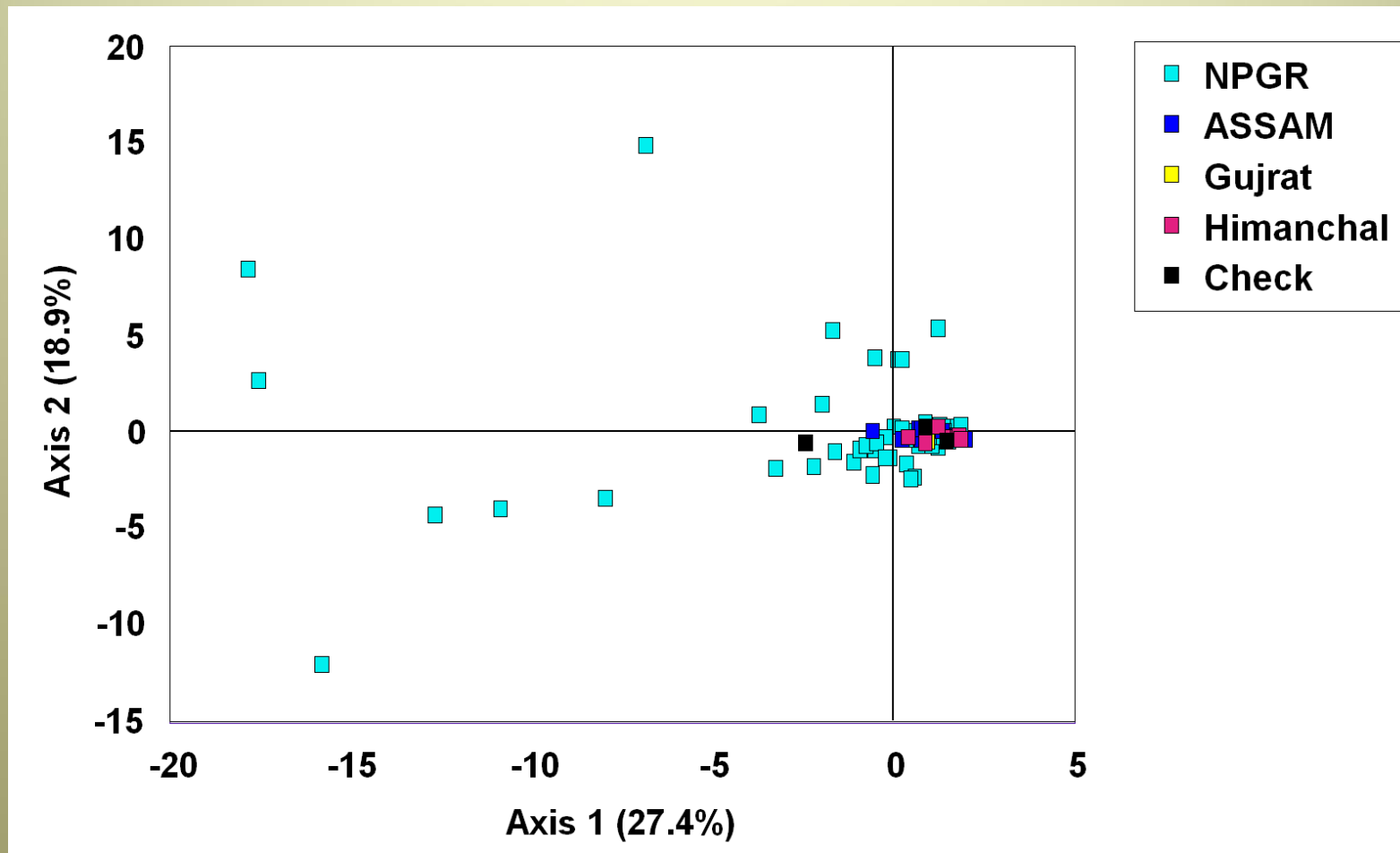
Primer	Total sample	alleles	SW	PIC
CEDG073	21	4	1.30	0.66
CEDG015	21	2	0.19	0.09
CEDG033	21	2	0.67	0.38
CEDG090	21	2	0.49	0.31
CEDG043	21	2	0.19	0.09
CEDG253	21	2	0.19	0.09
CEDG071	21	2	0.63	0.44
CEDG044	21	2	0.66	0.47
CEDG007	21	2	0.49	0.31
CEDG141	21	3	1.38	0.71
CEDG195	21	2	0.19	0.09
CEDG154	21	2	0.38	0.18
CEDG178	21	2	0.19	0.09
CEDG214	21	2	0.68	0.49
CEDAAG002	21	2	1.02	0.61
CEDG087	18	2	0.53	0.35
		2.2	0.57	0.34

Ricebean molecular diversity clustering and scatter plotting



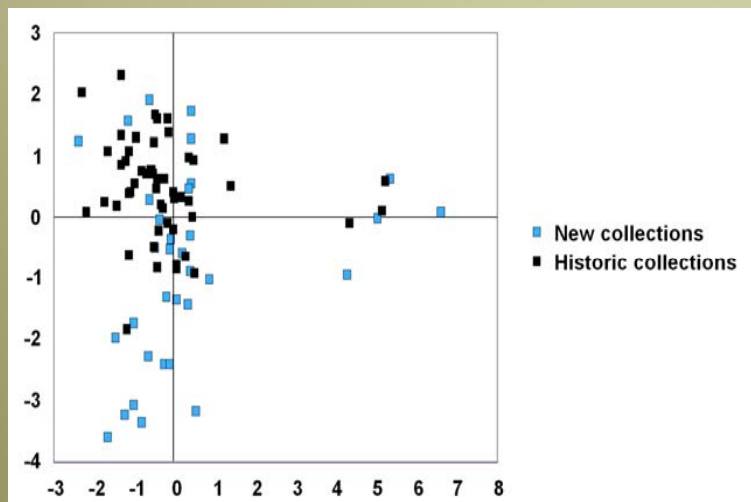
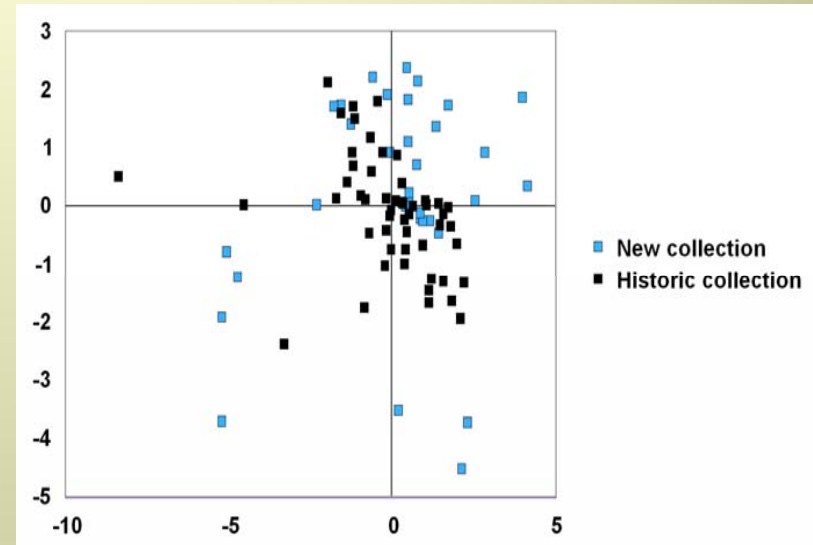
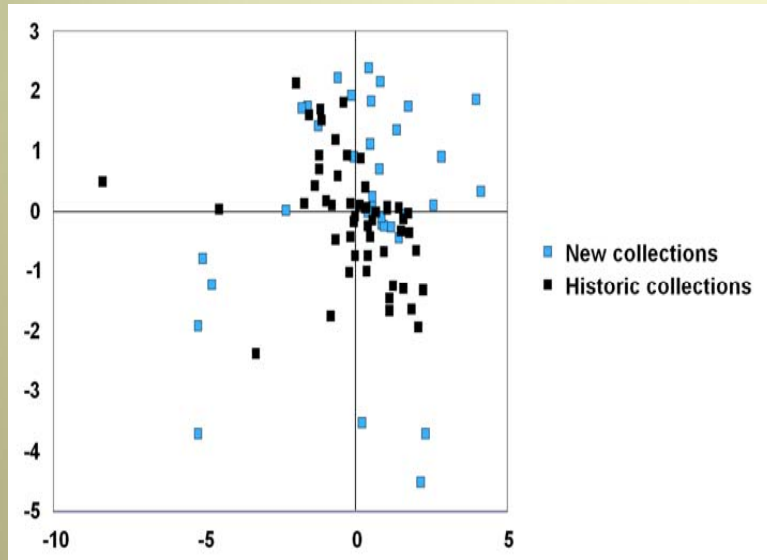
- 91 accessions grouped in 2 clusters
- Cluster I close association with checks of Adzuki bean
- Genotypes from Mugu distinct from rest and diverse from each other

Scatter plot of ricebean



Based on 79 polymorphic alleles PCA analysis

Principal component analysis



- Great variation observed in phenotypic traits
- Quantitative traits more informative
- No agro-ecological diversity observed

Relationship of molecular and agro-morphological similarity matrices

Variables	Percent of variation	Traits	Similarity matrices
Quantitative + Qualitative traits	41.0	Leaf measurement, plant height	
Quantitative traits	60.3	Plant height, Days to maturity, biomass	
Qualitative traits	38.4	Growth habit, twining tendency, hypocotyl colour, seed colour	
Qualitative + Quantitative + molecular trait	27.3	Days to maturity, HSW, Biomass, CEDG073, CEDAAG002	

Conclusions

- A high phenotypic diversity exist in Ricebean landraces
- Quantitative traits informative and described the diversity
- Plant height, Days to maturity, biomass, growth habit, twining tendency, seed colour important traits
- A small diversity in marker diversity observed

Conclusions

- Set of polymorphic loci useful in determining diversity of ricebean identified
- CEDG015, CEDG037, CEDG073, CEDAAG002, CEDG044 showed high PIC value on both sets of landraces.
- Indian germplasm showed comparatively high diversity, differ among the genotypes
- Nepalese germplasm clustered into two, some found close to Adzuki bean

Future research works on molecular markers

- Understanding of Ricebean genome along with other closely related legumes
- Research programmes on construction of microsatellite enriched library and development of ricebean markers

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