



Final Project Report

Date: 17 May 2007

Identification

GCP Project Number: 2005-CB05j

Project Name: Chickpea molecular marker reference kit

Principle Investigator: DA Hoisington, ICRISAT

Collaborators (name, affiliation): RK Varshney, HD Upadhyaya (ICRISAT)

Total Budget 2005: US\$6,300

Report

I. Executive summary

A chickpea SSR marker reference kit has been developed based on the genotyping of the global chickpea composite collection (3000 accessions) with 35 simple sequence repeat (SSR) markers. The kit consists of three pools of chickpea accessions along with supporting documentation on the SSR markers, PCR and detection conditions, and the allele sizes for each of the 35 SSR loci. All details on the marker such as the primer sequence, annealing temperature, source of the markers, diagnostic alleles in the pools of the reference genotypes and allelic patterns of the reference genotypes in pool have been posted on ICRISAT's web page at http://www.icrisat.org/gt-bt/Marker_Kits.htm. The developed SSR kit will have a wide range of applications, especially for genetic diversity studies in chickpea. By using the markers and reference accessions in the kit, scientists in other laboratories will be able to compare the genotypic data they obtain on their germplasm with that obtain using the global composite collection.

II. Introduction/Background

Chickpea ranks third among pulses, fifth among grain legumes, and 15th among grain crops of world. Chickpea accounts for 12% of the world pulse production, with a 1.9% annual growth rate of chickpea production during the last 20 years. In the framework of the Generation Challenge Program, an assessment of the

overall genetic diversity of 3000 chickpea accessions was assessed through the use of 35 SSR markers, distributed throughout the genome. This task was shared between two institutes: ICRISAT in Patancheru, India and ICARDA in Aleppo, Syria. The genetic diversity study provides an overview of the population structure as well as allelic diversity that can be associated ultimately with phenotypic traits. Based on genotyping data obtained with the 35 SSR markers, an effort has been made to provide the international community with a genotyping kit that could be used as a reference kit to compare with other genetic diversity studies in future.

III. Scientific activities (including tables and figures)

A set of 9 chickpea accessions (Table 1) representing the largest genetic diversity was chosen based on phenotyping data. After analysing the genotyping data for these genotypes in term of allele sizes, these genotypes were pooled into 3 groups, with each group comprised of three genotypes referred as control or reference genotypes (C1, C2 and C3). It is proposed to compare the allelic composition of these controls (genotype pools) with other genetic diversity studies.

For genotyping the composite collection of 3,000 chickpea genotypes (Upadhyaya et al. 2006), SSR markers were chosen from published and unpublished data from other colleagues (Table 2, Hüttel et al. 1999, Winter et al. 1999, Sethy et al. 2003). The amplified DNA fragments were separated and detected with fluorescence detection on the ABI 3700 sequencer (see Buhariwalla et al. 2005). The raw allele size was determined using the Genotyper version 3.1 software (ABI) and subsequently the data was analysed using an in-house developed programme “AlleloBin” and alleles determined as per the corresponding (expected) SSR length.

Allele size obtained by diagnostics alleles observed in the reference accessions as well as in control pools are given in Table 3. Allelic patterns of control and reference samples were documented for all 35 markers. For each marker, the 9 genotypes and the 3 mix controls were amplified and analyzed on ABI 3700 genetic analyzer. As an example, the allelic pattern for one marker CaSTMS15 has been shown in Figure 1. The figures for all 35 markers showing allelic patterns are available on ICRISAT's website <http://www.icrisat.org/gt-bt/Marker Kits.htm>. Distinct alleles amplified in each individual genotype with each of the markers with the called allele size can be visualized in figures to compare the allelic diversity among different germplasm collections.

IV. Deviation from the work plan

NIL

V. Data Availability

a. Description of data produced

- A kit of 35 SSR markers developed for chickpea
- Allelic data for the control and reference samples with all 35 SSR markers
- Documentation of all data in the forms of tables and figures

b Where the data sets have been posted, if not where and when they will be posted.

- All the above data has been submitted to GCP
- Details on chickpea SSR marker kit have been posted at http://www.icrisat.org/gt-bt/Marker_Kits.htm.

VI. Conclusions

The developed SSR marker kit of chickpea is recommended for use in assessing the diversity in chickpea germplasm. The diversity present in different germplasm collections can be compared with that of the global composite collection or mini core collection of ICRISAT. In fact, the application of the chickpea SSR marker kit has already been started. For instance, the subset of 20 markers of the kit has been used for genotyping the USDA core collection of chickpea.

VII. Acknowledgements

We acknowledge the support of K Eshwar, Seetha Kannan and VP Prasanth in generating and analysing the genotyping data. The study was performed under the Generation Challenge Program SP1 Chickpea genotyping project and SP5 Marker kit project.

VIII. References

- Buhariwalla H K, et al. (2005). Development of ESTs from chickpea roots and their use in diversity analysis of the *Cicer* genus. *BMC Plant Biology* 5: 16
- Hüttel B, et al. (1999) Sequence-tagged microsatellite site markers for chickpea (*Cicer arietinum* L.) *Genome* 42: 210- 217
- Sethy N K et al. (2003). Isolation and characterization of sequence-tagged microsatellite sites markers in chickpea (*Cicer arietinum* L.). *Mol. Ecol. Notes* 3:428- 430.
- Upadhyaya, H D et al. (2006). Development of a composite collection for mining germplasm possessing allelic variation for beneficial traits in chickpea. *Plant Genet. Resou.* 4: 13-19
- Winter P et al. (1999). Characterization and mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. *Mol. Gen. Genet.* 262: 90-101

Table 1: Reference accessions selected for molecular analysis

S. No.	Accession number	Accession holder	Accession origin	Pool (Reference ID)
1	ICC 14446	ICRISAT	Italy	C1
2	ICC 15996	ICRISAT	ICRISAT	C1
3	ICC 15994	ICRISAT	Spain	C1
4	ICC 11265	ICRISAT	Czechoslovakia	C2
5	ICC 6537	ICRISAT	Iran	C2
6	ICCV 2	ICRISAT	ICRISAT	C2
7	Annigeri	ICRISAT	India	C3
8	ICC 13454	ICRISAT	Iran	C3
9	ICC 4366	ICRISAT	Iran	C3

Table 2: Characteristics of the SSR markers of the marker kit

Marker name	Motif	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	Linkage group	T ^a	Source
CaSTMS2	(TAT)25	ATTTTACTTTACTACTTTTTTCCTTTC	AATAAATGGAGTGTAATTTTCATGTA	LG4	59	Huttel et al. 1999
CaSTMS15	(ATT)21	CTTGTGAATTCATATTTACTTATAGAT	ATCCGTAATTTAAGGTAGGTTAAAATA	LG4	59	Huttel et al. 1999
CaSTMS21	(CT)9 n(CTTT)2 (CT)4	CTACAGTCTTTTGTTCTTCTAGCTT	ATATTTTTTAAGAGGCTTTTGGTAG	LG6	59	Huttel et al. 1999
TA14	(TAA)22n(TAA)4T (A)3n(AAT)5n(A)3n(GAT) 4 (TAA)5	TGACTTGCTATTTAGGGAACA	TGGCTAAAGACAATTAAGTT	LG6	59	Winter et al. 1999
TA21	(TAA)51	GTACCTCGAAGATGTAGCCGATA	TTTTCCATTTAGAGTAGGATCTTCTTG	LG5	59	Winter et al. 1999
TA22	(ATT)40	TCTCCAACCCTTTAGATTGA	TCGTGTTTACTGAATGTGGA	LG4	59	Winter et al. 1999
TA27	(TAA)21	GATAAAATCATTATTGGGTGTCCTTT	TTCAAATAATCTTTCATCAGTCAAATG	LG7	59	Winter et al. 1999
TA28	(TAA)37n(TAA)30	TAATTGATCATACTCTCACTATCTGCC	TGGGAATGAATATATTTTTGAAGTAA	LG5	59	Winter et al. 1999
TA46	(TAA)22	TTTATTGCAATAAAACTCATTTCTTATC	TTCTTTTTGTGTGAAAAAAAAATATAGTGA	LG2	56	Winter et al. 1999
TA64	(TAA)39	ATATATCGTAACTCATTAATCATCCGC	AAATTGTTGTCATCAAATGGAAAATA	LG1	59	Winter et al. 1999
TA71	(AAT)32	CGATTTAACACAAAACACAAA	CCTATCCATTGTCATCTCGT	LG3	59	Winter et al. 1999
TA72	(ATT)36	GAAAGATTTAAAAGATTTTCCACGTTA	TTAGAAGCATATTGTTGGGATAAGAGT	LG2	59	Winter et al. 1999
TA76s	(AAT)7 (AAT)4 [ACT(AAT)11]2 n(AAT)3 n(AAT)2 (ATT)5	TCCTCTTCTTCGATATCATCA	CCATTCTATCTTTGGTGCTT	LG1	48	Winter et al. 1999
TA113	(TAA)26	TCTGCAAAAACACTATTACGTTAATACCA	TTGTGTGTAATGGATTGAGTATCTCTT	LG 1	56	Winter et al. 1999
TA116	(TAA)5n(A)3(TAA)20	AATTCAATGACGAATTTTATAAGGG	AAAAAGAAAAGGGAAAAGTAGGTTTTTA	NA	59	Winter et al. 1999
TA117	(ATT)52	GAAAATCCCAAATTTTTCTTCTTCT	AACCTTATTTAAGAATATGAGAAACACA	NA	56	Winter et al. 1999

TA118	(TAA)45	ACAAGTCACATGTGTTCTCAATA	GGAAAGGTTAAGAAATTTTACAATAC	NA	59	Unpublished (P. Winter)
TA130	(TAA)19	TCTTTCTTTGCTTCCAATGT	GTAATCCCACGAGAAATCAA	LG2	59	Winter et al. 1999
TA135	(TAA)17	TGGTTGGAAATTGATGTTTT	GTGGTGTGAGCATAATTCAA	LG1	56	Winter et al. 1999
TA142	(TTA)15	TGTTAACATTCCCTAATATCAATAACTT	TTCCACAATGTTGTATGTTTTGTAAG	NA	48	Winter et al. 1999
TA200	(TTA)37	TTTCTCCTCTACTATTATGATCACCAG	TTGAGAGGGTTAGAACTCATTATGTTT	NA	59	Winter et al. 1999
TA206	(TAA)25	GTCCCACTTCCACTTATAAAGGTT	TAACGTATCTTGCAGATTTCAAATAAA	NA	59	Winter et al., 1999
TAA58	(AAT)41	CATTGCTTAAGAACCAAATGG	CAATTTTACATCGACGTGTGC	LG5	56	Winter et al. 1999
TaaSH	(TAA)40	GGTAGACGCAAAGAGTGGG	GCCACATTGACCAGGAATG	LG3	59	Winter et al. 1999
TR2	(TTA)36	GGCTTAGAGTTCAAAGAGAGAA	AACCAAGATTGGAAGTTGTG	LG1	56	Winter et al. 1999
TR29	(TAA)8n(TAA)32	GCCCACTGAAAAATAAAAAG	ATTTGAACCTCAAGTTCTCG	LG3	59	Winter et al. 1999
TR31	(TAA)20 n(A)5 (TAA)9	CTTAATCGCACATTTACTCTAAAATCA	ATCCATTAACACGGTTACCTATAAT	LG1	59	Winter et al. 1999
TR43	(TAA)24	AGGACGAAACTATTCAAGGTAAGTAGA	AATTGAGATGGTATTAATGGATAACG	LG6	59	Winter et al. 1999
TR7	(TTA)25	GCATTATTCACCATTTGGAT	TGTGATAATTTTCTAAGTGT	LG4	59	Winter et al. 1999
TS84	(TTA)25	TTATAACAGCTTCCTTCTATTTGTTTTG	AAGGCAAAGTTTTTATCCCTTAATAG	NA	59	Winter et al. 1999
NCPGR4	(CT)16	TTACAGCTTGTGCTCAG	AGTCAGATTCTTATCCGA	NA	56	Sethy et al. 2003
NCPGR6	(CA)12	GACCAAGATTAGTAGAACCT	TATGTCTACACCTATGCATC	NA	56	Sethy et al. 2003
NCPGR7	(CA)14	GACCAAGATTAGTAGAACCT	CTTGATAAGGATGAGTCATG	NA	56	Sethy et al. 2003
NCPGR12	(CT)35	CCTTGTTAGTGTGTATAGGT	GTAATGACCAAGTGAACA	NA	56	Sethy et al. 2003
NCPGR19	(GA)19	TCCATTGTAGCTTAGCTTAG	TCTTACTCTTAGCTTACCTCTT	NA	56	Sethy et al. 2003

Table 3: Alleles identified for each SSR marker of the kit on reference accessions.

SSR marker name	Detection System	Molecular size (bp) of alleles identified								*Ref. 1 ("C1")	**Ref. 2 ("C2")	***Ref. 3 ("C3")
CaSTMS2	ABI	232	241	244	256	259	262	271		262,232,244	256,241,256	244,271,259
CaSTMS15	ABI	236	248	251	254	257	263	269		248,251,257	236,263,269	251,254,254
CaSTMS21	ABI	168	172							168,168,172	172,168,172	168,172,172
TA14	ABI	263	266	272	278					272,278,266	266,272,272	278,263,263
TA21	ABI	328	331	337	346	349	352	355	361	352,331,361	337,355,328	349,346,346
TA22	ABI	203	206	209	212	269	278			209,212,203	209,278,209	206,269,269
TA27	ABI	224	227	233	236	239	242			227,239,233	239,242,236	236,227,224
TA28	ABI	312	315	330	339	342	363	369	375	330,315,312	342,375,315	339,369,363
TA46	ABI	127	142	145	148	151	154			142,145,142	145,148,151	127,154,154
TA64	ABI	206	230	233	239	242	248	251	254	248,254,251	230,239,233	206,242,242
TA71	ABI	184	187	196	202	205	214	223		196,205,214	196,223,202	202,184,187
TA72	ABI	228	234	240	243	246	255	261		246,255,246	234,261,240	243,234,228
TA76s	ABI	203	212	218						203,212,218	218,218,212	212,218,218
TA113	ABI	189	192	195	201	204	207			189,201,204	201,201,195	207,192,192
TA116	ABI	179	182	194	212					179,179,182	194,179,179	179,212,212
TA117	ABI	227	230	236	245	248	251	254	260	251,230,260	236,254,227	248,245,245
TA118	ABI	188	191	200	215	218	230			215,191,200	188,191,230	200,230,218
TA130	ABI	206	221	224						224,221,221	221,206,224	221,224,224
TA135	ABI	175	190	193	199					199,193,193	193,199,193	175,190,190
TA142	ABI	125	128	131	134	137	140			131,131,131	137,128,125	134,140,140
TA200	ABI	271	274	286	292	295	298	298	307	271,292,271	295,286,298	298,307,274
TA206	ABI	373	376	382	385	388	394	406		406,382,394	382,388,376	376,385,373
TAA58	ABI	271	280	295	307	313				295,307,313	280,307,271	313,313,313
TaaSH	ABI	406	427	430	433					406,430,433	433,433,427	430,436,433
TR2	ABI	214	223	229	241	244	250			229,241,223	229,244,223	250,214,214
TR29	ABI	172	190	193	211	214	217	220		193,217,214	190,220,211	211,172,172
TR31	ABI	198	201	210	213	216				198,216,198	210,216,213	201,201,201

TR43	ABI	297	301	307	325	349	375			307,375,301	349,369,301	325,297,297
TR7	ABI	197	200	203	206	209	212	217	218	217,212,209	218,206,203	203,200,197
TS84	ABI	226	230	238	244					230,226,226	226,230,230	226,238,244
NCPGR12	ABI	213	225	253	255	259	261			259,261,225	253,253,213	255,235,235
NCPGR19	ABI	298	300	308	312					300,308,298	300,308,298	308,312,312
NCPGR4	ABI	180	194	196						194,180,194	194,196,194	198,194,194
NCPGR6	ABI	249	251	255						249,255,251	251,255,251	249,251,251
NCPGR7	ABI	217	219	223						217,223,219	219,223,219	217,219,219

*Ref. 1 ("C1") = ICC 14446, ICC 15996, ICC 15994

**Ref. 2 ("C2") = ICC 11265, ICC 6537, ICCV 2

***Ref. 3 ("C3") = Annigeri, ICC 13454, ICC 4366

Figure 1: An example of allelic pattern observed in case of SSR marker CaSTMS15. The locus image clearly shows 2-3 alleles in the pools of three reference genotypes.



**Allelic content
in the samples**

DNA_Sample	Control	Allele size
ICC14446	C1	248
IOC15996	C1	251
ICC15994	C1	257
ICC11265	C2	236
ICC6537	C2	263
ICCV2	C2	269
Annigeri	C3	251
ICC13454	C3	254
ICC4366	C3	254

**Allelic content
in the controls**

Control	Allele size
C1	248, 251, 257
C2	236, 263, 269
C3	251, 254