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A SSR kit to study genetic diversity in chickpea (*Cicer arietinum* L.)

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Abstract

A chickpea simple sequence repeat (SSR) marker reference kit has been developed based on the genotyping of the global chickpea composite collection (3,000 accessions) with 35 SSR markers. The kit consists of three pools of chickpea accessions along with supporting documentation on the SSR markers, polymerase chain reaction and detection conditions, and the expected allele sizes for each of the 35 SSR loci. These markers were selected based on quality criteria, genome coverage and locus-specific information content. Other important SSR selection criteria were quality of amplification products, locus complexity, polymorphism information content and well-dispersed location on a chickpea genetic map. The developed SSR kit has a wide range of applications, especially for genetic diversity studies in chickpea. Using the markers and reference accessions in the kit, scientists in other laboratories will be able to compare the genotypic data that they obtain for their germplasm with that obtained using the global composite collection.

Keywords: allele sizes; composite collection; polymorphism information content; simple sequence repeats

Introduction

Chickpea (*Cicer arietinum* L.) is a self-pollinated cool-season grain legume, cultivated on 13.2 million ha globally with a total production of 11.6 million metric tonnes (FAOSTAT, 2012). Chickpea is an important source of protein in the diets of the poor in the semi-arid tropics and West Asia and North Africa regions and is particularly important in vegetarian diets. Vast collections of chickpea germplasm are maintained at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, and the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria. The former contains 17,258 accessions (135 wild and 17,123 cultivated), while the latter houses 12,647 accessions (304 wild and 12,343 cultivated).

With the availability of large numbers of genomic simple sequence repeats (SSRs) in chickpea, it is now possible to conduct extensive molecular diversity studies in chickpea to identify diverse germplasm with beneficial traits for use in crop improvement programmes. Although a large number of SSR markers have been developed in recent years, inter-laboratory variations have made comparisons of results difficult. In the framework of the CGIAR Generation Challenge Program, the ICRISAT in India and the ICARDA in Syria assessed the genetic diversity of 3,000 chickpea accessions using 35 SSR markers distributed throughout the genome. Based on genotyping data obtained with the 35 SSR markers, a reference genotyping kit was developed to allow the international community to better integrate other genetic diversity studies.

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Table 1. Allele sizes obtained for each simple sequence repeat marker of the kit for reference accessions

	Allele size (bp)[a]		
Markers	C1	C2	C3
CaSTMS2	262, 232, 244	256, 241, 256	244, 271, 259
CaSTMS15	248, 251, 257	236, 263, 269	251, 254, 254
CaSTMS21	168, 168, 172	172, 168, 172	168, 172, 172
TA14	272, 278, 266	266, 272, 272	278, 263, 263
TA21	352, 331, 361	337, 355, 328	349, 346, 346
TA22	209, 212, 203	209, 278, 209	206, 269, 269
TA27	227, 239, 233	239, 242, 236	236, 227, 224
TA28	330, 315, 312	342, 375, 315	339, 369, 363
TA46	142, 145, 142	145, 148, 151	127, 154, 154
TA64	248, 254, 251	230, 239, 233	206, 242, 242
TA71	196, 205, 214	196, 223, 202	202, 184, 187
TA72	246, 255, 246	234, 261, 240	243, 234, 228
TA76 s	203, 212, 218	218, 218, 212	212, 218, 218
TA113	189, 201, 204	201, 201, 195	207, 192, 192
TA116	179, 179, 182	194, 179, 179	179, 212, 212
TA117	251, 230, 260	236, 254, 227	248, 245, 245
TA118	215, 191, 200	188, 191, 230	200, 230, 218
TA130	224, 221, 221	221, 206, 224	221, 224, 224
TA135	199, 193, 193	193, 199, 193	175, 190, 190
TA142	131, 131, 131	137, 128, 125	134, 140, 140
TA200	271, 292, 271	295, 286, 298	298, 307, 274
TA206	406, 382, 394	382, 388, 376	376, 385, 373
TAA58	295, 307, 313	280, 307, 271	313, 313, 313
TaaSH	406, 430, 433	433, 433, 427	430, 436, 433
TR2	229, 241, 223	229, 244, 223	250, 214, 214
TR29	193, 217, 214	190, 220, 211	211, 172, 172
TR31	198, 216, 198	210, 216, 213	201, 201, 201
TR43	307, 375, 301	349, 369, 301	325, 297, 297
TR7	217, 212, 209	218, 206, 203	203, 200, 197
TS84	230, 226, 226	226, 230, 230	226, 238, 244
NCPGR12	259, 261, 225	253, 253, 213	255, 235, 235
NCPGR19	300, 308, 298	300, 308, 298	308, 312, 312
NCPGR4	194, 180, 194	194, 196, 194	198, 194, 194
NCPGR6	249, 255, 251	251, 255, 251	249, 251, 251
NCPGR7	217, 223, 219	219, 223, 219	217, 219, 219

[a] C1: ICC 14 446, ICC 15 996, and ICC 15 994; C2: ICC 11 265, ICC 6537, and ICCV 2; C3: Annigeri, ICC 13 454, and ICC 4366.

Materials and methods

For genotyping, 35 SSR markers were chosen from published and unpublished data from other colleagues (Hüttel *et al.*, 1999; Winter *et al.*, 1999; Sethy *et al.*, 2006; Table S1, available online). The markers were selected based on quality criteria, genome coverage and polymorphism information content. Furthermore, the 35 selected markers are distributed across all the eight linkage groups, with a range of two to six markers per linkage group. Polymerase chain reaction (PCR) was carried out in a 5 µl volume containing 10 ng of DNA, 1 × buffer, 200 µM of dNTP, 2.5 mM $MgCl_2$, 1–5 pmol of forward (fluorescence dye labelled) and reverse primers, and 0.2 U of *Taq* DNA polymerase. PCR was carried out using Perkin Elmer 384-well Thermal Cyclers (Applied Biosystems, Foster City, CA, USA) using a touchdown PCR (65–60, 60–55 and 55–45°C) at corresponding annealing temperatures (Table S1, available online). The amplified DNA fragments were separated and detected using an ABI 3700 sequencer (Applied Biosystems, Foster City, CA, USA). Allele size data were base-called using the Genotyper version 3.1 software (Applied Biosystems, Foster City, CA, USA) and were subsequently analysed using an in-house-constructed program 'AlleloBin' and alleles were binned as per the corresponding SSR length.

Results and discussion

A set of nine chickpea accessions representing the largest genetic diversity was chosen based on phenotyping data. After analysing the allele sizes for these genotypes, three pools comprising three genotypes each were created (C1: ICC 14 446, ICC 15 996, and

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ICC 15 994; C2: ICC 11 265, ICC 6537, and ICCV 2; and C3: Annigeri, ICC 13 454, and ICC 4366). For each marker, the nine genotypes and three mix controls were amplified and analysed on an ABI 3700 genetic analyser (Applied Biosystems, Foster City, CA, USA). Allele sizes obtained in control pools are given in Table 1. The allelic patterns of control samples for all the 35 markers can be found at http://www.icrisat.org/gt-bt/chickpea/varshney-2013. Distinct alleles amplified in an individual genotype with each marker as well as control pools are given with the called allele size in Table S2 (available online).

The developed SSR marker kit of chickpea is recommended for use in assessing the diversity in chickpea germplasm. Using the kit, other germplasm collections can be more accurately compared with that of the global composite, reference and mini core collections.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000392

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