

Genotyping a composite germplasm set of lentil

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Abstract

The International Center for Agricultural Research in Dry Areas (ICARDA) is participating in a large-scale program that aims to explore the genetic diversity of the global germplasm collections held by the CGIAR's research centers. This project, Subprogram 1 of the CGIAR's Generation Challenge Program, will identify a 'composite collection' of germplasm for individual crops and characterize each composite set using anonymous molecular markers. The overall goal of this project is to study diversity across given genera and identify genes for resistance to biotic and abiotic stresses that can be used in crop-improvement programs.

ICARDA is responsible for creating the composite collection for lentil and to analyze these accessions for microsatellite diversity. ICARDA has the global mandate for lentil and houses the largest global collection of this crop, with over 10,800 accessions. From this collection, a global composite collection of 1000 lentil accessions was established (Furman, 2006). Accessions for the composite collection were compiled from landraces, wild relatives, and elite germplasm and cultivars.

The global composite collection developed at ICARDA has been analyzed using **30** SSR markers (Hamwieh et al., 2005). **[Something more here]** Plants grown for DNA analysis have been harvested and progeny will be evaluated under field conditions at ICARDA.

Introduction

Lentil (*Lens culinaris* Medik.) is an important cool-season crop in North Africa, West Asia, the Middle East, the Indian Subcontinent and North America (Erskine 1996). It is an important source of dietary protein (25 percent) in both human and animal diets, second only to soybeans as a source of usable protein (CGIAR, 2005). Lentil ranks seventh among grain legumes and is grown on over 3.5 million hectares in over 48 countries with a total production of over 3 million metric tons. The major lentil producing regions are Asia (58 percent of the area) and the West Asia-North Africa region (37 percent of the acreage of developing countries).

The genus *Lens* comprises seven taxa within four species including the cultivated type, *Lens culinaris* spp. *culinaris* (Ferguson *et al.*, 2000). Cultivated lentil includes two varietal types: small-seeded microsperma and large-seeded macrosperma (Barulina, 1930). *L. culinaris* subsp. *culinaris* is grouped with its three putative wild progenitors, *L. culinaris* Medikus subsp. *orientalis* (Boiss.) Ponert, *L. culinaris* Medikus subsp. *odemensis* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson, and *L. culinaris* subsp. *tomentosus* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson. The remaining wild species include *L. nigricans* (M.Bieb.) Godron, *L. ervoides* (Brign.) Grande, and *L. lamottei* Czefr. All members of *Lens* are self-

pollinating diploids ($2n = 2x = 14$; Sharma *et al.*, 1995). The haploid genome size of the cultivated genome is 4063 Mbp (Arumuganathan and Earle, 1991).

The International Centre for Agriculture in Dry Areas (ICARDA) has a global mandate for research on lentil improvement. As such, ICARDA houses the world collection of *Lens*, totaling over 10,800 accessions. The ICARDA collection includes 8860 accessions of cultivated lentil from 70 different countries, 1373 ICARDA breeding lines, and 581 accessions of 6 wild *Lens* taxa representing 24 countries (Figure 1). From this collection, a composite germplasm set of 1000 accessions was established and has been characterized utilizing molecular microsatellite markers.

Methods

- A global composite collection of 1000 lentil and wild *Lens* accessions was created (Furman, 2006).
- Plants were grown in the green house and leaves were harvested for DNA isolation.
- **Blah Blah Blah – DNA isolation, ABI, etc.**

Results

A global composite collection of 1000 lentil accessions was created at ICARDA consisting of landraces, wild relatives, and elite germplasm and cultivars (Figure 2). The composite collection represented the candidate set of accessions both in distribution, type and diversity (Furman, 2006).

This collection was planted for DNA extraction and analyzed for microsatellite diversity utilizing 30 SSR markers (Table 3, Figure 3; Hamwieh *et al.* 2005).

[OTHER RESULTS HERE]

Plants grown for DNA analysis will be harvested and progeny evaluated under field conditions at ICARDA.

Table (1): Lentil microsatellite codominant primer sequences used for analysis.

Primer No.	Primer sequence (F)	Primer sequence (R)	Tm	bp	Repeats
SSR13	GAAACAACACCGAAATACAC	CGAAGTCAGATGAAAGTTTG	53 °C	150	(CA)6
SSR 19	GACTCATACTTTGTTCTTAGCAG	GAACGGAGCGGTCACATTAG	58 °C	250	(TG)14
SSR 33	CAAGCATGACGCCTATGAAG	CTTTCACTCACTCAACTCTC	56 °C	289	(CA)21(GA)25
SSR 48	CATGGTGGAAATAGTGATGGC	CTCCATACACCACTCATTAC	57 °C	165	(TG)13
SSR 66	GGTAGTGGTGAGGAATGAC	GCATCACTGCAACAGACC	57 °C	253	(TG)10(AG)18
SSR 80	CCATGCATACGTGACTGC	GTTGACTGTGGTGTAAGTG	50 °C	155	(TC)14(AC)12(AT)2
SSR 96	GTTATCTTCCAGCGTC	GATATACAATCAGAGATG	48 °C	210	(TG)10
SSR 99	GGGAATTTGTGGAGGGAAG	CCTCAGAATGTCCCTGTC	57 °C	161	(TG)8TC(TG)2
SSR 107	GCGGCGAGCAAATAAAT	GGAGAATAAGAGTGAATG	51 °C	168	(TC)9+(AT)5C(AT)3(GT)14A(TG)7
SSR 113	CCGTAAGAAATAGGTGTC	GGAAAATAGGGTGGAAAG	46 °C	211	(AC)17(AT)13
SSR 119	GAAGTCAGTTTCTCATTG	GAACATATCCAATTATCATC	49 °C	266	(TA)4TT(TA)11(TG)19
SSR 124	GTATGTGACTGTATGCCTC	GCATTGCATTTACAAAACC	47 °C	174	(TGC)3+(GT)9TA(TG)2
SSR 130	CCACGTATGTGACTGTATG	GAAAGAGAGGGCTGAAACTTG	55 °C	196	(GT)9
SSR 151	GGTAGGTGAGATAGTTG	GGAGCAAGAAGAAGCAG	51 °C	134	(TG)4(TGTGTA)7(TG)4
SSR 156	GTACATTGAACAGCATCATC	CAAAATGGGCATGAAAAGGAG	53 °C	176	(TC)2(TG)13
SSR 167	CACATATGAAGATTGGTCAC	CATTTATGTCTCACACACAC	54 °C	160	(TA)16(TG)21
SSR 184	CCTAAAGCCTTGCCAGAC	CTGAAACCACCACATACG	55 °C	250	(GT)10(AT)15(GT)19
SSR 199	GTGTGCATGGTGTGTG	CCATCCCCTCTATC	51 °C	182	(GT)4GC(GT)8GC(GT)3
SSR 204	CACGACTATCCCACTG	CTTACTTTCTTAGTCTATTAC	52 °C	286	(TG)4+(AC)7
SSR 213	CACTCGCACCTCTATG	GAAATTGTCTCTTAGCAAG	46 °C	151	(TA)8(TG)5TA(GT)5
SSR 215	CATTAATATTTCTTTGGTGC	CTTTTCTTCTTTCCCC	50 °C	331	(CA)15(TA)25
SSR 233	CATTGTGGAATGCTTGATG	GCCGCCTACATTATGG	52 °C	111	(GT)9
SSR 302	CAAGCCACCCATACACC	GGGCATTAAGTGTGCTGG	50 °C	261	(TA)15(CA)11
SSR 323	AGTGACAACAAAATGTGAGT	GTACCTAGTTTCATCATTG	45 °C	250	(CG)3CC(CA)31(AT)22(CA)4
SSR 336	GTGTAACCCAACTGTTC	GGCCGAGGTTGTAACAC	49 °C	253	(TAA)6AGA(TAA)4
SSR 340	GTCATGTGCAATAGACACG	CCTTTTCCACCCCTACAC	49 °C	165	(GT)9
SSR 212-1	GACTCATTGTTGTACCC	GCGAGAAGAATGGTTG	44 °C	181	(AT)2(TC)26(AC)8
SSR 309-2	GTATGTCGTTAACTGTCGTG	GAGGAAGGAAGTATTCGTC	50 °C	182	(AT)3GT(TA)3T(TAT)6
SSR 317-1	GTGGGTGTAATATTGCTAC	GTATCAAACCTATGGTGAATC	48 °C	308	(AT)4(GT)16(GC)6GTGGC(GT)5A(TG)8+(TAA)5
SSR 317-2	CACGTAACATCTTGCTTATG	GTAGCAATAATTACACCCAC	48 °C	120	(TTG)2(AT)2A(AT)2G(TA)14ATC(GT)4
SSR 59-2	CCAAATACTGCAACACACCG	GTTCCTATCAGGCAGAAGG	58 °C	175	(CA)19(TA)19

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