CHAPTER 2

LENS BIODIVERSITY

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Abstract: The *Lens* genus includes the cultivated *L. culinaris,* and wild subspecies *orientalis* - the progenitor, *tomentosus,* and *odemensis,* are in the primary genepool, while *L. ervoides, L. nigricans* and *L. lamottei* are in the secondary – tertiary gene pool. The Middle East is the primary centre of diversity for the primary genepool, with distribution of *L. orientalis* extending to central Asia, and of *L. ervoides* extending along the Mediterranean to Spain. The largest *Lens* collection is held at ICARDA. *In situ* reserves of *Lens* diversity are in Turkey and Syria. Documentation and storage of *Lens* germplasm is discussed. An evaluation database covering a number of genebanks has been developed for lentil germplasm. Core collections are discussed in the context of the generation Challenge program. Application of DNA characterisation is outlined, along with the potential for allele mining for variation in key traits, the study of relationships within *Lens* and the use of mapping populations. Reference is made to the International treaty for Plant Genetic Resources for Food and Agriculture

1. SOURCES OF GENETIC DIVERSITY

The genus *Lens* is a member of the legume tribe Vicieae which includes the major legume crops of the classical Mediterranean civilizations, faba bean, pea along with lentil. The precise generic boundaries between *Lens* and the related Vicieae genera (*Vicia* L., *Lathyrus* L., *Pisum* L. and *Vavilovia* A. Fedorov) have been much debated, but *Lens* appears most closely related to *Vicia* (Kupicha 1981). *Lens* is a small Mediterranean genus which contains the cultivated lentil (*L. culinaris* Medikus subsp. *culinaris*) and six related taxa (Ferguson 2000), as shown:

S.S. Yadav et al. (eds.), Lentil: An Ancient Crop for Modern Times, 11–22.

L. culinaris Medikus
subsp. *culinaris*
subsp. *orientalis* (Boiss.) Ponert
subsp. *tomentosus* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson
subsp. *odemensis* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson
L. ervoides (Brign.) Grande
L. nigricans (M.Bieb.) Godron
L. lamottei Czefr.

The cultivated taxon, *Lens culinaris* Medikus subsp. *culinaris*, includes two varietal types: small-seeded *microsperma* and large-seeded *macrosperma* (Barulina 1930).

Lens culinaris subsp. *orientalis,* in the primary genepool (Ladizinsky and Alder 1976), is fully cross compatible with cultivated lentil (Muehlbauer and Slinkard 1981, Robertson and Erskine 1997). *L. nigricans,* in the secondary genepool, can also cross with the cultigen, yet seed set in hybrids is considerably lower than a *L. culinaris* subsp. *culinaris* x *L. culinaris* subsp. *orientalis* cross (Muehlbauer and Slinkard 1981). *L. culinaris* subsp. *orientalis* accessions have been found to contain resistance to drought (Hamdi and Erskine 1996), cold (Hamdi *et al.* 1996, Robertson et al. 1996), wilt (Bayaa et al. 1995), and Ascochyta blight (Bayaa et al. 1994, Robertson et al. 1996).

The Middle East is the primary centre of diversity for both the domestic *L. culinaris* and its wild progenitor *L. culinaris subsp. orientalis* (Cubero 1981, Zohary 1972), but taxa are found from Spain to Tajikistan. Although Ferguson et al. (1998) showed that for *L. culinaris* subsp. *orientalis* two centres exist, (a) south-eastern Turkey and north-western Syria, and (b) southern Syria and northern Jordan. The southern centre overlaps with the centre of diversity for *L. culinaris* subsp.*odemensis*. A region of diversity exists for *L. ervoides* along the eastern Mediterranean coast and for *L. nigricans* in south-western Turkey. Ferguson et al. 1998 also showed that *L. culinaris* subsp. *orientalis* accessions from Iran, central Asia and northern Turkey are all very similar to each other, and correspond to the common cytotype identified by Ladizinsky et al. (1984). *L. ervoides* populations from the coastal region of the former Yugoslavia have a narrow genetic base, as do *L. nigricans* accessions from the same region as well as those from France and Spain. Maps illustrating the distribution of variation can be found in Ferguson and Erskine (2001).

Wild populations of *Lens* are generally poor competitors and highly palatable to grazing animals (Ferguson and Erskine 2001). They are found predominantly in primary, ungrazed habitats where they are not subject to competition by aggressive coloniser plants. They usually form small disjunct populations. The density of plants may vary dramatically between years apparently because of climatic conditions. *L. culinaris* subsp. *orientalis*, subsp. *tomentosus*, subsp. *odemensis* and *L. nigricans* are generally found in open or partially shaded habitats, on shallow stony soils originating from calcareous, metamorphic or basalt rocks. *L. nigricans* and *L. lamottei* have also been found in habitats showing signs of earlier human disturbance such as abandoned terraces or plantations and ruins. Both *L. nigricans* and *L. ervoides* have been found from sea level to over 1000 m.*L. culinaris* subsp. *orientalis* and

subsp. *odemensis* have been found at even higher altitudes from 500 m up to 1700 m and from 700 m up to 1400 m respectively. Populations of *L. lamottei* have been located between 100 and 800 m. Although all wild species (except *L. ervoides*) have similar habitat preferences, they are rarely found together. *L. ervoides* usually inhabits shady places and is frequently associated with pine forests often growing on calcareous bedrock (Ferguson and Erskine 2001).

2. *EX SITU* COLLECTIONS

The International Center for Agricultural Research in Dry Areas (ICARDA) has a global mandate for research on lentil improvement. As such, ICARDA houses the world collection of *Lens*, totalling 10,578 accessions (Figure 1). The ICARDA lentil genetic resources collection includes 8858 accessions of landraces and cultivars from 69 different countries representing 4 major geographic regions (Figure 2), 1137 ICARDA breeding lines, and 583 accessions of 6 wild *Lens* taxa representing 24 countries (Table 1). The majority of the collection (48%) consists of accessions

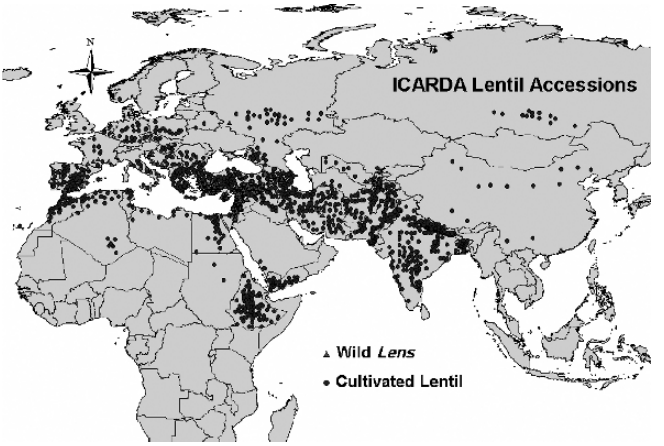

Figure 1. Distribution of ICARDA *Lens* accessions

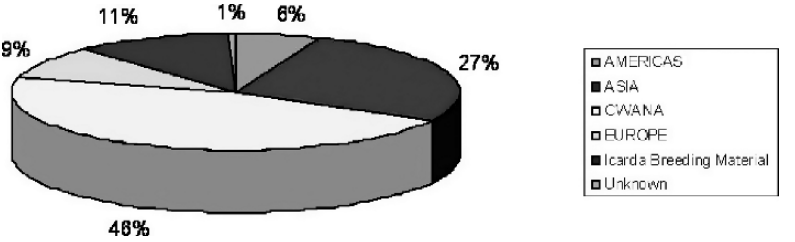

Figure 2. Geographic regions represented in ICARDA lentil collection

Table 1. Wild *Lens* accessions maintained at ICARDA

Taxonomic name	Number of countries represented	Number of accessions
Lens culinaris subsp. odemensis	4	65
Lens culinaris subsp. Orientalis	14	268
Lens culinaris subsp. tomentosus	2	11
Lens ervoides	15	166
Lens lamottei	3	10
Lens nigricans	8	63
Total	**46**	**583**

from Central and West Asia and North Africa, the centre of origin and primary diversity (Zohary and Hopf 1988; Ferguson and Erskine 2001), while South Asia represents an additional 25%.

Lentil accessions in the ICARDA collection were obtained from 113 ICARDA collection missions (46%), 56 donor institutions (44%) and ICARDA's breeding program (11%). Collection missions have yielded 1734 cultivated and 374 wild accessions. Recent missions (13 missions since 1999) to Central Asia and the Caucasus has substantially decreased geographic gaps from this region yielding a total of 122 cultivated and 27 wild *Lens* accession (Dr. Ken Street pers. Com. 2006.). Other important collections worldwide include those at Pullman USDA Agricultural Research Service (ARS) with 2797 accessions (http://www.ars.grin.gov/), N.I. Vavilov All-Russian Scientific Research Institute of Plant Industry (http://vir.nw.ru/) with 2396 accessions, the Australian Temperate Field Crops Collection in the Department of Primary Industries Victoria, Australia with 5250 accessions and the National Board of Plant Genetic Resource, India with 2212 accessions (Dwivedi et al. 2006).

Ferguson and Erskine (2001) conclude that based purely on the number of wild *Lens* accessions available, germplasm from North African countries such as Algeria, Libya, Sudan and Tunisia appear to be under-represented in the collection, as does germplasm from the new central and west Asian republics of the former Soviet Union. However the overall collection priority for the wild species based on the distribution of genetic variation (Ferguson et al. 1998a) remains south-west Turkey, particularly the provinces of Burdur, Isparta and Afyon.

3. *IN SITU* CONSERVATION

There has been no systematic attempt to conserve *Lens* diversity *in situ* using either genetic reserves or on-farm techniques. However, undoubtedly existing protected areas throughout the range of the genus contain *Lens* species. In these protected areas conservation may be referred to as 'passive' (*Lens* species presence and genetic diversity is not being actively managed or monitored) and therefore it is susceptible to further unobserved genetic erosion. More active conservation of Lens diversity is found in the managed genetic reserves of the Eastern Mediterranean (e.g. Ammiad

in Eastern Galilee, Israel; Kaz Dag, Aegean Region, Amanos, Mersin in Turkey and Ceylanpinar of South-east Turkey), all of which contain *Lens* diversity. The latter reserve is particularly important as Ferguson et al. (1998) showed that this area is one of the two centres of genetic diversity for *L. culinaris* subsp. *orientalis*. The second centre identified by Ferguson et al. (1998) is in southern Syria and Maxted (1995) proposed the establishment of a genetic reserve for Vicieae species in the region of Mimas, Djebel Druze in Southern Syria. Recently this area has been designated as a genetic reserve by the Syrian Scientific Agricultural Research Commission, as part of their Global Environment Facility funded "Conservation and Sustainable Use of Dryland Agrobiodiversity" project (Maxted et al. 2003). So although some progress has been made recently in *in situ* conservation in the areas of highest *Lens* genetic diversity, there remains an urgent need to systematically establish both reserves for the wild species of *Lens* and on farm projects to conserve the ancient land races of cultivated *Lens* species.

4. CONSERVATION AND STORAGE

The recommended storage facilities for genebanks (FAO/IPGRI 1994), would contain a Base collection of over 100 seed (FAO/IPGRI recommend over 1,000 seed) in −18°C–20°C, and an Active collection of up to 1,000 seed at 2°C – 5°C, respectively, for long term conservation and for medium term storage and distribution. Drying of seed at 10 – 25°C and 10–15% relative humidity (RH) is recommended before storage. The Base collection should aim to maintain the genetic integrity of the seed samples of accessions as originally received, and provide very long term conservation of genetic diversity. It also can replenish seed stocks in the Active collection in every 4th regeneration cycle, a precaution against genetic drift within samples over repeat cycles of regeneration (FAO/IPGRI 1994). A safety duplicate collection for long term storage in another location is recommended, also referred to as a Back-up collection.

Viability will decline in all stored seed, as a function of initial viability, seed moisture and storage temperature (Roberts and Ellis 1984). Seed harvest, drying, processing and pre-storage temperature and humidity conditions may all affect the initial viability, which is usually close to 100% for freshly harvested seed from well grown plants. An investigation of lentil seed longevity was initiated at ATFCC in 2003, with testing seed of 2 varieties at 5 temperatures (40°C, 22°C, 15°C, 2°C and −18°C) and 3 seed moisture levels (6–7%, 10–11%, 12–13%). An additional 8 genotypes are being tested at 22°C. This study will estimate the seed moisture and temperature storage constants for *Lens*, and examine whether these conform to a logarithmic formula as per the Roberts and Ellis (1984) prediction. Results are available for the 40°C treatments, in which the lentil variety Cumra with near 100% initial viability, at high (12.3%), medium (9.5%) and low (7.6%) seed moisture levels, took 200, 685 and 1060 days respectively to fall 50% seed viability.

These studies are important to enable better planning of intervals between regeneration cycles, and more strategic monitoring of viability with germination tests.

Seed in the Active 2°C collection may retain high viability for at least 30 years, given high initial viability, reduction of seed moisture and immediate storage post-harvest. Seeds should be stored in moisture proof containers. Vacuum and heat sealing of plastic lined foil envelopes is one of the common genebanks procedures. Opening and resealing of these packets for seed removal and distribution is best carried out in a drying room, e.g. 15°C and 15% RH as above. Alternately, 1–2 additional small seed samples per accession may be prepared in anticipation of requests for germplasm. A decision guide for seed regeneration, and precautions to maintain genetic integrity, are outlined by Sackville Hamilton and Chorlton (1997).

5. DOCUMENTATION

There are 3 main activities, described in the IPGRI handbook for germplasm collections (Reed 2004);

1. germplasm and site characterisation (passport) for an accession collected as a traditional landrace *in-situ*, for placement in an *ex-situ* genebank elsewhere,
2. genebank inventory with accession identifiers, taxonomic descriptors, country and site passport data, dates of accession, collector/donor details, synonyms for accessions acquired from other genebanks,
3. accession characterisation data. In the case of lentil standard trait descriptors are provided in IBPGR 1985. These describe discrete classifications for morphological traits such as colours of seed, stems and flowers; scales for disease susceptibility from 1 = none to 9 = lethal; and standards for quantitative traits such as flowering time, 100 seed weight, and seed yield, and others.

Full records are very important to check through synonyms for duplication of accessions, for precise collection site data with referencing by latitude, longitude and altitude whenever possible, and for quality assurance on identity. This can be an issue in genebanks with multiple sources of accessions, and large numbers of accessions and species.

Equally important are characterisation and evaluation data. Better targeted and more efficient utilisation of germplasm by plant breeders/researchers can be achieved if the trait characteristics of accessions are known. This can include the agronomic, disease reaction, yield and quality data of accessions in a particular study, and over the different studies for each accession. Evaluation data for lentil germplasm can now be queried for multiple traits from combined databases, such as constructed by ATFCC, using ICIS (International Crop Information Systems) platforms for digital search and retrieval across relational databases over countries and years. This database, the International Lentil Information System (ILIS) combines databases of ICARDA, USDA and ATFCC, enabling a more comprehensive search of the combined genetic resources over large collections for multiple trait expressions; http://149.144.200.51:8080/QMWebRoot

As far as possible, the internationally standard IPGRI descriptors are used for trait records in ILIS, as provided in IPGRI (1985). Additionally ILIS has options

for discretisation, with quantitative traits converted to a 1–9 scale based on arithmetic division of the range of values (outlying values quality checked and filtered out), or on statistical normalisation of data from each location/year. These conversions eliminate the environmental component from observed values, as well as the genotype X environment interaction, to provide genetic comparisons of landraces for trait expressions across locations, years, and genebanks. Breeders can request selected accessions on-line from genebanks, for validation in the respective target environments for farmer clients, and choice of parents for the breeding program.

There is increasing interest by breeders in exploiting the wider diversity usually available in the primary, secondary and tertiary gene pools of wild relatives. A number of genebanks provide well documented and up to date taxonomic guides for the *Lens* genus, eg. The GRIN database of USDA ARS; http://www.ars-grin.gov/cgi-bin/npgs/html/genform.pl

6. MOLECULAR DIVERSITY, INTRA-SPECIFIC AND INTER-SPECIFIC VARIATION

Two important new developments in molecular genetics have applicability to genebanks. One is the use of molecular markers for DNA 'fingerprint' characterisation of individual accessions. Spooner et al. (2005) reviewed choice of markers for particular investigations. These tools enable the identity of accessions to be confirmed between collections, detection of duplicates within collections, and analyses of genetic relatedness amongst accessions to define the structure of genetic diversity within a species/genus. This knowledge assists breeders seeking allelic diversity for particular traits. A second application is the capacity of allele mining for various traits using association genetics, both within species/wild relative collections, and across species with comparative genomics (Spooner et al. 2005). These approaches are scheduled for phases 2 and 3 of GCP with the lentil composite collection.

Molecular analysis corrected the taxonomy of *Lens* and resolved the relationships between the species and subspecies of cultivated and wild lentil (Ferguson 2000). This study confirmed the relationship results using morphology, isozymes and RAPDs on 404 accessions (100 *L. culinaris* subsp. *culinaris*, 128 *L. culinaris* subsp *orientalis*, 32 *L. odemensis*, 30 *L. nigricans*, 118 *L. evoides*, 32 *L. nigricans*, 7 *L. lamottei*, 8 *L. tomentosus).* Numerous previous studies also used molecular markers and proposed a mixture of different phylogenies for *Lens* species. These earlier studies used isozymes (Hoffman et al 1986; Ferguson and Robertson 1996); RFLPs (Harvey and Muehlbauer 1989); cpDNA (Muench et al. 1991; van Oss et al. 1997) and RAPDs (Abo-Alwafa et al. 2005; Sharma et al. 1995; Ford 1997 and 1999). However, far fewer accessions were used than the Ferguson (2000) study used and therefore the Ferguson (2000) classification, shown above, is now widely accepted (Sarker and Erskine 2006).

The *ex situ* collections of lentil and its wild relatives are serving as mapping populations using association mapping statistics as first demonstrated in maize (Thornsbury et al. 2001). First, the genetic structure of the germplasm mapping

population needs to be determined (Pritchard et al. 2000), and then an understanding of the amount of linkage disequlibrium (LD) needs to be determined as each crop species will vary. Large scale genotyping of the lentil germplasm collection held at ICARDA is complete (Furman 2006) and is underway on the lentil core collection held by the USDA Agriculture Research Service (ARS) in Pullman, WA USA using 39 mapped SSRs (Coyne personal communication). The ARS studies are for fine mapping of disease resistance QTL identification (Muehlbauer personal communication). The ARS plan is to sequence candidate genes in a core subset (32 pure lines) of the single plant core accessions and arrive at an estimate of the linkage disequilibrium in lentil.

7. CORE COLLECTIONS

The International Center for Agricultural Research in Dry Areas (ICARDA) is participating in the lentil project for the Generation Challenge Program (GCP). This project will identify a 'composite collection' of germplasm for individual crops, representing the range of diversity of each crop species and its wild relatives, and characterize each composite set using anonymous molecular markers, mainly SSRs. ICARDA was responsible for creating the composite (core) collection for lentil as part of the CGP, that aims to explore the genetic diversity of the global germplasm collections held by international research centers (http://www.generationcp.org). A global composite collection of 1000 lentil accessions was established representing the overall genetic diversity and the agro-climatological range of lentil. Entries included landraces, wild relatives, elite germplasm and cultivars representing the overall ICARDA collection in both distribution and type.

The methodology for establishing the composite collection combined classical hierarchical cluster analyses using agronomic traits and two-step cluster analyses using agro-climatological data linked to the geographical coordinates of the accessions' collection sites (Furman 2006). The hierarchical cluster analysis ensured that the level of variation found in the larger collection was maintained in the composite collection. In addition, scientists at ICARDA suggested 64 accessions of landraces, released cultivars, and breeding materials for their resistances to a number of stresses affecting lentil production to be included in the composite collection.

The composite core lentil collection for the GCP is both being evaluated for morphologic and agronomic traits, and analysed for molecular diversity. This collection has 522 landraces with latitude and longitude passport data. These are matched to climatic and soil maps in selecting for adaptation to crop moisture and abiotic stresses. This will enable trait association genetic mapping based on the combined phenotypic/molecular analyses of the consensus collection. The strategy is to identify sources of desired traits for development of drought tolerant varieties. The USDA ARS lentil collection totals 2839 accessions with 123 wild taxa. A core of 234 accessions was selected based on country of origin (Simon and Hannan 1995). Recently, the core was extended (384 accessions) to add mapping population parents, cultivars and wild accessions, and a subset of pure lines was created.

This pure line subset will be distributed to scientists interested in LD mapping in lentil.

8. UTILISATION OF LENTIL GERMPLASM

With provision of an evaluation/passport database covering the major world collections, targeted multiple trait searching of most of the available lentil germplasm can now be utilised more efficiently and effectively, with strategies such as allele pyramiding in key traits and sourcing of novel genes from wild relatives (see chapters 13, 16 and 17). Generally, breeders narrow the genetic diversity in their breeding populations in the process of selecting the required trait combinations for outputs of improved varieties (Maxted et al. 2000). Genebanks try to conserve the original landrace diversity for both current and for unforseen future needs in breeding, enabling survey and enhancement of germplasm diversity for key traits, and identification of novel genes from wild relatives.

The standard practice for genebanks is to document passport data on the origin including latitude/longitude/altitude (GPS data), synonyms, and collection data with details of source location and associated agriculture (IPGR 1985). This enables traceability of the accession with details of collection procedure and evolutionary status. However, without knowledge of the growth characteristics and reactions to abiotic and biotic stresses of accessions there is a risk that genebanks may become museums, unless conservation is linked to utilisation (Maxted et al. 2000). The example given is the collection of 30,000 pulse accessions at one national genebank with 'only 2–3% used in crosses, because of the lack of characterisation and evaluation data'. A breeder can only utilise a small number of accessions in practice, and is in need of all available characterisation and evaluation data. Given (1994) indicated that in general 80% of germplasm lacks characterisation data and 95% lack evaluation data. While FAO (1998) reports that many country's collections require further characterisation and the characterisation that has occurred is restricted to a few species, most commonly the major food or commodity crops.

ICARDA evaluates lentil germplasm for morpho-agronomic traits as per IBPGR (1985) descriptors, and has published a Lentil Germplasm Catalogue (Erskine and Witcombe 1984). This provides a listing of 20 trait evaluations for 4,550 lentil accessions. There has been a large increase in the size of the lentil collections in various genebanks, and also in the associated evaluation data which is often recorded with the regeneration of germplasm in various nurseries over many years. The combining of this information from many genebank sources is now possible with suitable electronic software for relational databases (Balachandra et al. 2006), as exemplified by the ILIS web site in section 2.5.

The ILIS database allows on-line users to run search-query interrogations for germplasm with multiple trait expressions, currently from the combined ICARDA, USDA and ATFCC collections. This is a very powerful search engine, to assist genebank clients, to add value to the collections, and to facilitate utilisation for

crop improvement. In addition, GPS passport data linked to climatic and stress maps may indicate adaptation characteristics of an accession. It is important to justify the expense of genebank operations, in both the short term with current crop improvement outcomes, and in the long term where a genetic insurance strategy may be very prescient to cope with a looming climate change.

Close linkage of lentil collections with breeders, pathologists and students, for evaluating key traits is becoming a significant genebank activity. ATFCC staff actively work with lentil breeders in germplasm enhancement for evaluation of tolerances to salinity, frost, herbicides, and disease (Redden et al. 2006). The ATFCC provided the Australian lentil breeding program with accessions ILL 2024 and ILL 213A identified for tolerance of high levels of boron. ICARDA has identified sources of resistance in the domestic genepool to rust, vascular wilt and to aschochyta, and in the wild progenitor *L.culinaris* subsp. *orientalis,* has found additional sources of resistance to vascular wilt and aschochyta and greater cold tolerance (Ferguson 2000).

9. INTERNATIONAL TREATY FOR PLANT GENETIC RESOURCES FOR FOOD AND AGRICULTURE (PGRFA)

Over 80 countries have now formally endorsed PGRFA, which is rapidly becoming the relevant international treaty for genetic resources of major food crops including lentil; http://www.fao.org/ag/cgrfa/itpgr.htm

The objectives of PGRFA are;

- Conservation and sustainable use of plant genetic resources – especially landraces and wild relatives as prime sources of diversity,
- Facilitated multi-lateral access and sharing of germplasm among genebanks,
- Associated provision of passport and evaluation data for better informed utilisation of germplasm,
- Fair and equitable sharing of benefits derived from use of germplasm –if there is restricted Intellectual Property (IP) access to derived commercial varieties,
- Harmony with the CBD, and benefit sharing principles for non-PGRFA crops.

The creation of ILIS in consistent with PGRFA, with collated evaluation data both adding value to collections and enabling efficiencies in germplasm utilisation with targeted search and request of germplasm for enhancement/breeding.

There are CGIAR guidelines for the application of a Standard Material Transfer Agreement under the PGRFA treaty, relevant to the world's largest collection of lentil landraces and wild relatives held by ICARDA.

It is hoped that this under-pinning framework for genetic resource diversity will increase the attraction of using available lentil germplasm to solve the need for novel genes and new QTL expressions, in lieu of transgenic solutions.

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