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The Italian Lentil Genetic Resources: A Worthy Basic Tool for Breeders

Gaetano Laghetti • Angela R. Piergiovanni • Gabriella Sonnante • Lucia Lioi • Domenico Pignone*

Institute of Plant Genetics, CNR, Via G. Amendola 165/A, 70126 Bari, Italy

Corresponding author: * domenico.pignone@igv.cnr.it

ABSTRACT

The present contribution reviews the lentil landraces traditionally cultivated in Italy. Due to its position in the middle of the Mediterranean, Italy represents a good territory for drawing more general conclusions. Literature regarding archaeobotanical studies, conservation, exploitation, and risk of genetic erosion or extinction of Italian germplasm is briefly discussed. Knowledge on agronomic evaluations, on variability assessed by genetic (AFLP, ISSR, SSR) and biochemical (SDS-PAGE) markers, and on relationships among the still cultivated landraces is reported. The distinctive traits of Italian lentil germplasm are evidenced, together with data on grain composition, due to its relevance for consumers and food industry. These data are discussed on the grounds of the necessity of adequate actions aimed at safeguarding these precious genetic resources. Three case studies are analysed in depth. The potential of lentil germplasm for breeding programmes aimed at the selection of ideotypes well adapted to Italian and Mediterranean environments are also discussed.

Keywords: biochemical traits, genetic diversity, ideotype, landraces, *Lens culinaris*

Abbreviations: AFLP, Amplified Fragment Length Polymorphism; **ISSR**, Inter Simple Sequence Repeat; **RAPD**, Random Amplified Polymorphic DNA; **SDS-PAGE**, Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis; **SSR**, Simple Sequence Repeat

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INTRODUCTION

Lentil (*Lens culinaris* Medik.) is a diploid (2n=2x=14), autogamous species cultivated since pre-historic times. This makes lentil one of the oldest crops in the world, together with cereals like barley and rye (Hopf 1986; Lev-Yadun *et al*. 2000). Its wild progenitor (*Lens culinaris* ssp. *orientalis* (Boiss.) Ponert) has a wide distribution, but the genetic stock from which the crop originated was probably found in the Near East (Zohary 1972). It is supposed that this crop is the result of a single domestication event (Zohary 1999).

The oldest carbonized remains of lentil were discovered in the Franchthi cave in Greece, dated 11,000 BC, and from Tell Mureybit in Syria, dated 8,500-7,500 BC (Hansen and Renfrew 1978). It is not possible to distinguish the state of domestication of these and other carbonized remains since small-seeded cultivated types are morphologically undistinguished from wild lentils. The oldest large store of lentil is dated to 6,800 BC, suggesting that domestication had already taken place (Zohary and Hopf 1988; Zohary 1992). The crop spread in Neolithic times to Cyprus, to South-Eastern Europe and, via the Danube, to Central Europe. Re-

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Invited Review

mains of lentils are found in early agricultural settlements in Europe dating since the 5th millennium BC. Archaeobotanical investigations provided evidence of the long-lasting tradition of lentil cultivation in the whole Italian peninsula as well as in its major islands. Remains of some edible grain legumes, among which lentil seeds, have been found in an archaeological site belonging to the early Iron Age (8th-7th century BC) and located in Liguria (Northern Italy) (Arobba *et al.* 2003). In the same period evidences of lentil cultivation in Sicily and Sardinia, the major Italian islands, have been reported by Costantini (1989) and Bakels (2002), respectively. Traces of lentil cultivation in more recent settlement have been found by Mercuri *et al.* (2006). The authors studying the people of the Terramara area (Middle-Late Bronze Age, 1,650-1,200 BC) around Po river plain (Northern Italy) found remains of cereals, lentil and other legumes. This implies a long story of adaptation of this crop to Italian agro-environments. The importance of lentil in historical times is testified by the importance that Columella, the famous Latin agriculture writer of the 1st century AD, gives to this crop. In the "*De Re Rustica*", precise prescriptions on lentil cultivation practices, harvesting and seed conservation to prevent weevil damage are reported. The value of lentils in Roman times is further evidenced by their presence as ritual offerings at Roman graves (Collis 1978) and remains near ancient Pompeii (Meyer 1980).

Presently, lentil is still cultivated under very traditional systems in the Mediterranean Basin and in Asia, while it has traits of intensive cultivation in the Americas. In Italy, lentil cultivation is still based on local landraces rather than on improved varieties, that usually take the name from the area where they are traditionally cultivated (Foti 1982). Few landraces are very appreciated as niche or speciality products, since they are mostly cultivated under low input or organic agricultural conditions, thus increasing their market potential, and consequently reducing the threat of extinction. Most landraces are still growing over small, marginal fields. Marketing and trade are very limited these landraces being generally grown for self- or local consumption. Their maintenance is often left to the care of elder farmers and this poses a big threat of extinction (Hammer *et al*. 1999; Negri *et al.* 2000). In relatively large areas of central Italy, appreciated lentil landraces are cultivated as field crops; they are effectively preserved *in situ* due to the extent of their cultivation and the relatively high income they give in these areas (Negri and Russi 1996).

The long lasting lentil cultivation has allowed not only the evolution of several local populations (**Table 1**), but also the development of cultural, historical and traditional knowledge threatened to be lost together with the abandon of lentil cultivation. The evolution of Italian agriculture, which occurred during the past 60 years, has transformed Italy from a producer to an importer of this grain legume. In the first half of the 20th century, Italy was among the major lentil producers of the Mediterranean basin (Piergiovanni 2000). In the first two decades of 1900 the cultivation of Onano lentil (Lazio region) had a great economic relevance, as evidenced by photos depicting local people attending to lentil cultivation. This landrace was exported outside Italy and its appreciation was confirmed by the attribution of gold medals at several international expositions. From 1930 to 1960 the intensive production of lentil was mainly based on two *macrosperma* landraces: Altamura (Apulia region) and Villalba (Central Sicily). Nowadays, only the Castelluccio landrace (Umbria region), a small-seeded biotype, has consolidated market opportunities, especially after the receiving in 1996 of the Protected Geographic Indication (PGI) mark by European Union (EU).

Due to its position, laying in the middle of the Mediterranean, studies carried out on the Italian territory may easily be transferred to other Mediterranean countries, which largely share the same climatic and geographic conditions.

Table 1 Local name, traditional cultivation area and morphological description of the major Italian traditionally cultivated lentil landraces.

Local name	Cultivation region	Morphotype	Coat colour
Castelluccio di Norcia	Umbria	*microsperma*	1+2+3+4+5+6
Colfiorito	Umbria	*microsperma*	1
Onano	Lazio	*microsperma*	1+2
Ventotene	Lazio	*microsperma*	6
S. Stefano in Sessanio	Abruzzo	*microsperma*	5
Altamura	Apulia	*macrosperma*	1
Villalba	Sicily	*macrosperma*	1
Ustica	Sicily	*microsperma*	4
Pantelleria	Sicily	*microsperma*	4
Linosa	Sicily	*microsperma*	4

Coat colour code: 1– light green; 2 – light brown; 3 green; 4 – brown; 5 – dark brown; 6 – pink

AGRICULTURAL TECHNIQUES USED IN ITALY

Present days lentils are divided into two morphotypes, based on seed shape and dimension, the small seeded (less than 6 mm in diameter) *microsperma* or Persian type and the large seeded (over 6 mm) *macrosperma* or Chilean type (Barulina 1930). The large-seeded has average seed size of 50 g or more per 1000 seeds, while the small-seeded has average seed size of 40 g or less per 1000 seeds. In both types seeds are lens-shaped which is at the origin of the name lentil. The two types differ not only for morphological features, but also for agronomic characters. For instance small seeded types have an earlier emergence and are therefore better fit to spring sowing, while large seeded types, being slower in emergence, are better fit to autumn sowing season. This occurrence implies that the two types are better adapted to different agro-environments. Seed coat colours range from white, light green, brown, grey, spotted purple to black. The cotyledons can be yellow, orange, or green.

Lentil seeds have a hypogeal germination, so offering some protection to the young seedlings. If the main shoot is damaged above ground, e.g. by a late spring frost, the plant can re-grow from buds below ground, since the first scale node is typically below the soil surface. The first leaf usually develops at the third node, and new leaves are produced on higher nodes every 4-5 days, under good growing conditions. The number of leaflets per leaf varies from 9 to 15. Lentil plants are typically short, but, according to growing conditions and variety, height can range from 20 to 75 cm. Just before flowering, leaves produce a tendril at the leaf tip. Early maturing varieties such as Eston, flower at about the 11th or 12th node stage. Later maturing types, such as Laird, flower at the 13th or 14th node stage. Flowers are self-pollinated so they do not require insects for pollination or seed formation. The flowers are borne in clusters of 2 to 3 on short stalks at the base of the upper leaves. The first few flower clusters on the main stem often suffer of abortion. This is more likely to occur if conditions favour vegetative growth over seed production such as with soil good moisture and high fertility.

Lentil plants have an indeterminate growth habit. Pods are small, usually less than 2.5 cm long, and generally contain 1 or 2 seeds. Vigorously growing lentil plants with adequate space will produce two or more primary shoots from the base. However, the main contribution to seed yield is made by secondary aerial branches, up to five, that arise from the uppermost node of the main stem just below the first flowering node. When growing conditions are extremely favourable, the secondary branches also produce additional seed-bearing branches.

Adaptation

Lentil is a cool season crop with moderate resistance to drought and high temperature. Seedlings are tolerant of light frost (-6°C); conversely, late summer or early fall frost will damage young pods and immature seeds. Lentils are susceptible to drought during flowering and seed ripening; a stress during these phases may drastically reduce yield.

In Italy lentil is diffused in disadvantaged areas with semi-arid temperate climate and mountain zones with a cooler one: in the former environments lentil is a winter crop, while in the latter it is sown in spring after ground de-frosting. Mountain environments are generally highlands of limited surface where climatic and soil conditions provide the crop with special organoleptic and cooking characteristic making them a sort of gourmet food (e.g. Castelluccio di Norcia lentil).

Rotation

Repeated lentil sowing in the same field can result in severe infections of *Ascochyta* blight and/or anthracnose and accelerate the breakdown of resistance. *Sclerotinia* and volunteer crops may be a problem, if lentil follows pea, faba bean, sunflower or mustard.

In Italy, lentil has its main diffusion in the areas characterized by semi-dry climate; in that situation lentils are improvers in crop rotation and usually they are sown prior and after cereals, as wheat and barley, or rye, at higher altitudes. This rotation is generally considered a good alternative to single-cereal system. The best rotational frequency for lentil is of 3 to 4 years (of 4 to 5 years with high anthracnose problems).

Soil

Lentil easily adapts to several soil types, and yield is not bad also in low fertility soils. Calcareous soils are not fit since the product is low quality with high cooking time. The best soils are clayey to muddy-sandy (also stony). The crop performs best on level or slightly rolling land, on fallow on medium to fine textured soils with pH of 6.0 to 8.0. Some researches indicate that lentil can be successfully grown on stubble; however yields will be lower than when grown on fallow (SPGM 2000). Lentil plants are short and must be cut near the soil surface, so fields should be free of surface stones and dirt lumps. To obtain the best possible surface, a land roller is used to smooth the soil surface after planting.

Lentil plants do not grow well on waterlogged soils and will not tolerate flooding or salinity. Although lentil plants are somewhat drought tolerant, they do require at least moderate moisture (15 to 25 cm) during the growing season to produce a full seed set. Excess moisture before the plant is in full bloom can delay and reduce seed set, and excess moisture near harvest time favours fungal diseases.

It is important to consider field history, since lentil is sensitive to herbicide residues (e.g. Ally, 2,4-D/MCPA, Curtail, Lontrel, Prestige); moreover it has poor competition with weeds, such as *Cirsium*, or volunteer crops like *Brassica* or *Sinapis*, and effective herbicide control methods for lentil fields are not available. Lentil is fit to direct seeding, especially through cereal crop residue, but volunteer cereal seeds, such as barley or wheat, are difficult to separate from lentils. In areas where anthracnose is widespread, it is better to avoid seeding lentil next to other lentil fields or lentil stubble to avoid the possibility of wind-blown disease transfer; the same in areas where *Ascochyta* is the main risk. A buffer strip of cereal at least 15 m wide may reduce the risk.

Seeding into tall standing stubble helps reduce soil moisture evaporation, particularly prior to flowering. This results in greater water use efficiency, an extremely important factor in dry lands. As stubble height increases, the height of the lowest pod is also increased, which can make swathing or combining easier and possibly reduce shattering losses. Anyhow, sowing into standing stubble or heavy crop residue increases the risk of late spring frost injury than under conventional tillage, as a consequence of a better temperature buffering effect of bare soils.

Seeding

Lentil production is highly dependent on seed quality. Lentil seeds are susceptible to mechanical damage during harvesting, handling, storage, and seeding, resulting in reduction of both germination and seedling vigour. Diseases can also be spread by infected seed. To avoid potential problems, it is best to have seed tested for germination, seedling vigour, weed contamination and seed-borne infections. Dry lentil seed (14% or less seed moisture) is very brittle and difficult to handle without chipping and splitting, so handling should be as gentle as possible. Even nearly invisible seed cracks can result in germination damage.

Anti-fungal seed treatment is generally not recommended. However, Crown (carbathiin and thiabendazole) is registered for the control of seedling blight, seed rot, and seed-borne *Ascochyta* infection in lentil. Crown should be considered if seed-borne *Ascochyta* infection levels exceed approximately 5%. Crown is safe to use with inoculants but should always be applied, and allowed to dry, prior to adding *Rhizobium*.

In Italy, inoculation is not used even though it has the potential to fix up to 80% of the nitrogen needed by the crop. In other countries seed coat-treated inoculants (peat-based or liquid) is thoroughly mixed with seeds just prior to seeding and after any fungicidal seed treatment.

As already said, lentil seedlings tolerate light frosts. This allows for early seeding which usually results in the best yield and quality. Seeding can begin when the average soil temperature, at the depth of seeding, reaches 5°C, providing soil is not excessively wet.

In Italy, in areas below 800-900 m asl, early seeding generally occurs in late October to early November. Early seeding increases plant height and the height of the lowest pods. Higher pods are desirable as they stay cleaner and are easier to swath resulting in higher grade. In Italian areas above 800-900 m asl or with a continental climate, seeding may be delayed to as late as March-April (after that the frost risk is ended), although late seeding rarely results in good yield as early seeding. Early seeding may help reduce flower abortion caused by high temperatures during flowering.

Plant development may be more rapid for plants that are seeded later because of the warmer growing conditions and longer days during early growth. Early maturing varieties may be able to nearly “catch up” with plants seeded at an earlier date, but late maturing varieties generally will not. The rate of plant growth and development generally increases with daily maximum temperatures up to about 27°C, after which heat stress starts reducing growth rate.

In an experiment in Sicily in 1979-81, based on 11 lentil cultivars, autumn sowing (December) gave yields of 1.32 and 1.98 t/ha in 1980 and 1981, respectively, compared with 0.36 and 0.78 t/ha, respectively from spring (Mar.-Apr.) sowing (Sarno *et al.* 1988a). Landrace Catanisa gave the highest seed yields from autumn sowing in both years, while cv. ‘FAO 1’ and landrace Villalba gave the highest seed yields from spring sowing in 1980 and 1981, respectively. The incidence of shrivelled seed and seed protein content were higher in spring sown crops.

De Franchi *et al.* (1998) in a field experiment in 1990-91 in a hilly area of southern Italy, studied bio-morphological and productive features of 4 lentil landraces as affected by sowing date. The cultivars were sown on February 2nd or March 3rd. Delaying sowing decreased pod yield. Landraces Pantelleria and Ventotene introduced from two small isles of south Italy showed low adaptability and produced lower yields than the landraces Corleto and Altamura.

Recommended seeding rates are 350-400 seeds/m^2 for a target of ca. 130 plants/ m^2. The actual seeding rate depends upon seed size (i.e. 70-80 kg/ha for *microsperma* and 130-150 kg/ha for *macrosperma*) and germination. Seeding rates should be adjusted for germination, since a number of plants lower than recommended will severely reduce the already weak competitive ability of lentil seedlings. Yield is often reduces by lower seeding rates or wider row spacing. Overall lentil yields were greater on tilled summer-fallow as opposed to direct-seeded stubble. Narrower row spacing will result in faster canopy closure and reduced soil moisture loss through evaporation from between the rows. Narrower row spacing also encouraged quicker rooting exploitation of the soil between the rows and the use of mid-row soil moisture. Differences due to row spacing would be less evident in regions with higher moisture. Higher than recommended seeding rates are often used as a hedge against expected losses. For instance, if harrowing is planned as an early season weed control measure and plant losses of 15% are anticipated, then a 15% boost in seeding rate will help offset these losses. Anyhow, plant stands higher than the target recommendation may facilitate rapid infection and spread of foliar diseases, particularly in wet years.

In an field trial at Agrigento (Sicily) in 1979-81, landrace 'Villalba' was sown at 5-100 kg/ha resulting in plant densities of 3.0-123.7 plants/m^2 in 1980 and 3.3-139.0 plants/m^2 in 1981 (Sarno *et al*. 1988b). Seed yields increased up to some 1.50 t/ha at a sowing rate of 40 kg/ha in 1980 but in 1981 seed yields was some 2.5 t/ha at 100 kg/ha sowing rate. 1000-seed wt decreased and seed protein content increased at higher sowing rates. In 1980 the crop matured after an average of 173.9 days, with sowing rate exerting little effect; in 1981 the crop matured in an average of 180.4 days, but took up to 184 days at the lowest sowing rates. Low yields in 1980 were attributed to a late application of herbicide during the early stages of crop development.

Seeding can be done with any type of seeders, including hoe drills, disc drills, discers and air seeders. When using air seeders, caution is advised as seed damage may occur if seeds are too dry (below 14%) or if air speed in the distribution system is too high. Adding water to dry seeds in an auger can reduce seed damage during handling and seeding.

Lentil seedlings can emerge from relatively deep seeding because the seeds are large, but large seeds are also prone to dry out. Deep seeding is not required providing the seed is placed in moist, firm soil. In direct seeding systems, the seed can be placed at a shallow depth compared to pre-tilled soils as soil moisture is usually much higher in untilled soils. A seeding depth of 3.8 to 7.6 cm is advised. Ideally, seed should be covered with 3.8 cm of moist packed soil. If the soil is not waterlogged, it should be firmly packed to ensure good soil contact with the seed. Letting a wet clay soil dry slightly will also help prevent surface crusting. Harrowing or further packing after seeding is not needed if seeders with on-row packing are used, but rolling will be required to smooth the surface. Seeding with discers or air seeders without packer wheels should be followed by harrowing and packing to level and firm the soil, and this should then be followed by rolling. Lentil seeds germinate quickly, so harrow packing and rolling should be completed very soon after seeding.

Weeds

Lentil is a short crop with a sparse canopy, which makes it a poor competitor with most weeds (van Emden *et al*. 1988). Yield losses due to weeds can be severe, a problem more important than in other crops, since no lentil-safe herbicides are available to control them (Yasin *et al*. 1995). For instance, *Solanum nigrum* L. produces a juicy fruit that, crushed during threshing, may stick dirt to the combine and to lentil seeds. In Italy, farmers commonly use to hoe several times the crop together with earthing ups.

However, some techniques for effective weed control are applicable to lentil. Post-emergence harrowing with a tine harrow can be used for weed control in lentil fields when plants are in the seedling stage (no more than 10 cm tall), provided the foliage is dry and the work is done on a warm sunny day. Some plant loss will occur, and it can be offset by using higher seeding rates (Boerboom and Young 1995; Ball *et al*. 1997).

Harvesting

Lentil plants have an indeterminate growth habit and will continue to flower until stopped by some stress, such as heat, frost or drought (Iannelli 1988). Large-seeded lentil varieties are more indeterminate than small-seeded varieties and in many years, crop maturity will not occur in a timely manner (Matranga *et al.* 1990). Because of this, many Italian farmers hasten the drying of their crop by swathing (chemical desiccants are not used). Lentil pods shatter easily when dry. Early swathing is used to hasten and make uniform drying, thus reducing shattering losses. Swathing when about 1/3 of the lower pods turn yellow to brown provides the best results. At this time, the upper pods are still green, but a further delay will increase the risk of shattering. Swathing is the most critical step in lentil production (Bozzini and Iannelli 1986). The bottom pods are close to the soil surface, so the cut-bar must travel close to the ground. The cutter bar should preferably be about 5 cm from the ground, at an angle of 20 to 30° to the ground. Cutting will be easier if the surface is flat, firm and dry. Fields rolled after seeding are easier to swath since rolling helps to level the soil surface. A properly equipped forage or grain swather can be used to cut lentil. Ideally, a swather should be equipped with a pickup reel and vine lifter guards for a good job of cutting, especially if the crop is lodged. To speed the drying process, after 36-48 hours (better in early morning), lentil swaths are turned by hand or with a swath turner, but, in this case, shattering losses may be high.

Threshing

In warm and windy weather lentils dry very rapidly. Correct timing can make a difference in the ease and success of the combining operation. Lentil seeds are safely stored at 14% moisture, and lower levels increase seed damage risk. If no drying equipment is available, it may be necessary to wait until the proper moisture is achieved before threshing. This may result in shattering losses and seed damage. It has been observed that when seed moisture is greater than 20%, the crop is hard to combine without smashing the seeds. Therefore, 20% and 14% are the recommended upper and low limits for the grain moisture content at harvesting, with 16% moisture being ideal. Rotor and cylinder speeds between 250 and 500 rpm are often used.

Cultivars vs. landraces

In two-year (2000-2001) field trials carried out in Sicily, Linosa, Pantelleria, Castelluccio, Pachino, Ustica, Palazzolo Acreide, Nissoria, Villalba and Maniace lentil landraces were tested in comparison with the Canadian commercial cultivars Laird and Eston (Avola *et al*. 2001). Pantelleria, Linosa, Castelluccio and Pachino showed the best agronomic performance while the foreign Laird and Eston resulted in the worse ones.

CHARACTERISTICS OF ITALIAN LENTIL LANDRACES

Morphological traits

The long lasting tradition of lentil cultivation in Italy allowed the selection of several local populations well adapted to their cultivation area. An overview of the current diffusion of lentils in Italy can be obtained by analysing the records of collecting missions jointly carried out over almost 40 years by the Institute of Plant Genetics (Bari, Italy) in collaboration with various Italian and European institutions. During these missions, lentil landraces were collected all over Italy and the cultivation conditions in the collection areas were recorded. Moreover, information was obtained on the extent of genetic erosion, encouragement to farmers to maintain landraces, agronomic, adaptive and qualitative traits of the retrieved accessions (Hammer *et al.* 1992, 1999; Hammer and Laghetti 2006).

The analysis of the geographic distribution of the collected materials showed that large-seeded samples were

Table 2 Seed quality traits of some important Italian lentil landraces. All the parameters are expressed on dry matter base. *

	1000 seed weight (g)	Protein (%)	Ash (%)	P (mg/100 g)	K (mg/100 g)	Ca (mg/100 g)	Na (mg/100 g)	Fe (mg/100 g)	Cu (mg/100 g)	Cooking time (min)
Castelluccio	26	26.8	2.9	405.4	919.9	59.79	23.00	8.05	1.61	28-30
Colfiorito	31	26.9	2.7	426.1	916.8	52.71	21.38	7.56	1.14	29-31
Capracotta	29	26.4	2.9	542.4	1071.8	57.80	10.85	8.82	0.92	28-30
Onano	33	25.8	3.4	486.0	962.6	55.35	19.24	8.66	0.96	31-33
Ventotene	28	28.6	3.1	456.3	949.6	56.97	18.98	9.50	0.95	31-33
Villalba	67	28.2	2.9	440.5	956.2	49.57	12.92	9.74	1.28	52-54

* based on data from Piergiovanni *et al.* 2003, 2006

more common in the semiarid environments of Sicily and southern regions. Only small-seeded types were collected in the small Italian islands. Small-seeded types were also predominant in the Apennine areas of central and southern Italy (Hammer *et al.* 1992, 1999; Piergiovanni and Pignone 2001). Few populations, that were admixtures of small and large seeds, were collected in Apulia and Basilicata (two samples each). Similar admixtures are usually cultivated in some countries of North Africa. Populations heterogeneous in cotyledon colour (yellow and orange in different ratios) were found in Apulia, Basilicata and Sicily regions (Piergiovanni 2000). Heterogeneity of cotyledon colour is a trait frequently observed in samples collected in Albania and Cyprus (Piergiovanni *et al.* 1998). The characterisation of the Italian populations collected by the Institute of Plant Genetics of Bari, Italy and the Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany, evidenced a significant morphological variation for both plant and seed traits (Di Prima *et al.* 1997; Piergiovanni *et al.* 1998; Monti *et al.* 1999).

Nutritional quality

Despite the long history of lentil cultivation in Italy, scientists have paid little attention to the exploitation of the nutritional and technological quality of Italian populations. The commercial value of traditional varieties is strictly related not only to their agronomic performances but also to their sensorial and nutritional quality. To this end it is crucial to acquire the appreciation of consumers, as well as to reach a good position in the market. Sporadic information is available in the literature, and consequently still today the nutritional and sensorial quality of Italian lentils is largely under-investigated. Senatore *et al.* (1992) compared the fatty acid and sterol composition of lentils from Altamura, Pantelleria and Ventotene against three cultivars. Altamura had the highest saponificable material content (12.3 g/kg), while Ventotene showed the lowest unsaturated/saturated fatty acid ratio (1.5). Recently, Gallo *et al.* (2006) evaluated the seed composition of lentil from Mormanno, an old landrace considered disappeared before that study (Gallo and Magnifico 2007). That lentil is characterised by a compositional profile (protein content 25.4% dm; ahs 2.8% dm, cooking time 30-32 min) close to the average observed for the other Italian landraces. Antonelli *et al.* (2005) quantified the level of free and conjugated phytoestrogens in lentils from Pantelleria. They found significant amounts only of genistin (0.4 μg/g) differentiating this landrace from the cultivar Eston, which showed a genistin level of 5.7 μg/g, together with daidzin and trihydroxyisoflavone (1.0 and 0.4 μg/g, respectively).

A more detailed comparison of some Italian lentil landraces appreciated in the past (such Villaba, Ventotene and Onano) or presently required by consumers (e.g., Castelluccio di Norcia or Colfiorito) was carried out by Piergiovanni *et al.* (2003). The compositional data collected in the present review, are far to be considered conclusive for each tested landrace (**Table 2**). In fact, that study compared materials cultivated under very different environments and adapting to traditional agro-techniques which varied from one local community to another. That study was aimed at an objective evaluation of the products that consumers found in a market. The comparison of data reported in **Table 2** clearly shows that each landrace has its own compositional profile. For example, Villalba was characterised by a high protein and iron content together with values of ash, phosphorus, calcium and sodium lower than those of the others samples; Ventotene had a compositional profile close to that of Villalba but, being a small-seeded type, it had the great advantage of a shorter cooking time; Capracotta was characterised by the highest phosphorous and potassium levels and intermediate protein content. These differences among the tested lentils should be regarded as a trade advantage rather than a disadvantage, since they could be able to satisfy different requirements of different segments of consumers, provided that an efficient strategy of valuation and distribution is devised for these productions which presently have in general only a local diffusion.

Electrophoretic screening of seed storage proteins

Crop genetic improvement is greatly favoured by the availability of a broad and well characterised genetic diversity. A single method is inadequate for assessing genetic variation in germplasm collections, because the different methods sample different levels of genetic variation and differ in resolution power and information content. Electrophoretic analysis of seed storage proteins using the SDS-PAGE method is an effortless method to study genetic variation in germplasm collections.

The SDS-PAGE study of seed storage proteins variation within and among 44 Italian lentil populations has been the object of two studies by Piergiovanni and Taranto (2003, 2005). These studies revealed that in the Italian lentil germplasm a considerable genetic variation is present. The comparison of the electrophoretic profiles relative to individual seeds (10 to 20) per each tested population showed that pattern variation regarded a number of bands. Over all SDS-PAGE profiles, the higher variation, detected as presence/absence and/or staining intensity, were found in bands around 97 kDa and molecular weight range between 55 and 45 kDa. Seven and ten patterns were associated with the ranges 97 kDa and 55-45 kDa, respectively. Large and small-seeded populations shared the same patterns but differed in their frequency. The detected patterns gave rise to 31 associations. Small-seeded types were more polymorphic than the large ones, showing 27 and 18 associations, respectively (Piergiovanni and Taranto 2005). More than one association was detected in 32 out of the 44 tested populations, while 3 associations were detected in seeds belonging to only one population. Intra-population variation, estimated as Shannon-Weaver Index (SWI), was changeable, ranging from 0.0 to 0.0815. No variation was detected between the seeds from Pantelleria and Linosa, two small islands near Sicily (**Fig. 1**). This results clearly differentiate these populations from all the others possessing an SWI always higher than 0.0. The complete similarity between the Pantelleria and Linosa lentil seeds populations is not surprising as the inhabitants of Linosa identify Pantelleria as the island where their forefathers came from (Hammer *et al.* 1997) and is in agreement with molecular data (Sonnante

Fig. 1 Italy and its regions. The spots indicate cultivation areas and names of the studied lentil landraces.

and Pignone 2007a). This suggests that the colonizers of Linosa introduced also plants and agricultural practices from their native island. The landrace from Castelluccio di Norcia (Umbria region) is the most appreciated and known landrace in Italy (**Fig. 1**). It has a very high morphological heterogeneity being a mixture of 10 biotypes (Bozzini *et al.* 1988). The electrophoretic analyses, however, evidenced that Castelluccio lentil a SWI equal to 0.0490, an intermediate value among those observed in the Italian landraces (Piergiovanni and Taranto 2005). The highest variation in seed storage proteins (SWI = 0.0815) was detected in lentil from S. Stefano di Sessanio, a small village in the Abruzzo region (**Fig. 1**), where lentils are cultivated in undulating highlands above 1000 m asl. Moreover, only individuals from this landrace showed the presence of a specific band immediately below 36 kDa differing for staining intensity from the other profiles. In fact, all the remaining populations analysed possessed at this mobility a band with strong staining intensity. This suggests that this band could be the result of a different allele fixed in the population of S. Stefano di Sessanio (Pergiovanni and Taranto 2005).

The most polymorphic bands among the profiles were used for statistical analysis by using cluster analysis. The 44 screened populations formed six clusters. It is interesting to notice that samples from close geographical origin did not always belong to the same cluster. For example, lentils from mainland Sicily were significantly distant from those collected in the small Sicilian islands (Ustica, Pantelleria and Linosa), these latter falling into different clusters. Similarly, lentils from Castelluccio di Norcia, Capracotta, S. Stefano di Sessanio and Colfiorito, which are geographically close villages of Central Italy (**Fig. 1**), belonged to different clusters. The estimation of the genetic richness (S) on regional bases, calculated according to Collwell and Coddington (1995), indicated that Central Italy (Abruzzo, Molise and Umbria regions) is the area were the most variable Italian lentil genetic resources are still present: for the populations collected in these regions the S value was equal to 17.278. Similar pattern of the genetic variation distribution was determined at molecular level (Sonnante and Pignone 2007a).

On a macro-geographical scale, some studies showed that lentils from the Mediterranean basin are characterised by a high genetic diversity (Erskine *et al.* 1998; Ferguson *et al.* 1998; Sonnante and Pignone 2001; Piergiovanni and Taranto 2003). The comparison of seed storage protein profiles of Italian lentils with those of 220 populations from 18 countries belonging to three continents (Asia, Africa and Europe) was carried out by Piergiovanni and Taranto (2003). This study evidenced that Italian lentils shared a large fraction of the detected electrophoretic associations with samples from other countries. Moreover, the Italian germplasm was characterised by a higher genetic richness (S = 34.026) than that observed in other Mediterranean countries, such as Cyprus (S = 27.859), Tunisia (S = 17.358), Algeria (S = 16.894) and Greece (S = 16.095). The dendrogram obtained analysing the electrophoretic data evidenced that 91.6% of Mediterranean populations are in the same cluster, while populations from countries located outside this area are clustered separately. In particular, Italy showed the lowest ranking in the tree, together with Albania, Algeria and Tunisia. This could be attributed to exchange of material among these countries probably favoured by similar climatic conditions, short distance and historical reasons, including human migrations and wars.

GENETIC ANALYSIS ON ITALIAN LENTIL GERMPLASM

Landraces are a particularly good biological system to study the diffusion and domestication of a crop, since they are the result of empiric selection operated by farmers over the centuries and adaptation to different agro-techniques, soils and climatic conditions, which alter, but do not eliminate, the genetic diversity of a landrace (Beebe *et al.* 2001). Moreover, the whole selection process of a landrace reflects preferences in the end uses of the crop. This evolution, guided by human selection as opposed to natural selection, mostly acts on few loci only (Doebley *et al.* 1997; Grandillo *et al.* 1999), a phenomenon that is generally known as the "domestication syndrome" (Hammer 1984; Harlan 1992). For this reason, morphological divergence does not always go along with genetic divergence, since molecular markers are more abundant and are presumably distributed all over the genome.

The survival of most Italian germplasm relies on the possibility of developing convenient support actions that include their precise identification in order to prevent frauds. Morphological traits are not sufficient to surely identify each landrace, or to describe the level of variation within each of them, for this reason the use biochemical or molecular markers is a requirement (Sonnante and Pignone 2001). Some lentil landraces from the Umbria region have been studied through both a morpho-physiological and molecular (RAPD) analysis with the aim of characterizing and protecting these rare autochthonous populations (Torricelli and Falcinelli 1997). Hammer *et al.* (2003) have stressed that some lentil landraces from small Italian islands (i.e. Linosa and Ustica) are of great scientific interest and that their identity and characteristics can be protected through community protection marks of origin and quality, thus boosting the farmers' income. Piergiovanni *et al.* (1998) characterized a lentil collection with various geographical origin. In that study a good correlation was found between morphological features and geographic origins of the accessions. Sonnante and Pignone (2001) evaluated a collection of lentil landraces, mostly from Italy, but including Mediterranean and foreign germplasm, using RAPDs, microsatellite-primed PCR and ISSRs, demonstrating that Italian samples showed to be quite differentiated from other samples of Mediterranean origin.

The eleven most important Italian lentil landraces were analysed by Sonnante and Pignone (2007a, 2007b) using ISSR markers on 15 randomly chosen individuals per landrace, to assess the level of genetic diversity within and among landraces and to ascertain their origin and genetic relationships. ISSR primers generated a high number of alleles in lentil, the most of which were polymorphic. That analysis demonstrated that 85% of the overall genetic diver-

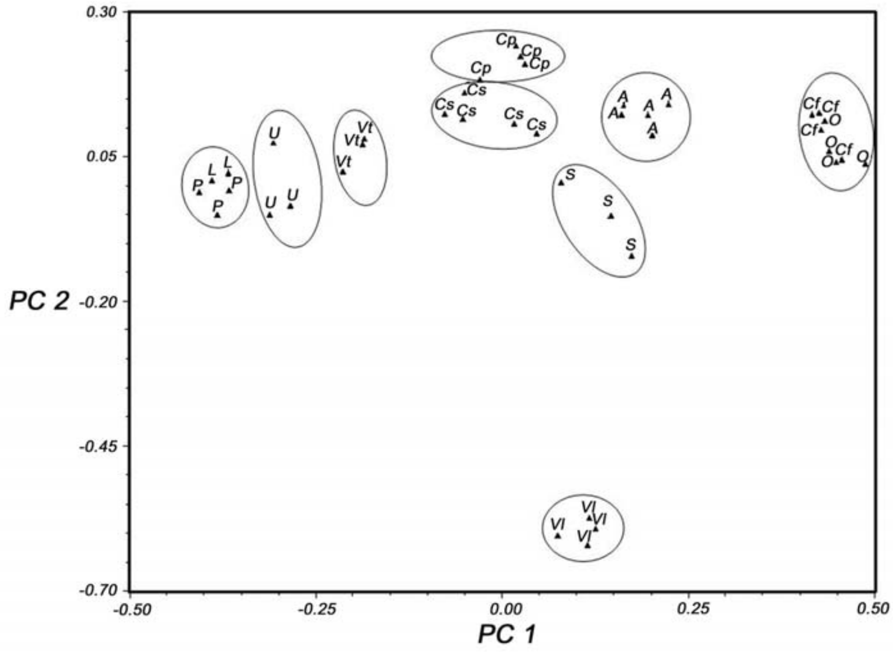

Fig. 2 Principal component scatterplot of Italian lentil landraces based on ISSR markers. PC1 and PC2: first and second principal component, respectively. A: Altamura; Cf: Colfiorito; Cs: Castelluccio; Cp: Capracotta; L: Linosa; O: Onano; P: Pantelleria; S: S. Stefano; U: Ustica; Vl: Villalba; Vt: Ventotene.

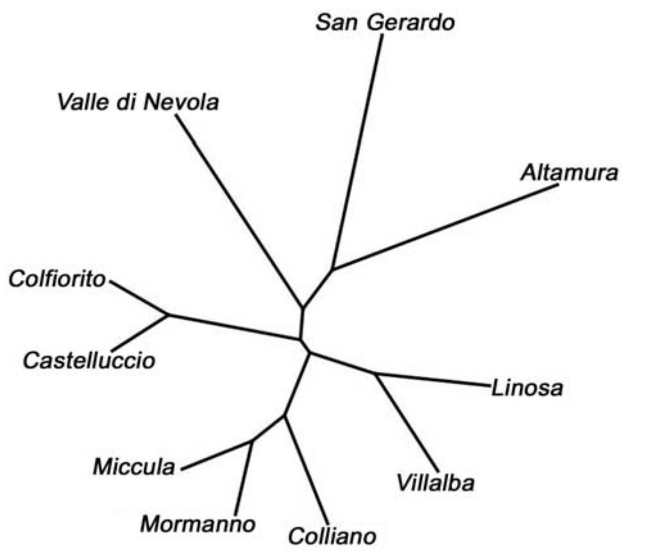

Fig. 3 UPGMA tree based on Nei's genetic distance obtained from SSR markers, and showing relationships among some Italian lentil landraces.

sity was found among landraces, while 15% resided within them (**Fig. 2**). Not all the landraces showed a similar distribution of the genetic variation; in fact, some of them revealed to be much more variable than others, showing a higher proportion of polymorphic loci and diversity indices. The highest levels of genetic diversity were observed in some landraces from the Apennine ridge (Colfiorito, S. Stefano, and Capracotta) and mainland Sicily (Villalba). Lower levels of genetic diversity were observed in landraces from the small Sicily islands Linosa and Pantelleria. Especially in the Linosa landrace, the genetic diversity indicators were much lower than the average over all landraces. The observed reduction in genetic diversity in small Sicily islands was interpreted as the result of a bottleneck effect occurred during the colonization of those isolated areas, which occurred from the mainland in different times, as also supported by historical data. On the contrary, the noticeable level of diversity observed in landraces from the Apennine was thought as an indicator of their older origin. Sonnante *et al.* (2007a, 2007b) assessed genetic diversity and relationships in ten local varieties using microsatellite (SSR) markers. The analyses were performed on a number of individual plants for each landrace, using sixteen primer pairs to amplify microsatellite regions in lentil, and the amplified fragments were scored using an automated sequencer. A total of 170 alleles were scored, with a range from 1 to 22 alleles per locus. The landraces analysed in those study showed quite high values for all the genetic diversity parameters, especially when compared to other legumes cultivated in Italy, such as common bean. The studies concluded that the examined landraces retain a high level of genetic diversity among and within the same landrace. The highest levels of genetic diversity were observed for Castelluccio di Norcia, Colliano, and Villalba landraces. A UPGMA dendrogram was constructed based on Nei's (1978) genetic distances (**Fig. 3**). The examined landraces were grouped according to their geographical origin. Moreover a correlation between phenological/bio-morphological data and SSR markers based analysis demonstrated that, in general, landraces showing a higher microsatellite diversity also possessed higher phenological (flowering and harvesting time) and bio-morphological diversity.

CASE STUDIES

Some elements of the Italian agricultural system, such as land ownership fragmentation, crop diversification in areas with peculiar landscape features, favor the establishment of landraces with peculiar characters. The small production scale makes these landraces niche products. The increasing interest of Italian and European consumers towards elite food, and consequently the increased market value of those productions, makes the cultivation of these landraces an economic opportunity for local populations and a valid strategy for *in situ* conservation local genetic resources.

This process of valuation cannot be estranged from a precise identification of the landraces, their specific agro-ecosystem, and the uses and traditions to them associated. To this end it is necessary to integrate knowledge at different levels, from the characterization of the territory to the production process. As for the identification of the landraces, besides the classical morphological features, biochemical and molecular markers can provide better insights into population structure, diversity, and quality, at the same time providing instruments for the defense of this local valued production from fraud.

Some recent research activities, carried out with the involvement of local communities and based on a multidisciplinary approach, have regarded the most appreciated lentil landraces from Southern Italy. They represent interesting case studies aimed at the protection of these materials from extinction and at the re-establishment of a cultivation scale able to safeguard the genetic integrity of the landraces and at the same time, providing farmers with an economically valued crop to improve their income.

Villalba lentil (Sicily)

Villalba is a village in inner Sicily, in Caltanissetta province, at 600 m asl, with a territory constituted of clay hills and steep calcareous relieves (**Fig. 1**). The town was established in the 17th century by the marquis Niccolò Palmieri, an able cereals producer and trader. Lentil cultivation was started at the beginning of the 19th century when the marquis Palmieri imposed on his lands a rotation including cereals, alfalfa and lentil, using the traditional cultivation techniques of those times. Lentil cultivation flourished between the years 1930-1960. Villalba lentil, a large seeded type, was the most traded Italian lentil variety, together with Altamura type. In 1956, 30% of the Italian production (some 130,000 metric tons) came from this area. This local variety was particularly appreciated for it sensorial properties, and for consumers' preference, at those times, for large seeded lentils.

In the traditional cultivation technique, lentils were sown from late November to mid December, based on rows 80 to 100 cm apart. Harvest was manual and made in June. The plants were sun desiccated for one week before threshing. The overall reduction of lentil cultivation in Italy and the changed consumers' preference towards small seeded types, have strongly reduced lentil cultivation to almost a compete abandon, which now is done mostly on a family scale for own consumption. The renewal interest toward Villalba lentil permitted its inclusion in the Italian Agriculture Ministry list of traditional products. Moreover, the Villalba local government is collaborating with public entities and research centres, like the Institute of Plant Genetics in Bari, in order to valuate and promote the cultivation of this landrace (Piergiovanni *et al.* 2006).

A recent study on the Villalba landrace evidenced a high uniformity among and between populations sampled *in loco* (Piergiovanni *et al*. 2003). The plants have a height between 37 and 45 cm, no pigment on stems and pods, small and glabrous leaves, small tendrils and white flowers. Generally pods bear a single seed, with only 15% bearing two; seeds are great and particularly flat, the testa is light green, and cotyledons are yellow. The 1000 seed weight over 18 populations cultivated during 1999-2000 using traditional agrotechnique varied from 61.3 to 72.6 g, with an average of 67.2 g (Piergiovanni *et al*. 2003). Grain quality was good (see nutritional quality section). A negative character of the Villalba lentil is cooking time, which is higher not only than small seeded types, but also than commercial large seeded varieties, such as cv. 'Laird'. A reduction of some 40% of cooking time can be obtained by soaking lentils in water for a couple of hours prior to cooking. It could be proposed the sale of this landrace in pre-cooked form, which could improve Villalba trade. This landrace showed to possess a very good attitude to semi-arid environments, in which rainfall is limited to winter and early spring, and with very dry summers. This trait is a desirable one for lentil breeders, especially in the perspective of the climatic change that will affect the agriculture of the Mediterranean region in the next future.

The electrophoretic analysis of seed storage protein of three Villalba samples provided good information. The analysis was based on 18 polymorphic bands of medium/strong intensity. On the basis of band presence/absence 10 different profiles were identified, 3 of which observed only in single seed. The 3 tested populations collected at Villalba proved to retain a certain level of diversity for seed protein profiles, one of them being more polymorphic. When a cluster analysis based on seed protein profiles was performed against commercial cultivars 'Eston' and 'Laird', used as reference, Villalba samples formed a compact and distinct cluster (Piergiovanni *et al*. 2003).

Five individuals per each population were also used to assess the level of genetic diversity using Simple Sequence Repeats (SSRs) analyses. The cultivars 'Eston' and 'Laird' were included as references. Genomic DNA was extracted from individual plant leaves and used as a template in PCR amplifications using the primer set described by Hamwich *et al*. (2005). The genetic diversity indices, (expected heterozygosity, average number of alleles per locus, percent of polymorphic *loci*), were estimated. As for electrophoretic data, one population showed a higher level of genetic diversity. From the same analysis it was possible to derive a matrix of genetic distances (Nei 1978). In this analysis it was evidenced that two populations are genetically closer to each other. Moreover molecular markers allow the complete discrimination between Villalba samples and commercial cultivars.

The results of this study demonstrate that, besides a high level of morphological similarity, molecular markers evidenced an appreciable genetic variation. Moreover molecular and biochemical makers produced highly comparable results. This means that both methods could be applied to the correct identification of seeds of Villalba landrace, providing useful tools for the protection of this landrace from frauds, an important pre-requisite if protection marks are to be issued.

Mormanno lentil (Calabria)

Mormanno is a mountain village in the ridges of the lower Apennine, with an average altitude around 700 m asl (**Fig. 1**). It was reported that this landrace was completely extinguished (Piergiovanni 2000) due to land abandon. Recently, in small farms own by elder farmers, few small plots of this old landrace were found (Gallo and Magnifico 2007). This landrace was cultivated only for self consumption. A collaborative project aimed at its safeguarding was started by the Institute of Plant Genetics of Bari, the Institute of Experimental Horticulture of the Research Council for Agriculture, Pontecagnano, Italy, and the Regional Extension Service of the Calabria Region.

To recover information on the traditional agricultural techniques together with other ethno-botanic information, a demographic survey was issued. One hundred people aged 35 to 90 and long living in the area were interviewed. Younger people may have no direct information, but have knowledge of the cultural heritage transmitted by their parents, grandparents, and older relatives. It has been established that the area of cultivation in former times was quite wide, and from the Pollino Mountains not far from Mormanno, to the border with the Basilicata region (some 70 km apart). The Mormanno lentils were not considered a primary production or a cash crop and were mostly grown for self or local consumption. It was confirmed that in the '70s its cultivation was abandoned, due to the availability of commerce of grains from abroad the area.

Traditionally, this landrace was sown in both late autumn and spring. Spring sowing took place between April and mid May, according the height of the field (600 to above 1000 m asl), resulting in a harvest time between late August and September. Autumn sowing was done at the same time as wheat, that it late October early November, depending on rains, resulting in a slightly earlier harvest respect to spring sowing, but better yields. In some cases lentil was consociated to wheat. This is very interesting since the consociation of lentil and cereals has been adopted in the early phases of lentil cultivation at prehistoric times. Production ranged from 0.2 to 0.4 metric tons per hectare depending on sowing time, soil fertility and climatic factors.

Weight of 1000 seeds is 37 g, since this is a small seeded landrace. Visually it appears as a mixture of seeds of different colour, but with high morphological uniformity (3.5 mm diameter). Seed and cotyledon colour heterogeneity is not rare in Italian landraces. In fact, other landraces are admixtures of seed morphotypes (Piergiovanni 2000), the most famous being Castelluccio di Norcia, possessing up to 10 different morphotypes in the same sample (Bozzini

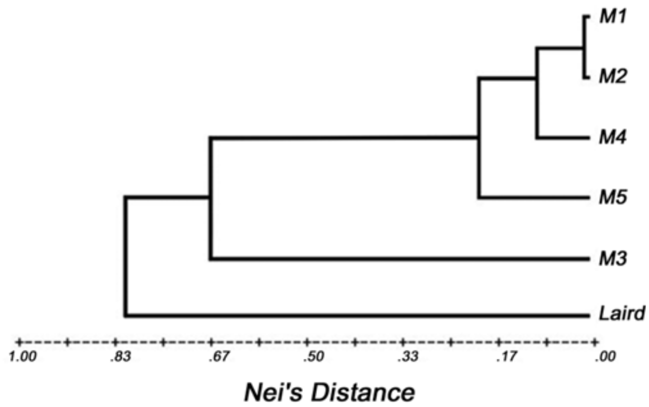

Fig. 4 UPGMA dendrogram showing genetic distances among the five morphotypes detected in Mormanno lentil landrace, based on SSR markers. M1: green; M2: mottled green; M3: pink; M4: beige; M5: mottled beige.

et al. 1988). On the basis of seed colour five morphotypes could be identified which showed a different frequency in the samples: green (M1 = 31%), mottled green (M2 = 8.0%), pink (M3 = 7.0%), beige (M4 = 47%) mottled beige (M5 = 7.0%).

For nutritional quality and protein content, the Mormanno landrace is in the average of Italian landraces (Piergiovanni *et al.* 2003). It has a short cooking time (30 min) and little tendency to seed disruptive at the end of cooking. These characters are highly appreciated by consumers and set an economical potential for this landrace.

The variability was appraised using seed protein electrophoresis. To this end 20 polymorphic bands of medium/ strong intensity were chosen. On the basis of presence/ absence of these marker bands, 20 different profiles were identified, 14 of which scored in single seeds only. The matrix derived by profiles was used to perform cluster analysis. It is interesting to notice that the pink morphotype is clearly differentiated from the remaining ones, thus suggesting a genetic distinctiveness of this morphotype.

The different morphotypes were also analysed using 14 SSRs markers (Gallo *et al.* 2006), similarly to the procedure used for the Villalba landrace. The genetic diversity indices from each morphotype showed that the green mottled morphotype has the highest diversity, while with beige and beige mottled seeds being the most uniform. A dendrogram based on Nei (1978) genetic distances (**Fig. 4**) shows that the pink morphotype (M3) is quite distinct from the others; moreover green (M1) and mottled green (M5) seeds showed almost genetic identity.

In conclusion, the finding of these small lots of Mormanno lentils demonstrates that extinction may not be a sudden event and that small amounts of old landraces can survive at least until older farmers survive. The valuation of the Mormanno landrace could be possible for its excellent cooking quality, but to this end it is important to involve the local community.

Altamura lentil (Apulia)

The Apulian large-seeded landrace Altamura, very popular and widespread in the past, presently is almost completely disappeared, although still present in the list of Italian traditional food. Formerly (1930s-1950s), some 20% of the Italian lentil production came from Apulia (Zucchini 1937). Altamura landrace was exported with very good economic results to many European countries, Canada, Australia and USA by notorious producers not only from Altamura, but also from other close towns of the Murgia area, a calcareous ridge with elevations around 400 m asl running parallel to the Adriatic coast in central Apulia (Castelli 1935). This landrace was reported in all old Italian agricultural books (e.g. Pantanelli 1944; Baldoni and Giardini 1981) and was also listed on the stock exchange of Bari and Bologna cities.

Since the 1970s, the Institute of Plant Genetics of Bari has acted to safeguard autochthonous Italian lentil traditional varieties, in collaboration with other Italian and international institutions, to assemble a good collection of Italian lentils. In recent years, a number of studies were carried out on the characterisation of specific landraces, being elite germplasm or at risk of genetic erosion or extinction. Recently, a project on the characterisation and reintroduction of the lost Altamura lentil was funded by some entrepreneurs (Sonnante *et al.* 2004).

This landrace is characterised by large, flat and pale green seeds. Ten populations collected in Altamura area in the 1970s were evaluated for morpho-agronomic traits in two localities for two years (2002 and 2003). They were morphologically similar to the late Altamura lentil, and two of them showed high yield (5.1 q/ha and 4.5 q/ha), similar to the modern commercial cultivars 'Laird', 'Eston' and 'Regular', used as controls. A low phenotypic intra-population variation was observed. The agronomic descriptors scored were: time to flowering, plant height and height of lowest pod. The agronomic trial also included two sowing time, autumn and spring. For the spring sowing, a yield decrease around 45% was observed, whereas the harvesting time was not significantly affected (Laghetti *et al.* 2006). Cluster analysis derived from morpho-agronomic data grouped all populations, except one, into three clusters.

In order to determine the level of genetic variation within and between populations, AFLP analysis was also performed on 15 individuals per each of the six populations which showed highest similarity to the records for the original landrace; cultivars Laird and Eston were used as controls. Three primer combinations were tested (E-ACT+M-CAT, E-AAG+M-CTC, E-AGC+M-CAT), producing a total of 204 fragments; in the Altamura populations 197 fragments were present, 84.3% of which were polymorphic. The presence or absence of each fragment was detected and a binary matrix was constructed. These data were used to calculate some indices of genetic diversity: number of observed alleles, Nei's index of gene diversity, Nei's total genetic diversity, genetic diversity within and among populations (Sonnante *et al.* 2004; Laghetti *et al.* 2006). The results revealed that most of the variation at the molecular level was present within populations, indicating that the six populations analysed are not well differentiated among them and could represent sub-populations derived from a single original population, thus supporting their pertinence to the same landrace. The data might also indicate that the observed level of gene flow among the sub-populations, unexpected for a self-pollinating crop, might be due to seed exchange among farmers. The comparison of the Altamura landrace with other major Italian landraces, on the basis of ISSR (Sonnante and Pignone 2001, 2007b) and SSR (Sonnante *et al.* 2007b) markers revealed that this landrace is quite differentiated from the others and therefore represents a peculiar genetic resource.

LENTIL IDEOTYPE FOR ITALIAN AREAS

Until the 1970s lentils received little attention from breeders, and only recently breeding programs have been developed at national and international levels, with the main objective of developing phenologically adapted, stress resistant and high-yielding cultivars with improved yield (Sarker and Erskine 2006). During the last decades, a large number of germplasm accessions has been collected, evaluated and preserved, the largest collection of cultivated and wild lentil germplasm being stored at the International Center for Agricultural Research in the Dry Areas (ICARDA). A major effort has been made to study the genetic variation in the world germplasm collection, in order to understand local adaptation and to develop specific research programs. Genotypes with resistance to various biotic and abiotic stresses, particularly resistance to vascular wilt, rust and

Ascochyta blight, have been identified, and directly exploited or used in breeding programs. New genotypes have been bred with good standing ability, suitable for mechanical harvest for West Asia and North Africa. Agronomic practices, including seeding time, seed rate, tillage requirements, soil type, and weed control, have been locally optimized and improved production packages have been developed to achieve higher yield. To date, a total of 91 improved cultivars have been released, mostly developed from genetic material supplied by ICARDA. Due to the outbreak of improved varieties in combination with improved production techniques, the average global productivity has increased from 611 kg/ha to 966 kg/ha in the last three decades (Bozzini and Chiaretti 1998; Katerji *et al*. 2001; Turk *et al.* 2003; Ford *et al.* 2007). Research at the molecular level, including construction of a lentil genetic linkage map, identification of molecular markers, and genetic transformation, has progressed considerably (Phan *et al.* 2002; Rubeena *et al.* 2003; Muehlbauer *et al.* 2006).

In Italy the major cause for the decline in the cultivation of grain legumes is the failure of plant breeders to produce new varieties resistant to plant diseases and suited to mechanized cultivation methods. Food fashion also plays a role (Abbate 2001). In particular lentil cultivation declined in the last decades owing to the low yield potential and the tendency to lodging of locally grown populations. Erect, higher yielding genotypes have been recently developed, which may renew the interest for this crop under Italian conditions (Faustini *et al*. 2004). Competitive ability appears to be mainly related to flowering earliness, which likely gives lentil a competitive advantage against the late emerging weeds (e.g. *Chenopodium* spp., *Atriplex* spp.). A wide variability in the competitive ability of genotypes was observed by these authors.

Bozzini and Rossi (1988) proposed a first lentil ideotype for Italy. Nowadays, the structural and physiological characters desired for lentil grown in Italy, for each cultivation area, could be: higher yield and 'harvest index' connected to more flower per stalks/plant, number of flower nodes, number of flowers per node, number of seeds per pod, 1000 seeds weight. These desirable traits are also related to a high LAI (Leaf Area Index) and LAD (Leaf Area Duration) mainly during the flowering-maturing period.

Other required characters are: seed vigour, fast growth of the young seedlings, strong and deep roots with many and early *Rhizobium* nodules, tall (40-60 cm) and lodging resistant stalks, determinate growth habit, lowest pod higher than 15-20 cm from the ground (for an effective mechanical harvesting), higher number of leaflets per leaf with many long and strong tendrils that, due to a 'net effect', might help decrease lodging problems, pods with very low shattering tendency and with a strong peduncle to avoid falling during harvesting, good adaptation to the environmental conditions of cultivation site, high diseases resistance, higher protein content (ca. 30%) with a well balanced amino-acid composition. It is also desirable that flowers are present in the last 4-5 internodes; in fact this apical position facilitates seed formation and decreases flowers fall, since this last problem is the main cause of low yield in several Italian areas.

About the ideal growth cycle for central-southern Italian zones, it advisable a lentil type adapted to autumn sowing time and with an early maturing time. Consequently this ideotype has to be resistant to autumn excess moisture, typical of many central-southern Italian zones, and to water deficiency and high temperate during the production phase. The usual flowering and maturing times of Italian landraces are late April to early May and late May and early June, for southern and central regions respectively, so that a earliness of at least 20-30 days would be required.

This ideotype has to be resistant to the most common diseases for lentil in Italy: *Fusarium oxysporum* f. sp. *lentis* and *Uromyces fabae* are the most dangerous. In some areas *Orobanche crenata* Forssk. and *Sitonia* spp. insects are also very injurious.

The best product quality relates to end use. Seeds for direct consumption should have high digestibility, so low or free tannin types (trait related to light coat colour) are the best. When the product is shelled lentils, orange or green cotyledon types are the best. Also cooking time is important even though it is generally related to the seed size.

All these traits characterising the ideotype may be present in the germplasm collections and in landraces, so that lentil breeders might start from this material to set up improvement strategies.

CONCLUSIONS

The most important ideal traits characterizing the proposed ideotype are present in Italian landraces, so that a breeding programme for new lentil cultivars for Italian areas might already begin starting from these autochthonous parents. Further studies at the molecular level, (e.g. a more detailed lentil genetic linkage map, the identification of specific molecular markers) can significantly help and accelerate the obtainment of high yield genotypes with high-quality characteristics.

In addition, several Italian lentil landraces may become of economical interest if considered as niche crops, especially in southern Italy.

In parallel with the creation of the ideotype, also cultural techniques have to be improved to suit the new cultivars and to meet the demand for organic food.

In conclusion lentil cultivation provides a number of advantages to producers. It can be used to diversify and lengthen crop rotation, reducing disease pressure and improving weed control. Lentil also improves soil tilth and reduces the requirement for nitrogen. Lentil ability in nitrogen and carbon sequestration improves soil nutrient status, which, in turn, provides sustainability in the production systems.

While lentil seeds are used mainly as food, the straw can also be used as a high quality animal feed or as a source of organic material for soil improvement.

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