Chapter 5
Tackling Lentil Biotic Stresses in the Genomic Era

Marcelino Pérez de la Vega, Pedro García García, Juan J. Gutierrez-Gonzalez, and Luis E. Sáenz de Miera

Abstract Lentils are already one of the main pulses of the world, as they are one of the main sources of protein for humans. As a crop, they are also gaining momentum because the rusticity and tolerance to water scarcity of some varieties are a good fit with the current global warming trend and climate change in general. However, while the harvested area and overall production have drastically increased over the last decades, yield has only experienced very modest increments. The reasons are two-fold. First, pathogens are affecting the crop as never before, likely due to not only the changing climate but also to the expansion of lentil cultivation to new geographic areas. Second, genomics-aided breeding is far behind many other crops. This is in partly due to the lack of genomic tools currently available to researchers. Progress is being made to adopt high-throughput genomic methods, and researchers will be able to tackle lentil gene discovery and breeding for pathogen resistance and other biotic stresses more efficiently in the coming years. We outline the current situation, novel findings, and prospects of lentil research for biotic stresses.

Keywords Lentil · *Lens culinaris* Medik · Biotic stress · Genomics · Resistance · Breeding

5.1 Introduction

Lentil (*Lens culinaris* Medik. subsp. c*ulinaris*) is one of the first domesticated species in the Fertile Crescent and along with barley, emmer wheat and einkorn wheat, pea, chickpea, and flax was part of the set of crops that defined the beginnings of the Neolithic transition to agriculture in this part of the World. The origin of the cultivated form is the wild *L. culinaris* subsp. *orientalis* (Boiss.) Ponert (syn. *L. orientalis* Boiss.). A recent publication by Liber et al. (2021) suggests that phylogenetics, population structure, and archeological data coincide in a lentil domestication prolonged in time in Southwest Asia, with two different domesticated gene pools. From the

M. Pérez de la Vega (✉) · P. García García · J. J. Gutierrez-Gonzalez · L. E. Sáenz de Miera
Área de Genética, Departamento de Biología Molecular, Universidad de León, León, Spain
e-mail: m.perez.delavega@unileon.es

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Fertile Crescent the crop expanded eastward, westward and southward in pre- and protohistoric times to almost all temperate areas of the Old World. The expansion of cultivated lentil most likely occurred simultaneously with other first-domesticated crops as the agriculture expanded fromthe Fertile Crescent to the rest of the Old World. The diffusion of lentils occurred during the earliest period of agriculture expansion since lentil remains have been recovered in several archaeological sites corresponding to the earliest agricultural sites in Old World geographical areas. For instance, in the ancient western end of its distribution, the Iberian Peninsula, there are lentil archaeological remains since the early Neolithic (Cubero et al. 2009; Pérez de la Vega et al. 2011). In the cave "de les Cendres" (Spain), there are remains of several crops (*Triticum monococcum*, *T. dicoccum*, *T. aestivum*, barley, pea, grass pea, lentil and faba bean, i.e. a typical Near East crop complex) dated by ^{14}C to 7540 ± 140 BP (Buxó 1997). Archeological data indicate that lentil reached the Atlantic Canary Island though North-African colonizers in prehistoric times, long before the first contact with Europeans in the XIV century (Henríquez-Valido et al. 2019). From the sixteenth century it was introduced in America and later in Australia.

Lentil has been grown and/or consumed in most temperate areas of the World during centuries. An indication of the extent of its diffusion among cultivated plants is that according to the compilation by Mikic (2019) there are more than 180 languages with a word of their own to designate lentil. According to Cubero et al. (2009), if lentils have been maintained by farmers through ages, it is most likely because they grow in poor soils, rough climates, and harsh conditions for humans, animals and crops. In many cases, they may be the only source of protein available to them.

The binary scientific name of lentil is attributed to Friedrich Kasimir Medikus (1736–1808), a German physician and botanist, hence the standard abbreviation of Medik. Medikus was a younger contemporary of Linnaeus (1707–1778) and reviewed some of the specific assignments of Linnaeus. Linnaeus included lentils into *Cicer* and later in *Ervum*. Thus, among the synonyms of *L. culinaris* are *Cicer lens* (L.) Willd., *Ervum lens* L., *Lens esculenta* Moench, *Lens lens* Huth, *Lentilla lens* (L.) W. Wight ex. D. Fairchild; *Vicia lens* (L.) Coss. & Germ. (Cubero et al. 2009; Mikic 2019). Among these binary names, only *L. esculenta* Moench is found in relatively recent scientific papers, or even *L. culinaris* Moench. Although the genus *Lens* had been recognized by earlier scientists, the authorized name of the genus is *Lens* Miller (Cubero et al. 2009).

The C (unreplicated haploid) genome size of lentil was determined by flow cytometry in an amount of 4.41 pg equivalent to 4,063 Mbp (Arumuganathan and Earle 1991), or by means of Feulgen's microdensitometry in 4.6 pg (Bennet and Smith 1976), a size similar to the size estimated to pea; and like in the pea genome, the lentil genome seems to be rich in transposable elements (Rey-Baños et al. 2017). It is worth mentioning that due to the repetition of transposon and other sequences the complexity of the lentil genome must be much lower than its size.

Lentil is the only cultivated species of the genus *Lens* in which all species have the same chromosome number, $2n = 14$, and share similar karyotypes (Ladizinsky 1993), but there are chromosomal rearrangements between species and sometimes intraspecific. Chromosomal rearrangements are observable partly by differences in

karyotype, but mainly by the occurrence of multivalents (translocation) at the first meiotic metaphase, or a bridge and fragment (paracentric inversion) at the first meiotic anaphase in pollen mother cells of intraspecific hybrids (Ladizinsky and Abbo 2015). The genus *Lens* is a relatively small genus that includes no more than six biological species *L culinaris* Medik., with two subspecies (*culinaris* and *orientalis*), *L. ervoides* (Brign.) Grande, *L. lamottei* Czefr., *L. odemensis* Ladiz., *L. nigricans* (M. Bieb) Godr., and *L. tomentosus* Ladiz. (Ladizinsky and Abbo 2015). The species or subspecies status of some of these taxa has been widely discussed. In part this is due to the use of two different species concepts: morphological and biological. On the basis of the biological species concept (a group of individuals that actually or potentially interbreeds and forms one genetic pool that is isolated by various reproductive barriers from individuals belonging to other species). Ladizinsky and Abbo (2015) rejected the subspecies status of *odemensis, lamottei* and *tomentosus.* According to the criterion of reproductive isolation, the only taxa that show high reciprocal crossability are *L. culinaris* and *L. orientalis*, hence the wide acceptance that *L. c. culinaris* and *L. c. orientalis* are two subspecies of a single biological species. Obtaining hybrids among the other taxa (in the vast majority of cases it has been tried between the cultivated lentil and some wild taxon) is difficult and sometimes it is only achieved through embryo rescue techniques; in addition, in many cases the existence of hybrid breakdown is evident (Fratini and Ruiz 2006, 2008; Singh et al. 2013, 2018). Therefore, it is very likely that in nature the reproductive isolation is total between these taxa.

According to a comparative analysis of DNA sequences, Alo et al. (2011) concluded that *L. nigricans* and *L. ervoides* are well-defined species at the DNA sequence level, while *L. odemensis*, *L. tomentosus*, and *L. lamottei* may constitute a single taxon pending verification with crossability experiments. Phylogenetic tree and STRUCTURE analysis of the genus *Lens* using genotyping-by sequencing (GBS) identified four gene pools (GP), namely *L. culinaris-L. orientalis-L. tomentosus*, *L. lamottei-L. odemensis*, *L. ervoides* and *L. nigricans* which form primary (GP1), secondary (GP2), tertiary (GP3) and quaternary (GP4) gene pools, respectively (Wong et al. 2015). However, Ladizinsky and Abbo (2015) included only subsp. *orientalis* in GP1 (likely limited to the accession with the same chromosome arrangement than subsp. c*ulinaris*), *L. odemensis, L. ervoides*, and *L. tomentosus* in GP2, and *L. nigricans* and *L. lamottei* in GP3. Phylogenetic analysis clustered carried out by Dissanayake et al. (2020) grouped the six traditional *Lens* taxa into four groups, namely, *L. culinaris/L. orientalis, L. lamottei/L. odemensis, L. ervoides,* and *L. nigricans*. Liber et al. (2021) confirmed previous studies proposing four groups within the genus *Lens.*

The genus *Lens* is included in the tribe Fabeae (formerly Vicieae) which comprises about 380 legume species, including some important grain legume crops such as pea, grasspea, and faba bean, in addition to lentil. In this tribe are also included the genera *Lathyrus* and *Vicia* (with around 150 species each one), *Pisum* (three species) and the monotypic genus *Vavilovia* (*V. formosa* (Stev.) Fed.). Phylogenetic analyses of the species in the tribe show that the genera *Vicia* and *Lathyrus* in their current circumscription are not monophyletic: *Pisum* and *Vavilovia* are nested in

Lathyrus, the genus *Lens* is nested in *Vicia* (into the Ervoid group of *Vicia*). According to ancestral character state reconstruction results, ancestors of Fabeae had a basic chromosomenumber of $2n = 14$, an annual life form, and evenly hairy, dorsiventrally compressed styles (Smykal et al. 2011; Schaefer et al. 2012). The close relationships between lentils and other Fabeae species ensure a good transferability of genetic and genomic information between species.

Some other taxa from close genera such as *Vicia* or *Lathyrus* have been assigned to *Lens*, for instance *Vicia montbretii* has been classified as *L. montbretii*, but there is a general agreement that they do not belong to *Lens* (Cubero et al. 2009; Ladizinsky and Abbo 2015; Smykal et al. 2015; Leht and Jaaska 2019).

Lentil has never been a model species in basic research; perhaps its greatest contribution is its inclusion among the species used by Vavilov (1922) in his seminal work on the Law of Homologous Series in Variation. Lentil (as *L. esculenta* Moench) was included in the legume species list of Vavilov's comparative study, together with pea (*Pisum sativum* L.), vetch (*Vicia sativa* L.), fava bean (*V. faba* L.), grass pea (*Lathyrus sativus* L.), chickpea (*Cicer arietinum* L.), and other legume species (Pérez de la Vega 2016). Ultimately, the law of homologous series indicates that the variation displayed among related species entails similar characteristics (morphological and also molecular) and that the equivalent characters are controlled by homologous genes (orthologs or paralogs). This law is in fact the basis of the comparative genetics and genomics (Pérez de la Vega 2016). From the practical point of view, what this law indicates is that any genetic or genomic information obtained in one species is always the first clue to be used in the research in any other phylogenetically close species.

Since lentils are cultivated in more than 70 countries this crop is subjected to different climatic conditions and culture practices such as winter or spring sowing; likewise, the biotic factors which affect yield and production are diverse. For instance, while Ascochyta blight (AB), a seed-borne disease, has been described in at least 16 countries in five continents making it the likely most widely distributed and devastating lentil disease, Stemphylium blight (SB) caused by *Stemphylium botryosum* Wallr. was once a minor disease with local significance in South Asia (its first outbreak was reported in Bangladesh in 1986), but is becoming a serious threat to lentil cultivation in many parts of the world such as Canada where it has become more prevalent (Mwakutuya and Banniza 2010; Das et al. 2019).

5.1.1 Economic Importance

Lentil is a predominantly self-pollinated diploid ($2n = 14$) annual grain legume species adapted to growth in dry-temperate climates, traditionally as a rainfed crop. Lentil (*Lens culinaris* ssp. *culinaris*) is a bushy annual herb with erect, semi-erect or spreading growth habit ranging from 25 to 30 cm in height for the majority of genotypes. The legume fruits usually contain one, two or rarely three seeds. They are lens shaped and weigh between 20 and 80 mg and are a rich source of protein and

dietary fiber. Seed diameter is the main characteristic of Barulina's classification of lentil genotypes into the large seeded macrosperma type (6 to 9 mm) or small and medium sized microsperma (2 to 6 mm) (Muehlbauer et al. 1995).

Lentils are consumed almost exclusively in the form of dry seeds and for human consumption, unlike some other nearby species that are also consumed as vegetables or are also used for animal feed (garden/field peas and faba beans). Normally, only damaged lentil grains, not suitable for human consumption, are destined for animal feed. Lentils are traditionally valued as a source of energy, proteins and iron in human nutrition. In addition, they are an important dietary source of fiber, minerals, vitamins and antioxidants (Pérez de la Vega et al. 2011). The amounts of these components vary among cultivars or accessions, thus the ranges for different components per 100 g of raw lentil dry matter are: energy 1483–2010 kJ, protein 20.6–31.4 g, fat 0.7–4.3 g, carbohydrates 43.4–69.9 g, fiber 5.0–26.9 g, ash 2.2–4.2 g (Urbano et al. 2007), although these values can vary depending on the lentil material and the cooking or precooking (e.g., dehulling) treatments (Petterson et al. 1997; Cuadrado et al. 2002; Almeida-Costa et al. 2007; Wang et al. 2009).

The average lentil production of the last five years (to minimize annual fluctuations) of which there are statistics (2014–2018) is 5.9 million tons, harvested in 5.2 million hectares; with an average yield of 1.1 t/ha (Table 5.1). The interest in the consumption of the lentil is shown in the constant and gradual growth of the production of this crop, although that growth is mainly due to the increase in the sown area. The lentil world production is now more than double that of 25 years ago, increasing since 1994 to 2018 from 2,818,469 tons to 6,375,732 tons (126.2%) (Fig. 5.1), but while its yield has moderately increased during this period (from 0.81 to 1.04 tons/ha; 28.4%) the harvested area has increased from 3,456,492 ha to 6,119,509 ha (77.0%). The key year in this change was 2009, in the previous 15 years the average yield was 0.85 t/ha while in the following 10 years it was 1.12 t/ha. Likewise, almost simultaneously the harvested area increased during these ten last years from roughly 3.5 million hectares to approximately 6 million hectares. According to FAOSTAT data, although there is a gradual increase in the surface sown with lentils in many countries and areas, such as the European Union, the most significant contribution to this increase is due to Canada and to more recently to India (Fig. 5.2).

Table 5.1 World lentil harvested area, yield and production from 2014 to 2018[1]

Year	Harvested area (ha)	Yield (hg/ha)	Production (Tons)
2014	4,017,683	11,697	4,699,562
2015	4,710,991	11,673	5,499,290
2016	5,444,686	12,055	6,563,805
2017	5,886,665	10,932	6,435,369
2018	6,119,509	10,419	6,375,732

[1]Data from FAOSTAT

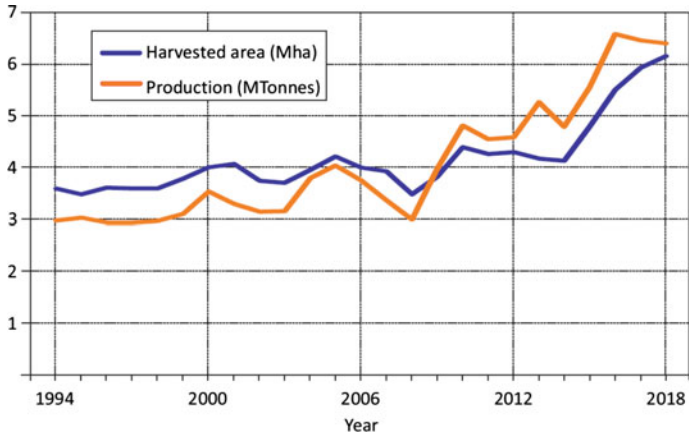

Fig. 5.1 Lentil production and harvested area from 1994 to 2018. Source FAOSTAT

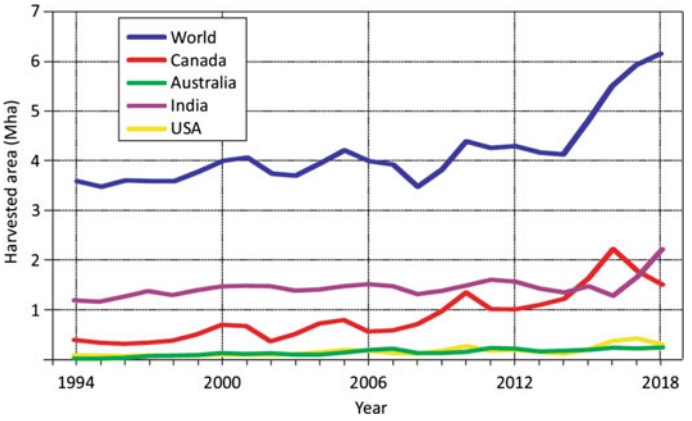

Fig. 5.2 Lentil production in some representative countries from 1994 to 2018. Source FAOSTAT

5.1.2 Reduction in Yield and Quality Due to Biotic Stresses

Yield losses caused by the different biotic stressors in lentils, as in other crops, are highly variable. They not only depend on the causative agent, but also on the environmental circumstances of the region and the year. Ascochyta blight (AB), caused by *Ascochyta lentis*, is probably the most generalized disease in lentil, and it has been reported to be the major lentil disease in many lentil-producing countries. The disease has considerable effects on both seed quality and yield. Yield losses have been estimated to reach of up to 40%, but inCanada economic losses from infected seed may reach more than 70%. In some cases, seed infection is so severe that the lentils are unmarketable (Gossen and Morrall 1983, 1984; Ye et al. 2002).

Virus diseases incidence and the losses they caused varied widely from extremely low (less than 1% incidence) to almost 100% with complete crop failure (Makkouk and Kumari 2009). A major lentil pest *Bruchu*s spp. also causes significant losses. Mean seed loss under organic farming was 15% and mean yield loss was 0.13 t/ha. Seed and yield losses were 2.6- and 8.4-fold higher, respectively, under organic than conventional farming. Valuable genotypic variability was observed with respect to both seed and yield losses. Farming system was the main source of variation for both losses, while early flowering and small seed size were traits associated with low losses (Vlachostergios et al. 2018). Lentil yield loss from the competition with weeds can range as high as 80% (Pala 2019).

Tests carried out in Australia showed that aphids and aphid-transmitted viruses cause appreciable yield reduction in pulse crops. Lentils were most affected by viruses, followed by faba beans, lupins (narrowleafed) and field peas, with yield reductions averaging 85% in lentils. Feeding damage on lentils averaged 4.5% (Valenzuela and Hoffmann 2015). Tests in the Palouse region of northern United States with the aphid *Acyrthosiphon pisum* and the associated *Bean leafroll virus* (BLRV) and *Pea enation mosaic virus* (PEMV) predicted lentil yield loses up to 100% by early virus infections (10 days after emergence) (Paudel et al. 2018), and a previous publication (Elbakidze et al. 2011) with these three species showed that aphid outbreaks have historically decreased pea and lentil yields by approximately 5% and 7% on average, respectively in the Palouse region (See also Sect. 5.2).

5.1.3 Growing Importance in the Face of Climate Change and Increasing Population

Global warming and other climatic changes associated with it are having and will have a clear effect on agriculture. The most evident direct effect of higher temperatures is on the growth and yield of crops, and the indirect effect more clearly associated with the increase in temperature is the increase in drought risk. Gupta et al. (2019) stated that it is anticipated that climate change is likely to exert a substantial effect on various insect pest management programs including host-plant resistance, natural plant products, bio-pesticides, natural enemies, and efficacy of synthetic chemicals. Several works have addressed the effect of global warming and climate change in lentil and other pulses (Cutforth et al. 2007; Bueckert and Clarke 2013; Bhandari et al. 2016; Bourgault et al. 2018). But the change in the geographical distribution in which crops and their possible pests and pathogens can grow is also important. The new challenges for crops in relation to biotic stresses are the spread of new diseases or pests, such as the recent spread of *Stemphylium* from a practically regional disease to extend to several main areas of lentil production (Mwakutuya and Banniza 2010; Das et al. 2019); or the ability to grow, and therefore infect, in areas that were relatively cold but are warmer now. An example of this is the northern enlargement of the

area where the parasitic weed *Orobanche* has been observed in lentil fields in Spain (Rubiales et al. 2008).

5.1.4 Limitations of Traditional Breeding and Rational of Genomic Designing

Breeding productive and resistant genotypes to diseases, pests and weeds is considered the most feasible and environmentally friendly method to manage major stressors (Rubiales et al. 2015; Keneni and Ahmed 2016). The use of resistant varieties against biotic stresses provides a number of comparative advantages particularly in reducing the use of environmentally unfriendly agrochemicals. The latest definition of the fundamental theorem of natural selection by Fisher (1941) says that the rate of increase in the average fitness of a population is equal to the genetic variance of fitness of that population. This is the reason why genetic variability is an indispensable initial condition for any selective breeding procedure. Natural genetic variability can be found in crop landraces and old varieties or can be gained or increased by sexual crosses, within the cultigene or through wide crosses with wild relatives, so that crossing is usually one of the ways to start a breeding program. Genetic maps and markers are invaluable genetic tools to advance in the rational use of variability in breeding, hence the advantage provided by the dense maps and the thousands of markers provided by the new genomic technologies (See Sect. 4.2).

Resistance genes to biotic stresses are often preferably or exclusively found in wild relatives, but transfer of genes from wild relatives to cultivated varieties can present interspecific cross-incompatibility hindering the use of this genetic variability. There is generally agreement in which landraces are sources of initial breeding materials since they have breeding values under suboptimal production as they contain valuable adaptive genes to different circumstances. Effective resistance against biotic stresses may be achieved from genetic improvement of the host species but genes for complete resistance to pests or disease agents may not exist in cultivated species of crop legumes as opposed to wild relatives which have coexisted with pests on an evolutionary time scale (Keneni and Ahmed 2016). Hence the frequent need to draw on wild materials. Another variable to consider is the genetic control of the resistance in question, monogenic, oligogenic or polygenic, which largely determines the breeding method to be followed (bulk, pedigree, backcrossing, etc.). When designing a breeding program, it is also appropriate to consider the convenience/possibility of pyramiding resistance genes for the same or for different pests or pathogens. When no broad spectrum resistance mechanisms are known in a host–pathogen-pest interaction, the convenience of pyramiding genes against the same biological agent arises from the fact that resistance in many cases is strain specific (or gene-for gene, see Flor 1971), and/or, therefore several resistance genes are needed to obtain a relatively broad resistance. On the other hand, pathogens and pests, as living beings, constantly evolve in such a way that new strains or pathovars can appear by mutation

or be selected between pre-existing variability by new environmental factors, which insists on the constant need to search for broad spectrum resistances, new resistance genes and/or pyramid genes already known. Nor can we forget that the globalization of the food trade helps the dispersal of pathogens and pests so that new agents or new stains can quickly colonize new territories. Another complication in breeding for resistance to biotic stresses is that the response to a biological agent may depend on other environmental factors (this could at least explain in part because many times a genotype is resistant under the controlled conditions of a greenhouse but susceptible in the field), in part because the signaling pathways to biotic and abiotic stresses are not completely independent (Atkinson and Urwin 2012; Ramegowda and Senthil-Kumar 2015). Last but not least, when improving for resistance to stresses, especially when using landraces or wild relatives, resistance genes can be linked to genes that are unfavorable for the yield or the characteristics of a domesticated crop (for example, pod shattering).

Transgenesis is a way to overcome interspecific cross incompatibility barriers and to extend gene sources to species from other biological kingdoms. Since the first transgenic crops in the 90's of the twentieth century, success has been obtained in achieving transgenic crops, in particular resistance to insects, viruses and herbicides. Legumes are a natural source of genes coding in particular for insecticidal proteins but there are only a few examples of transgenic pulses (Solleti et al. 2008; Kumar et al. 2018b; Sagar and Dhall 2018; Kumar and Jogeswar 2020), there is a lower number of examples of use in commercial production, and to date none in lentil (Gupta et al. 2020).Furthermore, as Kumar and Jogeswar (2020) stated, bio-safety issues and the possible effect of genetically modified crops on nutrition, growth, metabolism and health of people persists as a subject of public debate.

5.2 Description of Different Biotic Stresses

5.2.1 Fungi (See also Sect. 5.7)

Among the main threats to lentil production are several diseases caused by fungi. Ascochyta blight (AB), caused by *A. lentis*, is probably the most important and frequent disease of lentil throughout the world, and it can cause yield losses up to 70% in addition to seed damages (Gossen and Morrall 1983, 1984). *Ascochyta* spp. (teleomorphs: *Didymella* spp.) infect a number of legumes; including many economically crops, and the diseases they cause represent serious losses in legume production worldwide. *Ascochyta rabiei, A. fabae, A. pisi, A. lentis*, and *A. viciae-villosae* are pathogens of chickpea (*Cicer arietinum*), faba bean (*Vicia faba*), pea (*Pisum sativum*), lentil (*Lens culinaris*), and hairy vetch (*V. villosa*), respectively. Under controlled conditions *A. fabae, A. lentis, A. pisi, A. rabiei*, and *A. viciae-villosae* demonstrated to be host specific (Hernandez-Bello et al. 2006). These authors were able to obtain several interspecific hybrids between *Ascochyta* species, but

hybrids have shown a low pathogenic ability on the respective crop species. They conclude that the low pathogenic fitness of hybrids may be animportant speciation mechanism contributing to the maintenance of host specificity.

Other important diseases caused by fungi are: Stemphylium blight (SB) which is a defoliating disease in lentils, caused by the necrotrophic Ascomycete, *Stemphylium botryosum*. Rust originates by the infection of the biotrophic fungus *Uromyces viciafabae*. This pathogen is widespread and attacks the aerial parts of the plants. The root disease Fusarium wilt (FW) is caused by *Fusarium oxysporum*, a filamentous ascomycete fungus. Anthracnose is a disease caused by the hemibiotrophic fungus *Colletotrichum lentis*. Root rot disease is caused by the oomycete *Aphanomyces euteiches*.

5.2.2 Bacteria

Compared to fungal diseases, publications on bacterial diseases are very scarce. Most of the data is limited to the bacterial blight caused by *Pseudomonas syringae* pathovars. At least three pathovars have been associated to bacterial blight in lentil. Hunter and Taylor (2006) analyzed the patterns on interaction between *Pseudomonas* pathovars and several grain legume species. They found that lentil (nine accessions) showed patterns of interaction with isolates of *P. syringae* pv. *glycinea*, *P. syringae* pv. *phaseolicola,* and *P. syringae* pv. *pisi*. The minimum numbers of resistance (*R*) and avirulence (*avr*) gene pairs to account for the observed interactions were nine *R* genes in lentil, and the *avr* ranged from seven to nine among pathovars. It is likely that *P. syringae* pv. *syringae* can also cause bacterial blight in lentil since this pathovar is able to infect other close species of *Pisum* and *Lathyrus* (Martín-Sanz et al. 2011, 2012).

Phytoplasma naturally infecting lentil was first reported in 2016 (Akhtar et al. 2016). In April 2011, lentil plants were found with symptoms reminiscent of phytoplasma infection in Pakistan. Phytoplasma presence was confirmed by 16S rDNA PCR amplification, and experimental transmission was successful by grafting and by the leafhopper *Orosius albicinctus.*

5.2.3 Pest

Pests can cause a direct reduction in yield by feeding of plants and indirectly by transmitting pathogens. Many pest species inoculate pathogens such as viruses while feeding on plants, or open ways for further microbial infections through the wounds caused to plants; furthermore, these infections can reduce the plant ability to response to further abiotic stresses. For instance, the aphid *Acyrthosiphon pisum*is an infection vector of the *Bean leafroll virus* (BLRV) and the *Pea enation mosaic virus* (PEMV) in lentil. Thus, this section is related with Sect. 2.4 devoted to viruses.

Lentil is damaged by many types of insects and other pests. Among insects, major field pests are aphids (*Aphis craccivora, Acyrthosiphon pisum*), leaf weevil (*Sitona* spp.), lygus bugs (*Lygus* spp.) and the cutworm (*Agrotis ipsilon*) (Pérez de la Vega et al. 2011). Another major pest problem causing great seed losses areseed insect species: *Bruchus ervi* and *B. lentis* with *Callosobruchus chinensis* and *C. maculatus* (Stevenson et al. 2007). Rinehold et al. (2018) described as lentil pests the species bean aphid (*Aphis fabae*), green peach aphid (*Myzus persicae*), pea aphid (*Acyrthosiphon pisum*), lygus bugs (*Lygus* spp.), seed-corn maggot (*Delia platura*), western yellow-striped armyworm (*Spodoptera praefica*), and also included lentil as host of the pea moth (*Laspeyresia nigricana*). Rinehold et al. (2018) publication also includes pest description, monitoring and control. In a review on pest management in grain legumes, Sharma et al. (2010) listed the bruchid species *Callosobruchus chinensis* as a highly important pest in lentil; the pod borer *Etiella zinckenella*, weevil *Sitona* spp., the aphids *Aphis craccivora*, *Acyrthosiphon pisum*, and *Aphis fabae*, as moderately important; and pod borers of the genus *Helicoverpa*, pod sucking bugs of *Clavigralla*, defoliators of *Spodoptera*, and grasshoppers of *Empoasca* as occasional lentil pests. But the list of lentil pests is larger if local or regional pests are added to the list of global and general pests. For instance, chalky spot damage on red lentil, caused by the stink bugs *Piezedorus lituratus* and *Dolycorus baccarum*, is the most important problem waiting for a solution regarding plant health at lentil cultivation in Southeast Anatolia Region, Turkey (Mutlu et al. 2016). Although trips are less frequently mentioned as lentil pest, some trip species have been described as pea and lentil pest during spring in Eastern Europe (Pobozniak 2011).

Bruchids in particular are a legume pest that causes post-harvest damage by feeding inside the grains, decreasing their value. Although these pests are ancient, since there is evidence of bruchid infestation in lentils stored in ancient Egypt and preserved in the British Museum (radiocarbon dated 2,112 ± 48 BP, c. 162 BC) (Burleigh and Southgate 1975), little resistance has been achieved over centuries of cultivation and breeding. Clement et al. (1994), in a review on resistance to insect in cool season food legumes, indexed only three publications in relation to lentil, specifically against *Aphis craccivora*, *Bruchus lentis*, and *Sitona* spp. But, according to Clement et al. (1994), resistance to *B. lentis* was ecological, not genetics. In general, resistance against these pests seems to be scarce in all cool season food legumes. For instance, no resistance by antixenosis, antibiosis and/or tolerance was found after mass screening of 6,697 accessions of chickpea (to *Callosobruchus chinensis*) or 1,000 accession of broad bean, as summarized in the above mentioned review. In an evaluation of lentil varieties and farming system effect on seed damage and yield loss due to bruchid infestation in Greece, Vlachostergios et al. (2018) found that early flowering and small seed size were traits associated with low seed loss and yield loss. Among varieties, mean seed loss ranged from 8.5% to 29.2% and yield loss from 0.06 to 0.31 t/ha. Bruchid tolerance, revealed two types of promising varieties: varieties with high yield and low seed bruchid damage due to phenological escape, and varieties with high yielding potential despite the high seed loos and yield loos.

Other "generalist" insects, such as grasshoppers can reduce lentil yield. In field trials carried out in Canada using cages to evaluate the damage caused by grasshoppers to lentil flowers and pods, Olfert and Slinkard (1999) reported a decrease in yield from 28 to 57% in cages with two to 10 grasshoppers (*Melanoplus bivittatus*).

El-Bouhssini et al. (2008) reported the first sources of resistance to the coleopteran weevil *Sitona crinitus* in wild *Lens* taxa, namely, *L. ervoides, L. nigricans, L. odemensis*, and *L. orientalis*. Eight accessions were identified as resistant, with ≤ 10% nodule damage, compared to > 56% damage recorded on the cultivated lentil. Field evaluation and screening of lentil germplasm against black aphids (*Aphis craccivora*) resulted in the identification of 26 highly tolerant genotypes; Precoz was found to be a major source of resistance followed by LG 171 (Kumari et al. 2007).

5.2.4 Viruses

Table 5.2 summarizes the number of different virus species tested in four cool season legume species listed in the VIDE database (Brunt et al. 1996). The number of virus species tested in lentil and to which it was found susceptible (upper number in the diagonal) or unsusceptible (lower number) is clearly lower than the figures for the other three species. For instance, 27 susceptible in lentil in comparison with 124 in pea. These numbers are not absolute values because sometimes the same virus species is classified as susceptible and or unsusceptible, likely because this response is the results of particular interaction between a plant genotype and a virus strain. The table also indicate the number of common virus species; for instance, 22 virus species as been described trigging the susceptible response and four the unsusceptible one,

Table 5.2 Number of susceptible and unsusceptible virus species tested in four cool season legume species

	Lentil *Lens culinaris*	Sweet-pea *Lathyrus odoratus*	Pea *Pisum sativum*	Faba bean *Vicia faba*
Lentil *Lens culinaris*	27/14	9	22	20
Sweet-pea *Lathyrus odoratus*	7	57/25		
Pea *Pisum sativum*	4		124/164	
Faba bean *Vicia faba*	6			108/153

The diagonal indicates the virus species to which each of the four crop species has been described as susceptible/unsusceptible

Numbers above the diagonal indicate the number of common virus species to which they are susceptible

Numbers below the diagonal indicate the number of species to which the lentil is susceptible but unsusceptible the other species

in both pea and lentil. These data show that the available information on plant-virus interactions in lentil is limited in comparison to other closely related crops.

Surveys conducted in many countries in West Asia and North Africa during the last three decades established the most important aphid-borne viruses posing a significant limitation to legume production (and to cereals) (Makkouk and Kumari 2009). The list of the most important of these viruses affecting cool-season food legumes (faba bean, lentil, chickpea and pea) included: *Bean leafroll virus* (BLRV), *Beet western yellows virus* (BWYV), *Bean yellow mosaic virus* (BYMV), *Chickpea chlorotic stunt virus* (CpCSV), *Faba bean necrotic yellows virus* (FBNYV), *Pea enation mosaic virus-1* (PEMV-1), *Pea seed-borne mosaicvirus* (PSbMV) *and Soybean dwarf virus* (SbDV). All the above-mentioned viruses are persistently transmitted by aphids except BYMV and PSbMV which are occasionally transmitted by aphids. And the most important aphid species reported to transmit these legume viruses were *Acyrthosiphon pisum* (BLRV, BWYV, PEMV-1, FBNYV, SbDV), *Aphis fabae* (BLRV, FBNYV), *Aphis craccivora* (BLRV, PEMV-1, FBNYV, BWYV), *Myzus persicae* (PEMV-1, BLRV, BWYV), *Macrosiphum euphorbiae* (PEMV-1, BLRV), and *Aulacorthum solani* (PEMV-1, BWYV, SbDV) (Makkouk and Kumari 2009). This review also includes some sources of resistance to PEMV, BLRV, FBNYV and SbDV in lentil germplasm (ILL 75 had resistance to BLRV, FBNYV and SbDV, whereas ILL 74, ILL 85, ILL 213, ILL 214 and ILL 6816 were resistant to FBNYV and BLRV), in addition to procedures for the integrated management of these aphid-borne viruses. Makkouk et al. (2014) add information on Australia and listed the most important viruses reported to naturally infect lentil: *Alfalfa mosaic virus* (AMV), *Bean leafroll virus*, *Bean yellow mosaicvirus*, *Beet western yellows virus*, *Broad bean mottle virus* (BBMV), *Broad bean stainvirus* (BBSV), *Broad bean wilt virus* (BBWV), *Chickpea chlorotic dwarf virus* (CpCDV), *Chickpea chlorotic stunt virus*, *Cucumber mosaic virus* (CMV), *Faba bean necrotic yellows virus, Pea enation mosaic virus-1, Pea seed-borne mosaic virus*, and *Soybean dwarf virus.*

Sources of resistance seem to be scarce. All the 29 lentil lines tested in a pioneering work (Aydin et al. 1987) were susceptible to the PEMV strains PI 472,547 and PI 472,609 and showed significant yield reduction. The accessions of three wild *Lens* species tested were also susceptible. More promising results were obtained by Jain et al. (2014) who screened a total of 44 lentil accessions for resistance to PEMV. Two accessions (PI 431,663 andPI 432,028) were identified with resistance to PEMV in field tests while 14 accessions were found resistant or moderately resistant in greenhouse screenings. Most of the resistant accessions came from Iran. Thirty-six polymorphic simple sequence repeat (SSR) markers which produced 43 loci were used for genetic diversity analysis of this collection.

5.2.5 Nematodes

Askary (2017) listed the prominent genera of nematodes attacking pulses (including lentil): *Meloidogyne*, *Heterodera*, and *Paratylenchus*, the endoparasites,

Rotylenchulus, semi-endoparasites, and *Tylenchorhynchus* and *Helicotylenchus*, the ectoparasites. The root-knot nematode *Meloidogyne incognita*has been described as one of the major limiting factors affecting lentil growth and yield (Khan et al. 2017). Nine out of 300 lentil accessions were found to be resistant to *M. incognita.* Results suggested that the disease resistance in lentil accessions may be related to both post-infectional (nematode growth and development) as well as pre-infectional (penetration and establishment) defense mechanisms (Khan et al. 2017).

5.2.6 Weeds

Lentil growth and production are challenged by many weed species that depend in part on the region in which this crop is grown, and which are generally controlled by crop rotation and herbicides (Jurado Expósito et al. 1997). There are many weed species which largely depend on the geographic area in which the lentil is grown. In Southeastern Anatolia, Pala (2019) described as common weeds the species *Sinapis arvensis* L. (36%), *Ranunculus arvensis* L. (16%), *Galium aparine* L. (11%), *Cephalaria syriaca* L. (8%), and *Centaurea depressa* L. (8%). But one of the biggest challenges to the cultivation of lentils, and other crop legumes, in the Mediterranean and potentially dangerous in other temperate zones is broomrape, which unfortunately, and probably due to the general warming, is extending its range of distribution (Grenz and Sauerborn 2007; Rubiales et al. 2008). Broomrape could reduce the yield up to 90% in the Mediterranean region. Broomrape, *Orobanche crenata* Forsk., is a root holoparasitic weed and the main damaging weeds for temperate legumes, but other species such as *Orobanche foetida, Orobanche minor*, and *Phelipanche aegyptiaca*, can also induce high local damage (Rubiales and Fernandez-Aparicio 2012). Lentil can be severely infected by *O. crenata*, it can also be damaged although with less virulence by *O. aegyptiaca*, and can only be slightly infected by *O. foetida* (Fernández-Aparicio et al. 2009). Resistance to these parasitic weeds is difficult to access, scarce, of complex nature and of low heritability (Rubiales et al. 2009). Low infection rates seemed to be based on a combination of various escape and resistance mechanisms (Fernández-Aparicio et al. 2008). Resistance sources to broomrape have searched in cultivates and wild materials (Fernández-Aparicio et al. 2008, 2009). Fernández-Aparicio et al. (2010) proposed the use of berseem clover (*Trifolium alexandrinum*) as an intercrop with grain legumes to a significant reductionof *O. crenata* infection. Considerable internal variation within *O. crenata* populations parasitizing faba bean and lentil species was observed by molecular analyses, but significant divergence among the populations was detected (Ennami et al. 2017).

5.3 Genetic Resources of Resistance Genes

Domesticated lentil has a relatively narrow genetic base globally and most released varieties are susceptible to severe biotic and abiotic stresses. The crop wild relatives could provide new traits of interest for tailoring novel germplasm and cultivated lentil improvement (Singh et al. 2020a). There are a considerable number of global (mainly the ICARDA collection) and national collections of germplasm of land races and wild lentil relatives. GENESYS, the online platform that includes information on plant genetic resources for food and agriculture conserved in genebanks worldwide (https://www.genesys-pgr.org/), encompass records of 31,211 accessions named as lentil, although the information collected on lentil only refers to 70% of the total of 43,214 accessions conserved ex situ in all genebanks. References to lentil germplasm collections have been published in some review papers (Muehlbauer et al. 1995; Pérez de la Vega et al. 2011), and more recently extensive compilations of the cultivated and wild *Lens* germplasm collections can be found in the reviews by Singh et al. (2018) and Malhotra et al. (2019). The number of accessions varies widely between national collections, highlighting 10 collections with more than 1,000 accessions. Regarding the percentage of wild relatives, the high 70% of the Ethiopian collection stands out, and Canada, the Russian Federation, Chile, China and Spain have relevant values, around 10% up to 17%, although the number of wild relatives is unknown in approximately 50% of the collections. The percentage of land races varies between more than 90% in Pakistan, Nepal and Turkey, to 3–5% in Egypt and Hungary. The ICARDA collection maintains almost 11,000 accessions, of which 82% are local breeds and 583 wild relatives.

Three recent papers (Singh et al. 2018; Gupta et al. 2019, 2020) have reviewed the use of wild germplasm in relation to the response to stresses (cold, drought, salinity, diseases, etc.). Among the extensive amount of data compiled, these papers summarize the wild germplasm used to that date for the introgression of useful traits in cultivated lentil in relation to the response to several diseases (anthracnose, Ascochyta blight (AB), Fusarium wilt (FS), powdery mildew, and rust), to pests and parasitic plants (Sitona weevils, bruchid weevils, and orobanche), in addition to some abiotic stresses (drought, cold, and yield components). Also Ladizinsky and Abbo (2015) and Malhotra et al. (2019) summarized the potential of wild *Lens* resources as resistance sources. Likewise, Gupta et al. (2019) summarized the resistance sources found in the lentil cultivated gene from many different countries, namely to AB, anthracnose, rust, FW, Botrytis gray mold, and Stemphylium blight. Rana et al. (2016) summarized the pulse crop resources in the large national collection of India, listing lentil, and other pulses, accessions with resistance to diseases and pests and as sources of agro-morphological characters, quality, biochemical traits, and abiotic stresses.

Resistance to the pest *Callosobruchus chinensis* was evaluated in a germplasm collection of wild and cultivated *Lens* accessions (Gore et al. 2016). Accessions were categorized as highly resistant, resistant, moderately resistant, moderately susceptible, and susceptible. *L. ervoides* was highly resistant and *L. culinaris* was the most

susceptible species. Likewise, resistance against *Bruchus* spp. has been evaluated in a large collection of 571 cultivated and wild accessions from 27 countries (Laserna-Ruiz et al. 2012). A total of 32 accessions, including *L. culina*ris subsp. *culinaris*, *L. culinaris* subsp. *orientalis*, *L. nigricans*, and *L. lamottei*, showed lower infestation rates than the check and were selected as potential sources of resistance to this pest.

Resistance to AB has been found in several germplasm screens. In a wide experiment carried out under field and greenhouse condition in Canada,Tullu et al. (2010) found resistant accessions in all *Lens* wild taxa tested except in *L. tomentosus*, using a mixture of three monoconidial isolates of A*scochyta lentis* as the inoculum. Several consistently resistant accessions were found among entries of *L. ervoides* and *L. nigricans*. Thirteen accessions, previously reported as resistant to Syrian isolates of *A. lentis* were also resistant to the Canadian isolates. Furthermore, some of the wild accessions showed resistance to anthracnose. Cultivars and germplasm accessions of cultivated lentil showed a wide range of response between the resistant and susceptible controls. In a further study Dadu et al. (2017), in a search among 30 accessions from five wild *Lens* taxa, found resistance to AB to new highly aggressive Australian *Ascochyta* isolates in several wild *Lens* taxa, particularly in *L. orientalis*.

Dadu et al. (2018) analyzed the early response to two *Ascochyta lentis* isolates (FT13037 and F13082) of two cultivated lentil genotypes, ILL7537 (resistant) and ILL6002 (susceptible), and the recently identified AB-resistant *Lens orientalis* genotype ILWL180. Both isolates had significantly lower germination, shorter germ tubes and delayed appressorium formation on the resistant genotypes compared to the susceptible genotype; furthermore, these were more pronounced on the wild ILWL180 than on cultivated ILL7537. The authors concluded that the faster recognition of *A. lentis* is likely to be a major contribution to the superior resistance observed in genotype ILWL180 to the highly aggressive isolates of *A. lentis* assessed. Likewise, Dadu et al. (2019) using the focused identification of germplasm strategy, selected a subset of 87 landraces (originating from 16 countries) with highest likelihood for *A. lentis* resistance from 4,576 accessions held by the ICARDA. Significant variation for resistance was detected within the subset using completely randomized and replicated controlled climate bioassays with a highly virulent Australian *A. lentis* isolate, FT13037. Genotype IG 207 expressed the lowest percent area of symptomatic tissue and further 12 genotypes demonstrated moderate resistance. Furthermore, IG 207 recorded lowest mean disease score against four other highly aggressive fungal isolates and performed better than the currently used best resistance sources.

A recent study (Singh et al. 2020a) evaluated, under multi-location and multi-season, performances for several agronomic traits and resistance against rust (*Uromyces fabae*), powdery mildew (*Erysiphe trifolii*) and FW (*Fusarium oxysporum* f. sp. *lentis*) under field and controlled screening conditions. Genetic material comprised 96 wild lentil accessions, including samples of all *Lens* species, and two cultivated varieties. Results describe several donor accessions for their introgression against these three biotic stresses, in addition to lentil genetic improvement of important agronomic traits. Donor accessions for disease resistance breeding were found in all wild taxa. Moreover, some of the wild accessions from Syria and Turkey showed resistance against more than one disease indicating a rich diversity of lentil

genetic resources [accessions ILWL230 and ILWL476 of *L. orientalis* (rust and powdery mildew); ILWL9 and ILWL37 of *L. nigricans* (rust and powdery mildew); IG136639 of *L. ervoides* (powdery mildew and FW) and ILWL308 of *L. tomentosus* (rust and FW)]. Further, some stable gene sources were identified: ILWL203 of *L. odemensis* for rust and high pod number; ILWL230, ILWL476 of *L. orientalis* for rust and powdery mildew; ILWL191, ILWL9, and ILWL37 of *L. nigricans* for rust and powdery mildew, IG136639 of *L. ervoides* for powdery mildew and FW, and ILWL308 of *L. tomentosus* for rust and FW. The study has also identified some trait specific accessions, which could also be taken into the consideration while planning distant hybridization in lentil; but some belong to the most distant genepool from the lentil cultigen, such as ILWL18 and ILWL19 of *L. nigricans* promising for high seed yield per plant, or ILWL191, ILWL9, and ILWL37 for resistance, which makes their real use in breeding difficult.

Partial resistance to the parasitic weed *Orobanche crenata* was detected in in a collection of 234 Spanish cultivated accessions under field conditions, scoring a wide range of responses but no complete resistance. A range from complete resistance to susceptibility was found among 23 wild *Lens* accessions. The higher levels of resistance were observed in accessions of *L. ervoides, L. odemensis* and *L. orientalis* (Fernández-Aparicio et al. 2008, 2009).

5.4 Glimpses on Classical Genetics and Traditional Breeding

5.4.1 Breeding Objectives

The main breeding objective in relation to biotic stresses is to introduce genes that confer resistance to pathogens and pests, if possible, durable resistance. The complexity of the selection and breeding process is imposed by the great variety of stressors, which implies a large number of potentially useful qualitative (single-gene resistance) and quantitative genes (oligo- or polygene resistance), and by the convenience of their pyramiding.

The single-gene resistances have both advantages and disadvantages. The advantages, in addition to the fact that dealing with single gene genetics is much simpler than polygenic genetics, are complete protection against the parasite in question, and compatibility with breeding for wide climatic adaptation. The main disadvantage of single-gene or vertical resistance (genetic resistance that is effective at preventing successful attack only by certain races of a pathogen, also called specific) is its temporary nature, since it breaks down to new strains of the parasite. Other disadvantages include a loss of horizontal resistance (resistance that is effective at preventing successful attack by most/all races of a pathogen; also called general) while breeding for vertical resistance, and the fact that single-gene resistance cannot always be found.

Thus, it has appeared impossible to breed for vertical resistance to some species of crop parasites, including many of the insect pests of crops (Robinson1997).

Although theoretical and empirical studies comparing deployment strategies of more than one resistance gene are scarce, the REX Consortium (2016) concluded that that the overall durability of resistance genes may increase by pyramiding their presence in the same plant; and that data also suggests that the pyramiding of disease resistance genes is the most durable strategy. By extension, authors suggested that the combination of disease resistance genes with other practices for pathogen control (pesticides, farming practices) may be a relevant management strategy to slow down the evolution of virulent pathogen genotypes.

5.4.2 Classical Mapping Efforts

The inheritance of morphological and agronomical traits in lentil was first described at the end of 70's and early 80's (Pérez de la Vega et al. 2011). Several traits such as seed coat color, epicotyl and flower color, and pod dehiscence, were found to be controlled monogenically and thus appropriate to be used as morphological markers. Linkage analysis in lentil started in the 80's and was initially based on morphological and isozyme markers. However, the number of morphological and isozyme markers in lentil is relatively low, which made the classic genetic maps based on them of little use for breeding. The first genetic linkage analysis based on morphological and isozyme markers in lentil was reported by Zamir and Ladizinsky (1984). Muehlbauer et al. (1989) described the allozyme polymorphisms for 18 loci and the linkage relationships among them and with four genes controlling morphological traits. The linkage analysis resulted in six linkage groups (LGs), which contained 14 of the loci analyzed. This work also contributed to the first evidence of shared synteny between *Lens* and *Pisum* since several of the LGs were conserved between both species. Tahir and Muehlbauer (1994) were the first to use recombinant inbred lines (RILs) for mapping lentil markers. Kumar et al. (2015), Ates et al. (2018), and Gupta et al. (2019) have recently summarized the mapping efforts in lentil using from isozyme and morphological markers to molecular markers including random amplified polymorphic DNA (RAPD), SSR, inter simple sequence repeat (ISSR), single nucleotide polymorphism (SNP), etc., and from 1994 to 2017. The more recent list by Gupta et al. (2019) includes 20 different maps (23 references) generated from different segregant populations obtained from intra- and interspecific crosses. Likewise, this publication lists recombinant inbred line (RIL) populations obtained in the ICARDA, the National Bureau of Plant Genetic Resources, India, and the Indians Institute for Pulse Research, India. Further, some more RIL populations have been described (Suvorova and Ikonnikov 2014; Bhadauria et al. 2017a; Polanco et al. 2019).

5.4.3 Classical Breeding Achievements

According to the recent review by Gupta et al. (2020) breeding methods for incorporation of breeding traits employed in lentil majorly included pure line selection, hybridization, backcross, bulk, pedigree and single seed descent methods. As a result of these methodologies, a total of 146 cultivars have been released until 2017 across major lentil-producing countries with targeted traits. Pure line selection was extensively used to release cultivars with adaptability to wider areas and superior yield performance and disease resistance for Ascochyta blight (AB), rust and Fusarium wilt (FW). Certainly, in relation to biotic stresses, resistance to ascochyta is the main breeding targets for the newly released cultivars in all geographical areas of the world, followed by resistance to rust, stemphylium and fusarium. Cross-breeding is the widely chosen method in the recent past by breeders particularly to introgress special traits from exotic or other popular germplasm to the locally adapted cultivars, while single seed descent has often been used to produce RILs for use in constructing linkage maps and identification of quantitative trait loci (QTLs) controlling traits of interest such as resistance to AB, anthracnose and FW. Mutation techniques have been used in lentil as a complementary breeding strategy to introduce a desirable trait which is absent in the available germplasm. Several cultivars with different traits of interest have been developed and released worldwide using irradiation and ethyl methanesulfonate as a source of mutagens (Gupta et al. 2020). If we stick to biotic stresses, most of cultivars developed through mutation breeding registered in the Indian subcontinent and outside it have been improved for resistance to diseases such as to FW, AB, botrytis, rust and anthracnose (Laskar et al. 2019).

5.4.4 Limitations of Classical Endeavors and Utility of Molecular Mapping

Marker-assisted plant breeding based on the use of morphological markers had a slow development from the pioneering work of Sax (1923) due first to the limited number of morphological markers that could be simultaneously and independently genotyped. Additional drawbacks arose from epistatic effects among genes controlling related, an even apparently unrelated, traits, or due to the lack of observable markers during the first development stages hindering early selection. Likewise, the estimates of the recombination fraction were often limited by segregations in repulsion phase. The beginning of the use of isozymes (codominant markers) in plant genetics in the 70's of the twentieth century partially solved these problems, but again the number of isozyme markers that can be analyzed simultaneously was too low to be able to accurately locate most of the genes of interest, and even more so if they were QTLs. The incorporation of the molecular markers was a great step towards the solution of obtaining sufficiently saturated genetic maps and flanking markers of the locus of interest. The number of markers that could be studied simultaneously increased

dramatically and, above all, the use of codominant markers, such as SSRs and SNPs, solved the disadvantages of the dominant ones in estimating genetic linkage distances and confidence intervals. Isozyme and molecular markers have the additional advantage that they can be considered selectively neutral. Thus molecular markers allowed analyzing large segregating populations to attain much greater marker saturation with neutral phenotypic effects. However, the precision with which QTLs with minor effects are often located was still poor. The high-throughput sequencing techniques have contributed a definitive advance since marker numbers have gone from hundreds of codominant markers to thousands, fundamentally SNPs, which allows obtaining saturated maps with a good coverage of the entire genome, that in turn allows greater precision in locating QTLs and easily finding markers flanking the locus of interest for their use in marker-assisted selection (MAS).

The molecular information gained by the 'omics' techniques removes, although only partly, some of the limitations of selection on phenotype, by allowing selection at the genotype level, which results in more accurate, faster, and cheaper selection. It also provides a high number of markers for MAS. Ultimately, the use of MAS will be determined by the economic benefit relative to conventional selection. Further applications of MAS require the redesign of breeding strategies and their integration with other technologies, such as higher-resolution genetic maps and high-throughput genotyping technologies (Dekkers and Hospital 2002).

5.5 Brief on Diversity Analysis

5.5.1 Phenotype-Based Diversity Analysis

Numerous works have been published on the phenotyping of lentil germplasm collections in relation to the responses to the main diseases and pests and in the search for resistance genes. Many of these citations can be found in previous reviews, such as those by Pérez de la Vega et al. (2011), Singh et al. (2018) and Gupta et al. (2019), Malhotra et al. (2019), and Gupta et al. (2020). Phenotyping for response to several pest species has been carried out by El-Bouhssini et al. (2008), Kumari et al. (2007), Gore et al. (2016), and Laserna-Ruiz et al. (2012). Some sources for resistance to several viruses in lentil germplasm were reported by Makkouk and Kumari (2009). Resistance sources to broomrape weed were searched in cultivates and wild materials by Fernández-Aparicio et al. (2008, 2009). Recent phenotyping screenings for AB has been carried out by Dadu et al. (2018, 2019) and Singh et al. (2020a). This last publication describes the resistance against rust, powdery mildew and FW under field and controlled screening conditions. Podder et al. (2013) carried out a screening of wild and cultivated *Lens* germplasm for resistance to SB.

Some advances in phenotyping for resistance can help in accelerating the search for resistance and in obtaining new varieties. Lulsdorf and Banniza (2018) described a rapid generation cycling technique to cut the selection period in half. The technique

was tested on an F_2 population derived from a *L. culinaris* × *L. ervoides* cross in combination with a liable technique for the screening to aphanomyces root rot (ARR). Phenotyping of an F_2 population of more than 1,200 plants resulted in scores ranging from 2.4 to 4.0 on a scale from zero to five. Plants with scores lower than 4.0 were selected for advancement for five generations using a modified single-seed descent method and optimum growing conditions. Phenotyping of the F_7 population resulted in scores ranging from 1.4 to 4.0. Marzougui et al. (2019, 2020) applied phenomics technologies to evaluate ARR resistance in 547 lentil accessions and lines using Red–Green–Blue images of roots. Models were able to classify three disease categories with an accuracy of up to 0.91. The authors concluded that the image-based phenotyping approaches can help plant breeders to objectively quantify ARR resistance and reduce the subjectivity in selecting potential genotypes. The use of such technologies to the evaluation of other biotic stresses would certainly be of great help in phenotyping and plant breeding.

5.5.2 Extent of Genetic Diversity

As it was mentioned in Sect. 1.1, genomic studies suggest the existence of four gene pools in relation to cultivated lentils. The primary gene pool would include the taxa *orientalis* and *tomentosus*, the secondary *lamottei* and *odemensis*, and finally *L. ervoides* and *L. nigricans* would be the tertiary and quaternary gene pools, respectively (Wong et al. 2015), although there are different assignations of species to gene pools (Ladizinsky and Abbo 2015). Breeding lines and recombinant inbred lines have been obtained and described in the scientific literature at least from hybrids with *orientalis*, *odemensis* and *ervoides* (Suvorova and Ikonnikov 2014; Bhadauria et al. 2017a; Polanco et al. 2019).

A recent analysis (Khazaei et al. 2016) of the primary germplasm has indicated that cultivated lentils can be grouped into three agro-ecological zones. The study was based on the use of 1,194 SNP markers which span the lentil genome, analyzing 352 accessions from 54 countries obtained from three large germplasm collections. Accessions were categorized into three major groups, namely, South Asia (sub-tropical savannah), Mediterranean, and Northern temperate, which prominently reflected geographical origin (world's agro-ecological zones). The three clusters complemented the origins, pedigrees, and breeding histories of the germplasm. The study revealed that considerable genetic diversity for breeding can still be found in this primary pool, but that the South Asia and Canadian germplasms had narrow genetic diversity.

Pavan et al. (2019) analyzed a collection of lentil accession covering one of the first areas of distribution of this crop after domestication, the Mediterranean Basin countries, which holds large part of lentil biodiversity. They analyzed 184 *L. culinaris* accessions by high-throughput genotyping by sequencing of a Mediterranean collection. On the basis of 6,693 single nucleotide polymorphisms, the analysis of no redundant genotypes highlighted the occurrence of five highly differentiated

genetic clusters, related to geographic patterns and phenotypic traits, indicating that post-domestication routes introducing cultivation in Mediterranean countries and selection were major forces shaping lentil population structure. The identification of distinctive alleles across clusters suggested the possibility to set up molecular keys for the assignment of lentil germplasm to specific genetic groups, helping in lentil conservation genetics and breeding.

Dissanayake et al. (2020) carried out a wide analysis of the genetic variation and the relationships among the *Lens* taxa using a worldwide sample of 467 wild and cultivated accessions collected from 10 diverse geographical regions and 28 countries. *L. nigricans* exhibited the greatest allelic differentiation compared to all other species or subspecies, indicating that this species is the most distantly related to *L. culinaris*. Genetic distance matrices revealed a comparable level of variation within the gene pools of *L. culinaris, L. ervoides*, and *L. nigricans*. This work will be certainly a valuable source for the use of the wild germplasm in lentil breeding and gene introgression.

Liber et al. (2021) combined GBS of 190 lentil accessions (67 wild and 123 domesticated) from the Old World with archeological information to analyze the evolutionary history, domestication, and diffusion of lentils. GBS led to the discovery of 87,647 SNPs, which allowed inferring the phylogeny of genus *Lens*. The only gene flow detected was between cultivated varieties and their progenitor (*L. culinaris* subsp. *orientalis*) albeit at very low levels. Nevertheless, a few putative hybrids or naturalized cultivars were identified. Within cultivated lentil, three geographic groups were detected.

5.6 Association Mapping Studies

In the search for genetic variants linked to phenotypic differences, association mapping (AM) exploits long-term historic recombination in natural populations. Population based AM employs a sample of individuals from the germplasm collections or a natural population. With more accumulated recombination events it is considered to be more accurate than traditional mapping based on biparental crosses. The resolution of the AM depends on the extent of linkage disequilibrium (LD) across the genome, the number of accessions considered, and the number and distribution of markers employed. Lentils display extensive LD (Lombardi et al. 2014; Singh et al. 2017a; Kumar et al. 2019a; Ma et al. 2020), likely due to their high degree of self-pollination and the narrow genetic base of the breeding material, thus shirking the need for a large number of markers. The drawback of a wide LD is a lower resolution because a significant marker-trait association does not necessarily imply that a marker is in close proximity to the gene. The future release of a reference-quality genome assembly will allow quantifying the LD decay over the physical distance, and thus estimating the number of markers that are required for a particular scrutiny of the genome through AM.

The collection of cultivars, landraces and wild genotypes has been extensively reviewed and characterized (Coyneand McGee 2013; Lombardi et al. 2014; Laskar et al. 2019; Dissanayake et al. 2020; see also Sect. 5.3). Currently the whole cultivar collection amounts to a total of 43,214 accessions of the genus *Lens*, ICARDA being the institution that holds the most (24%). While wild accessions are genetically diverse, there is a reduced gene pool in the cultivated material that dates back to the bottleneck associated with domestication (Lombardi et al. 2014; Dissanayake et al. 2020). In order to introduce new variability into cultivars, hybridization with wild genotypes and with close species has been proposed (Singh et al. 2014).

Because of their large number and scattered distribution throughout genomes, SNPs are the most-used molecular markers for AM studies. Two SNP-based high-throughput approaches have been utilized in lentil research: SNP chips (microarrays) and genotyping-by-sequencing (GBS). Within the first, the Illumina® GoldenGate® assay has been the chosen genotyping platform for several studies (Gujaria-Verma et al. 2014; Lombardi et al. 2014), but it has been superseded by other microarray-based technologies and is now discontinued. The chip was able to interrogate up to 1,536 SNPs simultaneously. More modern microarrays, such as the customizable Infinium iSelect high definition (HD) and the Infinium iSelect high-throughput screening (HTS) custom genotyping BeadChips, are expected to grow in popularity as we gain more knowledge of the lentil genome and more trait-linked SNPs are discovered. Lentil researchers can now design a custom genotyping panel that supports up to 700 k custom targets among SNPs, indels, and copy number variations. The Infinium iSelect can be deployed in two options: either the HD with 3,072 to 90,000 custom markers, or HTS, able to screen between 90,001 and 700,000 markers. Creation of these custom assays enables focused, high-throughput genotyping applications tailored to specific project needs in a cost-effective manner.

Access to a genome assembly has facilitated GBS considerably, and, at the same time, has made GWA studies affordable. GBS (Elshire et al. 2011) is a high-density genotyping approach extensively used in breeding and genetics because of its low cost, high number and uniform distribution of SNP markers, and the capacity to simultaneously perform polymorphism discovery and genotyping. It has been proven effective in crops with large and repetitive genomes (Gutierrez-Gonzalez et al. 2019). In lentils, using GBS markers in a genome-wide association study (GWAS), Ma et al. (2020) identified 38 QTLs and 15 candidate genes that could be associated with aphanomyces root rot (ARR). Two of them, ABC transporter A family protein (ABCA), and pectin esterase (PE) were found differentially expressed at the early stages of infection, likely involved in the plant-defense mechanism against ARR. We expect GBS approaches to play increasing roles in highlighting plant defense mechanisms as more polished genome assemblies are being released.

5.7 Molecular Mapping of Resistance Genes and QTLs

Since the first efforts in lentil breeding, the main goal has been similar in all countries: to obtain larger and more stable seed yields (Pérez de la Vega et al. 2011). In order to reach this objective, the development of resistant cultivars to pathogens plays a crucial role in breeding programs, and the knowledge of the genetic basis of the resistance helps to design faster and more efficient breeding approaches. Traditionally, the genes involved in the resistance were studied by crossing two parental lines differing in the response to the pathogen and evaluating the segregating descendant population. The development of lentil RIL populations provided permanent materials that can be shared by many research groups, in which new additional markers and characteristics can be added along the time. Additionally, another advantage of RILs is that because the lines have gone through several rounds of meiosis before homozygosity is reached, the degree of recombination is higher compared to F_2 populations, and consequently, RIL populations show a higher resolution than maps generated from F_2 populations, and the map positions of even tightly linked markers can be determined (Schneider 2005).

Despite the advantages of RIL populations, the gained results may be relevant only for the studied material, and the validation in new germplasm is laborious. An improvement in the detection of relevant accessions and genes related with interesting breeding traits is the development of immortalized segregant populations obtained from the crossing of multiple parents (i.e. MAGIC populations). Some populations of this kind are being developed in ICARDA at the present time (Kumar et al. 2021). In addition, the availability of a high number of markers, in special after the genomic and transcriptomic studies published in lentil, has made possible to start some GWAS, such as the published by Kumar et al. (2018a) or Khazaei et al. (2018), but there are not published information on disease resistances so far.

Several diseases in lentil are due to fungus infections, such as AB, SB, rust, FW, anthracnose, ARR, collar rot, molds (*Botrytis cinerea*, *B. fabae* and *Sclerotinia sclerotiorum*). However, the genetic basis of the response to many of these pathogens has not been analyzed in a formal research. In the following paragraphs a review of the main data available to date is presented.

5.7.1 Genetics of Lentil Resistance to Ascochyta Blight

Ascochyta blight (AB) is one of the main lentil diseases in most lentil growing countries. This disease, caused by the fungus *Ascochyta lentis* (syn. *A. fabae* f. sp. *lentis*; teleomorph *Didymella lentis*), affects all above ground parts of the plant and is characterized by necrotic lesions, which on susceptible cultivars, in favorable conditions, can lead to breakage of the stems and severe yield reduction. Seed quality may also be reduced through seed discoloration or retardation of seed development. AB can be controlled by chemicals, but besides the environmental problems related

with the use of fungicides, the development of resistant cultivars is considered a more efficient and sustainable approach (Davidson and Kimber 2007). *Ascochyta lentis* is a host-specific pathogen (Peever 2007) and considered as a necrotrophic fungus, although a short biotrophic period cannot be completely excluded (Tivoli and Banniza 2007; Sari et al. 2017). The pathogenicity of this type of fungus and the resistance of the plant could be related to the production of specific fungal toxins and plant receptors or detoxifying molecules, and Kim et al. (2016) have described the presence of a set metabolites only found in *A. lentis*. The recent publication of the *A. lentis* genome sequence by Lee et al. (2021) will provide a powerful tool in order to identify the candidate genes involved in the pathogenicity of this fungus.

In lentil, the genetic control of the resistance response to the fungus were firstly studied in a qualitative way, by crossing susceptible and resistant cultivars obtaining various results, mainly one or two genes, dominant and/or recessive (see Pérez de la Vega et al. 2011; Sudheesh et al. 2016a; Rodda et al. 2017 for reviews). The different results may be due to the different genotypes used; however, the differences in screening methods or *Ascochyta* isolates employed cannot be ruled out. These initial studies allowed the identification of some major genes that have been used in the breeding programs, such as those found in the cultivar Indianhead, or in the ICARDA lines ILL5588 or ILL7537, although the molecular mechanisms for the resistance provided for these genes is still unknown. In the last years, pathogen isolates capable of overcoming the resistance provided for the major genes have appeared in Australia (Rodda et al. 2017), making a priority the identification of new genes and sources of resistance.

Although the qualitative classification of the resistance to AB provided the detection of some major genes, most of the results did not show a clear Mendelian segregation, and consequently, a quantitative analysis of the resistance response seems more appropriate to describe this trait (Rubeena et al. 2006; Gupta et al. 2012). QTL analyses on the response to *Ascochyta* in lentil using high-density maps based on gene SNPs have been carried out, allowing the identification of several QTLs (reviewed by Rodda et al. 2017), with magnitudes varying from 3 to 89% of the phenotypic variance evidenced, although it is common to find values between 20 and 50%. An important drawback of these studies is the lack of common markers between the genetic maps, and consequently the difficulty to establish a comparable nomenclature for the linkage groups (LG) in order to determine the QTL locations. Despite of that, some limited relationships have been done based in a few markers: for instance, the QTL named *AB_NF1* in LG6 in the study of Sudheesh et al. (2016a) is comparable in position to *QTL5* in LG1 of Rubeena et al. (2006), to *QTL1* in LG1 of Gupta et al. (2012), and to the three closely linked *AS-QTLs* detected in LG6 in Polanco et al. (2019) in an interspecific cross between *L. culinaris* and *L. odemensis*.

The use of QTL knowledge in breeding programs requires of the validation of the markers associated with the QTL in a diversity panel of genotypes. So far, only the allelic identity of the QTL *AB_IH1* (Sudheesh et al. 2016a) was found to predict the resistance response in more than 85% of the diversity panel, mainly composed by

Australian lentil germplasm. This relationship was not so conserved in the international germplasm panel, which suggests that there are new resistance genes or alleles to be detected.

All the lentil genotypes which showed resistance to AB so far known show a partial resistance or it is surpassed by new and more aggressive isolates (Dadu et al. 2017, 2018), and genetic and genomic studies point to that there are several response mechanisms to this pathogen. Genetic studies suggest that AB resistance genes in several partially-resistant lentil lines are nonallelic (Sari et al. 2017). Furthermore, these authors found that the partially resistant genotypes CDC Robin and 964a-46 differed in the timing and the magnitude of salicylic acid (SA) and jasmonic acid (JA) signaling pathway activation. The SA signaling pathway was only triggered in 964a-46, whereas the JA pathway was triggered in both partially resistant genotypes. The expression of JA-associated genes was lower in 964a-46 than in CDC Robin. These observations corroborate the existence of diverse AB resistance mechanisms in lentil genotypes carrying different *R*-genes (Sari et al. 2017; Khorramdelazad et al. 2018; Garcia-Garcia et al. 2020).

From a practical point of view in breeding programs, it is interesting to remark that some regions in which QTLs conferring resistance to AB are located also contains genes for resistance to other pathogens. For instance, a QTL that explained 41% of the variation in the reaction to AB found in the LG6 (Tullu et al. 2006) showed linkage to the *LCt2* gene for resistance to anthracnose (*Colletotrichum lentis*).

5.7.2 Stemphylium Blight Resistance

Stemphylium blight (SB) has recently emerged as a new important fungal defoliating disease in lentils. It is caused by the necrotrophic Ascomycete, *Stemphylium botryosum* Walr. (Pleosporales, Pleosporaceae) (teleomorph: *Pleospora herbarum* (Fr) Rab.), and it was firstly described in 1986 in Bangladesh, but the reports on yield losses caused by this disease have been increasing in the recent years all around the world (Das et al. 2019). The host range of *S. botryosum* is wide and includes a large number of ornamentals, horticultural and crops, including lentil, pea, tomato, alfalfa, lettuce or onion (Das et al. 2019). Usually the pathogen infects the lentil plants in the first stages of pod setting, when the spores germinate on the leaflet surfaces and the hyphae penetrate through the stomata or directly through the epidermis (Pierre and Millar 1965).

The first studies on the genetic basis of the resistance were done by Saha et al. (2010b), detecting several QTLs in two different years, although only one was significant in both, explaining between a 25% and a 46% of the phenotypic variation. The quite different results obtained in posterior crosses with *L. culinaris* genotypes made the inheritance of this resistance to be in an ambiguous stage (Das et al. 2019).

Deeper information on SB resistance is available from the wild species *Lens ervoides*. Because few sources of resistance were found in *L. culinaris*, a screening was carried out in wild genotypes, and *L. ervoides* was found to show high levels of

resistance, at higher frequencies than the other species (Podder et al. 2013). In the RIL population LR-66 derived from the cross between two *L. ervoides* accessions (L01-827A and IG 72815), Bhadauria et al. (2017a) detected three QTLs in the linkage groups LG2 and LG3 that together explained the 40.5% of the phenotypic variance. Because the *L. ervoides* genetic map in this experiment could be related with the *L. culinaris* genetic map of reference, and a high level of collinearity between the two genomes, especially in the identified QTL regions, the *L. culinaris* genome can be utilized to identify the candidate genes. Cao et al. (2019) analyzed two transgressive RILs derived from the *L. ervoides* RIL population LR-66 above mentioned in a search for candidate resistance genes against SB using transcriptome sequencing. In this work, three of the genes located in the QTLs have been chosen as the more promising candidate genes because of the expression changes showed after the infection in the resistant and susceptible RILs.

Additional information comes from the research of Adobor et al. (2020) with an interspecific RIL population (LR-26) developed from a cross between the moderately resistant parent *L. culinaris* cv. 'Eston' and the resistant parent *L. ervoides* accession IG 72,815. The plant resistance to SB was tested under controlled conditions and under field conditions. Although the distribution of disease severity scores for all RILs indicated a polygenic inheritance of SB resistance in the population, no resistant transgressive segregants were observed. Across all environments, 14 RILs consistently had resistance levels similar to the resistant parent IG 72,815, which makes them a promising material to be included in future breeding programs.

*5.7.3 Rust (*Uromyces viciae-fabae*) Resistance*

Rust disease in lentil is due to the infection by the biotrophic fungus *Uromyces viciae-fabae* (Pers.) J. Schröt. This pathogen is widespread and attacks the aerial parts of the plants, producing defoliation and the plant death. Although *U. viciae-fabae* infects several legume genera such as *Vicia, Lens, Pisum* or *Lathyrus*, the pathology studies have identified three specialized groups named *U. viciae-fabae* ex *V. faba* which infects only faba bean, *U. viciae-fabae* ex *V. sativa* which infects other species of *Vicia* and *U. viciae-fabae* ex *L. culinaris* which infects *L. culinaris* only (Rubiales et al. 2013b).

Lentil rust resistance seems to be under a simple Mendelian control. The results depend on the specific cross, but generally the segregation of a single gene explained the data, being the resistance dominant over the susceptibility; however, controls based on a recessive gene or duplicate dominant genes have also been founded (Chahota et al. 2002; Mishra et al. 2007, 2008; Saha et al. 2010a; Negussie and Pretorious 2012; Mekonnen et al. 2014; Dikshit et al. 2016; Singh et al. 2021). The names proposed for these genes are *Urf1, Urf2* and *urf3* (Sharma 2009). The relatively simple genetic control of the resistance has allowed the development of some molecular markers of potential utility in breeding programs, which will be described in the Sect. 5.8.

The two types of resistance (prehaustorial and posthaustorial) reported in the lentil germplasm suggest the existence of two different genetic mechanisms for the response to rust (Rubiales et al. 2013a). Prehaustorial resistance is usually connected to a non-host resistance and is generally based in a polygenic control. This kind of resistanceis expected to be more durable than the posthaustorial one, usually controlled by single genes. But unfortunately, so far no research on the genetic control of this type of resistance has been published.

5.7.4 *Wilt (Fusarium oxysporum f. sp. lentis) Resistance*

The wilt disease is one of the most important biotic stresses affecting the stability of production in lentil. It is caused by *Fusarium oxysporum*, a filamentous ascomycete fungus that produces spores protected by thick walls, making them able to survive in the soil by long periods, reason why is usually considered as saprophytic. When some nutrients are available, such as root exudates, the spores germinate and the hyphae grow and penetrate in the plant roots, invading the inter- and intra-cellular spaces. While the plant is alive, the fungus remains strictly limited to the xylem tissues and a few surrounding cells. After the host plant is killed by the pathogen, the fungus can invade the parenchymatous tissue, sporulate on the plant surface and release spores (Pouralibaba 2017). *F. oxysporum* infects a large number of plant species, and some strains have been adapted to colonize specific hosts, giving the named *formae speciales* (ff. spp.). More specifically, lentil wilt is caused by *Fusarium oxysporum* Schlecht. Emend Snyder and Hansen f. sp. *lentis* Vasudeva and Srinivasan.

The genetic studies on FW in lentil point to a simple control by a low number of genes, usually dominant (Choudhary and Kumar 2016). Thus, Kamboj et al. (1990) identified in total five dominant independently segregating resistance genes. More recently, the segregations usually detected only one dominant gene, named *Fw* (Eujayl et al. 1998; Hamwieh et al. 2005). This locus was mapped in the LG6 (Hamwieh et al. 2005) of their genetic map, linked to some SSR markers that seem to be located in the pseudochromosome 4 of the lentil genome v1.2.

New resistance genes of utility in lentil breeding have been detected in some transgressive segregants obtained for crosses between *L. culinaris* and *L. ervoides* (Singh et al. 2017c), although their characterization has not been published so far.

5.7.5 *Anthracnose (*Colletotrichum lentis*) Resistance*

Anthracnose is a disease attributed to the hemibiotrophic fungus originally identified as *Colletotrichum truncatum* [(Schwein.) Andrus & W. D. Moore] but since 2014 is attributed to the new species *Colletotrichum lentis* Damm, sp. nov. MycoBankMB809921 (Damm et al. 2014). When the pathogen infects the plant, initially it shows a biotrophic and symptomless stage, and afterwards changes to

a necrotrophic phase causing the death of plant cells. This switch seems to be the pathogen adaptive response to the defense mechanism of the plant, based on the cell death (Bhadauria et al. 2013). This disease has been described in many countries producing minor losses in production, however in western Canada has become the most important foliar fungal disease (Gela et al. 2020). There has been identified two pathogenic races of the fungus, race 0 and race 1 (Banniza et al. 2018), and the genetic resistance to anthracnose depends on the *C. lentis* race. While a partial resistance to race 1 is quite frequent in lentil, and it has been effectively transferred to elite cultivars, resistance to the highly virulent race 0 has not been identified. To date, the only sources of high levels of resistance to race 0 seems to be restricted to wild lentil species, especially *L. ervoides* (Gela et al. 2020).

The genetic resistance to race 1 appears to be under a single dominant gene (Tullu et al. 2003, 2006; Tar'an et al. 2003), although the different levels of resistance that has been detected in some crosses points to the existence of additional genes. Thus, Buchwaldt et al. (2013) explained their results as the interaction among two recessive genes, *ctr1* and *ctr2*, and three closely linked dominant genes, *CtR3*, *CtR4* and *CtR5*.

The genetics of the resistance to race 0 and race 1 has been analyzed in the same RIL population LR-66 derived of the cross between two *L. ervoides* accessions (Bhadauria et al. 2017a) mentioned in the SB resistance section. The results showed five QTLs with a significant association with resistance to race 0 and six QTLs to race 1 resistance. Three QTL for resistance to *C. lentis* races 1 and 0 co-localized, one in LG3 and two LG5, collectively explaining 47.58% and 54.82% of the variance in resistance response to *C. lentis* races 0 and 1, respectively. This suggests that a large proportion of the resistance to both races of *C. lentis* is regulated by genes at the same loci. The joint analysis of transcriptome studies and QTL mapping has allowed the identification of two genes as main candidates to be responsible of the resistance response, *Lc23518* (in LG5) coding for an LRR receptor-like kinase protein, and *Lc09295* (in LG2) coding for a MYB transcription factor, although these genes need further evaluation (Bawa 2020).

Recently, Gela et al. (2021) have analyzed a RIL population obtained from the cross between *L. culinaris* Eston and *L. ervoides* IG 72,815 to test the resistance to race 0 and 1 in an interspecific genomic background. Two QTLs conferring resistance to both races with a significant effect were consistently detected in the experiments, one in the LG3 that explained a 20.1–30.2% of the phenotypic variance, and the other in the LG7, explaining an 8.3–18.4%. The QTL in LG3 probably coincides with that found by Bhadauria et al. (2017a) since they map in the same genomic region. Bhadauria et al. (2017a) also detected a QTL in LG7, although in their research it was associated only with resistance to race 0. The co-localization of QTLs for resistance to both races detected in these studies suggests that the same genes are controlling some resistance responses common to both races or, alternatively, the race-specific defense genes to anthracnose are closely linked, according to the genomic distribution found in *Phaseolus* by Murube et al. (2019).The analysis by Gela et al. (2021) of the CDC Redberry genomic regions (assembly v.2.0; Ramsay et al. 2019) harboring the QTLs showed at least 22 genes in LG3 and 26 genes in

LG7 annotated as disease resistance/defense-related genes, supporting the clustering of resistance genes to different races, and making them candidates for new studies.

5.7.6 *Root Rot (*Aphanomyces euteiches*) Resistance*

Root rot (ARR) disease is caused by the oomycete *Aphanomyces euteiches* Drechs. This soil-borne pathogen has a wide host range within Fabaceae, including pea, lentil, faba bean and alfalfa (Gaulin et al. 2007). Although this pathogen was well known because is considered one of the most frequent in pea fields, in lentil it was not described as the cause of the root rot until 2008 and 2012 in U.S.A. and Canada, respectively. Nowadays is considered as a widespread pathogen in the American fields (Ma et al. 2020). Germplasm analyses showed that none of the lentil cultivars are resistant, which constitutes a threat because of the possible production losses.

In order to analyze the genetic basis of the resistance, a combination of classical and image-based phenotypic tools and a deep QTL mapping study using 2,880 SNPs has been recently carried out by Ma et al. (2020) in a RIL population. This RIL was obtained from the cross between a breeding line with a high level of partial resistance and a susceptible one. The results point to a classical polygenic inheritance of the resistance, because a high number of QTLs (19) were detected located on all the chromosomes except pseudochromosome 1, each QTL explaining a 5–12% of the phenotypic variance. It is worth noting than in this same research a complementary GWA study was undertaken, detecting 38 QTLs in a sample of 326 accessions from 60 countries on four continents (Asia, Europe, America, Africa). Notably, very limited co-localizations occurred among QTL detected in the RIL population and the association mapping population. As Ma et al. (2020) state "this highlight the importance of integrating QTL mapping and association mapping for a comprehensive assessment of genetics of the resistances". Despite the complexity of the genetic basis of the resistance to *A. euteiches*, two candidate genes have been identified combining these results with transcriptomic analysis (Ma et al. 2020).

5.8 Marker-Assisted Breeding for Biotic Stress Resistance

In recent years several reviews on the status of marker-assisted breeding in lentil have been published (Kumar et al. 2019b; Rana et al. 2019). Theoretically, MAS in breeding for disease resistance has a very important advantage over traditional methods because the phenotyping with artificial infections is influenced by the specific methodology used to measure the level of resistance and some subjective classification cannot be completely ruled out. MAS overcomes these problems associated with the selection based on the response to the pathogen, and additionally allows the selection in the very early stages of the development. Besides, MAS enables the pyramiding of several genes for the same or different resistances in an

elite cultivar and speeds up the breeding programs. In order to get these advantages, it is essential to develop locus-specific and highly reproducible markers that show a tight linkage (i.e., genetic distance <1 cM) with the genes controlling the character of interest. Frequently, the markers obtained do not accomplish these requirements. Many of the markers described in the early literature including RAPDs, amplified fragment length polymorphism (AFLP) or ISSRs, although contributed to a significant improvement in the QTLs mapped, were not easily transferred from one study to another. The increase in the development and use of SSR and SNP markers has allowed the identification of candidate genes in lentil for different resistances; nevertheless, very few have been progressed to the MAS level in lentil breeding (Rana et al. 2019).

Although not optimal and with limitations, there have been described markers that could be of practical interest for MAS, at least in some genetic backgrounds. For instance, the work of Shudheesh et al. (2016) identified three markers for *Ascochyta lentis* resistance relevant for de Australian breeding program, and one of those (AB_IH1) is also predictive in more than 85% of the germplasm tested. These markers also allow the selection of the two major resistance genes found in the cultivars Indianhead and Northfield (ILL5588).

A simple genetic basis of the resistance facilitates the use of markers in the breeding programs. For instance, the marker ME4XR16c is tightly linked to the major gene responsible of the SB resistance (Saha et al. 2010b), although there are not reports about the validation of the marker in different genetic backgrounds. The marker SSR59-2B (Hamwieh et al. 2005), closely linked to the *Fw* (Fusarium wilt), or the markers F7XEM4a (Saha et al. 2010a), SSR GLLC106 (Mekonnen et al. 2014), and SSR GLLC527 (Dikshit et al. 2016), linked to genes conferring rust resistance, are in the same stage. For this last disease, two markers (LcSSR440 and LcSSR606) flanking the resistance gene have recently been validated in a small set of resistant and susceptible genotypes (Singh et al. 2021).

When the resistance genes are located in different chromosomes or no common molecular markers are available, the pyramiding must involve a simultaneous selection for them. A favorable characteristic in lentil breeding programs is the linkage among some resistance genes for different pathogens, which facilitates the pyramiding of these traits. For instance, Tar'an et al (2003) obtained resistant lines to AB and anthracnose with a 55% efficiency using three markers, two linked to alleles conferring resistance to ascochyta ($RB18_{680}$, $UBC227_{1290}$) and one to anthracnose ($OPO6_{1250}$). When the markers were used in selecting only one resistance, the efficiency was higher than 80%. Tullu et al. (2006) identified a RAPD marker ($OP\text{-}P4_{400}$) linked to the major resistance gene to AB*AbR1* and to the *LCt2* responsible for resistance to anthracnose.

The development of high-density genetic maps based on genic markers obtained from transcriptomic studies provides a high number of useful markers for different traits, including resistances to pathogens. A clear example can be found in Polanco et al. (2019), in which several markers for morphological or agronomic traits are described, besides markers for QTLs related with AB resistance. It is clear that the integration of data from high-density linkage maps and the information available for

the lentil genome will speed the number of genic markers with a real utility in MAS programs.

A different approach for MAS is the named genomic selection, in which the genotypes of a high number of markers covering the genome are used to predict the final phenotype by means of mathematical models. In this way, it is supposed that all QTLs for a trait are detected. Recently, some initial studies on the genomic selection applicability in lentil breeding programs have been done (Haile et al. 2020); although no resistance traits have been analyzed.

5.9 Genomics-Aided Breeding for Biotic Stress Resistance

Recent advances in genomics have furthered research on plant resistance to pathogens. Genome-wide massive tools have come along to complement traditional breeding based on genetic linkage maps, expressed sequence tag (EST) libraries, gene-based markers, and comparative genomics (Rodda et al. 2017). The first release of the genome assembly, CDC Redberry v1.2 (Ramsay et al. 2016), was a significant breakthrough for lentil's genomics-aided breeding. It consisted of 7 pseudochromosomes and approximately 2.7 Gb of assembled sequence. Although a big leap from previous lentil pre-genomic era, this first assembly covers barely two thirds of the predicted size and displays high levels of fragmentation. A new improved draft, v2.0, is available upon request at https://knowpulse.usask.ca/ (Ramsay et al. 2019). The assembly has over 3.7 Gb, close to the expected lentil genome size of about 4 Gb. Currently, the use of next-generation sequencing (NGS) technology in lentil breeding programs is not widespread compared to other crops (Kumar and Gupta 2020). As improved assemblies are coming to light, researches will be able to tackle genome-wide approaches.

5.9.1 Transcriptome Analyses

Until a reference-quality genome sequence becomes available, de novo transcriptome assemblies are strategic in marker discovery and transcript profiling (Kaur et al. 2011; Verma et al. 2013; Sudheesh et al. 2016b; Gutierrez-Gonzalez and Garvin 2017). They have also proven to be an effective tool to unravel plant-pathogen interactions. Using RNA-seq, Khorramdelazad et al. (2018) compared AB resistant and susceptible lentil genotypes at 2, 6, and 24 h post-inoculation, with a focus on studying the physiology of the interaction between lentil and *A. lentis*. They found genotype- and time-dependent differential expression and identified genes with putative roles in primary, secondary and tertiary defense responses. Among these, there were genes coding for transcription factors (TFs), fungal elicitors' recognition, early signaling, structural and biochemical responses, hypersensitive reaction, and cell death and systemic acquired resistance.

Recently, Garcia-Garcia et al. (2020) were able to highlight the pathways that are most affected following *A. lentis* infection by using massive analysis of cDNA ends (MACE). The precise plant-pathogen recognition mechanism is not well understood for *A. lentis*. Nevertheless, some common patterns that are frequently seen after infection may give researchers a hint. For instance, authors demonstrated that the JA and lignin biosynthesis pathways were up-regulated in the resistant lines compared to the susceptible genotype. Conversely, the response to chitin, the SA pathway and the auxin response were activated in the resistant genotype. A majority of disease resistance genes in plants encode nucleotide-binding site leucine-rich repeat (NLR) as part of the R-protein mediated recognition of fungal effectors. Garcia-Garcia et al. (2020) found 42 tags that were assigned to the NLR gene family, although most of them did not show significant changes after the infection. Other transcriptomics research has been carried out by Cao et al. (2020) on resistance to BS and already described in Sect. 7.2, and by Sari et al. (2018) who found that lentil cultivars CDC Robin and 964a-46 activated cell surface receptors tentatively associated with pathogen-associated molecular patterns (PAMP) recognition and NLR upon *A. lentis* infection, and differedin their activation of SA and JA signal transduction pathways.

Anthracnose of lentil is another devastating fungal disease in some parts of the world. It is caused by pathogens of the hemibiotrophic species *Colletotrichum lentis*, where the transition from biotrophy to necrotrophy is critical for a successful infection. To shed light into the mechanisms regulating this transition, Bhadauria et al. (2013) assembled expressed tags into unique genes (unigenes). Among the assembled transcriptome, 387 unigenes were predicted to have stress and defense related roles. There were also membrane and transport associated sequences (101) and unigenes implicated in signal transduction (159), some of them thought to be part of the inducible plant response. The molecular mechanisms triggering the symptomatic phase of infection have also been investigated (Bhadauria et al. 2017b). Authors identified a total of 22 putative effectors, and 26 resistance genes implicated in the recognition of fungal effectors, signaling of pathogen perception, phytohormone level changes, and TFs. These resistance genes included both positive and negative regulators of plant immunity in an intricate molecular interplay between disease resistant proteins and effectors, in which, during a compatible interaction, the pathogen appears to exploit the defense responses mounted by the host.

5.9.2 Genomic Selection

Genomic selection (GS) is a promising approach in breeding programs as it provides opportunities to increase genetic gain of complex traits per unit time and cost (Bhat et al. 2016). It uses all marker data as predictors of performance to deliver more accurate predictions, but in turn requires the availability of genome-wide, high-throughput and cost-effective markers. A well-fitted statistical model is also required for the training population, which is phenotyped and genotyped. This model will be later applied to the breeding population that has been genotyped but not phenotyped.

Some SNP genotyping platforms, especially the GBS and SNP chips, as well as a polished genome assembly draft, have opened GS to lentil breeding programs. Haile et al. (2020) have tested several statistical prediction models specifically for lentil breeding. They suggested that GS can be implemented to make predictions within populations and across environments, as moderate to high accuracies were obtained. Across-population predictions were much lower, and thus, their use is discouraged when the population size is small. It is expected that GS will gain importance in the coming years.

5.9.3 Novel Genomic Tools in Other Plant Species

Genome-wide approaches successfully used to understand the responses to biotic stresses in other species could also be applied to lentils. Recently, Laflamme et al. (2020) designed a pangenome based analysis to unravel the complex interrelationship between pathogens and plants, supplying invaluable information about gene families involved in the resistance. The work was carried out on *Arabidopsis thaliana*, which was infected with one of the most common plant pathogens, the bacteria *Pseudomonas syringae*. Authors generated a *P. syringae* Type III Effector Compendium (PsyTEC) from 494 strains and identified the genes responsible for effector-triggered immunity in *Arabidopsis*. This pangenome analysis revealed that relatively few *A. thaliana* genes are responsible for recognizing the majority of *P. syringae* effectors. Furthermore, they identified new *Arabidopsis* immune NLR receptors able to recognize effectors expressed by most of the strains. These results provide insight into why most pathogenic microbes only infect specific plant species.

Multi-genome assemblies have also allowed identifying genetic differences between wheat lines that are important for breeding (Walkowiak et al. 2020). The research team was able to track the unique DNA signatures of genetic material incorporated into modern cultivars from several of wheat's undomesticated relatives. These wheat relatives have been used by breeders to improve disease resistance and stress resistance of wheat. For instance, a DNA segment from one of these relatives contains disease-resistant genes and provides protection against a number of fungal diseases. This segment can improve yields by as much as 10 per cent. The pangenome was also used to isolate an insect-resistant gene (*Sm1*) that enables wheat plants to withstand the orange wheat blossom midge, a pest which can cause millions in annual losses to producers. As more pangenomes are being announced this information could be validated and extrapolated to other plant species. Kumar and Gupta (2020) have highlighted the new opportunities of pangenome analysis in lentil breeding.

NGS was also used for large-scale pathogen diagnoses in soybean (Díaz-Cruz et al. 2019). Several bacteria, fungi, and viruses known to infect soybean were detected, as well as pathogens not previously identified. For some microorganisms, this technique was able to disentangle the different pathovars present and/or assemble their genome sequence. Since NGS generated data on the whole spectrum of flora

and fauna that thrive in leaves, it was possible to identify residual pathogens (i.e., pathogens of crops other than soybean) and multiple species of arthropod pests. Finally, the assembled NGS data allowed for the development of polymerase chain reaction-based diagnostics for some pathogens.

5.10 Recent Concepts and Strategies

The application of traditional breeding techniques to lentils, such as the development of molecular markers, QTL identification, and MAS, has led to important achievements. However, approaches that rely on the use of transgenic plants and plant tissue techniques are currently lagging behind. Lentils are long known to be recalcitrant to plant tissue culture, whole plant regeneration, and micropropagation (Polanco and Ruiz 1997; Fratini and Ruiz 2003; Khatib et al. 2011).

5.10.1 Research on Other Plant Species

Recent studies using model plant species have emphasized the complexity of the plant-pathogen response and have suggested novel and complementary pathways for resistance in crop species. For instance, in a genome-wide association mapping study in *Arabidopsis*, Aoun et al. (2020) dug into the genetic basis of the resistance to *Ralstonia solanacearum* under heat stress. They discovered multiple QTLs and the identity of the candidate genes underlying the 14 major QTLs. The nature of those genes is highly diverse, not matching the typical resistance genes encoding NLRs. Interestingly; the QTLs they found at 27 °C were different from those at 30 °C, indicating distinct genetic architectures for the responseto *R. solanacearum* at changing temperatures. Among non-classical defense-related candidate genes there is *SDS*, which encodes a meiotic cyclin-like protein related to cyclins previously described as being required for DNA repair. Its functional validation as a gene for susceptibility represents the first demonstration of the involvement of *SDS* in the plant response to a bacterial pathogen under heat stress. According to the authors, SDS acts together with other proteins to suppress unscheduled cell wall synthesis. Other candidate genes encode for proteins involved in cell wall and lignin polymerization. We think this genome survey reflects the complexity of the response pathways to biotic stresses, and, that despite of the progress made in the last years, *omic* approaches will have to provide further knowledge for us to fully understand the responses of crops to biotic and abiotic stresses.

Another example of this complexity is provided by Ngaki et al. (2021).They proved how a single gene (*Glycine max disease resistance 1; GmDR1; Glyma.10g094800*) can confer resistance to various pathogens and pestsi n soybean. Overexpression of its encoded plasma membrane protein led to enhanced resistance not only against the fungal pathogen *Fusarium virguliforme*, but also against

spider mites (*Tetranychus urticae*), soybean aphids (*Aphis glycines*) and soybean cyst nematode (*Heterodera glycines*). Authors also investigated if chitin, a PAMP, can significantly enhance defense pathways in *GmDR1*-overexpressed transgenic soybean lines. They concluded that chitin-induced SA- and JA-pathways could be involved in broad-spectrum resistance against pathogens and spider mites, for which no known resistance genes have been identified in soybean and in most crop species. It is likely that some of these results on *GmDR1* could be extrapolated to lentils, due to their taxonomic proximity.

Plant stomata play important roles in the response to stresses in plants. The perception of some biotic and abiotic stresses leads to stomatal closure. The flow of calcium ions (Ca^{2+}) across the plasma membrane is key in this response, but the calcium channel involved was not known. Thor el al. (2020) found that the *Arabidopsis thaliana* Ca^{2+}-permeable channel OSCA1.3 controls stomatal closure during defense response. In fact, OSCA1.3 is rapidly phosphorylated upon sensing PAMPs. Genetic and electrophysiological data revealed that OSCA1.3 is permeable to Ca^{2+}, and that BIK1-mediated phosphorylation increases this channel activity. Thus, OSCA1.3 and its phosphorylation by BIK1 are critical for stomatal closure during defense. Notably, OSCA1.3 does not appear to regulate stomatal closure upon sensing abscisic acid, a plant hormone associated with abiotic stresses. Their research suggests that there is specificity in the Ca^{2+} influx mechanisms in response to different stresses, opening new targets for pathogen resistance in crop plants.

The advent of NGS technologies has allowed the cataloging of genes, gene products and gene interactions within the biological context. TF-driven gene regulation underlies most aspects of organisms' biology, including the response to biotic stresses. High-throughput gene expression profiling is dramatically changing our views on how gene regulation networks are coordinated: from single-gene activities to gene interactions (Ko and Brandizzi 2020). Data gathered on interacting networks are valuable to integrate molecular communications and derive models to describe biological systems. Behind this is the idea of leveraging the interactions between genes and TFs over function of components alone (Ko and Brandizzi 2020). Thus, to understand the complex response of plants to pathogens and pests we will have to look at them as a whole.

Because they accumulate more recombination events, multi-parental segregating populations can offer better resolution than traditional biparental populations for the mapping of complex traits. They also have more genetic diversity and minimal population structure. Several multi-parent populations have been constructed in legumes, including peanuts, soybean, cowpea, and faba bean. Their utility ranges from being a tool for mapping quantitative trait loci to a means of providing germplasm for breeding programs (Scott et al. 2020).

Improved in situ hybridization (ISH) techniques have come out. One of them is RNAscope® (Wang et al. 2012), an ISH assay for detection of target RNA within intact cells through a novel signal amplification and background suppression. This method is capable of simultaneous detection of multiple target RNAs down to the single molecule level in individual cells, allowing researchers to study spatiotemporal patterns of gene expression. By applying confocal laser microscopy, Solanki

et al. (2020), designed an optimized method for RNAscope® detection to determine the spatial expression and semi-quantification of target RNAs. The generalization of RNAscope® method to lentils and other legumes will assist in gene expression studies, as researchers not only know the genes that are expressed, but also when and in which cells.

RNA transport and localization *in planta* represent important post-transcriptional regulation mechanisms. Plants have the capacity to transport mRNA molecules beyond the cell boundaries through plasmodesmata and over long distance by phloem. Peña et al. (2021) have described in plants an in vivo method for RNA-labelling which allows monitoring cell-to-cell transport of mRNA. Technical advances like these offer new and complementary alternatives for fine analysis of gene expression in various situations, including stress response.

5.10.2 Gene Editing

Precision gene editing by the CRISPR/Cas9 reagent is a powerful technique for the genetic manipulation of crop genomes and can be carried out by either targeted mutagenesis or gene targeting (Scheben et al. 2017). During the last years gene-editing methods have been established for some crop and model legumes species such as chickpea, cowpea, soybean, *Lotus japonicus* and *Medicago truncatula*, as reviewed by Bhowmik et al. (2021). However, the recalcitrance of other legumes to in vitro gene transfer and regeneration has posed a serious challenge to application of gene editing. Targeted mutagenesis, or gene knock-out, is the easier technique due to lower host plant transformation efficiency requirements. Gene targeting, or gene knock-in, is a more advanced technique that uses a donor template containing the desired DNA changes to be incorporated into the targeted region and requires a greater transformation efficiency to recover successfully edited plants.

Currently, the ability to manipulate DNA using CRISPR/Cas9 (Anzalone et al. 2019) exceeds the transformation technologies required to deliver reagents into the plant. Not surprisingly, improvements to the delivery of reagents has become a hot area of research which is attempting to address problems such as inefficient in vitro shoot regeneration, Agrobacterium-mediated T-DNA delivery, shoot regeneration from protoplast tissue and optimization of transgenic selection. Recent research has demonstrated the capability of morphogenic regulators to effectively generate transformed plants and this technology shows great promise for improvements to legume transformation and gene editing (Anand et al. 2018; Hoerster et al. 2020; Maher et al. 2020). As it is typical for many grain legumes, the lentil has a long and frustrating history of tissue culture and in vitro regeneration. In comparison with model plant species and many other crop species, lentil is a relatively recalcitrant species in relation to plant tissue culture, whole plant regeneration and micropropagation, hindering further biotechnological modifications (Pratap et al. 2018). Encouragingly, lentil plant transformation has been reported in several genotypes to date including Laird, Sultan and L-4076 at a transformation efficiency ranging between 0.9–3.1% (Gulati and McHughen, 2003; Akcay et al. 2009, 2015; Chopra et al. 2011). Improvements

to these procedures and/or the implementation of morphogenic regulators, combined with cultural practices such as micrografting transgenic shoots to non-transformed rootstocks to establish transgenic plants will likely improve transformation efficiencies and widen the range genotypes that can be transformed (Khatib et al. 2011). Genome editing technologies have been also reviewed by Gupta et al. (2020).

5.11 Role of Bioinformatics as a Tool

Most of the published lentil sequences are found in the National Center for Biotechnology Information (NCBI) and the European Bioinformatics Institute (EBI) databases, (in this chapter the information has been searched and referenced in the NCBI database). In the NCBI there are 33,503 entries of Nucleotides using "Lentil" as searching word. The vast majority of the sequences comes from the cultivated species, (29,240 entries), although numerous sequences from wild species can also be found: *L. orientalis*, 1,606; *L. ervoides*, 893; *L. nigricans*, 479; *L. odemensis*, 254; *L. tomentosus* 161; and *L. lamottei*, 86.

The most numerous entries related to a pathogen in the database refer to*Colletotrichum truncatum.* Data were obtained in a series of works analyzing the interactions between lentil and the pathogen (Bhadauria et al. 2011, 2013, 2017b).

Members of the Division of Crop Improvement of Indian Institute of Pulses Research from Kanpur analyzed the lentil genomic resources available in the public databases in a recent review (Kumar et al. 2020). Sequence-based markers are available from the NCBI databases. Among the first works used to obtain maps and markers is that by Kaur et al. (2011). They obtained 15,298 small-sized TSA (Transcriptome Shotgun Assembly) sequences from 6 lentil genotypes (BioProject PRJNA65667, 14-Apr-2011, Table 5.3). Sharpe et al. (2013) compared 11 genotypes (including two of *L. ervoides*). The raw data obtained with 454 GS FLX Titanium are found in the BioProject PRJNA192531 (6-Mar-2013). Yilmaz Temel et al. (2015) obtained 97,528 contigs of cDNAs from two genotypes (PRJNA210522, 7-Jan-2014). The entry of these BioProjects is shown in Table 5.3.

Without doubt the most important specialized database on pulse crops is KnowPulse (knowpulse.usask.ca) developed by the University of Saskatchewan (Sanderson et al. 2019). In it, numerous markers based on sequences obtained by the Sanger's technique and by NGS-based 454 and Illumina procedures are collected. These markers are located on the draft v1.2 of the *Lens culinaris* genome whose sequences come from the CDC Redberry variety. On that page it is possible to perform BLAST searches and browse the lentil genome with the JBrowse tool and perform other queries. The genes have been detected by comparing the genome with different lentil transcriptomes and the putative lentil orthologous genes to *Medicago* 4.0, *Arabidopsis* 10, chickpea 1.0 and soybean 2.75 genomes have also been located. Access to the data of this genome is limited and for a more complete use it is necessary to contact Dr. Kirstin E. Bett.

The v1.2 of the lentil genome consists of 2,748 Mb (38,998 genes) assembled in 7 large pseudomolecules corresponding to the 7 chromosomes of the species with 339,

317, 199, 246, 263, 210 and 247 Mb, respectively, in addition to another 128,639 small fragments or contigs containing the rest of the approximately 927 Mb. The raw data of sequence reads used in lentil genome construction is available from the NCBI in BioProject PRJNA343689 (21-Sep-2016, Hiseq 2000). The project includes 22 SRA experiments, with 1,087 Gb in raw data that are assembled in a total of 2,748 Mb of the 4,063 Mb of the haploid lentil genome (Table 5.3). Many of the annotated genes come from or have been verified with data from bioprojects focused on cDNAS (PRJNA434239, uploaded to the NCBI in February 2018). Both the raw data of the genome and the cDNAs used for their annotation were submitted by the research group at the University of Saskatchewan.

Other lentil cDNA sequences can be found in two BioProjects: PRJNA218843 is the oldest (11-Sep-2013, 4 Gb, submitted by India NIPGR) in which only one sample was analyzed; and PRJNA352096 (2-Nov-2016, 160 Gb) in which Sudheesh et al. (2016b) compare the transcripts of seven different tissues of the Cassab variety. The project with greatest sequencing effort, PRJNA497358 (18-Oct-2018, 207 Gb) of the Shadong Center of Crop Germplasm Resources (unpublished), includes six biological samples and 18 sequencing experiments. This project representsa new and significant contribution of new lentil transcripts, although it does not fully specify the data.

In addition to the nuclear genome, the NCBI database contains the lentil chloroplast genome sequence. The complete sequence can be found assembled in the BioProject PRJNA285561 submitted by the University of British Columbia (2-Jun-2015), although not much data of the technique used to obtain it is provided.

It is also possible to identify genome sequences from both prokaryotes and fungi that are part of the microbiota of the lentil root. Fungi are explored in the BioProject PRJNA470968 by analyzing the ITS1 spacer of ribosomal genes, the University of Saskatchewan is again participating in the project (10-May-2018). Prokaryotes were also studied by researchers at Assam University from 10 different samples. The analysis was based on the sequences of a fragment of the coding gene for ribosomal RNA that includes the variable regions V3 and V4 (PRNJNA622390, submitted at 8-Apr-2020). A new whole metagenomic analysis of two-samples has recently been performed by researchers at Bidhan Chandra Agricultural University (PRJNA639655, Jun-16–2020). Also, there are complete genomes of two of the most important lentil pathogens, *Colletotrichum lentis* (PRJNA407672, 14-Aug-2018, Bhadauria et al. 2019) and *Ascochyta lentis* (PRJNA506513, 22-Nov-2018, Curtin University) available from NCBI.

Numerous sequencing projects have focused their objectives on exploring the diversity of lentil at the genomic level. Among the firsts of them there is the study of 83 samples genotyped by sequencing (GBS) carried out by Wong et al. (2015), whose raw data can be obtained from the BioProject PRJNA261418(18-Sep-2014, 44 Gb). Two other GBS studies, based on genomic data, are included in the BioProjects PRJNA528610 (22-Mar-2019, 121 Gb) and PRJEB38912 (1-Oct-2020, 55 Gb). In the first one, Pavan et al. (2019) compared 349 lentil accessions, mostly landraces, while in the second, Liber et al. (2021) chose 190 genotypes of both cultivated and wild species to study the history of lentil domestication and spread. Ogutcen et al.

(2018) developed an exome capture array for lentil using 16 wild lentils and 22 cultivars accessions (PRJNA433205, 6-Feb-2018). The greatest effort in sequencing made to know on the diversity of lentil has been carried out by Dissanayake et al. (2020), although instead of GBS they studied RNAs from 467 accessions, including wild species (*L. culinaris* 304; *L. orientalis*, 57; *L. ervoides*, 57; *L. nigricans*, 24; *L. odemensis*, 22; *L. lamottei*, 1; two unidentified *Lens* accessions and no samples of *L. tomentosus*). The BioProject that collects the data from Dissanayake et al. (2020) is PRJNA625627 (16-Apr-2020, 1598 Gb).

The rest of the bioprojects devoted to lentil analyze the differential expression at the messenger level of lentil samples subjected to some type of stress, either abiotic or biotic. Although abiotic stress is not the main objective of this chapter, we must mention the two RNAseq studies in which the response to drought is analyzed, BioProjects PRJNA308969 (16-Jan-2016, 94 Gb) and PRJNA474098 (1-Jan-2018, 120 Gb) by Singh et al. (2017b) and Morgil et al. (2019) respectively, and the two studies dedicated to temperature, the one by Barrios et al. (2017) studies the effect of cold and uses the superSAGE technique (PRJEB14947, 9-Dec-2016, 1 Gb) and Shing et al. (2019) that analyses exposure to high temperatures (PRJNA423129, 20-Dec-2017, 63 Gb). Three other projects submitted by Lorestan University collect data on abiotic stresses, although the indications in the NCBI database are not too clear, they analyze the effect of temperature, drought and salinity (PRJNA378872, 12-Mar-2017, 43 Gb; PRJNA379217, 15 -Mar-2017, 60 Gb; and PRJNA379218, 15-Mar-2017, 52, Gb).

Several experiments analyze gene expression in relation to pathogens, affording messenger sequences to databases. They are all related to the infection of the fungus *Ascochyta lentis*. The first data come from the study by Khorramdelazad et al. (2018), in which three replicates were analyzed by treatment of the ILL7537 (resistant) and ILL6002 (susceptible) accessions at three times (2, 6 and 24 h after inoculation - hpi) with spores of the fungus or mock setting (PRJNA321618, 15-May-2016, 79 Gb). In the analysis by Garcia-Garcia et al. (2019) only the 3' terminal ends of the messengers were analyzed with the MACE technique 24 hpi with the fungus or mock setting, the genotypes chosen in the study are the susceptible cultivar 'Lupa', the moderately resistant 'ILL558' and the resistant wild accession of *L. orientalis* 'BG 16,880' (PRJNA356810, 9-Dec-2016, 1 Gb). Another study of RNAseq is that of Sari et al. (2018) that used the CDC Robin and 964a-46 lines as resistant and the Eston cultivar as sensitive. Samples were taken at eight different times after inoculation ranging between 0 and 60 h, the raw data were collected in the 24 SRA of the BioProject PRJNA422815 (18-Dec-2017, 40 Gb).

Finally, in the study by Polanco et al. (2019), the messengers of 78 RIL lines from the cross of the sensitive cultivar ALPO and the resistant ILWL235 accession of the wild species *L. odemensis,* the parents used for the cross, were analyzed 24 h after having been inoculated with spores of the *Ascochyta* isolate AL-84. Six replicates of each parent inoculated with spores or mock setting were analyzed from the parents to serve as a control, obtaining total of 6,306 polymorphic markers from the parents were used to obtain a high-density map. The raw data are found in the BioProject PRJNA523792 (22-Feb-2019, 416 Gb).

Table 5.3 Registered BioProjects in the NCBI Database with lentil nucleotide sequence public data

Bioproject	Registration date	# of SRAs	Biosamples (in NCBI)	Gbases	Technology	Strategy/Aims (Stress)	Publication
PRJNA65667	14-Apr-2011				454 GS	cDNA SSR markers	Kaur et al. (2011)
PRJNA192531	6-Mar-2013	11	1	2	454 GS	cDNA markers 6 lines	Sharpe et al. (2013)
PRJNA218843	11-Sep-2013	1	1	4	Illumina	RNAseq	India NIPGR
PRJNA210522	7-Jan-2014	2	2	11	Illumina	cDNA markers	Yilmaz Temel et al. (2014)
PRJNA261418	18-Sep-2014	166	83	44	HiSeq 2500	GBS	Wong et al. (2015)
PRJNA285561	2-Jun-2015					Chloroplast Genome	University of British Columbia
PRJNA308969	16-Jan-2016	5	1	94	HiSeq 2500	RNAseq/Drought	Singh et al. (2017a, b, c)
PRJNA321618	15-May-2016	36	2	79	Ion Torrent	RNAseq/Ascochyta	Khorramdelazad et al. (2018)
PRJNA343689	21-Sep-2016	1	22	1087	HiSeq 2000	Genome WGS	University of Saskatchewan
PRJNA352096	2-Nov-2016	7	7 tissues	160	HiSeq 2000	RNAseq	Sudheesh et al. (2016a, b)
PRJNA352318	3-Nov-2016	2	1	1	Illumina	cDNA/Various stresses	University of León
PRJNA356810	9-Dec-2016	6	3	1	HiSeq 2000	MACE/Ascochyta	García et al. (2019)
PRJEB14947	23-Dec-2016	2	1	1	454 GS	SuperSAGE/Cold	Barrios et al. (2017)
PRJNA378872	12-Mar-2017			43		RNAseq/Temperature	Lorestan University
PRJNA379217	15-Mar-2017		1	60		RNAseq/Drought and heat	Lorestan University

(continued)

Table 5.3 (continued)

Bioproject	Registration date	# of SRAs	Biosamples (in NCBI)	Gbases	Technology	Strategy/Aims (Stress)	Publication
PRJNA379218	15-Mar-2017		1	58		RNAseq/Salt	Lorestan University
PRJNA422815	18-Dec-2017	24	3	40	HiSeq 2500	RNAseq/Ascochyta-time	Sari et al. (2018)
PRJNA423129	20-Dec-2017	12	1	63	HiSeq 2000	RNAseq/Heat	Singh et al (2019)
PRJNA474098	1-Jun-2018	18	18	120	HiSeq 4000	RNAseq/Drought	Morgil et al. (2019)
PRJNA433205	6-Feb-2018	38	38	343	HiSeq 2500	cDNA Diversity	Ogutcen et al. (2018)
PRJNA434239	15-Feb-2018	17	17	261	Miseq	cDNA annotation	University of Saskatchewan
PRJNA470968	10-May-2018	112	112	4	MiSeq	ITS1 Fungi Microbiome	University of Saskatchewan
PRJNA407672	14-Aug-2018	1	1		HiSeq 2000; MiSeq	*Colletotrichum lentis* genome	Bhadauria et al. (2019)
PRJNA497358	18-Oct-2018	18	6	207	HiSeq 4000	cDNA	Shandong Center of Crop Germp. Resour
PRJNA506513	22-Nov-2018	6	6	14	NextSeq 500	*Ascochyta lentis* genome	Curtin University
PRJNA523792	22-Feb-2019	102	80	416	HiSeq 2500	cDNA Map/Ascochyta	Polanco et al. (2019)
PRJNA528610	22-Mar-2019	1	349	121	HiSeq 2500	GBS Diversity	Pavan et al. (2019)
PRJNA623690	8-Apr-2020	10	10	1	MiSeq	16S (V3-V4) Root Microbiome	Assam University
PRJNA625627	16-Apr-2020	467	467	1598	HiSeq 3000	cDNA Diversity	Dissanayake et al. (2020)
PRJNA639655	16-Jun-2020	2	2	14	HiSeq 2500	Metagenomic	Bidhan Chandra Agricultural University

(continued)

Table 5.3 (continued)

Bioproject	Registration date	# of SRAs	Biosamples (in NCBI)	Gbases	Technology	Strategy/Aims (Stress)	Publication
PRJEB38912	1-Oct-2020	190	190	55	NextSeq 550	GBS Diversity	University of Algarve

454 GS = GS-FLX Titanium shotgun; SRA = Sequence Read Archive; MACE = Massive Analysis of cDNA Ends; SuperSAGE = Supertag sequences; GBS Genotyping by sequencing

5.12 Future Perspectives

In a recent review on the status and prospects of biotechnological interventions for plant breeding the authors (Singh et al. 2020b) pointed out the following set of actions: (1) Deployment of genomic resources for trait discoveryand crop improvement by whole-genome sequencing, resequencing and pangenome analysis together with the development and deployment of molecular markers for breeding; (2) identification of QTLs associated with agronomic traits; (3) genomics-assisted breeding for trait improvement including marker-assisted backcrossing and recurrent selection, and genomic selection and speed breeding; (4) biotechnological interventions for crop improvement including the expression and overexpression of candidate genes for desired phenotype, RNA interference for in vivo knockdown of target genes, and gene and genome editing. While some significant advances have been achieved in the development of genomic resources, development of molecular markers and QTL identification and their use in lentil "molecular breeding", and many more are being and will be developed in the near future, most of the biotechnological interventions depend on the use of transgenic plants and plant tissue techniques, which represents a bottleneck in the application of the biotechnological interventions in current lentil breeding. Lentil is a relatively recalcitrant species in relation to plant tissue culture hindering further biotechnological modifications (See Sect. 5.12).

Third-generation single-molecule sequencing technologies reduce the cost of sequencing and can be used for sequencing the long DNA fragments expediting the assembling and scaffolding of complex genome. Hence use of these technologies can overcome problems associated with the large genome size of lentil and in coming years, use of NGS will boostgenetic gain in lentil (Kumar and Gupta 2020).

Recent publications on model species have again emphasized the enormous complexity of the response to pathogens in plants and suggest complementary or new pathways in the search for resistance in crop species.

References

Adobor S, Podder R, Banniza S, Vandenberg A (2020) Evaluation of resistance to stemphylium blight in interspecific recombinant inbred lines derived from *Lens culinaris* × *Lens ervoides*. Plant Genet Resour Charact Util 1–8. https://doi.org/10.1017/S1479262120000295

Akcay UC, Mahmoudian M, Kamci H, Yucel M, Oktem HA (2009) *Agrobacterium tumefaciens*-mediated genetic transformation of a recalcitrant grain legume, lentil (*Lens culinaris* Medik). Plant Cell Rep 28:407–417. https://doi.org/10.1007/s00299-008-0652-4

Akcay UC, Yucel M, Oktem HA (2015) Lentil (*Lens culinaris Medik.*) Agrobacterium Protocols. Method Mol Biol 1223:265–274. https://doi.org/10.1007/978-1-4939-1695-5_21

Akhtar KP, Dickinson M, Asghar MJ, Abbas G, Sarwar N (2016) Association of 16SrII-C phytoplasma with lentil phyllody disease in Pakistan. Trop Plant Pathol 41:198–202. https://doi.org/10.1007/s40858-016-0087-3

Almeida-Costa GE, Queiroz-Monici KS, Machado Reis SMP, Costa de Oliveira A (2007) Chemical composition, dietary fibre and resistant starch contents of raw and cooked pea, common bean,

chickpea and lentil legumes. Food Chem 94:327–330. https://doi.org/10.1016/j.foodchem.2004.11.020

Alo F, Furman BJ, Akhunov E, Dvorak J, Gepts P (2011) Leveraging genomic resources of model species for the assessment of diversity and phylogeny in wild and domesticated lentil. J Hered 102:315–329. https://doi.org/10.1093/jhered/esr015

Anand A, Bass SH, Wu E, Wang N, McBride KE et al (2018) An improved ternary vector system for *Agrobacterium*-mediated rapid maize transformation. Plant Mol Biol 97:187–200. https://doi.org/10.1007/s11103-018-0732-y

Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW et al. (2019) Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576:149–157. https://doi.org/10.1038/s41586-019-1711-4

Aoun N, Desaint H, Boyrie L, Bonhomme M, Deslandes L, Berthomé R, Roux F (2020) A complex network of additive and epistatic quantitative trait loci underlies natural variation of *Arabidopsis thaliana* quantitative disease resistance to *Ralstonia solanacearum* under heat stress. Mol Plant Pathol 21:1405–1420. https://doi.org/10.1111/mpp.12964

Arumuganathan K, Earle ED (1991) Nuclear DNA content of some important plant species. Plant Mol Biol Rep 9:208–218

Askary TH (2017) Diversity of plant parasitic nematodes in pulses. In: Ansari AA, Gill SS, Abbas ZK, Naeem M (eds) Plant biodiversity: monitoring, assessment and conservation. CABI, Wallinford, pp 239–274. https://doi.org/10.1079/9781780646947.0000

Ates D, Aldemir S, Alsaleh A, Erdogmus S, Nemli S, Kahriman A et al (2018) A consensus linkage map of lentil based on DArT markers from three RIL mapping populations. PLoS One 13:e0191375. https://doi.org/10.1371/journal.pone.0191375

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot 63:3523–3544. https://doi.org/10.1093/jxb/ers100

Aydin H, Muehlbauer FJ, Kaiser WJ (1987) Pea enation mosaic virus resistance in lentil (*Lens culinaris*). Plant Dis 71:635–638

Banniza S, Warale R, Menat J, Cohen-Skali A, Armstrong-Cho C, Bhadauria V (2018) The long path to understanding the host–pathogen interactions of *Colletotrichum lentis* on lentil. Can J Plant Pathol 40:199–209. https://doi.org/10.1080/07060661.2018.1451391

Barrios A, Caminero C, García P, Krezdorn N, Hoffmeier K, Winter P, Pérez de la Vega M (2017) Deep Super-SAGE transcriptomic analysis of cold acclimation in lentil (*Lens culinaris* Medik.). BMC Plant Biol 17:1–15. https://doi.org/10.1186/s12870-017-1057-8

Bawa PK (2020) Identification of candidate genes associated with resistance against race 0 of *Colletotrichum lentis* in *Lens ervoides*. PhD Dissertation, University of Saskatchewan

Bennet MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Phil Trans Roy Soc Lond B 274:227–274

Bhadauria V, Banniza S, Vandenberg A, Selvaraj G, Wei Y (2011) EST mining identifies proteins putatively secreted by the anthracnose pathogen *Colletotrichum truncatum*. BMC Genomics 12:327. https://doi.org/10.1186/1471-2164-12-327

Bhadauria V, Bett KE, Zhou T, Vanderberg A, Wei Y, Banniza S (2013) Identification of *Lens culinaris* defense genes responsive to the anthracnose pathogen *Colletotrichum truncatum*. BMC Genet 14:31. https://doi.org/10.1186/1471-2156-14-31

Bhadauria V, MacLachlan R, Pozniak C, Cohen-Skalie A, Li L, Halliday J, Banniza S (2019) Genetic map-guided genome assembly reveals a virulence-governing minichromosome in the lentil anthracnose pathogen *Colletotrichum lentis*. New Phytol 221:431–445. https://doi.org/10.1111/nph.15369

Bhadauria V, Ramsay L, Bett KE, Banniza S (2017) QTL mapping reveals genetic determinants of fungal disease resistance in the wild lentil species *Lens ervoides*. Sci Rep 7:3231. https://doi.org/10.1038/s41598-017-03463-9

Bhadauria V, Vijayan P, Wei Y, Banniza S (2017) Transcriptome analysis reveals a complex interplay between resistance and effector genes during the compatible lentil-*Colletotrichum lentis* interaction. Sci Rep 7:42338. https://doi.org/10.1038/srep42338

Bhandari K, Siddique KHM, Turner NC, Kaur J, Singh S et al (2016) Heat stress at reproductive stage disrupts leaf carbohydrate metabolism, impairs reproductive function, and severely reduces seed yield in lentil. J Crop Improv 30:118–151. https://doi.org/10.1080/15427528.2015.1134744

Bhat JA, Ali S, Salgotra RK, Mir ZA, Dutta S et al (2016) Genomic selection in the era of next generation sequencing for complex traits in plant breeding. Front Genet 7:221. https://doi.org/10.3389/fgene.2016.00221

Bhowmik P, Konkin D, Polowick P, Hodgins CL, Subedi M et al (2021) CRISPR/Cas9 gene editing in legume crops: opportunities and challenges. Legume Sci 2021:1–16. https://doi.org/10.1002/leg3.96

Bourgault M, Low M, Tausz-Posch S, Nuttall JG Delahunty AJ et al (2018) Effect of a heat wave on lentil grown under free-air CO2 enrichment (FACE) in a semi-arid environment. Crop Sci 58:803-812. https://doi.org/10.2135/cropsci2017.09.0565

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (eds) (1996 onwards). Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996. http://biology.anu.edu.au/Groups/MES/vide/

Buchwaldt L, Shaikh R, Adam J, Tullu A, Slinkard AE (2013) Recessive and dominant genes confer resistance to *Colletotrichum truncatum* in cultivated lentil. Can J Plant Pathol 35:222–231. https://doi.org/10.1080/07060661.2013.768296

Bueckert RA, Clarke JM (2013) Review: Annual crop adaptation to abiotic stress on the Canadian prairies: six case studies. Can J Plant Sci 93:375–385. https://doi.org/10.4141/CJPS2012-184

Burleigh R, Southgate BJ (1975) Insect infestation of stored Egyptian lentils in antiquity. J Archaeol Sci 2:391–392

Buxó R (1997) Arqueología de las Plantas. Crítica, Barcelona, Spain

Cao Z, Li L, Kapoor K, Banniza S (2019) Using a transcriptome sequencing approach to explore candidate resistance genes against stemphylium blight in the wild lentil species *Lens ervoides*. BMC Plant Biol 19:399. https://doi.org/10.1186/s12870-019-2013-6

Chahota RK, Gupta VP, Sharma SK (2002) Inheritance of rust resistance in lentil. Indian J Genet 62:226–227

Choudhary AK, Kumar S (2016) Genetic improvement of lentil for fusarium wilt resistance. In: Singh A, Bhakta N, Sangle U, Manibhushan, Sundaram PK, Kumar S, Khan YJ (eds) Scientific lentil production: Indian perspectives. Society for Upliftment of Rural Economy Varanasi, India, pp 59–72

Clement SL, El-Din Sharaf El-Dine N, Weigand S, Lateef SS (1994) Research achievements in plant resistance to insect pests of cool season food legumes. Euphytica 73:41–50

Coyne C, McGee R (2013) Lentil. In: Singh M, Upadhyaya HD, Bisht IS (eds) Genetic and Genomic Resources of Grain Legume Improvement. Elsevier Inc., London, UK, pp 157–180. https://doi.org/10.1016/B978-0-12-397935-3.00013-X

Cuadrado C, Grant G, Rubio LA, Muzquiz M, Bardocz S, Pusztai A (2002) Nutritional utilization by the rat of diet based on lentil (*Lens culinaris*) seed meal or its fractions. J Agri Food Chem 50:4371–4376

Cubero JI, Pérez de la Vega M, Fratini R (2009) Origin, phylogeny, domestication and spread. In: Erskine W, Muehlbauer FJ, Sarker A, Sharma B (eds) The Lentil: Botany, Production and Uses. CABI, Wallingford, UK, pp 13–33

Cutforth HW, McGinn SM, McPhee KE, Miller PR (2007) Adaptation of pulse crops to the changing climate of the northern Great Plains. Agron J 99:1684–1699. https://doi.org/10.2134/agronj2006.0310s

Dadu RHR, Ford R, Sambasivam P, Gupta D (2017) A novel *Lensorientalis* resistance source to the recently evolved highly aggressive Australian *Ascochyta lentis* isolates. Front Plant Sci 8:1038. https://doi.org/10.3389/fpls.2017.01038

Dadu RHR, Ford R, Sambasivam P, Gupta D (2018) Evidence of early defence to *Ascochyta lentis* within the recently identified *Lens orientalis* resistance source ILWL180. Plant Pathol 67:1492–1501. https://doi.org/10.1111/ppa.12851

Dadu RHR, Ford R, Sambasivam P, Street K, Gupta D (2019) Identification of novel *Ascochyta lentis* resistance in a global lentil collection using a focused identification of germplasm strategy (FIGS). Austral Plant Pathol 48:101–113. https://doi.org/10.1007/s13313-018-0603-7

Damm U, O'Connell RJ, Groenewald JZ, Crous PW (2014) The *Colletotrichum destructivum* species complex—hemibiotrophic pathogens of forage and field crops. Stud Mycol 79:49–84. https://doi.org/10.1016/j.simyco.2014.09.003

Das A, Dutta S, Jash S, Barman AR, Das R, Kumar, Gupta S (2019) Current knowledge on pathogenicity and management of *Stemphylium botryosum* in lentils (*Lens culinaris* ssp. *culinaris* Medik.). Pathogens 8:225. https://doi.org/10.3390/pathogens8040225

Davidson JA, Kimber RBE (2007) Integrated disease management of Ascochyta blight in pulse crops. Eur J Plant Pathol 119:99–110. https://doi.org/10.1007/978-1-4020-6065-6_10

Dekkers J, Hospital F (2002) The use of molecular genetics in the improvement of agricultural populations. Nat Rev Genet 3:22–32. https://doi.org/10.1038/nrg701

Díaz-Cruz GA, Smith CM, Wiebe KF, Villanueva SM, Klonowski AR, Cassone BJ (2019) Applications of next-generation sequencing for large-scale pathogen diagnoses in soybean. Plant Dis 103:1075–1083. https://doi.org/10.1094/PDIS-05-18-0905-RE

Dikshit HK, Singh A, Singh D, Aski M, Jain N et al (2016) Tagging and mapping of SSR marker for rust resistance gene in lentil (*Lens culinaris* Medikus subsp. *culinaris*). Indian J Exp Biol 54:394–399

Dissanayake R, Braich S, Cogan NOI, Smith K, Kaur S (2020) Characterization of genetic and allelic diversity amongst cultivated and wild lentil accessions for germplasm enhancement. Front Genet 11:546. https://doi.org/10.3389/fgene.2020.00546

Elbakidze L, Lu L, Eigenbrode S (2011) Evaluating vector-virus-yield Interactions for peas and lentils under climatic variability: a limited dependent variable analysis. J Agric Resour Econ 36:504–520

El-Bouhssini M, Sarker A, Erskine W, Joubi A (2008) First sources of resistance to Sitona weevil (*Sitona crinitus* Herbst) in wild *Lens* species. Genet Resour Crop Evol 55:1–4. https://doi.org/10.1007/s10722-007-9297-3

Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES et al (2011) A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PLoS One 6:e19379. https://doi.org/10.1371/journal.pone.0019379

Ennami M, Briache FZ, Gaboun F, Abdelwahd R, Ghaouti L, Belqadi L, Westwood J, Mentaga R (2017) Host differentiation and variability of *Orobanche crenata* populations from legume species in Morocco as revealed by cross-infestation and molecular analysis. Pest Manag Sci 73:1753–1763. https://doi.org/10.1002/ps.4536

Eujayl I, Erskine W, Bayaa B, Baum M, Pehu E (1998) Fusarium vascular wilt in lentil: inheritance and identification of DNA markers for resistance. Plant Breed 117:497–499

Fernández-Aparicio M, Emeran AA, Rubiales D (2010) Inter-cropping with berseem clover (*Trifolium alexandrinum*) reduces infection by *Orobanche crenata* in legumes. Crop Protec 29:867–871. https://doi.org/10.1016/j.cropro.2010.03.004

Fernández-Aparicio M, Sillero JC, Pérez-de-Luque A, Rubiales D (2008) Identification of sources of resistance to crenate broomrape (*Orobanche crenata*) in Spanish lentil (*Lens culinaris*) germplasm. Weed Res 48:85–94

Fernández-Aparicio M, Sillero JC, Rubiales D (2009) Resistance to broomrape in wild lentils (*Lens* spp.). Plant Breed 128:266–270. https://doi.org/10.1111/j.1439-0523.2008.01559.x

Fisher RA (1941) Average excess of and average effect of a gene substitution. Ann Eugen 11:53–63. https://doi.org/10.1111/j.1469-1809.1941.tb02272.x

Flor HH (1971) Current status of the gene-for-gene concept. Annu Rev Phytopathol 9:275–296. https://doi.org/10.1146/annurev.py.09.090171.001423

Fratini R, Ruiz ML (2003) A rooting procedure for lentil (*Lens culinaris* Medik.) and other hypogeous legumes (pea, chickpea and Lathyrus) based on explant polarity. Plant Cell Rep 21:726–732. https://doi.org/10.1007/s00299-003-0603-z

Fratini R, Ruiz ML (2006) Interspecific hybridization in the genus *Lens* applying *in vitro* embryo rescue. Euphytica 150:271–280. https://doi.org/10.1007/s10681-006-9118-3

Fratini R, Ruiz ML (2008) Micropropagation of intra and interspecific *Lens* hybrids. Euphytica 159:199–206. https://doi.org/10.1007/s10681-007-9473-8

Ganguly S, Ghosh G, Ghosh S, Purohit A, Chaudhuri RK, Das S, Chakraborti D (2020) Plumular meristem transformation system for chickpea: an efficient method to overcome recalcitrant tissue culture responses. Plant Cell Tiss Org Cult 142:493–504. https://doi.org/10.1007/s11240-020-01873-8

Ganguly S, Purohit A, Chaudhuri RK, Das S, Chakraborti D (2020b) Embryonic explant and plumular meristem transformation methods for development of transgenic pigeon pea. In: Jain M, Garg R (eds) Legume genomics: methods and protocols. Methods Mol Biol 2107. https://doi.org/10.1007/978-1-0716-0235-5_17

Garcia-Garcia P, Vaquero F, Vences FJ, Sáenz de Miera LE, Polanco C, González AI, Horres R, Krezdorn N, Rotter B, Winter P, Pérez de la Vega M (2020) Transcriptome profiling of lentil in response to *Ascochyta lentis* infection. Span J Agric Res 17:e0703. https://doi.org/10.5424/sjar/2019174-14982

Gaulin E, Jacquet C, Bottin A, Dumas B (2007) Root rot disease of legumes caused by *Aphanomyces euteiches*. Mol Plant Pathol 8:539–548. https://doi.org/10.1111/j.1364-3703.2007.00413.x

Gela TS, Banniza S, Vandenberg A (2020) Lack of effective resistance to the virulent race of *Colletotrichum lentis* in *Lens culinaris* Medikus subsp. *culinaris*. Plant Genet Resour Charact Util 1–7. https://doi.org/10.1017/S1479262120000027

Gela TS, Koh CS, Caron CT, Chen L-A, Vandenberg A, Bett KE (2021) QTL mapping of lentil anthracnose (*Colletotrichum lentis*) resistance from *Lens ervoides* accession IG 72815 in an interspecific RIL population. Euphytica 217:70. https://doi.org/10.1007/s10681-021-02804-0

Gore PG, Tripathi K, Chauhan SK, Singh M, Bisht IS, Bhalla S (2016) Searching for resistance in wild *Lens* species against pulse beetle, *Callosobruchus chinensis* (L.). Legume Res 39:630–636. https://doi.org/10.18805/lr.v0iOF.6778

Gossen BD, Morrall RAA (1983) Effects of ascochyta blight on seed yield and quality in lentils. Can J Plant Pathol 5:168–173. https://doi.org/10.1080/07060668309501620

Gossen BD, Morrall RAA (1984) Seed quality loss at harvest due to ascochyta blight of lentil. Can J Plant Pathol 6:233–237. https://doi.org/10.1080/07060668409501559

Grenz JH, Sauerborn J (2007) Mechanisms limiting the geographical range of the parasitic weed *Orobanche crenata*. Agric Ecosyst Environ 122:275–281. https://doi.org/10.1016/j.agee.2007.01.014

Gujaria-Verma N, Vail SL, Carrasquilla-Garcia N, Penmetsa RV, Cook DR, Farmer AD, Vandenberg A, Bett KE (2014) Genetic mapping of legume orthologs reveals high conservation of synteny between lentil species and the sequenced genomes of *Medicago* and chickpea. Front Plant Sci 5:676. https://doi.org/10.3389/fpls.2014.00676

Gulati A, McHughen A (2003) *In vitro* regeneration and genetic transformation of Lentil. In: Jaiwal PK, Singh RP (eds) Applied genetics of leguminosae biotechnology. Focus on Biotechnology, vol 10B. Springer, Dordrecht. https://doi.org/10.1007/978-94-017-0139-6_8

Gupta D, Dadu RH, Sambasivam P, Bar I, Azad M et al. (2020) Conventional and biotechnological approaches for targeted trait improvement in lentil. In: Gosal SS, Wani SH (eds) Accelerated plant breeding, Vol 3. Springer, Cham. https://doi.org/10.1007/978-3-030-47306-8_3

Gupta D, Dadu RHR, Sambasivam P, Bar I, Singh M, Beera N, Biju S (2019) Toward climate-resilient lentils: Challenges and opportunities. In: Kole C (ed) Genomic designing of climate-smart pulse crops. Springer Nature, Cham, Switzerland AG, pp 165–234

Gupta D, Taylor PWJ, Inder P, Phan HTT, Ellwood SR et al (2012) Integration of EST-SSR markers of *Medicago truncatula* into intraspecific linkage map of lentil and identification of QTL conferring resistance to ascochyta blight at seedling and pod stages. Mol Breed 30:429–439. https://doi.org/10.1007/s11032-011-9634-2

Gutierrez-Gonzalez JJ, Garvin DF (2017) *De novo* transcriptome assembly in polyploid species. In: Gasparis S (ed) Oats methods and protocols. Humana Press, New York, pp 209–221

Gutierrez-Gonzalez JJ, Mascher M, Poland J, Muehlbauer GJ (2019) Dense genotyping-by-sequencing linkage maps of two Synthetic W7984×Opata reference populations provide insights into wheat structural diversity. Sci Rep 9:1793. https://doi.org/10.1038/s41598-018-38111-3

Haile TA, Heidecker T, Wright D, Neupane D, Ramsay L, Vandenberg A, Bett KE (2020) Genomic selection for lentil breeding: empirical evidence. Plant Genome 13:e20002. https://doi.org/10.1002/tpg2.20002

Hamwieh A, Udapa SM, Choumane W,Sarker A, Dreyer F, Jung C, BaumM (2005). A genetic linkage map of lentil based on microsatellite and AFLP markers and localization of Fusarium vascular wilt resistance. Theor Appl Genet110:669–677. https://doi.org/10.1007/s00122-004-1892-5

Hoerster G, Wang N, Ryan L, Wu E, Anandet A et al (2020) Use of non-integrating Zm-Wus2 vectors to enhance maize transformation Non-integrating WUS2 enhances transformation. In Vitro Cell Dev Biol-Plant 56:265–279. https://doi.org/10.1007/s11627-019-10042-2

Hunter PJ, Taylor JD (2006) Patterns of interaction between isolates of three pathovars of *Pseudomonas syringae* and accessions of a range of host and nonhost legume species. Plant Pathol 55:46–53. https://doi.org/10.1111/j.1365-3059.2005.01314.x

Henríquez-Valido P, Morales J, Vidal-Matutano P, Santana-Cabrera J, Rodríguez Rodríguez A (2019) Arqueoentomología y arqueobotánica de los espacios de almacenamiento a largo plazo: el granero de Risco Pintado, Temisas (Gran Canaria). Trabajos de Prehistoria 76:120–137. https://doi.org/10.3989/tp.2019.12229

Hernandez-Bello MA, Chilvers MI, Akamatsu H, Peever TL (2006) Host specificity of *Ascochyta* spp. infecting legumes of the Viciae and Cicerae tribes and pathogenicity of an interspecific hybrid. Phytopathology 96:1148–1156. https://doi.org/10.1094/PHYTO-96-1148

Ijaz U, Ayliffe M, Adhikari K, Bariana H, Bansal U (2020) Australian *Uromyces viciae-fabae*; host and nonhost interaction among cultivated grain legumes. Plant Pathol 69:1227–1236. https://doi.org/10.1111/ppa.13222

Jain S, Porter LD, Kumar A, Mir RR, Eigenbrode SD, McPhee KE (2014) Molecular and phenotypic characterization of variation related to pea enation mosaic virus resistance in lentil (*Lens culinaris* Medik.). Can J Plant Sci 94:1333–1344. https://doi.org/10.4141/CJPS-2014-077

Jurado Expósito M, García Torres L, Castejón Muñoz M (1997) Broad bean and lentil seed treatments with imidazolinones for the control of broomrape (*Orobanche crenata*). J Agric Sci 129:307–314. https://doi.org/10.1017/S0021859697004723

Kamboj RK, Pandey MP, Chaube HS (1990) Inheritance of resistance to Fusarium wilt in Indian lentil germplasm (*Lens culinaris* Medik.). Euphytica 50:113–117

Kaur S, Cogan NOI, Pembleton LW, Shinozuka M, Savin KW, Materne M, Forster JW (2011) Transcriptome sequencing of lentil based on second-generation technology permits large-scale unigene assembly and SSR marker discovery. BMC Genomics 12:265. http://www.biomedcentral.com/1471-2164/12/265

Keneni G, Ahmed S (2016) Genetic options for combating biotic stresses in cool-season food legumes. Indian J Genet 76:437–450. https://doi.org/10.5958/0975-6906.2016.00062.6

Khan Z, Gautam NK, Gawade BH, Dubey SC (2017) Screening of lentil (*Lens culinaris* Medik.) germplasm for resistance to root-knot nematode *Meloidogyne incognita.* Indian J Genet 77:408–413. https://doi.org/10.5958/0975-6906.2017.00055.4

Khatib F, Makris A, Yamaguchi-Shinozaki K, Kumar S, Sarker A, Erskine W, Baum M (2011) Expression of the *DREB1A* gene in lentil (*Lens culinaris* Medik. subsp. *culinaris*) transformed with the *Agrobacterium* system. Crop Pasture Sci 62:488–495. https://doi.org/10.1071/CP10351

Khazaei H, Caron CT, Fedoruk M, Diapari M, Vandenberg A, Coyne CJ, McGee R, Bett KE (2016) Genetic diversity of cultivated lentil (*Lens culinaris* Medik.) and its relation to the world's agro-ecological zones. Front Plant Sci 7:1093. https://doi.org/10.3389/fpls.2016.01093

Khazaei H, Fedoruk M, Caron CT, Vandenberg A, Bett KE (2018) Single nucleotide polymorphism markers associated with seed quality characteristics of cultivated lentil. Plant Genome 11:170051. https://doi.org/10.3835/plantgenome2017.06.0051

Kim W, Peever TL, Park JJ, Park C-M, Ganget DR et al (2016) Use of metabolomics for the chemotaxonomy of legume associated *Ascochyta* and allied genera. Sci Rep 6:20192. https://doi.org/10.1038/srep20192

Ko DK, Brandizzi F (2020) Network-based approaches for understanding gene regulation and function in plants. Plant J 104:302–317. https://doi.org/10.1111/tpj.14940

Khorramdelazad M, Bar I, Whatmore P, Smetham G, Bhaaskaria V et al (2018) Transcriptome profiling of lentil (*Lens culinaris*) through the first 24 hours of *Ascochyta lentis* infection reveals key defence response genes. BMC Genomics 19:108. https://doi.org/10.1186/s12864-018-4488-1

Kumar H, Singh A, Dikshit HK, Mishra GP, Aski M, Meena MC, Kumar S (2019a) Genetic dissection of grain iron and zinc concentrations in lentil (*Lens culinaris* Medik.). J Genet 98:66. https://doi.org/10.1007/s12041-019-1112-3

Kumar J, Gupta D (2020) Prospects of next generation sequencing in lentil breeding. Mol Biol Report 47:9043–9053. https://doi.org/10.1007/s11033-020-05891-9

Kumar J, Gupta DS, Tiwari P (2020) Lentil breeding in genomic era: Present status and future prospects. In: Gosal SS, Wani SH (eds) Accelerated plant breeding Vol 3. Springer Nature, Switzerland, pp 193–209.

Kumar J, Gupta S, Gupta DS, Singh NP (2018) Identification of QTLs for agronomic traits using association mapping in lentil. Euphytica 214:75. https://doi.org/10.1007/s10681-018-2155-x

Kumar J, Agrawal SK, Gupta DS, Dubey S, Gupta S, Gupta P (2019b) Molecular marker assisted gene pyramiding. In: Singh M (ed) Lentils. Academic Press, pp 125–139. https://doi.org/10.1016/B978-0-12-813522-8.00007-8

Kumar J, Gupta DS, Baum M, Varsheny RK, Kumar S (2021) Genomics-assisted lentil breeding: current status and future strategies. Legum Sci e71. https://doi.org/10.1002/leg3.71

Kumar M, Yusuf MA, Nigam M, Kumar M (2018) An update on genetic modification of chickpea for increased yield and stress tolerance. Mol Biotechnol 60:651–663. https://doi.org/10.1007/s12033-018-0096-1

Kumar MS, Jogeswar P (2020) Expression of plant α-amylase inhibitor (*αAI*) genes revealing bruchid resistance in legume crops. Res J Biotechnol 15:145–153

Kumar S, Rajendran K, Kumar J, Hamwieh A, Baum M (2015) Current knowledge in lentil genomics and its application for crop improvement. Front Plant Sci 6:78. https://doi.org/10.3389/fpls.2015.00078

Kumari J, Ahmad R, Chandra S (2007) Identification and pedigree analysis of lentil (*Lens culinaris*) genotypes tolerant to black aphids (*Aphis craccivora*). Indian J Agric Sci 77:544–545

Ladizinsky G (1993) Wild lentils. Crit Rev Plant Sci 12:169–184

Ladizinsky G, Abbo S (2015) The genus *Lens*. In: Ladizinsky G, Abbo S (eds) The search for wild relatives of cool season legumes. Springer, Cham, Switzerland, pp 1–28. https://doi.org/10.1007/978-3-319-14505-1_1

Laflamme B, Dillon MM, Martel A, Almeida RND, Desveaux D, Guttman DS (2020) The pan-genome effector-triggered immunity landscape of a host-pathogen interaction. Science 367:763–768. https://doi.org/10.1126/science.aax4079

Laserna-Ruiz I, De-Los-Mozos-Pascual M, Santana-Meridas O, Sanchez-Vioque R, Rodriguez-Conde MF (2012) Screening and selection of lentil (*Lens* Miller) germplasm resistant to seed bruchids (*Bruchus* spp.). Euphytica 188:153–162. https://doi.org/10.1007/s10681-012-0752-7

Laskar RA, Khan S, Deb CR, Tomlekova N, Wani MR, Raina A, Amin R (2019) Lentil (*Lens culinaris* Medik.) diversity, cytogenetics and breeding. In: Al-Khayri JM, Jain SM, JohnsonDV (eds) Advances in plant breeding strategies: legumes. Springer, Cham. https://doi.org/10.1007/978-3-030-23400-3_9

Lee RC, Farfan-Caceres L, Debler JW, Williams AH, Syme RA, Henares BM (2021) Reference genome assembly for Australian *Ascochyta lentis* isolate Al4. Genes Genomes Genet 11:jkab006. https://doi.org/10.1093/g3journal/jkab006

Leht M, Jaaska V (2019) Phylogenetic position of *Vicia montbretii* and *Lens* species in relation to *Vicia* subgenus *Cracca* (Fabaceae): morphological and isozyme evidence. Feddes Repertorium 130:272–288. https://doi.org/10.1002/fedr.201800019

Liber M, Duarte I, Maia AT, Oliveira HR (2021) The history of lentil (*Lens culinaris* subsp. *culinaris*) domestication and spread as revealed by genotyping-by-sequencing of wild and landrace accessions. Front Plant Sci 12:628439. https://doi.org/10.3389/fpls.2021.628439

Lombardi M, Materne M, Cogan NOI, Rodda M, Daetwyler HD, Slater AT, Forster JW, Kaur S (2014) Assessment of genetic variation within a global collection of lentil (*Lens culinaris* Medik.) cultivars and landraces using SNP markers. BMC Genetics 15:150. https://doi.org/10.1186/s12863-014-0150-3

Lulsdorf MM, Banniza S (2018) Rapid generation cycling of an F_2 population derived from a cross between *Lens culinaris* Medik. https://doi.org/10.1111/pbr.12612

Ma Y, Marzougui A, Coyne CJ, Sankaran S. Main, D et al (2020) Dissecting the genetic architecture of Aphanomyces root rot resistance in lentil by QTL mapping and genome-wide association study. Intl J Mol Sci 21:2129. https://doi.org/10.3390/ijms21062129

Maher MF, Nasti RA, Vollbrecht M, Starker CG, Clark MD, Voytas DF (2020) Plant gene editing through de novo induction of meristems. Nat Biotechnol 38:84–89. https://doi.org/10.1038/s41587-019-0337-2

Makkouk KM, Kumari SG (2009) Epidemiology and integrated management of persistently transmitted aphid-borne viruses of legume and cereal crops in West Asia and North Africa. Virus Res 141:209–218. https://doi.org/10.1016/j.virusres.2008.12.007

Makkouk KM, Kumari SG, van Leur JAG, Jones RAC (2014) Control of plant virus diseases in cool-season grain legume crops. In: Loebenstein G, Katis N (eds) Advances in virus research 90:208–253, Academic Press, Burlington. ISBN: 978-0-12-801246-8

Malhotra N, Panatu S, Singh B, Negi N, Singh D, Rahul C (2019) Genetic resources: collection, conservation, characterization and maintenance. In: M Singh (ed) Lentils: potential resources for enhancing genetic gains. Elsevier, pp 21–41. https://doi.org/10.1016/B978-0-12-813522-8.00003-0

Martín-Sanz A, Pérez de la Vega M, Caminero C (2011). Resistance to *Pseudomonas syringae* in a collection of pea germplasm under field and controlled conditions. Plant Pathol 129:57–69. https://doi.org/10.1111/j.1365-3059.2011.02522.x

Martín-Sanz A, Palomo JL, Pérez de la Vega M, Caminero C (2012) Characterization of *Pseudomonas syringae* pv. *syringae* isolates associated with bacterial blight in *Lathyrus* spp. and sources of resistance. Eur J Plant Pathol 134:205–216. https://doi.org/10.1007/s10658-012-9980-x

Marzougui A, Ma Y, Zhang CY, Mcgee RJ, Coyne CJ, Main D, Sankaran S (2019) Advanced imaging for quantitative evaluation of Aphanomyces root rot resistance in lentil. Front Plant Sci 10:383. https://doi.org/10.3389/fpls.2019.00383

Marzougui A, Ma Y, McGee RJ, Khot LR, Sankaran S (2020) Generalized linear model with elastic net regularization and convolutional neural network for evaluating Aphanomyces root rot severity in lentil. Plant Phenom 2020:2393062. https://doi.org/10.34133/2020/2393062

Mekonnen F, Mekbib F, Kumar S, Ahmed S, Chahoe RK et al (2014) Identification of molecular markers associated with rust (*Uromyces vicia-fabae* Pers.) resistance genes in lentil (*Lens culinaris* subsp. *culinaris*). Can J Crop Prot 2:27–36

Mikic A (2019) Lexicon of pulse crops. CRC Press, Boca Raton, FL

Mishra GP, Mishra SK, Tiwari SK, Kumar A (2008) Inheritance of lentil (*Lens culinaris*) resistance to rust (*Uromyces fabae*). Indian J Agric Sci 78:994–996

Mishra SK, Sharma B, Sharma SK (2007) Genetics and cytogenetics of lentil. In: Yadav SS, McNeil D, Stevenson PC (eds) Lentil. An ancient crop for modern times. Springer, Dordrecht, The Netherlands, pp 187–208

Morgil H, Tardu M, Cevahir G, Kavakli İH (2019) Comparative RNA-seq analysis of the drought-sensitive lentil (*Lens culinaris*) root and leaf under short- and long-term water deficits. Funct Integr Genom 19:715–727. https://doi.org/10.1007/s10142-019-00675-2

Muehlbauer FJ, Kaiser WJ, Clement SL, Summerfield RJ (1995) Production and breeding oflentil. Adv Agron 54:283–332

Muehlbauer FJ, Weeden NF, Hoffman DL (1989) Inheritance and linkage relationships of morphological and isozyme loci in lentil (*Lens* Miller). J Hered 80:298–303

Murube E, Campa A, Ferreira JJ (2019) Integrating genetic and physical positions of the anthracnose resistance genes described in bean chromosomes Pv01 and Pv04. PLoS One 14:e0212298. https://doi.org/10.1371/journal.pone.0212298

Mutlu C, Karaca V, Eren S, Buyuk M, Gozuacik C, Duman M, Bayram Y, Bolu H, Kutuk H (2016) Chalky spot damage caused by stink bugs on red lentil seeds in Southeast Anatolia Region, Turkey. Legume Res 39:623–629. https://doi.org/10.18805/lr.v0iOF.9437

Mwakutuya E, Banniza S (2010) Influence of temperature and wetness periods on the development of Stemphylium blight on lentil. Plant Dis 94:1219–1224. https://doi.org/10.1094/PDIS-12-09-0804

Negussie T, Pretorious ZA (2012) Lentil rust: Present status and future prospects. Crop Protec 32:119–128

Ngaki MN, Sahoo DK, Wang B, Bhattacharyya MK (2021) Overexpression of a plasma membrane protein generated broad spectrum immunity in soybean. Plant Biotechnol J 19:502–516. https://doi.org/10.1111/pbi.13479

Ogutcen E, Ramsay L, von Wettberg EB, Bett KE (2018) Capturing variation in *Lens* (Fabaceae): developmentand utility of an exome capture array for lentil. Appl Plant Sci 6:e1165. https://doi.org/10.1002/aps3.1165

Olfert O, Slinkard A (1999) Grasshopper (Orthoptera: Acrididae) damage to flowers and pods of lentil (*Lens culinaris* L.). Crop Protec 18:527–530

Pala F (2019) A survey on weed management in dry lentil fields. Appl Ecol Envirom Res 17:13513–13521. https://doi.org/10.15666/aeer/1706_1351313521

Paudel S, Bechinski EJ, Stokes BS, Pappu HR, Eigenbrode SD (2018) Deriving economic models for pea aphid (Hemiptera: Aphididae) as a direct-pest and a virus-vector on commercial lentils. J Econ Entomol 111:2225–2232. https://doi.org/10.1093/jee/toy188

Pavan S, Bardaro N, Fanelli V, Marcotrigiano AR, Mangini G et al (2019). Genotyping by sequencing of cultivated lentil (*Lens culinaris* Medik.) highlights population structure in the Mediterranean gene pool associated with geographic patterns and phenotypic variables. Front Genet 10:872. https://doi.org/10.3389/fgene.2019.00872

Peever TL (2007) Role of host specificity in the speciation of *Ascochyta* pathogens of cool season food legumes. Eur J Plant Pathol 119:119–126. https://doi.org/10.1007/s10658-007-9148-2

Peña EJ, Robles Luna G, Heinlein M (2021) *In vivo* imaging of tagged mRNA in plant tissues using the bacterial transcriptional antiterminator BglG. Plant J 105:271–282. https://doi.org/10.1111/tpj.15035

Pérez de la Vega M (2016) Legumes' contributions to genetic research, a historical perspective from Mendelism up to massive sequencing. Arbor 192:a318. https://doi.org/10.3989/arbor.2016.779n3008.

Pérez de la Vega M, Fratini R, Muehlbauer F (2011) Lentil. In: Pérez de la Vega M, Torres AM, CuberoJI, Kole C (eds) Genetics, genomics and breeding of cool season grain legumes. CRC Press, Boca Raton, FL, pp 98–150

Pierre RE, Millar RL (1965) Histology of pathogen-suscept relationship of *Stemphylium botryosum* and alfalfa. Phytopathology 55:909–914

Pobozniak M (2011) The occurrence of thrips (Thysanoptera) on food legumes (Fabaceae). J Plant Dis Protec 118:185–193. https://doi.org/10.1007/BF03356402

Podder R, Banniza S, Vandenberg A (2013) Screening of wild and cultivated lentil germplasm for resistance to stemphylium blight. Plant Genet Resour 11:26–35

Polanco C, Sáenz de Miera LE, González AI, García P, Fratini R, Vaquero F, Vence FJ, Pérez de la Vega M (2019) Construction of a high-density interspecific (*Lens culinaris* x *L. odemensis*) genetic map based on functional markers for mapping morphological and agronomical traits, and QTLs affecting resistance to *Ascochyta* in lentil. PLoS One 14:e0214409. https://doi.org/10.1371/journal.pone.0214409

Polanco MC, Ruiz ML (1997) Effect of benzylaminopurine on in vitro and in vivo root development in lentil, *Lens culinaris* Medik. Plant Cell Rep 17:22–26. https://doi.org/10.1007/s002990050345

Petterson DS, Sipsas S, Mackintosh JB (1997) The chemical composition and nutritive value of Australian pulses (2nd edn). Grain Research & Development Corporation, Camberra, Australia, p 65

Pouralibaba HR (2017) Resistance to *Fusarium oxysporum* f. sp. *lentis* in lentil (*Lens culinaris):* mechanisms of resistance and pathogenic variability. PhD Dissertation, University of Córdoba

Pratap A, Prajapati U, Singh CM, Gupta S, Rathore M et al (2018) Potential, constraints and applications of in vitro methods in improving grain legumes. Plant Breed 137:235–249. https://doi.org/10.1111/pbr.12590

Ramegowda V, Senthil-Kumar M (2015) The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. J Plant Physiol 176:47–54. https://doi.org/10.1016/j.jplph.2014.11.008

Ramsay L, Chan C, Sharpe AG, Cook DR, Penmetsa RV, Chang P, Coyne C, McGee R, Main D, Edwards D, Kaur S, Vandenberg A, Bett KE (2016) *Lens culinaris* CDC Redberry genome assembly v1.2. https://knowpulse.usask.ca/genome-assembly/Lc1.2

Ramsay L, Koh C, Konkin D, Cook D, Penmetsa V, Dongying G, Coyne C, Humann J, Kaur S, Dolezel J, Bett KE (2019) *Lens culinaris* CDC Redberry genome assembly v2.0. https://knowpulse.usask.ca/genomeassembly/Lcu.2RBY

Rana JC, Gautam NK, Gayacharan SM, Yadav R et al (2016) Genetic resources of pulse crops in India: an overview. Indian J Genet 76:420–436. https://doi.org/10.5958/0975-6906.2016.00061.4

Rana M, Sood A, Hussain W, Kaldate R, SharmaTR et al (2019) Gene pyramiding and multiple character breeding. In: Singh M (ed) Lentils. Academic Press, pp 83–124. https://doi.org/10.1016/B978-0-12-813522-8.00006-6

REX Consortium (2016) Combining selective pressures to enhance the durability of disease resistance genes. Front Plant Sci 7:1916. https://doi.org/10.3389/fpls.2016.01916

Rey-Baños R, Sáenz de Miera LE, García P, Pérez de la Vega M (2017) Obtaining retrotransposon sequences, analysis of their genomic distribution and use of retrotransposon-derived genetic markers in lentil (*Lens culinaris* Medik.). PLoS One 12:e0176728. https://doi.org/10.1371/journal.pone.0176728

Rinehold J, Bell N, Waters T (2018) (Latest revision).Vegetable crops. Vegetable pests. K12-K212. PNW Insect Management Handbook. https://pnwhandbooks.org/insect

Robinson RA (1997) The acceptance of horizontal resistance in crops. Biotechnol Develop Monitor 33:2–4

Rodda MS, Davidson J, Javid M, Sudheesh S, Blake S, Forster JW, Kaur S (2017) Molecular breeding for Ascochyta blight resistance in lentil: Current progress and future directions. Front Plant Sci 8:1136. https://doi.org/10.3389/fpls.2017.01136

Rubeena, Taylor PWJ, Ades PK, Ford R (2006) QTL mapping of resistance in lentil (*Lens culinaris* ssp. *culinaris*) to ascochyta blight (*Ascochyta lentis*). Plant Breed 125:506–512. https://doi.org/10.1111/j.1439-0523.2006.01259.x

Rubiales D, Fernandez-Aparicio M (2012) Innovations in parasitic weeds management in legume crops a review. Agron Sustain Dev 32:433–449. https://doi.org/10.1007/s13593-011-0045-x

Rubiales D, Fernandez-Aparicio M, Rodriguez MJ (2008) First report of crenate broomrape (*Orobanche crenata*) on lentil (*Lens culinaris*) and common vetch (*Vicia sativa*) in Salamanca Province, Spain. Plant Dis 92:1368–1368. https://doi.org/10.1094/PDIS-92-9-1368B

Rubiales D, Fondevilla S, Chen W, Gentzbittel L, Higgins TJV, Castillejo MA, Singh KB, Rispail N (2015) Achievements and challenges in legume breeding for pest and disease resistance. Crit Rev Plant Sci 34:195–236. https://doi.org/10.1080/07352689.2014.898445

Rubiales D, Rojas-Molina MM, Sillero JC (2013) Identification of pre- and posthaustorial resistance to rust (*Uromyces viciae-fabae*) in lentil (*Lens culinaris*) germplasm. Plant Breed 132:676–680. https://doi.org/10.1111/pbr.12096

Rubiales D, Sillero JC, Emeran AA (2013) Response of vetches (*Vicia* spp.) to specialized forms of *Uromyces vicia-fabae* and to *Uromyces pisi*. Crop Protec 46:38–43

Rubiales D, Verkeleij J, Vurro M, Murdoch AJ, Joel DM (2009) Parasitic plant management in sustainable agriculture. Weed Res 49(Suppl. 1):1–5. https://doi.org/10.1111/j.1365-3180.2009.00741.x

Sagar D, Dhall H (2018) Legumes: potential source of entomotoxic proteins- a review. Legume Res 41:639–646

Saha GC, Sarker A, Chen W, Vandemark GJ, Muehlbauer FJ (2010) Identification of markers associated with genes for rust resistance in *Lens culinaris* Medik. Euphytica 175:261–265. https://doi.org/10.1007/s10681-010-0187-y

Saha GC, Sarker A, Chen W, Vandemark G, Muehlbauer FJ (2010) Inheritance and linkage map positions of genes conferring resistance to stemphylium blight in lentil. Crop Sci 50:1831–1839. https://doi.org/10.2135/crop-sci2009.12.0709

Sanderson L-A, Caron CT, Tan R, Shen Y, Liu R, Bett KE (2019) KnowPulse: a web-resource focused on diversity data for pulse crop improvement. Front Plant Sci 10:965. https://doi.org/10.3389/fpls.2019.00965

Sari E, Bhadauria V, Ramsay L et al (2018) Defense responses of lentil (*Lens culinaris*) genotypes carrying non-allelic ascochyta blight resistance genes to *Ascochyta lentis* infection. PLoS One 13:1–27. https://doi.org/10.1371/journal.pone.0204124

Sari E, Bhadauria V, Vandenberg A, Banniza S (2017) Genotype-dependent interaction of lentil lines with *Ascochyta lentis*. Front Plant Sci 8:764. https://doi.org/10.3389/fpls.2017.00764

Sax K (1923) The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552–560

Schaefer H, Hechenleitner P, Santos-Guerra A, Menezes de Sequeira M, Pennington RT, Kenicer G, Carine MA (2012) Systematics, biogeography, and character evolution of the legume tribe Fabeae with special focus on the middle-Atlantic island lineages. BMC Evol Biol 12:250. http://www.biomedcentral.com/1471-2148/12/250

Scheben A, Wolter F, Batley J, Puchta H, Edwards DC (2017) Towards CRISPR/Cas crops—bringing together genomics and genome editing. New Phytol 216:682–698. https://doi.org/10.1111/nph.14702

Schneider K (2005) Mapping populations and principles of genetic mapping. In: Meksem K, Kahl G (eds) The handbook of plant genome mapping genetical and physical mapping. Wiley-VCH Verlag GmbH Co KGaA, Weinheim, pp 3–21

Scott MF, Ladejobi O, Amer S, Bentley AR, Biernaskie J, Boden SA et al (2020) Multi-parent populations in crops: a toolbox integrating genomics and genetic mapping with breeding. Heredity 125:396–416. https://doi.org/10.1038/s41437-020-0336-6

Sharma B (2009) Genetics of economic traits. In: Erskine W, Muehlbauer FJ, Sarker A, Sharma B (eds) The lentil. Botany, production and uses. CAB International, pp 76-101

Sharma H, Srivastava C, Durairaj C, Gowda C (2010) Pest Management in grain legumes and climate change. In: Yadav S, Redden R (eds) Climate change and management of cool season grain legume crops. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-3709-1_7

Sharpe AG, Ramsay L, Sanderson LA, Fedoruk M, Clarke WE, Rong L et al (2013) Ancient orphan crop joins modern era: gene-based SNP discovery and mapping in lentil. BMC Genom 14:192–120. https://doi.org/10.1186/1471-2164-14-192

Singh A, Sharma V, Dikshit HK, Aski M, Kumar H, Thirunavukkarasu N et al (2017a) Association mapping unveils favorable alleles for grain iron and zinc concentrations in lentil (*Lens culinaris* subsp. *culinaris*). PLoS One 12:e0188296. https://doi.org/10.1371/journal.pone.0188296

Singh D, Singh CK, Taunk J, Jadon V, Pal M, Gaikwad K (2019) Genome wide transcriptome analysis reveals vital role of heat responsive genes in regulatory mechanisms of lentil (*Lens culinaris* Medikus). Sci Rep 9:1–19. https://doi.org/10.1038/s41598-019-49496-0

Singh D, Singh CK, Taunk J, Tomar RSS, Chaturvedi AK, Gaikwad K, Pal M (2017b) Transcriptome analysis of lentil (*Lens culinaris* Medikus) in response to seedling drought stress. BMC Genom 18:206. https://doi.org/10.1186/s12864-017-3596-7

Singh J, Sirari A, Singh H, Kumar A, Jaidka M, Mandahal KS, Kumar S, Singh S (2021) Identifying and validating SSR markers linked with rust resistance in lentil (*Lens culinaris*). Plant Breed 00:1–9. https://doi.org/10.1111/pbr.12917

Singh M, Bisht IS, Kumar S, Dutta M, Bansal KC, Karale M et al (2014) Global wild annual *Lens* collection: a potential resource for lentil genetic base broadening and yield enhancement. PLoS One 9:e107781. https://doi.org/10.1371/journal.pone.0107781

Singh M, Kumar S, Basandrai AK, Basandrai D, Malhotra N, Saxena DR, Gupta D, Sarker A, Singh K (2020) Evaluation and identification of wild lentil accessions for enhancing genetic gains of cultivated varieties. PLoS One 15:e0229554. https://doi.org/10.1371/journal.pone.0229554

Singh M, Rana JC, Singh B, Kumar S, Saxena DR et al (2017c) Comparative agronomic performance and reaction to *Fusarium* wilt of *Lens culinaris* x *L. orientalis* and *L. culinaris* x *L. ervoides* derivatives. Front Plant Sci 8:1162. https://doi.org/10.3389/fpls.2017.01162

Singh M, Sharma SK, Singh B, Malhotra N, Chandora R, Sarker A, Singh K, Gupta D (2018) Widening the genetic base of cultivated gene pool following introgression from wild *Lens* taxa. Plant Breed 137:470–485. https://doi.org/10.1111/pbr.12615

Singh M, Rana MK, Kumar K, Bisht IS, Dutta M et al (2013) Broadening the genetic base of lentil cultivars through inter-sub-specific and interspecific crosses of *Lens* taxa. Plant Breed 132:667–675. https://doi.org/10.1111/pbr.12089

Singh RK, Prasad A, Muthamilarasan M, Parida SK, Prasad M (2020) Breeding and biotechnological interventions for trait improvement: status and prospects. Planta 252:54. https://doi.org/10.1007/s00425-020-03465-4

Smykal P, Coyne CJ, Ambrose MJ, Maxted N, Schaefer H et al (2015) Legume crops phylogeny and genetic diversity for science and breeding. Crit Rev Plant Sci 34:43–104. https://doi.org/10.1080/07352689.2014.897904

Smykal P, Kenicer G, Flavell AJ, Corander J, Kosterin O et al (2011) Phylogeny, phylogeography and genetic diversity of the *Pisum* genus. Plant Genet Resour 9:4–18. https://doi.org/10.1017/S147926211000033X

Solanki S, Ameen G, Zhao J, Flaten J, Borowicz P, Brueggeman RS (2020) Visualization of spatial gene expression in plants by modified RNA scope fluorescent in situ hybridization. Plant Methods 16:71. https://doi.org/10.1186/s13007-020-00614-4

Solleti SK, Bakshi S, Purkayastha J, Panda SK, Sahoo L (2008) Transgenic cowpea (*Vigna unguiculata*) seeds expressing a bean α-amylase inhibitor 1 confer resistance to storage pests, bruchid beetles. Plant Cell Report 27:1841–1850. https://doi.org/10.1007/s00299-008-0606-x

Stevenson PC, Dhillon MK, Sharma HC, El-Bouhssini M (2007) Insect pest of lentil and their management. In: Yadav SS, McNeil D, Stevenson PC (eds) Lentil. An ancient crop for modern times. Springer, Dordrecht, The Netherlands, pp 31–348

Sudheesh S, Rodda MS, Davidson J, Javid M, Stephens A, Slater AT, Cogan NOI, Forster JW, Kaur S (2016) SNP-based linkage mapping for validation of QTLs for resistance to ascochyta blight in lentil. Front Plant Sci 7:1604. https://doi.org/10.3389/fpls.2016.01604

Sudheesh S, Verma P, Forster JW, Cogan NOI, Kaur S. (2016b) Generation and characterisation of a reference transcriptome for Lentil (*Lens culinaris* Medik.). Intl J Mol Sci 17:1887. https://doi.org/10.3390/ijms17111887

Suvorova GN, Ikonnikov AV (2014) Characteristics of recombinant lines of lentil *L. culinaris* × *L. orientalis*. Russian Agric Sci 40:22–26

Tahir M, Muehlbauer FJ (1994) Gene mapping in lentil with recombinant inbred lines. J Hered 85:306–310

Tar'an B, Buchwaldt L, Tullu A, Banniza S, Warkentin TD, Vandenberg A (2003) Using molecular markers to pyramid genes for resistance to ascochyta blight and anthracnose in lentil (*Lens culinaris* Medik). Euphytica 134:223–230. https://doi.org/10.1023/B:EUPH.0000003913.39616.fd

Thor K, Jiang S, Michard E, George J, Scherzer S et al (2020) The calcium-permeable channel OSCA1.3 regulates plant stomatal immunity. Nature 585:569–573. https://doi.org/10.1038/s41586-020-2702-1

Tivoli B, Banniza S (2007) Comparison of the epidemiology of ascochyta blights on grain legumes. Eur J Plant Pathol 119:59–76. https://doi.org/10.1007/s10658-007-9117-9

Tullu A, Banniza S, Tar'an B, Warkentin T, Vandenberg A (2010) Sources of resistance to ascochyta blight in wild species of lentil (*Lens culinaris* Medik.). Genet Resour Crop Evol 57:1053–1063. https://doi.org/10.1007/s10722-010-9547-7

Tullu A, Buchwaldt L, Warkentin T, Taran B, Vandenberg A (2003) Genetics of resistance to anthracnose and identification of AFLP and RAPD markers linked to the resistance gene in PI 320937 germplasm of lentil (*Lens culinaris* Medikus). Theor Appl Genet 106:428–434. https://doi.org/10.1007/s00122-002-1042-x

Tullu A, Tar'an B, Breitkreutz C, Banniza, Warkentin TD et al (2006) A quantitative-trait locus for resistance to ascochyta blight [*Ascochyta lentis*] maps close to a gene for resistance to anthracnose [*Colletotrichum truncatum*] in lentil. Can J Plant Pathol 28:588–595. https://doi.org/10.1080/07060660609507337

Urbano G, Porres, JM, Frías J, Vidal-Valverde C (2007) Nutricional value. In: Yadav SS, McNeil D, Stevenson PC (eds) Lentil. An ancient crop for modern times. Springer, Dordrecht, The Netherlands, pp 47–93

Valenzuela I, Hoffmann AA (2015) Effects of aphid feeding and associated virus injury on grain crops in Australia. Austral Entomol 54:292–305. https://doi.org/10.1111/aen.12122

Vavilov NI (1922) The law of homologous series in variation. J Genet 12:47–78

Verma P, Shah N, Bhatia S (2013) Development of an expressed gene catalogue and molecular markers from the *de novo* assembly of short sequence reads of the lentil (*Lens culinaris Medik*.) transcriptome. Plant Biotechnol J 11:894–905. https://doi.org/10.1111/pbi.12082

Vlachostergios DN, Lithourgidis AS, Baxevanos DV, Mavromatis AG, Noulas CS, Roupakias DG (2018) Evaluation of lentil varieties and farming system effect on seed damage and yield loss due to bruchid (*Bruchus* spp.) infestation. Crop Pasture Sci 69:387–394. https://doi.org/10.1071/CP17309

Walkowiak S, Gao L, Monat C, Haberer G, Kassa MT et al (2020) Multiple wheat genomes reveal global variation in modern breeding. Nature 588:277–283. https://doi.org/10.1038/s41586-020-2961-x

Wang F, Flanagan J, Su N, Wang LC, Bui S et al (2012) RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J Mol Diagn 14:22–29. https://doi.org/10.1016/j.jmoldx.2011.08.002

Wang N, Hatcher DW, Toews R, Gawalko EJ (2009) Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). LWT-Foof Sci Technol 42:842–848

Wong MML, Gujaria-Verma N, Ramsay L, Yuan HY, Caron C, Diapari M, Vandenberg A, Bett KE (2015) Classification and characterization of species within the genus *Lens* using genotyping-by-sequencing (GBS). PLoS One 10:e0122025. https://doi.org/10.1371/journal.pone.0122025

Ye G, McNeil DL, Hill GD (2002) Breeding for resistance to lentil Ascochyta blight. Plant Breed 121:185–191. https://doi.org/10.1046/j.1439-0523.2002.00705.x

Yilmaz Temel H, Gol D, Kahriman A, Tanyolac MB (2015) Single nucleotide polymorphism discovery through Illumina- based transcriptome sequencing and mapping in lentil. Turk J Agric for 39:470–488. https://doi.org/10.3906/tar-1409-70

Zamir D, Ladizinsky G (1984) Genetics of allozyme variants and linkage groups in lentil. Euphytica 33:329–336