

Lentil genetic improvement in Morocco: State of art of the program, major achievements and perspectives

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Lentil contributes to sustainable farming by biological fixation of nitrogen in soils, therefore reducing the use of chemical fertilizers. It enhances health and nutrition due to its grain rich in proteins, iron, zinc and other micronutrients. Furthermore, lentil is a staple food for a large proportion of the population, thus playing an important role in food security. In Morocco, lentil is grown in rainfed areas as part of the cereal-based cropping system. However, despite the importance of lentil, the country still relies on imports to cover local demand. Breeding towards high yielding, biotic and abiotic resistant and adapted varieties corresponding to farmer's needs are at utmost importance to enhance and sustain crop productivity. Clear review of the achievements, specific constraints analysis and improvement objectives definition could contribute to operational design of future prospects. Under this perspective, this paper is a review of lentil genetic improvement in Morocco.

Keyword: lentil, improved varieties, Morocco.

Introduction

Lentil (*Lens culinaris* Medik.) is one of the most important food legumes worldwide. It contributes to reduce malnutrition and to enhance health especially for low-income people in developing countries due to its grains consumed as staple food and are rich in proteins, fibers and some important micronutrients like iron and zinc (Carbonaro et al., 2015; Grusak 2009; Grusak and Coyne 2009). Furthermore, the crop provides a number of additional agronomic, environmental and socioeconomic benefits. As a leguminous crop, lentil is able to enhance soil fertility and thus contributes to farming sustainability by fixing atmospheric nitrogen in soils due to the symbiotic association of its roots with the bacterium *Rhizobium leguminosarum*.

In Morocco, lentil is currently grown as a rainfed crop in rotation with cereals, covering around 14 % of the area yearly dedicated to food legumes in the country. Within the legume group, it ranks third, after faba bean (42 %) and chickpea (20 %) (MAPM, 2015). Morocco is the second major producer of lentil in Africa, after Ethiopia, with an annual average production of about 38 200 t from an average cultivated area of 50 000 ha (FAOSTAT, 2017). Despite its importance in Morocco, lentil faces a number of constraints lowering its grain yield and profitability. To overcome these constraints and increase the production, a national breeding program aiming to develop improved varieties has been initiated by INRA-Morocco few decades before.

Constraints

Lentil production in Morocco is limited by several biotic and abiotic factors that result on low profitability causing serious decrease in cultivated areas dedicated to this crop. On a global scale, drought is a major constraint for crop production, especially in arid and semi-arid areas. In fact, as lentil is often cultivated in rainfed regions of Morocco, its productivity is frequently limited by irregular rainfall. Moreover, traditional farming based on poor adoption of efficient production techniques (sowing date, fertilizers and certified seeds of improved varieties) limits the potential of this crop and results in low production. Another major constraint is the limited use of mechanization due the weak adaptation of available traditional varieties and crop management used by farmers to the use of machines. This involves extensive needs for costly labor-workers for weeding and harvesting, decreasing the profitability of lentil. In addition, local populations and traditional varieties being used by farmers have long cycles in which flowering and pod onset are delayed toward late spring with low soil water availability and high temperatures causing significant increase of flower and pod abortion and thus reducing grain yield.

Among biotic constraints affecting lentil in Morocco, several diseases, parasitic plants and insects were reported to be important considering their impact on yield. Rust (caused by *Uromyces viciae fabae* (Pers.) J. Schröt), wilt (caused by *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen f. sp. *Lentis* Vasudeva and Srinivasan) and *Ascochyta* blight (caused by *Ascochyta fabae* Speg. f. sp. *lentis*) are the major damaging diseases.

The parasitic plant *Orobanche* (*Orobanche crenata*) is a major threat for lentil as well as for other food legumes such as faba bean and pea causing substantial damages. Complete crop failure due to *Orobanche* has been observed under severe infestations. To date, no accessible genetic resistance is available. Chemical control based on the use of low rates of a non-selective herbicide with a large spectrum (glyphosate) have shown effective success for controlling *Orobanche* in lentil fields. However, significant problems of phytotoxicity on lentil plants and other side effects of glyphosate remain major limitations for the extensive use of this herbicide (Tanji, 2013). On the other hand, as lentil is a dicotyledonous plant with high sensitivity to chemicals, the lack of the availability of selective herbicides resulted in weak chemical control of dicotyledonous weeds (Tanji, 2013). As a consequence, lentil fields in Morocco are often highly infested where both lentil biomass and grain yield are low as a result of high competition with weeds.

Bruchids cause substantial damages on lentil grains during storage limiting their quality and market value.

Improvement objectives

The overall objective of INRA-Morocco lentil breeding program has been to develop high-yielding varieties with improved characteristics. High grain yield, rust and *Ascochyta* blight resistance, early flowering and maturing, drought and cold tolerance as well as ability for machine harvesting were the main improvement objectives that have been targeted during first decades of the program.

Important results especially regarding high yield potential, rust and *Ascochyta* blight resistance, early flowering and maturing have been achieved (see the section below, Major results and achievements). However, a number of constraints that still limit lentil production need further breeding efforts to identify source of resistance/tolerance. In fact, priority is currently being given to such objective while keeping breeding for all other important characteristics as listed below:

- High yield
- Tolerance to abiotic stresses:

- High temperatures
- Drought
- Cold
- Tolerance to biotic stresses:
 - Orobanche
 - Rust
 - Fusarium
 - Anthracnose
 - Bruchids
- Ability for mechanical harvesting: Erect growth habit
- Plant architecture: less biomass, less ramifications, more pods/peduncle
- Height of the first pod to soil
- Specific adaptation to different agro-climatic zones of Morocco
- Herbicide tolerance
- Seed quality:
 - Nutritional (more proteins, iron, zinc, ...; less anti-nutritional factors: tannin, ...; reduced cooking time)
 - End-use quality requested for better use of lentil grains (end-users (processing, industry) new demands: seed shape, dimension, cotyledon color, ability of flour for making pasta and for mixing with wheat and other cereals...).

Among these objectives, tolerance to drought, high temperatures and Orobanche as well as the ability to machine harvesting are of utmost importance in the coming years. High priority is being given to design and to appropriate screening methods for these four characteristics. Combining these traits in high yielding, extra-early and diseases resistant varieties with good seed quality will contribute to enhance production potential and profitability of lentil in Morocco.

Breeding methodology

Conventional techniques have been used in Moroccan lentil breeding program. The breeding program is based on both modified pedigree and mass selection methods. The first step is germplasm development by collecting and introducing new genetic resources from international institutions (such as the International Center for Agricultural Research in the Dry Areas (ICARDA), and other gene banks and research institutes). These genetic resources are tested under specific nurseries (diseases, adaptation, and biotic and abiotic stresses). Selected lines from these nurseries could be used as parents in crossing blocks or directly introduced under yield trials after development of pure lines by single plant selection and auto-fertilization. After crossing, selection on segregation materials (F3-F7) based on single plant/row selection in superior families is carried

out following specific objectives. Advanced lines with fixed traits depending on targeted specific objectives from nurseries and segregating material are tested under multi-annual and multi-location yield trials (preliminary, advanced and national trials). Ultimately, few promising lines with confirmed improved characteristics compared to a local check are identified, selected and tested with farmers' participation in demonstration trials before being suggested for registration as varieties.

Three main contrasted agro-environments where lentil is being cultivated in Morocco were characterized as semi-arid areas (Abda-Chaouia region, western and north-central Morocco), favorable areas (Zair-Sais region, north-western Morocco) and high-altitude areas (middle Atlas Mountains, central Morocco) (Sakr, 2005). These environments are represented by INRA's experimental stations being used for screening and advancing genetic material under contrasted agro-climatic conditions: Dry areas (Jemaat Shaim and Sidi El Aidi Stations), favorable areas (Marchouch station) and high-altitude areas (Anneucer station). Prevalent biotic and abiotic stresses affecting lentil in each area were identified over 10 years of observations and survey (Sakr, 2005). Thus, drought, heat stress and rust were frequent in semi-arid areas while drought, rust, anthracnose and *Orobanche* were frequent in favorable areas. Cold stress and viruses were found to be present in high-altitude areas.

In addition to conventional breeding and characterization of genetic resources, it should be pointed out that recently, efforts have been deployed to incorporate the use of DNA markers for tagging economically important traits and for the characterization of landraces in the perspective of initiating marker-assisted selection protocols and enhanced use of genetic resources for breeding goals in the future (Idrissi et al., 2015a, 2015b, 2016b).

Major results and achievements

Considerable efforts of INRA Morocco have resulted in a strong national breeding program for lentil with wide international collaboration. Research activities under this program have yielded a number of improved varieties and advanced lines. Several selection criteria have been targeted under the three agro-environments (semi-arid, favorable and high-altitude areas) in field conditions and under greenhouse to overcome specific constraints. Significant genetic gain for important traits, for instance yield and rust resistance were obtained (Idrissi et al., 2019).

Rust and *Ascochyta* blight resistance have been successfully incorporated to the developed varieties and advanced lines through crossing and selection in prone environments. High yield and early maturity compared to local populations used by farmers were also combined with resistance to these diseases (Sakr et al., 2004 a, b; Sakr, 2005; Idrissi et al., 2019). Drought escape through early maturing varieties contributes to completing plant cycle (pod and seed filling) before the soil water decrease, a situation frequent in Morocco especially in semi-arid areas.

On the other hand, the use of biotechnological tools for breeding purposes has been initiated. In fact, important results regarding developed root systems, correlated above ground characteristics and associated genomic regions and linked DNA markers were obtained (Idrissi et al., 2015a, 2016b). These could help to enhance screening for drought avoidance-associated root traits in lentil improvement program in the perspective of development of drought tolerant varieties. Also, preliminary results about host differentiation and molecular variability of *Orobanche crenata* populations showing the specificity for lentil by lentil-grown *O. crenata* was reported by Ennami et al., (2017). This could be used in the future for designing screening studies.

Releases and germplasm

Registration of varieties

Continuous evaluation of germplasm and screening activities of INRA Morocco lentil breeding program have resulted in 9 improved varieties and several promising advanced lines. These varieties and their main characteristics are listed in table 1. Due to their respective improved characteristics, these varieties allowed an estimated breeding progress from 1989, the year of the first registration, to 2019, the year of the newest registration, of more than 35 kg ha⁻¹ yr⁻¹ as genetic gain for grain yield. This corresponds to an increase from 16% to 67% of the yield advantage over the local check (Idrissi et al., 2019).

Table 1: Lentil varieties registered in the official catalogue of plant varieties in Morocco

Varieties	Year of registration	Main characteristics
L241	1989	Small seeds, late maturing, yellow cotyledon
L561	1989	Large seeds, late maturing, yellow cotyledon
BAKRIA1	1989	Rust resistant, early maturing, large seeds, yellow cotyledon
BICHETTE2, 1	2000	High yield, moderate winter-hardy, resistant to rust, Ascochyta blight and wilt, yellow cotyledon
HAMRIA3, 1	2000	High yield, moderate winter-hardy, resistant to rust, Ascochyta blight and wilt, red-orange cotyledon
ZAARIA1	2003	High yielding, rust and Ascochyta blight resistant, red-orange cotyledon
ABDA1	2004	Early maturing, high yielding, rust resistant and semi erect growth habit, yellow cotyledon
CHAOUIA1	2004	Early maturing, high yielding, rust resistant and semi erect growth habit, yellow cotyledon
CHAKKOUF4, 1	2009	High yielding, Early maturing, rust and Ascochyta blight resistant, iron and zinc rich seeds, yellow cotyledon
Two promising advanced lines candidates for registration	2019	Extra early high yielding, resistant to rust and Aschochta blight, one with extra-large and the second with medium seed size

Table.

1: Idrissi et al., 2019; 2: Sakr et al. 2004a; 3: Sakr et al., 2004b; 4: Idrissi et al., 2012;

Characterization and valorization of local populations

The national gene bank held in INRA-Settat maintains a collection of Moroccan lentil landraces. Fifty-three landraces from this collection, together with 20 others from Mediterranean countries (Italy, Greece, and Turkey), were characterized using DNA markers (Simple Sequence Repeats and Amplified Fragment Length Polymorphism). Genetic differentiation according to agro-environmental origins (dry areas versus favorable and high-altitude areas) and preliminary results on marker-trait associations with drought tolerance were reported (Idrissi et al., 2015a,b; 2016a,b). Some of these local populations are being used as parents in crossing blocks of the breeding program. This allows oriented selection of genotypes to be included in breeding programs depending on specific objectives. Landraces from dry areas, especially those originating from Jemaât Shaim (one of the locations in Morocco where lentil has been grown under higher frequency of drought and heat), would result in greater genetic gain for drought and high temperatures' tolerance, while landraces from highlands (middle Atlas Mountains) would result in greater genetic gain for cold tolerance. On the other hand, genetic evidence for the differentiation of 'lentils of Ain

Sbit', grown in a small rural area known ancestrally for lentil cultivation, as a local product with a protected designation of origin (PDO) quality mark (produit de terroir) were obtained, thus offering efficient tools for enhanced valorization and for the protection of this landrace for the benefits of local farmers (Idrissi et al., 2015b).

Perspectives

Plant breeding programs are basically medium and long-term research programs that require designing appropriate working plans to solve current problems limiting crop production as well as future challenges. Thus, definition of breeding objectives depend on present constraints and demands but also on their expected evolution.

So far, lentil breeding efforts in Morocco have made resistant germplasm to two major diseases, namely rust and *Ascochyta* blight and early maturing germplasm with better adaptation to semi-arid areas available. These resistance and tolerance characteristics have been transferred to registered varieties. Moreover, the released varieties have high yield potential that could help to reduce gaps between demand and production and contribute to ensure national food security. Efforts on extension and seed increase and certification of these varieties are urgent in order to make them available for farmers use. Lentil is mainly grown by smallholder farmers in Morocco. Thus, seeds of improved variety are key production factors for this category of farmers relying on limited use of other inputs. Therefore, yield advantage that they could have from the use of improved varieties could significantly increase their incomes. Thus, contributing to rural development and helping to increase lentil production in Morocco.

On the other hand, continuous evolution of biotic and abiotic constraints especially in the context of climate change and global warming, recalcitrant abiotic and biotic stress and emergent grain quality and end-use requirements make continuous breeding of at utmost importance. Erect growth habit enabling machine harvesting is an important added-value trait for lentil. The frequency and intensity of drought and heat stresses are expected to increase in the coming years. Thus, drought and heat tolerance are important traits to be considered in the future. Given the complexity of these traits, multidisciplinary approaches taking in consideration physiology, agronomy and genetics will be adopted. *Orobanche* is still causing substantial damage limiting lentil production in Morocco. Among registered varieties, none has shown confirmed resistance for this parasitic plant. Furthermore, resistance mechanisms are currently under study. Thus, breeding for *Orobanche* tolerance/resistance is an important objective that should be targeted with high priority. Another biotic stress causing considerable damages for grains during storage that should be investigated is bruchids attacks. Exploring genetic solutions in breeding programs may help, in an environmentally friendly way, to solve this problem. Seed quality (nutritional richness, cooking time, shape and color...) should be also taken in consideration when developing new improved varieties that could correspond to consumers and food industry demand.

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