CHAPTER 15

BREEDING METHODS AND ACHIEVEMENTS

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Abstract: Lentil breeding has a relatively short history, however, since the inception of the ICARDA breeding program in 1977 substantial gains have been made in overcoming regional bottlenecks in germplasm diversity. This program has since been supplemented by breeding programs in both developing (eg India) and developed countries (Australia and Canada). These programs have had substantial success in improving tolerance to both biotic (disease) and abiotic stress as well as improving regional adaptation. The Australian breeding program is detailed indicating differences and similarities with other programs. In recent years the need to concurrently develop agronomic approaches and breeding has lead to a greater collaboration among breeders and agronomists

1. INTRODUCTION

Lentil breeding has a relatively short history compared to many of the major crops such as cereals. In many traditional lentil producing countries most of the lentil area is still occupied by landraces that are vulnerable to a range of biotic and abiotic factors (Sarker and Erskine 2002). However, regional breeding programs have now been established around the world and cultivars are being released that offer great advantages to farmers. Unfortunately, in many cases the distribution and adoption of new cultivars is still limiting the potential benefits of breeding in developing countries.

2. FORMATIVE YEARS FOR LENTIL BREEDING

The first major initiative to improve lentil began with the establishment of the International Center for Agricultural Research (ICARDA) in 1977. Based at Aleppo, Syria, it is a centre supported by the Consultative Group in International Agricultural

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Research (CGIAR), an international group of donor agencies, scientists, and administrators from developed and developing countries. Among its activities, ICARDA has the responsibility for lentil improvement internationally (Germplasm Program Annual Report for 1999.

ICARDA has played an essential role in the collection, characterization of landraces, the development of germplasm for countries around the world and cultivars for direct release. In order to understand the structure of the world lentil collection, landraces held at ICARDA were characterised into four major regional groups identified through analysis of variability in quantitative and qualitative morphological traits (Erskine *et al.* 1989). These were the Levantine group (Egypt, Jordan, Lebanon and Syria), the northern group (Greece, Iran, Turkey, USSR, Chile), the Indian subcontinent group and the Ethiopian group. Erskine *et al.* (1994b) later found that the dissemination of lentil around the world has resulted in the selection of different regionally specific balances between photoperiod and temperature for the control of flowering (Erskine *et al.* 1994b). For example, cultivated lentil spread from West Asia to the Indo-Gangetic plain around 2,000 BC (Erskine and Saxena 1993). Lentil landraces originating from West Asia flower much later in Pakistan and India than the local landraces, and their reproductive development begins when conditions are increasingly hot and dry in that environment (Erskine and Saxena 1993) (Table 1).

Although half the world area of lentil is found in South Asia, it is based on a narrow genetic base of exclusively pilosae lentil types with a reduced sensitivity to photoperiod and increased sensitivity to temperature than landraces from West Asia (Erskine *et al.* 1998). This "daylength bottle neck" limited the flow of lentil germplasm into the Indo-Gangetic plain and has been implicated in creating the low

Table 1. Lentil varieties emanated from ICARDA supplied materials and released by national programs (Sarker and Erskine 2002)

Region	Country	No. of varieties	Reason for release
Asia	Bangladesh, India, Nepal, Pakistan, China, Afghanistan, Iran, Iraq, Syria, Lebanon, Jordan, Yemen, Turkey	35	High yield; wilt, rust and ascochyta blight resistance; good standing ability, high biomass, early maturity, winter-hardiness
Africa	Ethiopia, Egypt, Morocco, Libya, Tunisia, Algeria, Lesotho, Sudan	24	High yield; wilt and rust resistance; early maturity, tolerance to excess moisture
The Americas	Argentina, Canada, Ecuador, USA	6	High yield; rust and ascochyta blight resistance; good standing ability
Oceania	Australia, New Zealand	9	High yield, ascochyta blight resistance; good standing ability
Europe	Portugal	2	High yield
Caucasus	Georgia	1	High yield

yield potential and disease susceptibility of lentil in that region. As in other regions, ICARDA was instrumental in expanding the gene pool for breeding in South Asia by intercrossing diverse lines and supplying inbred and segregating populations to South Asia for evaluation, selection and release.

ICARDA provides inbred lines, segregating populations and elite nurseries to lentil researchers around the world. As a result, ICARDA's lentil program has been very successful in changing the productivity of the crop across the world, with 77 of its lines having been released as local varieties in 29 countries by 2002 (Sarker and Erskine 2002). Lines were released in traditional lentil growing areas but were also the basis for new industries in developed counties such as Australia (Materne and Brouwer 1996). In the formative years of the ICARDA breeding program, Dr William Erskine, lentil breeder, conducted an international breeding program targeting all major lentil production regions and worked closely with scientists in these countries to identify constraints to production and in exploiting ICARDA germplasm. ICARDA scientists have also facilitated collaborative research programs with breeders in developed counties to the benefit of lentil improvement internationally.

3. BREEDING SUCCESSES

One of the major achievements of ICARDA's collaborative research is the breaking of an ancient 'bottleneck' as presented by the narrow genetic base of lentil in South Asia (Erskine *et al.* 1998). The genetic base of lentil in this region has been broadened through introgression of genes from ICARDA germplasm (Sarker and Erskine 2002). Early, high yielding and disease resistant varieties, such as Barimasur-2 and Barimasur-4 have been released in Bangladesh (Sarker *et al.* 1999a, 1999b), high yielding varieties with resistance to fungal diseases released in Pakistan (Tufail *et al.* 1995) and extra-early and extra-bold lines have been developed in India to fit in different cropping systems (Chauhan and Singh 1995, Sarker and Erskine 2002). The medium-maturity cultivar Shekher (ILL 4404) is now being grown in the mid-hills region of Nepal, a new area for lentils (Sarker and Erskine 2002).

3.1. Phenology

Lentils are grown in a broad range of climates and within each region there are variations in climate, soils, diseases and pests that impact on the performance of a genotype. The success of ICARDA in supplying landraces and breeding lines for countries around the world has been discussed. A major focus of initial evaluation was the identification of cultivars with regionally specific flowering responses to provide the basis for adaptation to the climatic variables of an environment. In traditional lentil growing regions the optimal time to flowering response was represented in the indigenous landraces for the growing system in use there. However, but in new production areas the optimal flowering response was unknown and needed to be demonstrated by looking at diverse responses over several years in evaluation trials where grain yield was measured. Where major changes in crop agronomy was also introduced (eg tolerance of diseases) there was also a need to reestablish the optimal flowering physiology under the altered circumstances. Key varieties in new production areas included Crimson and Eston in USA, Laird in Canada, and Digger and Northfield in Australia. Alternatively, a photothermal model and climatic data were used to select genotypes suited to winter sowing in the highlands of central and eastern Anatolia, Turkey (Keatinge *et al.* 1995, 1996).

3.2. Tolerance to Abiotic Stresses

In Syria and Australia, and most likely all other lentil growing areas, selection for yield under variable rainfed conditions has increased water use efficiency in lentil through an increased response to moisture availability (Murinda and Saxena 1983, Erskine and Saxena 1993, Materne 2003). However, breeding has increasingly focused on addressing abiotic and biotic constraints, particularly disease. In the USA and Turkey (Central Anatolia), large yield increases have been achieved by sowing lentil in winter rather than spring using genotypes tolerant to cold temperatures during winter (Saker *et al.* 1988, Erskine *et al.* 1981, Kusmenoglu and Aydin 1995, Hamdi 1996, Muehlbauer and McPhee 2002).

Although generally adapted to alkaline soils, lentil growth can be affected by hostile subsoil factors such as high pH, toxic levels of boron and salinity and sodicity (Yau 1999, Yau and Erskine 2000, Saxena *et al.* 1993, Hobson *et al.* 2006). Although variation in tolerance to these factors has been identified, breeding to target these stresses is to our knowledge relatively limited. Breeding lines with improved tolerance to boron, derived from ILL2024 have been developed in Australia and based on controlled environment experiments could improve yields by up to 91% in the target regions (Hobson *et al.* 2006). Similarly, lines with improved tolerance to NaCl have been developed and are soon to be released in Australia. These lines look to have great potential as they are the highest yielding entries in advanced yield trials (Materne *et al.* 2005). In contrast to Australia, boron deficiency has been identified as a limitation to lentil production on soils in Nepal and selection for tolerance occurs in this country (Srivastava *et al.* 1999, 2000).

3.3. Resistance to Disease

Of the diseases that occur and have proven destructive in lentils, breeding has had the greatest impact on delivering cultivars with improved resistance to Fusarium or vascular wilt, caused by *Fusarium oxysporum f.sp. lentis* Vasd. and Srin., rust, caused by *Uromyces vicia-fabae* (Pers.) Schroter, ascochyta blight caused by *Ascochyta lentis* Vassilievsky, Anthracnose caused by *Colletotrichum truncatum* (Schwein.) Andrus and Moore and stemphylium blight caused by *Stemphylium botrysum* Wallr. and botrytis grey mould, caused by *Botrytis cinerea* and *Botrytis fabae*.

Cultivars with resistance to Fusarium wilt have been developed by ICARDA and released in Middle Eastern countries such as Syria. ILL5588 (PI592998, Talia 2) has been the major source for resistance, registered as lentil germplasm resistant to vascular wilt (Fusarium wilt) and released as a cultivar (Erskine *et al.* 1996).

The rust resistant varieties Bakria (ILL4605), Bichette (ILL5562) and Hamira (ILL6238) have been released in Morocco and yields of up to 2.8 t/ha have been achieved (Sarker and Erskine 2002). In Ethiopia lentil varieties like Adaa and Alemaya have been released that have a high level of resistance to rust and to the wilt root rot complex (Sarker and Erskine 2002). Rust resistance breeding and/or evaluation is also important in India and South America.

Breeding for resistance to ascochyta blight has been a major success for lentil breeders internationally. ILL5588 (PI592998, Talia 2) has been registered as lentil germplasm resistant to vascular wilt (Fusarium wilt) and to ascochyta blight (Erskine *et al.* 1996) and as Northfield, an ascochyta blight resistant cultivar, in Australia (Ali 1995). Other cultivars released with improved resistance to ascochyta blight include Rajah (ILL6343) in New Zealand (Russell 1994a, b), Manserha 89 in Pakistan (Erskine and Saxena 1993, Erskine *et al.* 1994a), Pant L4 (Singh *et al.* 1994) and Masoor 93 (Tufail *et al.* 1995) in India and CDC Milestone, CDC Glamis, CDC Grandora, CDC Sovereign, CDC Vantage and CDC Robin were the first released in Canada (Vandenberg *et al.* 2001, 2002a, b, c, d, e) and Nipper in Australia.

In Canada, cultivars such as Robin have been released that have moderate resistance to anthracnose derived from Indianhead (Vandenberg *et al.* 2002e). The combination of resistance to anthracnose and ascochyta blight makes this cultivar a significant advance in breeding for Canada.

Breeding for resistance to Stemphylium blight has been a major success for collaborative breeding programs between ICARDA and the Bangladesh government. Bari-Masur varieties with resistance to Stemphylium blight are making a major impact in Bangladesh (Ashutosh Sarker pers. Comm.).

The first lentils with resistance to *Botrytis cinerea* were identified in the USSR (Khare 1981). The first cultivar with improved resistance to *Botrytis cinerea* was Masoor 93, which was released in Pakistan (Tufail *et al.* 1995). Genotypes with resistance to *Botrytis cinerea* and *Botrytis fabae* were identified in Australia (Materne *et al.* 2002a, Materne *et al.* 2006). Using Indianhead as a resistant source, the resistant cultivar Nipper was developed and commercialised in Australia in 2004 to provide farmers with a low risk cultivar in areas where botrytis grey mould can cause total crop loss in susceptible cultivars (Bretag 2000).

3.4. Harvestability

The large-scale production of lentil in countries such as Australia, Canada and USA has been achieved with mechanised harvesting systems, whereas in many traditional lentil producing countries lentil is still harvested by hand (Haddad *et al.* 1988, Sarker and Erskine 2002). Nonetheless, hand harvesting is considered a major constraint to lentil production in North Africa and the Middle East and its high cost has caused a large decrease in lentil production in Jordan and Syria (Erskine *et al.* 1991). Joint ICARDA and national breeding programs have produced new varieties suitable for mechanical harvesting, such as Idlib 1 and Idlib 2 in Syria, Rachyya in Lebanon, IPA 98 in Iraq and Sayran 96 in Turkey. Varieties suitable for mechanical harvesting, have been released in the Middle east and their use, combined with mechanised harvesting, has increased net returns to growers by an estimated A$200/ha (Sarker and Erskine 2002). Varieties with improved characteristics were a key reason for the development of a lentil industry in Australia (Materne and Brouwer 1996).

3.5. Weed Management

Due to their slow growth during winter and short stature, lentil competes poorly with weeds and weed control is a major limitation to growing lentil worldwide. Lentil production in many countries is dependent on herbicides for weed control. Cultivars with good early vigour, as well as improved tolerance to current herbicides that can cause crop damage (Materne *et al.* 2002b, Muehlbauer and Slinkard 1983) and herbicides that are used less frequently in lentils such as ALS-inhibitors (Holm *et al.* 2007) offer potential to improve weed control in lentil. Crop topping and weed wick wiping techniques have also been developed to control difficult weeds in Australia (Preston 2002) but will be dependent on having varieties with earlier uniform maturity and uniform height to maximize success.

4. BREEDING METHODOLOGY

Breeding methodology at ICARDA is based on a bulk population method with single plant selection at F4. F3 derived segregating populations and inbred lines are then distributed internationally. This low cost method has enabled ICARDA to develop populations for a diverse range of environments. This method has also been adopted in other countries around the world. In Australia this method is utilized along with single seed selection at F2 or F3 and summer increase prior to evaluation. In Canada single plants are selected as early as the F1 stage in complex crosses or at F2 in simple crosses (Albert Vandenberg pers. comm.). In most cases evaluation is conducted at diverse locations in the target growing region of a country with screening for major abiotic and biotic constraints (Figure 1).

Screening agronomic traits such as height and maturity and physical seed characteristics are usually a routine part of breeding programs during early generation multiplication and evaluation phases. Mass selection for physical seed characteristics is done by hand picking, using sieves or mechanization using small scale equipment such as gravity tables or colour sorters. Screening for biotic and abiotic stresses has been highly successful in lentil and is expanding. Screening may be a routine part of early generation multiplication and evaluation such as for ascochyta blight in Australia, where disease causes natural selection for resistant plants in the field and mass selection for seed resistance is done using a colour sorter to eliminate

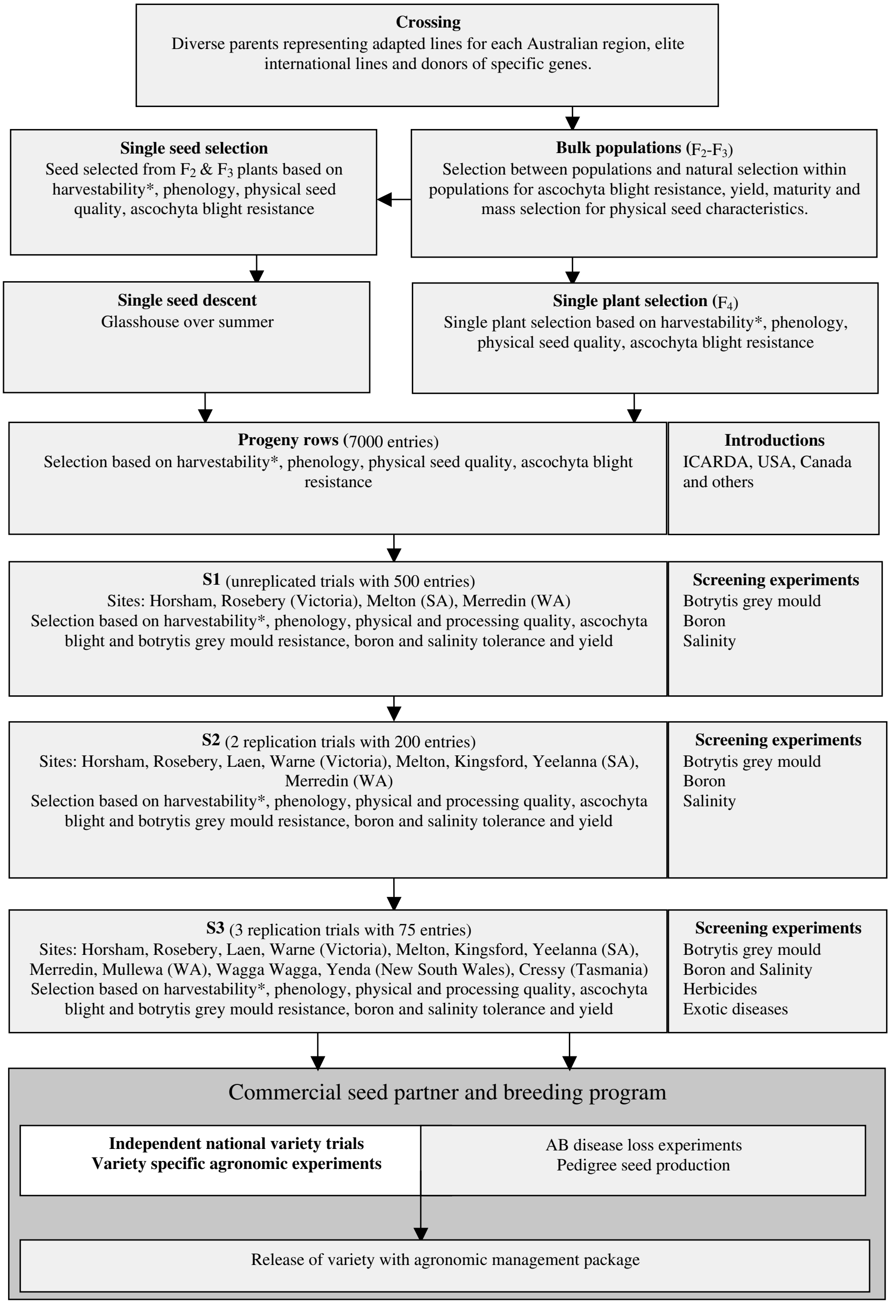

Figure 1. Representation of the lentil breeding program in Australia (high yield, tall, lodging resistant, non shattering, low pod drop, uniform maturity, disease & abiotic stress resistant)

seeds with ascochyta blight blemishes. In other cases, specific disease nurseries are grown in the field, such as for Fusarium wilt at ICARDA, Anthracnose in Canada and rust in northern India, or controlled environment screening is pursued such as with botrytis grey mould in Australia. Breeding for processing and cooking quality will become increasingly important as markets and consumers have more choice and become more sophisticated in their specifications.

Wild lentils have been investigated for some traits but their use in breeding has been limited as the wild species have usually not offered improvements that are significantly above the cultivated species, for example drought tolerance (Hamdi and Erskine 1996). Similarly, the use of molecular markers, transgenics and doubled haploids in lentil breeding has been limited by a lack of focused research (genomics Chapter 18).

In most countries breeding programs are funded by the government and much of the advisory and seed distribution roles are also controlled by government agencies. However, in more developed countries private investment is increasingly important. In Canada and Australia, farmers invest in lentil research, including breeding, through research levies collected on production. In these and other countries private companies are increasing investment in the variety release process by undertaking the multiplication of new varieties, distribution of seed and, in the case of Australia, collecting royalties for investment back into agricultural research. Private companies are also involved in the development and sale of agricultural products and information (extension) and as countries become more developed can be the major or only group supplying these services to farmers. Although components of lentil research are slowly becoming more attractive to commercial companies, there is no private company breeding lentils and it is unlikely that this will occur to the level that currently exits for crops such as oilseeds and the major cereals internationally. Lentil production is typically small and dispersed compare to the major crops and seed multiplication is slower and often more complicated, thus increasing the investment needed to get new varieties to market and reducing the desire of farmers to buy new seed regularly.

5. GERMPLASM ENHANCEMENT

Increasingly breeders have become aware of the need to better coordinate germplasm enhancement within regional breeding programs. Historically ICARDA has had a substantial involvement in this area for lentils as have other lentil research programs. However, in many instances the search for new characteristics and new tolerance sources has not been well linked to the breeding programs. Numerous instances exist in the literature of screening experiments for a range of potentially valuable characteristics that have not been directly taken up by breeding programs. To overcome this difficulty the Australian lentil breeding program has now developed a more formalised approach to incorporate germplasm enhancement using traditional and biotechnological approaches which is outlined in Figure 2. With this approach it is hoped to more rapidly locate and most importantly, incorporate, new

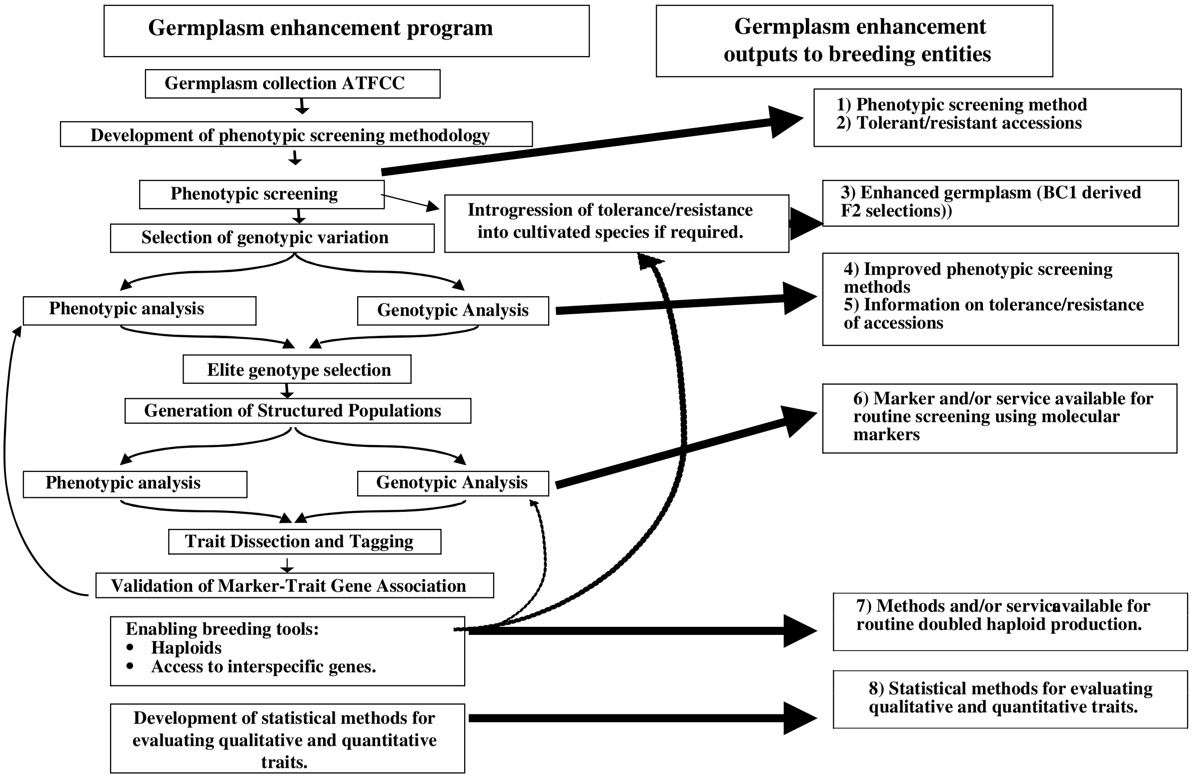

Figure 2. Representation of the lentil germplasm enhancement program and its links to the breeding program in Australia

characteristics into final cultivars. It is also intended that through this mechanism the breeding program is well positioned to collaborate with and provide adapted backgrounds for new biotechnological advances that may occur (eg Genetic Modification, doubled haploids etc.). It is also expected that by linking to breeding and germplasm enhancement approaches in other pulses that these technologies may be transferred to the lentil breeding efforts. For example developments in tissue culture of peas may indicate avenues for this method in lentils, isolation of sequence data from other crops related to a specific tolerance may aid in locating similar alleles in lentils.

6. GENOME SPECIFIC PACKAGES FOR LENTIL VARIETIES

Enhancements in breeding and agronomy have led to increased yields for lentils internationally. However, agronomic practices used in breeding programs can be quite specific and therefore the genetic gains made in breeding may not be realized in farmer's fields if the agronomy used does not enable the potential of the new variety to be realised. This will occur wherever genotype by environment, specifically management, interactions occur. For example lentil varieties differ in their response to sowing time for yield, disease severity and quality, disease management strategies based on resistance and tolerance to herbicides (Materne *et al.* 2002b, Materne 2003). Lentils are also likely vary in their response to farming practices that are being implemented such as a shift to wider row spacings, stubble retention and zero tillage systems, rolling of paddocks post sowing to enable easier harvesting and the use of specially formulated fertilizers (McNeil *et al.* 2006). Further progress in increasing lentil yields is thus likely to require increased concurrent development of genetics and agronomy. In this concurrent development paradigm for pulses, which are frequently used as rotation crops, the agronomy must take into account both the individual crop requirements and the agronomic needs of the cropping system (McNeil *et al.* 2006). In Australia the need for variety specific agronomy has been recognised and all new lentil varieties are evaluated for their response to the major agronomic practices used in Australia, and a variety management package made available with the new varieties to ensure that farmers can maximize the benefits of new lentil varieties. These packages contain the information to optimise grain yield and quality benefits of new varieties in a range of environments. The research concentrates on agronomic management aspects of new varieties for which we have limited knowledge. To maximise efficiencies and minimise time to release of the management package, research runs in parallel with the last stage of breeding, commercialisation of varieties (McNeil *et al.* 2006).

Development of variety specific packages involves interaction of breeders and agronomists late in the breeding cycle. However, substantial benefits may also occur from much earlier collaborations during germplasm development phases of breeding and two major opportunities exist. Firstly when new agronomy systems become plausible, genotypes must be identified which are optimally matched to the new agronomic practice, for example stubble retention, zero tillage systems with wide row spacings and sowing with satellite guidance and automatic steering in Australia (McNeil *et al.* 2006). Secondly, when new genes become available (for example, altered boron membrane transporters) that may provide possible improvements in traits of interest (either through GM, non-GM biotechnology or traditional selection approaches) their specific interactions with the environment, environmental requirements for desired trait expression and opportunities for agronomic application need elucidation (McNeil *et al.* 2006).

It is clear that to benefit from 'genome specific agronomy' approaches requires the concurrent input of agronomy and breeding research in collaboration with extension and farming systems experts to ensure maximal development for 'real world' situations.

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