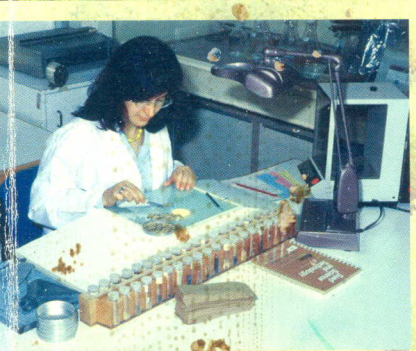
Seed Production of Cool-Season Food Legumes



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About ICARDA and the CGIAR

Established in 1977, the International Center for Agricultural Research in the Dry Areas (ICARDA) is one of 15 centers supported by the CGIAR. ICARDA's mission is to improve the welfare of poor people through research and training in dry areas of the developing world, by increasing the production, productivity and nutritional quality of food, while preserving and enhancing the natural resource base.

ICARDA serves the entire developing world for the improvement of lentil, barley and faba bean; all dry-area developing countries for the improvement of on-farm water-use efficiency, rangeland and small-ruminant production; and the West and Central Asia and North Africa (CWANA) region for the improvement of bread and durum wheats, chickpea, pasture and forage legumes, and farming systems. ICARDA's research provides global benefits of poverty alleviation through productivity improvements integrated with sustainable natural-resource management practices. ICARDA meets this challenge through research, training, and dissemination of information in partnership with the national, regional and international agricultural research and development systems.

The Consultative Group on International Agricultural Research (CGIAR) is a strategic alliance of countries, international and regional organizations, and private foundations supporting 15 international agricultural Centers that work with national agricultural research systems and civil society organizations including the private sector. The alliance mobilizes agricultural science to reduce poverty, foster human well being, promote agricultural growth and protect the environment. The CGIAR generates global public goods that are available to all.

The World Bank, the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Programme (UNDP), and the International Fund for Agricultural Development (IFAD) are cosponsors of the CGIAR. The World Bank provides the CGIAR with a System Office in Washington, DC. A Science Council, with its Secretariat at FAO in Rome, assists the System in the development of its research program.

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Faba bean, Chickpea, and Lentil

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Foreword

Cool-season food legumes are important crops throughout the world, grown for food and livestock feed, and as break crops. They are often the main source of protein for the majority of poor consumers, and they help maintain soil fertility by fixing atmospheric nitrogen. ICARDA, in partnership with national agricultural research systems, is developing new varieties of cool-season food legumes adapted to local farming conditions, with high and stable grain yield, improved grain and nutritional quality, and tolerance to biotic/abiotic stresses.

Agricultural research will impact on food security or crop productivity, if farmers have access to high-quality seed of new varieties. Seed is a fundamental 'renewable' resource in agricultural development; and the means by which new technologies (varieties) are disseminated and adopted by farmers. Availability and access to high quality seed of modern crop varieties is a prerequisite for the transformation of agriculture.

National and international research centers have developed improved varieties of faba bean, chickpea, and lentil – but seed production remains insufficient. Where efforts have been made to increase the availability of seed of new varieties, seed quantity is often low because of severe production constraints. Lack (or limited number) of new varieties, low mechanization levels, high incidence of parasitic weeds, and high production costs are major constraints to the development of a strong legume seed industry.

This publication blends technical, organizational, and managerial issues with the latest concepts in seed production and quality assurance; describing how to provide quality seed to farming communities at affordable prices. It is a useful reference for seed production and quality assurance officers, extension staff, or anyone involved in training or designing and implementing seed production programs.

Mahmoud Solh
Director General
ICARDA

Preface

Cool-season food legumes – faba bean, chickpea and lentil – have been grown in the dry areas of West Asia for millennia. Cultivation has now expanded as far north as western Canada, and as far south as Australia, with dramatic increases in area and production. Despite the long history of cultivation, crop improvement research began only recently, with the establishment of research centers such as ICARDA. Efforts are being made to increase production to meet increasing demand from national and international markets.

Provision of high quality seed of new crop varieties is one way of increasing production and productivity. To produce and disseminate high quality seed to farmers, production and quality control officers must have adequate technical knowledge of legume seed industry processes, from variety development to marketing and quality assurance. While there is a wealth of literature on crop improvement and grain production, technical information on seed production of cool-season food legumes is either unavailable or scattered among various sources; hence the need for a manual that will provide consolidated information with adequate detail on legume seed technology.

This manual provides such information for faba bean, chickpea, and lentil. It provides background information on variety description, for release of new varieties; variety maintenance, a major limitation to multiplication of improved varieties; and technical aspects of seed multiplication, cleaning, treatment, storage, and quality assurance, with special emphasis on mechanization problems and seed-borne diseases.

The manual is not intended to replace internationally established methods and procedures, but simply to make available, conveniently in one place, information on how to produce high quality legume seed in a developing-country context.

Acknowledgments

The authors wish to acknowledge the valuable contributions of those who helped produce this manual. Dr Mahmoud Solh, ICARDA Director General, contributed to the chapter on variety description of faba bean, chickpea, and lentil. Dr Bill Gregg, Senior Seed Industry Specialist, contributed to the chapter on seed processing, seed storage, and lot numbering. Dr Marlene Diekmann, former Head of ICARDA's Seed Health Laboratory, wrote the chapter on seed treatment, and contributed to the chapters on quality assurance and control of storage pests. Mr Abdoul Aziz Niane, ICARDA Seed Production Manager, provided comments and arranged pictures for some of the chapters. Finally, we wish to express our appreciation to the staff of ICARDA's Communication and Documentation Services for providing pictures and editorial assistance.

Chapter 1: Variety Description

Introduction

Modern crop varieties are central to the development of the formal seed industry. National Agricultural Research Systems (NARS) have primary responsibility for developing new varieties and associated technologies for commercial crop production. However, the new varieties must pass through a series of evaluations for registration and release before they can be produced commercially either for seed or grain. Principally, two types of evaluation are carried out – registration tests (distinctness, uniformity and stability, DUS) and performance tests (value for cultivation and use, VCU).

Although registration (DUS) and performance (VCU) tests are often used to evaluate, register and release a variety, many national seed programs in developing countries give priority to variety trials rather than variety description. Although both are essential, the benefits must be considered based on the country's immediate needs and the resources available. An effective, flexible, transparent, and participatory evaluation, registration and release system, involving all stakeholders, would assist in the development of a successful, efficient national seed industry.

Variety description is useful for implementing

- variety maintenance (purification)
- seed multiplication (roguing)
- seed certification (field inspection, control plots)
- consumer protection
- plant variety protection (PVP) rights.

A new variety can be granted PVP rights if it is clearly described; sufficiently distinct, uniform and stable; and novel (has not been commercialized before). Useful references for variety description are available in Ulvinen *et al.* (1973), Anderson (1984), van Gastel (1986) and UPOV (1993, 2002, 2003a, 2003b).

Definition of Variety

The definitions of a variety are many and varied. Carson provides a useful, practical definition. He defines a 'variety' as 'an agricultural unit developed and maintained by man, the first essential being that it should have an individuality which can be reproduced over a number of years, and secondly that it should be distinguishable by inherited morphological or physiological characters from other varieties.'

The varietal unit developed by man has evolved over the years through a combination of natural and human selection. At present, however, the degree of predictability inferred by the term 'variety' extends beyond the production field into the expectations of the industry and consumers. Purchasing seed of a known variety gives the farmer more assurance of performance than seed of unknown origin and identity. Authentic seed is a commercial product, valuable to the producer as well as the purchaser.

Requirements for Variety Release

The ability to distinguish and identify varieties of agricultural crops is fundamental to the operation of the modern seed trade (Cooke, 1998). The primary objective of any registration and release system, whether or not it is backed by legislation, is to recognize each variety as a unique entity. A variety name is of no value unless it can be associated positively with a particular kind of plant or group of plants capable of giving a more or less predictable response in a given environment. In order for the potential new variety to be recognized as an entity, it is essential that it is uniform and stable. Varieties that are distinct, uniform and stable, and have value for farmers and consumers, are the basis for a formal seed program. Two types of tests are carried out for variety release purposes.

Registration testing

Registration testing is carried out to establish the distinctness, uniformity, and stability (DUS) of the new variety. It is a descriptive assessment to establish the identity of the new variety using morphological, physiological, cytological, or chemical characters. Moreover, the test establishes whether the new variety is sufficiently uniform and stable. Registration tests are usually conducted for two independent growing seasons or years in at least one location.

Identification is on the basis of the inherited characters which are used to describe the variety. The new variety is compared with a wide range of existing varieties to establish its distinctness, after which the description and differences from other varieties are provided.

- *Distinctness*: A new variety should be morphologically different from existing ones and be recognizable during different stages of seed production to enable verification of its genetic/varietal purity and identity. For granting PVP, distinctness refers to a difference from any other variety whose existence is a matter of common knowledge.
- *Uniformity*: This relates to the degree of variability within the variety. The variety must be sufficiently uniform so that individual plants are alike, with guaranteed consistent quality. Uniformity makes field inspection more reliable.
- *Stability*: The new variety should be sufficiently stable in terms of its capacity to reproduce itself over several generations of seed multiplication and use for grain production, without losing its distinctive characters. The genetic make-up should remain the same over the years.

There is a close link between uniformity and stability. A variety is considered stable when individual plants within the variety are uniform and react similarly to a given environment or cropping practice.

Performance testing

Performance testing is done through multi-location trials focusing on the value for cultivation and use (VCU), i.e. the benefit the new variety provides to end users (farmers and consumers). These are referred to as variety trials or performance trials. The testing ensures that varieties that are superior in terms of grain yield and quality, agronomic characters (response to inputs, suitability for mechanization), tolerance to biotic and abiotic stresses, etc., are released for cultivation. The new varieties are evaluated and compared with existing standard commercial ones. Performance testing is conducted in different agro-ecological zones to assess the adaptation to different climatic conditions. In addition, the effects of different improved agronomic management practices are assessed in comparison with farmer practice. Performance trials are usually conducted for at least three years. In some countries, the varieties are further tested in on-farm verification trials under farmer management, before final release.

This manual focuses primarily on registration (DUS) testing rather than performance testing.

General Considerations

The mode of reproduction will determine the expression of inherited morphological characters. Self-fertilized crops are homozygous. Due to limited segregation, all plants are more or less alike, with little variation. The major sources of variation are environmental conditions, but some variations may result from small percentages of out-crossing, genetic drift, and spontaneous mutations. Chickpea and lentil have 1-2% out-crossing (Gowda, 1981; Wilson and Law, 1972; Singh and Saxena, 1999). Faba bean is also a self-fertilizing crop, but with high percentages of out-crossing; it is often regarded as a cross-pollinated crop. Bond and Poulsen (1983) reported an average of 35% cross-pollination (range 4 to 84%). Therefore, more variation is expected in faba bean than in strictly self-fertilizing crops such as chickpea and lentil.

It is important to note that no absolute or definite description will under all circumstances identify the variety. Whether the recorded assessment of the variety is a result of careful measurement or a visual assessment, the final decision involves personal judgment. It is also worth noting that some descriptions give the full

range of expression of the character, while others give only the midpoint of that expression.

Characters for Variety Description

Successful variety description requires adequate knowledge of the breeding habit of the variety, a comprehensive list of characters for distinguishing it from other varieties, and a system to record the expression of characters (Thomson, 1979). A character is any property in which we see similarities or differences (especially those of a heritable nature) between individuals. Any characteristic (morphological, physiological, cytological, chemical, etc) can be used for distinguishing varieties and for assessing uniformity and stability. According to UPOV (2002), the basic requirements that a characteristic should fulfill before it can be used for DUS testing are that its expression:

- result from a given genotype or combination of genotypes
- be sufficiently consistent and repeatable in a particular environment
- exhibit sufficient variation between varieties to be able to establish distinctness
- be capable of precise definition and recognition
- allow uniformity and stability requirements to be fulfilled.

Characteristics that are easily observable and less influenced by environmental conditions are preferred for variety description. The morphological characteristics used for description can be subdivided into qualitative, pseudo-qualitative, and quantitative (UPOV, 2002).

Qualitative characters

Qualitative characters

- have discontinuous variation
- are measured visually in discrete classes
- are affected little or not at all by the environment
- are usually controlled by a single or very few major genes.

Qualitative characters are often scored numerically. Examples include flower and seed color in chickpea and stem and cotyledon color in lentil.

Quantitative characters

Quantitative characters

- have continuous variation
- can be measured numerically or metrically
- are influenced by the environment
- are controlled by many genes.

Quantitative characters are often measured. Examples include plant height, days to flowering, and days to maturity.

Pseudo-qualitative characters

The expression of characters is partly continuous, but varies in more than one dimension. Similar to qualitative characteristics, each individual state of expression needs to be identified to adequately describe the range of the characteristic. Pseudo-qualitative characters, for example in chickpea, include seed shape: round (1), round to angular (2), or angular (3).

The International Union for the Protection of New Varieties of Plants (UPOV) has published test guidelines (www.upov.int) for the description of chickpea (UPOV, 1993), faba bean (UPOV, 2003a) and lentil (UPOV, 2003b). The guidelines describe the best characters to use for variety description, stage of scoring, and different states of expression of the characters. The International Board for Plant Genetic Resources (now Bioversity International) and ICARDA have also prepared lists of characters useful for variety description of cool-season food legumes.

Lists of descriptors are available for faba bean (IBPGR and ICARDA, 1985a, UPOV, 2003a), chickpea (IBPGR et al., 1993, UPOV, 1993), and lentil (IBPGR and ICARDA, 1985b; UPOV, 2003b). The combined lists for IBPGR, ICARDA and UPOV are provided in the annexes to this manual. All the characteristics listed should be used when initiating a DUS testing program or expanding to new crops. However, with more experience, we can limit the tests to only the most useful characters for distinguishing different varieties. To facilitate description, it is useful to establish reference varieties for different characters.

Experimental Arrangements

In well-developed seed programs, special experiments are conducted to assess DUS of new varieties before they are commercially released for seed production. Guidelines have been developed for such experiments (UPOV, 2002):

- Varieties should be planted in at least one location, with a minimum of two replicates. Two locations are preferable for technical and security reasons and to assess the expression of characters in different environments.
- The seeds should not be treated with chemicals, which could affect the expression of characters. If seed treatment is essential, the same chemical should be used for all entries.
- Planting should preferably be done by hand to avoid the risk of admixture, but small-plot planters are now available and can be used.
- Plots should be weeded, preferably manually, to avoid the use of herbicides that may influence the expression of characters.
- Entries should not be completely randomized; similar varieties should be grouped based on morphological characters for easier comparison. Randomization should be carried out within and between groups.
- A wide range of existing varieties should be included to confirm that the variety is distinct from all existing or new varieties.
- The experiments should be carried out for two independent growing cycles or years.
- To assess uniformity, single plant progenies may be planted in addition to the DUS trials.
- Seed of different generations may be planted, in order to assess stability of the variety.

Scoring characters

Qualitative characteristics are usually scored as consecutive numbers, starting with 1, with no upper limit (UPOV, 2002). Each state is different from the others. For example, flower color could be white (1), pink (2), or blue (3).

Quantitative characteristics are often scored on a 1 to 9 scale, where 1 = lowest state of the character, 9 = highest state, e.g. 1 = very short, 9 = very tall plant (UPOV, 2002).

Characters are scored using one of the following scales (UPOV, 2002):

- absent (1) and present (9)
- weak (3), medium (5), and strong (7)
- very weak (1), weak (3), medium (5), strong (7), and very strong (9)

The morphological characters and scales for scoring faba bean, chickpea, and lentil are provided in the annexes.

Reporting results

Distinctness

Two varieties are distinct if the difference is (i) observed in at least one important character, (ii) determined in at least one testing site, (iii) clear, (iv) consistent over the years (UPOV, 2002). For qualitative characteristics, the difference between two varieties is clear if the respective characteristics show expressions in two different states. For instance, lentil varieties A and B are distinct if variety A has orange cotyledon (scored as 1) and variety B has greenish yellow (scored as 2). In visually observed quantitative characters (e.g. plant height), a variety is considered distinct from another if the difference is more than one full class width on the 1–9 scoring scale (UPOV, 2002). For measured quantitative characteristics, two varieties are distinct if the difference between their averages is more than two standard deviations. The difference is considered clear if it occurs with 1% probability (LSD-test) (UPOV, 2002).

Uniformity

Tolerance levels for offtype plants differ according to the mode of reproduction and mutability of a variety. For self-fertilizing chickpea and lentil, the maximum permissible number of offtypes is suggested to be three plants per 100, whereas faba bean is treated like cross-pollinated crops (UPOV, 2002). Varieties with a larger percentage of offtypes than the standard variety used for comparison are considered non-uniform. For measured characteristics, the standard deviation is the criterion for comparison. A variety is considered not to be homogeneous if its variance exceeds 1.6 times the average of the variance of varieties used for comparison (UPOV, 2002).

Stability
A variety is considered stable when the characters remain the same from one generation to another during multiplication. Since it is not possible to test stability in the two growing seasons that are required for the DUS tests, a uniform variety is also considered stable.

Written descriptions
All results obtained from the examination of varieties are usually gathered and used to produce a written description. Descriptions have their undoubted uses – but they cannot substitute for reference materials.

Describing food legumes
Table 1.1 provides a sample of morphological descriptions. Table 1.2 summarizes the materials required for DUS testing in cool-season food legumes. To ensure uniformity, the seed should be taken from early-generation material.

Sample size and weight
The plot should be large enough to allow plants or plant parts to be removed for measurement and counting without prejudice to the observations, which must be made up to the end of the growing period. In each test, at least 160 plants for faba bean (UPOV, 2003a) and 100 each for chickpea (UPOV, 1993) and lentil (UPOV, 2003b) should be planted in two or more replicates.

Observations
All observations on flowers should be made when they have just opened, and on pods when they are fully developed at the central third of the plant. Observations on seeds should be made shortly after maturity (dry stage) and samples should be taken in the middle of the central three rows in each replicate. All observations determined by measurement or counting should be made on 40 plants or parts thereof for faba bean (UPOV, 2003a) and 20 plants or parts thereof for chickpea and lentil (UPOV, 1993, 2003b).

Single plant progenies
To assess uniformity, a number of plants should be harvested individually in the first year DUS plots and planted in progeny rows in the second year. The number of progeny rows to be planted is indicated in Table 1.2. If these progeny rows have more than one offtype, the variety is not sufficiently uniform.

Variety grouping characters
For a description to be meaningful, the candidate variety should be compared with established ones. Therefore, grouping characters are used for similar varieties (Table 1.2).

Characters and scoring
The characters that should be scored, and the scale for scoring, are provided in the annexes.

Choice of sites
The choice of sites for variety testing is governed by many factors, including:

- Soil type: the soil should be free draining, easily workable, as much as possible level, uniform, and free of stones, especially if the trial will be mechanically planted.
- Area: the area should be representative of the environment(s) in the country. It is often useful to select areas that are 'early', because the tests can be used for training.
- Accessibility: easy accessibility is an advantage, because DUS plots need to be examined almost daily to observe different morphological characteristics.
- Diseases and pests: the site should have no persistent soil-borne diseases and be free from cover such as trees sheltering birds, wild animals, etc.
- Economic factors: notwithstanding all of the above, economic factors may be the most important criteria for site selection.

Equipment and tools
The basic equipment for practical variety identification consists of:

- Field equipment: small-scale equipment for sowing, spraying, threshing, etc.
- Greenhouse facilities: these facilities will provide flexibility in managing DUS tests, and plants can be grown to maturity under semi-controlled conditions.
- Field equipment: pocket lens (10x and 20x magnification), penknife, scalpel, dissecting needles, black card, adhesives, etc.
- Laboratory equipment: stereomicroscope (5x to 60x magnification), flat field microscope, storage cabinets, adjustable light source, etc.

Table 1.1. Morphological variety description of two lentil varieties at ICARDA

	Idlib 1	Idlib 2
Scientific name	*Lens culinaris* (Medikus)	*Lens culinaris* (Medikus)
Common name	Lentil	Lentil
Crop type	Winter	Winter
Breeding institution	DASR-ICARDA	DASR-ICARDA
Country of release	Syria	Syria
Year of release	1987	2000
Vegetative stage		
Stem pigmentation	Absent	Absent
Leaf pigmentation	Absent	Absent
Stem pubescence	Absent	Present
Leaf pubescence	Present	Present
Leaflet size[1]	Large (≈2.1 cm^2)	Small (≈1.6 cm^2)
Tendril length	Long (3.3 cm)	Short (2 cm)
Plant height	Tall (33 cm)	Tall (32 cm)
Reproductive stage		
Emergence to flowering	72 days	76 days
Ground color of flower	White	White
Flowers per peduncle	High (3)	High (3)
Seeds per pod	Single (1.1)	Single (1.2)
Seed		
Size (1000 seed weight)	Large (40.4 g)	Medium (37.3 g)
Ground color of testa	Green	Reddish cream
Pattern of testa	Plain	Plain
Cotyledon color	Yellow	Red

[1]Width at the broadest part of randomly selected basal pairs of leaflets multiplied by length
Source: Seed Unit, ICARDA

Table 1.2. Requirements for DUS testing in faba bean, chickpea and lentil

	Faba bean	Chickpea	Lentil
Weight of sample (minimum)	2000 g (2000 seeds)	1000 g	500 g (10,000 seeds)
No. of plants to be planted	160	100	100
No. of plants examined (minimum)	40	20	20
Uniformity (offtypes per 100 plants)	7	3	3
Single plant progenies			
No. of progenies	10	35	50
Tolerance (uniformity)	1	1	1
Variety grouping characters	• Growth type • Melanin spot on wing • Color of testa	• Flower color • Seed color • Seed shape • Seed ribbing • Flowering time	• Cotyledon color • Anthocyanin coloration of plant • Color of standard • No. of colors of dry seed • Main color of testa of dry seed

This list is not exhaustive, but shows that DUS tests can be carried out using fairly simple equipment. Additional equipment can be acquired, based on experience and need.

Reference collections

Authentic samples of popular varieties should be available for comparison with unknown samples. It is most convenient if these samples are mounted on black cards, for easy accessibility and storage. Candidate varieties are compared with each other, and with varieties from the collection, using descriptive characters as well as direct comparisons in plots and beds. An 'authentic sample' (definitive stock) of the new variety, which is a portion of the seed for description, should be retained after the tests. It should be kept as part of the reference collection under safe storage conditions.

Molecular tools

The use of modern technology for variety identification is becoming increasingly important. Some methods have already been established and accepted by some sectors of the seed industry. Rapid, robust, reliable, and cost-effective techniques for identifying modern varieties are expected to become available soon.

Recent techniques such as randomly amplified polymorphic fingerprinting (DNA), amplified fragment length polymorphism (AFLP), microsatellites, etc. can be used to demonstrate the distinctiveness of plant varieties. However, none of these advanced methods has been recommended for use in variety description. To date, the electrophoresis test is the only regular tool used for identification.

Electrophoresis is a chemical test, which distinguishes varieties on the basis of a small amount of enzymes and/or seed storage proteins. It separates a mixture of enzymes or proteins into distinct bands. Such separation is possible because of the differences in the size and charge of proteins involved. Different types of electrophoresis – starch gel electrophoresis (SGE), polyacrylamide gel electrophoresis (PAGE), polyacrylamide gel electrophoresis using sodium dodecyl sulphate (SDS-PAGE), isoelectric focusing electrophoresis (IEFE) – are frequently used for protein characterization and to differentiate between varieties. However, such methods will not provide information on uniformity and stability.

Managing Variety Description

Responsibility for description

In general, variety description should be managed by an independent agency established for this purpose. Agencies vary from country to country and could be public organizations established by government, or separate institutions (public research institutes, agricultural universities) acting on behalf of that authority, or a private agency formed by the industry (e.g. associations representing breeders/seed producers/farmers), or in some cases the breeder. However, it is very important that the agency performs its task efficiently, without delays, using appropriate criteria, in a very transparent manner, and with the participation of stakeholders (Tripp *et al.*, 1997).

Harmonizing testing and release procedures

The procedures for testing varieties for registration, performance evaluation, and release are essentially similar in many countries. This provides an opportunity to develop testing protocols, share data, and adopt a flexible regionally or internationally harmonized variety release scheme. Adopting internationally acceptable UPOV guidelines will facilitate harmonization of variety registration and release procedures across countries and/or regions.

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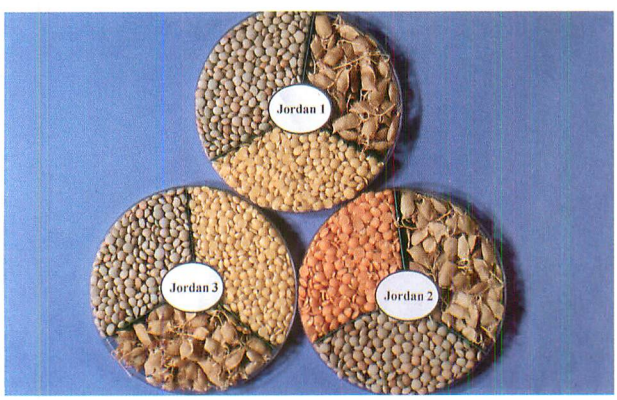
1.1 Legume variety description plots at ICARDA, Aleppo, Syria

1.2 Stem coloration in lentil varieties: left (absent) and right (present)

1.3 Lentil flower color of the standard: Left: (light) and right (deep blue)

1.4 Lentil leaflet size (left: large and right: small), color (left: light green and right: dark green) and tendrils (left: prominent and right: rudimentary)



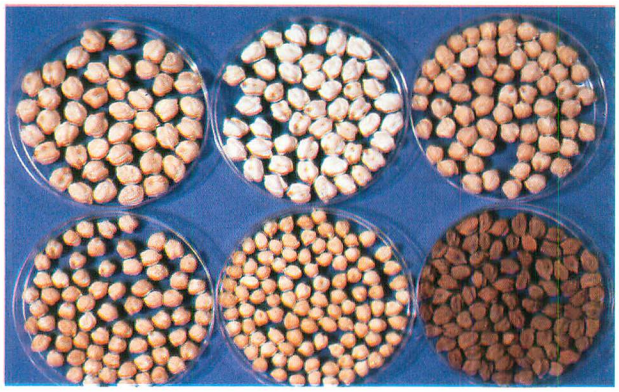
1.5 Jordanian lentil varieties with different pod, seed and cotyledon color

1.6 Lentil varieties with variation in pod, seed and cotyledon color

1.7 Chickpea varieties showing different maturity groups

1.8 Three chickpea varieties showing different plant height and vegetative growth

1.9 Stem coloration of chickpea: present (left) and absent (right)



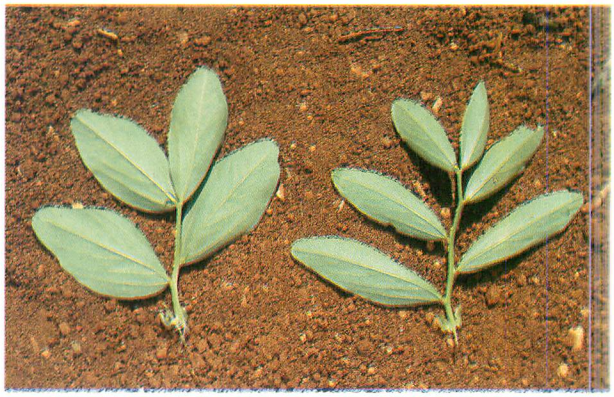
1.11 Variation in chickpea seed size and color

1.13 Anthocyanin coloration of stem in faba bean: present (left) and absent (right)

1.15 Variation in faba bean pod shape: fusiform (left) and parallel (right)

1.10 Variation in chickpea pod, seed and color

1.12 Faba bean varieties with different stem number (left: many and right: single), pod attitude (left: drooping and right: parallel)



1.14 Leaflet size in faba bean: large (left) and small (right)

1.16 Variation in faba bean seed size and color

Chapter 2: Variety Maintenance

Introduction

Newly released crop varieties need to be multiplied and made available to farmers as quickly as possible. During seed multiplication, strict attention should be paid to the maintenance of varietal purity and identity. If the breeder seed used for further multiplication is not of high purity, the contaminants (offtypes, other varieties, seed-borne diseases, etc) will increase in subsequent generations, resulting in low quality seed. In many developing seed programs, variety maintenance is carried out neither by the breeder nor by the seed producers. Consequently, varietal purity is low. Non-availability and lack of access to breeder seed remain major constraints in many national seed programs.

Variety maintenance procedures for legume crops have been described by Bouwman (1992), Drijfhout (1981), Julen (1983), Sharma (1987), and Singh and Saxena (1999). Procedures for variety maintenance and breeder seed production of faba bean, chickpea, and lentil are explained in this chapter, while the production of subsequent generations is described in Chapter 3.

Contamination of Variety

Seed production follows a generation system, where a small quantity of 'parental material' or 'nucleus seed' received from breeders is systematically multiplied into large quantities of certified seed for distribution to farmers. In the process, several factors may reduce the genetic, physical and health quality of the seed due to a progressive increase in the quantity of contaminants. Generally, three types of contamination are recognized: (i) genetic contamination, (ii) mechanical contamination, (iii) pathological contamination.

Genetic contamination

Genetic contamination arises from cross-fertilization and spontaneous mutations. Self-fertilizing crops often have small percentages of cross-pollination, which can cause genetic contamination of the seed crop. Undesirable cross-pollination is a common feature of all cross-fertilizing crops if fields are not adequately isolated. The spontaneous mutation rate is generally low, but increases significantly after long periods of seed storage. Since the mutations are usually micro-mutations and recessive, they are often difficult to detect. Natural selection, leading to genetic drift, may also change the genetic structure of a variety in cross-fertilizing crops if seed production takes place in an environment where the variety is not adapted.

Mechanical contamination

Mechanical contamination can result from several causes, but is mainly due to insufficient cleanliness of fields (cropping history, crop rotation), equipment (planters, combines, cleaners, vehicles), bags, stores, and inadequate measures to avoid such contamination (field selection, physical isolation, cleanliness).

Pathological contamination

Pathological contamination occurs due to infection with seed-borne diseases, which are exclusively seed transmitted. Pathogens from the same variety, other varieties, other crops, or from weeds, spread the disease to normal plants or contaminate the seed lot. Pathological contamination could also be a result of gradual loss of tolerance of the variety to specific seed-borne diseases.

Seed Classes

Seed production is based on a limited generation system in order to minimize the risk of contamination. The fewer the number of generations, the lower the chances of contamination. However, regardless of the number of generations, all certified seed originates from one or more generations of a known source called 'breeder seed'. Different seed certification schemes use different names for generations or seed classes (Table 2.1). This manual follows the OECD seed scheme.

Table 2.1. Nomenclature of legume seed classes in selected countries of West Asia and North Africa

Generation	OECD	AOSCA	Egypt	Ethiopia	Morocco	Syria
First	Breeder	Breeder	Breeder	Breeder	Epis-lignes (G_0)	Nucleus
Second	Pre-basic	Foundation	Foundation	Pre-basic	Prébase (G_1, G_2, G_3)	Foundation
Third	Basic	Registered	Registered	Basic	Base (G_4)	Registered
Fourth	Certified 1	Certified	Certified	Certified 1	Reproduction 1 (R1)	Certified
Fifth	Certified 2	–	–	Certified 2	Reproduction 2 (R2)	–

AOSCA = Association of Official Seed Certifying Agencies, OECD = Organization for Economic Cooperation and Development
Source: Madarati and Bishaw, 2002

Parental material, often called 'nucleus seed', is the initial seed obtained from bulking breeding lines to constitute the new variety. It is produced by the breeder and is used to produce breeder seed and later generations.

Breeder seed is the initial source of certified seed. It is produced by the breeder or his agent or a plant breeding institution from the parental material; and is then multiplied into pre-basic or basic seed. Breeder seed is usually not certified, but seed quality control staff should be involved in its production to familiarize themselves with the varietal characteristics.

Pre-basic seed is the progeny of breeder seed and is produced under the supervision of a breeder or his designated agency. It is commonly used for crops that have low multiplication rates, and where large quantities of certified seed are required.

Basic seed is the progeny of breeder or pre-basic seed. Production is supervised by the breeder (or his designated agency) and the seed quality control agency. The seed producer is responsible for the quality of seed produced, while the certification authority is responsible for ensuring quality through field inspection, seed testing, and certification.

Certified seed is the progeny of basic seed. It can be used to produce further generations of certified seed, or planted by farmers for grain production. The certification agency is responsible for inspecting the field, testing quality, and certifying the seed produced.

Commercial seed is usually not certified. It is produced without strict generation control, and may be produced based on internal quality guidelines without formal certification. It is often used in emergencies where regular seed supply is disrupted. Sometimes commercial grain is tested for purity and germination, and distributed to farmers for planting.

Each class of seed should be produced under strict supervision and must meet prescribed seed quality standards. Early generations (breeder and pre-basic seed) are usually produced on research farms and basic seed on specialized seed farms. Production of certified seed by private growers, who are contracted by the seed enterprise (public or private sector), is generally convenient and economical.

Purpose of Variety Maintenance

Laverack (1994) defines variety maintenance as 'the perpetuation of a small stock of parental material through repeated multiplication following a precise procedure'. Thus, the objective of variety maintenance is to maintain the genetic purity and identity of the variety, and produce a new lot of breeder seed, which is the basis for further multiplication. There are inter-relationships between variety development, testing, release, maintenance, and seed multiplication are all interrelated (Fig. 2.1).

Procedures for Variety Maintenance

To ensure a continuous supply of certified seed to farmers, all the different classes should be

produced regularly. A new lot of breeder seed should be used to start the multiplication cycle each year. The number of generations to be allowed after breeder seed depends on the mode of reproduction of the crop, risk of contamination, multiplication ratio, and the quantity of seed required.

Purification of varieties

Variety purification is an alternative procedure in situations where there is no organized variety maintenance program. The best seed field is selected, whereby the obvious contaminants are removed by negative mass selection. Offtypes and plants of other varieties are rogued, and the bulk of the crop is harvested as breeder seed and used for further multiplication. In purification, offtypes must be removed more strictly (larger numbers, stricter criteria) compared to normal seed production.

Mass selection

Mass selection is one of the most common methods of variety maintenance (Almekinders and Louwaars, 1999). In ***positive mass selection***, the best plants are selected, bulk-harvested and used for further multiplication; the remaining plants are discarded. In ***negative mass selection***, individual plants, which deviate from the description of the variety, are identified and rogued. The remaining plants are bulk-harvested and used as breeder seed for further multiplication. In ***stratified (grid) mass selection***, the seed field is divided into grids and an equal number of plants are selected from each grid and bulked. The bulked seed is used as breeder seed for further multiplication. Variety maintenance through mass selection is more appropriate for highly self-fertilizing crops such as chickpea and lentil than for faba bean.

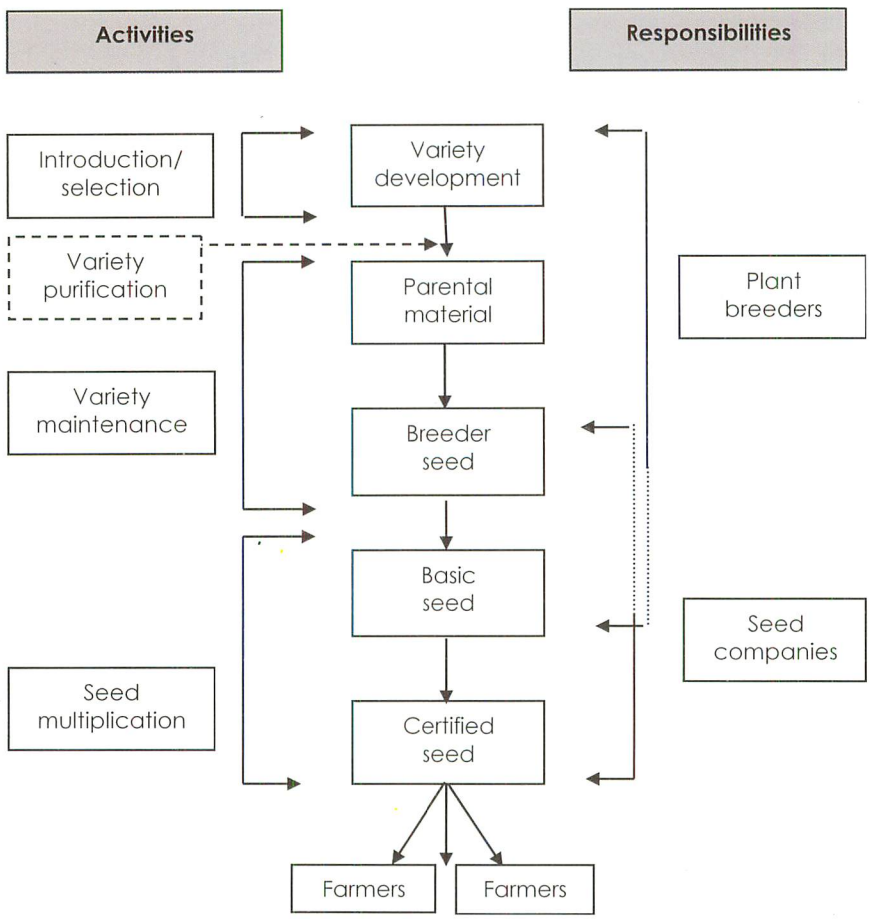


Figure 2.1. Interrelationships between variety development, maintenance, and seed multiplication

Plant-to-row

Plant-to-row is an effective method of variety maintenance for truly self-fertilizing crops such as chickpea and lentil. Single plants typical of the variety are selected and harvested separately. The seeds of each plant are planted in a progeny row, and the rows with offtypes are discarded. Only rows in which all plants conform to the varietal description are bulk-harvested as breeder seed. When varietal purity is in doubt or uniformity is insufficient, plant rows may be harvested individually and planted as small plots for a second season. Negative mass selection within these plots is then carried out before the plots are bulk-harvested as breeder seed.

Variety Maintenance in Chickpea and Lentil

A population of at least 300 plants is suitable for the maintenance of seed production in self-pollinated crops (Sharma, 1987). However, the number of plants must be modified based on the number of generations, the multiplication ratio, and the quantity of certified seed required. In legume crops, 500 plants are adequate to represent a variety for breeder seed production (Sharma, 1987). The following steps illustrate the plant-to-row procedure (Fig. 2.2).

Step 1

The initial parental seed supplied by the breeder is grown in a plot (year 0) and, for instance, 500 single plants typical of the variety, are selected. Select only plants that clearly exhibit all the characters of the variety, based on the characteristics discussed in Chapter 1. At maturity, harvest selected plants individually, thresh separately, and examine the seeds of each plant closely. Discard any plant that produces seeds not typical of the variety.

Step 2

Plant the seeds of selected plants (year 1) in individual progeny rows (plant rows). Carefully

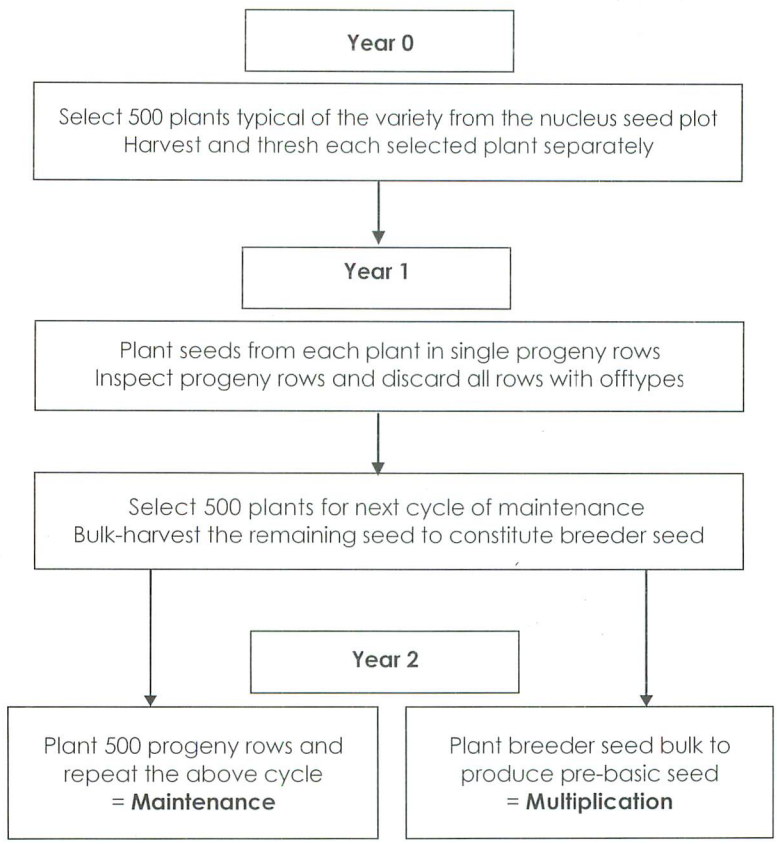


Figure 2.2. Variety maintenance in chickpea and lentil

observe the progeny rows during the entire growing season, from emergence to harvest, for any deviation from the description of variety. Observe the plants most carefully at flowering and maturity, because varietal characteristics are most clearly expressed at these stages. Discard any row (progeny) which contains offtype plants.

Step 3
Before bulk-harvesting the selected progeny rows as breeder seed, select 500 individual plants, based on morphological characters, for the next generation of breeder seed to initiate the next cycle of variety maintenance.

Step 4
After threshing and examining the seeds from each of the 500 selected plants, sow progeny rows as the next cycle of maintenance. Plant the breeder seed (year 2) to produce pre-basic seed.

Variety Maintenance in Faba Bean

The procedure for variety maintenance in faba bean is different, because faba bean has high percentages of out-crossing and suffers from many diseases. The 'rest seed' method is often used (Fig. 2.3).

Step 1
The first step is similar to that for self-fertilizing crops. Grow a small plot of parental seed, under strict isolation (at least 800 m). Inspect and rogue the plot carefully before flowering based on the morphological characteristics, to prevent offtypes out-crossing with true-to-type plants. Also discard the diseased plants. Finally, select 100–200 plants that exhibit all varietal characteristics (year 0). Harvest and thresh the selected plants individually. Carefully observe the seed characters including seed size, seed coat, and hilum color and discard all plants with offtype seeds. After selection, divide the harvested seed of each plant into two parts; use one part to plant the progeny rows (part 1) and store the remaining (part 2).

Step 2
In year 1, plant the seed in part 1 as individual progeny rows. Closely examine and select rows based on the variety description, and discard rows showing offtypes or diseased plants. This should be done before flowering, to prevent cross-fertilization between true-to-type and offtype plants. Harvest selected progeny rows separately and thresh individually. Carefully examine the seeds and discard plants producing offtype seeds.

Step 3
Take stored seed of plants that produced a good progeny in year 1, and plant them as individual progeny rows. Carry out strict selection before and afer flowering and remove deviating progenies and offtypes. Take material for the next cycle of variety maintenance before bulking the seed of selected progeny rows as breeder seed.

Step 4
Plant the small plot to start the next cycle of variety maintenance. Sow the breeder seed to produce pre-basic seed to initiate seed multiplication.

Production Arrangements

Production of breeder seed for variety lifetime

The best method of variety maintenance is to produce enough breeder seed for the lifetime of a variety (Bouwman, 1992). Each year a small portion of breeder seed is withdrawn and used to produce pre-basic or basic seed. This involves less work than producing breeder seed each year and minimizes the risk of any genetic shift, especially for cross-pollinated crops. However, such breeder seed should be stored under medium to long-term conditions (4°C and 40% relative humidity would be ideal). This could be done for varieties that are grown in small areas and have high multiplication factor, or where varieties have a short lifespan due to high rate of varietal turnover. It is not a viable option for legume varieties.

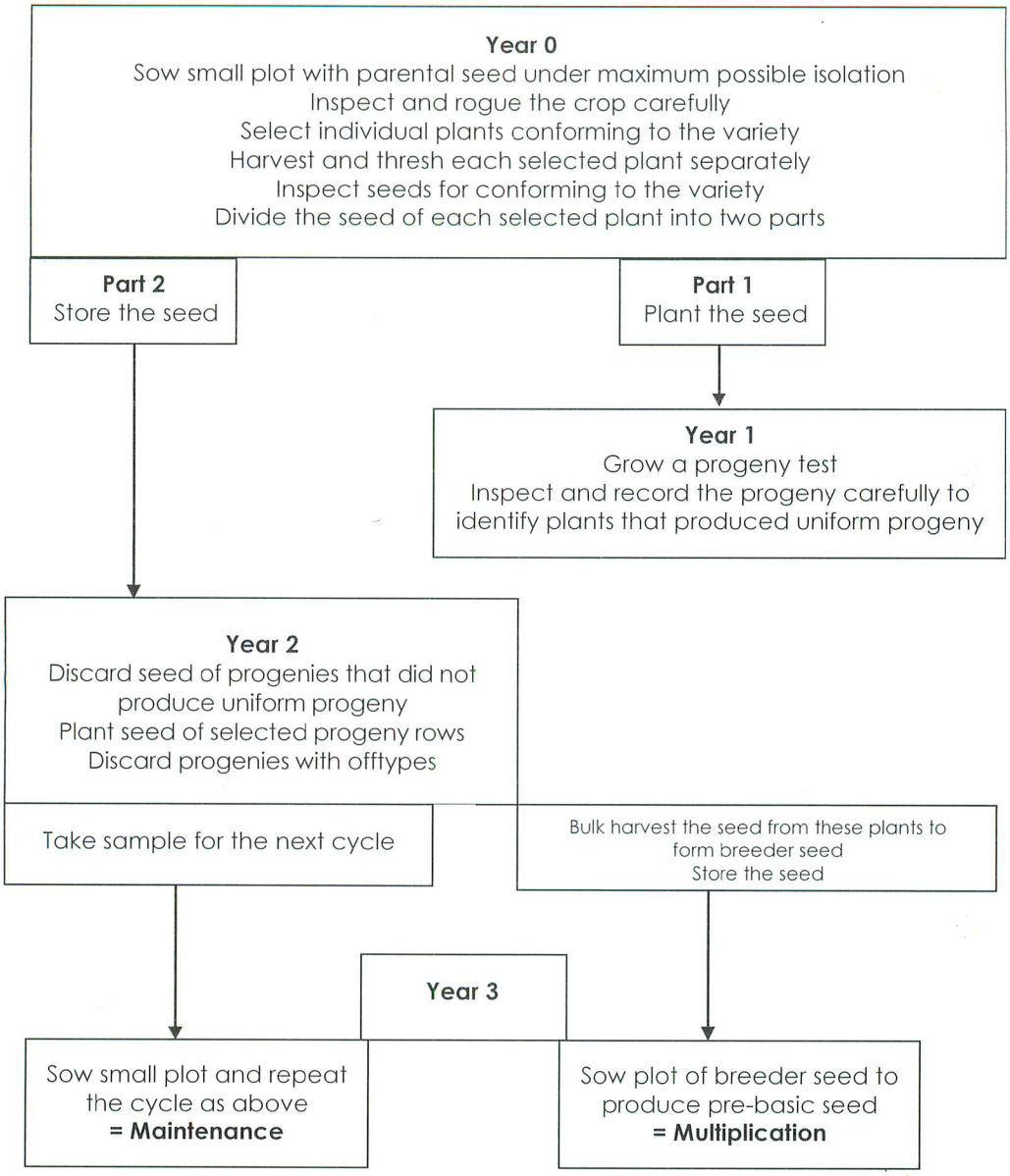


Figure 2.3. Variety maintenance in faba bean

Production of breeder seed periodically

An alternative strategy is to multiply the breeder seed at regular intervals. Initially, a sufficiently large quantity of breeder seed is produced for a few years, e.g. 4–5 years (Julen, 1983). If the variety remains in commercial production, breeder seed can be multiplied as needed, for the second and third time (Laverack, 1994). However, the breeder seed must be properly stored under relatively low temperature and relative humidity (20°C and 50% RH or 10°C and 60% RH).

Production of breeder seed regularly

It is impossible to store sufficient breeder seed of legume crops for the lifetime of the variety, because crop production areas are large, multiplication rates are low, the material is bulky, or

cannot be stored for long periods. Storage conditions are often not ideal. Therefore, breeder seed should be produced every year.

Agronomic Management Practices

In general, the crop management practices are similar to those used for producing pre-basic, basic, or certified seed (see Chapter 3). However, the best possible practices should be applied. Breeder seed is the earliest generation, and any mistake at this stage will be difficult to correct later. Moreover, losing breeder seed delays the availability of certified seed to farmers.

Planting

Variety maintenance plots can be planted by hand or machine if necessary. Plot planters designed for such purposes, are commercially available.

Isolation

Different methods can be used to ensure isolation during breeder seed production (see Chapter 3). Similar varieties of different generations can be grouped and grown side by side to minimize the risk of contamination. In Algeria and Morocco, for example, G_0 is surrounded by G_1 and then by later generations (G_2, G_3 or G_4) to prevent contamination from external sources. A wide border of the same cultivar is more important than a large distance between different cultivars to prevent contamination in faba bean (Nadal *et al.*, 2003).

Harvesting, cleaning and treatment

Individual plants and/or progenies can be harvested by hand and threshed using single-plant threshers or small-scale stationary or portable threshers. However, a small-plot combine can also be used for bulk harvesting the breeder seed. Breeder seed can be cleaned and treated manually or with laboratory-scale machines to avoid any admixture or contamination.

Seed storage

Some carry-over seed should be kept in case there is crop failure during breeder seed production. This seed should be properly stored to prevent damage by insect pests. Storage at 10°C and 40% RH will preserve the seed quality for up to five years. On the other hand, you can grow a large number of single plants and keep the carry-over seed for some time, as a precaution against genetic shift due to frequent multiplication.

Managing Variety Maintenance

Pre-release multiplication

Availability of breeder seed remains a major constraint to adoption and diffusion of new legume varieties. When a new variety is released, only a little seed is available because seed organizations often start production only after the official release. Consequently, it takes another 4–5 years before seed is available in large quantities for distribution to farmers. This could change if seed multiplication of the most promising varieties could begin before they are

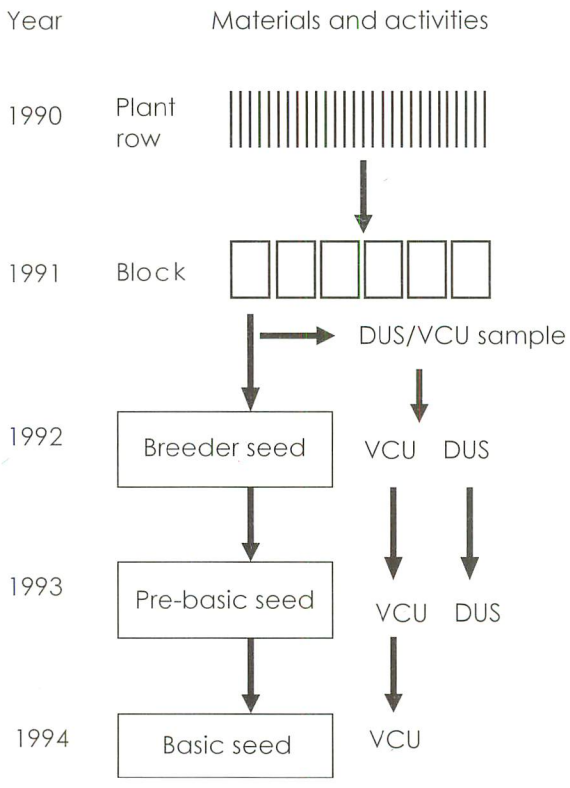


Figure 2.4. Variety release and seed multiplication scheme in a private seed company

officially released, i.e. during the final stages of evaluation (Fig. 2.4). This will ensure that enough breeder seed is available for immediate large-scale multiplication. The interest in new varieties is usually highest when they have just been released. The sooner seed is available, the faster farmers can benefit from the new variety.

Responsibility for variety maintenance

Plant breeders in the public sector may lose interest in a variety once it has been officially released. In many developing countries, neither the public plant breeding institutions nor the public seed producing agencies give sufficient attention to variety maintenance and breeder seed production. As a result, availability of breeder seed becomes a bottleneck in seed production. In Morocco, a separate Seed Unit within the Institut National de la Recherche Agronomique is responsible for maintaining and producing breeder seed of public varieties. In Ethiopia, the Ethiopian Seed Enterprise is responsible for the production of pre-basic and basic seed of the major crops on its own seed farm. Several useful management options for breeder seed production are available, and could be adopted in developing countries (Laverack, 1994). It would be helpful to integrate a breeder seed unit into plant breeding and seed production as in the private sector, or create a separate unit within the breeding institutions or seed producing organizations. Alternatively, an independent unit could be established, with a clear mandate to take over this responsibility.

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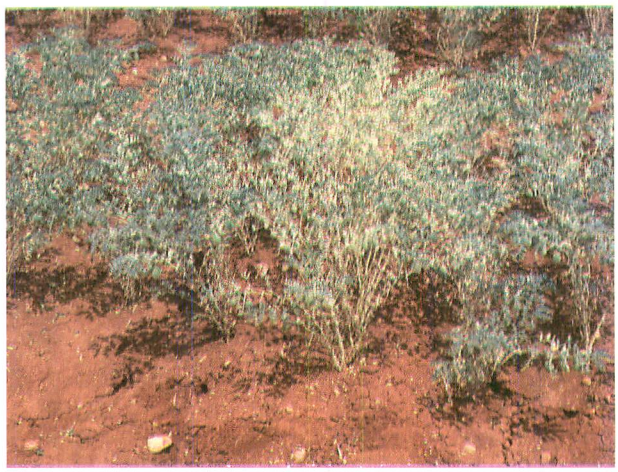
2.1 Lentil variety maintenance plot at ICARDA, Aleppo, Syria

2.2 Lentil maintenance plot with two offtype rows

2.3 Chickpea variety maintenance plot at ICARDA



2.4 Chickpea maintenance plot with one offtype row

2.5 Faba bean variety maintenance in isolated plots within legume field at ICARDA

2.6 Faba bean variety maintenance in isolated plots at ICARDA

Chapter 3: Seed Production

Introduction

Seed is one channel through which new technology is transferred to farmers, allowing them to benefit from agricultural research. Availability, access to, and use of quality seed is an important requirement for increasing agricultural production and productivity. Seed production requires special care to meet standard quality requirements (see Chapter 9). Both environmental conditions and cultural practices can affect seed quality. For example, environmental factors such as soil conditions, deficiency of water or nutrients, extreme temperatures, and pest infestation can reduce the viability and vigor of mature seed.

The key elements for the production of quality seed include:

- site selection to find suitable areas for seed production
- field selection to restrict volunteer plants and noxious weeds from preceding crops
- isolation from sources of contamination
- control of pollination to prevent out-crossing
- roguing to remove contaminants
- limiting the number of generations for multiplication
- cleanliness of farm machinery during planting, harvesting and transport
- cleanliness of equipment during cleaning, treatment, transport and storage
- seed production through specialized contract growers
- implementing quality assurance measures.

Development of an effective, efficient seed industry is more difficult for legumes, in comparison to cereals. There are several reasons: lack of improved adapted varieties, problems of mechanization, high disease and weed incidence, and high production costs. Moreover, farmers are reluctant to grow legumes either as seed or as grain because of lack of guaranteed prices (Erskine *et al.*, 1988).

Seed production of legume crops has been described in Agrawal (1985), Ahmed (1994), Doerfler (1976), Erskine (1986), Erskine *et al.* (1988), Khalil and Abdul-Kader (1988), Saxena and Singh (1987), Singh (1986), Singh and Saxena (1999), and Wellving (1984). This chapter deals with the production of the later generations, i.e. pre-basic, basic, and certified seed.

Selection of Seed Growers

Early generation seed (breeder, pre-basic, and basic) is often multiplied at research stations and/or in farms of seed companies, under the supervision of the breeder or other competent staff. Later generations (part of basic seed and certified seed) are usually produced on contract with seed growers. Contract growers should be carefully selected; only farmers with good reputations should be recommended for seed production.

Seed growers should be registered with the certification agency, which will assess their facilities and technical knowledge. Seed growers often form an association to advance the interests of their members and contribute to seed industry development.

Premium for Seed Growers

Contract seed growers usually incur additional costs because of the extra care and attention required to produce quality seed. If there is no price difference between seed and grain, contract growers have no incentive to apply management practices like isolation, roguing, cleanliness of machinery, etc. Seed growers therefore receive a premium over the grain price to cover the extra production costs. Since seed production in legumes requires more attention than for cereals, a substantially higher premium is suggested.

Monitoring Seed Quality

Before certification, the quality control agency should ensure that the seed crop is of the desig-

nated variety and desired quality. However, quality assurance is the primary responsibility of the seed producers and seed companies. They should have a thorough knowledge of the factors that affect seed quality during production. The grower should carefully inspect the seed in the field to ensure that it meets certification standards (Tables 1 and 3). If there are too many contaminants in the field, the grower should rogue them to bring the field to acceptable standards. The grower should ensure that the seed delivered to the company meets the standard specified in the contract. The seed company should also have an internal seed quality assurance system to check that seed fields meet the prescribed standards.

Seed Production Practices

Seed multiplication requires extra care to prevent genetic, mechanical and pathological contamination. The following section describes the most important management practices for producing seed of cool-season food legumes.

Choice of sites

Choice of area is the first step. Seed should be produced in: (i) areas where the variety is adapted, to prevent genetic shift, (ii) the right environment, to avoid climatic or natural hazards (frost, drought, flooding, salinity), (iii) areas with low pest incidence. For example, cool-season food legumes are susceptible to waterlogging (particularly during the seedling stage) and salinity compared to other crops (Salih *et al.*, 1996). Flat, non-stony land and leveled seedbeds are prerequisites for mechanization in all legumes, particularly for lentil because of its short stature. Warm areas are preferable because warm weather favors flowering, pollination, seed setting, and ripening (Thomson, 1979). However, very high temperatures may inhibit the development of ovules and fruits, and cause shedding of flower buds or young fruits.

Seed fields should be easily accessible for regular supervision, inspection, and immediate remedial action. This will also facilitate transportation to processing plants.

Previous cropping

Certain crops should not precede the seed crop in the field. It is important that the previous crop was appropriate. This will help control volunteer plants (which may reduce varietal purity) and prevent build-up of seed-borne diseases and noxious weeds. Previous cropping is different from rotation, which helps maintain soil fertility and control pests.

For pre-basic and basic seed production, the field should not have been planted with any other variety of the same species for at least two years. For certified seed, only one year is required between two seed crops of different varieties. However, a previous seed crop of the same variety and the same or higher seed class, is allowed.

For chickpea and lentil, avoid fields that were planted with *Vicia*, *Lathyrus*, pea, small-seeded faba bean, and phaseolus bean in the previous year. Seeds of these species are similar in size and difficult to remove during cleaning – therefore volunteer plants have to be rogued from the field. Similarly, volunteers of wheat and barley should be removed in the field because they are difficult to clean from lentil. Fields previously planted with *Lathyrus* and lupins are not recommended for faba bean seed production.

Many plant pathogens survive in the soil or on plant debris, while some (e.g. *Ascochyta* spp.) are seed-transmitted. Stem nematode (*Ditylenchus dipsaci*) is transmitted through both soil and seed. For some pathogens, e.g. *Fusarium*, *Rhizoctonia*, *Phoma* and *Botrytis* spp., seed-borne pathogen innocula are less important than soil-borne inocula. There are several effective methods to control these pathogens – crop rotation, i.e. cultivation of non-host plants for 3–5 years, burning of straw and stubble, flooding, or incorporation of debris into soil (Neergard, 1979).

Parasitic weeds such as broomrape (*Orobanche* spp.) and dodder (*Cuscuta* spp.) remain major problems in legume production. Broomrape seeds, for example, remain viable in the soil for over 10 years and may have several hosts. Dodder can remain viable in the soil for more than five years. An effective crop rotation may be difficult to implement. Fields infested with *Orobanche* are not acceptable for pro-

ducing seed of faba bean and lentil. Chickpea is less susceptible to *Orobanche* infestation, but is susceptible to dodder.

Seedbed preparation

Food legumes are very sensitive to waterlogging, particularly during the seedling stage. Therefore, it is important to provide adequate surface drainage during land preparation. Deep plowing to a depth of 15 cm is sufficient to obtain a good crop. However, where a hard pan exists in the rooting zone, sub-soiling will improve root development and infiltration rate. A well-tilled field may promote root and nodule development in seedlings. A deep seedbed is necessary for chickpea, because it has a long main root system. A firm weed-free seedbed on well drained soil is best for lentil production. Good seedbed preparation and weed control are prerequisites for combine harvesting.

In Egypt, sowing faba bean and chickpea on 60 cm wide ridges produces higher yields than sowing on flat beds, because the soil is better aerated. The broad bed (2.5 m) and furrow system improves drainage in lentil fields. Chickpea and lentil should be planted when the soil contains sufficient moisture to prevent crust, heavy weed infestation, and inoculum survival.

In lentil, rolling of fields provides a smooth and level surface for harvesting. It is preferable to roll the filed before crop emergence – but if the soil is dry this increases the risk of soil erosion. Rolling up to 5–7 node stages after crop emergence appears to have no effect on the crop, whereas rolling after that may damage the plants, increase the spread of foliar diseases, and reduce yield.

Planting methods

Row planting is generally preferable to broadcasting, because it requires less seed, facilitates mechanized weed control, roguing and field inspection (Galanopoulou *et al.*, 1996). In lentil, drilling gives a better seed yield than conventional methods (Saxena, 1981).

There should be roguing lanes (empty rows at intervals), which could be used by the seed grower for spraying or to walk through the field when rouging; or by the field inspector for inspecting the crop.

Lentil can be planted with a cereal drill whereas *kabuli* chickpea can be planted with a mechanical precision planter (Maxicorn). Small-seeded faba bean can be drilled with a maize planter or pneumatic precision plot planter (Erskine *et al.*, 1987). It is difficult to drill faba bean seeds that are heavier than 0.8 g. However, if a seeder has seed cups with large seed cells, it could be used for large-seeded faba bean without cracking of seeds. If different varieties are planted, planters should be properly cleaned between sowing, to avoid admixture or mechanical contamination.

Seed rates

Table 3.1 contains suggested seed rates for legume seed production. The optimum seed rate depends on crop, variety, location, seed size, time of sowing, and method of planting. However, for a new variety, the multiplication rate and yield per unit of seed planted are more important than maximum yield *per se*. With a high multiplication rate, the variety can be rapidly multiplied and distributed to farmers. Using lower seed rates and having large amounts of breeder seed initially is the best strategy for achieving faster seed multiplication. Experiments at ICARDA (unpublished data) have shown that decreasing the seed rate increases the multiplication rate in both chickpea and lentil.

Low seed rates also result in more open crop stands and lower disease incidence. However, low rates should only be used under close supervision, because it is not economical for seed growers (low yield per hectare).

The actual seed rates for a desired plant population can be calculated using the following formula:

Seed rate in kg ha^{-1} = [(plant density m^{-2} x 1000 seed weight in g) ÷ % field emergence or survival] x10

For example, consider a lentil variety with 1000 seed weight of 70 g, and 80% field emergence, planted at a population density of 12 plants m^{-2}. The seeding rate will be:

[(12 x 70) ÷ 80] x 10 = 105 kg ha^{-1}

Rhizobium inoculation

Legume crops can fix atmospheric nitrogen. The amount of nitrogen fixed varies between

Table 3.1. Suggested seed rates and row spacing for legume seed production

Crop	Seed rate (kg ha^{-1})	Spacing (cm) Row to row	Plant to plant
Faba bean	110	60	20
Chickpea	80	45	15
Lentil	60	30	4

and within species and is influenced by the environment. Faba bean is more efficient and fixes nitrogen throughout the growing season. Under favorable growing conditions, chickpea (60–80%) and lentil (>70%) can fix a significant proportion of their nitrogen requirements.

Leguminous root nodules are highly specialized structures formed because of a sequence of interactions between the host plant and the invading *Rhizobium*. Therefore, faba bean, chickpea, and lentil seeds must be inoculated with their specific *Rhizobium*, especially if they are planted in non-traditional areas. Both soil and seed inoculation increased chickpea seed yield in the semi-arid northern Great Plains of Canada (Gan *et al.*, 2005).

If the seed is dressed with chemicals, inoculation should be done after drying the treated seed. Granular inoculants are preferred, because the granules are separated from the seed and are less affected by chemical seed treatment. The seed must be planted immediately after inoculation; delays can reduce the efficacy of inoculants.

Fertilization

Since legumes can fix atmospheric nitrogen, they require less fertilizer than other field crops. High nitrogen levels in the soil can reduce nodulation and nitrogen fixation and delay maturity. Nitrogen fertilization is not required for legumes, except where a small quantity is recommended as a starter fertilizer.

Legumes require adequate amounts of phosphorus, potassium, calcium and other nutrients to ensure proper growth and development. They require phosphorus for healthy growth, faster maturity, and nitrogen fixation. For example, lentil has a relatively high phosphorus requirement for the development of an extensive root system and vigorous seedlings, thus promoting good nodulation and nitrogen fixation. Any fertilizer application should be based on soil analysis to determine the nutrient requirement of the crop under the prevailing growing conditions.

Weed control

Use of clean seed will help produce weed-free quality seed and avoid the introduction of weed species into the field. Faba bean competes poorly with the common annual grass and broadleaved species, especially at the seedling stage or under moisture stress. Chickpea is a poor competitor with weeds at all stages of growth and requires season-long weed management, because of slow growth during the seedling stage and relatively sparse optimum plant population with an open crop canopy. Lentil cannot compete with weeds, therefore it is essential to select weed-free fields. A good long-term weed control strategy is required, which considers the entire crop rotation. Annual weed species such as vetches (*Vicia* and *Lathyrus* spp.) and rough bedstraw (*Galium tricorne*) will not be efficiently weeded by manual labor because they have similar appearance, size, and time of maturity to lentils (Basler, 1981). Therefore, special attention should be paid to their control.

Orobanche spp. and *Cuscuta* spp. are two parasitic weeds, highly destructive to seed production of cool-season food legumes. *Orobanche* seeds are small (0.2–0.3 mm), and one gram contains 150,000–350,000 seeds. Faba bean is susceptible to *O. crenata* (Kukula *et al.*, 1985). Lentil is susceptible to several species such as *O. aegyptica, O. crenata*, and *O. ramosa* (Basler, 1981). *Orobanche crenata* is the most widespread and causes the greatest damage to faba bean in North Africa, from Egypt to Morocco (Gressel *et al.*, 2004). Chickpea seems to be less susceptible to *Orobanche* infestation but more susceptible to *Cuscuta*.

More information on the biology and control of *Orobanche* can be found in Borg (1986), Linke *et al.* (1989), Linke (1992), and Dahan and El-Mourid (2004). Khalil *et al.* (2004) and Abu Irmaileh (2004) have provided detailed information on the control of *Orobanche* in food legumes as follows:

Preventive methods

Prevent introduction and distribution of seeds from infested fields. Use clean seeds, clean farm equipment, and strict quarantine measures (at country, district or farm levels) to prevent introduction to non-infested areas.

Cultural practices

Hand weeding will reduce weed population and seed bank in the soil. It is useful when labor is cheap and there is no alternative strategy. Other methods to reduce *Orobanche* infestation are: adjusting planting date [late sowing in Egypt (mid-November to mid-December) and Spain], crop rotation, intercropping with trap crops (flax, fenugreek), fertilization (ammonium sulfate), deep plowing, and use of resistant/tolerant varieties.

Late planting reduces *Orobanche* infestation but also reduces crop yield (Kukula *et al.*, 1985; Linke, 1992).

Physical methods

Flooding for an extended period (weeks) may kill weed seeds in the soil. This has proved useful under irrigated conditions, especially in rice rotation, as reported in Iraq. Soil solarization with clear polyethylene sheets has been effective in reducing seed germination in Jordan, reducing *Orobanche* infestation, and increasing tomato yield in Sudan. However, it is relatively expensive.

Chemical control

Chemical control methods include: soil fumigation, seed dressing with selective herbicides, and pre-planting and/or pre-emergence application of herbicides (glyphosate, imidazolinones, and sulfonylureas) with repeated application at 2–4 week intervals. Knowledge of *Orobanche* phenology is essential for effective chemical control because the chemicals must be applied within a specific, limited period. Metham sodium, dazomet, and 1,3 dichloropropene are recommended for soil fumigation.

In faba bean, *Orobanche* can be controlled with Round-up (Lancer), applied as a post-emergence foliar spray. Apply three sprays at 86 g (a.i.) ha^{-1} each, at three-week intervals. The first spray should be at the beginning of flowering of the host plants. Spray 500 l ha^{-1} of the herbicide evenly with a knapsack sprayer (CP3). Glyphosate is effective for faba bean (three applications of 80 g a.i. ha^{-1} at 14-day intervals) and chickpea (two applications of 20 g a.i. ha^{-1}) but not for lentil. Imazethapyr is effective for faba bean (two applications of 30 g a.i. ha^{-1}) and lentil (two applications of 10 g a.i. ha^{-1}), but should not be used for chickpea.

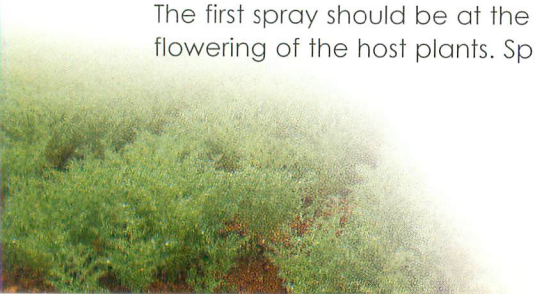
There is no single effective method for parasitic weed control in cool-season food legumes. Khalil *et al.* (2004) suggested an integrated approach for *Orobanche* control: use of resistant/tolerant varieties, crop rotation (trap or catch crops), land preparation (deep plowing or zero tillage), delayed sowing, fertilization (ammonium sulfate), frequent irrigation or flooding, biological control (*Phytomyza orobanchia*), solarization, and chemical control (glyphosate). There is an urgent need for an integrated control strategy combining cultural practices, chemical herbicides, and legislative procedures to limit the distribution of parasitic weeds with certified seed. For integrated *Orobanche* management in faba bean, Baya and Yahyaoui (2005) suggested the use of moderately susceptible or early maturing varieties, seed rate of 150 kg ha^{-1}, delayed sowing, and three foliar applications of glyphosate at 60 g a.i. ha^{-1} at flowering and then at two-week intervals. For lentil, they suggested delayed sowing, use of early maturing varieties adapted to late sowing, in combination with two post-emergence applications at a two-week interval, using either imazapic (5 g a.i. ha^{-1}) or imazethapyr (15x2 g a.i. ha^{-1}).

Irrigation

Irrigation is often necessary to provide sufficient water for prolific crop growth. Sprinkler irrigation should be minimized because it favors the development and spread of plant diseases. Irrigation should be applied early in the morning to combine good water-use efficiency with quick drying of leaves. Avoid passing irrigation water through plots of different varieties, because seed, pathogens and nematodes may be transported to another field and contaminate and/or infect the seed crops.

Isolation

Isolation is the growing of a seed crop away



from any source of contamination, whether genetic, mechanical, or pathological. Sufficiently large physical isolation in terms of distance, time, flowering date, etc. can reduce contamination. Minimum isolation distances usually depend on field size, pollination habit, direction and speed of wind, and the presence of natural barriers. Large fields are usually less liable to contamination than small ones, and the center portions are less susceptible than the borders. Therefore, larger isolation distances are required for smaller fields of early generation materials than for later generation certified seed.

Faba bean is a self-fertilizing crop, with an average of 35% outcrossing, and bees are the primary pollinators. Outcrossing decreases from 17% at 0.9 m to 1.2% at 92 m, and 0.6% at 184 m (Fig. 3.1) (Pope and Bond, 1975). Therefore, larger isolation distances are recommended: 400 m for pre-basic and 200 m for basic and certified seed (Table 3.2). However, a wide border of the same cultivar is more important than a long distance between different varieties, to avoid contamination (Nadal *et al.*, 2003).

On the other hand, chickpea and lentil are strictly self-fertilizing crops with very low percentage of outcrossing, therefore, a physical barrier between two fields could be sufficient. Singh and Saxena (1999) reported that outcrossing in chickpea is only 2%. However, slightly larger isolation distances are recommended, e.g. 5 m for pre-basic and 3 m for basic and certified seed (Table 3.2).

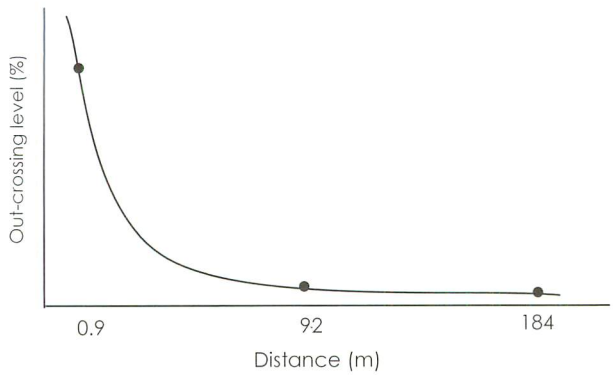


Figure 3.1. Isolation distance and level of outcrossing in faba bean

Table 3.2. Suggested isolation distances (m) for legume seed production

Crop	Pre-basic seed	Basic seed	Certified seed
Faba bean	400	200	200
Chickpea	5	3	3
Lentil	5	3	3

Roguing

Roguing is the systematic examination of seed fields and removal of undesirable plants (Gregg *et al.*, 1990; Laverack and Turner, 1995). The purpose is to maintain varietal purity and, to some extent, ensure freedom from virus (*faba bean necrotic yellow virus*, FBNYV) and seed-borne diseases. While roguing, take care not to carry out any selection that may change the genetic make-up of the variety. The breeder of the variety may assist in roguing early generation seed. Roguing can be carried out several times and at different growth stages. The following contaminants should be removed:

- Offtypes and other varieties (see Chapter 11)
- Other crop species (see Chapter 11)
- Weeds whose seeds cannot be separated by cleaning (see Chapter 11)
- Parasitic weeds such as *Orobanche* and *Cuscata* spp.
- Plants infected with seed-borne fungal and virus (FBNYV) diseases.

Always remove whole plants (including roots) during roguing to prevent re-growth. Remove rogued contaminants from the field and burn them; this is particularly important for *Orobanche*. Roguing is not very useful for most legume diseases except for viruses because of the long incubation period (plants are infected but do not show symptoms yet). For guidelines on roguing diseases in seed production fields, see Annex 4.

Roguing should be done at an appropriate growth stage of the crop. For example, in lentil, morphological characters used for roguing (plant height, vegetation color, fruit characteristics) are not easy to distinguish during late flowering, pod setting, and maturity. At these stages, the crop stand is too dense for a roguing crew to distinguish individual plants (Fig. 3.2). Thus, roguing should be done at flowering, although a crop is usually rogued more than once.

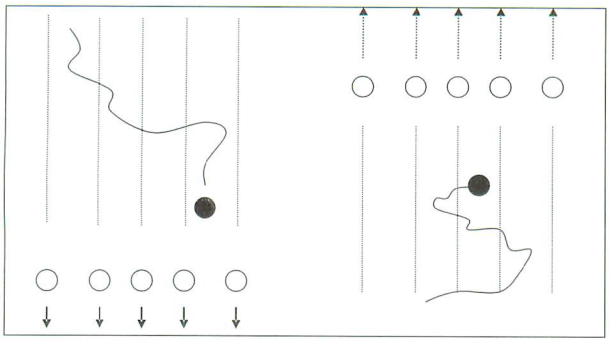


Figure 3.2. A five-member crew (o) following roguing lanes through a seed field monitored by the supervisor (●)

Roguing is more practical for breeder and pre-basic seed than for later generations (basic and certified seed), which are grown on large areas. It is important to remember that roguing is not a solution for poor seed crop management.

Disease control

The most important diseases are *Ascochyta* blight and chocolate spot in faba bean; *Ascochyta* blight in chickpea; and rust and wilt in lentil. The potential seed-borne diseases and insect pests of legumes are listed in Table 3.3 (Reddy, 1986: Diekmann, 1988). Guidelines on disease and pest management are provided in Annex 4.

Production of disease-free seed requires a combination of different practices. Since microorganisms and insects (as virus vectors) can move over large distances, isolation will not solve disease problems in legume seed production. The following section highlights some procedures for producing *Ascochyta*-free chickpea and wilt-free chickpea and lentil seed.

Ascochyta *blight of chickpea*

Ascochyta blight is the most widespread and economically devastating disease of chickpea. Yield losses are less than 10% in resistant genotypes but up to 100% in susceptible genotypes (Singh and Saxena, 1999). Kaiser (1984) recommended the following procedures for producing *Ascochyta*-free chickpea seed:

- Dry, warm weather impedes disease development. Seed should be produced in arid areas where there is little or no rainfall during flowering, fruiting, and harvesting.
- In areas where the disease reaches epidemic levels, plant chickpea for seed multiplication

Table 3.3. Important seed-borne diseases and insect pests of cool-season food legumes

Pests	Common name	Scientific name	Crop(s) affected
Viruses	Broad bean stain	BBSV	Faba bean
	Broad bean true mosaic	BBTMV	Faba bean
	Bean yellow mosaic	BYMV	Faba bean
	Pea seed-borne mosaic	PSbMV	Mostly pea, also faba bean
	Pea early browning	PEBV	Faba bean
	Vicia cryptic,	VCV	Faba bean
	Broad bean yellow band	BBYBV	
	Broad bean mild mosaic	BBMMV	
	Broad bean mottle,	BBMV	
	Broad bean wilt	BBWV	
Fungal diseases	*Ascochyta* blight	*Ascochyta* spp.	Faba bean, chickpea, lentil
	Chocolate spot	*Botrytis fabae*	Faba bean
	Gray mold	*Botrytis cinerea*	Faba bean, chickpea
	Wilt	*Fusarium oxysporum*	Lentil and chickpea
	Root rot	*Rhizoctinium solani*	Faba bean, lentil, chickpea
	Downy mildew	*Peronospora* spp.	Faba bean, lentil
Nematodes	Stem nematode	*Ditylenchus dipsaci*	Faba bean
Insects	'Field' bruchids	*Bruchus* spp.	Mostly faba bean and lentil
	'Storage' bruchids	*Bruchidius* spp., *Callosobruchus* spp.	Chickpea, lentil

only during spring – even though winter planting will give higher yield. The spring planted crop will need one or two supplemental irrigations to get adequate yield.
- It is highly desirable to grow chickpea in a three-year rotation to prevent build-up of the disease.
- *A. rabiei* can multiply on chickpea debris after harvest, and could be a source of inoculum. The residues should be burned or buried by deep plowing (10–15 cm) for faster decomposition and elimination.
- Use effective systemic fungicides to control possible surface and deep infections (Diekmann, 1986).
- Seed producers should inspect fields regularly to detect low level *Ascochyta* infection to take remedial action.

Wilt of chickpea and lentil

Wilt, caused by *Fusarium oxysporum,* is a seed- and soil-borne disease in both chickpea and lentil. In chickpea, the pathogen can survive for more than six years in the soil (Singh and Saxena, 1999). *Kabuli* type chickpea is more susceptible to soil-borne disease than the *desi* type. *Kabuli* chickpea has a thin (cream colored) seed coat, while the *desi* has a thick (dark colored) seed coat with some fungistatic properties due to tannin content.

Wilt is common in vertisols when temperatures are relatively high, often above 30°C. Areas infested with *Fusarium* should be avoided. Crop rotations with cereals will help prevent build-up of the pathogen. Infected plants should be destroyed and seeds should be dressed with an appropriate fungicide, especially in areas where wilt is a problem.

Combining biological control with seed treatment gives higher yield and better control of wilt than biocontrol or chemical seed treatment alone (De and Chaudhary, 1999). For example, use of *Bacillus subtilis* with carboxin (Vitavax) resulted in 66.8% wilt control and 145.4% increase in seed yield in lentil variety PPDL 2. In separate combinations with *Gliocladium virens, Trichoderma harzianum* or *T. virde* it reduced wilt by 79% and increased yield by 140, 224, and 241%, respectively.

Pesticide application

Chemical control is advisable to improve seed health and prevent yield losses. It is important to apply chemicals only if the incidence and severity of diseases are detrimental to seed health quality and/or reach economic thresholds. Seed infection in *Ascochyta*-susceptible chickpea and faba bean varieties can be prevented by spraying Chlorothalonil (Bravo) and Ortiva (Azoxystrobin) at 15-day intervals (Kharbanda and Bernier, 1979) when field conditions are above 15°C and 80% relative humidity. Two to three preventive sprays in March and April before infestation starts, are sufficient to control the disease in winter-sown crops. Application of fungicides to control chocolate spot is rarely economical; the most cost-effective fungicide is Mancozeb.

The source of *Bruchus* in lentil is infested seeds scattered in the field during harvest or infested seed used for planting (Pajni *et al.*, 1996). Field spraying may be necessary to control infestations in faba bean and lentil. Spraying fields with insecticides during oviposition can prevent infestation, but it is expensive unless other insects can be controlled at the same time. The selected insecticide should not be toxic to bees, since some plants will be flowering. Three insecticides – Methyl parathion, Alphacypermethrin, and Endosulfan – sprayed at pod setting and repeated two weeks later, have been effective in controlling *Bruchus dentipes* in faba bean fields (ICARDA, 1990). Endosulfan (Thiodan 35 EC) is recommended at 1.2 l ha^{-1} because it has relatively low toxicity to bees and beneficial insects and is also cheaper.

Harvesting

The main risk in mechanical harvesting of legumes is damage to seeds and physical admixture of varieties. Legumes are more prone to mechanical damage than most agricultural crops. Proper seed transport mechanisms (conveyor belts), adjustment of concave clearance, cylinder speed, sieves, and air blast will minimize seed damage during mechanical harvesting.

To prevent admixtures, clean the combines properly, run empty for some time or drive a few hundred meters to remove hidden seeds.

Attention should be paid to the threshing compartment, augers, elevators, and sieves. Harvest and discard the first two or three bags of seed. Or harvest the borders of the field first and discard this seed, because borders are often contaminated.

Legumes should be harvested at full maturity (moisture content of 12% and above). Combine harvesting should be done early in the morning when the moisture content of the seed is higher, to minimize cracking and losses.

Ellis *et al.* (1988) reported that faba bean seed harvested between 51% and 24% moisture content showed a decline in the proportion of broken seeds (from 19% to 2%) and an increase in germination (from 48% to 92%) with decrease in moisture content.

Chickpea has a low shattering potential, but pod drop and pod shattering may occur when harvesting is delayed or in unusually high temperatures. Delaying harvesting by one month reduced germination from 99% to 80%, and a further delay by one month reduced germination to 30% (Ellis *et al.*, 1988).

Lentil should be harvested at 100% pod maturity, because pods dehisce and drop if harvesting is delayed, resulting in up to 20% seed losses. Delaying harvesting by one week reduced germination by 20% (Ellis *et al.*, 1987).

Harvesting of faba bean should begin when the stems are slightly green and not dried out completely; usually within a few days after the upper pods turn completely black. This will minimize shattering and cracking during threshing. Faba bean plots are often mowed with a double-knife cutter bar in a swath (Erskine *et al.*, 1987). Small-seeded faba bean can also be combine harvested, but the large-seeded type (>0.8 g/seed) is still difficult to combine harvest. A drum speed of 300–500 revolutions per minute has been suggested. In Australia, the suggested settings are 400–600 rpm thresher speed, 15–35 mm concave clearance, 32–28 mm top sieve, 16–19 mm bottom sieve, and 700–900 rpm rotor speed.

Combines can be used to harvest large-seeded chickpea (Table 3.4). Winter-sown chickpea has an advantage over the traditional spring-sown crop because of its greater height. However, chickpea seeds have a characteristic small, protruding beak-like structure, which must not be damaged. Seed damage can be minimized by using conveyor belts (or by keeping the standard augers as full as possible) and operating at slower speeds. The cylinder speed should be adjusted to less than 500 rpm and the distance between concave and drum set close to its maximum. Upper sieves of 10–15 mm and lower sieves of 8–12 mm are used for chickpea, depending on seed size.

Lentil is more difficult to harvest than faba bean and chickpea because of the shorter height. However, rolled fields are easier to harvest mechanically using a combine equipped with a flex header or a pick-up reel and vine lifters when seed and pods are fully mature or after desiccation. Excessively dry seed will chip and peel during threshing. The drum speed and concave width should be adjusted to prevent splitting and decortication of seed. The cylinder speed should be adjusted to less than 500 rpm and the distance between concave and drum set close to its maximum. Standard cereal sieves are acceptable for lentils.

Table 3.4. Suggested combine settings for harvesting legumes

Drum speed (rpm)	Concave clearance	Sieves (mm)	References
Chickpea			
Plot combine <500	Maximum	10-15, 8-12	Erskine *et al.*, 1987
500-700	Inlet 19 mm Outlet 9.5 mm	16-19 9.5-12.5	Acikgoz & Kutlu, 1987
550-600	2x seed size	Depending on size	Saxena *et. al.*, 1987
Lentil			
Plot combine < 500	Maximum	Cereal sieves	Erskine *et al.*, 1987
Combine with special knife (15 cm longer) 400-500	Inlet 9 mm Outlet 3 mm		Hassan, 1987
Faba bean			
300-500			

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3.1 Lentil field infested with *Orobanche* spp.

3.2 Chickpea field infested with Cuscuta spp

3.3 Faba bean field infested with *Orobanche* spp.

3.4 Lentil and chickpea seed infected with *Aschochyta* blight

3.5 Faba bean leaves and pods infected with *Aschochyta* blight

3.6 Faba bean pods infected with *Aschochyta* blight

3.7 Clean chickpea seed production field at vegetative stage

3.8 Clean chickpea seed production field at flowering stage

3.9 Spraying a chickpea field against *Ascochyta* blight

3.10 Inter-row cultivation of lentil seed production field

3.11 Manual harvesting of lentil seed production fields

3.12 Mechanical harvesting machine suitable for lentil seed production

Chapter 4: Seed Cleaning

Introduction

Seed processing includes all steps involved in the preparation of harvested seed for marketing – drying, pre-cleaning, cleaning, upgrading, treating and packaging. After harvest, the seed should be transported as quickly as possible to the nearest processing plant, where it is pre-cleaned immediately to remove contaminants and dried, if necessary, to remove excess moisture. Effective separation of seed from contaminants depends on differences in physical properties, such as length, width, thickness, weight, shape, surface texture, and color. The seed is then treated (see Chapter 5) to prevent infections that could affect crop establishment. The process of cleaning and treating is referred to as seed processing.

Seeds enter and leave processing plants in lots. Therefore, it is very important to keep the seed lots separate, to maintain their identity during transportation, cleaning, and storage. Care should be taken to prevent admixture during processing. Further information on seed cleaning can be obtained from Boyd *et al.* (1975), Brandenburg (1977), Brandenburg and Park (1977), FAO (1981), Gregg *et al.* (1970), Gregg (1983, 1984), Grass and Gregg (2000), ISTA (1977), van der Burg (1986), and Vaughan *et al.* (1968).

Purpose of Seed Cleaning

Harvested seed contains undesirable materials, which have to be removed. The main undesirable materials are:

- inert matter such as plant parts, soil particles and stones
- weed seeds
- seeds of other crops
- seeds of other varieties
- immature, shriveled, broken, damaged, and deteriorated seeds of the same variety.

The following objectives are important (Gregg, 1977):

- Effective separation: removal of undesirable materials such as other crop seeds, weed seeds, and inert matter.
- Upgrading quality: improvement of quality by removing shriveled, diseased, broken and damaged seeds.
- Pesticide treatment: covering the seed with chemicals to eradicate existing infections or protect against future infections (during seedling emergence or crop establishment).
- Minimum seed loss: minimizing loss of good quality seed.
- Efficiency of machine: maintaining effective separation of contaminants consistent with the highest machine capacity.
- Minimum operation costs: minimize operation costs (which are variable and dependent on quantity) to an acceptable minimum.

Basis for Seed Cleaning

Seed cleaning is primarily based on the differences in physical properties between the desirable seed and the contaminants (Boyd *et al.*, 1975). It is not possible to separate seed and contaminants unless they differ in some physical characteristics. Seeds exhibit differences in length, width, thickness, weight, shape, surface texture, and color. Seed size, weight, and shape are commonly used characteristics for seed cleaning. Other characteristics are limited in application and require special machines for proper separation.

Size

Size is a highly variable characteristic, and the one most often used in seed cleaning. Size is described in three dimensions: length, width and thickness. Length is the biggest dimension, thickness the smallest. Most elongated seeds have three unequal dimensions. Theoretically, in round seeds length, width and thickness are equal, but seeds with near-perfect round shape may not exist.

Weight

Weight is the second most important characteristic used in seed cleaning. Materials of the

same size and general shape may differ in weight. Inert matter such as stones and soil clods are heavier, while immature, deteriorated, diseased and infested seeds are lighter than normal seeds.

Shape

Seeds of the same size are difficult to clean with screen and length separators; spiral separators can be used. Differences in shape are often used for effective separation. Round and flat seeds are separated using the differences in their ability to roll. Round seeds roll down a spiral plane faster, fall into the outer spiral, and are discharged separately, whereas flat seeds slide down the inner spirals and are carried to a different spout.

Texture

The outer coats of seeds differ in texture. Relative roughness and smoothness of the seed coat is used to separate seed on a belt grader. Smooth seeds roll down the inclined flat belt and fall off the lower end, whereas rough or irregular seeds are carried upward and fall off the upper end.

Color

Legume seeds differ in color due to immaturity, disease infection, early harvesting, and field weathering. Off-color seeds, mud balls and stones can be easily separated.

Principles of Seed Cleaning

Farmers traditionally use air and/or sieves to clean their harvest for seed. Modern seed plants integrate some of the traditional methods into an automated large-scale operation. Seed is cleaned using screens, cylinders, and air blasts. Screens and air blasts are most important for legumes, because cylinders are rarely used for round seeds. The principles of separation are as follows:

Screen separation

Screens are primarily used to separate seeds that are different in width and thickness. Round screens separate based on the diameter (width) of the kernel, while oblong (slotted) screens do thickness separation. Screens are of different sizes, and are made of several materials, most commonly iron sheet. Brushes mounted under the sieves or rubber balls placed in a frame of wire-mesh under the sieve help keep the screen clean during cleaning.

Air separation

Air separators work on the basis of the behavior of seeds in an air stream (terminal velocity, i.e. maximum speed attained by a particle in free fall). This speed depends on the density, shape, size, and surface texture of the seed. The most important characteristic in terms of air resistance is the weight. The lighter particles (dust, chaff, empty or partly filled seeds, pods) will be removed while the heavier seed will fall through the air stream.

Cylinder separation

Indented cylinders or disks – not often used in legume cleaning – are used for length separation. Depending on their size, the indent (cells or pockets) will lift seeds that fit into it. It will not lift long seeds, or may not lift them high enough to be separated. Cylinder pockets are usually hemispherical.

Density separation

Differences in relative density (weight) can be used for cleaning seeds. This involves two steps. First, the seed mixture is vertically stratified so that heavier seeds are at the bottom and lighter ones at the top. Second, the layers of seed that differ in density are separated as they move along the deck in different directions to the discharge spouts. Heavier seeds move to the upper end, lighter seeds to the lower end.

Basic Machines for Seed Cleaning

One machine can be used to clean seed of different crops, but one machine cannot separate seeds that differ in many physical characteristics. Hence, different machines must be combined in a specific sequence to separate seeds based on their physical characteristics. The

choice of machine depends on the crop, type of contaminants, and the quality standard that must be achieved (Boyd *et al.*, 1975).

Legumes are very susceptible to mechanical damage during seed cleaning and handling. It is therefore important to minimize the number of cleaning machines used, and avoid the seed falling over long distances in the machine.

Pre-cleaner

Pre-cleaners are high capacity machines with or without fans. They are mainly used to remove larger impurities from seed lots so that the seed can have maximum flow during subsequent cleaning operations. A pre-cleaner uses air and screens to remove the bulk of trash from the seed and facilitate elevating and cleaning. A pre-cleaner usually has one or two sieves with large round holes and a powerful air system. All commercial seed cleaning plants have a pre-cleaner. However, depending on the quality of seed lots received, a pre-cleaner may or may not be used.

Fine cleaner

The fine cleaner, also known as air-screen cleaner, is the most important and basic machine in all seed processing plants. It separates seeds according to their width, thickness, shape, and terminal velocity using a combination of screens and air blast. The screens, usually one to four, separate on size (width, thickness) and shape, while the air blasts (usually two) remove the lighter seed. In most cases, the air screen cleaner is sufficient to clean any seed lot because it separates based on the major physical characteristics, size, shape, and weight.

The air screen machine uses three principles. First is scalping, in which good seeds are dropped through screen openings while larger material is carried over the screens into a separate spout. Second is grading, in which good seeds pass over screen openings while smaller particles drop through. Third is aspiration, in which light material is removed from the seed mass.

Indented cylinder

The indented cylinder is another important machine. All cleaning plants usually have indented cylinders in the standard set-up. It separates mainly based on the length of the seed and can be used in two ways:

- Round grain application: short impurities are lifted and dropped into the tray while the longer seeds are left in the cylinder.
- Long grain application: the seed is lifted while longer impurities are left in the cylinder.

However, indented cylinders are less essential for the round seeds of many food legumes.

Gravity table

The gravity table is the best of the density separators. The essential parts include a porous deck, a fan that forces air through the deck, and assemblies that oscillate and incline the deck. After the seed has been cleaned by the air screen cleaner (and/or indented cylinder), it may be necessary to use a gravity table to remove seeds that have the same dimensions but different weights (relative density).

The seeds move along a shaking deck through which air is forced to flow. The heavier seeds, which are in contact with the surface of the deck, will be pushed towards the upper end of the table. The lighter seeds are not in contact with the deck and they tend to float downwards to the lower end of the table. Density separators are employed mainly to improve germination by removing deteriorated, diseased, or insect-damaged seed. The equipment requires careful setting and considerable experience to operate.

Legume Seed Cleaning

Faba bean

Faba bean seeds are very large compared to chickpea and lentil. The scalping sieve can remove the large impurities, the top sieve (round) will separate based on the width of the seed, while the bottom sieve will separate based on the thickness. Some loss of good seed is inevitable because there is substantial variation in size: large broken grains may be the same size as small complete or whole grains. The rejected material may be cleaned again to recover good seed. Faba bean does not often need an indented cylinder, but the gravity table can be used to grade the seed.

Chickpea

Chickpea can be properly cleaned by an air screen cleaner because the seeds are round, with only one dimension (diameter). If there are three sieves, the scalping deck will take out the larger impurities, the top sieve (round) will separate based on diameter, and the bottom sieve (oblong) will separate based on the thickness (remove broken and small grains).

Chickpea does not often need an indented cylinder. However, if unthreshed pods are of the same size (width and thickness) as large seeds, they may escape the scalping deck and may have to be removed by long grain application.

Lentil

Lentil has 'flat round' seeds, and in the air screen cleaner, a round sieve will separate based on diameter and an oblong sieve based on thickness. *Vicia, Lathyrus* and *Galium* seeds have similar seed shape to lentil and are, therefore, difficult to separate. A large proportion of these impurities can be removed by carefully choosing the right sieves.

Mechanically harvested lentils may contain many impurities, which justifies the use of an indented cylinder and a gravity table. An indented cylinder will remove smaller and shorter impurities (round grain application) that escaped separation in the air screen cleaner. Moreover, some of the larger vetches, cereals (mainly barley), and unthreshed lentil pods may be removed (long grain application).

The gravity table can be used to separate stones and soil particles of the same size. It is also very useful in removing bruchid infested seeds; seeds without seed coat; and *Galium* seeds, which have the same size as the smaller lentil seeds.

Table 4.1 presents the sizes of sieves and indented cylinders that can be used to clean cool-season food legume seeds. These can be modified depending on the variety, impurities, and growing conditions.

Optional Machines for Legume Seed Cleaning

Scarifier

Hard seed and impermeable seed coat are common in almost all legumes, including faba bean, chickpea, and lentil. A scarifier can reduce the hardness of the seed coat to improve germination. Seeds are fed through a drum, which has the inner wall lined with sandpaper or a brush. As the seeds pass through, they rub against the sandpaper or brush, and the coat may prickle, thus enabling the seeds to imbibe water and make them germinate more easily. However, caution should be exercised to avoid seed damage.

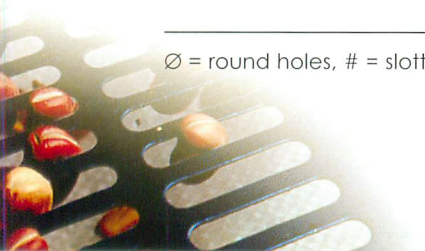
Needle indented cylinder

The needle indented cylinder is a special type of cylinder with needles on the indents. Theoretically, it removes insect-damaged legume seeds, but is rarely used in practice. Insect-infested grains are lifted in the tray as the needle fits into the hole made by the insect. However, a gravity table can also remove most of the insect-damaged seeds because of the differences in weight.

Table 4.1. Suggested sizes for screens and indented cylinders for cleaning legume seeds

Cleaner	Screen size, top (mm)	Screen size, bottom (mm)
Screens		
Faba bean	10–12 ∅	5.5–6 #
Chickpea	8–11 ∅	4–5 #, 5–5.5 ∅
Lentil (large)	8 ∅	5.5–6.5 ∅
Lentil (small)	5 ∅	3–3.5 ∅
Indented cylinders	**Indent size (mm)**	**Impurity removed**
Chickpea	9.5–10.5 (long grain)	Unthreshed pods
Lentil	6.5–7.5 (long grain)	Barley, wheat
	5.5 (round grain)	Small impurities

∅ = round holes, # = slotted (oblong) holes



Spiral separator

The spiral separator classifies seeds according to their shape and ability to roll. It consists of sheet metal strips fitted around a central axis in the form of a spiral. Round seeds roll easily. They accelerate as they roll down, until they roll over the edge of the inner flight into the outer flight, where they are collected separately. The slower flat seeds do not build up enough speed to escape from the inner flight.

The spiral separator is usually not included in a legume seed plant. It can, however, be used successfully to remove vetches, *Lathyrus* and *Galium* from lentil. In general, the spiral separator is very useful for removing broken grains from round crop seeds.

Color separator

Legume seeds can be discolored because of disease infection, early harvesting, high moisture during storage, etc. Discolored seeds are of lower quality and should be separated from the good seed. The color separator assesses the color of individual seed by means of photocells. Seeds that differ in color are detected and a jet of air blows them away from the stream. The color separator can be used to remove discolored seed from faba bean, pea, and beans. It can also be used to remove black vetch seed from the lighter lentil seed. However, this machine is expensive and the cost often does not justify the marginal increase in quality.

Picking belt

A picking belt is a conveyor belt on which the seed moves. It can be used to remove any abnormal seed that can be easily detected by the naked eye. Laborers who sit along the belt observe the seeds individually and remove the discolored, infected or infested ones. It can be used as an alternative to the color separator (to remove off-color seed) and the needle indented cylinder (to remove infested seeds).

Other Essential Equipment

Elevators and conveyors

During cleaning, the seed must flow between the different machines efficiently and without damage. Elevators are used to move seed vertically while conveyors are used to carry seed horizontally or at an angle. It is important to choose the right elevators and conveyors for legume seed crops, because they are sensitive to mechanical damage. Belt conveyors are preferred. Screw conveyors should never be used for seed because they easily damage the seed.

Elevators with a flat belt are generally recommended for legume seeds, rather than elevators with various types of chains or links. Avoid elevators that convey seed in an air stream flowing through sealed pipes, because they may cause mechanical damage to seeds. The speed of the elevators may also need to be adjusted for legume seeds.

Pipes connecting feeding machines should be placed at 45° to ensure that the seed is sliding (not falling) into the cleaning machines. For legume seeds, it is recommended to fit impact-absorbing materials (e.g. rubber) wherever the seed falls over longer distances.

Holding bins

In a processing line, different machines do not operate at exactly the same capacity. Without holding bins, all machines must run at the capacity of the slowest. Some cleaners do not separate seed effectively when operated below normal capacity. Holding bins are essential because they buffer the differences in capacity and permit each machine to operate at its most effective rate (it is a prerequisite for the gravity table). Every machine should have a bin that is large enough to permit efficient operation both of itself and the machine before or after it. The first bin in the cleaning line and the elevator serving it should have high capacity to fill the bin rapidly with enough seed for several hours. Similarly, the final bin receiving the processed seed and supplying the bagger-weigher should be large enough to hold all seed cleaned for several hours.

Air compressor and vacuum cleaners

Cleanliness is essential to avoid mechanical admixture. Seed cleaning machines should be thoroughly cleaned between seed lots, using an air compressor and industrial vacuum cleaner. The screens, cylinders, and decks should be

removed and cleaned separately, and the machines should be run empty at high speed to remove hidden seeds. The building and its surroundings should also be kept spotlessly clean.

Mechanical admixture can also be reduced by careful planning. For instance, cleaning early generation seed immediately after cleaning a later generation of the same variety (or very similar ones) will reduce the chances of contamination. The general recommendation is to clean seed in the following sequence: certified seed, basic seed, and pre-basic seed. Cleaning a crop with different characteristics (length, width, thickness, etc.) between two varieties of the same species will also help to avoid contamination.

Table 4.2 lists various types of equipment used for seed processing of legumes.

Management of Seed Cleaning

Seed cleaning operations consist of several steps in a specific sequence. Management of seed cleaning operations involves guiding the personnel to effectively operate the facilities (equipment), and produce high quality seed at the least possible cost.

Selection of appropriate machines

Seed cleaning involves the physical movement of seed through different machines, which perform specific operations. The efficiency of processing depends on the integration of all equipment into a complete line, proper seed flow, and execution of operations as a coordinated whole (Gregg 1977). The plant should be well designed, with options to shorten cleaning operations by bypassing machines that are not necessary.

Planning cleaning operations

Seed cleaning operations include receiving, pre-conditioning, basic cleaning, and finishing (separation and grading). They are an integral part of seed production, which involves personnel, equipment, and facilities to prepare quality seed for the market. It is important to prepare a realistic and flexible plan, paying attention to details. Develop a seed cleaning calendar, which matches the cropping calendar, to ensure timely distribution and planting. Plan for all aspects – machinery (maintenance and spare parts), supplies (packaging materials, seed treatment chemicals), personnel, labor requirement, etc.

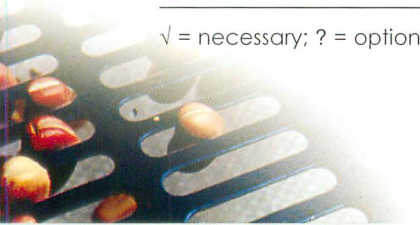
Monitoring seed quality

Seed processing plants should have an internal seed quality control laboratory that is adequately equipped and well staffed. The laboratory should conduct simple purity, germination, and moisture tests to monitor the quality of incoming materials, the cleaning and treating process, and storage. It should ensure that growers have met contractual agreements, ensure drying of incoming seed lots, selection of appropriate machines for cleaning operations, and maintenance of the desired quality standard. Such quality tests will ensure that no good seed is lost, that processed and stored seeds meet the desired standards, do not deteriorate or get damaged, and that the operations are efficient and cost-effective.

Table 4.2. Machines used for processing legume crops

Type of machine	Crops		
	Faba bean	Chickpea	Lentil
Pre-cleaner	√	√	√
Fine cleaner	√	√	√
Indented cylinder	–	–	√
Gravity table	√	?	√
Spiral separator	–	–	√
Color sorter	?	–	√
Scarifier	?	–	?
Treater	√	√	√
Bagger-weigher	?	?	?

√ = necessary; ? = optional, only required in certain cases



In summary, while cleaning the seed, it is important to:

- Avoid damage especially when lifting and dropping seed, because legumes are more vulnerable than other crops. Seeds damaged by impact usually have internal microcracks that cause loss of viability and may induce chemical injury during treatment.
- Avoid mechanical contamination of seeds by cleaning machines regularly while changing seed lots, varieties, or crops.

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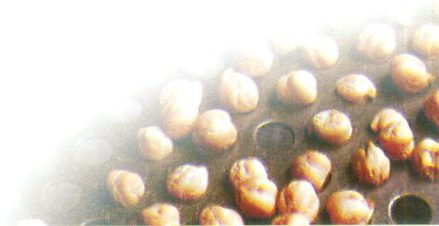
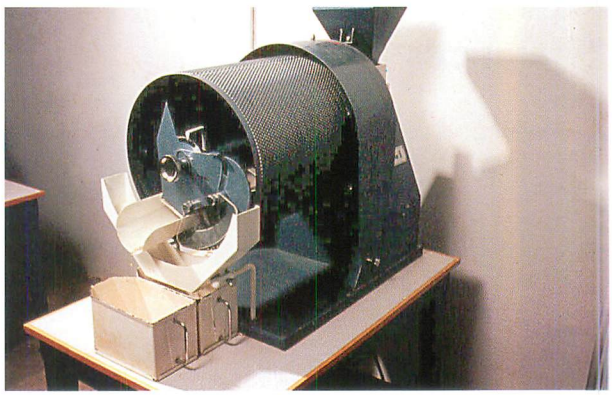


4.1 Seed processing plant ideal for cool season food legumes

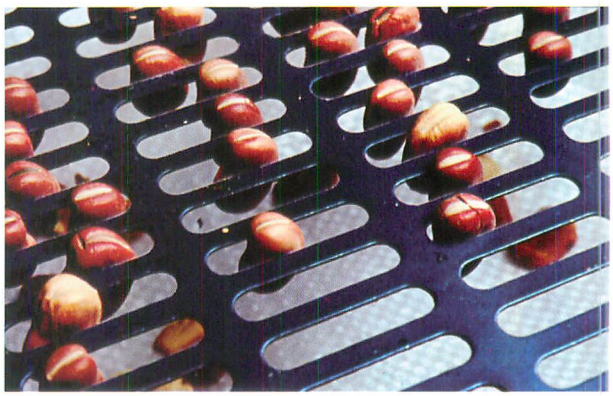
4.2 Internal seed laboratory with well equipped facilities

4.3 Air-screen cleaner with three layers

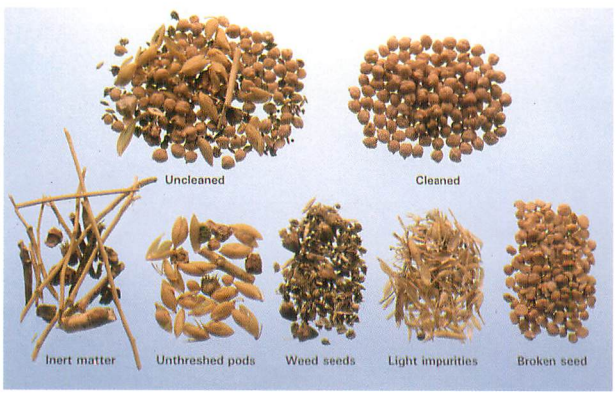


4.4 Indented cylinder for length separation

4.5 Screen with round holes with chickpea seeds



4.6 Screen with oblong holes with faba bean seeds



4.7 A sample of raw seed, clean seed and impurities

4.8 Hand picking of off-color legume seeds

Chapter 5: Seed Treatment

Introduction

Seed may be infected with fungi, bacteria, viruses, or infested with nematodes or insects, either on the surface or internally. Freedom from pathogens and parasitic weeds are key issues in seed health quality standards. Seed infection may lead to low germination, reduced field establishment, severe yield loss, or total crop failure. For example, *Ascochyta rabiei* (Maden, 1983) and *A. lentis* (Morrall and Sheppard, 1981) have been associated with reduced seedling emergence in chickpea and lentil, respectively. Moreover, seed is an important means of carrying over and dispersing plant pathogens.

To obtain healthy seed, it is important to produce in disease-free areas or under effective disease control and certification schemes (field inspection, laboratory testing). A combination of the following practices is required:

- use of disease-free seed lots
- zoning production in areas with low disease pressure
- off-season production
- isolation of fields
- roguing diseased plants
- spraying to control diseases
- field inspection and seed testing
- efficient cleaning of seed lots
- chemical seed treatment.

Understanding the epidemiology, transmission rate, and economic threshold of diseases, combined with seed health testing, helps in determining the need for chemical treatment. However, only certain groups of pests can be controlled effectively by seed treatment. There are good pesticides to control fungal pathogens and insect pests, but very few practical solutions for bacteria, viruses, nematodes, and parasitic weeds. General information on seed treatment is available in Diekmann (1993), Agrawal and Sinclair (1987), and Jeffs (1986).

Pathogens of Legume Seeds

Seed-borne transmission of diseases has some special features. There is a close association of the virulent pathogen with its host during the dispersal phase, a potential for long distance spread, and uniform distribution of inoculum throughout the crop. There are generally three types of pathogens in legumes:

- Pathogens that contaminate the seed superficially and infect the seedling after planting. These can be controlled with a wide range of broad-spectrum contact fungicides applied as seed treatment.
- Soil-borne pathogens that infect the seedling. Treatment of healthy seeds may be advisable (e.g. *Fusarium oxysporum*). These are rarely found on/in the seed, but survive in the soil. Other soil-borne pathogens that can be controlled by seed treatment include *Pythium* spp. and *Rhizoctonia* spp.
- Pathogens that infect many parts of the plant (leaves, stems, pods, seeds), e.g. *Ascochyta* spp. These produce many generations per year, depending on the environmental conditions. Seed infection is mostly internal, and systemic fungicides are recommended.

Fungicides for Disease Control

Chemical seed treatment is standard procedure for disease control in many crops. The choice of chemical(s) will depend on the target organisms. Chemicals are available in different formulations, e.g. dust, wettable powder for slurry treatment, or liquid concentrates. In general, dusts are applied at a rate of approximately 2 g kg^{-1} seed, and slurries or liquids at 5–10 ml kg^{-1} seed. Slurries or liquids are preferred to dust formulations because they are easier to measure, coat the seeds more effectively, and pose a low risk to operators. Different admixtures of dust formulations may be used for treatment to prevent

these problems. A cheap additive is 0.2% dextrine solution at the rate of 3–5 ml kg^{-1} seed. Special 'incrusters', such as Sacrust, are more effective, but also more expensive.

The following specific recommendations can be used for the control of legume diseases:

Chocolate spot

In faba bean, Benomyl + Thiram at 0.8 g kg^{-1} seed resulted in significantly less chocolate spot *(Botrytis fabae)* and significant yield increases, while Thiram alone at 0.8 g kg^{-1} did not prevent aggressive blotching of the lower leaves (Bainbridge *et al.*, 1985).

Ascochyta blight

Thiabendazole (Tecto WP) at 3 g a.i. kg^{-1} controlled *Ascochyta rabiei* on chickpea and *Ascochyta lentis* on lentil (Kaiser and Hannan, 1987, 1988). Vitavax-200 (Thiram and Carboxin) is used at 2–3 g kg^{-1} as a broad-spectrum chemical seed treatment at ICARDA. In North America, chickpea seed must be treated prior to planting with an effective fungicide such as Carbothiin, Thiabendazole, or Metalaxyl (Gan *et al.*, 2006). Tridemorph + Maneb (Calixin M) has been used against *Ascochyta*, but there are conflicting reports about the degree of efficacy (Reddy, 1980; Kaiser and Hannan 1988). Calixin M can be used to control *Ascochyta* in faba bean. Gan *et al.* (2006) gives a comprehensive review of options for *Ascochyta* blight management in chickpea.

Wilt, root rot, and damping-off

The fungi causing root rot and damping-off include *Pythium* spp., *Rhizoctonia solani*, *Fusarium oxysporum*, *F. solani*, *Aphanomyces euteiches*, *Thielaviopsis basicola*, etc. The following fungicides (or their combinations) provide effective control: Captafol, Chloroneb, Thiophanate-methyl, PCNB, ETMT, Triforine, Captan, Dowco 444, Prothiocarb, Metalaxyl, Thiabendazole, Benalaxyl, Thiram (Papavizas and Lewis, 1975; Kraft, 1982; Trapero-Casas *et al.*, 1990).

Thiram at 2 g a.i. kg^{-1} seed is a cost-effective seed treatment against seed rot and/or damping-off in faba bean, chickpea, and lentil.

It is difficult to control *F. oxysporum*, the pathogen causing wilt in legumes, with seed treatment. A good rotation is more effective. In practice, non-systemic fungicides can control other fungi, because fungal pathogens in legumes are mostly located in the seed coat (Ellis and Paschal, 1979; Tu, 1988).

The side effects of seed treatment on nodulation in legumes should be considered. Ram *et al.* (1984) found that *Rhizobium* inoculation followed by treatment with Dithane M-45 gave the best results. Chemicals reported to reduce nodulation are Oxycarboxin, PCNB, and copper fungicides, while the reports on Captan, Carboxin, Chloranil, Dichlone, Mercurials, and Thiram are inconsistent (Agarwal and Sinclair, 1987).

Insecticides for Pest Control

It is necessary to distinguish between the control of field pests and storage pests. Some field pests, e.g. wireworms or nematodes, can be controlled with pesticide applied as seed treatment (Table 5.1), which is generally more cost-effective than field application.

Mishra and Gaur (1984) found that Aldicarb at 2% a.i. w/w applied as dust after a thin coating with gum arabic, increased grain yield and significantly reduced the galling of *Meloidogyne incognita* and the final nematode population. Carbofuran at 3% was less effective, and Phorate (Thimet) even less so. However, these chemicals are extremely toxic and pose a high risk to those who apply them or handle the treated seed.

Table 5.1. Insecticides used for seed treatment to control field insects and nematodes

Trade name (manufacturer)	Active ingredient	Target pest/ pathogen	Crop(s)
Flowable Lindane 40% (Gustafson)	Lindane	Wireworms	Numerous
Curaterr (Bayer)	Carbofuran	*Sitona* spp., nematodes	Legumes
Promet (Ciba Geigy)	Furathiocarb	*Sitona* spp.	Legumes

Sitona spp. can be controlled by treating the seed with Promet (Furathiocarb) at 12 ml kg^{-1} seed (ICARDA, 1990).

Fumigation controls storage insect pests more effectively, but does not have a lasting effect. Contact insecticides, on the other hand, have a degree of persistence. It is preferable to treat seed with insecticide if fumigation is ineffective, for example airtight sealing of the storage area is not possible. Actellic and K-Othrine are relatively safe and do not affect seed viability (see Chapter 7).

Equipment for Seed Treatment

It is difficult, if not impossible, to achieve complete control even with the excellent chemicals currently available in the market. During treatment, some seeds may not receive adequate chemical load, while others may receive more than the recommended dosage. Choosing the right equipment and calibrating it properly can minimize these problems.

The latest seed treatment equipment ensures automatic measuring and mixing of chemicals with seeds. In the Gustafson treaters, for instance, the weight of the seed, measured in a weighing pan, is used to operate the chemical measuring system. By adjusting a counterweight, a fixed quantity of seed is treated with a fixed quantity of chemical, measured in standard cups, and operated with the trip of the weighing pan.

Most commercially available treaters are designed for slurry (suspension of a wettable powder in water) or liquid preparations. The solid particles may form sediments, therefore a stirring device is necessary. The particles may also clog nozzles, therefore the mixture should be applied from small measuring buckets on an endless chain. Liquid formulations are usually sprayed on the seed, as in the Mist-O-Matic treaters, which ensures even coverage of the seed.

Safety Precautions

It is essential to use chemicals that are safe for operators, farmers, and the environment. Very toxic substances, such as organic mercurials (Ceresan and others), and very persistent fungicides, such as Hexachlorobenzene (HCB), are being replaced by a new generation of chemicals that are less toxic and less persistent. Even with the new less toxic chemicals, the following safety precautions must be taken:

- Treated seed must be clearly labeled. If it remains unsold, it should not be used for feed or food under any circumstances.
- Treated seed should be planted immediately and not stored as carry-over seed.
- Seed with high moisture content (>16%) should not be treated with chemicals.
- Seed treatment should be non-toxic to the seed but effective against the target organism. The rates applied should be appropriate to prevent over-dosage leading to phytotoxicity and impairment of seed quality.
- Seed treatment should be carried out in a well-aerated area. There should be no contact with chemicals through breathing or skin. Protective clothing (overalls, boots, gloves, masks) should be worn when handling chemicals.
- Empty chemical containers should be properly disposed of and never re-used at home or on the farm.

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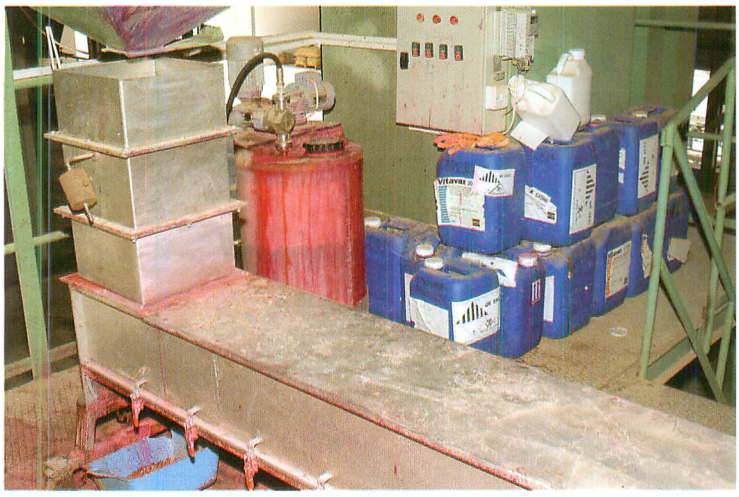
5.1 Uncleaned (left) and treated (right) chickpea seed

5.2 Cleaned (left) and treated (right) lentil seed

5.3 Cleaned (left) and treated (right) chickpea seed

5.4 A small mechanical batch seed treater



5.5 A partial view of commercial seed treater

Chapter 6: Seed Storage

Introduction

Seeds attain maximum germination capacity and vigor at physiological maturity. Legume seeds reach physiological maturity at seed moisture contents of 45–50% (Elllis *et al.*, 1988). Subsequently, the seed begins to deteriorate at a rate that is dependent on environmental conditions before and after harvest.

Long dry spells during maturation are important for seed quality, as unfavorable weather conditions (rain, high humidity, high temperature) or delayed harvest will affect seed quality. Once the seed is harvested, it is important to provide proper storage conditions to retard seed deterioration and minimize losses in physiological quality.

McDonald and Nelson (1986) have described the physiology of seed deterioration, and Justice and Bass (1978) have described the principles and practice of seed storage.

Seed deterioration

Loss of vigor and viability in seed is natural. All seeds lose viability during storage, with a loss of vigor preceding the loss in germination, which is manifested in various physiological and biochemical forms. These losses appear to be associated with the loss of membrane integrity, changes in the molecular structure of nucleic acid, and reduction in enzyme activity, which causes reduced germination rate, larger number of abnormal seedlings, and lower vigor and field emergence (Roberts, 1986; Roberts and Osei-Bonsu, 1988). Deterioration leads inexorably to death, and its effect can neither be reversed nor eliminated once it occurs – but can be slowed by proper storage.

A sample of seeds has a particular mean viability period under a set of conditions, and there is a random distribution of the viability period of the population around this mean. A mathematical model has been developed to relate seed viability to the storage environment, for the prediction of seed longevity (Ellis and Roberts, 1980).

Storage behavior of seeds

Roberts (1972) classified seeds into two major groups based on their physiological behavior: orthodox and recalcitrant. Orthodox seeds desiccate on the mother plant, and can be dried to low moisture contents without any damage. They are tolerant to sub-zero temperatures. Decrease in seed moisture and storage temperature increases their longevity.

Recalcitrant seeds lose viability on desiccation and are killed if their moisture content falls below a critical level. They have short viability periods even under moist conditions. Such seeds cannot withstand freezing temperatures; or sometimes just low temperatures.

Faba bean, chickpea, and lentil belong to the orthodox group of seeds and can be kept viable for many years depending on storage conditions. The mean viability period can be increased by decreasing the moisture content.

Inherent longevity

Several studies have demonstrated that seed longevity is an inherited characteristic that varies among species or cultivars (Agrawal, 1980; Harrington, 1972). Ellis *et al.* (1982) demonstrated the difference in longevity between different seed lots, in cowpea, soybean, and chickpea. Within the orthodox group, faba bean, chickpea, and lentil are moderate to relatively good storers (Delouche, 1988). In chickpea, *desi* types store better than *kabuli* types (Saxena, 1987).

General Considerations for Seed Storage

Pre-harvest and harvest factors

Pre-harvest and harvest conditions are important in seed storage. Weather conditions during the period between maturity and harvest can predispose the seed to deteriorative processes that reduce the storage potential (Delouche, 1988). Moreover, legumes are vulnerable to mechanical damage due to the nature of their seed coat. Seed moisture content and harvest-

ing and handling operations, e.g. delayed harvesting, have significant effects on the physiological quality of the seed (Ellis *et al.*, 1987, 1988).

Storage environment

Seed moisture content, storage temperature, and relative humidity are all important. Seeds with high moisture content deteriorate faster, and are more susceptible to damage from mechanical injury, extreme temperatures, chemical treatment, fumigation, heating, fungi, and insects. High temperature and relative humidity are detrimental.

Seed moisture content is a function of relative humidity at a particular temperature. High relative humidity will result in high seed moisture and vice versa (Table 6.1). Harrington's rules-of-thumb describe the effects of temperature and moisture on seed deterioration (Harrington and Douglas, 1970):

- The seed life doubles with every 5°C decrease in storage temperature, when temperatures are between 0°C and 50°C.
- Seed life doubles with every 1% decrease in seed moisture content, when moisture content is between 5% and 14%.
- These rules apply independently and, if combined, can produce geometric effects.

The effect of temperature and relative humidity on germination after different periods of storage for chickpea is illustrated in Table 6.2 (Ellis *et al.*, 1988). There was no loss of germination in good quality chickpea seed lots stored for 18 months at 20°C and 60% relative humidity. However, the higher the temperature and RH, the shorter the period for which seeds can be stored. Studies on lentil storage in India have shown that germination begins declining after

Table 6.1. Seed moisture equilibrium and relative humidity at about 25°C

Crop	Relative humidity (%)						
	10	20	30	60	75	80	85
Vicia faba	4.7	6.8	8.5	13.1	15.9	17.2	19.5
Pisum sativum	5.3	7.0	8.6	13.5	15.9	17.1	19.0

Source: Delouche, 1988 (citing Kreyger, 1972 and Harrington, 1972)

Table 6.2. Effect of different controlled environments on germination (%) of chickpea (accession BG 216) after different storage periods†

Storage period	20°C			33°C	
(days)	30 RH	60 RH	80 RH	30 RH	60 RH
62	96	96	94	92	100
100	92	96	87	94	100
165	93	95	46	92	63
255	96	97	10	100	–
373	96	96	–	95	–
531	95	94	–	95	–

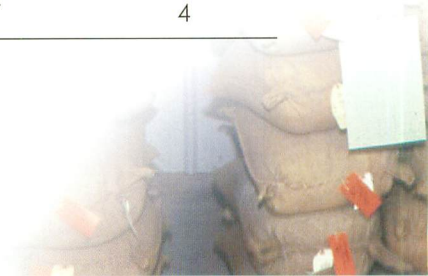
†Seed lot with initial germination of 95%

Source: Ellis *et al.*, 1988 (citing Agrawal and Kharlukhi, 1985)

Table 6.3. Estimated maximum storage periods (weeks) for faba bean seed

Temperature (°C)	Moisture content (%)					
	11	12	13	14	16	18
5	370	270	170	110	70	39
10	200	140	95	60	38	20
15	100	75	50	30	20	12
20	55	40	28	19	13	7
25	31	22	16	17‡	7	4

Source: Ellis *et al.*, 1988 (citing Hebblethwaite *et al.* 1983); ‡ Estimate may be erroneous



17 months of storage, falling to 25% after 37 months under insect-free ambient conditions (Agrawal, 1985).

Table 6.3 summarizes the effect of moisture content and temperature on the storability of faba bean seed. The storage period becomes shorter with higher seed moisture content and temperature, showing the combined effect of the two variables.

A computer program, based on the seed survival equation developed by Ellis and Roberts (1980), was developed to predict the storage period for several crops including cool-season food legumes (Kraak, 1992). The program can be used to calculate (i) initial viability, (ii) viability after storage, (iii) storage period, (iv) seed moisture content, or (v) temperature during storage, if three of the five parameters are known and one can be made variable.

Fungi, mites, and insects

Storage fungi can severely reduce seed quality by decreasing germination, causing heating, developing mustiness and caking, and causing total decay. They do not damage seed during storage if the moisture content is in equilibrium with 65–70% RH. Optimum conditions for insects are 30–35°C and 70–80% RH. Most storage fungi, mites, and insects do not develop below 0°C, 5°C, and 15°C, respectively.

Infestation with storage pests such as bruchids is a problem in grain legumes. Complete loss of viability can occur within 2–4 months of storage if the seeds are infested. In a survey of farmers' storage facilities in Morocco, it was found that infestation of faba bean with *Bruchus rufimanus* reduced germination and seed weight in proportion to the severity of infestation (Boughdad and Lauge, 1995). Germination dropped from 91% to 55% when the seed sample was infested by one and five adult bruchids, respectively. The dry weight of the seed correlated with severity, and the average weight loss due to one larva was 46.91 (±8.15) mg.

Mechanical damage

Testa quality is one of the principal components of legume seed quality, and is influenced by harvest, and specifically by mechanical damage. The amount of cracking caused by a given handling treatment depends on the moisture content. Mechanically injured seeds are less storable; they deteriorate faster and are more susceptible to damage by storage fungi and seed treatment. Faba bean, chickpea, and lentil embryos are surrounded by a testa; they should be harvested, threshed, processed, and handled carefully, to avoid damage to the testa.

The larger legume seeds are susceptible to physical injury, especially when the moisture content is less than 11–12% (Delouche, 1988). Faba bean seed harvested at 51% and 24% moisture content showed a decline in the proportion of broken seeds from 19% to 2%, and an increase in germination from 48% to 92% (Ellis *et al.*, 1988).

Spherical seeds are usually better protected than flat or irregularly shaped seeds (Roberts, 1972). Because of their lens shape, lentil seeds are more susceptible to mechanical damage than the rounder seeds of faba bean or chickpea (Muehlbauer *et al.*, 1985).

Storage periods

The species, genotype, and storage period determine the conditions for successful seed storage. Salih (1981) reported that storage under laboratory conditions had little effect on the germination of faba bean up to five years (92–87%), but longer periods had a drastic effect (20% after eight years). Similarly, the grain yield dropped from 1738 to 1105 kg ha^{-1} after five years of storage, and to 105 kg ha^{-1} after eight years (Table 6.4).

Requirements for safe storage differ according to the storage period, which may range from short term (e.g. storing seed to plant after a few months), to medium term (at least over one planting season), to long term (storage for genetic conservation). Delouche *et al.* (1973) and Ellis *et al.* (1988) summarized the requirements for storing cool-season food legumes.

Short-term storage

Generally, seeds are stored after harvest until the next planting season (up to a maximum of nine months). Storage conditions between 30°C, 50% RH and 20°C, 60% RH are satisfactory for

Table 6.4. Effect of seed age on germination, field emergence and yield of faba bean

Seed age (years)	Germination (%)	Field emergence (%)	Seed yield (kg/ha)
1	92.3	92.1	1738
2	91.3	86.1	1567
3	93.5	75.1	1410
4	94.8	71.3	1238
5	87.7	48.7	1105
6	44.0	40.8	210
7	34.6	–	–
8	19.9	13.7	105

Source: Salih, 1981

most species, including cool-season food legumes (Delouche, 1988). The equilibrium moisture contents for faba bean, chickpea, and lentil seeds are about 12%, 12%, and 14%, respectively (Ellis *et al.*, 1988).

Medium-term storage

In many seed production programs, nearly 100% of early generation seed and 20–25% of certified seed is carried over from one growing season to the next (up to 18 months) as a precaution against crop failures or other disasters. Ellis *et al.* (1988) recommended 30°C and 40% RH (9–10% moisture content), 20°C and 50% RH (10–12% moisture content), or 10°C and 60% RH (12–14% moisture content) for such storage periods of faba bean, chickpea, and lentil.

Long-term storage

Sometimes breeder seed may be produced at intervals, or only once during the lifetime of a variety, and needs to be stored for long periods. Storage at 10°C and 45% RH (9.5–10.5% moisture content) is recommended for 4–6 years. Authentic samples of new varieties, breeding materials, and seed for genetic conservation are also stored for long periods.

For long-term storage, FAO recommends a storage temperature of –18 to –20°C, with 5% moisture content, in equilibrium with 10–15% RH for starchy seeds, and 20–25% RH for oily seeds. Storage under such conditions is usually not necessary in a seed production program.

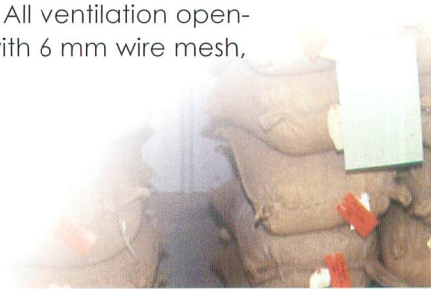
Safe seed storage facilities

Seed storage facilities must be specially designed, equipped, and managed to provide clean, cool, dry, and safe conditions. Well-constructed and well-ventilated stores are adequate for at least 9–12 months storage of faba bean, chickpea, and lentil in most production areas (Delouche, 1988). A good seed store should have no windows, and must be located in a dry, well-drained area. The floor should be one meter above the ground, or at truck-bed height, with a built-in vapor barrier equivalent to 0.25 mm polyethylene sheeting installed with hot-brushed bitumen. There should be only one or two doors in the middle of the building's short sides, to minimize floor space lost to aisles for handling seed.

The facilities should be protected from rain, moisture-vapor, and insects. There should be no cracks in walls or floors to facilitate cleaning and eliminate any cover for insect pests. The roof and walls should join without cracks, to prevent the entry of pests. There should be a rat-proof lip extending out about 20 cm around the building at about one meter height.

The facilities should be designed and constructed to minimize the entry of solar radiation and outside heat. The walls and ceiling should provide enough thermal insulation to minimize solar heat gain. Non-insulated wall construction may be adequate but some thermal insulation is usually necessary for the roof ceiling. The roof, walls, and doors should be painted with a light-colored reflective paint, to reduce solar heat intake. An extensive roof overhang will shade and cool the walls, and protect the ventilation openings from rain.

An exhaust fan may be used for ventilation when the outside temperature is lower than that of the seed store, but the relative humidity of the outside air should be considered when planning to ventilate the store. All ventilation openings should be screened with 6 mm wire mesh,



and located so as to remove hot air from the upper part of the building and moist air from the floor level.

Choice of seed storage site

Given the high agricultural and economic value of seed, the design and construction of safe storage facilities deserves special attention to minimize the influence of environment on the stored products. There are practical guides on how to choose a suitable storage site (Agrawal, 1976; Ellis, 1988). Ellis (1988) suggested the use of climatographs and isochrones, which will help identify potential sites and provide some indication of the expected losses in seed quality during storage (Fig. 6.1).

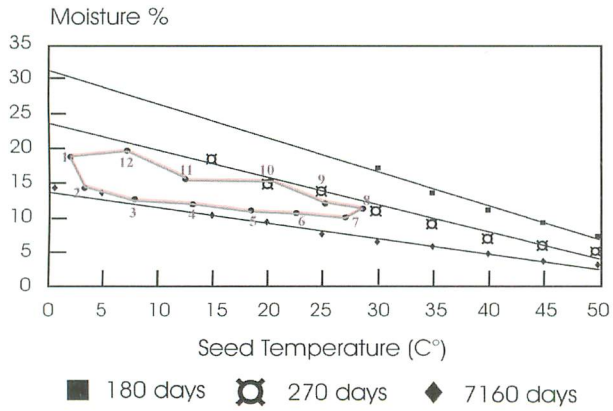


Figure 6.1. Isochrones showing germination decline in faba bean seed (from 95% to 85%), overlaid with climatograph

Climatographs are constructed from a combination of seed temperature and seed moisture (% wet basis) for a given site, based on detailed records of atmospheric temperature and RH. The data is then plotted and joined for each month of the year from January to December (Fig. 6.1). Isochrones are constructed from different combinations of storage temperature and seed moisture content (% wet basis), indicating the same time period it will take for seed viability to drop from a higher level (e.g. 95%)to a lower level (e.g. 85%) during storage. Overlaying the isochrones with climatographs of different sites will help identify a suitable site with minimum loss of viability, and help maintain physiological quality (Fig. 6.1).

Safe seed storage conditions

The storage facilities must be adequate to protect seed from damage and deterioration, and maintain its quality, i.e. vigor, germination, purity and identity. It is possible to minimize deterioration through proper planning and management, based on the expected storage period. If the moisture content does not exceed 10% during storage, most crop species can be stored under ambient conditions for at least 18 months, and will meet germination standards for certified seed.

Relative humidity and temperature are the two most important factors influencing seed viability during storage; and seed stored under low RH and temperature will lose viability very slowly. RH of 70% is the maximum for safe storage. A temperature of 20°C considered very safe, and an upper limit of 30°C is a reasonable compromise (Agrawal, 1976).

Adequate planning and management is essential to prevent losses and keep the seed free from insect pests. The storage should be clean, cool, and dry. The following specific preventive and remedial measures will be helpful.

- Clean all storage structures thoroughly and spray with contact insecticide. Follow a regular schedule of spraying a residual insecticide on walls, floors, ceilings, etc.
- Maintain proper sanitation in and around the seed store to deny insects any shelter for multiplication, and to control rodents.
- Clean the seeds and reduce the moisture content to a level that will allow safe storage for the required period.
- Use new bags, to prevent insect infestation and mechanical mixture. Legume seeds should preferably be packaged in a thick-weave cloth bag without any loose weaves. Bruchids do not easily penetrate such fabrics.
- Keep the seed bags on wooden pallets at least 50 cm away from the walls, with aisle space of 1 m.
- Inspect seeds upon entry. Store only seeds that are free from storage pests, with high germination and vigor.
- Apply a strict rodent monitoring and control program.

- Maintain seed identity by labeling each bag, keeping complete up-to-date records, and using stack cards.

Managing seed storage

Seeds are usually exposed to various deteriorative factors during storage. Therefore, an appropriate management strategy should be adopted to prevent losses or to take timely corrective action.

Keep the seed lot identity: Seeds enter or leave storage facilities in lots. It is essential to identify each bag with secure and proper labels. Keeping the identity will help in taking appropriate action.

Keep accurate records: For each seed lot, keep an accurate record of weight and number of bags, date and results of quality tests, and the quantity of seed sold or in store. Apply a first-in-first-out (FIFO) system in distributing seeds to avoid long storage periods. Laboratory tests will help identify seed lots with good quality and storability.

Prepare regular reports: The store manager should have an up-to-date report of the stock to facilitate marketing. The report should include the crop and variety, quantity of seed available, quality of the seed, and location of the storage facility. This will help plan for seed marketing.

Monitor insect infestation: Insect pests are highly destructive to stored seed. Seed should be inspected upon entry, and only seed that is free from pests should be stored. Check the stores for flying insects daily. Sample the seed at least once a week for the presence of insects, and if found, fumigate immediately with an appropriate insecticide.

Monitor seed quality: Loss of physiological quality is the most critical factor in seed storage. Such losses may be caused by prolonged storage periods, high temperature and RH, and insect infestation. It is important to monitor seed quality by conducting a germination test, and to store seed with high germination and vigor only. Alternatively, conduct vigor tests (accelerated ageing) to determine the suitability of seed lots for storage. The internal seed quality control laboratory plays a key role in ensuring that no loss of quality seed will occur once it has been processed and stored.

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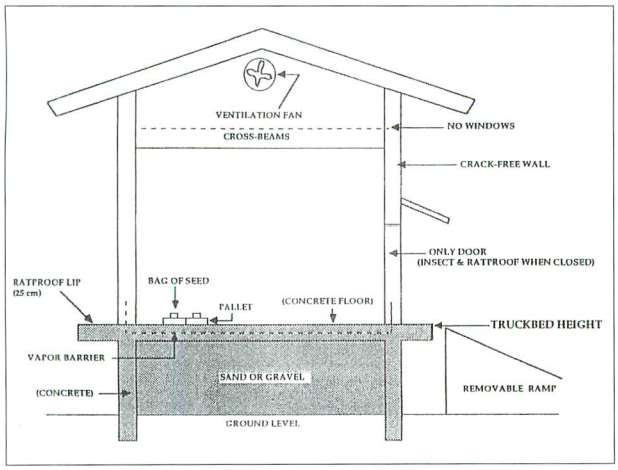
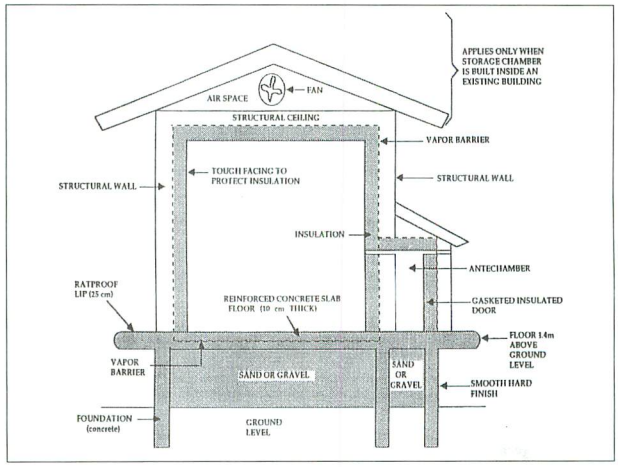
6.1 Poorly managed seed storage facility

6.2. Properly managed seed storage facility



6.3. Design for non-conditioned seed store



6.4. Design for conditioned seed store

Chapter 7: Control of Seed Storage Pests

Introduction

Legume crops are affected by a number of storage pests, including rodents, birds, insects, mites, fungi, and bacteria. Losses vary according to climatic conditions, crops, and storage facilities. Quantitative storage losses are estimated to reach up to 30% worldwide. Qualitative losses (losses in viability of seed) are more difficult to estimate and may have more severe consequences. More information on the control of storage pests can be found in Bond (1984), Diekmann (1988), Gwinner *et al.* (1990) and Sauer (1992).

Rodents

Rodents (rats and mice) can cause considerable losses. They contaminate seeds with urine and excrement, and damage seed, bags, fumigation sheets, electric wires, and buildings. They have a very high reproductive capacity, are extremely adaptable, and very difficult to control.

Monitoring

Seed stores should be inspected daily for the presence of rodents. Effective control depends on the species. Since rodents are not easily seen, indirect identification may be possible through the (i) shape, size and location of droppings, (ii) type of damage to seeds, bags, etc, (iii) footprints (Table 7.1). The brown rat (*Rattus norvegicus*) has round droppings, the roof rat (*Rattus rattus*) has more oblong, banana-shaped droppings, and mice droppings are smaller.

Tooth color, ear shape, size of eyes, snout, length, and body-tail length ratio can be used to identify the species. The behavior and habitat are direct indications of the species. The brown rat lives outside the store, using always the same runs to go to the store, as well as in the store. Roof rats live in store roofs and do not use the same runs around the store. Rats generally run along walls, pallets, bags, etc. Mice run around in all directions, but live in a very restricted area (1 m^2) and may never be seen by the storekeeper. Rats are suspicious of all new things, while mice are curious, investigating everything.

Rodents damage bags; and spilled grain under a stack (pallet) indicates their presence. The type of damage to the seed is also indicative of the species. The number of rodents identified during monitoring is indicative of the population size as shown in Table 7.2. These figures give an idea of the number of rats present in a store.

Preventive and curative measures

Prevention is very important, especially cleanliness of the seed store and its surroundings. The surroundings (5–10 m radius) should be spotlessly clean without vegetation, which could be hiding places for brown rats.

To prevent the entry of rats and mice, all openings should be screened with wire mesh and the stores should be rat-proof as much as possible. Biological control measures such as keeping cats in seed stores, and setting traps in and outside the store, would be helpful.

Rodenticides should only be used after preventive measures have been taken and the species of rodent is known.

Rodenticides

If the population of rats is high, acute poisons should be used, which are highly toxic and kill almost on the spot. However, rats are so clever that they can relate sickness or death with the poison and avoid touching the baits anymore. Pre-baiting can prevent bait shyness, whereby rats are first attracted by good quality baits without poison. After a few days, when the rats have been lured to the high quality food, the poison is added.

Chronic poisons are used for long-term control. The most frequently used chronic poison is zinc phosphide (a mixture of 975 g common cereal grain with 25 g poison). The 'old' chemicals, e.g. Warfarin and Cumatetralyl, need sev-

Table 7.1. Some tools for identifying rodents in storage facilities

	Brown rat *(R. norvegicus)*	Roof rat *(R. rattus)*	Mouse *(M. musclus)*
Behavior	Suspicious	Suspicious	Curious
Habitation	Outside store	Roof	Restricted area (1 m^2)
Droppings	Round	Banana shaped	Small
Movement	Same route	Around (different)	All directions

Table 7.2. Relating rodent population with number identified during monitoring

Population size	No. of rodents identified during monitoring
Zero to low	Few or no signs (0–10 rats)
Moderate	1–3 rats seen at night, none by day, old feces and gnawing common (10–30 active + up to 50 young in nests)
High	More than 3 rats seen at night, some by day, fresh feces, and damage common (>30 active rats)

eral intakes – up to seven times – to kill, whereas the 'new' generation chemicals (e.g. Difenacoum, Brodifacoun) require only one intake. The effect of the new chemiclas is not immediate and no bait shyness is developed. The chemicals act as anticoagulants and kill the rat through internal bleeding.

Rodenticides are available as (i) prepared bait, which is expensive), (ii) mixing powder, the best and cheapest formulation, (iii) wettable powder, to use in very dry areas where the rodents are in need of water, (iv) tracking powder, to use on the runs; when rats clean themselves they come into contact with the rodenticide and get killed. The bait should be of good quality, the mixing powder should be mixed with a grain that is common to the area and should be changed regularly. The rate for mixing powder is 18 parts of cracked grain to one part of poison and one part of sugar.

Mice are more difficult to control because they live in very small areas (1 m^2). They are curious but lose interest very quickly. Therefore, the bait should be densely distributed. Thirty kernels per bait station (small container) are necessary under the stack. The most widely used product is Calciferol.

Bait stations

Small wooden houselets, with entry and exit points, should be placed along the walls inside (for roof rat and mouse) and outside (for brown rat) the store. Inside the bait station, there should be a small barrier to prevent the poison from being taken out. For brown rats, one bait station per 200 m^2 with 200–400 g of bait is required, and three bait stations for 300 m^2 with 100–200 g of bait.

Mites and Insects

Mites mainly transmit spores of storage fungi, and cause skin irritation and allergies in persons handling infested seed. Insects can be very destructive. Different insect species feed on legumes, and their life cycles will determine the appropriate control measures.

Infestation with storage pests, especially Bruchidae, is a major problem in legumes (Bushara, 1988). The typical pests are *Callosobruchus* spp. and *Bruchidius* spp. Many workers have reported resistance to storage pests, especially *Callosobruchus* (Bushara, 1988). *Bruchus* spp. is important in the temperate zones of Europe, the Mediterranean, and Asia, while *Callosobruchus* spp. has worldwide distribution (Table 7.3).

Field infestation of faba bean and lentil by *Bruchus rufimanus* and *B. dentipes,* respectively, is common, whereas storage pests do not attack chickpea seeds in the field. Infestation of faba bean seeds starts in the field and the insects complete their life cycle in the store.

Table 7.3. Geographical distribution and importance of major legume storage pests

Pests	Distribution	Faba bean	Chickpea	Lentil
Univoltine species				
B. dentipes	Eastern Mediterranean to Afghanistan	++	–	–
B. lentis	Europe to Central Asia, Near East, India	–	–	++
B. ervi	Europe, North Africa, Near East	–	–	++
B. pisorum	Cosmopolitan	–	–	–
Multivoltine species				
C. maculatus	Cosmopolitan	++	+	+
C. chinensis	Cosmopolitan	+	++	++

++ major pest, + minor pest
Source: Bushara (1988)

There is only one generation per year, and no re-infestation can take place. Spraying the fields with insecticides during oviposition can prevent infestation. Visual inspection of the seeds after harvest can help estimate the level of infestation. Infested seeds show characteristic dark spots (holes) through which the newly hatched larvae penetrated into the seed. It is necessary to fumigate if there is infestation. This can stop larval feeding, maintain germination, and reduce the source of infestation for the next season – adults being carried with the seeds to the fields.

Callosobruchus spp. lay eggs on dry seeds, and several generations develop in the store through re-infestation. A complete seed lot may be damaged if no action is taken. There are many sources of infestation for the 'true storage pests', but the most important is contamination from stored infested seeds. The stores may also have crevices, corners, spilled seeds outside the store, empty sacks with some leftover seeds, etc, where insects can survive. It is desirable to detect infestation at the early stages through careful and regular inspection of stored materials. There are different methods of detecting infestations before they become clearly visible. Some of these methods are simple (flotation of grains), while others are very sophisticated (X-ray). However, insect infestation can be detected mainly by visual inspection.

Optimum conditions for mass population of insects are 30–35°C and 60–80% RH. About 35°C and 38°C are the maximum for development and survival, respectively. Most insects cannot survive for more than 2–3 weeks below 0°C. A certain moisture level is required for insect development: 10–11% is generally considered the minimum. The number of insects increases with increase in moisture content, up to a level where too many microorganisms develop (about 15–16%). Drying and storing seed at low temperatures, proper sanitation, and pesticide use can reduce or eliminate insect infestation.

Insecticide application

Since the seed is planted, insecticides do not constitute a residue risk to warm-blooded animals, but they may affect seed viability. However, insecticides should have low toxicity to humans, in order to protect users. They should also be affordable and have high and long-lasting effectiveness against several insects. Insecticides that kill the insect population and have a long lasting effect (pyrethroids, organo-phosphorus insecticides) should be differentiated from those that kill only the insects and have no residual effect (fumigants).

Storage insects are mostly controlled with chemicals, and there are many products in the market. Some alternative methods have been tried, e.g. olive oil and salt mixtures or neem extract (ICARDA, 1990).

There are different methods of applying insecticides – dusting, spraying, fogging, and evaporation. Dusting does not require much equipment: a powder formulation is either mixed with the seed, or applied in layers ('sandwich method'), or dusted over stacks. Dusting over stacks can only prevent re-infestation (e.g. after fumigation), since most powder insecticides do not penetrate well enough into the stacks to con-

trol internal infestation. A suspension (solid insecticide suspended in water, usually a wettable powder formulation) or solution (liquid insecticide diluted in water) can be used for spraying.

Insecticides can be applied with a knapsack sprayer. For suspensions, care should be taken to ensure that the particles remain suspended, by using special stirring devices or by shaking the container frequently. Fogging, which produces finer droplets than spraying, is used especially in stores, but special equipment is required. Evaporation can be used in special cases to control flying insects (moths), but it requires volatile insecticides and properly closed stores.

The following spraying scheme is recommended for effectiveness, low toxicity, long-lasting effect, and little side-effect on seed viability:

- use insecticide sprays once every 3–4 months as a preventive measure
- alternate Actellic with Malathion and K-Othrine
- use fumigation with Phostoxin when insects are detected.

You may apply 5 liters of 4% solution of Malathion 50 EC per m^2. Apply 0.25-0.5 g a.i K-Othrine per ton of seed either using the EC 'K-Othrine grain 25' at the rate of 1 liter in 99 liters of water for 100 tons of seed (for 6 months protection) or 1 liter with 49 liters of water per 100 tons of seed (for longer duration). You can use dust formulation K-Othrine grain pp^2 at 500 g per ton of seed (for 8–12 months protection). Table 7.4 shows the recommended application for Actellic.

Fumigation

The entire seed lot should be fumigated once insect infestation is observed. Use the proper fumigant and effective dosage for the required period. All fumigated stacks should be properly covered and sealed with gas-tight plastic cover, and all personnel should be properly protected during fumigation and when removing the fumigants. The main advantage of fumigation is that it can control all stages of the insect – eggs, larvae, pupae, adults – and also other storage pests, including rodents.

The two products mainly used for fumigation are methyl bromide and aluminum phosphide. They are active in the gaseous phase, have a good penetration capacity into piles of sacks or seed in silos, but are hazardous to human beings.

Methyl bromide

Methyl bromide is fast acting. Seed stacks can be aerated 12–24 hours after fumigating with methyl bromide. It is often used in seaports because storage is expensive, and fumigation has to be completed quickly. However, methyl bromide has been banned in many countries because of its toxicity.

For seed production, methyl bromide is less preferred because it can reduce gerination. Moreoer, (i) it is extremely toxic and accumulates in the human body, (ii) it is odorless, colorless, and difficult to detect without proper devices, (iii) residues will remain in the seed, (iv) it is heavier than air and fans are needed to recirculate the gas.

Phosphine

Phostoxin is slow-acting; fumigation may require a minimum of four days. Phostoxin releases a gas called phosphine, which has excellent penetration capacity because of the small size of the gas molecules. Its weight is similar to that of air and it has good circulation, therefore, no fans are necessary. Phosphine penetrates bags, cartons, boxes, and other containers. It is relatively easy to handle because the gas is released slowly, building up a lethal concentration after approximately one hour. It has no influence on germination and seed can be treated repeatedly. Phostoxin is inflammable at normal temperatures, and putting several tablets or pellets together may lead to ignition.

Table 7.4. Recommended application rate for Actellic

Trade name	Bulk seed/ ton	Bagged seed/ m^2	Empty stores/ m^2
Actellic EC	4–10 g a.i. in 1–2 l	250–500 mg a.i. in 50 ml	250–500 mg a.i.
Actellic 50 EC	8–20 ml a.i. in 1–2 l	0.5–1 ml a.i. in 50 ml	100 ml in 5 l per 100 m^2
Actellic dust 2%	200–500 g	12.5–25 g	1 generator (20 g).

***Formulation*:** Phosphine is available in solid (0.6 g pellets, 3 g tablets), sachets, and strips. The active ingredient is aluminum phosphide, mixed with ammonium carbonate and paraffin. After exposure to atmospheric moisture, the pellets decompose and release the active substance, hydrogen phosphide (PH3).

$$AlP + 3\ H_2O = PH_3 + Al(OH)_3$$

***Dosage and exposure time*:** Generally for fumigants, concentration multiplied by time remains constant (a concentration of 1000 ppm for 5 days equals a concentration of 500 ppm for 10 days). However, for phostoxin a lower concentration at a longer exposure time is more effective. The manufacturer usually indicates the dosage; usually 3–6 tablets per ton of seed (2–4 tablets m^{-2}). A higher dosage is needed for larger stacks or under sub-optimal conditions (low moisture content), or when eggs or pupae have to be killed. The FAO fumigation manual recommends 2–5 tablets per ton for silos, 3–6 for seed in heaps, and 2–4 tablets per cubic meter for seed stacks. The recommended dosage is 2.5 g m^{-3}, which is equivalent to 2.5 Phostoxin tablets of 3 g each or 12 pellets of 0.6 g (Bond, 1984).

The manufacturer also indicates the exposure time – usually three days – but it is recommended to always fumigate for at least five days. In general, the longer the exposure time the better, because this will kill the eggs and larvae. It is not recommended to raise the dosage because the insects may be knocked down but not killed. If fumigation is carried out outside the store, care should be taken that no condensation takes place under the fumigation sheets.

***Temperature*:** The dosage also depends on the temperature. The chemical reaction is faster at higher temperatures, and the metabolic systems of insects work better, resulting in faster and better intake of the gas. The following are generally recommended: Do not fumigate below 15°C. Expose for 10 days at 5–10°C, 5 days at 11–15°C, 4 days at 16–20°C, and more than 3 days at 20°C. Sometimes an extra day is recommended when tablets are used or when RH is low.

***Relative humidity*:** The relative humidity should not be less than 30%, because very little gas is released from the tablets when humidity is lower. The exposure time should be at least ten days at 30% RH. In very dry areas where sufficient moisture is not available, it may be necessary to place a container with water under the fumigation sheet.

***Equipment*:** To cover seed stacks for fumigation, firmly press airtight plastic sheets to the ground with sand snakes. Different types of fumigation sheets are available, but local availability, cost, and size will determine what type to use. Some examples of good fumigation sheets are: (i) unsupported polythene/polyvinyl chloride (PVC) film; (ii) woven polypropylene; (iii) laminated PVC with nylon/terylene scrims; (iv) multilayer laminates of thin films; (v) PVC-coated onto nylon/terylene scrims; and (vi) nylon cloth coated with neoprene.

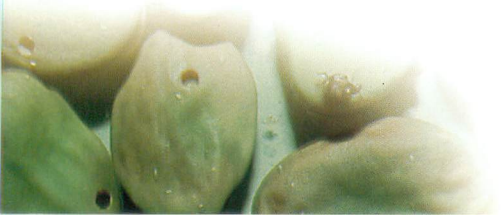
The escape of gas should be minimized, because it reduces insecticidal effect, leads to insect resistance, and is hazardous to users. Fumigation sheets should be completely airtight, sufficiently thick, and should be tightly fitted to the floor. A cemented floor is necessary to prevent the escape of gas through the soil, and the fumigation area should be properly aerated.

Conditions for fumigation

Smaller quantities of seed can be fumigated in airtight fumigation chambers. Large quantities of seed are fumigated in stacks under airtight fumigation sheets. To monitor the effect, live insects should be included in the fumigation area, preferably in a small bag or box. It is good practice to fumigate seed stacks that have been sprayed with a protective and persistent insecticide, because fumigation does not have a lasting effect and re-infestation may take place immediately after fumigation. If one stack is fumigated at a time, insects from non-fumigated stacks may re-infest the newly fumigated stacks.

Procedures

Necessary precautions should be taken before fumigation to ensure success. It is essential to clean the seed storage facilities, properly stack the bags, check and repair fumigation sheets, prepare sand snakes, trays, labels (warning signs), and apply the right dosage.



Place the seed (in bags or boxes) on pallets and cover the seed stack properly with the fumigation sheet. Place the tablets in trays under the pallets and seal the stack using sand snakes prepared for the purpose. Thereafter, place warning signs and lock the store to avoid any accidental entry until the end of the fumigation period.

Aerate the store immediately after the fumigation period. Wear a gas mask (B type) and open the sheets partially first at the corners before complete removal. Clean the storage facilities by collecting residues (bury or burn), removing warning signs, etc., and store the fumigation sheets properly.

Safety precautions

Wear a face mask with proper canister (Type B) especially during aeration. Record the time the canister has been used because canisters have a limited period during which they are effective. Wear cotton gloves when handling phosphine tablets or pellets.

Check the gas concentration for phosphine with a tube detector (Dr□ger). A warning sign should be clearly visible to prevent people from inadvertently removing plastic sheets or entering a fumigated building. Do not eat or drink during fumigation. Wash and shower after fumigating to prevent damage to the body by chemicals, and do not work alone.

In summary, based on the characteristics of fumigants, aluminum phosphide is recommended for use in seed production, while methyl bromide is not. It is very important to seal the area under fumigation, and to avoid whole store fumigation.

Fungi and Bacteria

Storage fungi require a high moisture content to grow (at least 14% seed moisture). Therefore, they do not play a major role in dry climates. The most important genera are *Aspergillus* and *Penicillium*. They are mostly saprophytes and cannot attack living tissues, but they grow on dead cells on the seed surface, where they produce toxins. They cause seed decay and may kill the embryo and reduce germination. The best way to control storage fungi is to maintain low moisture in seeds and storage facilities. Low temperature retards fungal growth. Fungicidal seed treatment does not often give the expected results because of the lack of free water required by many fungicides to become effective.

Bacteria do not affect stored seeds except when moisture content is very high or the temperature has been raised by fungal infection.

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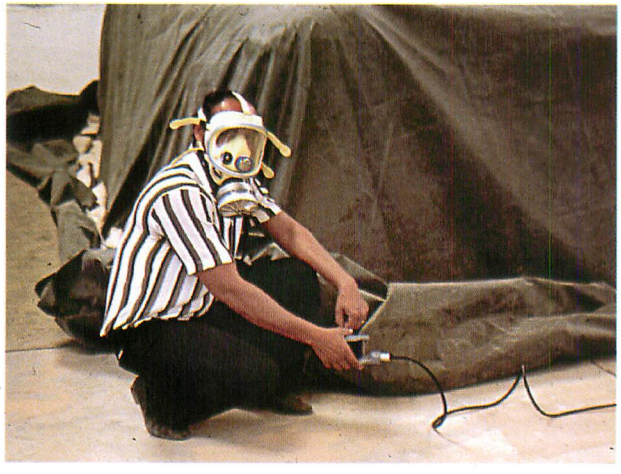
7.1 Lentil seed damaged by bruchids

7.2 Faba bean seed damaged by bruchids

7.3 Placement of phostoxin packages for fumigation

7.4 Sand snakes to ensure air tight fumigation



7.5 Measuring phostoxin concentration during fumigation

7.6 A diagram illustrating proper fumigation procedures

Chapter 8: Seed Quality Assurance

Introduction

A quality assurance system ensures that certified seed meets prescribed national and/or international quality standards. Technically, it ensures that the seed sold to farmers is of the designated variety and desired quality. This can be achieved through certification, i.e. field inspection, laboratory seed testing, and control plot testing. The system monitors the production of pre-basic, basic, and certified seed. This chapter discusses (i) field inspection (ii) seed quality tests, (iii) control plot tests, and (iv) lot numbering system.

Seed Certification Agency

The concept of quality assurance has evolved over time, shaped by the progressive development of the seed industry. It is a blend of technical and administrative procedures and guidelines supported by legislation to maintain and ensure that the seed offered for sale meets high standards of varietal (genetic), physical, physiological and health quality. It involves varietal certification through field inspection of crops, laboratory testing of quality attributes against a set of standards prescribed by the legislation, and labeling and sealing of seed in containers.

The organization, management and enforcement of seed certification and the division of responsibilities among participating institutions defines the nature of quality assurance schemes operating in both developed and developing countries. Any seed quality assurance program should have the following main features: (i) setting field and seed standards, (ii) monitoring and supervision of seed production to ensure quality, (iii) enforcement of quality standards during marketing. There is a need to increase the participation of producers in the decision-making process in ensuring quality while advocating for an independent and impartial agency for seed certification.

Field Inspection

An accurate, uniform, quick, and simple standard procedure is required to assess a seed production field based on the quality standards prescribed in the seed regulations. With standardized inspection procedures, all inspectors can uniformly assess the quality of all seed fields and ensure that the seed crop meets the prescribed standards.

Field standards

Field standards for seed production have to be set for offtypes, other varieties, other crops, parasitic weeds (*Orobanche*, *Cuscuta*), virus-infected plants, and *Ascochyta* blight (Table 8.1). Generally, the standards are higher for earlier seed classes than for subsequent generations. For example, a standard of 0.3% for offtypes and other varieties in basic seed of chickpea indicates that a seed field is not allowed to have more than one offtype or other variety in 333 plants of a given chickpea variety. Similarly, for

Table 8.1. Suggested field standards for seed production of cool season food legumes

	Pre-basic	Basic	Certified 1	Certified 2
Offtypes and other varieties (max. %)	0.1 (1:1000)	0.3 (1:333)	0.5 (1:200)	1.0 (1:100)
Other crops (max. %)	0.1 (1:1000)	0.3 (1:333)	0.5 (1:200)	1.0 (1:100)
Orobanche (max. %)	0.05 (1:2000)	0.1 (1:1000)	0.2 (1:500)	0.3 (1:333)
Cuscuta (max. %)	0.05 (1:2000)	0.1 (1:1000)	0.2 (1:500)	0.3 (1:333)
Virus diseases (max. %)‡	1.0 (1:100)	1.0 (1:100)	1.0 (1:100)	2.0 (1:50)
Ascochyta on pods (max. %)	0.3 (1:333)	0.4 (1:250)	0.5 (1:200)	1.0 (1:100)

Numbers in parentheses represent maxiumum limits, e.g. 1:100 indicates that maxiumum allowable limit is one contaminated plant per 100 plants

‡ For faba bean

Orobanche, a standard of 0.1% means that a seed field is not allowed to have more than one *Orobanche* plant in an area occupied by 1000 faba bean plants. The standards in this manual were adapted from the standards used in several countries in West Asia and North Africa (Madarati and Bishaw, 2002) and those given by Doerfler (1976).

Inspecting seed fields

Field inspections should be done at a time when potential contamination is likely to happen and contaminants can be identified. The inspector should verify whether the prescribed standards are met. At least two inspections should be made: one during flowering and another towards crop maturity. An extra inspection for *Ascochyta* blight should be made during the vegetative stage for faba bean and chickpea, while a special inspection for viruses in faba bean should be made at the six-leaf stage.

The field inspection methodology described in this manual is based on the method used by the Association of Official Seed Certifying Agencies (AOSCA, 1971), as described by Revier and Young (1970) and modified by Gregg *et al.* (1990). It consists of three steps: making the 'field overview', determining the 'field inspection sample' and taking the 'field counts'.

In the field overview, the seed production field is generally observed to assess the uniformity of crop stand. Field inspection sample involves determining the actual number of plants (or size of sample area) that statistically represents the field, for conducting the inspection. The field counts include inspecting the field and counting the actual number of each contaminant in a statistically determined number of plants. After counting and recording, the contaminants are compared with the standards, and if they are above the tolerance level, the field will be rejected.

Field overview

In field overview, the inspector walks through the field to see the entire crop area. He/she will determine if the field is uniform in quality, and assesses the following: (i) varietal identity, (ii) isolation distance, (iii) previous cropping, (iv) infection by plant diseases, including diseases that are not seed-borne, (v) infestation by weed plants, including weeds not in the standards, (vi) cultural practices, (vii) general crop stand, and (viii) yield estimate. The inspector should also count the number of plants to determine the plant population (density per m^2 or per m row length).

Variety identity: The inspector should make sure that the crop is of the designated variety, and should examine a reasonable number of plants very closely for positive identification. The characters described in Chapter 1 can be used for this purpose.

Isolation: Seed fields should be properly isolated from any possible source of contamination, as discussed in Chapter 3.

Previous cropping: The land for producing pre-basic or basic seed should have been free of any other variety of the same species for at least two years, unless if the previous crop was of the same variety and the same or higher category approved upon certification (see Chapter 3). For certified seed, one year is recommended.

Plant density: In each field, the number of plants in 10 randomly selected areas of 1 m row each should be assessed to estimate the average number of plants per meter row. This is necessary to enable the inspector to determine the area of the 'field inspection sample' and accurately assess whether the standards have been met.

Field inspection sample

To accurately assess whether the field meets the prescribed standards, the actual number of each contaminant in a given number of plants should be established. The inspector does not inspect the entire field, but assesses the quality by carefully inspecting a fixed number of plants in a representative sample area. This area, which comprises the number of plants to be inspected, is called the 'total field inspection sample'.

The sample is determined by using the strictest standard for all seed classes, which is 0.05% (Table 8.1); it allows one contaminant in 2000 plants Therefore, the 'total field inspection sample' for faba bean, chickpea and lentil should include 6000 plants (i.e. three times the

number of plants in which one contaminant is allowed). Using the number of plants per meter row length the inspector can calculate how many meters have to be inspected to include the required plants. Here, the inspector makes detailed observations and records the number of each contaminant listed in the standards (Table 8.1).

Field counts

To ensure that the 'total field inspection sample' accurately represents the field, it is divided into smaller areas called 'field counts'. The 'field counts' are located in randomly selected parts, and 5–6 counts are recommended. The total field inspection sample is then divided into six 'field counts' of 1000 plants each. Thus, each random field count includes 1000 plants.

The most practical approach is to use the average number of plants per meter row and calculate how many meters have to be inspected to include 1000 plants (one count). The number of meters is then divided by the number of rows the inspector can easily oversee (2, 4 and 6 rows in faba bean, chickpea and lentil, respectively).

A field is rejected if the total number of contaminants (in the six field counts) is larger than the number allowed by the standard (Table 8.2). For instance, a basic seed field of faba bean will be rejected if more than six *Orobanche* plants are found in an area of 6 counts, each of 1000 plants. Similarly, the field will be rejected if more than 18 offtypes or other varieties are found.

Walking pattern

A specific pattern of walking through the field is used so that the inspector can see almost all parts of the field, while minimizing the distance walked and time spent in the field (Fig. 8.1).

Contaminants

The inspector will assess the number of the following contaminants in the six field counts.

Offtype plants and other varieties: Offtypes are plants of the same variety but have one or more characteristics that are different from the original population. The field may also contain plants of the same crop species, which can be clearly and distinctively identified as other varieties.

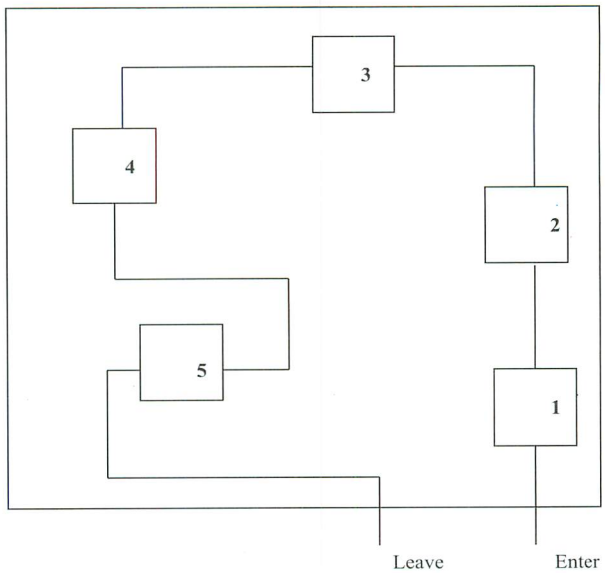


Figure 8.1. Suggested walking pattern through a seed field

'Other crop plants': This refers to species with similar growth habit and physical characters whose seed cannot be separated during processing. Such crops are specified in the field standards. For example, vetch and lupin in faba bean; bean and vetch in chickpea; and lathyrus, pea and cereals in lentil. The total number of these 'other crop plants' should not exceed the tolerance level, as presented in Table 8.2.

Weeds: A weed may be classified as common or noxious, restricted or prohibited. A weed plant is undesirable when it (i) competes with the seed crop, (ii) is difficult to eradicate, (iii) its seed is similar to that of the seed crop and is difficult to separate during cleaning, (iv) is parasitic. Parasitic weeds such as *Orobanche* and *Cuscuta* are most important in legumes because they can survive in the field for several years. Infested fields should not be used for seed production. The number of parasitic plants in the total field inspection sample should not exceed the tolerance level presented in Table 8.2.

Diseased plants: Seed-borne fungi, bacteria, viruses, or nematodes may be transmitted from one generation to the other through the seed. Such transmission can be eliminated by roguing diseased plants or isolating the seed crop from diseased plants.

Table 8.2. Rejection levels (number of plants) for different legume seed classes

	Pre-basic	Basic	Certified 1	Certified 2
Offtypes and other varieties	6	18	30	60
Other crops	6	18	30	60
Orobanche	3	6	12	18
Cuscuta	3	6	12	18
Virus diseases†	60	60	60	120
Ascochyta on pods	18	24	30	60

† For faba bean

Ascochyta blight is an important seed-borne fungal disease affecting faba bean, chickpea and lentil. The number of plants that are infected with *Ascochyta* is counted and used as the basis for accepting or rejecting the field. Similarly, the number of virus-infected plants is counted and compared with the standards (Table 8.2).

The inspector should also assess the number of plants infected with other seed-borne diseases such as chocolate spot (*Botrytis fabae*) and gray mold (*Botrytis cinerea*) in faba bean; wilt (*Fusarium oxysporum*) in chickpea and lentil; root rot (*F. solani*) in faba bean, chickpea and lentil; and downy mildew (*Peronospora* spp.) in faba bean and lentil (see Annex 4).

Reporting

The results of the field inspection should be recorded in the field inspection form (Annex 5), and should be signed by the inspector and the seed grower. The report should then be sent to the certification agency to determine whether the field will be accepted, re-inspected, or rejected.

Seed Quality Tests

Laboratory seed testing was developed to assess seed quality before sowing and minimize the risk of crop failure. There are different standard tests for testing the quality of samples taken from the seed lot (Fig. 8.2). The results represent the actual quality of the sample and by inference of the entire seed lot.

Seed quality reflects various attributes which affect the overall value of seed for planting. These include genetic (varietal purity and identity); physical (analytical, size); physiological (germination, vigor, moisture); health (freedom from seed-borne pests, parasitic weeds); and uniformity. All the attributes are important, but only three of them – physical purity, germination and moisture content – are routinely evaluated in the laboratory (Hampton, 2002).

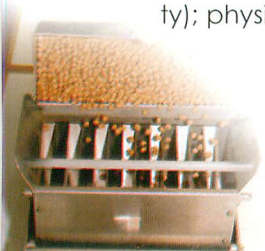
At least four tests should be conducted for legume crops: physical purity, germination, moisture content, and seed health. All the tests should be carried out using the International Seed Testing Rules (ISTA, 2003), but you may also consult the recommendations of the Association of Official Seed Analysts (AOSA, 1981). A description of an adequate seed testing laboratory is given by van der Burg *et al.* (1983).

Sampling seed lots

Principles of seed sampling

Sampling of seed lots is an important element of any quality assurance program. Sampling refers to the drawing of a representative sample from a seed lot of known size, variety, and generation using standardized methods and instruments. Sampling is necessary because the components of a seed lot may not be uniform. This variation may arise due to differences within the crop from which the seed was harvested, poor blending of seed of the same or different seed lots during processing and storage, and segregation of heavy and light seed components within the same bag.

No seed analysis, regardless of how carefully accomplished, is better than the sample on which it is performed. The size of the submitted sample is very small compared to the size of the total seed lot. Depending on the crop, a seed lot may contain several million individual seeds. However, the submitted sample may not exceed 25,000 seeds, and tests will be conducted on only a very small proportion of the sam-



ple. Up to a maximum of 2500 seeds will be tested for purity, and only 400 seeds tested for germination. Therefore, specific procedures should be strictly followed in order to ensure that a representative sample is submitted, which in turn will produce accurate test results. Otherwise the value of the analysis will be greatly diminished.

Procedures for seed sampling

Seed sampling is carried out at various stages of seed production and for different purposes, e.g. for internal quality control (monitoring) or for certification (Table 8.3).

Sampling involves a series of steps; at each level the size of the sample is further reduced. Many primary samples (small portions of seed) taken from different parts of the seed lot are combined together into a composite sample. After thorough mixing, the sample is reduced to the required weight of a submitted sample, which is sent to the seed testing laboratory. In the laboratory, the submitted sample is, through successive halving, divided into the working sample which will be analyzed for quality. To increase the accuracy of the results, the working sample is often sub-divided into replicates. Seed sampling procedures have been described by Bould (1986) and ISTA (2003).

Samples for laboratory testing should be taken from all seed lots (Fig. 8.2). The required weights of the submitted samples for official testing are presented in Table 8.4. Official sampling for quality assurance is usually carried out after the seed lot has been cleaned, treated, and packaged.

Sampling can be done by automatic samplers, triers, or by hand. Automatic samplers are generally attached to the seed processing plants and are not flexible. They take samples from different portions of the seed stream before packaging. Triers are the most common and efficient sampling instruments. They are used to sample seed in bags or small containers. A stick or spear trier can be used. The recommended triers for faba bean, chickpea, and lentil are listed in Table 8.4.

Maximum seed lot size

The prescribed maximum size of seed lot to be represented by one sample is 25 tons for *Vicia faba*, 20 tons for *Cicer arietinum*, and 10 tons for *Lens culinaris*, subject to 5% tolerance (ISTA, 2003). Any seed lot larger than these quantities should be sub-divided into smaller lots.

Sampling intensity

The prescribed number of primary samples for seed in bags/containers should be taken to obtain a representative sample, which will constitute the variation that exists in the seed lot. Samples must be taken randomly from more than one bag/container and from the top, middle, or bottom of the bags. The sampling frequency will depend on the number of the containers (15 kg up to 100 kg inclusive), as prescribed by ISTA rules (Table 8.5).

Table 8.3. Stages and purpose of seed sampling

Stage of sampling	Purpose and quality analysis conducted
Contract grower farm (ex-harvest sample)	Contract specification for moisture (for drying), purity, germination
On delivery to plant (pre-cleaning sample)	Confirm previous test results for payment, provide advice on special cleaning needs (moisture, purity, germination)
During seed processing (monitoring sample)	Monitor processing efficiency to meet seed standards (purity)
After seed processing (official sample)	Seed lot certification (purity, germination, health), post-control plot test (varietal purity)
Seed company store (monitoring sample)	Monitor loss of quality to update/dispose stocks and decide sale priority (germination, vigor)
Seed distribution store (enforcement sample)	Random sample to enforce standard for confirming quality of seed for sale (purity, germination)

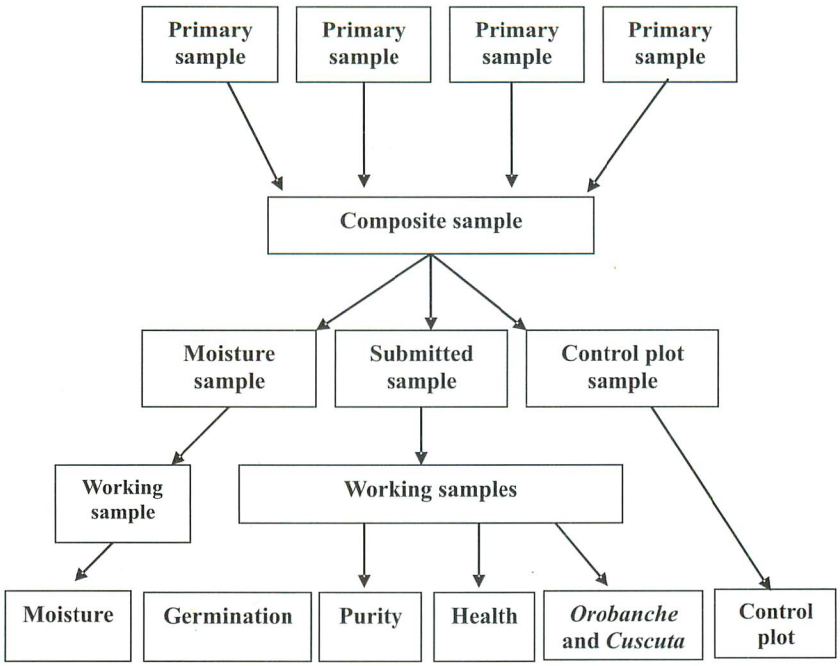


Figure 8.2. Procedure for seed sampling and testing

Table 8.4. Stick triers for sampling faba bean, chickpea and lentil

	Length (mm)	Outside diameter	No. of slots
Faba bean	1600	38.0	6 or 9
Chickpea	1600	38.0	6 or 9
Lentil	762	25.4	6

Table 8.5. Sampling intensity for seed lots in bags (A) and seed streams (B)

A: Maximum seed lot (no. of containers)	No. of bags to be sampled
1–4	3 primary samples from each container
5–8	2 primary samples from each container
9–15	1 primary sample from each container
16–30	15 primary samples from the seed lot
31–59	20 primary samples from the seed lot
≥ 60	30 primary samples from the seed lot
B: Lot size (kg)	**No. of primary samples**
≤ 500	5 primary samples from the seed lot
501–1500	1 primary sample for each 100 kg
1501–3000	15 primary samples from the seed lot
3001–6000	20 primary samples from the seed lot
≥ 6001	30 primary samples from the seed lot

Submitted sample

All primary samples are combined into the composite sample, but individual primary samples should be judged for heterogeneity (within sample and between samples). If the lot appears to be non-uniform, the primary samples should be sent directly to the laboratory for a heterogeneity test and no composite sample is prepared. If

the seed lot is uniform, the composite sample is thoroughly mixed using an appropriate divider (soil divider, etc) and is reduced to the weight of the submitted sample (1 kg for faba bean and chickpea, and 600 g for lentil), sealed, and dispatched immediately to the seed testing station.

At this stage, if desired, a separate submitted sample of 100 g for faba bean, chickpea, and lentil is prepared for the moisture tests. The sample should be packed in a vapor-proof container to maintain the moisture content of the seed. A separate submitted sample for post-control tests is also prepared at this stage: 2 kg for faba bean and chickpea and 1 kg for lentil.

Seed standards

Standards for seed quality attributes should be established (Hampton, 1998), and laboratory tests should be conducted to determine if these standards have been met. Standards for faba bean, chickpea, and lentil seed are provided in Table 8.6, based on Moroccan seed standards (Madarati and Bishaw, 2002) and suggestions by Doerfler (1976).

For example, an approved seed lot of pre-basic lentil seed should have: at least 98% physical purity, less than 0.2% of other crop seeds (vetch, *Lathyrus*, pea), less than 0.25% weed seeds (*Galium*), and at least 85% germination. Furthermore, it should not contain more than two seeds each of *Orobanche* and *Cuscuta* per 100 g seed. The seed lot should not have live insects, and not more than 3% of the seeds should be damaged by bruchids.

A seed lot should be sampled to assess whether these standards are met and the required tests conducted.

Physical purity test

The purity test has been described in detail in the International Seed Testing Rules (ISTA, 2003). To establish the physical purity of legume crops, the working sample should be classified into four components: pure seed, other crop seed, weed seed, and inert matter. However, for internation-

Table 8.6. Suggested seed quality standards for cool-season food legumes

	Pre-basic	Basic	Certified 1	Certified 2
Physical purity				
Purity (min. %)	98	98	98	97
Other crop seed (max. %)	0.2	0.2	0.2	0.5
Weed seeds (max. %)				
Faba bean	0	0	0	0
Chickpea	0	0	0	0
Lentil	0.25	0.25	0.25	0.5
Cuscuta (no. of seeds per 100 g)	1	2	2	2
Orobanche (no. per 100 g)	1	2	2	2
Germination				
Germination (min. %)	85	85	85	85
Hard seed expected to germinate (max. %)				
Faba bean	20	20	20	20
Chickpea	0	0	0	0
Lentil	0	0	0	0
Insects				
Live insects	0	0	0	0
Bruchid-damaged seed (max. %)	3	3	3	5
Diseases				
Ascochyta (max. %)	1	1	2	2
Seed moisture				
Seed moisture (max. %)	12	12	12	12

al trade, there is no distinction between seeds of cultivated species and weed seeds; both are classified as 'other seed'.

Pure seed: Felfoldi (1983) has defined a pure seed for all species. For legumes, the pure seed fraction includes:

- All intact seeds of botanical varieties and cultivars of the species. No distinction is made between different varieties of the same species.
- Immature, undersized, shriveled, diseased, or germinated intact seeds.
- Seed or pieces of seed that are larger than half the original size and still have a piece of the seed coat (half-seed rule). If no seed coat is present, the seed is classified as inert matter.

Other crop seed: Seeds or pieces of seed more than half the original size, which do not belong to the pure seed category, are classified as 'other seeds', taking into account the definitions of pure seed of the other crop species.

Weed seed: All seeds or pieces of seed more than half the original size, which do not belong to pure or other crop seed are classified as weed seeds.

Inert matter: This includes all seed units and other matter that do not belong to the other seed fractions. These include other crop and weed seeds that are equal to or smaller than half the original size, or, in the Leguminosae, seeds without a piece of testa.

Procedure

For faba bean and chickpea, the whole submitted sample (1000 g) is analyzed for purity (submitted sample = working sample). For lentil only 60 g of the 600 g submitted sample is used. For all three crops, the working sample should be divided in two equal sub-samples. The sub-samples should be carefully weighed before being separated into the four components: pure seed, other crop seeds, weed seeds, and inert matter.

After separation, each fraction is weighed and the percentage purity calculated to one decimal place and recorded. The difference in weight between the initial sample and the sum of fractions should not exceed 2%. The average of two subsamples should be calculated and the results compared with the tolerance table (ISTA, 2003; Mills, 1963).

Tolerance tables are used to check the accuracy of the test results from the two working subsamples (Table 8.7). If the physical purity of a chickpea seed lot was 98.15% and 97.50% for subsamples 1 and 2, respectively, the average purity will be 97.82% and the difference between the two subsamples will be 0.65. This difference should be compared with the figure in the tolerance table (1.54). If the difference is smaller than the maximum tolerance, the average physical purity of 97.82% is considered to be correct and reported. Comparison can also be made for each of the other seed purity components. If the difference is larger than allowed by the tolerance table, the test should be repeated.

The percentages of each fraction must be reported to one decimal place and they must add up to 100%. Components of less than 0.05% are reported as trace, and nil is reported as zero.

Washing test

National and international trade in contaminated seeds is the main factor for long-distance dispersal of *Orobanche* seeds in legume crops (Abu-Irmaileh, 2004). Seeds of parasitic weeds may mix with or adhere to the surface of crop seeds and infest clean fields. Since the seeds of parasitic weeds are very small, they are not easi-

Table 8.7. Use of tolerance table for purity test of chickpea seed lot

Components	Percentages				
	Sub-sample 1	Sub-sample 2	Average	Difference	Tolerance
Pure seed	98.15	97.50	97.82	0.65	1.54
Other seed	0.55	1.15	0.85	0.60	1.00
Weed seed	0.0	0.0	0.0	–s	–
Inert matter	1.30	1.34	1.32	0.04	1.26

ly detected in the purity analysis. Therefore, a washing test is necessary.

Submerge the seed sample in water (100 g seed in 100 ml water with a few drops of household detergent to eliminate surface tension) and shake thoroughly. Then pour the washing liquid over a sieve covered with white filter paper, which can be examined under a stereomicroscope for the presence of *Orobanche* or *Cuscuta* seeds.

Jacobsohn and Marcus (1988) developed a method to detect the presence of *Orobanche* seeds in vetch seeds. The *Orobanche* seeds are washed out of the vetch seeds using a combination of two sieves. The vetch seeds will remain in the top sieve while the broomrape seeds collect in the lower sieve, which has openings 100 µm square, too small for *Orobanche* seeds to pass through.

Germination test

Hard-seededness and impermeability of seed coat are common in food legumes including faba bean, chickpea, and lentil. Impermeability is associated with seed dehydration during the later stages of maturation, especially when RH is low during ripening (Agrawal, 1985; Ellis *et al.*, 1988). Hard-seededness is a serious problem in the drier regions. In India, 8–20% and 9–43% occurrence of hard seeds have been reported in chickpea and lentil, respectively (Anonymous, 1984). In lentil, the percentage of hard seeds falls from a maximum immediately after harvest to practically zero after 5–6 months (Agrawal, 1985).

El Bagoury (1975) reported an increase in the proportion of hard faba bean seeds due to high levels of potassium sulfate and delayed harvest in Egypt. Salih (1981) reported that apart from differences in variety and seed size, crop management practices such as delayed planting, early termination of irrigation water, population density, use of inorganic fertilizers (potassium sulfate), and delayed harvesting can cause hard-seededness in faba bean. All these factors, except delayed harvesting, increased the hard-seededness in the Sudan.

A detailed description of the germination test is available in the International Seed Testing Rules (ISTA, 2003). Laboratory germination is defined as the emergence and development of a seedling to a stage where the presence, absence, and formation of essential structures can be assessed, thus indicating whether the seedling is capable of developing into a normal plant under favorable conditions in soil. The following parameters could be used to evaluate seedlings of chickpea, lentil, and *Vicia* spp. (Bekendam and Grob, 1979; van Geffen, 1986).

Normal seedlings are seedlings that show the potential for continued development into normal plants when planted in good quality soil and under favorable moisture and temperature. A normal seedling should have intact:

- primary root, or with only slight defects, discolored or necrotic spots, healed cracks or splits, or cracks and splits of limited depth. (If the primary root is defective but a sufficient number of normal secondary roots have been developed the seedling is also considered normal)
- cotyledons, or with only slight defects (< 50% of the original tissue not functioning)
- epicotyl, or with only slight defects (discolored or necrotic spots; healed cracks, splits, or breaks; cracks or splits of limited depth; or loose twists)
- primary leaves, or with only slight defects (<50% of the area not functioning).
- terminal bud.

Abnormal seedlings are seedlings that do not show the potential to develop into a normal plant under favorable moisture, temperature, and light. All seedlings that are not normal are classified as abnormal.

Hard seeds are seeds of Leguminosae, which remain hard at the end of the prescribed test period because they could not absorb water due to an impermeable seed coat.

Fresh ungerminated seeds are seeds, other than hard seeds, which remain firm and apparently viable at the end of the test period.

Dead seeds are seeds that are neither hard nor fresh and have not produced any part of a seedling at the end of the test period.

Procedure

Germination tests are carried out on seeds from the pure seed fraction or from the submitted sample (Fig 8.2). About 450 seeds are randomly selected, from which four replicates of 100 seeds each are selected at random, counted, and planted in boxes. Faba bean and chickpea should be planted in sand, but lentil can be planted between pleated papers in boxes. The sand should be washed to remove toxic materials, and sterilized to kill foreign seed and micro-organisms. The number of seeds per box depends on the size of the box.

Sufficient water is added to each box for germination and initial development of the seedlings (approximately 60% of water-holding capacity for planting in sand), and placed under optimal temperature conditions (20°C). A germination cabinet/room, which can maintain a constant temperature of 20°C ± 2°C is required. More details are provided in Table 8.8 based on the ISTA rules (ISTA, 2003).

Evaluation

The first count of normal seedlings can be made after four days for faba bean, and after five days for chickpea and lentil. During the first count, the normal seedlings are counted and removed, while other seedlings are left in the box. No first count is usually made for faba bean and chickpea if they are planted in sand. At the end of the test period, the remaining seedlings are classified into normal or abnormal while the ungerminated seeds are classified as hard, fresh or dead. The results are categorized, estimated to the nearest whole number for the four replicates, and checked against the tolerance table (ISTA, 2003; Mills, 1963). The germination percentage is based on the number of normal seedlings.

Tolerance tables are also used to check the accuracy of the germination results from the four replicates (Table 8.9). If the germination percentages of a lentil seed lot are 97, 96, 73 and 98 respectively in four replicates, the average germination is 91% and the difference between the highest and lowest replicate is 25%. Since this difference is higher than the maximum tolerance (10), the test should be repeated. In the second test, germination is 96%, with a difference of 4 between the highest and lowest germination values, compared to the tolerance level of 7. Therefore, the result is acceptable.

To report the results, we consider both tests. Average germination of the two tests is 94%, and the difference is 5, compared to a tolerance of 9. The result is therefore considered correct and the average of the two tests is reported as the germination percentage. Separate comparisons can also be made for other components of germination test.

Moisture tests

The moisture content is an important determinant of seed viability: high moisture content at harvest will increase the susceptibility of seed to several deteriorative factors. Moisture content is

Table 8.8. Specifications for germination test of cool-season food legumes

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Treatment to break dormancy
Vicia faba	S; (BP)	20	4	14	Prechill
Cicer arietinum	S; (BP)	20; (20–30)	5	8	–
Lens culinaris	BP; (S)	20	5	10	Prechill

S = sand; BP = between paper

Table 8.9. Use of tolerance table for germination test of lentil seed lot

	Replicates				Average	Difference	Tolerance
	1	2	3	4			
Test 1	97	96	73	98	91	25	10
Test 2	98	94	97	94	96	4	7
Tests 1 and 2					94	5	9

also important in determining price in seed trade.

The moisture content of a sample is the loss in weight when dried under fixed temperature and over a fixed period of time. The ISTA rules provide specifications for moisture tests (ISTA, 2003).

The objective of the moisture test is to determine the moisture content of a seed lot at sampling. The submitted sample (100 g for faba bean, chickpea and lentil) should be packed in moisture-proof containers, sent to the testing station immediately, and analyzed upon arrival.

Procedure

The oven method is the standard procedure for measuring moisture content. The seeds are ground before drying. Coarse grinding (at least 50% of the ground material should pass through a 4 mm mesh sieve) is necessary for faba bean, chickpea and lentil. Moisture analysis is carried out on a duplicate 5 g working sample prepared from the submitted sample. The duplicate working sample is weighed to an accuracy of 1 mg. The containers are weighed with and without the samples and then dried for one hour at 130–133°C. The moisture content (MC wet weight basis), as a percentage by weight, should be calculated to one decimal place using the following formula (ISTA, 2003):

$$(M_2 - M_3) * \frac{100}{M_2 - M_1}$$

where

M_1 = weight of empty container and its cover

M_2 = weight of container, cover and seed before drying

M_3 = weight of container, cover and seed after drying

If the moisture content is more than 17%, pre-drying is obligatory, and the moisture content is calculated from the results obtained in the first and second stages of the procedure. If S_1 is the moisture loss in stage 1 (pre-drying) and S_2 the moisture loss in stage 2 (both are calculated and expressed in percentages), then the original moisture content of the sample, expressed in percentage, will be:

$$S_1 + S_2 - \frac{(S_1 * S_2)}{100}$$

The results of the duplicate analysis should not differ by more than 0.2%, otherwise it should be repeated.

Moisture meters can also be used for quick tests. Different brands and types of moisture meters are available to reduce the time needed for the analysis. The results of the quick tests should always be calibrated or checked against the standard air-oven method. Because of the uncertainties associated with using most quick methods, ISTA has designated the air-oven method as the only official method for the determination of moisture content.

Seed health tests

Adequate care should be taken to avoid introducing new pathogens and pests with imported seeds. For example, stem nematode (*Ditylenchus dipsaci*) has a high rate of transmission through seed and can cause serious damage to legume crops. Simple tests, such as covering a seed sample with water for 24 hours and then examining the suspension, are available to detect the pathogen. However, the sample should be large enough to detect low infestation levels.

In legumes, however, the transmission of most seed-borne pathogens, except *Ascochyta*, is a minor problem when compared to other sources of inoculum. A laboratory standard of 1% and 2% infection (based on testing 400 seeds) for basic and certified seed, respectively, is suggested (Table 8.6).

The analyses are carried out on separate working samples drawn from the submitted sample (Fig. 8.2). Two methods can be used, the agar plate test and the blotter test. ISTA recommends the blotter test, which is easy and cheap; while the agar plate test is more sensitive.

The blotter test

The method used for the blotter test is similar to the standard germination test, where seeds are placed on moistened absorbent paper (filter, towel) in petri dishes, germination boxes, or any other container that can be sealed. For routine testing, it is advisable to have a standard paper cut to a suitable size, and thick enough to absorb moisture for the duration of the test (e.g., Schleicher and Schüll filter paper No 8272 for faba bean and chickpea, or pleated strips for

lentil). A constant quantity of distilled water should be added based on the substratum, container size, crop species, etc.

Procedure: The seeds are incubated for 7–10 days at 20°C. To stimulate sporulation, near UV light at a cycle of 12/12 hours white light/darkness should be operated after four to five days incubation. Evaluation should be done under the stereomicroscope, but the diagnosis should be confirmed under a compound microscope until the analyst becomes familiar with the appearance of different pathogens on seeds. Surface sterilization suppresses saprophytes and affects the pathogens, therefore, it is generally not recommended.

Evaluation is easier when germination is suppressed. Therefore, for fungi which tolerate very low temperatures, the seeds are incubated for 24 hours at 20°C. They are then placed overnight in a deep freezer at –18°C, and afterwards incubated again at 20°C for seven days.

Agar plate test

The agar plate test requires special equipment and skills, and is conducted under sterile conditions to prevent contamination with saprophytes. An ideal working area is a laminar airflow bench, but if this is not available, a corner away from draft, thoroughly cleaned with 70% alcohol, will be adequate. Proper cleaning and sterilization of petri dishes, media, working area, and tools is very important. The petri dishes can be placed in special metal containers, or simply wrapped in aluminum foil and sterilized with either dry hot air (oven, 180°C for 20 minutes) or moist heat (autoclave or pressure cooker, 15 psi for 15 minutes).

The media should be sterilized in an autoclave as indicated. The media must be prepared using distilled water. The flasks should be filled to only 50% capacity. At about 45–55°C, the media can be poured into plates; the rim of the flask should be sterilized over a flame. The petri dishes should be opened in such a way as to prevent breathing on them. The media should be evenly distributed in the petri dish; the petri dishes can be stored upside-down after the medium has solidified, preferably in a refrigerator. Needles, tweezers, etc., can be sterilized by dipping them into 70% alcohol and then heating to redness over a Bunsen flame or alcohol burner.

Procedure: Surface-sterilize the seeds in sodium hypochlorite (e.g. 3–5 minutes in 10% solution of commercial Chlorox) to suppress saprophytes. Then place the seeds on the media, with sufficient space in-between to allow the development of distinct colonies (i.e. 5–7 faba bean seeds, 10 chickpea seeds, or 15–20 lentil seeds in a 9 cm petri dish). Incubate the seeds for seven days and expose to near UV light usually after three to four days, to increase sporulation. For evaluation, some colonies can be easily identified by their characteristic growth, color, fruiting bodies, etc. For others, it will be necessary to check at high magnification under a compound microscope.

Incubation media: The media for isolation of fungi, such as potato-dextrose agar (PDA) and malt extract agar (MEA) dehydrated culture media, are available at Merck, Difco, etc. Ox-gall agar is suitable mainly for *Fusarium* testing. Add to one liter of PDA, after autoclaving and cooling, 2 g ox-gall and 100 mg streptomycin sulfate. Host medium (legumes) is preferred for *Ascochyta* testing. Mix 40 g ground seeds with one liter of distilled water, autoclave for 40 minutes, filter the suspension with cheesecloth and add 20 g dextrose, 20 g agar, and water to one liter, then autoclave again for 15 minutes at 15 psi.

Control Plot Tests

A well-developed certification scheme usually includes control plot tests to further check on varietal identity, varietal (genetic) purity, and seed-borne diseases. The main purpose of control plots is to ascertain whether the certification system is satisfactory. Control plots are planted with samples taken from seed lots that were approved in the previous season. For example, seed samples from approved basic seed, planted as control plots, can be used as post-control for basic seed and as pre-control for certified seed production. Therefore, samples taken from each approved basic seed lot and 10–20% of

certified seed lots are planted to confirm that the varietal purity and identity have remained unchanged during seed multiplication. The description for cereal post-control plots (OECD, 2000) can be used as the basis for legume control plots.

Standard sample

It is important to have a reference sample of each variety under seed multiplication, especially for variety purity tests and control plot (pre/post) checks. The standard sample, which provides a living description of the variety, is the official authentic sample against which all other samples will be compared and judged. Variety descriptions (dead descriptions) may be helpful but do not always provide sufficient information.

The breeder should submit the standard sample at the time the variety is released, and it should be large enough to supply enough seed for reference during the lifetime of the variety. It should be stored under optimum conditions to avoid deterioration; 10°C and 45% RH is recommended. The weight of the standard sample could be 2 kg for faba bean and chickpea, and 1 kg for lentil. The seed quality control authority should store the standard sample for reference.

Growing methods

Plots should be sown in a clean field, free from legumes and weeds, taking the same precautions as during seed production (Chapter 2). Extreme care should be taken during planting to avoid admixture. Varietal purity, species purity, and seed-borne diseases of the seed lot are important factors to be scored.

Plot size

In control plot tests, samples of seed lots are grouped based on the variety and generation; all the lots of a variety/generation are planted next to each other. The standard sample is grown at a ratio of 1:15. Only one plot per seed lot is grown, and no replications are used.

Because the plots are small, only lots with high percentage of contamination can be detected; the plots cannot be used to decide whether the standards were met when the seed lot was certified.

Observations

Plots are observed throughout the growing season, with emphasis on the periods between flowering and harvest, to assess varietal identity, varietal purity (offtypes and other varieties), inseparable crops, diseased plants, and parasitic weeds. Many characteristics can be used, but it is impractical to observe each character in all plots (see Chapter 1). Based on the observations, the seed quality control authority can determine whether the certification system is satisfactory.

Many developing seed industries are not using control plots as part of the certification process. However, control plots can play a very important role in improving the quality of the seed. In addition to serving as a check on the certification process, the plots can be used as pre-control plots, i.e., seed samples from the basic seed lots can be used to plant pre-control for certified seed lots.

Control plots can also play a very important role in the training of field inspectors; samples of all varieties from approved seed lots are planted together in one field, providing an excellent opportunity to study differences and similarities. In addition, control plots can be used as an 'early warning system' if planted as early as possible. Field inspectors will be alerted to any problems encountered in the control plots, and advised to pay special attention to the field(s) that were planted from the same seed lot(s).

Lot Numbering

An effective seed quality assurance program should have a well established system to identify and monitor the movement of seed during production, processing, storage, and marketing. A lot numbering system should be designed in such a way that all seed lots can be traced back to the producer, processor, or distributor. Each seed lot is therefore given a unique number, which provides information on the year of production, grower, crop group, processor, and seed class. A lot numbering system for legumes could be made up of 16 digits (see Box 1), separated into eight groups by dashes as follows:

01-1-01-0001-01-1A-001

1. The first two digits identify the *crop*. Crops are numbered from 01 to 99. Each crop is assigned a number which never changes: e.g. 02 Faba bean, 03 Chickpea, 04 Lentil.
2. The second group (one digit) identifies the *generation*. Numbered from 1 to 6: Breeder seed = 1, Pre-basic seed = 2, Basic seed = 3, Certified seed 1 = 4, Certified seed 2 = 5, Commercial seed = 6.
3. The third group (two digits) identifies the *province or region*. The same province or region will always have the same number. If seed of two different provinces are bulked, the lower digit is used.
4. The fourth group (four digits) identifies the *seed grower*. Seed growers within a province or region are numbered from 0001 to 9999. Each grower has a unique number that never changes. If the seed grower stops growing seed, the number is discarded. If seed of the same variety and category is bulked from different growers, the resulting bulk is given the number of the numerically lowest crop identity number from those being included.
5. The fifth group (two digits) identifies the *processing plant* where the seed is cleaned. Each processing plant, either public or private, is assigned a number from 01 to 99. If a plant is closed, its number is discontinued.
6. The sixth group consists of one number and one letter. The digit is the last number of the *production year*; for example, seed produced in 2001 has digit 1. The letter identifies the *season*: A for seed produced in winter and B for summer.
7. The seventh group (three digits) identifies the *specific seed lot number*, sequentially numbered based on maximum lot size.

Managing Seed Quality Assurance

Responsibility for quality control

The seed certification authority should remain independent of seed production; and should be impartial in serving both seed producers and users. However, with the emergence of a diverse seed industry, it should be flexible and decentralized with more responsibility given to seed producers, rather than concentrating on comprehensive compulsory certification. Tripp *et al.* (1997) suggested that the agency could function more efficiently by using appropriate criteria and being transparent in involving the stakeholders. It should not be a barrier especially to the entry of small to medium indigenous private companies into the seed sector. Moreover, the seed certification agency should seek to educate farmers on how to produce good quality seed rather than acting as a simple law enforcement agency.

Establishing realistic standards

Field and seed standards are very common for cereals, but many national programs do not have standards for legume seed production. It is important to establish realistic standards that can be reviewed regularly, instead of introducing exceptionally high standards that will be difficult to achieve at national level. ICARDA has

Box 1. Numbering of seed lots

Seed lots are identified by a 16-digit alphanumeric system.
For example, seed lot number **02-3-06-0004-09-4A-003** would signify:

Digits 1 and 2:	Crop	**02**	Faba bean
Digit 3:	Generation	**3**	Basic seed
Digits 4 and 5:	Province	**06**	Sixth province
Digits 6 to 9:	Seed grower	**0004**	Grower number four
Digits 10 and 11:	Processing plant	**09**	Plant number 9
Digits 12 and 13:	Year	**4**	Year 2004
	Season	**A**	Winter season
Digit 14, 15 and 16:	Lot sequence number	**003**	Third lot bagged

collated field and seed standards from the WANA countries (Madarati and Bishaw, 2002) and FAO has suggested realistic standards for 'quality declared seed' (FAO, 1993).

Harmonizing seed certification

The procedures for seed certification are essentially similar in many countries except in seed classes, where there is variation in nomenclature. Moreover, most national seed regulations and standards are similar in many ways. Countries should work together to develop a flexible, harmonized, regionally or internationally acceptable seed certification scheme for the development of the seed industry.

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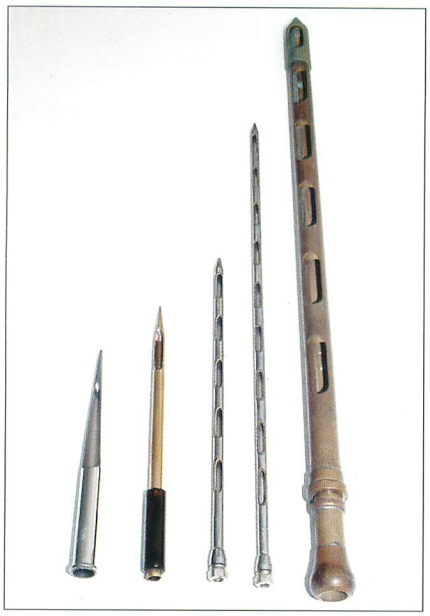
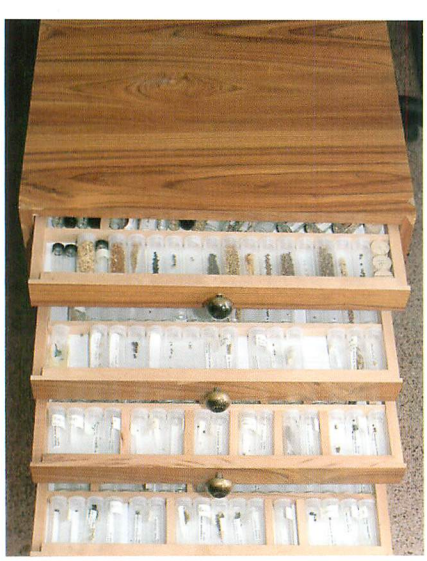


8.1 Different triers for seed sampling

8.2 Sampling a seed lot

8.3 A sample ready for dispatch to the seed testing laboratory

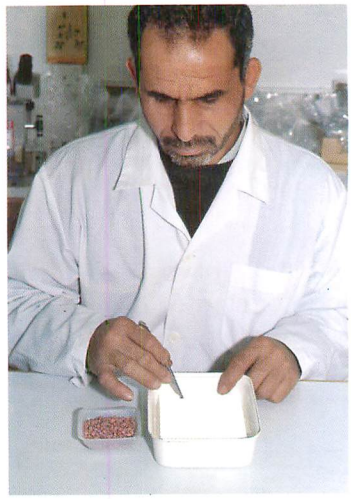
8.4 Preparing a chickpea working sample for purity test



8.5 Reference collection for identification of weed seeds

8.6 Purity analysis of lentil seed

8.7 Planting chickpea seed for germination test (counting board)



8.8 Planting lentil seed using pleated paper

8.9 Germination boxes in a germination room

8.10 Grinder and other equipment for moisture test

8.11 Oven and desiccators for moisture test

8.12 Balance for weighing purity and moisture samples

Annex 1. Morphological Characters of Faba Bean (based on UPOV and IPBGR)

Plant

- Growth type (always/essential): determinate (1), indeterminate (2)
- Height measured from ground to tip of the plant (always/essential): very short (1), short (3), medium (5), tall (7), very tall (9)

Stem

- Number of stems including tillers more than half the length of the main stem (always): few (3), medium (5), many (7)
- Number of nodes up to and including first flowering node: few (3), medium (5), many (7)
- Anthocyanin coloration (desirable): absent (1), present (9)
- ‡Intensity of anthocyanin coloration: slight (3), medium (5), much (7)
- ‡Stem color at maturity (desirable): light (1), dark (2)

Branching

- ‡From basal nodes, number of branches taken in late flowering stage (essential): very small (1), small (3), medium (5), large (7), very large (9)
- ‡From higher nodes (desirable): absent (1), present (9)

Foliage

- ‡Color: green (1), bluish green (2), greyish green (3)
- Greyish hue of green color: absent (1), present (9)
- Intensity of green color at flowering: light (3), medium (5), dark (7)
- ‡Number of leaflets on fully expanded leaves at median flowering node (desirable): very small (1), small (3), medium (5), large (7), very large (9)

Leaflet

- ‡Size observed on fully expanded leaves at intermediate flowering nodes (essential): very small (1), small (3), medium (5), large (7), very large (9)
- Length of basal pair at secondary node (always): short (3), medium (5), long (7)
- Width of basal pair at secondary node (always): narrow (3), medium (5), broad (7)
- Position of maximum width at secondary node (always): towards tip (1), at middle (2), towards base (3)
- Folding of the terminal pair along the main vein: weak (3), medium (5), strong (7)

Raceme

- Number of flowers at second or third flowering node (always): few (3), medium (5), many (7)

Flower

- Time of flowering, 50% of plants with at least one flower (always/essential): early (3), medium (5), late (7)
- ‡Ground color (standard petal flag) (essential): white (1), violet (2), dark brown (3), light brown (4), pink (5), red (6), yellow (7), other (8), mixed (9)
- ‡Intensity of streaks on standard petal (essential): weak (3), medium (5), strong (7)
- ‡Wing petal color (essential): uniformly white (1), uniformly colored (2), spotted (3), mixed (4)
- Length: short (3), medium (5), long (7)
- Melanin spot on the wing (always): absent (1), present (9)
- Color of melanin spot on the wing (always): greenish yellow (1), brown (2), black (3)
- Melanin spot on the standard: absent (1), present (9)
- Anthocyanin coloration of standard (always): absent (1), present (9)
- Extent of anthocyanin coloration of standard: slight (3), medium (5), much (7)

Pod

- ‡Days to maturity. Days from sowing until 90% of pods have dried (essential): early (3), medium (5), late (7)
- ‡Pod distribution (desirable): uniform (1), mainly basal (2), mainly terminal (3).

- Number of pods per truss: few (3), medium (5), many (7)
- ‡Number of pods per node on the second pod-bearing node (desirable): very small (1): small (3), medium (5), large (7), very large (9)
- Attitude on second or third pod-bearing node (always/desirable): erect (1), semi-erect (3), horizontal (5), semi-pendulous (7), pendulous (9)
- Length (without beak) (always/essential): very short (1), short (3), medium (5), long (7), very long (9)
- Width from suture to suture (always): narrow (3), medium (5), broad (7), very broad (9)
- ‡Shape (essential): subcylindrical (1), flattened constricted (2), flattened non-constricted (3), mixed (4), or flattened (1), angular (2), round (3), mixed (4)
- ‡Color at maturity (desirable): light (1), dark (2)
- Degree of curvature at green shell stage: absent or very weak (1), weak (3), medium (5), strong (7)
- Intensity of green color: light (3), medium (5), dark (7)
- Number of ovules including seeds (always/desirable): few (3), medium (5), many (7)
- Thickness of wall: thin (3), medium (5), thick (7)
- ‡Time to first fully developed pods: early (3), medium (5), late (7)

Seed

- Shape of cross section (essential): narrow elliptic (1), elliptic (2), broad elliptic (3)
- Shape of median longitudinal section (essential): elliptic (1), broad elliptic (2), circular (3), oblong (4), square (5), ovate (6)
- Color of testa immediately after harvest (always/essential): beige (1), green (2), red (3), violet (4), black (5)
- Black pigmentation of hilum (essential): absent (1), present (9)
- ‡Number of seeds per pod (essential): very small (1), small (3), medium (5), large (7), very large (9)
- Weight of largest seed from the largest pod for each plant sampled (always/essential): very low (1), low (3), medium (5), high (7), very high (9)
- Tannin: absent (1), present (9)

Note: ‡Morphological characters not included in UPOV guidelines

Annex 2. Morphological Characters of Chickpea (based on UPOV and IBPGR)

Plant

- Height when pods are fully developed (always/essential): very short (1), short (3), medium (5), tall (7), very tall (9)
- ‡Canopy width (desirable): small (3), medium (5), large (7)
- Attitude taken after flowering (always/essential): erect (3), semi-erect (5), prostrate (7)
- ‡Intensity of ramifications: weak (3), medium (5), strong (7)
- Hairiness of stems, leaves and pods (desirable): weak (3), medium (5), strong (7)

Stem

- Anthocyanin coloration (always/essential): absent (1), present (9)
- Height of insertion of first flower: low (3), medium (5), high (7)

Leaf

- Intensity of green color (always): light (3), medium (5), dark (7)
- ‡Size: small (3), medium (5), large (7)
- Number of leaflets per leaf (essential): 3 to 9 (1), 9 to 11 (3), 11 to 13 (5), over 13 (7)
- Leaflet size (always/essential): very small (1), small (3), medium (5), large (7), very large (9)
- ‡Area: small (3), medium (5), large (7)

Branch ‡

- Number of basal primary branches (essential): low (3), medium (5), high (7)
- Number of basal secondary branches (essential): low (3), medium (5), high (7)
- Number of apical primary branches (desirable): low (3), medium (5), high (7)
- Number of apical secondary branches (desirable): low (3), medium (5), high (7)
- Number of apical tertiary branches (desirable): low (3), medium (5), high (7)

Flower

- Time of flowering, 80% of plants have *at least* one flower (always/essential): very early (1), early (3), medium (5), late (7), very late (9)
- Color (essential/always): white (1), purplish pink (2)
- Peduncle length: short (3), medium (5), long (7)

Pod

- Time of maturity, when seeds are dry (always/essential): very early (1), early (3), medium (5), late (7), very late (9)
- Size (always/desirable): very small (1), small (3), medium (5), large (7), very large (9)
- Intensity of green color (always): light (3), medium (5), dark (7)
- Length of beak: short (3), medium (5), long (7)
- Number of pods per plant: mean of five randomly selected plants
- Predominant number of ovules (always): two (1), three (2)

Seed

- Color (essential/always): yellow (1), beige (2), ochre (3), brown (4), reddish brown (5), black (6)
- Intensity of color (always): light (3), medium (5), dark (7)
- Weight measured on two samples of 100 seeds (always/essential): very low (1), low (3), medium (5), high (7), very high (9)
- Shape (essential/always): round (1), round to angular (2), angular(3)
- Ribbing (always): absent or very weak (1), weak (3), medium (5), strong (7), very strong (9)
- Presence of minute black dots: absent (1), present (9)

Note: ‡Morphological characters not included in UPOV guidelines

Annex 3: Morphological Characters of Lentil (based on UPOV and IBPGR)

Plant

- Growth habit: erect (1), semi erect (3), prostrate (5)
- Anthocyanin coloration (always): absent (1), present (9)
- Height at flowering (always/essential): short (3), medium (5), tall (7), very tall (9)
- Intensity of ramification: weak (3), medium (5), strong (7)

Foliage

- ‡Leaf pubescence (desirable): absent (1), slight (3), medium (5), dense (7)
- Shape: elliptic (1), ovate (2), rectangular (3)
- Intensity of green color: light (3), medium (5), dark (7)
- No. of leaflets: few (3), medium (5), many (7)
- Leaflet size: small (3), medium (5), large (7)
- Tendril length (desirable): rudimentary (1), prominent (2)

Raceme

- No. of flowers per node: one (1), one to two (2), two (3), two to three (4), three (5), more than three (6)

Flower

- Time of flowering when 50% of plants are in flower (always/essential): very early (1), early (3), medium (5), late (7), very late (9)
- Size: small (3), medium (5), large (7)
- Color of standard (always): white (1), pink (2), blue (3)
- Violet stripes on standard: absent (1), present (9)
- Violet stripes on wings: absent (1) present (9)
- ‡Ground color (essential): white (1), white with blue veins (2), blue (3), violet (4), pink (5), other (6)
- ‡Maximum number of flowers per peduncle: few (3), medium (5), many (7)

Pod

- Pigmentation (desirable): absent (1), present (9)
- Color at dry harvest maturity (always): yellow (1), green (2)
- Intensity of color before dry harvest maturity: light (1), medium (5), dark (7)
- Number of ovules: mainly one (1) one to two (2), mainly two (3), two to three (4), mainly three (5)
- Length at dry harvest maturity (without beak) (always): short (3), medium (5), long (7)
- Width at dry harvest maturity: very narrow (3), narrow (3), medium (5), broad (7)
- Shape of apex at dry harvest maturity: truncate (1), truncate to pointed (2), pointed (3)
- ‡Number of seeds per pod (desirable): few (3), medium (5), many (7)
- ‡Height of lowest pod on non-lodged plants at harvest (desirable): low (3), medium (5), high (7)

Seed

- Time of maturity when 90% of pods are golden brown (essential): early (3), medium (5), late (7), very late (9)
- Width (always): very narrow (1), narrow (3), medium (5), broad (7), very broad (9)
- Profile in longitudinal section (always): elliptic (1), broad elliptic (2)
- Number of colors (always): one (1), two (2), more than two (3)
- Main color of testa (always/essential): white (1), greenish yellow (2), green (3), pink (4), ochre (5), black (6)
- Type of ornamentation (for varieties with more than one testa color only) (essential): patches (1), spots (2), marbled (3), complex (4)
- ‡Pattern of testa color (desirable): absent (1), olive (2), grey (3), brown (4), black (5).
- Cotyledon color observed in seeds less than three months old (always/essential): orange (1), greenish yellow (2), green (3)
- Weight of average of two samples of 100 randomly chosen seeds (always/essential): very low (1), low (3), medium (5), high (7), very high (9)

Note: ‡Morphological characters not included in UPOV guidelines

Annex 4: Key for Decisions Concerning Pests and Diseases in Seed Multiplication Fields (For Field Inspectors)

1 Identify the pest/pathogen, then: 2
2 The pest/pathogen is not known to be seed-transmitted: 3
2* The pest/pathogen is known to be seed-transmitted:
Take records and compare with standards.
After harvest, test seeds in laboratory
3 The pest/pathogen is not likely to affect yield or quality:
Do not do anything and hope for the best
3* The pest/pathogen is likely to affect yield or quality: 4
4 The causal agent is a fungus, insect, or nematode:
Select an appropriate pesticide and advise application at recommended dosage
4* The causal agent is a virus or bacterium:
Advise grower to rogue infected plants in order to prevent further spread (if feasible) and to spray insecticide if potential vectors are there
5 The causal agent is a virus: 6
5* The causal agent is not a virus: 7
6 The virus is not known to be insect transmitted:
Advise grower to rogue and burn infected plants (if feasible), test seeds after harvest
6* The virus is known to be insect transmitted:
Advise grower to rogue and burn infected plants (if feasible), and to spray insecticide to prevent further spread, test seeds after harvest
7 The pathogen has several generations per year, e.g. *Ascochyta* blight:
Advise application of appropriate pesticides at early stages of disease development, test seeds after harvest, treat infected seed lots

Annex 5: Seed Field Inspection Form

Grower's Application for Field Inspection and Certification

Instructions to applicant: Send to Provincial Certification Office before planting. Include bag tags. Keep one copy for your records. Do not fill in blanks between parentheses (__); these are to be filled by the inspector.

Crop: _____ **Variety**: _____ **Category:** _____

Applicant: Number (_____)

Name: _____

Address and tel.: _____

Date: _____ Signature: _____

Grower: Number (_____)

Name: _____

Address and tel.: _____

Seed:
Amount_____(_____) Category_____(_____) Origin_____(_____)

Lot no._____(_____) Seed treatment_____(_____)

Field: Number (_____) hectares_____(_____)

Describe exact location:

Previous crops: 1._____(_____)
2._____(_____)
3._____(_____)

Sketch Map of Field: **Remarks:**

N

W E

S

Annex 6: Legume Seed Field Inspection Report

Crop: _____ **Variety:** _____ **Category:** _____

Field: Number_____ hectares_____(___)

Grower: Number_____ Name_____

Plant density assessment: No. of plants per meter row length

Number of plants	1	2	3	4	5	6	7	8	9	10	Total	Average

Overview:

Field condition_____

Varietal identity_____

Isolation: North_____

East _____

South _____

West _____

Meets standards _____**Yes** _____**No**

Expected seed yield/hectare_____ Expected harvest date_____

	No of contaminants in field count							
	1	2	3	4	5	6	Total	Accepted
Offtypes & other varieties								Yes/No
Other crops (inseparable seed)								Yes/No
Orobanche								Yes/No
Cuscuta								Yes/No
Virus infected plants								Yes/No
Ascochyta (on pods)								Yes/No
Total diseases								Yes/No

Did grower or his representative accompany you during inspection? _____**Yes** _____**No**

Does field meet standards? _____**Yes** _____**No**

Re-inspection recommended _____**Yes** _____**No**

Remarks:

Grower: _____ Inspector: _____

Date: _____ Date: _____

Signature:_____ Signature: _____

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