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**A STUDY OF THE RELATIONSHIP BETWEEN SEED
QUALITY AND COMMERCIAL SPROUTING QUALITY OF
GREEN GRAM (*Vigna mungo* L. Hepper) AND
BLACK GRAM (*Vigna radiata* L. Wilczek)**

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ABSTRACT

Standard seed quality tests (seed moisture content, thousand seed weight, topographical tetrazolium, germination and seedling evaluation); vigour tests (accelerated ageing, conductivity, rate of germination and uniformity of germination) and industry based tests (oversoaks and sprouters) were evaluated for their ability to rank eight black gram (*Vigna mungo* L. Hepper) seed lots and seven green gram (*Vigna radiata* L. Wilczek) seed lots for the purpose of commercial sprouting. Each seed lot was sprouted using simulated commercial conditions (19°C water temperature; 20°C cabin temperature; dark; 5 days). Seed lots which performed well under these small scale commercial production (SSCP) conditions, in terms of total fresh yield and healthy sprout yield, were considered to be the best quality seed lots.

All tests were able to significantly determine differences among seed lots within each species. Linear regression analysis indicated that interim germination ($R^2 = 79.1\%$), final germination ($R^2 = 76.3\%$), seed moisture content (SMC) ($R^2 = 63.7\%$) and oversoak sprouters ($R^2 = 60.6\%$) were significantly related to total fresh yield in green gram seed lots only. No other significant linear relationships were found for either green gram or black gram. Incorporating interim germination, final germination, SMC and oversoak sprouters in a multivariate analysis reduced the level of unexplained variation in green gram total sprout yield. The best combination was interim germination and oversoak sprouters ($R^2 = 84.2\%$); $Y = 9.1(\% \text{interim germination}) - 8.1(\% \text{oversoak sprouters}) + 731.4$. Very similar to this was the combination of final germination and SMC ($R^2 = 83.8\%$); $Y = 4.7(\% \text{final germination}) + 15.3(\% \text{SMC}) + 165.4$.

The reason for the differing responses of black gram and green gram was not explained, but both genetic variation and differences in environment during seed development and handling prior to testing are likely causes. It was not possible to use any individual or combination of tests to predict sprouting performance for green or black gram with the accuracy the sprouting industry would require. However, the results have shown that it will be possible to eliminate many of the seed quality tests examined from further research. Refinement of test procedures for the relevant standard and industry based tests will be required to provide the accurate seed testing regime needed by the sprouting industry.

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CHAPTER 1

INTRODUCTION

Green gram and black gram (*Vigna radiata* L. Wilczek and *Vigna mungo* L. Hepper respectively) are just two of many legume species which are purposely sprouted, to produce a fresh vegetable for human consumption. These species are believed to be of Indian or Indo-Burmese origin, and have been cultivated in the Indian subcontinent and adjacent regions for several thousand years (Bailey, 1949; Jain & Mehra, 1980; Valvilov, 1926 - cited by Lawn & Imrie, 1991).

Generally, green gram is preferred for sprouting in China, Thailand, Europe, the United States of America and Australia, whereas black gram is preferred in Japan and New Zealand.

Regardless of whether sprout production is on a commercial basis or in the home, the sprouter aims to produce sprouts that are tasty, attractive in appearance and bacteriologically safe to eat. Factors affecting appearance include: sprout colour, sprout size, presence of roots, age and contaminants. The last two factors can also affect taste (Imrie, 1991). Many people think that sprout production is a simple germination process. However, anyone producing sprouts commercially (commercial sprouter) will always face at least three problems: 1. long roots and slender hypocotyls; 2. spoiling; and 3. anthocyanin formation in the cotyledons and hook region. Among these three, the most difficult is how to produce short-rooted and large diameter sprouts (Chang, 1978). For most Western markets, the ideal sprout has a 50mm long hypocotyl which is 3mm in diameter, (Ashley Berrysmith, Auckland pers. comm., 1996). A commercial sprouter also requires a high sprout yield (kg sprouts produced per kg

seed used) and sufficiently long shelf life to ensure extended consumer acceptance (Imrie, 1991). Although the procedure of sprouting seeds has been undertaken for centuries, and the general technique is simple, the consistent production of high quality sprouts has proven difficult.

Recently, sprouters in New Zealand, Australia, Europe and the USA have expressed concern regarding the quality of sprouts being produced. The emphasis has been on sprout uniformity within and between batches, with many sprouters finding it difficult to consistently produce uniformly short, stout, white sprouts. Both variability in the initiation of germination and the rate of seedling growth have been suggested as probable causes of poor sprout uniformity, (Robert Coulson, Feilding pers. comm., 1995), with variation between seed lots common. Yield losses from microbial spoilage and human health related problems are also of concern to sprouters. Quality is determined by the end-user, and problems arise when different end-users place emphasis on different quality attributes (Law & Law, 1991). This problem intensifies when aspects of sprout quality cannot be directly related to seed quality parameters, or the relationships between parameters are unknown.

Seed quality is determined by two parameters - genetic as determined by cultivar, and environmental as determined by the conditions under which the seed is produced (Copeland & McDonald, 1985). High seed quality is fundamental in the production of high quality sprouts. Most sprouters are not in a position to dictate seed production management, apart from demanding a particular cultivar. Therefore, accurate assessment of seed lot quality prior to purchase is imperative when selecting seed lots destined for sprout production. There are ten components of seed quality (Thompson, 1979): analytical purity, percentage of

weed seeds, germination capacity, seed size, seed health, species purity, cultivar purity, vigour, seed lot uniformity and seed moisture content. These components are not all of equal value, nor is their order of relative importance the same in all circumstances.

The importance of seed quality has long been known by those in the seed production business. This has led to the development of several rapid and reliable laboratory test procedures which have been standardised for most important plant species (completed and published by the International Seed Testing Association - ISTA, and the Association of Official Seed Analysts - AOSA) . Internationally accepted tests (ISTA, 1996) can be used to evaluate viability, moisture, seed weight, health and purity of seed lots, while new tests (not yet internationally standardised or agreed upon) have concentrated on assessing seed vigour. All these tests have one thing in common - they were devised in an effort to evaluate the field planting value of seed, not commercial sprout production. Germination, purity and health tests are presently used to evaluate the suitability of seed lots for sprouting, even though the relationships between test parameters and sprout quality are not well understood. Accurate selection of high quality seed for sprouting purposes will not be possible until these relationships are established.

The general objectives of this study were therefore:

1. To determine the quality of a number of available green and black gram seed lots - using the standard ISTA recommended test procedures.
2. To evaluate methods for determining uniformity in green and black gram seedling growth by the use of ISTA recommended seed vigour tests.
3. To determine the health status of submitted seed lots using standard ISTA procedures.
4. To evaluate the sprouting performance of seed lots using a 'small-scale' commercial sprouter, with emphasis on sprout yield and quality.
5. To determine the relationships between seed quality parameters and sprouting variability, correlating results from tests conducted in objectives one to three with small-scale commercial production results.

CHAPTER 2

LITERATURE REVIEW

2.1 THE BEAN SPROUTING INDUSTRY

On a commercial scale, sprout production is normally conducted under a shroud of secrecy. The fresh sprout market is very competitive, and most people within the industry (sprouters) are reluctant to supply information on either 'scale of systems' or 'method of production'. The following industry review is based on the few internationally published scientific papers available, and on comments from sprouters and researchers within the New Zealand and Australian industries.

2.1.1 The Sprouting Concept

The sprout is an intentionally, hydroponically germinated seed, produced for human consumption (Ahmad & Mohamed, 1987; O'Brien & Desmarchelier, 1991). Many different species of seeds are germinated, with the most popular being various types of legumes (Imrie, 1991). Sprout production may be done 'in-the-home' or on a 'commercial scale' - with large sprouters producing in excess of 200kg bean sprouts per day dominating domestic markets in western countries (Ashley Berrysmith pers. comm., 1996).

2.1.2 Bean Sprouts

The traditional and most popular sprout is commonly known as the 'bean sprout' or 'mungbean sprout'. Seeds of the genus *Vigna radiata* L. Wilczek (green gram, syn. *Phaseolus radiatus* L. and referred to in this thesis as green gram) and

Vigna mungo L. Hepper (black gram, syn. *Phaseolus mungo* L. and referred to in this thesis as black gram) are predominantly used in the production of bean sprouts (synonymous with 'mungbean' sprouts). While mungbean sprouts are a traditional oriental food, they have now become popular in western countries, where they are seen as a 'health' food. Sprouts are used fresh in salads or lightly cooked with other vegetables in various menus. Sprouts may be home grown from dry beans, purchased as fresh sprouts, or bought canned (Imrie, 1991).

2.1.3 Industry Size

The New Zealand sprout industry is dominated by one large producer based in Auckland, Sunsprouts NZ Ltd. They are the chief supplier of sprouts to national supermarket chains and restaurants. At present this company sprouts a large range of seed species (10-15 species), providing a range of sprout products for the niche health food market (Ashley Berrysmith pers. comm., 1995). Mungbean sprout production at present is based on black gram seed, although green gram seed has been used in the past. Black gram is preferred because the sprouts are considered to be whiter and therefore more acceptable to consumers than those produced from green gram seed. Daily production of mungbean sprouts is approximately 1200kg per day, from 180kg of seed at Sunsprouts NZ Ltd. This equates to an annual production of just under 440t of mungbean sprouts from 65.7t of seed. There are at least a further four commercial sprouters in the North Island and three in the South Island, producing sprouts for local restaurants and health food outlets. Their daily and/or annual production is not known, (Robert Coulson pers. comm., 1995).

Imrie (1990) in a report to the Australian Mungbean Association (AMA) discussed the Japanese mungbean sprouting industry. Annual seed imports of green gram

and black gram into Japan have ranged from 45,000 to 65,000t in recent years. About two-thirds of imports were of black gram seed with the remainder being green gram. The main suppliers of seed were Burma, China and Thailand. Almost all imported beans were destined for the sprouting industry. It has been estimated that there are about 1000 sprouters in Japan, with the larger sprouters using 100t of beans or more per year.

Australia is New Zealand's closest producer and supplier of mungbean seed. Green gram, which is the species used for sprouting in Australia is the dominant crop grown. Domestic consumption is estimated to be 2500t of sprouts per annum (Lawn *et al*, 1988). While the mungbean sprouting industry has recently grown (Bruce Imrie, Brisbane pers. comm., 1996) recent production data are not available. Sprouts are marketed in Australia in plastic bags, with some producers vacuum packing the product. There is estimated to be an average of one large sprouter and 6-12 smaller sprouters per major city in Australia.

The USA and Europe also have developing sprout markets, based on the increase in health conscious consumers. Their production systems tend to be advanced, and are dominated by large producers (Ashley Berrysmith, pers. comm., 1996). In 1987 it was reported that the USA sprouted between 8300t and 10,000t of mungbean seed (mostly green gram), of which 75% was imported (Lipton, Asai & Fouse, 1981; Cupka & Edwards, 1988). This volume of seed would produce 50,000t of fresh sprouts, assuming a 6-7kg sprout to seed ratio (Lipton, Asai & Fouse, 1981). No data relating to the European sprouting industry are available, although green gram seed is often purchased from Australia for the purpose of sprouting (Philip Smith & Bruce Imrie pers. comm., 1996).

Mungbean sprouts and other products derived from mungbean seed make up an important dietary component for people in many Asian countries. These include Bangladesh, Burma, China, India, Indonesia, Korea, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, and Thailand (Cheng, 1988; Navarro, 1988; Park & Hong, 1988; Singh, 1988). India appears to be the primary producer of mungbean and black gram seed in Asia, but none is exported, and it is seldom used for sprouting (Calkins, 1978; Singh *et al*, 1988). Even though large amounts of mungbean seed are produced and imported by other Asian countries, commercial production of sprouts on a large scale is uncommon. Most sprouts are produced in the home, providing a fresh vegetable all year round for the family. However, Tsou & Hsu (1978) reported a sprouter in Taiwan producing 1000kg mungbean sprouts per day in a 7m * 10m room, with production being doubled during the typhoon season when other vegetables are in short supply. National statistics on the volume of seed used for sprouting or the amount of sprouts produced appear non-existent.

2.1.4 Sprouting Methodology

The components of mungbean sprout production are described in the following section. Each sprouting operation will vary in production methodology, depending on system specialisation, size, sprouter expertise, tradition and consumer preference. However, all systems rely on water, controlled temperature, darkness, high seed quality and in some cases, chemicals. (An examination of seed quality and seed selection pertaining to sprout production is presented later in this review). The general process is as follows:

2.1.4.1 Washing of Seed

Before sprouting, seed is washed in either cold water or water plus sterilant (such as sodium hypochlorite). This aims to remove dust, inert material and surface microbial contaminants (Imrie, 1991). There are no recommended ratios of water to seed or length of washing period (Ashley Berrysmith pers. comm., 1995). However, several changes of water during the washing process increase the effectiveness of the procedure (Imrie, 1991). It is unclear whether there are any legislated chemical residue tolerances for mungbean sprouts in New Zealand, and the matter is complicated by the fact that, according to standard definitions, a bean sprout may be a fruit, a vegetable or both, depending on its stage of development at consumption (Goepfert, 1980 - cited by O'Brien & Desmarchelier, 1991). There is some debate in New Zealand as to whether any chemicals should be applied during sprout production, as consumption is lead by health conscious consumers (Robert Coulson pers. comm., 1995).

2.1.4.2 Soaking

Seed is normally soaked in warm water for six to eight hours prior to sprouting. Depending on the sprouter's preference, water temperature for soaking ranges between 32-45°C (Imrie, 1991). Some sprouters use temperature controlled facilities to maintain a constant soak water temperature, while others add heated water and allow it to cool naturally during the soak period (Ashley Berrysmith pers. com, 1995). Some sprouters also use sterilents such as sodium hypochlorite during this process. Imrie (1991) cites work by Robinson *et al* (1975) which reports that the inclusion of calcium as calcium nitrate at the rate of 0.25 milli-equivalents per litre in water applied to sprouts increased sprout recovery yields by 25-33%. Most sprouters are reluctant to divulge information on chemical use, and simply say they do not use any. The truth of this is

questionable. Soaking ensures that all viable seeds are fully imbibed (unless the seed lot contains hard seed) before entering the sprouting stage (Imrie, 1991), and supposedly increases the rate of germination and uniformity of the sprouts produced. The temperature, time and method of soaking are purely a personal preference, usually based on tradition and facilities alone. Once the soaking period is completed, the water is drained off, and the seed is placed in sprouting cabins (described below).

2.1.4.3 Sprouting

Commercially produced sprouts are usually grown in 1-2m deep cabins (1-2m³), with a perforated floor for drainage. Cabins are placed in sprouting rooms, which should be well sealed to maintain high relative humidity and prevent light entering (Ashley Berrysmith pers. comm., 1995). Smaller sprouters may use factory-produced sprouting cabinets or other 'home-built' sprouters (Imrie, 1991; Robert Coulson pers. comm., 1996). The sprouts must be watered regularly, at intervals of three to eight hours, until they reach marketable size. Sophisticated systems have traveling irrigators connected to electronic timers and temperature regulators. The form (width and length) of sprouts produced is also influenced by the physical pressure endured during growth. Increasing pressure (by increasing the amount of seed used or by adding a weight on top of the sprouting seeds) induces higher rates of ethylene production by the

sprouting seeds, resulting in plumper and crisper sprouts with short roots (Chang, 1978). For this reason, commercial sprouters use large sprouting cabins, capable of containing a large amount of sprouts.

The temperature of the irrigation water again varies between sprouters. Chang

(1978) reported that at a water temperature of 20°C, hypocotyl length reached 2.5cm in five days and 7.5cm in eight days, while the corresponding lengths at 25°C were 8.7cm and 16.8cm. Paul (1989) found that mungbean sprout growth was significantly accelerated at 30°C compared to 20°C, but the latter temperature induced secondary root development, therefore producing sprouts which were disliked by consumers. Imrie (1991) noted that under commercial conditions in Australia, sprouting is usually at 24-25°C, which produces acceptable sprouts in four days, whereas in Japan, sprouts are produced at 18-20°C and the sprouting duration is five to seven days. In England higher sprouting temperatures, ranging from 27-30°C are used (Imrie, 1991). New Zealand sprouters tend to use sprouting temperatures between 20-22°C, and take five to six days to produce marketable sprouts, (Ashley Berrysmith pers. comm., 1995). One pitfall in most publications on sprouting is the absence of classification of sprouting temperature. It is difficult to tell whether reports mean 1) temperature of water applied, or 2) temperature of the outer chamber (room), or 3) temperature within the sprouting cabin. It is important to know which temperature is meant, as the respiration of the sprouting seeds increases the cabin temperature an average of 2-3°C above the temperature of the irrigation water, (Bruce Imrie pers. comm., 1997).

Regular watering is important for several reasons. Heat is produced by the growing sprouts, encouraging an environment suitable for many micro-organisms. In conjunction with this, sprouts exude waste products during growth, again encouraging microbial activity. Regular, heavy watering flushes the sprouts, removing both heat and waste exudate, and hence reducing sprout spoilage. Of course, water is also required for sprout growth. There are few data available on the effects of watering procedures. Laboratory work conducted by Imrie (1991)

showed that sprout yield increased with increasing frequency of watering in trials conducted at temperatures in the range of 20-22°C, (details of trial unpublished). There are no data pertaining to the amount of water to be applied, either in total or per irrigation.

2.1.4.4 Harvesting and Packing

In most countries, sprouts of at least 5cm in length are the standard product. The time taken for a batch of sprouts to reach harvestable length will depend on the temperature of the water applied and the ability of the sprouting room to maintain the desired temperature and relative humidity. The higher the temperature, the shorter the growing period required.

When ready, sprouts are emptied from the sprouting cabins into a large water trough filled with clean water. Sterilents may again be used at this stage of production, in an attempt to lengthen shelf life. Some sprouters simply tip cabins straight into the troughs, while others load sprouts onto a conveyor belt (with a rough surface), which helps to remove unwanted seed coats. Sprouts are gently turned by hand to remove seed coats, and are then transferred to agitators or wringers, which remove excess water and much of the roots. Sprouts are then hand packed into plastic bags for distribution and sale. Packages should be refrigerated as soon as possible to stop further growth of both sprouts and spoilage organisms (Imrie, 1991; Ashley Berrysmith pers. comm., 1995).

2.1.5 Sprout Quality

Sprout quality is inevitably determined by the consumer. Variation occurs between countries, and indeed among people within the same country. For

simplicity, sprouts can be divided into two categories: (i) consumer acceptable sprouts (CAS), and (ii) consumer unacceptable sprouts (CUS). CAS sprouts in New Zealand, Australia, USA and the UK should ideally have a hypocotyl which is 50mm in length and 3mm in width, as well as being white, crisp and with a solitary short root (Imrie, 1991; Law & Law, 1991; Ashley Berrysmith pers. comm., 1995). Paul (1989) did a limited investigation on the effect of temperature, pressure and light on sprout taste and consumer acceptability of black gram sprouts, but there is no information regarding where the measurement requirements originated from. Another sprout product sold in New Zealand using mungbeans is 'baby-mung', where freshly germinated sprouts are marketed in a sprout 'combo'. Sprouts must have a radicle of 15mm or less (Ashley Berrysmith pers. comm., 1996).

Recently, sprouters in New Zealand and overseas have complained of variation in sprout uniformity within and between batches of sprouts (Robert Coulson pers. comm., 1995). Commercial sprouters ideally want high yields of sprouts which are uniform in size. Factors which determine uniformity are reported to be: (a) seed quality, (b) temperature of the sprouting chamber and cabin, (c) amount of pressure induced during sprouting, and (d) amount, temperature and frequency of water application during sprouting (Chang, 1978; Ahmad & Mohamed, 1987; Imrie, 1991, Law & Law, 1991; Ashley Berrysmith pers. comm., 1995). Information on the relationships between these input variables and sprout quality is sparse (Law & Law, 1991), and what little is available is often personal opinion, rather than researched information.

2.2 SEED QUALITY

The term seed quality has a number of meanings which are of varying importance to different sectors of the seed industry. Thompson (1979) defined seed quality as a combination of parameters; analytical purity, freedom from weeds, species purity, cultivar purity, seed size, seed lot homogeneity, germination capacity, vigour status, health status, and seed moisture content. Coolbear and Hill (1987) preferred to use just three categories to describe seed quality; (1) accurate description - species, cultivar, percentage of foreign and inert material, and seed weight, (2) hygiene - percentage of noxious weeds, microfloral contamination, bacterial and viral contamination, and pest infestation, and (3) viability and potential performance - percentage of normal seedlings, expected field emergence (and uniformity), seed vigour, and potential storability.

2.2.1 Factors That Affect Seed Quality

Seed quality is determined by two parameters - genetic as determined by cultivar, and environmental as determined by conditions under which the seed is produced (Copeland & McDonald, 1985). Seed quality can be influenced at any stage of the growing, processing and distribution of a crop. High quality seed is undamaged, has a high level of germination, and will produce uniform, vigorous seedlings without defects under various environmental conditions (Dickson, 1980).

2.2.1.1 Genetic Effects

Variation in seed quality occurs among species and among cultivars of the same species, due to genetic effects. Seeds of some cultivars have the ability to germinate and grow under what would normally be classed as marginal conditions (Delouche, 1992). A wide range of cultivars are often available within each country, each having been bred or selected to incorporate desirable production features, and often for use in specific environments. The success or failure of a newly introduced crop is dependent on the availability of well adapted cultivars (Imrie, Lawn & Yeates, 1991). Cultivar purity within a seed lot helps maintain uniformity of seed quality but unfortunately many commercial seed lots of green and black gram lack cultivar purity (Law & Law, 1991).

Mungbean breeding programs in Australia have resulted in the release of eight cultivars since 1969. The green gram cultivar which has formed the basis of an expanding industry producing beans for sprouting is cv. Berken. The favourable features of cv. Berken which led to its release were its large seed size, early maturity, synchrony of maturity, shattering resistance, and lack of hardseededness. However, cv. Berken is very susceptible to weather damage just prior to harvesting (Imrie, Lawn & Yeates, 1991). In 1975 an Australian black gram cultivar Regur was released, in recognition of the market potential in Japan and south-east Asia. Compared with green grams, cv. Regur is less prone to shattering and insect damage, produces higher yields and is more likely to produce a seed of potentially high sprouting quality when weather conditions are adverse during development. The main disadvantage of cv. Regur is its indeterminate growth habit which can cause harvest timing difficulties (Lawn *et al*, 1978 - cited by Imrie, Lawn & Yeates, 1991). The major objective, common to all production areas in present Australian breeding programs, is resistance to

weather damage (Imrie, Lawn & Yeates, 1991).

TABLE 1: Characteristics of green gram and black gram cultivars developed and released in Australia up until 1991.

SPECIES	CULTIVAR	SEED TRAITS
Green gram	Berken	green, shiny, oblong, TSW 60-70g
Green gram	Celera	green, shiny, spherical, TSW 30g
Green gram	King	green, shiny, oblong, TSW 65-75g
Green gram	Shantung	green, shiny, oblong, TSW 60-70g
Green gram	Emerald	green, shiny, oblong, TSW 65-75g
Green gram	Satin	green, dull, oblong, TSW 55-65g
Black gram	Regur	black, dull, cylindrical, TSW 55-65g

(Adapted from *New Crops - Agronomy and Potential of Alternative Crop Species* (1991), pp98).

The Asian Vegetable Research and Development Centre (AVRDC) in Thailand has the world's largest mungbean germplasm base. The AVRDC's breeding program over 15 years: increased the yield potential of mungbean to about 2.7t/ha from a low of 0.3-1.0t/ha; developed resistance to *Cercospora* leaf spot and powdery mildew; bred new lines which were less photoperiod sensitive, resulting in synchronised maturity; improved plant-type with pods above the canopy; and improved tolerance to lodging and pod shattering (Fernandez & Shanmugasundaram, 1987; Lin & Cheng, 1987; Tickoo *et al*, 1987). All of this work has ultimately resulted in higher yields of higher quality seed.

Dormancy provides a natural protection against seed deterioration, but breeders have often selected against dormancy because it is a production constraint in annual crops. Hard seed in legumes, a specific form of dormancy, is partially genetically controlled, although environmental conditions during ripening are also important (Dickson, 1980). Visible seed defects such as transverse cotyledon cracking occur widely in legumes. Rapid imbibition by very dry seed induces this rupture, and cultivars with a seed coat which allows only slow imbibition are less susceptible. Coloured seeds of many crops have been shown to be more resistant than white seed to fungi causing root and seed rots (Dickson, 1980).

Several diseases can adversely affect the growth, productivity and subsequent grain quality of any one crop, with particular diseases more prevalent in some locations and some seasons (Imrie, Lawn & Yeates, 1991). Resistance has been found to certain diseases in certain cultivars of many species. As an example, Australian green gram cultivars Satin and Shangtung show resistance to *Cercospora* leaf spot caused by the fungus *Cercospora canescens* (Imrie, Lawn & Yeates, 1991).

2.2.1.2 Environmental Effects

Plants have evolved a remarkable capacity to adjust seed production to the resources available. The typical response of plants to low soil fertility and/or chronic moisture stress is a reduction in the quantity of seed produced rather than in their quality. The fewer seeds produced under marginal conditions are usually as viable and vigorous as seeds produced under more favourable conditions (Delouche, 1980).

Mineral deficiencies in seed are rare, but they do occur. Cox and Reid (1964) found cotyledon discolouration and plumule discolouration in peanuts to be associated with boron and calcium deficiencies respectively. Fox and Albrecht (1957) cited by Delouche (1980) associated rapid germination and vigorous seedlings with high protein content in samples of wheat. This high protein content was in turn associated with increased levels of nitrogen fertilisation. Calcium deficiency results in hypocotyl collapse in beans. In sprouted beans, this occurs just below the cotyledon hook. Tissues become grey and translucent, followed by desiccation and shrinking. Hypocotyl collapse not only ruins affected sprouts, but the necrotic tissue allows the build-up of fungal and bacterial pathogens (Wick, 1991).

2.2.1.3 Seed Maturity

Seed development can be separated into three phases: (1) the growth stage - characterised by a rapid increase in both the fresh and dry weight, (2) the food reserve accumulation stage - rate of fresh weight increase may begin to slow down, increase in dry weight is effectively linear, seed moisture is constant or begins to fall, (3) the ripening stage - seed dry weight is now constant, but fresh weight and moisture content both fall rapidly as the seed tissues dehydrate. Seed is physiologically mature (PM) when it attains maximum dry weight (Harrington, 1972; Shaw & Loomis, 1950 - cited by Ellis & Filho, 1992) while still attached to the parent plant. It is generally assumed that at PM seeds reach maximum viability and vigour, and that any seed deterioration which occurs in the field commences from this point. More recent work by Ellis & Filho (1992), however, shows that this is not necessarily the case, with maximum viability and vigour occurring several days after PM in some species.

Not all seeds on a plant mature at the same time, especially if the parent plant has an indeterminate flowering habit. Thus, a harvested seed lot may contain seeds at different maturity stages. If seed is harvested too early, the percentage of immature seeds increases, resulting in an increase in abnormal seedlings and a reduction in germination and seed vigour (Singkanipa, 1996). Immature seed has a high moisture content, and mechanical harvesting of immature seed often results in bruising damage, while harvesting of mature but over-dry seed often results in cracking and loss of membrane integrity. Both types of damage result in a reduction in seed quality. Egli & TeKrony (1993) reported that immature soybean seeds need to take up more water before they can successfully germinate. Green seeds of mungbean which have not finished filling at harvest will wrinkle and discolour during drying (Lucy & Agnew, 1994).

2.2.1.4 Weathering Damage

Weathering damage is a general term used to describe the deleterious physical and chemical changes that can occur to seed following its exposure to rainfall and high humidity during ripening and prior to harvest (Imrie, Lawn & Yeates, 1991). Symptoms in mungbeans include seed testa discolouration, wrinkling, cracking of the testa, and loss of seed germination and vigour (Williams *et al*, 1995a). Weathering damage in mungbean develops when seeds are exposed to cyclic wetting and drying with the wetting phase being of sufficient duration to cause seeds to imbibe and/or germinate before subsequent drying. Exposure to even one cycle of weathering can also advance the timing and degree of seed damage during subsequent cycles (Williams *et al*, 1995a). Weathering damaged seed is more prone to fungal and bacterial infection (Imrie, Lawn & Yeates, 1991).

Prevention of weathering damage is particularly important in mungbean where the aim is to produce seed for sprout production, since the loss of seed quality due to weathering can render seed unsuitable for this purpose. There are two basic mechanisms by which crops can be protected from weathering damage; avoidance of unfavourable conditions, or resistance to those conditions (Imrie, Lawn & Yeates, 1991). Cultivars differ in their resistance to weathering, but selection for resistance based on field response has proven unsuccessful in Australia (Williams *et al*, 1995c). Further research has found that the degree of weathering damage is directly related to the level of hardseededness and pod wall thickness (Bruce Imrie pers. comm., 1996). Therefore breeding mungbean lines which are resistant to weathering, to help meet quality specification for sprouting, may, in fact, result in the development of cultivars which are then unacceptable to the sprouting industry on the basis of high hard seed content.

2.2.1.5 Mechanical Damage

Seed processing usually involves harvesting, drying and cleaning - all of which can cause mechanical damage - a common factor responsible for reducing seed quality (Singkanipa, 1996). Mechanical damage reduces membrane integrity and provides conditions that substantially favour infection by fungi, resulting in accelerated deterioration of seed quality (Neegaard, 1979).

Harvesting crops with a high level of wet green material will lead to staining and downgrading of mungbean seeds. Increased seed moisture content, which can lead to mould formation and quality reduction in storage, may also result when substantial amounts of green material from pods and stems of mungbean plants are present in the bulk seed (Walsh, 1991). In Australia the current recommendation is to commence harvesting when 85-95% of pods are black.

This should coincide with seed moisture contents below 12% (Easdown, 1989; Lucy & Agnew, 1994). Desiccation using chemicals may be required if a lot of green material is present just prior to harvest. Mungbeans are easily damaged during harvesting, with the risk of damage increasing significantly as the seed dries out. Two types of mechanical damage can occur: (a) severely damaged seed is usually split or badly chipped - these seeds may comprise between 5-20% of a harvested crop and must be (and can be) removed successfully during cleaning, (b) hairline cracks in the testa, which are hard to detect; seeds damaged in this way are not able to be removed by cleaning, resulting in a high percentage of oversoaks and downgrading (Lucy & Agnew, 1994). Rotary combine harvesters reduce seed cracking at similar drum and rotor peripheral speeds and levels of seed moisture content compared to conventional combines in soybeans (Newberry *et al*, 1980 - cited by Walsh, 1991). Rotary combines have been shown to produced 30% less splits than conventional combines in a Queensland Department of Primary Industries survey (Lucy & Agnew, 1994).

Bean ladders or belt conveyors, as opposed to augers, are recommended when transferring mungbeans from one site to another, (Philip Smith, Pitsworth & Bruce Imrie pers. comm., 1996). Said (1991) found that free-fall dropping of pea seed increased the percentage of bruised, split and broken seed, with the amount of damage closely correlated to seed moisture content. Mungbean seed crops seldom require artificial drying after harvesting and before storage, reducing the damage often incurred during this process. Aeration is recommended however, which redistributes moisture and cools down seed. This helps to reduce condensation and cracking, slow down mould development, prevents the development of 'hot-spots', and allows damp beans to be stored longer (GRDC News, 1995). The ideal seed moisture content for mungbean transport and

grading is 13%, while the maximum moisture content for storage is 12% (Lucy & Agnew, 1994).

Cleaning and grading usually involve both screen and gravity grading, seed sizing, and bagging. Mungbean seed produced in Australia which is destined for sprouting and exporting can only be cleaned and graded at an Australian Mungbean Association registered packing shed (Lucy & Agnew, 1994; Philip Smith pers. comm., 1996). Clean equipment is important when harvesting seed destined for sprouting, as this seed can receive little treatment (chemically) to prevent diseases caused by contaminants from harvesting through to eating (Agnote DPI/133, 1995).

2.2.1.6 Fungal Infection

Fungi that attack seeds can be grouped into one of two classes; field fungi, or storage fungi, based on their ecological requirements. For field fungi to infest and cause damage to seeds, the moisture content of the seed must be in equilibrium with relative humidities above 95%. Storage fungi are those fungi which grow in seeds when moisture contents are in equilibrium with relative humidities of 70-80%; and which have the ability to grow without free water (Christensen, 1965; Agarwal & Sinclair, 1987).

Common genera of field fungi which affect many plant species include:

Alternaria, *Fusarium* and *Drechslera* spp. (Agarwal & Sinclair, 1987).

Macrophomina phaseolina (Tassi) Goidanich (charcoal rot), *Cercospora cruenta* L. (cercospora leaf spot), *Sphaerotheca fuliginea* L. (powdery mildew), *Sclerotium rolfsii* L. (sclerotium leaf spot), *Pythium aphanidermatum* L. (damping-off), *Rhizoctonia solani* Kuhn (root rot/collar rot), *Rhizopus stolonifera* (Ehrenb:Fr) Vuill

(clump rot) and *Choanephora cucurbitarum* L. (choanephora blight) are also listed as common field pathogens of mungbean seed (Ilag, 1978; Quebral, 1978; Yang, 1978; Conde & Diatloff, 1991; Wick, 1991).

Macrophomina phaseolina (charcoal rot) is particularly important, as it causes damping off and stem rot both in the field and during sprouting. Charcoal rot appears as a firm dark brown to black lesion on the hypocotyl or roots of sprouts and by harvest, the entire sprout may be blackened and shrivelled. *Rhizopus stolonifera* rot of mungbean sprouts is commonly called 'clump rot', because during harvest, clumps of sprouts 5-10cm in diameter occur. The fungus produces tough mycelium, which effectively knits infected sprouts together. While *Rhizopus* is probably always present during sprout production, sprout infection will not occur except under favourable environmental conditions (Wick, 1991).

Common storage fungi that infect many species are those of the *Aspergillus* and *Penicillium* genera. Species of the former include *A. niger* Tiegh, *A. glaucus* Link:Fr, *A. candidus* Link., *A. ochraceus* K. Wilh and *A. flavus* Link:Fr. These fungi are relatively distinct from one another and therefore easy to identify, but the several species of *Penicillium* commonly found require specialists identification, and for this reason are often listed as *Penicillium* spp. (Christensen, 1974). Invasion by storage fungi can affect seed quality in a number of ways. The sequence of deterioration tends to progress from: decreased germinability, to discolouration of the embryo or entire seed, to production of mycotoxins, to heating, the development of mustiness and caking, and finally total decay (Christensen & Kaufman, 1969).

Inoculum can be considered to be any material capable of producing infection (Maude, 1996). Conidia and other resting spore structures of fungi are present on equipment used in the seed industry, such as bags, drills, combine harvesters, and seed cleaning equipment. Seeds can be invaded at any stage during development, harvesting, transporting, cleaning and storage (Agarwal & Sinclair, 1987). The likelihood of fungi successfully invading, developing and growing into pathogenic colonies on and in seeds is dependent on a number of factors: seed moisture content, temperature, degree of damage of seed, degree of present fungal infection, length of storage, and contamination of non-seed material (Christensen, 1974; Roberts, 1974; Agarwal & Sinclair, 1987; Agarwal, 1988). Seed does not have to be physically injured for fungal attack to be successful. Most storage fungi are ubiquitous in nature, but become parasitic when environmental conditions become favourable for their development and the seed itself provides the substrate for these pathogens to survive (Murray Hill, pers. com. 1996).

Mycotoxins are toxic secondary metabolites of fungi, some of which may cause pathological or undesirable physiological responses in humans and other animals (Christensen & Kaufman, 1974; Mirocha *et al*, 1985). Mycotoxicosis is the name given to diseases caused by the ingestion of foods or feeds which have been contaminated by mycotoxins (Goto, 1990). Brooks and White (1966) cited by Mirocha *et al* (1985) listed 96 species of fungi in 27 genera which, at that time, were known or suspected to cause mycotoxicosis. Of these fungi, 57% are in the genera *Aspergillus*, *Fusarium* and *Penicillium*. Aflatoxin contamination of grains occurs both in the field and after harvest. Drought has been shown to increase aflatoxin contamination in maize and peanuts (Christensen & Kaufman, 1974). Mycotoxins are a serious human health issue related to food products of plant

origin. So far there have been no confirmed reports of mycotoxin contamination of bean sprouts, although few investigations have been conducted.

2.2.1.7 Insect Damage

Insect attack in the field can be a serious problem, both for the parent plant and subsequent seed. Insects often have a major impact on seed quality as well as yield and can affect the overall profitability of a crop (Daglish, 1991; Ingram, 1991). The type of damage inflicted by insect pests on seeds is dependent on their feeding nature. Under Australian growing conditions, mungbean seed production and quality is jeopardised by at least six different insects: budworms (*Helicoverpa armigera* and *H. punctigera*), green vegetable bug (*Nezara viridula*), bean pod-borer (*Maruca testulalis*), thrips (*Megalurothrips* spp.), vegetable jassid (*Austroasca viridigrisea*), and mirids (*Creontiades* spp.). In other countries the bruchid beetle (*Callosobruchus phaseoli* and *C. maculatus*) can be serious storage insect pests (Daglish, 1991; Ingram, 1991; Lucy, 1994).

Budworms and bean pod-borers both attack the developing pod, their entrance hole allowing water from rainfall to enter the pod and surround developing seeds. This induces weathering and seed discolouration, and downgrading of seed lots (Ingram, 1991; Lucy, 1994). Those species which feed by piercing plant tissues and sucking sap eg. green vegetable bug, are most likely to directly cause seed quality loss. When they attack developing seeds, the young seeds may be aborted, partly grown seeds may shrivel, and fully expanded seeds may show discolouration (Ingram, 1991).

2.2.2 Seed Viability

Viability in the context of seed development occurs when a seed is capable of an independent existence separate from the parent plant. Normally, seeds become viable during stage two of development, although the ripening stage (stage three) also seems to be necessary in many seeds to 'switch-off' the processes of seed development and prepare the seed for germination. When discussing seed viability in terms of seed quality, viability and germination often become synonymous (McDonald, 1980). However, many legumes require specific conditions for germination, and dormancy (hardseededness) may prevent viable seeds from germinating at any particular time (Karen Hill, Palmerston North pers. comm., 1995). Seed viability is a major component of any assessment of seed quality, and germination testing remains the principle, and internationally accepted, criterion for seed viability (Perry, 1987; ISTA, 1995).

2.2.3 Seed Vigour

Seed vigour is 'the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. Seeds which perform well are termed high vigour seeds, and those which perform poorly are called low vigour seeds'. Seed vigour is not a single measurable property like germination, rather it is a combination of parameters (ISTA, 1981). In general, vigour at the individual seed level means one seed producing one normal seedling which will produce a plant of marketable quality. In this respect, vigour is a function of the rate of seedling emergence, of plant growth, and of development in relation to the maximum rate for that cultivar. Vigour at the level of a seed population may be expressed as rapid, uniform, and

high germination or field emergence. A vigorous seed lot continues to display these three properties as planting conditions deviate from ideal to stressful (Abdul-Baki, 1980).

Seed vigour can be considered a reciprocal of the deteriorative processes involved with seed ageing, both pre- and post-harvest, in that vigour decreases as the level of deterioration increases (Powell, 1988). The consequences of seed deterioration (physiological ageing) are a progressive reduction in germination rate, lowered germination, and consequently, increases in abnormal seedlings and dead seeds (Hampton & Hill, 1990). While the degree of tolerance or adaptability of germinating seed to environmental extremes is genetically predetermined, many seeds fall short of their maximum genetic potential. In this respect, seeds may fit into one of three broad categories: (1) those that never attain high vigour, (2) those that attain and maintain high vigour, or (3) those that attain high vigour, and then lose it partly or totally (Abdul-Baki, 1980). In any seed lot, losses of seed vigour are related to a reduction in the ability of seeds to carry out all the physiological functions that allow them to perform. The end point of this deterioration is ultimately death of the seed (Powell, Matthews & Oliveira, 1984; ISTA, 1995). Seed ageing has become recognised as the major cause of reduced vigour and viability (Ellis & Roberts, 1980 - cited by ISTA, 1995; Powell, 1988).

ISTA (1981) lists the following factors, which are considered to be associated with seed vigour. All are linked to the physiological status of the seed lot in question:

- (1) genetic constitution
- (2) environment and nutrition of the parent plant
- (3) stage of maturity at harvest

- (4) seed size, weight or specific gravity
- (5) mechanical integrity
- (6) deterioration and ageing

2.3 SEED QUALITY TESTING

The assessment of seed quality continues to attract increasing attention from seed and related industries. Farmers believe that seed quality information will enable them to make economic decisions regarding the cost of seeds, earliness of planting, quantity of seeds to plant and the anticipated uniformity of stand (McDonald, 1980). Sprout producers also feel that assessment of seed quality is important, for similar reasons to farmers (Ashley Berrysmith pers. comm., 1995). One of the greatest hazards in agriculture is sowing seed that does not have the capacity to produce an abundant crop. Seed testing has been developed to minimise this risk, by assessing the quality of seed before it is sown. Seed is a living biological product and its behaviour cannot be predicted with the certainty that characterises the testing of inert or non-biological material. The methods used must be based on scientific knowledge of seed and on the accumulated experience of seed analysts (ISTA, 1996). The following section reviews present pre-requisites for seed quality in the sprouting industry, and quantitative tests that were conducted in this research project.

2.3.1 Present Pre-requisites for Sprouting Seed

Three of the worlds more advanced sprouting countries (Australia, Japan and the United States of America) have clear pre-requisites with respect to seed quality,

when purchasing green and black gram seeds for sprouting. Sprouting markets in Australia and USA are dominated by green gram, while Japan prefers black gram (Cupka & Edwards, 1987; Lawn, Chay & Imrie, 1987; Imrie, 1990; Law & Law, 1991; Lawn & Imrie, 1994). Law & law (1991) presented the recommended minimum seed quality requirements of Australia, the USA and Japan, which are presented in Tables 2, 3 and 4 respectively. The Australian Mungbean Association (undated) also released the minimum seed quality standards for the three grades of mungbean seed they market. This information is presented in Appendix 5.

TABLE 2: The Australian Mungbean Association Standards for Premium Grade mungbeans (this includes both green and black gram).

Purity (ISTA)	99% minimum
Germination (ISTA)	94% minimum
Moisture (ISTA)	12% maximum
Oversoak AMA	5% maximum
Appearance	Sample must pass a standard comparative test.

The beans must be free of live insects excreta, mould, odours, noxious weeds and soil.

(Sourced from Law and Law, 1991).

TABLE 3: Seed quality requirements for black gram seed destined for the Japanese sprouting market.

Acceptability is based on the following tests of seed and sprouts with the sprouts having been produced using a 'Green Pony' Sprouter.

Admixture	1% maximum	
Seed size	5 - 6 g per 100 seeds (do not pass through 3.71 mm screen)	
Appearance	Damaged seeds	1% maximum
	Immature seeds	1% maximum
	Broken seeds	1% maximum
	Insect damaged seed	1% maximum
	Brown seed	1%
	Other seeds	1% maximum
	Total of all categories	1% maximum
Sprouting performance	<p>Minimum accepted standards are not known. Samples are compared on the basis of their germination percentage and their germination 'power', a vigour measurement.</p> <p>Method: 100 g seed is immersed in water at 45°C for 8 hours, then drained and let stand for 16 hours. Seed is transferred to the 'Green Pony' sprouter and after 55 hours classified as</p> <p>(a) germinated, hypocotyl >1 cm (b) germinated, hypocotyl <1 cm (c) seed not imbibed (d) seed imbibed but inviable.</p>	
Germination	$\frac{a + b + c}{\text{total}} \cdot 100$	
Germination power	$\frac{a + c}{\text{total}} \cdot 100$	

(The source of this information does not indicate whether the standards are for green gram solely, or for both green gram and black gram. Sourced from Law and Law, 1991).

TABLE 4: The International Sprout Growers Association (USA) Standards for No. 1 Grade sprouting mungbeans.

Germination	95% (including hard seeds) minimum. AOSA method.
Purity	99.5% minimum
Freshweight yield	8 lbs of sprouts per 1 lb of seed (5 days to yield at 27°C using AOSA germination test method)
Healthy sprouts	95% by weight minimum
Seed moisture content	12% by weight maximum
Oversoaks	5% by count maximum (1 hr at 32°C)
Colour	Uniform
Shelf life	Minimum 5 days of storage at 5°C
Seed size	Minimum 5 g per 100 seeds

(Sourced from Law and Law, 1991).

It is interesting to note the range of tests requested, many of which are not internationally standardised, and the precision quoted for many of these tests. There is no information or data available on the research and development of the industry based oversoaks test, or the requirements of a 'healthy' sprout versus an 'unhealthy' sprout. Sprouters usually admit a poor understanding of the relationships between sprout attributes and seed quality characteristics. Further problems arise when different end-users emphasise different quality attributes and when sprouting quality cannot be related directly to seed quality descriptors

(Law & Law, 1991). Commercial sprouters are often ill placed to define the exact cause of poor performance, with the 'cause and effect' syndrome often misinterpreted.

The seed quality tests listed for assessing mungbeans destined for sprouting fall into one of two categories: (a) Internationally standardised, ie. germination, purity, thousand seed weight, seed moisture content, seed health, or (b) Industry developed, ie. oversoaks, seed size range, fresh weight yield, healthy sprout yield, shelf life and appearance. There has been little work on vigour tests and their relationship with sprout quality.

2.3.2 Standardised Seed Quality Assessment

2.3.2.1 Germination

The objective of the germination test is to determine the maximum germination potential of a seed lot, which can then be used to compare the quality of different lots and also to estimate field planting value. Testing under field conditions is normally unsatisfactory, as the results cannot be repeated with reliability.

Laboratory methods have been developed in which external conditions are controlled to give the most regular, rapid and complete germination for most species. Germination of a seed in a laboratory test is the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil (ISTA, 1996). The procedure and definitions of seedling classification are listed in the International Rules for Seed Testing (ISTA, 1996).

The ISTA (1996) germination method is widely accepted by the sprouting industry as a primary test of seed quality for sprouting legume seed. However, in the USA, the International Sprout Growers Association (ISGA) has proposed modifications to the method, to include measurements of sprout quality such as 'uniformity of sprouts', 'yield ratio', 'sprout quality' and 'shelf life' (Law & Law, 1991). These changes have not yet been standardised, but are commonly quoted on USA seed trading certificates. In most cases, the environmental conditions under which seeds are sprouted are conducive to optimal germination and seedling growth. For this reason the germination test has a number of worthwhile attributes when assessing seed lots for sprouting (Ashley Berrysmith pers. comm., 1996).

2.3.2.2 Seed Lot Purity

Analytical purity is a basic component of seed quality. It indicates how much of the material in the seed lot submitted is intact seed of the species named on the label (Thompson, 1979). The purity test is therefore one of the most important tests to assess physical quality of a seed lot (Sakunnarak, 1985). ISTA (1996) describes the object of the purity test as being to determine (a) the percentage composition by weight of the sample being tested and by inference the composition of the seed lot, and (b) the identity of the various species of seeds and inert matter present in the sample.

In general, the separation of sample components is based on an examination of each particle in the sample, and is made in such a way as to ensure the capacity for germination is not impaired (Sakunnarak, 1985). The ISTA (1996) method of purity analysis is commonly used in assessing mungbean seed lots for sprouting, with 99% pure seed generally being regarded as the accepted minimum standard

(Law & Law, 1991). This methodology has some limitations with respect to the sprouting industry. For example, broken seeds greater than half average seed size, as well as mouldy and shriveled seeds are regarded as pure seed.

Obviously these are of no value to the sprouter (Law & Law, 1991), and can even encourage rapid deterioration of sprouts - as broken seeds often have high microbial loading and readily leak electrolytes, helping microbes to establish rapidly.

2.3.2.3 Seed Moisture Content

From the moment a seed reaches physiological maturity, until it is resown/sprouted, seed deterioration can occur. Seed moisture content (SMC) is a major factor influencing seed longevity (Harrington, 1972) which is influenced by two main environmental factors, ambient temperature and relative humidity. Water in seeds is present in various states, from free water to chemically bound water (Harrington, 1972). The principle of the laboratory SMC test is that all the free water is removed, while preventing oxidation, decomposition and the loss of other volatile substances (Thompson, 1979; ISTA, 1996). For safe harvesting, handling, grading, transporting and storing of mungbean seed, a SMC of 10-13% should be maintained (Lucy & Agnew, 1994; Agnote DPI/133, 1995; Mary-Ann Law, Toowoomba pers. comm., 1996).

2.3.2.4 Thousand Seed Weight

Thousand seed weight (TSW) is the standard descriptor of seed size (Thompson, 1979; ISTA, 1996). Seed size may affect germination, emergence, seedling vigour, seedling growth rate within cultivars, species and seed samples, and has often been used as a parameter of seed quality. Results have often been

conflicting however (Black, 1959; McKersie, Tomes & Yamamoto, 1981; Wang & Hampton, 1990; Egli, TeKrony & Wiralaga, 1990). Uniformity of seed size rather than size *per se* appears to be the major quality criterion for sprouters. The importance of uniformity could be related to the rate of water imbibition or evenness of growth, but these relationships are not well established (Law & Law, 1991). McKersie, Tomes & Yamamota (1981) reported that large seed when selected from within birdsfoot trefoil (*Lotus corniculatus* L.) lots tended to have better field emergence than the original seed lot.

2.3.2.5 Seed Health

The objective of a seed health test is to determine the health status of a seed sample, and by inference that of the seed lot. Health of seed refers primarily to the presence or absence of disease-causing organisms, such as fungi, bacteria and viruses, and animal pests, such as nematodes and insects, but physiological conditions such as trace element deficiency may also be involved (ISTA, 1996).

According to ISTA (1996), health testing of seed is important for three reasons:

- (1) Seedborne inoculum may give rise to progressive disease development in the field and reduce the commercial value of the crop.
- (2) Imported seed lots may introduce diseases into new regions. Tests to meet quarantine requirements may therefore be necessary.
- (3) Seed health testing may elucidate seedling evaluation and causes of poor germination or field establishment and thus be used to supplement germination test results.

The seedborne fungal disease commonly known as charcoal rot (caused by the pathogen *Macrophomina phaseolina*) is a major seed quality problem in mungbean sprout production (Scholefield & Griffin, 1979; Law & Law, 1991; Wick, 1991;

Ryley, 1992). Present recommendations for sprouting mungbean seed lot selection in Australia state that a 'zero' level of *Macrophomina phaseolina* only is acceptable (AMA Newsletter No. 2, undated; Law & Law, 1991; Philip Smith pers. comm., 1996). Charcoal rot is only a problem in high temperature sprouting, as used in Europe (Bruce Imrie pers. comm., 1996) where temperatures are above 25°C. However, the name charcoal rot is used by many in the sprouting industry to describe any disease present which causes sprouts to blacken in colour (Ashley Berrysmith pers. comm., 1995).

The presence of field and storage fungi gives an indication of a seed lots' history and present state of deterioration. There is a regular ecological succession of fungi in storage. Each of the species has its own rather sharply defined lower limit of SMC, below which it cannot grow (Christensen, 1974). The lower the microbial loading of a seed lot destined for sprouting the better, as the conditions under which sprouts are produced are also conducive to microbial propagation.

2.3.2.6 Topographical Tetrazolium Test

In the topographical tetrazolium test (TZ) a colourless solution of 2,3,5-triphenyl tetrazolium chloride is used as an indicator to reveal living tissue. This compound interacts with the reduction processes of the living cells, turning a non-diffusible red colour in the living cells. This makes it possible to distinguish the red-coloured living parts of the seed from the colourless dead parts (ISTA, 1996).

The object of this biochemical test is to make a quick estimate of the viability of seed samples in general, and it is often used to confirm the viability of dormant seeds after a germination test (ISTA, 1996). The TZ test can also quickly indicate if a seed lot suffers from mechanical or insect damage. McDonald (1980) lists

the TZ test as one of the most valuable tools of seed analysis. This test was originally designed as an indicator of seed vigour, but difficulties arose in standardisation of the method, due to the subjective nature of the assessment. A well trained analyst was required to accurately separate seeds into vigour categories, based on the intensity of the red stain. The TZ test also fails to detect seed treatment phytotoxicity, recent damage, injuries incurred from artificial drying, and fails to reveal seed dormancy (McDonald, 1980; Mason *et al*, 1982).

2.3.3 Seed Vigour Assessment

The major limitation of the germination test as an assessment of seed lot potential performance is its inability to detect quality differences among high germinating seed lots (Roberts, 1984). A vigour test is a reproducible laboratory method which distinguishes seeds of different vigour status, and which produces a result which should be more closely correlated with seed performance in the field under less than optimal growing conditions, than the germination test (Perry, 1987). Assessment of seed vigour is based on the physical or physiological performance of a seed lot. These performance parameters include: changes in biochemical processes; the rate and uniformity of germination and seedling growth, and; germination or emergence capability when exposed to stress conditions (Ferguson Spears, 1995). Vigour indicates seed deterioration, and one of the most important aspects of vigour, with respect to seed testing, is that the loss of vigour precedes loss of germination, (ISTA, 1981; Wang & Hampton, 1990).

Because seed vigour is not a single measurable property like germination, the events which precede loss of germination and could be associated with seed

vigour loss have been widely measured and developed into different tests, (Wang, 1989). To be internationally standardised and acceptable, a vigour test must meet certain requirements (McDonald, 1980; ISTA, 1987). A review by Hampton and Coolbear (1989) proposed the following as requirements which a successful vigour test must fulfil:

- 1) provide a more sensitive index of seed quality than the germination test
- 2) provide a consistent ranking of seed lots in terms of potential performance
- 3) be objective, rapid, simple and economically practical
- 4) be reproducible and interpretable.

The assessment of seed vigour is based on the physical or physiological performance of a seed lot. These performance parameters include: changes in biochemical processes; the rate and uniformity of germination and seedling growth, and; germination or emergence capability when exposed to stress conditions (Wang, 1989; Ferguson Spears, 1995).

To date, none of the vigour test procedures have proven sufficiently reliable and repeatable to be incorporated in the International Rules for Seed Testing (ISTA, 1996). The latest Handbook of Vigour Test Methods (ISTA, 1995) has placed two tests - Bulk Conductivity and Accelerated Ageing, into a 'recommended test' category, while all other tests remain in the 'suggested test' category. The following section details several of the developed vigour tests which may be useful in determining seed quality of sprouting legumes, in particular green and black gram.

2.3.3.1 Bulk Conductivity

The conductivity test provides a measure of electrolyte leakage from plant tissues, and was first recognised for seeds of several crop species by Hibbard and Miller (1928) - cited by Hampton (1995). The basis of the conductivity test is that poor membrane structure and leaky cells are associated with deterioration, and hence low vigour seeds (Abdul-Baki, 1980). The integrity of cell membranes and the ability to repair and reorganise themselves after damage or disruption is indirectly measured by electrolyte leakage during the conductivity test (Powell, 1988). Low vigour seeds have been shown to possess poor membrane integrity as a result of mechanical injury and storage damage (McDonald, 1980).

When seeds imbibe, cells having poor membrane integrity release cytoplasmic solutes into the imbibing medium. Those solutes with electrolytic properties carry an electrical charge which is detected by a conductivity meter (McDonald, 1980). Hampton, Johnstone and Eua-Umpon (1992b) examined the effect of SMC and conductivity testing procedures of ISTA (1987) and Loeffler *et al* (1988) on mungbean (*Vigna mungo* L.). Seed lot conductivity was increased as SMC fell below 10% and decreased as SMC rose above 14%. Soak temperature ranging from 20-25°C did not alter seed lot rankings, while soak water volume (75mL vs 250mL) had no significant effect on the variability of results. The lowest mean coefficient of variation was achieved when four replicates of 50 seeds were tested. Similar results have been obtained for soybean (Loeffler, TeKrony & Egli, 1988), red clover (Wang, 1989), garden pea (Hampton & Scott, 1982) and *Lotus* spp. (Hampton, Lungwangwa and Hill, 1994). Williams *et al* (1995b) used single seed conductivity readings in an attempt to evaluate the degree of weathering damage in green gram, for use in plant breeding selection. It was concluded that

the leachate conductivity technique can provide a reliable assay of weather damage in green gram.

The bulk conductivity test provides a rapid, objective assessment of seed vigour. It can be conducted easily in most seed testing laboratories with minimum expenditure on equipment and extra training of seed analysts (Ferguson Spears, 1995; Hampton, 1995). Eua-Umpon (1991) lists the following as being disadvantages of the conductivity test:

- (1) It is possible that one seed with severe damage or deterioration could produce a test result which does not truly represent the bulk seed sample. However, replication and tolerances will show whether the test needs to be repeated.
- (2) The test cannot distinguish between seed lots with a few very permeable seeds and other lots where most seeds are only moderately permeable (cites AOSA, 1983).
- (3) Hard seed has lower conductivity than non hard seed and a high hard seed content could give misleading results (cites Verma *et al*, 1987).
- (4) The test cannot differentiate dead seed from living seed (cites Hepburn *et al*, 1984).
- (5) The test has not always proved successful for predicting field emergence (cites Ram *et al*, 1989; Nuswantoro, 1988)

2.3.3.2 Accelerated Ageing

The accelerated ageing (AA) test exposes seeds for short periods of time to the two environmental variables which cause rapid seed deterioration; high temperature and high relative humidity (Delouche & Baskin, 1973; McDonald, 1980; TeKrony, 1995). Under these extreme conditions, it is proposed that the

decline in germination following AA is proportional to the initial physiological potential of the seed. High vigour seeds will deteriorate slowly under AA conditions and show only a small decrease in germination, while low vigour seeds will demonstrate a marked decrease in germination (TeKrony, 1995).

The AA test was first developed by Delouche (1965) to estimate the longevity of seed in storage. Subsequent tests have confirmed the accuracy of this test for such a purpose for several different species (Delouche & Baskin, 1973). Helmer (1962) cited by Baskin (1987) suggested that the AA test might have an application as a test for predicting seed performance other than storability - seed vigour. Recent work has used the AA test as an indicator of field performance under less than ideal conditions. Field emergence has been more highly correlated with AA than standard germination results for many species, such as French bean (Hampton, Johnstone & Eua-Umpon, 1992a), soybean (Perry 1981) sweet corn (Wilson & Trawatha, 1991; Wilson *et al*, 1992) and red clover (Wang, 1989). However, this trend has not held true for black gram (Hampton, Johnstone & Eua-Umpon, 1992b), who found no significant relationship between AA, controlled deterioration or standard germination and field emergence.

The AA test offers the advantages of being inexpensive, relatively easy and meaningful for predicting seed potential performance, both in the field and in storage (Wang, 1989). The major stress endured by mungbean seed under commercial sprouting conditions is pressure, which in turn increases surrounding ethylene levels (Chang, 1978; Ahmad & Mohamed, 1987). This factor, combined with the poor relationship found between AA and field performance, may suggest that the AA vigour testing technique may only be suitable for predicting the relative storability of mungbean seed.

2.3.3.3 Seedling Growth Evaluation

Seedling growth evaluation includes the 'speed of germination' and the 'rate of seedling growth'. A consequence of changes in metabolic processes is a reduction in the speed of germination and the rate of seedling growth. These alterations in the rate of growth and development of seedlings generally occur at a faster pace and prior to a reduction in the number of normal seedlings produced in a standard germination test (Ferguson Spears, 1995). Only seedlings judged to be morphologically abnormal are excluded from the germination test (Wellington, 1970 - cited by Perry, 1987; ISTA, 1996). No account is taken of the rate of germination or growth, nor of the strength or sturdiness of the seedling in making this decision, even though differences are frequently observed. A close examination of the rate of germination and seedling growth under the favourable conditions of the germination test can be used to assess seed vigour (Perry, 1987).

Rate of germination, uniformity of germination and uniformity of growth are very important in producing quality sprouts (Ashley Berrysmith pers. comm., 1995; Robert Couslon pers. comm., 1995). It appears that a test procedure that measures the rate and uniformity of germination or seedling growth may be valuable in assessing seed lots for sprouting.

2.3.3.4 Speed of Germination

The speed (or rate) of germination test is one of the oldest seed vigour tests. This test is based on the concept that more vigorous seeds germinate faster than less vigorous seeds. On a seed lot basis, individual seeds within a vigorous lot

normally germinate in a shorter time than those in a non-vigorous lot (Wang, 1989). There have been two main ways of assessing speed of germination:

- (a) First Count Method - the first count of the standard germination test is used.
- (b) Germination Rate - where seeds are placed under standard germination conditions and observed at set periods (daily or hourly), and those seeds which show radicle emergence are recorded each time.

The second of these tests appears to be more suited to green and black gram evaluation. This is because green and black gram seeds germinate relatively quickly (within 36 hours), and both rate and uniformity of germination would be more accurately assessed if counted over a shorter period than that of the interim count (Favaka, 1991), which is conducted on days 4 and 5 for black and green gram respectively (ISTA, 1996). Median germination time is determined by the T_{50} calculation, which is the time for 50% of the seeds tested to germinate. Uniformity of germination can be determined by $T_{90} - T_{10}$, the difference in time taken for 90% and 10% of the seeds tested to germinate respectively (Coolbear, 1990). Most sprouters are only interested in the interim germination count, as they think this reflects both the rate and uniformity of germination for a seed lot (Ashley Berrysmith pers. comm., 1995). It appears that most sprouters are unaware of any other methods useful for determining seedling uniformity or rate of germination.

In the past, this test has proved unreliable as a criterion of seed quality because of difficulties of standardisation and reproducibility (Verhey, 1960 - cited by Perry, 1987; McDonald, 1980). The speed at which a seed germinates may be affected by genetic variations in seed size, seed coat characteristics, and chemical

composition. It has been suggested that these tests be limited to comparisons within common genetic material (Ferguson Spears, 1995). Because of the nature of the sprouting procedure (ideal conditions and short production time), it appears that those seed lots which are disadvantaged due to genetically controlled factors in the short term of seedling development, are not suitable for sprouting, and therefore the test could be applied across all cultivars of the same species destined for sprouting. Small differences in moisture, light intensity and temperature can have a significant effect on the rate of germination, and these must be correctly standardised if results are to be reliable and repeatable (McDonald, 1980).

The length of a seedling after a specified period is the product of the time taken to germinate and subsequent rate of growth, and is more convenient to measure than a rate which requires frequent observations. Crop species which produce a single straight plumule or single roots are suitable subjects for this method of testing (Perry, 1987). Both linear measurements and dry matter accumulation can be assessed using this technique (McDonald, 1980; Perry, 1987).

The standard between paper (in paper rolls) germination method often results in slight distortions of seedlings and excessive elongation, and this effect is commonly seen in green and black gram seedlings (Karen Hill pers. comm., 1995). Growing seedlings on top of paper or in sand may be better for these species, making seedlings easier to measure. Seedling width is also a very important parameter for sprouts, with 3mm being optimal (Law & Law, 1991; Ashley Berrysmith pers. comm., 1995). The uniformity of sprouts produced is an area of concern to sprouters at present, and this test may help evaluate seed lots along these lines. The same problems are faced with the seedling growth

evaluation test as the rate of germination test. Measurement of seedlings is very time consuming and prone to inaccuracy as seedlings are not straight.

2.3.4 Industry Developed Test

2.3.4.1 Oversoaks & Sprouters

Oversoaks are the percentage of seeds fully imbibed plus the number of seeds partially imbibed with broken testas (% oversoaks) - after soaking in a water-bath (32°C) for one hour (AMA Newsletter No. 2, undated; Law & Law, 1991; Mary-Ann Law pers. comm., 1996). The oversoak test was developed in the USA, but no data appear to have been published concerning its development. In the original test the 'good' seed is returned to the water-bath for 24 hours, after which seeds are again removed, and the number of seeds which have produced a healthy radicle are recorded (% sprouters).

Law & Law (1991) reported that even though the effect of oversoaks on sprouting quality is not clearly defined, the test has found such universal acceptance that its importance cannot be disputed. Further research which provides scientific evidence of this relationship would be valuable.

CHAPTER 3

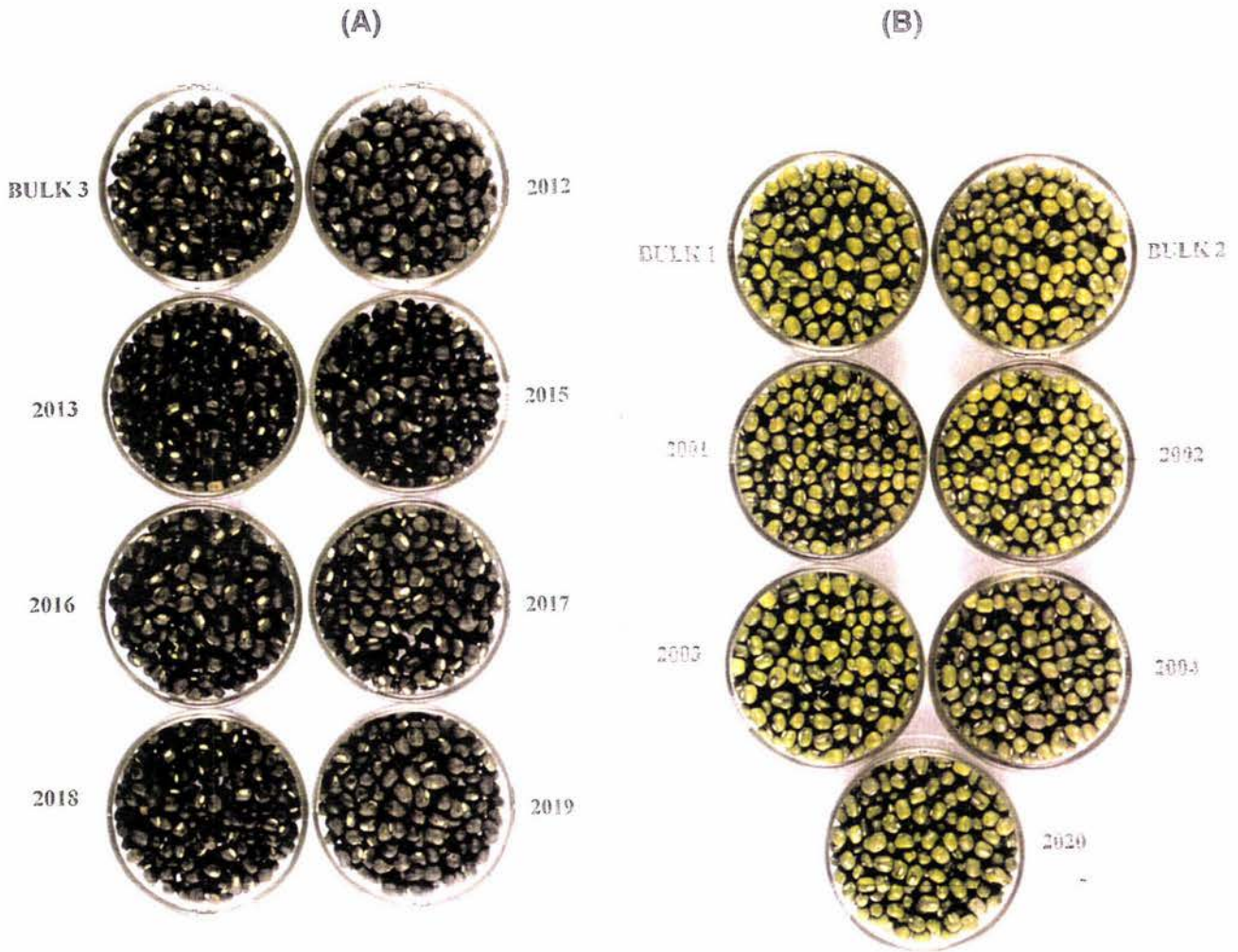
MATERIALS & METHODS

Samples of 15 mungbean seed lots were collected for seed quality analysis. Seven of them were green gram (*Vigna radiata*) and eight were black gram (*Vigna mungo*). On arrival, seed lots were weighed, numbered and recorded.

TABLE 5: Source, numbering and description (where available) of seed lots used throughout all laboratory seed quality tests and Small Scale Commercial Production (SSCP).

SAMPLE #	SEED SOURCE	OTHER INFORMATION
(Black Gram)		
BULK 3	Jamar Farming Ltd (Australia)	Harvest March 1996
2012	Sunsprouts NZ Ltd	
2013	Sunsprouts NZ Ltd	
2015	Jamar Farming Ltd (Australia)	cv. Regur Second Grade
2016	Jamar Farming Ltd (Australia)	cv. Regur
2017	Thailand Ag. Research Centre	cv. Phitsanuloke #2
2018	Thailand Ag. Research Centre	cv. Uthong #2
2019	Jamar Farming (Australia)	cv. Regur Harvested 1995
(Green Gram)		
BULK 1	Seedbank NZ Ltd	Jamar Farming Ltd, March 1996
BULK 2	Seedbank NZ Ltd	Chinese - Harvested March 1996
2001	Seedband NZ Ltd	cv. Berken
2002	Seedbank NZ Ltd	Chinese
2003	Sunsprouts NZ Ltd	
2004	Sunsprouts NZ Ltd	
2020	Selected Seeds Ltd (Australia)	cv. Emerald Harvested March 1996

PLATE 1: Photographs of seed samples from each seed lot used in this study
- (A) Black gram and (B) Green gram.



International seed testing rules and recommendations (ISTA, 1996) were adhered to as far as practical for all standard testing and vigour testing trials conducted to meet objectives two through to four (page 4) inclusive. Seed lot working samples were obtained from submitted samples after mixing and repeated halving using a Boerner centrifugal divider (ISTA, 1996). Between tests, seed samples were stored in paper bags inside large plastic bags in sealed plastic buckets at 3-5°C.

The following briefly describes the testing procedures and any variations to standard testing methodology used.

3.1 STANDARD SEED QUALITY TESTS

3.1.1 Seed Moisture Content (SMC)

Two 4-5g replicates of each seed lot were weighed, ground, placed in aluminum containers, reweighed and dried using the high temperature oven method (ISTA, 1996). ie. 130°C for 1 hour. After removal from the oven seeds were cooled in a desiccater for approximately 15 minutes and then reweighed. Seed moisture content was calculated on a wet weight basis and expressed as a percentage (ISTA, 1996).

3.1.2 Purity

Working samples of 120g for green gram and 700g for black gram (ISTA, 1996) were obtained using a centrifugal divider. Following the ISTA rules for the species, samples were divided into: pure seed, other seed, and inert matter fractions. Seeds which were less than half normal seed size, seeds with no testa, and seeds with separated cotyledons were classified as inert matter, along with pieces of seed, dirt, stones and chaffy material. Each fraction was weighed, and expressed as a percentage to one decimal place (ISTA, 1996). Any other seeds present and inert matter were identified and recorded.

3.1.3 Thousand Seed Weight (TSW)

Eight replicates of 100 seeds were counted from the pure seed fraction obtained during the purity test. Each replicate was weighed to three decimal places and

recorded. The average, variance, standard deviation and coefficient of variation were calculated. Thousand seed weight was calculated by multiplying the average 100 seed weight by 10 (ISTA, 1996) and results expressed in grams to one decimal place.

3.1.4 Topographical Tetrazolium Test

Four replicates of 50 seeds from the pure seed fraction of each seed lot were tested for viability using the topographical tetrazolium test (TZ test). Seeds were allowed to imbibe between paper (BP) at 5°C for 24 hours. They were then removed and chipped at the distal end with a scalpel, before being placed in plastic pottles and covered with 1% TZ solution. Seeds were soaked for 12 hours at 20°C and then placed in an incubator at 40°C for a further 2 hours. Seeds were assessed under standard rules (ISTA, 1996), with viable seeds requiring that at least one cotyledon, 50% of leaf, hypocotyl, radicle, and the junction between the radicle and hypocotyl must be stained red. Those seeds not meeting these requirements were classified as non-viable. Results were expressed as a percentage.

3.1.5 Germination Test (Interim and Final Counts)

The between paper (BP); 25°C; dark method was used to assess standard germination (ISTA, 1996). Four replicates of 100 seeds were counted from the pure seed fraction of the seed lot. Interim counts were conducted at day 4 and 5 for black gram and green gram seed lots respectively, and the final count for both was on day 7 (ISTA, 1996). No pretreatment or fungicide dusting was conducted in standard germination tests. The number of normal seedlings, abnormal

seedlings, hard seeds and dead seeds were counted, and results were expressed as a percentage (ISTA, 1996).

3.1.6 Seed Health Testing

The presence of field and storage fungi was evaluated using the agar plate method. Potato dextrose agar (PDA) was used to assess field fungi contamination, while PDA plus salt (NaCl) was used to assess storage fungi contamination. Two replicates of 5 seeds per seed lot for each test (PDA and PDA + salt) were used. Seeds were surface sterilised in 1% sodium hypochlorite solution for 5 minutes, then rinsed with tap water for 2 minutes. Plated seeds were incubated at 25°C for 7 days with alternating cycles of 12 hours UV light and 12 hours darkness. After 7 days colonies were examined under magnification and identified with the assistance of the following guides:

- a) *Pictorial Atlas of Soil and Seed Fungi*, (Watanabe, 1994).
- b) *Illustrated Genera of Imperfect Fungi*, (Barnett, 1960).
- c) *An Annotated List of Seedborne Diseases*, (ISTA, 1990).
- d) Prof. Murray Hill (Seed Technology Centre, Massey University)
- e) Dr Peter Long (Plant Science Department, Massey University)

Plates containing colonies that could not be identified at this stage were incubated for a further 4 days, and then re-examined. Results were recorded as the number of colonies per plate and the fungal species present.

3.2 INDUSTRY BASED SEED QUALITY TESTS

3.2.1 *Oversoaks*

This is an industry developed test, which originated from the USA and has been modified by the Australian Mungbean Association (AMA). The materials required and methodology for this test are as follows:

- MATERIALS:
1. A temperature controlled waterbath with agitator
 2. Mesh baskets, at least 5cm*5cm*5cm, to hold seed
 3. Analytical balance
 3. Timer with alarm to notify analyst when to count
 4. Recording material and calculator
1. Four replicates of 28g of seed were taken from the bulk section of each seed lot, (not the pure seed portion).
 2. Each sample was placed in a mesh basket and then immersed in water at a constant 32°C, for exactly one hour. It was important that the water temperature remained constant and that the water was continually circulating.
 3. After 1 hour, seeds were removed from the water bath, and the number of oversoaks counted, removed and characterised as.
 - (a) fully imbibed seed
 - (b) partially imbibed seed with broken or split testa
 - (c) pieces of seed
 4. The remaining seeds were returned to the water bath (at 32°C) for a further 24 hours. After this time, the following was counted and recorded:
 - (a) # Sprouters - Seeds with emerged radicles
 - (b) # Hard Seeds - Seeds which had not imbibed water
 - (c) # Dead Seeds - Seeds which had imbibed water but showed no radicle growth.

CALCULATIONS

$$(i) \quad \text{Total \# Seeds} = (\# \text{Oversoaks} + \# \text{Sprouters} + \# \text{Hards} + \# \text{Dead})$$

$$(ii) \quad \% \text{ Oversoaks} = \left(\frac{\# \text{Oversoaks}}{\text{Total \# Seeds}} \right) * 100$$

$$(iii) \quad \% \text{ Sprouters} = \left(\frac{\# \text{Sprouters}}{\text{Total \# Seeds}} \right) * 100$$

Replicates were repeated over time, and results were expressed as percentages.

(NB: This is the recommended procedure published by the Australian Mungbean Association in their Newsletter No. 2, undated).

3.3 SEED VIGOUR TESTS

3.3.1 Bulk Electrical Conductivity

Four replicates of 50 seeds from the pure seed fraction of each seed lot were counted and their weight recorded to two decimal places. Each replicate was placed in a 500mL conical flask, and 250mL of distilled water (equilibrated to 20°C prior to use) was added. Flasks were sealed with aluminum foil and placed in a controlled temperature room at 20°C for 24 hours, along with a control flask containing 250mL of distilled water only. Replicates were repeated over time, with a new control in each case. A Radiometer CDM-83 conductivity meter with dip cell was used to determine the level of electrolyte leakage. Seeds remained

in flasks, which were gently swirled before each reading (Hampton, Johnstone & Eua-Umpon, 1992b; ISTA, 1995). Two readings per replicate (and per control) were recorded, at 20°C. Results were expressed as $\mu\text{S}/\text{cm}/\text{g}$ of seed.

3.3.2 Rate of Germination & Uniformity of Germination

For these tests, radicle protrusion from the testa was the criterion of germination. Preliminary testing (conducted on seed lots BULK 1, BULK 2, BULK 3 and 2012) using the top of paper (TP); 25°C; light method was used. Seed lots in these preliminary tests showed radicle emergence after nine hours. Rate of germination was determined by calculating the T_{50} (the time taken for 50% of seeds to germinate), while uniformity of germination was determined by the calculation by $T_{90} - T_{10}$ (the time taken for 90% and 10% of seeds to germinate respectively). The following materials and methodology were used for these tests:

1. Blotters were labeled with seed lot number, and placed in germination boxes, two per box (25cm * 18cm * 18cm). Then 20mL of water was added to each box.
2. Four replicates of 50 seeds per seed lot were counted from the pure seed fraction, and then placed on wet blotters. The time when the first and last seed were applied was noted, and this was used to calculate the mean time, ie. the time when the test commenced. Seeds were placed on the blotters for testing nine hours before the first count was conducted.
3. Each box was enclosed in a clear plastic bag, and then placed in a germination cabinet at 25°C.
4. The first count was conducted nine hours after the mean time of placing seeds on the blotters. Counts were taken at hourly intervals, with

germinated seeds being removed and recorded each time. Boxes were counted in the same order each time, and placed back into sealed bags.

5. The final hourly count was made 21 hours after the seeds were placed on blotters. Remaining ungerminated seeds were left on the blotters in the germination chamber until the following day (36 hours after applying seeds to blotters). The number of germinated, hard and dead seeds left were then recorded.
6. Data were converted to a cumulative count and then a cumulative germination percentage for each replicate. The T_{10} , T_{50} and T_{90} for each was then calculated, using the equations below:

(a) T_{50} or Median Emergence Time:

$$T_{50} = \frac{t_i + \frac{N + 1}{2} - n_i}{(n_j - n_i)} * (t_j - t_i)$$

when: $n_i < (N + 1)/2 < n_j$

(b) $T_{90} - T_{10}$ or Uniformity of Germination:

$$T_{10} = \frac{t_i + \frac{N + 1}{10} - n_i}{(n_j - n_i)} * (t_j - t_i)$$

when: $n_i < (N + 1)/10 < n_j$

$$T_{90} = \frac{t_i + \frac{9(N + 1) - n_i}{10}}{(n_j - n_i)} * (t_j - t_i)$$

when: $n_i < 9(N + 1)/10 < n_j$

Where: N = the final emergence number

t_i = time of first count

t_j = time of last count

n_i = the cumulative emergence count at time t_i

n_j = the cumulative emergence count at time t_j

Tests were replicated over time, as the period for counting-off germinated seeds was too long for all replicates to be conducted together. Seeds with hilums facing the blotter appeared to germinate more rapidly than those with the hilum facing away from the blotter surface. After discussions with Karen Hill (Senior Tutor - Seed Technology Centre), it was agreed that seeds should be randomly placed (with respect to orientation) on the blotters. This problem would be reduced if the BP method was used, but this makes counting off more time consuming. The testas of black gram seeds tended to split several hours before the radicle emerged. This would mean some radicles were 3-4mm long before they protruded from the testa, compared to 1-2mm for green gram seeds. As this falsely increased the germinating period for this species, seeds with split testas had their testas removed, and if the radicle had swollen and grown over 1mm in length, a seed was classified as having germinated and was counted off.

3.3.3 Seedling Growth Uniformity

To assess seedling uniformity the following materials and methodology were used:

1. Four blotters per replicate were labeled with the appropriate seed lot number and placed (two thick) in germination boxes (25cm * 18cm * 8cm). Then 55mL of water was added to each box.
2. Four replicates of 50 seeds were counted from the pure seed fraction of each seed lot and were placed on the wet blotters. Boxes were then enclosed in sealed plastic bags and placed in the germination cabinet (25°C) in darkness.
3. After 120 hours (5 days), boxes were removed from the cabinet and the fresh weight of each replicate (including all sprouts, hard seeds, dead seeds and testas) was recorded to two decimal places . Weighed replicates were placed into a chiller (3-5°C) while awaiting measurement. The number of hard seeds and dead seeds were also recorded.
4. One replicate at a time was measured. Twenty sprouts were selected at random from each seed lot, and hypocotyl length - from top of hook to start of radicle (mm), total length - from top of hook to end of radicle (mm) and the diameter of the hypocotyl - within 10-15mm of the base of the hypocotyl (mm) were measured. Measurements took approximately 30 minutes per replicate.
5. All sprouts, dead seeds and testas per replicate were placed into a moisture tin. These were dried for 24 hours at 80°C. At the end of the drying period, the tins were removed from the oven and placed in a desiccator for 15 minutes to cool before reweighing to two decimal places.

6. The mean, minimum, maximum and range of total sprout length, hypocotyl length and hypocotyl diameter was calculated for each replicate and recorded using the equations below.

7. The total fresh weight (FRWT), and dry weight per sprout (DRWT) was calculated - assuming 100% germination, ie. 50 sprouts per replicate (refer below for equations).

CALCULATIONS

(i) Mean (mm) $x = \frac{(\text{Length Sprout 1} + \text{Length Sprout 2} \dots + \text{Length Sprout 20})}{20}$

(ii) Minimum (mm) = Lowest result per replicate

(iii) Maximum (mm) = Highest result per replicate

(iv) Range (mm) = Longest sprout length - Shortest sprout length

NB: The above four calculations were done for each parameter ie. total length, hypocotyl length and hypocotyl diameter.

(v) DRWT = $\frac{((\text{weight of tin} + \text{cover} + \text{sprouts after drying}) - (\text{weight of tin} + \text{cover}))}{50}$

Results for calculations (i) to (iv) were expressed in millimetres, and results for calculation (v) were expressed as grams per sprout.

3.3.4 Accelerated Ageing

Preliminary tests were conducted on four seed lots (BULK 1, BULK 2, BULK 3 and 2012). These seed lots were selected because there was sufficient seed in the submitted samples available for additional extra testing. Initial tests showed that the recommended 45°C for 96 hours (ISTA, 1996) was too severe, with the best seed lot only producing 12% normal seedlings. Seed lots were then aged for: 24hrs at 43°C; 48hrs at 43°C; 72hrs at 43°C; and 96hrs at 43°C.

Final test methodology utilised an ageing period of 96hrs at 43°C. Four replicates of 20g seed from the pure seed fraction were placed in mesh baskets within plastic box inner chambers. A standard 40mL of distilled water per replicate was used. Seed moisture post-ageing was determined by two stage drying using the high constant temperature method, (ISTA, 1996). After ageing 50 seeds per replicate were germinated under standard ISTA (1996) conditions, ie, BP; 25°C; light. Seeds were dusted with Thiram fungicide (ai 800g/kg thiram) before germination. Replicates were repeated over time, and results were expressed as a percentage.

3.4 SIMULATED COMMERCIAL PRODUCTION

3.4.1 Small Scale Commercial Production (SSCP) - Sprouting Methodology

In order to determine the relationship between each seed quality parameter tested and likely subsequent commercial sprouting quality of each seed lot, it was necessary to produce sprouts on a 'small-scale', mimicking commercial conditions as far as possible. The sprouter used was built, using a plan of a

prototype (refer appendix 4A & 4B for plan and photographs) developed by Dr Bruce Imrie and associates at the CSIRO Tropical Crops Division, St. Lucia, Brisbane, Australia. The following describes the materials and methodology used in this SSCP sprouting tests:

1. Four replicates of 120g of seed per seed lot were sprouted, with an outer chamber temperature of 20°C. Seeds were soaked at 35°C for 5 hours, rinsed with fresh tap water for 5 minutes and then placed in the sprouting tubes inside the outer sprouting chamber.
2. Seeds were sprouted for 5 days, with water, at a constant 19°C, being applied every 4 hours. There were 8 spray nozzles, with 2 tubes under each nozzle. The flowrate of water was 460mL per nozzle per minute. Water was applied for 4 minutes per application, applying 14.72L (approx. 15L) in total every 4 hours. Each tube was numbered and weighed prior to testing for use in assessing sprout weights.
3. After 120 hours, tubes were removed and weighed to determine total sprout weight (SSCP total yield). Each tube was then emptied, and a 60g sub-sample selected. This was separated into three categories: (a) SSCP healthy sprouts - sprouts with a hypocotyl at least 50mm in long and 2mm wide; (b) SSCP small and abnormal sprouts - sprouts with hypocotyl less than 50mm in length and 2mm wide, and abnormal (ISTA, 1996); (c) SSCP marked and rotten sprouts - any sprouts that had blemishes on roots, hypocotyl or cotyledon. Sprouts in category (a) are referred to as 'consumer acceptable sprouts' (CAS), while those in categories (b) and (c) are referred to as consumer unacceptable sprouts' (CUS).

4. The number of sprouts in each category was recorded. From the CAS, 20 sprouts were randomly selected and the following measurements made:
 - (i) total sprout length (mm); (ii) hypocotyl length - from top of hook to the base of the hypocotyl (mm) and; (iii) the hypocotyl diameter 10-15mm from the base of the hypocotyl (mm).

5. Sprouts were then returned to the total CAS sample and their dry weight determined, after drying at 80°C for 24 hours.

CALCULATIONS

$$\begin{aligned}
 \text{(i) } \quad \text{kgSprouts/kgSeed} &= \frac{\text{weight of sprouts + tube} - \text{weight of tube}}{\text{weight of seed sprouted}} \\
 \text{(SSCP ratio)} & \\
 &= \text{kg:kg}
 \end{aligned}$$

$$\begin{aligned}
 \text{(ii) } \quad \text{Sprout DryMatter} &= \frac{\text{weight of dried sprouts + bag} - \text{bag weight}}{\text{number of sprouts}} \\
 \text{(SSCP dryweight)} & \\
 &= \text{g/sprout}
 \end{aligned}$$

3.5 STATISTICAL ANALYSIS

For each seed quality test, and SSCP variable measured, a completely randomised design with four replicates was used, with the exception of seed health, SMC and TSW which had two (seed health, SMC) and eight (TSW) replicates (ISTA, 1996). An analysis of variance (ANOVA) was performed on the results of each seed quality test conducted (except for seed health and purity

which were not designed for this analysis), to compare the different quality of individual seed lots. Analysis of the two species was separate. The ranking of seed lots was determined in each test by Fisher's Least Significant Difference (protected LSD) at the $P < 0.05$ level of probability.

Linear regression analysis was used to determine whether any of the laboratory seed quality tests ranked seed lots (best to worst) in a similar order to quality parameters considered important by commercial sprouters. ie. SSCP total fresh yield and SSCP healthy yield. The SSCP variables were classified as the dependent variables (response variables) and the laboratory based seed quality tests were classified as the independent variables (predictor variables). Those regressions which produced a significant ($P < 0.05$) relationship are reported in this section and the corresponding coefficient of determination (R^2) and standard deviation (SD) values are also given.

Results which produced a significant relationship under linear regression analysis were then combined using multi-variate regression (multiple regression), in an effort to determine the most reliable laboratory based seed testing regime (model) - when evaluating the quality of mungbean seed destined for sprouting. (Summaries of those linear regressions which were not significant are listed in Appendix 3A and 3B).

The statistical computer program SAS (available at Massey University) was used to analyse all data.

CHAPTER 4

RESULTS

4.1 ANALYSIS OF VARIANCE (ANOVA)

4.1.1 Standard Seed Quality Tests

4.1.1.1 Purity

Purity was high in all seed lots (Table 6). The pure seed fraction ranged from 99.5% for lot BULK 3 to 99.9% for lots 2017 and 2018 in black gram seed lots, and from 99.6% for lot 2004 to 99.9% for lots BULK 2 and 2020 in green gram seed lots. All seed lots would meet export standards, for which there is a minimum of 99% pure seed (John Hampton pers. comm., 1995).

4.1.1.2 Thousand Seed Weight

Thousand seed weight ranged by 10g among black gram seed lots (Table 6). The heaviest (TSW = 64.80g) was lot 2013, while the lightest (TSW = 54.13g) was lot 2018. Lots 2012, 2013 and 2016 were significantly heavier than lots BULK 3 and 2019, which were significantly heavier than lots 2015 and 2017, and all of which were significantly heavier than lot 2018.

Thousand seed weight ranged by almost 20g among green gram seed lots (Table 6). The lightest lot was 2002 (TSW = 46.29g), while lot BULK 1 was the heaviest (TSW = 66.00g). The only two seed lots not significantly different from one another were BULK 1 and 2003.

TABLE 6: Purity, thousand seed weight (TSW), seed moisture content (SMC) and topographical tetrazolium (TZ) test results for all black and green gram seed lots.

SEED LOT	SEED TESTING PROCEDURE			
	PURITY	TSW	SMC	TZ
	(%)	(g)	(%)	(%)
(Black Gram)				
BULK 3	99.5	60.81	12.4	97.0
2012	99.8	64.75	12.5	99.0
2013	99.7	64.80	12.7	99.5
2015	99.8	57.10	10.0	96.0
2016	99.7	64.10	10.4	95.5
2017	99.9	57.39	11.8	94.0
2018	99.9	54.13	12.7	98.5
2019	99.8	60.72	9.3	95.0
LSD (P<0.05)	-	0.76	0.2	NS
(Green Gram)				
BULK 1	99.8	66.00	13.1	99.5
BULK 2	99.9	59.55	13.7	99.5
2001	99.7	52.36	11.1	99.5
2002	99.9	46.29	11.9	100.0
2003	99.9	64.79	11.1	100.0
2004	99.6	55.22	12.4	91.5
2020	99.9	62.29	9.2	99.5
LSD (P<0.05)	-	1.53	0.5	1.3

4.1.1.3 Seed Moisture Content

Seed moisture content of the eight black gram seed lots ranged from 9.3% for lot 2019 to 12.7% for lot 2013 and 2018 (Table 6). Although there were significant differences in SMC among lots, all lots fell within the recommended SMC for storage, handling and seed testing of black gram seed (Lucy & Agnew, 1994).

Seed moisture content of the seven green gram seed lots ranged from 9.2% for lot 2020 to 13.7% for lot BULK 2. Again there were significant differences among seed lots, but all fell within the recommended SMC for storage, handling and seed testing of green gram seed (Lucy & Agnew, 1994).

4.1.1.4 Topographical Tetrazolium Test (TZ)

The percentage of viable seeds as determined by the TZ test did not differ significantly among black gram seed lots (Table 6).

Only lot 2004 among the green gram seed lots tested had a TZ value below 99.5%, and was significantly lower than the other six lots (Table 6).

4.1.1.5 Standard Germination

Interim germination (% normal seedlings at day 4) among black gram seed lots ranged from 75% for lot 2015 to 94.5% for lot 2013 (Table 7). Interim germination was significantly lower for lots 2015 and 2016. Final germination (% normal seedlings) ranged from 87.5% to 98.5% for black gram seed lots (Table 7). Seed lots 2015 and 2016 had the lowest germination, although that for lot 2015 (91.5%) was not significantly different from that of lots BULK 3 (94%) and 2013 (95.5%). There were no significant differences in abnormal seedlings

among seed lots. A value of 5.5% abnormal seedlings may be considered agronomically important in sprouting seed as these are unacceptable to consumers and result in lower salable yields. Abnormal seedlings were the main reason why lot 2015 had a significantly lower germination. Both lots 2015 and 2016 had significantly higher levels of hard seed (3% and 10.5% respectively), compared to other seed lots. Dead seed ranged from 0% (lots 2012, 2015, 2017) to 2% (lot BULK 3). Black gram seed lots with germination above 90% are presently accepted for sprouting, which means only lot 2016 would have been rejected on the basis of germination performance (AMA Newsletter No. 2, undated).

Interim germination (% normal seedlings at day 5) ranged from 67% for lot 2020 up to 98.5% for lot BULK 2 among green gram seed lots (Table 7). Lot 2020 had a significantly lower interim germination than all other green gram seed lots, while lot 2004 was significantly lower than all but lot 2020. The remaining lots did not differ significantly. Final germination ranged from 78% to 99% for green gram seed lots (Table 7). Both lots 2004 and 2020 (with 88.5% and 78% germination respectively) had a germination percentage significantly lower than the other five seed lots. The lower germination of lot 2004 was due to a significant increase in both abnormal seedlings (5.5%) and dead seeds (6%), while lot 2020 had a significantly higher hard seed content (21%). No other significant differences among seed lots occurred. If the 90% germination standard had been applied, lots 2004 and 2020 would have been rejected for sprouting purposes.

Abnormal seedlings mainly comprised of seedlings with split primary roots, cotyledons remaining at the base of the hypocotyl, neck rot, stunted roots or stunted shoots.

TABLE 7: Germination results for black and green gram seed lots, including interim counts.

SEED LOT	STANDARD GERMINATION RESULTS				
	INTERIM	FINAL	FINAL	FINAL	FINAL
	(% normal)	(% normal)	(% abnormal)	(%dead)	(% hard)
(Black Gram)					
BULK 3	91.5	94.0	4.0	2.0	0.0
2012	93.0	98.5	1.5	0.0	0.0
2013	94.5	95.5	3.0	1.0	0.5
2015	75.0	91.5	5.5	0.0	3.0
2016	78.0	87.5	0.5	1.5	10.5
2017	92.0	96.5	3.5	0.0	0.0
2018	90.5	96.5	1.0	2.5	0.0
2019	86.5	98.5	0.0	0.5	1.0
LSD (P<0.05)	8.1	4.9	NS	1.7	2.3
(Green Gram)					
BULK 1	94.0	98.0	8.0	0.0	0.0
BULK 2	98.5	99.0	1.0	0.0	0.0
2001	89.5	99.0	1.0	0.0	0.0
2002	97.0	98.5	1.5	0.0	0.0
2003	91.5	96.5	3.5	0.0	0.0
2004	85.0	88.5	5.5	6.0	0.0
2020	67.0	78.0	1.0	0.0	21.0
LSD (P<0.05)	6.6	4.1	1.9	0.9	2.0

4.1.1.6 Seed Health

Alternaria alternata was most commonly detected in black gram seed lots, followed by *Fusarium* spp. and *Penicillium* spp. (Plate 2 and Appendix 1A). In the test for storage fungi *Aspergillus glaucus* was most common, followed by *Aspergillus flavus*. No fungal infection was found in lots 2013, 2017 and 2018, while lots 2016 and 2019 had the highest fungal loading (Appendix 1A). In green gram seed lots, both *Aspergillus glaucus* and *Penicillium* spp. were most commonly detected (Plate 2 and Appendix 1B), although the former is considered to be mainly a storage fungus. Lots BULK 2 and 2020 appeared to be free of field fungal infection. In general, the frequency of field fungi infection in green gram seed lots was lower than for black gram seed lots. Only lots BULK 2 (*Aspergillus glaucus*) and 2020 (*Aspergillus niger*) were infected with storage fungi (Appendix 1B).

4.1.2 Vigour Tests

4.1.2.1 Bulk Conductivity

Significant differences in conductivity were recorded among black gram seed lots (Table 8). Steep water leachate conductivity ranged from a high of 29.40 μ S/cm/g for lot 2017 to a low of 18.08 μ S/cm/g for lot 2016.

The range in seed leachate concentrations as measured by electrical conductivity was generally greater for green gram seed; ie. from 31.25 μ S/cm/g for lot 2004 down to 15.8 μ S/cm/g for lot 2020 (Table 8). The seed lots could be separated into two groups, ie. high conductivity seed lots (lots BULK 1, 2001, 2004) and lower conductivity seed lots (lots BULK 2, 2002, 2003, 2020).

4.1.2.2 Accelerated Ageing

Seed moisture content post-ageing ranged from 30.0% for lot 2012 to 36.2% for lot 2015 among black gram seed lots (Table 8). Only seed lot 2012 fell within the recommended post-ageing SMC range for black gram, which is 27-32% (ISTA, 1996). SMC for lot 2015 was significantly higher than that of lots 2018, 2019 and 2012, while there was no significant difference among seed lots 2017, 2016, 2013, BULK 3, 2018 and 2019. Lots 2019 and 2012 also did not differ significantly.

Accelerated ageing greatly reduced the post-ageing germination (percentage of normal seedlings) in black gram seed lots (Table 8). Germination was most affected in lot 2017 which had a final germination of 17% post-ageing (compared to 96.5% pre-ageing). Germination after ageing ranged from 65% for lot 2019 down to 17% for lot 2017. The final germination of lot 2017 was significantly

lower than that for all other black gram seed lots. Lot 2019 withstood AA significantly better than lots 2016, BULK 3 and 2017, but was not significantly different from lots 2013, 2012, 2018 and 2015. The next highest germinating seed lot was 2013 (61.5%), which was significantly higher than lots BULK 3 and 2017.

Differences in the percentage of abnormal seedling post-ageing among black gram seed lots were also significant, ranging from 4% for lot 2017 up to 17.5% for lot 2015 (Table 8). Dead seeds ranged from 21% for lot 2013 up to 79% for lot 2017. Again, lot 2017 was significantly higher than all other seed lots (Table 8).

Green gram lots ranged in SMC after ageing from 33.0% for lot BULK 1 to 36.3% for lot 2001. There were no significant differences among seed lots (Table 8). Germination was also greatly decreased post-ageing, ranging from 60% for lot BULK 1 down to 13% for lot 2004 (Table 8). Seed lots BULK 1 and 2003 did not differ, but maintained a significantly higher germination than the remaining five seed lots. Lots 2020 and 2002 again did not differ from one another, but were significantly superior to lots BULK 2, 2001 and 2004. Germination of lot BULK 2 was only significantly greater than lot 2004, the latter not being significantly different from lot 2001.

There were no significant differences among seed lots in the percentage of abnormal seedlings post-ageing. Variation in the percentage of dead seeds, which ranged from a low of 18% for lot BULK 1 up to 71% for lot 2004, was significant (Table 8). Lots 2001 and 2004 had a significantly higher percentage of dead seeds than all other green gram seed lots.

TABLE 8: Vigour test results - conductivity, accelerated ageing (AA), rate of germination (T50) and uniformity of germination (T90 -T10) for all black and green gram seed lots.

SEED LOT	VIGOUR TESTS						
	CONDUCTIVITY	ACCELERATED AGEING				RATE OF GERMINATION	UNIFORMITY OF GERMINATION
	(uS/cm/g)	(%SMC)	(%normal)	(% abnormal)	(%dead)	T50 (hrs)	T90 - T10 (hrs)
(Black Gram)							
BULK 3	22.65	34.1	52.0	16.5	31.5	11.43	7.55
2012	22.83	30.0	60.0	9.0	31.0	11.15	3.78
2013	19.85	34.3	61.5	17.5	21.0	12.93	3.3
2015	20.23	36.2	56.0	17.5	26.5	12.49	6.22
2016	18.08	34.8	55.5	10.5	34.0	14.73	8.66
2017	29.40	35.1	17.0	4.0	79.0	13.52	5.53
2018	22.68	33.1	56.0	9.5	34.5	14.03	5.07
2019	19.30	32.3	65.0	12.0	23.0	12.83	7.26
LSD (P<0.05)	1.73	3.0	9.5	8.5	10.0	2.08	2.06
(Green Gram)							
BULK 1	26.65	33.0	60.0	22.0	18.0	12.43	4.95
BULK 2	20.35	35.8	23.5	21.5	55.0	15.88	7.73
2001	29.50	36.3	18.0	12.0	70.0	13.75	8.21
2002	19.45	35.8	39.0	15.5	45.5	14.94	7.14
2003	21.03	36.2	54.0	20.0	26.8	14.01	5.37
2004	31.25	36.0	13.0	16.0	71.0	13.87	7.25
2020	15.80	34.1	44.0	16.5	34.5	17.57	18.64
LSD (P<0.05)	2.20	NS	9.5	NS	11.0	2.38	2.67

4.1.2.3 Rate of Germination and Uniformity of Germination

Rate of germination (T_{50}) for black gram seed lots varied between lots by up to 3.5 hours (Table 8), with lot 2012 having the shortest T_{50} time (11:15 hours), and lot 2016 having the longest (14.73 hours). Lot 2012 was significantly faster in reaching T_{50} germination than all lots except lot BULK 3. Lots 2013, 2015, 2017 and 2019 did not differ significantly, and neither did lots 2013, 2017 and 2018. The only lot not significantly faster than lot 2016 was lot 2018 (Table 8).

Uniformity of germination ($T_{50} - T_{10}$), ranged from 3.30 hours for lot 2013 to 8.66 hours for lot 2016 (Table 8). The order of ranking determined by the T_{50} changed when uniformity of germination was measured. Lot BULK 3, for example, had the fastest T_{10} and T_{90} time, but had the second slowest (worst) uniformity time. The slowest three seed lots to germinate (lots 2013, 2017, 2018) as measured by T_{50} , were three of the four most uniform seed lots (lot 2012 being the second most even). The following seed lots within each group did not differ significantly from one another, (in descending order of uniformity of germination ie. worst to best - BULK 3, 2016 and 2019; BULK 3, 2017, 2015, and 2019; 2015, 2017 and 2018; 2012, 2017 and 2018; 2012, 2013 and 2018 (Table 8).

Germination rate for green gram seed lots varied by approximately 5 hours, ranging from 12.43 hours for lot BULK 1 up to 17.57 hours for lot 2020 (Table 8). The time taken to reach T_{50} for lot 2020 was not significantly different from lot BULK 2, but was significantly greater than for lots BULK 1, 2001, 2002, 2003, and 2004, while lot BULK 1 was significantly faster in reaching T_{50} germination than lots BULK 2, 2002 and 2020. Uniformity of germination was poorest in lot 2020 at 18.64 hours, which differed significantly from all other seed lots. Lot BULK 1 has the most uniform germination period, but was not significantly different from lots

2002, 2003 and 2004. There was no significant difference among lots BULK 2, 2002, 2003 and 2004, nor among lots BULK 2, 2001, 2002 and 2004. There was no significant difference in the rate of initial germination (T_{10}) among seed lots, and those lots which had the shortest T_{90} time, were generally the most uniform.

4.1.2.4 Evaluation of Seedling Growth

The average total seedling length of black gram seed lots ranged from 138mm for lot 2017 up to 171mm for lot BULK 3 (Appendix 2). Lots BULK 3, 2012, 2013, 2015 and 2016 did not differ significantly, but all produced seedlings which were significantly longer than those from lots 2017 and 2018. Lots 2017, 2018 and 2019 did not differ significantly, and neither did lots 2012, 2013, 2018 and 2019. There were no significant differences in maximum, minimum or range of total seedling length among black gram seed lots (Appendix 2).

Average hypocotyl length ranged from 68mm for lot 2018 to 85mm for lot BULK 3. There were no significant differences among seed lots within the following groups - lots 2012, 2013, 2017, 2018 and 2019 or lots 2012, 2015, 2016, 2017 and 2019 or lots BULK 3, 2012, 2015, 2016 and 2017. Hypocotyl length of BULK 3 was significantly longer than lots 2013, 2018 and 2019, while lots 2015 and 2016 were significantly longer than lots 2013 and 2018. Again, there were no significant differences among seed lots for maximum, minimum and range of hypocotyl length. Hypocotyl diameter did not differ significantly for average, maximum, minimum or range measurements among black gram seed lots (Appendix 2).

Both total fresh weight and dry weight per sprout were also recorded. Only dry weight per sprout differed significantly among black gram seed lots, ranging from 0.029g for lot 2016 up to 0.049g for lot 2013 (Appendix 2). The dry weight of sprouts in lot 2016 was significantly lighter than that for all other seed lots. The only lots not significantly different from lot 2013 were lots 2012 and 2019, while lots 2012 and 2019 were not significantly heavier than lot BULK 3. There were no significant differences among lots 2015, 2017 and 2018 (Appendix 2).

The average total seedling length among green gram seed lots ranged from 101mm for lot 2020 to 137mm for lot BULK 2 (Appendix 2). On average, seed lots BULK 2, 2002 and 2004 were significantly longer than lots BULK 1, 2001, 2003 and 2020, while lots 2001, 2002 and 2003 did not differ, and neither did lots BULK 1, 2001, 2003 and 2020. Minimum total seedling length also produced significant differences among green gram seed lots, ranging from 50mm for lot BULK 1 to 84mm for lot 2002 (Appendix 2). The minimum total length of lots BULK 2 and 2002 were significantly longer than lots BULK 1, 2003 and BULK 1. There were no significant differences among lots BULK 1, 2001, 2002 and 2004 nor among lots BULK 1, 2001, 2003, 2004 and 2020. There were also no significant differences among green gram seed lots for maximum and range of total seedling length (Appendix 2).

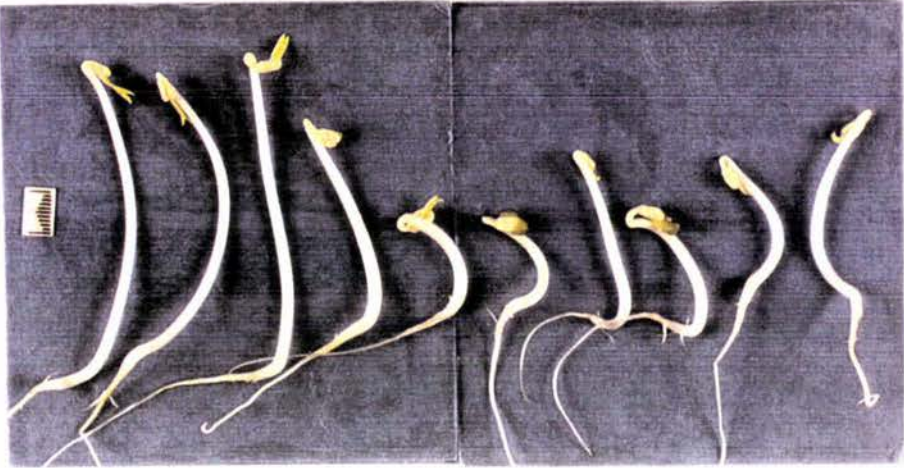
There were significant differences for minimum hypocotyl length among green gram seed lots, but not for maximum length or range (Appendix 2). Minimum hypocotyl length ranged from 13mm for lot 2020 to 30mm for lot 2001. Lot 2001 differed significantly from lots BULK 1, 2003 and 2020 while lots BULK 2, 2002 and 2004 had significantly higher minimum hypocotyl

length than lot 2020, while neither lots BULK 2, BULK 1, 2002, 2003 and 2004 nor lots BULK 1, 2003 and 2020 differed significantly from one another.

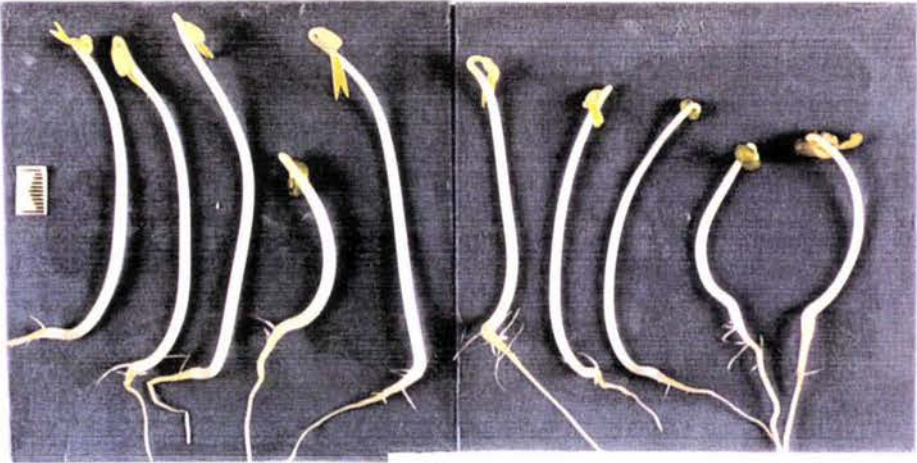
Significant variation in hypocotyl diameter of green gram seed lots was only found among maximum measurements (Appendix 2). The maximum hypocotyl diameter ranged from 2.6mm for lot 2003 up to 2.9mm for lot BULK 2. Maximum hypocotyl diameter was significantly greater for lot BULK 2 than for lots 2001, 2002, 2003 and 2020. Lots BULK 1, 2001, 2002, 2004 and 2020 did not differ significantly and lot 2003 did not differ significantly from lots 2001, 2002 and 2020.

Fresh yield differed significantly among green gram seed lots (Appendix 2), with weights ranging from 4.3g for lot 2020 up to 6.5g for lot 2002. The heaviest five seed lots (lots BULK 1, BULK 2, 2001, 2002 and 2004) did not differ significantly, but all were significantly heavier than lot 2020, while lot 2003 was only significantly different from lot 2002. Sprout dry weight also differed significantly among seed lots. Lots were divided into three significant groups, the heaviest being lots BULK 1, 2003 and 2020, the next lots BULK 2, 2001 and 2004, and all lots were significantly heavier than lot 2002 (Appendix 2).

PLATE 3: A series of photographs illustrating the differences in seedling growth, both within and among seed lots during seedling evaluation testing. (Each seed lot is numbered in the photographs)



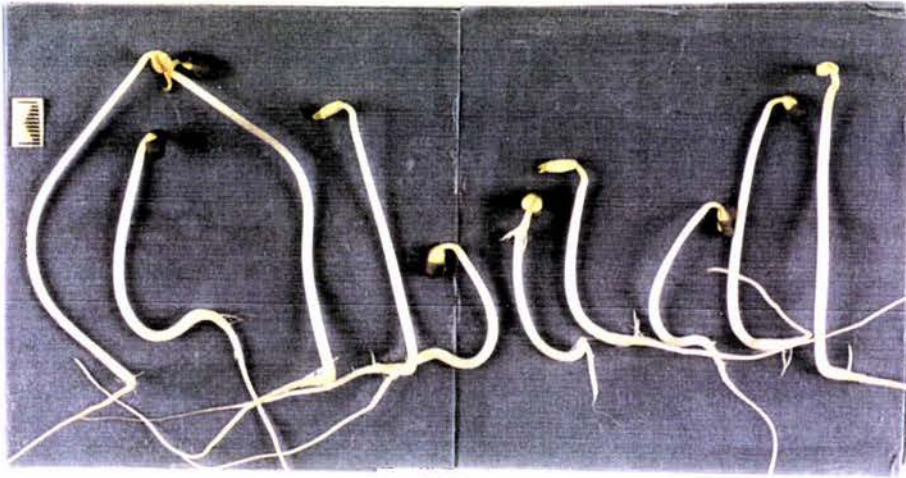
BUK 2



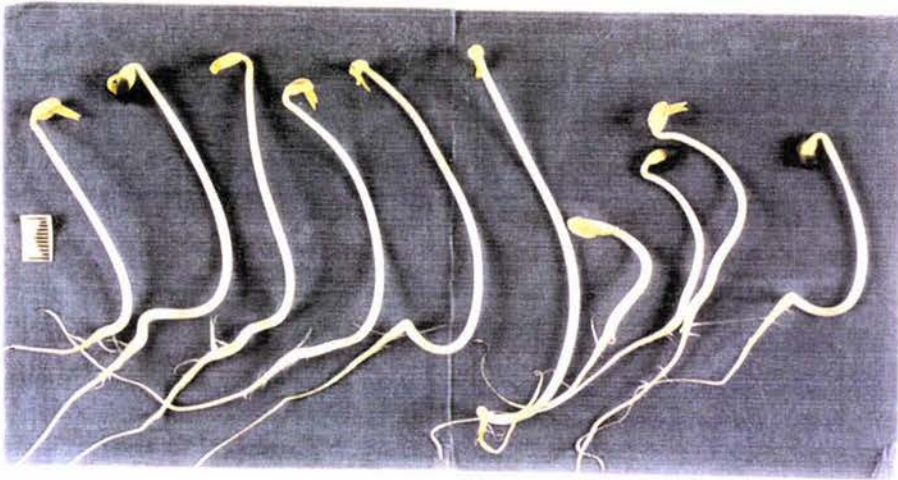
2004



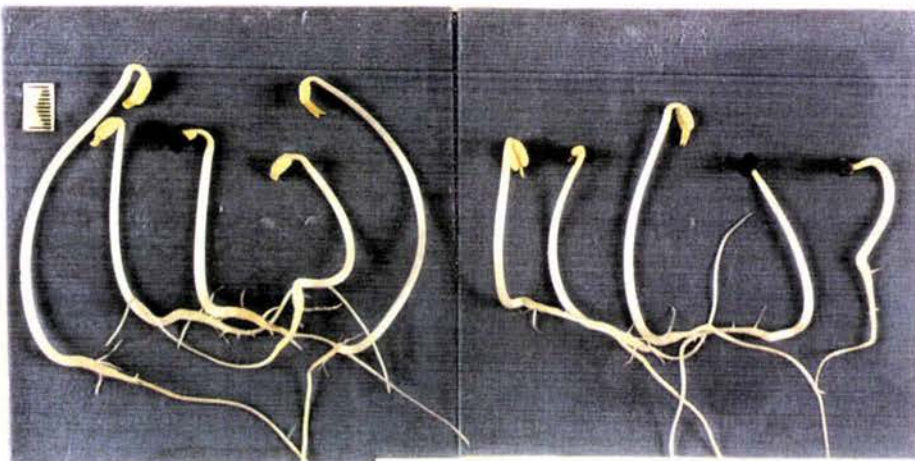
2002



2013



2014



2018

4.1.3 Industry Based Tests (the Oversoak Test)

4.1.3.1 Oversoaks and Sprouters

The percentage of oversoaks ranged from 0.8% for lot 2018 to 8.2% for lot 2015 among black gram seed lots (Fig. 1a), and there were significant differences among the seed lots (LSD=2.1%). Industry recommendations place an upper limit of 8% oversoaks on premium grade seed. Using this criterion, only lot 2015 would have been rejected.

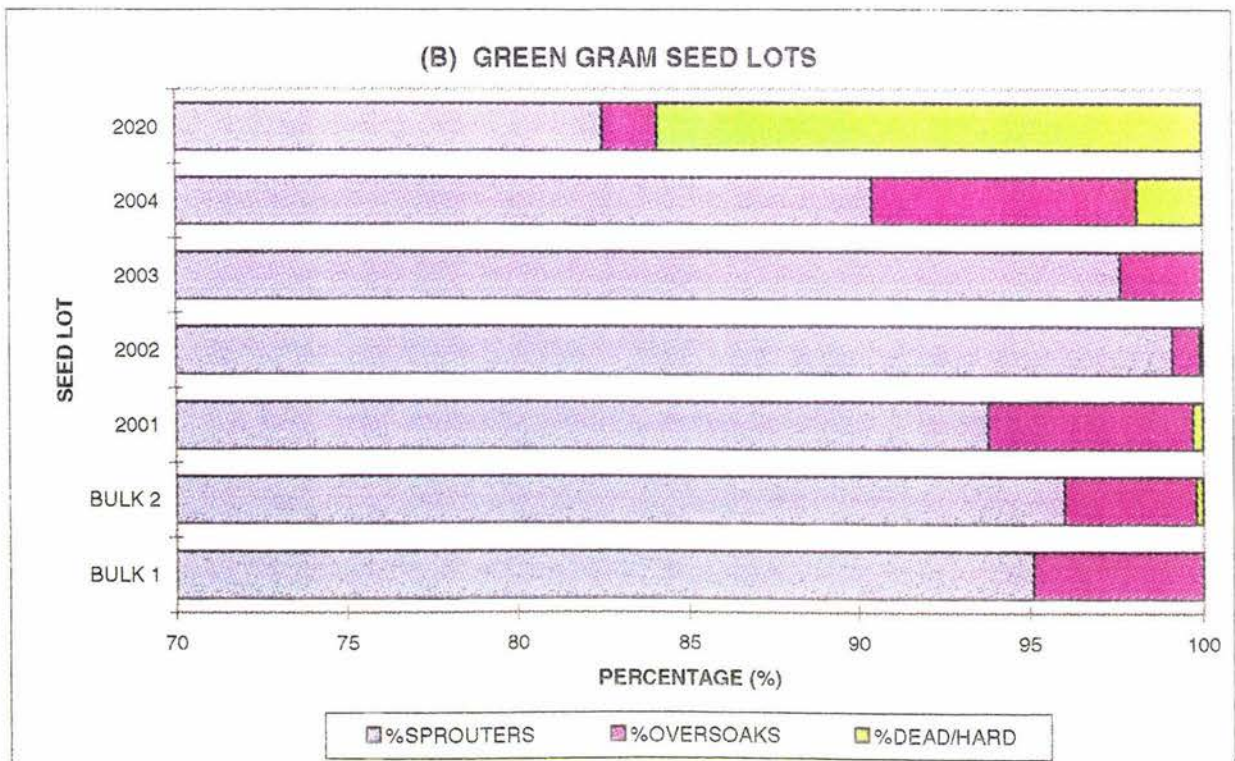
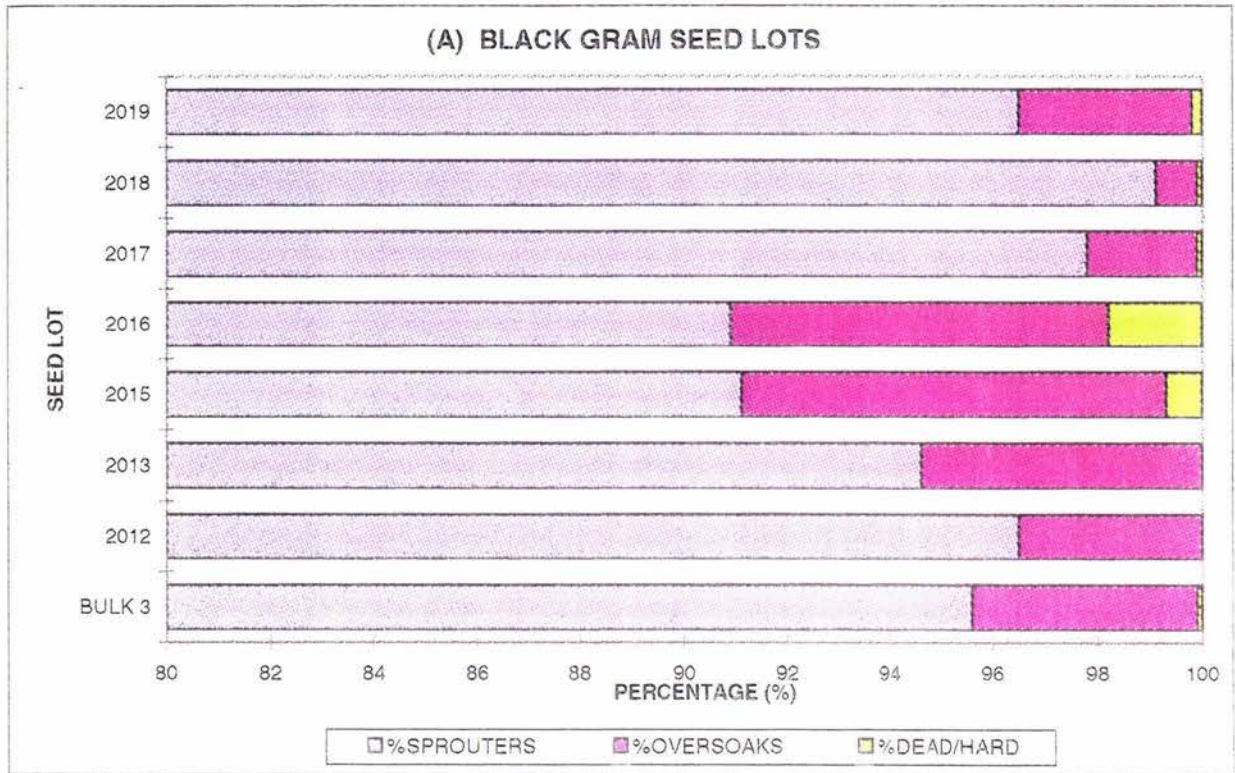
The percentage of sprouters in this test ranged from 90.9% for lot 2016 to 99.1% for lot 2018 (Fig. 1a), and again there were significant differences among seed lots (LSD= 2.1%). The difference between the percent of sprouters and 100% is made up by the total number of oversoaks, number of hard seeds and number of dead seeds (the latter two categories presented together as the 'remainder' in Fig. 1a). In all cases, dead seeds appeared to have the axis of the radicle and hypocotyl damaged, which prevented radicle elongation.

The percentage of oversoaks among green gram seed lots was similar to those of black gram. Oversoaks ranged from 0.8% for lot 2002 to 7.8% for lot 2004 (Fig. 1b). If the 8% rule was used in this species, all lots would have been accepted using this criterion. With a LSD of 2.9%, lots 2001 and 2004 had a significantly higher level of oversoaks than all other lots. Lots BULK 1, BULK 2 and 2003 did not differ significantly and neither did lots BULK 2, 2002 and 2003.

The percentage of sprouters ranged from 82.5% for lot 2020 to 99.1% for lot 2002 (Fig 1b). Seed lots 2001, 2004 and 2020 were the only lots below 95%. Lot 2020 was significantly lower than all other seed lots (LSD=3.3%), and lot

2004 was significantly lower than all but lot 2020. The highest three sprouters (lots 2002, 2003 and BULK 2) did not differ significantly. The high hard seed content of lot 2020 and the high oversoak content of lot 2004 were reflected in their low relative sprouting percentage result.

FIGURE 1: Breakdown of Oversoak test results for both black gram (A) and green gram (B) seed lots tested.



4.1.4 Small Scale Commercial Production (SSCP)

4.1.4.1 Total Yield (Fresh Weight)

Total yield differed significantly among black gram seed lots, ranging from a low of 670g for lot 2019 up to 816g for lot 2013 (Table 9). Both lots BULK 3 and 2013 were significantly higher yielding than the other six seed lots. Lots 2012, 2015, 2016 and 2017 did not differ significantly, but all except lot 2012 were significantly higher yielding than lots 2018 and 2019. The lowest yielding lot was 2019, which was significantly lower than all other lots except lot 2018. Lot 2018 was in turn significantly lower yielding than all other lots except lot 2012 (Table 9).

Variation in green gram total yield was significant and distinct among the seed lots, ranging from 689g for lot 2020 up to 885g for lot BULK 2 (Table 9). Lot BULK 2 was significantly higher in total yield than all other green gram lots, while lots BULK 1, 2001, 2002 and 2003 did not differ significantly, but were significantly higher yielding than lots 2004 and 2020. Finally, lot 2004 was significantly higher yielding than lot 2020.

4.1.4.2 Ratio of Yield:Seed

The total 'sprout yield:seed used' ratio differed significantly among black gram seed lots, ranging from 5.6 for lots 2018 and 2019 up to 6.8 for lots BULK 3 and 2013 (Table 9). The order of seed lots from best to worst was almost the same as for total yield, with only lots 2015, 2016 and 2017 interchanging. The theory is that the higher the ratio, the better the seed lot is for sprouting. This would mean lots BULK 3 and 2013 were significantly superior to all other black gram seed lots, while lots 2012, 2015, 2016 and 2017 were all significantly better than lot 2019. Lot 2018 was not significantly different from lots 2012, 2015, 2016, 2017 or 2019 (Table 9).

The ranking of green gram ratio of yield:seed used was also similar to the total yield ranking, with the only lots 2001 and 2003 interchanging. The significant differences generated were also the same as for total yield, ie. lot BULK 2 was significantly superior to all other seed lots, and lot 2020 was significantly inferior to all other seed lots (Table 9).

4.1.4.3 Yield of Healthy, Marked/Rotten and Small/Abnormal Sprouts

The yield of healthy sprouts (CAS) differed significantly among black gram seed lots, ranging from 290g for lot 2018 to 521g for lot 2013 (Fig. 2a). Lot 2013 differed significantly from all seed lots except BULK 3 and 2012, while the latter two lots were not significantly different from lot 2017. The remaining four lots did not differ significantly.

The yield of marked/rotten sprouts (CUS) ranged from 2g for lot 2018 up to 280g for lot 2016 (Fig. 2a). Lot 2018 had a significantly lower yield of marked/rotten sprouts than all other black gram seed lots. Lots 2012, 2013, 2017 and 2019 did not differ significantly, while lots 2013 and 2019 were not significantly different from lots BULK 3, 2015 and 2016.

Only lot 2018, with 400g of small/abnormal sprouts (CUS), was significantly different from the remaining seven black gram seed lots, which ranged from 95g for lot 2013 up to 159g for lot 2015 (Fig. 2a).

The yield of healthy sprouts (CAS) produced by green gram seed lots varied significantly among seed lots. The yields ranged from 384g for lot 2001 up to 631g for lot BULK 2 (Fig. 2b). The highest yield of healthy sprouts produced by lot BULK 2 was significantly different from lots BULK 1, 2001, 2004 and 2020, but

similar to lots 2002 and 2003. There was no significant difference among lots BULK 1, 2002, 2003 and 2020, with the lowest yielding lot (lot 2001) only significantly lower than lots BULK 2 and 2002.

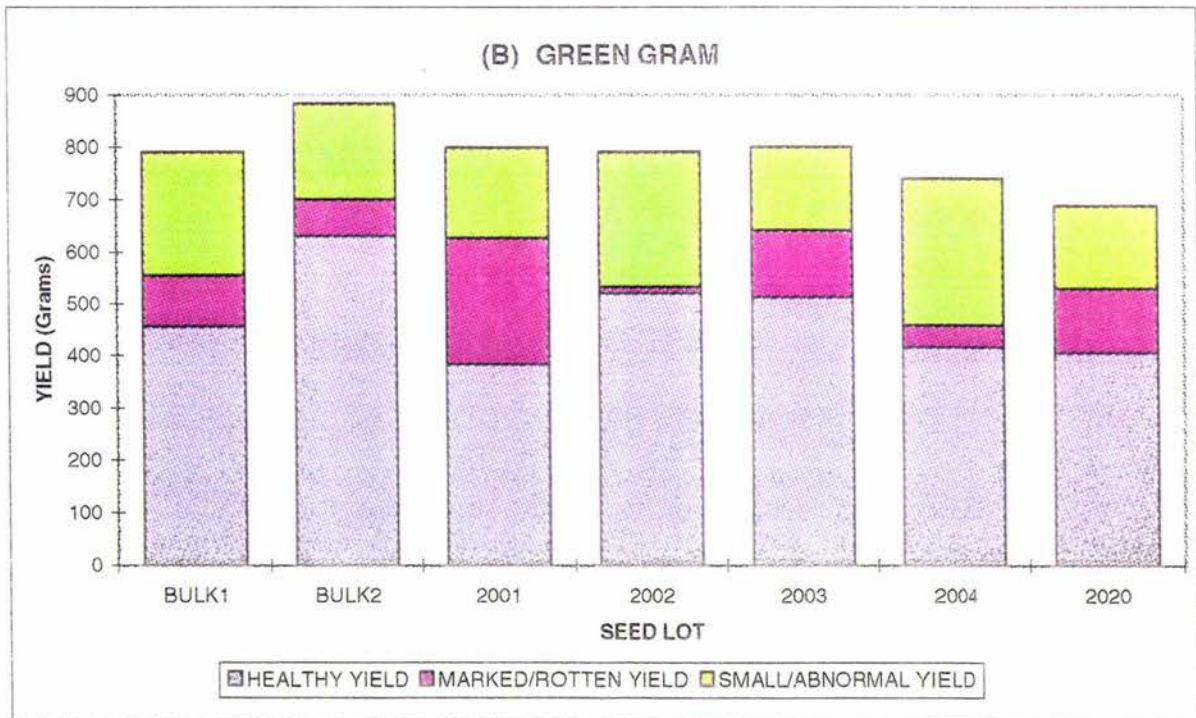
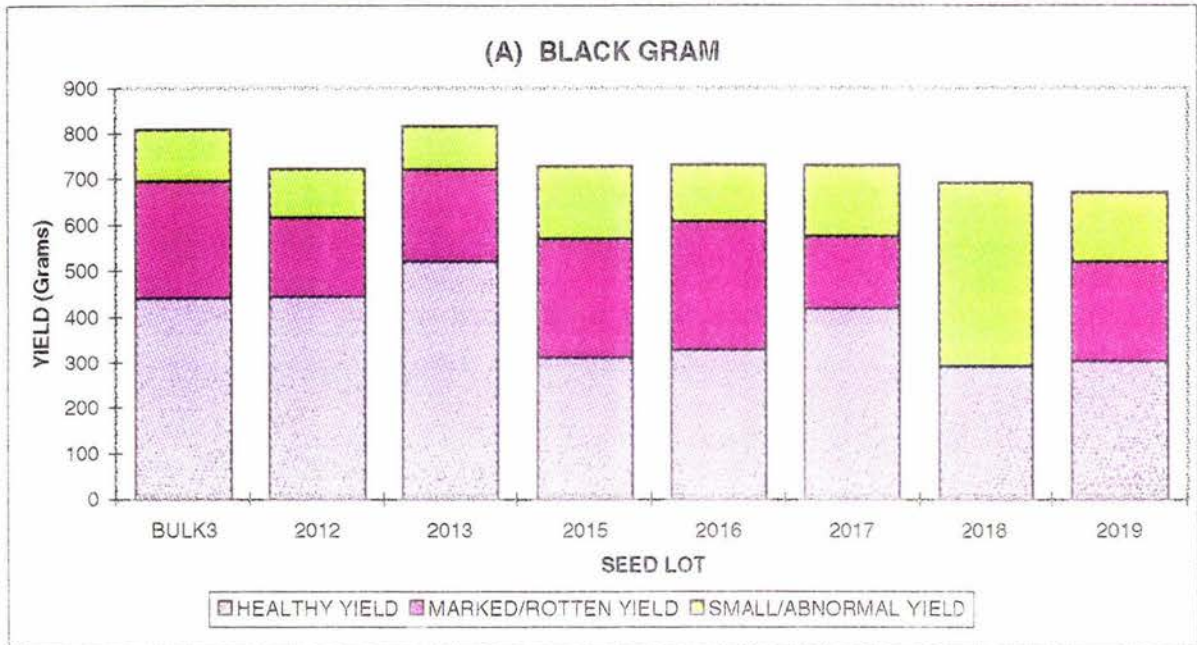
Variations in the yield of marked/rotten sprouts (CUS) were also significant, ranging from 12g for lot 2002 up to 243g for lot 2001 (Fig. 2b). The weight of marked/rotten sprouts was significantly heavier for lot 2001 than for any other green gram lot. Lots BULK 1, 2003 and 2020 did not differ significantly, but all except lot BULK 1 were significantly heavier than lots 2002 and 2004. Lots BULK 2 and 2001 did not differ significantly.

The yield of small/abnormal sprouts (CUS) also differed significantly among seed lots, ranging from 159g for lot 2003 up to 283g for lot 2004 (Fig. 2b).

TABLE 9: Total fresh yield and the ratio of sprouts:seed used (by weight) for all black and green gram seed lots.

SEED LOT	TOTAL FRESH YIELD (g)	YEILD:SEED RATIO (gSprouts:gSeed)
(Black Gram)		
BULK 3	809	6.8
2012	723	6.0
2013	816	6.8
2015	728	6.1
2016	728	6.1
2017	729	6.1
2018	675	5.6
2019	670	5.6
LSD (P<0.05)	49	0.4
(Green Gram)		
BULK 1	818	6.8
BULK 2	885	7.4
2001	800	6.7
2002	792	6.6
2003	800	6.7
2004	741	6.2
2020	689	5.7
LSD (P<0.05)	47	0.4

FIGURE 2: The composition of each seed lot sprouted under SSCP conditions in terms of 'healthy yield', 'small/abnormal yield' and 'marked/rotten yield' for both black gram (A) and green gram (B) seed lots tested.



4.1.4.4 Healthy Sprout Dryweight

The dryweight of healthy black gram sprouts differed significantly among seed lots, ranging from 0.031g for lot 2018 to 0.037g for lot 2013 (Table 9). Lots BULK 3, 2012, 2013, 2016, 2017 and 2019 did not differ significantly, but all differed from lots 2015 and 2018. The latter two lots were not significantly different from each other.

Significant differences in green gram healthy sprout dryweight were also demonstrated, ranging from 0.027g for lot 2002 to 0.038g for lot BULK 1 (Table 9). The two heaviest lots (BULK 1 and 2003) did not differ significantly from each other, but were both significantly heavier than all other seed lots. The remaining seed lots (lots BULK 2, 2001, 2002, 2004 and 2020) were all significantly different from one another.

4.1.4.5 Seedling Growth Evaluation

Sprouts produced under SSCP conditions were measured to determine average, minimum, maximum and range of total sprout length, hypocotyl length, and hypocotyl diameter.

All four sprout length parameters differed significantly length among the eight black gram seed lots (Table 10). Average sprout total length ranged from 120mm for lot 2018 up to 159mm for lot 2013. Sprouts from lots 2017 and 2018 were significantly shorter on average than from the remaining six lots. Conversely, lot 2013 produced significantly longer sprouts than lots 2015, 2017, 2018 and 2019, while lots BULK 3, 2012 and 2016 were not significantly different. There were no significant differences among lots BULK 3, 2012, 2015, 2016 and 2019.

Analysis of maximum total sprout length split seed lots into three significant groups. Maximum length ranged from 146mm for lot 2018 up to 196mm for lot 2012 (Table 10). Lots BULK 3, 2012, 2013, 2015, 2016 and 2019 did not differ significantly, but were significantly longer than lots 2017 and 2018, which differed significantly from one another.

Minimum total length ranged from 92mm for lot 2017 up to 121mm for lot 2016. Lot 2016 was significantly longer than lots 2012, 2015, 2017, 2018 and 2019 but not lots BULK 3 or 2013. Lots 2012, 2018 and 2019 were the shortest and did not differ significantly. Other lots not significantly different were BULK 3, 2013, 2015 and 2015 and 2019.

The range in total length was from 54mm for lot 2018 up to 100mm for lot 2012 (Table 10). Lots 2012 and 2019 had a significantly larger range than all other black gram lots. Lots BULK 3 and 2015 were more variable than lots 2016, 2017 and 2018, but not significantly different to lot 2013, which in turn was not significantly different to lots 2016 and 2017. Sprout length range was smaller for lot 2018 than for all other black gram seed lots (Table 10).

Hypocotyl length differed significantly among black gram lots for average length, maximum length and range (Table 10). Average hypocotyl length ranged from 57mm for lot 2018 to 79mm for lot BULK 3. Seed lots were divided into three significantly different groups; lots BULK 3, 2012, 2013, 2015, 2016 and 2019 did not differ, but were all significantly different from lots 2017 and 2018, and these latter two also differed significantly.

Maximum hypocotyl length ranged from 76mm for lot 2018 to 101mm for lot 2013 (Table 10). Lot 2013 was similar to lot 2012, but was significantly longer than all other lots. Lots BULK 3, 2012, 2015 and 2019 did not differ significantly, but were significantly greater than the remaining two lots (2017 and 2018).

The range in hypocotyl length varied by 23mm among black gram seed lots. Lot 2013 at 50mm had a significantly wider range than lots BULK 3, 2015, 2017 and 2018, but was not significantly different from lots 2012, 2016 and 2019 (Table 10). Lots 2017 and 2018 (both with a range of 27mm) were significantly less variable than all other lots. Lots 2012, 2015, 2016 and 2019 did not differ significantly, and neither did lots BULK 3 and 2015.

Only the average hypocotyl diameter varied among black gram seed lots, ranging from 2.2mm for lot 2015 to 2.4mm for lot 2013 (Table 10). Sprouts in lot 2015 were on average significantly thinner than those in lots 2012, 2013, 2018 and 2019, but did not differ from sprouts in lots BULK 3 and 2016. Lots BULK 3, 2012, 2013, 2016, 2017, 2018 and 2019 did not differ significantly.

All four total length parameters differed significantly among green gram seed lots (Table 10). Average total length ranged from 124mm for lot 2001 up to 138mm for lot BULK 2. Sprouts of lots BULK 2 and 2020 were significantly longer on average than lots 2001, 2002 and 2004, but not from lots BULK 1 and 2003. Lots BULK 1, 2001, 2002 and 2004 did not differ significantly and neither did lots BULK 1, 2002 or 2003.

Maximum total sprout length ranged from 155mm for lot 2001 up to 175mm for lot 2003 (Table 10). Sprouts from lots 2003 and 2020 were significantly longer than lots BULK 1, 2001 and 2004, but did not differ from lots BULK 2 or 2002. Sprouts from lot BULK 2 were significantly longer than those of lots BULK 1 and 2001, while those from lots 2002 were intermediate, and did not differ significantly from any of the green gram seed lots.

Minimum total sprout length ranged from 90mm for lot BULK 1 to 103mm for lot BULK 2 (Table 10). Sprouts in lot BULK 1 were more compact than those from all other green gram seed lots with the exception of lot 2003. Lots BULK 2, 2001, 2002, 2004 and 2020 did not differ significantly, and neither did lots 2001, 2002, 2003 and 2020.

The range in total sprout length was from 59mm for lot 2001 to 83mm for lot 2003 (Table 10). Total minimum sprout length of lot 2003 did not differ significantly from that of lot 2020, while that of lot 2020 did not differ from all other lots.

Neither the average or minimum hypocotyl length differed significantly among green gram seed lots (Table 10). Maximum hypocotyl length ranged by only 12mm among green gram seed lots; the minimum was 78mm (for lot 2004), which was not significantly different from lots BULK 1, 2001 and 2002, while lots BULK 1, BULK 2, 2001, 2003 and 2005 did not differ significantly from one another.

The range among hypocotyl lengths within each green gram seed lot was also significant (Table 10). Lot BULK 2 had the greatest range (41mm) which was significantly different from that for lots BULK 1, 2001, 2002 and 2004, but not from lots 2003 and 2020. Lots BULK 1, 2001, 2003 and 2020 did not differ

significantly and neither did lots BULK 1, 2001, 2002 and 2003.

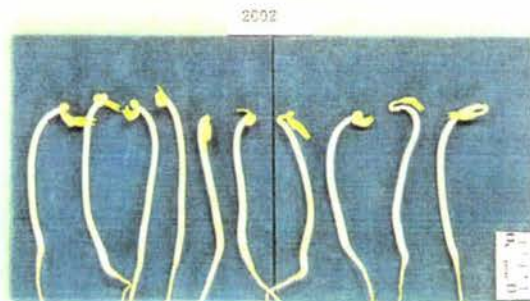
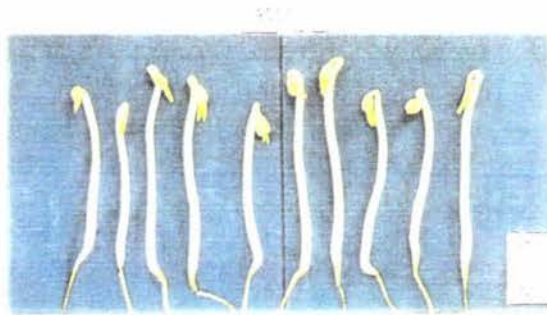
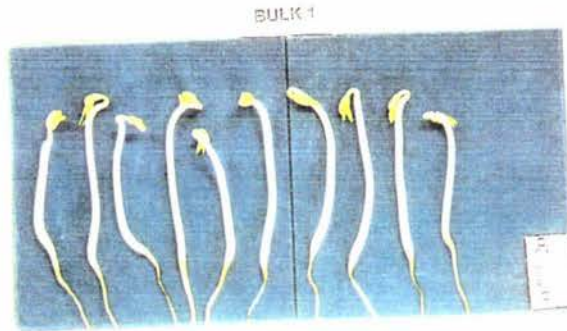
Both the average and range of hypocotyl diameters differed significantly among green gram seed lots (Table 10). Average hypocotyl diameter ranged from 2.5mm for lot 2002 up to 2.8mm for lot BULK 2. Green gram seed lots were divided into two significant groupings, lots BULK 1, BULK 2, 2003 and 2004 and lots 2001, 2002 and 2020.

The range of hypocotyl diameters within a seed lot ranged from 0.7mm for lot 2002 to 1.0mm for lot BULK 2 (Table 10). Lots BULK 1, BULK 2, 2001, 2003 and 2004 did not differ significantly, and neither were lots BULK 1, 2001, 2002, 2003 and 2020. There was significantly greater variation in sprout diameter in lots BULK 2 and 2004 compared to lots 2002 and 2020.

TABLE 10: Results of physical measurements of sprouts produced under SSCP conditions for all seed lots.

SEED LOT	SEEDLING GROWTH EVALUATION - SSCP SPROUTS											
	TOTAL LENGTH	TOTAL LENGTH	TOTAL LENGTH	TOTAL LENGTH	HYPOCOTYL LENGTH	HYPOCOTYL LENGTH	HYPOCOTYL LENGTH	HYPOCOTYL LENGTH	HYPOCOTYL DIAMETER	HYPOCOTYL DIAMETER	HYPOCOTYL DIAMETER	HYPOCOTYL DIAMETER
(Black Gram)	ave. (mm)	max. (mm)	min. (mm)	range (mm)	ave. (mm)	max. (mm)	min. (mm)	range (mm)	ave. (mm)	max. (mm)	min. (mm)	range (mm)
BULK 3	156	195	118	77	79	94	58	36	2.3	2.6	2.1	0.6
2012	150	196	96	100	76	98	53	45	2.4	2.7	2.1	0.6
2013	159	190	114	76	79	101	52	50	2.4	2.7	2.1	0.7
2015	149	188	108	80	77	94	53	41	2.2	2.5	2.0	0.5
2016	153	187	121	66	76	93	48	45	2.3	2.6	2.1	0.5
2017	127	157	92	62	67	80	53	27	2.3	2.7	2.1	0.5
2018	120	146	92	54	57	76	50	27	2.4	2.7	2.1	0.8
2019	149	191	98	93	76	94	50	45	2.4	2.7	2.1	0.5
LSD (P<0.05)	9	9	12	10	5	5	NS	7	0.1	NS	NS	NS
(Green Gram)												
BULK 1	131	159	90	69	69	82	32	32	2.7	3.1	2.3	0.8
BULK 2	138	171	103	67	71	90	41	41	2.8	3.3	2.3	1.0
2001	124	155	97	59	68	82	30	30	2.5	2.9	2.2	0.8
2002	130	165	97	67	69	84	29	29	2.5	2.9	2.2	0.7
2003	136	175	91	83	73	89	36	36	2.7	3.1	2.2	0.9
2004	127	160	99	60	65	78	28	28	2.7	3.1	2.1	1.0
2020	137	174	97	77	69	88	38	38	2.5	3.1	2.2	0.7
LSD (P<0.05)	8	11	6	14	NS	8	8	8	0.1	NS	NS	0.2

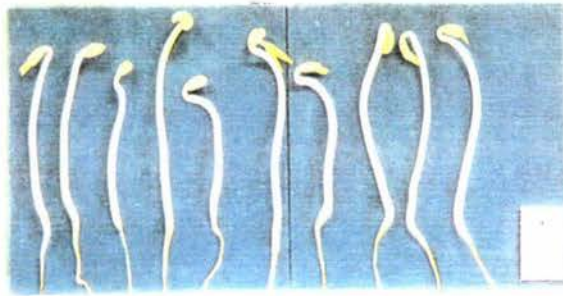
PLATE 4: A collection of photographs illustrating the differences in seedling growth under SSCP conditions, both within and among seed lots tested (each seed lot is numbered in the photograph).



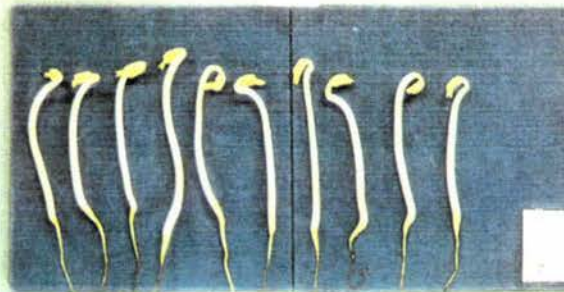
2016



91



2016



4.2 REGRESSION ANALYSIS

4.2.1 *Linear Regression*

4.2.1.1 Seed Moisture Content (SMC)

For green gram seed lots, SMC showed a significant relationship ($P < 0.05$) with SSCP total fresh yield. This standard seed quality test accounted for 63.7% (Table 11) of the variability in SSCP total fresh yield.

This relationship was not repeated among black gram seed lots.

4.2.1.2 Standard Germination (% Normal Seedlings)

Both interim and final counts were regressed against each of the SSCP variables for each species. Interim and final germination were both significantly related ($P < 0.05$) to SSCP total fresh yield among green gram seed lots. Interim germination accounted for 79.1% (Table 11) of the variability in SSCP total fresh yield, while final germination was slightly lower at 76.3% (Table 11).

This relationship was not repeated among black gram seed lots.

4.2.1.3 Oversoak Test - Percent Sprouters

The percent sprouters component of the oversoak seed quality test produced a significant relationship among green gram seed lots with respect to SSCP total fresh yield also. This test accounted for 60.6% (Table 11) of the variability in SSCP total fresh yield.

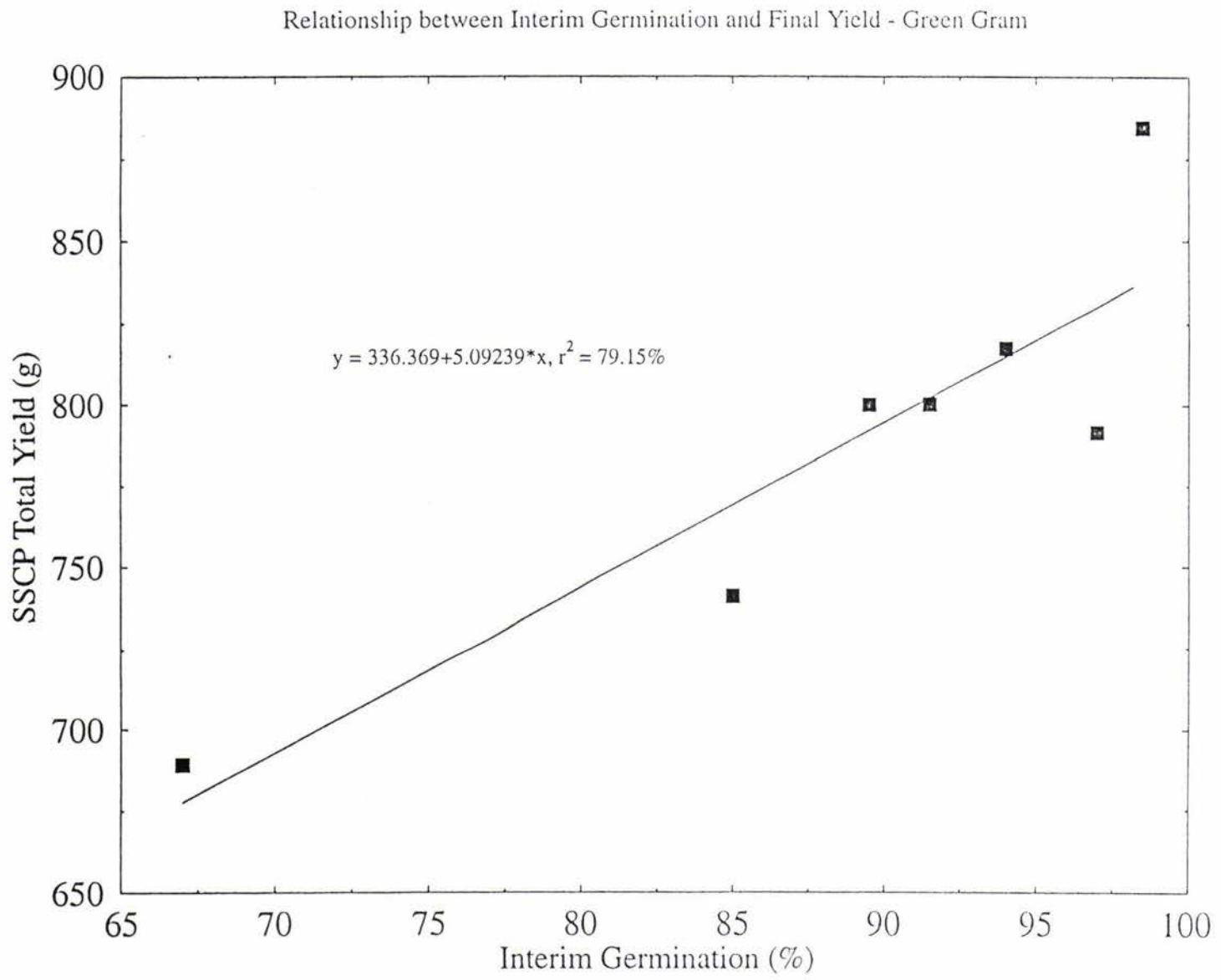
This relationship was not repeated among black gram seed lots.

TABLE 11: Summary of those laboratory based seed quality tests which produced a significant linear relationship with SSCP total fresh yield in green gram.

SEED QUALITY TEST	COEFFICIENT OF VARIATION (%)	Pr>F	SD (g)
INTERIM GERMINATION	79.1	0.01	32.6
FINAL GERMINATION	76.3	0.01	30.6
SEED MOISTURE CONTENT	63.7	0.03	40.4
OVERSOAK SPROUTERS	60.6	0.04	42.1

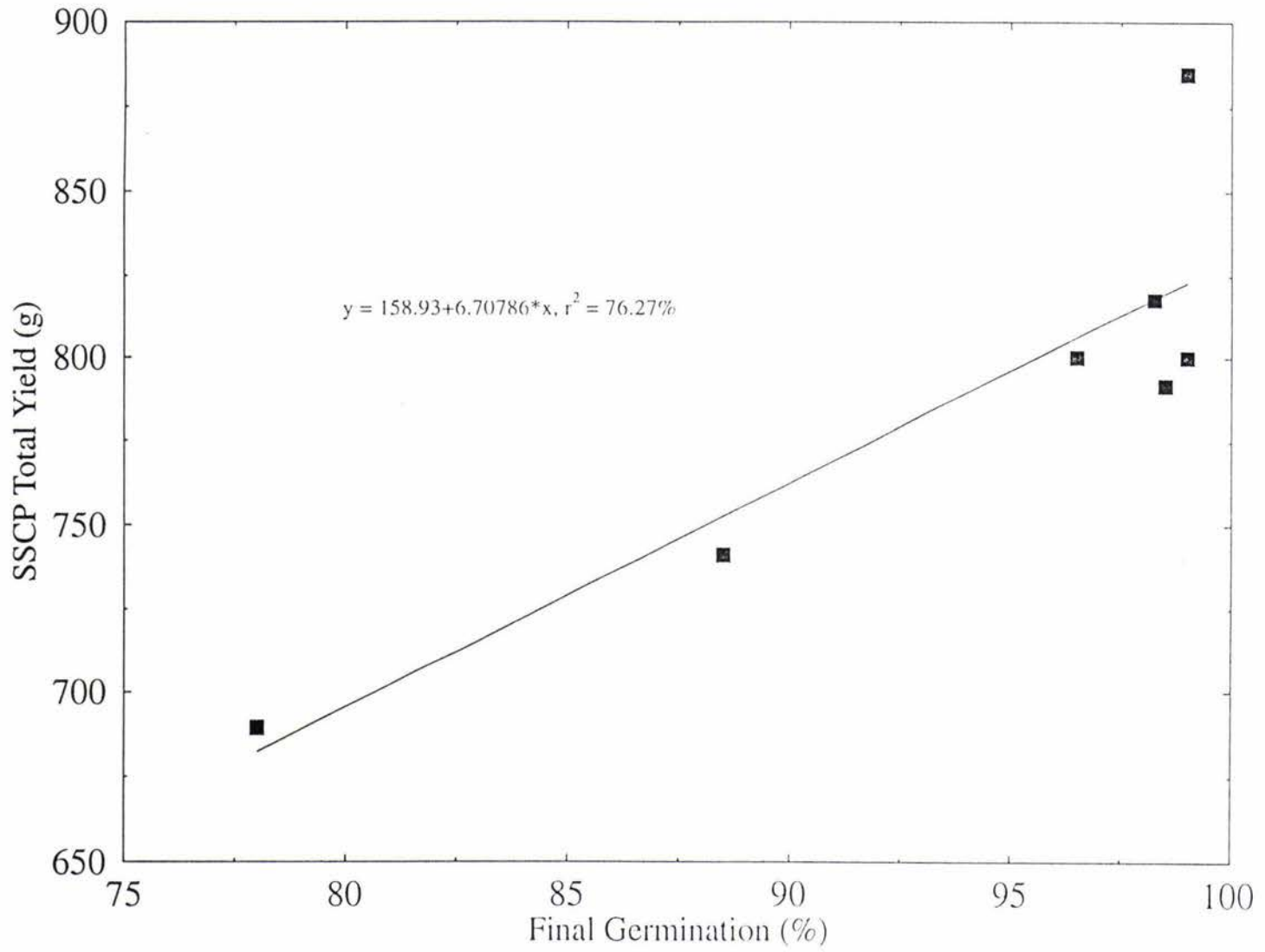
(NOTE: All other linear regression data (those relationships which were not significant for both black gram and green gram seed lots) are presented in Appendix 3A and 3B.)

A.

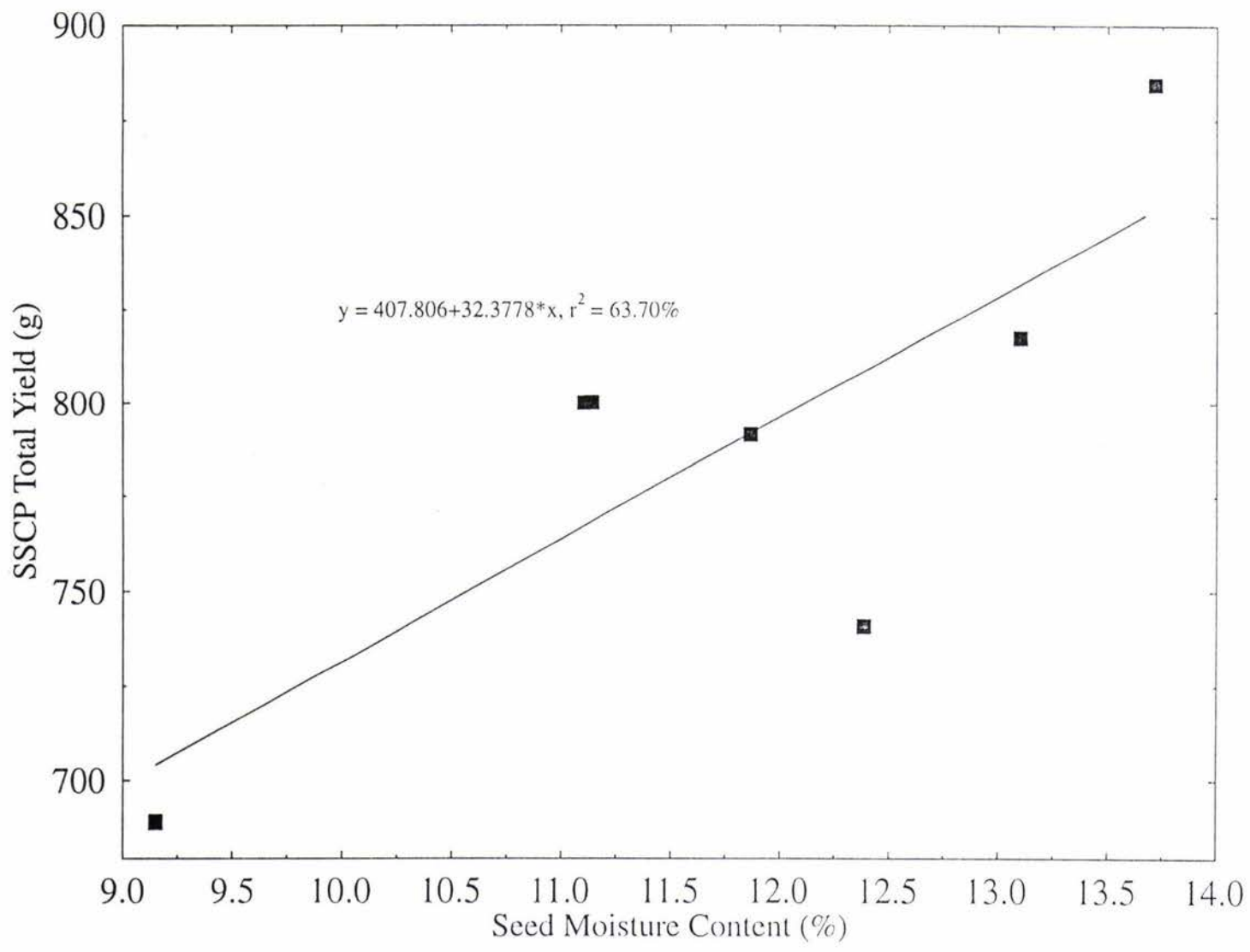


FIGURES 3A-D: Linear regression for seed quality tests which produced significant relationships with SSCP total fresh yield.

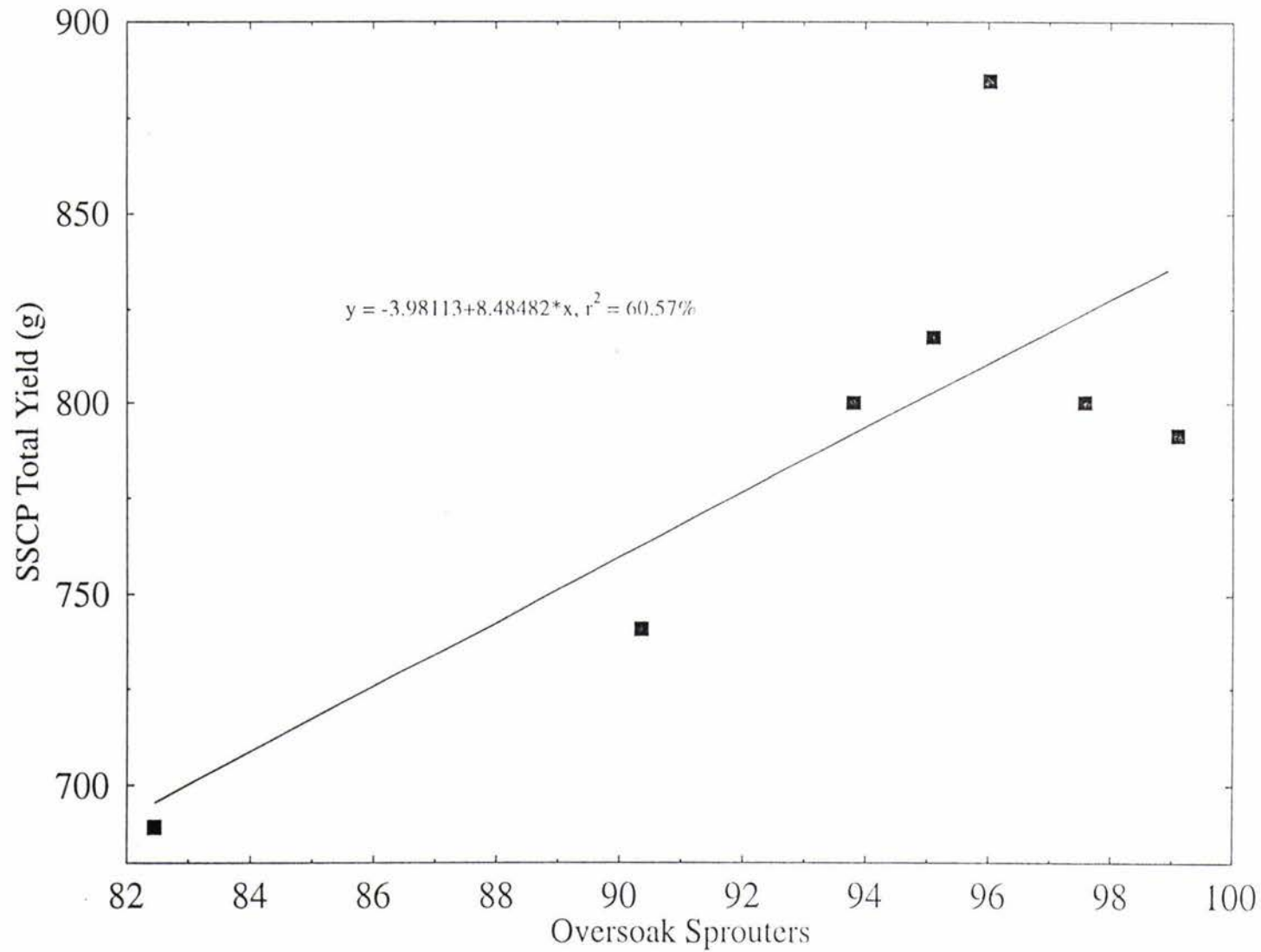
Relationship between Final Germination and Final Yield - Green Gram



Relationship between Seed Moisture Content and Final Yield - Green Gram



Relationship between Oversoak Sprouters and Final Yield - Green Gram



4.2.2 Multiple Regression

Multiple regression analysis was conducted among green gram seed lots and SSCP total fresh yield only, as there were no significant relationships among black gram seed lots and either SSCP total fresh yield or SSCP healthy yield. Multiple regression models are for estimating sprouting quality (potential) for green gram seed lots are expressed below, in the form of:

$$Y = aX_1 + bX_2 + C$$

Where: Y = Estimated Sprouting Performance
 aX_1 = Constant * Seed quality test #1
 bX_2 = Constant * Seed quality test #2
 C = Intercept

All multiple regression results analysed are listed in Table 12 (pp. 102).

4.2.2.1 Interim Germination + Final Germination

When interim and final germination (% normal seedlings) were regressed, the level of variability in SSCP total fresh yield explained was only slightly higher ($R^2=79.8\%$) than variability explained by using interim germination ($R^2=79.1\%$) alone. This is not surprising, as the germination rate of green gram is relatively fast, and the final germination count is conducted only two days after the interim germination count.

The model produced by this combination of standard seed quality test is:

$$Y = 3.6(\% \text{interim germination}) + 2.1(\% \text{final germination}) + 271.8$$

4.2.2.2 Interim Germination +Oversoak Sprouters

This combination of seed quality tests accounted for the highest proportion (at $P < 0.05$) of variability in SSCP total fresh yield among green gram seed lots ($R^2 = 84.2\%$). This combination accounted for just over 5% more of the variability in SSCP total fresh yield than interim germination alone.

The model produced by this combination of standard seed quality test is:

$$Y = 9.1(\% \text{interim germination}) - 8.1(\text{oversoak sprouters}) + 731.4$$

4.2.2.3 Interim Germination + Seed Moisture Content (SMC)

Again, this combination accounted for only a slightly greater percentage of variability in SSCP total fresh yield ($R^2 = 80.6\%$) than the interim germination alone ($R^2 = 79.1\%$). This combination was much better than using SMC alone ($R^2 = 63.7\%$) however.

The model produced by this combination of standard seed quality test is:

$$Y = 4.1(\% \text{interim germination}) + 8.6(\% \text{SMC}) + 323.4$$

4.2.2.4 Final Germination + Seed Moisture Content (SMC)

Not surprisingly, this combination produced a significant relationship with SSCP total fresh yield. With an R^2 of 83.8%, combining results from final germination (%normal seedlings) with SMC was the second best determinant of SSCP total fresh yield.

The model produced by this combination of standard seed quality test is:

$$Y = 4.7(\% \text{final germination}) + 15.3(\% \text{SMC}) + 165.4$$

TABLE 12: Results of multiple regression analysis among standard seed quality tests and SSCP total fresh yield for green gram seed lots.

SEED QUALITY TESTS	COEFFICIENT OF VARIATION (%)	Pr>F	SD (g)
INTERIM+FINAL+OVERSPR	86.5	0.08	31.8
INTERIM+OVERSPR+FINAL	86.5	0.08	31.8
INTERIM+FINAL	79.8	0.04	33.7
INTERIM+FINAL+SMC+OVER SPR	87.0	0.24	38.2
INTERIM+OVERSPR+SMC+FI NAL	87.0	0.24	38.2
INTERIM+OVERSPR+SMC	86.6	0.08	31.7
INTERIM+SMC+OVERSPR	86.6	0.08	31.7
INTERIM+OVERSPR	84.2	0.02	29.7
INTERIM+SMC	80.6	0.04	33.0
FINAL+SMC+OVERSPR	84.5	0.10	34.0
FINAL+OVERSPR	77.3	0.06	35.7
FINAL+SMC	83.8	0.03	30.2

WHERE: Interim = Interim germination (%normal seedlings)

Final = Final germination (%normal seedlings)

SMC = Seed moisture content (%)

Overspr = Oversoak sprouter (%sprouters)

CHAPTER 5

DISCUSSION

Regardless of whether sprout production is on a commercial basis or done in the home, the sprouter aims to produce a product which is tasty, attractive in appearance and safe to eat. Factors considered important in consumer acceptance include: sprout colour, sprout size and uniformity, absence of secondary roots, age and contaminants. Commercial sprouters in New Zealand, Australia, Europe and the USA have recently expressed concern regarding the quality of sprouts being produced. The emphasis has been on uniformity of yield and form, both within and between batches. Both general seed quality and the lack of understanding of relationships which may exist between seed quality parameters and subsequent sprout quality are regarded as major constraints to consistent high quality sprout production. The objective of this study was therefore to evaluate the ability of standard seed quality tests to select mungbean seed lots suitable for sprouting.

If results from standard tests for each seed lot as received are compared to the requirements set out by the Australian Mungbean Association (Appendix 5), then lots BULK 3, 2012, 2013, 2015 and 2018 of black gram and lots BULK 1, BULK 2 and 2004 of green gram would be rejected, mostly on the basis of seed moisture content. SMC recommendations are normally made to ensure safe storage and handling of seed lots. Most seeds can be handled safely at SMC less than 14%, but the AMA restricts SMC to 12% or less for mungbean seed. If the former SMC was required instead, only lots 2015 and 2004 would be rejected. Since SMC is a

seed quality parameter that can be rectified simply by drying in most cases, this would suggest that all but two lots (2015 and 2004) were of acceptably high quality and suitable for sprouting as determined by Australian standards for 'premium' sprouting mungbeans. Under the International Sprout Growers Association (ISGA - cited by Law & Law, 1991) requirements, again disregarding the 12% SMC limit, lots BULK 3, 2013, 2015 and 2016 of black gram and lots BULK 1, 2001 and 2004 of green gram would have failed, either due to lower than permitted germination or a higher than permitted proportion of oversoaks. Under the simulated commercial sprouting conditions (SSCP - 19^o C; 20^o C; 5 days) used in this trial to evaluate seed lot suitability for sprouting, neither the AMA or ISGA standards were consistently capable of identifying those seed lots which proved to be the best sprouting performers.

Commercial sprouters regard total fresh yield, yield of healthy sprouts and the ratio of sprouts produced per unit of seed used (kg:kg) as the three most important parameters of successful sprout production. In conjunction with these, the uniformity of sprout size and sprout colour are also important, although not often measured directly. In this trial, seed lots were sprouted under conditions maintained as close as possible to those experienced under large scale sprout production in New Zealand. Each sprouted seed lot underwent a series of 21 measurements in an attempt to understand the difference among lots and to rank lots in order of suitability for sprouting. Final rankings were based on the performance of each seed lot with respect to the three parameters considered to be of greatest importance by the commercial sprouter.

Seed lots within each species could be grouped into three categories: Best Performers - lots BULK 3 and 2013 (black gram) and lots BULK 2 and 2002 (green gram); Intermediate Performers - lots 2012 and 2017 (black gram) and lots BULK 1 and 2003 (green gram); Poor Performers - lots 2015, 2016, 2018 and 2019 (black gram) and lots 2001, 2004 and 2020 (green gram).

Although each seed quality test investigated was able to differentiate among seed lots (ANOVA), linear regression analysis indicated there was no single test capable of consistently ranking black gram seed lots in terms of either SSCP total fresh yield or SSCP healthy yield. However, there were four laboratory based seed quality assessment tests (interim germination, final germination, SMC and oversoak sprouters) which were significantly related to SSCP total fresh yield among green gram seed lots (again, no test related significantly to SSCP healthy yield for green gram seed lots either). These tests were then combined using multiple regression analysis in an attempt to improve the accuracy of rankings among green gram seed lots (ie. to decrease the level of variability in SSCP total fresh yield not accounted for by each individual test).

The following discussion concentrates on those seed quality tests which proved to be sufficiently significantly related ($P < 0.05$) to SSCP total fresh yield.

5.1 Seed Moisture Content

Seed moisture content (SMC) is very important in maintaining seed quality, especially viability and health. Seeds with high SMC are prone to bruising during harvesting and handling, and microbial attack during storage. Seeds which are too dry on the other hand are prone to testa fracturing and cracking during the same processes. A SMC of 14% is recommended for mungbean harvesting,

compared with 13% for handling and grading (Walsh, 1991; Agnew & Lucy, 1994), and less than 12% for storage and sprouting (AMA No. 2 Newsletter, undated).

SMC accounted for 63.7% of the variability demonstrated in SSCP total fresh yield among green gram seed lots. This relationship was the reverse of that which was expected, since a low SMC (<12%SMC) resulted in both lower yields and ratio of sprouts:seed used. In fact, two of the three seed lots which were above the AMA recommended SMC (12%SMC) were lots BULK 1 and BULK 2 (the other being lot 2004). The reason for this trend is unclear, but perhaps seeds which are too dry take longer to imbibe and hence germinate, resulting in smaller sprouts. It may also be possible that seed lots with low SMC have hard seed induced, eg. lots 2016 and 2020 in black and green gram respectively.

5.2 Germination (Interim and Final)

Germination in seed testing terms measures the ability of a seed to produce a normal seedling. Interim germination has been used in the past as an indicator of seed vigour (Perry, 1987), but difficulties in standardisation have limited the use of this test (McDonald, 1980; Perry, 1987). Many sprouters request information on the interim germination count, because they think this indicates the uniformity of a seed lot. Interim germination may also be associated with sprouting performance in mungbeans on the basis that interim counts are on days 4 and 5 for black gram and green gram respectively, a time period similar to that used by sprouters. Final germination describes the proportions of a seed lot in terms of normal seedlings, abnormal seedlings, dead seed and hard seed. All four of these components are important in sprouting. Seed lots with high dead or hard seed content are likely to produce poor yields, while a high content of abnormal

seedlings may result in poor quality sprouts. Abnormal seedlings and dead seeds are prone to microbial attack, again reducing sprout quality. Sprouters have expressed concern however, about the variation in the quality of sprouts which can often occur, even in seed lots which demonstrate a high germination prior to sprouting.

Interim germination was relatively strongly related to SSCP total fresh yield, with a coefficient of variation of 79.1% among green gram seed lots. Results were also similar for final germination, where the coefficient of variation was 76.3%. This was expected in both cases, since seed lots with high hard seed and dead seed components are identified in both tests, and both are detrimental to total sprout yield and the subsequent ratio of sprouts:seed used. In this species interim germination was counted after the same period of time as seeds were sprouted under SSCP conditions, but the temperature used was 5°C higher. After both interim and final germination, over 20% of the variability in total yield and ratio was still unexplained, again indicating a multi-parameter effect.

The fact that no similar relationships were found among black gram seed lots is surprising, considering the apparent importance of germination with respect to sprout yield and the generic similarity between the two species. The main difference between the species was that black gram germination was slightly less variable, (mainly due to the fact that lot 2020 of green gram had such a high hard seed content).

Both the Australian and ISGA seed quality requirements allow hard seed content to be added to final germination (normal seedlings), implementing an upper limit of 6% (Australia only) for all cultivars except cvs. Regur and Emerald, for which

there is no limit. This discretion between cultivars perhaps seems inappropriate for two reasons. Firstly, lot 2020 in this study was cv. Emerald harvested in March 1996, which had a hard seed content of 21% at the commencement of testing, and despite a period of 10 months of storage the hard seed content remained high (rate of germination test). This high level of hard seed and apparently slow breakdown of dormancy suggests lot 2020 was unsuitable for sprouting. Secondly, some seed lots which had lower levels of hard seed content (as low as 3% for lot 2015) were also poor sprout yielders. It would appear that values for germination and hard seed should not be combined, and that only seed lots with very low hard seed content should be accepted for sprouting.

5.3 Oversoak - Sprouters

This test is based on the principle that seeds which imbibe rapidly are of poor quality. Seeds which are either dead or have a damaged testa tend to take up water rapidly as a direct result of poor membrane integrity. The exact relationship between this test and sprout quality is not well understood (Law & Law, 1991), although seed lots with a high percentage of oversoaks could be low yielding (due to the number of dead seeds) or have a high percentage of marked sprouts (due to testa and cotyledon damage). Seeds which have been damaged by field weathering, during handling, or by insect attack, would be expected to be identified in the oversoak test because of the testa disruption. A high number of dead and damaged seeds may also increase the probability of microbial contamination during sprouting, directly affecting marketable yield. Dead and damaged seeds are often prone to high levels of microbial loading (Neergaard, 1979; Imrie, Lawn & Yeates, 1991).

Those seeds not removed during the prescribed oversoak period (1hr) were allowed to germinate over a further 24 hour period and were then counted. At the end of this extended period, seeds which had germinated (% sprouters) had an emerged radicle 5-15mm long. The number of sprouters is expressed as a percentage of the total number of seeds entering the oversoak test. Low sprouter value results may be due to a high level of oversoaks, a high number of dead seeds and/or high hard seed content. It appears that this test, being simply an extension of the oversoak test, gives a better overall picture of the quality of the seed lot, ie. hard seed content. Due to the nature of the test, the number of sprouters would not be expected to vary significantly from the combined total of normal and abnormal seedlings recorded during the germination test. However the former test does not differentiate normal from abnormal seedlings. There appears to be no information on the relationship between sprouters and sprout quality, or on the level of sprouters which are commercially acceptable. In Australia and the USA, only the oversoaks are currently recorded.

The coefficient of variation between the level of oversoak sprouters and SSCP total fresh yield for green gram seed lots was 60.6%. This was the SSCP variable expected to be most strongly related to the level of sprouters, since final yield is dependent on both the number of sprouts per unit of seed used and on sprout size. Seed lots which had a high percentage of sprouters would theoretically be more likely to produce a larger number of sprouts. However, an interaction must exist between the number of sprouts and the weight of sprouts produced. This theory of an interaction would appear to be the case, since just under 40% of the variability in SSCP total fresh yield could not be explained by the percentage of sprouters alone.

5.4 Combining Standard Seed Quality Test Results

At best, only 79.1% of the variability in SSCP total fresh yield was explained by a 'stand-alone' standard seed quality test - (% normal seedlings for the interim germination), leaving just over 20% of the variability in SSCP total fresh yield among green gram seed lots unexplained. Multiple regression of data for those tests which proved significantly related in the linear regression analysis, reduced this unknown component to just over 15% - when interim germination and oversoak sprouters were combined, ($Y = 9.1(\% \text{interim germination}) - 8.1(\% \text{oversoak sprouters}) + 731.4$). Just under 16% of the variability remained unexplained when final germination (% normal seedlings) and seed moisture content (SMC) were combined ($Y = 4.7(\% \text{final germination}) + 15.3(\% \text{SMC}) + 165.4$).

The latter model would probably be preferred at this stage as both these seed quality tests are frequently conducted in seed testing laboratories whereas the oversoak test is not, and would require extra equipment and training. Variability among laboratories is likely to be much lower for any one seed lot, when a common, frequently conducted test is used.

Although combining either interim germination and oversoak sprouters or final germination and seed moisture content produced a better predictor of sprouting quality than any individual test, neither combination accounted for enough of the variability to allow a commercial sprouter to confidently rank various seed lots based on the seed testing information available. It is for this reason also, that it is not practical to determine the limits between high/medium/low quality seed lots (eg. high >90% versus medium 75-89% versus low <74%) with respect to sprouting quality.

It is difficult to speculate what other factor(s) contribute to total fresh yield in green gram sprouts. However it is important to note that Scott and Close (1976b) tested 98 different seed lots before they determined the model for effective field emergence (EFE) in garden pea seed production, and this relationship was then not validated by Hampton and Scott (1982), when only five seed lots were evaluated. Further testing of green gram seed lots, of known origin, is required before all other seed quality tests can be eliminated from the seed testing regime.

5.5 Black Gram Versus Green Gram

One of the most surprising findings in this study was the pronounced differences demonstrated between the two species tested. In general, commercial sprouters have considered both black and green gram species to be very similar, although black gram sprouts are renowned as being whiter in colour, while green gram sprouts are easier to produce consistently (Ashley Berrysmith pers. comm., 1995). This study was not able to determine the reason for such differences demonstrated, but genetic differences would appear to be the obvious factor. Genetic variation can be reflected in many ways. Difficulties arise when trying to differentiate the effect of variation in genetic makeup and variation caused by environmental factors experienced during development and handling.

Previous studies conducted on green gram seed lots have shown that many cultivars have a strong predisposition to weathering (Williams *et al*, 1995b), especially cv. Berken, which is the Australian standard for sprouting mungbeans. The degree of weathering resistance is dependent on both pod wall thickness and level of hard seed content (Bruce Imrie pers. comm., 1996). Breeding for weathering resistance has produced cv. Emerald, which in this study proved to

have an extremely high hard seed content (lot 2020). The greater variation among green gram seed lots, and the fact that the oversoak test was developed based on tests conducted on green gram seed lots only (Mary-Ann Law pers. comm., 1996), may explain why there was a relationship between oversoak sprouters and SSCP total fresh yield for this species, and not black gram.

Under SSCP sprouting conditions, it was observed that black gram seed lots appeared to have a higher level of marked/damaged/rotten sprouts than green gram seed lots. Further analysis involving the relationships between seed quality tests and SSCP marked/rotten yield may have produced a better insight into this peculiarity. The level of marked/rotten sprouts may in fact be a better predictor of sprouting quality of black gram seed lots. One reason for this increased variation between species may originate from the fact that black gram has an indeterminate flowering pattern. At harvest, only a proportion of the seeds thrashed will be ripe and sufficiently dry, the remainder will be more susceptible to damage. The downfall of this theory, is that any damage to seed lots resulting in testa stress or damage to the seed's membrane integrity should have been picked-up in the oversoak test. Fine-tuning of this test using black gram seed lots may improve this test's ability to rank seed lots with respect to sprouting quality.

Results produced from this study suggest that perhaps present seed quality testing procedures may require reviewing for the black gram species.

CHAPTER 6

CONCLUSIONS & SCOPE FOR FUTURE WORK

6.1 Conclusions

Based on the results of this study, the following conclusions can be drawn:

- (i) Under the conditions used for SSCP of sprouts in this study, every single standard seed quality test was able to differentiate among seed lots within each species (ANOVA). However, these rankings were not consistent among the various seed tests, and there were no significant linear relationships found between seed quality tests and SSCP total fresh yield or SSCP healthy yield among black gram seed lots. The reason for this difference between species is unclear, but could possibly be due to genetic differences (ie. resistance to weathering damage) and/or production history (ie. irrigation and desiccation) of the various seed lots tested.

- (ii) Linear regression analysis indicated that SSCP total fresh yield was a multifactor variable. Combining test results from the interim germination and oversoak sprouter tests (multiple regression) for green gram seed lots reduced the unknown variability in SSCP total fresh yield down to 15.8%, which was 5.1% less than using interim germination results alone. There is still a relatively large proportion of the variability in SSCP left unexplained by the laboratory seed quality tests conducted.

- (iii) Although all vigour tests (AA, conductivity, rate of germination and uniformity of germination) could be used to separate seed lots within species, they were not able to consistently rank seed lots on the basis of their suitability for commercial sprouting.
- (iv) The percentage of sprouters in the oversoak test related better than most other seed quality tests to sprouting performance, and further refinement of this test appears to be warranted, especially for black gram.
- (v) It appears that there is no single seed quality parameter which is entirely responsible for total yield or uniformity of sprouts produced. The results suggest total yield and uniformity of sprouts produced are determined by the interaction of several quality parameters.

6.2 Limitations and Scope for Future Work

Currently there has been little work published on commercial sprout production. This is reflected in the poor understanding of relationships between input variables (ie. seed, water, temperature) and subsequent sprout quality amongst sprouters. An understanding of these relationships is paramount if consistent production of high quality sprouts is going to be achieved. Presently, New Zealand consumers have little choice as to their commercial source of sprouts, allowing producers to concentrate much more on total yield than on sprout quality. Increasing competition in the market will no doubt improve sprout quality.

The use of only one SSCP sprouting regime (water @ 19°C; sprouting chamber @ 20°C) was perhaps a limitation in assessing laboratory based tests in terms of their ability to rank seed lots for the purpose of sprouting in this study.

Relationships are likely to vary between seed quality characteristics and subsequent sprout quality at different sprouting temperatures. Regrettably the time limitation imposed on this study meant the effect of sprouting temperature could not be more fully assessed under SSCP conditions. The results obtained are likely to be of most use to Japanese and New Zealand sprouters, who use lower temperatures in commercial sprout production. On a more positive note, it would appear this study is the first in which commercial sprouting conditions have been simulated so closely for the purpose of assessing seed quality effects on sprout performance.

Definite conclusions about seed quality and subsequent sprout quality were made more difficult in this study due to the lack of 'pre-received' history details of the seed lots used. Differences expressed among seed lots may have been at least in part due to differences in agronomic management and practices which occurred during seed development and processing, or because of genetic differences among cultivars. Since this was an industry based study, with no prior research reported in this area, and because sprouters have little say over seed production practices, it had been decided to use as many seed lots from as many different sources as possible, to see if any one or a combination of laboratory test(s) was able to rank seed lots in order of suitability for sprouting as determined by SSCP.

This study is only the beginning of the work needed to assist the commercial sprouting industry. The next step in mungbean sprout research could be an attempt to study the effect of sprouting temperature, watering intervals and total water applied on SSCP sprouts. Perhaps fewer seed lots should initially be used, with selection based on similar pre-received histories. The oversoak test shows

the most promise as a single test able to determining sprouting quality of seed lots. Further refinement of this procedure may also increase the strength of this relationship, especially among black gram seed lots. This would seem to be an appropriate starting point for further research. Certainly, it would be expected that further work would result in changes to the present seed quality requirements as stipulated by various sprouting organisations (AMA , ISGA, etc...) which seem to be based on industry whims rather than any scientific evidence.

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APPENDIX 1A:

Summary of health test results of black gram seed lots tested, (A) Field fungi on PDA agar only, and (B) Storage fungi on PDA + Salt agar.

(A)

SEED LOT	REP. #	# SEEDS PER REP.	TOTAL # COLONIES	FUNGAL SPECIES IDENTIFIED
BULK 3	1	5	2	2 * <i>Alternaria alternata</i>
BULK 3	2	5	3	2 * <i>Alternaria alternata</i> ; 1 * <i>Aspergillus glaucus</i>
2012	1	5	2	1 * <i>Aspergillus niger</i> ; 1 * <i>Botrytis</i> spp.
2012	2	5	0	
2013	1	5	0	
2013	2	5	0	
2015	1	5	1	1 * <i>Fusarium</i> spp.
2015	2	5	0	
2016	1	5	4	4 * <i>Alternaria alternata</i>
2016	2	5	5	4 * <i>Alternaria alternata</i> ; 1 * <i>Penicillium</i> spp.
2017	1	5	0	
2017	2	5	0	
2018	1	5	0	
2018	2	5	0	
2019	1	5	7	4 * <i>Alternaria alternata</i> ; 2 * <i>Epicoccum purpurascens</i> ; 1 * <i>Fusarium</i> spp.
2019	2	5	2	2 * <i>Alternaria alternata</i>

(B)

SEED LOT	REP. #	# SEEDS PER REP.	TOTAL # COLONIES	FUNGAL SPECIES IDENTIFIED
BULK 3	1	5	1	1 * <i>Aspergillus glaucus</i>
BULK 3	2	5	1	1 * <i>Alternaria alternata</i>
2012	1	5	4	3 * <i>Aspergillus glaucus</i> ; 1 * <i>Aspergillus flavus</i>
2012	2	5	2	2 * <i>Aspergillus glaucus</i>
2013	1	5	0	
2013	2	5	0	
2015	1	5	0	
2015	2	5	1	1 * <i>Alternaria alternata</i>
2016	1	5	6	2 * <i>Aspergillus glaucus</i> ; 2 * <i>Aspergillus flavus</i> ; 3 * <i>Alternaria alternata</i>
2016	2	5	5	4 * <i>Aspergillus glaucus</i> ; 1 * <i>Alternaria alternata</i>
2017	1	5	0	
2017	2	5	0	
2018	1	5	0	
2018	2	5	0	
2019	1	5	3	3 * <i>Alternaria alternata</i>
2019	2	5	3	3 * <i>Alternaria alternata</i>

APPENDIX 1B: Summary of health test results of green gram seed lots tested, (A) Field fungi on PDA agar only, and (B) Storage fungi on PDA + Salt agar.

(A)

SEED LOT	REP. #	# SEEDS PER REP.	TOTAL # COLONIES	FUNGAL SPECIES IDENTIFIED
BULK 1	1	5	1	1 * <i>Chaetomium funicola</i>
BULK 1	2	5	1	1 * <i>Penicillium</i> spp.
BULK 2	1	5	0	
BULK 2	2	5	0	
2001	1	5	2	1 * <i>Aspergillus niger</i> ; 1 * <i>Penicillium</i> spp.
2001	2	5	0	
2002	1	5	0	
2002	2	5	1	1 * <i>Aspergillus glaucus</i>
2003	1	5	1	1 * <i>Aspergillus glaucus</i>
2003	2	5	0	
2004	1	5	2	2 * <i>Penicillium</i> spp.
2004	2	5	0	
2020	1	5	0	
2020	2	5	0	

(B)

SEED LOT	REP. #	# SEEDS PER REP.	TOTAL # COLONIES	FUNGAL SPECIES IDENTIFIED
BULK 1	1	5	0	
BULK 1	2	5	0	
BULK 2	1	5	1	1 * <i>Aspergillus glaucus</i>
BULK 2	2	5	0	
2001	1	5	0	
2001	2	5	0	
2002	1	5	0	
2002	2	5	0	
2003	1	5	0	
2003	2	5	0	
2004	1	5	0	
2004	2	5	0	
2020	1	5	1	1 * <i>Aspergillus niger</i>
2020	2	5	0	

APPENDIX 2: Results of physical measurements of sprouts produced in the 'Seedling Evaluation (Uniformity)' test for all seed lots.

SEED LOT	SEEDLING GROWTH EVALUATION - UNIFORMITY TEST											
	TOTAL LENGTH	TOTAL LENGTH	TOTAL LENGTH	TOTAL LENGTH	HYPOCOTYL LENGTH	HYPOCOTYL LENGTH	HYPOCOTYL LENGTH	HYPOCOTYL LENGTH	HYPOCOTYL DIAMETER	HYPOCOTYL DIAMETER	HYPOCOTYL DIAMETER	HYPOCOTYL DIAMETER
(Black Gram)	ave. (mm)	max. (mm)	min. (mm)	range (mm)	ave. (mm)	max. (mm)	min. (mm)	range (mm)	ave. (mm)	max. (mm)	min. (mm)	range (mm)
BULK 3	171	228	99	129	85	125	34	91	1.9	2.3	1.6	0.7
2012	160	213	113	100	75	124	42	83	2.0	2.3	1.6	0.7
2013	160	223	108	116	70	120	35	85	2.0	2.3	1.6	0.6
2015	169	215	115	101	82	121	39	85	1.8	2.0	1.4	0.6
2016	168	231	103	128	81	130	39	90	2.0	2.3	1.7	0.6
2017	138	185	92	93	77	130	38	92	1.9	2.1	1.6	0.5
2018	145	207	92	115	68	111	35	76	1.9	2.3	1.6	0.6
2019	150	216	98	118	71	120	37	83	2.0	2.2	1.6	0.6
LSD (P<0.05)	17	NS	NS	NS	11	NS	NS	NS	NS	NS	NS	NS
(Green Gram)												
BULK 1	105	172	50	123	52	104	16	88	2.4	2.8	2.0	0.8
BULK 2	137	196	82	114	64	103	28	75	2.6	2.9	2.2	0.7
2001	123	181	76	105	64	102	30	72	2.4	2.6	1.9	0.7
2002	132	196	84	113	67	112	28	84	2.3	2.7	1.9	0.8
2003	108	178	50	129	52	107	16	91	2.4	2.6	2.3	1.0
2004	135	193	75	118	66	103	26	77	2.5	2.8	2.2	0.6
2020	101	171	54	117	46	87	13	74	2.4	2.6	2.1	0.5
LSD (P<0.05)	25	NS	32	NS	NS	NS	12	NS	NS	0.2	NS	NS

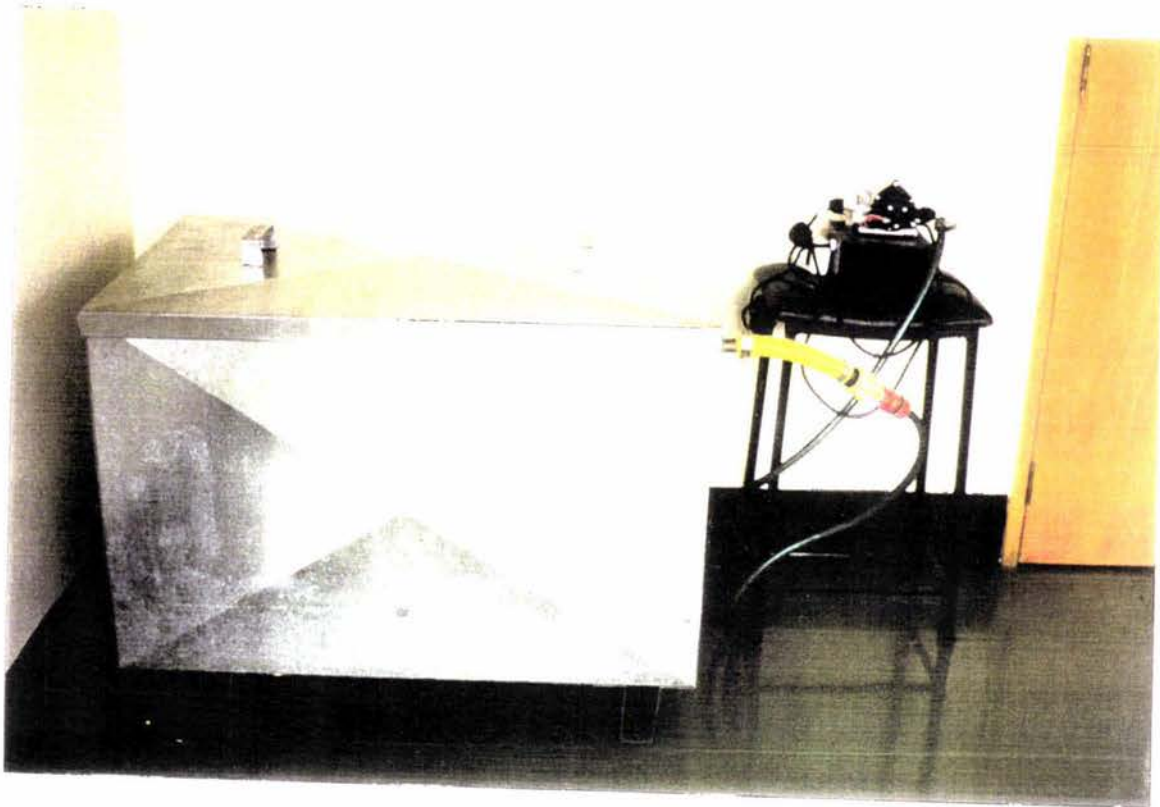
APPENDIX 3A: Summary of linear regression analysis for black gram, when laboratory based seed quality tests were regressed against both SSCP total fresh yield and SSCP healthy yield.

LABORATORY TEST	SSCP TEST	Pr > F	R ² (%)	SD (g)	SSCP TEST	Pr > F	R (%)	SD (g)
Interim germination		0.48	8.6	55.4		0.07	45.2	67.9
Final germination	S	0.56	6.0	56.2	S	0.59	5.2	89.3
Oversoak sprouters	S	0.50	7.7	55.7	S	0.81	1.1	91.3
Oversoak oversoaks	C	0.38	12.8	54.2	C	0.97	<0.1	91.8
SMC	P	0.38	12.8	54.2	P	0.09	40.6	70.7
TSW		0.24	22.1	51.2		0.13	33.8	74.7
T50	T	0.39	12.7	54.1	H	0.24	22.1	81.1
T90 - T10	O	0.68	3.0	57.1	E	0.16	30.2	76.7
Conductivity	T	0.98	<0.1	57.9	A	0.54	6.7	88.7
TZ	A	0.41	11.5	54.5	L	0.27	20.1	82.1
AA (SMC)	L	0.48	8.6	55.4	T	0.75	1.8	91.0
AA (%Normal seedlings)		0.93	<0.1	57.9	H	0.74	2.1	90.9
AA (%Abnormal seedlings)	F	0.16	30.2	48.4	Y	0.67	3.1	90.4
AA (%Dead)	R	0.79	1.3	57.6		0.87	0.5	91.6
Sprout length (ave.)	E	0.18	28.1	49.1	Y	0.74	0.2	90.9
Sprout length (max)	S	0.32	16.2	53.0	I	0.89	0.4	91.7
Sprout length (min.)	H	0.52	7.1	55.9	E	0.60	4.8	89.6
Sprout length (range)		0.53	6.8	55.9	L	0.85	0.6	91.6
Hypocotyl length (ave.)	Y	0.35	14.3	53.6	D	0.97	<0.1	91.8
Hypocotyl length (max.)	I	0.43	10.8	54.7		0.46	9.5	87.4
Hypocotyl length (min.)	E	0.34	15.5	53.3		0.67	3.2	90.4
Hypocotyl length (rge.)	L	0.16	30.4	48.3		0.29	18.1	83.1
Hypocotyl diameter (ave.)	D	0.76	1.6	57.5		0.57	5.8	89.1
Hypocotyl diameter (max.)		0.63	4.1	56.7		0.61	4.7	89.7
Hypocotyl diameter (min.)		0.99	<0.1	57.9		0.63	4.2	89.9
Hypocotyl diameter (rge.)		0.44	10.3	54.9		0.61	4.6	89.7
Seedling Dryweight		0.53	7.0	55.9		0.19	26.2	78.9

APPENDIX 3B: Summary of linear regression analysis for green gram, when laboratory based seed quality tests were regressed against both SSCP total fresh yield and SSCP healthy yield.

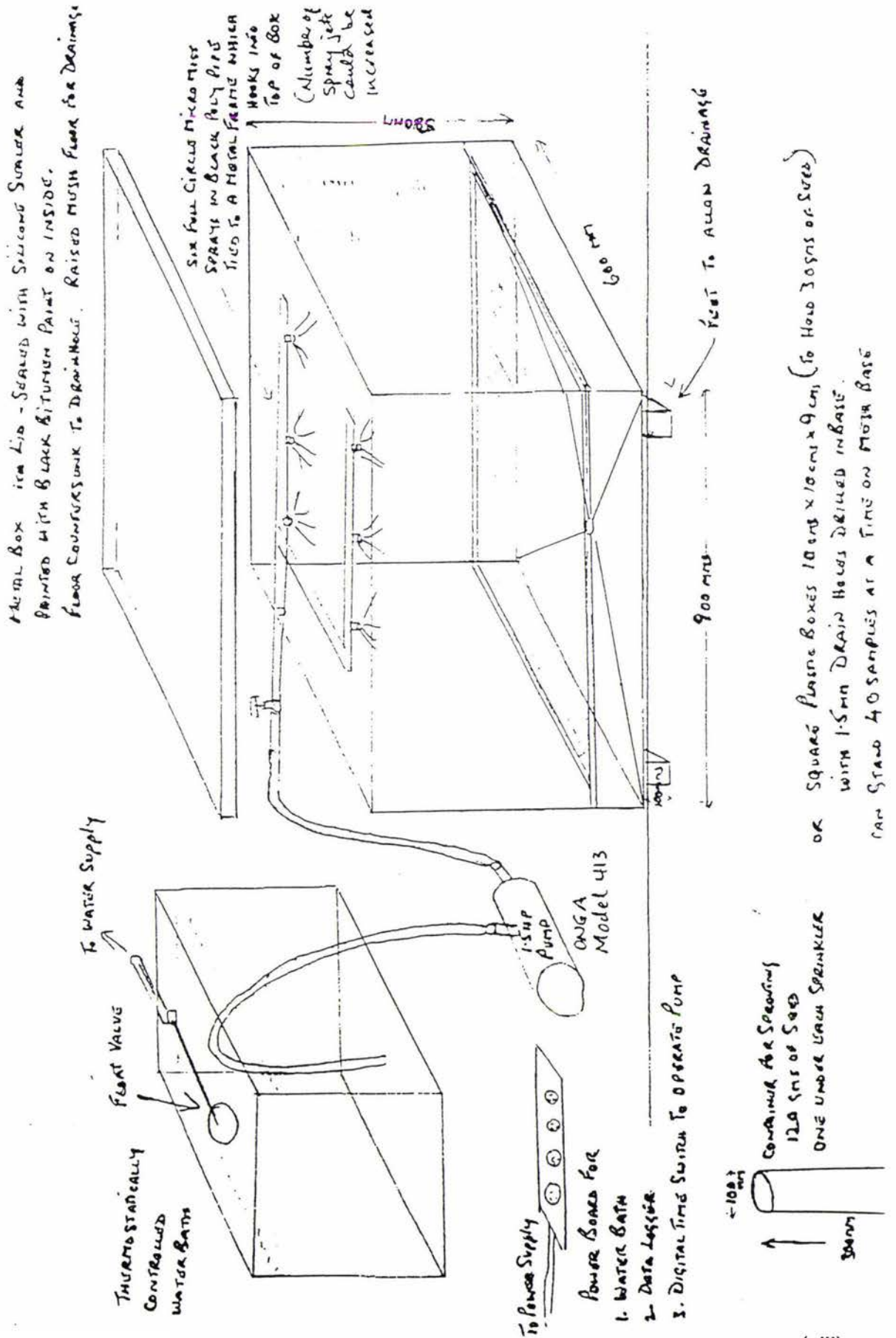
LABORATORY TEST	SSCP TEST	Pr > F	R ² (%)	SD (g)	SSCP TEST	Pr > F	R (%)	SD (g)
Interim germination		0.01	79.1	30.6		0.12	40.8	72.5
Final germination	S	0.01	76.3	32.6	S	0.28	22.5	83.0
Oversoak sprouters	S	0.04	60.6	42.1	S	0.17	33.6	76.8
Oversoak oversoaks	C	0.88	0.5	66.9	C	0.41	14.1	87.4
SMC	P	0.03	63.7	40.4	P	0.16	34.9	76.0
TSW		0.97	<0.1	67.0		0.94	0.1	94.2
T50	T	0.44	1.2	62.8	H	0.71	3.1	92.8
T90 - T10	O	0.09	47.0	48.8	E	0.45	11.7	88.6
Conductivity	T	0.83	1.0	66.7	A	0.32	19.3	84.7
TZ	A	0.45	1.2	62.9	L	0.48	10.2	89.3
AA (SMC)	L	0.24	2.6	57.7	T	0.49	10.0	89.4
AA (%Normal seedlings)		0.95	<0.1	67.0	H	0.85	0.7	93.9
AA (%Abnormal seedlings)	F	0.27	2.3	58.8	Y	0.13	40.2	72.9
AA (%Dead)	R	0.99	<0.1	67.0		0.74	2.4	93.1
Sprout length (ave.)	E	0.37	1.6	61.3	Y	0.37	16.3	86.2
Sprout length (max)	S	0.39	1.5	61.7	I	0.22	28.7	79.6
Sprout length (min.)	H	0.47	1.1	63.3	E	0.50	9.6	89.6
Sprout length (range)		0.85	0.7	66.8	L	0.74	2.4	93.1
Hypocotyl length (ave.)	Y	0.38	1.5	61.6	D	0.61	5.7	91.6
Hypocotyl length (max.)	I	0.16	34.9	54.1		0.34	18.0	85.4
Hypocotyl length (min.)	E	0.32	19.6	60.1		0.63	4.9	91.9
Hypocotyl length (range)	L	0.69	3.4	65.9		0.63	4.9	91.9
Hypocotyl diameter (ave.)	D	0.33	18.6	60.5		0.23	27.0	80.6
Hypocotyl diameter (max.)		0.23	27.3	57.1		0.27	23.8	82.3
Hypocotyl diameter (min.)		0.91	0.2	66.9		0.41	13.9	87.5
Hypocotyl diameter (range)		0.25	25.6	57.8		0.44	12.3	88.3
Seedling Dryweight		0.79	1.5	66.5		0.75	2.2	93.2

APPENDIX 4B: Photographs of the small scale commercial production (SSCP) sprouter made at Massey University, which was used in this study.



APPENDIX 4A:

Sketch of proposed small scale commercial production (SSCP) sprouter developed by Dr. Bruce Imrie (CSIRO, Brisbane), which was used as the basis for the SSCP sprouter used in this study.



APPENDIX 5: Seed quality standards for sprouting mungbeans, published by the Australian Mungbean Association (undated).



STANDARDS FOR EXPORT MUNGBEANS

	PREMIUM	NO. 1	PROCESSING
APPEARANCE	<p>BERKEN/SHANTUNG/EMERALD Equal to or better than the appearance of the standard sample. The standard sample has a bright appearance and no blemishes.</p> <p>SATIN Equal to or better than the appearance of the standard sample. The standard sample has an even bright appearance seed coat slightly dull compared with Berken and Shantung.</p> <p>CELERA Same as Berken/Shantung</p> <p>REGUR Same Satin</p>	<p>BERKEN/SHANTUNG/EMERALD Equal to or better than the appearance of the standard sample. The standard sample has a bright appearance but contains some offtypes.</p> <p>SATIN Equal to or better than the appearance of the standard sample. The standard sample has an even appearance but contains some offtypes and dull seeds.</p> <p>CELERA Same as Berken but 2% yellow acceptable</p> <p>REGUR Same as Berken but 2% brown acceptable.</p>	<p>BERKEN/SHANTUNG/SATIN/EMERALD Equal to or better than the appearance of the standard sample. Some mould-affected and weather damaged seed is the standard.</p> <p>CELERA Standard sample</p> <p>REGUR Standard sample</p>
SIZE RANGE	2mm (5/64") 98%	98%	
PURITY	99% No other seeds trace soil to be noted	99% 0.3% other seeds trace soil to be noted	98% 2% other seeds trace soil to be noted
GERMINATION (Including Hard)	94%	90%	—
Maximum hard seeds, Berken, Shantung, Satin (No Hard Seed Limit – Regur, Celera & Emerald)	6%	10%	—
OVERSOAKS	7%	10%	—
Maximum			
MOISTURE	12%	12%	12%
Maximum			
CHARCOAL ROT	ABSENT	ABSENT	—