# EFFECTS OF PLANT DENSITY ON SEED YIELD AND QUALITY IN DIFFERENT COMMON BEANS

(Phaseolus vulgaris L.)

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# ABSTRACT

Common beans (*Phaseolus vulgaris* L.) are an annual legume used for human consumption. While many cultivars/genotypes have long been a feature of New Zealand home gardens and the frozen food market, there has recently been an interest in the production of new genotypes of this crop legume suitable for use particularly in fresh and canned salads, as well as for other commercial purposes.

In New Zealand, little is known of the growth and performance of many genotypes of this plant as the agro-climatological conditions are different from the original native South American habitat. Therefore this study covered an evaluation of five unnamed but different seed coloured bean genotypes obtained originally from the CIAT collection by New Zealand Seed Bank Ltd. The objectives of this research were to: determine plant growth habit of the genotypes; describe plant growth habit of the genotypes; and assess the effect of plant density on vegetative and reproductive growth, seed yield and cooking quality. To facilitate the recognition of each genotype, they were named *white, mottled brown, mottled black, black and brown* according to their seed colour, after a visual selection of seeds at the beginning of this study.

Plant morphological characteristics were assessed in a trial conducted in a glasshouse at the Seed Technology Centre, Massey University, from September to November 1994. A field trial from November 1994 to March 1995 was aimed at determining the effects of plant density and genotype on seed production and quality for sowing and eating purposes.

The minimum and maximum temperatures in the glasshouse were 16°C and 25°C respectively. The daylength in September was around 11 h and gradually increased to about 14.5 h at the end of November. No supplementary illumination and no pesticides and insecticides were used in this trial. For this study, five plants of each colour group were used to determine plant morphological characteristics which included: leaf length and width for the 1st, 3rd and 8th trifoliolate leaves,

recorded from the terminal leaf; length of pedicellate bracts; flower (standard and wing) colour; pod colour, length and width; plant height and branch number; main stem internode number and internode length; pods per plant; and seeds per pod.

Trifoliolate leaf length was around 22 cm for all genotypes irrespective of leaf position, but leaf width increased from the 1st to the 8th trifoliolate leaf and differed with genotype. For example the 8th trifoliolate leaf width ranged from 11.0 cm in the mottled brown genotype to 14.6 cm in the brown genotype. Pedicellate bract length, main stem internode number and maximum internode length all varied with genotype, with the result that average plant height ranged from 166 cm for the brown genotype to 362 cm for the white genotype. None of the genotypes produced branches in the glasshouse.

Flower colour was assessed using the Dictionary of Colour Standards and the Horticultural Colour Chart from the British Colour Council. The standard and wing were white in the white, mottled brown and brown genotypes, mauve in the mottled black genotype, and were either white or mauve to rose purple in the black genotype. The colour of the wing was mauve in the mottled black genotype and was either white or mauve in the black genotype.

Pod colour for the white genotype was mimosa yellow to naples yellow, or mottled with either aster violet or hyacinth blue, while in the mottled brown genotype pod colour was predominantly naples yellow, mottled with china rose or also with chrysanthemum crimson. Pods from the mottled black genotype were mimosa yellow to amber yellow in colour, and sometimes mottled with purple brown. Pods from the black genotype were mimosa yellow or naples yellow and were either slightly mottled with lilac purple or with pansy violet, while pod colour from the brown genotype was erythrite red. Dried pod length varied from 9.3 to 12.1 cm in the brown and white genotypes respectively, while dried pod width ranged from 11.8 mm in the mottled black to 12.8 mm in the white genotype. The number of pods per plant varied from 13 in the mottled brown to 16 in the brown genotype, while seeds per pod varied from 4.4 in the brown genotype to 5.8 in the white genotype.

Daylength for the field trial ranged from 14.5 h (November) to around 12.3 h (March), with a maximum daylength of about 15 h in December. Seeds from the same seed colour groups used for the glasshouse studies were used in the field trial which was located at the Frewen's block, Massey University. Seeds were sown at three different rates (2.8, 5.6 and 8.4 g/$m^2$) by cone seeder on 28 November 1994 to obtain densities of 6.6, 13.3 and 20.0 plants/$m^2$ at row spacings of 60, 30 and 20 cm respectively. Within the rows a uniform space of 25 cm was maintained. Each treatment (plant density x genotype) was replicated four times in a split plot design.

For seed development studies, a total of 450 - 460 flowers per genotype (from the 13.3 plants/$m^2$ density) were randomly selected and labelled at anthesis, and 60 pods per individual genotype were harvested manually at 14, 20, 26, 32, 40 and 50 days after labelling for the determination of seed moisture content, fresh weight, dry weight and percentage seed germination. Seed yield and seed yield components (number of pods per plant and seeds per pod) were recorded after hand harvesting of 10 sample plants/plot.

The quality of seed for sowing purposes was assessed by germination, conductivity and accelerated ageing (AA) tests, while for cooking quality, seeds were assessed for their imbibition rate, seed texture and seed integrity after cooking. All the data acquired from this study were analyzed with the statistical analysis system of SAS with least significant differences at the 5% level.

The white and black bean genotypes produced 11 and 17% plants with indeterminate climbing characteristics respectively, while the other genotypes each produced 1 - 3% of plants with indeterminate climbing characteristics. All other plants were bush-indeterminate. Plant height in all bean genotypes at all the densities measured between 50 - 60 cm with a min./max. height of 40/85 cm.

The onset, peak and duration of flowering in all genotypes were not affected by plant density. The typical three phase sequence of seed development was recorded and physiological maturity, or the attainment of maximum dry weight, occurred at around 40 days after anthesis (d.a.a.) at more or less the same time for

all genotypes. Seed germination started around 20 d.a.a. and reached a maximum (of 100%) about the same time as the attainment of maximum seed dry weight at 40 d.a.a. However differences in seed coat permeability influenced the rate of seed desiccation and caused differences in seed moisture content (smc) among genotypes.

The number of branches per plant differed significantly from 4.6 in the brown genotype to 5.2 - 5.8 in other genotypes. At the 6.6 plants/$m^2$ density the number of branches per plant was 7.0 and decreased to 3.8 at the 20.0 plants/$m^2$ density. Flowers per plant varied from 46.9 to 63.9 in the brown and mottled brown genotypes respectively but did not differ with density.

Pods per plant were similar for all genotypes, and reached 32.2 at the 6.6 plants/$m^2$ density but decreased to 19.0 at the 20.0 plants/$m^2$ density. Seeds per pod varied slightly from 4.1 in the brown genotype to 4.6 in the mottled black genotype, but did not differ with density. Seed weight/100 seeds varied from 35.7 g in the mottled black genotype to 45.2 g in the black genotype, and was similar for all densities. The black genotype produced an average seed yield of 5,705 kg/ha (the highest), while the brown genotype had an average of 4,723 kg/ha (the lowest) at 10% smc. The average seed yield from the white, mottled brown and mottled black genotypes did not differ from that of the black genotype. There was no genotype x density interaction and the average seed yield for all genotypes was 3,800 kg/ha at the 6.6 plants/$m^2$ density, 5,366 kg/ha at the 13.3 plants/$m^2$ density and 6,715 kg/ha at the 20.0 plants/$m^2$ density.

The conductivity test result varied from 4.7 $\mu S\ cm^{-1}\ g^{-1}$ in the brown genotype to 15.5 $\mu S\ cm^{-1}\ g^{-1}$ in the white genotype, which demonstrated the differences in seed coat characteristics among genotypes. The germination before AA was 100% for all genotypes, and did not differ after AA (between 97 and 99%). The conductivity test result, as well as the percentage germination before and after AA did not differ with density. The brown genotype produced an average of 53.5% of seeds with 'delayed permeable' seed coats, while this property varied from 3.5 to 10.0% in the white and mottled brown genotypes respectively.

Seeds from all genotypes became soft after cooking for 20 min. However the force required to cut the seeds after cooking varied from 6.83 Newton in the white genotype to 15.23 Newton in the brown genotype. The high seed coat permeability in the white genotype caused 19.3% seed coat/cotyledonary damage, while the brown genotype had only 8.9%. The white, mottled brown, mottled black and black genotypes produced a high yield (between 5,136 and 5,705 kg/ha) of good quality seed for both sowing and eating purposes. The brown genotype had a lower seed yield (4,723 kg/ha), but the seed was also of good quality for sowing and eating purposes. Differences in the seed coat characteristic of different bean genotypes may mean a requirement for different lengths of cooking time to attain the same level of seed softness without seed coat splitting as required for human consumption.

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# CHAPTER 1
# INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an herbaceous annual legume which has epigeal germination, can be either determinate or indeterminate in plant growth, has pinnately compound trifoliolate leaves and flowers on axillary racemes. It is self-pollinated and the fruit is a pod, each of which contains several seeds (Chapman and Carter, 1976). The seeds vary in size, shape and colour.

For hundreds of years this crop has had the image of "poor man's meat" (Morrow, 1991) and was only consumed largely by people in developing countries (Aykroyd and Doughty, 1964). In many developing countries, common bean is consumed as a major supplementary source of protein. On average, common beans contain 22.1% protein; 1.7% fat; 61.4% carbohydrate; 0.137% calcium; 6.7 mg iron/100 g; 0.54 mg thiamine/100 g; 0.18 mg riboflavin/100 mg; and 2.1 mg nicotinic acid (niacin)/100 g (Aykroyd and Doughty, 1964), with a total soluble dietary fibre of between 1.7 - 4.2%, and 66 - 73% of insoluble fibre (Bressani, 1993). However since it was found that bean products have hypocholesterolemic effects (i.e. they can reduce blood cholesterol) (Uebersax et al., 1991), consumption has markedly increased in the USA and in other developed countries. In America, the USDA (1991) stated that the consumption levels of common beans in 1990 was increased by 30.9% over previous years. Bean products have now been introduced in restaurants in exotic salads, dips, pates and even as an essential ingredient in desserts (Morrow, 1991). Uebersax et al., (1991) reported that a daily consumption of 100 - 135 g of dry beans can reduce serum cholesterol levels by approximately 20% in the short-term, hypothetically reducing risk for coronary heart disease by 40%.

The world's total production of common bean is around 14 million t/year (Dessert and Bliss, 1991); Latin America is the leading bean producer, with approximately 30% or 4.0 million t/year. In New Zealand common beans have been

grown in private home gardens for over 100 years, but commercially they were initially trialled in the early 1970s in Wairarapa, Manawatu, Marlborough and Hawkes Bay. Common bean cultivars such as Great Northern (GN), Small Red (SR), Pinto and Black were first grown in 1975, and occupied an area of 4 ha for GN cultivar and 1.2 ha for each of the other three cultivars. The crop yield ranged from 1 to 4 t/ha with an average of 1.6 t/ha (Goulden and Malone, 1978). McKenzie (1989), reported that in New Zealand common beans were produced primarily for use in tinned baked beans. However more recently there has been an increasing interest in this country in the production of new varieties of this crop legume suitable for use in fresh salads as well as for canning (A. K. Hardacre, 1995, pers. comm.). The main beans used for these purposes are Red Kidney bean and Pinto. However a combination of several colours of bean will make bean salads more attractive which will lead to greater demand (R. Coulson, 1995, pers. comm.). This idea has encouraged bean producers and traders to introduce new common bean varieties with different colours.

Seed production is commonly related to cultivar, but growing conditions can markedly affect it. In New Zealand, little is known of the growth and performance of many genotypes of this plant in a habitat where the agro-climatological conditions and edaphic conditions are different from the original native South American habitat.

This study was an evaluation of five unnamed but different coloured bean genotypes obtained originally from the CIAT collection by New Zealand Seed Bank Ltd. The objectives were to:

1. determine plant growth habit of the genotypes;
2. describe plant growth habit of the genotypes;
3. assess the effect of plant density on vegetative and reproductive growth, seed yield and cooking quality.

# CHAPTER 2
# LITERATURE REVIEW

*Phaseolus vulgaris* L., an annual crop, is also known as French bean, kidney bean, haricot bean, salad bean, runner bean, snap bean, string bean and frijoles (Purseglove, 1968), and may also be called dry bean, food bean, field bean, common bean, phaseolus bean and dry edible bean (Voysest and Dessert, 1991). This crop is one of the most important grain legumes grown for human nutrition. Archaeological studies demonstrated that common bean originated in the Americas, but it was only some 30 to 40 years ago that wild-growing common bean populations were described for the first time (Gepts and Debouck, 1991). This crop possesses a wide range of adaptation to the most varied climatic conditions, which makes common bean relatively easy to spread/grow in many countries in the world.

## 2.1. Plant Morphology (a general description)

Axes (stem and branches) are generated by the integrated activity of the terminal meristem topping them. They are formed by a succession of nodes (where the foliar appendages and the lateral branches are inserted) and internodes. The main stem is easily distinguished from the lateral axes because of its large diameter and its link with the root system below it. The main stem derives from the axis of the seed embryo, of which the two parts below and above the two cotyledons 'the hypocotyl and the epicotyl, respectively' are still visible on the mature plant. Cotyledons generally fall within a fortnight of seed imbibition, leaving two scars in apparent opposite positions. The main stem starts at the insertion of the root system. The lower part of the stem tends to be erect without torsion. The upper part of the stem, in some cases (as in climbing genotypes) starts to twist at the second node. The stem twists around itself and also around the support. (Twisting is probably caused by the presence of fibres within the stem and is inherited; Debouck, 1991). The lateral axes are inserted at the nodes, in the axil of the leaves.

The primary leaves are initiated by the terminal meristem and have completed their full organogenesis during embryogenesis in the pod on the mother plant, while the trifoliolate leaves are formed during and after germination. The primary leaves are simple, cordiform, acuminate, symmetrical, and auriculate. The two lateral leaflets are asymmetrical while the unique terminal leaflet is symmetrical. The cotyledons are oppositely inserted on the main stem as well as the primary leaves, and the next to appear are trifoliolate leaves which are alternately inserted on the main stem and branches. A natural twisting of the stem, even in bush types, causes the trifoliolate leaves of the distichous succession not to over shading (Adapted from Debouck, 1991).

## 2.2. Taxonomy

Common bean belongs to the family of Leguminosae, sub-family Papilionoideae, tribe Phaseoleae (Isely and Polhill, 1980; Polhill, 1981), sub-tribe Phaseolinae (Lackey, 1977; 1981; Marechal et al., 1978), genus Phaseolus, and the species is *Phaseolus vulgaris* (Debouck, 1991).

## 2.3. Botanical Description

Common bean (*Phaseolus vulgaris* L.) is a twining or erect annual herb (Purseglove, 1968) with 3-foliolate leaves; stipels present, and with small stipules. The calyx is campanulate; bilabiate; corolla variously coloured; keel with a spirally coiled beak; stamens diadelphous; style hairy on the inside; stigma oblique. This kind of bean is an annual crop, up to 4 m. The racemes are shorter than leaves, up to 6-flowered. The corolla is 10 - 18 mm; white, pink, or purple; beak of keel forming 2 turns of a spiral (Adapted from Flora Europaea 2). The primary leaves are stipulate, simple, cordiform, acuminate, symmetrical, and auriculate. They have a rachis with 0 - 2 stipels and two pulvini. The prophylls are minute (about 1 mm or less), scale-like and membranaceous, triangular and have no rachis, stipules, stipels, or pulvini. The trifoliolate leaves have stipules, stipels, petioles, rachis and pulvini

(Adapted from Debouck, 1991). The two lateral leaflets are asymmetrical while the unique terminal leaflet is symmetrical.

The flowers are usually shorter than leaves and located at or near the apex of the peduncle. The pedicels are short, 5 - 8 mm long; the calyx bracts are leafy, ovate, as long as or exceeding the length of the calyx, up to 6 mm long; the calyx is 3 - 4 mm long, with 1 upper and 3 lower teeth. The corolla is white, yellowish, pink or violet; standard 9 - 12 mm in diameter, emarginate; claws of wings 5 - 6 mm long; keel spirally twisted; vexillary stamen free, rest connate; anthers small, globose, uniform, basifixed; style long, twisted, hollow; stigma capitate.

The pods are slender, narrower than in *Phaseolus lunatus*, 8 - 20 x 1 - 1.5 cm, usually with 4 -6 seeds, commonly glabrous, straight or slightly curved, edges rounded or convex, beak prominent, yellow in wax cultivars, light to dark green in green podded cultivars, sometimes with pink or purple splashes.

The seeds are variable in colour, shape and size. They are white, yellow, greenish, buff, pink, red, purple, brown or black; self-coloured, mottled, blotched or striped; some eyed with different colour round hilum; 0.7 - 1.6 cm long; kidney-shaped, oblong or globular, ratio of length, breadth and thickness are variable, often somewhat compressed; hilum usually white; endosperm absent. The weight of 100 seeds is between 20 - 60 g (Purseglove, 1968).

## 2.4. Ecology

Common bean is not suited to the over-wet tropics but does well in areas of medium rainfall from the tropics to the temperate regions. Excessive rain causes flower drop and increases the infestation of disease. However some rain is required for flowering and pod setting. Dry weather is required for harvest. They can be grown on most soil types from light sands to heavy clays and also on peat soils. They are sensitive to high concentrations of manganese, aluminium and boron (Purseglove, 1968). Common beans perform best in soils above pH 5.5, and Morrison (1973) reported that a pH between 6.0 and 6.5 will give the best yields.

This crop legume can be killed by frost. Common beans should not be sown until the soil temperatures are above 16°C. Optimum growth occurs at an average soil temperature of 25°C (Morrison, 1973). They are sensitive to fluctuating temperatures and dry conditions at flowering and pod-set.

Common beans require temperatures above 10°C for effective growth (Gane et al., 1975). Temperatures of 7°C can cause damage to floral primordia while temperatures of 35°C/20°C or 35°C consistently reduced pollen viability (Masaya and White, 1991) which may lead to low yield. The nightlength (the duration of the dark period) is crucial for internal control of the differentiation of tissues and organs that constitute plant reproductive units. Masaya and White (1991) reported that long days delay or stop the differentiation of flower buds, and the nature of this delay is obscure. For indeterminate genotypes of common beans long days or warm temperatures cause the development of branches on the axil of lower nodes (Masaya and White, 1991). However White and Laing (1989) stated that photoperiod not only affects time to flowering, but also affects node and branch formation, and photoperiod sensitivity increases with temperature.

White and Mansfield (1977) reported that the growth of lateral buds is markedly affected by light intensity, photoperiod and temperature. At low light intensity the apical dominance was greatest (Jackson and Field, 1972) and at that light intensity regime the main stem length is highly stimulated (Shein and Jackson, 1971). On the other hand White and Mansfield (1977) reported that the increase in radiant energy flux reduces main stem elongation. Jackson and Field (1972) found that the main stem growth occurred at a high intensity of 7000 lx and lowest at 21000 lx. The light intensity affects the apical dominance by altering the supply of carbohydrates to the buds (White and Mansfield, 1977). Similarly to light intensity, the light quality also exhibits its effect on the vegetative growth, especially on the main stem elongation in plants. Tucker (1976) reported that a high proportion of far-red light stimulated the main stem growth and enhanced the apical dominance in dwarf bean. It has been also reported that in common beans, red light reduced IAA content whereas far-red light increases the IAA content.

Light may differently affect hormone production (Shein and Jackson, 1971) and subsequently hormone balance within the plants. Tucker (1976) stated that the growth of the main stem and lateral shoots (buds) is dependent on the balance of different hormones at specific sites in the plant. Auxin which is synthesized in the stem tip is responsible for inhibiting the growth of the lateral buds (Krishnamoorthy, 1981). In common beans, a reduction of auxin by removing the young leaves encouraged the growth of laterals (Shein and Jackson, 1971).

The direction of movement of nutrients and metabolites inside the plant is controlled by auxin. Since the stem tip produces the auxin, it receives a major supply of nutrients and metabolites while the lateral buds are depleted of them leading to a continuous growth of the main stem (Krishnamoorthy, 1981).

## 2.5. Growth Habits

Beans are classified at flowering as determinate or indeterminate, based on whether a terminal reproductive or vegetative meristem is formed. A genotype is determinate if the main stem and lateral branches terminate in an inflorescence and indeterminate if the main stem and lateral branches are topped by a vegetative meristem capable of continuous organogenesis. In the indeterminate cultivars, flowers are located laterally, directly inserted at the nodes of the main stem and lateral branches (Debouck, 1991).

Evans (1973) reported the existence of two groups of common beans with determinate habits: the few-nodded types (with 3 - 7 trifoliolate leaves on the main stem) and the many nodded types (with 7 - 15 or even to 25 trifoliolate leaves on the main stem). Plants of the first group tend to be erect, whereas those of the second group tend to have climbing ability because of their long internodes. The indeterminate group is divided into the prostrate types with profuse branches, and the climbing types with reduced branches. These differences are genetically inherited and controlled by a few genes (Debouck, 1991). Climbing ability is also genetically inherited and controlled by a single dominant gene, and the gene action is controlled by light quality and photoperiod (Debouck, 1991).

In the vegetative phase, the terminal meristem of the main stem continuously produces leaves and axillary buds at the axil of each leaf. Branching can occur at two locations: at the lower part of the axis (i.e. at the axil of prophylls) and at each node of the normal subsequent trifoliolate leaves. Each node, particularly on the main stem, during the vegetative phase, constitutes a reserve for future branching.The number of leaves and their sizes are different between diverse genotypes. There are more leaves in the indeterminate cultivars. Cultivars with smaller leaves are more advantageous as shading on lower leaves is more limited (Gepts and Debouck, 1991).

## 2.6. Reproductive Organ Characteristics

### 2.6.1.Flowering and maturity

There are at least 1200 common bean cultivars which are different from each other in photoperiod sensitivity (Gniffke, 1985) and there is a range from 70 to 300 days to maturity (Voysest and Dessert, 1991). Flower initiation (especially the delay in flowering) was reported to be controlled either by one to three dominant genes, or by one to two recessive genes (Singh, 1991). It was also reported that the trait of early flowering and daylength-neutral is dominant during short days and it will become recessive during long days.

Debouck (1991) reported that strong competition between the young developing pods located at the lower parts on the first inflorescence limits further development of flower buds in the inflorescential part. The development of these flower buds consequently will be diminished and stopped because of abortion of these meristematic organs.

Ojehomon (1966) reported that within a simple raceme, internodes are usually short. The shortest ones are the first two which causes the first two flowers at the base of the simple raceme to be opposite and not alternate. Between these two flowers, one ovary will reach maturity before the other, and at lower temperatures one of the two flowers could open a day before the other.

### 2.6.2. Flower colour

Flower, stem and seed colours are controlled by genes that can have pleiotropic effects. Flower colour varies between white, yellow, red, purple (Robbins, 1924) and pink or violet (Purseglove, 1968). Colour can be intensified by the presence of an intensifying gene. Singh (1991) reported that pink flower colour was determined by gene R and the white colour was determined by its recessive allele r. Intensifying gene I, when present with R, changed flower colour from pink to purple. This shows how the combination of different genes control the diversity of colours in common bean flowers.

### 2.6.3. Pod characteristics

Common bean pods are straight, curved or compressed, different in colours (i.e. yellow, light to dark green, sometimes with pink or purple splashes), with different lengths of pod (i.e. between 8 and 20 cm) (Purseglove, 1968). In wild common beans, pods are generally dehiscent. When they reach maturity, the two pod valves separate and twist suddenly, causing a sharp slingshot-like movement which can project seeds to distances of several meters. This mechanism can be attributed to the presence of fibres surrounding the vascular bundles in the pod walls and a fibrous parchment layer lining the pod cavity (Roth, 1977). A decreased fibre content in the pod walls would reduce or avoid the dehiscence mechanism.

The length of pod differs among common bean varieties . Singh (1991) reported that a long pod was dominant over a small pod, and a single gene was responsible for this inheritance. The pod tip can be straight or curved and that curved tip shape is controlled by a single dominant gene.

### 2.6.4. S e e d

While wild common beans produce a high number of small seeds (average size of 6.2 - 13.9 g/100 seeds), the domestic common beans are much larger and can be from approximately 20 -100 g/100 seeds (Gepts and Debouck, 1991). Seed type (colour, size, shape, surface texture) is reported to show enormous diversity within

the species. Seed shape may vary from round to oblong, to kidney-shaped, with many combinations of colour. The surface texture may be shiny, opaque, or intermediate.

## 2.7. Importance of Photosynthesis

The accumulation of dry weight in common bean plants is a direct result of the balance among photosynthesis, respiration, and losses caused by senescence and abscission. Beans have a $C_3$ photosynthetic pathway, and maximum leaf photosynthetic rates at ambient $CO_2$ concentrations range from 12 mg $CO_2$ $dm^{-2}$ $h^{-1}$ to 35 mg $CO_2$ $dm^{-2}$ $h^{-1}$ (Shibles et al., 1975). Leaves increase in photosynthetic capacity as they expand, maintain a maximum for a highly variable period depending on cultivar and leaf position, and decline as the leaf becomes senescent (White and Izquierdo, 1991). The maximum photosynthetic rate in common beans is reached at an irradiance of 300 W $m^{-2}$ and the leaf photosynthetic rate reaches its maximum at the time of initial pod filling. Maximum leaf photosynthetic rate was positively correlated with leaf thickness, specific leaf weight, and the number of chloroplasts (White and Izquierdo, 1991).

Green pods also fix $CO_2$ at lower rates than adjacent leaves; this rate decreases with pod age (Tanaka and Fujita, 1979). Bean pods recycle substantial amounts of internally released $CO_2$, but are not an important photosynthetic source of dry matter for developing seeds (White and Izquierdo, 1991).

## 2.8. Starch Accumulation

Bean mesophyll cells are able to accumulate large amounts of starch without disrupting the chloroplasts. Sucrose-P synthetase is the key in legumes in regulating photosynthetic formation of sucrose, and hence, starch (White and Izquierdo, 1991).

## 2.9. Nitrogen Uptake

In bean seeds, a protein content of 20 to 24% implies a nitrogen content of approximately 4%, which means that every 1,000 kg of seed yield implies a need

for 40 kg nitrogen, not including the amounts needed to replace losses caused by leaching or residual nitrogen in other tissues (Izquierdo, 1981).

Although nitrogen can be introduced through the foliage, the normal route of uptake is through the roots, either as nitrate or $N_2$ which is converted by Rhizobium root nodule bacteria into ammonium ions. When nitrogen is absorbed as nitrate, it is firstly converted from nitrate to nitrite by nitrate reductase, and then from nitrite to ammonium by nitrite reductase. Barke (1978) found that common beans perform best when all or part of the nitrogen dressing is in the ammonium form. Thung (1991) reported that increasing nitrogen level increases K and Ca uptake, but nitrogen level does not influence the uptake of P and Mg.

Nitrogen can be obtained from the symbiosis with root nodule bacteria (Rhizobium sp.), but the amount of nitrogen supplied from that symbiotic association has not been found to be efficient as a source of nitrogen required by the plant (Barke, 1978). Thung (1991) observed that the highest demand for nutrient takes place during the pre-flowering stage, and early and pre-flowering nitrogen stress reduced the number of lateral branches as well as their development.

## 2.10. Growth of Reproductive Organs

### 2.10.1. Flowers

Common beans are self-pollinated (Purseglove, 1968). Anther dehiscence occurs within a few hours before anthesis, usually at night (White and Izquierdo, 1991; Gross and Kigel, 1994), and pollen germination and fertilization of most ovules are fast processes accomplished in about 4 hours (Gross and Kigel, 1994).

### 2.10.2. Pods and seeds

Pod growth is divided into three phases: pod wall growth, seed growth and desiccation (White and Izquierdo, 1991). Pods reach maturity 48 days after anthesis. The maximum pod length is reached in 24 days, and pod fresh weight drops only in the last 6 to 8 days, although seed weight continues to increase during the last phase of pod growth. During the stage of pod maturation, bean seeds enter a state

of reduced metabolic activity characterized by changes in dehydration of cellular membranes and a severe reduction in respiration rate.

Seed moisture content is influenced very little by environmental conditions during most of the sequence of seed development. However during the desiccation period, atmospheric conditions have a great effect on water loss (Howell et al., 1959). Izquierdo (1981) found that Physiological Maturity (PM) of common bean cv Black Turtle Soup was attained at 45 days after flowering. However the PM of other common bean cultivars such as Dwarf French bean, Dwarf Butter bean cv Choctaw Wax, and French bean cv Green Crop was attained successively at 23, 33 and 35 days after flowering (Mahamed, 1989; Dorji, 1989; Gil, 1989) suggesting that different cultivars of common bean posses different ranges of time between flowering and PM.

The term 'Physiological Maturity' has been used most frequently to describe the point where the seed reaches its maximum dry weight (Harrington, 1972). However, Andrews (1966) has suggested that PM should be judged in terms of maximum seed viability, vigour and dry weight. At Physiological Maturity the ovule vascular connection between the pod and the seed (the funiculus) is broken and for the first time the seed becomes a self contained unit (Browne, 1978).

Seed germination increases as the seed approaches PM and then generally declines slightly to the time of harvest (Muirhead and White, 1978). At Physiological Maturity the fresh weight and seed moisture content are decreasing. Following this stage, there is no further influx of inorganic nutrients, but conversion of simple organic reserves to storage products is occurring (Austin, 1972).

## 2.11. Abscission of Reproductive Organs

Causes of abscission vary greatly with growing conditions. Abscission of flowers and/or small pods (even older pods) may result from: water stress or competition among developing pods for nitrogen, other nutrients and carbohydrates; endogenous hormone balance; flower abnormalities; failures of pollination or fertilization; and disease problems (White and Izquierdo, 1991). Jiang and Egli

(1993) reported that flowers developing early in the reproductive period or flowers located at the base of a raceme abscise at lower frequencies than late developing flowers. Similarly, White and Izquierdo (1991) found that the basal and older pods regulate the abscission of new flowers and small pods at the distal end of the raceme. This senescence may be the result of increasing ABA concentration in those young reproductive organs, and the ABA may enhance senescence either directly or through the agency of ethylene (Tamas et al., 1979).

Stress caused by source-sink competition enhances bean pod abscission, but it was also found that flower buds with high probability of abscission showed a wide range of abnormalities, including large number of ovules with necrosis and 15% of buds lacking of embryo sac (White and Izquierdo, 1991). Pod abscission can continue until 45 days after anthesis and older pods may drop if adequate carbon assimilate is not available. Aguilar et al., (1977) stated that photosynthate supply controls the final pod number in common beans. The abscission of reproductive organs (flower and pods) in legume crops is often greater than 50% (White and Izquierdo, 1991).

### 2.12. Plant Density and Seed Yield

The production of common bean seeds can be influenced by edaphic and climatic conditions as well as agricultural management practices. In New Zealand, the average production of some common bean varieties in 1975 was 1.6 t/ha (Goulden and Malone, 1978). However McKenzie (1989) reported that there was a consistent production of around 3 t/ha of common bean seeds in the 1980s.

Thung (1991) stated that the potential yield of common bean in Latin America was 3 t/ha for bush beans, and 6 t/ha for climbing beans with artificial support. In another report, White and Izquierdo (1991) reported that the maximum yield for bush beans varies between 4 - 6 t/ha, and climbing beans on trellises have yielded 8 t/ha in small plots. Woolley and Davis (1991) found that the optimum plant density for climbing beans and bush beans in monoculture was approximately

12 plants/$m^2$ and 24 plants/$m^2$, respectively, and an intermediate between both populations is required for beans with semiclimbing types.

Shibles et al., (1975) reported that physiological processes (photosynthesis and respiration) have a direct effect on growth, and in addition to the morphological structure of the plant (i.e. with more or less branches) will influence the yield. Woolley and Davis (1991) found that a greater light penetration to the bean canopy improves bean yield. However a mutual shading decreases net photosynthesis, and excessive over shading of leaves might produce many nodes without pods (Singh, 1991).

Aguilar et al., (1977) stated that the seed weight is unlimited by photosynthate. White et al., (1992) reported that seed weight in common beans is quantitatively inherited, and the seed growth rates and duration is mostly controlled by sink effects (White and Izquierdo, 1991). In general there is no plant density effect on seed weight (Francis et al., 1982; Aguilar et al., 1977).

### 2.12.1. Growth of vegetative organs in response to plant density

The density of plants per unit area depends on the environmental resources available, soil fertility, agronomic management practices as well as the growth habit of the plant. The spatial arrangement and plant density affect the development of branches. Bennett et al., (1977) and Davis and Garcia (1987) found that the number of branches per plant in common beans was reduced by increased plant density. Bennett et al., (1977) observed that the number of branches per plant was significantly decreased as the density was raised from 17 to 63 plants/$^2$ while, Nienhuis and Singh (1985) reported that the number of branches per plant in several common bean genotypes was reduced from 5 - 6 to 3 - 3.5 as density increased from 5 to 30 plants/$m^2$. Similarly, the number of nodes per branch was depressed from 4 - 6 at a density of 5 plants/$m^2$ to 2.5 - 3 at a density of 30 plants/$m^2$. Changing plant arrangement can alter the structure of beans (Gane et al., 1975). Westermann and Crothers (1977) reported that increasing plant populations causes greater

*interplant* competition, which could increase the *intraplant* competition for assimilates. This condition results in fewer branches and pods per plant (De Moura and Foster, 1986) which causes a reduction of seed yield per unit area. McIntyre (1972) stated that at low nitrogen levels, the growth of buds on all common bean plants is completely arrested, but increasing the nitrogen supply to 210 ppm resulted in further increase in bud growth.

### 2.12.2. Seed yield and its components in response to plant density

Francis et al., (1982) and Davis and Garcia (1987) stated that higher bean densities resulted in fewer pods per plant and lower yield per plant, and the number of pods per plant generally affects seed yield (Zimmermann et al., (1984) as pods per plant are consistently and strongly correlated with yield (Husain et al., 1988). Aguilar et al., (1977) reported that pods present at maturity were only 48, 67 and 70% of the total pod potential at densities of 28.8, 7.2 and 3.2 plants/$m^2$. The increases in pods per plant and the smaller increases in seeds per pod were insufficient to compensate for lower plant numbers per unit area, so that seeds/$m^2$, and consequently seed yield, decreased with reductions in plant density (Aguilar et al., 1977).

Francis et al., (1982) found that bean yield reduction was associated with fewer pods per plant and fewer pods per $m^2$, but Gene et al., (1975) stated that by growing more plants per unit area higher yields can often be obtained. As observed by Jiang and Egli (1993), seeds per pod showed only minor changes with changes in plant density.

## 2.13. Harvest

Seeds are grown to be harvested and utilized for diverse purposes. Several aspects must be considered before harvest, such as the right time to harvest, seed moisture content and method of harvest. Inadequate precautions at harvest can result in seed damage which adversely affects germination and vigour, and reduces the

yield of marketable seed. Jones (1978), reported that harvesting the seeds prior to Physiological Maturity (PM) can result in seed of poor vigour. In contrast, any delay in harvest and the exposure of seeds/pods to rain or continual high relative humidity may cause fungal attack or damage to the testa which may induce rapid deterioration in seed vigour.

Common beans can be harvested manually or mechanically. For mechanical harvest, bean plants are cut at the roots just below ground level when seed moisture content is between 40 - 50% (Jones, 1978) to allow rapid dry down of leaves and other vegetative parts of the plant. The optimum moisture content for harvesting the Michigan Navy bean is around 18% to prevent excessive splitting and seed coat damage (Shea, 1978). However Jones (1978) reported that the greatest source of mechanical damage and impairment of seed vigour can be during threshing, and the optimum seed moisture content for that purpose is 11 - 14%. At higher moisture contents threshing may cause bruising of the cotyledons. Barriga (1961) observed that mechanical damage at low moisture content causes cryptic physiological changes which result in low seed vigour. Precautions are required during mechanical harvest and threshing (as well as processing) to avoid losses of seed vigour, because in common bean seeds the embryo is situated at the edge of the seed, and thus receives relatively little protection from the cotyledons as the areas of attachment are very small (Gane et al., 1975).

## 2.14. Seed Quality

### 2.14.1. Seed quality for sowing purposes (seed vigour)

High vigour seeds are mostly required for sowing and storage (for sowing) purposes as they have the great potential of rapid emergence in the field as well as their capability to produce uniform seedling emergence even under stress conditions. Under less than optimum conditions, high vigour seeds are more capable of remaining viable for a prolonged period than lower vigour seeds. ISTA (1987) has

defined vigour as "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".

When seeds are imbibed in water, a substantial amount of organic and inorganic solutes is released from the seeds. In many grain legumes, the intact testa forms a protection against membrane damage caused by rapid water uptake (Bruggink et al., 1991). When the testa is damaged or removed, the extent of imbibition damage increases and consequently increases the volume of solutes released from the seed. This increase is higher when seed membranes are aged (Bruggink et al., 1991). The increased leakage can be a result of a combination of ageing, seed damage and imbibition damage. Seeds that imbibe rapidly are of doubtful vigour because membranes through which water can pass most freely may be predisposed to large losses of cell solutes (Mullett, 1978). Cell membrane integrity can be considered to be a fundamental cause of differences in seed vigour (Hampton et al., 1992). High vigour seeds generally are able to rapidly reorganise their cells during the imbibition period to avoid further losses of minerals and nutrients from the seed (specially from the cotyledon). Gane et al., (1975), reported that a relatively high plant density had no adverse effects on the quality (vigour) of common bean seeds.

### 2.14.2. Seed quality for human nutrition (cooking quality)

There are a number of physical and chemical properties of dry and cooked bean seeds that influence consumer preference and processing standards. Hosfield (1991) reported that the preferences of consumers for beans include factors such as colour and appearance, easier of softening during cooking which shortens cooking time, wholesomeness, taste and texture. Bressani (1993) stated that seeds which are rapidly hydrated, as well as the production of a thick cooking liquor with good

flavour are preferred by consumers. The bean should have moderately split grains after cooking. However for industrial purposes, the integrity of the bean after cooking is required. Among all of the acceptable characteristics, cooking time is the most important. Cooking time under normal atmospheric pressure varies from 24 to 240 min, but most consumers reported values between 60 and 95 min (Bressani, 1993).

The cooking time of bean seeds is influenced by genetic and environmental conditions. Shellie-Dessert and Bliss (1991) stated that two traits can influence cooking time: hard seed coat (i.e. seeds that do not imbibe water normally and require a longer cooking) and hard-to-cook (i.e. seeds that imbibe water but the cotyledons do not soften sufficiently during cooking). Cultivars that develop an impermeable seed coat during the post harvest period often contain a high percentage of hard seed (hard-to-cook) that have a nonuniform texture when cooked.

The degree of hard seed coat in common beans increases as seed moisture content decreases, as occurs under conditions of high temperature and low relative humidity. This characteristic is highly heritable (Shellie-Dessert and Bliss, 1991). The hard-to-cook defect develops during storage under high temperature (above 21°C) and high relative humidity although 'high' for relative humidity was not defined (Dessert and Bliss, 1991). Storing beans with 12 - 14% moisture content at low temperature and relative humidity is the most efficient way to maintain the cooking quality of beans (Bressani, 1993). Growing site can also influence cooking time more than cultivar. High levels of K and Na in the seed are associated with good cooking quality (Bressani, 1993).

A preliminary soaking process is useful to moisten and soften the seed so that the cooking time can be reduced. Soaking facilitates uniform expansion of beans (for canning) and ensures product tenderness after cooking. During the soaking process, uncooked beans undergo an 80% increase in weight due to water imbibition and attain a moisture level between 53 and 57% (Hosfield, 1991). Traditionally, beans

have been soaked for 8 - 16 hours in cold water prior cooking. Hsieh et al., (1992) revealed that Azuki (*Vigna angularis*) seed soaking for 24 hours showed a small additional reduction in cooking time compared to soaking for 10 hours. However recently, high temperature soaking and other alternative soaking methods have been developed to accelerate hydration. The seed soaking and tenderization processes are influenced by seed coat permeability and starch/protein characteristics. The degree and rate of hydration of the starch/protein influences the cooking rate and the final texture of cooked beans. Cooking rate of common bean seeds is also highly dependent on cooking temperature (Uebersax et al., 1991).

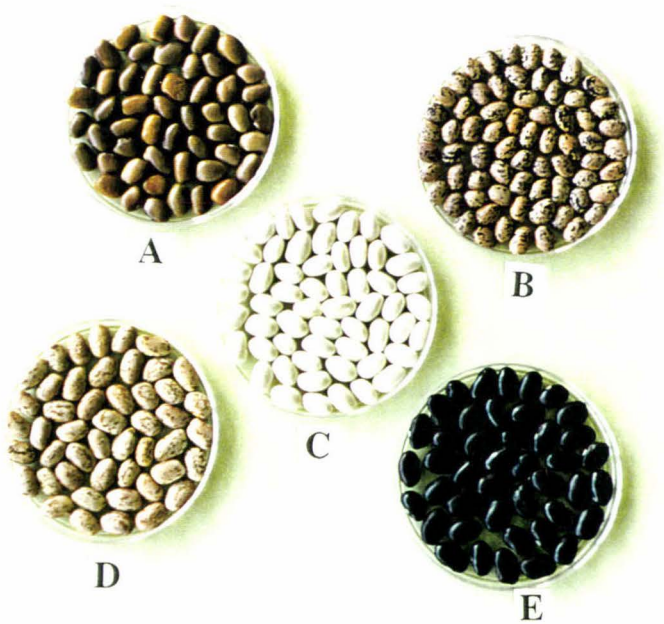
Acidity of soaking medium affects bean hydration characteristics, and at low pH, starch and protein swelling is inhibited, causing a reduction in hygroscopicity (Uebersax et al., 1991). Hosfield (1991) reported the existence of considerable genetic variation in cooked bean texture. Bean texture after cooking is expressed by its firmness and softness and thus serves as an index for consumer acceptance for cooked bean seeds. Bean seeds may be unacceptable if they are perceived as too firm ("tough beans") or too soft ("mushy beans") after cooking.

# CHAPTER 3
# MATERIALS AND METHODS

## 3.1. Plant characteristics in the glasshouse

Bean seeds used in this study were obtained from the CIAT collection by New Zealand Seed Bank Ltd. Initially seeds were visually sorted according to their colours and five colour groups were obtained: white, black, brown, mottled black and mottled brown, (Fig.1) but these were of unknown cultivar or genotype. To facilitate the recognition of each group, names such as white bean, mottled brown bean, etc, were given according to their seed colours.

Fig.1 Bean seeds from the five colour groups used in this study




A = brown bean C = white bean E = black bean

B = mottled black bean D = mottled brown bean

The following study was conducted in a glasshouse at the Seed Technology Centre, Massey University from September to November 1994. The minimum and maximum temperatures in the glasshouse were automatically adjusted to 16°C and 25°C respectively. The daylength from September was around 11 h and gradually increased to about 14.5 h at the end of November. No supplementary illumination was used in this study.

The trial in the glasshouse was conducted in the following way. Twenty five small plastic pots (15 cm diameter x 11.5 cm high) with small holes at the bottom, were filled with a commercial 'seed raising mix medium' of unknown composition, a product from Yates New Zealand Ltd. Seeds of individual groups were planted in five separate pots, at around 2 cm deep and with two seeds per pot. After seedling emergence, pots with two seedlings were thinned to one seedling per pot. The seedlings were watered daily with around 200 to 300 ml water using a spray nozzle and hand-held hose. Two weeks after seedling emergence, the main stem of each plant was supported by an individual bamboo pole 1.5 - 2 cm in diameter and 2.5 - 3 m in height, which was inserted into the pot. Beginning 30 days after seedling emergence, plants were supplied each week with a solution of Liquid Lush fertilizer which supplied each pot with approximately 0.087 g Nitrogen, 0.027 g Phosphorus, 0.051 g Potassium and traces of Magnesium, Iron, Copper, Zinc, Manganese, Molybdenum and Boron, in a chelate form. No pesticides and insecticides were used in the glasshouse trial as there were no significant infestations of pathogens or insects.

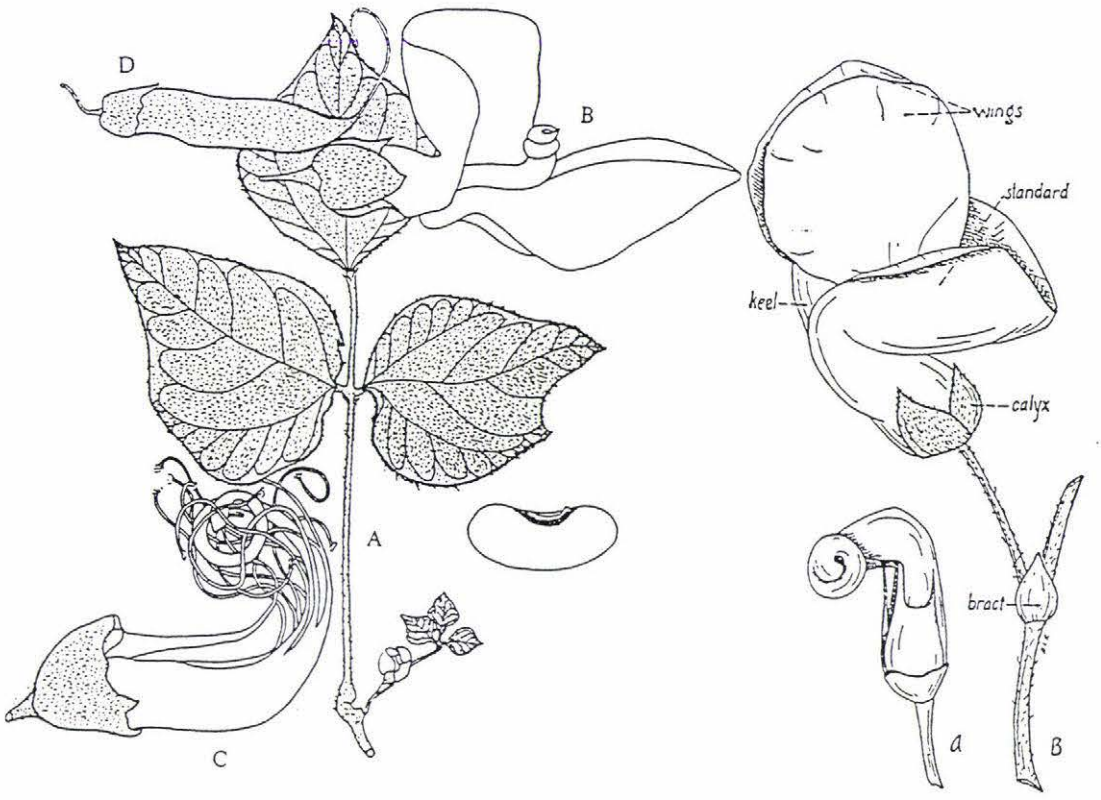
Five plants of each colour group were used to determine the morphological characteristics of the plants, including the colour of the flowers, the main stem and internode lengths, the length and width of the primary and trifoliolate leaves, and the length or length and width of smaller organs such as the primary bracts, pedicellate bracts, stipules and stipels.

Leaf length and width measurements were carried out 5 - 7 days after the appearance of the first inflorescence on each plant, as at that time the leaf area had mostly reached its maximum size. The length and width of both primary leaves of individual plants were measured without being removed from the plant. The primary leaf length was measured from the base of the leaf (i.e. at the junction between the leaf and the petiole) to the tip of the leaf. The leaf width was measured at the point of its largest distances from the side of the leaf to the main leaf vein.

The size of the 1st, 3rd and the 8th trifoliolate leaves of each plant was also measured. These leaves successively represented the smallest, medium and largest size of trifoliolate leaves below the 10th trifoliolate leaf. Most of the trifoliolate leaves located beyond the 10th were gradually reduced in size. Trifoliolate leaves are generally defined as compound leaves with three leaflets arising from the same point (Lane, 1984). In bean genotypes, the trifoliolate leaves are formed by two leaflets which are oppositely located along the base of the rachis, and with a single terminal leaflet at the furthest tip of the leaf raceme. The enumeration of the trifoliolate leaves began from the base of the plant. The length and width of the 1st, 3rd and the 8th trifoliolates were only taken from the last leaflet, as it was the only symmetrical leaflet among the three.

The colour of the flowers of each original seed colour group was determined between the first and second weeks of flowering. From 5 - 6 flowers per plant were used to determine flower colour. The colour of both parts of the flower (standard and wing) (Fig.2) was determined by using the Dictionary of Colour Standards (1951) from the British Colour Council and the Horticultural Colour Chart (1942) from the British Colour Council in collaboration with the Royal Horticultural Society.

Fig.2 Some different parts of the bean plant and bean flower




Phaseolus vulgaris : COMMON BEAN. A, leaf (× ¼); B, flower (× 3); C, flower with corolla removed (× 4); D, young pod (× 1); E, seed (× 2).

Common kidney bean (Phaseolus vulgaris). *A*, spiral keel; *B*, entire flower. × 2½.

Adapted from Purseglove, 1968 (left) and Robbins, 1924 (right)

Flower colours were determined between 9 - 12 am. (under clear and shiny conditions). These flowers were not removed from the plants and their colours were determined by checking against the colour standards.

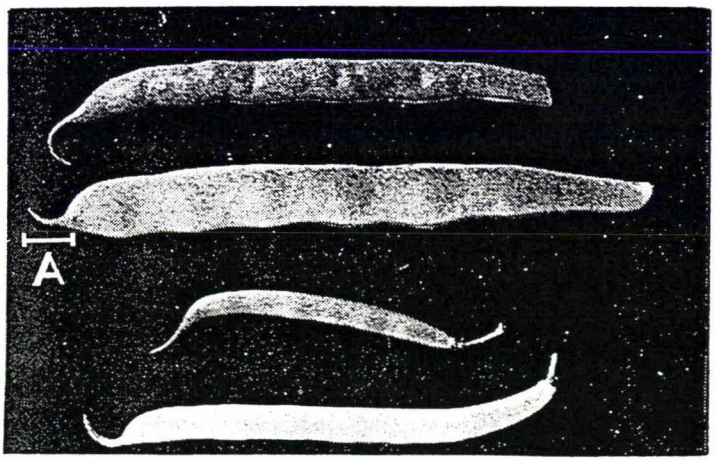
The size (i.e. the length and width) of smaller organs such as primary bracts, pedicellate bracts, bractioles, stipules and stipels was measured between the first and second week of flowering. From 10 - 15 of each of the organelles were removed

from individual plants of each genotype, and their length or length and width were determined. For the thinnest and membranaceous organelles, such as the pedicellate bracts, only length was measured. The length of each organelle, such as the length of the primary bracts, was measured from the base to the opposite tip; the width was measured at the largest point.

Pod colour was determined around three weeks after pod formation, as by this time most pods were highly coloured. Five pods from each plant were used (without being removed from the parent plants) to determine the pod colour using the Dictionary of Colour Standards (1951) from the British Colour Council and the Horticultural Colour Chart (1942) from the British Colour Council in collaboration with the Royal Horticultural Society.

Pods were left to dry on the parent plants before they were manually harvested. Pods were mostly harvested on 26 November 1994, and those which remained were progressively harvested over the following two weeks. The length and width of all pods from 5 plants/seed colour was measured after harvest. Pod length was measured from the base (part which is generally covered by a pair of bractioles) to the base part of the pod bic which is located at the opposite site of the pod. Pod width was measured at the widest point of the pod. Pod bic can be described as an extension of pod material, without seeds. In appearance it is like a spine, variable in length and shape, which is located at the tip of the pod (Fig.3).

Fig.3 Pod bic of the green bean.




A = pod bic

Adapted from Gane, 1975 (with some modifications)

All pods and seeds contained in each pod from each plant of the individual genotypes in the glasshouse were counted to obtain the number of pods and seeds per pod for the individual genotype. The length and width of the seeds were measured after harvest (at smc of between 10 -12%) by taking a random measurement of 50 seeds/colour group.

The length of the main stem and the number of internodes, as well as the internode lengths were measured after harvest. The main stem of each plant was cut to 1 cm above the medium contained in the pots, and measured from the cotyledonary scars (where the two cotyledons were held before being dropped) to the last trifoliolate leaf. The number of internodes on the main stem was counted from the first node to the last trifoliolate leaf, while the internode lengths were measured from node to node along the main stem.

## 3.2. Field trial

### 3.2.1. Land preparation and experimental design

Another trial was conducted in the field, from November 1994 (14.5 h daylength) to March 1995 (daylength of around 12.3 h). The maximum daylength between this period was about 15 h (December). The air maximum/minimum temperatures (in the field) from this period were respectively around 17.3/10.1 and 23.3/14.5 C°.

Seeds from the same five unknown bean genotypes grown in the glasshouse were used in the field trial which was located at the Frewen's block, Massey University with an approximate altitude of 33 m above mean sea level. The soil at the experimental site was a fine sandy loam (Cowie, 1974), and a soil analysis on 24 November 1994 showed that Phosphorous, Sulphur, Exch. Potassium, Exch. Calcium and Exch. Magnesium were successively 13 µg/g, 5.5 µg/g, and 0.49, 7.2, 1.39 meq/100g. The exchangeable cations (CEC) was 21 meq/100g with a pH of 5.3. The experimental site had previously grown a crop of ryegrass (*Lolium perenne* L.) for 6 months prior to this trial.

The site was desiccated (glyphosate), ploughed and harrowed, followed by an application of 125 kg/ha of Nitrophoska fertilizer (BASF), with a 12:10:10:2 ratio of N:P:K:S. Three days before sowing (i.e. on 25 November 1994), the experimental site was sprayed with Triflur 40 (a grass pre-emergence herbicide with 400g a.i./l of Trifluralin) at a rate of 1 l/ha which was followed by a second harrow to allow the incorporation of the herbicide into the soil. A second application of Basagran herbicide (480 g a.i./l of Bentazone) at a rate of 1.44 l/ha was made on 4 January 1995. Before sowing, bean seeds were mixed with Thiram (Thiram 80 W) in a proportion of 1% a.i. by weight. Seeds from each genotype were sown at three different rates (around 2.8, 5.6 and 8.4 g/m$^2$) by cone seeder on 28 November 1994 to obtain densities of 6.6, 13.3 and 20 plants/m$^2$ with 60, 30 and 20 cm respectively between the rows. Within rows a uniform space of 25 cm was maintained. Each main plot (plant density) was split into subplots (genotype) which were four rows 3.0 m long. Each density was randomly assigned to the main plots, and genotypes

were randomly assigned to subplots. Each treatment (density x genotype) was replicated four times. No irrigation was provided after sowing. Seedling emergence began 7 - 9 days after sowing. Some seedling spacing problems within rows caused by a seeder malfunction were rectified within 3 - 5 days after seedling emergence by removing the wrongly spaced seedlings from the soil and immediately transplanting them to establish the appropriate plant density. The whole planting area was watered daily with around 250 - 300 ml/plant by using a spray nozzle and hand-held hose each day for 7 days from transplanting. No fungicide or insecticide was applied during the field trial as there was no evidence of significant damage caused by pathogens and insects.

Thirty days after seedling emergence, 3.15 t/ha of hydrated lime [$Ca(OH)_2$] was applied to elevate the soil pH to approximately 1 unit above the initial pH of 5.3. The addition of lime was followed by an irrigation to allow its rapid movement and incorporation into the soil. Four days after liming (i.e. approximately five weeks after seedling emergence), 240 kg of urea (46% N)/ha was broadcast on the experimental area. The soil pH from the experimental site 6 months from harvest (beans were harvested in March 1995) was 5.6.

### 3.2.2. Data collection and seed development

For data collection, ten plants were randomly selected from rows 2 and 3 of each subplot. Plants from row 1 and 4 were used as border plants. The parameters recorded in this study were the number of flowers per plant, pods per plant, seeds per pod, 100 seed weight, seed yield per plant and seed yield per unit area ($g/m^2$). All data measurements had four replicates, except for the number of flowers per plant which was only based on three replicates. The non-selected plants in both inner rows of each subplot were used for seed development studies.

In all bean genotypes, the colour of flowers was generally brighter and fresher at the first day of flower emergence but had become pallid (a reduction in colour brightness and freshness) in the days following. Flowers were almost dry

around 5 - 7 days after the first day of emergence. The degradation of colour brightness and freshness increased as the number of days after flower emergence increased. These differences in colour brightness and freshness among flowers were used to distinguish between flowers with different flower emergence dates, and simultaneously served to avoid double counting of flowers from the same sample plant at the different times of flower counting.

In this trial the number of flowers from each of the 10 sample plants per genotype at different plant densities was counted every two days from the beginning of inflorescence emergence (16 Jan.1995) to the appearance of the last inflorescence (22 Feb.1995) to obtain the final number of flowers per plant. To facilitate in counting, the number of flowers per plant in each sample plant was recorded from the individual branches located along the base of the main stem to the furthest tip of the plant.

The number of secondary branches per plant of individual bean genotypes at different plant densities were recorded after harvest. All secondary branches of the 10 sample plants per genotype at the different plant densities were counted to obtain the final number of secondary branches/plant. The determination of the number of plants with indeterminate climbing characteristics (i.e. with longer and profuse branches, able to climb and to continuously produce new flowers) and the indeterminate non-climbing (i.e. bush indeterminate) per genotype was conducted in the field by using all the plants in each subplot (a total of 160 - 180 plants per seed colour group) from the density of 13.3 plants/$m^2$. The same number of plants was used to determine the average of the main stem length (i.e. plant height) of the determinate plants, and also to determine the percentage of seed colour mutation (changes/variation in seed colour) that occurred in individual bean genotypes during the field trial. These measurements were conducted after harvest.

Other plant morphological characteristics such as the length of primary leaves, bractioles, pedicellate bracts, etc. were not measured in the field trial.

*Seed development*

For the seed development studies, at least a total of 450 - 460 flowers/ seed colour group (from the 13.3 plants/$m^2$ density of plants that were not used for density studies) were randomly selected and labelled at anthesis (18 to 23 Jan) to obtain at least 60 pods per individual genotype at each harvest time. Pods produced from the selected flowers were harvested manually 14, 20, 26, 32, 40 and 50 days later for the determination of seed moisture content (smc), fresh weight, dry weight and the percentage seed germination.

*Seed moisture content* (smc)

For the determination of smc (wet basis), duplicates of 30 whole seeds were dried at 103°C for 17 hours (ISTA, 1993), and moisture content was calculated as:

$$\text{smc (\%) (wet basis)} = \frac{\text{Initial seed weight - Final seed weight}}{\text{Initial weight seed}} \times 100$$

*Fresh seed weight*

The fresh seed weight was obtained from duplicate measurements of 30 whole seeds immediately after being removed from the pods.

*Dry weight*

The dry weight of seeds was determined as follows:

$$\text{Dry seed weight (g)} = \text{Fresh seed weight} \times \frac{100 - \text{smc}}{100}$$

*Percentage germination*

Entire fresh seeds were allowed to dry at room temperature for 2 - 3 weeks and then a standard germination test (ISTA, 1993) was carried out using the between paper method at 25°C. First count was done at 5 days and a final count after 9 days. Seeds were dusted with Thiram (1% a.i. by weight) prior to germination.

*Harvest*

*Pods per main stem and pods per secondary branches*

All pods from the main stem as well as from the secondary branches from each of the 10 sample plants per genotype at the different plant densities, were counted at the day before the first harvest (20 March 1995) to obtain the number of harvested pods/main stem and harvested pods/secondary branch from different densities without being removed from the plant. The sample plants remained in the field until all the pods were harvested.

*Branches per plant (secondary branches)*

For this purpose, all the secondary branches of the sample plants at different densities were counted in the field at the day before the first harvest (20 March 1995).

*Harvested pods per plant*

Pods from the sample plants that were located around the base of the main stem dried earlier and were harvested by hand on 20 March 1995 while the others on the upper part of the plant were harvested by hand (30 March 1995). The pods per plant obtained from both harvest dates were placed in the same paper bag. All pods from the sample plants (at different densities) were counted to obtain the final number of pods/plant.

*Seeds per pod*

Seeds were harvested from the field at around 21 to 22% smc. After harvest, they were removed from the individual pods by hand and then counted before being placed in the paper bags to ambient air dry to 11 - 12% smc. All seeds from each of the pods from different densities were recorded to obtain the number of seeds/pod.

*Seed yield per plant*

The seed weight per plant was determined by weighing the seeds from each plant. This weight was then converted to 10% smc.

*Seed yield per unit area*

The yield per unit area (i.e. kg/ha) of different bean genotypes at different densities was obtained from the yield per plant of each genotype times plant population per unit area.

*Hundred seed weight*

To determine 100 seed weight, five lots of 100 seeds were drawn randomly from each seed sample with known smc and weighed. The 100 seed weight is presented at 10% seed moisture content.

## 3.3.Seed Quality

### 3.3.1. Seed vigour test

#### 3.3.1.1. Conductivity test

Four replicates of 50 seeds of each bean genotype were weighed to two decimal places before being placed in 500 ml conical flasks to which 200 ml of distilled water at 20°C was added. A control flask containing distilled water only was set up with each test run. All flasks were covered with parafilm sheet to reduce

evaporation and dust contamination, and maintained at 20°C for 24 hours after which conductivity was recorded. All measurements were taken at 20°C in accordance with ISTA (1987) recommendations.

### 3.3.1.2. Accelerated Ageing (AA)

Fifty grams of seeds at 11 - 12% smc were weighed and placed in a single layer on a wire-mesh tray (10 x 10 x 3 cm) and subsequently placed above 40 ml distilled water in a 11 x 11 x 3.5 cm plastic box which was then covered securely with a lid (not sealed). Plastic boxes contained seeds were then placed into a dry air incubator running at 42°C for 72 hours (ISTA, 1987). Inner chambers were arranged in groups of six on the upper and middle shelf in the incubator, avoiding over-crowding and allowing for air circulation. The incubator door remained shut for the duration of the ageing period. This trial was conducted with four replicates and at the end of the ageing period, seeds were removed and germination tested (BP at 25°C) using four replicates of 50 seeds (ISTA, 1993). Seeds were dusted with Thiram (1% a.i. by weight) prior to germination. The percentage of normal seedlings after AA was recorded at day 5 and day 9.

## 3.3.2. Cooking quality

### 3.3.2.1. Imbibition rate

Cooking quality, as characterized by the ability of the seeds to imbibe quickly, cooking time and softening after cooking, was assessed for seeds from bean genotypes harvested from the field trial (March 1995) and Haricot bean seeds obtained from WoolWorths Super Market (Palmerston North). Only seeds from the 20 plants/$m^2$ density were used in this study. The seed moisture content of seeds from each bean genotype and Haricot bean were determined according to the ISTA (1993) rules, while the percentage of the seeds with seed coat cracks was visually determined using four replicates of 50 seeds which were taken randomly from each seed sample.

For the imbibition rate measurement (i.e. water absorption rate), three replicates of 10 g seed were soaked in 50 ml of distilled water for 2,4,8,14 and 24 hours at 25C°. After soaking for the appropriate time, the water was drained and surface water removed by blotting. Gain in weight was taken as the amount of water absorbed and expressed as a percentage of the dry weight (Sefa-Dedeh et al., 1979; Hincks and Stanley, 1986) which can be represented as follows:

$$\text{S W I (Seed Weight Increase)} = \frac{\text{Last Seed Weight - Initial Seed Weight}}{\text{Initial Seed Weight}} \times 100\%$$

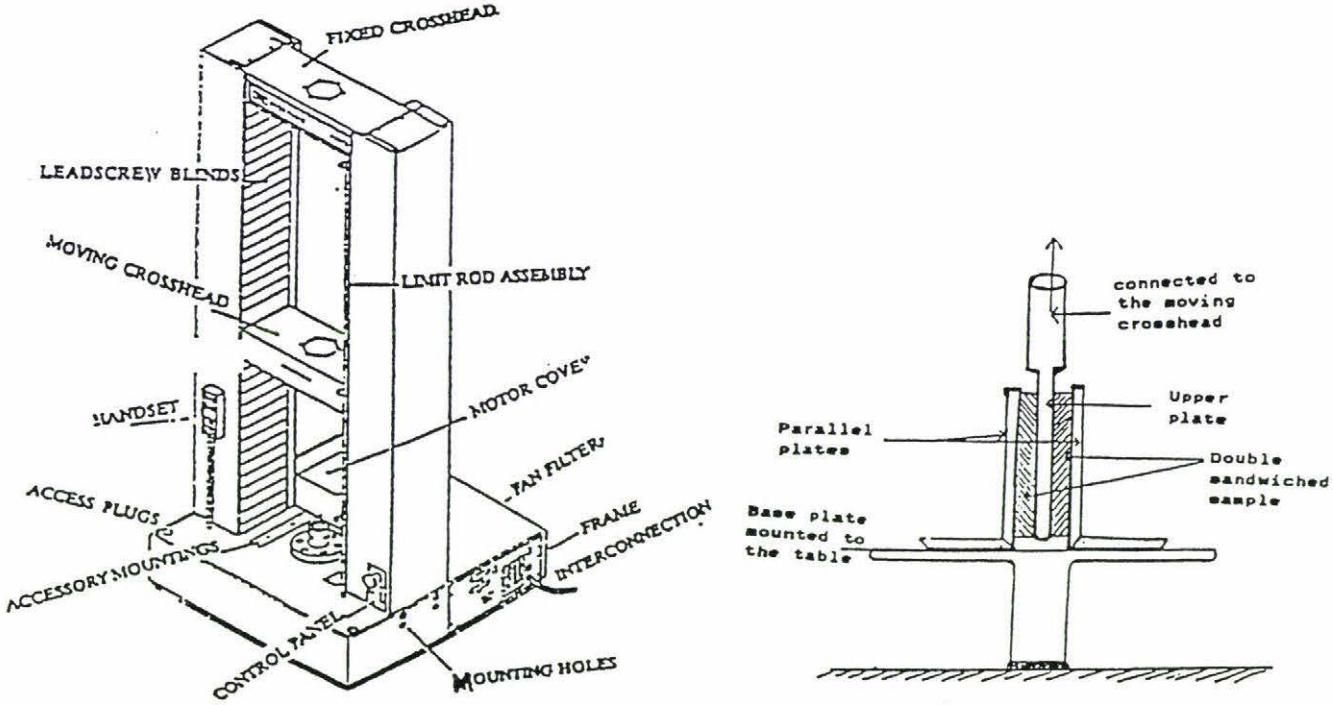
**3.3.2.2. Seed texture (softness) after cooking**

Before cooking, seeds from each bean genotype and Haricot bean (as a control) were soaked at 25C° for 14 hours in a volume of distilled water five times their dry weight (Hsieh, et al., 1992). Seeds that failed to imbibe during soaking were removed from the others before cooking.

Seeds of all genotypes were cooked for 20 minutes in boiling water (between 103 - 104 C° which was measured by a thermometer while they were cooking) under normal atmospheric pressure. Cooktime commenced when the water started boiling. The cooked bean samples were drained and allowed to cool for at least 1 hour to facilitate accurate mass measurements for hardness (or softness) (Hincks and Stanley, 1986). For the measurement of seed texture, cooked seeds that still remained intact were randomly selected and measured by using the Instron Universal Testing Machine (model IX series 4502 manufactured by Instron Company of Coronation Road, High Wycombe, Bucks. England) (Hincks and Stanley, 1986; Faraay, 1995). For this purpose, individual samples was placed on a flat plate (base plate mounted to the table) (Fig.4) to be cut (compressed) by the upper plate (blade

around 1 mm thick) that was connected to the moving crosshead which was operated in the Instron Testing Machine. The crosshead speed used in this study was 5 cm/min. All tests were performed at room temperature (20-21 C°) (Shama and Sherman, 1973) with four replicates of 12 seeds in each. The texture of beans was expressed by the force (Newton) required to cut through their seed coat and cotyledons.

Fig.4 Diagram of the Instron Universal Testing Machine




Adapted from Faraay, F. M., 1995.

### 3.3.2.3. Seed integrity after cooking

After cooking, seeds were gradually cooled, and the number of damaged seeds such as those with cotyledons almost or already broken in different parts, and seeds with smashed or with loose seed coats, were determined by a simple visual counting. This observation was done with four replicates of 50 seeds.

All the data acquired from the study were analyzed with the statistical analysis system of SAS with least significant differences at the 5% level.

# CHAPTER 4
# RESULTS

## 4.1. Plant characteristics measured in the glasshouse

### 4.1.1. Primary leaf

Table 1. Effect of genotype on the primary leaf characteristics

| The characteristics | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| leaf length (cm) | | | | | |
| range | 11.0 -12.4 | 10.0 -13.4 | 10.8 -13.0 | 10.4 - 12.8 | 13.2 - 14.5 |
| mean | 11.6 ± 0.1 | 11.2 ± 0.2 | 11.8 ± 0.2 | 12.0 ± 0.2 | 13.8 ± 0.2 |
| leaf width (cm) | | | | | |
| range | 10.9 -12.4 | 10.4 -13.0 | 8.0 - 13.4 | 9.0 - 11.3 | 12.3 - 15.7 |
| mean | 11.4 ± 0.2 | 11.0 ± 0.1 | 10.1 ± 0.2 | 10.0 ± 0.3 | 13.9 ± 0.5 |
| stiple (mm) | | | | | |
| range | without | 1.2 - 1.4 | 2.0 - 2.5 | without | 1.2 - 1.5 |
| mean | stiple | 1.3 ± 0.05 | 2.2 ± 0.2 | stiple | 1.3 ± 0.05 |

Primary leaf length was largest for the brown genotype (Table 1) and smallest for the mottled brown genotype. Small differences existed among the white, mottled black and black genotypes. The range in the primary leaf lengths was greatest for the mottled brown genotype, while being similar for the other genotypes. The brown genotype had the largest width for the primary leaf (Table 1) while the smallest size was recorded from the mottled black and black genotypes. The range in this parameter was highest for the mottled black genotype and did not differ with other genotypes. No stiple was recorded for the white and black genotypes, while it was bigger for the mottled black than for the two other genotypes, both of which were similar in their range.

### 4.1.2. Trifoliolate leaves

Table 2. Effect of genotype on the 1st trifoliolate leaf characteristics

| The characteristics | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| length (cm) | | | | | |
| range | 12.5 - 13.9 | 13.0 -16.0 | 11.9 -14.7 | 11.8 - 15.0 | 20.1 - 23.8 |
| mean | 12.9 ± 0.1 | 14.1 ± 0.2 | 13.3 ± 0.6 | 3.4 ± 0.2 | 21.7 ± 0.7 |
| width (cm) | | | | | |
| range | 7.2 - 8.7 | 7.1 - 10.4 | 5.6 - 9.5 | 6.3 - 7.9 | 11 - 13.6 |
| mean | 7.8 ± 0.2 | 8.7 ± 0.2 | 7.1 ± 0.6 | 7.1 ± 0.2 | 12.2 ± 0.3 |
| stipel (mm) | | | | | |
| range | 1.4 - 2.1 | 1.6 - 2.4 | 1.3 - 2.4 | 1.2 - 2.8 | 2.2 - 2.9 |
| mean | 1.8 ± 0.1 | 2 ± 0.1 | 1.9 ± 0.1 | 1.9 ± 0.1 | 2.5 ± 0.2 |

The length of the 1st trifoliolate leaf was largest for the brown genotype (Table 2) while small differences were recorded among the other four genotypes. The range in this measurement was lowest for the white genotype while being similar for the others. The brown genotype had the largest width for the 1st trifoliolate leaf (Table 2), while small differences existed among the other genotypes. The range in this parameter was lower for the white and black genotypes than for the others. The size of the stipel was largest for the brown genotype (Table 2), and small differences were recorded among the other genotypes. The range in the stipel sizes was greatest for the black genotype while the others were similar in their range.

Table 3. Effect of genotype on the 3rd trifoliolate leaf characteristics

| The characteristics | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| length (cm) | | | | | |
| range | 10.5 - 13.5 | 12.5 -15.9 | 13.2 -16.1 | 11.6 - 13.2 | 20.4 - 23.8 |
| mean | 12.4 ± 0.1 | 14.1 ± 0.4 | 14.7 ± 0.3 | 12.4 ± 0.1 | 22.0 ± 0.7 |
| width (cm) | | | | | |
| range | 8.0 - 9.6 | 8.6 - 10.3 | 8.4 - 11.0 | 8.2 - 9.2 | 13.1 - 16.0 |
| mean | 9.0 ± 0.2 | 9.6 ± 0.4 | 9.5 ± 0.2 | 8.6 ± 0.1 | 14.1 ± 0.2 |
| stipel (mm) | | | | | |
| range | 1.7 - 2.3 | 1.9 - 3.3 | 2.0 - 3.2 | 1.6 - 2.6 | 2.4 - 3.7 |
| mean | 1.9 ± 0.1 | 2.5 ± 0.1 | 2.2 ± 0.2 | 1.9 ± 0.1 | 2.9 ± 0.2 |

The length of the 3rd trifoliolate leaf was largest for the brown genotype (Table 3), while the smallest was for the white and black genotypes. The range in this parameter was lowest for the black genotype while the other genotypes were similar in their range. The brown genotype was the one with largest width for the 3rd trifoliolate leaf (Table 3), and the black genotype had the smallest width. The range in this parameter was lower for the black genotype than for the others. The stipel size was biggest for the brown genotype (Table 3), with the white and black genotypes having the smallest stipels. The range in the stipel sizes was similar among the different genotypes.

Table 4. Effect of genotype on the 8th trifoliolate leaf characteristics

| The characteristics | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| length (cm) | | | | | |
| range | 13.5 - 16.9 | 12.8 -15.9 | 11.9 -20.0 | 12.0 - 17.3 | 16.0 - 24 |
| mean | 15.0 ± 0.6 | 14.0 ± 0.4 | 16.1 ± 0.4 | 15.5 ± 0.3 | 21.8 ± 0.5 |
| width (cm) | | | | | |
| range | 10.3 - 12.2 | 9.7 - 12.3 | 8.8 - 14.2 | 9.6 - 12.7 | 11.5 - 16.5 |
| mean | 11.5 ± 0.2 | 11.0 ± 0.2 | 11.9 ± 0.1 | 11.4 ± 0.1 | 14.6 ± 0.8 |
| stipel (mm) | | | | | |
| range | 2.5 - 2.8 | 2.6 - 3.1 | 2.5 - 3.5 | 2.3 - 3.4 | 3.0 - 3.9 |
| mean | 2.7 ± 0.1 | 2.9 ± 0.1 | 2.8 ± 0.2 | 2.7 ± 0.1 | 3.4 ± 0.2 |

The length of the 8th trifoliolate leaf was largest for the brown genotype (Table 4) while the other genotypes were similar in their length. The range in this parameter was highest for the mottled black and brown genotypes while it was similar for the others. The width of the 8th trifoliolate leaf was largest for the brown genotype (Table 4) but no differences were recorded for the others. The range in this measurement was highest for the mottled black and brown genotypes although small differences were also recorded among the other genotypes. The stipel size was biggest for the brown genotype (Table 4) but did not differ for the other genotypes. The range in the stipel sizes was lowest for the white and mottled brown genotypes while no differences existed among the other genotypes.

### 4.1.3. Main stem

Table 5. Effect of genotype on the main stem characteristics

| main stem charact. | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| length (cm) | | | | | |
| range | 273 - 411 | 115 - 349 | 170 - 364 | 253 - 367 | 117 - 217 |
| mean | 362 ± 37 | 197 ± 24 | 253 ± 37 | 321 ± 12 | 166 ± 37 |
| total nodes/main stem | | | | | |
| range | 17 - 21 | 9 - 23 | 12 - 22 | 16 - 22 | 11 - 16 |
| mean | 19 ± 1 | 14.5 ± 2 | 17 ± 3 | 19 ± 1 | 13 ± 2 |

The main stem length was greatest for the white and black genotypes (Table 5) while the brown one had the lowest stem length. The mottled brown and brown genotypes were similar for their main stem length, which the mottled black genotype was intermediate. The range in this parameter was highest for the mottled brown and mottled black genotypes while the others were similar in their range. The highest number of nodes/main stem was recorded from the white and black genotypes (Table 5) while the brown genotype had the lowest number of nodes/main stem. The other two genotypes were similar for this measurement. The range in this parameter was greatest for the mottled brown and mottled black genotypes, the three others being similar in their range.

### 4.1.4. Bracts

Table 6. Effect of genotype on the primary bract, bractiole and pedicellate bract characteristics

| The characteristics | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| Primary bract | | | | | |
| length (mm) | | | | | |
| range | 3.1 - 4.0 | 3.2 - 4.7 | 4.1 - 5.0 | 3.2 - 4.6 | 3.9 - 5.1 |
| mean | 3.5 ± 0.2 | 3.9 ± 0.1 | 4.5 ± 0.2 | 3.8 ± 0.1 | 4.5 ± 0.2 |
| width (mm) | | | | | |
| range | 2.2 - 2.8 | 2.4 - 3.6 | 2.5 - 3.9 | 2.3 - 3.7 | 3.1 - 4.5 |
| mean | 2.4 ± 0.1 | 2.8 ± 0.2 | 3.0 ± 0.1 | 2.8 ± 0.1 | 3.8 ± 0.2 |
| Bractiole | | | | | |
| length (mm) | | | | | |
| range | 5.9 - 6.9 | 6.0 - 7.9 | 6.6 - 7.8 | 5.1 - 7.0 | 5.2 - 7.5 |
| mean | 6.3 ± 0.1 | 6.9 ± 0.2 | 6.9 ± 0.1 | 6.2 ± 0.1 | 6.1 ± 0.3 |
| width (mm) | | | | | |
| range | 5.1 - 5.9 | 5.3 - 7.4 | 5.4 - 6.1 | 3.7 - 6.1 | 4.1 - 6.6 |
| mean | 5.5 ± 0.1 | 6.0 ± 0.1 | 5.7 ± 0.1 | 5.0 ± 0.2 | 5.2 ± 0.2 |
| Pedicellate bract | | | | | |
| length (mm) | | | | | |
| range | 0.8 - 1.6 | 1.2 - 1.9 | 1.5 - 2.2 | 1.2 - 1.8 | 3.1 - 4.2 |
| mean | 1.1 ± 0.05 | 1.4 ± 0.1 | 1.8 ± 0.1 | 1.6 ± 0.1 | 3.6 ± 0.2 |

The length of the primary bract was largest for the mottled black and brown genotypes (Table 6) but did not differ for the other genotypes. The range in this parameter was similar for all genotypes. The white bean had the smallest width for the primary bract while the brown bean had the largest (Table 6). The mottled brown, mottled black and black genotypes were similar for this measurement. The range in the width of the primary bract was lowest for the white genotype but did not differ with the other genotypes.

The length of the bractiole was largest for the mottled brown and mottled black genotypes (Table 6) but did not differ for the other genotypes. The range in the bractiole length was greatest for the brown genotype, although small differences were also recorded among the other genotypes. The black and brown beans had the smallest width for the bractioles (Table 6) but this did not differ for the other genotypes.The greatest range in this measurement was recorded from the black and brown beans while the other genotypes had similar ranges.

The length of the pedicellate bract was largest for the brown genotype (Table 6) and smallest for the white genotype, although some small differences existed among the others. The range in this measurement was similar for all genotypes.

### 4.1.5. Pods

Table 7. Effect of genotype on pod characteristics

| Pod characteristics | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| harvested pods/plant | | | | | |
| range | 13 - 20 | 7 - 14 | 8 - 20 | 12 - 17 | 14 - 17 |
| mean | 15 ± 1.3 | 13 ± 2 | 14 ± 1 | 13.5 ± 1 | 16 ± 1 |
| pod length (cm) | | | | | |
| range | 8 - 15 | 8 - 13 | 8 - 13 | 8 - 14 | 6 - 10.5 |
| mean | 12.1 ± 0.7 | 10.4 ± 0.1 | 10.7 ± 0.1 | 11.3 ± 0.7 | 9.3 ± 0.5 |
| pod width (mm) | | | | | |
| range | 11 - 14.6 | 11.1 - 14 | 11 - 13 | 11.2 - 13.8 | 10.7 - 14.5 |
| mean | 12.8 ± 0.05 | 12.7 ± 0.1 | 11.8 ± 0.2 | 12.6 ± 0.4 | 12.6 ± 0.4 |
| the length of the pod bic (mm) | | | | | |
| range | 6.7 - 15.5 | 3.7 - 13.4 | 3.1 - 14.5 | 4.4 - 18.1 | 3.4 - 18.3 |
| mean | 10.9 ± 0.3 | 9.7 ± 0.4 | 8.2 ± 0.8 | 11.7 ± 1.1 | 11.9 ± 0.4 |

The mean number of harvested pods/plant was highest for the white and brown genotypes (Table 7) but did not differ for the other genotypes. However the

range in this measurement was highest for the mottled black genotype. The white genotype had the largest pod length and the brown genotype had the smallest (Table 7), while small differences were also recorded among the other genotypes. The range in pod length was lowest for the brown genotype.

The pod width was smallest for the mottled black bean (Table 7) while the other genotypes were similar in their size. The range in this measurement did not differ with genotype. The pod bic length was largest for the brown bean and smallest for the mottled black bean genotype (Table 7). The range in pod bic length was lowest for the white genotype and highest for the brown genotype but was variable for all genotypes.

### 4.1.6. Seeds

Table 8. Effect of genotype on seed characteristics

| Seed characteristics | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| seeds/pod | | | | | |
| range | 4 - 8 | 4 - 7 | 3 - 7 | 4 - 7 | 3 - 6 |
| mean | 5.8 ± 0.4 | 5.1 ± 0.2 | 5.1 ± 0.1 | 5.3 ± 0.3 | 4.4 ± 1.3 |
| seed length (mm) | | | | | |
| range | 10.3 - 15 | 9.7 - 14.5 | 10 - 14.5 | 11 - 15.6 | 10.7 - 14.3 |
| mean | 13.4 ± 0.5 | 13.1 ± 0.2 | 12.3 ± 0.4 | 13.9 ± 0.1 | 13.1 ± 0.1 |
| seed width (mm) | | | | | |
| range | 7 - 9.4 | 7.1 - 9.5 | 7.2 - 9 | 7.3 - 9.6 | 7.1 - 9.3 |
| mean | 8.3 ± 0.1 | 8.5 ± 0.2 | 8.2 ± 0.2 | 8.7 ± 0.2 | 8.4 ± 0.3 |

The white genotype had the greatest number of seeds per pod (Table 8), while the brown genotype had the lowest. The other three genotypes did not differ for seeds/pod.

Seed length and seed width were largest for the black genotype and smallest for the mottled black genotype (Table 8), although differences among the white, mottled brown, black and brown genotypes were very small. The range in seed length was lowest for the brown genotype, the other four genotypes being similar in their range. The range in the seed width was lowest for the mottled black genotype but did not differ with other genotypes.

### 4.1.7. Internode length

Table 9. Effect of genotype on the internode length (max. internode length)

| Internode length (cm) | white | mottled brown | mottled black | black | brown |
|---|---|---|---|---|---|
| range | 19.0 -28.5 | 16.0 -20.0 | 15.0 -22.5 | 17.0 -23.0 | 14.0 -21.0 |
| mean | 22.1 ± 0.2 | 17.5 ± 0.2 | 18.0 ± 0.5 | 19.5 ± 0.4 | 18.3 ± 0.7 |
| between internodes | 13 - 16 | 10 - 17 | 6 - 11 | 13 - 16 | 6 - 10 |

The maximum internode length of the main stem was greatest for the white genotype (Table 9) while being similar for the other genotypes. The range in this measurement was highest for the white genotype and lowest for the mottled brown genotype, the other genotypes being similar in their range. The maximum internode length for the mottled black and brown genotypes was recorded from the internodes that were located around the base of the main stem (between internodes 6-11) (Table 9), while the maximum internode length for the other genotypes was obtained from internodes at higher positions on the main stem.

### 4.1.8. Flower colour

Only two parts of the flower (*standard* and *wing*) (Fig.2) were determined in this trial.

a. The standard colour

The standard (part of the corolla) was white in the white, mottled brown and brown genotypes while it was mauve 633/3 in the mottled black genotype. However in the black genotype, the standard was either white or mauve 633/3 to rose purple 533/3 on different plants.

b. The colour of the wing

The white, mottled brown and brown genotypes produced flowers with white wings (wing petals) while they were mauve 633/1 to 633 in the mottled black genotype and either white or mauve 633/1 to 633 in different plants of the black genotype.

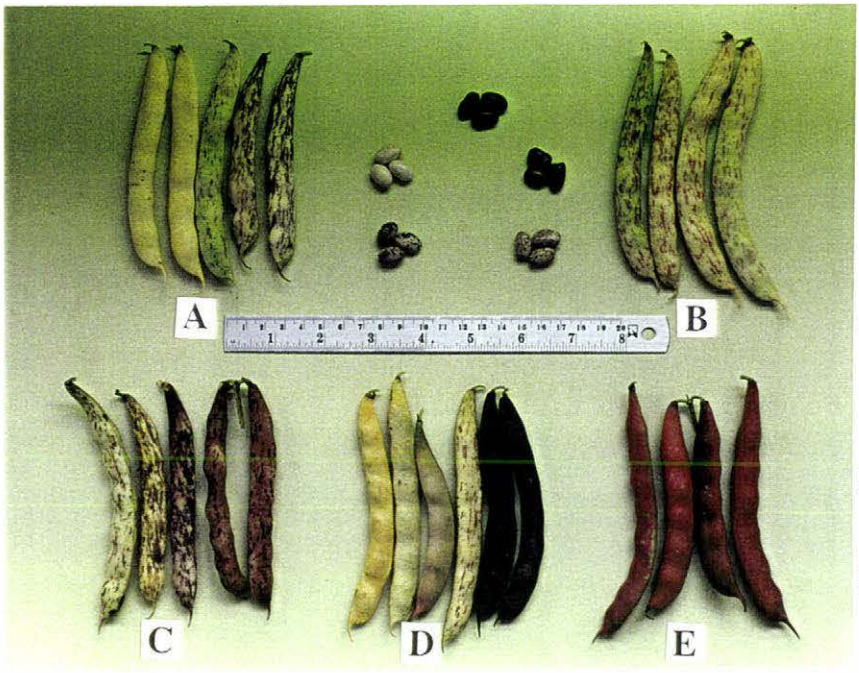
### 4.1.9. Pod colour

Three of 5 plants from the white genotype produced pods which were mimosa yellow 602/3 to naples yellow 403/3 - 403/2 while others produced pods with the same colour but mottling with either aster violet 38/3 or hyacinth blue 40/3. In the mottled brown genotype, the pods were predominantly naples yellow 403/3 in colour, mottled with china rose 024/3 - 024/1 or also with chrysanthemum crimson 824/3. The background colour in pods of the mottled black genotype was mimosa yellow 602/3 to amber yellow 505/3 (sometimes mixed with venetian pink 420/3). The pods of this genotype could also be mottled with purple brown (B.C.C. 136).

The black genotype had pods which were mimosa yellow 602/3 or naples yellow 403/3 mixed with light rose bengal 25/3 or crimson 22/3 (covering mostly the bottom part of the pod). They were either slightly mottled with lilac purple 031/3 - 031/1 or with pansy violet 033/3 - 033/1. However there were around 2 to 4% of

this genotype (in the field trial) that produced pods which were plum (B.C.C. 29) to egg plant (B.C.C. 30) or burgundy (B.C.C. 40) in colour. The brown genotype produced pods which were erythrite red 0027/3 - 0027/1 in colour. The same pod colours from individual genotypes (in the glass house) were also observed in the field trial (Fig. 5).

Fig.5 Colour of pods from five bean genotypes harvested from the field trial




A = white bean

B = mottled brown bean

C = mottled black bean

D = black bean

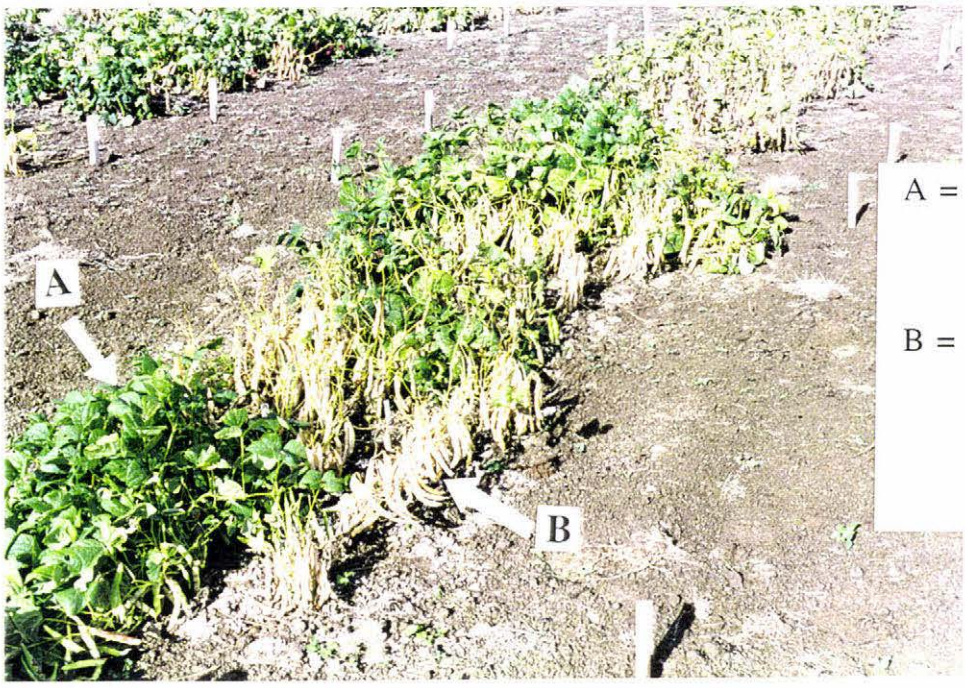
E = brown bean

## 4.2. Field Trial

### 4.2.1. General characteristics (segregation level in the field)

The black and white bean genotypes produced an average of 17 ± 2.4% and 11 ± 2% of plants with indeterminate climbing characteristics respectively whereas the mottled brown, mottled black and brown genotypes each produced an average of between 1 - 3% of plants with indeterminate climbing characteristics (Fig.6).

Fig.6 The coexistence of the indeterminate climbing and indeterminate non-climbing beans from the white genotype at a density of 20 plants/$m^2$ before harvest.



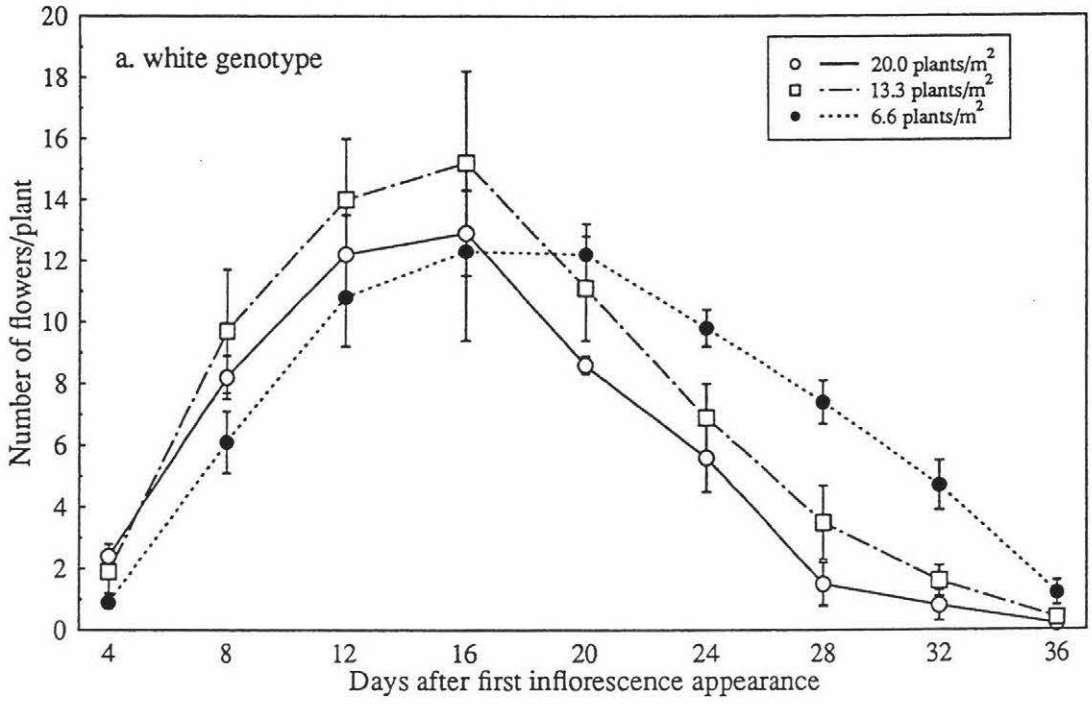

Between 1 - 3% of plants from the white genotype (with white seeds) produced pods with black seeds whereas 16 ± 2% of the plants from the black genotype (with black seeds) produced pods with white seeds.

The average plant height in all bean genotypes at different densities was between 50 - 60 cm with a min./max. height of 40/80 cm.

### 4.2.2. Flowers/plant

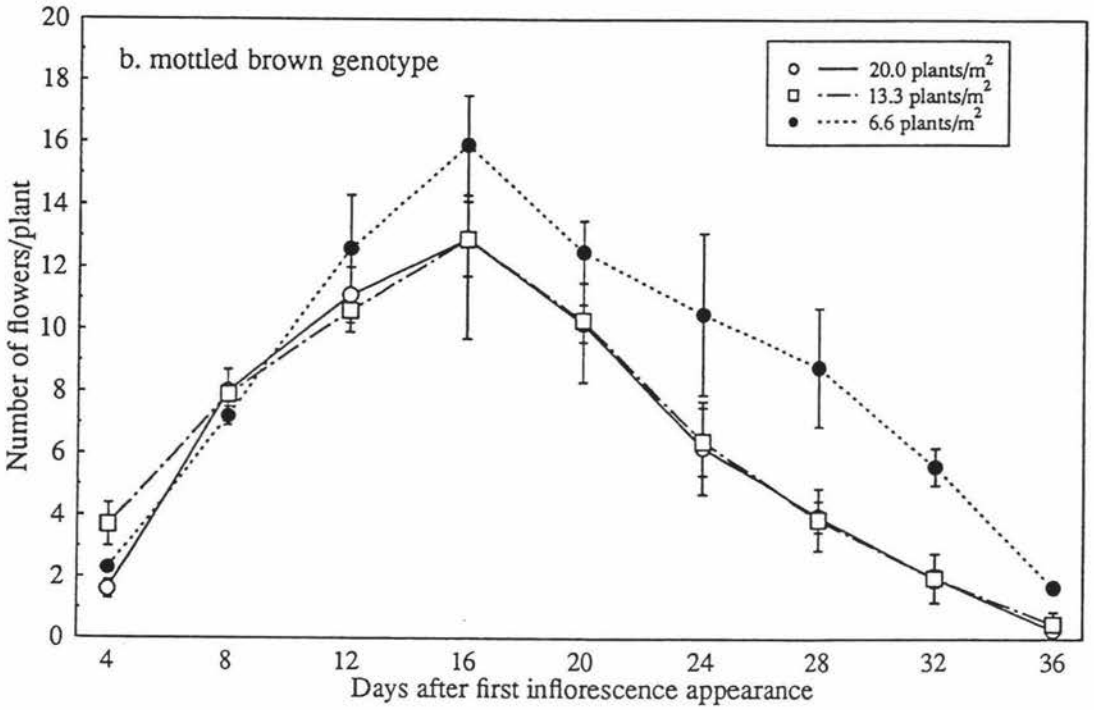
The number of flowers/plant of individual bean genotypes at four day intervals from the appearance of the first inflorescence until the appearance of the last inflorescence at each density is presented in Fig. 7a-e.

Fig.7a-e Number of flowers/plant from individual bean genotypes at different densities

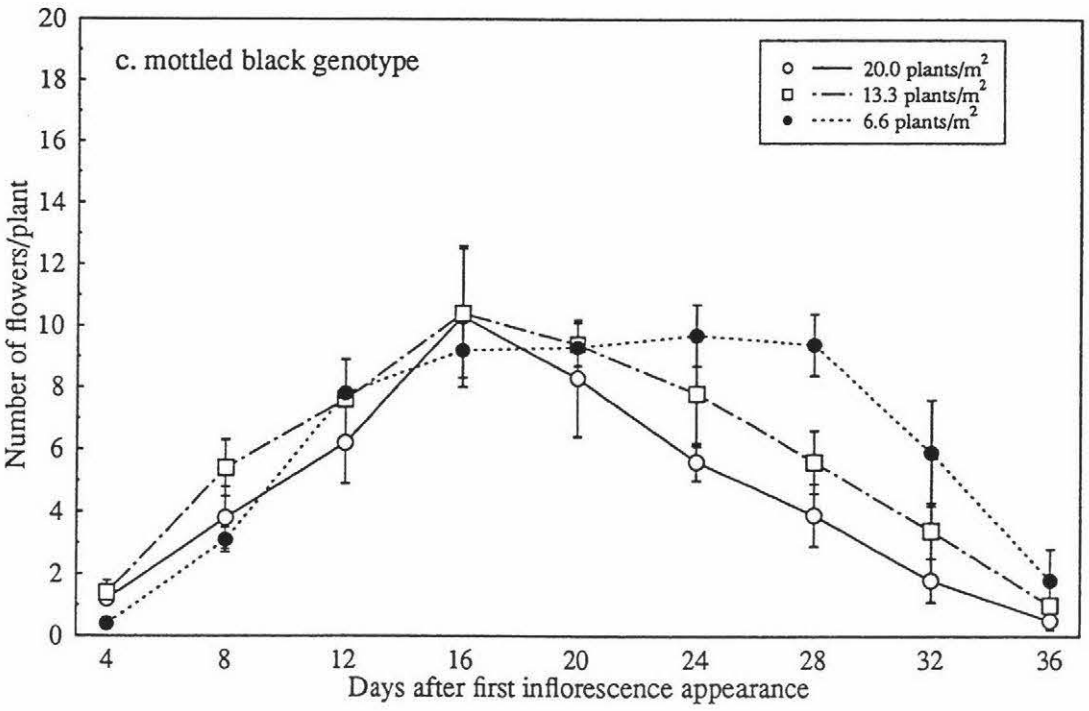



Flowers/plant in the white bean genotype did not differ with density at 4 days after first inflorescence appearance (f.i.a.) . However at 8 days from the f.i.a. the number of flowers/plant was higher for the 20.0 and 13.3 plants/$m^2$ density than that for the 6.6 plants/$m^2$ density (Fig. 7.a), although that difference was very small. Flowers/plant from all densities reached their peak at 16 days after f.i.a. and did not differ with density at 12 and 16 days after f.i.a (Fig. 7.a).

Flowers/plant from the 20.0 and 13.3 plants/$m^2$ densities decreased rapidly from 16 to 36 days after f.i.a. With the exception of the 20 days after f.i.a., the number of flowers/plant was highest for the 6.6 plants/$m^2$ density from 24 to 36 days after f.i.a. (Fig. 7.a). Flowers/plant were similar between the 20.0 and 13.3 plants/$m^2$ from 4 to 36 days after f.i.a.

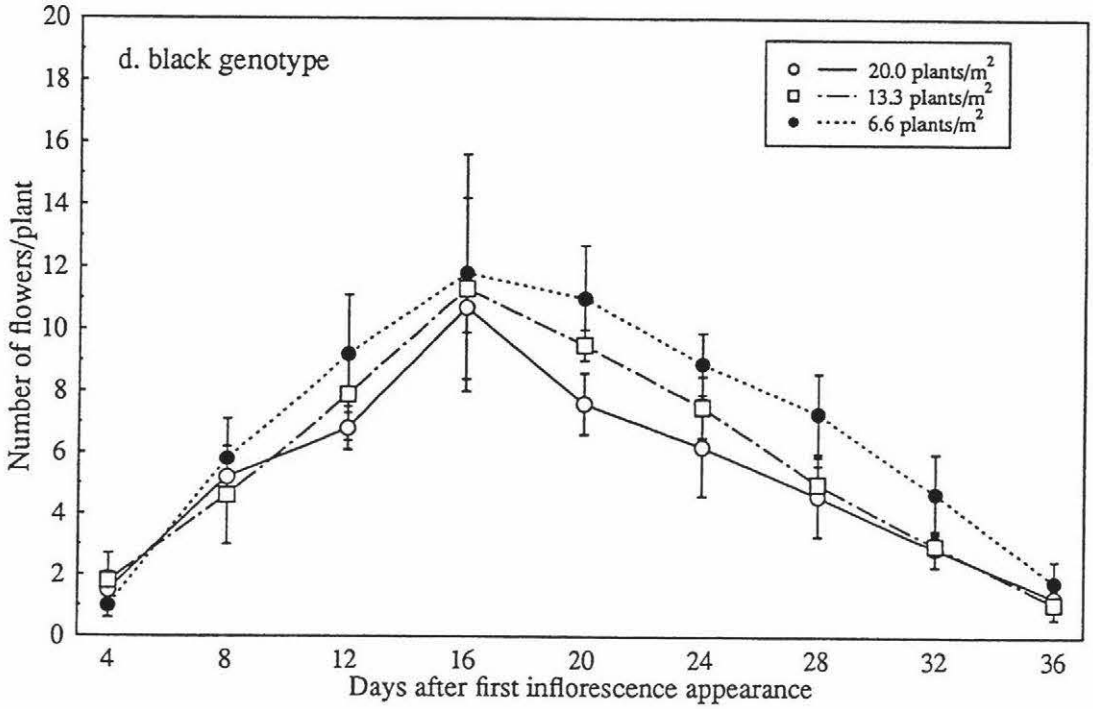



The mottled brown genotype had small differences in flowers/plant among densities at 4 days after f.i.a. but no differences at 8 days after f.i.a. (Fig. 7.b). The number of flowers/plant was maximum at 16 days after f.i.a. and did not differ with density from 12 to 16 days after f.i.a. Flowers/plant from densities of 20.0 and 13.3 plants/$m^2$ decreased rapidly from 16 to 36 days after f.i.a. (Fig. 7.b). At this period the number of flowers/plant from the density of 6.6 plants/$m^2$ was higher than the number of flowers/plant from the other two densities. The 20.0 and 13.3 plants/$m^2$ density did not differ in the number of flowers/plant from 4 to 36 days after f.i.a.

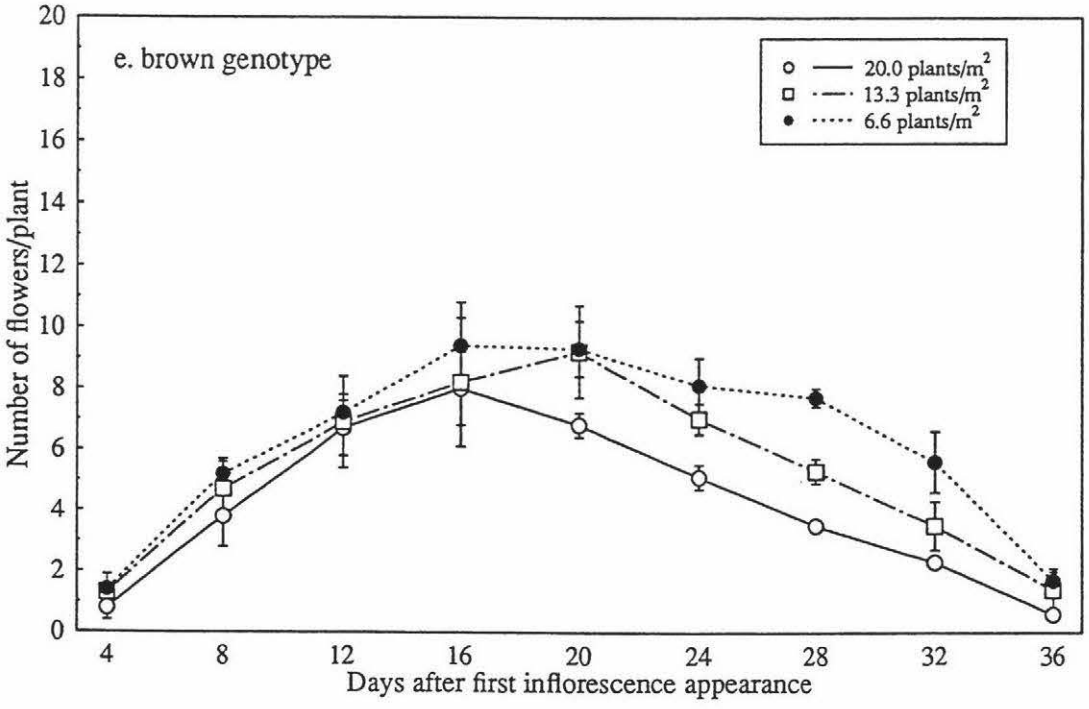



Flowers/plant in the mottled black genotype were lower for the 6.6 plants/$m^2$ density at 4 days after f.i.a. but only differed from the 13.3 plants/$m^2$ density at 8 days after f.i.a. (Fig. 7.c). With the exception of the 6.6 plants/$m^2$ density, flowers/plant reached their peak at 16 days after f.i.a. and did not differ with density from 12 to 20 days after f.i.a.

Flowers/plant were higher for the 6.6 plants/$m^2$ density than for the 20.0 plants/$m^2$ density at 24 and 32 days after f.i.a. (Fig. 7.c) and were similar between the 20.0 and 13.3 plants/$m^2$ density. Flowers/plant were highest for the 6.6 plants/$m^2$ density at 28 days after f.i.a. but similar for all genotypes at 36 days after f.i.a.

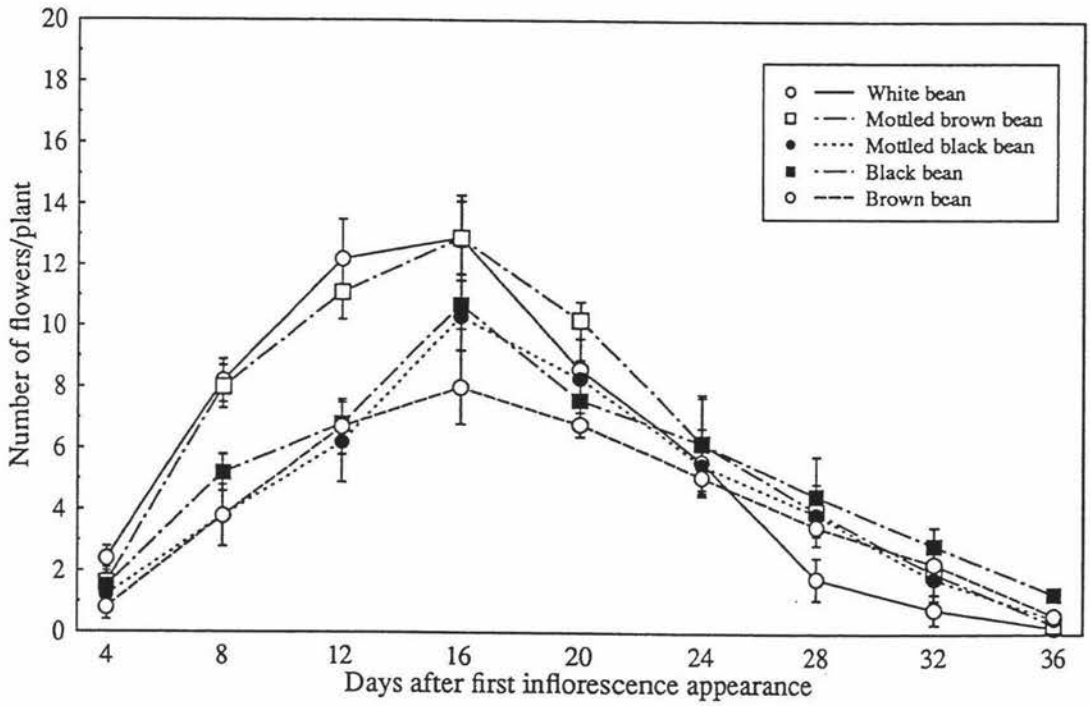



With the exception of 20 days after f.i.a., flowers/plant in the black genotype did not differ with density from 4 to 36 days after f.i.a. Flowers/plant were highest for the 6.6 plants/$m^2$ density and lowest for the 20.0 plants/$m^2$ density at 20 days after f.i.a (Fig. 7.d) while the maximum number of flowers/plant for each density was recorded at 16 days after f.i.a.



Flowers/plant in the brown genotype did not differ with density from 4 to 16 days after f.i.a. The maximum number of flowers/plant for the 6.6 and 20.0 plants/$m^2$ density was recorded at 16 days after f.i.a., while the maximum number of flowers/plant for the 13.3 plants/$m^2$ density was attained at 20 days after f.i.a. (Fig. 7.e). Flowers/plant were higher for the 6.6 plants/$m^2$ density than for the 20.0 plants/$m^2$ density from 20 to 24 days after f.i.a. but did not differ between the two lower densities (Fig. 7.e). The 6.6 plants/$m^2$ density had the highest number of flowers/plant from 28 to 32 days after f.i.a. while the 20.0 plants/$m^2$ density had the lowest (Fig. 7.e). Flowers/plant were similar for each density at 36 days after f.i.a.

Fig.8 Number of flowers/plant from different bean genotypes at a density of 20.0 plants/$m^2$




No significant differences between flowers/plant for the white and mottled brown genotypes were recorded from 4 to 16 days after f.i.a. Flowers/plant in both these genotypes from 8 to 12 days after f.i.a. were significantly higher than those from the other genotypes (Fig.8). At 16 days after f.i.a., no significant differences existed between flowers/plant from the white, mottled brown, mottled black and

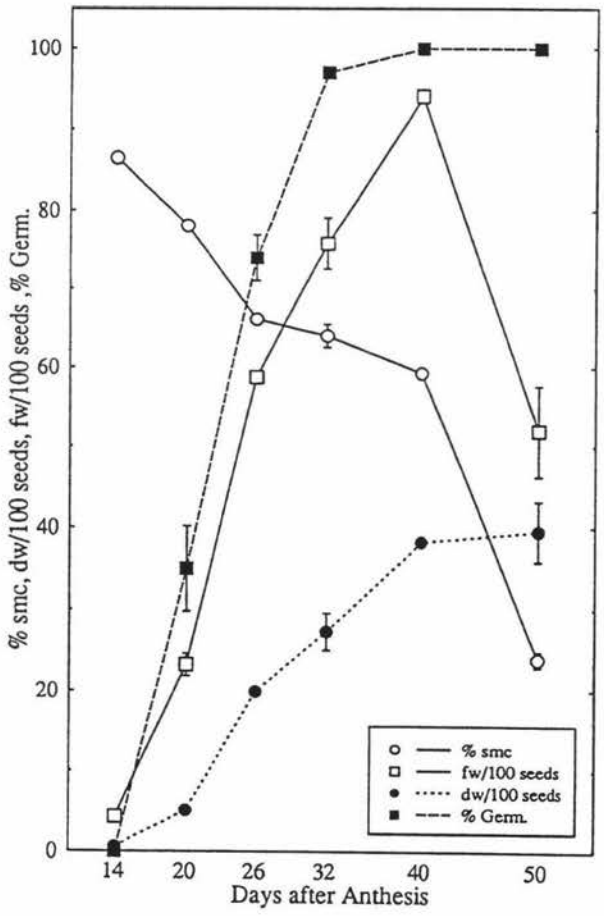
black genotypes, but the number of flowers/plant from the white and mottled brown genotypes was significantly higher than those from the brown genotype.

No significant differences were found among flowers/plant from different bean genotypes after 16 days from f.i.a., with the exception that the number of flowers/plant in the white genotype was significantly decreased at 28 and 32 days after f.i.a. (Fig. 8).

### 4.2.3. Seed development

The pattern of seed development, including changes in seed moisture content (smc), fresh weight (fw), dry weight (dw) and the percentage seed germination from 14 to 50 days after anthesis (at the 13.3 plants $m^2$ density) was similar among bean genotypes and is represented by the pattern of seed development for the white genotype (Fig. 9). The results of the data analysis on each of those characteristics from individual genotypes are presented in Tables 10a - 13b.

Fig.9 Sequences of seed development in the white bean genotype



Seed moisture content

In all bean genotypes, the percentage seed moisture content (smc) was around 86% at 14 days after anthesis (d.a.a.) and decreased gradually to around 60% at 40 d.a.a. (losing around 1% smc/day). However after this period, the percentage smc decreased rapidly from around 60% to around 25-30% at 50 d.a.a. (losing approximately 3 to 3.5% smc/day) (Fig. 9).

Fresh weight

The fresh weight/100 seeds in the white genotype (as well as in other bean genotypes) increased rapidly from around 4.3 g at 14 days after anthesis (d.a.a.) to around 91.8 g at 40 d.a.a. In the next 10 days, the fresh weight decreased rapidly until it was around 45.3 g at 50 d.a.a. (date of the last observation) (Fig. 9).

Dry weight

The dry weight/100 seeds increased slowly from around 0.55 g at 14 d.a.a. to around 5.1 g at 20 d.a.a. (with an increase of around 0.7 g/day), while the increase of the seed dry weight was faster from 20 d.a.a. (with around 5.1 g) to around 37.3 g at 40 d.a.a. (with an increase of 1.6 g/day). However there were no significant changes in the seed dry weight between 40-50 d.a.a. (Fig. 9).

Percentage seed germination

At 14 d.a.a. seeds were not able to germinate. They had begun to germinate by 20 d.a.a. The percentage germination increased rapidly after this period to approximately 90-95% germination at 32 d.a.a. The maximum percentage germination (100%) was obtained around 40 d.a.a. and was maintained until 50 d.a.a. (last observation date).

The results of the data analysis on the percentage smc, fresh weight, dry weight and the percentage germination of seed from each genotype at different days after anthesis are presented in the following tables (Table 10.a-13.b).

Table 10.a. Percentage seed moisture content from different bean genotypes at different days after anthesis

| Bean genotypes | days after anthesis 14 | 20 | 26 |
|---|---|---|---|
| white | 86.5 a | 78.1 a | 66.1 |
| mottled brown | 86.1 ab | 75.9 b | 67.0 |
| mottled black | 86.4 a | 78.8 a | 67.2 |
| black | 85.8 b | 75.9 b | 66.5 |
| brown | 85.8 b | 75.1 b | 67.6 |
| LSD P < 0.05 | 0.45 | 1.8 | NS |

Figures within each column followed by same letter are not significantly different at the 5% level.

Table 10.b. Percentage seed moisture content from different bean genotypes at different days after anthesis (CONTINUATION)

| Bean genotypes | days after anthesis 32 | 40 | 50 |
|---|---|---|---|
| white | 64.0 | 59.4 bc | 22.6 b |
| mottled brown | 63.2 | 59.0 bc | 21.5 b |
| mottled black | 62.0 | 58.4 c | 23.7 b |
| black | 63.8 | 60.8 a | 32.3 a |
| brown | 65.5 | 60.1 ab | 31.0 a |
| LSD P < 0.05 | NS | 1.2 | 4.1 |

Figures within each column followed by same letter are not significantly different at the 5% level.

At 14 days after anthesis (d.a.a.). the percentage smc in the white and mottled black genotypes was significantly higher than that measured from the black and brown genotypes. This was also the case at 20 d.a.a., but the percentage smc in the mottled brown genotype was also significantly lower than the percentage smc of the white and mottled black genotypes.

No significant differences existed between the percentage smc from different bean genotypes at 26 and 32 d.a.a. Differences in the percentage smc were found only between the mottled black and black genotypes at 40 d.a.a. At this time the first genotype had a significantly lower percentage smc than the other. However at 50 d.a.a., the percentage smc in the black and brown genotypes was significantly higher (between 31-32%) than the percentage smc from other genotypes (between 21-23%) (Table 10 a,b).

Table 11.a. Fresh weight (g) of 100 seeds from different bean genotypes at different days after anthesis

| Bean genotypes | days after anthesis 14 | 20 | 26 |
|---|---|---|---|
| white | 4.34 | 23.2 bc | 58.8 |
| mottled brown | 3.47 | 27.4 ab | 59.3 |
| mottled black | 4.32 | 19.5 c | 58.6 |
| black | 4.83 | 25.5 b | 51.2 |
| brown | 4.68 | 30.7 a | 58.3 |
| LSD P < 0.05 | NS | 4.2 | NS |

Figures within each column followed by same letter are not significantly different at the 5% level.

Table 11.b. Fresh weight (g) of 100 seeds from different bean genotypes at different days after anthesis (CONTINUATION)

| Bean genotypes | days after anthesis 32 | 40 | 50 |
|---|---|---|---|
| white | 75.9 | 91.8 a | 45.3 c |
| mottled brown | 72.6 | 93.6 a | 47.0 c |
| mottled black | 71.3 | 88.0 ab | 48.7 bc |
| black | 76.7 | 83.7 b | 54.7 a |
| brown | 71.9 | 84.4 b | 51.7 ab |
| LSD P < 0.05 | NS | 6.0 | 4.5 |

Figures within each column followed by same letter are not significantly different at the 5% level.

The fresh weight (fw) of 100 seeds from different bean genotypes did not differ significantly at 14 d.a.a., but it was significantly higher in the brown genotype compared to the black, white and mottled black genotypes at 20 d.a.a. The fw of 100 seeds did not differ among genotypes at 26 and 32 d.a.a., but was significantly higher in the white and mottled brown genotypes than in the black and brown genotypes at 40 d.a.a.

No significant difference between the fw of 100 seeds for the black and brown genotypes was recorded at 50 d.a.a. However at this time, the fw of 100 seeds of both genotypes was significantly higher than the white and mottled brown genotypes.

Table 12.a. Dry weight (g) of 100 seeds from different bean genotypes at different days after anthesis

| Bean genotypes | days after anthesis 14 | 20 | 26 |
|---|---|---|---|
| white | 0.55 | 5.1 bc | 19.9 |
| mottled brown | 0.45 | 6.6 ab | 21.4 |
| mottled black | 0.58 | 4.9 c | 18.8 |
| black | 0.65 | 6.2 abc | 18.4 |
| brown | 0.65 | 7.6 a | 18.9 |
| LSD P < 0.05 | NS | 1.5 | NS |

Figures within each column followed by same letter are not significantly different at the 5% level.

Table 12.b. Dry weight (g) of 100 seeds from different bean genotypes at different days after anthesis (CONTINUATION)

| Bean genotypes | days after anthesis 32 | 40 | 50 |
|---|---|---|---|
| white | 27.3 | 37.3 | 37.0 |
| mottled brown | 27.1 | 38.3 | 36.9 |
| mottled black | 27.1 | 35.7 | 36.5 |
| black | 27.8 | 34.9 | 37.7 |
| brown | 24.9 | 35.2 | 35.5 |
| LSD P < 0.05 | NS | NS | NS |

Figures within each column followed by same letter are not significantly different at the 5% level.

The dry weight (dw) of 100 seeds did not differ significantly among genotypes at 14 d.a.a. It was significantly higher in the brown genotype compared to the white and mottled black genotypes at 20 d.a.a., but at the same period no significant differences among the dw of 100 seeds for the mottled brown, black and brown genotypes were recorded.

No significant differences in the dw of 100 seeds among genotypes were recorded at 26, 32, 40 and 50 d.a.a.

Table 13.a. Percentage seed germination from different bean genotypes at different days after anthesis

| Bean genotypes | days after anthesis 14 | 20 | 26 |
|---|---|---|---|
| white | 0.0 | 35.0 b | 74.0 b |
| mottled brown | 0.0 | 60.0 a | 87.5 a |
| mottled black | 0.0 | 63.8 a | 86.0 a |
| black | 0.0 | 61.0 a | 80.0 ab |
| brown | 0.0 | 62.0 a | 80.0 ab |
| LSD P < 0.05 | NS | 10.7 | 8.6 |

Figures within each column followed by same letter are not significantly different at the 5% level.

Table 13.b. Percentage seed germination from different bean genotypes at different days after anthesis (CONTINUATION)

| Bean genotypes | days after anthesis 32 | 40 | 50 |
|---|---|---|---|
| white | 97.0 | 100 | 100 |
| mottled brown | 97.0 | 100 | 100 |
| mottled black | 97.0 | 100 | 100 |
| black | 97.0 | 100 | 100 |
| brown | 96.0 | 100 | 100 |
| LSD $P < 0.05$ | NS | NS | NS |

Figures within each column followed by same letter are not significantly different at the 5% level.

No germination occurred in all bean genotypes at 14 d.a.a. The percentage germination from 20 to 26 d.a.a. was significantly lower in the white genotype than for the other bean genotypes. No significant differences occurred among the different genotypes at 32, 40 and 50 d.a.a.

### 4.2.4. Seed yield and its components

The effects of different plant densities (6.6, 13.3 and 20 plants/$m^2$) and different bean genotypes on the number of branches/plant, pods/main stem and pods/secondary branch are presented in Table 14 while the effects of plant densities on plant structure and pod position in different bean genotypes are demonstrated in Fig.10.

Table 14. Effects of plant densities and bean genotypes on the number of branches/plant, pods/main stem and pods/secondary branch

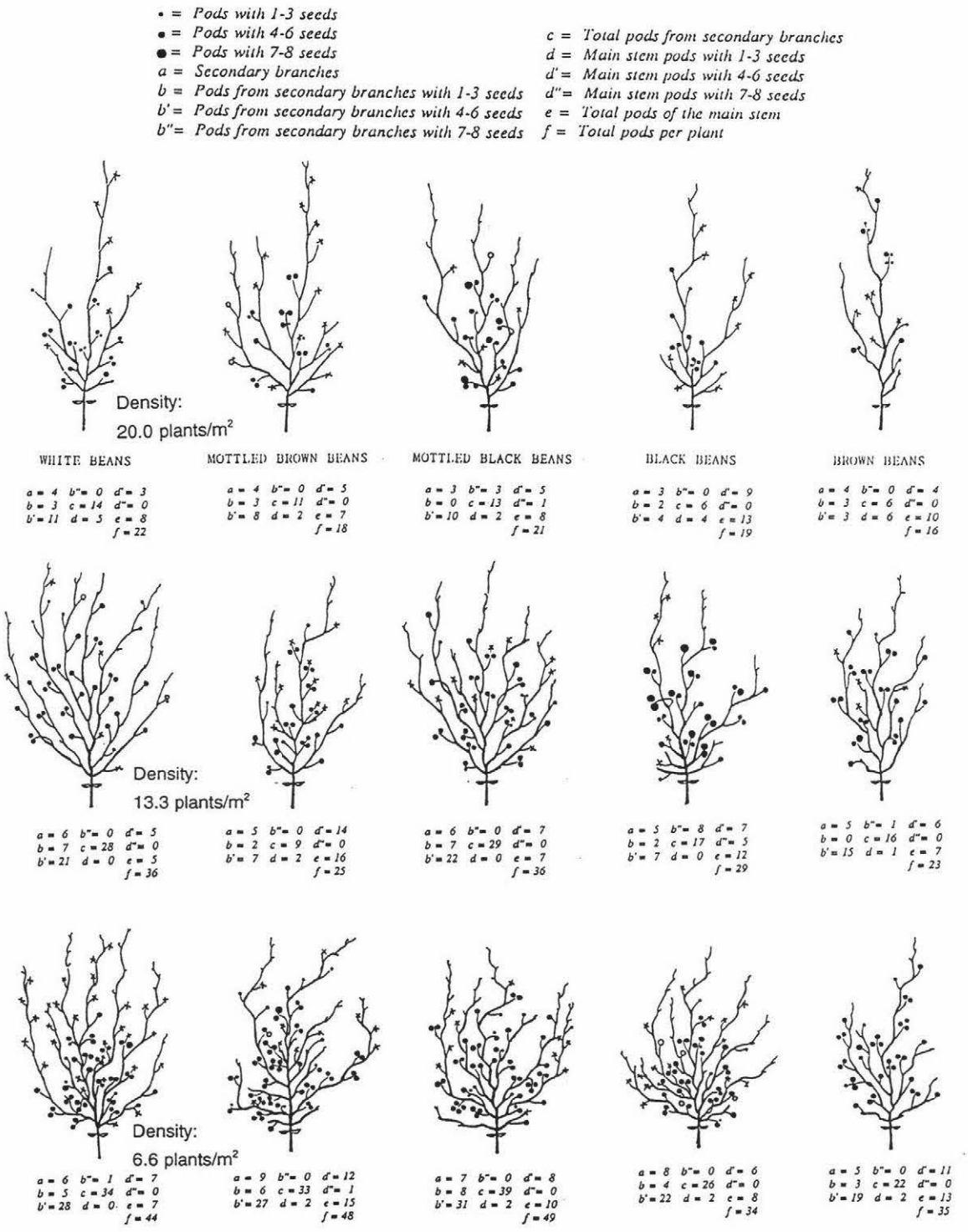
| Bean genotype and plant density | Branches/ plant | Pods/ main stem | Pods/secondary branch |
|---|---|---|---|
| white bean | 5.8 a | 10.0 | 16.9 ab |
| mottled brown | 5.7 a | 8.5 | 17.5 ab |
| mottled black | 5.2 b | 8.5 | 18.9 a |
| black bean | 5.4 ab | 9.0 | 14.3 b |
| brown bean | 4.6 c | 8.6 | 14.7 b |
| LSD P < 0.05 | 0.4 | NS | 2.6 |
| 6.6 plants/$m^2$ | 7.0 a | 8.9 | 23.2 a |
| 13.3 plants/$m^2$ | 5.2 b | 8.8 | 14.1 b |
| 20.0 plants/$m^2$ | 3.8 c | 9.4 | 10.4 c |
| LSD P < 0.05 | 0.5 | NS | 2.9 |

Figures within each column followed by same letter are not significantly different at the 5% level.

The number of branches/plant did not differ for the white, mottled brown and black genotypes, while the mottled black and black genotypes did not differ. The brown genotype had a significantly lower number of branches/plant than all other genotypes (Table 14). No significant differences were found for the number of pods/main stem. However the number of pods/secondary branch differed significantly between the mottled black and black and brown genotypes, as the mottled black genotype produced a greater number of pods/secondary branch than the other two genotypes.

No significant difference was found for the number of pods/main stem at the different densities. The number of branches/plant and pods/secondary branch was significantly higher at the lowest density (6.6 plants/$m^2$) and lowest at the highest density (20 plants/$m^2$). No significant interactions were found between bean genotypes and densities for these three parameters.

Fig.10 Diagrams of vegetative framework of pod position and branches at different plant densities




Branches/plant increased in each genotype as density decreased from 20 to 13.3 and 6.6 plants/m$^2$ and more pods/plant were recorded as the number of branches/plant increased (data in Table 14). Pods were more dispersed along the

secondary branches than on the main stem (especially at lower densities).
The seed yield and its components were mostly influenced by plant genotype and density. However no significant interactions were found between bean genotypes and plant densities in this trial.

Table 15. Effects of bean genotypes and densities on flowers per plant, pods per plant and seeds per pod

| Bean genotype and plant density | Flowers/ plant | Pods/ plant | Seeds/ pod |
|---|---|---|---|
| white bean | 60.8 ab | 24.4 | 4.4 b |
| mottled brown | 63.9 a | 24.1 | 4.5 ab |
| mottled black | 50.1 c | 26.9 | 4.6 a |
| black bean | 53.4 bc | 23.9 | 4.4 b |
| brown bean | 46.9 c | 23.8 | 4.1 c |
| LSD P < 0.05 | 12.4 | NS | 0.25 |
| 6.6 plants/$m^2$ | 63.3 | 32.2 a | 4.6 |
| 13.3 plants/$m^2$ | 54.7 | 22.6 b | 4.4 |
| 20.0 plants/$m^2$ | 47.0 | 19.0 c | 4.3 |
| Lsd P < 0.05 | NS | 3.4 | NS |

Figures within each column followed by same letter are not significantly different at the 5% level.

The number of flowers/plant in the white genotype was not significantly different from the mottled brown genotype or the black genotype (Table 15). Both of the first two genotypes produced a significantly higher number of flowers/plant than the mottled black and brown genotypes. The number of pods/plant did not differ significantly among genotypes.

There were no significant differences in seeds/pod between the white, mottled brown and black genotypes. Seeds/pod from the mottled black genotype were significantly higher than those from the white, black and brown genotypes. However no significant differences existed between the mottled black and mottled brown genotypes (Table 15).

The number of flowers/plant and seeds/pod did not differ significantly with plant density, but the number of pods/plant was decreased significantly as density increased (Table 14). No significant interactions existed between bean genotypes and densities for flowers/plant, pods/plant or seeds/pod.

Table 16. Effects of bean genotypes and densities on seed dry weight, yield per plant and yield per hectare

| Bean genotype and plant density | 100 seed weight (g) at 10% smc | Seed yield/ plant (g) | Seed yield/ha (kg/ha) |
|---|---|---|---|
| white bean | 42.2 b | 43.4 ab | 5,387 ab |
| mottled brown | 42.9 b | 45.7 a | 5,516 a |
| mottled black | 35.7 d | 43.3 ab | 5,136 ab |
| black bean | 45.2 a | 46.7 a | 5,705 a |
| brown bean | 40.8 c | 38.8 b | 4,723 b |
| LSD P < 0.05 | 1.7 | 7.2 | 929 |
| 6.6 plants/$m^2$ | 40.6 | 57.0 a | 3,800 c |
| 13.3 plants/$m^2$ | 42.1 | 40.2 b | 5,366 b |
| 20.0 plants/$m^2$ | 41.4 | 33.5 c | 6,715 a |
| LSD P < 0.05 | NS | 6.7 | 527 |

Figures within each column followed by same letter are not significantly different at the 5% level.

The 100 seed weight in the black genotype was significantly higher than that from other genotypes in this trial. The white and mottled brown genotypes were not

significantly different; both had significantly higher weight/100 seeds compared to the brown and mottled black genotypes. This last genotype was the one with the lowest weight/100 seeds.

The yield/plant and the yield/ha differed significantly only between the mottled brown, black and brown genotypes. The mottled brown and black genotypes did not differ from each other. However the yield/plant and the yield/ha from both these genotypes were significantly higher than from the brown genotype (Table 16).

No significant differences existed for weight/100 seeds among the densities. The yield/plant decreased as density increased. However in contrast, the yield/ha increased significantly as density increased. There were no significant interactions between bean genotypes and densities for 100 seed weight, yield/plant or yield/ha.

### 4.3. Seed Quality

#### 4.3.1. Seed quality for sowing (Seed vigour)

Table 17. Conductivity, Accelerated Ageing and standard germination results from different bean genotypes and different densities

| Bean genotype and plant density | Conduct. results ($\mu S\ cm^{-1}\ g^{-1}$) | Germination before AA (%) | Germination after AA (%) |
|---|---|---|---|
| white bean | 15.7 a | 100 | 99.6 |
| mottled brown | 13.8 b | 100 | 98.1 |
| mottled black | 10.7 c | 100 | 98.1 |
| black bean | 7.6 d | 100 | 98.0 |
| brown bean | 4.7 e | 100 | 97.8 |
| LSD P < 0.05 | 1.8 | NS | NS |
| 6.6 plants/$m^2$ | 9.9 | 100 | 98.4 |
| 13.3 plants/$m^2$ | 10.2 | 100 | 98.0 |
| 20.0 plants/$m^2$ | 11.4 | 100 | 98.0 |
| LSD P < 0.05 | NS | NS | NS |

Figures within each column followed by same letter are not significantly different at the 5% level.

The conductivity of all bean genotypes differed significantly (Table 17). The white genotype was the one with the highest conductivity value and was successively followed by the mottled brown, mottled black, black and brown genotypes. The AA and the standard germination results did not show any significant differences among different bean genotypes, and also no significant differences were found for the conductivity, AA and standard germination results among genotypes at different densities. No significant interactions were found between bean genotypes and densities for these three parameters.

### 4.3.2. Cooking quality

Table 18. Physical characteristics of raw seeds from different bean genotypes (before cooking)

| Bean genotype | Initial % smc | Seed coat cracks (%) | Unimbibed seeds after soaking/32 h (%) |
|---|---|---|---|
| haricot bean | 12.8 | 39 | 1.0 d |
| white bean | 10.4 | 0 | 3.5 cd |
| mottled brown | 10.5 | 0 | 10.0 b |
| mottled black | 10.6 | 0 | 4.5 cd |
| black bean | 10.8 | 0 | 7.5 bc |
| brown bean | 10.7 | 0 | 53.5 a |
| LSD P < 0.05 | - | - | 4.7 |

Figures within each column followed by same letter are not significantly different at the 5% level.

With the exception of the haricot bean seeds (12.8% smc), the initial smc of other bean genotypes before cooking was between 10.4 - 10.8% (Table 18). The proportion of seed coat crack/damage was around 39% in the haricot bean. No cracks were found in seeds from other bean genotypes.

The brown bean was the only genotype with a significantly higher amount of unimbibed seeds compared to other genotypes after soaking for 32 h. No significant differences existed between the haricot bean, white bean and mottled black bean genotypes, all of which had a significantly lower percentage of unimbibed seeds compared to the other genotypes. The percentage of unimbibed seeds produced by the mottled brown and black genotypes was similar. Both genotypes had a significantly lower percentage of unimbibed seeds compared to the brown genotype but significantly higher if compared to the haricot bean genotype (Table 18).

Table 19. Percentage seed weight increase from different bean genotypes after soaking for different periods of time

| Bean genotype | Soaking time 2 h | 4 h | 8 h | 14 h | 24 h |
|---|---|---|---|---|---|
| haricot | 39.1 a | 68.0 a | 95.7 a | 105.7 a | 108.0 a |
| white bean | 9.3 b | 26.5 b | 74.2 b | 98.7 b | 106.4 a |
| motl. brown | 5.9 bc | 19.8 c | 53.1 c | 84.9 c | 101.0 b |
| motl. black | 4.3 c | 9.0 d | 30.7 d | 73.8 d | 100.7 b |
| black bean | 5.2 c | 9.1 d | 23.9 e | 57.4 e | 92.3 c |
| brown bean | 4.2 c | 6.3 d | 13.4 f | 27.0 f | 49.1 d |
| LSD P < 0.05 | 3.9 | 4.2 | 3.6 | 4.3 | 4.7 |

Figures within each column followed by same letter are not significantly different at the 5% level.

After 2 h soaking, the percentage seed weight increase (% swi) in the white bean genotype was significantly lower compared to the haricot bean but was significantly higher compared to the mottled black, black and brown genotypes (Table 19). No significant differences existed between the white and mottled brown

genotypes at this time. However after 4 h soaking, the % swi in the white bean was lower compared to the haricot bean but was significantly higher than for other genotypes.

The % swi differed significantly among individual genotypes after 8 and 14 h of soaking. In both periods, the haricot bean had the highest % swi and was followed successively by the white, mottled brown, mottled black, black and brown genotypes. However after 24 h of soaking, the % swi from the haricot and white bean genotypes was not significantly different. Both genotypes had significantly higher % swi compared to other genotypes. The % swi from the mottled brown and mottled black genotypes did not differ at this period. Both had lower % swi than the haricot and white genotypes but significantly higher than the black and brown genotypes. The brown bean was the genotype with the lowest % swi after 24 h of soaking.

Table 20. Percentage seed weight increase and conductivity test results after 14 h soaking (prior to cooking), seed texture and seed coat/cotyledonary damage after cooking for 20 minutes

| Bean genotype | % Seed wt. increase | Conduct. results ($\mu S\ cm^{-1}\ g^{-1}$) | seed texture (Newton) | % Seed coat/ cotyledonary damage |
|---|---|---|---|---|
| haricot | 105.7 a | 20.90 a | 6.16 d | 34.6 a |
| white bean | 98.7 b | 14.68 b | 6.83 cd | 19.3 b |
| motl. brown | 84.9 c | 9.33 c | 9.10 bc | 19.9 b |
| motl. black | 73.8 d | 6.95 d | 11.48 b | 10.9 c |
| black bean | 57.4 e | 4.50 e | 14.10 a | 8.7 c |
| brown bean | 27.0 f | 1.55 f | 15.23 a | 8.9 c |
| LSD P < 0.05 | 4.2 | 0.95 | 2.4 | 6.8 |

Figures within each column followed by same letter are not significantly different at the 5% level.

The percentage seed weight increase after 14 h of soaking and the conductivity results showed that the haricot bean had significantly higher values compared to the other genotypes. These values decreased significantly for the white, mottled brown, mottled black, black and brown genotypes respectively.

Cooked beans from the black and brown genotypes had a significantly harder texture compared to the other genotypes. The mottled brown and mottled black genotypes had no differences in texture hardness after cooking. Both had significantly lower texture hardness than the black and brown genotypes but were significantly harder if compared to the haricot and white genotypes. No differences in texture hardness existed between the haricot and white bean genotypes after cooking.

Seed coat and/or cotyledonary damage after cooking was significantly higher in the haricot bean (as control) compared to other genotypes. However it did not differ between the white and mottled brown genotypes (Table 20). Both had significantly lower amount of seed coat and/or cotyledonary damage after cooking compared to the haricot bean but a significantly higher amount if compared to the mottled black, black and brown genotypes (Table 20).

# CHAPTER 5
# DISCUSSION

## 5.1. Plant characteristics and some implications for seed production

The differences in leaf size among bean genotypes caused by the differences in leaf length and width did not affect the number of pods/plant. This suggests that the photosynthate supply which controls the final pod number in common beans (Aguilar et al., 1977) was not related to the leaf size but depends on the accumulation and translocation of assimilate from other vegetative organs to the pods and seeds. For instance, after flowering some legumes (e.g. lupin, *Lupinus albus*) continue to allocate photosynthate to the roots and nodules, and the requirements for pod and seed production are met by remobilization of the assimilate (C- and N containing compounds) in the vegetative parts of the shoot (Bewley and Black, 1994).

Differences in leaf size among genotypes have also been reported by other researchers (e.g. Debouck, 1991). In dicotyledonous plants, the leaf size is determined by cell number and not by cell size (Volkenburgh, 1987) which suggests that genotypes with greater leaf size (such as the brown genotype) possess higher cell numbers than genotypes with smaller leaf size. The differences for the main stem length among bean genotypes were caused by differences in the number of nodes/main stem. However these differences in the number of nodes/main stem had no effect on the number of pods/main stem (Table 14), as pods produced from the nodes on the upper part of the main stem generally aborted in response to the intra-competition caused by the older pods and flowers which were located at the nodes on the lower parts of the main stem.

Variability in the number of nodes/main stem is an intrinsic characteristic of an individual genotype, and the gene action which controls this is far from being well understood (Debouck, 1991). The differences in the size of other organelles

such as the length and width of the primary bract, the bractiole width and the length of the pedicellate bract among genotypes might be controlled by genetic factors, most of which still not well understood. Adams et al., (1985) reported that information on the linkage of genes in *Phaseolus vulgaris* is limited, because of the difficulties of classifying individual chromosomes.

In the glasshouse, harvested pods/plant differed for different bean genotypes, which indicates that different genotypes possess different abilities to produce pods under the same environmental conditions. The number of pods/plant is genetically inherited (Singh, 1991).

Generally, pods with greater length and width contain more seeds of a larger size than pods with smaller length and width. Therefore differences in pod length and pod width among genotypes are important, and in conjunction with the number of pods/plant, determine the seed yield per plant. Differences in pod length and width among genotypes might be determined by genetic factors, as Singh (1991) stated that pod length is genetically inherited.

The differences in pod bic length among bean genotypes are unlikely to have any effect on seed production. The diversity in pod bic length might be controlled by intrinsic factors such as the curved tip shape in pods (for instance) which is controlled by a single dominant gene (Singh, 1991).

Differences in the maximum number of seeds/pod among bean genotypes are genetically controlled. Izquierdo (1991) reported that there was a genetic variability for the yield potential among bean genotypes. The variability in the number of seeds/pod for different genotypes might depend on the response of individual genotypes to environmental factors such as water availability and temperature before and during the flowering period. Bewley and Black (1994) reported that in cereals, for instance, water stress during development of the inflorescence reduces the number of primordia that are produced, resulting in a reduction in the total number of grains that can be formed. Little is known about beans. However this evidence from the cereals can suggest that the formation of the flower primordia in beans may

also be controlled by environmental factors, and different genotypes might respond differently to the same environmental conditions, leading to the production of flower buds (especially the ovary) with different numbers of ovules. The ovule number indicates the potential seed number (White and Izquierdo, 1991). Differences in seed size (length and width) among genotypes caused differences in their seed weight.

The diversity of the maximum internode length among genotypes (Table 9) may be attributed to intrinsic factors such as the genetic material and hormone levels within each genotype. Debouck (1991) indicated that there are several genes responsible for controlling internode length in common beans. However some environmental factors such as the effects of light are also involved in this process. Shein and Jackson (1971) reported that low light intensity significantly stimulated stem length, and the promotive effect of low light intensity was enhanced under $GA_3$ treatments. Krishnamoorthy (1981) stated that gibberellin causes stem elongation by enhancing rapid elongation of internodes that is due to both cell division and cell elongation, while Shein and Jackson (1971) reported that light may differently affect hormone production. This suggests that different genotypes might produce different levels of intrinsic hormones under the same growing conditions which subsequently may produce different internode lengths for different bean genotypes.

The differences in flower colour (colour of the standard and wing) among genotypes are controlled by different genes. Singh (1991) stated that flower colour is controlled by some genes that can have pleiotropic effects (i.e. the occurrence of diverse phenotypic effects originating from the action of a single gene). Flower colour can also be intensified by the presence of an intensifying gene. However the existence of plants from the black genotype with different flower colours (white or mauve 633/3 to rose purple 533/3) might be due to the genetic heterogeneity of the seeds used in the trial (i.e. there was more than one genotype with the same seed colour). This contributes to the existence of variability in flower colours as well as the variability in pod colours that were produced from the same seed colour group.

The existence of both growth habits (indeterminate climbing and indeterminate non-climbing or bush indeterminate) among bean plants/genotype in the field was attributed to the differences of their internode length and node numbers/main stem. The internode length and node numbers are genetically inherited (Debouck, 1991; Singh, 1991).

Plants with indeterminate non-climbing characteristics might have been different genetic material from those with indeterminate climbing characteristics. For this reason light may differently affect hormone production and hormone balance within the plants (Shein and Jackson, 1971) which eventually regulates the growth of the main stem and lateral shoots (buds) of the plant (Tucker, 1976). This condition offers to the plants different growth behaviours in the field. From these results it is obvious that bean seeds/genotype that were used for this trial were not genetically homogenous.

There were some variations in seed colour from individual genotypes; for example between 1-3% of plants from the white genotype produced pods with black seeds whereas 14 - 18% of plants from the black genotype produced pods with white seeds. This seed colour variation in both genotypes might be influenced by the genetic heterogeneity of the original seeds/colour group used in this trial. These seeds with inappropriate colour might have been produced from parent plants with different genetic expression, such as from plants with different flower colour and/or from different pod colour. Purseglove (1968) stated that common beans are self-pollinated, and anther dehiscence occurs within a few hours before anthesis, usually at night (White and Izquierdo, 1991; Gross and Kigel, 1994). This indicates that there is no chance for natural cross-pollination to occur. Therefore the production of different seed colours from a single original colour group is most likely due to genetic characteristics of the parent plants. The genetic heterogeneity among parent plants can also be observed from the differences in their flower colour as well as differences in pod colour. Therefore further work for regrouping the seeds into sub-

groups according to plant genetical expression is important to avoid genetical heterogeneity among seeds from the same seed colour group.

**5.2. Plant height** (a comparison between bean genotypes grown in the glasshouse and bean genotypes grown in the field at different planting dates)

The average plant height recorded in the field from 28 November 1994 to 20 March 1995 (i.e. during summer) for all bean genotypes at different densities was between 50 - 60 cm, with a min./max. height of 40 - 85 cm. However the average plant height for each genotype recorded from the glasshouse trial from September to November 1994 (i.e. during spring) ranged between 166 - 362 cm for the white and brown genotypes respectively (Table 5), while the other genotypes were intermediate in height. This morphological instability might be due to the effects of the environmental conditions, such as the photoperiod, light intensity and some physiological events within the plant. As reported by Laing and White (1989), photoperiod affects node and branch formation, while Masaya and White (1991) stated that for indeterminate genotypes of common beans, long days (or warm temperatures) cause the development of branches on the axil of lower nodes. These branches are competitive organs which compete with the main stem for nutrients and assimilate for further growth and development. As the competition increases, the amount of assimilate which is normally acquired by the main stem will be diminished, which consequently reduces the further growth of the meristematic parts of the main stem. Debouck (1991) reported that the indeterminate bean characteristic can be broken off under certain conditions, such as competition for carbohydrate, which may turn the habit into a determinate one. Moreover the high light intensity during summer may contribute its effect in suppressing the main stem growth in beans. An effect of light intensity was also observed by White and Mansfield (1977) who found that the increase in radiant energy flux reduces main stem elongation. This in conjunction with the effects of photoperiod might have caused the reduction

of plant height in the field during the summer season. Light intensity has often been shown to affect apical dominance and it may act by altering the supply of carbohydrate to the buds (White and Mansfield, 1977). In contrast, bean plants grown in the glasshouse during the spring generally received lower light irradiation/intensity, and thus produced greater stem length than beans grown in the field. Jackson and Field (1972) observed that at low light intensity, apical dominance was greatest. This condition will direct most part of the nutrients and metabolites to the stem tip while the lateral buds are depleted of them. For this reason the main stem length was highly stimulated and the production of branches was totally arrested for all bean genotypes. Furthermore the existence of bean plants without branches (in the glasshouse) encouraged a greater growth of the main stem than the branched bean plants in the field.

### 5.3. Flowers/plant

The number of flowers in all genotypes were significantly higher for the 6.6 plants/$m^2$ density from 24 to 32 days after first inflorescence appearance (f.i.a.) (Fig. 7a-e). This was due to the appearance of some new branches which could produce additional flowers at that time, as well as the continuous formation of new flowers along the tip of the flower racemes which were located along the terminal part of the branches. This extended flower formation gives rise to the greater amount of late flowers. In contrast, the number of flowers/plant for the 13.3 and 20.0 plants/$m^2$ density decreased more rapidly from 24 to 32 days after f.i.a. as the emergence of new flowers along the tip of the flower racemes located at the edges of the branches was arrested. This might be due to the increase of inter-competition among bean plants as well as intra-plant competition among the reproductive organs that were formed at the base of the flower racemes and at the lower parts of the plant. Debouck (1991) reported that strong competition between the young developing pods located at the lower parts on the first inflorescence limits further development of flower buds in the inflorescential part. As a result, the development of these

flower buds consequently becomes diminished, and is stopped because of abortion of these meristematic organs. A reduction of the number of flowers/plant at higher densities was also recorded from soybean and broadbean (Mejico, 1983).

The time of emergence for the first and the last inflorescence was similar among genotypes, and did not differ with density, which suggests that all bean genotypes in this trial had the same photoperiod sensitivity. Kretchmer et al., (1979) reported that time of flowering is photoperiodically sensitive. For this reason changes in plant density did not exert an effect on the duration of the flowering period.

Differences in the number of flowers/plant among genotypes at the same density (such as from the 20.0 plants/$m^2$ density) (Fig. 8) were due primarily to the differences in the number of branches/plant (Table 14), as under the same growing conditions, genotypes with more branches/plant provide more nodes/plant, which eventually increases the number of flowering sites. Flowers/plant in the white genotype at the 20.0 plants/$m^2$ density were significantly lower at 28 and 32 days after f.i.a. than the number of flowers/plant from the other genotypes (Fig. 8). This rapid reduction of flowers/plant for the white genotype was due to the heterogeneity of plant size and the ability to produce flowers. Some white bean plants with shorter or smaller stature, with relative shorter branches (i.e. with less nodes/branch) which might be caused by genetic heterogeneity, produced less flowers or even did not produce flowers after 28 d.a.a. as the sites responsible for flower emergence were limited. Therefore the average number of flowers/plant produced by this genotype was reduced from 28 to 32 days after f.i.a.

### 5.4. Seed development

The pattern of seed development for individual bean genotypes was similar to the pattern of seed development in broadbean reported by Hyde et al., (1959). This consisted of three distinct phases:

Phase I : commonly known as the growth stage

Phase II : commonly known as the food reserve accumulation stage

Phase III: commonly known as the ripening stage or the desiccation phase

Phase I of seed development (s.d.) in all the bean genotypes ended around 20 days after anthesis (d.a.a.). This phase was characterized by a very slow increase in seed dry weight (Fig. 9).

No physiological studies were done during seed development in this trial. However Bewley and Black (1994) reported that during seed development there is commonly an increase in the ABA content of a seed, both within the embryo itself and in the surrounding structures, and the increasing ability of the dwarf bean seeds to germinate along the period of seed development is accompanied by a steep decrease in seed ABA content. It is generally accepted that ABA plays an important role in preventing germination during seed development, that it declines during late maturation, and that at the same time there is frequently also a decrease in sensitivity to its presence (Bewley and Black, 1994).

The duration of phase I of seed development for all genotypes was similar to peas where the length of time occupied by this phase is from 8 to 20 d.a.a. (Flinn and Pate, 1977). However the duration of phase I of s.d. in other bean genotypes such as the French bean cv Green Crop, Dwarf French bean cv Early Green and Dwarf Butter bean cv Choctaw Wax grown at the same site was successively 10, 12 and 14 d.a.a. (Gil, 1989; Mahamed, 1989; Dorji, 1989). These differences in the length of time for phase I of s.d. (i.e. the duration of time to complete cell division in a developing seed) might be determined by environmental factors, particularly temperature, species and cultivar. In grains, for example, the total endosperm cell number differs markedly between species (Palmer, 1989). Environmental variables, such as temperature, irradiance and water supply, may affect the rate of endosperm cell division (Duffus and Cochrane, 1982), which means that cell division can be perturbed if these factors are unfavourable. Therefore seeds from different cultivars

that were planted at the same growing sites, even under the same or different planting dates generally possess different durations for phase I of s.d.

During phase I of s.d., the increase in seed dry weight is controlled by cell division. Loewenberg (1954) reported that at 12 d.a.a. (early stage of phase I) the number of the cotyledonary cells/seed in the Black Valentine bush bean (*Phaseolus vulgaris* L.) increased rapidly from 360,000 to 2,400,000 at 20 d.a.a. (end of phase I), while only a few cell divisions occurred after this time. This indicates that the slow increase in seed dry weight during phase I of s.d. was mostly due to the increase in the cell number.

At the beginning of this phase (14 d.a.a.), seeds were not able to germinate, suggesting that at this time the seed embryo was not completely developed (immature embryo). The inability of seeds to germinate at this time is due to embryo immaturity and the condition of hormones within the seed. Ihle and Dure (1972) stated that abscisic acid (ABA) is believed to play a regulatory role in suppressing germination at that time (i.e. inhibiting precocious germination) in order to allow the occurrence of normal development of the embryo. Meanwhile at 20 d.a.a. (end of phase I of s.d.) the percentage germination was around 35% (Fig. 9), indicating that around 35% of seeds had completed their embryo development.

Phase II of s.d. (known as the dry matter accumulation stage) started when phase I ended, and proceeded until the maximum dry matter was attained. In all genotypes, the time occupied by this phase was from around 20 to 40 d.a.a. (Fig. 9) (i.e. the accumulation of dry matter initiated from around 20 until 40 d.a.a). In other genotypes such as Dwarf French bean cv Early Green and Dwarf Butter bean cv Choctaw Wax (grown at Massey University experimental field in 1989), the duration of phase II of s.d. was from 13 to 23 and 14 to 33 d.a.a. respectively (Mahamed, 1989; Dorji, 1989), while Adams et al., (1985) stated that for some bean genotypes, the duration of phase II of s.d. was from 23 to 50 d.a.a. These differences in the

duration of time for phase II of s.d. among bean genotypes are also determined by environmental factors such as temperature. In general, an increase in temperature to a certain range results in an acceleration of nutrient absorption, while lowering of temperature from that range will slow down any process of nutrient absorption by slowing down any process dependent on free diffusion (passive absorption) and/or slowing down the biochemical reactions which occur in active transport (Devlin and Witham, 1983). Thus when the rate of nutrients and assimilate translocation from the parent plant to the seeds is faster (as influenced by temperature) the seeds will grow faster and consequently they will be fully developed and attain their maximum size within a shorter length of time than seeds that develop under lower temperature regimes.

The duration of seed filling in phase II of s.d. might be also depend on the number of seed cells responsible for the accumulation of dry matter. Cell division and cell enlargement following fertilization determine the size and storage capacity of a seed (Bewley and Black, 1994), and bean mesophyll cells are able to accumulate large amounts of starch without disrupting the chloroplasts (White and Izquierdo, 1991). Other results, for instance, indicate that the duration of seed filling is shortened at high (over 30°C) temperatures in wheat (Bewley and Black, 1994). White and Gonzalez (1990) reported that the main increase in seed size during domestication of common beans was obtained through increased cell number. This suggests that differences in seed size among genotypes might be due to differences in cell number. Therefore the differences in seed size caused by differences in cell number, in conjunction with differences in grain filling rate and/or grain filling duration as a response of an individual genotype to the environmental conditions at the growing site may cause differences in the duration of phase II of s.d. for different bean genotypes.

The rapid increase of seed dry weight from 20 to 40 d.a.a. (Fig. 9) is a characteristic of phase II of s.d. Loewenberg (1954) reported that during bean s.d., the greatest rate of growth occurs after cell division had ceased. For this reason, the

rapid increase of seed dry weight from 20 to 40 d.a.a. was due to the accumulation of reserves such as starch. Bean mesophyll cells are able to accumulate large amounts of starch (White and Izquierdo, 1991). Sucrose-P synthetase is the key in legumes in regulating photosynthetic formation of sucrose, and hence, starch. The growth of the seeds during this phase is mainly by the increase in cell volume (Wang and Hedley, 1991). As stated by Dure (1975), phase II of s.d. is characterized by the deposition of food reserves or is known as the seed-filling period (Adams et al., 1985). The percentage germination of individual bean genotypes was around 90-95% at 32 d.a.a. (before the attainment of maximum dry weight) (Fig. 9) which indicates the approach of physiological maturity (PM). At 40 d.a.a. the percentage germination for all genotypes was 100% and it coincided with the attainment of maximum seed dry weight.

Phase III of s.d., which is known as the ripening stage or the desiccation phase, began for all bean genotypes at 40 d.a.a. (end of phase II) and continued until 50 d.a.a. (end of observation in the field). There was a rapid decrease of seed fresh weight and %smc (seeds losing approximately 3 to 3.5% smc/day) in this phase, with no changes in seed dry weight (Fig. 9). The seed dry weight was constant during this phase as at PM the ovule vascular connection between the pod and the seed (funiculus) is broken (Browne, 1978) and the translocation of nutrients from the parent plant to the seed ceases. The rapid decrease of seed fresh weight during this phase was due to the rapid loss of seed moisture. The rapid decrease of smc itself might due to the total suppression of water supply from the parent plant in response to the breakdown of the funiculus at PM and the release of water from the internal parts of the seed through the seed coat.

### 5.4.1. Seed moisture content (smc)

The significant differences in the percentage smc among bean genotypes at 14 and 20 d.a.a. (Table 10.a) might have been due to random error during the selection of pods/seeds from the field, because most pods harvested for this purpose

were not homogenous in size as they were harvested from different parts of the plant. Moreover the number of pods that were taken from individual sample plants were variable, as the distribution of young pods through the sample plants was not homogenous. Significant differences in smc among bean genotypes at 40 d.a.a. (Table 10.b) may be attributed to changes in physiological activities and differences in seed coat characteristics which involves the behaviour of the micropyle, raphe and the thickness of cells along the outermost zone of the seed coat. Lush and Evans (1980) reported that the arrangement of cells into layers within the seed coats of most legumes (including cowpeas and beans [*Phaseolus vulgaris* L.]) was similar, and the rough-seeded cowpeas (for instance) differ from smooth-seeded ones in the following ways: the micropyle is open, the raphe is less conspicuous and the palisade cell walls are relatively thin. These properties of the seed may differ among genotypes which may explain the difference in smc at 40 d.a.a.

At 50 d.a.a. the percentage smc in the black and brown genotypes was significantly higher than the percentage smc from the other genotypes (Table 10.b). This might be due to differences in seed coat thickness of both genotypes. Harrington (1973b) observed that even within one species, the moisture content of individual seeds in equilibrium with a given relative humidity will vary, depending on the seed size and the thickness of the seed coat, while Lush and Evans (1980) reported that the variation in the seed coat thickness is associated with differences in the size of cells.

### 5.4.2. Fresh weight (fw)

The significant differences in seed fresh weight among genotypes at 20, 40 and 50 d.a.a. may be attributed to differences in the percentage smc. At 50 d.a.a. the amount of water lost from the internal parts of the seed was mostly controlled by the permeability of the seed coat.

### 5.4.3. Dry weight (dw)

The dw/100 seeds only differed significantly among genotypes at 20 d.a.a. (Table 12a). This difference might have been due to random error during the selection of pods in the field, as previously explained.

### 5.4.4. Seed germination

Seeds from each bean genotype did not germinate at 14 d.a.a. as their embryos were not fully developed. However the low percentage germination in the white genotype recorded at 20 and 26 d.a.a. (Table 13a) might be due to low tolerance of the young seeds to desiccation.

## 5.5. Seed yield and its components

### 5.5.1. Branches/plant

Significant differences in branches/plant among bean genotypes (Table 14 and Fig. 10) were due to the characteristics of individual genotypes. However differences in the number of branches/plant among densities, such as the increase in the number of branches/plant as the density decreased and vice-versa is a classic plant density response (Bennett et al., 1977; Davis and Garcia, 1987). Differences in branches/plant among densities were induced by the availability of soil nutrients and minerals, and space, as well as the environmental factors required for plant growth and development. Westermann and Crothers (1977) stated that increasing plant populations causes greater interplant competition, which can increase the intra plant competition for assimilates. Nitrogen stress (for instance) reduces the number of lateral branches/plant and their development (Barke, 1978), while McIntyre (1972) stated that at low nitrogen levels, the growth of buds on all common bean plants is completely arrested. For this reason, at highest density the interplant and/or intraplant competition for nutrients, space and light are important factors that control the formation, growth and development of branches.

At the 20.0 plants/$m^2$ density, the competition among plants might have suppressed any expression of the branches i.e. either the branches never grew or they died shortly after their development. In contrast the increase in branches at the 6.6 plants/$m^2$ density was caused by less competition for nutrients, space and light. Less competition presumably allowed the full expression of the initiated branches and their development (Mejico, 1983).

### 5.5.2. Flowers/plant

Significant differences in the total number of flowers/plant among genotypes (Table 15) were attributed to differences in plant size, the number of reproductive branches/plant and nodes/plant. Nodes/plant are important as they provide sites for flower emergence. Jiang and Egli (1993) stated that flowers/plant is determined by flowers/node and nodes/plant, and the variation in plant size and flowers/node contributes to the variation in flowers/plant.

### 5.5.3. Pods/main stem and pods/secondary branch

The number of pods/main stem did not differ with genotype and density whereas the number of pods/secondary branch differed significantly among genotypes and density. The significant differences in pods/secondary branch among bean genotypes (Table 14) were caused by the variation in branch development and the number of nodes/branch (data not collected). Under optimal conditions, genotypes with more reproductive branches and nodes/branch are more beneficial as they provide more sites for flowers and pod formation.

Pods/secondary branch were significantly higher at the 6.6 plants/$m^2$ density (Table 14 and Fig. 10). This was induced presumably by less competition among plants for nutrients, space and light. On the other hand, pods/secondary branch were lowest at the 20.0 plants/$m^2$ density (Table 14 and Fig. 10) which was caused by high competition among plants for nutrients, space and light, and subsequently may enhance the intra-competition among the reproductive organs for nutrients. White

and Izquierdo (1991) reported that competition among the developing pods for nitrogen, other nutrients, and carbohydrates may result in abscission of flowers and small pods, and causes poor pod set. For this reason high density generally produces a lower number of pods/secondary branch.

### 5.5.4. Pods/plant

Pods/plant did not differ with genotype but differed significantly among densities. The number of pods/plant (harvested pods/plant) was highest at the 6.6 plants/$m^2$ density (Table 15), with approximately 49% of flowers that failed to develop into mature pods. This amount of reproductive organ abortion is lower than that observed in other legume crops where it is often greater than 50% (White and Izquierdo, 1991), or as many as two-thirds of the total flowers (Adams et al., 1985). In contrast, the number of pods/plant was lowest at the 20.0 plants/$m^2$ density, with 59% of flowers that failed to develop into mature pods.

The higher percentage of reproductive organ abortion at the 20 plants/$m^2$ density caused the greatest reduction in the number of pods/plant. The abortion of reproductive organs at high density might be due to the increase of interplant competition for nutrients and light, as well as the stress caused by the intraplant competition among the reproductive organs. Westermann and Crothers (1977) reported that increasing plant populations causes great interplant competition, which could increase the intraplant competition for assimilates, while White and Izquierdo (1991) stated that stress caused by source-sink competition enhances bean pod abscission. Therefore high density increases plant competition which subsequently increases the number of reproductive organs aborted and reduces the number of harvested pods/plant. As reported by Jiang and Egli (1993) pods/plant is determined by flowers/plant and the proportion of flowers that develop into mature pods.

### 5.5.5. Seeds/pod

The number of seeds/pod did not differ with density but differed significantly with genotype. Jiang and Egli (1993) reported that seeds/pod showed only minor changes with changes in plant density, while Westermann and Crothers (1977) stated that seeds/pod did not differ with plant population. The significant differences in seeds/pod among genotypes might be due to individual plant characteristics and the nature of the formation of flower primordia which might control the number of ovules/flower (data not available) prior to their development into mature seeds. White and Izquierdo (1991) stated that the ovule number indicates the potential seed number.

### 5.5.6. Seed weight (g)

Seed weight/100 seeds (at 10% smc) did not differ with density and this is in agreement with the results reported by Aguilar et al., (1977); Westermann and Crothers (1977) and Francis et al., (1982). Seed weight/100 seeds did not respond to changes in density as it is not controlled primarily by the amount of assimilate. White and Izquierdo (1991) stated that seed weight is controlled by the sink effects rather than source effects, and this characteristic in common bean is quantitatively inherited (White et al., 1992).

The significant differences in seed weight/100 seeds among genotypes was attributed to the differences in seed size. Thus, genotypes with smaller seeds (smaller length and width) such as the mottled black (Table 8) possessed significantly lower seed weight/100 seeds, whereas genotypes with larger seeds such as the black genotype (Table 8) possessed significantly higher seed weight/100 seeds (Table 16).

### 5.5.7. Seed yield/plant

Significant differences in seed yield/plant among genotypes were determined by the differences in seed weight and seeds/pod, while the significant differences in seed yield/plant among densities was determined by differences in pods/plant. Thus,

the highest number of pods/plant at the lowest density (6.6 plants/$m^2$) produced the highest seed yield/plant, and the lowest number of pods/plant at the highest density (20.0 plants/$m^2$) produced the lowest seed yield/plant (Table 16).

### 5.5.8. Seed yield/ha

Significant differences in seed yield/ha among genotypes were determined by the differences in seed weight and seeds/pod, while differences in seed yield/ha among densities was determined by differences in pods/plant. However at lower densities the increase in pods/plant was insufficient to compensate for lower plant number per unit area, so that seed yield/ha, consequently decreased with reductions in plant density (Table 16). Therefore under the same environmental conditions, the 20.0 plants/$m^2$ density was able to produce a higher seed yield/unit area than the lower densities.

From a density study in Mexico using an indeterminate bush variety (Michoacan 12-A-3), Aguilar et al., (1977) reported a yield of 4,210 kg/ha at 14% smc from a density of 28.8 plants/$m^2$. McKenzie (1991) reported that the optimum plant population of beans in Canterbury (New Zealand) was about 60 plants/$m^2$ with a production of around 3,000 kg/ha, while previous studies carried out at Massey University using the Dwarf Butter bean cv Choctaw Wax (Dorji, 1989) gave 2,915 kg/ha at 14.4% smc at a density of 33.2 plants/$m^2$. From the same experimental site, Mahamed (1989) indicated that for the Dwarf French bean cv Early Green, a density of 15 plants/$m^2$ was able to produce 3,273 kg/ha at 10% smc. However the seed yield obtained from the white, mottled brown, mottled black, black and brown genotypes at the 20.0 plants/$m^2$ density at Massey University experimental field averaged 6,715 kg/ha, higher than the seed yield obtained by Dorji (1989), Mahamed (1989) and others.

These reports differ from each other for maximum seed yield and optimum plant density mainly because of the diversity of bean cultivars/genotypes being used for the studies. Furthermore the instability of plant morphological characteristic in

response to different environmental conditions in different seasons would cause a source of uncertainty in the determination of a consistent plant density able to produce high seed yield with high quality for sowing and/or for eating purposes. It is important therefore to determine the morphological characteristics of individual genotypes in different seasons as well as their optimum density for seed production. The seed yield obtained from the 20.0 plants/$m^2$ density may still have been able to be increased because even at this density, seed dry weight did not differ with the seed dry weight from other lower densities. This indicates that the availability of nutrients and assimilates for seed filling were not a limiting factor for this density. Therefore studies with plant populations higher than the 20.0 plants/$m^2$ density could be useful to increase seed production for all bean genotypes used in this trial.

## 5.6. Seed quality

### 5.6.1. Seed vigour

The significant differences for the conductivity results among bean genotypes (Table 17) are likely to be attributed to differences in seed coat permeability, because of the nature of the seed coat (such as seed coat thickness). Lush and Evans (1980) stated that the seed coat is important in controlling the exchange of water between seeds and their environment, as a rapid imbibition of water by seeds is accompanied by an equal rapid efflux of solutes including sugars, ions, amino acids, and proteins (Bewley, 1986). This suggests that genotypes with a thinner seed coat imbibe more rapidly than genotypes with a thicker seed coat. Consequently the water inrush velocity is higher, and reaches injurious levels which causes the exodus of higher amount of food reserves and other nutrients out of the seed very quickly. Lush and Evans (1980) moreover reported that because seeds with thin coats imbibe rapidly, their coats are more easily separated from the cotyledons. However the low level of the conductivity result for the brown genotype was probably due to a high number of 'delayed-permeable' seeds (Kuo, 1989). This kind of seed require a longer time to imbibe than other seeds because of the low permeability of the seed coat. Because they imbibe more slowly, imbibition injury to the seed is reduced. As

a result, the amount of solutes released from the seed is reduced, and this is indicated by the results of the conductivity test. This characteristic of the brown genotype did not affect the percentage germination. The only difference was that seeds from the brown genotype required more time (more days) to imbibe and to germinate.

Differences in conductivity results which ranged from 4.7 to 15.7 $\mu S\ cm^{-1}\ g^{-1}$ for the brown and white bean genotypes respectively (while the other genotypes had intermediate values) did not affect the percentage germination for the same genotypes before and after AA (Table 17). Hampton et al., (1992) also observed that within species, seed lots of the French bean differed for the conductivity results, ranging from 17.0 to 21.8 $\mu S\ cm^{-1}\ g^{-1}$, but did not differ for germination. This suggests that bean seeds with a conductivity result of up to 15.7 $\mu S\ cm^{-1}\ g^{-1}$ are still considered as having high vigour.

The conductivity results did not differ with density which indicates that differences in seed coat characteristics are controlled by genetic factors and not by plant density. Gane et al., (1975) reported that a relatively high plant density had no adverse effects on the quality (vigour) of common bean seeds.

### 5.6.2. Cooking quality

#### 5.6.2.1. Delayed-permeable seeds

The significant differences in the percentage of 'delayed-permeable' seeds among genotypes (after soaking for 32 h) (Table 18) was due to the nature of the seed coat as well as the characteristics of the raphe. The low percentage of 'delayed-permeable' seeds in the haricot bean was attributed to its high percentage of seed coat cracks which facilitated water entrance to the seed. However the significant differences in the percentage of 'delayed permeable' seeds among other genotypes that did not have seed coat cracks was due to the permeability of their seed coat and the characteristics of the raphe and hilum. Lush and Evans (1980) reported that the permeability of the raphe itself and the rest of the testa varies, and the mechanism of action of the hilum and raphe are not known.

### 5.6.2.2. Percentage seed weight increase after soaking

The percentage seed weight increase was significantly higher for the haricot bean after soaking for 2 h (Table 19). This rapid increase of seed weight was caused by the highest percentage of seed coat cracks (39%) which consequently allowed the seeds to imbibe more rapidly, while differences in seed weight increases among other genotypes after soaking for 2 h were due to the differences in seed coat permeability and the characteristics of the raphe and hilum.

The percentage seed weight increase was lowest for the brown genotype at 8, 14 and 24 h soaking, suggesting that this genotype had a seed coat which resisted the rapid uptake of water. However seed weight increase did not differ significantly between the haricot and white bean genotype after 24 h of soaking (Table 19) as at this time the haricot bean cells were almost fully imbibed, so that the imbibition rate began to decrease for this genotype.

### 5.6.2.3. Seed texture

Seeds from all bean genotypes became soft after cooking for 20 minutes under normal atmospheric pressure. Stanley (1987) stated that the cause of the textural softening in soaked beans after cooking is thought to be the influence of heat in promoting depolymerization of calcium and magnesium salts of pectic substances composing the middle lamella to produce separation of intact cells. However results from this study demonstrated that genotypes with the highest conductivity result such as the haricot bean (20.90 $\mu S\ cm^{-1}\ g^{-1}$) required a lower force (6.16 Newton) to cut through the seed after cooking (Table 20) whereas the genotype with the lowest conductivity result i.e. the brown genotype (1.55 $\mu S\ cm^{-1}\ g^{-1}$) required the highest force (15.23 Newton) to cut through the seed after cooking (Table 20), while the other genotypes had intermediate values for both parameters. These results suggest that the variability of water inrush velocity through the internal parts of the seed might cause different levels of cell membrane injury, disfunction and disruption which consequently not only produces the leakage of nutrients and

ions but also affected seed texture after cooking. Uebersax et al., (1991) reported that the degree and rate of hydration of the starch and proteins influences the cooking rate and the final texture of cooked beans.

### 5.6.2.4. Percentage seed coat and cotyledonary damage

The significant differences in the percentage seed coat/cotyledonary damage among genotypes were due to the morphological conditions of the seed coat and cotyledons. For this reason, the highest percentage of damage recorded from the haricot bean was primarily attributed to the high amount of seed coat cracks (39%) before cooking, while the variability for this kind of damage among other genotypes might be attributed to the differences in their seed coat characteristic such as seed coat thickness and the cotyledonary condition after soaking (prior to cooking). Lush and Evans (1980) stated that seeds with thin seed coat imbibe rapidly and their seed coats are more easily separated from the cotyledons. Furthermore Matz (1962) reported that lignin can prevent softening and disintegration of the tissue during cooking, and it binds the cellulose fibre together (raw bean seed coat contains 70.7% of fibre) in a layer that is not disintegrated by boiling water. For this reason, genotypes with a higher amount of lignin in their seed coat might have a seed coat with higher resistence to damage during the cooking process and vice-versa. The results from this trial indicate that the morphological appearance of the beans, such as the integrity of the seed coat is essential in determining the integrity of the seed after cooking, and seeds that imbibe faster possess softer texture after cooking than seeds with a poor ability to imbibe. For this reason, under normal conditions and with the exception of the seeds with 'hard to cook' characteristic, seeds that imbibe faster require less time to cook.

Results from this study indicated that the white, mottled brown, and mottled black genotypes were the best genotypes for cooking, as the seeds imbibed rapidly and therefore required less time to cook and to attain a desirable seed texture. The black and brown genotypes have slightly harder texture and therefore required more cooking time to increase seed softness.

# CHAPTER 6
# CONCLUSION

The environmental conditions, particularly the differences in daylength and (possibly) light intensity/irradiation in the late winter to spring and late spring to summer (in New Zealand) caused the differences in bean plant height between the glasshouse and field trial, as well as the formation and development of branches, which are responsible for the production of flowers and pods.

The five bean genotypes (*white, mottled brown, mottled black, black and brown*) can all be grown in New Zealand. Because of their sensitivity to the differences in daylength, they should not be sown before November. Earlier sowing may allow the production of plants with long main stems (i.e. tall plants with no branches), which would require extra cost for artificial support to protect the plants from lodging caused by rain and/or wind, as well as inconveniences for harvest either by hand or by harvester.

The genetic heterogeneity which exists in the present genotypes could be reduced initially by removing the "off type" plants (i.e. plants within a genotype which have a different morphological appearance). Once this roguing process was complete, genetic purity could be assessed by an electrophoretic procedure which would provide information on the level of genetic heterogeneity in plants that are morphologically undistinguishable by eye.

Differences in seed desiccation and imbibition rates among genotypes which were found are not yet clearly understood i.e. whether these properties are controlled by differences in seed coat thickness or by differences in the structure of the hilum, raphe or micropyle. A further study in this area is required because an understanding

the main cause (or causes) of these differences, will provide information for breeders to select the genotypes with certain desiccation or imbibition abilities that may satisfy consumer preferences.

The black and brown bean genotypes had a lower desiccation rate than the other genotypes. It is not known for how long both genotypes should be dried down in the field to attain the same level of seed moisture content (smc) as the other genotypes, and what the consequences may be for seed quality in response to their longer time in the field. A further study to determine the length of time needed to dry down seeds from different genotypes to a specific smc (e.g. 10%) is necessary, as this factor will determine the right time for harvest in individual genotypes, without losing seed quantity and quality.

The brown genotype had lower seed yield than the other four genotypes, even though the yield of this genotype was still higher than seed yields reported for other genotypes in New Zealand. However, it should be remembered that these yields were from hand harvesting, and lower yields would be expected from machine harvesting. In addition, these data were from only one season, and one in which insects and/or pathogens were not a problem. Yields may be reduced by particularly pathogen infestation. The same bean genotypes in this study may also produce different yields in response to different temperature regimes in different regions of New Zealand. A further study of the effects of the environment on seed yield and quality will be useful, to determine the suitability of individual genotypes for different regions of New Zealand where the climatic conditions are not uniform (e.g. between the North and South Islands).

The length of time required to cook each bean genotype to a certain level of seed texture softness without producing damage such as cotyledonary cracks and/or seed coat splitting as required for food processing and bean fresh salads is still

unknown. A further study in this area is therefore required, to determine the appropriate length of time for cooking the different genotypes so that they are suitable for fresh salads, as well as for other food processing purposes for human consumption. The differences in the seed coat characteristics of the genotypes may play a major role in the response to this processing.

All five bean genotypes used in this study can be grown in New Zealand for human consumption, and these genotypes can be used in combination with other genotypes such as the red kidney and pinto bean to make fresh bean salads and other bean foods more colourful and more attractive, which can simultaneously give satisfaction to bean consumers as well as bean producers and food traders.

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# APPENDIX

1. Graphic of Daylength Changes in Palmerston North (adapted from Dr. Ian Gordon, 1992).