SEED GROWTH AND ASSIMILATE REMOBILISATION IN CHICKPEA (Cicer arietinum L.) AS AFFECTED BY WATER DEFICIT

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Chickpea (*Cicer arietinum* L.) has become an important pulse crop in the Western Australian (WA) grain-growing region due to its suitability to alkaline soils. This region has a Mediterranean-type climate characterised by a winter-dominant rainfall pattern. Winter-sown chickpea crops inevitably encounter terminal drought near the end of the growing season which not only reduces yield, but also affects seed quality by limiting seed filling. Small seed is unacceptable or less preferred by consumers. The aim of this thesis is to examine the effect of terminal drought on seed filling in chickpea and to determine physiological mechanisms to maximise seed filling under drought.

In the first study, pod and seed growth was determined in three contrasting genotypes under rainfed and irrigated field conditions. Tyson, a small-seeded (121 mg/seed) desi cultivar, ICCV88201, a large-seeded (194 mg/seed) desi breeding line, and Kaniva a large-seeded (422 mg/seed) kabuli cultivar were grown in Merredin, WA, which on average receives 200 mm of growing-season rainfall. Midday leaf water potential (LWP) and photosynthetic rates were measured on the uppermost expanded leaf in both the irrigated and rainfed plants. Pods were selected at pod set on a number of individual plants of each genotype and treatment. Seed filling in these selected pods occurred over a period of increasing water deficit in rainfed plants which was coupled with declining leaf photosynthesis. Measurement of seed and pod wall dry weight (DW) indicated genotypic differences in maximum rate and duration of seed growth exist in chickpea, although both were reduced by drought irrespective of genotype. Overall, average seed weight was reduced in rainfed plants by 19, 23 and 34% and yield by 47, 40 and 64% in Tyson, ICCV88201 and Kaniva, respectively. Pod wall DW reached a maximum by the time the seed entered rapid filling and then decreased implying that remobilisation of pod wall dry matter (DM) may be an important assimilate source for seed filling, contributing between 9 and 15% of seed DW.

In light of the very low rates of current photosynthesis during seed filling in rainfed plants, a glasshouse study was conducted to quantify DM remobilisation to the seed in both well-watered and water-stressed chickpea. Pod number was reduced in water deficit plants by 66, 59 and 91% in Tyson, ICCV88201 and Kaniva, respectively. The contribution of DM remobilisation to seed filling was determined in Tyson and Kaniva using $^{13}$C and $^{15}$N stable isotope labelling of vegetative tissue prior to podding. Remobilised pre-podding C contributed 9 and 16% of the total seed C in well-watered and water-deficient Tyson plants, respectively, and 7 and 8% of the total seed C in Kaniva. Remobilised pre-podding N was a vital N source for the seed accounting for 85% of total seed N in well-watered and 97% in water-deficient Tyson and 62 and 91% of the total seed N in Kaniva. Kaniva had the lowest harvest index and the highest proportion of unfilled pods compared to either desi genotype, regardless of treatment.
indicating poor DM partitioning, remobilisation and drought tolerance for the kabuli genotype.

In a second glasshouse study the importance of remobilisation was further examined to assess the source and destination of remobilised DM in ICCV88201 chickpea. Remobilisation was estimated using the same technique as above, but the plants were divided into various components in order to determine which seeds were most dependent on remobilised assimilates in both well-watered and water-deficient plants. The proportion of remobilised pre-podding C and N was lower in later-formed seeds compared to earlier-formed seeds. Earlier formed seeds may well have been supplied with C and N remobilised from vegetative tissues formed after $^{13}\text{C}$ and $^{15}\text{N}$ labelling and therefore was not measured in these studies. Overall remobilised pre-podding C and N contributed 9% of the seed C and 55% of the seed N in well-watered plants and 13% of the seed C and 93% of the seed N in water deficient plants. Remobilised pre-podding C and N was primarily derived from the leaves in chickpea and was related to leaf senescence as a consequence of the breakdown of photosynthetic proteins.

High yield under water deficit was associated with high harvest index and maintenance of pod number across the three genotypes. Genotypic variation in the remobilisation of C and N in the higher yielding desi genotypes implies that selection for improved remobilisation ability may aid yield maintenance and seed size in plants subject to water deficit. Genotypic differences in pod number and seed size were maintained in plants subject to water deficit implying that increased seed size in chickpea in water-limited environments requires selection of genotypes with large potential seed size and high source to sink ratio. Remobilisation increases harvest index and helps to compensate for low assimilate production by the leaves in chickpea subject to water deficit.
Abstract ...........................................................................................................(ii)
List of figures ..................................................................................................(v)
List of tables ...................................................................................................(vii)
Acknowledgements .........................................................................................(viii)
Declaration ......................................................................................................(viii)
Thesis related publications ...............................................................................(ix)

CHAPTER 1 - General Introduction .................................................................1

CHAPTER 2 - Literature Review .................................................................4
  2.1 Origin and spread of chickpea ...............................................................4
  2.2 Assimilate supply to developing seed ..................................................8
  2.3 Plant adaptation to water deficits .........................................................19
  2.4 Remobilisation .....................................................................................21
  2.5 Pod set in pulses ..................................................................................28
  2.6 Pod and seed development .................................................................29
  2.7 Water deficit and seed growth ............................................................36
  2.8 Conclusion ..........................................................................................37

CHAPTER 3 - Seed Growth of Desi And Kabuli Chickpea in a Short-Season Mediterranean-Type Environment .............................38
  3.1 Introduction ..........................................................................................38
  3.2 Materials and methods ........................................................................39
  3.3 Results ..................................................................................................41
  3.4 Discussion ..........................................................................................48

CHAPTER 4 - Remobilisation of Carbon and Nitrogen in Desi and Kabuli Chickpea Subject to Water Deficit ........................................51
  4.1 Introduction ..........................................................................................51
  4.2 Materials and Methods .........................................................................52
  4.3 Results ..................................................................................................56
  4.4 Discussion ..........................................................................................67

CHAPTER 5 - Distribution of Remobilised Carbon and Nitrogen in Chickpea Subject to Water Deficit .........................................................73
  5.1 Introduction ..........................................................................................73
  5.2 Materials and Methods .........................................................................74
  5.3 Results ..................................................................................................76
  5.4 Discussion ..........................................................................................92

CHAPTER 6 - General Discussion ..................................................................96
References .....................................................................................................103
Appendix ........................................................................................................126
LIST OF FIGURES

Figure 3.1  Daily and cumulative rainfall (a) and maximum and minimum air temperatures (b) over the growing season at Merredin, Western Australia, in 1995. The arrows indicate the mean time of the first flowers (F) and pods (P). The gap in the air temperature data was due to failure of the data logger.

Figure 3.2  Change with time in the (a) water potential and (b) rate of net photosynthesis of the uppermost expanded leaves of Tyson (■, □), ICCV88201 (●, ○) and Kaniva (▲, Δ) chickpeas grown under irrigated (closed symbols) and rainfed (open symbols) conditions at Merredin, Western Australia, in 1995. Bars give +/- one standard error of the mean of 12 leaves where the values are greater than the size of the symbols.

Figure 3.3  Change with time in the dry weight of the seed (●, □) and pod wall (■, ○) of (a) Tyson, (b) ICCV88201 and (c) Kaniva chickpeas grown under irrigated (closed symbols) and rainfed (open symbols) conditions at Merredin, Western Australia, in 1995. Bars give +/- one standard error of the mean of 4 replicates where values are greater than the size of the symbols.

Figure 4.1  Changes with time in the pre-dawn water potential of the uppermost fully expanded leaves of Tyson (■, □), ICCV88201 (●, ○) and Kaniva (▲, Δ) chickpea grown under well-watered (closed symbols) and water deficit (open symbols) conditions in the glasshouse. Bars represent +/- one standard error of the mean of 4 replicates where values are greater than the size of the symbols.

Figure 4.2  Changes with time in pod number in (a) well-watered and (b) water-deficient Tyson (■, □), ICCV88201 (●, ○) and Kaniva (▲, Δ) chickpea grown in the glasshouse. Bars represent +/- one standard error of the mean of 8 replicates where values are greater than the size of the symbols.

Figure 4.3  (a) Average seed dry weight, number of (b) filled and (c) unfilled pods and (d) seed yield (g/plant) in glasshouse-grown Tyson (■, □), ICCV88201 (●, ○) and Kaniva (▲, Δ) chickpea subject to both well-watered (control) and water deficit (stressed) conditions. Bars represent one standard error of the mean of 4 replicates.

Figure 4.4  Change in total seed dry weight (a,c and e) and growth rate (b,d and f) with time (DAS, days after sowing) in well-watered (solid line) and water-deficient (dashed line) glasshouse-grown Tyson (a and b), ICCV88201 (c and d) and Kaniva (e and f) chickpea. Data, derived from parameters estimated by fitting sigmoid curves to dry weight data (not shown).

Figure 4.5  Decreases in the dry weight (g/plant) of the stems, leaves, roots and pod walls between 81 and 123 days after sowing. Bars represent one standard error of the mean of 4 replicates.

Figure 5.1  Changes with time in pre-dawn water potential of the uppermost expanded leaves in well-watered (●) and water-deficient (○) chickpea grown in the glasshouse. Bars represent +/- one standard error of the mean of 4 replicates where values are greater than the size of the symbols.
Figure 5.2  Changes with time in pod (■, □) and seed number (●, ○) in well-watered (control, ■, ●) and water-deficient (stressed, ○, □) glasshouse-grown chickpea. Bars represent +/- one standard error of the mean of 8 replicates where values are greater than the size of the symbols.

Figure 5.3  Changes with time in pod wall (■, □) and seed (●, ○) dry weight (a and b) and leaf (▼, ▼), stem (▲, △) and root (●, ●) dry weight (c and d) in well-watered (a and c) and water-deficient (b and d), glasshouse-grown chickpea. Bars represent +/- one standard error of the mean of 8 replicates where values are greater than the size of the symbols.

Figure 5.4  Change with time in (a) total seed dry weight and (b) growth rate in well-watered (control) and water-deficient (stressed) glasshouse-grown chickpea. Data, including +/- standard error (dotted line), derived from parameters estimated by fitting sigmoid curves to dry weight data.

Figure 5.5  Average seed weight (a and b) filled (c and d) and unfilled (e and f) pod number, and seed yield (g and h) of pods set on different dates in glasshouse grown chickpea plants grown under well-watered (control) and water deficient (stressed) conditions. Bars represent one standard error of the mean of 3-4 replicates.

Figure 5.6  Final seed dry weight (a and b), maximum seed growth rate (c and d), estimated average seed growth rate (e and f) and duration of seed growth (g and h) of seed in pods which had set at different times throughout reproductive development in well-watered (control) and water-deficient (stressed) glasshouse-grown chickpea. Bars represent one standard error of the mean of 4 replicates.

Figure 5.7  Changes with time in allocation of dry matter (a and b), ¹³C (c and d) and ¹⁵N (e and f) to seed, pod walls, stems, leaves and roots as a proportion of total dry matter, ¹³C and ¹⁵N in well-watered (control) and water-deficient (stressed) glasshouse-grown chickpea.

Figure 5.8  Changes with time in concentration (%) of enriched ¹³C in leaves of different age (defined by the timing of pod set except the basal leaves with no pods) from glasshouse-grown chickpea under well-watered (a, closed symbols) and water-deficit (b, open symbols) conditions. Bars represent +/- one standard error of the mean where values are greater than the size of the symbols.

Figure 5.9  Changes with time in concentration (%) of enriched ¹⁵N in leaves of different age (defined by the timing of pod set except the basal leaves with no pods) from glasshouse-grown chickpea under well-watered (a, closed symbols) and water-deficit (b, open symbols) conditions. Bars represent +/- one standard error of the mean where values are greater than the size of the symbols.

Figure 5.10 Changes with time in concentration (%) of leaf nitrogen in leaves of different age (defined by the timing of pod set except the basal leaves with no pods) from glasshouse-grown chickpea under well-watered (control, ●) and water-deficient (stressed, ○) conditions. Bars represent +/- one standard error of the mean where values are greater than the size of the symbols.

Figure 5.11 Changes with time in the contribution of post-podding assimilated (solid line) and pre-podding (dashed line) remobilised C and N as a proportion of the final, total seed C and N in well-watered (a and c) and water-deficient (b and d) glasshouse-grown chickpea.
**LIST OF TABLES**

| Table 2.1 | Reported contributions (%) of remobilised carbon (C), dry matter (DM) and/or nitrogen (N) to seed in cereals and pulses grown under a range of conditions. | 24 |
| Table 3.1 | Yield components at final harvest of three chickpea genotypes grown under irrigated and rainfed field conditions at Merredin, Western Australia, in 1995. Different letters within rows indicate statistically significant differences at $P = 0.05$. | 42 |
| Table 3.2 | Average seed weight (mg), at final harvest, of clean chickpea seed according to branch order from three chickpea genotypes grown under irrigated and rainfed field conditions at Merredin, Western Australia, in 1995. Different letters within rows indicate statistically significant differences at $P = 0.05$. Data within columns was not significantly different at $P = 0.05$. | 42 |
| Table 3.3 | Seed growth characteristics determined from fitted sigmoidal growth curves of seeds of three chickpea genotypes grown under irrigated and rainfed field conditions at Merredin, Western Australia, in 1995. Different letters within rows indicate statistically significant differences at $P = 0.05$. | 45 |
| Table 3.4 | Seed growth characteristics determined from fitted sigmoidal growth curves of seed yield data of three chickpea genotypes grown in the glasshouse under well-watered (control) and water deficit (stressed) conditions. Different letters within rows indicate statistically significant differences at $P = 0.05$. | 61 |
| Table 4.1 | Total, vegetative (including pod walls) and seed above-ground dry matter and harvest index of Tyson, ICCV88201 and Kaniva chickpea grown under control and stressed conditions at final harvest. Different letters within rows indicate significance at $P = 0.05$. | 65 |
| Table 4.2 | Proportion of plant dry weight (%) in the stems, leaves, roots, pod walls and seed in glasshouse grown Tyson and Kaniva chickpea under well-watered and water deficit conditions at each harvest. Different letters within rows indicate significance at $P = 0.05$. | 65 |
| Table 4.3 | Proportion of $^{13}$C (%) in the stems, leaves, roots, pod walls (P. wall) and seed in glasshouse grown Tyson and Kaniva chickpea under well-watered and water deficit conditions at each harvest. Different letters within rows indicate significance at $P = 0.05$. | 66 |
| Table 4.4 | Proportion of $^{15}$N (%) in the stems, leaves, roots, pod walls and seed in glasshouse grown Tyson and Kaniva chickpea under well-watered and water deficit conditions at each harvest. Different letters within rows indicate significance at $P = 0.05$. | 66 |
| Table 4.5 | Total seed C and N (mg/plant) and the C and N arising from post-podding fixation and uptake or from pre-podding remobilisation in glasshouse grown Tyson and Kaniva chickpea under both well-watered (control) and water deficit conditions (stressed). | 67 |
| Table 5.1 | Contributions of remobilised pre-podding C and N and post-podding C and N to total seed C and N in seed from pods of different age on well-watered (control) and water-deficient (stressed) glasshouse-grown plants. Percentage contribution of remobilised pre-podding C and N to total seed C and N shown in brackets. | 91 |
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DECLARATION

I declare that this thesis is my own composition and the result of my own research. All sources used herein are acknowledged.

Stephen L. Davies


CHAPTER 1

GENERAL INTRODUCTION

Chickpea (*Cicer arietinum* L.) ranks as the world’s third most important pulse crop. World production of chickpea in 1997 was 8.8 million tonnes, compared to field pea, 11.4 million tonnes, and common bean, 18.8 million tonnes. India produced 6 million tonnes or 68% of world chickpea production in 1997 and Turkey and Pakistan contributed 8.2 and 6.7%, respectively. Australia, the world’s sixth-biggest producer in 1997, produced 2.4% of total production (FAO, 1998). The area sown to chickpea in Australia has increased substantially from 3100 ha in 1983 to over 200,000 ha since 1994 (ABARE, 1998; FAO, 1998). Chickpea is the third most important pulse crop in Australia behind lupins and field pea and is worth about $80 million on farm (Siddique, 1998).

In 1997-98 a total of 256 kt of chickpea were exported from Australia and this was worth $118 million to the Australian economy (ABARE, 1998). In recent years Australia has become the world’s biggest exporter of chickpea, predominantly exporting desi chickpea. In 1996 Australia exported around 217 kT of chickpea while Turkey exported only 193 kT of predominantly kabuli-type chickpea. Turkey, a long-term exporter of chickpea, dominated export up to 1993 when it was surpassed by Australia (FAO, 1998). The Indian subcontinent incorporating India, Pakistan, and Bangladesh is the biggest importer of chickpea (Siddique *et al.*, 1998a,b) and in 1996 these countries imported 122, 76 and 52 kT of chickpea, respectively (FAO, 1998).

In Western Australia the importance of chickpea has increased substantially over the past decade with large increases in chickpea production (Siddique and Sykes, 1997). These increases have occurred despite variable yields due to unfavourable seasonal conditions. For example, widespread drought in 1994-95 resulted in an average yield of 0.6 t/ha across Western Australia and only 0.4 t/ha across the whole of Australia (ABARE, 1996). Overall production in Western Australia has increased at an almost exponential rate, largely due to the increase in the area sown to chickpea. The production area in Western Australia has increased from 500 ha in 1990-91 to an estimated 40,000 ha in 1997-98 (ABARE, 1998). This increase was due to considerable effort by agricultural research and extension agencies to develop production strategies and lift the profile of chickpea and some of the other pulse crops as alternative crops for fine-textured, neutral-alkaline soils. Improved agronomic packages, marketing and access to information on the potential of chickpea coupled with reasonable prices and the flow on benefits to a subsequent cereal or oilseed crop in the rotation have all led to increased production (Siddique, 1998; Siddique *et al.*, 1998a). Recent release of locally
adapted chickpea cultivars, Sona and Heera, that mature early and have large pale seed desired by overseas markets, is likely to result in continued increases in WA chickpea production (Siddique et al., 1998a), unless the spread of *Ascochyta* blight reduces farmer acceptance.

Chickpea is sown following the break-of-season in late autumn and grows throughout the winter ultimately maturing in spring with the onset of high temperatures and declining soil water (Thomson et al., 1997a). Continued improvement of chickpea seed yield and quality in southern Australia is subject to a number of biological and environmental constraints. Substantial potential remains for improved varieties with early flowering, better pod set, improved disease resistance and better seed quality (Siddique and Sykes, 1997). The foliar diseases, *Botrytis* grey mould, sclerotinia, *Phoma* blight and *Ascochyta* blight and root lesion nematode are the principal disease constraints reducing yield and seed quality (Siddique and Sykes, 1997; Siddique et al., 1998a). Cold and high temperature stress, and drought are the major environmental constraints. Chickpea is particularly sensitive to cool, spring temperatures which results in flower and pod abortion (Siddique and Sedgley, 1986). Mean daily temperatures below 15°C cause pollen sterility and failure of pollen tube growth leading to pod set failure in winter (Clarke and Siddique, 1998; Lawlor et al., 1998) while temperatures above 35°C in spring can also reduce yield by increasing flower abortion and shortening the duration of the reproductive period (Clarke and Siddique, 1998). Terminal drought and decreasing soil moisture in spring is the major constraint (Turner, 1992; Loss and Siddique, 1994; Singh et al., 1997; Thomson et al., 1997a) as chickpea can tolerate high temperatures and continue pod set when adequate water is available (Clarke and Siddique, 1998).

Terminal drought lowers crop yields but it can also decrease seed quality by reducing seed size. Importers of chickpea prefer large seed size and will pay higher prices for large seed size in kabuli chickpea (Siddique and Sykes, 1997; Siddique et al., 1998c). In drought-affected plants, reduced assimilate supply can limit seed filling, resulting in small seed in these short-season environments (Singh et al., 1987; Hooda et al., 1989; Turner and Henson, 1989; Dracup and Kirby, 1996). In other legumes subject to terminal drought, reductions in seed size can be confined to later formed seed (Dracup and Kirby, 1996). In chickpea seed size has been observed to decline acropetally (Khanna-Chopra and Sinha, 1987), but there has been little research on the effect of drought on the size of seed in relation to their spatial distribution.

Seed size is a function of the rate and duration of seed growth (Egli et al., 1978; Egli, 1981). Seed growth rate is maintained in soybean subject to water deficit (Westgate and Crafts-Brandner, 1989), while increased, decreased or unchanged growth rates have been associated with drought in lupin (French and Turner, 1991; Dracup and Kirby,
1996). Drought decreases the duration of crop growth shortening the duration of seed growth (Loss and Siddique, 1994; Dracup and Kirby, 1996). Chickpea seed development and the effect of terminal drought on the rate and duration of seed growth has not been studied.

In cereal crops seed growth in drought-affected plants is largely maintained through increased remobilisation of non-structural assimilates from the vegetative components to the seed (Pheloung and Siddique, 1991; Nicolas and Turner, 1993; Palta et al., 1994). While remobilisation of dry matter to seeds has been shown to occur in a number of pulses, its contribution to seed growth is variable (Pate et al., 1983; Bushby & Lawn, 1992). Genotypic variation in the extent of remobilisation has been identified in cereals and pulses (Papakosta and Gagianas, 1991; Bushby and Lawn, 1992; Nicolas and Turner, 1993; Kurdali et al., 1997), but the contribution and importance of remobilised assimilates to seed growth in pulses subject to water deficit has not been determined.

The aim of this thesis is to understand the effects of terminal drought on the rate and duration of seed growth in chickpea and how this affects seed size. The importance of assimilate remobilisation from the vegetative tissue as an alternative source of assimilates for seed filling in chickpea will be examined and genotypic variation for this trait sought under both well-watered and water deficit conditions.
CHAPTER 2

LITERATURE REVIEW

2.1 ORIGIN AND SPREAD OF CHICKPEA

Chickpea is likely to have originated in south-eastern Turkey and is thought to be one of the first grain legumes domesticated in the Old World (van der Maesen, 1987). The first domesticated types were small seeded with characteristic purple flowers and angular, coloured seeds. These types have since become known by the hindi name, desi. Desi chickpea was present in the Mediterranean region between 4,000 and 3,000 BC. The newer large-seeded type, known as kabuli chickpea, was subsequently domesticated in this region. Kabuli types are believed to have been derived from the older desi types through mutation followed by conscious selection (Jana and Singh, 1993). Kabuli-type chickpea is characterised by white flowers (Nene 1987; Singh et al., 1991) and large, rams-head shaped seeds (Jana and Singh, 1993).

Chickpea spread from its Turkish origin to the Indian subcontinent in the East and to California and Chile in the West (van der Maesen, 1987; Jana and Singh, 1993). The effect of this spread can still be seen today in the Indian subcontinent where desi types dominate and are well adapted compared to kabuli types. Kabuli chickpeas are well adapted to the Mediterranean and to the countries in the New World, such as California and Chile (Jana and Singh, 1993). These regions are now the major producers and consumers of chickpea and form the principal chickpea market, primarily consuming those types which are grown in their respective regions. In these countries chickpea and other pulse crops provide high quality protein in diets which are cereal-dominated and where many of the consumers are either vegetarians or meat is largely unavailable to them (Singh, 1997).

Chickpea morphology

Chickpea (Cicer arietinum L.) is a self-pollinated, annual pulse crop. Chickpea grown in Mediterranean-type climates is sown in winter and relies on the winter dominant rainfall pattern associated with this climate (Siddique et al., 1998a). However, chickpea can also be grown with very little rainfall on stored receding soil moisture (Indian sub-continent, northern New South Wales, southern Queensland) or under irrigation (Nene, 1987) as is the case in the Ord river irrigation area in Western Australia (Siddique, 1998; Siddique et al., 1998a).

Leaves are produced alternately along the branch. Each leaf has five to eight pairs of serrated leaflets attached to a central rachis with a small petiole (Clarke and Siddique,
Development of leaf area is slow in chickpea and increased rates of leaf area development and biomass occur after flowering (Clarke and Siddique, 1998; Khanna-Chopra and Sinha, 1987). The leaves, pods and stems are also densely covered with fine acid-secreting hairs, known as trichomes. This highly acidic secretion consists primarily of malic, oxalic and citric acids which play a role in protecting the plant from pests such as red-legged earth mite, lucerne flea and aphids. The roots also secrete acids which can solubilise some nutrients (Clarke and Siddique, 1998).

Unlike some other species, such as lupin, the main stem does not tend to dominate the latter formed primary branches. Generally the primary stems arise from the basal nodes of the main stem although some stems do develop from the apical nodes. The primary basal stems tend to be thick and can sometimes be difficult to distinguish from the main stem without careful examination. Secondary branches develop from buds on the primary branches. Again these branches tend to form from buds low down on the stem but occasionally from buds closer to the apex (van der Maesen, 1987). The secondary branches are less vigorous than the primary branches and are consequently thinner, they do however contribute substantially to yield (Siddique, 1998). Tertiary branches that form from secondary branch buds are fewer in number and are less important in terms of yield. Branches which develop from buds closer to the apex tend to be short but produce flowers and pods along most of their length making a major contribution to seed yield.

Chickpea has various growth habits defined by the angle of branches from vertical, these habits are classified as: erect, semi-erect, semi-spreading, spreading and prostrate (Pundir et al., 1985). Most of the modern varieties tend to be erect or semi-erect to enable the use of mechanical harvesters (Siddique, 1998).

Chickpea has an indeterminate growth habit which results in the production of a succession of flowers and pods being set and filled. Floral bud initiation occurs in the axils of compound leaves supported on a jointed peduncle, 6-13mm long. Generally, only one pea-like flower is set per flowering node. Pollination takes place while the flower is still in bud with pollen and the receptive female style remaining enclosed within a fused petal, known as the keel. Chickpea produces many flowers but only 50-80% of these develop into mature pods (Clarke and Siddique, 1998), due to pod set failure and high levels of pod abortion (Zaiter and Barakat, 1995). The proportion of flowers that form pods is dependent upon variety, time of sowing and environmental factors. Pod set in chickpea is sensitive to temperature and the first flowers do not set pods in southern Australia due to the colder winter temperatures. Low temperatures during pollen development can result in infertile pollen and can inhibit pollen tube growth and slow pollen tube growth increases the risk of flower abortion (Clarke and Siddique, 1998; Lawlor et al., 1998). Pod set can be delayed for up to month after initial flowering due to cold-inhibition. A consequence of this low temperature sensitivity is
that early flowering lines have been shown to provide little yield advantage in certain Australian conditions. About six days is required from fertilisation of the ovule to the appearance of the pod wall beyond the senescing petals. After pod set the pod wall grows rapidly, while the seed undergoes a cell division phase with little increase in dry matter (Clarke and Siddique, 1998).

Once podding starts pods are set sequentially up the stem with continued production of new flowering nodes as stem growth continues. Consequently, there is a gradation of pod maturity with older pods lower on the stem and younger pods higher on the stem. However, all the pods mature at the same time when the plant enters senescence so that filling duration of seeds in pods formed by the first flowers is longer than pods that develop from later formed flowers (Eser et al., 1991). Chickpea pods tend to contain 1 to 3 seeds per pod (Clarke and Siddique, 1998).

Chickpea tends to be deep rooted with a strong tap root and relatively few lateral roots (Singh, 1997). Root growth occurs rapidly before flowering but will continue until maturity under favourable conditions (Siddique and Sedgley, 1987). In deep soils with good structure chickpea roots can penetrate more than three metres contributing to the ability of chickpea to tolerate drought conditions. Nodulation of chickpea roots is brought about by a symbiotic relationship with *Rhizobium* bacteria. The fan-like nodules are visible after approximately one month and contain the bacteria which subsequently fix atmospheric nitrogen while being supplied with carbohydrates and sugars from the plant (Clarke and Siddique, 1998).

**Chickpea production in southern-Australia**

Southern Australia experiences a Mediterranean-type climate characterised by hot, dry summers with cool, wet winters (Turner, 1992). On average 60-70% of the rain falls during the growing season (Rovira, 1992). Narrow-leafed lupin (*Lupinus angustifolius* L.) is the dominant pulse crop grown in Western Australia. Lupins are well adapted to the most common soil types, which are deep, coarse textured, acid-to-neutral soils (Siddique et al., 1993; Thomson et al., 1997). Lupins, however, are poorly adapted to the fine textured, neutral-to-alkaline soils that constitute about 60% of the WA wheatbelt (Siddique et al., 1993; Thomson et al., 1997). Consequently, alternative pulse crops, better adapted to these soils have been sought and developed. Chickpea (*Cicer arietinum* L.) has been shown to be one of a number of pulses that are more suited to the fine-textured, neutral-to-alkaline soils of the eastern cropping zone of Western Australia (Siddique and Sedgley, 1986; Siddique et al., 1993; Siddique et al., 1999a).

The soils of southern Australia are primarily derived from weathered ancient rocks and are inherently low in organic matter and many of the plant nutrients, particularly
phosphorus and trace elements (Rovira, 1992). Consequently, in a relatively short period of cropping significant yield reductions can occur due to nutrient depletion. However, there are other factors, in particular drought, which reduce crop yield. In this type of environment drought is by far the most important abiotic stress. This is because the crop is grown in an environment where soil moisture declines near the end of the season (Singh et al., 1997). This terminal drought is often accompanied by increasing temperatures often to 30°C or higher which can interfere with pod filling (Johansen et al., 1994).

Benefits of chickpea production

Declining profits from wheat and wool, the benefits of pulses in reducing soil disease in following cereal crops (Hamblin and Kyneur, 1993), the opportunity to use selective herbicides in a broad leaf pulse crop to control grassy weeds, usefulness of pulse stubble for feeding, declining wheat protein levels using traditional rotation methods and the relative profitability of pulses in their own right have all combined to provide chickpea and other pulses a place in Australian farming systems (Rees et al., 1994, Siddique et al., 1998a).

The ability of pulse crops and pasture legumes to fix nitrogen (N) has long been recognised as being beneficial in terms of the soil N status. However, when this perception is scrutinised more closely the actual N benefit the soil receives is highly variable depending on each situation. Chickpea and other pulse crops tend to leave less residual soil N than pasture species because much of the fixed N is harvested with the grain (Holford and Crocker, 1997). Chickpea generally produces less dry matter than other grain legumes which results in lower N fixation in this species (Siddique et al., 1998a). However, cereal crops following a chickpea crop generally have a yield benefit (Armstrong et al., 1997).

Marketing and utilisation of chickpea

Nutritionally, chickpea is known to be one of the most digestible of the pulses (Byth et al., 1980) and it is a good source of carbohydrates, protein, minerals and trace elements. Its protein content is similar to or better than other pulses (Jambunathan and Singh, 1990). Singh et al. (1991) looked at the cooking quality and nutritional attributes of five desi and five kabuli cultivars and found that kabuli chickpea may be preferred to desi cultivars in terms of cooking time and sensory properties. The calcium content of chickpea was found to be higher in desi types than in kabuli. Levels of magnesium, iron, copper and zinc were similar for both types. There were no substantial differences in the protein and amino acid contents of desi and kabuli lines but kabuli types contained more utilisable protein than the desi types and so may be nutritionally superior (Singh et al.,
Australian grown chickpea are mostly desi types. They are exported for human consumption to the Indian subcontinent due to the inability of these countries to produce sufficient chickpea to meet expanding demand (Siddique, 1993). In 1994-1997 India produced 6-6.4 million tons of chickpea per year and production is unlikely to get much higher (FAO, 1998). Large increases in the production of cereals have occurred in India with the adoption of improved varieties, disease control and superior cultural practices. Consequently cereal prices have fallen and the consumption of pulses has decreased per capita (Parkin, 1993). However due to the expanding population, demand for chickpea continues to rise resulting in increased imports into the Indian subcontinent. Consequently, there are excellent prospects for continued export of Australian chickpea (Siddique, 1993; Rees et al, 1994; Hawthorne, 1993).

Physical characteristics of the seed are also important in terms of consumer acceptance with the principal quality parameters for export being size, uniformity, colour and shape, as well as freedom from external damage and foreign material, and ease of processing if it is required (Saini, 1993). Seed size is an important quality factor in chickpea with large seed tending to fetch a higher price than smaller seed especially for kabuli chickpea (Eser et al., 1991). However pulses, including desi chickpea, are often dehulled and split before they are consumed (Saini, 1993; Parkin, 1993). For processing, not only is average seed size important but also the uniformity of seed size. A large range of seed sizes makes processing more difficult, and decreases splitting yield (Williams and Singh, 1987).

2.2 ASSIMILATE SUPPLY TO DEVELOPING SEED

Source can be defined as all those organs that supply assimilates to the sink, which in turn can be defined as all those organs which utilise and have a demand for assimilates (Ho, 1988). Within the concept of source the term assimilate will be used to encompass both nitrogenous and photosynthetic (carbohydrate) compounds. Where only products of photosynthesis are referred to, the terms photosynthate or photoassimilate will be used.

The stage of development of an organ can be an important determinant of whether it is a net provider or consumer of assimilate. During its initiation and early expansion a leaf is a net importer of assimilate but when it reaches a point where assimilate production exceeds demand it becomes a net exporter of assimilate. This has important implications for chickpea because it is an indeterminate species and production of vegetative tissue, such as leaves and stems, can be in direct competition with developing seeds during reproductive development (Khanna-Chopra and Sinha, 1987).
Changes in assimilate supply and partitioning affect final seed yield and seed size. Variation in assimilate partitioning occurs both spatially and temporally. During seed filling the demand for assimilate changes and different source organs can assume greater or lesser importance. In pulses seed filling is often associated with plant senescence and its associated decreases in photosynthesis and N fixation. Consequently the current assimilate supply can be decreasing at a time of increasing assimilate demand from the filling seed (Egli and Crafts-Brandner, 1996). This process can be exacerbated by water deficit (Turner and Henson, 1989; Devries et al., 1989; Hooda et al., 1989; French and Turner, 1991; Sinclair and Serraj, 1995; Swaraj et al., 1995). When current assimilation is unable to meet the demands of the growing seed, alternative sources of assimilate must be found to maintain seed filling. This can occur through the movement of non-structural C and N from existing vegetative tissue to the filling seed, a process known as remobilisation.

Consequently, assimilate supply can be derived from two distinct sources. The first source consists of current assimilation of C and N while the second consists of remobilisation of stored reserves. While the importance of these sources varies with plant development and environment, they can occur simultaneously and independently of each other.

**Photosynthesis and carbon fixation**

Photosynthesis has been the subject of much study as it is the process by which atmospheric CO\textsubscript{2} is assimilated by plants. In summary, photosynthesis involves the oxidation of H\textsubscript{2}O, resulting in the release of O\textsubscript{2}, and the reduction of CO\textsubscript{2} to form organic compounds such as carbohydrates. This energy-requiring process uses specialised pigments, including the chlorophylls, in order to ‘capture’ the light energy which drives the reaction. The carbohydrates formed in the process can then be used in the production of numerous structural compounds or alternatively can be used as an energy source essential for maintaining life. This breakdown of carbohydrates for energy known as respiration is essentially the reverse reaction of photosynthesis. During respiration H\textsuperscript{+} and O\textsubscript{2} are combined to form H\textsubscript{2}O. Overall photosynthesis can be summarised in the following reaction:

\[ n\text{CO}_2 + n\text{H}_2\text{O} + \text{light} \rightarrow (\text{CH}_2\text{O})_n + n\text{O}_2 \]

Photosynthesis can be divided into two groups of reactions, the light-capturing reaction and the CO\textsubscript{2}-fixing reaction. Both groups of reactions occur in the chloroplast which are plastids with an outer membrane surrounding a system of internal membranes (Beadle et al., 1985). The internal membrane consists of stacks of thylakoids which are pouch-like, flattened disk membranes. The light reactions occur on and in the thylakoid membranes.
and are the reactions responsible for light harvesting. The thylakoids contain pigments which are responsible for absorption of sunlight energy.

Energy captured in the light reactions is used to fix atmospheric CO₂ to form carbohydrates. The key enzymes involved in carbon fixation are ribulose bisphosphate carboxylase/oxygenase (Rubisco) and in some species, phosphoenolpyruvate carboxylase (PEP-C; Beadle et al., 1985). While photosynthesis can be directly measured by the net uptake or production of CO₂, measurements of the activity of Rubisco and PEP-C can also provide a useful measure of the potential photosynthetic activity of a tissue.

Stage of plant development and photosynthesis

Transport of photosynthate shifts direction with stage of development. Early in vegetative growth much of the photosynthate that a crop produces is directed towards the establishment of the plant canopy and the growth and development of the roots and nodules. In pulses, the roots and nodules are a major sink for photosynthate in the vegetative phase not only for development but also for the maintenance of N fixation (Herridge and Pate, 1977). Photosynthate produced later in vegetative growth by the lower leaves is utilised by the roots, while the growing shoot apex is supplied with photosynthate from the upper leaves (Flinn and Pate, 1970). Shortly after flowering commences and pods start to develop, there are major shifts in photosynthate movement. Generally at this time roots, including nodules, receive less C (Herridge and Pate, 1977; Hume and Criswell, 1973) resulting in a decrease in the DW of the roots and nodules during early podfill (Herridge and Pate, 1977; Rao et al., 1984, Hooda et al., 1986).

In pulse crops, leaf photosynthesis is the main source of photosynthates for seed filling (Flinn and Pate, 1970; Singh and Pandey, 1980; Atkins and Flinn, 1978; Sheoran et al., 1987). In adequately watered plants, photosynthate fixed after anthesis dominates seed filling contributing 96-98% of final seed C. By mid to late seed filling the seeds are the largest and most active sink for current photosynthate (Yamagata et al., 1987; Pate et al., 1980). This represents a major shift in C partitioning from vegetative to reproductive development and results in an increase in the rate of development of reproductive structures at the expense of stem and root growth. Leaf initiation and development tends to be maintained albeit at a slower rate (Geiger and Shieh, 1988).

Leaf photosynthesis

The leaves are the most important photosynthetic organ in chickpea (Singh and Pandey, 1980) with a higher photosynthetic rate than both pods and stems (Prasad et al., 1978). Furthermore during reproductive development, the upper leaves which bear flowers and
pods in their axils, are more important than the lower leaves which do not possess flowers or pods. When the upper leaves of chickpea are removed, plant assimilation is reduced by 68% of the $^{14}$C assimilation of intact plants while removal of the lower leaves reduces $^{14}$C assimilation by only 30% (Singh and Pandey, 1980). Better C assimilation by upper leaves is primarily due to the fact they are younger than lower leaves and have higher photosynthetic rates (Harvey, 1977). The presence of axillary pods at upper leaf nodes increases their photosynthetic rate due to increased sink demand (Flinn, 1974; Harvey, 1977; Prasad et al., 1978). The position of upper leaves in the canopy would also suggest that they intercept more light compared to lower shaded leaves. Shading, including clouds blocking out sunlight, substantially reduces the rate of transpiration, stomatal conductance and CO$_2$ assimilation in soybean leaves. Recovery after placement of plants into full light takes 8 min as stomata re-open (Fay and Knapp, 1995).

In many well watered pulses subtending leaves are an important source of photosynthate for their associated pod. In field pea the subtending leaf accounts for approximately 43% of the C invested in its associated seed (Flinn and Pate, 1970) and is an important source of photosynthate for seed growth. Of the C assimilated by chickpea subtending leaves between 41-64% of the C is transported to their associated pod (Singh and Pandey, 1980). However, removal of the subtending leaf of a particular pod caused no reduction in seed yield of that pod (Sheoran et al., 1987) suggesting that other sources of photosynthate can compensate for the loss of the subtending leaf.

Improving photosynthesis in chickpea may allow for higher biomass production and the potential for higher yields (Mythili and Nair, 1996). Substantial genotypic variation in CO$_2$ fixation suggests that considerable potential exists for selecting for improved photosynthetic efficiency (Prasad et al., 1978; Mythili and Nair, 1996).

Increased temperatures have little effect on the maximum photosynthetic rate of chickpea leaves at saturating light and CO$_2$ concentrations. However, long term exposure to high temperature can result in leaves becoming acclimatised to higher temperatures. Chickpea plants grown in low temperature (25°C day; 17°C night) conditions achieve maximum photosynthetic activity when temperatures are increased to 30-35°C. However, in chickpeas developing in high temperatures (40°C days; 25°C nights) maximum photosynthetic rates occur between 35-40°C. These increases in photosynthesis were associated with increased Rubisco activity (Laurie and Stewart, 1993). Chickpea grown in Western Australia is sown in late-autumn and grows throughout winter with seed filling occurring in spring with increasing temperature so that this acclimation capacity is likely to be advantageous.
Stem Photosynthesis

The photosynthetic efficiency of chickpea stems varies according to genotype. However, in all genotypes photosynthetic efficiency is much higher in leaves than stems. Stem photosynthesis ranges from 26-84% of leaf photosynthesis (Prasad et al., 1978). In completely defoliated chickpea plants, high incorporation of \(^{14}\)C label by stems indicates significant assimilation of \(\text{CO}_2\) and this \(\text{C}\) is used to produce leaves at the apical meristem (Singh and Pandey, 1980).

Pod photosynthesis

Many legume pods are photosynthetically active and contribute to seed development. Pod photosynthesis has two components, firstly assimilation of external \(\text{C}\) by the pod wall, as measured by net \(\text{CO}_2\) exchange over a period of time, and secondly, fixation of \(\text{CO}_2\) from inside the pod cavity by the inner layers of the pod wall. In order to simplify terminology, assimilation will be used to describe the uptake of \(\text{CO}_2\) from the atmosphere while fixation will refer to the recycling of respired \(\text{CO}_2\).

Pod photosynthesis and its significance relative to whole plant photosynthesis has been the subject of many studies in numerous pulse species (Atkins et al., 1977; Sambo et al., 1977; Flinn et al., 1977; Singh and Pandey, 1980; Sheoran et al., 1987). Its importance, however, is probably dependent on the morphology and physiology of the pods of each species. In field pea the pod wall has two distinct layers. The outer layer is characterised by a thick cuticle covering the epidermis with some stomata allowing for gas exchange and transpiration. Beneath the epidermis is a densely packed mesophyll cell layer which may prevent gaseous losses from the pod cavity to the atmosphere. The inner epidermis, which lines the pod cavity, has a thin cuticle and the cells contain many chloroplasts which contain 20% of the pod’s chlorophyll. It is estimated that 66% of the respired \(\text{CO}_2\) could be re-fixed by these chloroplasts (Atkins et al., 1977).

The photosynthetic efficiency of the pod walls of field pea is higher than the leaves (Sinha and Sane, 1976), while in chickpea the photosynthetic efficiency of the pod walls is lower than leaves (Singal et al., 1986; Singh and Pandey, 1980). Measurements of the activity of key photosynthetic enzymes, including Rubisco, in the pod walls, seed coats and subtending leaves of chickpea 10 days after flowering, reveal that their activities are much higher in leaves than in pod walls and seed coats. However, activity of the key enzymes in \(\text{C}_4\) metabolism, including PEP-C, are higher in pod walls than leaves (Singh, 1987) suggesting that considerable ability for \(\text{C}\) fixation is retained by the pod walls of chickpea despite the lower relative importance. Activity of Rubisco in the pod wall peaks during the phase of rapid seed filling while the activity of PEP-C peaks during mid-seed filling (Sheoran et al., 1987).
Like field pea, the pod walls have external stomata but do not have internal stomata (Sheoran et al., 1987). The ability of chickpea pods to assimilate $^{14}$CO$_2$ decreases with age. In young pods, 9 days after anthesis (DAA), 49% of the $^{14}$C assimilated by the pod wall is translocated to the seed. However, in older pods (36 DAA) only 8.1% of the $^{14}$C is directed to the seed (Singh and Pandey, 1980). Translocation from the pod wall to the seed is closely related to the stage of seed development. At pod set most of the CO$_2$ assimilated is utilised in active pod wall growth. Seed growth, which at that time consists primarily of cell division, requires little C relative to the phase of rapid filling when the pod wall is close to the end of its rapid growth phase and much more of the C it assimilates is likely to be translocated to the seed (Singh and Pandey, 1980).

Net CO$_2$ uptake, indicating CO$_2$ assimilation, by chickpea pods reaches a maximum at 18 days after anthesis (DAA) which continues up to 21 DAA. This contribution is estimated to be 20% of the final seed DW (Sheoran et al., 1987). However, this assumes that all of the CO$_2$ uptake by the pod is utilised for seed growth, whereas pod wall growth constitutes the most important sink for assimilated C early in development. Preventing light interception by covering chickpea pods with aluminium foil, which prevents both assimilation and fixation of CO$_2$, reduced seed yield in a range of genotypes by 11-21% apart from in one genotype in which seed yield was reduced by only 1% (Sheoran and Singh, 1987). Therefore there appears to be genotypic differences in the contribution of pods to seed yield indicating that there may be potential for selecting for genotypes whose pods contribute more to yield.

Potentially, fixation of respired CO$_2$ is of more significance to seed growth than assimilation of atmospheric CO$_2$. Puncturing the pod wall of chickpea pods prior to 14 DAA renders them permeable to gas exchange and results in cessation of seed growth. Seed growth continues for a short duration if pods are punctured 21-28 DAA, but there was a 29% decrease in pod dry weight (Sheoran and Singh, 1987). This may be due to the pod’s inability to re-fix respired CO$_2$ and hence reduced source capacity for the seed. Pod abortion may be a response to wounding, or change in water vapour concentration inside the pod may affect turgor and hence seed growth (Shackel and Turner, 1998).

Absence of stomata in the inner epidermis suggests that the integrity of the pod wall which prevents gas escape from the pod cavity is important in enabling the efficient recycling of CO$_2$ respired by the seed. Pod cavity CO$_2$ concentration increases from 9 DAA, half way through the seed’s cell division phase, to a maximum near the beginning of seed filling (18 DAA) and then remains constant throughout seed filling (Sheoran et al., 1987). In field pea, pod cavity CO$_2$ concentration was 50-150% higher than the CO$_2$ concentration of ambient air and was found to be consistently lower in illuminated pods compared to pods kept in darkness (Flinn et al., 1977), indicating utilisation of the
cavity CO₂ in fixation. However, while re-fixation of respired CO₂ in chickpea pods occurs, its contribution to seed filling is yet to be quantified. In soybean, CO₂ assimilation by the pod wall makes little contribution to seed growth even though 50-70% of respired CO₂ is fixed (Sambo et al., 1977).

Hormones and photosynthate supply

Several hormones promote various aspects of assimilate supply. The auxin, indoleacetic acid (IAA) may increase stomatal opening thereby increasing CO₂ gas exchange and photosynthesis (Tamas et al., 1973), although this effect is variable (Robinson et al., 1978). This contrasts with stomatal closure by ABA. Cytokinin application increases photosynthesis due to increased chlorophyll content and accelerated chloroplast development (Caers and Vendrig, 1986). IAA and gibberellic acid (GA) application also appear to promote sucrose export from leaves. Phloem loading is enhanced by IAA while application of GA to excised, mature faba bean leaves resulted in increased sucrose export (Baker, 1985; Aloni et al., 1986).

Water deficit and photosynthesis

Water deficits decrease photosynthesis, but the extent depends on species, timing and the severity of water deficits (Daie, 1996). Plants respond to drought by closing or partially closing their stomata, thereby decreasing the amount water lost through transpiration and at the same time decreasing CO₂ exchange. Water deficits also lead to a decline in shoot growth and leaf expansion (Munns and Sharp, 1993; David et al., 1998; Lecour and Guilioni, 1998). This decline is not solely triggered by reduced turgor as decreases in shoot growth and leaf expansion can also occur prior to any measurable decrease in leaf water status (Munns and Sharp, 1993). This response indicates the presence of a water deficit stress signal that is perceived by the plant prior to reduced turgor in the leaf tissue. Abscisic acid (ABA) appears to be the primary signal in plants responsible for triggering the response to low water availability (Davies and Mansfield, 1983).

Water deficit may also directly influence assimilate transport. Fructose-1,6-bisphosphate (FBPase) is one of a number of enzymes involved in the formation of sucrose prior to its export from the leaves (Brenner and Cheikh, 1995). When plants are subject to a water deficit or to exogenous ABA, the functioning of FBPase is inhibited (Cheikh and Brenner, 1992; Harn and Daie, 1992). Furthermore, FBPase activity is inhibited by fructose-2,6-bisphosphate, which increases in leaves of plants subject to water deficit (Quick et al., 1989). Although no relationship between ABA and the regulation of fructose-2,6-bisphosphate has been established, water deficits do appear to inhibit sucrose synthesis in the leaf (Brenner and Cheikh, 1995). Furthermore, ABA application inhibits the phloem loading of sucrose (Baker, 1985) although whether this is an indirect effect of disrupted sucrose synthesis is unknown.
ABA synthesis occurs in both leaves and roots (Zhang and Davies, 1987; 1989; Wolf et al., 1990). ABA synthesised in roots can move to leaves in the xylem stream stimulating stomatal closure and reducing transpiration (Davies et al., 1994; Turner, 1996). Increased leaf ABA content is closely correlated with stomatal closure in wheat and lupins grown under water deficit (Henson et al., 1989a,b; Henson and Turner, 1991). An increased concentration of leaf ABA is not a pre-requisite for stomatal closure, which can also be induced by a redistribution of ABA in the leaf tissue (Hartung and Slovik, 1991) without a corresponding increase in the concentration leaf ABA. This suggests that ABA may have particular sites of action (Bray, 1993). Stomatal sensitivity to ABA may also be increased at low leaf water potential (Tardieu and Davies, 1992).

Stomatal closure as a result of water deficits leads to reduced CO₂ exchange.

Photosynthesis can also be reduced from prolonged and direct effects of water deficits on the photosynthetic apparatus. Photosynthesis declines in chickpea subject to water deficit (Hooda et al., 1989; Singh et al., 1987; Leport et al., 1998; Leport et al., 1999). In WA irrigated chickpea and lupin (Lupinus albus L.) have net photosynthetic rates in the uppermost expanded leaves of about 30μmol/m²/s, 20μmol/m²/s in lentil (Lens culinaris L.), and 10-20μmol/m²/s in grass pea (Lathyrus sativus L.), field pea (Pisum sativum L.) and faba bean (Vicia faba L.). In plants subject to water deficit there was little variation in the photosynthetic rate across these species and it uniformly fell to 5μmol/m²/sec or less. Falling soil water content occurred during podding in all species. Species differences in photosynthesis were not reflected in yield with faba bean always yielding the highest regardless of water status (Leport et al., 1998). Terminal drought, common in Mediterranean-type environments (Turner, 1986a,b) decreases photosynthesis and this frequently occurs during seed filling.

Further reductions in canopy photosynthesis occur through reductions in leaf area. In plants subjected to water deficit there is a reduction in the time from flowering to physiological maturity. Corresponding with this shortened duration is a reduction in leaf area (Singh, 1991; Andriani et al., 1991; Muchow et al., 1986; Lecour and Guilioni, 1998). This is particularly critical when drought begins in the vegetative phase. Reduced leaf area can be a result of slower rates of leaf production and expansion and increased rates of leaf senescence and abscission (Muchow et al., 1986). This may result in increased assimilate partitioning to the reproductive parts which has been shown to increase yield under particular conditions (Singh, 1991; French and Turner, 1991). This is dependent, however, upon the timing and degree of water deficit in relation to plant development. Drought during the reproductive phase, particularly terminal drought, generally results in yield reductions (Ney et al., 1994).
Nitrogen Fixation

The N content of seeds of pulse crops is high, most of which is in the storage proteins (Sinclair and de Wit, 1975). The supply of N to the developing seed comes from soil uptake and/or N fixation. Nitrogen fixation is the process by which atmospheric N, N₂, is converted to NH₃. This is a consequence of a symbiotic relationship between legume species and *Rhizobia* bacteria. Symbiosis between the host plant and the bacteria is dependent upon an exchange of C and N. Before the onset of N fixation, C and N substrates are supplied by the host plant to support the growth of the developing root nodule which consists of plant cells infected with the bacteria (Schubert, 1986). The bacteria in this enlarged non-motile state are referred to as bacteroids and there can be several thousand in a single root nodule. Leghemoglobin, a red coloured protein found in the cytosol of the nodule, probably facilitates oxygen transport to the bacteroids which is essential for their operation, however, too much oxygen will denature components of the nitrogenase enzyme complex which catalyses N fixation. Nitrogen fixation is summarised in the following reaction:

\[ \text{N}_2 + 8 \text{ electrons} + 16 \text{ MgATP} + 16 \text{ H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{H}_2 + 16\text{MgADP} + 16 \text{ Pi} + 8 \text{ H}^+ \]

The original source of electrons and protons for this reaction come from carbohydrate supplied by the plant. Electron transport involves a number of stages before the ultimate conversion of N₂ → NH₃. NH₃, probably as NH₄⁺, is translocated out of the bacteroids prior to further metabolism in the cytosol of the bacteroid-containing cells. Prior to being transported to other regions of the plant, NH₄⁺ is converted to glutamine, glutamic acid, asparagine and in many species, N-rich ureides (Schubert, 1986). The amides are found to be the predominant form in temperate legumes such as field peas, lupins, faba beans and chickpeas, while ureides tend to be the dominant form in tropical legumes (Sinclair and Serraj, 1995). From the bacteroid-containing cells, asparagine and ureides move to the pericycle cells which are found adjacent to the vascular bundles surrounding the nodules. In many species these pericycle cells are modified as transfer cells which appear to actively secrete N compounds into the xylem cells. These N-rich compounds are then transported in the xylem to the upper parts of the plant (Lewis and Pate, 1973). In the leaves these compounds are degraded back to NH₄⁺ before being incorporated into amino acids, amides and protein for further plant development (Schubert, 1986).

Total N fixation varies among genotypes over the growing season. In lentils N fixation ranges from 111 to 154 kg N ha⁻¹ over the season. Cultivars and their N uptake post-flowering can be divided into three groups: those that have continuous N₂ fixation and soil N uptake; those with low N₂ fixation but high N uptake from the soil and those with negligible N assimilation from either source (Kurdali et al., 1997).
Rhizobial strain also has a significant effect on N fixation with some strains having better competitive ability, improved N fixation efficiency or better ability to be effective in specific soils, such as those with high acidity (Hungria and Neves, 1987; Beck, 1992). Soil nitrate levels can also effect N fixation. Low levels of soil nitrate can enhance N fixation. This can be a consequence of improved plant growth after emergence but prior to N fixation becoming established (Da Silva et al., 1993). Starter N added at low rates to improve seedling performance may not necessarily translate into improved yields despite higher total N accumulation (Doughton et al., 1993). At the other extreme, high levels of mineral N can suppress N fixation, although final yield may not be affected (Da Silva et al., 1993). The suppression of N fixation by mineral N appears to vary with species. Soil nitrate levels at establishment and the percentage of N derived from N fixation was found to be inversely related in chickpea (Doughton et al., 1993).

Genotypic variation in the pattern of N fixation occurs in a number of species including chickpea (Beck, 1992; Vessey, 1992; van Kessel, 1994; Kurdali et al., 1997; Hardarson, 1993). In chickpea and several other pulses, N fixation peaks during flowering and declines during pod filling (Kurdali, 1996; Hooda et al., 1986; Herridge and Pate, 1977; Bethlenfalvay and Phillips, 1977; Vikman and Vessey, 1992; Lawn and Brun, 1974). Decreased N fixation during seed filling may be the result of several factors. Development of seeds as the primary sink can result in reduced supply of photosynthate to nodules, thereby decreasing N fixation (Herridge and Pate, 1977; Saxena, 1984). High rates of root respiration during seed filling may exceed C supply and remobilisation will also lead to decreases in root DW (Harvey, 1977; Herridge and Pate, 1977; Hooda et al., 1986). Decreased root and nodule DW is accompanied by a decline in nodule activity. Furthermore, low soil moisture at this time in Mediterranean-type climates can directly decrease nodule function and indirectly reduce N fixation by reducing photosynthesis and the C supply.

Thus as in the case of photosynthesis, the supply of current fixed N can be decreasing at the time when N demand for seed filling is increasing. Consequently, other sources of N, apart from current N fixation, are important for the plant to meet this high N demand (Egli and Crafts-Brandner, 1996). Under water deficit, particularly terminal drought, this problem can be exacerbated.

**Water deficit and nitrogen fixation**

Like photosynthesis, N fixation is sensitive to water deficit (Devries et al., 1989; Hooda et al., 1989; Sinclair and Serraj, 1995; Swaraj et al., 1995). Nitrogenase activity in chickpea subject to water deficit decreases at flowering while in well-watered plants the decline did not start until seed filling (Hooda et al., 1989). This decrease in response to
water deficit is rapid and can occur prior to any visible stress symptoms, such as leaf wilting (Devries et al., 1989). Recently, the activity of sucrose synthase, the principal sucrose hydrolytic enzyme in soybean nodules, has been shown to decline under water deficit (Gonzalez et al., 1995; Gordon et al., 1997). Nitrogenase activity correlates with sucrose synthase activity (Gordon et al., 1997). In chickpea, subject to water deficits during the vegetative and flowering phases, decreased N fixation appears to be related to an inability of the nodules to utilise assimilate rather than a shortage of photosynthate, whereas when the water deficit occurs during seed filling, decreased N fixation is due to photosynthate shortage (Hooda et al., 1989).

The sensitivity of N fixation to drought varies between pulses (Devries et al., 1989; Sinclair and Serraj, 1995). This variation is related to the form in which a particular legume species transports fixed N. Nitrogen fixation in the tropical species which transport N as ureides are much more sensitive to drought, whereas the temperate species which transport N, almost exclusively as amides, are considerably more drought tolerant. Low solubility of ureides may restrict transport from the nodules when xylem flow decreases as a consequence of drought. Increased ureide concentration in the nodules would reduce N fixation (Sinclair and Serraj, 1995).

Drought-induced reductions in N fixation are also related to the permeability of the nodule membrane to oxygen. Oxygen is required by the bacteroids for respiration in order to provide energy for nitrogenase activity (Purcell and Sinclair, 1995) and drought-induced reductions in N fixation are correlated with a decrease in membrane permeability (Pankhurst and Sprent, 1975). Prolonged moisture stress in chickpea results in some breakdown in nodule form (Swaraj et al., 1995). This includes decreases in nodule size as a result of reductions in nodule branching and the extent of nodule invasion into the root. Furthermore, nodule vacuolation is decreased so that nodule cells are more densely packed which could potentially result in reduced O₂ permeability. These structural changes are accompanied by decreases in nitrogenase activity and leghemaglobin content which may be a consequence of premature nodule senescence (Swaraj et al., 1995). It should be noted however, that these observed effects are a consequence of prolonged drought and do not explain reductions in nitrogenase activity in the short term. Exposure of soybean roots to a polyethylene glycol (PEG) solution to simulate drought resulted in a reduced nitrogenase activity prior to any observed reduction in membrane permeability (Purcell and Sinclair, 1995). Further investigation has found that decreases in nodule activity caused by short term drought can be reversed by exposing the nodules to increased O₂ while longer term drought, which causes more substantial decreases in nodule activity, cannot be reversed (Serraj and Sinclair, 1996). This suggests that other components of N fixation may also be affected or that there has been some form of drought-induced damage that cannot simply be reversed by increased
O₂ exposure. Rhizobial death and nodule senescence may accompany longer and more severe periods of drought, due not only to a lack of water, but an inability of the plant to supply the C required for bacterial respiration (Serraj and Sinclair, 1996). Genotypic variation in the response of N fixation to water deficits occurs (Hungria and Neves, 1987; Serraj et al., 1997) indicating that selection for improved N fixation may be possible.

The effect of terminal drought, coupled with high demand and competition for C from filling pods, will lead to earlier and greater reductions in N fixation than the normal decreases in N fixation observed during pod filling in well watered plants. This suggests an increased reliance on N remobilised from vegetative tissues for the completion of pod filling under conditions of terminal drought.

2.3 PLANT ADAPTATION TO WATER DEFICITS

In plants numerous mechanisms are employed which enable the plant to adapt to conditions of low water availability. In this review the framework used to classify the various adaptive mechanisms will follow that used by Turner (1986a,b). According to this framework plant adaptation mechanisms can be classified as either drought avoidance, sometimes called drought escape, dehydration postponement or dehydration tolerance mechanisms.

Drought avoidance

Drought avoidance mechanisms involve either fast phenological development or developmental plasticity. Utilising genotypic variation for fast phenological development in pulses involves selecting genotypes with early flowering, podding and maturity (Turner et al., 1999). In environments where terminal drought is likely, short crop growth duration enables plants to mature prior to the onset of drought (Turner, 1986a,b; Turner, 1996).

In chickpea, genotypic variation for early flowering and podding has been utilised for selection of short-duration genotypes. However chickpea is susceptible to cold temperature stress and successful fertilisation of flowers requires an average daily temperature of 15°C or higher (Lawlor et al., 1998; Srinivasan et al., 1998). In WA the benefits of early flowering are negated with pod set being delayed by cool winter temperatures (Subbarao et al., 1995; Clarke and Siddique, 1998; Leport et al., 1999).

Furthermore, reductions in the duration of crop growth can result in reduced potential seed yield as a consequence of lower biomass production and fewer reproductive nodes (Fischer, 1979, Arnon, 1992). Maximising potential seed yield requires rapid crop growth in order to maximise DM production coupled with increased partitioning to the
seed (Fischer, 1979; Siddique et al., 1998). Biomass accumulation during the vegetative phase is slow in chickpea relative to other pulses such as faba bean and field pea, particularly in environments like southern Australia, where vegetative growth occurs when temperatures are low (Siddique et al., 1993; Thomson and Siddique, 1997; Siddique et al., 1998).

Plants with high developmental plasticity are able to adapt their phenology to the seasonal environment and can respond to both decreases and increases in soil water availability (Subbarao et al., 1995). Indeterminancy in chickpea and other pulse crops gives them a high degree of developmental plasticity. Indeterminate plants in a good season are able to continue growth and production of new reproductive nodes during favourable seasons thereby increasing yields (Turner et al., 1999). In less-favourable seasons continued growth and development is stopped and the crop matures early.

**Dehydration postponement**

Dehydration postponement involves traits which both maximise water uptake and water use efficiency (WUE) in plants already subject to water deficit (Subbarao et al., 1995).

Deep rooting is a common mechanism enabling plants to maximise water uptake by extracting water stored at a greater depth in the soil profile. This can result in increased biomass production and seed yield (Sponchiado et al., 1989) with deep-rooted plants being able to maintain an uninterrupted supply of water for a longer time (Gregory, 1988). Continued root growth during pod filling is particularly beneficial in environments where plants are grown on stored, receding soil moisture (Subbarao et al., 1995). Chickpea has a strong tap root and tends to be deep rooted which is likely to be a beneficial adaptation in many drought-prone environments (Singh, 1997). Identification of chickpea lines with increased rooting depth and improved drought resistance indicates significant potential for this adaptive mechanism to be utilised (Saxena et al., 1994; Singh et al., 1996).

Increased rooting depth, however, will only be of benefit in soils in which roots can grow deep into the soil profile. In Western Australia, rooting depth is often limited in duplex soils where chickpea is commonly grown with roots being unable to penetrate the clay B horizon apart from using sparsely distributed channels left from previous roots (Turner, 1992; Passioura, 1992). Consequently, the depth of soil water extraction is limited by soil characteristics rather than rooting depth (Leport et al., 1999).

Efficient use of water tends to involve shoot characteristics which maximise the biomass or yield gain for every unit of water used. Following germination the ability of a crop to rapidly produce leaf area, thereby minimising evaporation is critical for maximising light interception and WUE (Subbarao et al., 1995). Slow early growth in
chickpea in the South-west of Australia, compared to other pulses, is coupled with a slow development of leaf area taking a longer time to fully intercept the incoming radiation (Mwanamwenge et al. 1997). The canopy architecture and leaf size, shape and reflectance are important in determining the radiation use efficiency (RUE; Subbarao et al., 1995). In chickpea the presence of acid secreting glandular hairs on the leaf surface may increase leaf reflectance and the boundary layer adjacent the leaf (Ehleringer, 1980). Humidifying of this boundary layer through transpiration would lower the vapour pressure deficit resulting in an increase in transpiration efficiency (Farquhar et al., 1989).

Osmotic adjustment, or osmoregulation, is the mechanism by which plants, in response to drought, synthesise and accumulate sugars and other small molecules and ions in plant tissues. This results in a lowering of the osmotic potential and maintenance of turgor (Morgan, 1984; Arnon, 1992). Lowering osmotic potential enables plants to continue to extract water from soils with low water potential (Subbarao et al., 1995) and to maintain turgor, stomatal conductance, photosynthesis and plant growth despite fluctuating soil water potentials (Turner and Jones, 1980; Wright et al., 1983; Ludlow, 1987; Subbarao et al., 1995; Turner, 1996).

Osmotic adjustment can be positively correlated with yield in plants subject to water deficit (Morgan et al., 1986; Blum, 1989; Morgan et al., 1991; Rodriguez-Maribona et al., 1992). Osmotic adjustment improved field pea yields when grown in dry years that were prone to drought (Rodriguez-Maribona et al., 1992). Similarly, in chickpea, seed yield is positively correlated with osmotic adjustment in genotypes grown at drier sites with no benefit at wet sites. Seed yields at dry sites are improved by as much as 20% as a result of osmotic adjustment but there is no correlation between seed yield and osmotic adjustment in field pea or chickpea when mild water deficit is experienced (Rodriguez-Maribona et al., 1992; Morgan et al., 1991).

**Dehydration tolerance**

Plants with a high degree of dehydration tolerance are able to continue metabolism at the cellular level when plant water status is low (Turner et al., 1999). This is achieved through the maintenance of membrane stability and protein function which may occur through the accumulation of compatible solutes such as proline in the cytosol (Stewart and Hanson, 1980; Aspinall and Paleg, 1981).

### 2.4 REMOBILISATION

Inevitably, despite these adaptations to water deficit, plants can still be left with insufficient current assimilate to meet the demands of developing seed. In these circumstances plants generally reduce sink demand by reducing pod number, seed
number and seed size while supplementing assimilate supply through remobilisation. The term remobilisation, as used in this context, refers to the mobilisation of C and N, assimilated prior to reproductive development, stored in the vegetative tissues and redistributed to developing seed. Assimilate remobilisation enables a plant to maintain assimilate supply to seed during periods of low current assimilate availability (Wardlaw, 1990).

Measurements of remobilisation have largely been made in cereal species, primarily wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and maize (*Zea mays* L.). Consequently, much of our understanding with regard to remobilisation was developed in relation to these species. The concept of remobilisation was then extended to pulse species.

**Remobilisation in cereals**

Remobilisation in cereals relies upon the temporary storage of C in water soluble carbohydrates (WSC; Schnyder, 1993). Reserve C is stored primarily as fructans (Winzeler et al., 1990) which can represent up to 85% of the WSC in wheat stem internodes (Blacklow et al., 1984). Storage of WSC in the internodes occurs when the demands of developing sinks have been met and there is excess photosynthate (Schnyder, 1993). Storage is most rapid when the internode has completed its extension growth (Bonnett and Incoll, 1992; 1993a; 1993b). With the progressive growth of the stem the pattern of reserve storage changes. The lower internodes have a considerably longer period over which they can store WSC but the rate at which they are stored is lower than for upper internodes. Consequently lower internodes generally contain reserve C stored in the vegetative phase while the upper internodes tend to have reserve carbon that has accumulated in the reproductive phase (Bonnett and Incoll, 1992). Under good conditions the accumulation of WSC in the stems can continue up to 10-20 days after anthesis (Bonnett and Incoll, 1992; Kühbach and Thome, 1989).

Remobilisation of WSC from the stems to the grain usually starts when grain filling enters its rapid DM accumulation phase. This strong sink demand often coupled with decreased photosynthesis results in a shortfall in photosynthate supply that is met by remobilisation of stem reserves (Spieritz and Ellen, 1978; Blacklow et al., 1984; Kühbach and Thome, 1989). Remobilisation usually results in an increase in the concentration of free fructose in the stem (Blacklow *et al.*, 1984; Kühbach and Thome, 1989; Bonnett and Incoll, 1993b) which suggests that enzymes such as fructan exohydrolase(s) are responsible for fructan degradation (Henson, 1989). Fructan accumulation indicates that the rate of resynthesis of fructose into sucrose and export to the sink is less than the rate of degradation of fructans (Schnyder, 1993).
Grain yield and the contribution of remobilisation to grain yield is highly variable as it is dependent upon both genetic and environmental factors (Table 1.1). Variation in remobilisation ability occurs within species and attempts have been made to determine whether this variation is associated with particular traits. As storage of the WSC predominantly occurs in the stems the relationship between stem height and remobilisation has been examined. Increased stem height confers no remobilisation advantage in some studies (Rawson and Evans, 1971; Austin et al., 1980) but has significant benefit in others (Blum et al., 1997). Genotypic variation does exist and is closely linked with high yield potential and high harvest index (HI; Pheloung and Siddique, 1991). Modern tall and semi-dwarf wheat varieties have higher yields and utilise more of the stem’s non-structural dry matter for seed filling than older tall cultivars (Pheloung and Siddique, 1991).

Drought tolerance is also related to increased storage of C reserves available for remobilisation (Nicolas et al., 1985a). Hence the ability of genotypes to partition more DM to the seed by measuring HI, has been examined as a possible trait for better remobilisation ability (Gent and Kiyomoto, 1989). Harvest index is correlated with more efficient C partitioning during grain filling (Gent and Kiyomoto, 1989) but it is not consistently related with a better ability to remobilise DM (Papakosta and Gagianas, 1991).

Remobilisation is related to the source to sink ratio with reductions in source tending to increase remobilisation (Reed et al., 1988; Uhart and Andrade, 1995) while sink reductions can result in greater accumulation of carbohydrates in the stem and lower remobilisation (Uhart and Andrade, 1995). Furthermore, any stress such as water deficit which reduces the plant’s ability to meet the sink demand with current photosynthate can result in increased dependency on remobilisation (Hossain et al., 1990; Pheloung and Siddique, 1991).
Table 2.1  Reported contributions (%) of remobilised carbon (C), dry matter (DM) and/or nitrogen (N) to seed in cereals and pulses grown under a range of conditions

<table>
<thead>
<tr>
<th>Species</th>
<th>% Contribution</th>
<th>Growing conditions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seed DM</td>
<td>Seed N</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Irrigated, field</td>
<td>Bidinger et al., 1977</td>
</tr>
<tr>
<td>Barley</td>
<td>17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Droughted, field</td>
<td>Bidinger et al., 1977</td>
</tr>
<tr>
<td>Barley</td>
<td>44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Hot, dry year</td>
<td>Austin et al., 1979</td>
</tr>
<tr>
<td>Barley</td>
<td>11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cool, wet year</td>
<td>Austin et al., 1979</td>
</tr>
<tr>
<td>Wheat</td>
<td>2.7-12.2</td>
<td>Well-watered, pots</td>
<td>Rawson &amp; Evans, 1971</td>
</tr>
<tr>
<td>Wheat</td>
<td>13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Irrigated, field</td>
<td>Bidinger et al., 1977</td>
</tr>
<tr>
<td>Wheat</td>
<td>27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Droughted, field</td>
<td>Bidinger et al., 1977</td>
</tr>
<tr>
<td>Wheat</td>
<td>21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Well-watered, field</td>
<td>Borrell et al., 1989</td>
</tr>
<tr>
<td>Wheat</td>
<td>6-73&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Winter, field</td>
<td>Papakosta &amp; Gagianas, 1991</td>
</tr>
<tr>
<td>Maize</td>
<td>4.7</td>
<td>46.5</td>
<td>Irrigated field</td>
</tr>
<tr>
<td>Wheat</td>
<td>64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Rapid drought, pots</td>
<td>Palta et al., 1994</td>
</tr>
<tr>
<td>Wheat</td>
<td>36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Slow drought, pots</td>
<td>Palta et al., 1994</td>
</tr>
<tr>
<td>Wheat</td>
<td>60</td>
<td>Irrigated pots</td>
<td>Nicolas et al., 1985</td>
</tr>
<tr>
<td>Wheat</td>
<td>70</td>
<td>Droughted pots</td>
<td>Nicolas et al., 1985</td>
</tr>
<tr>
<td>Wheat</td>
<td>47-73</td>
<td>Well-watered, field</td>
<td>Takahashi et al., 1996</td>
</tr>
<tr>
<td>Lupin</td>
<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Field, rainfed</td>
<td>Pate et al., 1980</td>
</tr>
<tr>
<td>Cowpea</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Well-watered, pots</td>
<td>Pate et al., 1983</td>
</tr>
<tr>
<td>Soybean</td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Pots</td>
<td>Yamagata et al., 1987</td>
</tr>
<tr>
<td>Common Bean</td>
<td>9</td>
<td>Well-watered</td>
<td>Geiger &amp; Shieh, 1988</td>
</tr>
<tr>
<td>Mungbean</td>
<td>11</td>
<td>Released cultivar</td>
<td>Bushby &amp; Lawn, 1992</td>
</tr>
<tr>
<td>Mungbean</td>
<td>42</td>
<td>Landrace line</td>
<td>Bushby &amp; Lawn, 1992</td>
</tr>
<tr>
<td>Mungbean</td>
<td>10</td>
<td>Wild Accession</td>
<td>Bushby &amp; Lawn, 1992</td>
</tr>
<tr>
<td>Cowpea</td>
<td>60</td>
<td>Well-watered, pots</td>
<td>Peoples et al., 1983</td>
</tr>
<tr>
<td>Chickpea</td>
<td>81</td>
<td>Rainfed, field</td>
<td>Kurdali, 1996</td>
</tr>
<tr>
<td>Soybean</td>
<td>55-60</td>
<td>Irrigated, pots</td>
<td>Warembourg et al., 1982</td>
</tr>
<tr>
<td>Lentil</td>
<td>43-94</td>
<td>Rainfed cultivars</td>
<td>Kurdali et al., 1997</td>
</tr>
<tr>
<td>Common Bean</td>
<td>39</td>
<td>Rainfed, field</td>
<td>Foster et al., 1995</td>
</tr>
<tr>
<td>Common Bean</td>
<td>73</td>
<td>Droughted, field</td>
<td>Foster et al., 1995</td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of seed C  
<sup>b</sup> Pre-anthesis DM or C  
<sup>c</sup> Percentage of ear dry weight  
<sup>d</sup> Post-anthesis DM

Remobilisation in pulses

In chickpea it has been estimated that over the season about 15% of the assimilates produced prior to pod initiation is translocated to the pods (Singh, 1991), with DW changes indicating that remobilisation contributes about one third of the pod DW or 20% of seed DW (Saxena, 1984; Khanna-Chopra and Sinha, 1987). However, this estimate is high compared to other pulses such as lupin, where an estimated 2% of the pods C came from remobilisation in association with N used for protein synthesis (Pate et al., 1980). In pulse species leaves and pod walls tend to lose more DW than the stems suggesting that leaves are a more important source of remobilised assimilates compared to cereal species (Flinn and Pate, 1970; Dure, 1975; Singh, 1991; Rao et al., 1984; Geiger and Shieh, 1988).
Carbon remobilised from leaves is primarily derived from the breakdown of leaf starch (Hammond and Burton, 1983; Fader and Koller, 1983; de Veau et al., 1992). Soybean leaf starch accumulates predominantly within the chloroplasts of the inner layers of palisade parenchyma tissue (de Veau et al., 1992). Starch accumulates when leaf photosynthesis is high so that sucrose production exceeds sucrose export from the leaves. Diurnal changes are particularly important with starch accumulation occurring during the day with subsequent degradation at night which enables sucrose export to be maintained (Hammond and Burton, 1983). Similarly, throughout plant development increased carbon fixation rates can result in both increased starch accumulation and sucrose export (Fader and Koller, 1983; Hammond and Burton, 1983). In soybean, leaf starch concentration and sucrose export increased in pod bearing plants as a result of increased CO₂ exchange rates (CER). Cultivar variation in soybean leaf starch accumulation and concentration enables cultivars with high leaf starch concentration to maintain high sucrose export rates when carbohydrate production rates are low (Fader and Koller, 1983). This implies that selection for improved C remobilisation in chickpea could be achieved by selecting for increased leaf starch accumulation during early plant development. This needs to be coupled with increased photosynthesis in order to maximise starch accumulation whilst maintaining sucrose export and DM production.

Like soybean CER in chickpea peaks during the late vegetative and maximum pod filling phases of plant development and significant genotypic variation in CER exists in chickpea. High CER is positively correlated with specific leaf mass (SLM) and leaf N content per unit area (Mythili and Nair, 1996). This implies that thicker leaves contain more photosynthetic proteins and increased photosynthetic capacity. However, the relationship between SLM and CER was not obligatory and high CER was not always associated with high DM production (Mythili and Nair, 1996).

N is also remobilised from vegetative to reproductive parts. In soybean, 85% of the total fixed N is found in reproductive parts at maturity suggesting considerable remobilisation occurs from vegetative parts (Warembourg et al., 1982). So despite the ability of leguminous plants to fix atmospheric N, they remain heavily dependent on remobilised N (Table 1.1). Estimated remobilisation of N is much greater than C in all pulses. This dependence is due to the high N requirement of pulse seeds for protein (Sinclair and De Wit, 1975) and substantial decreases in N fixation common during seed filling (Herridge and Pate, 1977; Hooda et al., 1986; Kurdali, 1996).

In pulses most of the remobilised seed N is derived from leaves and is closely related to plant senescence with translocation of N, in particular, into the developing seed (Peoples et al., 1983; Egli and Crafts-Brandner, 1996). Remobilised N is predominantly derived from the breakdown of photosynthetic proteins (Feller and Fischer, 1994; Crafts-Brandner et al., 1998) including chlorophyll, which results in chlorosis of the
photosynthetic organs (Egli and Crafts-Brandner, 1996; David et al., 1998). In chickpea, leaf senescence is linked to pod development and removal or failure to set pods delays leaf senescence (Saxena, 1984). Consequently, as pods set and develop acropetally along a branch, older leaves, near the base of the branches, senesce first with senescence continuing acropetally up the branch. During senescence leaflets abscise from the petiole which remains attached to the stem. Senescence may be regulated by a hormonal signal produced in the seed and transported to the leaves (Noodén and Guiamét, 1989) or due to high assimilate demand by the developing seeds which leaves insufficient assimilate for leaf maintenance and respiration (Egli and Crafts-Brandner, 1996).

Intraspecific variation in N remobilisation occurs (Kurdali et al., 1997; Sanetra et al., 1998) which can be related to seed filling duration (Vasilas et al., 1995). Genotypes with short seed-filling periods remobilise more N than those with long seed-fill periods. This has no correlation with seed yield (Vasilas et al., 1995) but may be due to a faster decline in N fixation once rapid seed filling has begun with more assimilate diverted away from nodule maintenance towards pod filling (Kurdali, 1996).

In pulses, more than half of the total N can be fixed in the vegetative phase (Rao et al., 1984; Herridge and Pate, 1977). In chickpea, N fixation peaks between flower bud initiation and maximum flowering, so that by early seed filling 81% of the total N was fixed (Hooda et al., 1986; Kurdali, 1996). In cowpea, N fixed after anthesis contributed 40% of N in pods, whilst 60% was derived from mobilisation of N fixed prior to anthesis (Peoples et al., 1983). In adequately watered chickpea remobilised N from the roots, leaves and nodules accounted for 21, 31 and 12% of the seed N. However, these estimates do not take into account the N lost to the soil from senescing nodules (Hooda et al. 1986). The HI for N is nearly double the HI for C, in chickpea pointing to the efficient remobilisation of N. However, in well-watered plants most of the seed DM is derived from current photosynthesis rather than being mobilised from vegetative parts and there is no decrease in shoot DW and only minor decreases in root DW (Hooda et al., 1986). In rainfed chickpea mobilisation of N from the roots and shoots accounts for 81% of the N in the pods (Kurdali, 1996).
Water deficit and remobilisation

Water deficits increase a plant’s dependency on remobilisation for grain filling (Rawson and Evans, 1971; Bidinger et al., 1977; Austin et al., 1980; Nicolas et al., 1985; Palta et al., 1994). In barley pre-anthesis assimilates contribute 44% to the grain dry matter in a hot, dry year compared to just 11% in a cool, wet year (Austin et al., 1980). This increase under water deficit, however, may not be the result of more reserves remobilised in absolute terms but an increase in the proportion of reserve C in the grain (Rawson et al., 1977; Palta et al., 1994).

The rate of water deficit development and its timing in relation to plant development is critical in determining the importance of remobilisation to seed filling (Palta et al., 1994; Pheloung and Siddique, 1991). When the rate of water deficit development is slow, remobilised DM makes less of a contribution to grain DM than when water deficit development is rapid (Palta et al., 1994). Remobilisation is also increased when there is more filling seed resulting in a lower source:sink ratio (Schonbeck et al., 1986; Griffith, 1992; Munier-Jolain et al., 1996). This has implications for terminal drought in chickpea which usually occurs during seed filling when the number of reproductive units are already largely determined resulting in a low source:sink ratio.

Terminal drought increases a plant’s dependency on remobilised C. In field grown lupins, water deficit decreases stem DW during pod filling which implies remobilisation (French and Turner, 1991). Stem DW decreases occur predominantly in the higher order apical branches which appear to be a source of remobilised assimilates for filling seed on the dominant main stem (French and Turner, 1991; Dracup and Kirby, 1996b) which indicates that plant architecture can be an important factor in remobilisation. In chickpea, there is little translocation of \(^{14}\)C label from individually \(^{14}\)CO\(_2\) labelled branches to other branches (Singh and Pandey, 1980). This implies that in chickpea there is no order of priority between branches. Remobilisation is also related to sink size and plants with high seed numbers remobilise more DM from the vegetative parts than those with fewer seed (Andriani et al., 1991).

Similarly, water deficit reduces N fixation resulting in N remobilisation becoming more important in plants subject to water deficit compared to well-watered plants (Devries et al., 1989; Sinclair and Serraj, 1995; Swaraj et al., 1995; Purcell and Sinclair, 1995; Serraj and Sinclair, 1996; Hooda et al., 1989; Hooda et al., 1990). In common bean, with adequate water, 27% of the seed N was remobilised from the leaves and 12% from the stem while in plants subject to water deficit 55 and 18% is remobilised from the leaves and stems, respectively. However, under severe water deficit, N remobilisation can decrease (Foster et al., 1995) due to large reductions in sink size and the possible...
inhibition of N transport in the plant. The contribution of remobilised N to seed N appears to be more dependent upon the amount of N available for remobilisation at the onset of seed filling than on the availability of soil N or N fixation during seed filling (Egli et al., 1983, Vasilas and Fuhrmann, 1993; Vasilas et al., 1995).

Measuring remobilisation

While DW decreases of vegetative plant components during seed filling can be used as indicators of C remobilisation, the technique is limited due to DW losses caused by respiration. Thus, if respiration is not taken into account, determining remobilisation using DW losses results in an overestimation of the contribution by remobilisation. Other methods have been developed which enable the contribution of remobilisation to be quantified and the importance of particular plant organs as sites of assimilate storage determined. These methods utilise isotopes of C ($^{13}\text{C}$, $^{14}\text{C}$) and N ($^{15}\text{N}$) to label C and N pools within the plant and then track the subsequent movement of these assimilates (Warembourg et al., 1982; Palta et al., 1994; Deléens et al., 1994).

2.5 POD SET IN PULSES

Pod number in most pulses, including chickpea, represents only a small proportion of the number of flowers (Subhadrabandhu et al., 1978; Downes and Gladstones, 1984; Duc et al., 1994; Zaiter and Barakat, 1995). This is due to the failure to form pods and the high degree of pod abortion in early pod set (Srivastava et al., 1996). Additionally, the production of new flowers can be modified. However, this and the indeterminate nature of growth gives chickpea a high degree of plasticity in the ability to modify pod and seed set.

Pod set failure can be due to failure of fertilisation, temperature extremes, assimilate competition and hormonal factors. Infertile pollen and pollen incompatibility can cause fertilisation failure (Duc et al., 1994) and, in addition, failure of pollen tube growth at low temperatures affects chickpea (Lawlor et al., 1998). In southern Australia chickpea flowers in winter but pod set is delayed due to the sensitivity of fertilisation to cold temperature. High temperatures can also make pollen infertile (Duc et al., 1994) and suppress floral bud development and pod set (Downes and Gladstones, 1984; Ahmed and Hall, 1993).

In pulses, seed abortion is highest in the later formed pods (Subhadrabandhu et al., 1978; Tamas et al., 1979; Heitholt et al., 1986a; Mauk et al., 1987) which may be due to competition for assimilates or hormonal regulation. In a Mediterranean-type environment high temperatures or water deficits late in the season may result in increased pod and seed abortion of later-set pods, an interaction between abiotic stress and the timing of pod/seed development.
In pulses, including chickpea, the critical stage for seed abortion has been observed and defined (Duthion and Pigeaire, 1991; Turc et al., 1994; Ney et al., 1994; Genter et al., 1997). The critical stage occurs during rapid cell division prior to the cell expansion, assimilate storage phase. In pulses measurements of seed length characterise this stage due to its correspondence with cotyledon elongation (Duthion and Pigeaire, 1991; Ney et al., 1994). It is also known as the final stage in seed abortion as it represents the point beyond which seed abortion is unlikely to occur (Pigeaire et al., 1986). Termination of seed abortion appears to be correlated to the end of cell division and may be associated with carbohydrate metabolism (Schussler and Westgate, 1991a; Zinselmeier et al., 1995a,b,c) but the concentration of soluble carbohydrate in fully open flowers and undeveloped pods is not related to the likelihood of abortion (Heitholt et al., 1986b).

Fewer abortions occur in low plant density than high plant density. This was node dependent and is most likely related to light interception (Mauk et al., 1987). Supplemental light reduced flower and pod abscission and shading of both flowers and pods increased abscission. Light appears to have a role in regulating flower and pod abscission as well as having an influence on their ability to attract and utilise photosynthate.

Water deficits cause decreases in pod and seed number (Palta and Ludwig, 1996; Ney et al., 1994) and flower and seed abortion is high in chickpea in Australia where terminal drought is common (Turner, 1986a, b), the effects of which can be reduced by irrigation (Mauk et al., 1987). Increased rates of pod and seed abortion in plants subject to water deficits may be a consequence of either insufficient assimilate supply to the aborting pods and seed or some hormonal signal which increases pod and seed abortion when its production is stimulated by water deficit.

2.6 POD AND SEED DEVELOPMENT

A seed is a storage sink, the primary function of which is to store imported assimilates as sugar, starch, proteins and oils (Herbers and Sonnewald, 1998) which can be mobilised during seed germination. Upon establishment of a seed, the competitive ability of that seed, the sink strength, is determined by the seed’s intrinsic ability to attract and utilise assimilates. Potential sink strength is determined by physical and physiological constraints and is genetically determined. Potential sink strength is expressed when there is no source limitation and the sink has peaked in metabolic activity with optimum environmental conditions (Ho, 1988).

Following assimilate movement from the source, via the vascular system, the subsequent utilisation of assimilate by a sink usually involves a number of steps which can serve to regulate or constrain sink activity. Following assimilate unloading from the
phloem and transport to the sink cells, subsequent uptake by the sink cells often involves some initial metabolism. Having taken up the assimilate it then becomes available for the maintenance of growth, development and/or storage (Herbers and Sonnewald, 1998). Consequently, the physiological constraint on sink strength is determined by numerous factors associated with the efficiency of assimilate transport and utilisation.

Seed morphology has implications for development, particularly in relation to assimilate import which largely regulates seed growth (Weber et al., 1997). Pulses develop seed within pods, however, the number of seeds contained within a pod, thickness of the pod wall and volume of air surrounding the seed vary (Flinn et al., 1977; Dracup and Kirby, 1996b). Chickpea pods have one to two seeds per pod, which are surrounded by a relatively large volume of air for most of their development and the pod wall tends to be thin and leathery (Cubero, 1987). In contrast lupin pods can contain up to five seeds in separate compartments, throughout much of development the pod wall is thick and succulent and the volume of air around the seed is low (Dracup and Kirby, 1996b).

Early in legume pod development the pod wall grows more rapidly than the seed such that pod wall DW is almost at its maximum by the time seed filling begins (Pate and Flinn, 1977; Clarke and Siddique, 1998). Legume pod walls are photosynthetic although the photosynthetic efficiency varies considerably (Sinha and Sane, 1976; Atkins et al., 1977; Sambo et al., 1977; Flinn et al., 1977; Singh, 1987).

Within legume pods seed is attached to the pod wall via the funiculus which contains both phloem and xylem responsible for transporting assimilates and water to seed. In pulses several types of seed coat vascular distribution exist (Patrick and Offler, 1995). However, regardless of the type of vascularisation, assimilate is delivered to the entire surface of the cotyledons, although possibly at uneven rates (Patrick and McDonald 1980; Thorne, 1980; Offler et al., 1989; Patrick and Offler, 1995). The seed coat vascular bundles are capable of providing a greater carrying capacity than the maximum capacity required (Offler et al., 1989) which indicates that transport of assimilates within the seed coat does not limit seed growth.

Seed development in pulses is characterised by developmental changes in the embryo, consisting of a cell division phase, followed by a cell expansion and filling phase which incorporates maturation (Dure, 1975; Smith, 1984).

Cell division is a phase of high metabolic activity despite the fact that during this time there is little increase in seed DM (Dure, 1975). During this lag phase, 80% of the final embryo cell number is formed (Duc et al., 1994) and the seed consists primarily of undifferentiated, rapidly dividing liquid endosperm cells. Cell division continues and differentiation and cotyledon development begins (Walbot et al., 1972). Cotyledons
lengthen, but only 15% of the seed DW is accumulated before the storage phase begins (Duc et al., 1994; Smith and Denyer, 1992).

The storage phase is characterised by the rapid accumulation of seed DM which is a consequence of the synthesis and storage of starch, proteins and lipids (Ambrose et al., 1987). Cotyledon cell expansion results in rapid growth and consumption of the endosperm as the cotyledons fill the seed coat. Seed carbohydrate content increases throughout seed development due to starch synthesis and storage (Singh et al., 1981) but the starch content and DW of the pod wall decreases (Fountain et al., 1989; Rochat and Boutin, 1989). Later, during pod senescence, non-structural pod wall N is remobilised and accumulates in the developing seed (Rochat and Boutin, 1989). Continued storage and accumulation of starch, protein and lipid is associated with a decrease in the seed water content ultimately resulting in desiccation and maturation (Dure, 1975; Singh et al., 1981; Pate, 1984).

Assimilate transport to the developing embryo

Assimilate supply may regulate the rate of seed growth but assimilate utilisation by the seed can also modify the rate of seed growth. Assimilates move to the seed through the phloem. All assimilate entering the seed must first come via the funiculus and be unloaded into the seed coat (Thorne, 1981; Wang and Hedley, 1993). Between the maternal seed coat and the embryo there are no vascular or symplastic connections and assimilate from the seed coat must pass through the apoplast into the embryo (Wang and Hedley, 1993, Weber et al., 1997; Patrick and Offler, 1995).

The apoplast is effectively a small pool through which all assimilate destined for the seed must pass (Patrick and Offler, 1995). Changes in the rate of assimilate uptake by the cotyledons have an immediate impact on the concentration of assimilates in the apoplast pool which alters the osmotic potential of the apoplast solution and the seed coat turgor (Patrick, 1990; Patrick, 1994b; Wolswinkel, 1990; Patrick, 1993a; Thorpe et al., 1993). Seed coat turgor is determined by difference between the osmotic potentials of the seed coat and apoplast and any change in the osmotic potential of either will effect seed coat turgor and the rate of assimilate unloading into the apoplast (Patrick, 1994b).

This implies that a homeostatic mechanism is in operation which matches the rate of assimilate utilisation by the embryo to the rate of assimilate efflux by the seed coat maintaining a constant turgor pressure in the seed coat (Patrick, 1993b). Sucrose is the principal carbohydrate imported into the embryo (Weber et al., 1997) and the rate of influx increases with concentration of assimilates in the apoplast (Patrick, 1994b).
Carbohydrate metabolism in the developing seed

Sucrose imported into the embryo must be hydrolysed before further metabolism. This cleavage reaction can be catalysed by a number of enzymes including invertase, sucrose synthase or sucrose phosphate synthase (Quick and Schaffer, 1996; Weber et al., 1997).

Invertase appears to be related to early seed development. In maize, early seed growth is inhibited in the mn-1 mutant which lacks the invertase enzyme (Cheng et al., 1996). In the parenchyma cells of the seed coat responsible for assimilate unloading, a cell wall-bound invertase gene is expressed in the cell division phase (Weber et al., 1995). High invertase activity has been shown to be associated with early seed growth in rice, sorghum, canola, pea and faba bean (Kato, 1995; Patel and Mohapatra, 1996; Maness and McBee, 1986; Déjardin et al., 1997; Weber et al., 1995; King et al., 1998) and with high apoplastic glucose and fructose concentrations (Déjardin et al., 1997). In faba bean cotyledons high invertase activity is associated with high hexose concentration (Weber et al., 1995) and consequently, cell division is related to a high hexose to sucrose ratio (Weber et al., 1997). Hydrolysis of sucrose in the seed coat by extra-cellular invertase in the apoplast may facilitate seed coat unloading of sucrose and provide hexose sugars for cell division (Weber et al., 1995).

The transition from cell division to the cell expansion phase is characterised by a rapid increase in seed DW and sucrose synthase activity (Quick and Schaffer, 1996). Sucrose synthase and to some extent alkaline invertase, catalyzes the reaction in which sucrose and uridine diphosphate (UDP) is cleaved to form fructose and UDP-glucose, which are then used in starch synthesis (Quick and Schaffer, 1996). The rate of seed filling and seed size is positively correlated with sucrose synthase activity (Kato, 1995; Sung et al., 1994; Patel and Mohapatra, 1996; Lowell and Kuo, 1989).

Increased sucrose concentration is correlated with increased sucrose synthase activity and starch content in the cotyledons (Sung et al., 1994; Ross et al., 1996). The affinity of sucrose synthase for sucrose is low (Ross and Davies, 1992) and so the high hexose to sucrose ratio in the cell division phase inhibits sucrose synthase activity. To achieve the high sucrose synthase activity associated with the storage phase there is a decrease in the hexose to sucrose ratio. Sucrose-phosphate synthase activity can be induced by hexose sugars in vitro, and high activity of this enzyme increases the sucrose concentration. Furthermore, decreased invertase activity, possibly as a consequence of the cotyledons filling the seed coat and physically preventing wall-bound invertase from operating, will lower the hexose concentration (Weber et al., 1996; Weber et al., 1997). The developmental switch from cell division to the storage phase may be a consequence of decreases in the hexose to sucrose ratio and changes in carbohydrate metabolism in the seed may be involved in the regulation of its development (Weber et al., 1997).
The formation of protein

After carbohydrates, protein is the second largest component and up to a quarter of pulse seed DW can be protein. This protein is made up of globulin, a water insoluble storage protein and albumin which is water soluble (Casey et al., 1993). Globulin is synthesised throughout seed development and stored in organelles called protein bodies. During seed germination these protein bodies are hydrolysed releasing C skeletons and N for the developing seedling. Accumulation of storage protein starts early in the expansion of the cotyledon (Wang and Hedley, 1993) but protein deposition does not begin until after the start of starch deposition (Wang and Hedley, 1993).

In early seed development the free amino acids represent temporary reserve materials that are stored in the endosperm. Later in development, free amino acids are translocated to the apoplast of the seed coat and the cotyledons where they are used for protein synthesis. As a consequence soluble N decreases throughout seed development and protein N increases (Singh et al., 1981; Rochat and Boutin, 1989).

Initially the pod wall is the dominant site for the processing and incorporation of N, but as the pod develops the activity of the seed coat and embryo in processing the nitrogenous compounds increases. Ureides tend to be metabolised primarily in the pod and seed coat, while amides are metabolised in all pod tissues but predominantly in the embryo (Peoples et al., 1985). In chickpea seed the proportion of amides decreases from 14 DAA to 28 DAA as it is converted to protein N which increases up to 35 DAA. The principal amino acids which compose the seed proteins of chickpea are: glutamic acid, aspartic acid, leucine, lysine, arginine and phenylalanine (Singh et al., 1981).

Embryo cell number and cell size

In seeds, embryo cell number can be used as a measure of the physical constraint on seed size (Ho, 1988). Differences in final seed weight are related to embryo cell number which determines the rate of seed growth (Egli et al., 1981, Guldan and Brun, 1985). Embryo cell number is under genetic control but can be affected by environmental factors that influence the supply of assimilates to the cotyledonal cells during cell division (Ho, 1988). Increased assimilate supply results in more cells while decreased supply due to water deficit, results in fewer cells, starch granules and smaller seed (Egli et al., 1989; Nicolas et al., 1984; Nicolas et al., 1985a; Ober et al., 1991). Environmental modification of cell number may also be in response to changes in carbohydrate metabolism and may lead to early seed abortion. For example, water deficit early in seed development may effect invertase activity and hence, cell number, which would ultimately effect the final size of the seed. Genotypic differences in seed size are largely due to differences in cotyledon cell number, while variation in seed size within a genotype is due to variation in the size of the cotyledonal cells (Hirshfield et
al., 1993).

**Hormones and seed growth**

The role of hormones in seed growth in contrast to their involvement in maturation is equivocal but ABA, cytokinins and gibberellins have all been implicated. Cytokinins induce cell division (Fosket *et al.*, 1977) and the concentration of cytokinins in developing pulse seed is highest during cell division (Davey and van Staden, 1977; Van Staden, 1983). The increase in cytokinin concentration begins after fertilisation (Davey and van Staden, 1977) and peaks when the endosperm volume is at its maximum (Burrows and Carr, 1970; Davey and Van Staden, 1979). Pod set in lupin is increased and pod abortion reduced by the application of exogenous cytokinins (Atkins and Pigeaire, 1993; Palta and Ludwig, 1996). As completion of cell division is considered to be a pre-requisite for continued pod and seed development, the enhancement of cell division by cytokinins will enable pod and seed set to occur.

Cytokinins induce cell wall invertase and enhance glucose and sucrose uptake via hexose monomers (Ehness and Rotisch, 1997). Activation of invertase and a hexose transporter correlates well with the high hexose:sucrose ratio during cell division (Weber *et al.*, 1997). Decreasing cytokinin concentrations may result in lower activities of cell-wall bound invertase and hexose transporter resulting in decreases in the hexose:sucrose ratio and for the switch to the storage phase of seed development. However, while the concentration of cytokinins in developing seeds decrease during the storage phase (Van Staden, 1983), they may indirectly enhance seed filling by increasing cell division in early seed development (Michael and Beringer, 1980), thereby increasing sink strength.

Increased ABA accumulation during water deficit correlates with decreased cell division in seeds during early development (Ober *et al.*, 1991) but it does not provide any evidence that ABA is the causal agent. ABA may be involved in the regulation of carbohydrate metabolism and therefore act indirectly. Removal of older pods decreases the abortion rate in young pods which is coupled with lower ABA concentrations compared to the concentration in pods of the same age on intact plants. Older pods have lower ABA concentrations than younger pods but the ABA concentration in aborting pods varied widely (Tamas *et al.*, 1979). When pods are removed it becomes difficult to separate assimilate supply and ABA factors as pod removal will also affect assimilate distribution and the source:sink ratio.

In soybean, the highest concentration of ABA is found in the embryonic axis and seed coat (Hein *et al.*, 1984) and generally peaks during the time of rapid seed filling (Brenner and Cheikh, 1995). ABA does move from the site of production in the leaves
to the seeds (Dewdney and McWha, 1979; Setter et al., 1981) and in soybean depodding prevents ABA movement from leaves which subsequently accumulate ABA resulting in stomatal closure (Setter et al., 1981). This implies that ABA translocation from the leaves to the developing seeds is necessary to continue leaf photosynthesis. Subjecting soybean plants to drought results in increased concentrations of ABA in the leaves which is translocated to the seeds following rewatering. Increased ABA concentration in seed can enhance sucrose uptake compared to cotyledons from seeds on well watered plants (Brenner and Cheikh, 1995) but promotion of sucrose uptake by excised soybean cotyledons in response to exogenous ABA is genotype specific (Schussler et al., 1984). However, whether or not high ABA contents are simply the result of higher growth rates and increased accumulation of solutes, including ABA, is not known. In ABA-deficient and ABA-insensitive, pea and *Arabidopsis* mutants, there is no significant decrease in assimilate uptake (de Bruijn and Vreugdenhill, 1992), indicating that ABA is not obligatory for seed filling.

Other hormones such as gibberellin and auxin (Swain et al., 1993; Kuiper, 1993) may also be important in developing seeds but are beyond the scope of this thesis.

**Rate and duration of seed growth**

Final seed size is a product of the rate and duration of seed growth (Westgate and Thomson-Grant, 1989b). Seed growth rate is usually positively correlated with final seed size (Egli et al., 1978; Egli, 1981; Egli et al., 1981). However, this relationship is not causal as differences in seed size can occur despite similar seed growth rates (Gbikpi and Crookston, 1981; Guldan and Brun, 1985; Hanson, 1986; Swank et al., 1987). Genotypic differences in seed growth rate are correlated with both the number and volume of cotyledonary cells (Egli, 1981; Egli et al., 1981; Guldan and Brun, 1985; Sexton et al., 1997). Increased seed growth rate due to more or larger cells is primarily a result of increased surface area over which assimilates can be transferred. However, there is no difference in DM accumulation rate per unit seed coat area and per unit seed DW in genotypes with varying seed size when assimilate supply is not limiting (Hanson, 1986; Hanson and Burton, 1994).

Assimilate supply can affect seed growth rate indirectly via changes in cotyledon cell number during the cell division phase or directly via cell expansion and DM accumulation during the phase of rapid DM accumulation (Nicolas et al., 1984; Egli et al., 1985; Egli et al., 1989). Seed growth rate can respond to changes in source:sink ratio. Increased source:sink ratio results in faster rates of seed DM accumulation (Egli et al., 1985; Fader and Koller, 1985) and reductions in seed size caused by water deficit can be partially reversed by increasing the source:sink ratio (De Souza et al., 1997). Within a genotype, increased seed size as a result of improved nutrient availability
during reproductive growth is a result of larger rather than more cotyledonary cells (Hirshfield et al., 1993).

The duration of seed filling also influences final seed size (Egli et al., 1984). Seed filling duration is closely related to plant senescence which is largely determined by N remobilisation (Hayati et al., 1995; Vasilas et al., 1995; Munier-Jolain et al., 1996). Seed filling generally ends when N availability from remobilisation is exhausted, although when source:sink ratio is very high seed filling ends when the seeds reach their maximal size (Munier-Jolain et al., 1996). Reduced N availability reduces the duration of seed fill by increasing the requirement for remobilised N from the vegetative tissues thereby hastening senescence (Egli et al., 1981; Hayati et al., 1995). Similarly, water stress during seed filling accelerates plant senescence and shortens the duration of seedfill (Muchow, 1985; Muchow et al., 1986; Desclaux and Roumet, 1996; De Souza et al., 1997). Reduced seed size as a consequence of water deficit appears to be predominantly a result of decreased seed growth duration rather than seed growth rate (Eck et al., 1987; Westgate et al., 1989a,b) though long term water deficits may also reduce seed growth rate (Westgate et al., 1989b).

Within a plant the rate of DM and protein accumulation can be higher for late maturing seeds (Gbikpi and Crookston, 1981) or can be relatively constant (Egli et al., 1978), while the duration of seed filling tends to be reduced (Egli et al., 1978; Spaeth and Sinclair, 1984a).

2.7 WATER DEFICIT AND SEED GROWTH

In maize, water deficit decreased kernel set (Schussler and Westgate, 1991a,b) despite the ovary being buffered from water deficit. DM accumulation by the ovary is inhibited but the levels of sucrose and glucose increases in the ovaries compared to those on well-watered plants. Water deficit appears to disrupt carbohydrate metabolism and the ability of ovaries to convert soluble sugars to starch (Schussler and Westgate, 1991b; Zinselmeier et al., 1995a,c). Furthermore, inhibition of starch synthesis as indicated by the accumulation of soluble sugars, decreases sink strength and reduces assimilate flux to the developing ovary (Schussler and Westgate, 1995). In spite of sugar metabolism being altered, supplementary stem infusion of sugars, in particular sucrose, restored kernel set and growth in plants subject to water deficit. Starch synthesis, however, remained low (Zinselmeier et al., 1995c).

A decrease in water potential may directly reduce seed expansion, however, the seed growth rate can be maintained under water deficit (Westgate and Thomson-Grant, 1989a, b). The water status of developing seed is independent of the water status of maternal plant tissues. In plants exposed to severe water deficit, soybean seed is able to
maintain seed water potential in spite of low leaf water potential (Westgate and Thomson-Grant, 1989a, b) but this only occurs when the seed is in the storage phase and seed abortion is unlikely (Duthion and Pigeaire, 1991; Ney et al., 1993).

2.8 CONCLUSION

The Mediterranean-type environment of south-west Australia is characterised by having hot, dry summers and cool, wet winters (Sedgley et al., 1990; Turner, 1992). The growing season can extend from April to October (Rovira, 1992) and is determined by the opening rains of the season in autumn and the last rains in spring, which are associated with high temperatures and terminal drought (Loss and Siddique, 1994). While annual rainfall can range from 300 up to 600 mm, only 60-70% falls in the growing season (Rovira, 1992). Consequently, the pattern of rainfall distribution throughout a year is important (Mwanamwenge et al., 1997) as even in seasons with above average rainfall, low rainfall in September and October can still result in crops experiencing substantial terminal drought (Siddique et al., 1996). In these environments grain filling commonly occurs under increasing soil water deficit (Turner and Nicolas, 1987) as evaporative demand greatly exceeds rainfall near the end of the season (Hamblin et al., 1986; Palta and Fillery, 1995). Water deficit reduces source size through reductions in leaf area and the rate of net photosynthesis. This source reduction commonly occurs in spring with the onset of terminal drought and corresponds to seed filling in chickpea (Siddique and Sedgley, 1985). Subsequently, in this environment drought also reduces sink size through decreasing the rate of flower and pod production and increasing the extent of flower and seed abortion. Seed quality can also be reduced through reductions in seed size which is an important quality parameter for overseas markets. A short fall in assimilate supply as a consequence of drought when demand due to seed filling is high can be partially compensated for by the remobilisation of non-structural DM from the vegetative components to the seed.

This thesis will examine the effect of water status on seed growth in an integrated way in whole plants in the field and glasshouse. Seed growth will be studied in the field in plants with different final seed size. The redistribution of DM, C and N will be examined using $^{13}$C and $^{15}$N in an attempt to understand the role of vegetative assimilates on seed filling under terminal water deficit.
CHAPTER 3

SEED GROWTH OF DESI AND KABULI CHICKPEA IN A SHORT-SEASON MEDITERRANEAN-TYPE ENVIRONMENT

3.1 INTRODUCTION

Chickpea (Cicer arietinum L.) has been shown to be one of a number of pulses that are suited to the fine-textured, neutral-to-alkaline soils of the eastern cropping zone of Western Australia and southern Australia where narrow-leafed lupin (Lupinus angustifolius L.) is poorly adapted (Siddique and Sedgley, 1986; Siddique et al., 1993). Recently the area sown to chickpea in Western Australia has increased from 500 ha in 1991 to about 60000 ha in 1996 (Siddique and Sykes, 1997). Both cereals and pulses are subjected to terminal drought in this environment (Turner, 1992).

Recent studies have shown that pollination and pod development are inhibited by low temperatures in chickpea, so that under the winter conditions present in southern Australia, pod set and seed filling are delayed until spring when leaf photosynthetic rates are low as a consequence of soil water depletion (Siddique et al., 1993; Leport et al., 1998). One of the major consequences of this is that the terminal drought reduces the size of the seed, particularly in late-formed seeds (Dracup and Kirby, 1996). As seed size and uniformity are important in determining the market price, particularly in kabuli chickpea, any variation among genotypes in maintaining seed size under conditions of terminal drought will be important in breeding for improved yield and quality in chickpea for drought-prone environments.

Seed development in pulses has been shown to follow a sigmoidal pattern of growth (Dure, 1975). Initially the pod wall expands rapidly, and in chickpea achieves its maximum dry weight while the embryo is very small and is in the phase of cell division (Pate and Flinn, 1977; Wang and Hedley, 1993). Seed growth is then characterised by a high rate of metabolic activity (Smith, 1984) associated with the rapid, linear accumulation of dry matter, principally as starch and storage protein (Dure, 1975). At the end of this phase, a period of dehydration and maturation follows, by which time there is little endosperm left with the embryo filling the seed coat (Pate and Flinn, 1977; Wang and Hedley, 1993). The maximum potential size of a seed is a function of the rate and duration of embryo growth. Variation in the rate and duration of seed growth has been shown between species (Egli, 1981) and within crops such as field pea (Pisum sativum L.) and soybean (Glycine max L.) (Dumoulin et al., 1994; Egli et al., 1984; Hanson, 1986). Environmental factors such as temperature and water availability affect seed growth rate and final seed size in soybean and lupin (Egli et al., 1989; Dracup and
Kirby, 1996a). While seed growth has been carefully studied in lupin (Dracup and Kirby, 1996a, b) there have been few studies on seed growth in chickpea and no studies on the effect of drought on chickpea seed growth.

This first study was designed to determine the influence of terminal drought on seed growth under field conditions in three genotypes of chickpea differing markedly in seed size. The study aimed to determine whether terminal drought decreased the duration and/or rate of seed growth in chickpea, thereby reducing seed size, and whether there is genotypic variation in seed growth in response to terminal drought.

It is hypothesised that in chickpea subject to terminal drought the duration of seed growth is reduced but the rate of growth is maintained.

3.2 MATERIALS AND METHODS

Three genotypes of chickpea, cvs. Tyson and Kaniva, and ICCV88201, a line introduced from the International Crops Research Institute for the Semi Arid Tropics (ICRISAT), were grown under rainfed and irrigated conditions in a neutral-to-alkaline, fine-textured soil (Calcic Haploxeralf) at Merredin, Western Australia (31°30'S, 118°12'E). These genotypes represent the large diversity in chickpea seed size available in Australia. Tyson is a desi cultivar with small seed (average weight of sown seed, 121mg, range 119-126mg), ICCV88201 is a desi-type advanced breeding line (sister line to the recently-released cultivar, Sona) with medium-sized seeds (194mg, range 191-199mg), while Kaniva is a kabuli cultivar with large seed size (422mg, range 417-428mg). The three genotypes were grown in plots 30 by 1.44 m, each containing eight rows with 18 cm between the rows. Five metres at the end of each plot was drip irrigated twice weekly from flowering (108 days after sowing, DAS) onwards, with the irrigation volume being equivalent to that lost through pan evaporation. Irrigation was terminated when the seeds in the rainfed plot reached physiological maturity at 172 DAS. There were 4 replicate plots for each genotype in a randomised complete block design. At sowing (13 May 1995), double superphosphate was drilled with the seed (72 kg/ha) which had been inoculated with a commercial group-N chickpea Bradyrhizobium. Seeding rates were based on average seed weight and germination percentage (Tyson, 97%; ICCV88201, 98%; Kaniva, 77%), to give a target density of 40 plants/m². Final plant densities were 32 plants/m² for Tyson and ICCV88201 and 35 plants/m² for Kaniva.

An automatic weather station recorded daily maximum and minimum air temperature at the experimental plots and rainfall was measured daily with a manual rain gauge.

Phenological observations were made weekly on two randomly-selected plants in each irrigated and rainfed section of every plot. Flowering was defined as the time when 50%
of the plants in each plot had at least one open flower. Podding was defined as the time when 50% of the plants had at least one visible (~3mm long) pod.

After seedling emergence, 25 plants in the irrigated and rainfed section of each plot were randomly marked with a numbered flag. On these plants, individual pods were tagged when the length of the pod was 8-10mm (6-7 days after flowering). Tagging was done 136-137 DAS for all marked plants and where possible four pods were tagged per plant. Six sequential harvests were carried out at weekly intervals from tagging to maturity. At each harvest, 3 entire plants were harvested from both irrigated and rainfed sections of each plot. At the final harvest (192 DAS), pods from (i) the main stem, (ii) the apical primary branches arising from the apical nodes of the main stem, (iii) the basal primary branches from the basal nodes of the main stem and (iv) secondary and tertiary branches were kept separate and the average weight of seed from these pods determined. Additionally, at final harvest, a 0.5m² quadrat of plants and all remaining tagged plants were harvested. Whole plants and selected pods were dried individually to constant weight in a fan-forced oven at 70°C. Dry weights of each pod and each seed were measured. The seed dry weight data for the selected pods were fitted by non-linear regression to a logistic curve (Darroch and Baker, 1990):

\[ \text{seed DW} \approx A/(1+e^{B-C/d}) \]

where \( A \) estimates the final grain weight, \( B \) is related to both the duration and rate of seed filling, and \( C \) is related to the rate of seed filling. Parameter estimates were obtained using the non-linear regression procedure (nlin) in the SAS® System (SAS® Institute Inc., Cary, N.C., USA) software package. Duration of growth was defined as the time required for each seed to reach 95% of its final dry weight and it was calculated using: \( t = (B+2.944)/C \) (Darroch and Baker, 1990). The maximum rate of seed growth (R) was calculated as: \( R = CA/4 \) (Darroch and Baker, 1990). At final harvest the number of pods per plant was calculated from the 0.5m² quadrat cut from each plot and the seed number per pod and average seed weight calculated from selected individual plants. Yield was calculated from these components.

From 95 DAS to 174 DAS, the rate of net photosynthesis in the uppermost expanded leaf was measured using a portable open gas exchange system (Model LCA3, ADC, Hoddesdon, U.K.) on a weekly basis between 10:30h and 14:30h on cloud-free days when the photosynthetically active radiation was above 1500 \( \mu \text{mol/m}^2/\text{s} \), the level at which photosynthesis is saturated (Singh et al., 1987). Leaf water potential (LWP) was measured using the pressure chamber technique (Scholander et al., 1964) with the precautions recommended by Turner (1988). LWP measurements were made simultaneously with leaf photosynthesis. The uppermost expanded leaf was covered with a small plastic bag, removed using a razor blade at the point where it joined the
stem and the first four leaflets removed before the midrib and remaining leaflets were inserted into the pressure chamber. Measurements were made on 3 plants/plot and were replicated 4 times.

Data analysis

Seed yield and yield components (Table 3.1) and average seed DW and branch order (Table 3.2) data were analysed using the GLM procedure in the SAS® system software. In each case a two-factor multivariate ANOVA was performed. For seed yield and yield components the significance of genotype, treatment (irrigated and rainfed) and the genotype x treatment interaction was determined for each of the dependent variables. For average seed DW the significance of genotype, treatment and branch order and relevant interactions were determined.

3.3 RESULTS

For the month of August the average maximum monthly temperature was 17.0°C while the minimum was 5.1°C (Fig. 3.1b). These temperatures increased marginally for the month of September with a maximum of 19.2°C, a minimum 6.3°C, while for October the increase in temperatures was considerably more, to 24.8°C, 9.3°C, respectively. The average growing-season (May-October) rainfall for Merredin is 210 mm and in 1995 there was 313 mm (Fig. 3.1a). Most rain fell in May to July with 275 mm before first pod set and only 38 mm during seed filling. Of this 38 mm, 28 mm fell in one event on 19 October (159 DAS) which was close to physiological maturity in the desi chickpeas. The irrigated plants received 152 mm of supplemental water between 108 and 172 DAS.

The rates of LWP and net photosynthesis in the irrigated plants were about -1.2 MPa and 21-29 μmol CO₂/m²/s until near maturity with little difference among genotypes (Fig. 3.2a,b). The low rates of photosynthesis in the irrigated chickpeas, particularly Kaniva, on 151 and 167 DAS were associated with extremely windy conditions (Fig. 3.2b). In mid-September (130 DAS), LWP and the rate of net photosynthesis in rainfed plants began to rapidly decrease compared to the irrigated plants (Fig. 3.2). By 135 DAS the rate of net photosynthesis and LWP in all rainfed plants had decreased to below 10 μmol CO₂/m²/s and below -2.5 MPa, respectively. LWP increased in response to the rainfall event on 159 DAS, but this did not result in a corresponding increase in the rate of photosynthesis.

At the final harvest, in both irrigated and rainfed conditions Tyson had the greatest number of pods/plant and Kaniva the least (Table 3.1). The number of pods/plant was affected by water status in all three genotypes with 59, 33 and 53% reductions in pod number for Tyson ICCV88201 and Kaniva, respectively, in the rainfed compared with the irrigated plants. All genotypes had more than one seed per pod on average. In Tyson
and ICCV88201 one third to two thirds of pods had two seeds, whereas in Kaniva only 5% of pods had two seeds. Water stress caused a 16% reduction in the number of seeds/pod in Tyson, but did not affect the number of seeds/pod in ICCV88201 and Kaniva. Water shortage reduced the average seed weight by 19, 23, and 34% in Tyson, ICCV88201, and Kaniva, respectively. Yield/plant and yield/m² also decreased as a result of water shortage by 74% in Tyson, 52% in ICCV88201, and by 72% in Kaniva compared to irrigated plants.

Table 3.1  Yield components at final harvest of three chickpea genotypes grown under irrigated and rainfed field conditions at Merredin, Western Australia, in 1995. Different letters within rows indicate statistically significant differences at \( P = 0.05 \).

<table>
<thead>
<tr>
<th></th>
<th>Tyson</th>
<th>ICCV88201</th>
<th>Kaniva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irrigated</td>
<td>Rainfed</td>
<td>Irrigated</td>
</tr>
<tr>
<td>Number of pods/plant</td>
<td>89a</td>
<td>36b</td>
<td>59c</td>
</tr>
<tr>
<td>Number of seeds/pod</td>
<td>1.57c</td>
<td>1.32b</td>
<td>1.35b</td>
</tr>
<tr>
<td>Av. seed weight (mg)</td>
<td>133a</td>
<td>108a</td>
<td>214b</td>
</tr>
<tr>
<td>Weight of seed/plant (g)</td>
<td>19a</td>
<td>5b</td>
<td>17a</td>
</tr>
<tr>
<td>Weight of seed/m² (g/m²)</td>
<td>595</td>
<td>164</td>
<td>545</td>
</tr>
</tbody>
</table>

While seed size was always reduced in the rainfed compared to irrigated plants, there were no differences in average seed weight from the different branch orders in all three genotypes in either irrigated or rainfed treatments (Table 3.2).

Table 3.2  Average seed weight (mg), at final harvest, of clean chickpea seed according to branch order from three chickpea genotypes grown under irrigated and rainfed field conditions at Merredin, Western Australia, in 1995. Different letters within rows indicate statistically significant differences at \( P = 0.05 \). Data within columns was not significantly different at \( P = 0.05 \).

<table>
<thead>
<tr>
<th></th>
<th>Tyson</th>
<th>ICCV88201</th>
<th>Kaniva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irrigated</td>
<td>Rainfed</td>
<td>Irrigated</td>
</tr>
<tr>
<td>Main Stem</td>
<td>134a</td>
<td>111a</td>
<td>217c</td>
</tr>
<tr>
<td>1° Order/Apical</td>
<td>130ab</td>
<td>109a</td>
<td>207c</td>
</tr>
<tr>
<td>1° Order/Basal</td>
<td>137ab</td>
<td>110a</td>
<td>220c</td>
</tr>
<tr>
<td>2°/3° Order</td>
<td>124ab</td>
<td>106a</td>
<td>198c</td>
</tr>
</tbody>
</table>
Figure 3.1. Daily and cumulative rainfall (a) and maximum and minimum air temperatures (b) over the growing season at Merredin, Western Australia, in 1995. The arrows indicate the mean time of the first flowers (F) and pods (P). The gap in the air temperature data was due to failure of the data logger.
Figure 3.2. Change with time in the (a) water potential and (b) rate of net photosynthesis of the uppermost expanded leaves of Tyson (■, □), ICCV88201 (●, ○) and Kaniva (▲, △) chickpeas grown under irrigated (closed symbols) and rainfed (open symbols) conditions at Merredin, Western Australia, in 1995. Lines drawn for Tyson cultivar only. Bars give +/- one standard error of the mean of 12 leaves where the values are greater than the size of the symbols.
Seed growth was measured on pods labelled shortly after pod set, that is on the first formed pods on each branch. The final average seed weight of the selected seeds was therefore larger than for the whole plant (Table 3.3) which included later-formed smaller seeds. Also, as the seed number per pod was always more than one on average, but varied from pod to pod, among genotypes and was reduced in the rainfed treatment only in Tyson, the results are presented as seed weight per pod rather than per seed. Those pods with more than one seed per pod had smaller seed than those with only a single seed per pod. Because Tyson and ICCV88201 had more double-seeded pods than Kaniva the individual seed weight was considerably lower in the desi genotypes and showed larger variation on a per seed basis than in Kaniva (data not shown). The general pattern of seed and pod wall growth was consistent for all three genotypes (Fig. 3.3). The pod wall grew first and had almost reached its maximum dry weight before seed growth began. In the seed, there was an early period when there was little increase in dry matter, then a period of rapid seed fill with an effectively linear increase in dry weight, followed by a period with a decrease in the rate of seed growth as the seed neared the completion of filling. Tyson had the lowest final seed dry weight/pod with 254 mg in irrigated plants and 169 mg in rainfed plants (Table 3.3), a 34% reduction in the rainfed compared to irrigated plants. Kaniva had the highest seed dry weight/pod with 454 mg in irrigated plants and 331 mg in rainfed plants, a reduction of 28%. ICCV88201 was intermediate in seed dry weight/pod with 345 mg in irrigated plants and 189 mg in rainfed plants, a 45% decrease. In rainfed Kaniva there was an increase in seed dry weight at the end of the growing period in response to the 28 mm rainfall received on 159 DAS that did not occur in the other genotypes (Fig 3.3c). Fitted curves revealed that in all three genotypes, the maximum rate and duration of seed fill in rainfed plants was always considerably less than in irrigated plants (Table 3.3) with a reduction of 5 to 12 days in the duration, and a decrease of 20 to 30% in the maximum rate of seed filling. The maximum rate of seed filling in Kaniva was twice that of Tyson and 1.5 times that of ICCV88201 regardless of treatment.

Table 3.3  Seed growth characteristics determined from fitted sigmoidal growth curves of seeds of three chickpea genotypes grown under irrigated and rainfed field conditions at Merredin, Western Australia, in 1995. Different letters within rows indicate statistically significant differences at \( P = 0.05 \)

<table>
<thead>
<tr>
<th></th>
<th>Tyson</th>
<th>ICCV88201</th>
<th>Kaniva</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Irrigated</td>
<td>Rainfed</td>
<td>Irrigated</td>
</tr>
<tr>
<td>Final seed weight (mg/pod)</td>
<td>254b</td>
<td>169a</td>
<td>345c</td>
</tr>
<tr>
<td>Duration of seed fill (days)</td>
<td>37d</td>
<td>26a</td>
<td>39d</td>
</tr>
<tr>
<td>Max rate of seed fill (mg/pod/day)</td>
<td>11.3c</td>
<td>9.1a</td>
<td>16.0d</td>
</tr>
</tbody>
</table>

For all three genotypes there was a significant decrease in the weight of pod walls in
rainfed plants as seed filling proceeded (Fig. 3.3). This decrease was 19.4 mg/pod in Tyson, 26.9 mg/pod in ICCV88201 and 28.3 mg/pod in Kaniva. These decreases represented 28, 32 and 24% of the maximum pod wall dry weight for Tyson, ICCV88201 and Kaniva, respectively.
Figure 3.3. Change with time in the dry weight of the seed (■, □) and pod wall (●, ○) of (a) Tyson, (b) ICCV88201 and (c) Kaniva chickpeas grown under irrigated (●, ■) and rainfed (○, □) conditions at Merredin, Western Australia, in 1995. Bars give +/- one standard error of the mean of 4 replicates where values are greater than the size of the symbols.
3.4 DISCUSSION

Although flowering began at the end of August (106-108 DAS), first pod set did not occur for another 25 to 30 days (130 DAS in the desi chickpeas and 135 DAS in Kaniva), presumably due to the low temperatures throughout September. Mean daily temperatures below 15°C are considered to prevent fertilisation and pod set (Clarke 

et al., 1998). Terminal drought is a feature of Mediterranean type-environments in southern Australia and water deficits developed in the rainfed chickpeas during mid- to late-September (125-149 DAS, Fig. 3.2a) despite higher than average seasonal rainfall. The rate of leaf photosynthesis under these conditions decreased rapidly resulting in plants having little current leaf-derived photosynthate available to meet the demands of the growing pods. In irrigated plants, LWP and photosynthesis were maintained at much higher levels throughout seed filling. This supports earlier research (Singh 

et al., 1987) in which photosynthetic rate, in droughted chickpea plants at the time of flowering, was already less than 5 μmol CO₂/m²/s, and it continued to decrease with time. This low rate of leaf photosynthesis when seed filling was initiated has been observed previously in several grain legume species, including chickpea in a drier-than-average season (Leport 

et al., 1998). The LWP in the kabuli cultivar, Kaniva, decreased slightly earlier than in the other two genotypes, but there were no consistent differences in the rate of leaf photosynthesis between the three genotypes.

In order to compensate for reduced assimilate supply to actively growing sinks due to drought, plants must reduce the demand for photosynthate, or maintain assimilate supply through the remobilisation of stored reserves (Chapter 2; Pheloung and Siddique, 1991). In this study chickpea utilised both methods to balance assimilate supply and demand. In rainfed plants, sink size was reduced in all three genotypes. Kaniva was the most sensitive to drought in terms of yield. In particular, pod number and average seed weight were greatly reduced. As chickpea has an indeterminate growth habit, there was a cessation of stem and branch growth, and consequently a reduction in the number of new pods that developed in all three genotypes. Kaniva was about 5 days later in pod initiation and this may have resulted in a lower proportion of its potential pod number being set prior to the onset of the water deficit. In a short-season Mediterranean environment this requirement for a 5-day longer period before pod initiation may be detrimental for a plant trying to escape terminal drought. On the other hand, Kaniva was visually greener for longer than the desi genotypes and was able to capitalise on the late rainfall event 159 DAS. The other genotypes in the rainfed plots had reached physiological maturity by this time and were unable to initiate any regrowth and utilise the rainfall.

The rate of seed filling was clearly related to the final size of the seed, with the fastest
rate of seed filling in the large seeded Kaniva and the slowest rate of seed filling in the
small-seeded Tyson. Terminal drought induced a 20% reduction in the rate of seed
filling in Tyson and a 30% reduction in both ICCV88201 and Kaniva. Thus the
reduction in the rate of seed filling as a result of water deficit was not related simply to
seed size or type of chickpea, with the mid-sized desi ICCV88201 having the same
reduction in the maximum rate of seed filling as the larger-seeded kabuli-type. The
duration of seed filling was significantly shorter in the irrigated Kaniva than in the two
desi genotypes indicating that larger seeds do not take longer to fill and duration, while
reduced by drought, was similarly reduced in all three genotypes.

Despite seed weight/pod being more strongly affected by drought in Kaniva and
ICCV88201 than in Tyson, Kaniva still had the highest average seed weight,
ICCV88201 was still intermediate in weight and Tyson was still the smallest seed under
terminal drought conditions, particularly as a third of the pods had two seeds per pod.
Seed weight of Kaniva under rainfed conditions at Merredin in 1995 was 285 mg, well
below the optimum desired for export markets (>400 mg). Despite ICCV88201
displaying a poorer ability to maintain seed size under drought than Tyson, its potential
seed size was larger than Tyson regardless of treatment, thereby making it a more
acceptable desi genotype. Therefore, although drought does affect the average seed
weight, the genetically-controlled potential for seed weight exerts a significant influence
in determining the final seed weight.

In chickpea, maintenance of assimilate supply through remobilisation of stored reserves
appeared to be important under terminal drought. Dry matter remobilisation from the
pod wall could account for up to 12% of the dry weight of the seed in Tyson, 15% in
ICCV88201, and 9% in Kaniva assuming that all the dry weight loss measured in the
pod walls was remobilised to the seed. Similar decreases in pod wall dry weight during
the rapid seed filling stage have also been measured in pea and lupin (Pate and Flinn,
1977; Dracup and Kirby, 1996b). In pea up to 50% of the pod wall DW is lost during
pod senescence (Pate and Flinn, 1977). In lupin the thick, fleshy pod walls appear to
serve as a nutrient reservoir for the filling seed (Dracup and Kirby, 1996b). The
contribution of the pod walls of chickpea, which are thinner and have lower moisture
content than those of lupin, to the filling seed may be less significant than in lupin.
Further work as outlined in Chapters 3 and 4 is required to establish whether these
decreases in pod wall DW under rainfed conditions were the result of remobilisation or
respiration losses and to quantify the contribution of remobilisation to seed fill. The
remobilisation of reserves from other vegetative tissues is likely to be another important
source of assimilate (Leport et al., 1999). In the remobilisation of reserves, each branch
may behave as an independent unit with little transfer of assimilates with the rest of the
plant. C14 labelling studies (Singh and Pandey, 1980) indicate that there is little transfer
of assimilate between branches in well-watered chickpea. Our data showing the same
effect of drought on branches of different order, in terms of average seed weight in all
genotypes (Table 3.2), suggests a similar response in rainfed as in irrigated chickpea.

Unless it is possible to improve pod set at cool temperatures in chickpeas, avoidance of
terminal drought is not possible in the short-season Mediterranean-type environments of
southern Australia. A comparison of groundnut (*Arachis hypogaea* L.) cultivars subject
to terminal drought revealed that the genotype with the highest yield set a large number
of early pods, thereby maintaining a high sink strength and rapid dry matter
accumulation prior to the water deficit being imposed. This strategy resulted in the
genotype having the highest HI of those studied (Chapman *et al.*, 1993). In chickpea,
Kaniva podded marginally later than the other genotypes and set considerably fewer
pods consequently suffering the highest yield penalty. However, increased pod set in
lupin, induced by exogenous cytokinin application, did not result in increased yield but
rather resulted in a decrease in seed number and yield due to an inability of the plant to
maintain assimilate supply under both well watered and water deficit conditions (Palta
and Ludwig, 1996). This suggests that lupin seed growth is source limited or the number
of seeds set is determined by the size of the source of assimilates. One way to increase
the source of assimilates is to increase the remobilisation of assimilates stored prior to
seed development. This study has shown that there appears to be from 9 to 15%
retranslocation of dry matter from the pod wall to the seed when water deficits occur in
the seed-filling phase. Secondly, this study has shown that there is variation in the
degree of reduction in seed size in response to drought in chickpea. Despite this the
inherently larger-seeded genotypes still had larger seeds when stressed. This suggests
that selection for large seed under favourable conditions may aid in the selection for
large seed size under terminal drought.

The decrease in DW of the pod walls under rainfed conditions in this study compared to
that in irrigated plants, together with DW losses observed in leaves and stem of rainfed
chickpea in a complementary study (Leport *et al.*, 1999) suggests considerable
remobilisation of assimilates to the seed during terminal drought. Thus the next chapter
details a study in which the remobilisation of pre-podding labelled C and N was
examined during seed filling under well-watered and terminal drought conditions.
CHAPTER 4

REMOBILISATION OF CARBON AND NITROGEN IN DESI AND KABULI CHICKPEA SUBJECT TO WATER DEFICIT

4.1 INTRODUCTION

In the Mediterranean-type environment of south-western Australia, winter-sown, rainfed chickpea crops generally experience terminal drought. Terminal drought decreases the rate of net photosynthesis during seed filling, a period of high assimilate demand (Chapter 3; Leport et al., 1998). Furthermore, N fixation also decreases during seed filling in chickpea (Hooda et al., 1986; Kurdali, 1996), a response which is exacerbated by water deficit (Hooda et al., 1989; Swaraj et al., 1995). Strong demand for assimilate when the supply of current assimilate is decreasing results in an assimilate shortfall (Pate et al., 1980; Egli and Crafts-Brandner, 1996). Consequently, alternative sources of assimilate are required to maintain seed filling.

Terminal drought in field grown chickpea leads to increased rates of flower and pod abortion with significant decreases in pod and seed number. In Chapter 3 both the maximum rate and duration of seed growth were reduced in chickpea subjected to terminal drought. This resulted in average seed weights being 19% lower in Tyson, 23% lower in ICCV88201 and 34% lower in Kaniva in stressed compared to well watered chickpea. Seed yield in Tyson and Kaniva plants subject to drought was decreased 72 to 74% compared to the well-watered plants with less decrease in ICCV88201 (52%; Chapter 3). Chickpea, like other indeterminate species, also responds to water deficit by decreasing stem elongation, the rate of leaf emergence and expansion and the establishment of new reproductive nodes (Muchow et al., 1986; Mwanamwenge et al., 1999). Therefore, sink size was reduced in all three genotypes, although there was still a demand for assimilate from the remaining pods.

In a number of species the remobilisation of pre-anthesis stored reserves of C and N can be important assimilate sources for seed filling, particularly when plants are subject to water deficit (Pheloung and Siddique, 1991; Palta et al., 1994; Bidinger et al., 1977; Nicolas et al., 1985a,b; Foster et al., 1995). In cereals remobilisation has been reported to contribute between 3-64% of seed DM (Chapter 2; Rawson and Evans 1971; Palta et al., 1994), while in pulse species the contribution of remobilised DM ranges from 2-42% (Pate et al., 1980; Bushby and Lawn, 1992). In chickpea, DW decreases in the stems, leaves, and pod walls of field grown plants is an indicator of DM remobilisation and an important alternate assimilate source to current photosynthate (Chapter 2; Khanna-Chopra and Sinha, 1987; Leport et al., 1999). In field grown chickpea it was
estimated (based on DM decreases in the vegetative tissue) that one-third of the accumulated pod DM was derived from the remobilisation of DM from vegetative tissue (Khanna-Chopra and Sinha, 1987). Decreases in pod wall DW in Chapter 3 indicate that about 10% of the seed DW could be derived from DM remobilised from the pod walls.

A second experiment was undertaken to confirm and quantify the remobilisation of DM as a source of assimilate for seed filling in chickpea exposed to water deficit. Well-watered and water-stressed chickpea were compared for leaf water potential (LWP), photosynthesis, seed yield, pod abortion, and DW changes including seed growth and development. Additionally, the remobilisation of C and N from vegetative tissues to filling seed was quantified using the stable isotopes, $^{13}$C and $^{15}$N (Palta et al., 1994) in contrasting desi and kabuli cultivars. The aims of the experiment were to quantify the contribution of pre-podding C and N to seed filling in both well-watered and water-stressed chickpea and to determine whether variation in the contribution of remobilised pre-podding C and N to seed filling existed in contrasting chickpea genotypes differing in their ability to maintain seed yield under terminal drought.

4.2 MATERIALS AND METHODS

Soil preparation, sowing, and watering regimes

The soil, a reddish-brown, sandy clay loam (Calcic Haploxeralf, pH 7.0 in CaCl$_2$), used in the experiment was obtained from Merredin, Western Australia in the eastern wheatbelt by collecting the upper 10 centimetres of topsoil from an area of undisturbed native soil. The soil was prepared by sieving it through an 8 mm sieve to remove any large soil aggregates and pieces of organic matter. Following sieving, 47.5 kg of Merredin soil, was thoroughly mixed with 2.5 kg of sand and 34 g fertiliser for each 50 kg batch of soil.

The fertiliser included: 1.0 g of “Richgro”™ trace elements (11% potassium as potassium sulphate, 5% iron as ferrous sulphate, 5% calcium as calcium carbonate, 2% magnesium as magnesium sulphate, 2% manganese as manganese sulphate, 1.5% copper as copper sulphate, 1% zinc as zinc oxide, 0.2% boron as borax, and 0.1% molybdenum as sodium molybdate), 7.51g of potassium nitrate, 7.13 g of ammonium nitrate, 10.67 g of calcium nitrate and 7.67g of triple superphosphate (20.0% phosphorus). Granular components of the fertiliser were ground to powder prior to mixing. Each pot contained 6.0 kg of soil. Pots consisted of polyvinyl chloride (PVC) pipe, 150 mm long and 60 mm in diameter with end-caps as bases with four 10-mm drainage holes.

The same three contrasting genotypes as used in Chapter 3 were grown: Tyson a small seeded desi variety; ICCV88201 a medium seeded desi breeding line from the
International Crops Research Institute for the Semi-Arid Tropics (ICRISAT); and Kaniva, a large seeded kabuli variety. Prior to sowing, the seeds were hydrated overnight by placing them in beakers of water which were aerated to prevent seed death. At sowing the seeds were inoculated with commercial, group N Bradyrhizobium inoculum. Plants were grown in a temperature controlled glasshouse operating at 23°C during the day and decreasing with ambient temperature at night (approximately 12-15°C). Four seeds were sown into each pot on 3 May 1996 at a depth of 3.0 cm. Two weeks after sowing the seedlings were thinned to two per pot. On 23 July (81 days after sowing, DAS) two watering regimes were applied: water was withheld from half of the pots (hereafter referred to as stressed plants) while the rest were kept well-watered (hereafter referred to as control plants). Additionally, four pots of each genotype and each treatment were used for photosynthesis and LWP measurements.

From the onset of the watering regimes, pre-dawn LWP measurements were taken at 0530 h, every 4-5 days as water deficits developed. For each measurement four uppermost fully-expanded leaves per genotype were taken from plants grown specifically to measure LWP using the pressure chamber technique (Turner, 1988). Photosynthesis measurements were made when weather permitted, between 1100-1500 h on cloudless days. On the 9 August (98 DAS) and 16 August (105 DAS) water deficit pots were watered with 250 mL of water per pot in order to reduce the severity of the water deficit. Due to there being relatively few days suitable for measuring photosynthesis, measurements were made on only 4 days throughout development, on 56 and 69 DAS during vegetative growth and 96 and 98 DAS during reproductive growth in each of the genotypes. The final watering for all plants was 16 August (105 DAS).

**Plant development and seed yield**

There were six harvests each of 4 replicates i.e. 8 plants per treatment per cultivar at each harvest. The first was carried out at the imposition of the watering regimes on the 23 July (81 DAS), and then weekly for the next four harvests on 88, 95, 102 and 110 DAS. Final harvest was at physiological maturity on 3 September (123 DAS). At each harvest leaves, stems, and pods were separated and dried in an oven at 70°C for 48 hours. Roots were carefully washed from the soil, partially dried with paper towel and then oven dried. Most of the fallen leaves were collected in a net made of shade cloth attached around the top of each pot. The number of filled and unfilled pods was counted and the seeds were removed and counted. Dry weight measurements of all the components, leaves, fallen leaves, stems, roots, filled and unfilled pods, pod walls and seeds were recorded at each harvest. Unfilled pods included those pods which had senesced or died prior to pod expansion and those which expanded but did not contain a seed. Sigmoidal growth curves were fitted to the total seed DW (g/plant) data using the same equation and procedure as used in the chapter 2 for seed in selected pods.
**13C and 15N assimilation and remobilisation**

For this part of the study only Tyson and Kaniva were used and there were 3 harvests at 81, 95 and 123 DAS. At each harvest 4 replicate pots were used per genotype. Prior to imposing the watering regime and the start of podding, all of these plants were labelled three times with 13C and 15N. The labelling techniques used were modified from procedures described by Palta *et al.* (1994).

15N labelling was carried out by leaf feeding with a 0.4% 15N-urea solution. At each labelling time two upper, fully-expanded leaves were selected from each plant and three leaflets at the leaf tip were cut in half under water to aid uptake and prevent dehydration. The leaf tip was then quickly placed into a small 1.5 mL Eppendorf tube containing the 0.4% 15N-urea solution. Two tubes were used for each plant. The leaf was held in place and evaporation prevented by using a foam earplug as a stopper in the mouth of the tube. A piece of thin wire pushed into the earth with a loop at the other end was used to support the tube and stopper. Labelling started at 0900h in the morning and was allowed to continue for the rest of the day or until the plants had taken up all the solution from the tube. Then 0.5mL of water was placed in the tube to ensure complete uptake of the 15N. At each labelling the amount of 15N fed to each plant was increased as plant DW increased at each time with 2.4, 4.8 and 7.2 mg 15N urea/plant used at 19, 40 and 47 DAS, respectively. In total 14.4 mg 15N urea was applied per plant over three labellings.

13C labelling was carried out using a canopy feeding technique. Pots were prepared for labelling by placing a layer of plastic beads over the soil surface to help isolate the soil atmosphere from the canopy atmosphere. A clear plastic mylar film chamber, 2.3m long, 0.9m wide and 0.9 m high, with an aluminium frame was assembled inside a large growth cabinet. The 48 pots of chickpea were placed in the chamber. At one end of the chamber a small electric fan helped circulate and mix the air inside the chamber which was sealed with tape. The CO2 concentration inside the chamber was measured using an infra-red gas analyser (IRGA, LI-6251, LI-COR, Inc., Lincoln, NE) placed outside the chamber. Plastic tubing connected the IRGA to each corner of the chamber. A small pump was included in the line after the IRGA resulting in a constant flow of chamber air through the IRGA. A larger pump was used to draw air from the chamber through a column and back into the chamber. When the chamber was first sealed the column contained soda lime to decrease the CO2 concentration to 150ppm. At this point the 13CO2 was released into the atmosphere of the chamber by injecting lactic acid into a beaker containing 13C-sodium bicarbonate (NaH13CO2) taped to the inside of the chamber. Prior to releasing the 13CO2, the column was replaced with a silica column to remove excess moisture from the air as a result of transpiration. The column was replaced regularly and the silica re-dried in a 70°C oven. Temperature in the growth cabinet was maintained at 20°C with full lighting for maximum assimilation of 13CO2.
When all of the NaH\textsuperscript{13}CO\textsubscript{2} had reacted and the CO\textsubscript{2} concentration had again fallen to 150 ppm, CO\textsubscript{2} was blown directly into the chamber through a port and the plants were allowed to continue assimilation to ensure maximum uptake of any remaining \textsuperscript{13}CO\textsubscript{2}. This was repeated a second time prior to opening the chamber for plant removal. Following labelling, the plants were moved back to the glasshouse. Labelling was carried out on three occasions at 27, 41 and 48 DAS with 44.3, 103.2 and 147.5 mg \textsuperscript{13}C being applied per plant at each time. Over the three labelling periods a total of 295 mg \textsuperscript{13}C was applied per plant.

The first harvest, was carried out when the watering regime was imposed on 23 July (81 DAS). Second and final harvests of the labelled plants were carried out on 6 August (95 DAS) and on the 3 September (123 DAS). At each harvest the plants were divided into roots, stems, leaves (plus any flowers), pod walls, and seeds. Samples were snap frozen in liquid N then stored in a freezer (-20°C). The samples were freeze dried, weighed and ground to powder in a mill. The samples were placed in sealed plastic vials after grinding and kept in the dark. Samples were kept dry and 5 mg sub-samples were weighed into small aluminium capsules which were then rolled into balls using tweezers. These balls containing the labelled sub-samples were then loaded into a dual head mass spectrometer (VG-Micromass Sira-10, V-G Isogas Ltd., Middlewich, England) connected to a Europa Roboprep C-N Analyzer (Europa Scientific Ltd., Crewe, England). Total C, total N and % \textsuperscript{13}C and % \textsuperscript{15}N were measured for each of the sub-samples.

At the first harvest eight additional unlabelled plants of each genotype were used for natural \textsuperscript{13}C and \textsuperscript{15}N abundance measurements in leaf, stem and root tissue. Harvested samples were prepared as described above and natural abundance levels of \textsuperscript{13}C and \textsuperscript{15}N were measured using the mass spectrometer. There was no significant variation in natural abundance of either stable isotope in any of the plant components.

The amount of C (\textit{C}_g) or N (\textit{N}_g) in the seed that was derived from the vegetative parts prior to podding and that which was derived from post-podding assimilation and fixation was calculated. This calculation was made using the \textsuperscript{13}C and \textsuperscript{15}N content in the seed at maturity (\textsuperscript{13}C\textsubscript{gm}, \textsuperscript{15}N\textsubscript{gm}) and the contents in the vegetative parts (vp) of unlabelled C (\textsuperscript{12}C) and \textsuperscript{13}C (\textsuperscript{13}C\textsubscript{vp}) and unlabelled N (\textsuperscript{14}N) and \textsuperscript{15}N (\textsuperscript{15}N\textsubscript{vp}) at podding according to the following:

\[
\textit{C}_g = \textsuperscript{13}C_{gm}(\textsubscript{vp}/\textsuperscript{13}C_{vp})
\]

\[
\textit{N}_g = \textsuperscript{15}N_{gm}(\textsubscript{vp}/\textsuperscript{15}C_{vp})
\]
It was assumed that there was no discrimination in the remobilisation of $^{12}$C and $^{13}$C or $^{15}$N and $^{14}$N when C and N were translocated from the vegetative tissues to the seed. Consequently it is assumed that unlabelled C and N are remobilised in a consistent ratio with their stable isotopes from podding onwards. In this way the total contribution of remobilised C and N to the seed was estimated.

Data analysis

Seed yield and yield component results were analysed using the GLM procedure in the SAS® system software. A two-factor multivariate ANOVA was performed to test the significance of genotype, treatment (well-watered and water deficit) and the genotype x treatment interaction for each of the dependent variables. Results of the ANOVA are presented in Appendix.

4.3 RESULTS

Leaf water potential and photosynthesis

Pre-dawn LWP (Fig. 4.1) was maintained at -0.3 to -0.7 MPa in the control plants regardless of genotype. Shortly after the imposition of the water deficit treatment (81 DAS) the LWP of the stressed plants fell rapidly to -2.0 to -2.5 MPa at 91 DAS. Rewatering (92 DAS) following this rapid onset of water deficit resulted in a recovery in LWP to -1.1 in Tyson and -1.3 MPa in Kaniva which was maintained in the stressed plants with two subsequent rewaterings at 98 and 105 DAS. After 105 DAS watering was stopped for all plants and LWP decreased.

Prior to the imposition of water deficit and in the adequately watered plants the net photosynthesis in the uppermost expanded leaf was around 20 µmol CO$_2$/m$^2$/s in the three genotypes. Withholding water induced a substantial decrease in the rate of net photosynthesis to about 5 µmol CO$_2$/m$^2$/s at 96 DAS and between 6 and 11 µmol CO$_2$/m$^2$/s shortly after rewaterting on 98 DAS (data not shown).

Pod, seed and dry matter production

One week after water treatments were imposed (88 DAS), Kaniva had set significantly fewer pods in both the control (Fig. 4.2a) and stressed (Fig. 4.2b) plants than Tyson and ICCV88201. Pod numbers in both treatments increased over the next week and then were stable but the increase in pod numbers was much less in the stressed than in the control plants.

Average seed weight also varied substantially depending on genotype and treatment. Average seed weight increased in stressed ICCV88201 and Kaniva plants by 24 and
37%, respectively, while in Tyson there was no significant increase compared to the controls (Fig. 4.3a). The stressed treatment decreased the number of filled pods by 66, 59 and 91% compared to control plants in Tyson, ICCV88201 and Kaniva, respectively (Fig. 4.3b). The proportion of unfilled pods in control and stressed plants, was 13 and 36% in Tyson, 14 and 47% in ICCV88201 and 30 and 79% in Kaniva, respectively (Fig. 4.3c). Seed yield reflected the number of filled pods and consequently was highest in the control plants of each genotype and was higher in the desi genotypes compared to the kabuli genotype (Fig. 4.3d). Seed yield was reduced by 61% in Tyson, by 56% in ICCV88201 and by 80% in Kaniva in stressed compared to control plants. There was no significant difference in seed yield between the two desi genotypes in either treatment.
Figure 4.1. Changes in pre-dawn water potential of the uppermost fully expanded leaves with time of Tyson (■, □), ICCV88201 (●, ○) and Kaniva (▲, △) chickpea grown under well-watered (closed symbols) and water deficit (open symbols) conditions in the glasshouse. Bar represent +/- one standard error of the mean of 4 replicates where values are greater than the size of the symbols.
Figure 4.2. Changes in pod number with time in (a) well-watered and (b) water-deficient Tyson (■, □), ICCV88201 (●, ○) and Kaniva (▲, △) chickpea grown in the glasshouse. Bar represent +/- one standard error of the mean of 8 replicates where values are greater than the size of the symbols.
Figure 4.3. (a) Average seed dry weight, number of (b) filled and (c) unfilled pods and (d) seed yield (g/plant) in glasshouse-grown Tyson, ICCV88201 and Kaniva chickpea subject to both well-watered (control) and water deficit (stressed) conditions. Bars represent one standard error of the mean of 4 replicates.
The effect of water deficit on seed development varied between the different genotypes. In the two desi genotypes early seed development in stressed plants was similar to control plants (80-95 DAS), but later development was curtailed (Fig. 4.4a,c). The water deficit reduced the duration of seed growth from 54 to 32 days in Tyson and from 32 to 15 days in ICCV88201 with water deficit (Table 4.1). The seed yield calculated using all the data from the fitted curves was significantly reduced by water deficit in all genotypes. In control plants seed yield was higher in Tyson (8.2 g/plant) than in ICCV88201 (5.6 g/plant) and Kaniva (3.4 g/plant). However, the estimated decrease caused by water deficit was greater for Tyson than it was for ICCV88201, resulting in a similar total seed DW in stressed Tyson and ICCV88201 plants. The seed yield in Tyson control plants as determined by the fitting of the seed growth curves (Table 4.1) was considerably higher than the measured seed yield (6 g/plant; Table 4.2).

Table 4.1  Seed growth characteristics determined from fitted sigmoidal growth curves of seed yield data of three chickpea genotypes grown in the glasshouse under well-watered (control) and water deficit (stressed) conditions. Different letters within rows indicate statistically significant differences at $P = 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>TYSON</th>
<th>ICCV88201</th>
<th>KANIVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Stressed</td>
<td>Control</td>
</tr>
<tr>
<td>Seed yield (g/plant)</td>
<td>8.2a</td>
<td>2.6b</td>
<td>5.6a</td>
</tr>
<tr>
<td>Duration of seed fill (days)</td>
<td>54a</td>
<td>32a</td>
<td>32a</td>
</tr>
<tr>
<td>Max. rate of seed fill (mg/plant/day)</td>
<td>206a</td>
<td>98a</td>
<td>216a</td>
</tr>
</tbody>
</table>

In stressed Tyson plants rapid seed filling commenced at a similar time to the well-watered plants (Fig. 4.4a) but both the duration and maximum rate of seed fill was substantially reduced. In comparison, rapid seed growth of stressed ICCV88201 and Kaniva plants began earlier than in well-watered plants (Fig. 4.4d,f), however, the duration of seed fill in Kaniva was halved from 28 to 14 days with little increase in seed DW. The maximum seed growth rate in the control plants was 269 mg/plant/day in Kaniva followed by ICCV88201 (216 mg/plant/day) and Tyson (206 mg/plant/day). Water deficit substantially reduced the maximum rate of seed growth in all three genotypes with the largest decrease occurring in Kaniva.
Figure 4.4. Change in total seed dry weight (a,c and e) and growth rate (b,d and f) with time in well-watered (solid line) and water-deficient (dashed line) glasshouse-grown Tyson (a and b), ICCV88201 (c and d) and Kaniva (e and f) chickpea. Data derived from parameters estimated by fitting sigmoid curves to dry weight data (not shown).
Increased seed DW occurred in conjunction with decreases in the DW of some of the vegetative components. Leaf DW decreased substantially across all treatments (Fig 4.5a,b) although some of the loss of leaf DW may be a consequence of inaccuracy in collecting fallen leaflets. Leaf DW decreased by 26% in control and 30% in stressed Tyson plants, 30 and 18% in ICCV88201 and 24 and 17% in Kaniva. Tyson was the only genotype in which the leaf DW decrease in the well-watered plants was not significantly larger than that in the stressed plants. Root DW decreased to a lesser extent than leaf DW and there were no significant differences between any of the genotypes or treatments. Pod wall and stem DW decreases were insignificant in all three genotypes regardless of treatment but in Kaniva stem DW continued to substantially increase throughout plant growth in both control and stressed plants which did not occur in either desi genotype (data not shown). In control plants, the overall net decrease in vegetative DW could potentially account for up to 53% of seed DW in Tyson, 81% in ICCV88201 and 17% in Kaniva while in stressed plants the decrease in vegetative DW was greater than the increase in seed DW regardless of genotype. The decrease in net vegetative DW in stressed plants was highest for Tyson followed by ICCV88201 and least for Kaniva.

Total above-ground DM production was similar across all genotypes under well-watered conditions (Table 4.2). The water deficit resulted in a decrease in above-ground DM production in all three genotypes, although this decrease was least for ICCV88201 so that it produced significantly more above-ground DM than Tyson. Similarly, vegetative DM was decreased by 36% in stressed Tyson, 28% in stressed ICCV88201 and 36% in stressed Kaniva plants relative to the control plants. Seed yield was 2.4 g/plant in the stressed Tyson and ICCV88201 plants so the higher above-ground biomass in ICCV88201 under water deficit was a consequence of more vegetative DM (6.7 g/plant) compared to Tyson (5.0 g). By comparison, Kaniva, despite producing similar biomass to ICCV88201 in both treatments, had lower seed yields than the desi genotypes regardless of treatment (Table 4.2). These differences were reflected in higher harvest indices in both desi genotypes compared to the kabuli genotype, in both treatments. Water deficit decreased HI in all three genotypes.
Figure 4.5. Decreases in the dry weight (g/plant) of the stems, leaves, roots and pod walls between 81 and 123 days after sowing. Bars represent one standard error of the mean of 4 replicates.
Table 4.2  Total, vegetative (including pod walls) and seed above-ground dry matter and harvest index of Tyson, ICCV88201 and Kaniva chickpea grown under control and stressed conditions at final harvest. Different letters within rows indicate significance at $P = 0.05$.

<table>
<thead>
<tr>
<th>Above-ground DM (g/plant)</th>
<th><strong>TYSON</strong></th>
<th><strong>ICCV88201</strong></th>
<th><strong>KANIVA</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Stressed</td>
<td>Control</td>
</tr>
<tr>
<td>Total</td>
<td>13.9a</td>
<td>7.4b</td>
<td>14.7a</td>
</tr>
<tr>
<td>Vegetative</td>
<td>7.8a</td>
<td>5.0b</td>
<td>9.3ad</td>
</tr>
<tr>
<td>Seed</td>
<td>6.1a</td>
<td>2.4b</td>
<td>5.4a</td>
</tr>
<tr>
<td>Harvest Index</td>
<td>0.44a</td>
<td>0.32b</td>
<td>0.37a</td>
</tr>
</tbody>
</table>

$^{13}C$ and $^{15}N$ Assimilation and Remobilisation

At the first harvest, enriched $^{13}C$ was well distributed throughout the plant with the proportions in the various plant components mirroring the distribution of DM. In both genotypes the roots, leaves and stems each contained about a third of the total plant DM (Table 4.3). Similar proportions of the total enriched $^{13}C$ had been assimilated into each of these components (Table 4.4). At final harvest, 9% of the total enriched $^{13}C$ was found in the seed of control Tyson plants, and 7% in stressed Tyson plants (Table 4.4), while the seed DM represented 38% of the total plant DM in control plants and 29% in stressed plants (Table 4.3). In Kaniva, 4% of the total enriched $^{13}C$ was in control seeds and 2% in seed of stressed plants (Table 4.4), while the seed DM at maturity represented 20% and 9% of the total plant DM, respectively (Table 4.3). The proportion of $^{13}C$ in the stems at maturity was significantly lower in both treatments in either genotype than at first harvest but the proportion in the roots increased regardless of genotype or treatment (Table 4.4).

Table 4.3  Proportion of plant dry weight (%) in the stems, leaves, roots, pod walls and seed in glasshouse grown Tyson and Kaniva chickpea under well-watered and water deficit conditions at each harvest. Different letters within rows indicate significance at $P = 0.05$.

<table>
<thead>
<tr>
<th></th>
<th><strong>81 DAS</strong></th>
<th><strong>123 DAS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tyson</td>
<td>Kaniva</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Stressed</td>
</tr>
<tr>
<td>Stems</td>
<td>28d</td>
<td>26d</td>
</tr>
<tr>
<td>Leaves</td>
<td>33d</td>
<td>34d</td>
</tr>
<tr>
<td>Roots</td>
<td>36d</td>
<td>36d</td>
</tr>
<tr>
<td>Pod walls</td>
<td>2a</td>
<td>2a</td>
</tr>
<tr>
<td>Seed</td>
<td>2a</td>
<td>2a</td>
</tr>
</tbody>
</table>

-65-
Table 4.4  Proportion of $^{13}$C (%) in the stems, leaves, roots, pod walls (P. wall) and seed in glasshouse grown Tyson and Kaniva chickpea under well-watered and water deficit conditions at each harvest. Different letters within rows indicate significance at $P = 0.05$.

<table>
<thead>
<tr>
<th></th>
<th>81 DAS</th>
<th>123 DAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tyson</td>
<td>Kaniva</td>
</tr>
<tr>
<td>Stems</td>
<td>31b</td>
<td>38c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>36b</td>
<td>28a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>33b</td>
<td>33bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pod walls</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{15}$N was not as evenly distributed as the $^{13}$C. In both genotypes approximately two-thirds of the total enriched $^{15}$N was found in the leaves, 18% in the stems with the remaining 15% in the roots and pod tissues (Table 4.5). By maturity, 59 and 47% of the total enriched $^{15}$N was located in the seed of control and stressed Tyson plants, respectively. The proportion of $^{15}$N in the stems and leaves decreased over this same period with the biggest decrease occurring in the leaves which contained only 24 and 29% of the enriched $^{15}$N at maturity in control and stressed plants, respectively. In Kaniva, 33% of the enriched $^{15}$N was in the seed of control plants by maturity and 11% in the seed of stressed plants. Like Tyson there were significant reductions in the proportion of enriched $^{15}$N in the stem and leaf tissues, but there was also an increase in the proportion of $^{15}$N in the roots of both control and stressed plants (Table 4.5).

Table 4.5  Proportion of $^{15}$N (%) in the stems, leaves, roots, pod walls and seed in glasshouse grown Tyson and Kaniva chickpea under well-watered and water deficit conditions at each harvest. Different letters within rows indicate significance at $P = 0.05$

<table>
<thead>
<tr>
<th></th>
<th>81 DAS</th>
<th>123 DAS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tyson</td>
<td>Kaniva</td>
</tr>
<tr>
<td>Stems</td>
<td>17d</td>
<td>19d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td>68d</td>
<td>66d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roots</td>
<td>12b</td>
<td>12b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pod walls</td>
<td>1a</td>
<td>1a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed</td>
<td>2a</td>
<td>2a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Using the $^{13}$C and $^{12}$C data, the contribution of pre-podding and post podding C to seed C can be derived. Overall, the total mean seed C in Tyson was 3.36 g in control plants, 3.1 g of this was derived from C assimilated after podding began and 0.3 g remobilised from C assimilated prior to podding. In stressed Tyson plants the seed contained 1.8 g of C, 1.5 g assimilated after podding and 0.3 g remobilised pre-podding C from vegetative tissue. In Kaniva the seed of the control plants seed contained 2.3 g of C, 2.1 g
assimilated after podding and 0.2 g of remobilised pre-podding C. Stressed seed contained 0.6 g of C, 0.5 g derived from post-podding assimilation and 0.05 g derived from the remobilisation of pre-podding C (Table 4.6).

Like C, the $^{15}$N and $^{14}$N data was used to derive the contribution of pre-podding and post podding N to seed N. Comparatively, the contribution of pre-podding N was much higher (Table 4.6). Of the total 200 mg N in seed from well-watered Tyson plants, 170 mg was pre-podding N and 30 mg from post podding N, whereas in seed from stressed Tyson plants almost all of the N was remobilised pre-podding N. Similarly in seed from well-watered Kaniva, 90 mg of the 150 mg of total seed N was from pre-podding remobilisation and this contribution was substantially higher in seed from stressed plants (Table 4.6).

Table 4.6  Total seed C and N (mg/plant) and the C and N arising from post-podding fixation and uptake or from pre-podding remobilisation in glasshouse grown Tyson and Kaniva chickpea under both well-watered (control) and water deficit conditions (stressed). Percentage contribution of remobilised pre-podding C and N to total seed C and N shown in brackets.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total C</th>
<th>Post-pod fixation</th>
<th>Pre-pod remobilised</th>
<th>Total N</th>
<th>Post-pod uptake</th>
<th>Pre-pod remobilised</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td></td>
<td></td>
<td>Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TYSON</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3360</td>
<td>3058</td>
<td>302 (9)</td>
<td>200</td>
<td>30</td>
<td>170 (85)</td>
</tr>
<tr>
<td>Stressed</td>
<td>1800</td>
<td>1508</td>
<td>292 (16)</td>
<td>155</td>
<td>4</td>
<td>151 (97)</td>
</tr>
<tr>
<td>KANIVA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2284</td>
<td>2130</td>
<td>154 (7)</td>
<td>148</td>
<td>56</td>
<td>92 (62)</td>
</tr>
<tr>
<td>Stressed</td>
<td>626</td>
<td>574</td>
<td>52 (8)</td>
<td>53</td>
<td>5</td>
<td>48 (91)</td>
</tr>
</tbody>
</table>

4.4 DISCUSSION

In pot-grown plants there is usually only small volumes of soil moisture available for plant roots to explore and when water is withheld there can be a rapid decrease in plant water status and hence greater stress than under field conditions (Jordan and Ritchie, 1971; Ritchie, 1981). In this experiment the water deficit, in particular the initial rapid decrease in plant water status, had important implications for plant development with large decreases in pod number and seed yield when compared to those observed under field conditions (Chapter 3). The impact of water deficit on plants is modified by the speed at which water deficit develops and the stage of development when plants become stressed (Palta et al., 1994).

By the time the water deficit was imposed, ICCV88201 and Tyson had set more pods than Kaniva due to the faster rate of pod set in the desi genotypes compared to Kaniva. This faster rate of pod set in the desi genotypes was probably due to their smaller pods and seeds which require less assimilate per podding node. Faster rates of pod set are beneficial for tolerating water deficits. Although the water deficit largely prevented further pod set across all genotypes, Tyson and ICCV88201 had already set around 15
pods per plant compared to Kaniva which had only set 4 pods per plant by 88 DAS.

The proportion of unfilled pods was lower in stressed Tyson (36%) and ICCV88201 (47%) compared to Kaniva (79%). Therefore, Kaniva plants, with a slower rate of pod set, fewer pods and a high proportion of unfilled pods, suffered the greatest yield penalty with water deficit, yielding only 15% of the control compared to 39% and 43% in Tyson and ICCV88201, respectively. This confirms the importance of matching phenology in relation to the environment (Siddique et al., 1998a; Siddique et al., 1999b; Loss and Siddique, 1999). In Mediterranean-type environments, it is beneficial for chickpea subject to terminal drought to be able to set pods early when conditions are likely to be more favourable in a short growing season (Siddique and Sedgley, 1986; Sedgley et al., 1990). However, in the field, pods were set at a similar time for all plants regardless of genotype with pod set being limited by temperature rather than genotype (Chapter 2). Chickpea requires a daily mean temperature above 15°C for successful pod set (Siddique and Sedgley, 1986; Clarke and Siddique, 1998; Clarke et al., 1998).

Between the two desi genotypes there was no significant difference in seed yield in either control or stressed plants. Even under well-watered conditions Kaniva seed yield was only equivalent to the seed yield of stressed Tyson and ICCV88201. Low seed yield may be a consequence of low biological yield as high seed yield is closely correlated with high biological yield in field grown chickpea (Siddique and Sedgley, 1986). In this experiment, however, there were no differences in the total above-ground biomass produced between any of the genotypes within each treatment. Consequently, seed yield under well-watered conditions and maintenance of seed yield in plants subject to water deficit was closely associated with DM partitioning. Harvest index was decreased by 27% in Tyson, 29% in ICCV88201 and by 52% in Kaniva by the water deficit. Overall Tyson and ICCV88201 partitioned more DM into seed than Kaniva under both well-watered and water deficit conditions, resulting in higher harvest indices. The ability of the desi genotypes to set and produce pods earlier and more rapidly than the kabuli genotype enabled greater partitioning to the reproductive components.

Average seed weight was increased in both ICCV88201 and Kaniva under water stress which implies that C and N supply was increased on an individual seed basis in these two genotypes. The HI was significantly lower in stressed plants compared to well-watered regardless of genotype. This indicates that seed DW was decreased to a greater extent than the DW of the vegetative components in stressed compared to control plants. The magnitude of the increase in seed size in stressed plants reflected the size of the decreases in HI. Thus there was no significant increase in seed size in Tyson, a 31% increase in seed size in ICCV88201 and a 62% increase in seed size in Kaniva. The HI in stressed Kaniva decreased by 52% compared to the well-watered Kaniva plants. In Tyson and ICCV88201 the decrease in HI was 27 and 30%, respectively. Water deficit
greatly decreased the number of filled pods but by re-watering, to prevent premature plant death, photosynthesis was able to recover marginally resulting in increased assimilate to the remaining pods and hence larger seed.

Smaller decreases in HI in stressed Tyson compared to Kaniva suggests that differences in HI between stressed and well-watered plants may indicate greater remobilisation in stressed Tyson than stressed Kaniva. This is confirmed by the greater measured remobilisation in Tyson compared to Kaniva. Stable isotope studies, carried out concurrently on Tyson and Kaniva enabled the remobilisation of non-structural, vegetative C and N to be quantified. Changes in DW of vegetative components can also be used as an indicator of remobilisation. Water deficit can result in significant DW decreases from the vegetative plant parts (Pheloung and Siddique, 1991; Palta et al., 1994) indicating increased DM remobilisation. In this study both water deficit and genotype had an impact on the occurrence and extent of DW decreases. The contribution of pre-podding remobilised C to seed C was relatively low with the majority of seed C being derived from post podding assimilation. Remobilised pre-podding C contributed 9 and 16% of the seed C in control and stressed Tyson, and 7 and 8% in control and stressed Kaniva. In well-watered wheat and barley the contribution of pre-anthesis remobilised DM to grain DM is generally 10-12% (Bidinger et al., 1977; Austin et al., 1979), although, significant intra-specific variation has been reported (Rawson and Evans, 1971; Papakosta and Gagianas, 1991). In cereals subject to water deficit the contribution of pre-anthesis remobilised C to grain C was quite variable (17-64%) depending on timing, degree and rate of water deficit (Bidinger et al., 1977; Austin et al., 1979; Palta et al., 1994). The relative contribution of remobilisation in chickpea compares favourably with that reported in other well-watered pulses. For example, in well-watered common bean remobilisation contributed to 9% of the seed DM (Geiger and Shieh, 1988) and only 4% in soybean (Yamagata et al., 1987). However, the small increases in remobilisation in stressed chickpea is lower than that in stressed cereals. Whether this is common to other pulses is not clear as there are no reports of remobilisation of pre-podding assimilates in stressed pulses.

Water deficit decreased the absolute amount of C remobilised in Kaniva from 154 mg in control plants to 52 mg of remobilised C in stressed plants. Despite this, the relative contribution remained similar due to a similar sized reduction in total seed C. In Tyson the total amount of C remobilised to the seed was similar in both control and stressed plants, but the relative contribution of remobilised C was higher which is a pattern repeated in other studies (Bidinger et al., 1977). Clearly Tyson was better able to remobilise C than Kaniva when subject to water deficit with only a minor decrease in the amount remobilised compared to the 66% decrease in Kaniva. A water-deficit-induced decrease in the amount of post-podding C assimilated of 51% in Tyson and
73% in Kaniva reveals the relative inability of the kabuli genotype to tolerate water stress.

In cereals, the stems are the most important source of remobilised DM for grain filling (Pheloung and Siddique, 1991; Schnyder, 1993; Nicolas and Turner, 1993) and consequently decreases in stem DW during seed filling can be a useful indicator of remobilisation (Rawson and Evans, 1971; Blum et al., 1997). In cereals C is stored in the form of water-soluble carbohydrates (Schnyder, 1993) and higher contributions from remobilisation to seed filling can be closely associated with increased carbohydrate storage in the stems (Blum et al., 1997). In this study with relatively small amounts of remobilised C, it was difficult to determine the principal source of remobilised C. It appears, however, that the stems were the only components that significantly and consistently showed decreases in the proportion of $^{13}$C at the final harvest, although this does not correlate well with the small stem DW decreases.

Compared with C, the remobilised pre-podding N was vital for seed filling and seed protein content and played an even more important role in plants subject to water deficit. Remobilised pre-podding N contributed 85 and 97% of total seed N in control and stressed Tyson plants and 62 and 91% in Kaniva plants, respectively. This correlates well with rainfed chickpea in which 81% of the seed N was derived from remobilisation (Kurdali, 1996). Previous studies have shown that in many cereal and pulse species the contribution of remobilised N to seed N is high (Nicolas et al., 1985; Palta et al., 1994; Takahashi et al., 1996; Bushby and Lawn, 1992; Foster et al., 1995; Kurdali et al., 1997).

Post-podding fixed N made a relatively small contribution (15-30%) to seed N in well-watered plants and contributed less than 10% of seed N in stressed plants. This suggests that N fixation and uptake by the roots is particularly sensitive to developmental stage, water deficit and competition from other sinks. In well watered chickpea, N fixation was found to peak between flower bud initiation and flowering and then decreased markedly during podding and seed filling (Kurdali, 1996). The *Rhizobia* bacteria responsible for N fixation require a source of C for survival and continued N fixation (Herridge and Pate, 1977; Schubert, 1986), and competition for C by developing pods may explain the decrease in N fixation over this period (Hooda et al., 1989). Water deficit may exacerbate this effect on N fixation as a consequence of reduced C supply to the *Rhizobia* nodules, but prior to podding N fixation can decline prior to any visible stress symptoms such as wilting (Devries et al., 1989a). Consequently water deficit may also have a direct effect on N fixation and in chickpea drought has been implicated as being responsible for the inability of nodules to utilise assimilates (Hooda et al., 1989; Swaraj et al., 1995).
During their production and expansion, the leaves form a large and important sink for N. Leaves are the major site of photosynthesis in the plant and consequently contain high amounts of the photosynthetic enzyme Rubisco which contains high levels of N (Egli and Crafts-Brandner, 1996). During seed filling there is a large requirement for N (Sinclair and De Wit, 1975; Herridge and Pate, 1977) so that as seeds fill the N and chlorophyll content of the leaves decrease. This degradation of leaf protein ultimately leads to leaf senescence (Egli and Crafts-Brandner, 1996). Following the degradation of leaf protein the N and associated C can be translocated to developing seed (Peoples et al., 1983).

In chickpea, the leaf N content at the first harvest was about 4% of leaf DW which made up 37% of the plant DW while the N content of the stems and roots was only 2% of their DW which made up 63% of the total DW. With subsequent harvests, the N content of the leaves fell to 1-2% of leaf DW. This decrease was also reflected in a large decrease in the proportion of total plant enriched $^{15}$N found in the leaves. In Tyson leaf $^{15}$N decreased from 68% at 81 DAS to 24 and 29% at maturity in the control and stressed plants, respectively. Over this same period, seed $^{15}$N increased to 59 and 47% of the total plant $^{15}$N by the final harvest. For Kaniva the decrease in the proportion of $^{15}$N in the leaves was similar to that in Tyson from 66% at 81 DAS to 27 and 34% of the enriched $^{15}$N at maturity in control and stress plants, respectively. By maturity seed $^{15}$N accounted for 33 and 11% of the total plant enriched $^{15}$N.

Stressed plants of both genotypes retained a higher proportion of $^{15}$N in the leaves and a lower proportion of $^{15}$N was remobilised to the seed. Low sink demand as a consequence of a large number of unfilled pods in the water-stressed plants can result in a reduced demand for remobilised N (Devries et al., 1989). Increased proportions of $^{15}$N in the stems and roots of water-stressed Kaniva suggests that though much of the N from the leaves was mobilised, partitioning of this N was limited by low sink strength, leading to an accumulation of N in the root and stem tissues. The concentration of N in the seed at 123 DAS was significantly higher in stressed Tyson (3.8%) and Kaniva (3.6%) than in the control plants (2.8%) of either genotype, suggesting that transport and partitioning of N to Kaniva seed was sufficient to meet the low sink demand in the stressed plants.

In both control and stressed Tyson plants, there was an increase in the proportion of $^{15}$N in the pod walls from 81 to 95 DAS followed by a decrease at the final harvest, 123 DAS. N may potentially increase in the pod wall as a temporary N pool during pod development and then be moved to the seed during rapid seed filling and pod maturation. In field pea it is estimated that up to 20% of the N in the seed is derived from the pod wall (Rochat and Boutin, 1989). However in chickpea, the amount of N moved from the pod wall to the seed appeared to be relatively small.
This experiment confirmed that genotypic variation for tolerance to water deficit exists in chickpea. Although a limited number of genotypes (3) were used in the study, they represent the best performing commercial cultivars and advanced breeding lines tested and selected in a wide range of rainfed environments in Australia. Kaniva, the kabuli genotype, is less able to tolerate water deficit than the desi types (ICCV88201 or Tyson). The proportion of unfilled pods was higher in Kaniva and the yield loss for water stressed plants was greater. Similar observations have been recorded in the field and generally kabuli types yield 30 to 50% less than the best desi genotypes especially in low rainfall environments (Siddique et al., 1999). In Tyson the seed DW was 44 and 32% of the total plant DW in control and stressed plants, respectively. For ICCV88201 this fell to 37 and 26% and in Kaniva to 21 and 10% of the total plant DW in control and stressed plants, respectively. In the case of the stressed plants this result is strongly influenced by the high proportion of unfilled pods. However, even in the control plants Kaniva invested less in the seed. Poor DM partitioning to the seed is further exemplified by poor remobilisation of C and N from the vegetative components to the seed in control and stressed Kaniva plants. Tyson and ICCV88201 set pods faster than Kaniva and were able to partition a greater proportion of their DM, C and N to the seed.

Although net decreases in the DW of the vegetative parts over-estimate the contribution of remobilisation they appear to be correlated with remobilisation. Kaniva, while still having some DW decreases in vegetative tissue, was estimated to have less remobilisation than Tyson, as much of the DW loss could be accounted for by increases in stem DW. Therefore, it may be possible to use DW changes as an indicator in the selection of genotypes with better ability to remobilise vegetative DM for seed filling. Selection for varieties that are better able to partition C and N to the seed, whether it be from remobilisation or current assimilation and fixation, would be a useful strategy in improving the performance of chickpea, particularly when subjected to water stress.
CHAPTER 5

DISTRIBUTION OF REMOBILISED CARBON AND NITROGEN IN CHICKPEA SUBJECT TO WATER DEFICIT

5.1 INTRODUCTION

Water deficit is accompanied by a substantial decrease in the rate of net leaf photosynthesis in chickpea grown both in field and glasshouse environments (Chapters 3 and 4). In response to a decreasing assimilate supply and a high demand from the developing seed, the remobilisation of C and N assimilated prior to flowering can become an important source of total seed C and N in wheat (Palta et al., 1994). In Chapter 4 the contribution of remobilised pre-podding C and N to total seed C and N was quantified in two contrasting chickpea genotypes using $^{13}$C and $^{15}$N stable isotopic techniques. Overall the contribution of remobilised pre-podding C was 7-9% of seed C in well-watered plants and increased to 8-16% in those subject to water deficit. In cereals, C remobilised from the pre-anthesis vegetative parts to seed can account for up to 64% of seed C in plants subject to water deficit (Palta et al., 1994). N remobilisation contributed between 62-85% of seed N in well-watered plants, which is similar to levels reported by Kurdali (1996), and 91-97% in chickpea plants subject to water deficit.

In Chapter 4 changes in the proportion of $^{12}$C and $^{15}$N in the leaves, stems, pod walls and roots of chickpea indicated that leaves were the most important source of remobilised C and N. In chickpea, C fixed by the subtending leaves is primarily transported to its associated pod with less C movement to other pods (Singh and Pandey, 1980). Photosynthate movement from one branch to another is relatively small so each branch on the plant works as a partially independent sub-unit of the whole plant (Singh and Pandey, 1980). Movement of remobilised assimilates is probably subject to the same transport limitations implying that C and N remobilised from subtending leaves may be preferentially directed to their associated pods (Chapter 4).

Chickpea has an indeterminate growth habit and flowers, pods and seeds develop sequentially along branches (Khanna-Chopra and Sinha, 1987). Under terminal drought, pods and seeds that are formed later in the upper parts of the canopy may be subject to more severe water deficit during seed filling than pods formed early in development in the lower parts of the canopy. Consequently, the distribution of remobilised C and N may be higher in seeds filling later when competition for assimilates is high and current C assimilation is very low. This hypothesis therefore requires investigation.
In this Chapter sequential pod and seed development of the desi chickpea line ICCV88201 was analysed to determine whether patterns of seed development alter with pod age (position) and whether water deficit affects these patterns. The changing importance of C and N remobilisation to seed was examined by analysing the movement of $^{13}$C and $^{15}$N to seed set at different times during growth under well-watered or water deficit conditions. Seeds were grouped to represent different ages and positions in the canopy. Pod walls and leaves were grouped according to the same categories and analysed separately. So that the importance of subtending leaves in C and N remobilisation could be assessed, basal leaves without pods and upper leaves with pods were measured separately.

The hypotheses tested were: that seeds filling later in the reproductive phase have faster seed growth rates and shorter duration’s of seed growth than seeds filling earlier, particularly in chickpea subject to water deficit; seeds that develop later are more dependent upon remobilised assimilates to maintain seed filling in chickpea subject to water deficit than lower seed which fill earlier; the subtending leaves are the most important source of remobilised assimilates for the associated seed.

5.2 MATERIALS AND METHODS

In this study ICCV88201, an ICRISAT-bred sister line to the recently-released cultivar Sona (tested as ICCV88202), was used. Soil and pot preparation was carried out according to the procedures used in Chapter 4 with only one variation. Instead of sowing seed directly into the prepared pots the imbibed seed were first germinated in moist potting mix. Once the shoot had emerged two similar-sized germinated seedlings were transplanted to each of 104 pots on 1 July 1997. Plants were well watered and grown in a temperature-controlled, evaporatively-cooled glasshouse. Temperature in the glasshouse was maintained at 23°C during the day and decreased with ambient temperatures at night (approximately 12-15°C). Plants started flowering at 59 DAS and started podding on 71 DAS. At 93 DAS water was withheld from half the pots while the other half were kept well-watered.

Four well-watered and four droughted pots were used to measure pre-dawn LWP and midday photosynthesis using the same techniques as in Chapter 4. Pre-dawn LWP was measured eight times following the commencement of the two watering treatments at 94, 101, 105, 107, 112, 116, 119 and 123 DAS. The rate of net photosynthesis was measured when weather permitted on cloudless days between 1100 and 1500hrs. Measurements were made at 95 and 107 DAS. Droughted pots were watered with 200 mL of water five times after the start of watering regimes, at 104, 107, 111, 114 and 118 DAS, in order to prevent premature plant death.
In 24 control and 24 stressed pots, pods were tagged when the pod walls became clearly visible (size 6 mm long) beyond the senescing petals (6-7 days after flowering). All pods set on the 30 September (91 DAS), 7 October (98 DAS), 14 October (105 DAS), and 21 October (112 DAS) were tagged.

Six harvests were carried out at weekly intervals at 93, 99, 106, 113, 120 and at maturity, 156 DAS. At each harvest, the date of pod set of every node was determined based on the rate of addition of new podding nodes and the date of pod set of the labelled pods. Pods were grouped according to their pod set date and dried separately. Dry weights of pod walls and seeds were measured individually. All leaves and stems were dried and weighed for each plant.

Dry weight increases for each group of seeds (where enough replicates were available) was fitted to the equation utilised previously (Chapter 4). Seed development was analysed on a per plant and per seed basis.

Plants in a second set of 24 control and 24 stressed pots were labelled with stable isotopes using the same technique used in Chapter 4. Stable isotope labelling was carried out three times prior to podding. Plants were labelled with $^{13}$C at 53, 64 and 77 DAS and $^{15}$N at 57, 69 and 78 DAS. Pod labelling and harvesting was carried out on the same dates with plants sampled for DW. At each harvest, seeds, pod walls and leaves were placed in groups on the basis of their position along the branch and these categories were measured separately in order to quantify any differences in the importance of remobilisation to pods and seeds of varying age. Five categories were defined:

1. Lower pods, set prior to 86 DAS, prior to the start of watering regimes (92 DAS);
2. Mid-lower pods, set between 86-90 DAS;
3. Mid pods, set at 91 DAS, when water regimes started;
4. Mid-upper pods, set 92-98 DAS;
5. Upper pods, set 99 DAS onwards.

The pods and leaves from each of these categories were harvested, dried and measured separately for DW and then analysed for $^{13}$C and $^{15}$N. Plants were further divided into their remaining components, basal leaves without associated pods, basal stems without podding nodes, and upper stems with podding nodes, and roots and were frozen in liquid nitrogen and freeze dried (Dynavac, Model FD12). Seed growth rate and duration of categorised seed was also measured using the technique used previously in Chapter 3.

After DW measurement, the various plant components were ground to powder (particles less than 1 mm). Samples were kept dry and 5 mg sub-samples were weighed into small
aluminium capsules which were then rolled into balls. Balls containing the labelled subsamples were loaded into a dual head mass spectrometer (VG-Micromass Sira-10, V-G Isogas Ltd., Middlewich, England) connected to a Europa Roboprep C-N Analyzer (Europa Scientific Ltd., Crewe, England) which measured total C, total N and % $^{13}$C and % $^{15}$N for each sub-sample.

Four plants which were not labelled with stable isotopes were also harvested at 93 DAS and divided into leaves, stems, roots and pods. These samples were prepared as described above and natural abundance levels of $^{13}$C and $^{15}$N were measured using the mass spectrometer. The amount of enriched $^{13}$C and $^{15}$N was calculated in each of the samples using was calculated using the equations described in Chapter 4 (Palta et al., 1994).

5.3 RESULTS

Leaf water potential and pod number

It was intended that restricted re-watering of the stressed plants would result in the pre-dawn LWP being maintained at a low and constant level. Pre-dawn LWP were lower in stressed plants compared to control plants (Fig. 5.1), but stressed plants went through changes in pre-dawn LWP as a result of re-watering. Pre-dawn LWP in stressed plants ranged from -1.0 to -2.1 MPa (average -1.4 MPa) which was significantly lower than the -0.65 MPa maintained in control plants. After 120 DAS there was no further watering and LWP fell to -2.3 MPa in control and stressed plants (Fig. 5.1).

Pod setting commenced at 71 DAS and by the first harvest (93 DAS; Fig. 5.2) 30-35 pods per plant had set, before the commencement of water stress treatment. Pods continued to set in both control and stressed plants although the number of new pods was reduced in stressed plants resulting in a decrease in filled pod number from 49 to 33 pods per plant in the period from 107 to 120 DAS. This followed the initial drop in pre-dawn LWP for stressed plants from -0.6 MPa to -1.5 MPa by 98 DAS with a second decrease to -2.0 MPa by 107 DAS (Fig. 5.1). In well-watered plants pod number stabilised from 115 DAS onwards at about 75 pods per plant. Final pod number was reduced in stressed plants by 55% compared to control plants (Fig 5.2).

Seed development and yield

Reduced pod and seed number resulted in the stressed plants only attaining half the yield of control plants. On average, control plants yielded 12.3 g of seed/plant (Fig. 5.3a) compared to 6.2 g seed/plant for stressed plants (Fig. 5.3b). Continued pod production in well-watered plants was accompanied by increased pod wall DW to 115 DAS (Fig. 5.3a). By comparison pod wall DW peaked earlier in stressed plants at about 105 DAS (Fig. 5.3b). Similarly, seed filling commenced later, at about 107 DAS, in the well-watered control plants (Fig. 5.3a) compared to 101 DAS in stressed plants (Fig. 5.3b).
Figure 5.1. Changes with time in pre-dawn water potential of the uppermost expanded leaves in well-watered (●) and water-deficient (○) chickpea grown in the glasshouse. Bars represent +/- one standard error of the mean of 4 replicates where values are greater than the size of the symbols.
Figure 5.2. Changes with time in pod (■, □) and seed number (●, ○) in well-watered (control, ■, ●) and water-deficient (stressed, ○, □) glasshouse-grown chickpea. Bars represent +/- one standard error of the mean of 8 replicates where values are greater than the size of the symbols.
Figure 5.3 Changes with time in pod wall (■, □) and seed (○, ◦) dry weight (a and b) and leaf (▽, △) stem (▲, △) and root (●, ◆) dry weight (c and d) in well-watered (a and c) and water-deficient (b and d), glasshouse-grown chickpea. Bars represent ± one standard error of the mean of 8 replicates where values are greater than the size of the symbols.
During the seed filling phase the leaf DW decreased in control plants from 7 to 5 g/plant (Fig. 5.3c) and in stressed plants (Fig. 5.3d) from 6 to 4 g/plant between maximum observed DW and DW at maturity. Root DW decreased from 4 to 3 g/plant in both control and stressed plants. In control plants stem DW increased to almost 7 g/plant and in stressed plants it increased to 6 g/plant after which the stem DW remained stable irrespective of treatment (Fig. 5.3c,d). Assuming no DW loss through respiration and organ senescence, an estimated 3 g of plant DM was remobilised from the vegetative tissues. This suggests that up to 23 and 48% of the seed DW could be remobilised from the vegetative tissues in control and stressed plants, respectively.

From fitted logistic curves, rapid seed growth occurred between 108-123 DAS in well-watered plants and between 100-118 DAS in stressed plants (Fig. 5.4a). Maximum seed growth rate was lower in stressed plants (0.19 g/plant/day; Fig. 5.4b) compared to control plants (0.56 g/plant/day; Fig. 5.4b), but the duration of seed growth was similar regardless of treatment (days). Estimates of final seed DW from fitted curves were lower compared to those measured directly with an estimated yield of 11.7 g seed in control plants and 5.2 g/plant in stressed plants, a 46% reduction (Fig. 5.4a).

Sequential seed development on both a per plant and per seed basis was examined according to the time of pod set. At maturity, the average seed weight in the control plants was similar in all pods regardless of when they were set (Fig. 5.5a), but it was smaller than in seed from stressed plants (Fig. 5.5b). In stressed plants, average seed weight was consistent for pods set between 77 and 99 DAS, but there was some variation in pods set prior to and after this period (Fig. 5.5b).

For each date of pod set, the average number of filled and unfilled pods per plant was determined at final harvest. Those pods that set but later abscised were not included. On control plants, fewer filled pods were set at podding times prior to 85 DAS, following which there was an increase in the number of filled pods at each podding time (Fig. 5.5c). In stressed plants there were considerably fewer pods set after 77 DAS compared to the control plants (Fig. 5.5d), despite watering treatments not being imposed until 93 DAS. Unfilled pod number increased with later pod set times in both control and stressed plants (Fig. 5.5e,f). Despite a lower absolute number of unfilled pods on stressed plants at final harvest, the proportion of unfilled pods was higher (Fig 5.5f). In both control and stressed plants those pods set on the earliest dates always filled.
Figure 5.4. Change with time in (a) total seed dry weight and (b) growth rate in well-watered (control) and water-deficient (stressed) glasshouse-grown chickpea. Data, including +/- standard error (dotted line), derived from parameters estimated by fitting sigmoid curves to dry weight data.
Figure 5.5. Average seed weight (a and b) filled (c and d) and unfilled (e and f) pod number, and seed yield (g and h) of pods set on different dates in glasshouse grown chickpea plants grown under well-watered (control) and water deficient (stressed) conditions. Bars represent one standard error of the mean of 3-4 replicates.
On average, seed DW for each date of pod set tended to reflect the number of pods set in control plants (Fig. 5.5g). In stressed plants, the seed DW obtained was similar for pods set between 75 and 102 DAS (Fig. 5.5h). In stressed plants pods set after treatment (93 DAS onwards) bore one-third (33%) of the total seed DW in stressed plants compared to control plants in which these pods contributed almost half (47%) of the total seed DW.

Seed growth was also analysed for each date of pod set on a per seed basis. Parameters were estimated from curves fitted to the data. The seed DW in control plants was consistent across the range of pod set times (Fig. 5.6a), with there being only slightly more seed DW in the earliest set pods whereas in stressed plants seed DW varied considerably with different pod set dates (Fig. 5.6b) which was probably a consequence of rewatering at different times after drought was imposed. Maximum seed growth rate did not substantially vary for seed from control plants irrespective of when the pods were set (Fig. 5.6c) while in stressed plants the maximum seed growth rates were higher in pods set later (Fig. 5.6d). For well-watered plants the average rate of seed growth decreased in pods set later (Fig. 5.6e) while the duration of seed growth also gradually increased in later set pods (Fig. 5.6g). In stressed plants there was no consistent pattern for average seed DW (Fig. 5.6f), while, seed growth duration was similar regardless of when the pods were set (Fig. 5.6h).

Remobilisation and distribution of dry matter

At the first harvest (93 DAS) 40% of the total plant dry matter was in the leaves, 30% in the stems and 22% in the roots (Figs. 5.7a,b). At maturity (156 DAS) 40% of plant DM was partitioned to the seed in control plants (Fig. 5.7a) and 30% in stressed plants (Fig 5.7b). At the first harvest the allocation of enriched $^{13}$C mirrored the distribution of DM with about 43% in the leaves, 35% in the stems and 15% in the roots but at the final harvest only 16% (Fig. 5.7c) and 10% (Fig. 5.7d) of the total enriched $^{13}$C was in the seed of the control and stressed plants, respectively. By comparison, the majority (70%) of enriched $^{15}$N was in the leaves at 93 DAS with only 15% in branches (Figs. 5.7e,f), but by final harvest 66% of the enriched $^{15}$N was in the seed of well-watered plants (Fig. 5.7e) and 60% in stressed plants (Fig. 5.7f). Corresponding to this large increase in seed-enriched $^{15}$N was a fall in the proportion in the leaves which contained 16-18% regardless of treatment. There was no significant variation in natural abundance of $^{15}$N (0.378±0.001%) and $^{13}$C (1.080±0.0004%) in any of the plant components.
Figure 5.6. Final seed dry weight (a and b), maximum (Max.) seed growth rate (c and d), estimated average (Av.) seed growth rate (e and f) and duration of seed growth (g and h) of seed in pods which had set at different times throughout reproductive development in well-watered (control) and water-deficient (stressed) glasshouse-grown chickpea. Bars represent one standard error of the mean of 4 replicates.
Figure 5.7. Changes with time in allocation of dry matter (a and b), $^{13}$C (c and d) and $^{15}$N (e and f) to seed, pod walls, stems, leaves and roots as a proportion of total dry matter, $^{13}$C and $^{15}$N in well-watered (control) and water-deficient (stressed) glasshouse-grown chickpea.
The distribution of the stable isotopes and DM varied in the leaves along a branch. In the early formed leaves (with pods set between 87-90 DAS), the concentration of enriched $^{13}$C was substantially higher than that in later formed leaves (those with pods set between 92-98 DAS and after 99 DAS) regardless of treatment (Fig. 5.8a,b). Higher concentrations of enriched $^{13}$C in the leaves with pods set between 87-90 DAS is representative of the basal leaves and all the leaves with pods set up to 91 DAS (data not shown). Similarly the concentration of enriched $^{15}$N was lower in the youngest leaves compared to the concentration in those which were present during labelling (as represented by leaves with pod set between 87-90 DAS) in both control (Fig. 5.9a) and stressed plants (Fig. 5.9b).

The concentration of total N (%) was examined in leaves of different age as an indicator of senescence and leaf N remobilisation. The pattern of leaf N decline was similar for all the leaves of different age (Fig 5.10). However, by the first harvest the older leaves already contained only 3-4% leaf N (Fig 5.10a,b) while the concentration of N in the younger leaves was still 4-5% (Fig. 5.10c,d). While the decline in leaf N tended to occur earlier in plants subject to water deficit, this difference was generally insignificant and by physiological maturity most of the leaves contained about 1.5% leaf N regardless of treatment.

In well-watered plants the later formed seed from pods set after 92 DAS, contained 71% of the total seed C at maturity compared to 43% in the equivalent seed from stressed plants (Table 5.1). In well-watered plants pre-podding C contributing only 6% of the total seed C in pods set after 99 DAS compared to 16% in early formed seed from pods set before 86 DAS. Similarly, in stressed plants pre-podding C contributed only 8% of the total seed C of later-formed seed compared to 19% of the C in early-formed seed. The proportion of seed C derived from pre-podding was always higher in stressed plants compared to control plants regardless of pod age so that overall remobilised pre-podding C contributed 9% of the total seed C in well-watered plants and 13% in water stressed plants (Table 5.1).
Figure 5.8. Changes in concentration (%) of enriched $^{13}$C with time in leaves of different age (defined by the timing of pod set except the basal leaves with no pods) from glasshouse-grown chickpea under well-watered (a, closed symbols) and water-deficit (b, open symbols) conditions. Bars represent +/- one standard error of the mean where values are greater than the size of the symbols.
Figure 5.9. Changes in concentration (%) of enriched $^{15}$N with time in leaves of different age (defined by the timing of pod set except the basal leaves with no pods) from glasshouse-grown chickpea under well-watered (a, closed symbols) and water-deficit (b, open symbols) conditions. Bars represent +/- one standard error of the mean where values are greater than the size of the symbols.
Figure 5.10. Changes in concentration (%) of leaf nitrogen with time in leaves of different age (defined by the timing of pod set except the basal leaves with no pods) from glasshouse-grown chickpea under well-watered (control, •) and water-deficit (stressed, ○) conditions. Bars represent +/- one standard error of the mean where values are greater than the size of the symbols.
Figure 5.11. Changes with time in the contribution of post-podding assimilated (solid line) and pre-podding (dashed line) remobilised C and N as a proportion of the final, total seed C and N in well-watered (a and c) and water-deficient (b and d) glasshouse-grown chickpea.
Table 5.1. Contributions of remobilised pre-podding C and N and post-podding C and N to total seed C and N in seed from pods of different age on well-watered (control) and water-deficient (stressed) glasshouse-grown plants. Percentage contribution of remobilised pre-podding C and N to total seed C and N shown in brackets.

<table>
<thead>
<tr>
<th>Category</th>
<th>SEED C (mg/plant)</th>
<th>SEED N (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Post-pod assimil.</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>Stressed</td>
</tr>
<tr>
<td>Upper</td>
<td>1775</td>
<td>332</td>
</tr>
<tr>
<td></td>
<td>1938</td>
<td>705</td>
</tr>
<tr>
<td>Mid-Lower</td>
<td>444</td>
<td>269</td>
</tr>
<tr>
<td>Mid-Lower</td>
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<td>411</td>
</tr>
<tr>
<td>Lower</td>
<td>531</td>
<td>705</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5295</td>
<td>2423</td>
</tr>
</tbody>
</table>
The contribution of pre-podding N to seed N was much higher than for C (Table 5.1). In control plants pre-podding N contributed 39 and 100% of the seed N in the latest and earliest formed seed, respectively. In stressed plants remobilised N accounted for 42% of the N in the latest-formed seed and 100% of the N in the earliest-formed seed. Overall 55% of the seed N in control plants and 93% in stressed plants was derived from pre-podding N (Table 5.1).

The relative contribution of remobilised pre-podding C and N to total seed C and N was also examined over time (Fig. 5.11). In both control and stressed plants small amounts of remobilised C were invested in the seed from the beginning of seed fill until maturity (Fig. 5.11a,b). The fastest rate of total seed C gain occurred between 106-120 DAS in well-watered plants (Fig. 5.11a). This high demand for C was primarily met by post-podding assimilation, but there was a small increase in the contribution of pre-podding C over this period. In stressed plants the proportion of pre-podding C to total seed C was marginally higher throughout seed development compared to the control plants. Pre-podding C reached its maximum at around 113 DAS after which most of the seeds C continued to be supplied by post-podding assimilation (Fig. 5.11b).

The contribution of pre-podding N to seed N in control plants increased rapidly from the start of seed filling, but its contribution slowed after rapid seed filling was completed at 120 DAS (Fig. 5.11c). By comparison, in stressed plants, the contribution of pre-podding N increased steadily throughout seed filling such that by maturity most of the seed N was derived from remobilised pre-podding N (Fig. 5.11d).

5.4 DISCUSSION

Being an indeterminate annual grain legume, chickpea under favourable environmental conditions is able to produce more flowers, pods, seeds and leaves while pod and seed abortion restrict pod and seed number particularly in response to environmental stress. This plasticity in pod and seed number in response to the environment has important implications for both C and N remobilisation to the seed and seed growth.

In well-watered plants the average seed DW was consistent regardless of when the pod was set despite differences in pod number over time. The number of pods set on any particular day increased over time, but these increases were tempered by increases in the rate of seed abortion. Consequently, a substantial number of the pods formed later in plant development remained unfilled at maturity. High rates of seed abortion in later formed pods are probably due to high levels of competition for the available assimilates and the effect of older pods in inhibiting the development of younger pods (Tamas et al., 1979).
In the well-watered plants the average seed DW appeared to be maintained in pods of different age through modification to the duration of seed growth which may also be determined by competition. In the earlier set pods, the duration of seed growth was shorter and the average seed growth rate higher than in those pods set later. Pods set earlier have less competition for assimilates from other pods possibly enabling them to be filled more rapidly than those set later in which the competition for assimilates is intense. Under these conditions the flexibility of the well-watered plants to increase seed growth duration in the later set pods enabled the average seed DW to be maintained over the whole plant. In stressed plants, the average seed DW was more variable indicative of the variation in assimilate supply in these plants. Despite this variation, the average seed DW was generally higher in the stressed plants compared to the control plants presumably due to an increase in the source:sink ratio. Sink size was reduced through both a decrease in the rate of pod and seed set, and a proportionally greater number of unfilled pods. In the stressed plants, the duration of seed growth did not vary with pod age so that the seed size reflected differences in seed growth rate. The large seed size in stressed plants suggests that the assimilate supply may not have been limiting in relation to reduced sink size (seed number), so that the final seed size reflected the maximum potential size of the seed.

Seed filling was largely completed by 135 DAS in both the control and stressed plants and the end of seed filling can be associated with the depletion of N reserves available for remobilisation (Munier-Jolain et al., 1996). Declining leaf N was used as indicator of senescence, however, the pattern of leaf N loss did not vary substantially in plants subject to water deficit. The decline in leaf N was largely completed by 120 DAS except in the youngest leaves in which the N concentration at this time was continuing to decline. In the previous study (Chapter 4) the larger seed in stressed plants had a higher N concentration than that in control seed indicating that N was not limiting. This also appears to be the case in this study with the N concentration in both the stems and roots being lower in control plants compared to the stressed plants. Seed N (%) was also higher in the stressed plants (4.0%) compared to the control plants (3.4%; data not shown).

From these results it can be concluded that the final seed size is determined by assimilate supply and the extent of competition between sinks within a plant, i.e. source:sink ratio. In pulses, the losses in seed yield due to reduced pod and seed number can be partially compensated for by an increase in average seed DW (Spaeth and Sinclair, 1984b; Schonbeck et al., 1986; Zaiter and Barakat, 1995; Palta and Ludwig, 1998). Where assimilate supply is not limiting, the seed size obtained will be determined by the maximum potential seed size as determined by the number and volume of cotyledonary cells (Egli et al., 1981; Guldan and Brun, 1985; Sexton et al.,
Furthermore, individual seed growth rate is highly correlated with cotyledon cell number (Egli et al., 1981; Guldan and Brun, 1985; Wang and Hedley, 1993) and seed size in pulses (Egli and Crafts-Brandner, 1996). Assimilate supply influences cell division and consequently the final cotyledonary cell number such that increased supply results in more cells (Egli et al., 1989) which indicates that the differences measured in seed growth rate and size may be due to the influence of assimilate supply at an earlier stage on cell number.

Remobilisation of vegetative dry matter enables assimilate supply to the developing seed to be maintained when assimilate supply from current photosynthesis is limited. Dry matter remobilisation in crop species has been estimated by measuring DW changes in the vegetative plant components (Khanna-Chopra and Sinha, 1987; Leport et al., 1998). This technique has limitations because DW decreases are also a consequence of respiration and organ abscission (Chapter 4). In this study, the DW of the leaves and roots decreased during the seed filling phase implying that assimilates were remobilised from these tissues in order to provide assimilates for seed filling. The contribution of remobilised vegetative DM to seed DM was estimated to be 35% in control and 54% in stressed plants which compares reasonably well with field grown chickpea, in which it was estimated that 33% of pod DM was derived from remobilisation (Khanna-Chopra and Sinha, 1987). However, increases in enriched $^{13}$C in the seed of both control and stressed plants indicate that remobilisation of pre-podding C to total seed C ranged from 9% in the controls to 16% in stressed plants. In this study the leaves were the primary source of remobilised $^{13}$C and were the only vegetative components in which the proportion of enriched $^{13}$C substantially declined in both control and stressed plants.

N fixation is more sensitive to drought than photosynthesis (Devries et al., 1989; Hooda et al., 1989) and so N remobilisation is a vital source of seed N. Leaves with their high N content relative to the other vegetative components are the primary source of remobilised N. Leaf senescence is an indicator of the breakdown of the N-rich leaf proteins (Rubisco) into amino acids which are then available for translocation to the seed (Peoples et al., 1983; Egli and Crafts-Brandner, 1996). N concentration was highest in the youngest leaves, regardless of treatment, which implies they were more photosynthetically active during early reproductive development and consequently had higher concentrations of protein N or may be due to less N remobilisation from the younger leaves. In albus lupin (Lupinus albus L.), subject to water deficit younger leaves were found to contain higher concentrations of Rubisco and protein than older leaves (David et al., 1998). Overall, leaf N concentrations decreased to similar low levels in all leaves indicating the remobilisation of N from all the leaves. Small decreases in the proportion of N in the pod walls, branches and roots indicates that they contribute relatively small amounts of remobilised N (Hooda et al., 1986; Peoples et al., 1983).
It was expected that seeds set later in reproductive development when assimilate supply and competition was high would be more dependent upon remobilised DM, but the proportion of remobilised C and N was higher in seed from earlier set pods compared to that from later-set pods, regardless of treatment. The relative significance of pre-podding C remobilisation was considerably higher in earlier formed seed (pods set before 86 DAS) with an estimated 19% of seed C coming from pre-podding remobilisation in stressed plants and 16% in control plants. In later formed seed (pods set after 99 DAS) the relative contribution was lower, representing only 6% of total seed C in well-watered and 8% in water-stressed plants. Similarly, all of the seed N was derived from remobilisation in the earliest set seed, while for seed formed in pods set after 99 DAS only 39% of seed N was from pre-podding N in well-watered plants and 42% in water-stressed plants.

Stable isotope labelling enables the total contribution of pre-podding remobilised C and N to the seed to be measured (Palta et al., 1994; Chapter 4). However, the technique does not take into account the remobilisation of N from leaves, pod walls and stems formed post-podding in indeterminate species in which vegetative growth occurs after isotope labelling. The later formed leaves were not present during isotope labelling and consequently they contained lower concentrations of $^{13}$C and $^{15}$N than the early formed leaves present during labelling. This will result in an underestimation of the contribution of remobilisation to the seed in later formed pods. Furthermore, it explains why remobilised pre-podding N contributed most of the seed N until mid-way through seed development in control plants, after which its contribution decreased significantly when N taken up after podding was subsequently being remobilised from the later formed leaves. Overall, in well-watered plants 43% of the seed N was derived from post-podding assimilation compared to 12% in stressed plants, but some of this would also be remobilised from vegetative tissues formed after podding (Zeiher et al., 1982; Egli et al., 1983).

Clearly pre-podding assimilate remobilisation from vegetative tissues to seed is an important source of assimilates significantly contributing to filling seed in early formed pods. However post-podding assimilate remobilisation from vegetative tissues formed after pod set has begun may also be an important assimilate source particularly from later formed leaves to their associated pods and seeds and this requires further examination.
Pod set and seed filling in chickpea grown in the Mediterranean-type environment of south-west Australia occur with increasing drought and spring temperatures (Leport et al., 1998, 1999). The decline in plant water status in spring results in a decrease in stomatal conductance and photosynthesis to low levels in chickpea regardless of genotype (Leport et al., 1999). Consequently, in this environment photosynthesis is low when seed filling commences resulting in reduced seed yield and seed size.

The adaptation of chickpea to terminal drought could be improved by selecting for genotypes with improved drought avoidance. Drought avoidance involves development of genotypes with shorter phenological stages so that plants can mature prior to being significantly affected by drought or by increasing developmental plasticity enabling plants to respond according to seasonal conditions (Subbarao et al., 1995; Turner et al., 1999). In pulses, selection for rapid phenological development has been successfully achieved by selecting for early flowering and podding (Thomson et al., 1997; Siddique et al., 1999b). Adaptation of faba bean to Mediterranean-type climates has been improved by selecting genotypes which flower early (Mwanamwenge et al., 1998). However, early flowering in chickpea does not result in early podding due to pod set being delayed until the mean daily average temperature reaches 15°C or higher regardless of flowering time (Lawlor et al., 1998; Srinivasan et al., 1998; Leport et al., 1999). Furthermore, slow rates of leaf area and DM production in chickpea compared to other pulses (Siddique et al., 1993; Thomson and Siddique, 1997; Mwanamwenge et al., 1997) suggests that selection for shorter phenological development is likely to restrict plant biomass and hence seed yield potential. Successful pod set and rapid rates of vegetative DM production at low temperatures need to be improved to maximise seed yield in chickpea before the duration of plant development can be decreased.

With an indeterminate growth habit, chickpea does have a high degree of developmental plasticity with a large capacity to respond to seasonal conditions whether they be favourable or unfavourable (Turner et al., 1999). Chickpea seed yield is substantially improved in wetter-than-average seasons (Leport et al., 1999) compared to drier-than-average seasons (Leport et al., 1998) in terminal drought-prone environments. In the field study, chickpea produced substantially more pods and seeds with higher average seed weight and seed yield when irrigated during flowering and podding compared to rainfed chickpea, demonstrating the ability of chickpea to respond to improved environmental conditions.

Reductions in seed size due to terminal drought have significant implications for seed quality. In kabuli chickpea in particular, larger seeds attract higher prices (Siddique and
Sykes, 1997). Maintenance of seed size in environments which commonly experience terminal drought would be beneficial for seed quality, market acceptance and increased profit.

Improved drought adaptation can also be achieved through maximising water uptake and water use efficiency by minimising water loss, thereby postponing plant dehydration (Subbarao et al., 1995). Increased rooting depth is one of the most common ways of increasing the uptake of soil water (Gregory, 1988), but in south-west Australia, especially on fine textured duplex soils, the depth of soil available for root exploration is generally shallow so that selection for deep-rooting in chickpea in these environments is rarely beneficial (Turner, 1992; Passioura, 1992). Furthermore, in Mediterranean-type environments chickpea is dependent on seasonal rainfall rather than on stored, receding soil moisture and in drier seasons the depth of wetting in the soil profile is unlikely to extend below 40 to 50 cm (Smith and Harris, 1981). Additionally, water loss can be reduced by stomatal closure. The decrease in stomatal conductance, that caused the observed decrease in photosynthesis (Chapters 3 and 4), is indicative of stomatal closure which is important in minimising water loss through transpiration (Turner et al., 1999).

Plant dehydration can also be postponed by accumulating solutes in plant tissues, thereby increasing the osmotic potential and maintaining tissue turgor. Osmotic adjustment enables soil water extraction to be increased (Morgan and Condon, 1986) and in chickpea, while not preventing the decline in photosynthesis, it may enable low photosynthetic rates to be maintained (Leport et al., 1998). In a complementary study genotypic variation in osmotic adjustment was observed in chickpea subject to drought (Leport et al., 1999). However, we observed no benefits for seed yield associated with high osmotic adjustment (Leport et al., 1999) as reported previously (Morgan et al., 1991).

In this environment low current photosynthate supply during seed filling in droughted chickpea is unlikely to be substantially improved through the incorporation of these drought avoidance and dehydration postponement characteristics. Decreased leaf photosynthesis occurs despite the fact that a number of these characteristics are already incorporated in chickpea. Therefore, the ability of chickpea to augment assimilate supply through the redistribution of non-structural C and N is likely to be very important under terminal drought conditions. This was examined in the present thesis as a means of maintaining seed size and yield. However, the results were complicated by the fact that water deficits also reduced the number of pods and seeds (sink size).

When assimilate supply is not limiting, the final seed size obtained reflects the maximum potential seed size as determined by cell number and volume. In the field study reported in this thesis, terminal drought reduced leaf photosynthesis with a
corresponding decrease in seed size. Measurement of the growth of seed from selected pods indicates that this reduction was a consequence of both a lower maximum rate and a shorter duration of seed filling. The smaller seed size observed in this field study occurred despite fewer pods and seeds per pod. However, in glasshouse-grown chickpea subject to water deficit, pod and seed number were reduced to such an extent that the source:sink ratio and average seed DW increased compared to well-watered plants. This was exacerbated by the need to re-water those plants subject to water deficit in order to ensure their survival, which increased the source of C and N although not to the level maintained in well-watered plants.

In chickpea reductions in seed number in droughted plants compared to well-watered plants can be a consequence of increased rates of pod and seed abortion and reduced rates of pod and seed production (Desclaux and Roumet, 1996). In chickpea seed, abortion occurs during cell division, prior to the cell expansion and rapid DM accumulation phase during which seed abortion is unlikely to occur. Assimilate supply (Heitholt et al., 1986), hormonal regulation (Tamas et al., 1979; Atkins and Pigeaire, 1993; Swain et al., 1993; Palta and Ludwig, 1996; Swain et al., 1997) and shading (Heindl and Brun, 1983) have all been implicated as determinants of reproductive abortion in pulses. Pod initiation and development appears to be dependent upon a critical level of assimilate supply and new pods will fail to develop should assimilate supply fall below this minimum requirement (Muchow and Charles-Edwards, 1982; Beech et al., 1989). In the present experiments, Kaniva, the large-seeded kabuli genotype, was more sensitive to pod abortion than the desi genotypes which is likely to be a consequence of an increased assimilate requirement by each individual pod. Furthermore, the rate of pod production was higher for the desi genotypes enabling them to maintain relatively more pods and seeds compared to Kaniva when subject to drought. Establishment and maintenance of a high sink demand, through increased pod and seed number, promotes DM partitioning to the seed as indicated by the desi genotypes having higher harvest indices compared to the kabuli genotype.

One of the main thrusts of this thesis was to study the contribution of C and N stored during vegetative growth to seed C and N and the ability of these remobilised assimilates to maintain seed growth and seed size. The remobilisation of pre-podding C from the vegetative tissues to seed C in the present study (Chapters 3 and 4) was less than the contribution of pre-anthesis C to grain C reported in wheat using an identical method (Palta et al., 1994). Compared to other pulses such as lupin (Pate et al., 1980) and soybean (Yamagata et al., 1987), where 2 and 4% of the seed C was derived from remobilisation of pre-anthesis C, the 15% contribution of pre-podding C to seed C is relatively high in chickpea. However, substantial variation exists within cereal and pulse species in the extent

Although, substantial declines in current leaf photosynthesis occur in droughted chickpea, resulting in significant reductions in photosynthate supply, more than 80% of the seed C is still derived from post podding assimilation. This may be partly derived from continued photosynthetic activity in the pod walls. While net uptake of CO₂ from the air by pods is of minimal importance in chickpea (Saxena and Sheldrake, 1980; Sheoran et al., 1987, Leport et al., 1999), there is evidence that re-fixation of seed-respired CO₂ by the pod wall may be an important source of photosynthate for the seed (Atkins and Pate, 1977; Flinn et al., 1977; Sheoran et al., 1987). This recycling of respired CO₂ may continue in droughted chickpea, particularly as water loss with this system would be minimal. Additionally, more leaves were produced and in these leaves photosynthesis was not zero at midday and this low but positive photosynthesis must have contributed to seed C. Furthermore, this study did not consider any contribution of stem photosynthesis to seed C.

Relative to C, the contribution of pre-podding N to seed N was very high in chickpea, particularly in plants subject to drought. Nitrogen remobilisation is an important source of seed N in many pulses most of which experience decreases in N fixation during podding as C is redirected from N fixation to seed filling (Kurdali, 1996; Hooda et al., 1986; Herridge and Pate, 1977; Bethlenfalvay and Phillips, 1977; Vikman and Vessey, 1992; Lawn and Brun, 1974). During vegetative growth much of the C supply supports N fixation and so the accumulation of N in the vegetative tissue is often rapid during this period (Herridge and Pate, 1977). During flowering and podding most of the available C is utilised by the developing pods and seeds leading to declining N fixation, a process which is exacerbated when plants are subject to drought (Hooda et al., 1990). As demonstrated in this thesis, decreasing N fixation and uptake of soil N, coupled with a high N demand for the filling seed results in substantial N remobilisation in chickpea. In well-watered chickpea the contribution of pre-podding N to the seed was 60% which increased to 90% in droughted chickpea. In plants subject to water deficit, it is likely that C supply to the nodules declines earlier and more rapidly leading to an increased dependence on N remobilised from vegetative tissues.

In chickpea, most of the remobilised C and in particular N, is derived from the leaf tissues. Carbon is mobilised from the leaves following the breakdown of leaf starch reserves (Hammond and Burton, 1983; Fader and Koller, 1983; de Veau et al., 1992) with some C entering the seed in association with remobilised N. Remobilised N is derived from the breakdown of photosynthetic proteins (Feller and Fischer, 1994; Egli and Crafts-Brandner, 1996; Crafts-Brandner et al., 1998; David et al., 1998), the highest concentrations of which are found in the leaf tissues in chickpea (Singh and Pandey,
In these studies this was indicated by a steady fall in the concentration of leaf N during seed growth.

The extent of C and N remobilisation is also partly dependent on the source:sink ratio. When the source:sink ratio is low the requirement for remobilised assimilates is high, so remobilisation is maximised, however, when the source:sink ratio is high, less remobilised assimilate is required and seed fill can be completed without using all the assimilate available for remobilisation (Schonbeck et al., 1986; Munier-Jolain et al., 1996). In Kaniva, pre-podding C and N remobilisation to the seed was lower than that measured in Tyson, regardless of treatment. In droughted Kaniva, pre-podding C and N contributed 8% of the seed C and 91% of the seed N compared to droughted Tyson in which 16% of the seed C and 97% of the seed N was derived from remobilisation. This difference may be due to low sink demand in Kaniva which has relatively poor DM partitioning to pods and seeds. In cereals attempts have been made to relate remobilisation ability with plant height, as much of the reserves are remobilised from the stems (Schnyder, 1993). However, the results of this approach have revealed a lack of consistency in the relationship between plant height and remobilisation ability (Rawson and Evans, 1971; Austin et al., 1980; Pheloung and Siddique, 1991; Blum et al., 1997). Similarly in chickpea, there are no obvious morphological reasons why remobilisation should be lower in the kabuli genotype. Therefore, lower DM remobilisation in Kaniva is likely to be due to lower sink demand from the fewer pods. This is particularly true in droughted chickpea where the amount of N required from remobilisation was clearly limited by low sink demand as evidenced by higher N (protein) contents in the seed from stressed plants.

In droughted chickpea the contribution of pre-podding C and N to the seed was decreased to a lesser extent than the supply of current assimilates. Consequently, the relative importance of pre-podding C and N was increased when plants were subjected to water deficit despite the absolute amounts of C and N remobilised being lower in these plants. This may be due to a higher source:sink ratio in droughted plants, with seed filling completed prior to all the C and N available for remobilisation being required (Munier-Jolain et al., 1996). In this instance seed filling is limited by the maximum physiological capacity of the seed to store starch and protein.

Variation in C and N remobilisation within genotypes of chickpea indicates that selection for increased remobilisation may be useful when plants are subject to drought. Use of stable isotopes to measure C and N remobilisation is too expensive and time consuming to be used with a large number of genotypes. In cereals, selection for remobilisation ability has been achieved by measuring DM decreases in the vegetative tissue (Blum et al., 1997). This method does not take into account losses due to respiration or ammonia volatilisation from vegetative tissues and consequently
overestimates the contribution made by remobilisation (Blum et al., 1997; Francis et al., 1997). However, despite these inaccuracies, Leport et al. (1999) has recently measured vegetative DM decreases in the field in a range of chickpea genotypes and those genotypes with high remobilisation ability as estimated from DM changes correlated well with the results from isotope studies presented in this thesis for the same genotypes. Remobilisation in kabuli types may be improved by crossing them with genotypes that have high remobilisation ability. In Bumper, a kabuli by desi cross, remobilisation, as indicated by DW changes, was substantially improved over Kaniva (Leport et al., 1999).

Selection for improved remobilisation in plants subjected to drought can be achieved by using chemical desiccants and senescing agents to prevent current photosynthesis in a manner similar to that caused by terminal drought, thereby increasing the plants dependency on remobilised DM (Blum et al., 1983a,b). Using this method genotypes with increased remobilisation ability can be selected as they are better able to maintain seed size and yield (Nicolas and Turner, 1993). However, unlike determinate cereals selection for maintenance of seed size in indeterminate chickpea may not be as convenient because increased remobilisation may result in maintenance of more pods and seeds, rather than seed size.

In chickpea both seed size and pod and seed number are highly plastic and subject to considerable variation in response to environmental conditions. Maintenance of large seed in chickpea subject to terminal drought is best achieved by having a genotype with large potential seed size, intermediate pod number and high remobilisation to achieve a high source:sink ratio. Restricting pod number in droughted chickpea might be achieved through decreasing the rate of pod production and increasing the sensitivity of pod and seed abortion to environmental stresses. The compromise for such a genotype, however, would be lower potential seed yield, particularly in drought prone environments. In these studies the sensitivity of pod and seed abortion in Kaniva under water deficits in the glasshouse, was too high resulting in large reductions in seed yield. However, high sink demand (i.e. low source:sink ratio) is essential for maximising both seed yield and the utilisation of remobilised assimilates for seed filling. In these studies low pod number is associated with poor DM partitioning to the seed and low harvest index. Kaniva has large potential seed size and relatively few pods compared to the desi genotypes, but in the field, plants subject to terminal drought had low average seed DW. Increased assimilate supply through improved remobilisation and DM partitioning to the seed in droughted Kaniva may increase seed size. Remobilisation of vegetative C and N to the filling seed should be maximised in chickpea subject to terminal drought in low rainfall areas as a means of maximising assimilate supply and partitioning to filling seed.
Selection for increased seed size has been achieved in drought-prone Mediterranean-type environments by selecting for genotypes with larger potential seed size. Sona, sister line to ICCV88201, and Heera are both recently-introduced desi genotypes which produce larger seed than Tyson in the low rainfall areas which have less than 350 mm annual rainfall. This suggests that although terminal drought prevents the potential seed size from being attained in chickpea, relative differences between genotypes can be maintained regardless of terminal drought. In drought-prone environments, consistent production of a high proportion of large marketable kabuli seed (greater than 9 mm diameter) while still producing economical yields is likely to be a difficult objective (Siddique et al., 1999a), but may be achieved by selecting for the traits discussed above. Further studies should use a wide range of desi and kabuli chickpea genotypes with different pod and seed number and seed size for this purpose. Since, an increase of 1 mm in seed diameter in kabuli chickpea can be worth at least an extra $50 per tonne, there are clearly situations in which a yield penalty would be economical provided larger seed is produced.
REFERENCES


## Chapter 3

### Table 3.2 - Branch order ANOVA

The SAS System

General Linear Models Procedure - Class Level Information

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<th>Class</th>
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<th>Values</th>
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<td>I K T</td>
</tr>
<tr>
<td>TMT</td>
<td>2</td>
<td>Con Str</td>
</tr>
<tr>
<td>BRANCH</td>
<td>4</td>
<td>A F L M</td>
</tr>
<tr>
<td>REP</td>
<td>4</td>
<td>1 2 3 4</td>
</tr>
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</table>

Number of observations in data set = 96

General Linear Models Procedure

Dependent Variable: SDWT

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
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<tbody>
<tr>
<td>Model</td>
<td>23</td>
<td>1.19012752</td>
<td>0.05174467</td>
<td>52.70</td>
<td>0.0001</td>
</tr>
<tr>
<td>Error</td>
<td>72</td>
<td>0.07069090</td>
<td>0.00098182</td>
<td></td>
<td></td>
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<tr>
<td>Corrected Total</td>
<td>95</td>
<td>1.26081842</td>
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<td></td>
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</tbody>
</table>

\[ R^2 = 0.943933 \]
\[ C.V. = 13.94288 \]
\[ \text{Root MSE} = 0.0313340 \]
\[ \text{SDWT Mean} = 0.2247310 \]

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Type I SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENO</td>
<td>2</td>
<td>1.02404909</td>
<td>0.51202454</td>
<td>521.51</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
### Chapter 4

**Figure 4.3 - Yield component ANOVA**

The SAS System

General Linear Models Procedure

Class Level Information

<table>
<thead>
<tr>
<th>Class</th>
<th>Levels</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENO</td>
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<td>I K T</td>
</tr>
<tr>
<td>TMT</td>
<td>2</td>
<td>C S</td>
</tr>
<tr>
<td>REP</td>
<td>8</td>
<td>1 2 3 4 5 6 7 8</td>
</tr>
</tbody>
</table>

Number of observations in data set = 48

General Linear Models Procedure

Dependent Variable: YIELD

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<tr>
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<th>Type III SS</th>
<th>Mean Square</th>
<th>F Value</th>
<th>Pr &gt; F</th>
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</thead>
<tbody>
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<td>GENO</td>
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<td>1.02404909</td>
<td>0.51202454</td>
<td>521.51</td>
<td>0.0001</td>
</tr>
<tr>
<td>TMT</td>
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<td>0.10676291</td>
<td>0.10676291</td>
<td>108.74</td>
<td>0.0001</td>
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<tr>
<td>BRANCH</td>
<td>3</td>
<td>0.00128939</td>
<td>0.00042980</td>
<td>0.44</td>
<td>0.7267</td>
</tr>
<tr>
<td>GENO*TMT</td>
<td>2</td>
<td>0.05637295</td>
<td>0.02818647</td>
<td>28.71</td>
<td>0.0001</td>
</tr>
<tr>
<td>GENO*BRANCH</td>
<td>6</td>
<td>0.00080129</td>
<td>0.00013355</td>
<td>0.14</td>
<td>0.9911</td>
</tr>
<tr>
<td>TMT*BRANCH</td>
<td>3</td>
<td>0.00045614</td>
<td>0.00015205</td>
<td>0.15</td>
<td>0.9262</td>
</tr>
<tr>
<td>GENO<em>TMT</em>BRANCH</td>
<td>6</td>
<td>0.00039576</td>
<td>0.00006596</td>
<td>0.07</td>
<td>0.9987</td>
</tr>
</tbody>
</table>

Source      | DF | Type III SS | Mean Square | F Value | Pr > F |
---          |----|-------------|-------------|---------|--------|
GENO        | 2  | 1.02404909  | 0.51202454  | 521.51  | 0.0001 |
TMT         | 1  | 0.10676291  | 0.10676291  | 108.74  | 0.0001 |
BRANCH      | 3  | 0.00128939  | 0.00042980  | 0.44    | 0.7267 |
GENO*TMT    | 2  | 0.05637295  | 0.02818647  | 28.71   | 0.0001 |
GENO*BRANCH | 6  | 0.00080129  | 0.00013355  | 0.14    | 0.9911 |
TMT*BRANCH  | 3  | 0.00045614  | 0.00015205  | 0.15    | 0.9262 |
GENO*TMT*BRANCH | 6  | 0.00039576  | 0.00006596  | 0.07    | 0.9987 |

Sum of Squares | Average | F Value | Pr > F
---            |---------|---------|--------|
Source         | DF      | Squares | Square | F Value | Pr > F |
Model 42 174.61064375 34.92212875 14.41 0.0001
Error 47 101.76858750 2.42306161
Corrected Total 47 276.37923125

R-Square C.V. Root MSE YIELD Mean
0.631779 47.84995 1.5566186 3.2531250

Source DF Type I SS Mean Square F Value Pr > F
GENO 2 52.36346250 26.18173125 10.81 0.0002
TMT 1 120.61850208 120.61850208 49.78 0.0001
GENO*TMT 2 1.62867917 0.81433958 0.34 0.7165

Source DF Type III SS Mean Square F Value Pr > F
GENO 2 52.36346250 26.18173125 10.81 0.0002
TMT 1 120.61850208 120.61850208 49.78 0.0001
GENO*TMT 2 1.62867917 0.81433958 0.34 0.7165

Figure 4.4 - Curve fitting

The SAS System
Total Seed DW Increase 1996

--------------------------------------------- GENOTYPE=TYSON ---------------------------------------------
---------NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable STRESS

Source DF Sum of Squares Mean Square
Regression 3 111.88609865 37.29536622
Residual 37 20.51010135 0.55432706
Uncorrected Total 40 132.39620000
(Corrected Total) 39 45.84256000

Parameter Estimate Asymptotic Asymptotic 95 %
Std. Error Confidence Interval

W 2.60033079 0.3403868856 1.9106451701 3.290016419
B 15.02095132 5.2228937427 4.4384204242 25.603482214

-128-
Chapter 5

Figure 5.4 - Curve fitting

The SAS System

Total Seed DW Increase 1997

-------------------------------------------------------------------------------
| TMT=Control |  NOTE: Convergence criterion met. |
-------------------------------------------------------------------------------

Non-Linear Least Squares Summary Statistics

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Sum of Squares</th>
<th>Mean Square</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression</td>
<td>3</td>
<td>1129.4279928</td>
<td>376.4759976</td>
</tr>
<tr>
<td>Residual</td>
<td>44</td>
<td>145.9200140</td>
<td>3.3163640</td>
</tr>
<tr>
<td>Uncorrected Total</td>
<td>47</td>
<td>1275.3480069</td>
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</tr>
<tr>
<td>(Corrected Total)</td>
<td>46</td>
<td>724.8421671</td>
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Parameter Estimate

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Asymptotic Std. Error</th>
<th>Asymptotic 95% Confidence Interval</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>W</td>
<td>9.73976873</td>
<td>0.6558214917</td>
<td>8.418051969 11.061485496</td>
</tr>
<tr>
<td>B</td>
<td>21.93161215</td>
<td>5.0470577724</td>
<td>11.759971014 32.103253289</td>
</tr>
<tr>
<td>C</td>
<td>0.18692867</td>
<td>0.0438052540</td>
<td>0.098645292 0.275212053</td>
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</table>

Asymptotic Correlation Matrix

<table>
<thead>
<tr>
<th>Corr</th>
<th>W</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>W</td>
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<td>-0.224319475</td>
<td>-0.250203743</td>
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<tr>
<td>B</td>
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<td>0.9988684082</td>
</tr>
<tr>
<td>C</td>
<td>-0.250203743</td>
<td>0.9988684082</td>
<td>1</td>
</tr>
</tbody>
</table>