PHYSIOLOGICAL ASPECTS OF THE RESPONSES OF GRAIN FILLING TO HIGH TEMPERATURE IN WHEAT

by

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Declaration

I hereby declare that the thesis presented here contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text.

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ABBREVIATIONS

ADPG	adenosine diphospho glucose
°C	degree Celsius
et al.	and others
Fig.	figure
GBSS	granule bound starch synthesis
GF	grain filling
DAA	days after anthesis
HPLC	high-performance liquid chromatography
hr	hour
HMW	high molecular weight
inc	incorporated
LMW	low molecular weight
HSPs	heat shock proteins
K	potassium
Km	affinity of the enzyme for substrate
min	minute
µL	micro litre
N	nitrogen
nmol	nanomole
P	phosphorus
Q_{10}	temperature quotient
SE	size-exclusion
SDS	sodium dodecyl sulphate
SSS	soluble starch synthesis
Trim	trimming
Tem	temperature
UDMSO	urea-dimethylsulphoxide
Var	variety
Vmax	maximum velocity
Vmax/ Km	enzyme efficiency

Abstract

High temperature during grain filling is one of the major limitations for grain yield in many wheat growing areas. The effects of a sustained period of moderately high temperature on physiological and biochemical aspects of grain development were investigated in wheat cultivars grown under controlled environment conditions. The effect of variation in plant nutrition on the response of cultivars to high temperature was also studied. The plants were grown in pots in a growth cabinet set at 20/15°C. After anthesis, half of the pots were shifted to another growth cabinet set at 30/25°C and the rest of the plants were kept at 20/15°C as control. The supply of nitrogen to the grains was altered either by changing the level of nitrogen applied to the plants or by trimming of the ears.

There was a substantial reduction in individual grain weight ranging from 21 to 40% at the higher compared to the lower temperature. Genotype variation existed for both rate and duration of grain filling but the variation was small for the duration at high temperature. There was a small increase in the rate of grain filling in response to an increase in growth temperature. The small positive response of the rate of grain filling was not associated with the availability of sucrose in the grains. Nor was the differential response of the two cultivars in response to temperature associated with the changes in the level of sucrose or in the amounts of the precursor of starch synthesis, ADP-glucose, in the grains.

The affinity of soluble starch synthesis (SSS) for its substrate amylopectin, and the efficiency of the enzyme were decreased at high temperature *in vitro*. The differential

responses of the efficiency of SSS in Kavko and Lyallpur to an increase in temperature *in vitro* accounted to some extent for differences in the temperature sensitivity of grain filling. However, the most remarkable difference between the two cultivars *in vivo* was in the absolute values of the SSS efficiency as the values were greatest in the more tolerant cultivar Kavko. Compared to SSS the activity of granule bound starch synthase (GBSS) was less sensitive to high temperature. The activity of SSS was substantially lower at later stages of grain development but there was little developmental change in the activity of GBSS.

The variation in nitrogen supplied to the plants modified the response of the cultivars to temperature. Only at the standard level of nitrogen (which treatment included post anthesis nitrogen application) was the reduction in final grain weight at high temperature greater in Lyallpur than in Kavko; there was no difference between the two cultivars at low nitrogen. The effect of nitrogen on the differential responses of cultivars to temperature was due to its effect on starch weight and the effect was significant only for amylose but not for amylopectin. The difference between the two cultivars in terms of the rate of grain protein accumulation in response to temperature was also dependent on the level of nitrogen.

Trimming of the ears increased the weight of the remaining grains only in Lyallpur and not in Kavko. The increase in grain weight in the trimmed ears was associated with a simultaneous rise in the rate of grain filling. However, trimming did not significantly influence the response to high temperature. It seems that trimming does not alter the delivery of sucrose to the grains but instead appears to influence processes in the pathway of the conversion of sucrose to ADP-glucose. The efficiency of SSS was greater in the

trimmed than in the untrimmed ears but there was no straightforward relationship between grain filling rates and efficiency of SSS.

The composition of protein and starch were influenced by high temperature. The contribution of the HMW-glutenin was substantially higher at high temperature and also at the standard compared to the low level of nitrogen. However, the effect of the nitrogen level on the temperature response of HMW-glutenin was not big enough to be statistically significant. The grain amylose% was higher at high temperature only in Kavko. The increases in amylose% at high temperature were evident at low but not at the standard level of nitrogen.

Chapter 1

General introduction

Wheat is a temperate crop best adapted to areas where cool temperatures predominate. The optimum post anthesis temperature for maximum kernel weight in wheat is about 15°C (Chowdhury and Wardlaw 1978). The optimum temperature for photosynthesis is about 10°C higher than the optimum for grain development (Kobza and Edwards 1987). The mean daily temperatures during grain filling often exceed the temperature optimum for grain filling in most wheat growing areas of the world (Paulsen 1994). In the Australian cereal belt maximum daily temperatures in some occasions often rise above 30°C and can even reach 40°C (Wardlaw and Wrigley 1994). Long term records of temperature show a mean number of 5 to 7 days above 30°C in October in marginal cereal growing regions of South Australia (Australian Bureau of Meteorology 1996 in Wallwork 1997). Furthermore, there is a progressive change in global temperature. As expected with current trends in greenhouse gas emissions there would be a possible 3°C increase in the global annual mean temperature by the end of the 21st century (Warrick and Farmer 1990), compared to 1-2°C variation in the earth's mean surface temperature in the last 10,000 years (Parry *et al.* 1990). The rise in global temperature will increase the incidence of possible periodic drought and high temperatures (Wigley and Raper 1992). The likelihood of a temperature above 35°C would, for example, be doubled by a 1.7°C increase in mean temperature in an area in Iowa, USA (Mearns *et al.* 1984).

The vegetative and reproductive stages of growth in wheat can be adversely influenced by high temperature (Rawson 1986; Wardlaw *et al.* 1989a) but the periods of high temperature are most frequent during grain filling and can reduce grain yield substantially.

After anthesis the main effect of high temperature is on grain size. Controlled-environment studies have shown that there is a reduction of about 3-4% in single grain weight with 1°C rise in temperature above the optimum (Wardlaw *et al.* 1989a). Under field conditions the losses of grain yield from high temperature are even greater as yield components other than grain size may also be influenced by rising temperatures (Wardlaw and Wrigley 1994). A yield penalty of 10-15% per year is estimated from the above optimum temperatures prevailing in the field in Australian wheat growing areas. Even a short period of very high temperature of as little as few days can substantially reduce grain yield (Stone and Nicholas 1994) and quality (Blumental *et al.* 1991). Although frequent periods of drought aggravate the effect of high temperature on grain development (Nicolas *et al.* 1984) even in the regions where enough water is available high temperature can reduce grain yield substantially (McDonald *et al.* 1983). Genotype variation has been reported for the response of grain filling in wheat cultivars to high temperature (Rawson 1986; Wardlaw *et al.* 1989a,b; Hunt *et al.* 1991). Attention must therefore be paid to a better understanding of the physiological and biochemical bases of the response to high temperature in order to develop varieties that can maintain their performance under unfavourable temperature conditions occurring during grain development.

Investigations on temperature responses of grain filling in wheat have been carried out within two temperature ranges: (A) sustained periods of moderately high temperatures, and (B) short periods of very high temperatures above 30°C. The temperatures in the range between 20°C and 30°C are typical during grain filling and have therefore been used for the investigation in this study. Two types of the response mechanism are distinguishable between the two temperature ranges. At moderate temperatures there is a progressive diminution in grain size that results from the effect of shortened duration of grain filling

due to hastened development of the grains at elevated temperatures (Hunt *et al.* 1991). The increase in the rate of grain filling as the temperature rises is usually too small to counterbalance the adverse effect of shortened duration of filling on grain weight. At temperatures in excess of 30°C the rate of grain filling drops and this is mainly associated with a reduction in starch synthesis (Jenner 1994). Supply of assimilates to the grains does not seem to be a major limitation for the rate of grain filling at high temperatures (Jenner 1991). In other words, factors that operate inside the grain itself are mainly limiting the rate of grain growth at high temperatures. It now seems clear that the reduction in starch synthesis at temperatures above 30°C is mainly due to the reduced activity of soluble starch synthase (SSS) catalysing the synthesis of the starch in developing grains (Jenner *et al.* 1993; Keeling *et al.* 1993). However, little attention has been paid to the temperature responses of starch synthesis at the biochemical level at moderately high temperatures.

The main objectives of this study were: (a) to evaluate the relationships between grain-filling parameters and high temperature tolerance in wheat cultivars, (b) to investigate whether or not the nutritional status of the plants can regulate the response to high temperature, and (c) to find out if the difference in the temperature sensitivity of the wheat cultivars could be explained by the availability of substrate and/ or the activity of the enzymes involved in starch synthesis. The results from this study are expected to provide more information for wheat breeders to develop cultivars that are suitable for areas subjected to unfavourable temperature conditions.

To achieve the above mentioned goals first the characteristics of dry matter accumulation over the entire period of grain filling were studied at two growth temperatures (at 20/15°C and 30/25°C day/ night) in four wheat cultivars known to differ in their response to high

temperature. Then, the temperature responses of starch and protein accumulation was evaluated under two different levels of supplied nitrogen and in the trimmed and untrimmed ears of Kavko and Lyallpur chosen respectively as tolerant and sensitive cultivars. Finally, the responses of the two cultivars to temperature were compared in terms of the availability of substrates and the activities of starch synthases (SSS and granule bound starch synthesis, GBSS) in developing grains at high temperature. An investigation of the effects of high temperature and grain nitrogen level on starch and protein composition also formed part of the investigations in this study.

Chapter 2

Literature review

2.1 Grain growth and development in wheat

The growth of grain in wheat and other cereals as increase in dry matter usually follows a sigmoidal pattern with three recognized phases: lag phase, linear phase, and final phase. During the lag phase, the cells of the endosperm divide, the starch and protein bodies are formed, and the growth potential of the grain is determined (Buttrose 1963; Evers 1970; Brocklehurst 1977; Briarty *et al.* 1979). This period extends from fertilization up to 14 to 20 days after anthesis (DAA) at medium temperatures (Briarty *et al.* 1979). At higher temperatures the duration of cell division is shortened and the rate of cell division is increased, but the final number of cells formed in mature grains does not vary significantly with change of temperature (Wardlaw 1970; Nicolas *et al.* 1984). However, cell enlargement does not keep pace with division and thus smaller cells are produced over time (Hoshikawa 1962; Singh 1982). There is a variable relationship between the duration of the lag phase and final grain weight in different cultivars of wheat. Cultivars having similar lengths of lag phase may differ greatly in their final grain weights (Herzog and Stamp 1982). Cultivars with large grain dry matter may even have short lag phases (Bauer *et al.* 1985). Yet, to realise the importance of this period, it is worth mentioning that the alterations of sink capacity of grains by experimental means can be successful only during the first two to three weeks after heading (Fisher and Hille RisLambers 1978; Jenner 1979; Herzog 1982; Herzog 1986).

During the second phase, characterised by a nearly linear growth rate (increase of dry matter), most of the starch and protein are deposited in cells and organelles already formed

in the endosperm (Jenner *et al.* 1991). This period starts from about day 14 DAA and continues until about day 35 (Briarty *et al.* 1979). In this phase, the rate of dry matter accumulation in the grain is usually considered to be constant. However, the data from several experiments indicate that there is a continuous increase in the rate of dry matter accumulation from the beginning of grain filling towards a point at which the rate is maximum, thereafter a steady decline is recognised (Spiertz 1974; Chowdhury and Wardlaw 1978; Herzog 1986). The exact timing of the maximum rate of grain growth is not fixed but variable and cultivar dependent (Herzog 1982). Due to the inconstancy of growth during the linear phase, the slope of the linear regression equation has therefore been used to estimate the mean growth rate more correctly (Sofield *et al.* 1977a; Simmons and Crookston 1979; Van Sanford 1985; Hunt *et al.* 1991).

Two parameters characterise the linear phase of grain growth, the rate and the duration of grain filling (GF) (Jenner *et al.* 1991). They are controlled by different mechanisms. The rate of GF is a reflection of biochemical reactions involved in the synthesis of starch and protein, largely determined by genetic factors, whereas the duration of GF is related to the developmental mechanisms of the grain, mainly controlled by environment (Hunt *et al.* 1991; Jenner *et al.* 1991). Final grain weight may positively be associated with both rate and duration of GF (Gebeyehou *et al.* 1982), but in most cases grain weight is closely correlated with the rate but not with duration (Nass and Reiser 1975; Van Sanford 1985; Bruchner and Frohberg 1987). Cultivars with a longer grain filling period may take advantage of environments more favourable for grain growth than those with shorter duration (Perry and D'Antuono 1989). However, grain weight becomes proportional to the rate of grain growth in areas where the period of GF is shortened by environmental stress (Wiegand and Cuellar 1981).

The final phase of grain development is referred to as physiological maturity at which grain growth is terminated and maximum dry matter has accumulated in the grain. The cessation of dry matter may occur with a clear discontinuity in the rate of growth at physiological maturity, but there is generally a gradual decline in growth rate before grain growth is terminated. Chalabi *et al.* (1988) reported that in some cultivars the rate of dry matter accumulation fell to one-third of the maximum before an abrupt cessation of grain growth. The termination of dry matter accumulation corresponds to the stage when lipids are deposited in the phloem strands and the flow of assimilates to the grain ceases (Zee and O'Brien 1970). This is not caused by a lack of substrate supply to the grains (Jenner and Rathjen 1977; Sofield *et al.* 1977a). However, the declining rate of grain growth towards physiological maturity may be more pronounced when the supply of assimilates is limited by environmental stresses in the late stage of grain growth (Loss *et al.* 1989).

It is therefore concluded that the potential capacity of the grain to accommodate dry matter is determined during the first phase of grain development. However, the final grain weight is predominantly determined by the rate of dry matter accumulation during the linear phase of grain growth. This is more evident in areas where duration of grain filling is shortened by adverse environmental conditions.

2.2 Accumulation of carbohydrate compounds in the grains

2.2.1 Supply of photoassimilate to the grains

The photosynthate for grain filling is derived from two sources: current photosynthate and the mobilization of the carbohydrate stored in the vegetative parts of the plants. In old genotypes of wheat the assimilates stored in vegetative organs were very critical for grain yield, but in new genotypes current photosynthate is the main source of assimilates for

developing grains and accounts for over 90% of grain growth under normal condition (Evans *et al.* 1975; Herzog 1986). Several plant organs contribute current photosynthate to grain filling, however, under adequate growing conditions almost all of the photoassimilate required for grain growth can be provided by the flag leaf and the ear (Jenner *et al.* 1991). The contribution of the ear is considerably higher in awned genotypes (Evans *et al.* 1972) or under water stress where the stress sensitivity of ears is less than that of flag leaves (Wardlaw 1971).

Carbohydrates deposited in vegetative parts of wheat plants are a potential source of assimilate for grain filling when current photosynthesis becomes limiting (Austin *et al.* 1977; Blacklow *et al.* 1984; Borrell *et al.* 1989; Bell and Incoll 1990; Pheloung and Siddique 1991; Schnyder 1993; Blum *et al.* 1994; Tahmasebi 1995). When the rate of photosynthesis exceeds the needs of plant growth, the surplus photosynthate is temporary stored mainly in the extended internodes of the stem in the form of water soluble carbohydrates (WSC). Storage of assimilates in vegetative organs continues even after anthesis during the first part of grain growth when the demands of grains for assimilates is low (Judel and Mengel 1982; Kuhbauch and Thome 1989). During the later stages of grain filling, at a time when current photosynthate is less than the needs of grains, some of the WSC stored in the vegetative parts are remobilized to sustain grain growth. The onset of WSC mobilization usually coincides with the initiation of flag leaf senescence and the period of near constant rate of dry matter accumulation in the grains, when the demand of grains for assimilates is high and the net assimilation rate decreases substantially (Herzog 1986: Schnyder 1993). The contribution of remobilized carbohydrates to grain yield increases and becomes more important under water stress (Bidinger *et al.* 1977; Blum 1988), where photosynthesis may be decreased much more than assimilate consumption by

the grains (Wardlaw 1971). Under irrigation and warm conditions, however, the remobilization of carbohydrate does not seem to have significant contribution to grain filling, even throughout the period of maximum grain growth (Jenner *et al.* 1991).

Sucrose supplied to the grains from either current photosynthesis or remobilization is the primary substrate for starch synthesis (Duffus 1979), and its concentration in the endosperm is considered as an indication of the availability of assimilates for grain growth. The amount of sucrose in the endosperm is partly regulated by factors controlling the movement of sucrose into and through the grain. For instance, the concentration of sucrose in the endosperm was much lower than it was in the vascular bundle supplying the grain (Jenner 1974; Ho and Gifford 1984). Also, trimming ears after anthesis increased the sucrose content in the rachis, but the increase was not observed to the same extent in the endosperm (Jenner 1980), implying that the flux of sucrose into the grain is restricted (Fisher and Gifford 1986). Stem transport does not restrict the supply of assimilates to the grains; cutting half of the vascular bundles of the peduncle did not affect grain growth (Wardlaw and Moncur 1976). Wang *et al.* (1995) reported that the cellular pathway of photosynthate transfer can have a major impact on the control of carbon transport in the developing grain. Consequently, the poor demands of grains for assimilates during cell division is related to a weak driving force produced by the mechanism involved in photosynthate transfer in the developing grains. On the other hand, during the grain filling stage, the consumption of carbohydrate produces a demand force to drive photosynthate transfer to and within the developing grain.

The rate of starch synthesis is, to some extent, associated with the sucrose content of the endosperm (Jenner 1970; Jenner and Rathjen 1978; Rijven and Gifford 1983; Armstrong *et*

al. 1987). At high concentrations of sucrose in the grain, the rate of starch synthesis becomes less responsive to the increasing levels of sucrose and more associated with the performance of the synthetic system in the endosperm (Jenner *et al.* 1991,1993). The maximum catalytic activity of soluble starch synthase is one of the most important factors controlling the rate of starch synthesis in the endosperm (Jenner and Hawker 1993). The relationship between the supply of sucrose for grain filling and the rate of starch synthesis, therefore, is the outcome of the amount of sucrose available for distribution to the grain, the resistance of cellular pathway to the movement of sucrose into the endosperm, and the capacity of synthetic system to convert sucrose to starch (Jenner *et al.* 1991).

The photosynthetic activity of wheat plants is significantly affected by environmental factors such as water stress (Wardlaw 1971) and high temperature (Al-Khatib and Paulsen 1989). However, studies have shown that the supply of assimilates to the grains and also the availability of assimilates inside the endosperm are not the major limitation to the rate of grain growth under heat stress. For example, no change in the response of grains to high temperature was observed when the potential of supply of assimilate was increased by decreasing the number of the grains per ear (Wardlaw *et al.* 1980). Warming the ear alone had the same effect on grain growth as warming the whole plant (Bhullar 1984), implying that the effect of heat on the pattern of grain growth was independent of supply of assimilates to the ears. In a study by Jenner (1991) on wheat cultivar Kalyansona, the amount of sucrose in the endosperm was even raised when temperature was increased from 21°C to 35°C. On the other hand, the interaction between light intensity and the response of grain filling to high temperature (Wardlaw *et al.* 1989a) indicates that the response of grain growth to high temperature may be partially modified by the supply of assimilates. To what extent the differences between wheat cultivars in their rate of grain growth at

moderately high temperature can be explained by differences in the levels of assimilates in the grain is not yet well documented.

2.2.2 Starch accumulation in the grains

Starch is deposited as granules in the amyloplast in wheat endosperm. Starch granules consist of two main classes, large A-type and small B-type (Briarty *et al.* 1979; Parker 1985; Morrison and Gadan 1987; Bechtel *et al.* 1990). The A-granules appear in the amyloplast within the first few days after anthesis and the number peaks when cell division ceases; thereafter the number remains constant but the size increases until maturity. B-type granules are formed later during cell enlargement in amyloplasts already containing A-granules. B-granules increase in number very quickly so that at maturity they represent in some cultivars more than 90% of endosperm granules (Brocklehurst and Evers 1977), yet A-granules contribute 60-80% of endosperm starch weight (Hughes and Briarty 1976; Evers and Lindley 1977). The size and the number of starch granules is affected by environmental conditions such as water stress (Brooks *et al.* 1982) and temperature (Batey *et al.* 1989; Tester *et al.* 1995). High temperature during grain development results in a reduced size of starch granules and a decrease in the number of B-type granules (Hoshikawa 1962; Bhullar and Jenner 1985; Shi *et al.* 1994). The relative stability in the number of A-granules has been related to their early initiation in grain development when environmental stress is not severe enough to reduce the number of A-type granules (Bhullar and Jenner 1985). Batey *et al.* (1989) reported that the weight percentage of only B-granules was reduced in a location where a hot period occurred during the last weeks of wheat grain development.

Granule size distribution in the endosperm may affect starch composition, which in turn influences its quality. Some workers have shown that the amylose content is higher in large A-granules than in small B-granules (Duffus and Murdoch 1979; Soulaka and Morrison 1985; Morrison and Gadan 1987). However, some have found no difference (Bathgate and Palmer 1972; Evers *et al.* 1974). Nevertheless, in both types of granules, starch synthesised at successive stages of grain development contains progressively higher proportions of amylose than does the starch initially produced (Duffus and Murdoch 1979; Morrison and Gadan 1987). As a result, the amylose content of endosperm starch increases during grain development (Matheson 1971).

The ratio of amylose to amylopectin is an important parameter affecting the starch quality and functionality. The amylose content of starch in the endosperm of wheat cultivars is normally from 18-26% (Batey 1991). However, there are some identified wheat mutants which contain less amylose and lack one or other of the three waxy genes encoding GBSS responsible for amylose synthesis (Nakamura *et al.* 1993a,b; Yamamori *et al.* 1994). These partially waxy mutants have been used to produce some wheat lines, called waxy wheats, which lack all waxy genes and have essentially no amylose (Nakamura and Yamamori 1995; Hoshino *et al.* 1996; Yasui *et al.* 1997). As amylopectin is the main contributor to starch quality and gives a higher viscosity and elasticity to the starch paste (Martin and Smith 1995), the flour of low amylose or amylose-free wheats might improve the quality of end-products, such as white salted noodles, in which quality is negatively correlated with the amylose content (Oda 1980). The application of transgenic technology to change the structure of amylopectin and hence to alter its crystallisation pattern could also be another approach to improve starch quality without much modification in starch chemistry (Tester and Karkalas 1997).

The proportion of amylose in the endosperm is controlled by cultivar and also in some extent by environment (Moss and Miskelly 1984). It is now clear that high temperature during grain development increases the proportion of amylose in wheat flour (Shi *et al.* 1994; Tester *et al.* 1995) and results in a lower flour quality obtained from areas experiencing hot weather during grain filling (Blumental *et al.* 1993). However, more information is necessary about the pattern of amylose and amylopectin accumulation throughout the period of grain development in different cultivars under high temperature. This is considered as a part of the investigations in this study.

2.2.3 Starch biosynthesis

Starch is synthesised from sucrose translocated from photosynthetic organs or remobilized from stem reserves in the grain endosperm where it is converted into starch through a number of enzymatic reactions (Preiss 1991; Okita 1992; Morell *et al.* 1995; Martin and Smith 1995; Smith *et al.* 1995; Preiss and Sivak 1996). The first reaction occurs in the cytosol where sucrose is degraded to UDP-glucose and fructose by the activity of sucrose synthase. UDP-glucose is converted to glucose-1-phosphate (G-1-P), in a reaction catalysed by UDP-glucose pyrophosphorylase. G-1-P is also produced via G-6-P through the action of hexokinase on fructose. G-1-P is transported into the amyloplast and there it is converted to ADP-glucose by ADP-glucose pyrophosphorylase (ADPase), the key substrate for starch synthesis. The location of ADPase shows variation between species: both plastidial and cytosolic activities of ADPase isoforms have been reported in maize (Denyer *et al.* 1996) and barley (Thorbjørnsen *et al.* 1996). In wheat, however, the studies of isolated amyloplasts from endosperm has shown that the activity of ADPase is mostly plastidial (Smith *et al.* 1997).

The glucose from ADP-glucose is used for starch synthesis through the activities of starch synthase and branching enzyme (Preiss and Levi 1980; MacDonald and Preiss 1983; Preiss 1991; Morell 1995). There are two forms of starch synthase: granule bound starch synthase (GBSS), involved in the synthesis of amylose, and soluble starch synthase (SSS) which, in association with branching enzyme, catalyses the synthesis of amylopectin. Amylose consists of sparsely branched linear chains of glucose residues linked by ∝-(1,4) bonds, while amylopectin consists of shorter chains of ∝-(1,4)-linked glucose residues much more highly branched by ∝-(1,6) cross linkages. Starch synthase links the glucose units of ADP-glucose to the ∝-1,4-glucan primer to extend the chain and branching enzyme forms the ∝-1,6-linkage in amylopectin (Fekete *et al.* 1960). The precise location of GBSS on the granule is unclear, however, the enzyme is likely active in areas of the granule which are not accessible to active branching enzyme (Morel *et al.* 1995) where amylose is produced.

It is widely believed that ADPase is the rate-limiting enzyme in the pathway of starch synthesis (Stark *et al.* 1992) and controls the deposition of starch. Although ADPase exerts considerable control over the rate of starch synthesis in photosynthetic organs (Neuhaus and Stitt 1990), this may not apply to all non-photosynthetic storage organs (Smith *et al.* 1995). In wheat endosperm for example there is evidence that the soluble forms of starch synthase predominantly control the rate of starch synthesis (Jenner *et al.* 1993; Keeling *et al.* 1993).

2.3 Accumulation of nitrogenous compounds in the grains

2.3.1 Sources of nitrogen for grain filling

Nitrogen for grain filling originates from two main sources: remobilization from vegetative tissues and uptake from the soil after anthesis. Some work has been done to improve the efficiency of the N-related traits, but the interaction between these components and the environment has always been a major limitation.

Most of the nitrogen taken up from soil is transported to the leaves and there fixed mainly as proteins. During remobilization these proteins are degraded to amino acids and transported from the leaves to the grains (Dalling *et al.* 1976; Feller 1979; Dalling 1985). Other plant organs also contribute some of the remobilized N (Spiertz and Ellen 1978; Simpson *et al.* 1983). A significant proportion of the N exported from leaves is cycled to other plant organs before final transport into the grain. In a study by Simpson *et al.* (1983), in which the entire amount of grain N was derived from remobilization, leaves contributed 40%, glumes 23%, stem 23%, and roots 16% of grain N. Less than half of the N exported from the leaves in the phloem was translocated directly to the grains, the rest was transported to the roots and then back to the other plant organs before incorporation into the grains.

N remobilization is controlled by environmental conditions and is also genetically controlled. N relocation is accelerated by N deficiency (Morris and Paulsen 1985) and temperature (Spiertz 1977). Guitman *et al.* (1991) reported that the rate of proteolytic activity was faster in plants with an interrupted supply of N, and this increased the amount of N transported to the ear. Yet, N remobilization started immediately after anthesis even when a high rate of nitrogen was applied late in development (Spiertz and Ellen 1978).

Translocation of N was also increased as temperature was elevated from 10°C to 25°C where the rate of leaf senescence and N incorporation into the grains was higher (Spiertz 1977). Variation exists among cultivars in the efficiency of N translocation (McNeal *et al.* 1966; Halloran 1981; Cox *et al.* 1886), and in some studies the relationship between grain protein concentration and N translocation has not been consistent (Mikesell and Paulsen 1971; McNeal *et al* 1972). Moreover, due to the interactions between genotype and environment, the performance of cultivars may differ from one environment to another. Van Sanford and MacKown (1987) found that in the field some cultivars were more efficient than others in remobilizing N but under N stress in the growth chamber the differences were not observed.

The amount of nitrogen taken up from the soil after anthesis varies depending on growth conditions (Pavlov 1969; Spiertz and Ellen 1978; Gregory *et al.* 1981) and genotype (Austin *et al.* 1977; Cox *et al.* 1985). When temperature is low and plants have an adequate supplies of soil water and nitrogen, the senescence and remobilization of N is slow and a high proportion of final grain N is taken up from the soil after anthesis (Pavlov 1969; Austin and Jones 1975; Spiertz and Ellen 1978). About 50% of grain N was absorbed from the soil after anthesis when soil N was not limiting (Spiertz and Ellen 1978). On the other hand, in semi-arid regions, where the level of water and nitrogen is low during grain filling, N remobilization contributes more than 80% of the grain N yield (Dalling 1985).

2.3.2 Nitrogen accumulation in the grains

The availability of N during plant growth is a factor regulating grain yield and grain nitrogen concentration in wheat. The magnitude of the effect of N on yield and protein

depends on the time and the rate of N application as well as the environment under which the crop is grown.

The accumulation of nitrogen in the grain is mainly source limited. Unusually high accumulation of grain N has been observed in different instances: when detached wheat culms were grown in liquid culture, the N concentration in the grain was dependent on the levels of N in the culture medium (Barlow *et al.* 1983). The N content of the grains is also increased by trimming of the ears after anthesis (Jenner 1980) or when nitrogen is supplied directly to the top parts of the plants (Finney *et al.* 1957; Blacklow 1982).

The extent to which the applied nitrogen affects yield and grain protein depends also on the timing of N application. The supply of nitrogen at an early stage of the crop growth generally increases yield, but it can be associated with low grain protein concentration (Langer and Liew 1973; Sarandon and Gianibelli 1990). The later nitrogen is applied, the greater is the effect on grain protein concentration. The application of N from heading to post anthesis increases the grain protein content but usually at the expense of grain yield (Langer and Liew 1973; Pushman and Bingham 1976; Smith *et al.* 1989; Sarandon and Gianibelli 1992). Accordingly, an inverse relationship between grain protein concentration and grain yield is usually observed (Terman *et al.* 1969; McNeal *et al.* 1972; Austin *et al.* 1977; Payne 1983), which is to some extent dependent on location and year (Terman *et al.* 1969) and the rate of N application (Langer and Liew 1973). However, in some studies an even weak but positive correlation between grain yield and grain protein concentration has been reported (Brunori *et al.* 1980; Cox *et al.* 1985). Also simultaneous increase in both traits has been achieved by application of N during late stages of growth in some

circumstances (Finney *et al.* 1957; Spiertz and Ellen 1978; Morris and Paulsen 1985; Banziger *et al.* 1994).

It has been argued that the inverse relationship between grain yield and grain protein could be explained on the basis of a bioenergetic competition between N accumulation and carbohydrate synthesis (Penning de Vries *et al.* 1974). Nitrogen assimilation and carbohydrate synthesis are both energy consuming and the cost of energy for N assimilation is higher than that of carbohydrate synthesis (Penning de Vries *et al.* 1974; Bhatia and Rabson 1976; Le Van Quy *et al.* 1991). Therefore, when N is applied to the crop some energy is used for N assimilation, which could otherwise be consumed for carbohydrate synthesis. This has been shown to occur by some workers. Champigny *et al.* (1991) reported that high rate of nitrate applied to detached leaves of N starved wheat seedlings significantly decreased the rate of sucrose synthesis in the leaves. Banziger *et al.* (1994) also reported that the uptake and assimilation of N between heading (late N application) and anthesis were accompanied by a significant but transient decrease in the amount of water soluble carbohydrates (WSC) of the shoot. For each 1 g kg^{-1} increase in shoot N concentration there was a decrease of 8.5 g kg^{-1} in the respective shoot WSC concentration.

On the other hand, the assimilation of CO_2 is positively associated with the N status of crop tissue (Evans 1983), and the assimilate for grain filling under non-stressful conditions is mainly derived from current photosynthate (Evans *et al.* 1975). Also, with high soil N fertility, a substantial amount of grain N is provided by root uptake (Neales *et al.* 1963), N fertilization decreases the remobilization of N from vegetative tissues, which is associated with leaf senescence, thereby maintaining and prolonging the photosynthetic capacity of

the leaves (Evans 1983). The duration of photosynthetic area after anthesis is positively correlated with grain yield (Simpson 1968). Therefore, the net effect of N application on grain yield would depend on the balance between the energy invested for N assimilation and the benefits from the improved photosynthetic capacity of the canopy due to better N status of the crop. In the study of Banziger *et al.* (1994), the simultaneous increase in grain yield and grain protein concentration was achieved by late N application for all genotypes. Although a transient reduction in water soluble carbohydrates of the shoot occurred, carbon exchange rate and leaf area duration were increased by N application, and this enhanced the photosynthetic capacity of the canopy during grain filling. The investment of energy for N assimilation and also into late infertile tillers was, therefore, compensated by the benefits of a higher plant N status on photosynthesis rate and leaf area duration. However, these results were obtained under conditions where N uptake during grain filling was significant and also grain growth was primarily dependent on current photosynthate. The same results were obtained by Morris and Paulsen (1985) where N fertilizer was applied from booting until post anthesis to the wheat plants grown under controlled conditions. They concluded that the increase in both grain yield and grain protein concentration was achieved because a high rate of N fertilizer was applied and also other resources such as moisture and temperature were in favour of crop growth. In semi-arid environments where N uptake is low (Heitholt *et al.* 1990), or carbohydrate reserves are the major source for grain filling (McCaig and Clark 1982), or under conditions where leaf area duration after anthesis is limiting (Simpson 1968), the supply of N during late growth stages may have little effect on grain yield.

As reviewed before, under high temperature during grain filling, grain weight is limited mainly by factors operating inside the grain rather than by the supply of assimilates (Jenner

1994). Thus, the advantage of a higher plant N status on photosynthetic capacity of the crop would be of less importance under warm conditions compared with normal conditions. It would, therefore, be expected that the relationship between the increased N concentration and grain yield at high temperature mainly results from interrelationships between protein and starch synthesis inside the grain and also the effect on the concentration and the activity of enzymes involved in starch synthesis. However, available information is lacking to show whether higher plant or grain N concentration can change these relationships under high temperature and if there are any differences among cultivars in this regard.

2.4 Effect of high temperature on grain filling

High temperature during grain filling of wheat is an important factor limiting grain yield in many wheat growing areas, even where enough water is available (Wrigley *et al.* 1994; McDonald *et al.* 1983). The extent to which yield reductions occur depends on the variety and the intensity, duration and time of exposure to high temperature.

2.4.1 Effects of high temperature on grain growth

The main effect of high temperature after anthesis is a reduction in kernel size. High temperature during booting and grain formation, especially during the first 3 days after anthesis (DAA), can reduce the number of kernels per ear, but after grain formation, which may be up to 7 DAA kernel number is not significantly reduced 30/25°C (Tashiro and Wardlaw 1990). However, very high temperatures (35-40°C) during early stage of grain development can cause a small reduction in grain number (Corbellini *et al.* 1997). Reductions in grain yield due to high temperature after anthesis, therefore result predominantly from reductions in kernel size rather than in kernel number (Wardlaw *et al.*

1989b). Accordingly variation in individual kernel weight has been used to determine the changes in grain yield in the experiments conducted under high temperature conditions.

Studies have shown that the optimum average post anthesis temperature for maximum kernel weight in wheat is about 15°C (Chowdhury and Wardlaw 1978) and there is a reduction in single grain weight of 3-5% for each 1°C rise in temperature above this (Wiegand and Cuellar 1981; McDonald *et al.* 1983; Wardlaw *et al.* 1989a). Even short periods of very high temperature, which are common during wheat grain filling, can significantly affect wheat grain yield (Randall and Moss 1990; Hawker and Jenner 1993; Stone and Nicolas 1994) and cooler, post shock conditions do not improve the ability of wheat to recover from short periods of very high temperature (Stone *et al.* 1995b). The extent of the reduction in grain weight due to high temperatures varies depending on variety and the duration, intensity and time of exposure to heat stress (Tashiro and Wardlaw 1990; Randall and Moss 1990; Stone and Nicolas 1995a,b; Corbellini *et al.* 1997).

The time and duration of exposure to high temperature significantly influences the response of grain filling to high temperature (Stone and Nicolas 1995a). Individual kernel mass at maturity was most sensitive to heat stress (5 days of max. 40°C) applied early in grain filling and kernel growth became progressively less sensitive as grain filling progressed. In both heat tolerant and heat sensitive cultivars the effect of heat stress on mature individual kernel mass was reduced by 1% for each 2-day delay in the start of heat treatment. The decrease in sensitivity to high temperature with time during grain filling was also observed for both moderately high (Tashiro and Wardlaw 1989) and very high temperature ranges (Randall and Moss 1990). However, different results were later

obtained by Corbellini *et al.* (1997) for early exposure to high temperature. They reported that a heat shock (35-40°C) of 5 days during the early stage of grain filling reduced the final kernel mass by only 4% while a shock of the same duration applied at medium or late stages produced greater reductions in kernel mass (13% and 14% respectively). However, when plants were exposed to a heat shock with longer duration (10 days) the sensitivity to high temperature decreased with time. The contrasting results in these two experiments was interpreted by Corbellini *et al.* (1997) in the following way. They concluded that plants subjected to a short period (5 days) of early heat shock acquired thermotolerance to further heat shocks. They also suggested that in their study because the plants experienced a progressive rise of temperature during grain filling before and also during the application of the stress, so the exposure to high temperature was gradual, leading to an acquisition of thermotolerance, while in the work presented by Stone and Nicolas (1995a) the plants were kept at constant low temperature before heat treatment and they could not become naturally acclimated. In another study by Stone *et al.* (1995), a short period of very high temperature applied early in grain filling also reduced the sensitivity to the subsequent moderately high temperature.

The interactions between moderately high and very high temperature stresses were investigated by Stone *et al.* (1995). They reported that the effects of short periods of very high temperature on individual grain yield followed by sustained periods of moderately high temperature were not additive. The lack of additivity of the effects of high temperature was also observed for moderately high temperature (Tashiro and Wardlaw 1990), suggesting that a kind of adaptation to further heat stress may be developed following a high temperature exposure at the early stage of grain filling.

The effect of rapid and gradual exposure to very high temperature during grain filling on the responses of two wheat varieties differing in heat tolerance was examined by Stone and Nicolas (1995b). A rapid increase in temperature from 20°C to 40°C induced a greater reduction in individual kernel mass than a gradual heat treatment in a heat sensitive variety. However, the more heat tolerant variety responded similarly to both rapid and gradual heat treatments. They concluded that for some genotypes, gradual heat acclimation may reduce yield loss at very high temperature, and that selection for tolerance to short periods of high temperature should be undertaken on gradually heat acclimated plants, particularly as this most closely resembles field conditions.

Given that individual kernel weight is the most important yield component of wheat after anthesis (Wardlaw *et al.* 1989b) and that the final grain weight is a function of rate and duration of grain filling (Sofield *et al.* 1977), the question was raised whether flexibility in the rate or duration of grain filling, or a combination of the two, is important in maintaining kernel size under high temperature conditions (Wardlaw and Moncur 1995).

2.4.2 Effect of temperature on grain-filling components

Both rate and duration of grain filling (GF) are influenced by high temperature (Jenner 1994). Temperatures above 15°C shorten the duration of GF. The rate of GF, compared with the duration, is much less responsive to temperature variation in the range of 20-30°C, and above 30°C the rate drops as temperature is increased in some circumstances. The increase of the rate of GF at temperatures above 20°C is not always large enough to compensate for the reduction in grain weight due to decreased duration.

Numerous studies in wheat have shown an inverse relation between temperatures over 15°C and duration of grain filling. Sofield *et al.* (1977) found that the duration of grain filling was reduced as temperature increased from 15/10°C (day/night) to 30/25°C. Reductions of 3.1 (Wiegand and Culler 1980) or 2.2 (Vitkare *et al.* 1990) days in the duration of grain filling have been reported for each 1°C increase in mean daily temperature.

The rate of grain filling displays different responses to variation in temperature. When temperature was elevated from 15/10°C to 21/16°C, there was an increase in the rate of GF which was large enough to compensate for the reduced duration (Sofield *et al.* 1977). However, as temperature rose from 21/16°C to 30/25°C there was a little change in the rate of GF so that grain weight was decreased at maturity. In a study by Tashiro and Wardlaw (1989), the grain growth rate of wheat was increased by a rise in temperature from 21/16°C to 24/19°C, but it was relatively stable from 24/19°C to 30/25°C, and started to decreased above 30/25°C.

The reduction in final grain weight under high temperature, therefore, is due either to the reduction in the duration of grain filling, which is not compensated by an increased rate of grain filling, or to reduction in both the rate and duration of grain filling.

2.4.3 Genotypic variation in response to high temperature

Genotypic differences in sensitivity of grain weight and both rate and duration of grain filling to high temperature exist among wheat cultivars. The variation for duration of grain filling among the genotypes is small in relation to the responses of rate of GF to high temperature (Hunt *et al.* 1991).

There is a considerable degree of variation in response to high temperature during grain filling among wheat cultivars. In one study, Wardlaw *et al.* (1989b) evaluated the responses of 66 Australian cultivars and breeding lines of wheat to a sustained moderately high temperature (30°C). They found that single grain weight was reduced by about 60% in the most sensitive cultivars but by only 30% in the least sensitive cultivars as temperature was increased from 18/13°C to 30/25°C. Genetic variability in wheat has also been reported in response to a short period (3 days) of very high temperature (Stone and Nicolas 1994).

Genetic variation has been reported for both rate and duration of grain filling (Nass and Resier 1975; Bruckner and Frohberg 1987; Darroch and Baker 1990; Hunt *et al.* 1991; Wardlaw and Moncur 1995). In a study by Nass and Resier (1975) the duration of grain filling ranged from 38 to 46 days in the cultivars tested in the field, however, this variation was not significantly different for cultivars that reached anthesis at the same time. Wiegand and Cuellar (1981), working in an area that experiences high temperature during wheat grain development, reported that the duration of GF was affected by temperature so that the final kernel weight in cultivars became proportional to the rate of grain filling. Wardlaw and Moncur (1995) analysed the rate and duration of grain filling in seven cultivars of wheat and showed that cultivars most tolerant of high temperature during grain filling were those in which the rate of kernel filling was most enhanced by high temperature. Similar results were obtained previously by Hunt *et al.* (1991).

Considering the results from the work of Hunt *et al.* (1991), the duration and rate of grain filling are seen to change independently in response to high temperature. Cultivar Nacosari, for example, had the biggest reduction in the duration of GF in relation to high

temperature, while it had the greatest Q_{10} (increase in weight between 20°C and 30°C) for the rate of GF among cultivars examined. As rate and duration of GF respond separately to high temperature, simultaneous selection for high GF rate and high kernel weight become possible without lengthening GF duration in environments prone to postanthesis drought and high temperature stresses (Bruckner and Frohberg 1987).

The fact that the rate of grain filling is important in the sensitivity of cultivars to high temperature validates the studies of the physiological and biochemical factors that are limiting the rate of grain growth and creating differences in the responses of cultivars to high temperature.

2.4.4 Factors regulating the response to high temperature

Some factors may modify the effect of high temperature on wheat plants during grain filling. It is therefore important to consider the interactions between these factors and high temperature while selecting for heat tolerance under different growth conditions. Light is one important factor that may change the response of cultivars to temperature. The sensitivity to high temperature during grain filling is increased under low light condition and results in a greater reduction in kernel size (Wardlaw *et al.* 1989a; Wardlaw 1994). The extent of these reductions varies among wheat cultivars. The sensitivity of both cultivars Banks and Kalyansona to high temperature, for example, increased as radiation was reduced by 50% (Wardlaw *et al.* 1989a), but the reductions in kernel weight occurred to a greater extent in Kalyansona than in Banks. Pre-anthesis conditions may also influence the postanthesis response to high temperature. The sensitivity of developing grains to high temperature was reduced by high temperature or low light applied during ear development (Wardlaw 1994).

There has been erratic variation in the response of cultivars to high temperature from one experiment to another (Wardlaw 1994). A variance of 12% is reported in reduction of kernel weight of cultivars Banks and Kalyansona in response to temperature in several experiments conducted in different seasons over many years. Although, it may not change the order of tolerance across cultivars, these interactions may affect breeding programs selecting for high temperature tolerance if selection is done over a number of generations and under different growth conditions. It was, therefore, suggested that screening of plants under high light conditions would be appropriate for the Australian wheat belt where high temperature is likely to be associated with high light condition (Wardlaw *et al.* 1989a). However, this may not be appropriate for the selection of heat tolerant cultivars adapted to the dense canopy situation of an irrigated crop, where high leaf area index may induce poor conditions of light inside the canopy.

Water content of the grains drops at high temperature, although there is no substantial change in water and osmotic potentials of the grains (Bhullar and Jenner 1983). There were no differences in the response of grain water content to high temperature when heat was applied to the whole plants, intact ears only, or ears in solution culture (Bhullar and Jenner 1985). This suggests that water potential of the grain is largely controlled by factors inside the grain (Barlow *et al.* 1980). The effect of high temperature on grain dry matter accumulation, therefore, does not seem to be mediated through an influence on water relations of the grain (Jenner 1994).

There is little information to show the extent to which the nutritional status of the plants can modify the effect of high temperature on wheat cultivars. A study by Dawson and Wardlaw (1984) showed no interaction between the level of nutrients and the response of

grain weight to high temperatures. However, in this thesis an investigation of the effects of a change made in the nutritional level of the grains (by trimming of the ears) was undertaken to reveal any effect of the variation in nutrients on the regulation of the temperature response of wheat cultivars.

2.4.5 The heat shock response

Plants respond to high temperature stress by the synthesis of specific proteins known as heat shock proteins (HSPs). This phenomenon, called heat-shock response (HSR), is conserved among all biological organisms (Kimpel and Key 1985; Lindquist 1988). These proteins can be classified on the basis of their molecular weight into two groups; High molecular weigh HSPs (68-110 KDa) which are widespread among all organisms and low molecular weight HSPs (15-27 KDa) which are prominent in higher plants. The optimal temperature for induction of the HSPs depends on the normal growing temperature of the organism. In plants, a heat shock of 8°C to 10°C above normal growing temperatures induces the synthesis of HSPs. During a heat shock event, the synthesis of HSP is accompanied by a decrease in the synthesis of 'normal' proteins (Ougham and Stoddart 1986). The synthesis of HSPs is rapid and transient; it occurs after a few minutes exposure of tissue to heat shock (Weng and Nguyen 1992). When tissues are returned to the normal growing temperature the synthesis of HSPs ceases and the synthesis of normal proteins recovers gradually (Howarth 1991). HSPs are also produced by stresses other than heat including various chemicals such as arsenite (Lin *et al.* 1984). Several studies have confirmed a positive correlation between the synthesis of HSPs and the development of thermal tolerance (Vierling 1991). However, the actual role of HSPs in the acquisition of thermal tolerance is not fully understood (Harrington *et al.* 1994).

The synthesis of heat shock proteins has been reported in several field crops such as cotton (Fender and O'Connell 1989), soybean (Altschuler and Mascarenhas 1985; Lin *et al.* 1984), corn (Cooper *et al.* 1984; Ristic *et al.* 1991), sorghum (Howarth 1991; Howarth and Skot 1994), and wheat plants (Krishnan *et al.* 1989; Hendershot *et al.* 1992; Weng and Nguyen 1992; Nguyen *et al.* 1994). Genotype variability in the synthesis of HSPs and in the ability to develop thermal tolerance has been found between cultivars of some field crops. Ougham and Stoddart (1986) observed genotype differences in the level of HSP synthesis in the seedlings of grain sorghum. The differences were related in some lines to acquired thermotolerance during germination. Heated plants of the drought and heat resistant line of maize synthesised a band of HSPs which was not found in heated plants of the drought and heat sensitive line (Ristic *et al.* 1991). However, Fender and O'Connell (1989) found no quantitative differences in HSPs between two heat resistant and heat sensitive lines of cotton.

The duration of HSP synthesis and the synthesis of normal proteins during recovery from heat shock in the seedlings of sorghum were both dependent on the severity of the initial heat shock (Howarth and Skot 1994). They reported that following an extreme heat shock of 48°C normal protein synthesis did not resume following 6h at 35°C and HSPs synthesis continued. Following a 45°C heat shock, however, the pattern of protein synthesis recovered much more quickly. It was concluded that once HSPs have accumulated to a level which copes with the heat stress their synthesis is then repressed and the 'normal' pattern of protein synthesis resumes. Similarly, the transcription of heat shock genes in soybean plants continued for longer duration recovery from a more severe heat shock (Kimpel *et al.* 1990).

The effect of gradual and rapid exposure to heat stress on the induction of HSPs were studied by Altschuler and Mascarenhas (1985). They reported that HSPs were synthesised at higher temperatures in soybean seedlings exposed to a gradual temperature increase as compared to a rapid heat shock. The mRNAs for HSPs were found at temperatures 6°C to 9°C higher after a gradual temperature increase than after a rapid heat shock. The synthesis of 'normal temperature' proteins also occurred at higher temperatures after a gradual increase in temperature than after a rapid heat shock.

In wheat, heat shock proteins are synthesised in different parts of the plant in response to heat stress. A set of 13 new proteins was produced in the coleoptiles and roots of 3-day-old seedlings of wheat cultivars Chinese Spring and Cheyenne in response to a heat shock of 40°C (Necchi *et al.* 1987). The seedlings and the flag leaves of flowering wheat plants synthesised both low and high molecular weight HSPs when leaf temperature increased about 10°C above the optimal growth temperature (Hendershot *et al.* 1992). HSPs were also present in the endosperm when wheat heads were exposed to a rapid increase in temperature to 40°C (Bernardin *et al.* 1994).

Genetic differences in the synthesis of HSPs and in the ability to induce an acquired cellular thermal tolerance mechanism has been reported in some wheat cultivars. Significant quantitative differences in the synthesis of HSP were observed between the seedlings of two winter wheat cultivars Mustang (heat tolerant) and Sturdy (heat sensitive) in response to a heat shock of 37°C (Krishnan *et al.* 1989). The levels of synthesis of low molecular weight HSPs in two cultivars were positively correlated to their differences in thermal tolerance. In another study (Weng and Nguyen 1992), there were not only quantitative differences of individual HSPs between Mustang and Sturdy, but also some

unique HSPs were only found in Mustang. The heat tolerant cultivar Mustang had higher steady-state levels of low molecular weight HSP mRNAs and faster transcript accumulation than Sturdy in response to heat stress. They suggested that some of the unique HSPs in Mustang may play an important role in the regulation of heat shock gene expression and may result in differences of thermosensivity between these wheat lines under heat stress.

The synthesis of HSPs in leaves and stems of wheat plants may play some unique roles in maintaining functions of these tissues during heat stress allowing higher source activity (Nguyen *et al.* 1994), and so ameliorate harmful effects of high temperature during the vegetative phase. Heat shock responses in the endosperm during grain filling are dealt with in section 2.8.4 below.

2.5 Effects of high temperature on starch synthesis

Starch is the major component of the wheat kernel and reduction in grain weight at high temperature is mainly due to the effects on starch deposition (Bhullar and Jenner 1985). Supply of assimilates to the grains does not seem to restrict the production of starch under high temperature (Chowdhury and Wardlaw 1978; Wardlaw *et al.* 1980; Nicolas *et al.* 1984). The conversion of sucrose to starch in the endosperm is impaired at high temperature and causes the major limitation to starch synthesis in wheat (Bhullar and Jenner 1985) and also in barley (MacLeod and Duffus 1988). However, the transfer of sucrose from the crease vascular system of the kernel into the endosperm might be another possibility to explain part of the temperature response in wheat cultivars (Wardlaw *et al.* 1995).

The reduction in starch synthesis in the endosperm at high temperature is largely due to the reduced activity of soluble starch synthase (SSS) (Denyer *et al.* 1994; Jenner 1994). The maximum activity of SSS is low compared with the other enzymes involved in the pathway of starch synthesis and is close to the rate of starch synthesis, whereas the activity of other enzymes are between 10 to 50-fold greater (Hawker and Jenner 1993). Therefore, a reduction in the activity of SSS can significantly decrease the rate of starch synthesis (Jenner *et al.* 1993; Keeling *et al.* 1993). This enzyme compared to the other enzymes in the metabolic pathway is much more sensitive to heat inactivation and has a low optimum temperature for maximum activity (Keeling *et al.* 1993; 1994). Keeling *et al.* (1993) reported that the maximum activity of SSS was achieved at about 25°C then decreased, whereas the activities of several other enzymes involved in the pathway were not affected by elevated temperature. They found a close correlation between the loss in the activity of SSS and loss in starch synthesis in the endosperm. The maximum rate of grain growth (Tashiro and Wardlaw 1989) or starch production (Bhullar and Jenner 1985) is also achieved at temperatures below 30°C. Even a short period of exposure to temperatures in the excess of 30°C can decrease the activity of SSS, and the loss of activity can be irreversible if the time of exposure is extended (Jenner *et al.* 1993; Keeling *et al.* 1993). In conclusion, SSS is the primary flux-controlling enzyme in the pathway of starch synthesis in wheat endosperm. However, in some storage organs the other enzymes may be more important in limiting starch synthesis at high temperature, such as ADPglucose pyrophosphorylase (ADPase) in potato tubers (Lafta and Lorenzen 1995). In wheat, the activity of ADPase may also be decreased at elevated temperature, but to a lesser extent and more slowly than SSS (Jenner *et al.* 1993). The loss of the activity of ADPase in maize grown at high temperature influences starch synthesis through a reduction in the duration, but not on the rate of grain growth (Singletary *et al.* 1994).

To explain the irreversible effect of heat inactivation on SSS, it is proposed that the tertiary structure of the enzyme may somehow be changed by elevated temperature so that the ability of SSS to interact with its substrate decreases (Keeling *et al.* 1994). This can account for the loss of activity of SSS at high temperature so it is assumed to be an intrinsic property of the enzyme alone (Denyer *et al.* 1994). In a study by Jenner *et al.* (1995), the affinity of the SSS for both substrates, amylopectin and ADPglucose, was decreased by high temperature. As the effects of temperature on the K_m for amylopectin were much greater, it was concluded that the major action of temperature involves interaction of the enzyme with amylopectin. Yet, it is not revealed whether temperature acts on the enzyme, or on its substrate, or on both.

A time course of heating showed that heating isolated wheat grains for only 30 minutes at 35°C caused more than 50% reduction in the activity of SSS (Jenner *et al.* 1993). A greater reduction occurred by further heating, but the effects of heating diminished gradually such that the activity of the enzyme was maintained at low but almost steady level for 3 hours. As there are several isoforms of SSS identified in wheat endosperm (Denyer *et al.* 1995), one form or some forms might be more tolerant to heat than others or might have a higher affinity for the substrate, allowing the enzyme to stay active and compensate for the loss of activity (Jenner 1994; Jenner and Sharma 1997).

Only the soluble form of starch synthase is sensitive to heat inactivation; elevated temperature does not significantly affect the activity of granule bound starch synthase (GBSS), involved in amylose synthesis (Jenner *et al.* 1993; Hawker and Jenner; 1993; Keeling *et al.* 1993). As a result, the proportion of amylose in starch increases at high temperature (Shi *et al.* 1994; Tester *et al.* 1995). The heat sensitivity of GBSS seems not

to be intrinsically different from SSS, but being bonded into the structure of the starch granule seems to provide some protection for GBSS from heat inactivation. This view is supported by the fact that the solubilised form of GBSS, released by treatment of the starch with ∝-amylase, is also sensitive to heat at temperatures under which the activity of GBSS in the intact starch granule is not affected (Denyer *et al.* 1994).

At temperatures below 30°C, the loss of the activity of SSS is not large enough to account for the temperature responses of starch deposition in wheat endosperm. Only an 8% reduction in the activity of SSS was observed by exposing wheat plants to 30°C for 2 hour, compared to 69% loss of activity at 35°C during the same period (Rijven 1986). This indicates that at temperatures in excess of 30°C the reduction in starch synthesis can be explained satisfactorily by the loss of activity of SSS. Yet, the kinetic properties of the SSS are very sensitive to temperatures above 20°C and may to some extent account for the temperature response of starch synthesis in the range 20-30°C (Jenner *et al.* 1995; Jenner and Sharma 1997). The increase in the K_m of SSS for amylopectin is associated with rising temperature, indicating that the affinity of enzyme for its substrate reduces at high temperature. The highest affinity of SSS for amylopectin in wheat and barley was observed in the temperature range of 15-20°C (Jenner and Sharma 1997). The differences in the temperature response of cultivars Trigo (tolerant) and Lyallpur (sensitive) at 30°C compared to 20°C was also explained by temperature response of the kinetic properties of SSS (Jenner and Sharma 1997). The superior performance of cultivar Trigo at 30°C was related to the higher efficiency of SSS, a characteristic obtained from the combined effects of growth temperature on two kinetic properties of the enzyme, V_{max} and K_m .

In conclusion, limitation of starch synthesis in wheat endosperm at high temperature is mainly due to a loss in the activity of SSS or to changes in the kinetic properties of the enzyme. At temperatures below 30°C, the loss of activity is small and the temperature response of starch synthesis is more dependent upon the changes in the kinetic properties of SSS. However, the extent to which the differences in temperature responses of cultivars, in terms of the rate of grain filling, can be explained by changes in the kinetic properties of SSS in the range of 20-30°C still remains to be examined.

2.6 Effect of sink manipulation on N and sugar accumulation

The manipulation of source and sink has long been used to understand the physiology of yield and the relationship between sink and source under various growth conditions. In this part the effect of altering the provision of nutrients and manipulating the capacity of the ear to accommodate them on the development of wheat plants is briefly reviewed.

2.6.1 Nitrogen accumulation

The nitrogen concentration in wheat grains can be modified by different manipulations such as N fertilizer application (Sofield *et al.* 1977b) or ear trimming (Jenner 1980; Perez *et al.* 1989). Removal of some grains from the ear increases the supply of amino acid and the accumulation of nitrogen in the remaining grains in the ear (Simmons and Moss 1978; Jenner 1980; Thorne 1981; Radley and Thorne 1981; Perez *et al.* 1989). In a study by Perez *et al.* (1989), removal of the upper half of the ear increased the nitrogen content of the grains remaining in the lower half of the ear. The increase in grain nitrogen content was because of a change in nitrogen distribution within the shoot and was not due to an increase in the nitrogen uptake of the shoots. The reduction in the size of the ear reduced the total aboveground nitrogen content as a result of a reduction in nitrogen uptake by

shoots, as also reported by Thorne (1981). The authors concluded that the decreased uptake may be either because of limited capacity of the grains, or an indirect effect of the decreased size of the ear. For example, the reduction in nitrogen uptake as a result of ear removal in maize was suggested to be caused by stomatal closure which, in turn, would reduce the flux of nitrate into the shoot (Christensen *et al.* 1981). In wheat plants, removing half the grains from ear also decreased flag leaf stomatal conductance and transpiration which maintain higher leaf water potential especially under drought stress, and resulted in a reduction in carbon exchange rate and stem reserve mobilization (Blum *et al.* 1988). Ghildiyal *et al.* (1995) also reported that the stomatal resistance of the flag leaf in wheat was higher in plants with the spike removed particularly at the post-anthesis stage. In contrast with these results, increased uptake of nitrogen by shoots due to the removal of the upper half of the ear in wheat plants has also been reported (Martinez-Carrasco and Thorne 1979). There have also been varietal differences in the capacity of grains to store the supplement of nitrogen at various stages of development (Perez *et al.* 1989). Trimming at the early stage (anthesis and 5 DAA) increased grain nitrogen content more than trimming late (15 and 25 DAA) in cvs. Splendour and Hobbit, but there were no significant differences among trimming times in cv. Maris Huntsman. Some studies (Mackown and Sandford 1988; MacKown *et al.* 1989; Osaki *et al.* 1995) have also shown that the nitrogen content decreases in leaves and increases in stem or tillers by sink manipulation. The relatively uncontrolled inflow of nitrogen into the wheat grains has also been shown in wheat ears grown in liquid culture. For instance, the grain N content of detached wheat culms increased with increasing levels of glutamine in the culture medium (Barlow *et al.* 1983), indicating that the accumulation of nitrogen in wheat grains is predominantly source limited.

2.6.2 Sugar accumulation

The accumulation of soluble sugars in leaves increases as a result of trimming the ears (Herold 1980; Blum *et al.* 1988; Labrana and Araus 1991; Lazan *et al.* 1993). However, there was no significant increase in the concentration of soluble sugars in the remaining grains of trimmed ears, despite an increased supply of sucrose available for distribution to the grains (Jenner 1980). On the other hand, a decrease in source/sink ratio due to source excision (leaf removal) decreases the leaf sugar concentration (Araus and Tapia 1987; Guitman *et al.* 1991).

Some workers (Herold 1980; Lazan *et al.* 1993) have suggested that the accumulated sugars in the leaves due to a reduction in sink size produces a feedback reduction in photosynthesis and accelerates leaf senescence. While others (Blum *et al.* 1988; Labrana and Araus 1991) indicated that the level of these carbohydrates in leaves would not trigger or accelerate flag leaf senescence. Inhibition by carbohydrate of photosynthesis was shown by Morcuende *et al.* (1996) who suggested that carbohydrate accumulation in leaves when sink demand is low may cause phosphate limitation of photosynthesis under non-extreme natural conditions. Wang *et al.* (1997) reported that sink reduction decreased the leaf net photosynthetic rate of irrigated wheat, but it had a very little effect on the production of photosynthates of rainfed wheat. Sink reduction decreased photosynthate translocation into grains, and increased it into other aboveground parts of rainfed wheat. The results supports the idea that under non-stress conditions the grain growth in wheat is sink-limited (Jenner and Rathjen 1977; Barlow *et al.* 1983), and thus a reduction in sink size would lead to an accumulation of soluble sugars in leaves.

2.7 Grain protein fractions

2.7.1 Classification

The first classification of wheat and other plant proteins was presented by Osborne (1924). This classification was based on solubility and consists of four protein groups; albumins (water-soluble), globulins (soluble in dilute saline), prolamins (soluble in aqueous alcohols) and glutelins (soluble only in dilute acid or alkali). Glutelins have later been considered as prolamins as they are soluble in aqueous alcohol after reduction of inter-chain disulphide bonds (Kreis *et al.* 1985). Prolamins, gliadins and glutenins are major components of wheat gluten and account for about half of the total grain nitrogen. Gliadins mainly consist of single polypeptide chains (monomers) while the glutenins are polymers consisting of subunits crosslinked by disulphide bounds. There is much overlap between the different solubility fractions in Osborne's classification because each fraction is a complex mixture of different polypeptides. To minimise any overlap, the prolamins (gliadins and glutenins) were classified according to molecular relationships (electrophoretic mobility and amino acids sequence) rather than on extractability and solubility properties (Shewry *et al.* 1986). In this classification prolamins are recognised in three groups of proteins called; S-rich (α-, β-, γ-gliadins) and low molecular weight (LMW) glutenin subunits, S-poor prolamins (ω-gliadins), and high molecular weight (HMW) prolamins (HMW subunits of glutenin).

Size-exclusion high performance liquid chromatography (SE-HPLC) has recently provided a molecular size based classification, which is used to separate different wheat protein fractions based on their molecular weights (Singh *et al.* 1990; Batey *et al.* 1991; MacRitchie 1992). In this method proteins are separated in order of elution into three main groups: polymeric proteins, gliadins, monomeric albumin/ globulins. The difficulty of

solubilizing of unreduced large polymeric proteins is mainly overcome by using sonication of the flour dispersed in SDS (sodium dodecyle sulfate-polyacrylamide) buffer solution. The polymeric protein fraction consist of high and low molecular weight glutenin subunits (about 85%) and a HMW portion of albumin and globulin subunits (about 15%) (MacRitchie 1992; Gupta *et al.* 1993).

2.7.2 Functionality in flour

Proteins are the most important of the components of wheat grain that determine the functional properties of wheat flour. Wheat gluten proteins in particular are largely responsible for the visco-elastic properties of dough that are associated with good breadmaking performance (Hoseney 1994).

A considerable amount of work has been done to determine the molecular basis of the physical properties of gluten. Studies have shown that, of the two components of gluten, the glutenins (polymers) are primarily responsible for elasticity and the gliadins (monomers) for viscosity and extensibility of wheat dough (Shewry 1994). Within the glutenin fraction, the HMW glutenin subunits have a much greater effect on dough strength than LMW glutenin subunits. Popineau *et al.* (1994) using isogenic lines, found a direct relationship between HMW subunits of glutenins and gluten visco-elasticity. Gupta *et al.* (1995) reported that the deletion of HMW subunits of glutenins reduced gluten elasticity more than did the deletion of LMW subunits. They explained that the greater effect of HMW glutenin subunits on dough strength was not due to differences in the amounts of the two types of subunits as the LMW glutenin subunits have a significantly greater contribution in total polymeric protein than did the HMW glutenin subunits (MacRitchie

1992). Their relative effects on the size distribution of the polymeric protein was proposed (Gupta *et al.* 1993) to be important on determination of dough strength.

The direct relationship between the amount of glutenin subunits, especially HMW glutenin subunits, and dough strength implies that wheat quality can be improved further without increasing grain protein levels and thus without reducing grain yield (Gupta *et al.* 1989; Gupta *et al.* 1994a).

2.7.3 Accumulation of protein fractions

The accumulation of protein fractions in the wheat grain is asynchronous; as the grain develops the proportion of each fraction changes in the protein complex. Most studies have shown that the accumulation of storage proteins in the grain is in such a way that the average molecular size of grain proteins increases during grain filling. In a study by Kapoor and Heiner (1982), the proportion of albumin and globulin decreased and that of gliadin and glutenin increased as the wheat cultivars matured. Stone and Nicolas (1996a) also reported that the pattern of accumulation of albumin/ globulin proteins was different from that of storage proteins. Albumin/ globulin accumulated most rapidly in the very early stages of grain development while the storage proteins accumulated more in the mid to later stage of grain growth. In this study the synthesis of monomers was followed by that of SDS-soluble polymer and finally by the SDS-insoluble polymer. As the SDS-insoluble fraction was shown to have a significantly greater proportion of larger polymers than the SDS-soluble fraction, Gupta *et al.* (1993) concluded that the average molecular size of grain protein increases throughout grain filling. Suter *et al.* (1994) concluded that during grain filling, gliadin synthesis is initially rapid and then levels off, whereas glutenin

synthesis increases after an initial lag period. During this synthesis the glutenin polymers go through an aggregation process, increasing in average molecular weight.

There is a rapid change in the size distribution of the polymeric proteins during the late stage of grain development. The development of polymeric glutenin is initiated with the synthesis of a backbone structure of high molecular weight glutenins (Panozzo *et al.* 1994). The glutenin subunits are detectable as early as 7 days after anthesis (Gupta *et al.* 1994b) but they exist largely as small polymers until the middle stage of grain development. They are converted into large polymers very rapidly during the later stage of grain filling. The period of most rapid accumulation of SDS-insoluble polymers also coincided with that of most rapid decrease in the amount of SDS-soluble polymers (Stone and Nicolas 1996a). These results are consistent with the general concept that the gliadin subunits are incorporated into glutenin polymers during the late stage of grain development when the monomer subunits are in sufficient amounts, as shown by Lew *et al.* (1992).

The order of accumulation of the different protein fractions in the grain, which leads to an increased proportion of larger polymer subunits during the late stage of grain development, would suggest that an increase in grain quality and dough strength towards maturity would be expected (Gupta *et al.* 1993). However, any change in gliadin/ glutenin ratio imposed by environmental factors such as temperature (Blumental *et al.* 1993) or nitrogen application (Dubetz *et al.* 1979) may significantly modify the protein composition and as a consequence the quality of mature grain.

2.7.4 Effect of temperature on protein composition

Temperature is the most important environmental factor modifying grain quality during grain filling in the wheat growing areas of Australia (Randall and Moss 1990; Wrigley *et al.* 1994). The response of grain quality to temperature varies depending on the extent to which high temperatures occur during grain filling. An increase in temperature during grain filling up to 30°C generally increased grain quality measured as dough strength, however, temperatures above 30°C applied even for a short period (3 days of 36°C maximum), tended to decrease dough strength (Randall and Moss 1990). Wrigley *et al.* (1994) reported that dough strength was greater for wheat grown in the northern region of the Australian cereal zone, where temperatures during grain filling are higher, compared with wheat from southern Australia. They found that some of the annual fluctuations in dough properties were associated with occasional heat stresses (>35°C) at times when the developing grain was sensitive. Similar results were obtained by Borghi *et al.* (1995) in southern Europe. They were also able to relate fluctuations of temperature during grain filling, in an area with Mediterranean climate in Italy, to rheological characteristics of bread and durum wheat cultivars. Earlier Peterson *et al.* (1992), when evaluating the general interaction between genotype and the environment for Hard Red Winter wheat in USA, also emphasised the importance of environmental factors on grain quality compared with genotype and genotype-environment interactions.

The improvement in wheat quality at moderately high temperatures (up to 30°C) seems to be due to an increase in grain protein percentage rather than to a change in protein composition. As temperature increases in the range of about 20-30°C the accumulation of starch in the grain is reduced to a greater extent than that of protein (Jenner 1994). This

results in an increase in the proportion of protein in the mature grain and increases the bread-making quality of wheat (Randall and Moss 1990; Wrigley *et al.* 1994).

A considerable number of studies have been conducted to formulate a molecular mechanism to explain the observed changes in grain quality due to heat stress (> 30°C) during grain filling. Changes in protein composition have been known to be responsible for the variation in grain quality under heat stress. The effects of heat stress on protein composition have been detected as changes in the glutenin to gliadin ratio (Blumenthal *et al.*1993,1994; Stone and Nicolas 1996), in the size of glutenin polymers (Blumenthal 1995; Borghi *et al.* 1995; Corbillini *et al.* 1997), or in the amount of heat shock proteins (Blumental *et al.* 1990; Bernardin *et al.* 1994). Blumenthal *et al.* (1993) showed that during a period of heat stress the synthesis of gliadin continued at a greater rate than glutenin synthesis and as a consequence the ratio of gliadin/ glutenin increased in the mature grain and produced weaker dough. Stone *et al.* (1996) reported that heat treatments reduced the polymer: monomer ratio because the reduction in the accumulation of monomer was less than that of polymer. High temperature may also affect the size distribution within protein classes. Corbellini *et al.* (1997) found that a period of 5 days of heat shock (35-40°C) increased the SDS-soluble polymeric proteins and low molecular weight gliadins while there was a reduction in the formation of more complex SDS-insoluble polymeric proteins. Heat shock proteins which are synthesised in developing wheat grains during exposure to high temperature (Blumenthal *et al.* 1990; Giorini and Galili 1991) are implicated in the heat-related loss of dough strength (Bernardin 1994).

The time of the exposure to heat stress has a significant effect on the accumulation of different protein fractions (Stone and Nicolas 1996b). This may be due to the

asynchronous accumulation of the protein fractions during grain development. Variation among cultivars may also exist in the response to timing of the exposure to high temperature in terms of protein fraction accumulation. Stone and Nicolas (1996b), for example, reported that the effect of heat stress on the accumulation of SDS-insoluble polymer diminished with time from anthesis for cultivar Oxley, but increased towards maturity for cultivar Egret.

The availability of nitrogen in the grain may regulate the effects of high temperature on grain protein composition. Shewry *et al.* (1994) showed that the effect of temperature on protein composition was modified by the level of the nitrogen in the grain. The proportion of prolamins (gluten protein) nitrogen was increased at high temperature at high nitrogen availability, but there was little effect of temperature on prolamin nitrogen at low nitrogen availability. They did not specify the effect of temperature at different levels of nitrogen on gluten components separately. It needs to be considered in further studies.

2.8 Conclusion

High temperature during grain filling (GF) is an important factor limiting grain yield in wheat growing areas. The reduction in final grain weight under high temperature is due either to the reduction in the duration of GF, which is not compensated by an increased rate of GF, or to reduction in both the rate and duration of GF. Cultivars show variation for both characters, but variation between cultivars for duration of GF is small in relation to temperature. The reduced rate of dry matter accumulation at high temperature is mainly due to the effects on starch deposition. Supply of assimilates to the grains does not seem to restrict the starch deposition under high temperature. Limitation of starch synthesis at high temperature is mainly due to a loss in the activity of SSS. At temperatures blow 30°C, the

loss of activity is small and the temperature response of starch synthesis is more dependent upon the changes in the kinetic properties of SSS. The extent to which the differences in temperature responses of cultivars, in terms of the rate of GF, can be explained by changes in the kinetic properties of SSS the range of 20-30°C still remains to be examined.

Compared to the deposition of starch protein accumulation is less affected by variation in temperature, especially in the range below 30°C. With increase in temperature the rate of protein accumulation seems to be accelerated to a greater extent than starch deposition. However quantitative data on the responses to temperature of the rate of accumulation of different protein fractions are lacking and there is little information on variation among cultivars. The heat shock response mechanism appears to play little or no part in the effects of temperature on grain filling in the temperature range below 30°C.

Higher plant N status during grain development may, in some circumstances, improve grain yield by increasing the photosynthesis capacity of the crop. The advantage of a higher plant N status on photosynthetic capacity is of less importance under warm conditions. Therefore, the relationship between the increased N concentration and grain yield at high temperature may largely result from interrelationships between protein and starch synthesis inside the grain. However, available information is lacking to show whether the temperature response of cultivars can be regulated by N status of the grain.

Chapter 3

Materials and Methods

3.1 Selection of cultivars

Four spring cultivars of wheat (*Triticum aestivum* L.) were used for this study; Trigo (AUS-4073), Lyallpur (AUS-18804), Kavko (AUS-25114), and Sun 27B (AUS-20007). They were chosen to represent a range of cultivars with wide responses to high temperature. Trigo had performed as a tolerant and Lyallpur as a sensitive cultivar in response to an increase in temperature from 18/13°C to 30/25°C in a study conducted by Wardlaw *et al.* (1989b). Kavko and Sun-27B had been tested by Hunt *et al.* (1991) and found to be tolerant and semitolerant cultivars respectively in response to an increase in temperature from 20/15°C to 30/25°C.

3.2 Growth conditions

Pre-anthesis

Plants were grown in 25 cm (6.5L soil) diameter pots and kept in an environment-controlled growth cabinet. The seedlings were thinned one week after emergence to 20 plants per pot. Tillers were removed as they appeared. Pots were well watered and fertilised with 200 mL of soluble fertiliser containing 2.6 g Aquasol^{TM} (N:P:K ratio of 23:4:18), applied fortnightly from one week after emergence until anthesis. No fertiliser was applied to the plants after anthesis. For experiment 3 two levels of nitrogen were used and the method has been explained in detail in Chapter 5. The temperature of the growth cabinet was set at 20°C during the day (14h) and 15°C at night. Plants were illuminated with high pressure sodium lamps, supplemented with fluorescent tubes provided a photosynthetic photon irradiance of 310-320 $\mu mol.m^{-2}.sec^{-1}$ at ear level. Experiments 3

and 4 (reported in Chapters 5 and 6) were conducted in the growth rooms illuminated by two banks of 5 metal halide lamps, adjusted to provide constant irradiance at canopy level similar to the first and second experiment.

Post anthesis

At day 2 after anthesis, half of the pots of each cultivar were shifted to another growth cabinet, adjusted to 30/25°C (day/ night) as the heat stress treatment. The rest of the plants were kept in the growth cabinet at 20/15°C (day/ night) temperature. The pots were transferred to the new growth cabinet individually. The beginning of anthesis for each pot was considered when 4 or more plants flowered at the same day. The day length for both treatments was the same as pre-anthesis conditions. In experiments 3 and 4 the ears on plants in half of the pots of each cultivar were trimmed by removing spikelets from the top and bottom of the ears leaving only the central four spikelets on each side of the ear (Jenner 1980). Trimming was done at days 8 and 16 after anthesis at 30°C and 20°C respectively. To avoid any effect of water stress, the plants at 30/ 25°C were watered two times a day.

3.3 Sampling

Individual plants were tagged for subsequent sampling when the first anther in the ear emerged. At the time of sampling, one ear from each pot was taken every 2 and 4 days from pots at 30/25°C and 20/15°C respectively, started from day 2 after anthesis. In the experiment 1, the samples were taken every 4 days at both temperatures. The grains were sampled from the spikelets in the middle of the ears.

3.4 Grain weight measurement

The grains were weighed immediately after sampling and the fresh weight was recorded. The grains were then oven-dried at 80°C for 48 hours and cooled over silica gel and grain dry weight was determined. The difference between grain fresh weight and grain dry weight was considered as the water content of the grain. The dried grains were ground into a fine flour suitable for nitrogen, protein, or starch measurement, using a laboratory pulverising mill (Labtechnics model LM1-P). The grains were ground in the smallest ring and roller bowl (50g size) fitted to a pulverising mill for 4 minutes. In experiment 1, a pestle and mortar were used to crush and grind the grains into fine flour suitable for nitrogen analysis.

3.5 Estimation of grain growth parameters

The parameters of grain filling were estimated by the ordinary logistic model. This model along with the other growth models tested in this study is explained in Chapter 4.

3.6 Analysis of grain nitrogenous compounds

3.6.1 Grain nitrogen

The amount of grain nitrogen was measured by using the Dumas Total Combustion method (American Society of Brewing chemist, 1996) utilising a Carlo Erba 1500 Nitrogen Analyser. The nitrogen percentage of flour samples (5mg) in aluminium folds was determined through a complete combustion at 800°C. The assay was calibrated by using samples of standards (oatmeal and alfalfa) with a known nitrogen quantity with each set of analysed samples.

3.6.2 Grain Protein

The amount of grain protein was measured by using Bio-Rad protein Assay based on the method of Bradford (Bradford 1976). 10 mg of grain flour was suspended in 1ml of 50% n-propanol containing 1% dithiothreitol (DTT) in a 1.5 mL Eppendorf tube. The sample was mixed and incubated in a water bath set at 60°C. After 1 hour the sample was removed from the water bath, mixed and centrifuged for 5 min at full speed (Beckman Microfuge E^{TM}). An aliquot of 0.1 mL of the supernatant was transferred to a spectrophotometer tube, 4.9 mL of diluted Bradford reagent (1 part dye reagent concentrate diluted with 4 parts distilled water) was added, mixed, and left at room temperature. After 5 min the optical density was read at 595 nm against water. With each set of measured samples one sample of wheat gluten (Sigma) was measured. The amount of gluten protein per sample was calculated from a standard curve prepared by using different concentrations of Bovine Serum Albumin and by correcting the data for the differences in extinction values of the two proteins.

3.6.3 Protein fractions

Protein fractions were first extracted by using a protein denaturing solvent containing sodium dodecyl sulphate (SDS) and then separated by Size Exclusion High Performance Liquid Chromatography (SE-HPLC).

Protein extraction

Eleven mg grain wheat flour was suspended in 1 mL of SDS-phosphate buffer (50 mM Na_2HPO_4 and 50 mM NaH_2PO_4 in 0.5% SDS solution, pH 6.9) in a 1.5 mL Eppendorf

tube. The suspension was stirred with a stainless steel wire and vortexed for 5 seconds. The sample was then stirred by a stirrer for 5 min at 2000 rpm and centrifuged for 30 min at 10000 g. The supernatant was transferred into another 1.5 mL Eppendorf tube and filtered through a 0.45 μm filter (Minispilce, Gelman Science) and used for the measurement of SDS-soluble proteins. The pellet was resuspended in 1 mL SDS-buffer as above by stirring with a stainless wire and then sonicated for 30 seconds at power setting 5 (output 40 W) with Branson Sonifier, model B-12 with a 3-mm diameter stepped microtip probe. After sonication the sample was centrifuged for 30 min at 10000 g. The supernatant was then filtered as described before for the SDS-insoluble protein fraction. With each set of measured samples one sample of Spear flour was used as a standard with a known quantity of protein.

Protein separation

Size-exclusion SE-HPLC was carried out on a Waters Protein-Pak 300 Size-exclusion column, using a Waters HPLC system comprising two model 510 pumps, a WISP 71OB automated sample injector, and a model 481 UV-visible detector at 214nm. Pump control and data acquisition were achieved with a Millennium chromatography program. Protein fractions were separated by injecting 20 μL of the sample into the column and running for 40 min. A mixture of 50% (v/v) acetonitrile and water containing 0.05% (v/ v) trifluoroacetic acid was used as the eluent with a flow rate of 0.5 ml min^{-1}. The amount of protein in injected samples was determined by using a sample of Spear flour with a known quantity of protein as following:

Eleven mg of Spear flour contained 1.6852 mg protein. The amount of protein in injected Spear sample was $(1.6852 \times 20)/ 1000 = 0.0337$ mg.

0.0337 mg Spear protein gave an area of 60.9 area units under the chromatogram peaks.

1 mg Spear protein therefore gave an area of 60.9/ 0.0337 = 1807 units (area).

The HPLC peak area of sample A, for example, was 37.6 units.

Amount of protein in sample A per 20 μL injection was 37.6/ 1807 = 0.0208 mg.

Amount of protein per sample A (11.8 mg) was (0.0208 × 1000/ 20) = 1.04 mg.

3.6.4 Grain soluble amino acids

Extraction of soluble amino acids

The freshly sampled grains were dropped into a tube containing 10 mL of boiling 80% ethanol, gently boiled for 10 minutes, cooled and stored at 4°C while it was capped. Just before the time of extraction, the sample was transferred to an oven set at 80°C. After 5 minutes the tube was removed from the oven and the supernatant was decanted into a 25 mL volumetric cylinder. The tissue was transferred to a mortar, a little sand was added, and the tube was rinsed with 2 mL of 80% ethanol. The tissue was crushed and ground with a pestle to a fine suspension. The contents of mortar and pestle were then rinsed into a volumetric cylinder and the volume was adjusted to 15 mL with water. After shaking, the extract was transferred to a concial disposable centrifuge tube and centrifuged at 3,000 rpm for 5 minutes. Portions of supernatant were sampled for analysis of amino acids and soluble carbohydrates.

Grain soluble amino acids analysis

The amount of grain soluble amino acids was measured based on the method of Lee and Takahashi (1966), using ninhydrin dissolved in citrate buffer pH 5.75 prepared as follows: 44.1 g of Analar sodium citrate dihydrate (294 Mwt) was dissolved in approximately 270 mL distilled water. The pH of the solution was adjusted to 11.0 with addition of 5N NaOH

and boiled for 5 min. When the sample had cooled, the pH was adjusted to 5.75 with concentrated AR HCL and volume was made up to 300 mL with distilled water.

A portion (0.2 mL) of grain extract was mixed with 3.8 mL of a reaction mixture (0.1 g ninhydrin dissolved in 14 mL of citrate buffer pH 5.75 and 24 mL glycerol) in a test tube and placed in a boiling water bath for 12 min. When it had cooled, the sample was mixed and the optical density was read against water at 570 nm within one hour. The amount of soluble amino acids in the liquid was estimated by using a standard curve of glycine.

3.7 Analysis of grain carbohydrates

3.7.1 Total starch assay

The amount of starch in the endosperm was measured using a modified procedure of Megazyme Total starch method as described by McCleary *et al.* (1994). Starch was first solubilized by using urea-dimethylsulphoxide (UDMSO) solution prepared according to the method of Morrison and Laignelet (1983): 100 mg of grain flour was suspended in 10 mL of UDMSO in a glass test tube (16×100 mm). The tube was put in a boiling water bath for 15 min, and then transferred to an oven set at 100°C for 90 min. The tubes were allowed to cool to room temperature and the starch was hydrolysed by using reagents from the Megazyme Total Starch Assay. An aliquot (0.8 mL) of starch-UDMSO solution was weighed into a plastic conical centrifuge tube. Thermostable α-amylase in MOPS buffer was added and the tube was incubated in a boiling water bath. After 5 min the tube was moved to another water bath set at 50°C, followed by the addition of sodium acetate buffer containing amyloglucosidase. After 30 min the tube was taken out of the water bath and the volume was adjusted to 10 mL with distilled water. The resulting solution was centrifuged for 10 minutes at 3000 rpm. An aliquot of the diluted solution was mixed with GOPOD (Glucose oxidase/peroxidase) reagent and incubated in the water bath set at 50°C

for 20 min. Glucose standards were also assayed with each set of measured samples. The absorbance was read at 510 nm against reagent blank. Total starch was calculated as follows:

Total starch = DE × F × 1000 × 1/1000 × 100/W × 162/180

where DE = absorbance (read against reagent black)

F = conversion factor from absorbance to mg

1000 = volume correction for aliquot assayed

1/1000 = μg to mg

100/W = express starch as % (w = weight in mg)

162/180 = adjustment from free glucose to anhydroglucose.

3.7.2 Amylose assay

Starch was dissolved in UDMSO as described above in the starch assay. After removing the samples from the oven they were allowed to cool, and an aliquot (1 mL) of the starch-UDMSO solution was weighed into the bottom of a glass test tube (16×100 mm). Nine-mL of ethanol were added and the tube was capped and vortexed for a few seconds. After standing for 15 min, the tube was centrifuged for 10 min at 2000 g. The supernatant was discarded allowing a few seconds drainage. Starch was redissolved by adding 1mL UDMSO. The tube was then capped and transferred to an oven set at 100°C. After 30 min the tube was removed from the oven and the starch-UDMSO solution was rapidly washed via a wide funnel into a 100-mL flask using approximately 95 mL water. Two-mL of iodine-potassium iodine solution (2 mg iodine + 20 mg KI/ mL) were added, the flask's contents were mixed immediately, and the volume was adjusted to 100 mL. After 15 min at room temperature the optical density was read at 635 nm against water in a temperature-controlled spectrophotometer set at 20°C. Amylose percentage was calculated as follows:

Amylose (%) = (28.414×Blue Value)- 6.218 (Morrison and Laignelet 1983).

Blue value was defined as the absorbance at 635 nm of 10 mg anhydrous starch in 100 mL dilute I-KI solution at 20°C.

3.7.3 Grain soluble carbohydrates

Aliquots of 0.1 mL of ethanolic extract, explained in section 3.6.4, were pipetted into 150×18 mm glass tubes. The volume was adjusted to 1.0 mL with distilled water. Another set of 5 tubes was also included as a standard curve containing 0,10,30,60 and 100 μg of sucrose per tube adjusted to the same volume with water. Portions of 1 mL of 5% aqueous phenol were added to the samples including standards and mixed (See Dubois *et al.* 1956). A jet of concentrated AR sulphuric acid (5 mL) was pumped into the centre of each sample and the contents were mixed while still hot. When the samples had cooled to room temperature, the liquid was carefully transferred to a spectrophotometer tube and the optical density was read against water at 490 nm. The amount of soluble carbohydrate in each sample was estimated using the standard curve.

3.7.4 Sucrose assay

The amount of sucrose in the endosperm was determined using a Waters HPLC system as described in section 3.6.3. Samples of six grains were selected from the basal florets of spikelets in the middle of the ear. The endosperms were squeezed out onto a piece of aluminium foil and dropped into tubes containing 10 mL of boiling 80% ethanol. After boiling for 10 minutes, the samples were removed from the bath and stored at 4°C in a refrigerator. Just before the time of measurement, the tubes were taken out of the refrigerator and allowed to warm to room temperature. The supernatant was transferred to a 25-mL cylinder and the residue was crushed with a glass rod which was then rinsed with

2.5 mL of 80% ethanol. The tubes were heated in a sand bath until boiling, then removed from the bath and left at room temperature to be cooled. The contents were washed into the 25-mL cylinder by another 2.5 mL 80% ethanol. The volumes were adjusted to 15 mL and mixed thoroughly. Portions of 10 mL were transferred into 10-mL centrifuge tubes which were then capped and centrifuged for 5 minutes at 3000 rpm. Portions of 3.5 mL of supernatant were dried in vacuo cooled with liquid N. The dried material of each tube was dissolved in 1 mL distilled water, mixed and transferred to a HPLC vial. Separation was carried out using a Waters HPLC system with a Waters Dextro-Pak Column with water as elutant and a Waters Model 410 differential refractometer (Ugalde and Jenner 1990). Sucrose was separated from other sugars by injecting 100 µL of sample into the column running for 60 min. The amount of sucrose per injection was calculated using the quantified samples of standard sucrose.

3.8 Analysis of starch synthetic enzymes

3.8.1 Soluble starch synthase

Enzyme extraction

Extraction of Soluble Starch Synthase was carried out according to the procedure describe by Jenner *et al.* of (1995). Endosperms were squeezed out (100 endosperms, about 2 g) from freshly harvested grains, weighed and homogenised with a pestle in a mortar cooled on ice in 10 mL (3 x 3.33 mL) of extraction buffer containing 50 mM MOPS, 3-(*N*-morpholino)-propanesulfonic acid, (Sigma M-1254) (pH 7.0), 2 mµ EDTA (ethylendiamine tetra-acetic acid, BDH), 1 mM DTT (dithiothreitol, Sigma D-0632) containing three protease inhibitors: AEBSF (4-2-aminoethyl)-benzenesulfonyl fluoride, at 0.1 µmole mL^{-1} (Sigma A-5938), leupeptin (Sigma L-2023) at 0.5 µg mL^{-1}, and 0.014 mL

of a 1 mg mL $^{-1}$ solution of pepstatin (Sigma P-4265) dissolved in pure ethanol, in a final volume of 20 mL of extraction buffer.

The homogenate was centrifuged at 10 000 g for 10 min in a temperature controlled centrifuge (Sorvall RC-5B refrigerated superspeed centrifuge, Du Pont Instruments) set at 4 °C. The supernatant was transferred into a measuring cylinder kept on ice, and the volume was adjusted to 11.2 mL with extraction buffer. The contents were poured into a 50 mL beaker on ice and mixed. A portion, 0.2 mL, was transferred to an Eppendorf tube for assaying crude enzyme activity, and the remaining 10 mL of solution was brought to 40% saturation by the addition of 2.49g ammonium sulphate, $(NH_4)_2SO_4$, with stirring on ice until the ammonium sulphate was dissolved completely. The suspension was centrifuged at 10 000 g for 30 min and the supernatant was discarded. The pellet was redissolved in 1 mL of extraction buffer and desalted by applying to a Sephadex PD 10 Column, (G-25 M, Pharmacia Biotech Sweden) which was equilibrated with column buffer containing 50 mM MOPS (pH 7.0), 2 mM EDTA (pH 7.0), and 1 mM DTT, while it was kept at 4°C in the refrigerator. The extract was allowed to pass through the column and the eluent was discarded. Extraction buffer, 1.5 mL, was added to the column and the eluent was again discarded. A further 1.5 mL of extraction buffer was applied to the Sephadex column and a 1.5 mL fraction was collected in a 2 mL Eppendorf tube kept on ice to be used as the enzyme for the soluble starch synthase assay.

Soluble starch synthase assay

The enzyme activity was estimated by measuring the rate of incorporation of [^{14}C] glucose from ADP[U-^{14}C]glucose into starch using the resin method described by Jenner *et al* (1994). Soluble starch synthase was assayed at temperatures 20°C and 30°C for 2 and 4 min intervals as follows. Assay mixture, 100 µL, was pipetted into tubes containing 80

μL of amylopectin solution, which were then put in racks in the water bath to be equilibrated to the assay temperature. The assay mixture contained 50 μL extraction buffer (above) 0.134 mg ADPG (adenosine 5'-diphosphoglucose, Sigma A-0627), 0.15 μL ^{14}C (0.14-KBq) ADPG (Amersham) and 50 μL water. Amylopectin, (Sigma potato amylopectin A-8515), solutions were prepared to give final concentrations from 0.02 to 2 mg mL^{-1} for the 20°C assay and from 0.1 to 10 mg mL^{-1} for the 30°C assay. To start the assay 20 μL of enzyme was added to each Eppendorf tube containing the assay mixture, vortexed for 5 seconds , and incubated in the water bath for 2 or 4 minutes. The reaction was stopped by heating the reaction tubes in a boiling water bath (maintained at 95°C) for 2 min. The samples were allowed to cool to room temperature for further processing.

Two types of blank assays were carried out. Zero-time blanks were transferred to the 95°C water bath immediately after the addition of the enzyme. Zero amylopectin blanks, continuing all constituents except amylopectin, were incubated for 2 min or 4 min as detailed above.

Preparation of resin columns

Ion exchange resin columns were prepared as follows. Collars, made by cutting Eppendorf tubes, held 1 mL pipette tips plugged with cotton wool over scintillation vials arranged in a rack. To make the resin suspension, 100 g Dowex 1-X8 resin 200-400 mesh, chloride form (Sigma 1x 8-400) was placed in a 500 mL beaker and washed thoroughly with nanopure water until the supernatant became clear after the resin bed was settled. The supernatant was decanted off and the resin was suspended in water in the ratio 100 g resin to 200 mL water. 1 mL of resin suspension was pipetted into the column placed in the scintillation vials, and centrifuged for 1 min at 1000 rpm and the liquid draining into the vial was

discarded. Each column was washed with 1 mL nanopure water and centrifuged for 1 min at 1000 rpm just before using.

Processing the reaction product for counting

Portions, 180 µL, of the 200 µL of assay mixture were pipetted onto the top of the resin bed in each column which had been placed in a fresh scintillation vial. The columns were centrifuged at 1000 rpm for 2 min and washed twice with 50 µL of nanopure water followed by centrifugation between washings. The highly radioactive columns containing unreacted [^{14}C] ADPG were removed from the vials and discarded. Beckman Ready Value scintillation fluid (2 mL) was added to each vial which was shaken thoroughly and counted by liquid scintillation spectrometry. The radioactivity was also counted in vials with 100 µL of assay mixture containing 0.14 KBq of [^{14}C] ADPG and in the zero amylopectin blanks and the zero time blanks.

The enzyme activity was calculated as follows;

Enzyme activity =(counts per minute / specific activity of [^{14}C] ADPG)5(200/180)5(1/ incubation time)5(1/ sample fresh weight)5(1.5 mL enzyme/ 0.02 mL enzyme per assay)

Specific activity =((counts per minute in 100 µL assay mix of standard) /(nano moles ADPG in 100 µL assay mix))

3.8.2 Granule bound starch synthase

Enzyme extraction

The assay was performed using the method for granule bound starch synthase described in Hawker and Jenner (1993) with some modifications as follows. Ten endosperms were

squeezed from grains taken from the same wheat ears used for soluble starch synthase assay, weighed and homogenised in 2 mL of extraction buffer (as prepared for soluble starch synthase assay) for 40 seconds in an all glass homogenizer (Kontes) driven with a power drill (Black and Decker, Australia). The homogenate was filtered through Miracloth into a small beaker. Filtered homogenate, 30 µL, was pipetted into a scintillation vial containing 1.8 mL of column buffer (as prepared for soluble starch synthase assay), and the vial was centrifuged at 3000 rpm for 4 minutes. The supernatant was decanted and the pellet was kept on ice for using in the granule bound starch synthase assay. Another portion of 100 µL of filtered homogenate was pipetted into a 2 mL Eppendorf tube containing 1.8 mL of column buffer, and the tube was centrifuged at full speed with a micro centrifuge for 1 minute. The supernatant was decanted and the pellet in this tube was frozen to be used for starch determination.

Enzyme assay

Twenty µL of extraction buffer and 80 µL of amylopectin (12.5 mg/ mL) were added to each vial containing the enzyme pellet and the vials were vortexed promptly. The tubes were put into the water bath (s) to be equilibrated with the temperature of the assay. To start the assay 100 µL of the same assay mixture as prepared for the soluble starch synthase assay was added into each vial, vortexed for 5 seconds, and incubated in a water bath for 10 minutes at 20°C or 30°C. The reaction was stopped by heating the reaction vials in a boiling water bath (maintained at 95°C) for 2 minutes. The samples were left to be cooled at room temperature for further processing. Blank assays were performed with the enzyme pellet that had been heated before starting the assay for 2 minutes at 95°C.

Processing the reaction product and counting

Three-mL methanolic KCl, prepared by mixing 250 mL of 4% aqueous KCl to 750 mL of methanol, was added into each assay vial, vortexed, and centrifuged for 5 minutes at 4000 rpm. The supernatant (highly radioactive) was decanted into a waste bottle. The pellet was suspended in 0.2 mL nanopure water, and the starch precipitated with another 3 mL of methanolic KCl and centrifuged as above. This washing procedure was repeated once more. Finally the pellet was suspended in 0.2 mL water. Two-mL of Ready Value scintillation fluid was added to each vial, shaken thoroughly and counted for ^{14}C by liquid scintillation spectrometry.

3.9 Analysis of ADP-glucose

The amount of ADP-glucose (adenosine diphosphoglucose) in the endosperm was determined using a Waters HPLC system as described in section 3.6.3. The analytical procedure was based on the method described by Jenner (1991). Samples of four grains were selected from basal florets of spikelets in the middle of the ear. The grains were peeled and the embryos were removed. Each peeled grain was put between two pieces of glass fibre filter, crushed in clamps cooled in liquid N, cut into small pieces to fit in a centrifuge tube kept on ice. The frozen material was then broken up with a glass rod in 1 mL of 1.41 M of cold perchloric acid. The sample was crushed several times with the rod over a period of 30 min. One mL of HPLC water was added, the sample was crushed and mixed again and centrifuged at 15000 rpm for 2 min (the time started when the speed reached at 14000 rpm). The supernatant was decanted into a corresponding clean centrifuge tube. The residue was re-extracted three times with 1 mL portions of HPLC water and centrifuged as above. The supernatant was decanted between washes. After the final wash, each sample was neutralised, using indicator paper, by incremental addition of

5M K_2CO_3. For the first lot about 600 µL of potassium carbonate was added into the tube, which was followed by the addition of 10 µL lots until pH 7 was achieved. The tube was centrifuged at 15000 rpm for 2 minutes as before, the supernatant was transferred into a 5 mL volumetric, and the volume was adjusted to 5 mL. The extract was filtered through 0.22-µm Adelab filter and transferred into HPLC vials for analysis. ADP-glucose was separated by injecting 100 µL of sample into the column running for 60 min. With each set of samples one sample with a known quantity of ADP-glucose was injected for analysis as a standard.

3.10 Statistical analysis

Pots were arranged inside the growth rooms using a randomised complete design. Four replications were used for each treatment in all experiments. Standard analysis of variance was applied to analyse the data. The Least Significant Difference (LSD) values were calculated and used to compare the treatment means; non-significant differences are abbreviated n.s. in all captions to tables and figures throughout this thesis.

Chapter 4

Effects of high temperature on grain filling of some wheat cultivars.

4.1 Introduction

High temperature during grain filling is an important factor limiting wheat yield in Australia and in many other wheat growing areas over the world (Wardlaw and Wrigley 1994). Several stages of wheat growth can be affected adversely by high temperature (Rawson 1986; Wardlaw *et al.* 1989a). However, under field conditions periods of high temperature occur more frequently during grain filling than at earlier stages, and can limit grain yield substantially (McDonald *et al.* 1983). Reduction in grain yield due to high temperature following anthesis results predominantly from decreases in kernel size (Wardlaw *et al.* 1989b). The optimum average post anthesis temperature for maximum kernel weight in wheat is about 15°C (Chowdhury and Wardlaw 1978), and each 1°C rise in temperature above the optimum can cause a 3-5% reduction in single grain weight under both controlled-environment (Wardlaw *et al.* 1989a) and field conditions (Wiegand and Cuellar 1981). Even a period of very high temperature as short as 3 days can have a marked effect on grain yield (Stone and Nicolas 1994).

Genetic variation exists among wheat cultivars in the response of grain filling to high temperature (Rawson 1986; Wardlaw *et al.* 1989a,b; Hunt *et al.* 1991). Wardlaw *et al.* (1989b) examined the response of 66 wheat cultivars during grain development to exposure to high temperature (30/25°C day/night). In their study single grain weight was reduced by about 60% in the most sensitive cultivars but by only 30% in the least sensitive cultivars compared to cooler conditions (18/13°C day/night). Genetic variability in wheat

has also been reported in response to a short period of very high temperature (>35°C; Stone and Nicolas 1994).

Both rate and duration of grain filling are independently influenced by high temperature (Jenner 1994). Sustained periods of moderately high temperature (up to 30°C) reduce the grain weight predominantly through shortening the duration of grain filling (Sofield *et al.* 1977a). Each 1°C increase in mean temperature can decrease the duration of grain filling by about 3 days (Wiegand and Cuellar 1981). The rate of grain filling is much less responsive than duration to temperature variation in the range of 20-30°C. The magnitude of the changes in the rate of grain filling in this temperature range is dependent on genotype (Hunt *et al.* 1991) and environmental factors such as nutrients (see Chapter 5). The rate of grain growth declines at temperatures in excess of 30°C (Tashiro and Wardlaw 1989). The decline in the rate of grain growth is mostly due to a decrease in the rate of starch accumulation. Protein deposition is less temperature sensitive (Bhullar and Jenner 1985). The increase in the rate of dry matter accumulation in the range 20-30°C is not always large enough to compensate for the reduction in grain weight due to shortened duration, resulting in a significant decrease in final grain weight (Wardlaw 1980; Tashiro and Wardlaw 1989).

Genetic variation among wheat cultivars has also been reported for both rate and duration of grain filling (Nass and Reiser 1975; Bruckner and Frohberg 1987; Daroch and Baker 1990; Hunt *et al.* 1991). However, the differences among genotypes for the duration of grain filling are small in relation to high temperature effects on final grain weight (Hunt *et al.* 1991). In the study of Hunt *et al.* (1991) the final kernel weights of several wheat cultivars tested at 30/25°C were highly correlated with the rate but not with the duration of

dry matter accumulation. The sensitivity of wheat cultivars to high temperature was therefore associated with temperature responses to the rate of grain filling.

Several mathematical models have been used to estimate grain filling parameters in wheat cultivars (Gebeyehou *et al.* 1982; Bruckner and Frohberg 1987; Loss *et al.* 1989; Darroch and Baker 1990). Measurement of the rate of grain filling is not a straight forward matter because the rate changes with time, and there is no non-destructive method for evaluating it. For these reasons there is not a generally accepted way for the estimation of the rate of grain filling. Bruckner and Frohberg (1987) and Gebeyehou *et al.* (1982) have calculated the average rate of grain growth as the ratio of maximum grain dry weight to duration estimated from quadratic and cubic polynomial curves. Assuming the rate to be constant during the linear phase, some workers (Van Sanford 1985; Hunt *et al.* 1991) have used linear regression to estimate the rate of grain growth for the linear part of growth curve. These types of calculations do not consider the nonlinearity of grain growth. Loss *et al.* (1989) and Darroch and Baker (1990) have estimated the maximum rate of grain filling from a logistic curve and found that the logistic curve could describe grain filling in the tested cultivars very well. Darroch and Baker (1990) suggested that polynomial functions could be appropriate only if grain weight decreases after reaching a maximum which was not the case for the wheat cultivars used in their study, while in a logistic curve grain weight does not necessarily decrease when maximum dry matter is achieved. In barley, both logistic and Gompertz models fitted the grain filling data equally well (Koesmarno and Sedcole 1994).

The objectives of this study were to: (a) evaluate the effects of high temperature on grain development of some wheat cultivars in terms of grain dry matter and nitrogen

accumulation; (b) examine the relationships between estimated grain-filling parameters and high temperature tolerance, and (c) to see if any perceived varietal differences in the temperature sensitivity of dry matter accumulation can be explained on the basis of differences in grain nitrogen accumulation under high temperature.

A number of growth curves were also fitted to the grain filling data and the most appropriate model was chosen and used to describe grain filling processes in all experiments.

4.2 Materials and Methods

Two experiments were conducted in environment-controlled growth cabinets using four wheat cultivars differing in their response to high temperature. The plants were grown in pots of recycled soil and kept in a growth cabinet set at 20/15°C (day/night). At day 2 after anthesis, half of the pots of each cultivar were shifted to another growth cabinet, adjusted to 30/25°C as the high temperature treatment. The rest of the plants were kept in the growth cabinet at 20/15°C as control. Pots were arranged inside the growth rooms under a randomised complete design. Four replications were used for each treatment. Samples were taken every 2-4 days throughout grain filling period. At each sampling date, 10 grains were taken out from the spikelets in the middle of the ears, oven-dried and grain weight was determined. Grain nitrogen content was measured using the Dumas Total Combustion. Selection of cultivars, growth conditions, sampling, grain nitrogen analysis have been described in detail in Chapter 3 (Materials and Methods).

Five growth models were fitted to the grain filling data using the Genstat statistical program for all eight data sets. Grain dry weight was used as the response variable and

time (days after anthesis) was used as the independent variable. Three standard sigmoid curves (France and Thornley 1984) and two growth curves, outlined in Darroch and Baker (1995) were fitted to the data. The three growth curves were:

Generalised logistic	$W(t)= c/ [1+d \exp (-b (t-m))]^{1/d}$
Ordinary logistic	$W(t)= c/ [1+\exp (-b (t-m))]$
Gompertz	$W(t)= c \exp (-\exp (-b (t-m)))$

where c estimates the final dry weight, b estimates the rate of growth (a slope parameter), m is the inflexion point for time, and d is the power-law parameter.

Koesmarno and Sedcole (1994) have used all three models to describe grain growth in barley. Loss *et al.* (1989) used the ordinary logistic model to fit grain filling data in some wheat cultivars, but in their equation a constant (a) related to the initial size of the grain is also considered, $W(t)= a+c/ [1+\exp (-b (t-m))]$.

In developing the logistic equation it is assumed that the amount of growth machinery is proportional to dry weight (W) and the growth rate is also modified by the availability of substrate. In the derivation of the Gompertz equation the assumption is that the substrate is not limiting, the quantity of growth machinery is proportional to the dry weight, but the efficiency of the catalytic growth decays with time due to enzyme degradation or senescence. The logistic growth curve has a smooth sigmoid behaviour but the Gompertz curve shows a longer linear period about the inflexion point. The Gompertz equation involves three parameters but the inflexion point does not occur at the half point way of the growth curve, as occurs with logistic curves (France and Thornley 1984).

The two growth curves outlined in Darroch and Baker (1995) are:

Darroch (1) $W(t) = W_f / [1+(W_f - W_o)/ W_o)\exp(-rt)]$

Darroch (2) $W(t) = W_f / [1+(W_f -1)\exp(-rt)]$

where W_f estimates the final dry weight (analagous to 'c' above); W_o estimates the dry matter at time t = 0; and r estimates the relative rate of growth during early exponential phase (Darroch and Baker 1995). Darroch (2) is a modified form of Darroch (1) equation in which a constant value of 1.0 is used for W_o.

According to Darroch and Baker (1990), theoretically grain weight will never reach its maximum (C), because of the asymptotic shape of the logistic curve. Therefore, the duration to maximum weight (physiological maturity) is considered the time when W=0.95C.

The duration and maximum growth rate for each curve fitted are calculated as follows:

model	duration	maximum growth rate
Generalised logistic	$m - (1/ b) \ln [(1.053^d - 1)/ d)$	$[bc/ (1+d)^{1+1/d}]$
Ordinary logistic	$(bm + 2.944)/ b$	$bc/ 4$
Gompertz	$(bm + 2.970)/ b$	$bc \exp^{(-1)}$
Darroch (1)	$(1/ r) \ln [(19 W_f - 19 W_o)/ W_o]$	$r W_f / 4$
Darroch (2)	$(1/ r) \ln (19 W_f - 19)$	$r W_f / 4$

4.3 Results

4.3.1 Experiment 1

In this experiment, the response of grain filling of two spring wheat cultivars Lyallpur and Trigo to high temperature was compared in terms of grain dry matter and nitrogen accumulation. A comparison was made between some growth models in fitting grain filling data derived from experiment 1 and 2. The results are presented (in Section 4.3.2.1) only for the second experiment that included two more cultivars.

4.3.1.1 Effect of high temperature on parameters of grain filling

The results of the analysis of variance and the estimated values of grain filling parameters for cultivars Lyallpur and Trigo grown at 20/15°C and 30/25°C are shown in Tables 4.1, 4.2, and 4.3. These parameters were all estimated by the ordinary logistic model which was shown to fit the grain filling data adequately.

Final grain weight

As the main effect of high temperature after anthesis is a reduction in kernel size, the variation in individual grain weight is usually used to determine the changes in grain yield in the experiments conducted under high temperature conditions. The interaction between temperature and cultivar was marginally significant in terms of final grain weight (Table 4.1). Final grain dry weights in both cultivars were significantly lower in the plants grown at 30/25°C compared to those grown at 20/15°C (Table 4.2). The decreases in grain weight at high temperature in Lyallpur were due to the reductions in both rate and duration of grain filling (Table 4.3). In Trigo the rate of grain filling was significantly increased at high temperature and the reduction in grain weight was due to the effect of significantly shortened duration of grain filling that was not compensated for by the effect of increased

Table 4.1 P values obtained from the analysis of variance table for grain growth parameters in cultivars Lyallpur and Trigo grown at 20/15°C and 30/25°C.

Source	Final grain dry weight	Maximum rate of grain filling	Duration of grain filling	Time to inflection point
Tem	.0001	.4340	.0001	.0001
Var	.3823	.0565	.9521	.2672
Tem * Var	.0459	.0045	.0098	.0686

rate of grain filling on grain weight.

It is necessary to note that there was a compressor failure in the growth room set at 30/25°C, which caused temperature to rise more than 10°C above the adjusted temperature for a few hours. It happened on day 17 after anthesis at the time when only the plants of Trigo were left inside the growth room. This hastened the development of the grains in Trigo at a faster rate than expected and may have resulted in a larger reduction in the grain filling period at high temperature than was anticipated. However, as the temperature failure happened a few days after the time when the rate of grain filling reached its maximum, the estimation of the maximum rate of dry matter accumulation in both cultivars is assumed to be reliable. Due to the unusual reduction in the duration of grain filling in Trigo the final grain weight in this cultivar at 30/25°C was probably underestimated (see Stone and Nicolas 1995a,b) and as a consequence the percentage reduction in final grain weight at 30/25°C was overestimated.

Maximum rate of grain filling

The maximum rate of grain filling represents the maximum rate of grain dry matter accumulation at the inflection point when the rate of grain dry matter accumulation starts

Table 4.2 Grain growth characteristics of cultivars Lyallpur and Trigo grown at 20/15°C and 30/25°C estimated by the ordinary logistic model.

Cultivar	Tem. (°C)	Final grain dry weight (mg)	Maximum rate of grain filling (mg/ day)	Duration of grain filling (days)	Time to inflection point (day)
Lyallpur	20	59.1	2.13	44.5	23.9
	30	37.8	1.73	29.8	13.5
Trigo	20	63.4	2.00	49.4	26.1
	30	36.0	2.26	24.7	12.9
LSD 5%		4.2	0.29	5.0	2.1 ns

LSD 5% for Temperature × Variety

Table 4.3 Effects of growth at 30/25°C compared to 20/15°C on grain growth parameters of cultivars Lyallpur and Trigo, calculated from Table 4.2.

Cultivar	Reduction in grain weight (%)	Q_{10} for the rate of grain filling (30°C / 20°C)	Reduction in duration of grain filling (%)	Reduction in time to inflection point (%)
Lyallpur	36	0.81	33	44
Trigo	43	1.13	50	51

to decline gradually. Physiologically it represents the maximum achievable rate of filling under the prevailing conditions. There was a significant interaction between temperature and cultivar for the maximum rate of grain filling (Table 4.1). The rate in Trigo was increased at high temperature (Table 4.2), with a Q_{10} (increase in the rate at 30/25°C compared to 20/15°C) equal to 1.13 (Table 4.3), whereas Lyallpur had a lower rate at high temperature with a Q_{10} of 0.8. There was no significant difference between the two

cultivars at 20/15°C but the rate was significantly higher in Trigo (2.26 mg/day) than in Lyallpur (1.73 mg/day) at 30/25°C.

Duration of grain filling

There is commonly observed an inverse relationship between the duration of grain filling and growth temperature, a consequence of accelerated grain development at high temperature. High temperature hastened the development of the grains and resulted in a significantly shorter grain filling period in both cultivars at 30/25°C compared to 20/15°C (Table 4.2). There appeared a greater reduction in the duration of grain filling at high temperature in Trigo (50%) than in Lyallpur (33%; Table 4.3) and the interaction between temperature and cultivar was significant (Table 4.3).

Time to inflection point

The inflection point of time for the course of dry matter accumulation represents the instant of time when dry matter is accumulated in the grain at its maximum rate. From the physiological point of view it represents the greatest rate achieved in the process of grain filling after which dry matter accumulation begins to slow down. The main effect of temperature was significant on the time to the inflection point (Table 4.1). The number of days from anthesis to the maximum rate of grain filling was significantly reduced in plants grown at 30/25°C compared to those grown at 20/15°C (Table 4.2). The percentage reduction in time to the inflection point at high temperature was higher in Trigo (51%) than in Lyallpur (44%; Table 4.3) but the interaction between temperature and cultivar was not significant. The time to inflection point was significantly shorter in Lyallpur (24 days) than in Trigo (26 days) at 20/15°C but both cultivars reached the inflection point at the same time about 13 days after anthesis at 30/25°C.

4.3.1.2 Effect of high temperature on grain nitrogen accumulation

The results of the analysis of variance and the data for the grain nitrogen accumulation in Lyallpur and Trigo grown at 20/15°C and 30/25°C are shown in Table 4.4 and 4.5. Linear regression was used to estimate the rate of grain nitrogen accumulation. Fig. 4.1 demonstrates that the grain nitrogen accumulation gives a reasonable fit to a linear pattern in both cultivars under two temperatures. The main effect of temperature was significant on the amount at maturity and the rate of grain nitrogen accumulation (Table 4.4).

Table 4.4 P values obtained from the analysis of variance table for the components of grain-N accumulation in cultivars Lyallpur and Trigo grown at 20/15°C and 30/25°C.

Source	N weight at maturity	Sustained rate of N accumulation
Tem	.0001	.0013
Var	.6854	.2799
Tem * Var	.4815	.7126

Table 4.5 Grain-N accumulation in cultivars Lyallpur and Trigo grown at 20/15°C and 30/25°C. The rate was estimated by linear regression.

Cultivar	Tem. (°C)	Final grain N weight (mg)	Reduction in grain N weight at 30°C (%)	Rate of N accumulation (mg/ day)	Q_{10} (30°C /20°C)
Lyallpur	20	1.69	24.9	0.035	1.29
	30	1.27		0.045	
Trigo	20	1.71	29.2	0.033	1.27
	30	1.21		0.042	
LSD 5%		0.15 ns		0.007 ns	

LSD 5% for Temperature × Variety

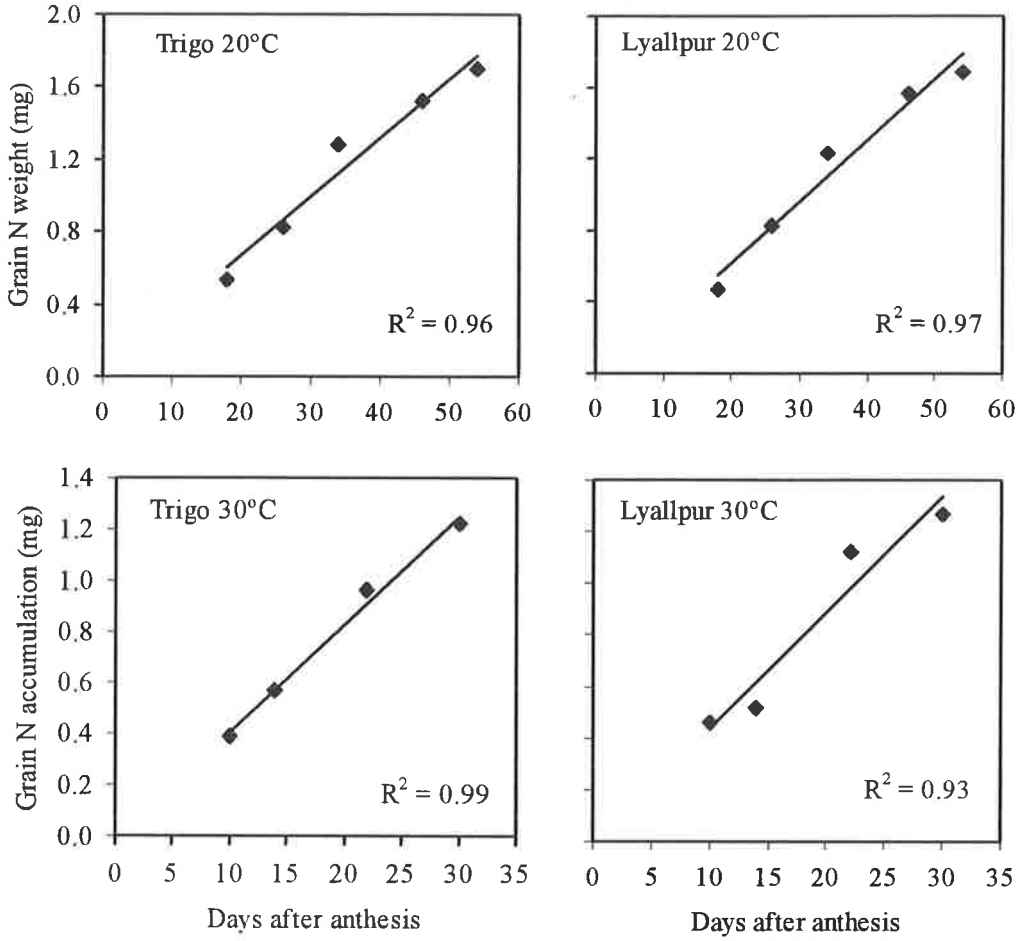

Fig 4.1 Grain nitrogen accumulation in cultivars Trigo and Lyallpur grown at 20°C and 30°C fitted to linear regression to determine the rate of N accumulation.

The elevated temperature reduced the grain nitrogen weight in both cultivars (reductions of 25% and 29% in Lyallpur and Trigo respectively; Table 4.5) but the difference between the two cultivars in response to temperature was not statistically significant. The grain nitrogen weight in both cultivars was not significantly different within each temperature. The rate of grain nitrogen accumulation was increased in both cultivars at high temperature. The increases at high temperature (values of Q_{10}) were almost the same in both cultivars (1.29 and 1.27 mg/ day in Lyallpur and Trigo respectively). There were no significant differences in the rate of grain nitrogen accumulation between two cultivars

within each temperature. As the rate was enhanced at high temperature in both cultivars, the decreases in final grain nitrogen weight must be related to the reductions in duration of grain nitrogen accumulation which were not estimated in this experiment.

4.3.2 Experiment 2

In the first experiment cultivars Trigo and Lyallpur showed some differences in their response to high temperature. However, the question was raised whether or not the unplanned rise in temperature due to growth cabinet technical problems had led to an overestimation of the sensitivity of Trigo to high temperature. Therefore, it was decided to test again the response of these two cultivars to high temperature and to include two other cultivars which had become available, Sun 27B and Kavko, under the same conditions as for the first experiment.

4.3.2.1 Growth models

The data of the grain dry matter accumulation in wheat cultivars Lyallpur, Sun27B, Kavko, and Trigo grown at 20/15°C and 30/25°C were fitted to five growth models in order to select a model that could describe grain-filling processes in the cultivars appropriately. The growth models have been described earlier in the Material and Methods section.

4.3.2.1.1 Growth models and fitting grain filling data points

The values of the adjusted R^2 and the residual mean squares (residual MS) derived from the growth models were used to compare the models in fitting grain filling data (Table 4.6). There were small differences in adjusted R^2 between the models. Except for the Darroch (2) model, the other growth models fitted the data with R^2 values higher than 99%. Some differences appeared between the growth models when residual mean squares were

Table 4.6 The comparative statistics for five growth models fitted to the grain filling data of four wheat cultivars grown at 20/15°C and 30/25°C.

			Adjusted R^2		Residual mean squares	
Cultivar	Model	Number of parameters	20°C	30°C	20°C	30°C
Lyallpur	Generalised logistic	4	99.5	99.4	1.575	0.569
	Ordinary logistic	3	99.5	99.4	1.491	0.518
	Gompertz	3	99.2	99.2	2.543	0.692
	Darroch (1)	3	99.5	99.4	1.491	0.518
	Darroch (2)	2	96.3	95.4	10.76	4.006
Sun 27 B	Generalised logistic	4	99.9	99.8	0.288	0.153
	Ordinary logistic	3	99.7	99.8	0.572	0.141
	Gompertz	3	99.9	99.5	0.265	0.291
	Darroch (1)	3	99.7	99.8	0.572	0.141
	Darroch (2)	2	96.5	96.4	6.773	2.287
Kavko	Generalised logistic	4	99.8	99.5	0.339	0.440
	Ordinary logistic	3	99.7	99.5	0.527	0.410
	Gompertz	3	99.0	99.4	1.861	0.488
	Darroch (1)	3	99.7	99.5	0.527	0.410
	Darroch (2)	2	99.2	96.3	1.510	3.181
Trigo	Generalised logistic	4	99.5	99.5	1.289	0.604
	Ordinary logistic	3	99.5	99.5	1.291	0.551
	Gompertz	3	99.5	99.3	1.398	0.869
	Darroch (1)	3	99.5	99.5	1.291	0.551
	Darroch (2)	2	98.9	97.6	2.999	2.833

considered. The residual MS for Darroch (2) were notably higher than those of other models in all cases with the exception of Kavko at 20/15°C. Gompertz ranked second after Darroch (2) for having higher values of residual MS. Only in Sun 27B at 20/15°C was the residual MS for Gompertz less than those recorded for the other models. Results obtained for the ordinary logistic and Darroch (1) were similar for both adjusted R^2 and residual MS. As a comparison, the fitted growth curves are illustrated for the case of dry matter

accumulation in the grains of cultivars Lyallpur and Sun27B at both temperatures (Fig. 4.2). As shown, throughout the grain filling period the deviation of the predicted grain dry matter from those observed were much larger with the Darroch (2) model than with the other four models. The dry matter accumulation estimated by Darroch (2) compared to the observed values was underestimated in the early phase of grain filling period and overestimated in the second half of the linear phase. The other models, especially the logistic models, generally fitted the data points very well over the whole period of grain filling.

4.3.2.1.2 Growth models and grain-filling estimation

A comparison was made between different growth models in estimating the rate of grain filling, duration of grain filling and final grain weight in four cultivars grown at 20/15°C and 30/25°C (Table 4.7). In all cases, similar results were obtained for the ordinary logistic and Darroch (1) models to estimate the grain filling parameters. The values of the rate of grain filling estimated by Darroch (2) were considerably higher than for the other models in all varieties under both temperatures. The other four models gave values that differed little from each other for the rate of grain filling especially at high temperature.

The differences between the growth models in estimating the duration of grain filling were greater than observed for the estimates of the rate of grain filling. Under both temperatures and in all varieties there was a consistent trend for the Gompertz model to overestimate the duration of grain filling compared to the ordinary logistic model. The reverse trend was observed for Darroch (2), which gave the shortest estimates of grain filling duration in all cases. The differences between the growth models were very small in estimating the final grain weight. Darroch (2), for example, which had a considerably different estimates of

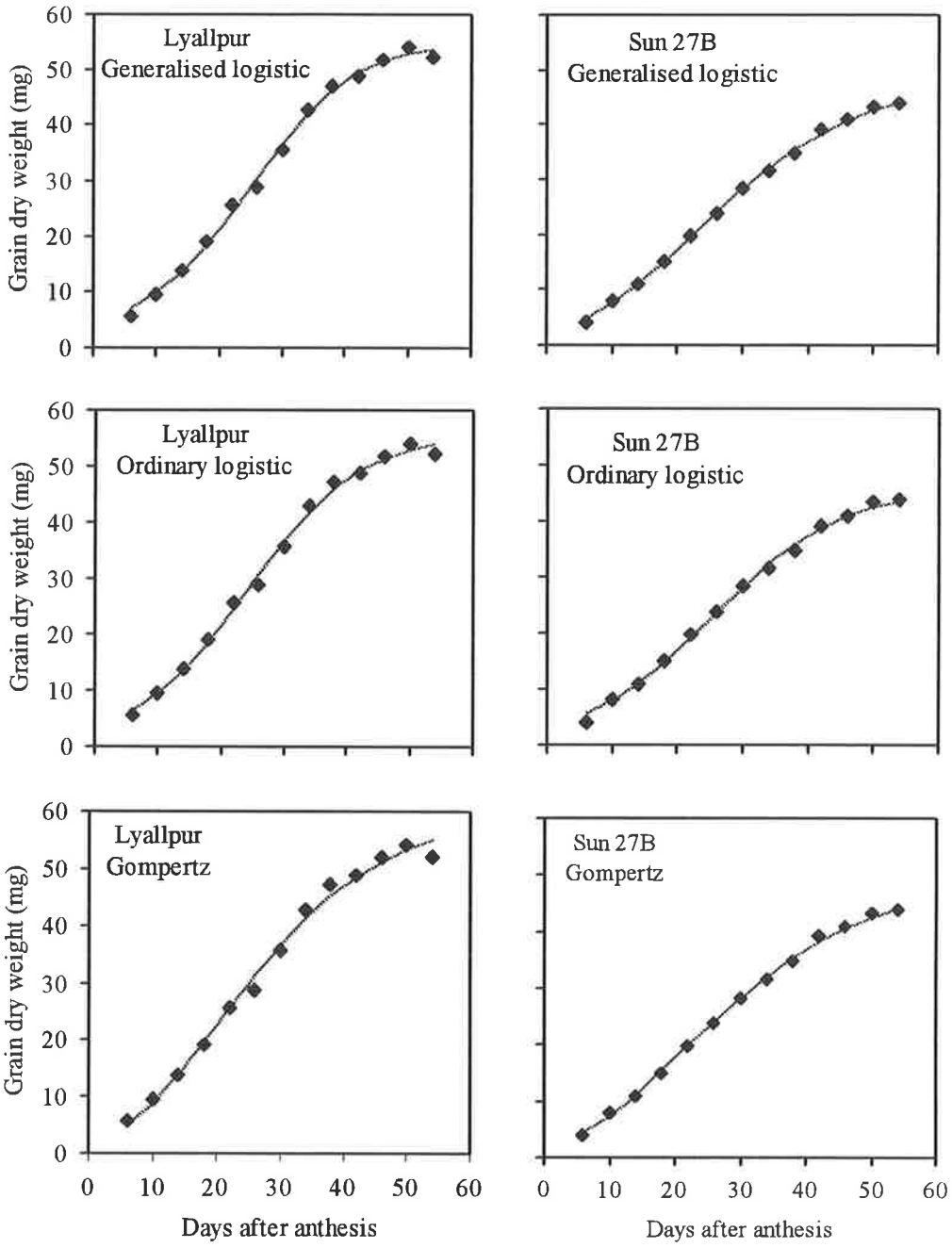

Fig 4.2 (a) Grain dry matter accumulation in cultivars Lyallpur and Sun 27B grown at 20/15°C fitted to the generalised logistic, ordinary logistic and Gompertz model.

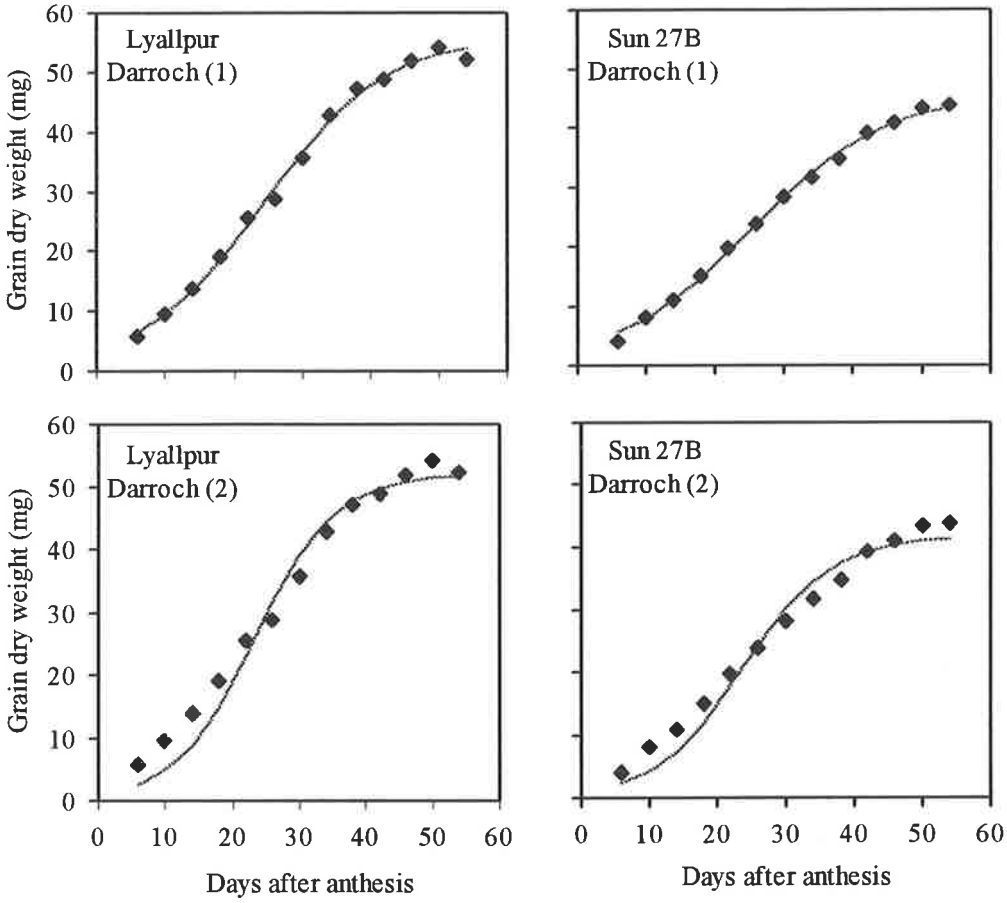

Fig 4.2 (b) Grain dry matter accumulation in cultivars Lyallpur and Sun 27B grown at 20/15°C fitted to Darroch (1) and Darroch (2) model.

the rate and duration of grain filling from the other models, gave very close values for the final grain weight to those estimated by the ordinary logistic. This was because the effect of the overestimated rate of grain filling on final grain weight was counterbalanced by the effect of the underestimated duration on grain weight. The ordinary logistic was therefore chosen as the most appropriate model to estimate grain-filling parameters in tested cultivars.

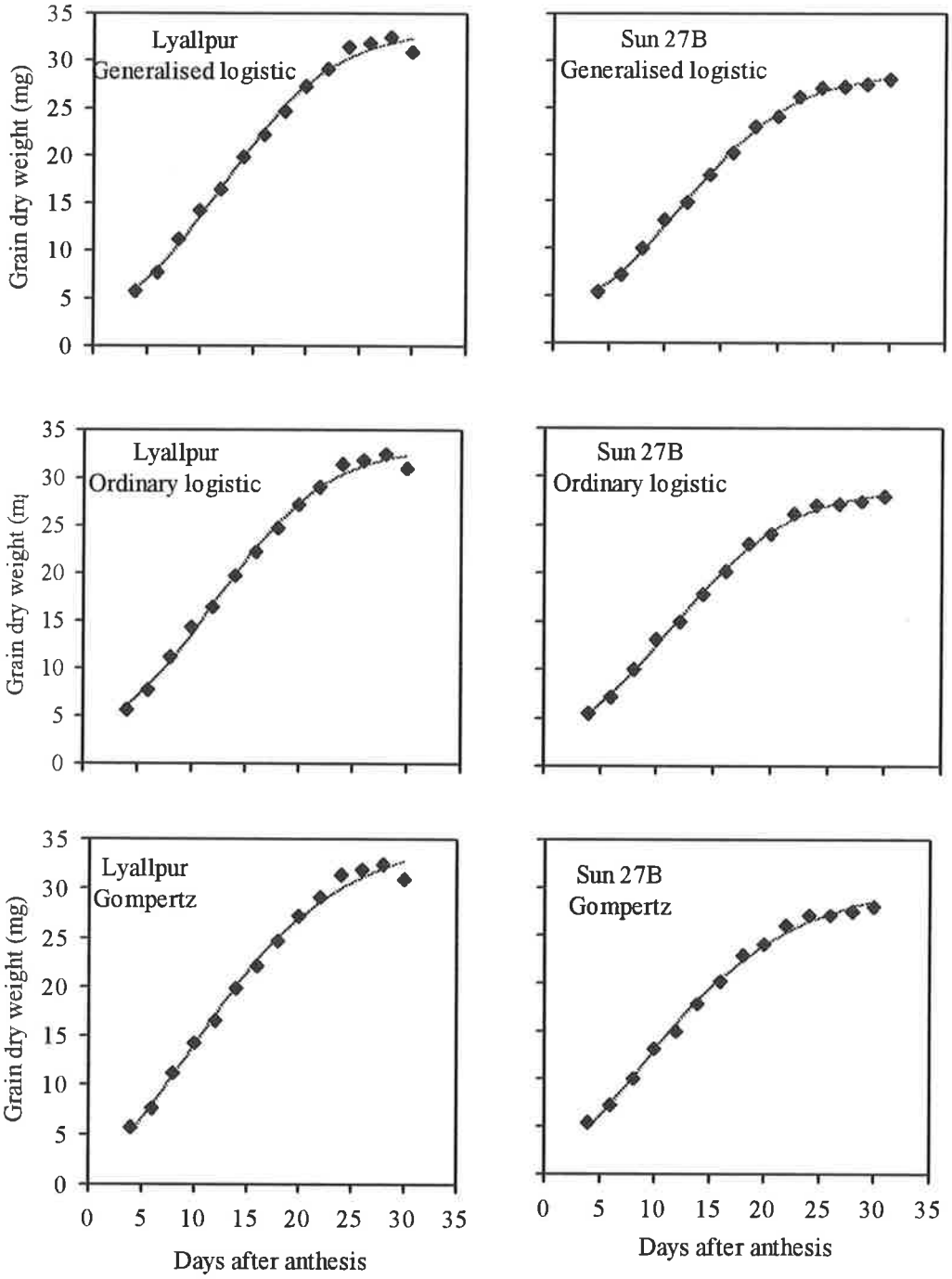

Fig 4.2 (c) Grain dry matter accumulation in cultivars Lyallpur and Sun 27B grown at 30/25°C fitted to the generalised logistic, ordinary logistic and Gompertz model.

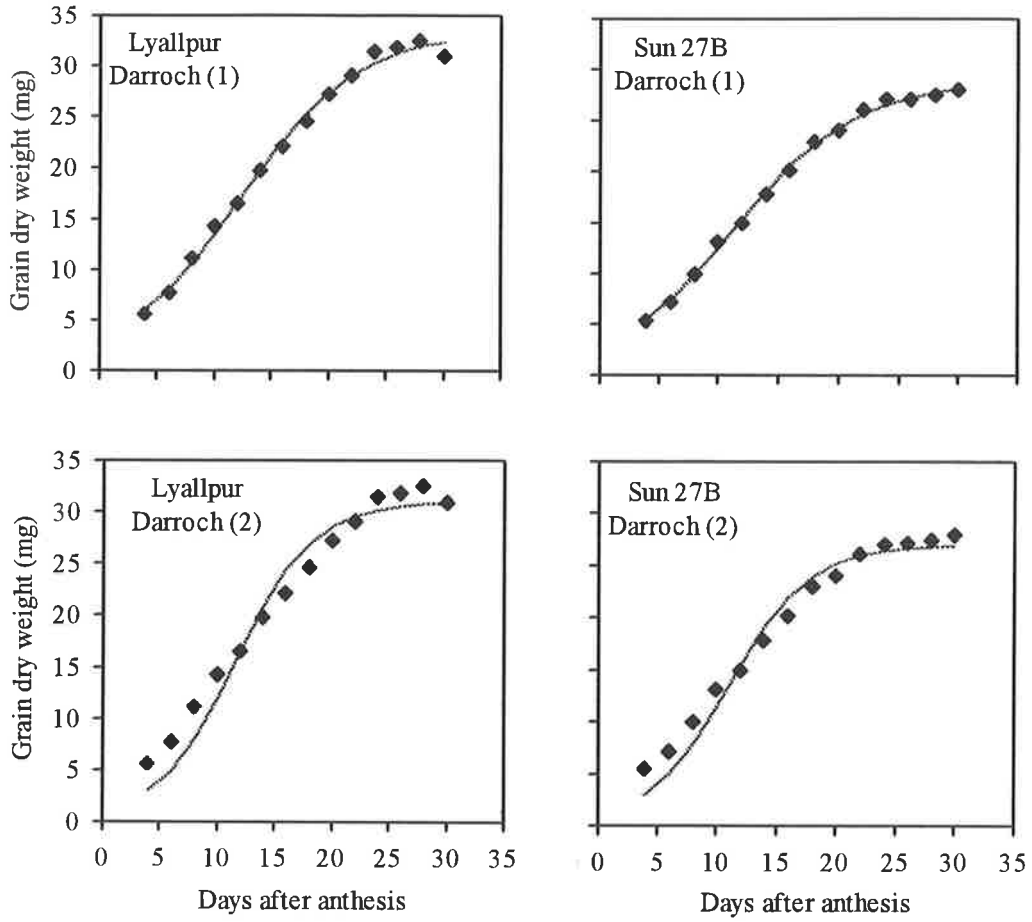

Fig 4.2 (d) Grain dry matter accumulation in cultivars Lyallpur and Sun 27B grown at 30/25°C fitted to Darroch (1) and Darroch (2) model.

4.3.2.2 Effect of high temperature on parameters of grain filling

The results of the analysis of variance and the estimated values of grain filling parameters for wheat cultivars Lyallpur, Trigo, Kavko, and Sun 27B grown at 20/15°C and 30/25°C are shown in Tables 4.8, 4.9, and 4.10. Apart from the sustained rate of grain filling, these parameters were all estimated by the ordinary logistic model which was shown to fit the grain filling data adequately. The sustained rate of grain filling was estimated by linear regression analysis.

Table 4.7 A comparison between the growth models in estimating grain filling parameters of four wheat cultivars grown at 20/15°C and 30/25°C.

		Rate of grain filling (mg/day)		Duration of grain filling (days)		Final grain weight (mg)	
Cultivar	Model	20°C	30°C	20°C	30°C	20°C	30°C
Lyallpur	Generalised logistic	1.58	1.58	49.0	27.8	55.4	33.7
	Ordinary logistic	1.57	1.58	50.6	27.6	56.0	33.5
	Gompertz	1.49	1.58	63.9	34.2	60.9	36.0
	Darroch (1)	1.56	1.58	50.5	27.6	56.0	33.5
	Darroch (2)	2.19	2.28	40.6	21.7	51.8	31.2
Sun 27 B	Generalised logistic	1.13	1.42	64.7	25.7	49.0	28.6
	Ordinary logistic	1.17	1.42	54.1	26.4	45.8	29.0
	Gompertz	1.12	1.43	70.5	31.7	50.9	30.4
	Darroch (1)	1.17	1.42	54.1	26.2	45.8	29.0
	Darroch (2)	1.62	1.98	42.8	21.1	41.6	27.0
Kavko	Generalised logistic	1.46	1.63	40.5	27.4	40.1	33.1
	Ordinary logistic	1.40	1.67	44.4	26.0	41.2	32.6
	Gompertz	1.33	1.65	54.1	31.9	43.6	34.6
	Darroch (1)	1.40	1.64	44.2	26.4	41.2	32.6
	Darroch (2)	1.63	2.29	40.4	21.2	40.0	30.6
Trigo	Generalised logistic	1.66	1.91	50.4	26.1	52.9	36.1
	Ordinary logistic	1.73	1.91	45.7	26.5	51.0	36.4
	Gompertz	1.60	1.86	59.6	32.5	57.1	38.8
	Darroch (1)	1.72	1.90	45.8	26.4	51.0	36.4
	Darroch (2)	2.00	2.49	40.9	22.2	48.2	34.3

Table 4.8 P values obtained from the analysis of variance table for grain growth parameters of four wheat cultivars grown at 20/15°C and 30/25°C.

Source	Final grain dry weight	Maximum rate of grain filling	Duration of grain filling	Time to inflection point
Tem	.0001	.0043	.0001	.0001
Var	.0001	.0001	.0035	.0642
Tem * Var	.0001	.4610	.0174	.0411

Final grain weight

The results of the analysis variance and the estimated values for the grain filling parameters in four wheat cultivars are shown in Tables 4.8, 4.9, and 4.10. The interaction between cultivars and temperatures for final grain weight was highly significant (Table 4.8). The individual kernel weight was significantly reduced in all cultivars at 30/25°C compared to 20/15°C (Table 4.9). Kavko appeared to be the most tolerant cultivar to high temperature with 21% decline in its grain weight and Lyallpur the most sensitive one with having 40% reduction (Table 4.10). The reduced grain weight in Lyallpur at high temperature was due to the effect of the significantly shortened duration of grain filling on grain weight and no change in the rate of grain filling. In the other three cultivars there was an increase in the rate of grain filling but it was not able to compensate for the reduced grain filling period. Although there was a significant increase in the rate of grain filling of Sun27B at high temperature, the large reduction in the duration of grain filling of this cultivar at high temperature (28 days) caused a considerable decline in final grain weight (38%). This made Sun27B rank second after Lyallpur in terms of grain yield sensitivity to high temperature. Among the four cultivars the individual kernel weight varied from 41 mg (in Kavko) to 56 mg (in Lyallpur) at 20/15°C and from 29 mg (in Sun27B) to 36 mg (in Trigo) at 30/25°C. The grain weight at maturity over cultivars was positively but not significantly correlated with both rate ($r = 0.41$) and duration of grain filling ($r = 0.33$) at 20/15°C. At 30/25°C, the correlation between the rate of grain filling and final grain weight ($r = 0.67$, $P < 0.01$, $n = 16$) was much larger than the correlation between grain filling duration and final grain weight ($r = 0.25$, n.s.). The final grain weight was also positively correlated with the number of days from anthesis to the inflection point but the correlation coefficient was much greater at 30/25°C ($r = 0.69$, $P < 0.01$) than at 20/15°C ($r = 0.35$, n.s.).

Table 4.9 Grain growth characteristics in four wheat cultivars grown at 20/15°C and 30/25°C, estimated by ordinary logistic model.

Cultivar	Tem. (°C)	Final grain dry weight (mg)	Maximum rate of grain filling (mg/ day)	Duration of grain filling (days)	Time to inflection point (days)
Lyallpur	20	56.0	1.57	50.6	24.2
	30	33.5	1.60	28.1	12.3
Sun 27B	20	46.4	1.16	55.2	25.8
	30	29.0	1.42	26.8	11.5
Kavko	20	41.2	1.40	44.4	22.7
	30	32.6	1.67	26.0	11.5
Trigo	20	51.0	1.73	45.7	24.0
	30	36.4	1.91	26.5	12.4
LSD 5%		3.1	0.24 ns	4.6	1.6

LSD 5% for Temperature × Variety

Table 4.10 Effects of growth at 30/25°C compared to 20/15°C on grain growth parameters of four wheat cultivars calculated from Table 4.9.

Cultivar	Reduction in grain weight (%)	Q_{10} for the maximum rate of grain filling (30/20°C)	Reduction in duration of grain filling (%)	Reduction in time to inflection point (%)
Lyallpur	40	1.02	45	49
Sun 27B	38	1.22	51	55
Kavko	21	1.19	41	49
Trigo	29	1.10	42	48

Maximum rate and sustained rate of grain filling

The main effect of temperature and cultivar on the maximum rate of grain filling was significant but the interaction was not significant (Table 4.8). The maximum rate of grain filling in all cultivars, with the exception of Lyallpur, was increased at 30/25°C compared to 20/15°C (Table 4.9). Lyallpur maintained the same rate at both temperatures with Q_{10} equal to one (Table 4.10). The rate ranged from 1.16 to 1.73 (mg/ day) at 20/15°C and from 1.42 to 1.91 (mg/ day) at 30/25°C. Averaged over both temperatures, Trigo had the highest and Sun27B the lowest values for the maximum rate of grain filling (1.82 and 1.29 mg/ day respectively). At each temperature separately the highest and lowest rates were also observed in Trigo and Sun27B.

The sustained rate of grain filling, as defined here, was estimated by linear regression and represents the rate sustained for a period during which between 80 and 90 % of the final grain dry matter was accumulated. The sustained rate was calculated by sequential omission of data points from both ends of grain growth curves as shown in Table 4.11 for plants grown at 20/15°C and in Table 4.12 for plants grown at 30/25°C. As expected, estimates of this attribute increased in rate as the number of points was reduced tending at the extremes towards the values of the maximum rates appearing in Table 4.9. There was also a tendency for values R^2 to increase in the same direction, but in general the estimates of the average rates sustained during the accumulation of between 80% and 90% of the grains total dry matter appear only a little less precise than estimates derived from shorter periods. A comparison was made between the maximum and sustained rate of grain filling of the cultivars at two temperatures (Table 4.13). The sustained rate was smaller than the maximum rate of grain filling in all cases, as expected. Cultivars maintained the same ranking for the sustained rate as they did for the maximum rate of grain filling. Ranking

Table 4.11 The sustained rate of grain filling (mg/day) of four wheat cultivars grown at 20/15°C estimated by linear regression.

Cultivar	Days after anthesis	Number of points	Rate of grain filling	Confidence interval	R^2	Standard errors
Lyallpur	6 – 50	12	1.18	1.08 – 1.28	98.3	0.046
	10 – 46	10	1.24*	1.12 – 1.36	98.5	0.051
	14 – 42	8	1.31	1.16 – 1.46	98.7	0.060
	18 – 38	6	1.38	1.18 – 1.58	98.7	0.071
	22 – 34	4	1.47	0.76 – 2.18	96.0	0.171
Sun 27B	6-54	13	0.89	0.82 – 0.96	98.5	0.032
	10-50	11	0.92*	0.86 – 0.98	99.1	0.028
	14-46	9	0.96	0.89 – 1.03	99.3	0.029
	18-42	7	0.98	0.91 – 1.05	99.6	0.026
	22-38	5	0.95	0.78 – 1.12	99.3	0.039
Kavko	6-46	11	0.99	0.87 – 1.11	97.2	0.053
	10-42	9	1.08*	0.94 – 1.22	97.7	0.058
	14-38	7	1.18	1.00 – 1.36	97.8	0.072
	18-34	5	1.35	1.19 –1.51	99.3	0.058
Trigo	6-46	11	1.25	1.12 – 1.38	97.9	0.057
	10-42	9	1.37*	1.26 – 1.48	99.1	0.046
	14-38	7	1.43	1.28 – 1.58	98.9	0.061
	18-34	5	1.51	1.20 – 1.82	97.8	0.113

The values identified by (*) represent the rate sustained for the period indicated during which 80 % to 90 % of the final grain dry matter accumulated.

for the increases in the rate of grain filling at 30/25°C compared to 20/15°C (Q_{10}) was also identical for the maximum and sustained rates of grain filling. The increases in rates of grain filling at high temperature (values of Q_{10}) in all cultivars was proportionally about 4 to 5% smaller for the sustained rate than for the maximum rate of grain filling.

Duration of grain filling

There was a significant interaction between cultivars and temperatures (Table 4.8). The duration of grain filling was significantly reduced in all cultivars in the plants grown at

Table 4.12 The sustained rate of grain filling (mg/day) of four wheat cultivars grown at 30/25°C estimated by linear regression.

Cultivar	Days after anthesis	Number of points	Rate of grain filling	Confidence intervals	R^2	Standard errors
Lyallpur	4-28	13	1.20*	1.09 – 1.31	98.0	0.049
	6-26	11	1.24	1.13 – 1.35	98.6	0.046
	8-24	9	1.27	1.20 – 1.34	99.6	0.028
	10-22	7	1.27	1.17 – 1.37	99.5	0.035
	12-20	5	1.33	1.21 – 1.45	99.6	0.044
Sun 27B	4-26	12	1.06*	0.94 – 1.18	97.1	0.056
	6-24	10	1.13	1.02 – 1.24	98.5	0.047
	8-22	8	1.16	1.05 – 1.27	99.0	0.044
	10-20	6	1.17	1.00 – 1.34	98.7	0.060
	12-18	4	1.34	1.20 – 1.48	99.8	0.033
Kavko	4-26	12	1.23*	1.09 – 1.37	97.2	0.064
	6-24	10	1.27	1.12 – 1.42	97.7	0.065
	8-22	8	1.34	1.21 – 1.47	98.9	0.054
	10-20	6	1.42	1.21 – 1.63	98.6	0.077
	12-18	4	1.38	0.52 – 2.24	93.9	0.200
Trigo	4-26	12	1.44*	1.29 – 1.59	97.7	0.067
	6-24	10	1.48	1.28 – 1.68	97.0	0.087
	8-22	8	1.58	1.33 – 1.83	97.2	0.101
	10-20	6	1.71	1.38 – 2.04	97.7	0.117
	12-18	4	1.98	1.08 – 2.88	96.8	0.207

The values identified by (*) represent the rate sustained for the period indicated during which 80 % to 90 % of the final grain dry matter accumulated.

30/25°C in comparison with those grown at 20/15°C (Table 4.9). The reductions in the duration of grain filling at high temperature varied from 18 days (41%) in Kavko to 28 days (51%) in Sun27B (Table 4.10). Significant genotypic variation existed at 20/15°C (about 10 days difference between the longest and the shortest grain filling duration), but the variation was small at 30/25°C and no significant differences (less than 2 days) were observed between cultivars at this temperature. Sun27B had the longest period of grain filling at 20/15°C compared to the other three cultivars.

Table 4.13 A comparison between the maximum and sustained rate of grain filling in four wheat cultivars grown at 20/15°C and 30/25°C, estimated by ordinary logistic model and by linear regression respectively.

Cultivar	Tem. (°C)	Maximum rate of grain filling (mg/ day)	Sustained rate of grain filling (mg/ day)	Q_{10} for the maximum rate (30°C /20°C)	Q_{10} for the sustained rate (30°C /20°C)
Lyallpur	20	1.57	1.24	1.01	0.97
	30	1.58	1.20		
Sun 27B	20	1.17	0.92	1.21	1.15
	30	1.42	1.06		
Kavko	20	1.40	1.08	1.19	1.14
	30	1.67	1.23		
Trigo	20	1.73	1.37	1.10	1.05
	30	1.91	1.44		

Time to inflection point

The interaction between temperatures and cultivar was significant for the time from anthesis to maximum rate (inflection point) of grain filling (Table 4.8). The time to inflection point was significantly reduced at 30/25°C compared to 20/15°C in all cultivars (Table 4.9). In Sun27B the reduction (55%) was larger than the other cultivars (48% in Trigo and 49% in Lyallpur and Kavko; Table 4.10). The time to the inflection point at 20/15°C ranged from 23 days (in Kavko) to 26 days (in Sun27B) but at 30/25°C all cultivars reached inflection point at the same time about day 12 after anthesis.

4.4 Discussion

All growth models tested here generally fitted the grain growth curves very well, with the exception of a logistic model modified by Darroch and Baker (1995) named here as Darroch (2). In some cases the deviations of the predicted grain dry matter from the observed values (residual mean squares) were larger for the Gompertz model than for the logistic models. In other studies logistic models had also provided a good fit to grain filling data in wheat cultivars (Loss *et al.* 1989; Darroch and Baker 1990) and barley (Koesmarno and Sedcole 1994). Koesmarno and Sedcole (1994) reported that both logistic and Gompertz models fitted the grain filling data equally well in barley. In the present study also the differences between the two models in estimating the final grain weight were very small, but considerable differences appeared between the two models when they were used to estimate the duration of grain filling (Table 4.7). According to these results it seems that overall the logistic models describe grain filling in the tested wheat cultivars better than the Gompertz model. The generalised logistic model compared to the ordinary logistic model involves one extra parameter. Therefore, the simpler ordinary logistic was chosen as the most appropriate model to estimate grain-filling parameters in tested cultivars.

As the time course of the accumulation of dry matter fitted a logistic model better than a linear regression it could be inferred that the shape of the growth curve itself might reflect something of the nature of the processes involved in grain filling. For example, the inflection point is an estimate of the maximum (instantaneous) rate at or close to the mid point of grain filling. This attribute therefore might be an indication of an upper limit to the rate of grain filling for a given cultivar under the conditions of the experiment. Such a

limit might reflect the maximum activity of synthetic system and/ or an upper limit to the influx of nutrients into the grain.

In several studies genetic variation among wheat cultivars has been reported in the responsiveness of grain filling to high temperature (Rawson 1986; Wardlaw *et al.* 1989a,b; Hunt *et al.* 1991). In this study there was a significant decrease in kernel weight in response to an increase in temperature from 20/15°C to 30/25°C, which ranged from 21% (in Kavko) to 40% (in Lyallpur; Table 4.10). Lyallpur (Wardlaw *et al.* 1989b) and Kavko (Hunt *et al.* 1991) had also been identified respectively as sensitive and tolerant cultivars to high temperature among the tested wheat cultivars. The identification of cultivars that exhibited a clear contrast in their response provided an opportunity to conduct further experiments to study the physiological and biochemical basis of the temperature sensitivity of grain filling. The greater grain-yield sensitivity of Trigo to high temperature in experiment 1 compared to Lyallpur was unexpected because in other studies (Wardlaw *et al.* 1989b; Wardlaw and Moncur 1995) Trigo has been shown to be more temperature tolerant than Lyallpur. Wardlaw *et al.* (1989b) recorded a 22% reduction in the grain weight of Trigo compared to 51% in Lyallpur with an increase in temperature from 18/13°C to 30/25°C. Greater reductions in the grain weight for Kavko and Sun 27B at high temperature were observed in the current study compared to those reported in the work of Hunt *et al.* (1991) under the same range of temperature. Factors such as light intensity or quality, temperature or shoot density during the pre-anthesis period, or the postanthesis light conditions can change the level of the postanthesis sensitivity of cultivars to high temperature from one experiment to another (Wardlaw *et al.* 1989a; Wardlaw 1994). Although the absolute values of the responses may be affected by such factors, the

relative order of tolerance across cultivars seen in these experiments may not be so changeable.

Significant genotypic variation was observed for the duration of grain filling, but the variation between cultivars was small in relation to temperature effects. Little variation among cultivars for duration of grain filling in relation to temperature effects has also been reported in other studies (Hunt *et al.* 1991; Wardlaw and Moncur 1995). These results as well as the non-significant correlation between final grain weight and duration of grain filling at high temperature ($r = 0.25$) means that the role of duration of grain filling in the selection of temperature tolerant cultivars is of less importance than the responses of the rate of grain filling. However, there was a significant positive correlation between final grain weight and the time from anthesis to maximum rate of grain filling (inflection point) at high temperature ($r = 0.69$), indicating that under high temperature conditions the longer is the period to the inflection point the more dry matter may be accumulated in the grains.

The rate of grain filling was increased in three of the four cultivars at moderately high temperature but the reduction in grain yield due to shortened duration was not compensated for completely by the increased rate of grain filling, as has been reported by others (Sofield *et al.* 1977a; Wardlaw *et al.* 1980; Tashiro and Wardlaw 1989). However, the significant positive correlation between final grain weight with the rate ($r = 0.67$), but not with duration of grain filling ($r = 0.25$, n.s.) at high temperature showed that the response of the rate of grain filling to temperature was more important in determining the temperature sensitivity of the cultivars. This supports the results from the work of Wardlaw and Moncur (1995). They reported that the most tolerant cultivars to high temperature during grain filling were those in which the rate of grain filling was increased

most by high temperature. They also reported that at high temperature the association between kernel weight at maturity with the rate of kernel filling was much stronger (r = 0.94) than with the duration of kernel filling (r = 0.51). Similar results were obtained in the field studies conducted in the areas experiencing high temperature during grain filling (Bruckner and Frohberg 1987). The simultaneous selection for high grain filling rate and high grain weight was therefore suggested to be possible without extension of the grain filling period. These investigations validate the studies of the factors that limit the rate of grain growth in sensitive cultivars under high temperature conditions.

The temperature quotient (Q_{10}) is a measure of the response to an increase in temperature of 10°C. Most values of Q_{10} observed within the normal temperature range for physiological processes in temperate crops normally fall within the range 1.3 to 1.6. Values of Q_{10} for the rate of grain filling reported in Table 4.3 are well below the lower end of this range and that for Lyallpur is below 1.0 indicating that the rate at 30/25°C was lower than that at 20/15°C. Q_{10} values of 1.29 and 1.27 for grain nitrogen accumulation on the other hand (Table 4.5) were at the lower end of this range, and there was no difference between the responses of Lyallpur and Trigo.

The maximum rate of grain filling is not sustained for long enough to be a meaningful measure of the rate of grain growth during the major phase of grain filling. Calculation of the average rate is a more pertinent estimate of the overall performance of the grain filling processes. Linear regression analysis of data taken during an arbitrarily specified portion of the grain's development was chosen as a measure of this attribute: the average rate maintained during the accumulation of 80% to 90% of the final grain dry weight. This attribute was termed the sustained rate of dry matter accumulation (Tables 4.11, 4.12 and

4.13). Although the physiological significance of this attribute is not self evident, it may be a reflection of the overall functioning of the synthetic processes and/ or the delivery of nutrients to the grains.

Whether or not these two estimates of the rate of grain filling are indicative of different aspects of the grain filling process, two things are clear from Table 4.13. Judging from the Q_{10} values there are similar temperature responses for both attributes and that, by either estimation Kavko and Sun27B show a greater positive temperature response than does Lyallpur, with Trigo intermediate between the two extremes.

High temperature depressed grain dry matter accumulation much more than grain nitrogen accumulation. The rate of dry matter accumulation in Lyallpur, for example, was significantly decreased by elevated temperature (Table 4.2) while a significant increase was observed in the rate of nitrogen accumulation (Table 4.5). This indicates that protein deposition is less temperature sensitive than dry matter accumulation overall and the reduction in the grain weight at high temperature is mainly due to the reduction in the starch accumulation (also see Bhullar and Jenner 1985).

Dry matter accumulation is the sum of all of the components deposited in the grain; the Q_{10} for nitrogen (mostly protein) is within the range normally expected for plant processes. Thus the abnormally low values for Lyallpur must be due to an exceptional response of starch deposition to the effects of high temperature, and that the basic reason for the poor performance of Lyallpur at high temperature is related to the failure of the rate of starch deposition to respond positively to an increase in temperature from 20/15°C to 30/25°C.

In conclusion, a sustained period of moderately high temperature (up to 30°C) significantly decreased grain weight mainly through shortening the duration of grain filling combined with small or no positive effects on the rate of grain filling. Genotypic variation were observed for both rate and duration of grain filling, but the variation was small at high temperature for the duration of grain filling. The significant correlation found between single grain weight with the rate, but not with the duration, of grain filling at high temperature indicated the important role of the rate of grain filling in the temperature sensitivity of wheat cultivars. Cultivars maintained the same ranking for both the sustained rate and the maximum rate of grain filling. Nitrogen accumulation was less sensitive to high temperature than dry matter accumulation and the reduction in grain weight at high temperature is probably due to a reduction in starch accumulation, which was not measured directly in this experiment. The ordinary logistic model was found as the most appropriate model and used to describe grain filling in the tested cultivars.

Chapter 5

The effect of grain nutrition level on the response of two wheat cultivars to high temperature

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5.1 Introduction

The results of the last experiment showed that a sustained period of moderately high temperature significantly decreased grain weight in wheat cultivars mainly through shortening the duration of grain filling combined with small or no positive effects on the rate of grain filling. The small genotypic variation for duration of grain filling at high temperature and the significant correlation between single grain weight with the rate of grain filling at high temperature indicated that the response of the rate of grain filling was important in determining the temperature sensitivity of the cultivars (Hunt *et al.* 1991; Wardlaw and Moncur 1995). Cultivars most tolerant of high temperature during grain filling have been those in which the rate of kernel filling was most enhanced by high temperature (Wardlaw and Moncur 1995). Therefore, it is important to search for the physiological and biochemical aspects that control the rate of grain filling for an explanation for differences between cultivars in their response to high temperature.

Starch and protein accumulation are both affected by high temperature, but reductions in dry matter accumulation are due mainly to a decrease in starch deposition (Bhullar and Jenner 1985). The supply of assimilates to the grains and also the availability of assimilates inside the endosperm are not the major limitation to the rate of grain growth at high temperature (Wardlaw *at al.* 1980; Nicolas *et al.* 1984; Jenner 1991). No change in the response of grains to high temperature was observed when the potential supply of assimilate was increased by decreasing the number of the grains per ear (Wardlaw *et al.*

1980). The response of the rate of grain filling at high temperature, therefore, could be mainly due to the factors that operate inside the grains.

Grain yield generally is negatively correlated with grain nitrogen concentration (James *et al.* 1990). This has been attributed to the bioenergetic competition between N accumulation and carbohydrate synthesis, which use the same source of energy from photosynthetic assimilates (Austin *et al.* 1977; Dhugga and Waines 1989). The cost of energy for N assimilation is higher than that of carbohydrate synthesis (Penning de Vries *et al.* 1974; Bhatia and Rabson 1976; Le Van Quy and Champigny 1991). Therefore, when N is applied to the crop some energy is used for N assimilation, which could otherwise be consumed for carbohydrate synthesis. Simultaneous increase in grain yield and grain protein concentration has been achieved by late N application (Morris and Paulsen 1985; Banziger *et al.* 1994). In these cases the investment of energy for N assimilation was compensated for by the benefits of a higher plant N status on the rate of photosynthesis. However, under high temperature during grain filling, grain weight is limited mainly by factors operating inside the grain rather than by the supply of photosynthetic assimilates (Jenner 1994). Thus, the advantage of a higher plant N status on photosynthetic capacity of the crop does not appear to be important. The relationship between the increased N concentration and grain yield at high temperature may therefore result from interrelationships between protein and starch synthesis inside the grain. There is no information available in the literature on relationships at high temperature between the availability of the substrates for starch and protein synthesis within the grains and the responses of grain filling to high temperature.

This study investigated the effects of trimming the ear and varying the level of soil nitrogen, treatments designed to alter the availability of substrates within the endosperm, on the responses to high temperature of the deposition of starch and protein in the grains of two wheat cultivars Kavko and Lyallpur. These varieties were chosen because they showed in the last experiment to differ in their responses to elevated temperature.

The accumulation of different protein fractions is also influenced by high temperature (Blumenthal *et al.* 1991, 1993), and an interaction between genotype and temperature has been reported for fractional protein accumulation (Stone and Nicolas 1994, 1996). The monomer/ polymer ratio in the grain protein generally increases at high temperature and reduces dough strength (Blumental *et al.* 1993, Stone and Nicolas 1995a). The increased proportion of monomers compared to polymers has been attributed to an increase in the synthesis of monomers (Blumental *et al.* 1993; Stone and Nicolas 1994) or to the lower temperature sensitivity of the synthesis of monomers compared to the other protein fractions (Stone *et al.* 1996). The grain protein composition can be changed in response to nitrogen fertilisation and is also associated with an increase in grain protein percentage (Salomonsson and Larsson-Raznikiewicz 1985; Stenram *et al.* 1990). To what extent the effect of grain nitrogen level on protein composition can be modified by a sustained period of moderately high temperature is another matter dealt with in this study.

The ratio of amylose to amylopectin determines the quality of starch in wheat grain. Amylopectin is the main contributor to starch quality and gives a higher viscosity and elasticity to the starch paste (Martin and Smith 1995). The flour of low amylose starch for example with its higher than normal viscosity is suitable for products such as white salted noodles (Oda 1980). The ratio of amylose to amylopectin is controlled by genotype and

also to some extent influenced by the environment (Moss and Miskelly 1984; Tester *et al.* 1995). High temperature during grain filling has been shown to increase the proportion of amylose in the flour (Shi *et al.* 1994). The pattern of amylose and amylopectin accumulation throughout grain development has therefore been considered as part of this study in the two cultivars tested under high temperature.

5.2 Materials and Methods

5.2.1 Growth conditions

This experiment was conducted in 25-cm diameter pots kept in environmentally controlled growth rooms. Two temperature regimes and two levels of nitrogen were applied to the plants with trimmed and untrimmed ears of two wheat cultivars. Pots were arranged inside the growth rooms under a randomised complete design. Four replications were used for each treatment. The condition inside the growth rooms and the individual methods for the measurements of grain nitrogen, protein, starch, amylose, soluble carbohydrates, soluble amino acids, and also HPLC analysis of the protein fractions have been explained in detail in Chapter 3 (Materials and Methods).

5.2.2 Cultivars

Two wheat cultivars differing in their response to temperature were used in this experiment, Kavko and Lyallpur. Among the cultivars tested in the last experiment Kavko and Lyallpur were respectively the least and the most sensitive cultivars in response to an increase in temperature from 20/15°C to 30/25°C.

5.2.3 Soil preparation

University of California (UC) soil lacking in nitrogen was prepared and used in this experiment as follows: 400 L of washed sand (Golden Grove) were sterilised at 100°C for 0.5 hours. A 300 L Bale of peatmoss (Eorotorf Peat) was added and mixed with the sand for 30 seconds (the sand particles ranged from 0.5 to 5 mm, 85% from 0.5 to 2 mm). The resultant mixture dropped to about 85°C and the following nutrients were added straight away:

Calcium hydroxide (hydrated lime or Limil)	700 gm
Dicalcium phosphate (super phosphate)	560 mg
Calcium carbonate (agricultural or ground limestone)	480 gm
Calcium sulphate (Plaster of Paris)	400 gm
Magnesium carbonate (magnesite)	120 mg
Potassium sulphate (sulphate of potash)	100 mg

The pH of this mixture was about 6.8.

Micro nutrients

Three micro nutrient solutions were prepared separately and applied to the pots one week after emergence. 5 ml of solution 1 containing (g/ L) 1.4 $MnSO_4.4H_2O$, 0.1 $H_2MoO_4.H_2O$, 0.2 $CoSO_4.7H_2O$, 0.03 $NiSO_4.7H_2O$, 2.0 $CuSO_4.5H_2O$, and 0.2 H_3BO_3 and 2 ml of solution 2 containing (g/ L) 0.350 $FeSO_4.7H2O$ and 2 ml of solution 3 containing (g/ L) 0.440 $ZnSO_4.7H_2O$ were applied to each kg of pot dry soil.

5.2.4 N treatments

Two levels of nitrogen as sodium nitrate (Na_2NO_3) were applied to the pots. For the low level, 60 mg nitrogen for each plant were added to the pots (1200 mg nitrogen per pot) split between two growth stages: 40% was applied one week after the seedlings emerged and 60% at the time of ear formation. For the standard level of nitrogen, in addition to nitrogen provided for the low level described above, another 60 mg nitrogen per plant were applied at ear emergence and a further 60 mg during the grain filling period (a total of 2400 mg nitrogen per pot), on days 8 and 16 after anthesis for plants growing at 30/25°C and 20/15°C respectively.

5.2.5 Trimming of the ears

The ears on plants in half of the pots of each cultivar were trimmed by removing spikelets from the top and bottom of the ears leaving only the middle four spikelets on each side of the ear (Jenner 1980). Trimming was done at days 8 and 16 after anthesis at 30/25°C and 20/15°C respectively.

5.2.6 Sampling

One plant from each pot was taken every 2 days (at 30/25°C) and every 4 days (at 20/15°C) starting from day 2 after anthesis. The grains of spikelets in the middle of the ears (corresponding to the positions remaining in the trimmed ears) were removed and 10 grains (out of 16) were chosen at random and were put into an oven set at 80°C for 48 hours. The grains were allowed to cool in a desiccator over silica gel and grain dry weight was determined. The remaining six grains were boiled in ethanol for 10 minutes and stored in a cold room for the measurement of grain soluble carbohydrates and soluble

amino acids. The dried grains were ground into fine flour suitable for laboratory measurements using a laboratory pulverising mill explained in Chapter 3.

5.3 Results

5.3.1 Grain growth parameters

The accumulation of grain dry matter in the grains of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen application and trimming are shown in Fig. 5.1. The grain growth parameters, except for the sustained rate of grain filling, were estimated by the ordinary logistic model which was shown in the last chapter to fit the grain filling data adequately. The sustained rate of grain filling was estimated by linear regression analysis as explained in Chapter 4. The results for the analysis of variance and the estimated values of the parameters are shown in Tables 5.1, 5.2, and 5.3. Although none of the third order interactions was statistically significant many of the lower order interactions and the main effects were significant.

Final grain weight

Final grain weight was significantly decreased in the plants grown at 30/25°C compared to those grown at 20/15°C in all treatments (Fig. 5.1 and Table 5.2). The interactions between temperature, cultivar and nitrogen were highly significant (Table 5.1). The reductions in grain weight at high temperature were higher in Lyallpur (40%) than in Kavko (24%) at the standard level of nitrogen but the decreases were similar for both cultivars (about 30%) at the low level of nitrogen (Table 5.3 and Fig. 5.4A). The reductions in grain weight in both cultivars were due to the significant reduction in the duration of grain filling at high temperature (Table 5.3). The increased rate of grain filling at high temperature, which occurred in some treatments, could not compensate for the

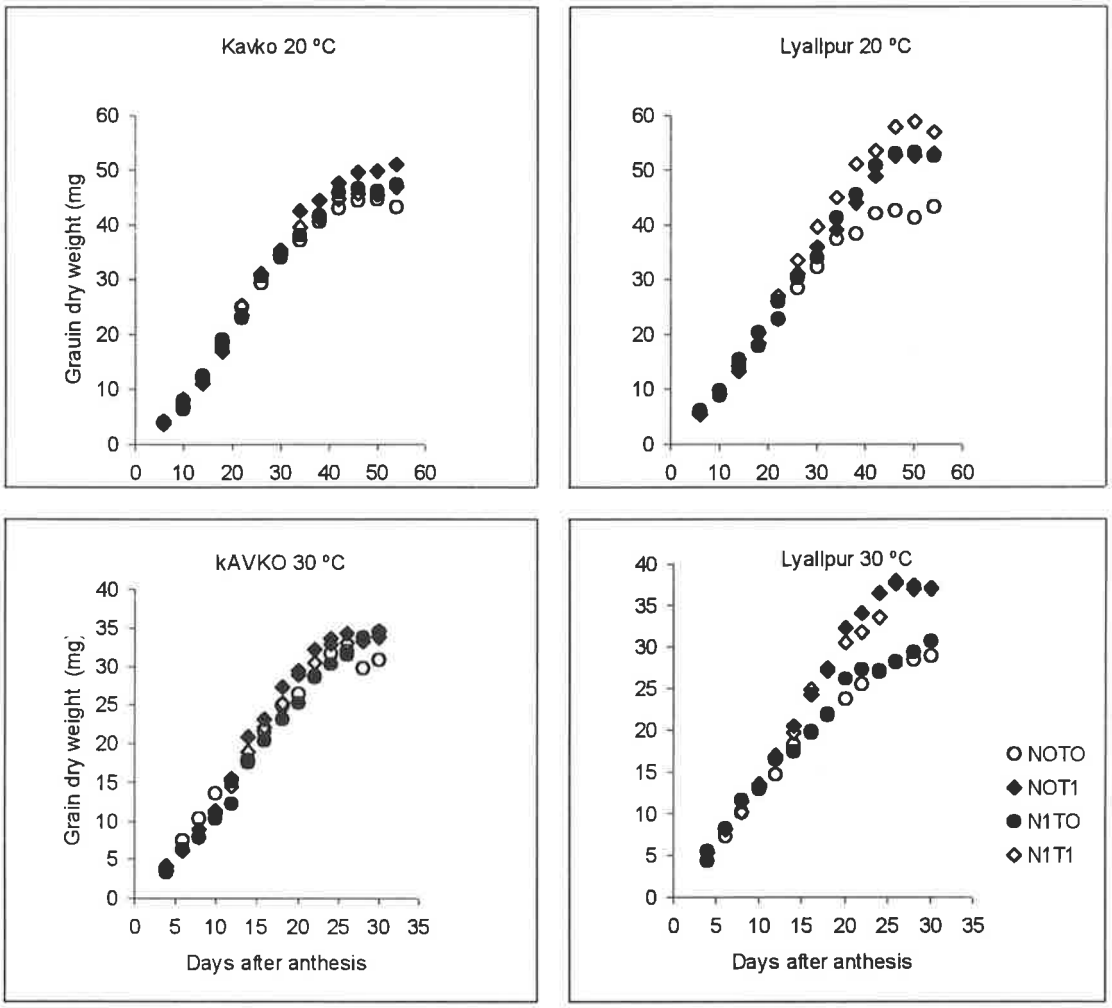

Fig. 5.1 Grain dry matter accumulation in Kavko and Lyallpur grown at 20°C and 30°C subjected to two levels of nitrogen and trimming (NO= low level of nitrogen; N1= standard level of nitrogen; TO = untrimmed ears; T1 = trimmed ears).

effect of reduced grain filling duration on final grain weight, which was observed in all cases.

There were significant interactions between trimming and cultivar and also between trimming, temperature and nitrogen (Table 5.1). In Lyallpur the grain weight was 21%

Table 5.1 P values obtained from the analysis of variance table for grain growth components of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming (Tem: temperature; Var: variety).

Source	Final grain dry weight	Sustained rate of grain filling	Maximum rate of grain filling	Duration of grain filling	Time to inflection point
Tem	.0001	.0001	.0001	.0001	.0001
Var	.0001	.0001	.0001	.0001	.1134
N	.0001	.3997	.8047	.0492	.0238
Trimming	.0001	.0001	.0001	.7119	.0102
Tem * Var	.0001	.0048	.0173	.0008	.0005
Tem * N	.0220	.0025	.0108	.5407	.6427
Tem * Trimming	.6733	.0001	.0001	.0513	.1229
Var * N	.0017	.0711	.0599	.7826	.8001
Var * Trimming	.0001	.0001	.0070	.1053	.0102
N * Trimming	.0001	.2837	.0846	.0068	.0005
Tem * Var * N	.0001	.3997	.1369	.0524	.0175
Tem * Var * Trimming	.2209	.1014	.2866	.8333	.7677
Var * N * Trimming	.9192	.7931	.3665	.6303	.8992
Tem * N * Trimming	.0178	.8841	.1082	.0112	.0264
Tem * Var * N * Trimming	.3858	.7050	.8047	.1553	.1229

Table 5.2 Grain growth characteristics of cultivars Kavko (K) and Lyallpur (L) grown at 20/15°C and 30/25°C as estimated by the ordinary logistic model (NO= low level of nitrogen; N1= standard level of nitrogen; TO = untrimmed ears; T1 = trimmed ears).

Treatment	Tem. (°C)	Final grain dry weight (mg)		Sustained rate of grain filling (mg/ day)*		Maximum rate of grain filling (mg/ day)		Duration of grain filling (days)		Time to inflection point (days)	
		K	L	K	L	K	L	K	L	K	L
NOTO	20	44.9	43.9	1.14	1.00	1.54	1.34	42.8	45.3	21.3	21.1
	30	32.8	30.5	1.35	1.01	1.66	1.32	27.1	29.1	12.5	12.3
NOT1	20	51.0	56.4	1.20	1.17	1.71	1.54	45.3	52.2	23.2	25.2
	30	35.2	40.0	1.54	1.51	2.13	1.99	25.1	28.3	12.9	13.4
N1TO	20	47.5	56.9	1.20	1.16	1.57	1.50	44.6	52.6	22.5	24.5
	30	36.4	31.2	1.27	1.02	1.70	1.34	30.8	29.2	15.1	12.3
N1T1	20	46.8	60.7	1.20	1.29	1.65	1.76	42.3	49.8	21.7	24.2
	30	35.5	39.8	1.46	1.45	1.92	1.85	27.2	29.4	13.6	13.5
LSD 5%		3.3 ns		0.12 ns		0.17 ns		3.8 ns		1.7 ns	

*The sustained rate of grain filling was estimated by linear regression model as explained in Chapter 4.
LSD 5% for Temperature × Variety × Nitrogen × Trimming

Table 5.3 Effects of growth at 30/25°C compared to 20/15°C on the final grain weight and the rate and duration of grain filling in cultivars Kavko (K) and Lyallpur (L) calculated from Table 5.2. (Treatments as specified in Table 5.2).

Treatment	Reduction in grain weight (%)		Q_{10} of the sustained rate of grain filling (30°C /20°C)		Q_{10} of the maximume rate of grain filling (30°C /20°C)		Reduction in duration of grain filling (%)		Reduction in time to inflection point (%)	
	K	L	K	L	K	L	K	L	K	L
NOTO	27	31	1.18	1.01	1.08	0.99	37	36	41	42
NOT1	31	29	1.28	1.29	1.25	1.29	45	46	44	47
N1TO	23	45	1.06	0.88	1.08	0.89	31	45	33	50
N1T1	24	34	1.22	1.12	1.16	1.05	36	41	37	44
Mean	26	35	1.19	1.08	1.14	1.06	37	42	39	46

heavier in the trimmed than in the untrimmed ears but trimming had no significant effect on the grain weight in Kavko (Fig. 5.7A). The sensitivity of grain weight to temperature was not significantly changed by trimming at low level of nitrogen but at the standard level of nitrogen the plants with trimmed ears compared to those with untrimmed ears were less sensitive to high temperature (see later in Fig. 5.10A).

Sustained rate and maximum rate of grain filling

Although estimates of the sustained rate of grain filling give smaller values than the corresponding maximum rates, responses of these attributes are similar (Table 5.1). There was a significant interaction between temperature and cultivar for the rate of grain filling. Overall, the maximum rate of grain filling was greater in Kavko (1.85) than in Lyallpur (1.63) at 30/25°C but the rate was similar in both cultivars at 20/15°C (1.62 and 1.55 in Kavko and Lyallpur respectively: Fig. 5.4B). The positive effect of temperature on the rate of grain filling was overall greater in Kavko; the values of Q_{10} (30/20°C) were 1.14 and 1.06 for Kavko and Lyallpur respectively (Table 5.3). Regardless of cultivar, the response to temperature was dependent on the level of nitrogen as the interaction between temperature and nitrogen was significant (Table 5.1). The rate of grain filling was increased at high temperature to a greater extent at the low (Q_{10} = 1.16) than at the standard (Q_{10}= 1.05) level of nitrogen but the effect of nitrogen on the response to temperature was similar in both cultivars.

The interaction between temperature and trimming of the ears was highly significant (Table 5.1). In both cultivars alike, the maximum rate of grain filling in the trimmed ears was greater at 30/25°C than at 20/15°C with a Q_{10} of 1.18 but the rate was similar at both temperatures in the untrimmed ears (Fig. 5.8A). Although the highest Q_{10}'s for the rate

were achieved for both cultivars in the trimmed ears treated with a low level of nitrogen (Table 5.3), this interaction was not significant. There was a significant interaction between cultivar and trimming. Overall the maximum rate of grain filling was increased in the trimmed ears compared to the untrimmed ears to a greater extent in Lyallpur (29%) than in Kavko (14%; Fig. 5.7D).

Duration of grain filling

There was a highly significant interaction between temperature and cultivar (Table 5.1). The duration of grain filling was significantly decreased at 30/25°C compared to 20/15°C (Table 5.2). The period of grain filling was shorter in Kavko (44 days) than in Lyallpur (50 days) at 20/15°C but there was no difference between the two cultivars at high temperature (Fig. 5.4C). The extent of the reduction in duration at high temperature was overall greater in Lyallpur (42%) than in Kavko (37%; Table 5.3). The interactions between temperature, nitrogen and trimming were significant (Table 5.2). Regardless of cultivar, the reduction in duration of grain filling at high temperature was greater at the low (46%) than at the standard (39%) level of nitrogen in the trimmed ears but the level of nitrogen did not affect the response to temperature in the untrimmed ears. In general, the duration of grain filling was significantly longer in the grains raised at the standard than those raised at the low level of nitrogen but both cultivars responded similarly to the level of nitrogen.

Inflection point of time

The time from anthesis to maximum rate (inflection point) was significantly decreased at high temperature (Table 5.2). The interactions between temperature and cultivar and the level of nitrogen were significant (Table 5.1). The reductions at high temperature were

greater in Lyallpur (47%) than in Kavko (35%) at the standard level of nitrogen but there was no significant difference between the two cultivars in this attribute at the low level of nitrogen (Table 5.3). The effect of trimming on interactions between temperature and nitrogen was similar to that of duration of grain filling.

5.3.2 Characteristics of grain protein accumulation

The results of the analysis of variance and the estimated values for the parameters of protein accumulation in the grains of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming are shown in Tables 5.4, 5.5, and 5.6. These parameters were all estimated by the ordinary logistic model. None of the third order interactions was statistically significant.

Final grain protein weight

There were significant interactions between temperature with cultivar and the level of nitrogen (Table 5.4). Except for Kavko at low nitrogen, the final grain protein weight appeared to be lower at 30/25°C than at 20/15°C and the reductions were greater at the standard compared to the low level of nitrogen (Table 5.5 and 5.6). However, the effect of nitrogen on the interaction between temperature and cultivars was not significant. The reductions in protein weight at high temperature were overall greater in Lyallpur (28%) than in Kavko (14%; Fig. 5.4F). Regardless of cultivar, the grain protein weight was decreased at high temperature in the plants raised at the standard level of nitrogen but no significant differences were there between the two temperatures at the low level of nitrogen (Fig. 5.10B). At the low level of nitrogen the effect of shortened duration of protein accumulation on final grain protein weight at high temperature was almost compensated for by the increase in the rate of grain protein accumulation (Table 5.6). At

Table 5.4 P values obtained from the analysis of variance table for the components of grain protein accumulation in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming.

Source	Final grain protein weight	Maximum rate	Duration	Time to inflection point
Tem	.0001	.0001	.0001	.0001
Var	.0043	.0001	.0001	.4230
N	.0001	.0001	.0754	.0722
Trimming	.0001	.0001	.3185	.0771
Tem * Var	.0017	.0046	.0177	.0082
Tem * N	.0001	.0002	.0132	.0001
Tem * Trimming	.1462	.0008	.6241	.5604
Var * N	.1112	.0006	.1483	.3385
Var * Trimming	.0008	.7353	.1215	.0002
N * Trimming	.0134	.7353	.0054	.0038
Tem * Var * N	.5404	.0173	.2569	.3156
Tem * Var * Trimming	.4761	.8657	.8394	.7054
Var * N * Trimming	.5056	.8657	.7572	.9623
Tem * N * Trimming	.0160	.2398	.0016	.0004
Tem * Var * N * Trimming	.2974	.6123	.9151	.8254

Table 5.5 The characteristics of grain protein accumulation of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C as estimated by the ordinary logistic model. (Treatments as specified in Table 5.2).

Treatment	Temperature (°C)	Final grain protein weight (mg)		Maximum rate of protein accumulation (mg/ day)		Duration of protein accumulation (days)		Time to inflection point (days)	
		K	L	K	L	K	L	K	L
NOTO	20	4.36	4.87	0.16	0.14	41.7	48.1	23.1	23.5
	30	5.28	4.21	0.24	0.21	31.0	30.0	17.6	14.7
NOT1	20	5.69	7.14	0.21	0.20	45.7	55.3	25.2	28.5
	30	6.11	6.23	0.36	0.34	29.3	30.3	16.9	16.7
N1TO	20	8.29	8.53	0.24	0.20	54.2	60.4	28.9	28.5
	30	4.80	4.70	0.31	0.20	27.6	31.5	15.7	14.2
N1T1	20	7.11	9.57	0.30	0.27	42.6	53.9	25.0	28.0
	30	5.75	6.55	0.42	0.30	25.7	33.2	15.4	17.0
LSD 5%		1.04 ns		0.04 ns		6.6 ns		2.2 ns	

LSD 5% for Temperature × Variety × Nitrogen × Trimming

Table 5.6 Effects of growth at 30/25°C compared to 20/15°C on the characteristics of grain protein accumulation in cultivars Kavko (K) and Lyallpur (L), calculated from Table 5.5 (Treatments as specified in Table 5.2).

Treatment	Ratio of grain protein weight (30°C /20°C)		Q_{10} for the maximume rate (30°C /20°C)		Reduction in duration at 30°C (%)		Reduction in time to inflection point at 30°C (%)	
	K	L	K	L	K	L	K	L
NOTO	1.21	0.86	1.50	1.50	26	38	24	37
NOT1	1.07	0.87	1.71	1.70	36	45	33	41
N1TO	0.58	0.55	1.29	1.00	49	48	46	50
N1T1	0.81	0.68	1.40	1.11	40	38	38	39
Mean	0.92	0.74	1.48	1.33	38	42	34	42

the standard level of nitrogen, however, the increase in the rate of protein accumulation was not big enough to counterbalance the effect of reduced duration on grain protein weight, and resulted in a significant reduction at high temperature. Trimming of the ears decreased the sensitivity of both cultivars to temperature at the standard nitrogen level but this effect was not evident at the low level of nitrogen (Fig. 5.10B).

Maximum rate of protein accumulation

There were significant interactions between temperature, cultivar and nitrogen (Table 5.4). The maximum rate of grain protein accumulation was significantly increased at 30/25°C compared to 20/15°C except in Lyallpur at the standard level of nitrogen (Fig. 5.6A). The extent of the increases at high temperature (values of Q_{10}) was greater in Kavko (1.33) than in Lyallpur (1.04) at the standard level of nitrogen but the temperature response of both cultivars was similar at the low level of nitrogen (the values of Q_{10} were 1.58 and 1.65 for

Kavko and Lyallpur respectively). The interaction between temperature and trimming was significant as the positive effect of temperature on the maximum rate of protein accumulation was overall greater in the trimmed (Q_{10} = 1.40) than in the untrimmed (Q_{10}= 1.26) ears (Fig. 5.8B). In no case was the rate of protein accumulation lower at 30/25°C than at 20/15°C and the Q_{10} values for grain protein (Table 5.6) were all greater than the corresponding values for grain dry matter (Table 5.3).

Duration of protein accumulation

High temperature significantly decreased the period of grain protein accumulation (Table 5.5). The reductions at high temperature were overall greater in Lyallpur (42%) than in Kavko (38%; Table 5.6). The level of nitrogen had no significant effect on the differential response of the two cultivars to temperature. There were significant interactions between temperature, level of nitrogen, and trimming. Averaged over the two cultivars, the extent of the reduction in duration at high temperature was smaller at the low (29%) than at the standard (49%) level of nitrogen in the untrimmed ears but there was no significant effect of nitrogen level on the response to temperature in the trimmed ears (about 40% reduction at both level of nitrogen). The magnitude of the decrease in the duration of protein accumulation at high temperature was the same as that of dry matter accumulation.

The time from anthesis to maximum rate of grain protein accumulation (inflection point) was significantly reduced at high temperature (Table 5.5). The interaction between temperature and cultivar was significant (Table 5.4). The reductions in the time to the inflection point were proportionally greater in Lyallpur (42%) than in Kavko (34%) at high temperature (Table 5.6). The pattern of the effect of nitrogen level and trimming on the response to temperature was similar to that of the duration of protein accumulation.

5.3.3 Grain-N accumulation

As the grain nitrogen accumulation did not follow a sigmoid pattern in all cases and the data did not fit well to the logistic model, linear regression was used to estimate the sustained rate of grain-N accumulation. For this purpose the rate was calculated by sequential omission of data points from both ends of the nitrogen curve and those data points that fitted the linear regression model with R^2 more than 0.95 were used to estimate the sustained rate of grain-N accumulation.

Grain-N content

The interaction between temperature and cultivar and also between temperature and nitrogen was significant but level of nitrogen or trimming of ears did not significantly change the differential response of cultivars to temperature (Table 5.7). Overall, the amount of grain-N at maturity was lower in the grains developed at 30/25°C compared to those raised at 20/15°C and the reductions at high temperature was greater in Lyallpur (26%) than in Kavko (15%; Table 5.8). The magnitude of the response to temperature was dependent upon the level of supplied nitrogen. Averaged over cultivars, the reduction in grain-N content at high temperature occurred to a greater extent at the standard (29%) than at the low (6%) level of nitrogen. Trimming the ears had no significant effect on the response to temperature.

Grain-N content was significantly higher at the standard compared to the low level of nitrogen applied to the soil at 20/15°C but these increases were not statistically significant in the trimmed ears of either cultivar raised at 30/25°C (Table 5.8). Trimming of the ears had a significant and positive effect on grain-N weight except in Kavko raised at 20/15°C at the standard level of nitrogen where there was little difference between the trimmed and

Table 5.7 P values obtained from the analysis of variance table for the components of grain nitrogen accumulation in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming.

Source	Grain nitrogen weight at maturity	Sustained rate of grain nitrogen accumulation
Tem	.0001	.0001
Var	.6550	.0002
N	.0001	.0001
Trimming	.0001	.0001
Tem * Var	.0006	.0001
Tem * N	.0001	.0047
Tem * Trimming	.2910	.0178
Var * N	.3584	.2453
Var * Trimming	.1929	.1194
N * Trimming	.0062	.2453
Tem * Var * N	.6550	.5764
Tem * Var * Trimming	.1665	.8787
Var * N * Trimming	.0333	.6114
Tem * N * Trimming	.2786	.6474
Tem * Var * N * Trimming	.0658	.1899

untrimmed ears. However, the interaction between cultivar and trimming was not significant (Table 5.7).

A comparison was made between the grain protein as measured by the Bio-Rad reagent and the protein calculated from the nitrogen data (Table 5.9). The values of the protein weight converted from nitrogen data in all cases were greater (overall 1.23 times) than those of the measured protein. Regardless of cultivar and treatments, the differences between the two estimates of protein were greater at 30/25°C (1.28) than at 20/15°C (1.18)

Table 5.8 Nitrogen accumulation in the grain of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C. (Treatments as specified in Table 5.2).

Treatment	Temperature (°C)	N weight at maturity (mg)		Ratio (30°C /20°C)		Sustained rate of N accumulation (mg/ day)		Q_{10} (30°C /20°C)	
		Kavko	Lyallpur	Kavko	Lyallpur	Kavko	Lyallpur	Kavko	Lyallpur
NOTO	20	0.87	0.98	1.02	0.85	0.017	0.018	2.64	1.78
	30	0.89	0.83			0.045	0.032		
NOT1	20	1.19	1.24	1.00	0.90	0.025	0.027	2.16	1.85
	30	1.19	1.12			0.054	0.050		
N1TO	20	1.59	1.50	0.69	0.64	0.033	0.030	1.55	1.30
	30	1.09	0.96			0.051	0.039		
N1T1	20	1.55	1.76	0.84	0.67	0.035	0.037	1.77	1.35
	30	1.30	1.18			0.062	0.050		
LSD 5%		0.12 ns				0.007 ns			

LSD 5% for Temperature × Variety × Nittogen × Trimming

especially at the standard level of nitrogen. Except for Kavko at the low level of nitrogen, the reductions in the grain protein weight at high temperature were slightly smaller in the protein calculated from nitrogen than in the Bio-Rad measured protein.

The rate of grain-N accumulation

The main effects of cultivar and treatments as well as the second order interactions between temperature and cultivar, nitrogen, and trimming were significant; none of the third order interactions was significant (Table 5.7). The rate of grain-N accumulation was significantly increased in the plants grown at 30/25°C compared with those grown at 20/15°C (Table 5.8) and the magnitude of the temperature effect was greater in Kavko (Q_{10} = 1.89) than in Lyallpur (Q_{10} = 1.54). The positive effect of temperature on the rate of grain-N accumulation was greater at the low (Q_{10} = 2.05) than at the standard (Q_{10} = 1.50) level of nitrogen. Although the interaction between temperature and trimming was significant the increase in the rate of grain-N accumulation at 30/25°C compared to 20/15°C was almost similar in the trimmed (Q_{10} = 1.74) and untrimmed (Q_{10} = 1.68) ears. The effect of nitrogen or trimming on the differential response of cultivars to temperature was not significant (Table 5.7). The Q_{10}'s (30/20°C) values in Table 5.8 for the rate of grain-N accumulation were all greater (overall 1.29 times) than the corresponding values for the maximum rate of grain protein accumulation seen earlier in Table 5.6. These differences were greater in Kavko (1.52) than in Lyallpur (1.15) at the low level of nitrogen but not at the standard level of nitrogen.

5.3.4 Effect of high temperature on starch accumulation

The accumulation of starch and its components, amylose and amylopectin, throughout grain development was analysed in cultivars Kavko and Lyallpur only in the untrimmed

Table 5.9 A comparison between grain protein content (mg) as measured by the Bio-Rad method and values converted from grain nitrogen data. (Treatments as specified in Table 5.2).

Treatment	Temperature (°C)	Measured protein		Ratio (30°C/ 20°C)		Converted protein		Ratio (30°C/ 20°C)	
		Kavko	Lyallpur	Kavko	Lyallpur	Kavko	Lyallpur	Kavko	Lyallpur
NOTO	20	4.21	4.67	1.05	0.80	4.94 **(1.17)**	5.59 **(1.20)**	1.03	0.85
	30	4.40	3.75			5.07 **(1.15)**	4.75 **(1.27)**		
NOT1	20	5.58	6.45	0.98	0.82	6.77 **(1.21)**	7.07 **(1.10)**	1.00	0.90
	30	5.45	5.29			6.78 **(1.24)**	6.38 **(1.21)**		
N1TO	20	7.57	7.53	0.59	0.54	9.05 **(1.20)**	8.54 **(1.13)**	0.69	0.64
	30	4.44	4.09			6.20 **(1.40)**	5.47 **(1.34)**		
N1T1	20	6.96	8.82	0.78	0.61	8.85 **(1.27)**	10.05 **(1.14)**	0.84	0.67
	30	5.42	5.42			7.39 **(1.36)**	6.71 **(1.24)**		
LSD 5%		0.67 ns				0.72 ns			

The values in the parentheses refer to the ratio of the data derived from grain nitrogen values compared to those of grain protein measured by the Bio-Rad method.

ears raised at the standard level of nitrogen (e.g. the similar conditions used for other chapters of this thesis). The final weight and the rate and duration of accumulation for each component were estimated by the ordinary logistic model from data taken throughout grain filling period. The analysis of variance and the estimated values for these parameters are shown in Tables 5.10, 5.11, and 5.12.

Final grain starch weight

The main effects of temperature and cultivar and also the interactions were significant on the final weight of starch, amylose, and amylopectin (Table 5.10). The weights of grain starch and its components were significantly less in Kavko than in Lyallpur at 20/15°C (Table 5.11) but there were no significant differences between cultivars at 30/25°C. The amounts of grain starch and its components were significantly decreased at high temperature and the reductions occurred to a greater extent in Lyallpur than in Kavko (Table 5.12). [The depressing effect of high temperature in both cultivars was relatively greater on grain starch than on grain dry matter accumulation (N1TO treatment in Table 5.3]. The difference between the response of starch and that of dry matter was relatively greater in Kavko than in Lyallpur.

The temperature responses of the grain amylose and amylopectin content were similar to that of starch (Table 5.12). The reductions in the weight of starch and its components in Kavko at high temperature was due to the significantly reduced duration of accumulation which was not completely compensated for by the effect of the increased rate of accumulation. In Lyallpur, however, the reductions were due to a combination of shortened duration and reduced rate of accumulation.

Table 5.10 P values obtained from the analysis of variance table for the accumulation of starch components in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming.

	Source	Final weight	Maximum rate	Duration	Time to inflection point
Starch	Tem	.0001	.7276	.0001	.0001
	Var	.0097	.0672	.0066	.8597
	Tem * Var	.0023	.0265	.2554	.0067
Amylose	Tem	.0001	.4041	.0001	.0001
	Var	.0095	.0356	.0100	.8782
	Tem * Var	.0005	.0406	.0643	.0124
Amylopectin	Tem	.0001	.4341	.0001	.0001
	Var	.0205	.0866	.0322	.7590
	Tem * Var	.0067	.0301	.5165	.0059

Table 5.11 The characteristics of starch, amylose and amylopectin accumulation in cultivars Kavko (K) and Lyallpur (L) in grains taken from the plants with untrimmed ears raised at the standard level of nitrogen (N1TO), estimated by the ordinary logistic model.

	Temperature (°C)	Final weight (mg)		Maximum rate (mg/ day)		Duration (days)		Time to inflection point (days)	
		K	L	K	L	K	L	K	L
Starch	20	27.9	34.0	1.00	1.04	44.3	49.8	23.6	25.5
	30	17.9	17.2	1.17	0.82	26.0	28.5	14.6	12.4
Amylose	20	8.4	10.0	0.31	0.31	45.3	51.8	25.5	27.7
	30	5.5	5.1	0.39	0.27	26.4	27.7	15.7	13.7
Amylopectin	20	19.7	24.1	0.70	0.73	43.7	48.9	22.8	24.7
	30	12.4	12.0	0.79	0.55	25.7	28.7	14.1	11.7
LSD 5% for starch		2.7		0.23		3.8 ns		1.9	
LSD 5% for amylose		0.6		0.07		3.9 ns		2.2	
LSD 5% for amylopectin		2.3		0.17		5.2 ns		2.0	
LSD 5% for Temperature × Variety									

Table 5.12 Effects of growth at 30/25°C compared to 20/15°C on the characteristics of starch, amylose and amylopectin accumulation in the grains of cultivars Kavko (K) and Lyallpur (L), calculated from Table 5.11.

	Reduction in finalweight at 30°C (%)		Q_{10} for maximum rate (30°C /20°C)		Reduction in duration at 30°C (%)		Reduction in time to inflection point at 30°C (%)	
	K	L	K	L	K	L	K	L
Starch	36	49	1.17	0.79	41	43	38	51
Amylose	35	49	1.26	0.87	42	47	38	51
Amylopectin	37	50	1.13	0.75	41	41	38	53

Maximum rate of accumulation

There was a significant interaction between temperature and cultivar for the rate of grain starch, amylose, and amylopectin (Table 5.10). The rate of starch deposition was increased at high temperature in Kavko with a Q_{10} (30°C/20°C) equal to 1.17 (although the difference between the values of the rate in Kavko at 20/15°C and 30/25°C was not statistically significant; Table 5.12), whereas in Lyallpur the rate was reduced at high temperature (Q_{10} = 0.78). The accumulation of starch was more temperature sensitive than that of dry matter in Lyallpur as the Q_{10} for the maximum rate of starch accumulation was smaller than the corresponding value (0.89) for dry matter accumulation (N1TO treatment in Table 5.3). In contrast, the accumulation of starch in Kavko was more positively responsive to temperature than was dry matter; the Q_{10} for dry matter accumulation was equal to 1.08.

The responses of the rate of amylose and amylopectin accumulation to high temperature were similar to that of starch, but the extent of the changes were slightly different (Table 5.12). The depressing effect of temperature on the rate of amylose accumulation in

Lyallpur was less than that of starch accumulation, the values of Q_{10} for amylose in Kavko and Lyallpur being 1.26 and 0.87 respectively. Amylopectin deposition compared to amylose was less enhanced by the rise in temperature and the changes in the maximum rate of amylopectin synthesis were closer to those of starch than to amylose as might be expected since amylopectin is the major component of starch.

Duration of accumulation

Generally the duration of starch, amylose, and amylopectin deposition was significantly different for temperature and cultivar but the interaction was not significant (Table 5.10). The duration of the grain starch, amylose, and amylopectin accumulation was significantly longer in Lyallpur than in Kavko at 20/15°C (Table 5.11) but the differences between two cultivars at 30/25°C were not significantly different. [This was also evident for the duration of grain dry matter accumulation (Table 5.2)]. The period of starch accumulation and its components were significantly decreased at high temperature approximately to the same extent in each case (Table 5.12).

The time from anthesis to maximum rate (inflection point) was decreased at high temperature for grain starch, amylose, and amylopectin (Table 5.11) and the interaction between temperature and cultivar was significant for all components (Table 5.10). High temperature reduced the time to the inflection point to a greater extent in Lyallpur (52%) than in Kavko (38%, Table 5.12). The reductions in the time to the inflection point of starch components in Lyallpur at high temperature were greater than for their duration of accumulation.

5.3.5 Developmental changes in grain amylose %

The grain amylose percentage during grain development was calculated only in the untrimmed ears raised at the standard level of nitrogen and the results are illustrated in Fig 5.2. As shown, the grain amylose percentage increased during grain development towards maximum values at maturity. The rate of increase in grain amylose percentage was higher at 30/25°C than at 20/15°C. Within each temperature the rate was faster at early than at later stages of grain development. The pattern of the changes in amylose percentage over the grain filling period at 20/15°C was similar in the grains of both cultivars. At 30/25°C, however, the grain amylose percentage was significantly lower in Kavko than in Lyallpur at the early stages of grain development but the rate of increase in Kavko at this stage was faster than in Lyallpur so that the amylose percentage was the same in both cultivars by

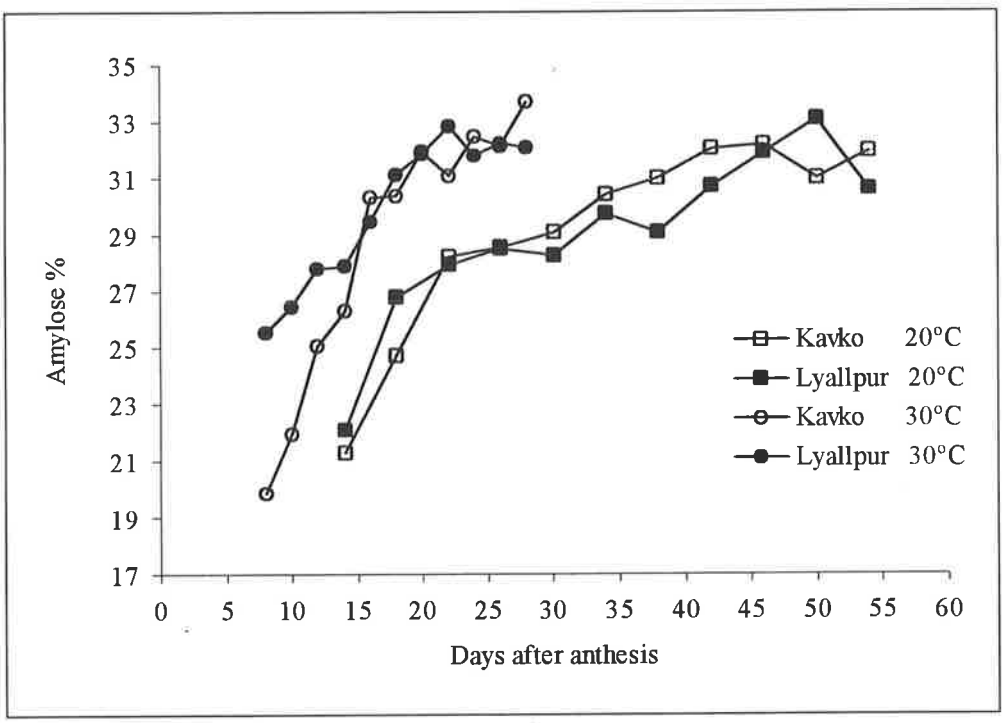

Fig 5.2 Developmental changes in amylose percentage in the grains taken from untrimmed ears of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to the standard level of nitrogen.

about day 16 after anthesis. The grain amylose percentage at maturity was not significantly different at the two temperatures in either cultivar.

5.3.6 Effect of nitrogen and trimming on the temperature response of starch accumulation

The amounts of grain starch, amylose, and amylopectin were measured for all treatments only for the samples taken on 50 and 26 days after anthesis at 20/15°C and 30/25°C respectively. The results of the analysis of variance and the measured values for grain starch and its components are shown in Table 5.13, 5.14, and 5.15.

5.3.6.1 Grain starch content

There were significant interactions between temperature and cultivar and the level of nitrogen (Table 5.13). High temperature significantly reduced the grain starch weight in all treatments (Table 5.14). The grain starch content was decreased at high temperature to a greater extent in Lyallpur (47%) than in Kavko (39%) at the standard level of nitrogen but there was no difference between the temperature response of the two cultivars at the low level of nitrogen (about 36% reduction for both cultivars; Table 5.15 and Fig. 5.4E). Averaged over two temperatures, the amount of starch was higher in the trimmed than in the untrimmed ears of Lyallpur and the magnitude of increase was greater at low (37%) than at the standard (12%) level of nitrogen. Trimming of ears did not affect the accumulation of starch in the grains of Kavko. The reductions in the grain starch weight at high temperature (Table 5.15) were in all cases greater than the corresponding values in the weight of grain dry matter (Table 5.3).

5.3.6.2 Grain amylose content

The amount of grain amylose was significantly decreased at high temperature to a greater extent in Lyallpur (48%) than in Kavko (36%) at the standard level of nitrogen but there was no difference between the temperature response of the two cultivars at the low level of nitrogen; a response similar to that of total starch (Table 5.15). However, the effect of nitrogen level on amylose accumulation was more evident than for total starch accumulation as the interaction between temperature and nitrogen was only significant for amylose (Table 5.13). Overall trimming the ears increased the accumulation of amylose in the grains only in Lyallpur (23%) but the effect was not significantly different at two levels of nitrogen.

The amylose% was increased at high temperature in Kavko but not in Lyallpur (Table 5.14 and Fig. 5.5F). The effect of nitrogen on the differential response of the two cultivars to temperature was not significant but the interaction between temperature and nitrogen was significant (Table 5.13). Overall, the grain amylose% was greater at 30/25°C compared to 20/15°C at the low level of nitrogen but no difference was observed between two temperatures at the standard level of nitrogen.

5.3.6.3 Grain amylopectin content

As grain amylopectin content accounts for more than two thirds of the total starch content, the responses in terms of amylopectin were almost identical to those of total starch (Table 5.13). Differences in the temperature and cultivar, and temperature and nitrogen interactions were reflected in effects on grain amylose percentage, which have already been referred to above.

Table 5.13 P values obtained from the analysis of variance table for the accumulation of starch components in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming.

Source	Grain starch weight	Grain amylose weight	Grain amylopectin weight	Grain amylose percentage
Tem	.0001	.0001	.0001	.0155
Var	.0182	.0004	.0345	.0147
N	.1343	.5402	.0792	.3145
Trimming	.0001	.0001	.0001	.1838
Tem * Var	.0304	.0018	.0901	.0343
Tem * N	.0745	.0040	.2111	.0221
Tem * Trimming	.2274	.6583	.4815	.0680
Var * N	.0005	.0002	.0002	.7570
Var * Trimming	.0001	.0001	.0001	.1078
N * Trimming	.1073	.1365	.6068	.2511
Tem * Var * N	.0361	.0182	.0511	.4840
Tem * Var * Trimming	.4628	.4493	.8678	.2348
Var * N * Trimming	.0224	.2071	.0901	.2861
Tem * N * Trimming	.4374	.0682	.1022	.6064
Tem * Var * N * Trimming	.9830	.5184	.3090	.9835

Table 5.14 Grain starch, amylose and amylopectin content in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C subjected to two levels of nitrogen and trimming, measured in grains taken on days 50 and 26 after anthesis at 20/15°C and 30/25°C respectively. (Treatments as specified in Table 5.2).

Treatment	Temprature (°C)	Grain starch weight (mg)		Grain amylose weight (mg)		Grain amylopectin weight (mg)		Grain amylose (%)	
		K	L	K	L	K	L	K	L
NOTO	20	29.5	24.4	8.02	7.20	21.5	17.2	27.3	29.6
	30	19.7	15.4	5.74	4.60	14.0	10.8	29.2	29.9
NOT1	20	29.7	33.4	8.33	9.34	21.4	24.1	28.0	28.0
	30	18.5	21.2	5.54	6.30	13.0	14.9	30.0	29.8
N1TO	20	26.3	30.8	7.57	9.59	18.7	21.3	28.9	31.1
	30	16.0	14.5	4.70	4.32	11.3	10.2	29.4	29.2
N1T1	20	26.5	34.2	7.39	9.95	19.1	24.3	27.9	29.1
	30	16.5	20.6	4.82	5.93	11.7	14.7	29.2	28.8
LSD 5%		3.3 ns		0.95 ns		2.2 ns		1.7 ns	

LSD 5% for Temperature × Variety × Nittogen × Trimming

Table 5.15 Effects of growth at 30/25°C compared to 20/15°C on starch and its components in cultivars Kavko (K) and Lyallpur (L) measured in grains taken on days 50 and 26 after anthesis at 20/15°C and 30/25°C respectively. (The ratios are calculated from Table 5.14; treatments as specified in Table 5.2).

Treatment	%reduction in starch weight at 30°C		%reduction in amylose weight at 30°C		% reduction in amylopectin weight at 30°C		Ratio of amylose % (30/ 20°C)	
	K	L	K	L	K	L	K	L
NOTO	33	37	28	36	35	37	1.07	1.01
NOT1	38	37	34	33	39	38	1.07	1.06
N1TO	39	53	38	55	40	52	1.02	0.94
N1T1	38	40	35	40	39	40	1.05	0.99
Mean	37	42	34	41	38	42	1.05	1.00

5.3.7 Grain soluble carbohydrates

In order to measure the amount of soluble carbohydrates, the grains were sampled at three sampling dates on days 22, 34, and 50 from the plants grown at 20/15°C and on days 12, 16, and 26 after anthesis at 30/25°C. As the amounts of grain soluble carbohydrates in general were not statistically different at the first two sampling dates, the data from samplings 1 and 2 were merged together and the average was considered as harvest A and the results of sampling 3 were considered as harvest B. The results for the analysis of variance and the measured amounts of grain soluble carbohydrates are respectively shown in Tables 5.16 and 5.17.

The amount of soluble carbohydrates per grain was significantly lower in the plants grown at 30/25°C compared to those grown at 20/15°C (Table 5.17) but there were no significant

Table 5.16 P values obtained from the analysis of variance table for the amounts of grain soluble carbohydrates and amino acids in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming.

Source	Grain soluble carbohydrates	Grain soluble amino acids	Source	Grain soluble carbohydrates	Grain soluble amino acids
Tem	.0001	.0075	Tem * Var * N	.5472	.8963
Var	.0012	.0001	Tem * Var * Trimming	.1189	.7786
N	.0368	.0001	Tem * Var * Harvest	.1456	.0004
Trimming	.5829	.0001	Var * N * Trimming	.9736	.3048
Harvest	.0001	.0001	Var * N * Harvest	.4471	.6072
Tem * Var	.1100	.9235	Var * Trimming * Harvest	.4792	.0373
Tem * N	.5560	.4512	Tem * N * Trimming	.1492	.0259
Tem * Trimming	.4874	.2187	Tem * N * Harvest	.3448	.4847
Tem * Harvest	.0368	.0001	Tem * Trimming * Harvest	.2761	.9126
Var * N	.1071	.3626	N * Trimming * Harvest	.9314	.1701
Var * Trimming	.4238	.3772	Tem * Var * N * Trimming	.9209	.1108
Var * Harvest	.0007	.0001	Tem * Var * N * Harvest	.4162	.0177
N * Trimming	.1252	.4151	Tem * N * Trimming * Harvest	.3123	.4720
N * Harvest	.0026	.1108	Var * N * Trimming * Harvest	.1896	.3113
Trimming * Harvest	.8790	.0005	Tem * Var * N * Trimming *Harvest	.0511	.0149

Table 5.17 Grain soluble carbohydrates (mg/ grain) in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming. (Treatments as specified in Table 5.2).

Treatment	Tem. (°C)	Harvest A		Ratio (30/20°C)		Harvest B		Ratio (30/20°C)	
		K	L	K	L	K	L	K	L
NOTO	20	1.39	1.26	0.66	0.74	1.26	1.56	0.79	0.58
	30	0.92	0.93			1.00	0.90		
NOT1	20	1.42	1.44	0.65	0.64	1.47	1.53	0.59	0.67
	30	0.93	0.92			0.87	1.02		
N1TO	20	1.28	1.40	0.75	0.66	1.54	1.78	0.66	0.58
	30	0.96	0.92			0.99	1.04		
N1T1	20	1.36	1.34	0.65	0.66	1.40	1.73	0.66	0.68
	30	0.89	0.88			0.93	1.18		

LSD 5% Temperature × Variety × Nittogen × Trimming × Harvest is 0.20.

Table 5.18 P values obtained from the analysis of variance table for the concentration of grain soluble carbohydrates and grain soluble amino in untrimmed plants of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and at the standard levels of nitrogen.

Source	Grain soluble carbohydrates	Grain soluble amino acids
Tem	.6081	.3548
Var	.0221	.0034
Harvest	.0283	.0008
Tem * Var	.6565	.9395
Tem * Harvest	.0650	.0127
Var * Harvest	.6307	.0109
Tem * Var * Harvest	.2052	.0376

interactions between temperatures and cultivars (Table 5.16). Neither nitrogen level nor trimming of the ears significantly affected the response to temperature. The amount of soluble carbohydrates was overall greater in the grains sampled at harvest B compared to those sampled at harvest A at both temperatures. The difference between the two harvests was greater at 20/15°C than at 30/25°C so that the Q_{10} (30/20°C) was slightly decreased at harvest B compared to that of harvest A.

As the grain size was significantly smaller at high temperature, the differences between two temperatures in terms of the amount of soluble carbohydrates per grain may be influenced by changes in the size of the grain. An estimation of the concentration of soluble carbohydrates in the grain water was possible but only in the untrimmed ears raised at the standard level of nitrogen. The results of analysis variance and the values for the measured concentration of soluble carbohydrates are shown in Tables 5.18 and 5.19. The main effects of cultivar and harvest were significant (Table 5.18). The concentration of soluble carbohydrates was greater in Lyallpur than in Kavko and also it was greater at harvest B than harvest A especially in Lyallpur at high temperature but it was not significantly different at the two temperatures (Table 5.19).

5.3.8 Grain soluble amino acids

The samples were taken at three sampling dates as described for soluble carbohydrates. The results from the first sampling date were considered as harvest X. As the amounts of grain soluble amino acids in general were not statistically different at the last two sampling dates, an average was taken from the two sampling dates and the result were considered as harvest Y. The results of the analysis of variance and the measured amounts of grain soluble amino acids are respectively shown in Tables 5.16 and 5.20.

Table 5.19 The concentration of grain soluble carbohydrates (mg/ mL of grain water) in the plants with untrimmed ears raised at standard level of nitrogen (N1TO treatment).

Cultivar	Tem. (°C)	Harvest A	Ratio (30/20°C)	Harvest B	Ratio (30/20°C)
Kavko	20	45.2	0.96	49.0	1.04
	30	43.5		51.1	
Lyallpur	20	53.9	0.87	52.6	1.25
	30	46.9		65.5	

LSD 5% for Temperature× Variety × Harvest is 12.6.

The amount of soluble amino acids per grain was significantly lower at 30/25°C compared to 20/15°C in both cultivars at harvest X (Table 5.20). The extent of reductions at high temperature was greater in Lyallpur (35%) than in Kavko (14%). The depressing effect of temperature in Lyallpur was greater at the standard (40%) than at the low (28%) level of nitrogen but there was no significant effect of nitrogen on the temperature response of Kavko. At 20/15°C, the amount of soluble amino acids in the grains was significantly lower at harvest X than at harvest Y. At 30/25°C, this was evident only in the grains taken from the trimmed ears of Kavko but in other cases there were no significant differences between two harvests. At harvest Y, the amount of soluble amino acids in the grains was higher at 30/25°C compared to 20/15°C and the difference between the two temperatures was more evident in Lyallpur than in Kavko. Overall, the decreases at high temperature were greater in the untrimmed than in the trimmed ears at the standard level of nitrogen but there appeared no effect of trimming at the low level of nitrogen.

The results of analysis variance and the values for the concentration of soluble amino acids

Table5.20 Grain soluble amino acids (μ mol/ grain) in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming. (Treatments as specified in Table 5.2).

Treatment	Tem. (°C)	Harvest X		Ratio (30/20°C)		Harvest Y		Ratio (30/20°C)	
		K	L	K	L	K	L	K	L
NOTO	20	1.82	1.52	0.80	0.77	0.72	1.00	1.57	1.11
	30	1.45	1.19			1.13	1.11		
NOT1	20	2.33	1.93	0.85	0.67	1.16	0.97	0.98	1.48
	30	1.98	1.29			1.14	1.44		
N1TO	20	2.28	1.93	0.69	0.58	1.60	1.27	0.93	1.28
	30	1.58	1.12			1.48	1.62		
N1T1	20	2.44	2.26	1.06	0.63	1.36	1.24	1.02	1.55
	30	2.58	1.41			1.39	1.92		

LSD 5% for Temperature × Variety × Nittogen × Trimming × Harvest is 0.37.

Table 5.21 The concentration of grain soluble amino acids (mg/ mL grain water) in the plants with untrimmed ears raised at standard level of nitrogen (N1TO treatment).

Cultivar	Tem. (°C)	Harvest X	Ratio (30/20°C)	Harvest Y	Ratio (30/20°C)
Kavko	20	79.8	1.01	58.4	1.07
	30	80.9		62.5	
Lyallpur	20	68.1	0.83	50.0	1.36
	30	56.4		67.8	

LSD 5% for Temperature× Variety × harvest is 12.4.

in the grain water estimated for the grains of untrimmed ears raised at the standard level of nitrogen are shown in Tables 5.18 and 5.21. The interaction between temperature, cultivar and harvest was significant but the main effect of temperature and the interaction between temperature and cultivar were not significant (Table 5.18). High temperature did not significantly affect the concentration of grain soluble amino acids in Kavko at either harvest. In Lyallpur the concentration was significantly increased at 30/25°C compared to 20/15°C at harvest Y only.

5.3.9 Effect of high temperature on grain protein composition

Due to their important role in protein quality the effects of temperature on the high molecular weight (HMW) glutenins were investigated and are reported in this section. Size exclusion HPLC analysis of protein factions was done for the grains sampled at days 50 and 26 after anthesis at 20/15°C and 30/25°C respectively. The results of the analysis of variance and the measured values of protein fractions are shown in Tables 5.22, 5.23, and 5.24.

5.3.9.1 Total HPLC protein per grain

The amount of total protein per grain overall was not significantly different at 30/25°C compared to 20/15°C (Table 5.23). The difference between the temperature response of the two cultivars overall was not quite big enough to be significant (Table 5.22; Fig.5.6C). The response to temperature was different at the two levels of nitrogen as the interaction between temperature and nitrogen was significant. At the standard level of nitrogen the protein weight was overall about 14% lower in the grains raised at 30/25°C compared to those developed at 20/15°C but there was no significant difference between the two

temperatures at the low level of nitrogen. Trimming the ears did not significantly affect the response to temperature.

5.3.9.2 HMW-glutenins per grain

The main effects of temperature, nitrogen, and trimming as well as the interaction between cultivar and trimming were significant (Table 5.22). The amount of HMW-glutenins per grain was significantly increased (overall 20%) at 30/25°C compared to 20/15°C (Table 5.23). The magnitude of the increases at high temperature was higher in Kavko (27%) than in Lyallpur (12%; Fig. 5.6E) but the interaction between temperature and cultivar was not significant (Table 5.22). Despite the apparently greater positive effect of high temperature at the low compared to the standard level of nitrogen, the interaction did not quite achieve significance at the 5% level. The values of Q_{10} (30°C/20°C) were in all cases greater for the amount of HMW-glutenins per grain than for total protein per grain. The interaction between cultivar and trimming was significant but this interaction was not significant for total protein per grain measured by HPLC. The amount of HMW-glutenins per grain was overall about 33% higher in the trimmed than in the untrimmed ears of Lyallpur but the effect of trimming was not significant in Kavko. Trimming had no significant effect on the temperature response of the cultivars.

5.3.9.3 HMW-glutenin %

As a percentage of total protein measured by HPLC, the level of the HMW-glutenins fraction was increased at high temperature (Table 5.23). The increases at 30/25°C compared to 20/15°C were overall similar in Kavko (27%) and Lyallpur (31%). In the untrimmed ears the magnitude of the increases in HMW-glutenins percentage at high temperature were greater at the standard (34%) than at the low (21%) level of nitrogen. In

Table 5.22 P values obtained from the analysis of variance table for the HPLC analysis of protein composition in cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming.

Source	Total protein per grain	HMW glutenins per grain	HMW glutenins %	Concentration of total protein (area/ mg)*	Concentration of HMW glutenins (area/ mg)*	HMW glutenins% (area/ mg)*
Tem	.1402	.0018	.0001	.0001	.0001	.0001
Var	.0001	.3365	.0001	.0001	.0024	.8787
N	.0001	.0001	.0001	.0001	.0001	.0001
Trimming	.0001	.0008	.9047	.0434	.3395	.3493
Tem * Var	.0506	.2630	.1269	.0227	.1208	.7338
Tem * N	.0024	.0521	.8961	.2955	.5736	.9957
Tem * Trimming	.8827	.8141	.8618	.2808	.4824	.5798
Var * N	.2861	.7664	.2520	.0092	.0221	.0379
Var * Trimming	.1050	.0430	.2184	.6062	.8392	.6763
N * Trimming	.0701	.2380	.4099	.2844	.2925	.2928
Tem * Var * N	.4311	.6650	.8024	.2461	.9331	.6094
Tem * Var * Trimming	.4427	.6740	.9740	.7635	.8089	.7683
Var * N * Trimming	.9428	.5286	.1647	.9250	.5329	.4387
Tem * N * Trimming	.2126	.4663	.0106	.2736	.0344	.0140
Tem * Var * N *Trimming	.5798	.9015	.6955	.1342	.3683	.4262

*arbitrary units: area under chromatogram peaks per mg of flour

the trimmed ears the situation was reversed; the increases were greater at the low (42%) than at the standard (19%) level of nitrogen. The percentage of HMW-glutenins per grain was overall higher in Lyallpur than in Kavko.

5.3.9.4 Concentration of total HPLC protein in the flour

The concentration of total protein in the flour was significantly higher in the grains developed at 30/25°C compared to those raised at 20/15°C (Table 5.24). The extent of the increases at high temperature was overall greater in Kavko (41%) than in Lyallpur (32%) as the interaction between temperature and cultivar was significant (Table 5.22 and Fig. 5.6D). The concentration of total protein was decreased at low compared to the standard level of nitrogen to a greater extent in Kavko (20%) than in Lyallpur (10%). However, the level of nitrogen had no significant effect on the temperature response of the cultivars. The concentration of protein was significantly higher in Kavko than in Lyallpur and the difference was overall greater at the standard than at the low level of nitrogen. Trimming of ears had overall a positive effect on the concentration of protein in the flour but trimming did not significantly influence the response to temperature.

5.3.9.5 Concentration of HMW-glutenins in the flour

The concentration of HMW-glutenins in the flour was overall 2.3 times higher in the grains developed at 30/25°C than those raised at 20/15°C (Table 5.24). The extent of the increases at high temperature was similar for both cultivars (Fig. 5.6F) as the interaction between temperature and cultivar was not significant. The concentration of HMW-glutenins in the flour was overall 30% greater in Kavko compared to Lyallpur but the difference was only significant at the standard level of nitrogen. There were significant interactions between temperature, level of nitrogen, and trimming. Regardless of cultivar,

Table 5.23 HPLC analysis; amounts of total protein and high molecular weight glutenins in the grains of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming. (Treatments as specified in Table 5.2).

Treatment	Temperature (°C)	Total protein (mg/grain)		Ratio (30/ 20°C)		HMWglutenin (mg/grain)		Ratio (30/ 20°C)		HMWglutenin %		Ratio (30/ 20°C)	
		K	L	K	L	K	L	K	L	K	L	K	L
NOTO	20	3.63	2.63	1.18	1.13	0.60	0.52	1.40	1.39	16.7	19.5	1.17	1.25
	30	4.27	2.96			0.84	0.72			19.5	24.3		
NOT1	20	4.69	4.29	1.06	0.96	0.64	0.86	1.58	1.31	13.7	20.0	1.48	1.38
	30	4.96	4.11			1.01	1.13			20.3	27.6		
N1TO	20	5.47	4.90	0.88	0.77	1.00	1.04	1.14	1.04	18.1	21.1	1.31	1.36
	30	4.79	3.76			1.14	1.08			23.8	28.7		
N1T1	20	5.40	5.74	1.02	0.79	1.04	1.28	1.14	0.95	19.2	22.0	1.13	1.22
	30	5.49	4.54			1.19	1.22			21.7	26.9		
LSD 5%		0.89 ns				0.28 ns				3.3 ns			

Amounts of protein were estimated from the size exclusion chromatographic data and areas converted to amounts of protein from the spear standards (see Materials and Methods).
LSD 5% for Temperature × Variety × Nittogen × Trimming

Table: 5.24 Estimates of protein composition of the flour milled from grains taken from the plants of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C and subjected to two levels of nitrogen and trimming. (Treatments as specified in Table 5.2).

Cultivar	Temperature (°C)	Total protein (area/ mg)		Ratio (30 /20°C)		HMWglutenin (area/ mg)		Ratio (30 /20°C)		HMWglutenin% (area/ mg)	
		K	L	K	L	K	L	K	L	K	L
NOTO	20	156	157	1.68	1.27	41	51	3.37	1.92	26	32
	30	262	200			138	98			51	49
NOT1	20	188	163	1.45	1.39	48	53	3.15	2.77	26	33
	30	273	227			151	147			55	63
N1TO	20	231	169	1.32	1.44	99	61	2.30	2.85	42	36
	30	306	244			228	174			73	70
N1T1	20	246	192	1.26	1.18	122	88	1.73	1.57	48	43
	30	310	226			211	138			68	61
LSD 5%		34 ns				51 ns				13 ns	

Data were obtained by dividing the area of the size exclusion chromatograph by the weight of flour injected.
LSD 5% for Temperature × Variety × Nittogen × Trimming

the magnitude of the increases in HMW-glutenins in the flour at high temperature in the trimmed ears were greater at the low (2.9 times) compared to the standard (1.7 times) level of nitrogen but in the untrimmed ears the increases were similar at both (about 2.5 times) level of nitrogen.

Differential effects of the experimental variates on the total protein concentration and the glutenin concentration are reflected in variation in the ratio of these two attributes (Table 5.24) where the effects of the temperature are especially prominent. The relative concentration of HMW-glutenins in the flour (the ratio) was significantly increased at high temperature in both cultivars but the interaction between temperature and cultivar was not significant. Significantly more (72%) of the total protein was composed of HMW-glutenins at the standard compared to the low level of nitrogen (50%) but this effect of nitrogen level was significant only at 30/25°C and in the untrimmed ears.

5.4 Discussion

The aim of this experiment was to investigate whether or not factors that influence the nutritional supply to the grain modify the temperature response and in particular alter the differential response of the two selected cultivars. Wardlaw *et al.* (1989a) gave evidence that carbohydrate nutrition could change the response to temperature in wheat cultivars. They compared the temperature responses of cultivars Banks and Kalyansona at different levels of light and concluded that there was an interaction between light level and the temperature response of the cultivars. This investigation used a different approach i.e. altering the nutritional supply to the grain by a) reducing the amount of nitrogen supplied to the plant and b) reducing the number of grains on the ear for the same supply of carbohydrates and nitrogen to that ear.

5.4.1 Grain nutrition status

The nitrogen concentration in wheat grains can be modified by different manipulations such as N fertilizer application or ear trimming. Removal of some grains from the ear increases the supply of amino acid and the accumulation of nitrogen in the remaining grains in the ear (Jenner 1980; Perez *et al.* 1989; Ma *et al.* 1990). The accumulation of soluble sugars in leaves increases as a result of trimming the ears (Blum *et al.* 1988). However, there was no significant increase in the concentration of soluble sugars in the remaining grains of trimmed ears (Jenner 1980). In the present study there appeared a significant effect of nitrogen level and trimming on soluble amino acids concentration and the effect was dependent on temperature, cultivar, and the stage of grain development (Tables 5.20 and 5.21) but trimming had no significant effect on the level of soluble carbohydrates (Table 5.20). Although the amount of grain soluble carbohydrates was overall lower at low nitrogen, the amount of nitrogen supplied to the plants did not change the concentration of soluble carbohydrates at the time when the rate of grain filling was maximum (at harvest 1; Fig. 5.3A). Moreover, cultivar and temperature did not influence the effect of nitrogen on soluble carbohydrates (Table 5.20). These results may therefore indicate that effects of the nitrogen level or trimming on the response of grain development to temperature do not seem to be mediated through changes in the availability of photoassimilate in the grains.

The amount of soluble carbohydrates per grain was overall less at 30/25°C than at 20/15°C (Fig. 5.3B) whereas the concentration of soluble carbohydrates in the grain water was not significantly different at the two temperatures (Fig. 5.3C). The reduction in the amount of soluble carbohydrates per grain at high temperature was therefore due to the reduction in grain size. In other words, the diminished weight of grain at high temperature does not

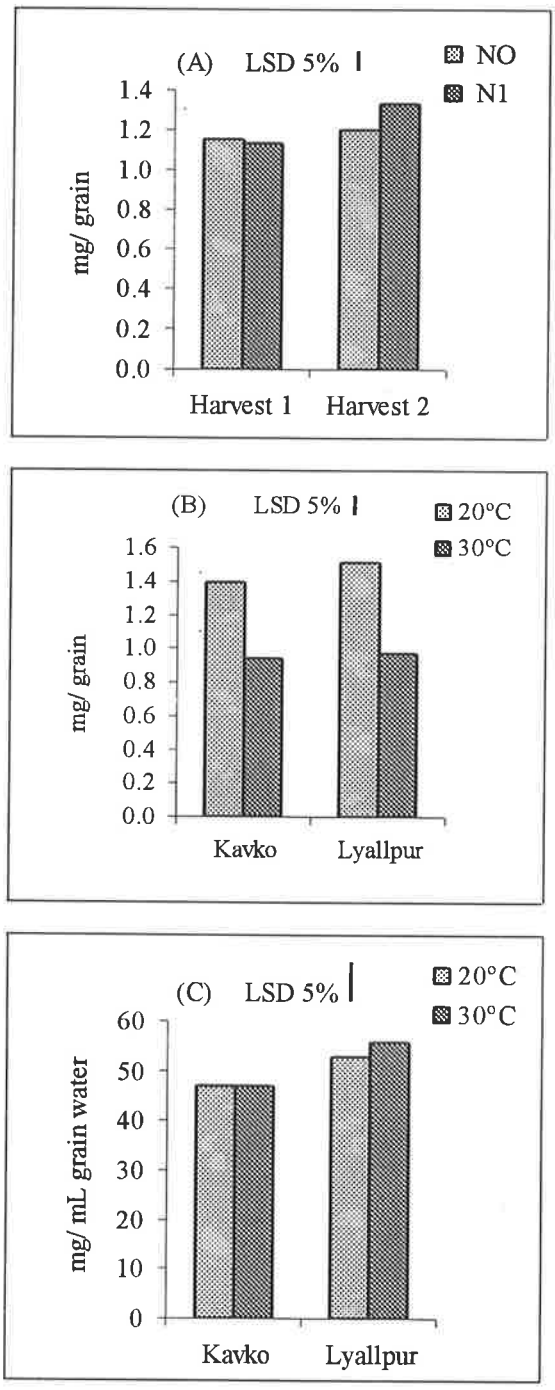

Fig. 5.3 The amount of soluble carbohydrates per grain (A and B) and per mL grain water (C) in the plants of Kavko and Lyallpur grown at 20/15°C and 30/25°C at low (NO) and standard (N1) level of nitrogen sampled at two harvests.

seem to be a result of the depression of the availability of photoassimilate within the grain. On the contrary, the concentration of soluble carbohydrates was increased in the grains of Lyallpur at high temperature at the later stage of grain development (at Harvest 2; Table 5.17). This may imply that the rate of the conversion of soluble carbohydrates to starch in the grains of Lyallpur at high temperature might be slower than the rate of the delivery of soluble carbohydrates to the grain. However, as sucrose is the main precursor for starch synthesis the response to temperature can not be reliably explained by the changes in the level of total soluble carbohydrates. The contribution of sucrose itself to the temperature response of grain development and starch synthesis will be examined in Chapter 6.

5.4.2 Effect of nitrogen on the response to temperature

The variation in nitrogen nutrition affected grain development and in particular the differential response of the two cultivars to temperature. Only at the standard level of nitrogen did high temperature reduce final grain weight more in Lyallpur (40%) than in Kavko (24%); the response of the two cultivars was the same at the low level of nitrogen (Fig 5.4A). In order to understand the effect of nitrogen on the response to temperature two questions will be reviewed here: (a) which attribute of grain filling, rate or duration, has been affected (b) which component of dry matter, starch or protein, responds in the same way as dry matter does.

The rate of grain filling was increased at high temperature to a greater extent in Kavko than in Lyallpur (Fig 5.4B), and the duration of grain filling in Lyallpur was most depressed by temperature (Fig 5.4C). Although the differences between the two cultivars in response to temperature for both attributes were greater at the standard than at the low level of nitrogen, the effect of nitrogen was not big enough to be significant (Table 5.2).

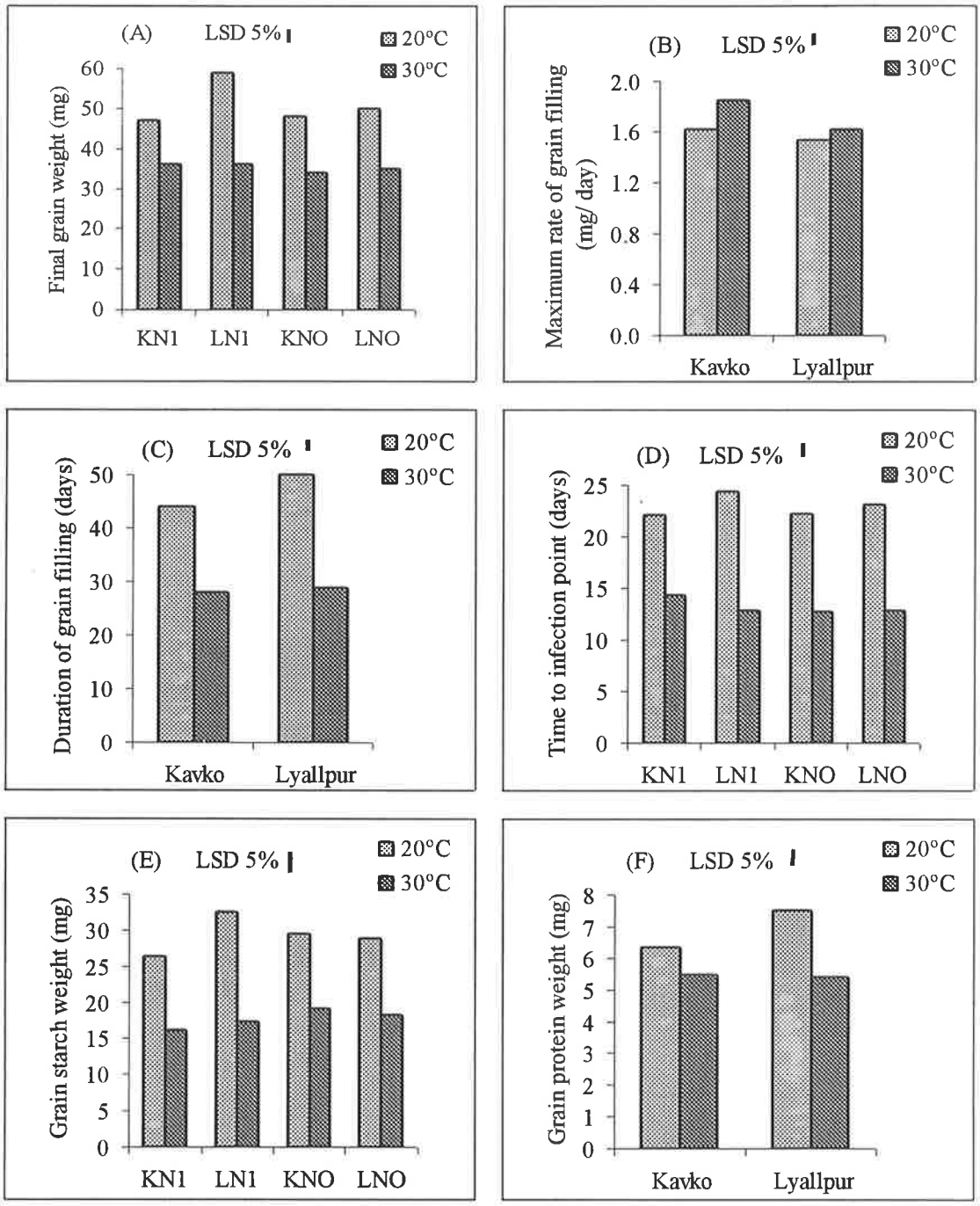

Fig. 5.4 Effects of temperature on (A) final grain weight, (B) maximum rate of grain filling, (C) duration of grain filling, (D) time to inflection point, (E) grain starch weight, and (F) grain protein weight in the plants of cultivars Kavko (K) and Lyallpur (L) grown at 20/15°C and 30/25°C at low (NO) and standard (N1) level of nitrogen.

Therefore, according to these results the effect of nitrogen on the temperature response of grain weight can not satisfactorily be explained on the basis of the rate or duration of grain filling alone. There appeared to be some contribution also from the time to the inflection point. The time to the inflection point was decreased at high temperature to a greater extent in Lyallpur than in Kavko but only at the standard level of nitrogen (Fig 5.4D). So the effects of temperature on final grain weight then are partly bound up with the effects of nitrogen on the temperature response of duration, but also partly with effects on rate, and in particular on the time to the inflection point. The greater reduction in the time to the inflection point indicates that the rate of grain filling at high temperature starts to slow down earlier in Lyallpur than in Kavko. Therefore, the rate of grain filling at high temperature is affected in two ways a) the instantaneous maximum rate of grain filling is relatively slower in Lyallpur than in Kavko at the standard level of nitrogen, and b) the rate starts to slow down at high temperature sooner in Lyallpur than in Kavko.

The temperature response of starch accumulation to nitrogen level was similar to that of final grain weight. In other words, only at the standard level of nitrogen was the reduction in starch accumulation at high temperature significantly greater in Lyallpur than in Kavko (Fig. 5.4E). Although the interaction between cultivar and nitrogen level was significant for both grain amylose and amylopectin (Table 5.14), the effect of nitrogen level on the differential responses of the two cultivars to temperature was significant only for amylose (Fig 5.5B) but not for amylopectin (Fig 5.5C). These results indicate that the effect of nitrogen on the temperature response of starch accumulation resulted mainly from the response of amylose accumulation to nitrogen. Differing temperature responses for the two cultivars were also observed for the rate of starch accumulation where the instantaneous rate at high temperature was increased in Kavko while it was decreased in

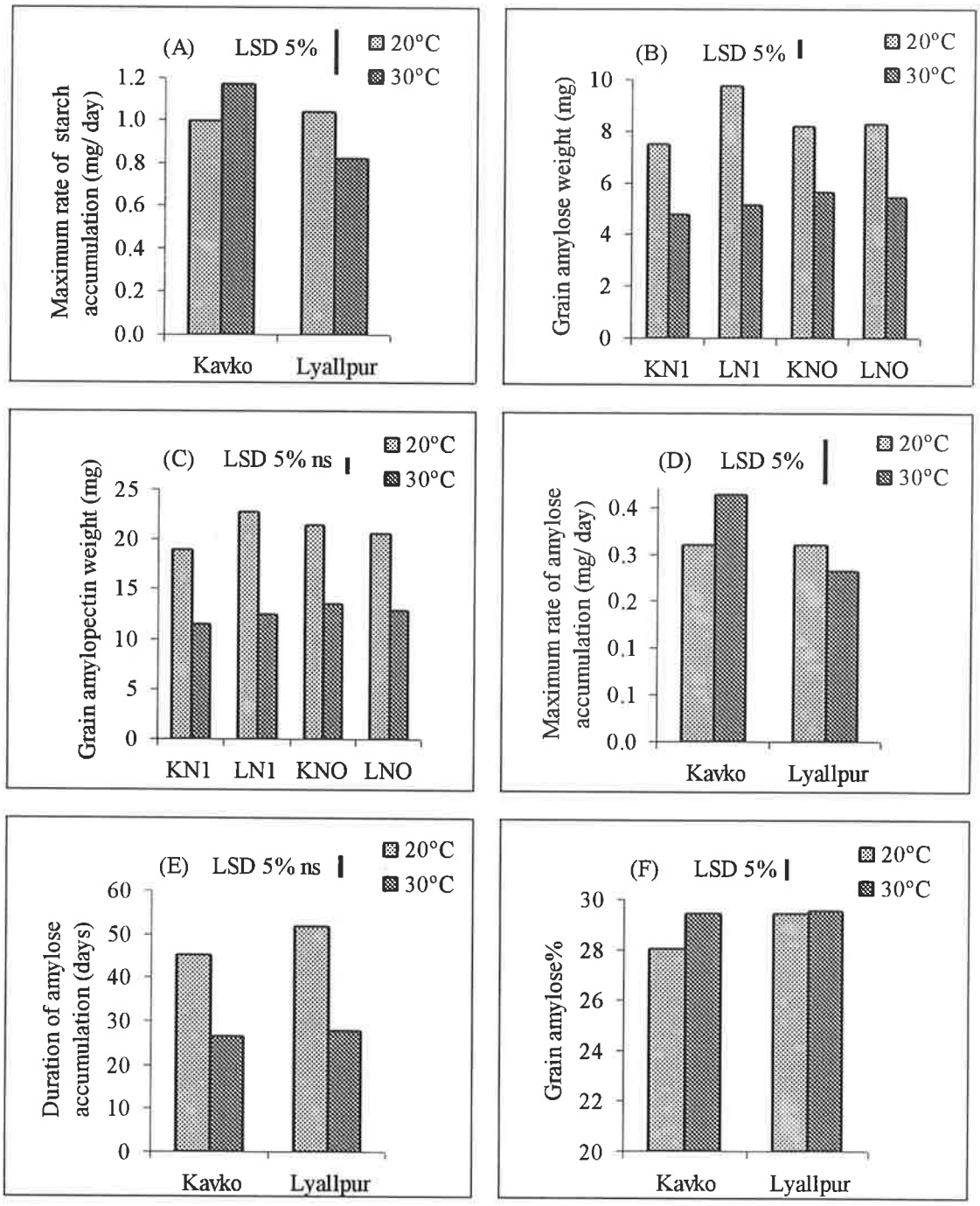

Fig. 5.5 Effects of temperature on (A) rate of starch accumulation, (B) grain amylose weight, (C) grain amylopectin weight, (D) rate of amylose accumulation, (E) duration of amylose accumulation, and (F) grain amylose% in the plants of cultivars Kavko (K) and Lyallpur (L) grown at 20/15°C and 30/25°C at low (NO) and standard (N1) level of nitrogen.

Lyallpur (Fig. 5.5A), and the same effect was observed for amylose synthesis (Fig. 5.5D). Overall the rate of amylose synthesis appeared to be the most temperature responsive as the rate of amylose accumulation was increased at high temperature to a greater extent compared to that of amylopectin accumulation (Table 5.12).

The reduction in grain protein weight at high temperature was overall greater in Lyallpur than in Kavko (Fig. 5.4F). There was a significant interaction between temperature and nitrogen level but the effect of nitrogen on the differential response of the two cultivars was not significant (Table 5.5). Thus, the temperature response of the two cultivars to nitrogen in respect of grain weight can not be explained by the response of final grain protein weight. However, the temperature response of the rate of grain protein accumulation to nitrogen level was significantly different in the two cultivars. The value of Q_{10} (30°C/20°C) for the rate of grain protein accumulation was significantly lower in Lyallpur (1.06) than in Kavko (1.35) at the standard level of nitrogen while at low nitrogen the value of Q_{10} was quite similar for both cultivars (Fig. 5.6A). The reductions in grain protein weight at high temperature were due to significant effects on reduced duration (Fig.5.6B) which was not compensated for by the increased rate of protein accumulation.

There appeared some differences in the response of the two cultivars in terms of the grain protein measured by the Bio-Rad method (Fig. 5.4F) and the grain protein measured by HPLC (Fig. 5.6C) where the interaction between temperature and cultivar was only significant for the grain protein measured by Bio-Rad. As the measurement of protein by the Bio-Rad method is based on a reaction between the dye and certain amino acids this method is sensitive to any changes in protein composition. Therefore, the differences in the responses of the two cultivars between the two different protein measurements might

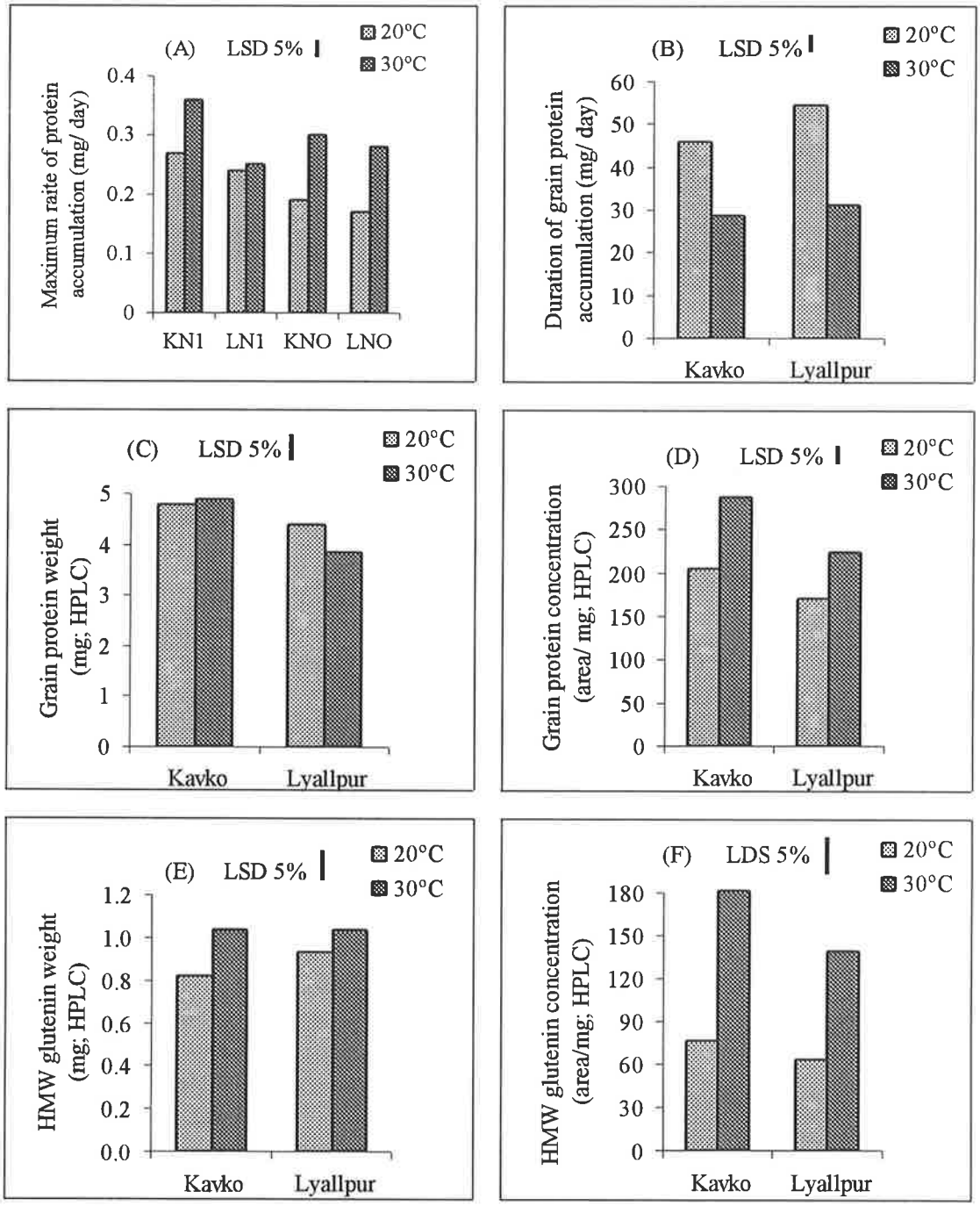

Fig. 5.6 Effects of temperature on (A) rate of protein accumulation, (B) duration of protein accumulation, (C) grain total protein weight, (D) grain protein concentration, (E) HMW-glutenin weight, and (F) HMW-glutenin concentration in the plants of cultivars Kavko (K) and Lyallpur (L) grown at 20/15°C and 30/25°C at low (NO) and standard (N1) level of nitrogen.

have been created by differences in their protein composition.

The concentration of HMW-glutenin in the flour was increased at 30/25°C compared to 20/15°C to a greater extent (2.3 times) than that of the total protein (1.4 times), indicating that the polymer: monomer ratio was increased at high temperature. In a study by Stone *et al.* (1996), there was also an increase in polymer: monomer ratio in mature grains developed at moderately high temperature (27/22°C) imposed from day 20 after anthesis until maturity. At 30/25°C, however, the ratio rapidly increased after day 35 but it was not sustained and at maturity the ratio was less compared with the control at 21/16°C. The decreases in the ratio at high temperatures are related to a relative increase in monomer (Blumenthal *et al.* 1990) or a decrease in polymer (Stone *et al.* 1996). The temperature response of total protein concentration was different in the two cultivars (Fig. 5.6C) but the varietal differences for HMW-glutenin concentration were not significant (Fig. 5.6E). The difference between the two cultivars in response to high temperature in terms of total protein accumulation was therefore associated with the response of the monomer fraction.

There was a higher HMW-glutenin concentration at the standard level compared to low nitrogen: 76% higher in Kavko and 32% in Lyallpur. The corresponding increases for total protein concentration were smaller: 25% in Kavko and 11% in Lyallpur indicating that the contribution of HMW-glutenin in the flour was increased with an increase in protein concentration. The change in protein composition in response to high N application has been attributed to a simultaneous increase in both gliadins and glutenin but to greater extent in gliadins (Dubetz *et al.* 1979), an increase in gliadins only (Doekes and Wennekes 1982), or an increase in gliadins together with a decrease in glutenin (Stenram *et al.* 1990). In these reports the contribution of polymer has been decreased under higher nitrogen

supply which is not in accordance with the present results. The dissimilarity between reports may be due to the differences between the fractionation methods used and/ or the different quantities of protein found in the grains developed under different growth conditions. The increase in the concentration of HMW-glutenin at standard compared to low nitrogen tended to be greater at 20/15°C than at 30/25°C but the interaction between temperature and nitrogen was not statistically significant (Table 5.24).

5.4.3 Effect of trimming on grain filling

The effect of sink manipulation on grain weight varies depending on the variety (Ma *et al.* 1990) and growth condition (Martinez-Carrasco and Thorne 1979). In the present study grain weight was increased by 20% in trimmed compared to untrimmed ears in Lyallpur but not in Kavko (Fig. 5.7A). Similar responses were observed for the grain starch (Fig. 5.7B) and grain protein weight (Fig. 5.7C). The effect of trimming on grain weight was associated with the response of the rate of grain filling as the extent of the increase in the rate in the trimmed ears was greater in Lyallpur than in Kavko (Fig. 5.7D). In addition, the time to the inflection point in Lyallpur was longest in the trimmed ears (Fig. 5.7E) meaning that the rate of grain filling in this cultivar continues for a longer period in the trimmed ears before it begins to decline.

The effect of trimming on grain growth varied with temperature and the level of supplied nitrogen. The rate of grain filling was increased by trimming by 30% at 30/25°C but only by 10% at 20/15°C (Fig. 5.8A) and the rate of protein accumulation responded similarly (Fig. 5.8B). Also the increase in grain weight in the trimmed ears was greater at the low (20%) than at the standard (6%) level of nitrogen (Fig. 5.9A). The corresponding increases in grain protein weight were 34% and 10% respectively for the low and standard nitrogen

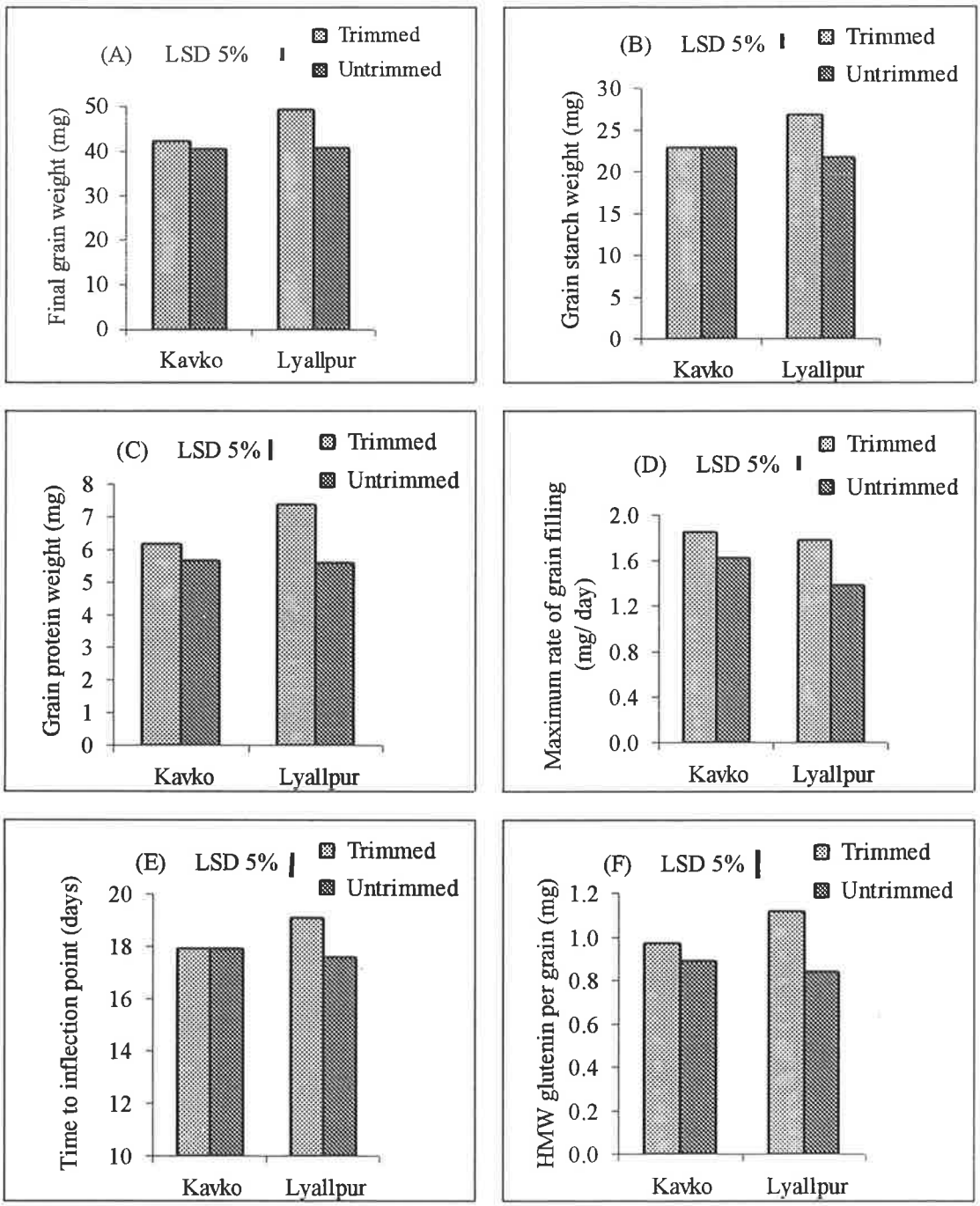

Fig. 5.7 Effects of trimming of ears on (A) final grain weight (B) grain starch weight, (C) grain pertain weight, (D) maximum rate of grain filling, (E) time to inflection point, and (F) HMW-glutenin per grain in trimmed and untrimmed ears of the plants of cultivars Kavko and Lyallpur.

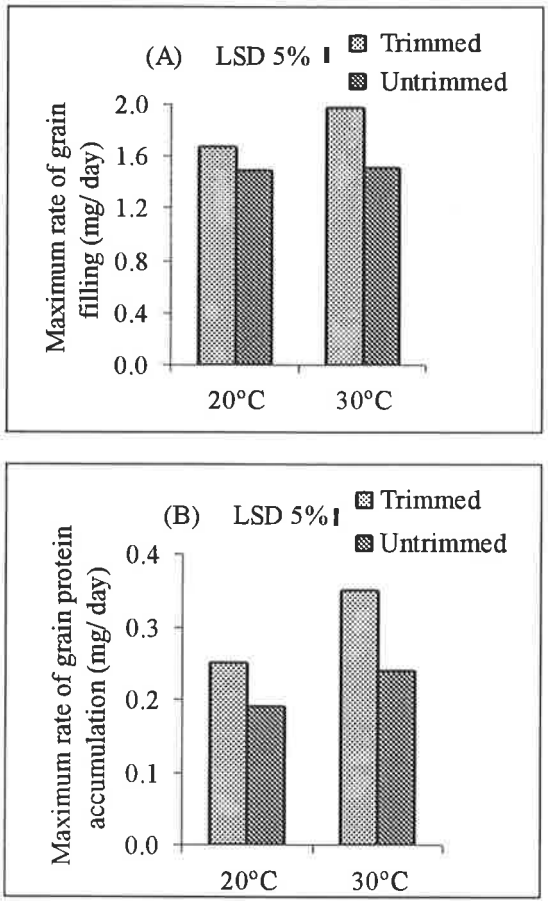

Fig. 5.8 Effects of the interaction between trimming of ears and temperature on (A) maximum rate of grain filling and (B) maximum rate of grain protein accumulation in trimmed and untrimmed ears of plants (both cultivars combined) grown at 20/15°C and 30/25°C.

(Fig. 5.9B). However, there was no significant interaction between the nitrogen and trimming for grain starch weight (Table 5.14) indicating that the effect of nitrogen level on the response to trimming was related to changes in grain protein weight. Martinez-Carrasco *et al.* (1993) reported that the partial removal of spikelets increased photosynthesis under low N supply in two cultivars while at high N supply trimming inhibited photosynthesis in a cultivar that was unresponsive at low N level. These results therefore show that the effect of trimming on grain filling or photosynthesis tends to be

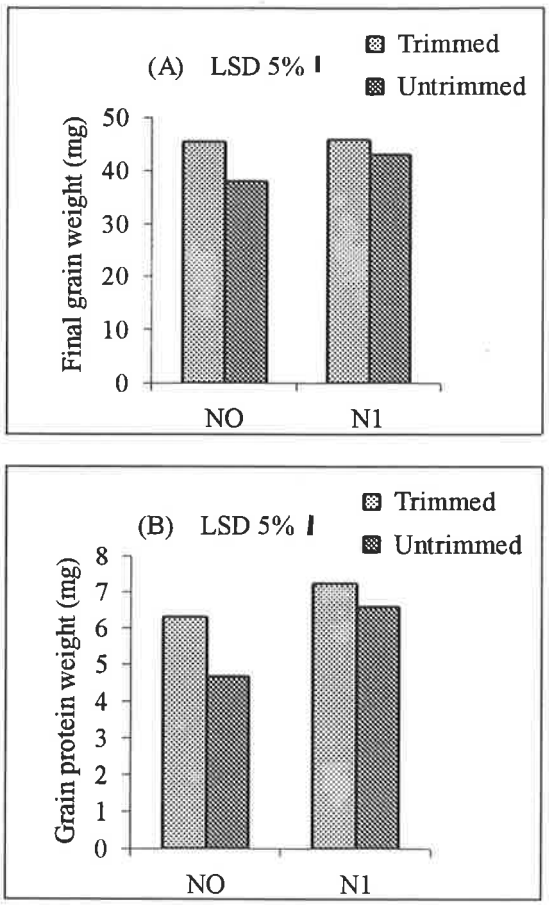

Fig. 5.9 Effects of the interaction between trimming of ears and the level of nitrogen on (A) final grain weight and (B) grain protein weight in the trimmed and untrimmed ears of the plants of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C at low (NO) and standard (N1) level of nitrogen.

smaller under conditions more favorable for grain growth.

In both cultivars alike, only at the standard level of nitrogen was the reduction in grain weight at high temperature smaller in the trimmed than in the untrimmed ears (Fig. 5.10A). This response was more evident for grain protein accumulation (Fig. 5.10B). However, trimming did not change the temperature response of starch accumulation (Table 5.14). Under an adequate supply of nitrogen that included post anthesis nitrogen application the

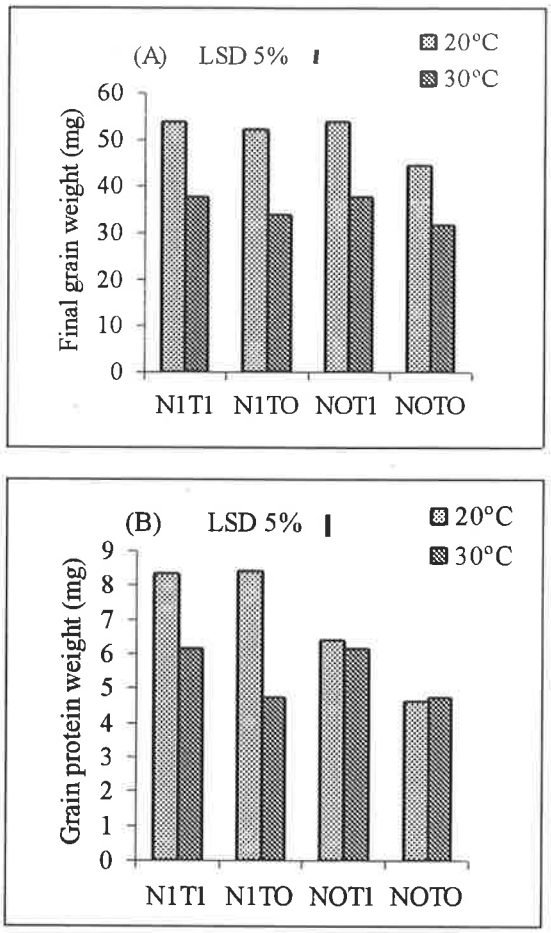

Fig. 5.10 Effects of the interactions between temperature, nitrogen level, and the trimming of ears on (A) final grain weight, (B) grain protein weight in trimmed and untrimmed ears of plants (means of both cultivars) grown at 20/15°C and 30/25°C at the low (NO) and standard (N1) levels of nitrogen.

effect of trimming on the temperature response of the grain dry matter accumulation was therefore related to the changes in the accumulation of proteins in the grains.

Trimming did not influence the composition of starch in the grains (Table 5.14) but it affected grain protein composition. The amount of HMW-glutenin proteins per grain was 33% more in the trimmed than in the untrimmed ears of Lyallpur but the effect was not significant in Kavko (Fig. 5.7F). Although the amount of grain protein measured by HPLC

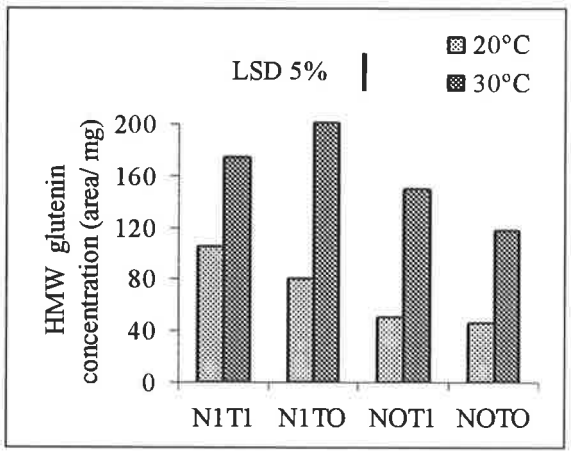

Fig. 5.11 Effects of the interactions of temperature, nitrogen, and trimming on the grain HMW-glutenin concentration in the trimmed and untrimmed ears of the plants of cultivars Kavko (K) and Lyallpur (L) grown at 20/15°C and 30/25°C at low (NO) and standard (N1) level of nitrogen.

was overall more in the trimmed ears, the interaction between cultivar and trimming was not significant (Table 5.24). In other words, it is the high molecular weight fraction that responded to trimming differently in the two cultivars. Only at the standard level of nitrogen was the positive effect of temperature on the concentration of HMW-glutenin proteins smaller in the trimmed than in the untrimmed ears (Fig. 5.11).

5.4.4 Conclusions

The differential response of the two cultivars to temperature was significantly influenced by the nutritional condition of the grains. Only at the standard level of nitrogen that included post anthesis nitrogen application did the two cultivars respond differently to high temperature. The varietal difference was associated with the temperature response of the rate of grain growth as the Q_{10} (30/20°C) value for the rate was greater in Kavko than in Lyallpur also the rate started to slow down at high temperature sooner in Lyallpur than in

Kavko. The temperature response of starch (only amylose but not amylopectin) accumulation to nitrogen level was similar to that of dry matter accumulation. The differential response of the two cultivars to temperature at the standard level of nitrogen was positively correlated with the changes in the rate of protein accumulation. Trimming influenced grain growth only in Lyallpur and the effect was smaller under conditions more favourable for grain growth. The effect of nitrogen level or trimming on the temperature response of grain development did not appear to be through changes in the availability of photoassimilate in the grains, nor did the diminished weight of the grain at high temperature result from the depression of the photoassimilate within the grain. A further experiment was therefore conducted to evaluate the physiological and biochemical factors that control the rate of grain growth inside the grains, and is reported in Chapter 6.

Chapter 6

Effects of high temperature on grain growth and on the metabolites and enzymes in the pathway of starch synthesis in the grains of two wheat cultivars differing in their responses to temperature.

6.1 Introduction

The results of previous experiments showed that the differences in the temperature responses of cultivars Kavko (tolerant) and Lyallpur (sensitive) in terms of single grain weight could mainly be explained by their differences in the rate of grain filling. Also, the responses of these two cultivars to temperature were changed when the relationship between supply and demand of the grains for the substrates involved in the synthesis of the grain storage substances was altered by manipulating the ears (trimming). The fact that nutritional status appeared to affect the sensitivity of these cultivars to high temperature justified a further study to evaluate the physiological and biochemical factors that control the rate of grain growth and resulted in differences in the responses of these two cultivars to high temperature.

Photosynthetic assimilates are mostly transported from different parts of the plant into the wheat grains in the form of sucrose. Sucrose is then converted to starch in the grains through a number of biochemical activities involving metabolites and different kinds of enzymes. ADP-glucose is one of those metabolites which is involved in the final section of the starch synthesis pathway and is the direct precursor for starch. Thus, the amounts of sucrose and ADP-glucose in the developing grains were measured in order to find out if the levels of these metabolites that play a key role in starch synthesis can explain the differential response of the two cultivars to temperature.

A number of enzymes are involved in the pathway of sucrose to starch in wheat endosperms. Of these soluble starch synthase (SSS) catalyzing the incorporation of ADPglucose into amylopectin (the major component of starch) has been found to be the most sensitive one to high temperature (Hawker and Jenner 1993; Jenner *at al.*1993; Keeling *at al.*1993). The reduction in the rate of starch accumulation is highly associated with the decreased activity of soluble starch synthase at temperatures above 30°C. The optimum temperature for the rate of catalysis by SSS was reported (Keeling *et al* 1993) between 20°C and 25°C and the rate decreased with a further increase in temperature above 25°C. The kinetic properties of SSS are also sensitive to temperature. In a study by Jenner *et al.* (1995) the K_M for amylopectin was minimal at 20°C and rose exponentially between 25°C and 45°C. Genetic variation exists for both the level of SSS activity and kinetic responses to high temperature (Jenner and Sharma 1997). In the findings published to date, the activity of granule bound starch synthase (necessary for the synthesis of amylose) is less sensitive to the effects of temperature than is SSS (Hawker and Jenner; 1993; Keeling *et al.* 1993).

Soluble starch synthase has two substrates, ADPglucose and a carbohydrate primer, in this case amylopectin. When assayed at saturating amylopectin concentration and variable ADP-glucose concentration V_{max} and K_M were both affected by temperature (Jenner *et al.* 1995). The K_M for ADPglucose at 45°C was approximately 2.7 times higher than that for the assay conducted at 25°C. However, effects of temperature on the K_M for amylopectin were much greater. Assayed at 45°C the K_M for amylopectin was about 30-fold greater than at 25°C. Therefore, increasing temperature results in a much greater decrease in the affinity of the enzyme for amylopectin than its affinity for ADPglucose. For this reason it was decided to confine attention to the effects of growth temperature on the kinetics of

SSS based on variable amylopectin levels, and in the presence of saturating levels of the other substrate ADP-glucose.

The main aim of this study was to characterize the temperature responses of soluble starch synthase (SSS) and granule bound starch synthase (GBSS) extracted from the endosperm in order to investigate the possibility that any variation in the thermal responses of the enzymes involved in synthesis of starch in the grains might explain the differential responses of Kavko and Lyallpur to high temperature.

6.2 Materials and Methods

This experiment was conducted in 25-cm diameter pots with recycled soil (20 plants per pot) kept in environmentally controlled growth rooms. Plants were fertilised with Aquasol^{TM} as explained in Chapter 3 (Materials and Methods). Two temperature regimes (20/15°C and 30/25°C day/ night) were applied during grain development to the plants with trimmed and untrimmed ears of two wheat cultivars differing in their response to high temperature Kavko (tolerant) and Lyallpur (sensitive). Pots were arranged inside the growth rooms under a randomised complete design. Four replications were used for each treatment. Trimming of the ears and the sampling and determination of the grain dry weight throughout grain development were done as explained in Chapter 5. The activities of soluble starch synthase (SSS) and granule bound starch synthase (GBSS) and also the amounts of sucrose and ADP-glucose in the endosperm were measured at two stages of grain development. The first sampling corresponded with the anticipated time of the inflection point, when grain filling was at its maximum rate, on days 12 and 22 after anthesis at 30/25°C and 20/15°C respectively. The second set of samples was taken on days 16 and 30 after anthesis. The individual methods for enzymes, sucrose, ADP-glucose

and also the conditions inside the growth rooms before and after anthesis have been explained in detail in Chapter 3.

6.3 Results

6.3.1 Effect of high temperature on parameters of grain filling

The results of the analysis of variance and the estimated values of grain filling parameters for cultivars Kavko and Lyallpur in the plants with trimmed and untrimmed ears grown at 20/15°C and 30/25°C are shown in Tables 6.1, 6.2, and 6.3. Apart from the sustained rate of grain filling, these parameters were all estimated by the ordinary logistic model. The sustained rate of grain filling was estimated by linear regression analysis as explained in Chapter 4.

Final grain weight

There was a significant interaction between temperature and cultivar (Table 6.1). The final grain weight was significantly decreased in the plants grown at 30/25°C compared to those grown at 20/15°C (Table 6.2). The reductions at high temperature were greater in Lyallpur (39%) than in Kavko (33%; Table 6.3 and Fig. 6.2A). The interaction between cultivar and trimming was highly significant. Lyallpur responded positively to the trimming of the ears and produced significantly heavier (11%) grains in the trimmed than in the untrimmed ears; trimming did not significantly affect Kavko's grains at either temperature (Fig. 6.3A). However, the reductions of final grain weights at high temperature were similar in the trimmed and untrimmed ears (Table 6.3).

Table 6.1 P values obtained from the analysis of variance table for grain growth components of trimmed and untrimmed plants of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C (Tem: temperature; Var: variety).

Source	Final grain dry weight	Sustained rate of grain filling	Maximum rate of grain filling	Duration of grain filling	Time to inflection point
Tem	.0001	.0001	.0001	.0001	.0001
Var	.0001	.0001	.0001	.1353	.5539
Trimming	.1419	.0001	.0001	.0115	.0175
Tem * Var	.0026	.2347	.1789	.4177	.8819
Tem * Trimming	.7576	.0375	.2647	.2392	.2195
Var * Trimming	.0049	.0001	.0082	.1701	.0046
Tem * Var * Trimming	.7576	.1587	.1980	.2519	.0795

Table 6.2 Grain growth characteristics of cultivars Kavko (K) and Lyallpur (L) with trimmed and untrimmed ears grown at 20/15°C and 30/25°C as estimated by the ordinary logistic model.

Treatment	Temperature (°C)	Final grain dry weight (mg)		Sustained rate of grain filling (mg/ day)		Maximum rate of grain filling (mg/ day)		Duration of grain filling (days)		Time to inflection point (days)	
		K	L	K	L	K	L	K	L	K	L
Untrimmed	20	58.3	65.5	1.07	1.23	1.38	1.74	62.3	56.7	31.1	28.9
	30	39.0	39.1	1.59	1.57	2.21	2.24	26.2	25.1	13.0	12.1
Trimmed	20	55.7	71.0	1.11	1.52	1.50	2.05	54.9	55.1	27.5	29.3
	30	37.9	44.5	1.67	2.10	2.31	2.86	24.2	23.6	11.9	12.1
LSD 5%		4.9 ns		0.15 ns		0.25 ns		4.7 ns		1.7 ns	

* The sustained rate of grain filling was estimated by linear regression model as explained in Chapter 4.
LSD 5% for Temperature × Variety × Trimming

Table 6.3 Effects of growth at 30/25°C compared to 20/15°C on the final grain weight and rate and duration of grain filling in cultivars Kavko (K) and Lyallpur (L) calculated from Table 6.2.

Treatment	Reduction in grain weight (%)		Q_{10} of the sustained rate (30°C /20°C)		Q_{10} of the maximum rate (30°C /20°C)		Reduction in duration of grain filling(%)		Reduction in time to inflection point (%)	
	K	L	K	L	K	L	K	L	K	L
Untrimmed	33	40	1.49	1.28	1.60	1.29	58	56	58	58
Trimmed	32	37	1.50	1.38	1.54	1.40	56	57	57	59
Mean	33	39	1.50	1.33	1.57	1.35	57	57	58	59

Rate of grain filling

The maximum rate of grain filling was increased at high temperature in both cultivars (Table 6.2 and Fig. 6.2B). The increase (Q_{10}) in the rate at high temperature was greater in Kavko (1.57) than in Lyallpur (1.35; Table 6.3) but the interaction between temperature and cultivar was not significant (Table 6.1). Trimming had a significant and positive effect on the rate of grain filling in Lyallpur; the rate was about 23% higher in the trimmed than in the untrimmed ears (Fig. 6.3B). In Kavko there was no difference between the trimmed and untrimmed ears in this respect. Although the interactions were not significant, Lyallpur responded to temperature more positively compared to Kavko in the trimmed than in the untrimmed ears (Table 6.3). Thus, the differences in the temperature responses of the two cultivars appeared to be greater in the untrimmed than in the trimmed ears. The responses of the sustained rate and maximum (instantaneous) rate of grain filling were in most cases similar (Table 6.1). However, the positive effect of trimming on the temperature response was significant only for the sustained rate of grain filling.

Duration of grain filling

High temperature hastened grain development and shortened the duration of grain filling in both cultivars (Table 6.2). There was no significant difference between the temperature response of the two cultivars as the interaction was not significant (Table 6.1). The duration of grain filling was overall shorter in the trimmed than in the untrimmed ears but the interactions between trimming with temperature or cultivar were not significant. The differences between the two cultivars in terms of the temperature response of grain weight, therefore, were not due to an effect of temperature on the duration of grain filling.

Time to inflection point

The period between anthesis and the maximum rate of dry matter accumulation (inflection point) was significantly decreased at high temperature (Table 6.2) but there was no significant interaction between cultivar and temperature (Table 6.1). Trimming decreased the time to the inflection point in Kavko (11%) but it had no effect on Lyallpur. In other words, in the grains of Kavko the rate of grain filling started to slow down sooner in the trimmed than in the untrimmed ears. However, the interaction between trimming and temperature was not significant. Except for the significant interaction between cultivars and trimming for the inflection point the similarities between the responses of the inflection point and the duration of grain filling are notable (Table 6.1).

6.3.2. Grain sucrose concentration

The results for the analysis of variance and the data on the measured concentration of sucrose in the developing grain (expressed on a fresh weight basis of the endosperm) are shown in Table 6.4 and 6.5. There were significant interactions between temperature, cultivar, and harvest (Table 6.4). The amount of sucrose in the grains was significantly higher in Kavko than in Lyallpur except in the grains developed at 20/15°C and sampled at harvest 2 (Table 6.5). Only in Kavko at 20°C was there a significant difference between the levels of sucrose in the grains between the two harvests. There were overall no significant differences between the amounts of sucrose in the grains at the two temperatures in both cultivars (see Fig. 6.2C). However, at harvest 1 (12 and 22 days after anthesis at 30/25°C and 20/15°C respectively) less sucrose was observed at 30/25°C than at 20/15°C in both cultivars. Regardless of the trimming effect, the reductions at 30/25°C were greater in Kavko (23%) than in Lyallpur (14%). At harvest 2 (16 and 30 days after

Table 6.4 P values obtained from the analysis of variance table for the concentration of sucrose and ADP-glucose in the grains of Kavko and Lyallpur grown at 20/15°C and 30/25°C.

Source	Grain sucrose concentration	Grain ADP-glucose concentration
Tem	.9652	.0001
Var	.0001	.0001
Trimming	.0935	.0021
Harvest	.0299	.5001
Tem * Var	.9131	.8978
Tem * Trimming	.0284	.8021
Tem * Harvest	.0001	.7786
Var * Trimming	.9478	.0026
Var * Harvest	.0033	.0423
Trimming * Harvest	.1603	.2454
Tem * Var * Trimming	.8273	.4593
Tem * Var * Harvest	.0446	.0577
Var * Trimming * Harvest	.8958	.0955
Tem * Trimming * Harvest	.1667	.0290
Tem * Var * Trimming * Harvest	.4726	.1103

anthesis at 30/25°C and 20/15°C respectively) in all except the untrimmed ears of Lyallpur more sucrose was observed at the higher temperature. Although of possible biological importance, this difference in the temperature response between the two cultivars was not statistically significant (Table 6.4). Averaged over cultivars and harvests, the level of sucrose in the grains was overall higher in the trimmed than in the untrimmed ears at 30/25°C but there were no significant differences between the level of sucrose in the trimmed and untrimmed at 20/15°C.

Table 6.5 Grain sucrose concentration (mg/gFw): response of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C in trimmed and untrimmed ears.

Treatment	Tem. (°C)	Harvest 1*		Ratio (30/20°C)		Harvest 2*		Ratio (30/20°C)	
		K	L	K	L	K	L	K	L
Untrimmed	20	8.75	5.62	0.72	0.84	5.83	5.98	1.21	0.93
	30	6.30	4.70			7.08	5.57		
Trimmed	20	9.27	6.40	0.82	0.89	5.07	4.80	1.55	1.46
	30	7.60	5.68			7.88	7.03		
Mean		7.98	5.60			6.47	5.85		

LSD 5% for the interaction of Temperature × Variety × Trimming × Harvest is 1.62 ns.
Harvest 1 : Sampling on days 12 and 22 after anthesis at 30°C and 20°C respectively.
Harvest 2 : Sampling on days 16 and 30 after anthesis at 30°C and 20°C respectively.

6.3.3 Grain ADP-glucose concentration

The concentration of ADP-glucose in the grains was measured at two stages of grain development as explained for grain sucrose concentration. The results, expressed on a fresh weight basis of the endosperm, are shown in Table 6.6 and Fig. 6.2C. The grains of Kavko contained less ADP-glucose compared to those of Lyallpur and the concentration of ADP-glucose in the developing grains of both cultivars was lower at 30/25°C compared to 20/15°C. The response of the two cultivars to temperature was not different as the interaction between cultivar and temperature was not significant (Table 6.5). The level of ADP-glucose was significantly higher in the trimmed than in the untrimmed ears of Lyallpur but trimming had no effect on the level of ADP-glucose in the grains of Kavko (Fig. 6.3D).

Table 6.6 Grain ADP-glucose concentration (nmol/g Fw): response of cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C in trimmed and untrimmed ears.

Treatment	Tem. (°C)	Harvest 1*		Ratio (30/20°C)		Harvest 2*		Ratio (30/20°C)	
		K	L	K	L	K	L	K	L
Untrimmed	20	126	119	0.73	0.92	101	156	0.80	0.65
	30	92	110			81	101		
Trimmed	20	119	163	0.64	0.79	113	163	0.83	0.91
	30	76	129			94	148		
Mean		103	130			97	142		

LSD 5% for the interaction of Temperature × Variety × Trimming × Harvest is 24 ns.
Harvest 1 and Harvest 2 as specified in Table 6.5

6.3.4 Effects of growth temperature on the amount of SSS extracted from the grains of Kavko and Lyallpur grown at different temperatures.

The grain samples for the measurement of the enzymes were taken at two stages of grain development as explained for grain sucrose concentration. The two sampling dates are referred as harvest 1 and harvest 2 in the related tables and figures. The kinetic parameters of soluble starch synthase were calculated from the Eadie-Hofstee plots. The plots for harvest 1 are shown in Fig. 6.1. All the data fitted closely the expected linear relationship between V and V/ [S] so the kinetic parameters could be calculated with confidence.

The maximum catalytic activity of soluble starch synthase (V_{max}) per single grain was calculated in order to provide comparisons between the enzyme activities and the amount of the end product, starch, on a per grain basis. The most pertinent data for examining the effects of growth temperature on the catalytic activity of SSS are data for assays conducted at the same temperature as the daytime growth temperature, and these data are shown in

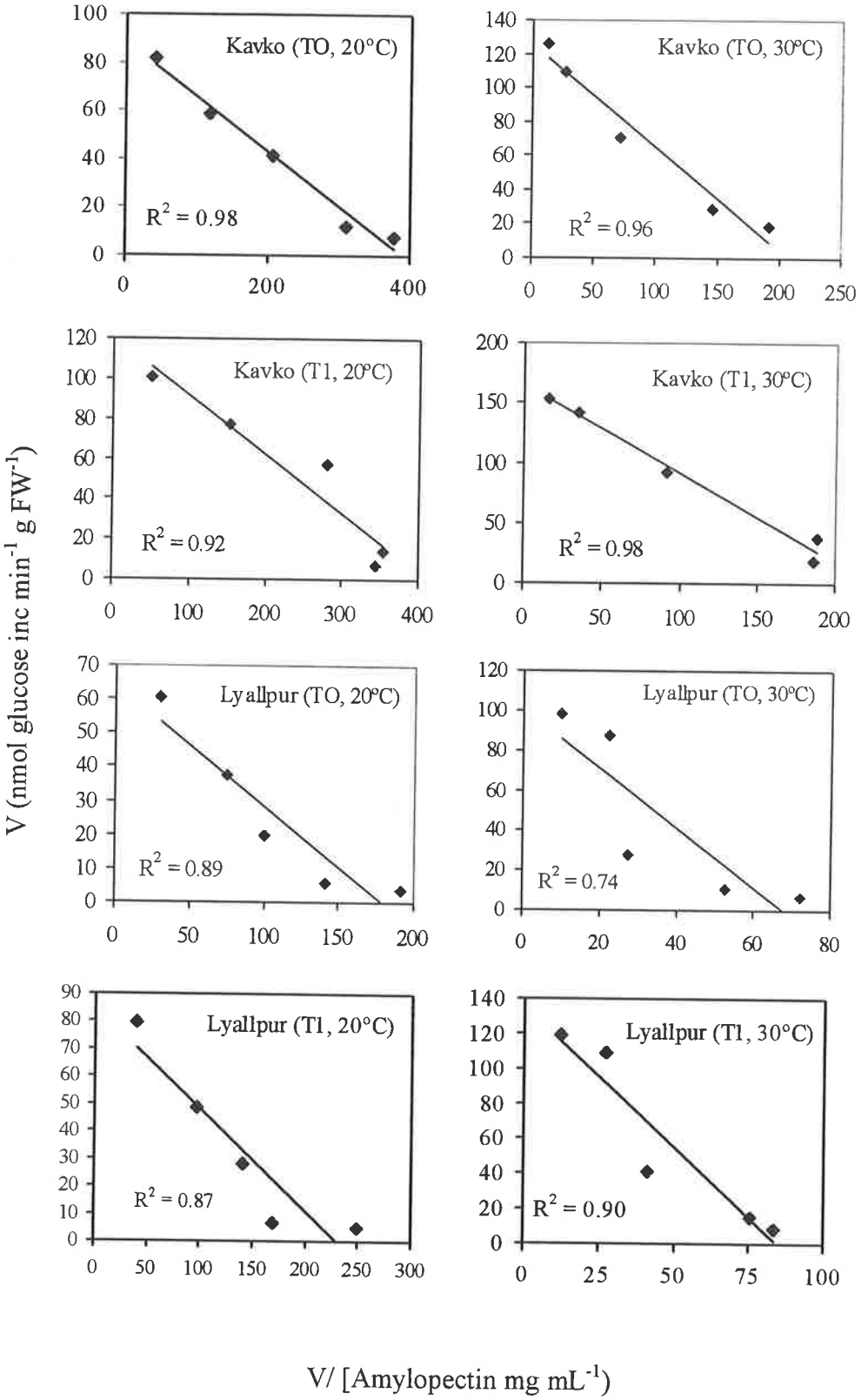

Fig. 6.1 (a) Eadie-Hofstee plots for dependence of velocity on the concentration of amylopectin in the grains sampled at day 22 after anthesis in trimmed (T1) and untrimmed (TO) ears of Kavko and Lyallpur raised at 20/15°C and assayed at 20°C and 30°C.

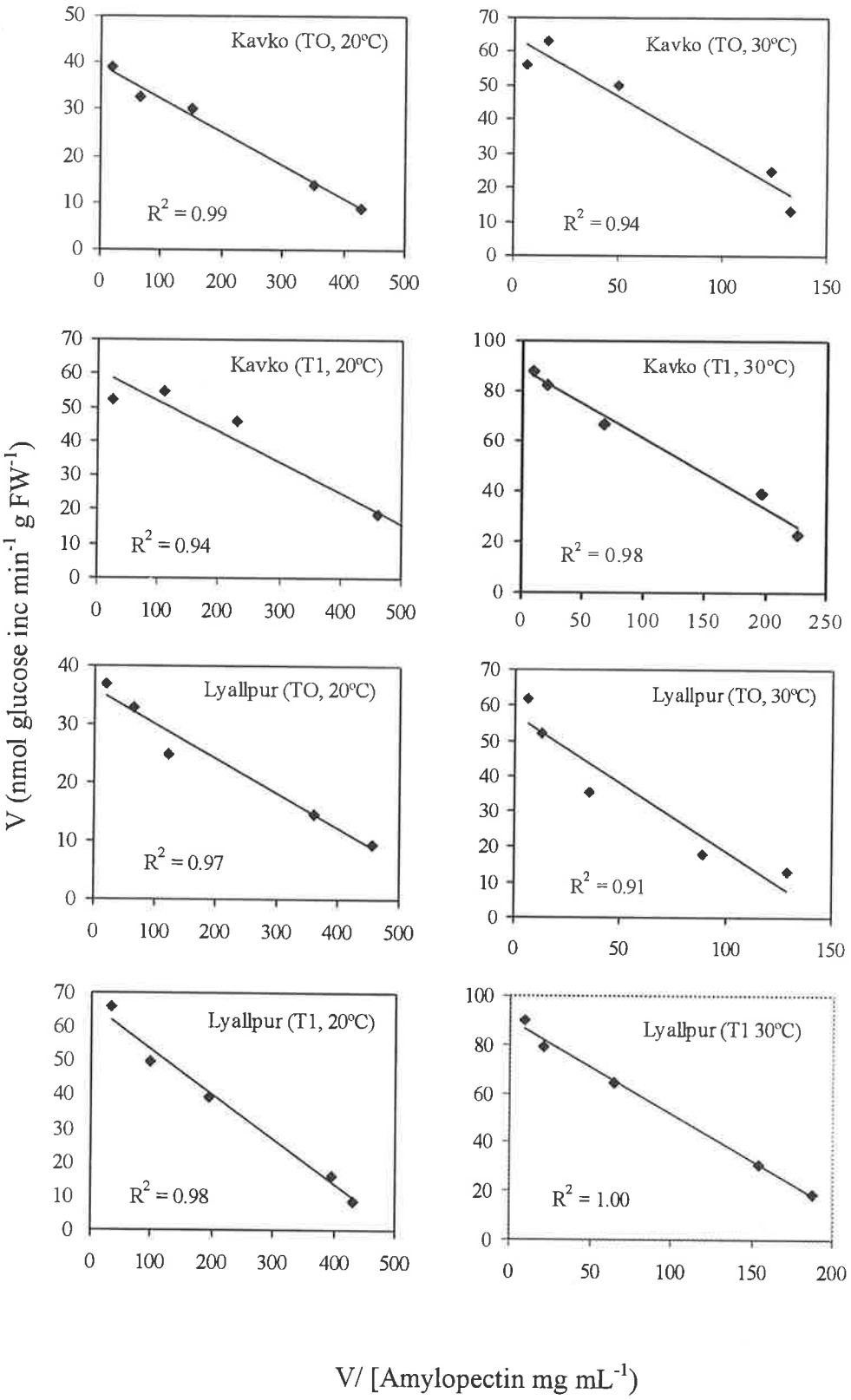

Fig. 6.1 (b) Eadie-Hofstee plots for dependence of velocity on the concentration of amylopectin in the grains sampled at day 22 after anthesis in trimmed (T1) and untrimmed (TO) ears of Kavko and Lyallpur raised at 30/25°C and assayed at 20°C and 30°C.

Table 6.7 Effects of growth temperature on the amount of SSS activity (V_{max}) extracted from the grains of Kavko (K) and Lyallpur (L) developed in plants with trimmed and untrimmed ears grown at 20/15°C and 30/25°C and assayed at the same temperature as the day time growth temperature. V_{max} is expressed as nmole ADPG per min per grain.

Treatment	Day time temperature (°C)	Harvest 1				Harvest 2			
		V_{max}		Q_{10} (30/20°C)		V_{max}		Q_{10} (30/20°C)	
		K	L	K	L	K	L	K	L
Untrimmed	20	2.72	2.21	0.47	0.44	0.53	0.40	1.28	1.53
	30	1.29	0.98			0.68	0.61		
Trimmed	20	3.79	3.08	0.57	0.67	1.38	0.62	0.93	1.37
	30	2.15	2.06			1.29	0.85		

Table 6.7 and Fig. 6.4A. Compared to growth temperature 20/15°C, the amounts of activity of SSS per grain were reduced at 30/25°C in both cultivars at harvest 1. At harvest2 V_{max} was much smaller than at harvest 1 and, except for the trimmed ears in Kavko, the response of the activity of SSS per grain to temperature was also changed resulting in higher values of V_{max} at 30/25°C than at 20/15°C. In both cultivars at harvest 1 the values of Q_{10} (the proportional change between the two growth temperatures) were higher in the trimmed ears compared to those of the untrimmed ears. At harvest 2 the higher values of Q_{10} for V_{max} were observed in the untrimmed ears, the opposite response to that observed in harvest 1. In all comparisons the values of V_{max} for Kavko were greater than those for Lyallpur. As noted above, the activities of SSS per grain of both cultivars fell with time. The overall reduction at harvest 2 compared to harvest 1 was greater in Lyallpur (78% and 54% at 20/15°C and 30/25°C respectively) than in Kavko (65% and 40% at 20/15°C and 30/25°C respectively).

Table 6.8 The activity of SSS (V_{max}) extracted from the grains of Kavko and Lyallpur developed in trimmed and untrimmed ears; V_{max} is expressed as nmole ADPG per min per grain.

Cultivar	Treatment	H1*	Ratio*	H2	Ratio
Kavko	Untrimmed	2.00	1.49	0.61	2.20
	Trimmed	2.97		1.34	
Lyallpur	Untrimmed	1.60	1.61	0.50	1.48
	Trimmed	2.58		0.74	

H: Harvest 1; H2: Harvest 2; Ratio: (Trimmed / Untrimmed)

The data for the response of cultivars to trimming in terms of the activity of SSS per grain is shown in Table 6.8 and Fig. 6.5A. In both cultivars the amount of activity was increased in the grains taken from the trimmed ears compared to those from untrimmed ears. The increase overall was greater in Kavko (1.9 fold) than in Lyallpur (1.5 fold) but the difference between the two cultivars in response to trimming was only evident at harvest 2 where Kavko was 38% more responsive than Lyallpur.

6.3.5 Effect of growth temperature on kinetic aspects of SSS extracted from the endosperm of Kavko and Lyallpur grown at different temperatures.

Kinetic attributes of SSS were also evaluated in the conventional manner of expressing activity on the basis of the fresh weight of the tissue extracted, in this case the endosperm.

6.3.5.1 Growth temperature and V_{max}

V_{max} is an estimate of the velocity of the reaction at infinitely high substrate concentrations where the enzyme molecules become saturated with respect to the substrate. The data for the kinetic parameters of soluble starch synthase extracted from the grains of Kavko and

Lyallpur grown at 20/15°C and 30/25°C, assayed at the same temperature as the day-time growth temperature, are shown in Tables 6.9 and 6.10 and Fig. 6.4B) with amylopectin as the variable substrate. The activities of SSS (V_{max}) were in general higher in Kavko than in Lyallpur. One apparent exception to this trend was the trimmed ears of Lyallpur at 30/25°C at harvest 1, in which the value of V_{max} was similar to that of Kavko. The values of V_{max} in the grains of Kavko were reduced under high growth temperature at harvest 1 and the Q_{10} for V_{max} was 0.73 in both trimmed and untrimmed ears. There was no or little effect of temperature on V_{max} in Lyallpur and the values of Q_{10} were close to unity in both treatments.

At harvest 2 compared to harvest 1, the values of V_{max} were substantially lower and were reduced to a greater extent in the grains at 20/15°C (4.5 fold) than at 30/25°C (2.4 fold) resulting in higher values of V_{max} at high temperature. The relative increases in the values of V_{max} at high temperature in both cultivars were higher in the untrimmed than in the trimmed ears. The Q_{10} for Lyallpur at the later harvest was greater than Kavko only in the trimmed ears; similar results being obtained for both cultivars in the untrimmed ears.

The results for the effect of the interaction between the two cultivars and trimming of the ears on the activity of SSS are shown in Table 6.11 and Fig. 6.5B. Trimming of the ears increased the values of V_{max} in both cultivars at both harvests. The responses of the cultivars to trimming were dependent on the stage of grain development. Kavko had slightly greater response to trimming at harvest 2 than at harvest 1 while Lyallpur responded positively only at harvest 1.

Table 6.9 The kinetic parameters of soluble starch synthase extracted from grains of Kavko and Lyallpur grown at 20/15°C or 30/25°C, assayed at the same temperature as the day time growth temperature. [(K) Kavko; (L) Lyallpur; (TO) untrimmed ears; (T1) trimmed ears]. V_{max} is expressed as nmole ADPG per min per g fresh weight of endosperm.

	Day time temperature	V_{max}				K_m				V_{max} / K_m	
	(°C)	H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	H2
K TO	20	88	0.73	16.7	2.07	0.23	1.52	0.04	9.00	390	375
	30	64		34.5		0.35		0.36		184	95
L TO	20	64	0.89	13.5	2.07	0.36	1.06	0.17	1.71	179	79
	30	57		28.0		0.38		0.29		150	95
K T1	20	121	0.73	35.5	1.18	0.30	0.93	0.09	3.78	410	380
	30	88		42.0		0.28		0.34		320	125
L T1	20	85	1.06	18.2	1.43	0.37	1.03	0.15	2.27	230	118
	30	90		26.0		0.38		0.34		235	77

H1: Harvest 1; H2: Harvest 2; Ratio: (30/ 20°C)

Table 6.10 Effects of temperature on kinetic parameters of soluble starch synthase (SSS) extracted from the grains of Kavko and Lyallpur Kavko and Lyallpur (averaged over trimming) at two harvests for assay done at the same daytime growth temperature. Enzyme activity expressed as nmole ADPglucose per min per g fresh weight of endosperm.

	Day time temperature (°C)	V_{max}				K_m				V_{max} / K_m	
		H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	H2
Kavko	20	105	0.72	26.1	1.47	0.27	1.19	0.07	5.00	400	378
	30	76		38.3		0.32		0.35		252	110
Lyallpur	20	75	0.99	15.9	1.70	0.37	1.03	0.16	2.00	205	99
	30	74		27.0		0.38		0.32		193	86

H1: Harvest 1; H2: Harvest 2; Ratio: (30/20°C)

6.3.5.2 K_M

K_M represents the concentration of substrate at which the rate of the enzyme reaction is at half its maximum velocity. It is associated inversely with the strength of binding (affinity) of substrate to enzyme. The values of K_M for amylopectin at two temperatures were in general smaller for Kavko than for Lyallpur at harvest 1, but at harvest 2 and at 30/25°C the reverse was the case in the untrimmed ears (Tables 6.9 and 6.10; Fig. 6.4C). K_M was considerably increased in both cultivars at high temperature at harvest 2; smaller effects of temperature were seen in harvest 1. In Kavko the values of K_M at high temperature were smaller in the trimmed ears than in the untrimmed ears at both harvests. In Lyallpur the differences between trimmed and untrimmed ears in terms of the changes in K_M at 30/25°C were observed only at harvest 2 at which the smallest value of K_M at high temperature was achieved in the untrimmed ears. The data for the responses of the two cultivars to the trimming of the ears for K_M are shown in Table 6.11 and Fig. 6.5C. At harvest 1, the values of K_M were similar in the trimmed and untrimmed ears but at harvest 2 the values in both cultivars were about 10% higher in the trimmed ears.

6.3.5.3 V_{max}/ K_M

The ratio of V_{max}/ K_M is the combined effects of temperature on V_{max} and K_M and is used as an indication of the efficiency of the enzyme for catalyzing the reaction (Fersht 1985). The data for the efficiency of SSS calculated as V_{max}/ K_M for both cultivars are shown in Tables 6.9 and 6.10 and Fig. 6.4D. In all comparisons except one (harvests 2, 30/25°C, untrimmed ears) the efficiency of SSS was greater in Kavko than in Lyallpur, the average values overall being 285 and 145 respectively. The efficiency of SSS in Kavko was lower at 30/25°C than at 20/15°C in all cases and the proportional reductions at high temperature were greater at harvest 2 than at harvest 1. In Lyallpur the efficiency was lower at 30/25°C

Table 6.11 The values of V_{max}, K_m, and V_{max} / K_m of SSS in the trimmed and untrimmed ears of Kavko and Lyallpur grown at 20/15°C or 30/25°C; V_{max} is expressed as nmole ADPG per min per g fresh weight.

Cultivar	Treatment	V_{max}				K_m				V_{max} / K_m	
		H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	H2
Kavko	Untrimmed	76	1.38	26	1.50	0.29	1.00	0.20	1.10	287	235
	Trimmed	105		39		0.29		0.22		365	253
Lyallpur	Untrimmed	61	1.44	21	1.05	0.37	1.03	0.23	1.09	165	87
	Trimmed	88		22		0.38		0.25		233	98

H1: Harvest 1; H2: Harvest 2; Ratio: (Trimmed / Untrimmed)

at harvest 1 only in the untrimmed ears but at harvest 2 the efficiency was reduced at high temperature only in the trimmed ears. In all comparisons the reductions in efficiency at high temperature were relatively greater in Kavko than in Lyallpur. Even so, at 30/25°C the enzyme from Kavko was 30% more efficient than that from Lyallpur, and Kavko was more efficient at 30/25°C than Lyallpur was at the lower temperature (Table 6.10). Trimming of the ears reduced the effects of high temperature on the efficiency of the enzyme in Kavko at both harvests. In Lyallpur the positive effect of trimming on the temperature response was only evident at harvest 1 and the reverse was the case at harvest 2. The results for the effect of the interaction between the two varieties and trimming on the efficiency of SSS are shown in Table 6.11 and Fig. 6.5D. In both cultivars the efficiency of the enzyme was higher in the trimmed than in the untrimmed ears and the positive effect of trimming was more evident at harvest 1 than at harvest 2.

6.3.6 Temperature response of SSS in vitro

To evaluate the immediate responses of the enzyme to an upward shift of temperature a comparison was made between the kinetic properties of SSS in the grains developed at growth temperature of 20/15°C and assayed at 30°C with those assayed at 20°C. Data derived from assays conducted at 30°C from grains grown at 20/15°C give an indication of the intrinsic properties of the enzyme in its response to an upward shift of temperature *in vitro*.

6.3.6.1 Vmax

The maximum velocity of SSS was increased with an increase in the assay temperature from 20°C to 30°C (Tables 6.12 and 6.13; Fig. 6.6A). The values of V_{max} were greater in Kavko than in Lyallpur at both assay temperatures. The activity of the enzyme dropped at

Table 6.12 The kinetic parameters of soluble starch synthase extracted from grains of Kavko and Lyallpur grown at 20/15°C and assayed at 20°C and 30°C. [(K) Kavko; (L) Lyallpur; (TO) untrimmed ears; (T1) trimmed ears]. V_{max} is expressed as nmole ADPG per min per g fresh weight of endosperm.

	Assay temperature	V_{max}				K_m				V_{max} / K_m	
	(°C)	H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	H2
K TO	20	88	1.42	16.7	1.86	0.23	2.60	0.04	9.50	390	375
	30	125		31		0.60		0.38		208	83
L TO	20	64	1.58	13.5	2.07	0.36	4.14	0.17	7.71	179	79
	30	101		28		1.49		1.31		68	21
K T1	20	121	1.36	35.5	1.86	0.30	2.43	0.09	7.22	410	380
	30	165		66		0.73		0.65		225	101
L T1	20	85	1.59	18.2	1.92	0.37	4.30	0.15	6.00	230	118
	30	135		35		1.59		0.90		85	38

H1: Harvest 1; H2: Harvest 2; Ratio: (30/20°C)

Table 6.13 Effects of temperature on kinetic parameters of soluble starch synthase (SSS) extracted from grains of Kavko and Lyallpur grown at 20/15°C and assayed at 20°C and 30°C (averaged over trimming). Enzyme activity expressed as nmole ADPglucose per min per g fresh weight of endosperm.

	Assay temperature	V_{max}				K_m				V_{max} / K_m	
	(°C)	H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	H2
Kavko	20	105	1.38	26.1	1.86	0.27	2.48	0.07	7.43	400	378
	30	145		48.5		0.67		0.52		217	92
Lyallpur	20	75	1.57	16.9	1.98	0.37	4.16	0.16	6.94	205	99
	30	118		31.5		1.54		1.11		77	30

H: Harvest 1; H2: Harvest 2; Ratio: (30/20°C)

harvest 2 compared to harvest 1 by about 3.5- and 4.1-fold in Kavko and Lyallpur respectively and the reductions occurred in a greater extent at 20°C (4.2 fold) than at 30°C (3.4 fold).

6.3.6.2 Km

The Km of SSS for its substrate, amylopectin, was considerably increased in both cultivars as a result of shifting assay temperature from 20°C to 30°C (Table 6.12 and 6.13; Fig. 6.6B). The decreases in the affinity of the enzyme (e.g. increases in Km) at higher assay temperature were greater in Lyallpur (4.2-fold) than in Kavko (2.5-fold) at harvest 1 but the reductions were similar in both varieties (about 7-fold) at harvest 2. In all comparisons the affinity of the enzyme was higher in Kavko than in Lyallpur. The differences between the two cultivars were greater at 30°C than at 20°C at harvest 1 but the differences were similar at both assay temperatures at harvest 2. The affinity was higher at the later than at the first harvest and the difference between the two harvests was greater at 20°C than at 30°C.

6.3.6.3 Vmax/ Km

The efficiency of SSS calculated as V_{max}/ K_M was decreased in both varieties at 30°C compared to assay at 20°C (Tables 6.12 and 6.13; Fig. 6.6C). The reductions in the efficiency at higher assay temperature were slightly greater in Lyallpur (62%) than in Kavko (46%) at harvest 1. At harvest 2, the proportional decreases in the efficiency of reaction at high temperature were greater than at harvest 1 but the extent of the reductions were similar in both varieties (about 70%). Corresponding values of V_{max}/ K_M for Kavko were all higher than those for Lyallpur and in all comparisons Kavko's efficiency at 30^0 was as high as, or not appreciably lower than, Lyallpur's efficiency at 20°C.

6.3.7 Effects of growth temperature on the amount of activity of granule bound starch synthase (GBSS)

6.3.7.1 GBSS activity per grain

The amount of the activity of GBSS per single grain was decreased in the plants grown at 30/ 25°C compared to those grown at 20/15°C and almost to the same amount in both cultivars at harvest 1 (Table 6.14 and 6.15; Fig. 6.7A). At harvest 2, however, the activity in Lyallpur was highest at 30/25°C and the positive effect of temperature was greater in the trimmed than in the untrimmed ears. The adverse effect of temperature on enzyme activity in Kavko was smaller at the later harvest than at the earlier one but the values of Q_{10} for Kavko were less than unity in all cases. The extent of the reductions in GBSS activity in Kavko at high temperature was greater in the trimmed than in untrimmed ears, the opposite response to that observed in Lyallpur. Corresponding values of GBSS activity were all higher in Lyallpur than in Kavko except for the trimmed ears at 20/15°C where the situation was reversed. The enzyme activity was higher at harvest 2 compared to harvest 1 at 30/25°C and the developmental changes were similar in both cultivars. At 20/15°C, however, this trend was only observed in the trimmed ears of Kavko. The results for the interaction between the two cultivars and the trimming of the ears on the GBSS activity are shown in Table 6.16. Trimming had positive effect on the enzyme activity in Kavko but the effect of trimming was not so noticeable in Lyallpur.

6.3.7.2 GBSS activity per fresh weight of endosperm

Compared to the activity calculated per grain basis, the adverse effect of high temperature appeared smaller when the of activity GBSS was calculated on the basis of fresh weight of endosperm (Table 6.14 and 6.15; Fig. 6.7B) because fresh weight was reduced at high temperature. Only in Kavko was the enzyme activity dropped at high temperature and in

Table 6.14 The activity of granule bound starch synthase (GBSS) extracted from the grains of Kavko and Lyallpur grown at 20/15°C or 30/25°C, assayed at the same temperature as the day time growth temperature. [(K) Kavko; (L) Lyallpur; (TO) untrimmed ears; (T1) trimmed ears]. Enzyme activity expressed as nmole ADPglucose per min on the basis of: the fresh weight of the endosperm, the weight of starch per grain and as total activity per grain.

	Day time temperature	Enzyme activity per grain				Enzyme activity per g fresh weight				Enzyme activity per g starch weight			
	(°C)	H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	Ratio	H2	Ratio
K TO	20	2.49	0.55	2.30	0.96	84	0.74	85	1.13	102	0.79	96	1.14
	30	1.37		2.20		62		96		81		109	
L TO	20	3.29	0.49	2.61	1.09	117	0.94	78	1.23	165	0.80	105	1.12
	30	1.62		2.85		110		96		132		118	
K T1	20	3.45	0.42	4.53	0.60	101	0.52	113	0.79	123	0.54	149	0.77
	30	1.44		2.74		53		89		66		114	
L T1	20	2.80	0.62	2.65	1.31	74	1.38	77	1.23	96	1.29	95	1.27
	30	1.74		3.48		102		95		124		121	

H1: Harvest 1; H2: Harvest 2; Ratio: (30/20°C)

Table 6.15 Effects of temperature on the activity of granule bound starch synthase (GBSS) extracted from the grains of Kavko and Lyallpur Kavko and Lyallpur (averaged over trimming) at two harvests for assay done at the same daytime growth temperature. Enzyme activity expressed as nmole ADPglucose per min on the basis of: the fresh weight of the endosperm, the weight of starch per grain and as total activity per grain.

	Day time temperature	Enzyme activity per grain				Enzyme activity per g fresh weight				Enzyme activity per g starch weight			
	(°C)	H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	Ratio	H2	Ratio
Kavko	20	2.97		3.42		93		99		113		123	
			0.47		0.72		0.62		0.94		0.65		0.91
	30	1.41		2.47		58		93		74		112	
Lyallpur	20	3.05		2.63		96		78		131		100	
			0.55		1.21		1.10		1.23		0.98		1.20
	30	1.68		3.17		106		96		128		120	

H1: Harvest 1; H2: Harvest 2; Ratio: (30/20°C).

Table 6.16 The activity of granule bound starch synthase (GBSS) in the trimmed and untrimmed ears of Kavko and Lyallpur grown at 20/15°C or 30/25°C, assayed at the same temperature as the day time growth temperature; Enzyme activity expressed as nmole ADPglucose per min on the basis of: the fresh weight of the endosperm, the weight of starch per grain and as total activity per grain.

Cultivar	Treatment	Enzyme activity per grain				Enzyme activity per g fresh weight				Enzyme activity per g starch weight			
		H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	Ratio	H2	Ratio
Kavko	Untrimmed	1.93	1.27	2.25	1.61	73	1.05	91	1.11	92	1.03	103	1.28
	Trimmed	2.45		3.64		77		101		95		132	
Lyallpur	Untrimmed	2.46	0.92	2.73	1.12	114	0.77	87	0.99	149	0.74	112	0.96
	Trimmed	2.27		3.07		88		86		110		108	

H1: Harvest 1; H2: Harvest 2; Ratio: (Trimmed/ Untrimmed)

Lyallpur the activity was overall greater at 30/25°C than at 20/15°C. The highest values of Q_{10} were belonged to the grains of Lyallpur taken from the trimmed ears. Except for the trimmed ears of Lyallpur, the effects of temperature were more positive at later harvest compared to harvest 1. There was a minor effect of trimming on the enzyme activity in Kavko (Table 6.16; Fig. 6.8) while in Lyallpur the activity at harvest 1 was even lower (about 25%) in the trimmed than in untrimmed ears. Overall the effect of trimming was more evident at 20/15°C than at 30/25°C.

6.3.7.3 GBSS activity per starch weight of endosperm

As GBSS is involved in the synthesis of amylose, and is bound tightly to the starch granule, the activity of GBSS may be related to the amount of starch available in the developing endosperm. Thus, the enzyme activity was calculated on the basis of the starch weight of endosperm to find out if there is variation in the specific activity of GBSS which is independent of the amount of starch in the endosperm. Overall the pattern of the response of the enzyme to temperature was similar to that calculated on the basis of fresh weight of endosperm (Fig. 6.7C). There almost 50% difference between the highest and lowest values, and greater variation was noted at harvest 1 than at harvest 2 in the effects of temperature.

6.3.8 Temperature response of GBSS in vitro

The results of the activity of GBSS extracted from the grains developed at 20/15°C and assayed at 20°C and 30°C are shown in Table 6.17 and 6.18 and Fig. 6.9. The activity of the enzyme was increased overall to the about same extent (1.5 times) in both varieties with a shift of assay temperature from 20°C to 30°C. Assayed at 30°C the values for the amount of the enzyme activity for Kavko were lower than those for corresponding values

Table 6.17 The activity of granule bound starch synthase (GBSS) extracted from the grains of Kavko and Lyallpur grown at 20/15°C and assayed at 20°C and 30°C. [(K) Kavko; (L) Lyallpur; (TO) untrimmed ears; (T1) trimmed ears]. Enzyme activity expressed as nmole ADPglucose per min on the basis of: the fresh weight of the endosperm, the weight of starch per grain and as total activity per grain.

	Assay temperature	Enzyme activity per grain				Enzyme activity per g fresh weight				Enzyme activity per g starch weight			
	(°C)	H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	Ratio	H2	Ratio
K TO	20	2.49	1.50	2.30	1.73	84	1.50	85	1.72	102	1.50	96	1.74
	30	3.73		3.97		126		146		153		167	
L TO	20	3.29	1.66	2.61	1.37	117	1.65	78	1.36	165	1.65	105	1.36
	30	5.45		3.58		193		106		273		143	
K T1	20	3.45	1.30	4.53	1.61	101	1.30	113	1.59	123	1.28	149	1.60
	30	4.48		7.27		131		180		158		239	
L T1	20	2.80	1.32	2.65	1.77	74	1.31	77	1.77	96	1.32	95	1.78
	30	3.70		4.70		97		136		127		169	

H1: Harvest 1; H2: Harvest 2; Ratio: (30/ 20°C)

Table 6.18 Effects of temperature on the activity of granule bound starch synthase (GBSS) extracted from grains of Kavko and Lyallpur grown at 20/15°C and assayed at 20°C and 30°C (averaged over trimming). Enzyme activity expressed as nmole ADPglucose per min on the basis of: the fresh weight of the endosperm, the weight of starch per grain and as total activity per grain.

	Assay temperature	Enzyme activity per grain				Enzyme activity per g fresh weight				Enzyme activity per g starch weight			
	(°C)	H1*	Ratio*	H2	Ratio	H1	Ratio	H2	Ratio	H1	Ratio	H2	Ratio
Kavko	20	2.97	1.38	3.42	1.64	93	1.39	99	1.65	113	1.38	123	1.65
	30	4.11		5.62		129		163		156		203	
Lyallpur	20	3.04	1.51	2.63	1.57	96	1.51	78	1.55	131	1.53	100	1.56
	30	4.58		4.14		145		121		200		156	

H1: Harvest 1; H2: Harvest 2; Ratio: (30/20°C)

of Lyallpur at harvest 1 and the reverse was observed at harvest 2. However, there were no major differences between the two cultivars when assayed at 20°C. There was no notable developmental change in the activity of the enzyme expressed either as per gram fresh weight or on the basis of starch weight of endosperm.

6.4 Discussion

Starch accounts for about 75% of grain dry matter and the reduction in grain weight at high temperature is due to the diminished starch deposition (Bhullar and Jenner 1985). Temperatures in excess of 30°C reduce the activity of soluble starch synthase in the endosperm (Hawker and Jenner 1993) and the rate of starch synthesis above 30°C is almost entirely controlled by the activity of SSS (Jenner *et al.* 1993; Keeling *et al.* 1993). Little attention has been paid to the temperature responses of starch synthesis at the biochemical level in the moderate temperature range (20-30°C). The main purpose of this experiment was to characterize the temperature response of starch synthase in developing endosperm in the range between 20°C and 30°C and also to investigate if the difference in the temperature sensitivity of the wheat cultivars Kavko and Lyallpur could be explained by the availability of substrate and/ or the activity of the enzymes involved in the starch synthesis.

6.4.1 Grain growth and substrate availability

The rate of grain filling increases with a rise in temperature above 20°C but the increase is normally small (Sofield *et al.* 1977a; Wardlaw *et al.* 1980; Tashiro and Wardlaw 1989; Hunt *et al.* 1991). In 10 cultivars tested by Hunt *et al.* (1991) the Q_{10} values for the rate of grain filling, calculated by Jenner (1994), were low and in the range from 1.0 to 1.5 between 20°C and 30°C. The temperature optimum is also low for the rate of starch

production in developing wheat endosperm (Bhullar and Jenner 1986). In the present study the rate of grain filling increased respectively by 50% and 33% and the grain weight dropped by 33% and 39% respectively in Kavko and Lyallpur as postanthesis temperature was raised from 20/15°C to 30/25°C (Table 6.3; Fig. 6.2A and B). The reduction in the duration of grain filling at high temperature was similar in both cultivars (57%), therefore the difference in the responses of the two cultivars appeared to be due to the response of the rate of grain filling to high temperature.

Although raising the temperature above 20°C has a big effect on the rate of grain respiration with a Q_{10} about 2.0, the respiratory loss of the ear accounts for only 12-14% of the rate of grain growth at 30/25°C (Wardlaw *et al.* 1980). Furthermore, the weak responsiveness of the rate of grain growth to an increase in temperature has not been associated with lack of supply of assimilates to the ear or the reduced availability of substrate within the grain (Wardlaw *et al.* 1980; Nicholas *et al.* 1984; Bhullar and Jenner 1986; Jenner 1991). The results of the current study are consistent with these reviews. Although the concentration of sucrose was higher in the grains of Kavko compared to those of Lyallpur, high temperature overall had no effect on the amounts of sucrose extracted from the endosperm of either cultivar (Fig. 6.2C). There appeared to be no correlation between the changes in sucrose concentration and ADP-glucose concentration at high temperature. The concentration of ADP-glucose was reduced in the heated grains as also reported by Jenner (1991) and the extent of the reductions was slightly greater in Kavko (26%) than in Lyallpur (19%; Fig. 6.2D). ADP-glucose is the immediate substrate for starch synthesis. There are larger amounts of ADP-glucose detected in the grains of Lyallpur than of Kavko, and there was a smaller decline in ADP-glucose concentration with increase of temperature in Lyallpur than in Kavko. These results therefore indicate

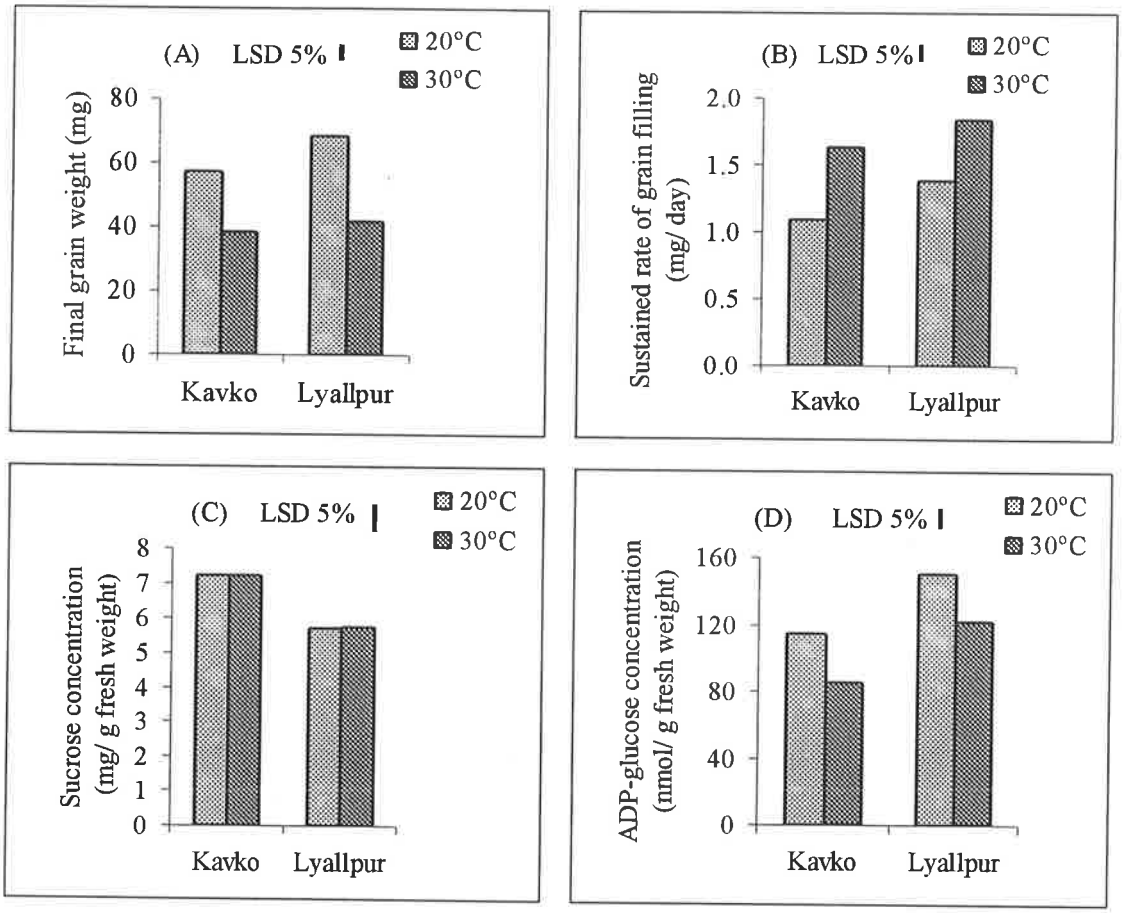

Fig. 6.2 Effects of temperature on (A) final grain weight, (B) the sustained rate of grain filling, (C) the concentration of sucrose and (D) ADP-glucose in the grains taken from the plants of Kavko and Lyallpur grown at 20/15°C and 30/25°C.

that neither the supply of assimilate (sucrose) nor the availability of direct substrate for starch synthesis (ADP-glucose) could explain why the positive response of the rate of grain growth to increase in temperature is smaller in Lyallpur compared to Kavko.

6.4.2 The activity of soluble starch synthase (SSS)

6.4.2.1 Response in vivo

Vmax

The optimum temperature for the rate of catalysis by SSS is reported to be about 25°C and

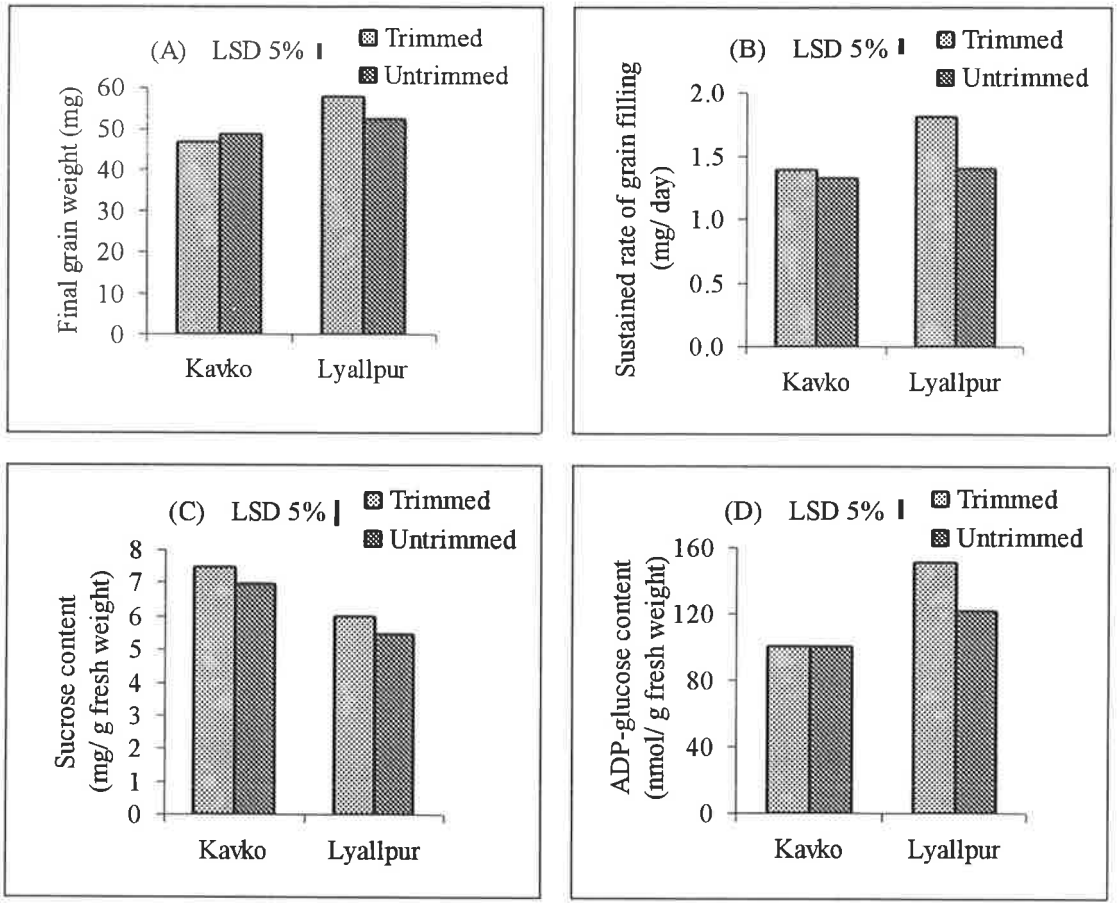

Fig. 6.3 Effects of trimming of ears on (A) final grain weight, (B) the sustained rate of grain filling, (C) the concentration of sucrose and (D) ADP-glucose in the grains taken from the plants of Kavko and Lyallpur grown at 20/15°C and 30/25°C.

above that point the rate decreases with a further increase in temperature (Keeling *et al.* 1993). The maximum velocity for the catalysis of SSS calculated per single grain basis was lower at 30/25°C than at 20/15°C at harvest 1 at the stage that the rate of grain filling was at its maximum (Fig. 6.4A). When measured on the basis of fresh weight of endosperm (Fig. 6.4B), V_{max} was decreased at high temperature in Kavko but not in Lyallpur and there was not much difference between the two cultivars at 30/25°C. The greater temperature sensitivity of Lyallpur compared to Kavko therefore could not be explained on the basis of the temperature response of V_{max} for SSS at harvest 1. There was

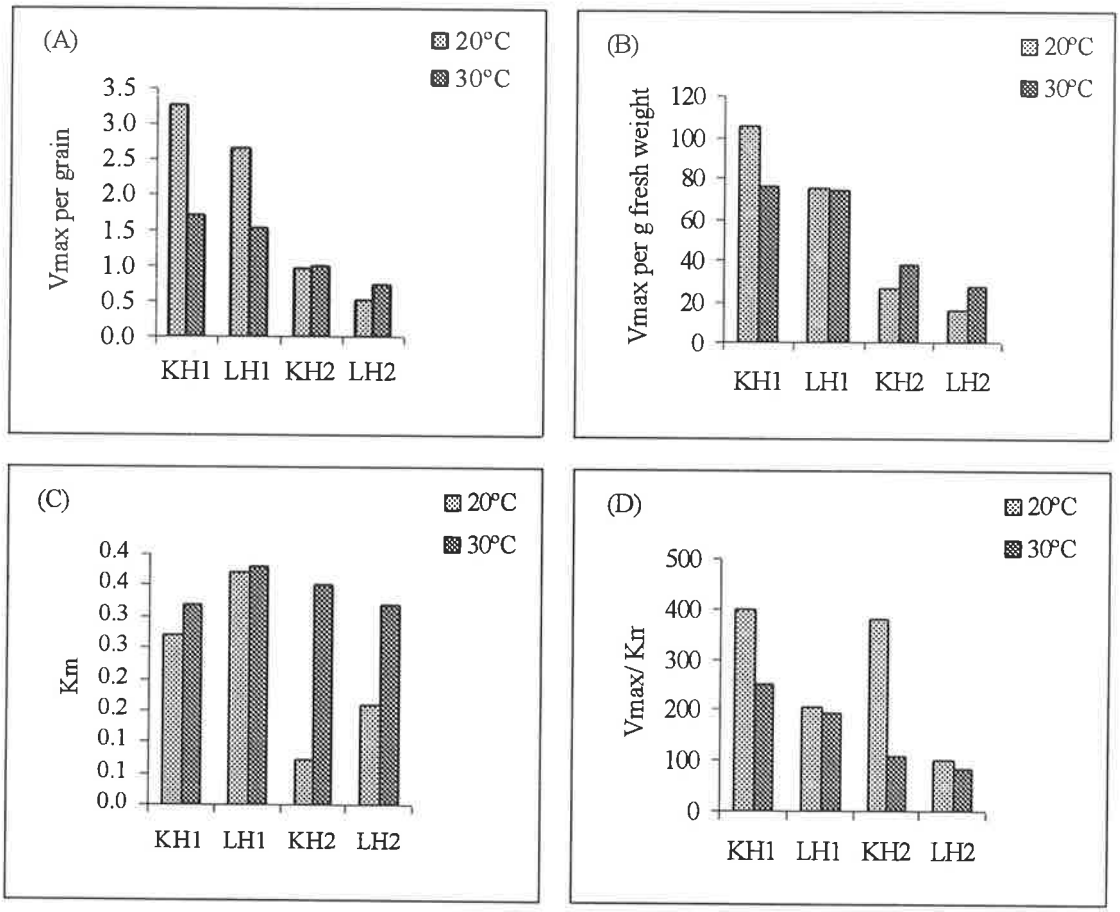

Fig. 6.4 Effects of temperature on the kinetic parameters of soluble starch synthase; (A) V_{max} per grain, (B) V_{max} per gram fresh weight of endosperm, (C) K_m, and (D) V_{max} / K_m extracted from grains of wheat cultivars Kavko (K) and Lyallpur (L) grown at 20/15°C and 30/25°C (H1: harvests 1, H2: harvest 2).

a large drop in V_{max} at the later stage of development, but V_{max} was higher at 30/25°C than at 20/15°C.

K_M

K_M is associated inversely with the affinity of the substrate for the enzyme and is independent of enzyme concentration (Wilson and Walker 1994). The affinity of an enzyme for its substrate diminishes at temperatures above the optimum for plant growth

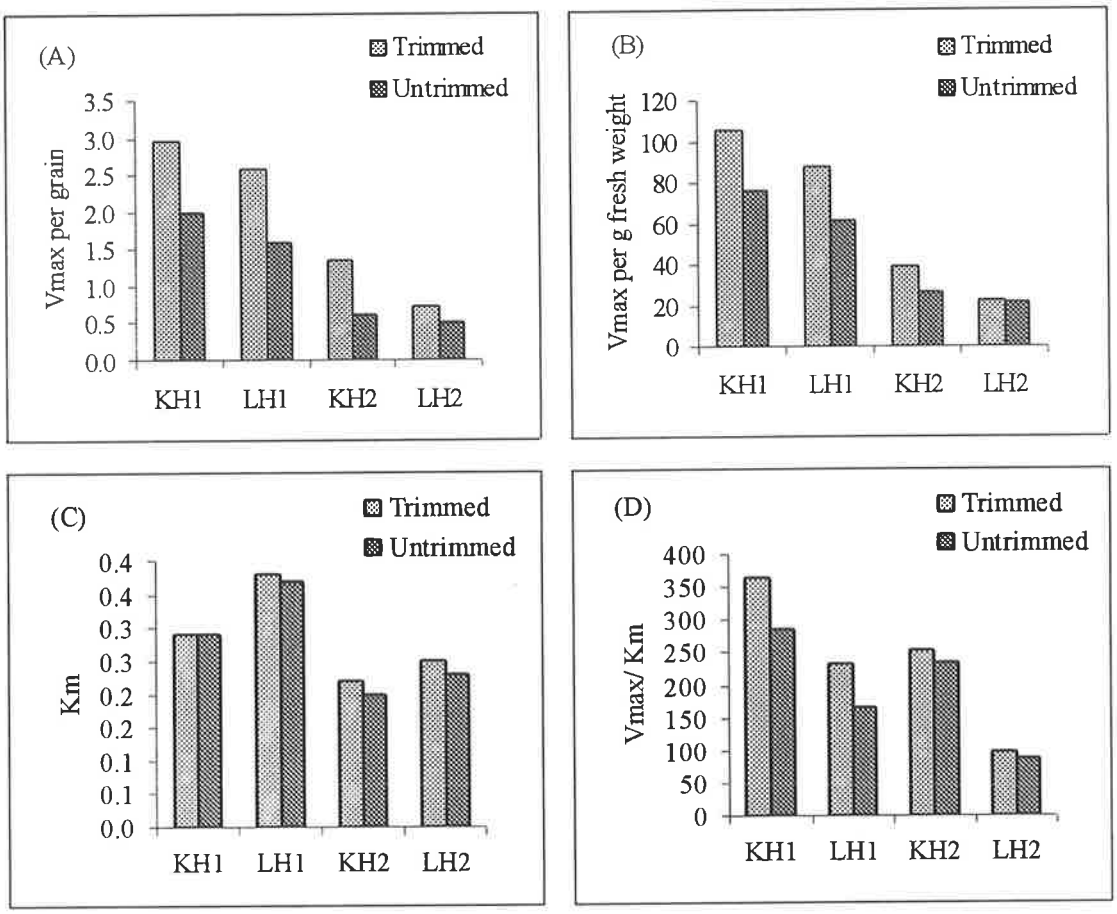

Fig. 6.5 Effects of the trimming of ears on the kinetic parameters of soluble starch synthase (SSS) extracted from endosperms of wheat cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C.

(Burke *et al.* 1988). In wheat, the K_M of SSS for amylopectin was increased 28-fold between 25°C and 45°C and the highest affinity was observed at 20°C with little change between 15°C and 25°C (Jenner *et al.*1995). In the present study the affinity of SSS for amylopectin was slightly higher in Kavko than in Lyallpur at both temperatures in harvest 1 (Fig. 6.4C). The temperature response of K_M was highly dependent on the stage of grain development as the affinity remained almost unchanged between the two harvests at 30/25°C while it had increased by about 3 fold at the later harvest at 20/15°C. The temperature response of K_M was therefore not associated with differential responses of the

two cultivars to high temperature in terms of the response of the rate of grain filling.

Vmax/ Km

The ratio of V_{max}/ K_M, termed the efficiency of the enzyme, is an indication of the performance of the enzyme in the plant (Wilson and Walker 1994). At harvest 2 the efficiency of SSS was almost 4 times greater in Kavko than in Lyallpur at 20/15°C, and a smaller difference (about 20%) existed between the two cultivars at 30/25°C (Fig. 6.4D). The response of the efficiency of SSS therefore might explain at least partly the better performance at high temperature of Kavko compared to Lyallpur in terms of the rate of grain filling.

In this experiment the plants were growing under elevated temperature for an extended period they might, therefore, have had chance to adapt to the high temperature conditions. As there are several isoforms of SSS identified in wheat endosperm (Denyer *et al.* 1995) one form or some forms might be more tolerant to heat than others or might have a higher affinity for the substrate, allowing the enzyme to stay active and compensate for the loss of activity. In an earlier study by Jenner and Sharma (1997) the superior performance of Trigo at high temperature compared to Lyallpur was found to be associated with the temperature response of the efficiency of SSS. In their study the plants were exposed to high temperature from day 9 after anthesis and the samples were taken 4 days later whereas in the current experiment the plants were under high temperature from day 2 after anthesis for 10 days before sampling.

6.4.2.2 Response in vitro

In order to evaluate the immediate responses of the enzyme to an upward shift of temperature the kinetic properties of SSS were measured in the grains developed at growth temperature of 20/15°C and assayed at 20°C and 30°C. The V_{max} and K_m for SSS were increased at higher assay temperature (Fig. 6.6A and B). The values of V_{max} were larger in Kavko than in Lyallpur at both assay temperatures (Fig. 6.6A). The reductions in the affinity of the enzyme at high temperature were much greater in Lyallpur than in Kavko (Fig. 6.6B). Compared to 20°C the efficiency of the enzyme assayed at 30°C was reduced by 46% and 62% in Kavko and Lyallpur respectively (Fig. 6.6C). However, the most conspicuous difference between the two varieties was in the absolute values of their efficiency as the values were substantially greater for Kavko than they were in Lyallpur. Thus the immediate response to an upward shift of temperature is a fall in the affinity of SSS for its substrate amylopectin, and an associated fall in efficiency. The differential responses of the two varieties to an increase in temperature may also account for differences in the temperature sensitivity of grain filling.

6.4.3 The activity of granule bound starch synthase (GBSS)

In comparison with the soluble form of starch synthase, the activity of granule bound starch synthase, catalysing the synthesis of amylose, is less affected by high temperature (Jenner *et al.* 1993; Hawker and Jenner; 1993; Keeling *et al.* 1993). This may explain why the proportion of amylose in starch increases at high temperature in wheat (Shi *et al.* 1994; Tester *et al.* 1995). The activity of GBSS varied with cultivar in response to a rise in growth temperature from 20/15°C to 30/25°C. While there was a drop in the activity of GBSS in Kavko at high temperature the response of the enzyme to temperature was positive in Lyallpur (Fig. 6.7B). There appeared to be some recovery in the activity at high temperature over time in Kavko so that at later stage of development (harvest 2) the

activity was almost the same in both temperatures. There was little developmental change in the activity of GBSS expressed either as per gram fresh weight or on the basis of starch

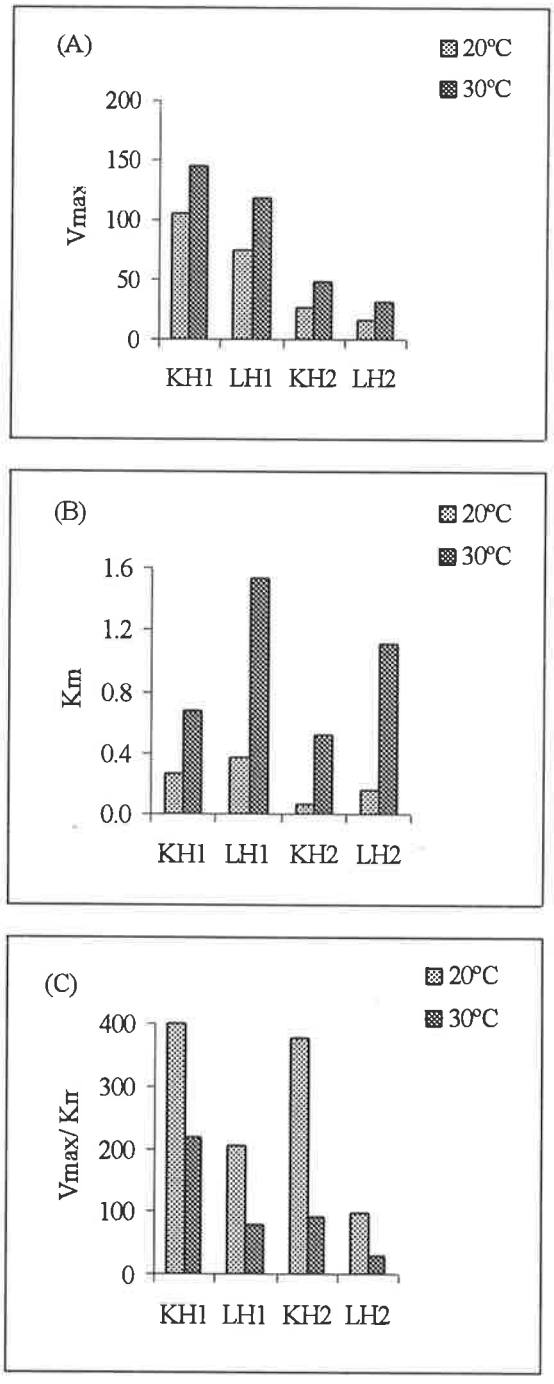

Fig. 6.6 Effects of assay temperature on the kinetic parameters of soluble starch synthase, (A) V_{max} (B) K_m, and (C) V_{max} / K_m extracted from endosperms of wheat cultivars Kavko (K) and Lyallpur (L), grown at 20/15°C, assayed at 20°C and 30°C, (H1: harvests 1, H2: harvest 2).

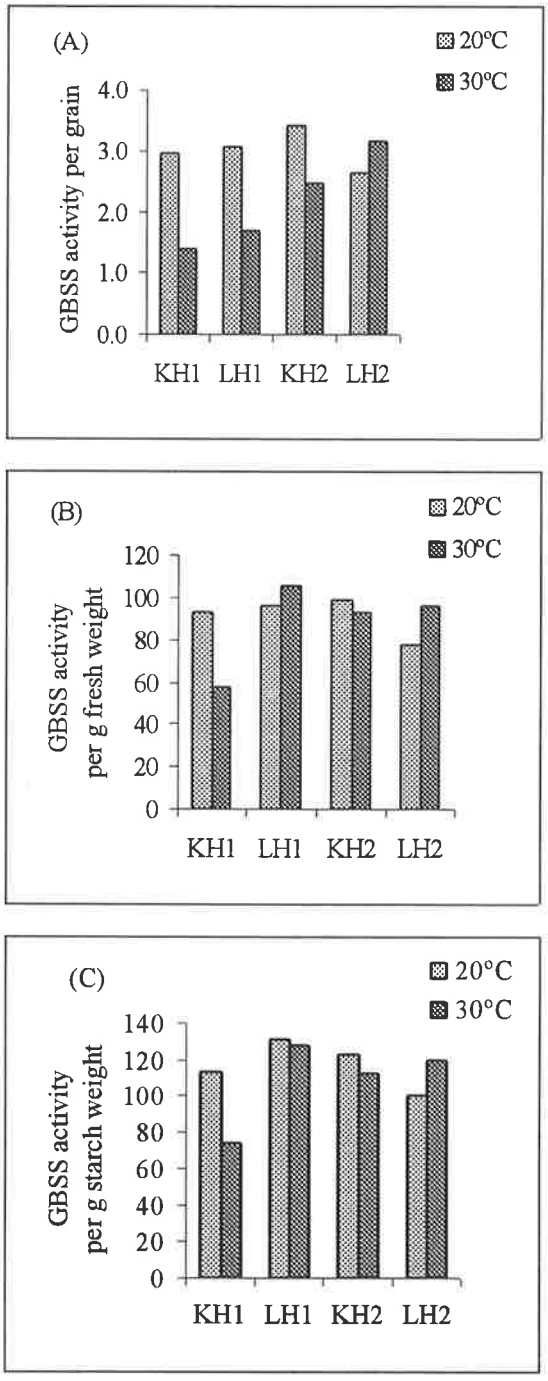

Fig. 6.7 Effects of temperature on the activity of granule bound starch synthase (A) per single grain, (B) per gram fresh weight, and (C) per gram starch weight of endosperm extracted from the grains of wheat cultivars Kavko (K) and Lyallpur (L) grown at 20/15°C and 30/25°C (H1: harvests 1, H2: harvest 2).

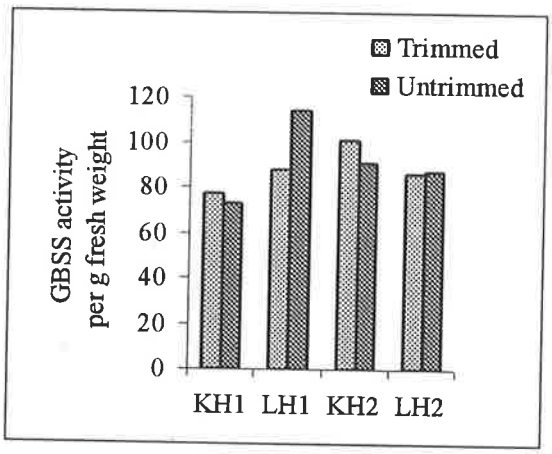

Fig. 6.8 Effects of the trimming of ears on the activity of granule bound starch synthase (GBSS) extracted from endosperms of wheat cultivars Kavko and Lyallpur grown at 20/15°C and 30/25°C. (H1: harvests 1, H2: harvest 2).

weight over the period between the two harvests in contract to a large shift downward in the activity of soluble starch synthase (Fig. 6.4A and B) at the later harvest. Being bonded to the granule, the activity perhaps stays more stable in the granule-bound than in the soluble form of starch synthase. As a result the relative activities of the two enzymes changes over time in the direction of relative increase in the activity of granule bound starch synthase. This may result in a progressive increase in the grain amylose percentage over the grain filling period especially at high temperature as found in the last experiment.

Assay at 30°C compared to 20°C (Fig. 6.9) of extracts from grains developing at 20/15°C resulted in higher rates of catalysis by GBSS in both cultivars and at both harvests. Clearly,

reduced starch synthesis at high temperature does not arise from temperature effects on GBSS activity, but changes in starch composition might be associated with these effects.

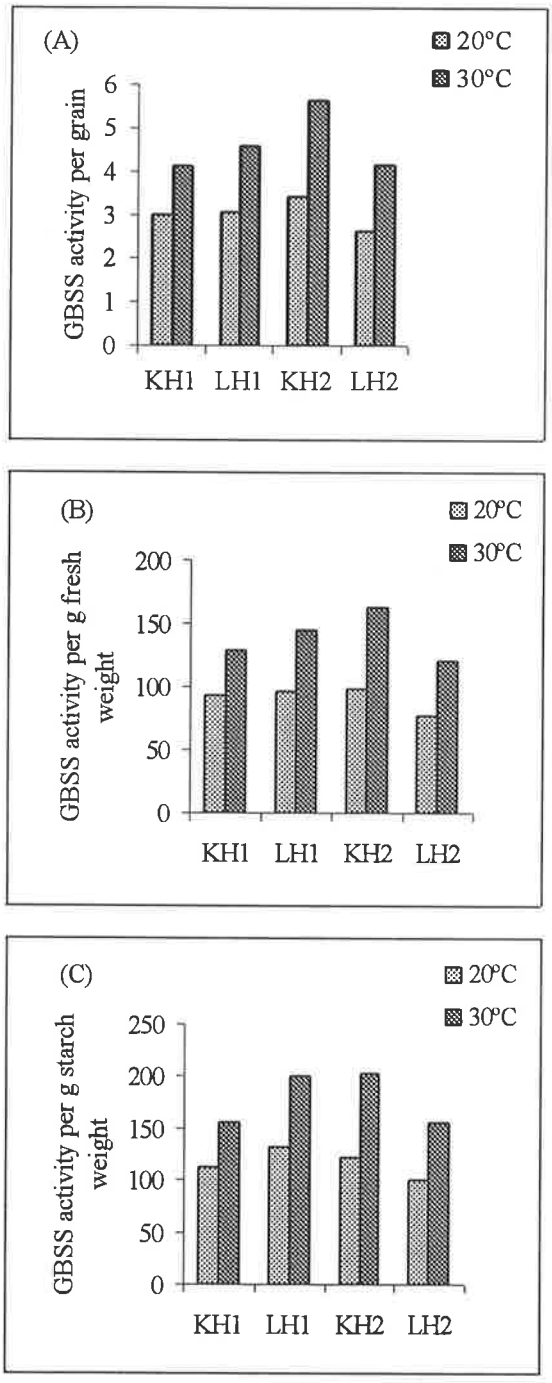

Fig. 6.9 Effects of assay temperature on the activity of granule bound starch synthase (A) per single grain, (B) per gram fresh weight, and (C) per gram starch weight of endosperm extracted from grains of wheat cultivars Kavko (K) and Lyallpur (L) grown at 20/30°C and assayed at 20°C and 30°C sampled at harvests 1 (H1) and harvest 2 (H2).

6.4.4 Effect of Trimming on grain growth

Trimming had a positive effect on grain weight and in particular on the rate of grain filling in Lyallpur but not in Kavko (Fig. 6.3A and B) while the concentration of sucrose in the endosperm was not significantly affected by trimming in either cultivar (Fig. 6.3C). However, the amount of ADP-glucose extracted from the grains of Lyallpur was higher in the trimmed than the untrimmed ears (Fig. 6.3D), a response similar to that of the rate of grain filling (Table 6.3). These results therefore may indicate that trimming of the ears does not alter the delivery of sucrose to the grains but trimming does influence processes in the pathway of the conversion of sucrose to ADP-glucose.

The efficiency of SSS was positively influenced by trimming but the response was similar in both cultivars (Fig. 6.5A and B). As trimming had no effect on the affinity of the enzyme for its substrate, the increased efficiency was therefore due to an increase in the maximum velocity of the enzyme in the trimmed ears. Since the V_{max} for SSS was estimated at saturating substrate concentration and the affinity of the enzyme was not affected by trimming, the activity of the enzyme may have been increased through increasing the enzyme concentration in the remaining grains of the trimmed ears. Compared to SSS, the activity of GBSS was less affected by trimming but the trimming effect was to some extent dependent on the cultivar (Fig. 6.8).

Chapter 7

General discussion

7.1 Introduction

Several stages of wheat growth can be affected adversely by high temperature (Wardlaw *et al.* 1989a) but under field conditions periods of high temperature are more frequent during grain filling than at earlier stages. Thus, the temperature treatments in all experiments conducted in this study were imposed on the plants after anthesis and during grain development. Although a short period of very high temperature can also influence grain yield significantly (Stone and Nicholas 1994) the temperatures in the range of 20-30°C, used in the present study, are typical during grain filling in wheat growing areas.

The existence of genetic variation among wheat cultivars in the response of grain filling to high temperature (Rawson 1986; Wardlaw *et al.* 1989a,b; Hunt *et al.* 1991) provided an opportunity to conduct experiments to evaluate the physiological and biochemical aspects that could explain the differences between cultivars in their responses to high temperature. The main objectives of this study were therefore: (a) to examine the relationships between grain-filling parameters and high temperature tolerance in wheat cultivars, (b) to investigate the relationships at high temperature between the availability of the substrates for starch and protein synthesis within the grains and the responses of grain filling to high temperature, and (c) to characterize the temperature responses of the enzymes (SSS and GBSS) involved in the synthesis of starch in the grains. The purpose of (c) was to investigate the possibility that any perceived variation in the thermal responses of these enzymes might explain the differential responses of Kavko and Lyallpur to high temperature.

7.2 Grain growth and substrate availability

A sustained period of moderately high temperature (30/25°C) substantially decreased the kernel weight in all cultivars. The variation among cultivars in response to temperature was considerable and the reductions in grain weight ranged from 21% to 40% (Table 4.10). High temperature depressed grain weight through a shortened duration of grain filling not being compensated for completely by an increased rate of grain filling. There was genotypic variation for rate and duration of grain filling but the variation was small for duration at high temperature as reported by Hunt *et al.* (1991). In addition, the correlation between final grain weight with the rate ($r = 0.67$) but not with duration ($r = 0.25$) of grain filling was significant at high temperature. The results therefore indicate that under high temperature conditions final grain weight becomes proportional to the rate of grain filling. These findings are in agreement of the results of Wardlaw and Moncur (1995) who showed that the most tolerant cultivars to high temperature were those in which the rate of grain filling was increased most by elevated temperature. Despite non-significant association with the entire period of grain filling at high temperature, final grain weight was significantly associated ($r = 0.69$) with the period between anthesis and the time to maximum rate (inflection point) at high temperature. Thus, cultivars that sustain a longer period between anthesis to the inflection point at high temperature may have better chance to accumulate dry matter in their grains at faster rates for a longer period.

There is usually a small increase in the rate of grain filling with a rise in temperature above 20°C with Q_{10} ranging from 1.0 to 1.5 between 20°C and 30°C (Sofield *et al.* 1977a; Tashiro and Wardlaw 1989; Hunt *et al.* 1991). The Q_{10}s calculated in this study were also within this range (Table 4.9, 5.3, and 6.3). Although the increase in the rate of grain respiration at temperatures above 20°C is substantial, the respiratory loss of the ear

accounts for only 12-14% of the rate of grain growth at 30/25°C (Wardlaw *et al.* 1980). Therefore the question was raised if the low response of the rate of grain growth to rising temperatures would be associated with either the availability of assimilates or the efficiency of synthetic system in the grains or with both.

The concentration of sucrose in the endosperm is considered as an indication of the availability of assimilates in the grains. Neither the concentration of total soluble carbohydrates (Fig. 5.3C) nor the concentration of sucrose in the grains (Fig. 6.2C) was significantly different at 30/25°C and 20/15°C. Nor were the varietal differences in terms of the response of the grains to high temperature explainable by the availability of sucrose in the grains. The results from this study therefore indicate that the low response of the rate of grain growth to an increase in temperature is not mainly associated with supply of assimilates to the grains as reported previously (Wardlaw *et al.* 1980; Nicholas *et al.* 1984; Bhullar and Jenner 1986; Jenner 1991). However, Wardlaw *et al.* (1995) suggested that greater attention should be given to the effect of temperature on the transfer of substrate from the crease vascular system of the grains. In other words, there might be a simultaneous increase in the rate of delivery of sucrose into the grains and an increase in starch accumulation without any change in the pool size of the sucrose inside the grains.

ADP-glucose is the immediate precursor for starch synthesis. There was a higher level of ADP-glucose in the endosperm of Lyallpur: the heat sensitive genotype compared to Kavko: the tolerant genotype. Moreover, although the concentration of ADP-glucose in the grains of both cultivars was decreased at high temperature the extent of reduction was slightly greater in Kavko than in Lyallpur (Fig. 6.2D). The change in the concentration of

ADP-glucose at high temperature can not therefore explain why the positive response of the rate of grain growth to an increase in temperature is smaller in Lyallpur than in Kavko.

7.3 The activity of starch synthase

The reduction in the rate of grain growth at temperatures in excess of 30°C is mainly due to the reduced activity of soluble starch synthase (SSS) (Jenner *et al.* 1993; Keeling *et al.* 1993). This enzyme has a low optimum temperature of 25°C for its maximum activity (Keeling *et al.* 1993;1994), and its K_M for amylopectin, which is inversely associated with the affinity of the enzyme for its substrate amylopectin, was increased 28-fold between 25°C and 45°C (Jenner *et al.* 1995). At temperatures below 30°C, however, the loss of the activity of SSS is not large enough to account for the responses of starch deposition to temperature (Rijven 1986). However, the kinetic properties of the SSS are very sensitive to changes in temperature (Jenner *et al.* 1995; Jenner and Sharma 1997).

The maximum velocity (V_{max}) for the catalysis of SSS on the basis of the fresh weight of endosperm was about 30% lower at 30/25°C compared to 20/15°C in Kavko at harvest 1 but there appeared no difference between two temperatures in Lyallpur (Fig. 6.4B). On the other hand at harvest 2, although at 30°C the V_{max} in Lyallpur was smaller than that recorded for Kavko, V_{max} in Lyallpur was 70% higher at 30/ 25°C than at 20/15°C whereas the comparable value in Kavko was only 47% higher (Table 6.10). *In vitro*, the V_{max} in the grains developed at 20/15°C and assayed at 20°C and 30°C was about 1.6 and 1.8 times greater at 30°C than at 20°C in Kavko and Lyallpur respectively (Fig. 6.6A). Accordingly, the greater temperature sensitivity of Lyallpur compared to Kavko (Fig. 5.5A) was not associated with the temperature response of V_{max} for SSS.

The ratio of V_{max}/ K_M, termed the efficiency of the enzyme, is an indication of the performance of the enzyme in the plant. The efficiency of SSS in the grains of Kavko was overall 2.2 times higher at 20/15°C than at 30/25°C but in Lyallpur the efficiency was similar at both temperatures (Fig. 6.4D). *In vitro*, however, compared to 20°C the efficiency of the enzyme assayed at 30°C was reduced in both cultivars and to a greater extent in Lyallpur (62%) than in Kavko (46%; Fig. 6.6C). The reduction in the efficiency of the enzyme in vitro was due to a fall in the affinity of SSS for its substrate amylopectin. The reductions in the affinity of the enzyme at high temperature were greater in Lyallpur than in Kavko. The differential responses of the two varieties to an increase in temperature *in vitro* may therefore account for differences in the temperature sensitivity of grain filling. However, the most remarkable difference between the two varieties *in vivo* was in the absolute values of their efficiency as the values were substantially greater for Kavko than they were in Lyallpur.

In a study by Jenner and Sharma (1997) the superior performance of Trigo at high temperature compared to Lyallpur was associated with the temperature response of the efficiency of SSS. The efficiency of SSS in Lyallpur was substantially decreased at high temperature in both *in vivo* and *in vitro* conditions while in this study the efficiency of the enzyme was the same *in vivo* at both temperatures. In their study the plants were exposed to high temperature for 4 days whereas in the current experiment exposure was for 10 days before sampling. Furthermore, the difference between Kavko and Lyallpur in terms of SSS efficiency at high temperature was smaller *in vivo* (Fig. 6.4D) than *in vitro* (Fig. 6.6C). The results may therefore imply that there has been a chance for SSS in Lyallpur to adapt to temperature in the plants growing under high temperature for a longer period. *In*

vitro, the immediate response of the enzyme to temperature does not provide the opportunity for such a time dependent adaptation to elevated temperature.

Compared to the soluble form of starch synthase (Fig. 6.4B), granule bound starch synthase (GBSS) responds more positively to high temperature (Fig. 6.7B). Only in Kavko at harvest 1 was the activity of GBSS lower at high temperature but there was a recovery in the activity of the enzyme at high temperature over time so that at the later stage the activity was similar at both temperatures. Being bonded to the granule, the activity perhaps stays more stable in the granule-bound than in the soluble form of starch synthase. The positive response of the activity of GBSS at moderately high temperatures may result in an increase in the percentage of amylose in the grains (Fig. 5.5F). At temperatures over 30°C it may be the lesser sensitivity of GBSS compared to SSS (Jenner *et al.* 1993; Keeling *et al.* 1993) that increases the proportion of amylose in starch at high temperature (Shi *et al.* 1994; Tester *et al.* 1995).

There was a considerable developmental change in the activity of soluble starch synthase in developing grains. The maximum velocity (V_{max}) for the catalysis of SSS was 4.5-fold (at 20/15°C) and 2.4-fold (at 30/25°C) lower at harvest 2 compared to harvest 1 (Fig. 6.4B). The affinity of SSS for its substrate amylopectin remained unchanged in the grains raised at 30/25°C but the affinity was several times higher at the later stage at 20/15°C (Fig. 6.4C). In contrast, there was little developmental change in the activity of GBSS observed over the period between the two harvests (Fig. 6.7B). As a result the relative activities of the two enzymes change over time in the direction of relative increase in the activity of GBSS. This could result in a progressive increase in the grain amylose percentage over the grain filling period especially at high temperature (Fig. 5.2).

7.4 Plant nutritional status and response to temperature

In this study the supply of nitrogen to the grains was altered either by changing the level of nitrogen applied to the plants or by reducing the number of the grains in the ear (trimming). Although removing half of the grains doubled the ratio of source to sink only the concentration of soluble amino acids (Tables 5.20), but not that of soluble carbohydrates (Tables 5.17), was increased in the remaining grains of the trimmed ears. Also the increase of nitrogen supply to the plants did not significantly change the amount of the grain soluble carbohydrates at the time when the rate of grain filling was maximum (Fig. 5.3A). The effect of nitrogen level or trimming on the response of grain filling to temperature does not seem to be through changes in the availability of photoassimilate in the grains.

The variation in supplied nitrogen substantially influenced the differential responses the cultivars to high temperature. Only at the standard level of nitrogen (that included post anthesis nitrogen application) was the reduction in final grain weight at high temperature greater in Lyallpur (40%) than in Kavko (24%); there was no difference in temperature response of the cultivars at the low level of nitrogen (Fig. 5.4A). The effect of nitrogen level on the differential responses of these cultivars to temperature was due to its effect on the final starch weight (Fig. 5.4E) and the effect was significant only on amylose (Fig. 5.5B) but not on amylopectin (Fig. 5.5C). Amylose contributes only a minor portion to the grain starch weight but any change in its proportion does influence starch composition and may influence the starch quality.

Variation in the level of nitrogen did not significantly affect the differential response of cultivars to temperature in terms of final grain protein weight (Table 5.4). However, the

difference between cultivars Kavko and Lyallpur in terms of the rate of grain protein accumulation in response to temperature was dependent on the level of nitrogen. The rate of grain protein accumulation at the standard level of nitrogen in Kavko was 33% higher at 30/25°C compared to 20/15°C, but in Lyallpur the rate was similar at both temperatures (Fig. 5.6A). At the low level of nitrogen the rate was increased at high temperature approximately to the same extent in Kavko (58%) and Lyallpur (65%).

Other environmental factors such as light intensity may also regulate the differential response of wheat cultivars to high temperature. Wardlaw *et al.* (1989a), for example, reported that the effect of high temperature on grain weight was enhanced under low light conditions to a greater extent in cultivar Kalyansona than in Banks. Alteration of light intensity was not used in present work to influence assimilate supply to the grain; ear trimming was used instead. Trimming of the ears increased the weight of the remaining grains in Lyallpur but trimming had no significant effect on grain weight in Kavko (Fig. 5.7A and 6.3A). The increase in the grain weight in the trimmed ears of Lyallpur was associated with a simultaneous rise in the rate of grain filling (Fig. 5.7D and 6.3B) and also with the extended time from anthesis to maximum rate (Fig. 5.7E). The increase in the rate of grain filling in the trimmed ears was in turn associated with an increase in the concentration of ADP-glucose, the immediate substrate for starch synthesis (Fig. 6.3D). The amount of sucrose in the grains was not affected by trimming in either cultivar (Fig. 6.3C). This indicates that trimming does not alter the delivery of sucrose to the grains but instead appears to influence processes in the pathway of the conversion of sucrose to ADP-glucose.

At 20/15°C, across cultivars and trimming treatments, the level of ADP-glucose in the grains and the sustained rate of grain filling were correlated (Tables 6.6 and 6.2), implying that ADP-glucose level may be a factor involved in controlling the rate of grain filling. Also trimming at 30/25°C increased the level of ADP-glucose in Lyallpur relatively more than it did at 20/15°C, with similar responses evident in grain filling. However, the relationship between the sustained rate of grain filling and ADP-glucose level was not the same at the two temperatures: at 30°C, and for a given level of ADP-glucose, the rate of grain filling in the trimmed ears of Lyallpur was lower than the estimate for Kavko (1.51 vs. 1.96 $mg.day^{-1}.nmol^{-1}.g^{-1}$ fresh weight of ADP-glucose respectively). Thus although trimming might have reduced the difference in grain filling performance between the two cultivars at 30/25°C, the effect of trimming could not have been wholly mediated through an increased level of ADP-glucose in Lyallpur at high temperature. Another factor must be involved.

If the rate of grain filling was simply dependent upon the efficiency of SSS for starch deposition then there should be a simple relationship between efficiency and rate. Comparison between Lyallpur and Kavko shows this is not the case, because efficiency in Lyallpur is lower than in Kavko. There are evidently differing relationships between efficiency, ADP-glucose levels and grain filling rates between the two cultivars.

Trimming resulted in an increased rate of grain filling in Lyallpur (Table 6.2) and also in an increase in the efficiency of SSS (Table 6.11). However, at 30/25°C there was no greater increase in efficiency in Lyallpur than in Kavko which could have accounted for the relatively greater increase in grain filling rate in Lyallpur than in Kavko at 30/25°C. Moreover, in untrimmed ears the efficiency of Kavko relative to that of Lyallpur fell more

with the temperature increase from 20/15°C to 30/25°C despite the higher Q_{10} in Kavko than in Lyallpur. Thus effects of temperature and trimming on efficiency do not parallel the effects on grain filling. There is too little information on the relationships between kinetic characteristics of SSS and starch deposition to allow an interpretation of these results. As stated above, there is no straightforward relationship between grain filling rates and efficiency of SSS. Nevertheless, in the two comparisons that have been examined (this work and that of Jenner and Sharma 1997) the most tolerant cultivars have the greatest efficiency of SSS.

7.5 Grain protein and starch composition

Grain quality may be modified at moderately high temperatures and at very high temperatures as a result of a change in the concentration and composition of grain protein (Randall and Moss 1990; Stone *et al.* 1996). Among different protein fractions, high molecular weight glutenins are known to have a significant role in the determination of dough strength (Gupta *et al.* 1993). In this study a shift in growth temperature from 20/15°C to 30/25°C increased the concentration of total protein and to a greater extent the concentration of HMW-glutenin in the flour (Table 5.24); the ratio of polymer: monomer was therefore increased at high temperature. The varietal differences for HMW-glutenin concentration were not significant (Fig. 5.6E); the differential temperature response of the two cultivars in terms of protein accumulation (Fig. 5.6C) was therefore associated with the response of the monomer fraction. An increase in the ratio was also reported by Stone *et al.* (1996) for the grains developed at 27/22°C but at 30/25°C the ratio at mature grains was less compared to 21/16°C. The decrease in polymer: monomer ratio at high temperatures has been related to a relative increase in the synthesis of monomer

(Blumenthal *et al.* 1990) or to a greater decrease in the synthesis of polymer (Stone *et al.* 1996a).

At the standard compared to the low supply of nitrogen the contribution of HMW-glutenin in the flour was elevated to a greater extent in Kavko (76%) than in Lyallpur (32%) (Table 5.24). The increases at the standard level of nitrogen tended to be greater at 20/15°C than at 30/25°C but the interaction between temperature and nitrogen was not statistically significant (Table 5.24). Other reports, however, indicate that there has been a decrease in the contribution of polymer under higher nitrogen supply. The decrease in polymer contribution in response to high N application has been attributed to a greater increase in gliadins (Dubetz *et al.* 1979), an increase in gliadins only (Doekes and Wennekes 1982), or an increase in gliadins together with a decrease in glutenin (Stenram *et al.* 1990). The dissimilarity between reports may be due to the differences between the fractionation methods used and/ or the different quantities of protein found in the grains developed under different growth conditions.

Amylopectin is the main contributor to starch quality and gives a higher viscosity and elasticity to the starch paste (Martin and Smith 1995). Only small changes in the ratio of amylose to amylopectin have big effects on starch gelatinisation which is important for noodle production (Oda 1980). High temperatures increase the amylose% (Shi *et al.* 1994; Tester *et al.* 1995) and lowers flour quality obtained from hot areas (Blumental *et al.* 1993). In the current study the grain amylose% was also higher (5%) at high temperature in Kavko but the amylose% was similar at both temperatures in Lyallpur (Fig. 5.5F). The effects of nitrogen on amylose% were dependent on temperature, as the increases in amylose% at high temperature were evident at the low but not at the standard level of

nitrogen. Although the interaction was not statistically significant, the amylose% was lower in the trimmed than in the untrimmed ears at 20/15°C but there appeared no effect of trimming on amylose% at 30/25°C.

7.6 Selection for heat tolerance and grain quality

The results of this and the other studies (Hunt *et al.* 1991; Wardlaw and Moncur 1995) show that there is a poor association between duration of grain filling and grain weight at high temperature and genotypic variation for the duration was small in relation to temperature. Therefore, the extension of the duration of grain filling does not seem to be a promising strategy to increase grain yield in areas with high temperatures during grain filling. Selection for earliness would be a possible way to avoid heat damage (Loss *et al.* 1989; Austin *et al.* 1989) but these varieties do not take advantage from the longer period of filling during cooler seasons (He Zhong-hu and Rajaram 1994). It would therefore be desirable to combine high yield potential in the absence of stress and the selection of characteristics that provide heat tolerance. In connection with the significant correlation of both attributes with individual grain weight at high temperature, a combination of a high rate of grain filling and a longer period between anthesis and the maximum rate (inflection point) would be appropriate in selection of cultivars for warm environments. At the molecular level, it seems that wheat cultivars in which soluble starch synthase (SSS) posses a higher efficiency may perform better under high temperature conditions. The evidence to support this idea is that the superior performance of Kavko and Trigo at high temperature compared to Lyallpur, in this study and that of Jenner and Sharma (1997), was associated with the efficiency of SSS. As there are several isoforms of SSS (Denyer *et al.* 1995), the identification of heat stable forms would also provide significant cultivar improvement in relation to heat tolerance (Wardlaw and Wrigley 1994). Heat shock

proteins (HSPs) play some unique roles in maintaining functions of vegetative parts in wheat plants during heat stress (Nguyen *et al.* 1994). As grain growth of wheat under high temperature is mostly limited by factors inside the grain (Jenner 1994), a study of the kinetics of HSPs and their role in thermotolerance in developing grains could result in improvement in heat tolerance in wheat.

The role of high molecular weight glutenins in determining grain quality and dough strength is now well evident. The development of genetic transformation for bread wheat is now allowing the manipulation of the amount of HMW-glutenin in transgenic plants (Shewry *et al.* 1997). In order to increase the proportion of amylopectin, which is associated with starch quality, wheat mutants that contain less amylose have recently been used to produce wheat lines that have essentially no amylose (Nakamura and Yamamori 1995; Hoshino *et al.* 1996). The application of transgenic technology to change the structure of amylopectin could also be another approach to improved starch quality (Tester and Karkalas 1997).

7.7 Conclusions

1) Significant variation existed among wheat cultivars in their response of grain filling to a sustained period of moderately high temperature.

2) Variation for duration of grain filling was small in relation to changes in temperature and final grain weight at high temperature was proportional to the changes in the rate of grain filling.

3) Neither the low response of the rate of grain filling to an increase in temperature nor the varietal differences in terms of the response of the grains to high temperature were associated to the availability of assimilates in the grains.

4) The nutritional status of the plants modified the response to temperature; only at the standard level but not at low nitrogen was the reduction in grain weight at high temperature greater in Lyallpur than in Kavko.

5) Trimming of the ears increased the weight of the remaining grains in Lyallpur but trimming had no significant effect on grain weight in Kavko.

6) Trimming of ears did not alter the delivery of sucrose to the grains but instead it appeared to influence processes in the pathway of the conversion of sucrose to ADP-glucose.

7) The affinity of SSS for its substrate amylopectin was significantly decreased at high temperature *in vivo* and to a greater extent in Lyallpur than in Kavko.

8) *In vitro* the differential responses of the efficiency of SSS in two cultivars to an increase in temperature were in parallel with differences in the temperature sensitivity of grain filling.

9) The most remarkable difference between the two varieties *in vivo* was in the absolute values of SSS efficiency as the most tolerant cultivar had the greatest efficiency of SSS.

10) The activity of SSS was substantially decreased at later stages of grain development but there was little developmental change in the activity of GBSS.

11) The concentration of HMW-glutenin in the grains was substantially higher at elevated temperature and also at the standard compared to the low level of nitrogen.

7.8 Comments for further study

1) The loss of grain yield at high temperature in intact plants under simulated field populations has been recently reported (Gibson and Paulson 1999) to be much greater than the losses reported in plants trimmed to a single culm. Therefore, it would be worthwhile to evaluate the tested cultivars under simulated field populations to evaluate the possible contribution of tillers on the differential response of cultivars to high temperature.

2) In this study like other related studies, the pool size of sucrose in the grains was considered as an indication of the availability of assimilates in developing grains. This does not take into account the possibility that the rate of the delivery of sucrose into the grains may change independently of the pool size. The study of the effect of temperature on the transfer of substrate from the crease vascular system of the grains as proposed by Wardlaw *et al.* (1995) may therefore give a more complete picture of the supply of assimilate into the grains at high temperature.

3) The efficiency of SSS in Lyallpur was lower at high temperature *in vitro* but the efficiency was the same at both temperatures *in vivo* indicating a possible adaptation under prolonged exposure to high temperature, which might be related to heat tolerant forms of SSS identified by Denyer *et al.* 1995. The characterisation of these isoforms may give a

better understanding of the differences in the response of the efficiency of SSS *in vitro* and in developing grains, and the involvement of kinetic properties of SSS in heat tolerance.

4) In a study by Wardlaw *et al.* (1989a) there was an interaction between light level and the temperature response of the cultivars. Alteration of light intensity was not used in present work. It would be therefore advisable to evaluate the effect of light level on the differential response of Kavko and Lyallpur to high temperature.

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