BRUIN, FREDERIEKE SOPHIA MATHILDA

THE EFFECT OF TEMPERATURE ON THE GERMINATION AND RESPIRATION OF KERNELS OF WHEAT *(TRITICUM AESTIVUM L.)*

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THE EFFECT OF TEMPERATURE ON THE GERMINATION AND RESPIRATION OF KERNELS OF WHEAT (*TRITICUM AESTIVUM* L.)

by

Frederieke Sophia Mathilda Bruin

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Supervisor: Prof. H.A. van de Venter

Co-supervisor: Mr. S. de Meillon

Opgedragen aan mijn liefhebbende Ouders...

DECLARATION

I hereby certify that this research, unless specifically indicated to the contrary in the text, is the result of my own investigation.

Frederieke S.M. Bruin

31st October 1994

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ADP	:	adenosine 5'-diphosphate disodium salt
Adn	:	total adenosine nucleotides
AEC	:	adenylate energy charge
AMP	:	adenosine 5'-monophosphate disodium salt
ATP	:	adenosine 5'-triphosphate disodium salt
BSA	:	bovine serum albumin
CO_2	:	carbon dioxide
cv.	:	cultivar
E_a	:	activation energy
EDTA	:	1 mM ethylenediaminetetraacetic acid (disodium salt)
GC	:	germination coefficient
K_{eq}	:	equilibrium constant
LSD	:	lowest significant difference
mm Hg	:	millimetre mercury
mRNA	:	messenger ribonucleic acid
m/v	:	mass per volume
nmol gas.min^{-1}.(13 kernels)$^{-1}$	:	nanomoles of gas per minute per 13 kernels
nmol O_2. min^{-1}.(13 kernels)$^{-1}$	:	nanomoles of oxygen per minute per 13 kernels

O_2	:	oxygen
PEP	:	phosphoenolpyruvate
$\%.h^{-1}$	:	percentage per hour
r	:	regression coefficient
RQ	:	respiratory coefficient
SHAM	:	salicylhydroxamic acid
TCA	:	aqueous solution containing 1 M trichloroacetic acid
TES	:	N-tris (hydroxymethyl) methyl-2-aminoethanesulfonic acid
TRIS-H_2SO_4-EDTA medium	:	20 mM tris(hydroxymethyl) aminomethane containing 1 mM EDTA and with the pH adjusted to 7.75 with sulphuric acid
2X TRIS-H_2SO_4-EDTA	:	as previous medium but containing 40 mM TRIS and 2 mM EDTA
UF	:	ultra free
v	:	volts
V_{alt}	:	alternative pathway
V_{cyt}	:	cytochrome oxidase pathway
V_{res}	:	residual pathway
V_t	:	total respiration activity
v/v	:	volume per volume

1. INTRODUCTION

Although the biochemical and physiological effects of heat shock have received much attention during the last decade, the scientific literature in general has been dominated by studies on the effect of sub-optimal rather than supra-optimal temperatures on germination (Segeta 1964; Taylor & Roweley 1971; Lyons 1973; Simon *et al.* 1976; Miedema 1982). This is probably due to the fact that the occurrence of sub-optimal temperatures is a bigger problem than that of supra-optimal temperatures in seedbeds in the northern hemisphere, where most research is done.

Seeds are often the first to encounter stress during a plant's life-cycle. Besides moisture, temperature is one of the more prominent environmental factors affecting seed germination (Murphy & Noland 1982a), with sub- and supra-optimal temperatures regimes being important environmental constraints. In general, the effects of temperature on germination are complex because it affects each stage of the germination process in a different way and is interdependent on other factors (Mayer & Poljakoff-Mayber 1982).

In commercial crop production, high temperatures generally result in lack of uniformity and poor stand (Guedes & Cantliffe 1980; Riley 1981a) placing a limitation on crop potential in many arable areas of the world (Basra *et al.* 1989).

Seed germination is strongly temperature dependent (Bewley & Black 1983; Kamaha & Maguire 1992), and germination capacity is expected to follow a normal distribution over a wide temperature range (Abernethy *et al.* 1989; Hampson & Simpson 1990). The optimal temperature for germination of wheat kernels is about 20°C although differences are encountered among varieties and seed lots (International Seed Testing Association 1985). Germination declines sharply as temperature rises above the optimum (Mayer & Poljakoff-

Mayber 1975; Hampson & Simpson 1990; Ojeda & Trione 1990; Small *et al.* 1993). In some seeds the inhibition of germination at supra-optimal temperatures is released after the ungerminated seeds are transferred to optimal temperatures (Ching 1975; Emerson & Minor 1979; Wurr & Fellows 1984). This type of inhibition of germination is called thermo-inhibition (Vidaver & Hsiao 1975).

The limiting process that causes inhibition of wheat, *Triticum aesivum* L. cv. Betta germination is, as yet, unidentified.

Previously, a reduction in the ability to germinate has been explained by the inability of seed membranes to function as efficient semi-permeable membranes (Leopold 1980; Murhpy & Noland 1982b; Suganuma *et al.* 1985). An increase in temperature was thought to increase the rate of water uptake thereby increasing germination rates (Becker 1960; Lafond & Baker 1986), and thus affecting other metabolic processes leading to germination (Allerup 1958; Abernethy *et al.* 1989). According to Lindstrom *et al.* (1976) and Lafond and Baker (1986) the differences in the rate of germination could not be attributed to the differences in the rate of water uptake in wheat kernels (*T. aestivum*).

Several authors suggested that respiratory metabolism was intricately involved in the induction of thermo-inhibition (Woodstock & Grabe 1967; Suganuma *et al.* 1985; Small *et al.* 1993). The rate of respiration of seeds during incubation is also one of the most frequently used criteria for determining the rate of metabolism, because seed respiration rate during early incubation is directly related to the percentage germination (Woodstock & Grabe 1967). In studies by Morohashi and Shimokoriyama (1975a,b) on *Phaseolus mungo* L. seeds, the respiration rate seemed to be inhibited during early imbibitional stages causing inhibition of germination. The data of Woodstock and Grabe (1967), Mukherejee *et al.* (1973) and Suganuma *et al.* (1985), studying germinating wheat (*Triticum aestivum* L.) rice

rice (*Oryza sativa* L.) and spinach (*Spinacia oleracea* L.) seeds respectively, also supported these results. Unpublished results of a technique study on ground wheat kernels which preceded the report by Van de Venter and Grabe (1989), showed that the rate of oxygen uptake increased as temperature increased to 40°C (personal communication: Prof. H.A. van de Venter, Department of Botany, University of Pretoria). In agreement with the findings on *Zea mays* L. embryos (Riley 1981a), the respiratory oxidation of these wheat kernels was not thought to be the limiting factor causing the inhibition of germination at supra-optimal temperatures (40°C). The preliminary study of Van de Venter and Grabe (1989) motivated the further investigation on wheat respiration conducted in this research project.

If indeed the respiration of seeds was affected by supra-optimal temperatures, it could be expected that the changes in the respiration rate would cause changes in the ATP content, since ATP production in aerobic organisms is associated with mitochondrial oxidation (Haferkamp *et al.* 1977). Maize kernels which failed to germinate when exposed to a supra-optimal temperature (41°C), did not have a lower ATP content than kernels at the optimal temperature (25°C), which implies that the energy metabolism was not the factor limiting germination at supra-optimal temperatures (Riley 1981a). On the other hand, inhibition of germination of lettuce seeds at supra-optimal temperatures was thought to be due to a disruption of respiratory activity, and therefore, decreased energy levels (Small *et al.* 1993). Osborne (1983) found that the ATP content of isolated wheat embryos was sufficient for early biosynthetic processes necessary for early germination, thereby implying that factors other than the energy metabolism were causing the decrease in germination percentage at supra-optimal temperatures. Factors other than energy supply would therefore appear to pose a limit upon the germination process in wheat embryos, such as, for instance, protein synthesis (Osborne 1983).

The aim of the present study was to establish whether the respiratory activity of wheat, *Triticum aestivum* cv. Betta, was a limiting factor at supra-optimal temperatures which inhibited germination.

2. THE RESPONSE OF WHEAT KERNEL GERMINATION TO TEMPERATURE

2.1 INTRODUCTION

Temperature is one of the more prominent environmental factors affecting seed germination (Mayer & Poljakoff-Mayber 1982; Murphy & Noland 1982a). According to Ching (1975) and Kamaha and Maguire (1992), seed germination is strongly temperature dependent. For the temperatures to which seeds are normally exposed in nature (*ca.* 0 to 40°C), a temperature optimum is found where the highest percentage germination is attained in the shortest time (Bewley & Black 1983). The optimum temperature may differ for seeds of various species, but normally falls in the 25 to 30°C region. Supra-optimal temperatures for germination refer to temperatures higher than the optimal temperature range for germination.

Little research has been conducted on the germination response of wheat to supra-optimal temperatures. In general, a low percentage germination is found in seeds incubated at supra-optimal temperatures (Emerson & Minor 1979; Wurr & Fellows 1984). If seeds that do not germinate at supra-optimal temperatures in neither light or dark, do so after being transferred to optimal temperatures, the seeds are said to have been thermo-inhibited (Vidaver & Hsiao 1975). As temperatures increase, germination rates may increase, possibly due to increased imbibitional rates (Allerup 1958; Abernethy *et al.* 1989). Metabolic processes then proceed more quickly until the optimum temperature is reached (Hampson & Simpson 1990), resulting in an increase of all metabolism leading to the germination process (Abernethy *et al.* 1989). At supra-optimal temperatures however, some metabolic processes are inhibited, leading to the inhibition of germination. Germination percentage is, therefore, expected to follow a normal distribution, enabling the identification of the cardinal

temperatures; the minimum, optimum and maximum temperatures.

In this investigation, the effect of temperature, ranging from 10-40°C, on the germination response of wheat kernels, *T. aestivum* cv. Betta, was investigated, in order to determine the optimum temperature. The supra-optimal temperature range could then be defined for the purpose of designing subsequent experiments.

2.2 MATERIALS AND METHODS

2.2.1 Wheat kernels

Triticum aestivum L. cv. Betta kernels, harvested at Reitz during December 1990, were obtained from the Wheat Board, and stored in sealed bottles at ambient temperature. Kernels of uniform size were selected (> 2.5 mm diameter), as it is known that seed size has an effect on the rate of water uptake and germination (Lafond & Baker 1986).

2.2.2 Germination assay

A kernel was regarded as germinated when the radicle appeared through the coleorhiza.

Fifty kernels were placed in 90 mm diameter Petri dishes, containing two layers of Schleicher & Schuell number 0860 filter paper and 10 cm^3 of distilled water. Each temperature treatment was replicated four times and Petri dishes randomized in a Labcon growth chamber at constant temperature with a maximum temperature fluctuation of *ca.* 0.5°C. There was no light inside the incubator but kernels received diffused light daily when counted. The time interval from the addition of water to the Petri dish to the appearance of

the radicle, was regarded as the time taken for germination.

Kernels that failed to germinate before the pre-selected time, were then placed under optimum temperature conditions to check viability.

2.2.3 Temperature treatments

To ensure that equilibrium conditions were reached at the relatively high temperatures used, a pre-equilibration period of 12 h was applied. Prepared Petri dishes, distilled water and wheat kernel samples, in paper bags, were pre-equilibrated at the respective temperatures. Incubation temperatures of 10, 15, 20, 25, 28, 35 and 40°C were chosen, since high temperatures were investigated and the upper limit of 40°C was regarded as a temperature too extreme for normal germination.

2.2.4 Germination variables

2.2.4.1 Cumulative germination

Germination was scored cumulatively every 30 min for the first 2.5 h, and thereafter, at 4.5, 5.5, 7.5, 11.5, 24, 48.5 and 72.5 h after the start of imbibition. This was expressed as percentage germination.

2.2.4.2 Germination capacity

The final percentage germination was determined after 72.5 h and was represented as a percentage of the total number of grains incubated.

2.2.4.3 Rate of germination

The rate of germination can be represented in many ways. Mean germination time, i.e., the rate-index of Ellis and Roberts (1980), was used in this study. Non-viable kernels are excluded from this formula (Ellis & Roberts 1980).

$$\textit{Mean germination time (hours)} = \frac{\sum (h \,.\, n)}{\sum n}$$

$\sum$ = *the summation of*
n = *number of kernels germinated at hour h*
h = *incubation time, hours*

2.2.4.4 Germination coefficient

Germination coefficient (GC) is the final germination percentage divided by the mean germination time (Chern & Sung 1991), and is expressed in $\%.h^{-1}$. It provides a comparable picture of the rate to obtain the germination capacity.

2.2.4.5 Viability Check

After 72.5 h, all non-germinated kernels were transferred to the optimum temperature, 28°C, to check for viability. At 40°C germinated, non-germinated viable, and dead kernels were expressed as a percentage of the original amount of kernels incubated.

2.2.5 Statistical Analysis

The experiment was planned as a completely random design with a 7 x 7 factorial (temperature and hours of imbibition as factors) arrangement of treatments. Four replicates were used. The least significant difference (LSD) (Student's t-test) was calculated for data for the cumulative germination, germination capacity, mean germination time, and the germination coefficient.

2.3 RESULTS

According to the cumulative germination curve (Figure 2.1), showing the effect of temperature on germination at various time intervals (Scott *et al.* 1985), 28°C may be regarded as the optimum temperature for wheat kernel germination. The curves for 24 and 48.5 h of imbibition are so broad that one temperature cannot be singled out of the optimum temperature range (10-28°C) (Figure 2.1). A significant decrease in the percentage germination occurred at 40°C (Figure 2.1).

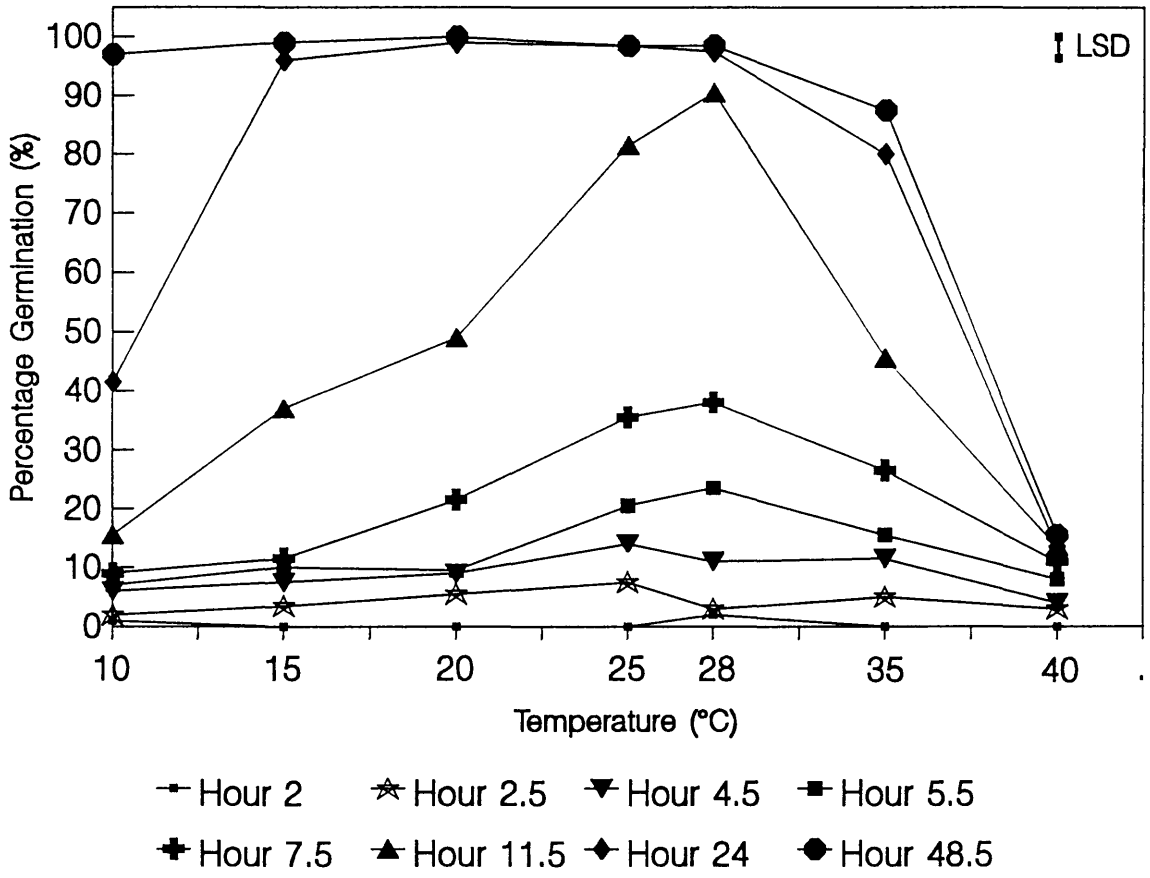

Figure 2.1 The effect of temperature on the germination of *Triticum aestivum* cv. Betta kernels as determined after various incubation periods.

The germination capacity was not significantly affected within the temperature range of 10-28°C (Table 2.1). Germination capacity diminished significantly when seeds were exposed to a temperature of 35°C and above (Table 2.1). At 40°C physical splitting of some wheat kernels occurred, and a final germination capacity of 15 % resulted. Distinction between dead and viable non-germinated kernels at 40°C was achieved by transferring non-germinated kernels to the optimum temperature, 28°C, after 72.5 h. Forty seven percent of these kernels were found to be viable, while 37 % were dead.

Table 2.1 Final germination percentage, mean germination time, and the germination coefficient of kernels of *Triticum aestivum* cv. Betta incubated at different temperatures for 78.5 h

Temperature (°C)	Germination capacity (%)	Mean Germination Time (h)	Germination Coefficient ($\%.h^{-1}$)
10	97.0*c**	27.281*d*	3.555*b*
15	99.5*c*	18.822*c*	5.286*c*
20	100.0*c*	16.358*b*	6.113*c*
25	98.5*c*	11.064*a*	8.902*d*
28	98.5*c*	9.498*a*	10.370*e*
35	91.0*b*	18.665*bc*	4.875*c*
40	15.5*a*	8.645*a*	1.793*a*

*Values followed by the same letter are not significantly different at $p = 0.05$.

The mean germination time, which is a measure of the average time it takes for seeds to germinate, decreased with temperature (Table 2.1). The only exception was the mean germination time at 35°C, which had a relatively high value (Table 2.1).

The rate of germination, represented as mean germination time, was found to be misleading in the case of the 40°C data because, although the average time to germinate was 8.6 h, only 15.5 % of the kernels germinated. The germination coefficient, which is the ratio of the final germination capacity and the mean germination time, was then calculated. The germination coefficient (GC) increased progressively from 10 to 28°C by 6-10 $\%.h^{-1}$, and then decreased at 35 and 40°C to 5 and 2 $\%.h^{-1}$, respectively (Table 2.1). The inhibitory effect of high temperatures on germination percentage and GC was evident (Table 2.1).

2.4 DISCUSSION

Germination curves for wheat kernels can be quantitatively described by three parameters; the length of the lag period before the first kernels germinate, the maximum percentage germination attained and the rate of germination (Meyers et al. 1984; Garcia-Huidobro *et al.* 1985). Temperature may affect all of these parameters (Meyers *et al.* 1984).

The length of the lag period, before the first germination of the wheat kernels at the optimum temperature of 28°C commenced, was 2 h after the start of imbibition. As the incubation period increased, more kernels germinated at temperatures below and above 28°C, so that broad cumulative curves were obtained from which the optimal temperature was not easily distinguished.

A maximum percentage germination of 97% was obtained for all temperature treatments between 10 and 28°C, which agrees with the results of Addae and Pearson (1992) who studied wheat germination over the temperature range of 8-25°C. Although, germination percentages were similar (91-100 %) for the various temperature treatments (10-35°C), except for the 40°C treatment where a mere 15 % of the kernels germinated, the germination rates differed between the temperature treatments. According to the literature, the mean germination time decreases with temperature until the optimum temperature for germination is reached; in wheat this was at 28°C. Information on how the temperature/mean germination time curve would progress in the region of supra-optimal temperatures, could not be found. One would expect the mean germination time to increase with temperature in the supra-optimal temperature range, as a consequence of the retarding effect which such temperatures have on germination. This expectation was not realized for the 40°C treatment, probably because the treatment was so harsh that only 15 % of the kernels could germinate. Thus the mean germination time on its own is not sufficient to draw reliable conclusions on the effect of supra-optimal temperature on the rate of germination. Therefore the germination coefficient which takes both the germination percentage and the mean germination time into account, was considered. The detrimental effect of supra-optimal temperature is now evident, as the germination coefficient increases gradually with temperature until the optimum temperature is reached, thereafter decreasing sharply in the supra-optimal temperature region. This tendency is in agreement with Garcia-Huidobro *et al.* (1982) and Chern and Sung (1991).

The extreme temperature of 40°C inhibited wheat germination and Hampson and Simpson (1990), and Ojeda and Trione (1990) reported that the germination of seeds of many species are inhibited at temperatures as high as 40°C. Contradictory to findings on rice

seeds, wheat germination was more severely affected by high temperature during the later phase and less in the early phase of germination (Mukherjee *et al.* 1973). In this study, ungerminated wheat kernels incubated at 40°C were found to be viable even after 48 h of heat treatment where 47 % germinated after transfer to optimal temperature, therefore being thermo-dormant. This means that 37 % of the kernels were killed by the supra-optimal temperature treatment (40°C). It is possible that, during at least the initial hours of imbibition, the rate of events leading to germination was not significantly altered by the extreme temperature, as stated by Abernethy *et al.* (1989) who worked with heat shock treatments up to 52°C. To investigate this statement, one of the most important early events, the imbibition of the seeds (Chapter 3), was investigated to ascertain whether imbibition was responsible for germination differences at various temperatures.

3. THE EFFECT OF TEMPERATURE ON THE WATER UPTAKE OF WHEAT KERNELS

3.1 INTRODUCTION

Although it has frequently been demonstrated that the rate of imbibition of seeds is temperature-dependent (Brown & Worley 1912; Murphy & Noland 1982a,b; Vertucci & Leopold 1983), the reasons for this phenomenon have remained controversial (Murphy & Noland 1982b). In seeds of radish (*Raphanus sativa* L.) and sugar pine (*Pinus lambertiana* L.), which have permeable seed coats, bulky storage tissue and low starch contents, water uptake followed the change in the viscosity of water as the temperature changed (Murphy & Noland 1982b). In seeds with a high starch content, however, no relationship between the rate of water uptake and its viscosity was found, and the effect of temperature on the rate of water uptake varied with time of imbibition (Allerup 1958; Mayer & Poljakoff-Mayber 1975; Leopold 1980; Riley 1981a). Dramatic changes with time in the activation energy (E_a) of water uptake (Blacklow 1972) suggested that there were several mechanisms governing water uptake and each may have a different temperature coefficient (Vertucci 1989).

Slow germination in a low temperature range (10-25 °C) has been related to a low rate of water uptake (Addae & Pearson 1992). According to Bewley and Black (1985), the membrane protein composition or minor membrane components (e.g., sterols), may differ in different temperature tolerant cultivars. Changes such as temperature variation, could therefore result in changes in properties of the membranes including their permeability and consequently affect the ability of the seeds to germinate (Nola & Meyer 1986). Supra-optimal temperatures cause a reduction in the ability to germinate, possibly due to the

inability of seed membranes to function as efficient semi-permeable membranes beyond critical temperatures (Leopold 1980; Murphy & Noland 1982b; Suganuma *et al.* 1985). Imbibition at supra-optimal temperatures was therefore investigated.

Although an investigation of aerobic respiration was the main aim of this study, the effect of temperature on water uptake was determined since moisture content and the respiration rate of seeds are closely associated (Bakke & Noecker 1933). During the pre-germinative phase of 0-2 h of imbibition, the water uptake rate was determined and an Arrhenius plot drawn up. A temperature range of 10-40°C was used.

3.2 MATERIALS AND METHODS

3.2.1 Wheat kernels

Kernels of *T. aestivum* cv. Betta as described under 2.2.1 were used.

3.2.2 Imbibition Assay

Air-dry kernels (as described in section 2.2.1) in paper bags and Petri dishes (90 mm diameter), containing two layers of Schleicher & Schuell number 0860 filter paper and 10 cm^3 of distilled water, were temperature pre-equilibrated for 12 h at temperatures of 10, 15, 20, 25, 28, 35, and 40 °C. Four replicates of 50 kernels per temperature were used. The initial fresh mass of each 50 kernel sample was determined after temperature-equilibration and the samples were incubated in prepared Petri dishes in Labcon growth cabinets at the constant temperatures listed above, with fluctuations of *ca.* 0.3°C. Water absorbtion was measured gravimetrically every 15 min and recorded as a percentage of the original fresh

mass. After 2 h of imbibition, germination commenced at the higher temperatures (28 and 35°C) and the measurements for water uptake were terminated. Radicle emergence may be associated with renewed water uptake which is to be distinguished from imbibitional water uptake (Trione & Cony 1990).

3.2.3 Arrhenius plot

The effect of temperature on the activation energy for water uptake was deduced from the Arrhenius plot. For setting up Arrhenius plots, it was assumed, according to findings of Leopold (1980), that water uptake during the first 15 min was linear at all temperature treatments. The logarithm of the average water uptake rates obtained for the temperature treatments were plotted against the reciprocal of the absolute temperature (1/T), to obtain the Arrhenius plot (Vertucci & Leopold 1983). Activation energies were calculated using the Arrhenius equation as quoted by Glasstone and Lewis (1963).

$$E_a = -[\, k \,.\, (2.303 \,.\, R)]$$

E_a = *Activation energy, cal.mol* $^{-1}$
k = *slope of Arrhenius plot, degree Kelvin*
R = *gas constant*, 1.987 *cal.degree Kelvin* $^{-1}$*.mole* $^{-1}$

3.2.4 Statistical analysis

The experiment was planned as a random design with a 7 x 7 factorial (temperature and minutes of imbibition as factors) arrangement of treatments. Four replicates were used. The least significant difference (LSD) (Student's t-test) was calculated for the data of water uptake over time and the water uptake over the square root of time, at the 5% level.

3.3 RESULTS

The time course shown in Figure 3.1 illustrates that the rate of water uptake by wheat kernels increased significantly with temperature, and was therefore a temperature-dependent process. The uptake of water was marked by an initial, rapid water uptake (0-15 min) which decelerated (from 15 min to 2 h after the start of imbibition) such that the data fitted a regression curve with a correlation coefficient of 0.991 (Figure 3.1). Although the water content of the seeds increased continuously with imbibition time, the rate of water uptake decreased with time (Figure 3.1).

At the onset of germination at 28°C, 2 h after the start of imbibition, wheat kernels incubated at 10°C contained 21 % moisture on a dry mass basis, whilst those at 40°C contained 34 % moisture.

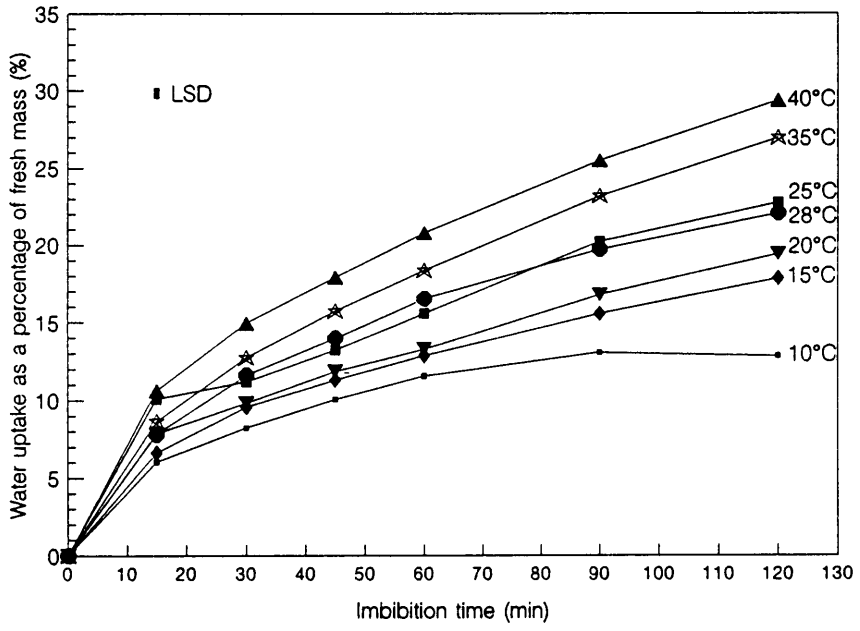

Figure 3.1 The effect of temperature on the water uptake of *Triticum aestivum* cv. Betta kernels during the first 2 h of incubation.

The Arrhenius plot permits one to compare more specifically the water uptake responses at a specific time to temperature (Figure 3.2), and allows the calculation of the apparent activation energies (E_a) for the imbibition process. According to the Arrhenius plot (Figure 3.2), the rate of water uptake increased at higher temperature treatments, with a slope of -642.2 (Figure 3.2).

Activation energy (E_a) for water uptake from 10 to 40°C was approximately 2.9 Kcal.mol^{-1}.

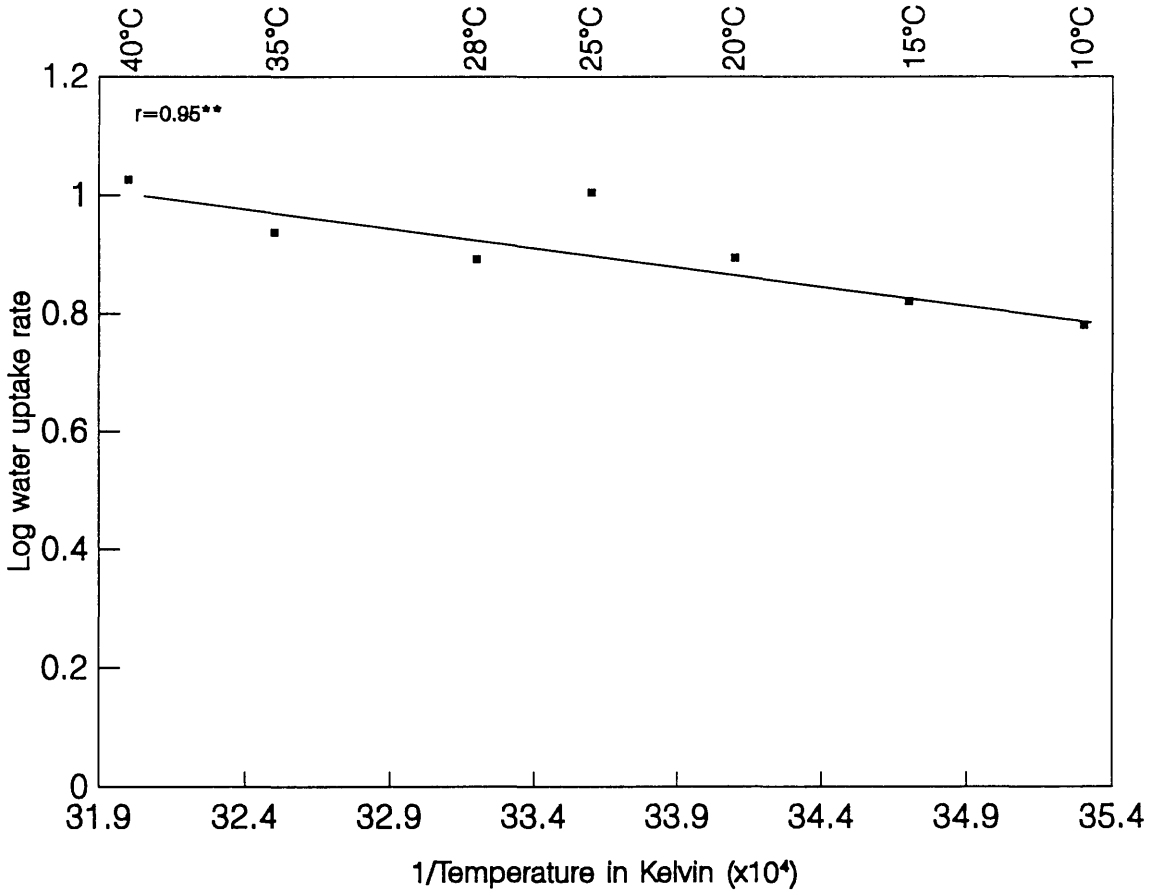

Figure 3.2 The Arrhenius plot of the rate of imbibition by *Triticum aèstivum* cv. Betta kernels during the first 15 min of imbibition.

The moisture gain of wheat in water should be approximately proportional to the square root of the absorption time, if the diffusion equation is applicable (Becker 1960). The moisture gain of wheat was indeed found to be proportional to the square root of the imbibition time over 2 h (Figure 3.3).

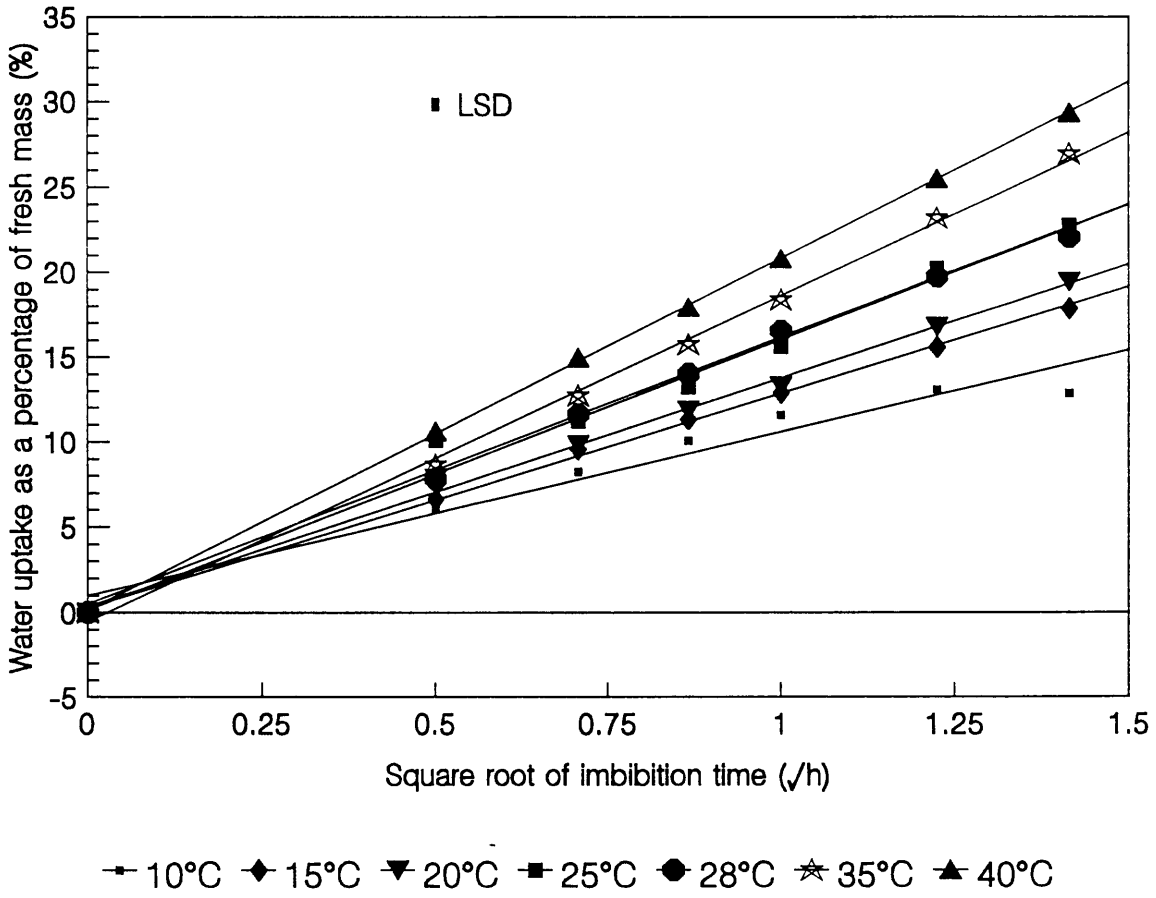

Figure 3.3 The relationship between moisture gain and the square root of imbibition during the initial 2 h of imbibition of *Triticum aestivum* cv. Betta kernels.

3.4 DISCUSSION

Water uptake in kernels of *T. aestivum* cv. Betta was found to be temperature dependent and increased with temperature, supporting the findings of Allerup (1958), Becker (1960) and Lafond and Baker (1986) on wheat. Blacklow (1972), Murphy and Noland (1982b), Vertucci and Leopold (1983) and Addae and Pearson (1992), found similar tendencies in seeds of several other species.

The fact that the rate of wheat kernel water uptake decreased with time supported the Continuity equation, which states that, as the concentration of water in the seed increases with time, the flux decreases as water penetrates the seed (Vertucci 1989).

The rapid initial wetting phase was found to be highly dependent on temperature (Clarkson & Sanderson 1969). Vertucci and Leopold (1987) suggested that the wetting phase was affected by temperature because biopolymers unfolded at faster rates allowing more sites for absorption at higher temperatures, the extent depending on seed species and tissue type. In seeds, the chief component which imbibes water is protein, while starch only swells after treatment at high temperatures (Mayer & Poljakoff-Mayber 1982). Wheat represents kernels rich in starch and poor in proteins and fats (Allerup 1958). At 40°C, starch swelling may have occurred, which may have increased the amount of tissue imbibing water. This may explain the physical splitting of wheat kernels observed at this temperature and the low final germination percentage of 15 %. Similar findings have been documented in several legume species (Roos & Pollock 1971 in Chern & Sung 1991; Woodstock & Tao 1981 in Chern & Sung 1991).

The mechanism by which the rate of imbibition is affected by temperature has remained controversial. The E_a value reported here (2.9 Kcal. mol^{-1}) for water uptake by

wheat kernels during the initial wetting phase, is lower than the E_a value for the viscosity change of water (Vertucci & Leopold 1983). It may thus be concluded that the increase in water uptake of wheat kernels with temperature in the range 10-40°C, is probably not only due to changes in the viscosity of water. The moisture gain of wheat kernels reported here, correspond to findings of wheat by Becker (1960), that the moisture gain of wheat is proportional to that of the square root of the imbibition time. This shows conformity with Fick's Law of diffusion. However, while the E_a for diffusion processes is represented by the range of E_a values from 4 to 5 kcal.mol^{-1}, an E_a lower than this (3 kcal.mol^{-1}) was obtained in wheat kernel imbibition during the first 15 min of imbibition.

It is probable that higher temperatures affect not only water uptake, but also protein denaturation and the rate of chemical reactions. The rate of chemical reactions are expected to increase with temperature until the optimum temperature is reached (Salisbury & Ross 1992). It is difficult to distinguish between direct effects of temperature on metabolism and indirect effects via increased water uptake. For instance, Woodstock and Grabe (1967) who studied maize, found differences in respiration to occur within 2 h after the start of imbibition due to temperature treatments. During this initial phase of water uptake the rate of O_2 uptake has been found to be directly related to subsequent seed germination (Woodstock & Grabe 1967). The initial aim of this study, was to determine the effect of temperature on aerobic respiration of wheat kernels. Thus, after characterizing water uptake, a study of respiration was undertaken (Chapter 4).

4. THE EFFECT OF TEMPERATURE ON WHOLE WHEAT KERNEL RESPIRATION

4.1 INTRODUCTION

Aerobic respiration is an oxidation process, with oxygen as final electron acceptor, accompanied by ATP production (Hackett 1959), a usable energy source within the cell (Hackett 1959). Under anaerobic conditions, fermentation, is another, less effective process for obtaining ATP or energy (Bewley & Black 1985). Germination is an energy requiring process and is therefore dependent on the respiration of the seed (Mayer & Poljakoff-Mayber 1982; Bewley & Black 1983; Stewart *et al.* 1990). As respiration has often been incompletely defined, it is emphasized that respiration consists of both oxygen uptake and carbon dioxide evolution.

A sharp rise in respiration rate was detected during early imbibition (Opik & Simon 1963; Paul & Mukherji 1972), and attributed in part to activation of mitochondrial enzymes already present in the dry seed (Paul & Mukherji 1972; Riley 1981a), and in part to the growth and development of the seed tissue (Opik & Simon 1963). Striking correlations were noticed between respiratory rate during the early imbibition phase and subsequent seed germination (Woodstock & Grabe 1967). Respiration during imbibition may therefore serve as an index of overall metabolic activity (Mukherejee *et al.* 1973), and respiration could be used for determining the effect of temperature on metabolism.

It is not yet clear how high temperatures, resulting in poor germination, affect respiration during early imbibition (Suganuma *et al.* 1985), and two main views have been presented in the literature. Firstly, because a direct correlation exists between germination

and respiration rates (Woodstock & Grabe 1967), and germination rates decrease at high temperature treatments, Woodstock and Grabe (1967), Mukherejee *et al.* (1973), Morohashi and Shimokoriyama (1975a,b) and Suganuma *et al.* (1985) suggested that inhibition of respiration was the cause of inhibition of germination. On the other hand, Ching (1975) suggested germination failure at 30°C, in clover (*Trifolium incarnatum* L.) seeds, to be due to uncontrolled biosynthetic processes depleting the energy pool. According to Weidner and Ziemens (1975) breakdown of metabolism then occurs by increased proteolytic activity and other enzyme-inactivating metabolic activities. In germinating maize embryos, protein synthesis was found to be an especially temperature-sensitive process (Riley 1981b), while respiration was not impaired at the same supra-optimal temperature, and was therefore probably not the factor resulting in reduced germination percentages at high temperatures (Riley 1981a; Ismail *et al.* 1989).

A mechanism of adaptation to supra-optimal temperatures has been recognised in some plants. Contradictory to expectations, some plants were able to maintain high rates of protein synthesis at supra-optimal temperature. This phenomenon was called "inverse compensation" by Weidner and Ziemens in 1975. Plants that exhibited this mechanism, were found to cope better with heat stress. This mechanism of adaptation to relatively high temperatures represents a stimulation of one or more physiological process (Weidner & Ziemens 1975). Support for this hypothesis comes from a study of the rate of protein synthesis of wheat seedlings, which increased with temperature, thereby probably improving the ability of wheat to cope with heat stress (Weidner & Ziemens 1975). Respiration rates of wheat kernels, *T. aestivum* cv. Betta, were therefore expected to increase with temperature, if the inverse compensation hypothesis is valid.

In the investigation reported in this chapter, the effect of temperature on the rate of

O_2 uptake and CO_2 emission was determined, 2 and 6 h after the start of imbibition, in order to investigate whether respiration was the cause of inhibited kernel germination at supra-optimal temperatures.

4.2 MATERIALS AND METHODS

4.2.1 Wheat kernels

Kernels of *T. aestivum* cv. Betta as described under section 2.2.1 were used in this study.

4.2.2 Respiratory assay

Kernels were incubated in Petri dishes (90 mm in diameter) in the dark, for 1 and 5 h prior to respiratory assays, in Labcon chambers at temperatures indicated in the following section (4.2.3). The Gilson differential respirometer was used for the determination of gas exchange. Reaction flasks were lined with one layer of filter paper to which 1 cm^3 of distilled water was added (de Visser *et al.* 1990). Twenty five intact, ungerminated kernels were then transferred to the prepared reaction flasks.

A fluted paper wick was placed in the centre well of each reaction flask, which increased the surface area of exposure of the solution used in the centre well. Potassium hydroxide (KOH) (0.2 cm^3, 15% m/v) was placed in the centre well of the reaction flask to absorb the carbon dioxide in the system when oxygen uptake was measured, and to standardize experimental conditions, 0.2 cm^3 of distilled water was used when gas exchange was

measured.

After the respiration flasks were connected to the respirometer, they were shaken at 100 oscillations per min in a water bath kept constant at the appropriate temperature. A period of 30 min was allowed for equilibration. The manometer valves were closed and readings were taken at 10 min intervals over a period of *ca.* 2 h.

Gas exchange rates were calculated after transformation of the data to conditions of standard temperature and pressure. Results were expressed in nmol gas.min^{-1}.(13 kernels)$^{-1}$.

4.2.3 Temperature treatments

To ensure that equilibrium conditions were reached at the relatively high temperatures used, a pre-equilibration period of 12 h was applied. Prepared Petri dishes, distilled water, and wheat kernel samples, in paper bags, were pre-equilibrated at the respective temperatures. Incubation temperatures for respiratory measurements were 22, 25, 28, 32, 35, 40, and 44°C, for the 2 h imbibed kernels. For the 6 h treatment, 25, 30, 35 and 40°C temperature treatments were used. Kernels were imbibed for 2 or 6 h at the corresponding temperatures after which respiratory experiments were commenced. Unlike similar experiments reported in the literature (Opik & Simon 1963; Murphy & Noland 1982b; Lecat *et al.* 1992), the respiratory measurements were performed at the same temperatures as the incubation temperatures.

4.2.4 Respiratory variables

4.2.4.1 Oxygen uptake

Measurements were made using 15 % potassium hydroxide (KOH) in the centre well of the reaction flasks. The correction factor was used to correct readings to standard temperature and pressure, according to the formula used by Umbreit *et al.* (1972).

$$\Delta V_{g\cdot} = \frac{273.(P - P_w) \cdot \Delta V_g}{P\cdot \cdot T_w}$$

$\Delta V_{g\cdot}$ = *mm* ^{3} *at STP*
ΔV_g = *experimental readings mm* ^{3}
P = *Air pressure, mm Hg*
P_w = *Vapour pressure of water at temperature* T_m
$P\cdot$ = *Standard pressure,* 760 *mm Hg*
T_m = *Micrometer temperature, degree Kelvin*

Conversions were then made to express the oxygen uptake in nmol $O_2.min^{-1}.(13\ kernels)^{-1}$.

4.2.4.2 Carbon dioxide emission

Measurements of gas exchange were made using distilled water in the centre well of the reaction flasks. To obtain the value for carbon dioxide release the formula of Umbreit *et al.* (1972) was used:

Uncorrected CO_2 release = net gas release + uncorrected O_2 uptake

The following formula of Gregory and Winter (1965) was used for the correction of volume CO_2 release to standard temperature and pressure.

$$\Delta V_{g\cdot} = \Delta V_g \cdot \left(\frac{P - P_r}{P\cdot \cdot T}\right).\left(\frac{[T\cdot + T_w \cdot v \cdot (BC - 0.0355)]}{V_g}\right)$$

$\Delta V_{g\cdot}$ = *mm ^{3} at STP*
ΔV_g = *uncorrected CO_2 release, mm ^{3}*
P = *Air pressure mm Hg*
P_r = *Vapour pressure of water at room temperature, mm Hg*
$P\cdot$ = *Standard pressure, 760 mm Hg*
T = *Room temperature, degree Kelvin*
$T\cdot$ = *Standard temperature, 273 Kelvin*
T_w = *Temperature of water bath, degree Kelvin*
V = *Volume of fluid of respirometer, mm ^{3}*
BC = *Bunsen coefficient for CO_2 at water bath temperature*
V_g = *Total gas volume of respirometer at beginning of experiment, mm ^{3}*

Conversions were then made to express the carbon dioxide released in nmol $CO_2.min^{-1}.(13\ kernels)^{-1}$.

4.2.4.3 Respiratory quotient (RQ)

Respiratory quotient (RQ) is the ratio of CO_2 emission to O_2 uptake, and is often near unity. If carbohydrates such as sucrose, fructose, or starch are the respiratory substrate, and if they are completely oxidized, the volume of O_2 taken up exactly balances the volume of CO_2 released (Salibury & Ross 1992).

4.2.4.4 Arrhenius Plot

Arrhenius plots of the logarithm of respiration rate against the reciprocal of the absolute temperature (Pomeroy & Andrews 1975) were obtained from the respiration data by calculating regression lines after 2 and 6 h of imbibition. An inflection in the Arrhenius plot for a physiological process at high temperature is usually indicative of the denaturation of proteins or enzymes above the inflection temperature and indicate an alteration of that process (Salibury & Ross 1992). The effect of temperature on the E_a for aerobic respiration was deduced from the Arrhenius plots. Activation energies were calculated using the equation in Glasstone and Lewis (1963).

$$E_a = - [k \cdot (2.303 \cdot R)]$$

E_a = *Activation energy, cal.mol* $^{-1}$
k = *Slope of Arrhenius plot, degree Kelvin*
R = *Gas constant*, 1.987 *cal.degree Kelvin* $^{-1}$*.mol* $^{-1}$

4.2.5 Statistical analysis

The experiment was planned as a completely random design. The mean of six replicates of gas exchange at each temperature was determined. Analyses of variance were performed and least significant differences (LSD) (Student t-test), calculated at the 5% significance level.

4.3 RESULTS

The results of O_2 uptake and CO_2 emission after 2 h (Figure 4.1) and 6 h (Figure 4.2) of imbibition can be interpreted in two ways. Firstly, the rate of O_2 uptake after 2 h of imbibition increased with temperature to a maximum at 35°C, followed by a slight decrease at 40 and 44°C, while the rate of CO_2 emission tended to increase to a maximum at 44°C (Figure 4.1). This interpretation assumes that the values for O_2 uptake at 32°C, and those for CO_2 emission at 32 and 40°C, represent anomalous experimental variations from the trends described.

After 6 h of imbibition, the rate of O_2 uptake increased with temperature to a maximum at 40°C, while the CO_2 emission rate increased to 30°C after which it decreased (Figure 4.2). In this case the O_2 uptake at 35°C is interpreted as being anomalously low.

Secondly, the results can be described as being biphasic, where the O_2 uptake and CO_2 emission rates after 2 h of imbibition, followed an increase from 22°C to an optimum at 28-32°C. At 35°C however, O_2 uptake showed a second peak, followed by a slight decrease at 40 and 44°C (Figure 4.1). CO_2 emission, increased to a plateau at 35 and 40°C, followed by an increase at 44°C (Figure 4.1).

The second interpretation of the data after 6 h of imbibition, is that O_2 uptake and CO_2 emission rates increased from 25°C to an optimum at 30°C, so O_2 uptake showed a second peak at 40°C (Figure 4.2). Acceptance of a biphasic pattern implies that two systems of gas exchange (possibly respiratory oxidation and some other oxidative system, residual oxidation) have different temperature optima.

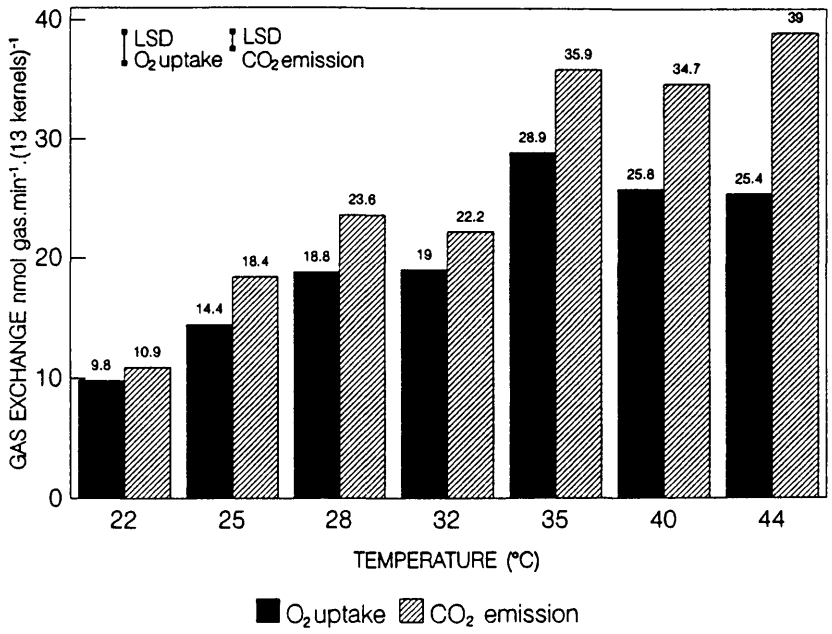

Figure 4.1 The effect of temperature, after 2 h of incubation, on the rates of O_2 uptake and CO_2 emission of *Triticum aestivum* cv. Betta.

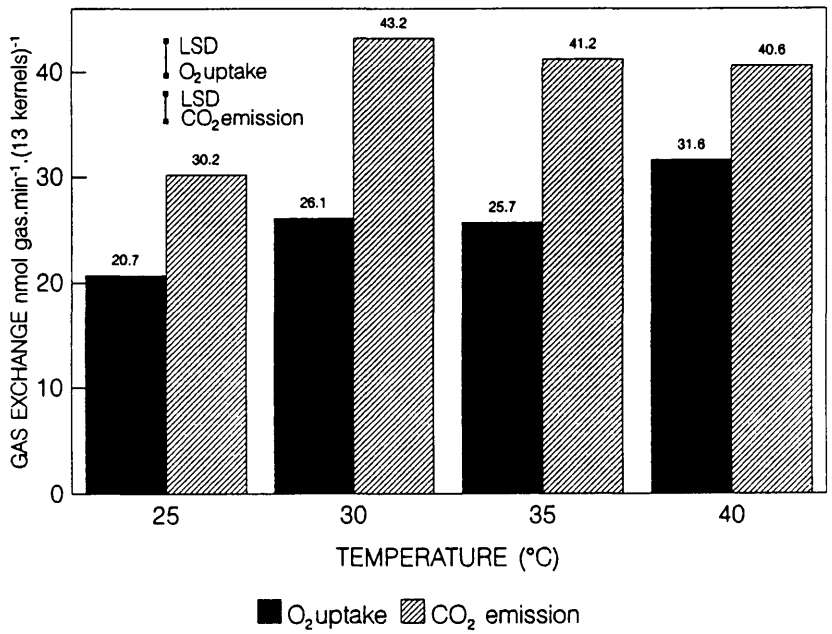

Figure 4.2 The effect of temperature, after 6 h of incubation, on the rates of O_2 uptake and CO_2 emission of *Triticum aestivum* cv. Betta.

RQ values were calculated, after 2 h and 6 h of incubation varied with temperature between 1 and 1.5 (results not shown).

Arrhenius plots were constructed to describe the relationship between temperature and E_a for enzymes of the respiration process (Figures 4.3 & 4.4). The results will be described with reference to the temperatures in degrees Celsius, represented on the second Y-axis. The Arrhenius plot of the rates of O_2 uptake, 2 h after the start of imbibition (Figure 4.3), was discontinuous between 35 and 40°C, indicating substantial changes in the E_a of reactions utilizing O_2 as temperature increased. The activation energy (E_a) calculated for O_2 consumption rates, at 2 h of imbibition, measured at temperatures below the inflection point was 13.3 Kcal.mol^{-1}, while the E_a at temperatures greater than the inflection point were markedly lower, at 2.9 Kcal.mol^{-1} (Figure 4.3). Rates of CO_2 emission on the Arrhenius plot measured after 2 h of imbibition (Figure 4.3) indicated a similar break between 35 and 40°C. The E_a for the rate of CO_2 emission at temperatures below the inflection point was 9.9 Kcal.mol^{-1}, while above the inflection point the E_a was -13.1 Kcal.mol^{-1} (Figure 4.3).

After 6 h of incubation, the Arrhenius plot of O_2 uptake rates was linear (Figure 4.4) with an E_a of 4.7 Kcal.mol^{-1} whilst that of the rate of CO_2 emission, had a break at approximately 32°C (Figure 4.4). The E_a calculated for CO_2 emission rates measured at temperatures below the inflection point was 5.7 Kcal.mol^{-1}, while above the inflection point the E_a was markedly lower at 0.5 Kcal.mol^{-1} (Figure 4.4).

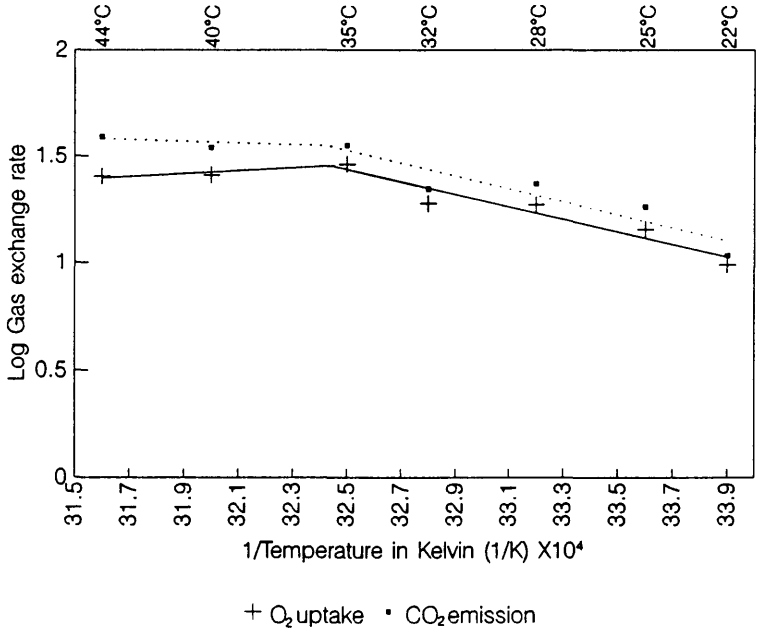

Figure 4.3 Arrhenius plots of the rate of O_2 uptake and CO_2 emission, after 2 h of imbibition, of whole wheat kernels, *Triticum aestivum* cv. Betta.

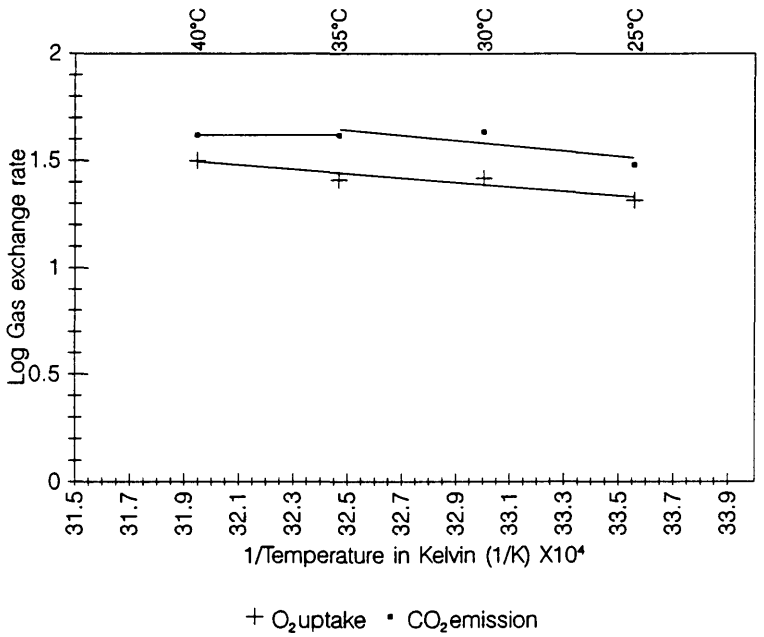

Figure 4.4 Arrhenius plots of the rate of O_2 uptake and CO_2 emission, after 6 h of imbibition, of whole wheat kernels, *Triticum aestivum* cv. Betta.

4.4 DISCUSSION

Neither O_2 uptake nor CO_2 emission of winter wheat kernels *T. aestivum* cv. Betta were markedly inhibited at supra-optimal temperatures. Superficially, it does not appear that the significant decrease in germination at 40°C (Figure 2.1) can be ascribed to a decrease in O_2 uptake after 6 h. This is in agreement with the findings of Riley (1981a) on maize kernels.

The nature of the substrate used in respiration at different temperatures could not be distinguished from the RQ values of wheat, both fermentation and carbohydrate metabolism seem to have occurred. This is in agreement with James (1953), who observed that RQ values during the first 24 h of incubation, depend so much on physical factors, that the respiratory substrate can not be identified.

A single break in the Arrhenius plots of wheat kernel O_2 uptake and CO_2 emission rates, 2 h after the start of imbibition, supported the findings of discontinuous two-phase plots by Pomeroy and Andrews (1975), Weidner and Ziemens (1975) and Vettucci and Leopold (1987). This was not supportive of the "Inverse Compensation" hypothesis, but indicated that substantial changes in reactions utilizing O_2 and emitting CO_2 occurred as temperature was increased.

From the Arrhenius plots for cucumber (*Cucumis sativus* L.) fruit, Eaks and Morris (1956) deduced that, after chilling temperature treatments, the respiration rate initially decreased, but thereafter increased to a value higher than normal. They regarded this phenomenon as indicative of chilling injury to the tissues. The above phenomenon observed for cucumber fruit, was however not found for fruit tissue resistant to chilling (Eaks & Morris 1956). Since no information on Arrhenius analyses of the effect of supra-optimal temperatures on respiration could be found, it was tentatively concluded from the Arrhenius

plots presented here, that injury occurred in the wheat kernels when subjected to supra-optimal temperature (40°C). From Figures 4.3 & 4.4, it is evident that at the relatively lower temperatures (25, 30 and 35°C), the Arrhenius plots for 2 and 6 h incubation periods have similar gradients. While the plot for the 6 h incubation treatment is linear for the whole temperature range, a break occurs for the 2 h treatment. This indicated that a change in the activation energy (E_a) occurred during the early incubation periods but not the longer period, and is in agreement with the data of Eaks and Morris (1956), for cucumber fruit tissue. Raison and McMurchie (1974) and Pomeroy and Andrews (1975), interpreted changes in the Arrhenius plot to be due to a temperature-induced change in the lipid component of mitochondrial membranes and the kinetics of oxidative enzymes which were modified.

Whether one regards the wheat kernel gas exchange as a biphasic reaction to temperature or not, the rate of O_2 uptake and CO_2 release at supra-optimal temperatures are higher than at the optimum temperature, although the percentage germination at the supra-optimal temperature is lower than at the temperature optimum. This is true for 2 and 6 h imbibed wheat kernels. It cannot however, be categorically stated that respiration was not the limiting factor causing thermo-inhibition of germinating wheat kernels at a supra-optimal temperature of 40°C, because the gas exchange could have emanated from systems other than from respiratory oxidations (examined in Chapter 6). Furthermore, the increase in respiration at 40°C was perhaps not sufficient for the ATP demand at supra-optimal temperatures.

One of the problems with interpreting respiratory data of whole kernels, is that the various tissues of the seed may hydrate at various rates (Madden 1991). It was therefore, thought appropriate to study the effect of temperature on the uptake of oxygen by embryonic axes and endosperm tissue separately (Chapter 6), especially as axes are regarded as being ideally suited for observing changes in metabolic events (Obendorf & Marcus 1974; Helm *et al.* 1989).

5. THE EFFECT OF TEMPERATURE ON EMBRYONIC AXES AND ENDOSPERM RESPIRATION OF WHEAT

5.1 INTRODUCTION

Respiration patterns for most imbibing seeds have been characterized as tri-phasic over time (Bewley & Black 1985). As the rate of O_2 uptake was found to be directly related to the rate of tissue hydration, during Phase I, and differences in the rate of hydration of embryonic and endosperm tissues (Madden 1991) exist in whole kernels, respiration measurements of whole seeds were difficult to interpret. Excised embryonic axes, which complete hydration within the first few minutes of imbibition (Obendorf & Marcus 1974; Helm *et al.* 1989; Ehrenshaft & Brambl 1990; Petruzzelli *et al.* 1992), and endosperm tissues, which hydrate much slower, were used separately to determine the rate of oxygen uptake. The embryonic axes is thought to be an especially effective indicator of the metabolism of the seed, since the respiration of embryonic axes comprises the greatest percentage of the entire seed respiration in wheat kernels (Barnell 1937; Stiles 1960 in Labouriau 1972).

In maize, the embryo is the site of greatest sensitivity to supra-optimal temperature (Riley 1981a,b). This is in contradiction to what has been found for wheat by Abernethy *et al.* (1989) and Helm *et al.* (1989) who found that wheat embryos are usually tolerant to high temperatures prior to 6 h of imbibition, in that germination was unaffected after exposure to 52°C for the first 2 h of imbibition. For wheat kernels of cv. Betta used in this study however, germination was already reduced by incubating the kernels at 40°C for 6 h (Chapter 3). The rate of O_2 uptake by wheat embryonic axes and endosperm tissue was studied to determine whether the lower percentage germination found at supra-optimal

temperature could possibly be ascribed to an effect of temperature on respiration.

This chapter deals with an investigation of the effect of temperature (25-40°C) on the oxygen consumption of the embryonic axes and endosperm of wheat kernels, 2 and 6 h after the start of imbibition. Oxygen uptake by the embryonic axes and endosperm at different temperatures was determined using a polarograph and the Gilson differential respirometer, respectively.

To determine the most appropriate method of embryonic axis removal for respiratory measurements, it was necessary to compare two methods of embryonic axes isolation: mechanically isolated embryonic axes from dry kernels, and manually excised embryonic axes from imbibed kernels.

5.2 MATERIALS AND METHODS

5.2.1 Wheat Kernels

T. aestivum cv. Betta kernels, harvested at Reitz during December 1991, were obtained from the Wheat Board, and stored in sealed containers at 4°C. Kernels of uniform size were selected (> 2.5 mm in diameter), as it is known that the seed size has an effect on the rate of water uptake and germination (Lafond & Baker 1986).

5.2.2 Temperature treatments

To ensure that equilibrium conditions were reached at the relatively high temperatures used, a pre-equilibration period of 12 h was applied. Prepared Petri dishes, distilled water and wheat kernels in paper bags, were temperature pre-equilibrated at the respective temperatures. Imbibition prior to and after excision, as well as determinations, were conducted at these temperatures. The supra-optimal temperature used (40°C) was the maximum temperature tolerance of the polarograph, therefore the effect of higher temperatures was not studied.

5.2.3 Embryonic axes

5.2.3.1 Embryonic axes isolation

Two methods were used for isolating embryonic axes. Firstly, intact embryonic axes were manually dissected from imbibed grains of wheat using a dissection microscope. Fifteen minutes before the respiratory assay, which started 2 and 6 h after the commencement of imbibition, samples of 13 embryonic axes were separated from the endosperm of kernels pre-incubated at the different temperature treatments. Embryonic axes were placed in a Petri-dish lined with a single layer of Schleicher and Schuell number 0860 filter paper and 5 cm^3 distilled water (McCrate *et al.* 1982), pre-equilibrated at a temperature corresponding to the specific treatment.

Secondly, wheat germ preparations were made according to the method of Johnston and Stern (1957). Approximately 250 g of wheat mixed with 150 cm^3, *ca.* 12 mm^2 lumps

of dry ice (solid CO_2) in an aluminium Waring blender jar, was equilibrated for 2 min before the it was ground for 20 s. The mixture was screened using a set of sieves, 10-, 14-, 28-mesh (Tyler scale) (openings of 2.00, 1.00 and 0.60 mm, respectively). The portion that remained on the 10-mesh sieve and an additional 50 cm^3 of dry ice, was returned to the blender jar, and ground for 10 s. The agitation and sieve cycles were repeated twice. Embryos and large fragments pass through the 10- and 14-mesh sieves, while many embryos connected to small fragments of endosperm pass through the 10- but not through the 14-mesh sieve. After thorough shaking of the sieves, only the 14- and 28-mesh portions were therefore collected.

The bran in these fractions was removed by means of a Hearson-seedblower. The embryonic axes were then separated from the endosperm fragments by stirring the mixture into a cyclohexane-carbon tetrachloride solution, (10/25 v/v), allowing the suspension to settle, and skimming the embryos off the surface. This step was repeated three times.

Embryonic axes were washed three times using a layer of filter paper in a Buchner funnel to drain the ice water rapidly, after which the sample was air dried. Samples were stored for 12 h at 4°C with silica gel as desiccant (Noubhani & Gidrol 1992). Undamaged embryonic axes were selected under a stereo microscope just prior to use.

Since experiments lasted only a few hours, no special precautions were required to ensure sterility.

5.2.3.2 Embryonic axes germination assay

Germination tests were carried out in plastic disposable Petri dishes, 90 mm in diameter, containing sterile agar (0.9%), glucose (1%) and streptomycin (0.01%) (Johnston & Stern

1957). Thirteen embryonic axes were transferred to the agar plates in a laminar-flow bench to prevent further contamination. Each treatment was replicated four times and randomized in a Labcon growth chamber at a constant temperature of 25°C with a maximum temperature fluctuation of *ca.* 0.3°C.

For the purpose of this experiment, germination was defined as a 2 mm increase in length of the embryonic axis, and was determined using a stereo microscope, equipped with a calibrated scale.

5.2.3.3 Embryonic axes oxygen uptake assay

The O_2 uptake of embryonic axes isolated in two ways was measured polarographically after a subsequent 2 and/or 6 h period of imbibition, using a Clark-type oxygen electrode connected to a YSI monitor model 53 (Yellow Springs Instruments Co.) which operated at 0.7 v. Embryonic axes were transferred to a water jacketed glass cuvette (10 cm^3), containing 3 cm^3 of air-saturated distilled water which was continuously stirred. Oxygen depletion was measured, in the dark at the different temperature treatments, for 30 min and the rate of oxygen uptake expressed as nmol $O_2.min^{-1}.(13 \text{ embryonic axes})^{-1}$.

In agreement with the findings of Brown (1943), no difference in oxygen uptake was detected whether a reaction medium (50 mM TES buffer, pH 7.4, 0.4 mM mannitol, 2 $mg.cm^{-3}$ BSA, 5 mM KH_2PO_4 and 5 mM $MgCl_2$) or distilled water was used in the determinations (results not shown), and therefore air-saturated distilled water was used.

5.2.4 Endosperm

5.2.4.1 Endosperm isolation

Fifteen minutes before the respiratory assay, which started 2 h after the start of imbibition, samples of 13 embryos were manually dissected from imbibed wheat kernels, and the remaining endosperm portion was used in the determinations. This remaining tissue consisted of endosperm tissue as well as the aleuron layer and the pericarp. The latter two will be referred to as associated tissues. The endosperm tissues were placed in a Petri dish lined with a single layer of Schleicher & Schuell number 0860 filter paper and 5 cm^3 distilled water pre-equilibrated at the specific temperature corresponding to the specific treatment.

5.2.4.2 Endosperm oxygen uptake assay

Respiratory assays were carried out with the aid of the Gilson differential respirometer as described in section 4.2.2.

5.2.5 Statistical analysis

The experiment was planned as a completely random design. The mean of six replicates at each temperature was determined. Analyses of variance were preformed and LSD (Student t-test), calculated at the 5% significance level.

5.3 RESULTS

5.3.1 A comparison of two methods of embryonic axes removal

Isolating embryonic axes with the Johnston and Stern (1957) mass isolation method entailed the storage of these embryonic axes overnight because the time necessary for embryo isolation was so long that it was impractical to do the respiratory assay immediately afterwards. Mass isolation of embryonic axes was found to be a slow process with a low yield of undamaged embryonic axes. The final germination percentage of non-stored manually dissected axes was 94 % while the germination percentage of stored, mass isolated axes was 86 %, after 48 h of imbibition. The rate of O_2 uptake of stored, mass isolated embryos was 30 % less than rate of freshly isolated embryos (Table 5.1). In the subsequent studies, it was therefore decided to use only embryonic axes which were manually dissected just prior to respiratory measurements.

Table 5.1 The rate of O_2 uptake of mass isolated embryonic axes (stored) and manually dissected embryonic axes (non-stored) of wheat *Triticum aestivum* cv. Betta, at various temperatures

Temperature treatments °C	Manually isolated embryonic axes (non-stored) nmol $O_2.min^{-1}$.(13 embryonic axes)$^{-1}$	Mass isolated embryonic axes (stored) nmol $O_2.min^{-1}$.(13 embryonic axes)$^{-1}$
25	4.39ab*	4.14a
30	5.44ef	5.12cde
35	5.64f	5.32def
40	4.90cd	4.70bc

* Values followed by the same letter are not significantly different at p = 0.05.

5.3.2 The effect of temperature of the oxygen uptake of wheat embryonic axes and endosperm tissues

In embryonic axes imbibed for 2 h, the rate of O_2 uptake tended to increase with temperature from 25 to 35°C, although there was no significant difference between the O_2 uptake rate at 30 and 35°C. At 40°C however, a significant decrease occurred (Figure 5.1). The O_2 uptake rate at 40°C was not lower than the rate at 25°C after 2 h (Figure 5.1). After 6 h of incubation, on the other hand, embryonic axes O_2 uptake rates did not differ significantly from 25 to 35°C but a significant decrease was also detected at 40°C (Figure 5.1).

In the case of the endosperm (referring to the endosperm and associated tissues), the rates of O_2 uptake increased with temperature, from 25 to 40°C, although there was no significant increase in O_2 uptake between 35 and 40°C (Figure 5.2). Endosperm O_2 uptake after 6 h of imbibition was not determined. The endosperm O_2 uptake contributed over 60% of the total rate of whole kernel O_2 uptake at all measured temperatures, as seen if Figure 4.1 (whole kernel O_2 uptake) is compared to Figure 5.2 (endosperm O_2 uptake).

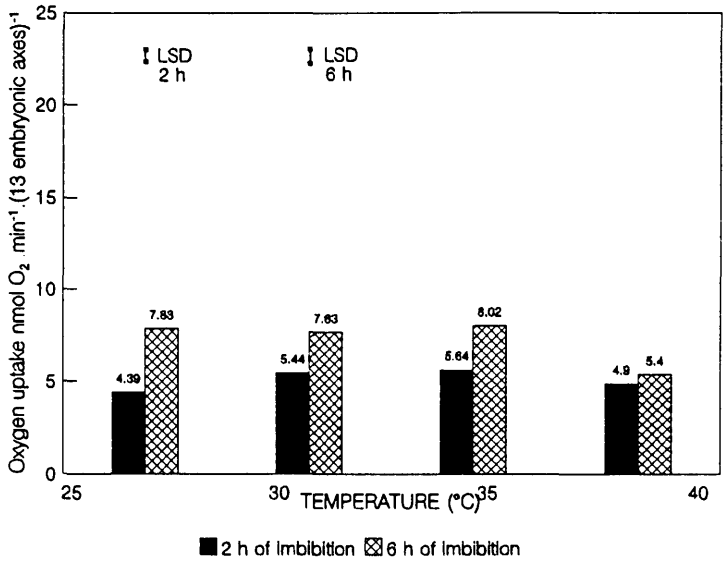

Figure 5.1 The rate of oxygen uptake of embryonic axes of wheat, *Triticum aestivum* cv. Betta, excised from the intact kernels after 2 and 6 h of imbibition.

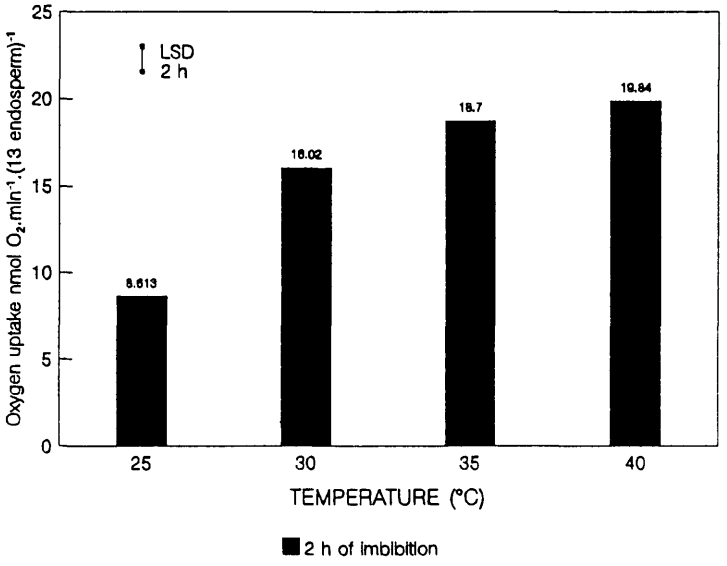

Figure 5.2 The rate of oxygen uptake of endosperm and associated tissues of wheat, *Triticum aestivum* cv. Betta, excised from the intact kernels after 2 h of imbibition.

5.4 DISCUSSION

5.4.1 A comparison of two methods of embryonic axes removal

During early stages of imbibition, wheat embryonic axes not stored after excision from imbibed grains exhibited greater viability after excision than embryonic axes removed from dry wheat kernels (stored). This is in consensus with findings on rye seeds by DeRopp (1939) according to Dure 1960. The rates of O_2 uptake of manually dissected, non-stored embryonic axes were slightly higher than those of axes removed from dry wheat kernels.

5.4.2 The effect of temperature on the oxygen uptake of wheat embryonic axes and endosperm tissues

The O_2 uptake of isolated embryonic axes of wheat increased with temperature over the range 20 to 35°C for 2 h incubated axes, but not for 6 h incubated axes. This may partly be because the rate of O_2 uptake, during early imbibition (2 h), could be directly related to the rate of tissue hydration according to Madden (1991), and hydration has been found to be temperature dependent (Chapter 2). After 6 h, hydration is expected to be complete and the rate of O_2 uptake is therefore expected to level off (Bewley & Black 1985). On the other hand, temperature not only influences the rate of imbibition but also the rate of chemical reactions and the effectivity of enzymes. Therefore it may be that at the higher temperatures, 35 and 40°C, enzymes are denatured and the rate of O_2 uptake is slowed down. In accordance with the findings of Murphy and Noland (1982a), the rate of O_2 uptake in the embryonic axes increased from 2 to 6 h of imbibition, although the difference in O_2 uptake

between 2 and 6 h imbibed axes was not as prominent at the higher temperatures used in this study. After 2 h of imbibition the rate of O_2 uptake at 40°C was still above the rate measured at 25°C, although the percentage germination at 40°C was already lower than at 25°C, after 4.5 h. In nature, 40°C is not an unnatural occurrence, though it is not found for long periods. Its effect in general could lead to some impairment of respiratory activity by failure of the enzyme levels to increase (Riley 1981a), as also reported by Madden (1991) for the embryos of maize. Another possible explanation for the lower rate of O_2 uptake at 40°C, may be related to a decrease in protein synthesis as found in maize kernels (Riley 1981a & b).

No direct comparisons between whole kernel O_2 uptake and that of embryonic axes can be made from these results (Abernethy *et al.* 1989), because removing the embryonic axes from its natural environment may affect its metabolism and therefore distort the results, which may lead to faulty deductions.

Oxygen uptake measurements of isolated parts of the wheat kernel, namely the embryonic axes and the endosperm, showed that the O_2 uptake occurred mainly in the endosperm, after 2 h of imbibition. Previous data of O_2 uptake of isolated parts of the wheat kernel have shown that the respiratory activity is concentrated in the embryo (Barnell 1937; Stiles 1960 as described by Labouriau 1972), and the endosperm and associated tissues comprised only 5% of the entire seed respiration (Brown 1943). The aleuron layer, which adheres to the inside of the seed coat, and becomes metabolically active, was thought to contribute to the high rates of O_2 uptake, according to Bewley and Black (1985) and Livesley *et al.* (1992). As the majority of the cells in the endosperm of cereals are non-living at maturity (Bewley & Black 1985), it is possible that non-mitochondrial oxidation could be responsible for most of the increase in O_2 uptake with temperature. The contribution of non-

mitochondrial oxidation is examined in Chapter 6.

From these results it can be deduced that the O_2 uptake of embryonic axes imbibed for 6 h are probably negatively affected by an incubation temperature as high as 40°C. Endosperm tissue O_2 uptake increased with temperature, after 2 h of imbibition. According to Rameshwar and Steponkus (1971), it would seem that the cotyledonary metabolism of intact kernels are not only directly affected by high temperature, but also indirectly via the effect that a relatively high temperature has on the metabolism of the axes.

Oxygen uptake of intact wheat kernels when imbibed at supra-optimal temperature in comparison to O_2 uptake at the optimum temperature (28°C), may possibly be ascribed to oxidative processes other than mitochondrial oxidation, as was found for soybeans (*Glycine max* L.) and barley (*Hordeum* sp. L.) by Parrish and Leopold (1977) and Rychter and Ostrowska (1985) respectively. It was therefore decided to investigate the effect of temperature on non-mitochondrial oxidations of whole kernels, embryonic axes and endosperm tissues (Chapter 6).

6. THE EFFECT OF TEMPERATURE ON THE NATURE OF OXIDATIVE PROCESSES IN WHEAT KERNELS

6.1 INTRODUCTION

The mitochondrial electron transport system in plants consists of a cytochrome and/or alternative pathway of respiration (Palmer 1976; Solomos 1977; Laties 1982; Lambers 1985; Lance *et al.* 1985). A significant part of oxygen uptake of whole tissue respiration however, remains unaffected by inhibitors of these pathways and is known as residual oxidation (Bahr & Bonner 1973; Rychter & Ostrowska 1985). Residual oxidation is thought to originate from oxidation enzymes not involved in the mitochondrial oxidation process, of which lipoxygenase, peroxidase, tyrosinase and phenol oxidase are examples (Theologis & Laties 1978).

Some residual oxidation enzymes are thought unlikely to be active in intact, undamaged tissues because compartmentation prevents oxidation enzymes to have access to suitable substrates (Laties 1982). The fact that some residual oxidation enzymes are indeed active follows from the results of Rychter and Ostrowska (1985), who demonstrated residual O_2 uptake to occur in whole seeds, where it appeared necessary in the germination process of pea (*Pisum sativum* L.), barley and sunflower (*Helianthus annus* L.) seeds. In the case of wheat kernels imbibing at 40°C, it is possible that the heat treatment may cause disruption of compartments, thus leading to residual oxidation exceeding the normal level. Residual oxidation as studied by Theologis and Laties (1978) and Rychter and Ostrowska (1985) was found to be constant with temperature.

In this study, the contribution of residual oxidation to oxygen uptake by whole wheat kernels, embryonic axes and the endosperm with its associated tissues, at normal and supra-optimal temperatures, was determined by the simultaneous addition of inhibitors of both the cytochrome pathway and the alternative pathway of respiration. Mitochondrial oxygen uptake was then obtained by calculation, to determine to what extent mitochondrial oxygen uptake was inhibited at supra-optimal temperatures.

6.2 MATERIALS AND METHODS

6.2.1 Wheat kernels

The kernels of *T. aestivum* cv. Betta as described under section 2.2.1 were used in this study.

6.2.2 Inhibitor mixtures

Salicylhydroxamic acid (SHAM) and propyl gallate were the inhibitors chosen to inhibit the alternative pathway, while azide (NaN_3) was used to inhibit the cytochrome pathway. Accordingly, inhibitor mixtures SHAM/azide or propyl gallate/azide were used to inhibit both pathways simultaneously. Residual oxygen uptake could then be determined. Unfortunately both SHAM and propyl gallate are inhibitors of lipoxygenase, an active non-mitochondrial oxidant, found to be active in wheat (Laties 1982). The results would therefore not be representative of the complete activity of non-mitochondrial oxidation enzymes.

All commercially available reagents were of the highest grade and were used without further purification. Salicylhydroxamic acid (SHAM) was dissolved in small amounts of methoxyethanol (5 cm^3/75 cm^3 v/v methoxyethanol/water solution). Twenty mM SHAM solution (pH 6.5) was mixed in equal volume with 10 mM azide solution, resulting in a solution with a pH of 6.5. In the propyl gallate/azide solution, equal volumes of 10 mM propyl gallate and 10 mM azide were mixed. Solutions were made up daily and temperature-equilibrated before use.

Oxygen uptake was studied, using KOH and inhibitors in Gilson respiration flasks. It was found that the volatility of the SHAM/azide solution was temperature dependent, since pure hydrogen azide is believed to reach its boiling point at 35.7°C (Manson 1967). Through experimentation a correction factor for azide volatility was determined for each temperature, and used in the calculation of the O_2 uptake at each specific temperature.

6.2.3 Respiratory assay

Whole kernels were imbibed at different temperatures in the appropriate inhibitor solution, either SHAM/azide or propyl gallate/azide. After 1 h and 45 min, 13 whole kernels or the endosperm tissues of 13 kernels, manually isolated according to section 5.2.3.1, were transferred to a prepared respiration-flask for each replicate. The Gilson differential respirometer was used to determine the O_2 uptake, as described in section 4.2.2. Thirteen embryonic axes were manually isolated as described in section 5.2.2.1, for each replicate, and the O_2 uptake was determined using the polarograph described under section 5.2.2.3. The appropriate inhibitor solution was used instead of water for both the incubation and measurement procedures described in sections 4.4.2.2 and 5.2.2.3.

All procedures were performed under a green safe-light to prevent the disruption of the chemical bond between azide and cytochrome a_3 of the terminal electron transfer chain in the inner membranes of the mitochondria (Goodwin & Mercer 1983).

6.2.4 Temperature treatments

To ensure that equilibrium conditions were reached at the relatively high temperatures used, a pre-equilibration period of 12 h was applied. Prepared Petri dishes, inhibitor solution, and wheat kernel samples, in paper bags, were pre-equilibrated at the respective temperatures. Incubation temperatures for O_2 uptake measurements were 25, 30, 35, and 40°C. Unlike similar experiments reported in the literature (Opik & Simon 1963; Murphy & Noland 1982a; Lecat *et al.* 1992), the O_2 uptake measurements were preformed at the same temperatures as incubation temperatures.

6.2.5 Analysis

Analysis of the oxygen uptake data, was done following the method of Bahr and Bonner (1973) as modified by Theologis and Laties (1978), to determine the relative importance of the various respiratory pathways. The total respiration activity (V_t) is:

$$V_t = V_{cyt} + V_{alt} + V_{res} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Where V_{cyt} is the component of respiration which is mediated by the cytochrome oxidase pathway and is sensitive to azide, V_{alt} is that component mediated by the alternative pathway and is sensitive to SHAM and to propyl gallate, and V_{res} is the component mediated by non-mitochondrial, residual oxidation and is insensitive to the combination of NaN_3 and SHAM, or NaN_3 and propyl gallate. V_{res} is considered to be constant as temperature increases (Theologis & Laties 1978), provided that the inhibitors have no side effects on respiration (Mϕller *et al.* 1988). Equation 1 was converted to:

$$(V_{cyt} + V_{alt}) = V_t - V_{res} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

where $(V_{cyt} + V_{alt})$ is the mitochondrial O_2 uptake and V_{res} is residual oxidation, in Equation 2.

6.2.6 Statistical analysis

The experiment was planned as a completely random design. The mean of six replicates at each temperature was determined. Analyses of variance were performed and least significant differences (LSD) (Student t-test), calculated at the 5% significance level.

6.3 RESULTS

Whole kernel mitochondrial O_2 uptake rate increased with temperature (Figure 6.1), to an optimum at 35°C, followed by a significant decrease at 40°C. The residual rate of O_2 uptake increased linearly as temperature increased from 25 to 40°C (Figure 6.1), the slope was however so gradual that the increase in residual oxidation between 30 and 40°C was not significantly different. The residual O_2 uptake made up a mere 2 % of the total O_2 uptake at 25°C, while at the temperatures 30, 35, and 40°C, the residual oxidation made up 9 % of the total oxidation.

Similar results were obtained using propyl gallate/azide and SHAM/azide, and consequently results obtained with the former inhibitor mixture are not shown.

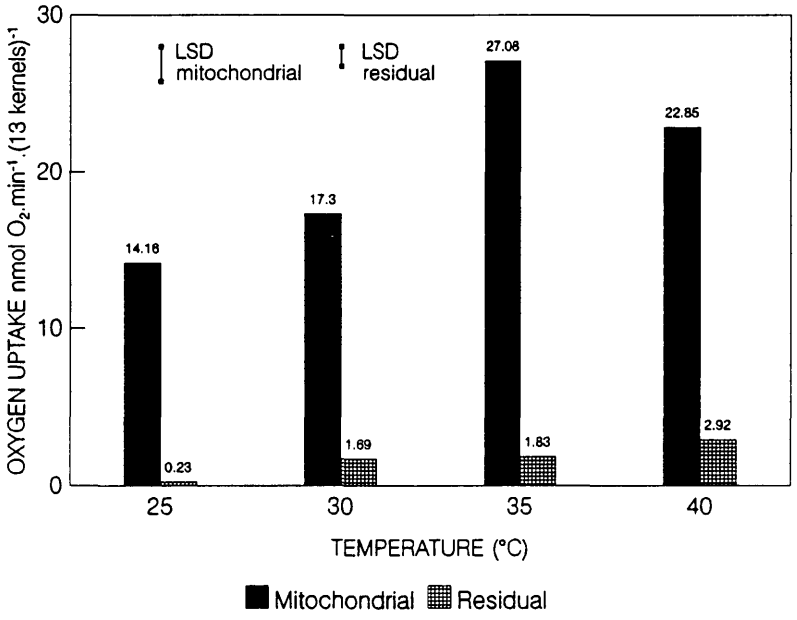

Figure 6.1 The effect of temperature on the residual and mitochondrial O_2 uptake of whole wheat kernels, *Triticum aestivum* cv. Betta, using a mixture of SHAM and azide as inhibitors.

In embryonic axes, the rate of mitochondrial O_2 uptake increased with temperature from 25-35°C, at 40°C it was lower and did not differ significantly from the rate of mitochondrial O_2 uptake at 25°C (Figure 6.2). In the case of the residual oxidation of embryonic axes, statistical analysis indicated a linear increase in residual oxidation with temperature but the slope was so gradual that, for all practical purposes, the residual oxidation may be regarded as being relatively constant over the 25-40°C temperature range (Figure 6.2). The residual O_2 uptake averaged 20.5 % of the total embryonic axes O_2 uptake.

A similar phenomenon was observed for the endosperm with its associated tissues: The mitochondrial oxidation rates also increased with temperature from 25-35°C and decreased slightly at 40°C, to a value not significantly different from mitochondrial O_2 uptake rate at 30°C (Figure 6.3). The rate of residual O_2 uptake of the endosperm with its associated tissues increased linearly with temperature from 25 to 40°C (Figure 6.3). Residual O_2 uptake in the endosperm made up 14 % of the endosperm O_2 uptake.

Comparing the rate of residual O_2 uptake of embryonic axes (Figure 6.2) with that of endosperm and associated tissues (Figure 6.3), it is evident that the majority of residual oxidation took place in the endosperm and associated tissues. It should be noted that the summation of the values for the residual O_2 uptake of the embryonic axes (Figure 6.2) and the endosperm tissues (Figure 6.3), gives a value larger than the value found for the residual O_2 uptake of intact wheat kernels (Figure 6.1).

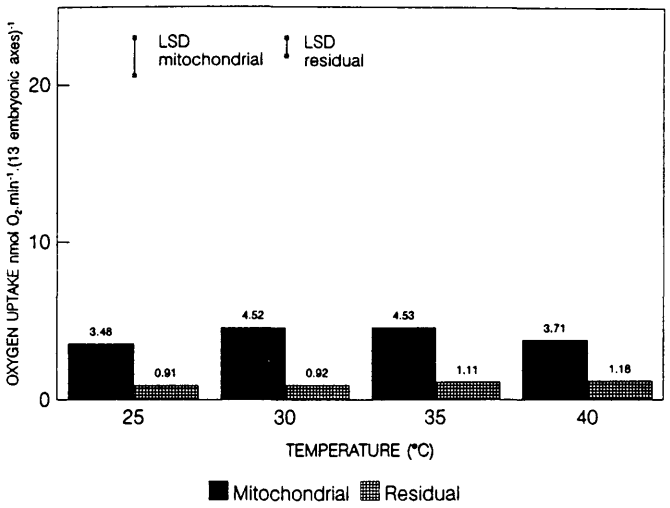

Figure 6.2 The effect of temperature on the residual and mitochondrial O_2 uptake of embryonic axes of *Triticum aestivum* cv. Betta, measured in a solution of SHAM/azide, after excision from intact wheat kernels which were imbibed in the inhibitor mixture.

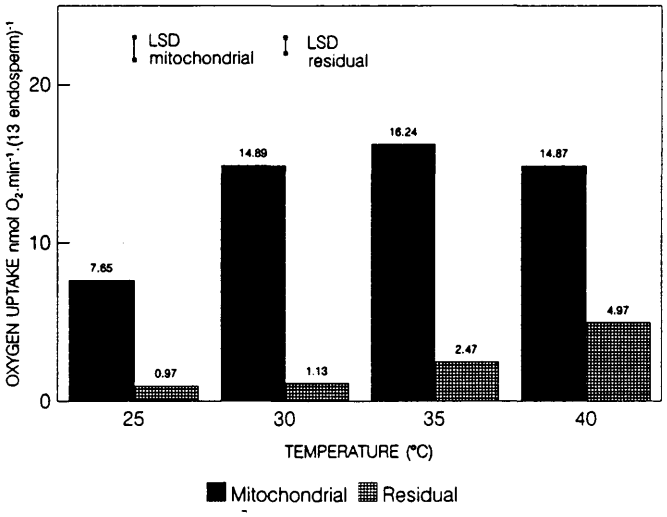

Figure 6.3 The effect of temperature on the residual and mitochondrial O_2 uptake of the endosperm with its associated tissues of *Triticum aestivum* cv. Betta, measured in a solution of SHAM/azide, after excision from intact wheat kernels which were imbibed in the inhibitor mixture.

6.4 DISCUSSION

In whole kernels, mitochondrial O_2 uptake was predominantly responsible for the observed increase in total oxidation with temperature. At 40°C, a slight decrease in mitochondrial oxidation occurred to a value similar to that at 30°C. These results are in agreement with the findings for maize embryos at 41°C (Riley 1981a).

Residual O_2 uptake rate of whole wheat kernels tended to increase with temperature. According to Purvis (1988), this phenomenon may be due to the fact that azide is less inhibitory to respiration at the lower than the higher temperatures, causing the mitochondrial respiration to apparently decrease with temperature, and the calculated residual respiration to apparently increase with temperature. In this study, the percentage residual O_2 uptake of the total O_2 uptake remained constant with temperature (30-40°C), which may indicate that no damage to compartmentation occurred at 40°C after 2 h of imbibition. Had compartmentation been disrupted by relatively high temperatures, increased levels of residual oxidation could be predicted (Laties 1982).

In isolated wheat embryonic axes and endosperm tissues, a slight increase in residual O_2 uptake was detected with an increase in temperature. Mitochondrial O_2 uptake was, however, mainly responsible for the total increase in O_2 uptake in the temperature range 25 to 40°C, for both embryonic axes and endosperm tissues. At 40°C a slight decrease in the mitochondrial O_2 uptake occurred in both embryonic axes and endosperm tissue of wheat. In agreement with the findings of Riley (1981a), the decrease in mitochondrial respiration was so slight that it seems improbable that low germination at 40°C can be ascribed to insufficient energy provision by mitochondrial respiration.

According to Yu *et al.* (1979), who studied lettuce (*Lactuca sativa* L.) seed O_2 uptake, the higher rate of residual oxidation found for excised tissues as compared to that of whole seeds, may be the result of damage caused by excision. Excision is thought to disrupt compartmentation in the cells (Laties 1982), therefore bringing substrate and enzymes into contact, resulting in residual oxidation. In wheat embryonic axes and endosperm tissues, this phenomenon may have occurred. The pericarp is known to retard O_2 diffusion up to six times (Becker 1960), and it may thus be speculated that the excision of the endosperm from the kernel, increased O_2 diffusion through the endosperm to such an extent that residual oxidation was stimulated in this tissue.

Two hours after the start of imbibition, the effect of temperature on the total O_2 uptake rate could partially be attributed to the rise observed in the rate of residual O_2 uptake with temperature, although residual respiration was not the main component of the O_2 uptake. The mitochondrial O_2 uptake rate of isolated embryonic axes and endosperm, as well as that of whole kernels, was not inhibited at 40°C to the extent that this process could be suspected to be the cause of reduced germination at this temperature. As the inhibitors used in this study are not specific only to the mitochondrial electron transport chain (Laties 1982), it was decided that determinations of adenylate levels may provide a more valid reflection of the effect of supra-optimal temperatures on respiration of wheat kernels (Chapter 7).

7. THE EFFECT OF TEMPERATURE ON THE ADENYLATE CONTENT OF WHEAT KERNELS

7.1 INTRODUCTION

It is accepted that an adequate supply of high-energy adenosine phosphates is essential for seed germination (Obendorf & Marcus 1974; Ching 1975; Riley 1981a; Adkins & Ross 1983). ATP is the main, direct energy providing substance for biological activities.

Respiration is an ATP (energy) producing process and its rate is controlled by the metabolic demand for ATP in the short-term. This is called the ATP-demand hypothesis which operates via effects of adenylates on glycolysis and oxidative phosphorylation (Williams & Farra 1990; Bingham & Stevenson 1993). As adenine nucleotides are a major factor in the regulation of early wheat embryo germination (Ching 1973; Obendorf & Marcus 1974), the action of supra-optimal temperatures on the energy supply of seeds was investigated. Studies on maize kernels have shown that the energy metabolism proceeds normally at 41°C but that some anabolic reactions necessary for germination are inhibited (Riley 1981a,b). Small *et al.* (1993), however, found thermo-inhibited germination in lettuce seeds to be due to a reduction in aerobic respiration and hence, a reduction in ATP synthesis.

Besides the ATP concentration, the ADP and AMP concentrations must also be taken into account to predict the energy status of seeds. The adenylate energy charge (AEC), is the molar fraction of adenylate energy-rich compounds in the adenylate pool (Pradet & Raymond 1983).

According to Pradet and Raymond (1984), ATP generation and utilization processes

are both affected by temperature. The AEC in biological systems should therefore remain unchanged at different temperatures (Pradet & Raymond 1983). In this study on wheat, it was found that the respiration rate increased with temperature (Chapters 4 & 5) which may probably be attributed to the action of regulatory mechanisms that take into account the cell energy requirements to maintain homeostasis (Varakina *et al.* 1991). The respiration rate of wheat kernels did not appear to be insufficient for supporting germination at 40°C (Chapters 4 & 5), but germination was indeed inhibited at 40°C over the first 6 h of imbibition (Chapter 3). Although respiration (O_2 uptake) appears to be sufficient, the O_2 uptake at supra-optimal temperatures might be unrelated to ATP production, and accordingly the latter must be determined, as reported here.

7.2 MATERIALS AND METHODS

7.2.1 Wheat kernels

Kernels of *T. aestivum* cv. Betta as described under section 5.2.1 were used in this study.

7.2.2 Extraction of adenylates

The technique of Raymond and Pradet (1980) as modified by De Meillon *et al.* (1994) was used for adenylate extraction. Wheat kernels incubated at different temperatures: 25, 30, 35 and 40°C, for 2 and 6 h, were rapidly removed from Petri-dishes, lightly blotted, and within 8 s transferred to liquid diethyl ether (ca. -100°C) for 5 min. Thirteen kernels were

homogenized in 150 cm^3 TCA-EDTA medium for six periods of 20 s at -15°C, using an Ultra-Turrax homogeniser. The extract was centrifuged at 24,000 xg for 10 min at 0°C, the pellet was resuspended in 35 cm^3 of TCA-EDTA using a Vortex vibrator, and recentrifuged as above. The two supernatants were combined and the clear solution was made up to 250 cm^3 with TCA-EDTA. A 10 cm^3 aliquot was extracted three times with 30 cm^3 cold diethyl ether to remove TCA, after which the solution was aerated to remove the diethyl ether. The pH of 5 cm^3 of this solution was adjusted to 7.0 with 0.2 $mol.dm^{-3}$ KOH, and 2x TRIS-H_2SO_4-EDTA medium was added to bring the final volume up to 10 cm^3. The final solution was frozen in liquid nitrogen and stored at -20°C.

7.2.3 Bioluminescence assay

For ATP determinations a stock solution was defrosted and used to prepare ATP standards ranging from $1x10^7$ to $1x10^9$ $nmol.cm^{-3}$ which was kept on ice. Each standard (50 mm^3), or defrosted sample was mixed with 50 mm^3 cold sterile TRIS-H_2SO_4-EDTA in a cuvette, the reaction was started by injection of 50 mm^3 luciferin-luciferase mixture (Lumit PM reagent; Lumac System, The Netherlands) and bioluminescence determined at 23°C in a Packard Picolite bioluminescence apparatus. To determine the quenching, the TRIS-H_2SO_4-EDTA was replaced with 50 mm^3 internal standard having twice the apparent concentration of the sample. No sign of quenching was observed in the samples. The bioluminescence was determined for 10 s immediately after mixing the standard or sample with the luciferin-luciferase mixture. A linear regression equation ($r = 0.999^{**}$; $n = 7$) of the log [ATP] over the log [counts] was calculated after correcting for background.

7.2.4 Determination of ADP and AMP content

The enzymatic procedure of Holm-Hansen and Karl (1978) was used to convert ADP and AMP of the samples to ATP. Reagents A, B and C were freshly prepared from biochemicals obtained from Boehringer Mannheim, and kept on ice. The composition of reagents A, B and C for ATP determination was; reagent A, 75 mM potassium phosphate buffer at pH 7.4, 15 mM $MgCl_2$; reagent B, 75 mM buffer, 15 mM $MgCl_2$, 0.24 cm^3 pyruvate kinase (Boehringer Mannheim 128155), 0.16 cm^3 phosphoenolpyruvate (Boehringer Mannheim 108278), in a final volume of 6 cm^3, and reagent C, 75 mM buffer, 15 mM $MgCl_2$, 0.18 cm^3 pyruvate kinase, 0.12 cm^3 phosphoenolpyruvate as for B, and 1.125 cm^3 adenylate kinase (Boehringer Mannheim 127272), in a final volume of 4.5 cm^3.

Prior to use, the ammonium sulphate ($(NH_4)_2SO_4$) should be removed from the enzyme preparations, as $(NH_4)_2SO_4$ affects the luciferase reaction (Saglio *et al.* 1979). This was done using microcentrifuge filters (Millipore ultrafree-MC, filters with a polysulfone UF membranes only) with a specific molecular mass cut off point. A 10 000 molecular mass cut off was used for adenylate kinase and a 30 000 cut off for pyruvate kinase. After the enzymes, suspended in $(NH_4)_2SO_4$, were centrifuged for 2 min at 10 000 xg, the precipitate was resuspended in a 10 mM phosphate buffer (personal communication by Boehringer Mannheim Research and Development Department, Germany). The resuspended enzymes were transferred to the respective ultrafree-MC filters, and centrifuged for 30 min at 10 000 xg, at 4°C. After further addition of phosphate buffer to the sample cup, the whole was recentrifuged as described above. The enzyme suspension remaining in the sample cup was then made up to the original volume using 10 mM phosphate buffer.

For each reagent A, B or C, 200 mm^3 of sample or standard was mixed with 50 mm^3

of reagent, incubated for 30 min at 30°C, heated for 2,5 min at 93°C and cooled.

Bioluminescence was determined by counting for 10 s immediately after mixing 50 mm^3 of the former mixture, 50 mm^3 TRIS-H_2SO_4-EDTA and 50 mm^3 luciferin-luciferase. The counts obtained by reacting standards, dilute samples or samples with reagent A, B or C, were corrected by subtracting the counts for the appropriate blanks (determined by processing the mixture of TRIS-H_2SO_4-EDTA and reagent A, B and C and injecting luciferin-luciferase).

A regression equation was calculated for ATP standards reacting with reagents A, B or C, as well as mixtures containing known concentrations of ATP, ADP and AMP. This was then used to calculate the ATP concentration of the samples, which was corrected for by the appropriate conversion factor for ADP/ATP or AMP/ATP conversion. AMP, and ADP conversion to ATP relies on the following principle:

$$AMP + ATP \rightleftharpoons 2ADP \quad \ldots\ldots 1$$

$$2ADP + 2phosphoenolpyruvate \rightleftharpoons 2ATP + 2pyruvate \quad \ldots\ldots 2$$

where reaction 1 is catalysed by adenylate kinase and reaction 2 catalysed by pyruvate kinase. In the reaction mixture B, pyruvate kinase was used to realise ADP conversion to ATP in the samples, reaction 2, while in reaction mixture C both enzymes were used to convert AMP to ATP in the samples, according to reactions 1 and 2.

7.2.5 Efficiency of turn-over and techniques

To determine the percentage recovery, 1 cm^3 of 33 mM ATP standard was added to the TRIS-H_2SO_4-EDTA prior to homogenisation. The recovery of this ATP was 110 %.

In addition to ATP concentration, the ADP and AMP concentrations of the extracts were determined. The efficiency of the conversion of ADP to ATP and AMP to ATP was determined using various mixtures of the three standard adenylates AMP, ADP and ATP, and was found to be 109 % and 104 %, respectively. Correction factors were used to obtain the correct concentrations. The AEC of samples was calculated using the three adenylate values according to the formula of Atkinson (1968).

$$AEC = \frac{[ATP] + 0.5[ADP]}{([ATP] + [ADP] + [AMP])}$$

7.2.6 Statistical analysis

The experiment was planned as a completely random design. The mean of three replicates at each temperature was determined. Regression analyses were performed on the data, and the significance level of the regression coefficients calculated and indicated with one asterisk in the case of a significant ($p=0.05$) correlation, and two asterisks if a highly significant ($p=0.01$) correlation was obtained.

7.3 RESULTS

7.3.1 ATP content

Following imbibition at 25, 30, 35 and 40°C, the ATP content after 2 and 6 h of imbibition increased linearly with temperature, such that data fitted the regression curves with correlation coefficients of 0.95^{**} and 0.89^{**}, respectively (Figure 7.1). The ATP content after 6 h of incubation was significantly higher than the ATP content after 2 h for all temperature treatments (Figure 7.1).

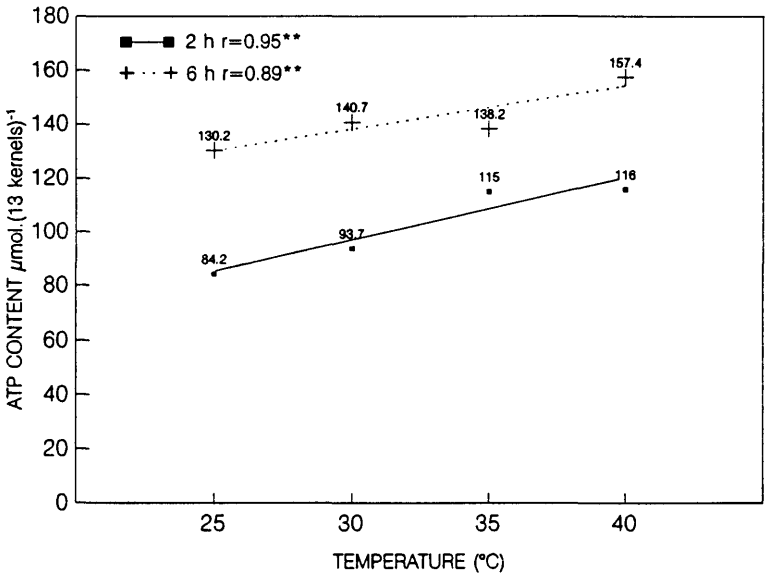

Figure 7.1 The effect of temperature on the ATP content of *T. aestivum* cv. Betta, extracted 2 or 6 h after the start of imbibition.

7.3.2 ADP and AMP content

The ADP profile for 2 h incubated seeds, is similar to the corresponding ATP profile already determined. Although the linear increase in ADP content with temperature was significant for the 2 h treatment, the ADP content of the 6 h incubated seeds did not show a linear relationship with temperature in the range 25 to 40°C (Figure 7.2). The overall tendency seemed to indicate a decrease in ADP content with temperature (Figure 7.2). On the whole, the ADP content of the 6 h incubated seeds was higher than that of seeds incubated for 2 h, except for the 40°C treatment (Figure 7.2).

In contrast to the increase with temperature of ATP and ADP contents after 2 h of incubation, the AMP content decreased linearly (r=0.95**) with temperature (Figure 7.3). In comparison to the 2 h AMP content, the AMP content of seeds incubated for 6 h, increased with temperature (r=0.89**) (Figure 7.3). Thus, at the optimal temperature of 25 and 30°C, the AMP content of the 6 h incubated kernels was lower than that of the 2 h incubated kernels, while at supra-optimal temperatures (35 and 40°C) its AMP content was higher (Figure 7.3).

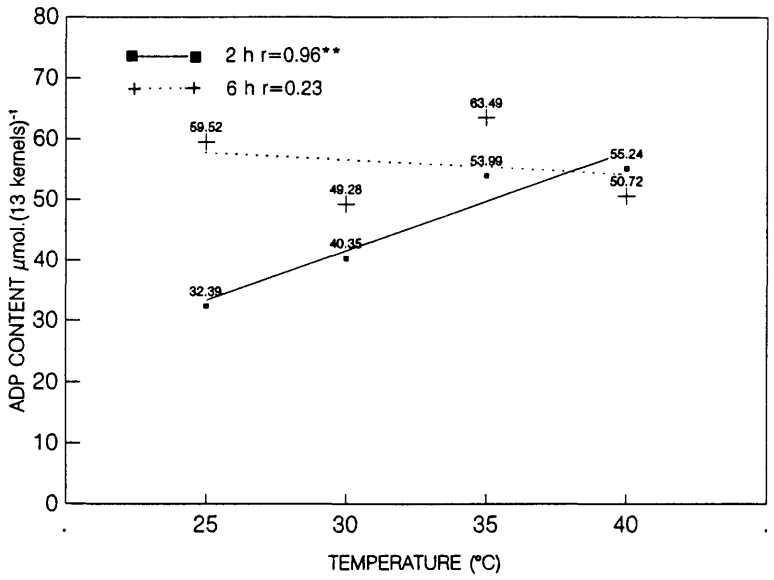

Figure 7.2 The effect of temperature on the ADP content of *Triticum aestivum* cv. Betta kernels, extracted 2 or 6 h after the start of imbibition.

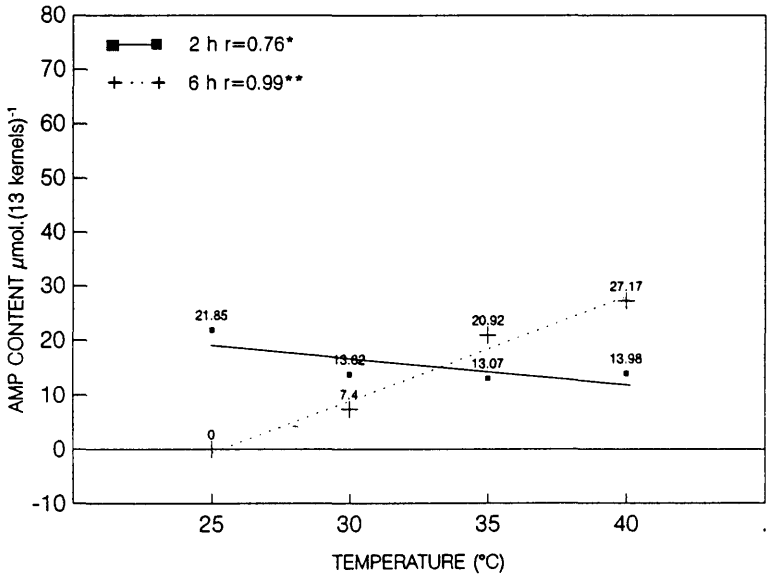

Figure 7.3 The effect of increasing temperature on the AMP content of *Triticum aestivum* cv. Betta kernels, extracted 2 or 6 h after the start of imbibition.

7.3.3 Total adenylates (Adn)

The total adenylate pool is correlated linearly with temperature for both the 2 and 6 h imbibed kernels (Figure 7.4). Not only was the adenylate pool size increased by *ca.* 28 μmol.(13 kernels)$^{-1}$ when increasing the imbibition time from 2 to 6 h, but a similar increase in the pool size was observed by changing the imbibition temperature of the 2 h incubated seeds from 25 to 40°C (Figure 7.4). By comparing Figure 7.1 with Figure 7.2, it is clear that the ATP content was the main contributor to the adenylate pool after 2 h of imbibition.

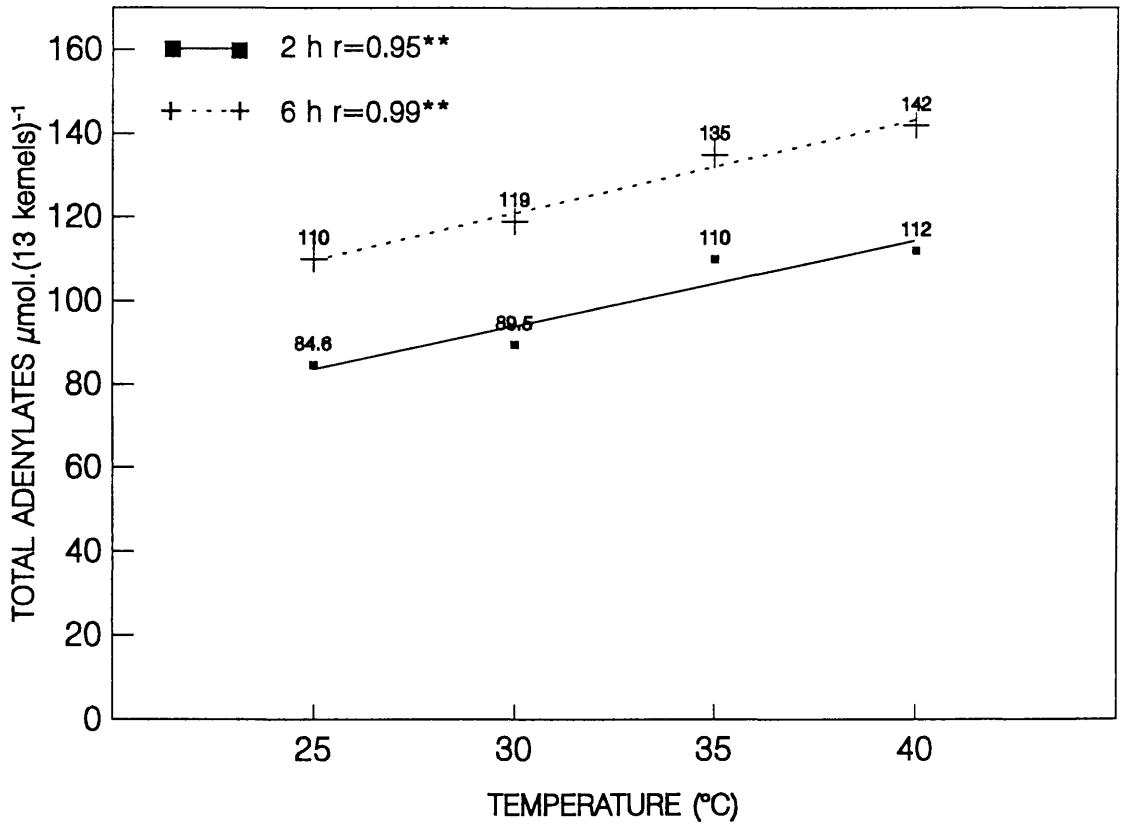

Figure 7.4 The effect of temperature on the total adenylate content of *Triticum aestivum* cv. Betta kernels, extracted 2 or 6 h after the start of imbibition.

7.3.4 Adenylate energy charge (AEC) and ATP/ADP ratios

The AEC of wheat kernels incubated for 2 h, increased linearly with temperature (r=0.85**) from a value of 0.72 at 25°C to a value of 0.78 at 40°C (Figure 7.5). After 6 h of incubation, the AEC value at 25 and 30°C was lower, compared to values for kernels incubated for 2 h, and on the whole a linear decrease of AEC value with temperature (r=0.85**) was observed for the 6 h incubated kernels (Figure 7.5).

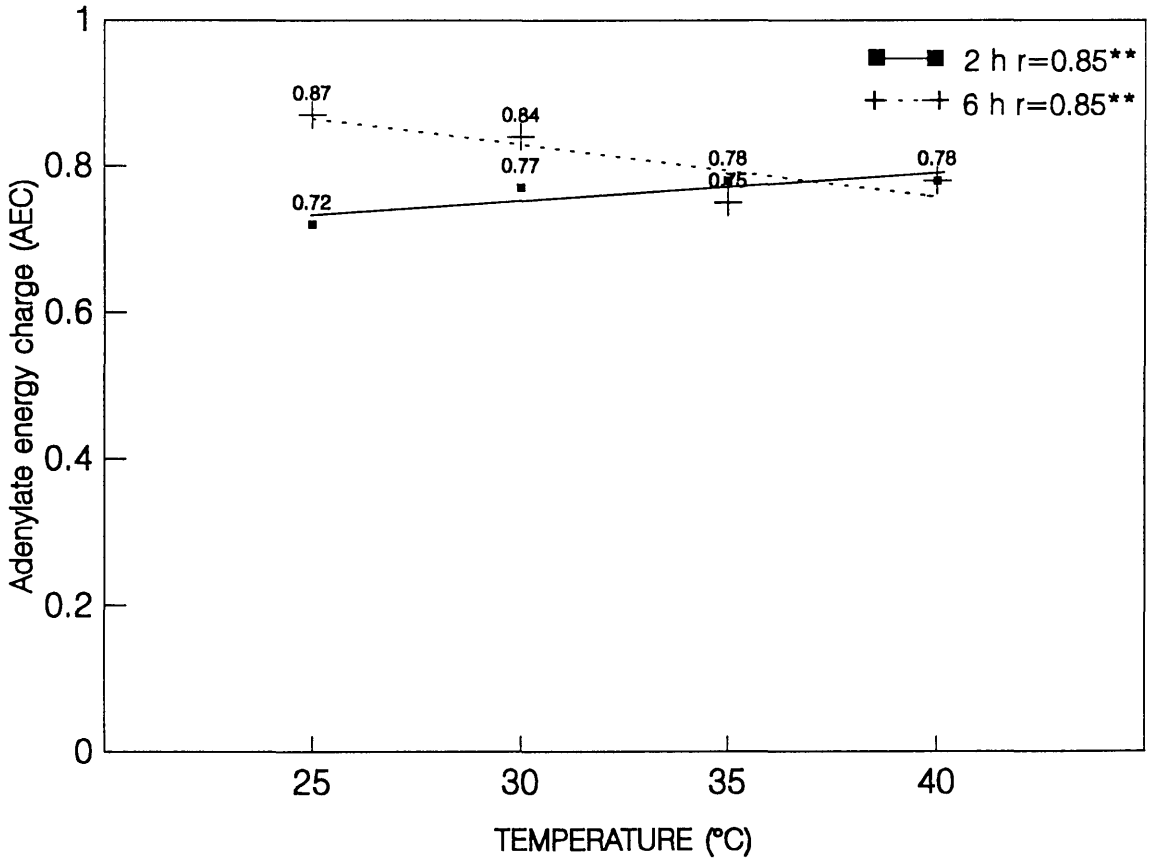

Figure 7.5 The effect of temperature on the adenylate energy charge of *Triticum aestivum* cv. Betta kernels, extracted 2 or 6 h after the start of imbibition.

Use of the AEC concept rests on the assumption that the adenylate pool is equilibrated by adenylate kinase (Raymond & Pradet 1980). Using the K_{eq} values as an indicator of the adenylate kinase activity, calculations of the K_{eq} indicated that, except for kernels incubated for 6 h at 40°C, all K_{eq} values were between 0.4 and 1.4 (Table 7.1). At 40°C the value was much higher, namely 2 (Table 7.1).

The criticism which Pradet & Raymond (1984) have on the AEC, is that a large variation of the ATP/ADP ratios cause small variations in the AEC values, and consequently the ATP/ADP ratios were also calculated in this study. The ATP/ADP ratio as calculated for the 2 h treatment, tended to decrease linearly with temperature, although it did not change significantly from a value of 2.2 for the different temperature treatments (Table 7.1). After 6 h of incubation however, the ratio at 30 and 40°C increased significantly to 3, while the values at 25 and 35°C remained similar when compared to measurements after 2 h of incubation (Table 7.1).

Table 7.1 Calculated K_{eq} values for adenylate kinase catalysed reaction of wheat kernels incubated for 2 and 6 h at high temperatures. The error values for sums, products and ratios were calculated according to Aikens *et al.* (1978)

Kernel incubation temperature (°C)	K_{eq} values		ATP/ADP ratio	
	2 h	6 h	2 h	6 h
25	1.45±0.34	0.00±1.33	2.49±0.23	2.19±0.10
30	0.80±0.22	0.43±0.94	2.32±0.10	2.86±0.09
35	0.52±0.72	0.72±0.39	2.13±0.29	2.18±0.12
40	0.53±0.38	2.05±0.18	2.10±0.05	3.10±0.12

7.4 DISCUSSION

The ATP content of an incubated seed represents the surplus energy after repair and reactivation, that can be used for biosynthetic processes (Khan 1982). In this study, not only did the ATP content of wheat kernels increase linearly with temperature, but the total adenylate content increased linearly with temperature as well over the range 25-40°C. The energy demand was therefore thought to be higher at 40°C, than at the lower temperatures. Although it is not clear if this increase in ATP content was sufficient to meet the energy demand at the supra-optimal temperatures, ATP was not thought limiting to biosynthetic processes at a temperature treatment of 40°C, at which temperature wheat germination was inhibited. Radicle protrusion at 30°C commenced at an ATP content of *ca.* 93.7 μmol.(13 kernels)$^{-1}$, whereas at 40°C the ATP content was higher than this throughout the period 2 to 6 h. It may be however, that the high ATP content at supra-optimal temperature could still not be sufficient to satisfy the demands of the germination processes. A similar phenomenon regarding the ATP content at a supra-optimal temperature, was found for maize embryos exposed to 41°C (Riley 1981a), and findings with both maize embryos and wheat kernels thus support the idea that homeostasis is maintained at these supra-optimal temperatures. Contradictory to these findings, thermo-inhibitory studies of lettuce seeds by Small *et al.* (1993), indicated decreased ATP levels at temperatures where germination was inhibited.

It is not possible to differentiate between ATP synthesized by fermentation (Raymond *et al.* 1985; Côme *et al.* 1988), and that synthesized by aerobic respiration from the available data. Wheat kernel mitochondrial oxidation however, seemed to contribute significantly to the increased respiration with an increase of temperature (Chapter 4, 5 & 6). Although some fermentation was probably present according to the RQ values (Chapter 4), anaerobic

respiration was thought not to contribute significantly to ATP production during the early stages of wheat germination, which is in agreement with a statement made by Raymond *et al.* (1985), working on wheat kernels.

In addition to the ATP concentration *per se*, there are additional possible regulatory measures that involve the adenine nucleotides (Obendorf & Marcus 1974), and which can be used to interpret the data. The first of these is that a high ATP concentration stimulates protein synthesis while high levels of ADP and AMP inhibit protein synthesis (Freudenberg & Mager 1971). Thus, under conditions where ADP and AMP concentrations are low, stimulation of protein synthesis could be expected. The two mechanisms are however, not mutually exclusive; a high ATP concentration would inevitably mean that the other two adenine nucleotide components (ADP and AMP) are at low concentrations.

With regard to the 6 h incubated kernels, the ATP content increased with temperature, which is known to stimulate protein synthesis. The ADP content of wheat kernels tended to decrease with temperature, while the AMP content increased. Values for the ADP content were noticeably higher than for the AMP content, while the ATP content was much higher still. It can be speculated that although high ATP contents may stimulate protein synthesis, the threshold for the AMP content was overridden, and protein synthesis could possibly have been inhibited. In agreement with findings by De Meillon *et al.* (1994), who studied the dormancy of *Strelitzia juncea* Ait. seeds, the increase in ATP content was higher than the decrease found in AMP content. In wheat, this phenomenon was observed for all temperature treatments, and the total adenylate content thus increased with temperature. This demonstrates that, like other non-dormant seeds after a short incubation period (Pradet 1982), *de novo* synthesis of adenylates in wheat also contributes to the total adenylate pool.

The second type of regulation, advanced by Chapman *et al.* (1971), considers the AEC as the primary regulator and states that germination can only occur at an AEC of about 0.8 (Obendorf & Marcus 1983). The AEC is the molar fraction of adenylate energy-rich compounds of the adenylate pool (Pradet & Raymond 1983). The size of the adenylate pool is determined by both regenerating and utilizing pathways. ATP is regenerated mainly through respiratory processes, while protein synthesis is the main ATP utilizing pathway (Hackett 1959; Karl & Holm-Hansen 1978; Hourmant & Pradet 1981; Fader & Koller 1984). According to Atkinson (1968) and Pradet and Raymond (1983), an increase or a decrease in temperature can induce a marked limitation of respiration without causing a variation in the AEC, because it affects both ATP-utilizing and ATP-regenerating pathways. The AEC value of wheat kernels imbibed for 2 h, increased with temperature. After 6 h of incubation however, the AEC value decreased linearly with temperature, indicating that at the higher temperatures more energy was used. At 35 and 40°C the AEC values were similar, which should indicate that sufficient energy is available for germination to take place at 40°C.

The AEC concept rests on the assumption that the adenylate pool is equilibrated by adenylate kinase (Raymond & Pradet 1980). Calculation of the K_{eq} for wheat kernels indicated that, except at 40°C after 6 h of imbibition, the pool could have been equilibrated by adenylate kinase, with values ranging between 0.4 and 1.4, which is in accordance with acceptable values as reported by Raymond and Pradet (1980). Conclusions based on a high AEC value calculated for the unacceptably high K_{eq} value obtained for 40°C, indicated that the conclusion must therefore, only be provisionally made. In this study, it was found that germination occurred at AEC values above the lower limit of 0.5, associated with viability in normal hydrated plant tissue (Atkinson 1969). Germination of cocklebur (*Xanthium pennsylvanicum* Wallr.) seeds also took place at AEC values lower than 0.8 (Eshashi *et al.*

1983).

As small variations in AEC values correspond to large variations in the ratio of ATP/ADP, the latter was calculated. ATP/ADP values, generally found *in vivo*, vary from 3.8 to 10.8 (Pradet & Raymond 1984), while a ratio of 5 is considered to indicate normoxic metabolism (Raymond *et al.* 1985). In agreement with findings of Standard *et al.* (1983) on wheat kernels, ATP/ADP ratios lower than 5 have been recorded in actively germinating seeds after 5 h of incubation.

Wheat kernel germination was inhibited at 40°C. In wheat some of the reactions of germination may proceed normally at supra-optimal temperatures, but further development may be prevented because an essential process or component may be particularly temperature sensitive. In agreement with data in the literature (Min-Tze-Wu & Wallner 1983; Varakina *et al.* 1991), the ATP content regenerated by respiratory oxidation was found to be higher at supra-optimal temperatures than optimal temperatures. This phenomenon can probably be due to one of two reasons. Firstly, a relatively greater ATP required for growth at supra-optimal temperatures compared to the energy (ATP) needed under normal temperature conditions will result in an increased respiration rate, resulting in a greater ATP content. Secondly, a reduced utilization of ATP will result in a greater ATP content. According to Helm *et al.* (1989) and Varakina *et al.* (1991), heat shock proteins are synthesized at supra-optimal temperatures during early imbibition for the survival and eventual germination of wheat. In agreement with the results of Osborne (1983) studying rye (*Secale cereale* L.), the ATP content is unlikely to be a limiting factor in the early biosynthetic events in germinating wheat kernels at supra-optimal temperature. Factors other than energy supply would therefore appear to pose a limit upon the germination process during early germination in wheat at supra-optimal temperatures.

8. GENERAL DISCUSSION

The establishment of respiration, an ATP generating system, is an important metabolic event during normal germination (Bewley & Black 1985). Therefore, if germination does not occur, respiration is one of the first metabolic events which should be examined. Experiments conducted on wheat kernels over a temperature range from 25 to 40°C, showed an inhibition of germination at 40°C, and this phenomenon was referred to as thermo-inhibition, because 59 % germination took place when the kernels were transferred back to an optimum temperature. According to the literature, supra-optimal temperatures are thought to affect membrane properties, leading to reduced O_2 uptake, reduced cytochrome oxidase electron transport activity, and lowered ATP levels (Murphy & Noland 1982a; Small *et al.* 1993). The exact mechanism causing inhibition of germination is, however, not known.

In this study, membrane properties were not examined, although water uptake was determined. The effect of temperature on water uptake, determined during the first 2 h of imbibition, led to the conclusion that the effect which temperature had on germination rate could not be attributed to its effect on the rate of water uptake, except if the water uptake at 40°C was not sufficient to satisfy the demand. These findings are supported by the results of Lafond and Baker (1986) on wheat.

The effect of temperature on the rate of gas exchange during the first 6 h of imbibition of whole wheat kernels indicated that O_2 uptake and CO_2 emission were not the limiting factors causing thermo-inhibition of wheat at a supra-optimal temperature of 40°C, and that the higher gas exchange may be related to the maintenance of homeostasis in the seeds. Closer examination of the gas exchange of embryonic axes and endosperm tissues indicated a slight decrease of O_2 uptake by embryonic axes at 40°C after 6 h of imbibition,

while the rate of O_2 uptake in the endosperm and associated tissues, increased with temperature. This supports the findings of Riley (1981a) on maize embryo respiration where a slight decrease in O_2 uptake occurred at 40°C. Whether this decrease of respiration was due to the effect of temperature on the embryonic axes or whether it was due to an injurious reaction caused by excision of the embryonic axes from the kernels, is not clear. Respiration was, however, not thought to inhibit germination.

Gas exchange could have emanated from systems other than respiratory metabolism. In this study, mitochondrial oxidation was found not to be so restrictive as to cause the inhibition of wheat germination at supra-optimal temperatures (40°C). Residual oxidation increased linearly with temperature but contributed only a small percentage to the total oxidation of wheat. As respiratory oxidation was found to increase at supra-optimal temperatures, a greater energy pool was probably required for growth than would be the case under normal conditions (Varakina *et al.* 1991).

The ATP content as well as the total adenylate pool size of wheat kernels increased linearly with temperature. It would therefore seem that the energy supply was adequate at 40°C and failure of wheat to germinate at this temperature was not due to any disruption of the energy metabolism. The energy metabolism proceeded normally but some other anabolic reaction leading to germination was probably inhibited.

Although the energy metabolism of wheat was not thought to be limiting, Ching (1975) reported that the energy charge of crimson clover embryos was negatively influenced by high temperatures after 6 h of imbibition. Ching (1975) concluded that clover seeds failed to germinate at the supra-optimal temperature of 30°C because of excessive use of energy for uncontrolled biosynthesis and turnover. Furthermore, Small *et al.* (1993) reported thermo-inhibition of lettuce seeds at 38°C to be due to limiting respiratory metabolism, and

that the ATP content failed to reach a sufficient level to satisfy the requirement for germination.

Wheat kernels, however, reacted similar to the maize embryos studied by Riley (1981a), who found that no restriction of respiration or ATP content could be found when germination was inhibited at 41°C. Although the adenylate content and respiration rates were highest at 40°C, there is no indication whether even these high values are sufficient for the demand at the supra-optimal temperature. The energy metabolism is, however, thought to maintain homeostasis at supra-optimal temperatures, and it is more likely that some anabolic reactions leading to germination are inhibited. In maize, the underlying cause of this appeared to be a decreased rate of protein synthesis; it is possible that high levels of AMP in wheat kernels at supra-optimal temperatures may cause a similar phenomenon. This too, is consistent with the idea that some of the reactions of germination proceed normally, but further development is prevented because an essential process or component, such as an enzyme, is especially temperature sensitive.

These experiments indicate that the respiratory metabolism was not the primary site of high temperature sensitivity in wheat kernels. Failure to germinate at 40°C was thought not to be due to the disruption of energy metabolism.

Although prolonged periods of continuous exposure to supra-optimal temperatures are unlikely to occur in nature, supra-optimal temperature during early imbibition will lead to a poor, uneven emergence and a low yield. Future studies should investigate the synthesis of proteins and the molecular basis thereof, since the literature has indicated temperature sensitive enzymes in the protein synthetic pathway. The identification of the site(s) of sensitivity to supra-optimal temperature, will promote the progress of breeders in establishing crops successfully in a world where global heating has become prominent.

9. SUMMARY

The germination of wheat kernels, *Triticum aestivum* cv. Betta, occurred optimally at a temperature of 28°C, while germination was inhibited at the supra-optimal temperature of 40°C.

The rate of water uptake increased with temperature, and it was concluded that the effect which temperature had on germination could not be attributed to an insufficient rate of water uptake.

The respiratory metabolism, one of the most frequently used criteria for the determination of the metabolic state of seeds, was then investigated. The rate of oxygen uptake and carbon dioxide emission by intact kernels increased with temperature. Closer examination of gas exchange by embryonic axes, indicated that the rate of oxygen uptake was slightly lower at 40°C, in relation to that at 25°C. The rate of oxygen uptake by the endosperm and associated tissues, increased with temperature over the 25-40°C range.

Gas exchange could have emanated from systems other than respiratory metabolism. The rate of residual oxygen uptake was found to increase slightly with temperature, but was only a minor contributor to the total rate of oxygen uptake of wheat kernels. Mitochondrial oxygen uptake of isolated embryonic axes, endosperm tissues and whole kernels, were not restricted to such an extent that it could be suspected to be the cause of reduced germination at supra-optimal temperatures. As the rate of mitochondrial oxidation was found to increase with temperature, a greater amount of energy was probably required for germination than would be the case under normal conditions.

A linear increase in ATP content and total adenylate content was found by subjecting seeds to temperatures ranging from 25 to 40°C. It would therefore seem that the energy supply was adequate at 40°C, and failure of wheat to germinate at this temperature was probably not due to the disruption of the energy metabolism.

In conclusion, 40°C inhibited wheat kernel germination, but not respiration. The high rate of oxygen uptake observed at 40°C, is probably related to a higher ATP production in order to satisfy a higher energy demand for germination processes at 40°C compared to that at 25°C.

10. OPSOMMING

Die ontkieming van koring, *Triticum aestivum* cv. Betta, was optimaal by 28°C, terwyl ontkieming by die supra-optimale temperatuur van 40°C sterk afgeneem het.

Die tempo van wateropname deur die sade het toegeneem met temperatuur, en dit was dus onwaarskynlik dat die invloed van supra-optimale temperature op ontkieming aan 'n onvoldoende wateropname-tempo toegeskryf kan word.

Respiratoriese metabolisme, een van die mees algemene kriteria om die metaboliese toestand van saad mee te bepaal, is gevolglik ondersoek. Die suurstofopname- en koolstofdioksiedvrystellings-tempo van die intakte saad het toegeneem met temperatuur. By nadere ondersoek het dit geblyk dat die suurstofopname-tempo deur die embrionale assies by 40°C laer as dié by 25°C was. Suurstofopname-tempo deur die endosperm en meegaande weefsels het toegeneem met temperatuur oor die 25-40°C gebied.

Verdere karakterisering van die gaswisseling het getoon dat die residuele suurstofopname-tempo 'n geringe toename met temperatuur getoon het, maar die relatiewe bydrae wat dit tot die totale suurstofopname-tempo gemaak het, was gering. Mitochondriale suurstofopname in geïsoleerde embrionale assies, endospermweefsel, en intakte saad, was nie tot so 'n mate beperkend by supra-optimale temperature dat 'n onvoldoende mitochondriale suurstofopname-tempo as die rede vir swak ontkieming aangevoer kon word nie. Omdat mitochondriale oksidasie met temperatuur toegeneem het, is dit waarskynlik dat daar 'n groter behoefte aan energie vir ontkieming by supra-optimale temperature as by normale toestande benodig word.

'n Liniêre toename in ATP- en totale adenilaat-inhoud is waargeneem vir temperatuur -behandelings wat van 25 tot 40°C gestrek het. Dit wil dus voorkom of die energie-voorsiening by 40°C voldoende was, en dat die onvermoë van koringsaad om by hierdie temperatuur te ontkiem, waarskylik nie as gevolg van die ontwrigting van energie-metabolisme is nie.

Die slotsom word gemaak dat swak ontkieming by 40°C nie die gevolg van beperkte respirasie was nie. Die hoë suurstofopname-tempo by 40°C in vergelyking met dié by 25°C, hou waarskynlik verband met 'n hoë ATP produksie, wat aan die veronderstelde hoër aanvraag vir metaboliese energie vir die ontkiemingsproses by 40°C sal kan voldoen.

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