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Effect of heat stress during seed development and maturation on wheat (Triticum durum) seed quality

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Effect of heat stress during seed development and maturation on wheat (*Triticum durum*) seed quality

Grass, Lahcen, Ph.D.
Iowa State University, 1994
Effect of heat stress during seed development and maturation on wheat (Triticum durum) seed quality

by

Lahcen Grass

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

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Major: Crop Production and Physiology

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For the Graduate College

Iowa State University
Ames, Iowa

1994
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>v</td>
</tr>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Explanation of the Dissertation Format</td>
<td>3</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>Physical Seed Quality</td>
<td>6</td>
</tr>
<tr>
<td>Physiological Seed Quality</td>
<td>9</td>
</tr>
<tr>
<td>Biochemical Seed Quality</td>
<td>12</td>
</tr>
<tr>
<td>Mechanism of Heat Stress on Seed Quality</td>
<td>16</td>
</tr>
<tr>
<td>Seed Vigor Measurement</td>
<td>18</td>
</tr>
<tr>
<td>PAPER 1. EFFECT OF HEAT STRESS DURING SEED DEVELOPMENT AND MATURATION ON WHEAT SEED GERMINATION AND VIGOR</td>
<td>21</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>22</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>24</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>28</td>
</tr>
<tr>
<td>Plant Material</td>
<td>28</td>
</tr>
<tr>
<td>Physical Traits of Mature Seed</td>
<td>29</td>
</tr>
<tr>
<td>Seed Germination and Seedling Vigor</td>
<td>29</td>
</tr>
<tr>
<td>Embryo Culture</td>
<td>29</td>
</tr>
<tr>
<td>Embryonic Respiratory Measurements</td>
<td>30</td>
</tr>
<tr>
<td>Statistical Treatment</td>
<td>31</td>
</tr>
<tr>
<td>RESULTS</td>
<td>32</td>
</tr>
<tr>
<td>Physical Properties of Mature Seed</td>
<td>32</td>
</tr>
</tbody>
</table>
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GENERAL INTRODUCTION

The ability of the seed to germinate, emerge into a uniform and vigorous field stand is a direct function of its quality. Evidence that the parental growth environment may influence the quality of the seed produced is quite strong. Several environmental stresses such as freezing, high temperature, drought, nutrient deficiencies, and/or diseases are known to effect seed viability and vigor. In the present study, controlled environment facilities were used to investigate the effect of different temperature regimes during wheat grain development and maturation on subsequent seed quality.

In the semi-arid regions of Morocco, drought and high temperatures are frequently found together, and limit the productivity of most important agricultural crops grown in these regions. Ambient air temperatures of 28 to 40 °C are common during spring and summer periods which coincide with grain development and maturation.

The effect of heat stress on wheat growth, yield and yield components has been the subject of considerable research. However, seed quality was often ignored in most of these studies. Furthermore, existing information on the influence of temperature on seed quality is related to either physical quality, chemical composition or to seed dormancy.
Comparatively little attention has been given to the physiological quality or metabolism of the seed as they are effected by the maternal temperatures.

Factors that contribute to the establishment, germinability and vigor of the mature seed begin in the field. There is general agreement that the viability and the performance of the seed during its early stages of germination may be related to the conditions under which that seed had formed, developed, and matured. In fact, differences in seed vigor have been reported to result from events early during seed formation and development.

Seed germination is an energy requiring process. Recently it has been agreed that most of the energy needed during early germination is supplied by mitochondria via oxidative phosphorylation. The amount of the energy produced will, in turn, determine the performance of the subsequent germination events. Evidence of the influence of the environmental stress, in this case high temperatures, on nucleotide levels and mitochondria is quite strong. However, most of the reported studies have focused on plant growth and development and little has been done on the plant progeny, the seed. Furthermore, most studies carried out on the seed itself were mainly interested in the after-harvest stages. Thus, much remains to be investigated relative to the effect of temperatures during pre-harvest stages on different
metabolic processes of the produced seed.

The objectives of this study were:

(1) To examine the effects of high temperatures during seed development and maturation on wheat seed quality as measured by different parameters including physical properties, seed germination, seedling vigor, and excised embryo growth potential and oxygen uptake.

(2) To investigate the effects of parent growth temperatures on the biochemical metabolism of the produced wheat seed during its early stages of germination as assessed by mitochondrial activity and adenine nucleotide levels of the isolated embryos.

A better understanding of the physiological and biochemical basis of seed response to parent growth temperatures will contribute to a better management and production of high yield and high quality seed.

Explanation of the Dissertation Format

This dissertation includes two papers which will be submitted for publication in Seed Science Research. The first paper entitled "Effect of heat stress during seed development and maturation on wheat seed germination and vigor" examines physical seed characteristics, seed
germination and seedling vigor as affected by different temperature regimes. The second paper entitled "Mitochondrial respiration and nucleotide pools during early germination in wheat embryos affected by heat stress during seed development and maturation" investigates the effect of parent growing temperature on the metabolism of the seed during early stages of germination. These two papers are preceded by literature review and followed by general conclusions, references cited outside papers and appendix.
LITERATURE REVIEW

Most agriculturally important crops start from seeds. Therefore, high seed quality is important for both the producer and the user. Seeds in their lifetime are exposed to three different environments (Harman and Stasz, 1986): the seed production field environment, where seeds form, develop and mature; the storage environment, where seeds are conditioned and kept until needed; and the soil environment, where seeds are put in the soil to regenerate new plants. To produce and preserve a high quality seed, optimum conditions should be maintained at each level. Because this is unlikely to occur, decreases in seed quality are inevitable. Deterioration can take place either during development and maturation of the seed on the mother plant or while the seed is in storage (Helm et al., 1989). It has been reported that vigor and viability differences arise early during seed development (Dell‘Aquila and Toritto, 1991).

It is well known that the germination and viability of seeds may vary greatly from year to year and from one production site to another. Much of this variation has been attributed to the environmental conditions prevailing during the formation, development and maturation while the seed is still attached to the mother plant. Different studies have shown that extensive damage to developing and maturing seeds
can be caused by different types of environmental stress such as, freezing, high temperature, inadequate water supply and mineral deficiencies (Austin, 1972; Delouche, 1980; Dornbos et al., 1989; Frey, 1981; and Tekrony, 1981). Mild climatic conditions during the pre-harvest period usually contribute to high quality seed, whereas adverse conditions often result in rapid losses of viability and vigor of the mature seed (Delouche, 1980).

Depending on the stage of development, high temperatures may strongly influence the quality of mature seed. For the purpose of this study, we divided seed quality into three major categories: physical quality (seed size, seed mass, and seed chemical composition); physiological quality (seed dormancy, seed germination, and seed vigor); and biochemical quality (respiratory system, nucleotide pools, and protein and nucleic acid synthesis). This literature review will examine the physical, physiological and biochemical changes which can take place as result of heat stress while seed is still attached to the mother plant.

Physical Seed Quality

It is well established that temperature has a major influence on the final yield of grain cereals. The response, however, varies with stage of development. High temperatures
during booting stage, or before anthesis, greatly reduce grain number per ear (Wardlaw et al., 1989). Tashiro and Wardlaw (1990a) reported that seed set adjustments mostly occur within 10 days after anthesis. An increase in temperature from 21 to 30 °C immediately following anthesis resulted in a decrease in seed number. After the seed number has been determined, cereal grain yield becomes proportional to kernel weight which is function of the rate and duration of grain filling period (Wiegnand and Cuellar, 1981). High temperatures during or following anthesis adversely affect both these parameters (Wardlaw et al., 1980). Field and growth chamber experiments confirm that heat stress, at this period of seed development, accelerates initial grain growth rate but shortens the grain filling period (Bauer et al., 1985; Bruckner and Frohberg 1987; and Sofield et al., 1977). However, such compensation occurs only under mild stress (15 to 21 °C) and not under severe stress (21 °C and above). Under severe stress the decrease in duration of grain filling period is no longer balanced by an increase in the grain growth rate (Bhullar and Jeuner, 1983; Sofield et al., 1977; and Wardlaw et al., 1980). As a consequence, the mature seed mass at higher temperatures is much reduced.

Heat stress may also alter other physical seed traits while it is still on the mother plant. The specific type and extent of damage is dependent on the timing of the stress
treatment in relation to the stage of grain development. Tashiro and Wardlaw (1990ab) reported a wide range of grain damage when they applied high temperature treatments during the period from head emergence to the early stages of seed development. High temperatures two to three days before anthesis resulted in a high frequency of sterile grains. Parthenocarpic, abortive, and shrunken grains were induced by high temperatures during and three days after anthesis, whereas high temperature treatments ten days after anthesis resulted in notched, split, and opaque grains. The same authors (1990b) reported that heat stress strongly influenced wheat grain dimensions. Grain length at maturity was most sensitive to high temperatures imposed seven days after anthesis, while grain width was most sensitive at 12 to 21 days after anthesis. Similar results have been reported in beans by Abdus Siddique and Goodwin (1980). They also noted a high frequency of irregularities in the final appearance of the seeds subjected to high temperatures immediately after pod set. Khan and Laude (1969) had observed that the thickness of barley grain seed coat decreases with longer exposure of the developing seed to warmer temperatures. Keigley and Mullen (1986) found that a high temperature environment during soybean seed fill increased the percentage of small, etched and discolored seeds, and reduced the seed mass.
Seed chemical composition also can vary with stage of development and prevailing environmental conditions. Bewley and Black (1985) described the time course of carbohydrate, lipid, and protein composition changes during the development of cereal seeds. Starch and proteins are the major constituents of mature wheat seed. Their content and proportion per grain are determined by the interaction of genotype with the environment. The increase in protein content of the seed is positively correlated with environmental stresses such as high temperature and inadequate water supply (Bhullar and Jeuner, 1985; and Spiertz, 1977). However, this increase is mostly due to a reduction in starch content of the grain and not to a change in the quantity of nitrogen per se (Bhullar and Jeuner, 1985).

Physiological Seed Quality

The physiological quality of the seed has also been reported to be influenced by parent seed environment. One of the aspects of physiological quality is seed dormancy. Differences in seed dormancy may be genetic in origin but are also influenced by the environmental conditions during seed development and ripening (Hagemann and Ciha, 1987; and Sawhney and Naylor, 1978). It is well established that the
degree and extent of post-harvest seed dormancy are strongly affected by the combination of the environmental conditions under which seed had developed and matured. With very few exceptions, a decrease in seed dormancy is positively correlated with high temperature during seed maturation (Fenner, 1991). Koller (1962) showed that warm temperatures during the maturation of lettuce seeds reduced dormancy. Similarly, Gray et al. (1988) reported that high temperatures alleviate or prevent the occurrence of dormancy in lettuce. This reduction in dormancy by warmer temperatures during seed development has also been demonstrated in the wild oats (Sexsmith, 1969), barley (Khan and Laude, 1969) and wheat (Reddy et al., 1985). Several studies have reported that high temperatures at the time of seed development and ripening shortens the dormancy period of wheat seeds (Olsson and Mattsson, 1975; and Lalluka, 1976). For these species that exhibit post-harvest dormancy, high temperatures during seed development often result in higher germination percentages due to a decrease in seed dormancy (Mullen, 1992). The exceptions to this general observation are those species which express little or no post-harvest seed dormancy. In this case, high parent growth temperatures result in a decrease in germination and/or vigor of mature seed. Keigley and Mullen (1986) reported that high temperatures during soybean seed fill reduced germination and
vigor of mature seeds. High temperature stress during corn seed maturation also affected subsequent seedling emergence and reduced seedling vigor as indicated by root and shoot dry weight (Frey, 1981). Fussel and Pearson (1980) found that the temperature at which the Pearl millet grain had developed did not affect seed viability, but grains developed at low temperatures produced more vigorous seedlings than the grains which had developed at high or very high temperatures. Mohamed et al. (1985) found that the temperature conditions during millet seed development affected seed size, and subsequently, germination rate and seed viability. Similarly, Steiner and Opoku-Boateng (1991) demonstrated that high air temperature (daily maximum >35 °C) shortly before and after anthesis reduced vigor of mature lettuce seed. Similar results have also been reported for crisp lettuce seeds (Gray et al., 1988). Datta et al. (1972) reported that the temperature regime under which the mother plants grew affected germination speed and germination percentage of the Aegilops caryophyllea. In their study on oat, Sawhney and Quick (1985) demonstrated that even the temperatures during vegetative growth (up to anthesis) can influence the germination behavior of the resulting seeds. Walter and Jensen (1970) reported that air and soil moisture regimes during alfalfa seed production not only affected seed yield, size and germination but also influenced vigor of subsequent
seedlings. Moss and Mullett (1982) demonstrated that seed vigor in beans can be modified by varying the temperature under which the seed is produced over several generations. In contrast to this negative response, an increase in temperature during seed development has been reported to increase seed germination and vigor in some species, such as barley (Khan and Laude, 1969), bracted plantain (Stearns, 1960), onions (Gray and Steckel, 1984) and sugar beet (Wood et al., 1980).

These differences in seed germination and vigor were mainly attributed to the effect of high temperatures on seed weight and composition of the seed, particularly nitrogen content (Gray et al., 1988; Fussel and Pearson, 1980; and Halmer and Bewley, 1984).

**Biochemical Seed Quality**

Physiological differences in seed performance are usually dependent on the biochemical metabolism of seed during the early stages of imbibition. One of the apparent biochemical changes that occurs at this stage of the germination process is an increase in oxygen uptake. It is well known that respiration is necessary for growth. Respiration plays at least two important roles during germination: provision of the required energy to the
germinating seed; and as an index of both integrity and overall activity of the metabolic machinery of the seed (Dell'Aquila and Toritto, 1991). Compared to imbibed seed, dry seed usually have very low respiration. Oxygen uptake is readily detectable very soon after the initiation of imbibition and continues to increase until the seed tissue is fully hydrated. This suggests that a functional respiratory mechanism is present in dry seed and rapidly activated upon hydration and implies an increase in mitochondrial activity. Regardless of seed quality, mitochondria in quiescent seeds are functionally and structurally deficient, they are poorly differentiated internally and exhibit low respiratory control and ADP:O ratios (Harman and Stasz, 1986; Pradet, 1982; and Bewley and Black 1985). As soon as seeds are wetted, mitochondrial activity begins. Ultrastructural studies show that during the first hours of imbibition, mitochondria become enlarged and exhibit a more complex inner membrane structure (Morohashi et al., 1981; and Solomos et al., 1972). Several physiological studies also demonstrated an increase in the quantity of mitochondria, activity of mitochondrial enzymes, state 3 respiration rate, and respiratory control and ADP:O ratios during the early phases of germination (Pradet, 1982; Morohashi et al., 1981). Beevers and Hanson (1964) found that both respiration and phosphorylation in corn seedling roots and shoots were affected by high
temperatures. Also, Madden (1992) reported that high temperature treatment during desiccation resulted in mitochondrial damage of maize seed axes. Seed deterioration during storage or accelerated ageing can also result in a delay in mitochondrial activation, a decrease in respiration rates and respiratory control ratio values, and a lower phosphorylative efficiency (Ferguson et al., 1990; and Woodstock et al., 1984).

Seed germination is a biological process that requires energy. This energy, in the form of nucleotide, plays a major role in germinating seed metabolism; nucleotide participate in and regulate all phases of carbohydrate, lipid, protein and nucleic acid metabolism (Mangat, 1982). ATP is regarded as the main form of the available energy. However, dry seed do not accumulate any ATP. Therefore, ATP production at the early stages of seed imbibition is essential for successful germination and for direction of growth and developmental processes that occur in germinating seed (Moreland et al., 1974; and Perl, 1986). Different environmental stresses have been shown to influence the ATP level and/or the adenylate energy charge. Mangat (1982) reported that a combination of high air and soil temperatures resulted in low levels of total free nucleotide, RNA, and protein in soybean seedlings. Similar metabolic changes occur during other environmentally stressful conditions.
Turner and Wellburn (1985) showed that the ATP levels in leaves of sweet pepper were very sensitive to extremely mild water stress, where a small change in leaf water potential resulted in a significant reduction in leaf ATP content to 70-80% of the control. Mendelssohn and McKee (1985) reported that nutrient stress resulted in greatly reduced adenylate pools in marsh plant leaves. Ching (1982) stated that any chemical or herbicide treatment that inhibits respiration, photosynthesis or growth tends to reduce ATP content in seeds and young seedlings. Madden (1992) showed that desiccation temperature over 40 °C resulted in a reduction of all the high energy nucleotide levels during the early stages of maize seed germination. Several investigators have also reported that ATP levels increase only slowly during the hydration of aged seeds (Ching, 1973; Ching and Danielson, 1972; and Lunn and Madsen, 1981).

The adenylate energy charge (AEC), which characterizes the energy status of living cells, follows almost the same pattern as nucleotide pools. The AEC of quiescent seed is extremely low, but it rapidly increases as soon as seed imbibes water (Moreland et al., 1974). AEC of 0.5 or less is often indicative of a quiescent state, whereas AEC of 0.8 or more is indicative of a metabolically active state (Bewley and Black, 1985). This increase in AEC reflects an increase in ATP and a corresponding drop in both AMP and ADP.
concentrations. Also, AEC has been used as an index of stress (Ivanovichi and Weibe, 1981).

**Mechanism of Heat Stress on Seed Quality**

Many hypotheses have been proposed regarding the mechanisms of heat injury to cultivated plants. These hypotheses have been ranked by Levitt (1972) as direct or indirect heat injuries. Direct heat injury involves both lipid and protein changes which result in membrane damage. Loss of membrane integrity at high temperatures could be due to either excessive fluidity and phase transition of lipids or to denaturation and aggregation of membrane proteins. Indirect heat injury involves primarily damage of major organelles such as mitochondria and chloroplast. In seeds, the precise mechanism of heat stress during seed development on physiological and biochemical metabolism of the resulting mature seed is still unknown. However, a number of theories and processes have been formulated regarding seed deterioration either in the field or in storage. One of the extensively studied mechanisms is membrane integrity and its chemical composition. Halmer and Bewley (1984), in their review of seed vigor, suggested membrane integrity as an important determinant of seed vigor. Excess solute leakage from seeds often indicates reduced vigor (AOSA, 1983).
Basavrajappa et al. (1991) provided evidence for the loss of membrane integrity as the probable first deteriorative change during ageing in maize seeds. Several studies have reported that environmental stress during seed growth, development and/or maturity strongly alter the chemical composition of the seed membranes. Dornbos et al. (1989) have shown that drought stress and high temperature during seed fill strongly influenced the fatty acid composition of membrane phospholipids in soybean seeds. Dornbos (1988) in his review of the effect of high parent growth temperature on seed phospholipid composition, concluded that membrane lipid composition and fluidity may represent one of the mechanisms of heat injury on seed quality.

Other hypotheses of heat injury to seed quality may involve alteration in the expression of one or several components of seed metabolism such as enzymatic machinery, gene expression, desiccation tolerance and/or heat shock proteins (HSP) (Mullen, 1992). Bewley and Marcus (1990) reported that the expression of some developmentally regulated genes can be altered by environmental factors during seed development. Heat stress is known to induce synthesis of heat shock proteins. Abernethy et al. (1989) suggested that HSPs could be synthesized as a normal part of seed development and retained during seed desiccation, or they can be synthesized only in response to high temperature
stress during seed maturation. Howarth (1990) reported that long-lived RNAs in the dry seed, including mRNAs encoding HSPs, can be synthesized during the seed-ripening period. The author suggested that the environmental conditions prevalent during development and maturation of the seed on the parent plant modify the subsequent seed thermosensitivity during germination. In other words, these environmental conditions can affect the capacity for the HSP synthesis in the early stages of germination. Helm et al. (1989), in their study on wheat embryos, concluded that low vigor embryos are deficient in their ability to synthesize a number of HSPs that are normally synthesized by high vigor embryos. The authors suggested that this decrease in HSP response is due to specific lesions in the gene expression in low vigor seeds.

Seed Vigor Measurement

Seed quality is commonly evaluated by the standard germination test, which is designed to provide optimal conditions for early plant growth and development. Unfortunately, optimum conditions are rarely encountered in the field. Seed lots that exhibit a higher percentage germination under the optimal conditions of standard germination test often show reduced germination in the field.
Vigor is defined as "those properties of the seed that determine the potential for rapid, uniform emergence and development of normal seedling under a wide range of field conditions" (AOSA, 1983). Different tests have been developed and used to measure seed vigor (AOSA, 1983; and ISTA, 1987). Widely used tests include: seedling growth evaluation, stress tests, some biochemical tests such as tetrazolium chloride, and electrical conductivity. Recently there has been increasing interest in using physiological and biochemical measurements to evaluate seed vigor (Halmer and Bewley, 1984). Respiratory metabolism of germinating seeds, and more specifically mitochondrial activity, have been shown to be important in the expression of seed quality and seedling vigor (McDaniel, 1969; and McDaniel, 1973). McDaniel (1969) reported that both the quantity of mitochondria and their activity directly influence seed vigor in barley. A decrease in both the number and efficiency of mitochondria have been also reported in low vigor germinating soybean seed axes (Woodstock et al., 1984). A positive relationship between seed vigor and oxygen uptake by embryos and/or whole seed have been also reported (Tluczkiewicz, 1980; Woodstock, 1973; and Woodstock et al., 1984).

Similarly, the use of ATP as an index of seed vigor has been reported by many investigators. Significant correlations were found between seed vigor and ATP levels in
germinating seeds of various crop species including lettuce, rape, rye grass and crimson clover (Ching, 1973), wheat (Standard et al., 1983), cauliflower (Lunn and Madsen, 1981) and lettuce (Ching and Danielson, 1972); where low vigor seeds exhibit low ATP levels during the early stages of imbibition. Standard et al. (1983) reported that the analysis of nucleotide levels reflects the metabolic state of the seed and provides a useful tool for the assessment of growth potential of the seed. Lunn and Madsen (1981) demonstrated a drastic drop in the ATP levels after seven hours of imbibition of cauliflower seeds following accelerated aging, without any significant change in the germination percentage. The authors suggested that severe impairment in seed metabolism machinery may take place before it is possible to detect any decrease in viability as measured by the conventional germination test.
PAPER 1. EFFECT OF HEAT STRESS DURING SEED DEVELOPMENT AND MATURATION ON SEED GERMINATION AND VIGOR
ABSTRACT

Two wheat cultivars "Marzak" and "Oum-rabia" were subjected to three temperature regimes (20/15, 28/21, 36/29 °C) beginning 10 days after anthesis to maturity. As expected, high temperature resulted in low values of both seed yield and physical traits of seed quality. The effect of temperature on seed germination was not consistent among the two cultivars. High temperature during seed development and maturity had no effect on seed germination of Oum-rabia, whereas it decreased seed germination of Marzak. In contrast to seed germination, seed vigor was adversely affected by heat stress. This decline in seed vigor was reflected in reduced shoot and root dry weight, increased shoot/root ratio, reduced root length, low root number per seedling, and high seed conductivity. Excised embryo culture showed a marked differences in the embryo growth potential. Although embryos from all treatments had germinated, a delay of 24 to 48 hours was observed in the germination of embryos excised from seeds grown under high temperature conditions. Also, their shoot and radicle development over time lagged behind that of embryos isolated from seeds grown under cool temperature conditions. Exposing seeds to high temperature during development and maturity resulted also in low embryo oxygen uptake. Results presented in this study show that the
growing conditions in this case temperature, of parent plant affect the quality of its seed.
INTRODUCTION

Seeds obtained from different seasons or different geographical areas often vary in germination capacity and viability. Much of this variation has been attributed to differences in environmental conditions prevailing during the formation, development and maturation of the seed while still on the mother plant (Peacock and Hawkins, 1970; and Datta et al., 1972). In their review on seed quality, Roberts and Black (1989) reported that seed quality may be affected before the seeds are mature and ready for collection from the mother plant. Several workers have reported on the influence of different types of environmental stress on seed quality, e.g., freezing, high temperatures, water stress, and mineral deficiencies (Austin, 1972; Delouche, 1980; Dornbos et al., 1989; Frey, 1981; and Tekrony, 1981).

In the semi-arid regions of Morocco, drought and high temperatures occur frequently during late spring and early summer. Ambient field temperatures of 28 to 40 °C are common during this season, which coincides with grain development and maturation. Temperature is one of the most important environmental factors affecting not only plant growth and development but also the quality characteristics of the progeny. Evidence consistently shows that exposure of the parent plant to high temperatures affects the quality of the
High temperatures following anthesis adversely affect grain development in wheat (Tashiro and Wardlaw, 1990). High temperatures accelerate initial grain growth rate while shortening the grain growth period (Bauer et al., 1985; Bruckner and Frohberg, 1987; and Sofield et al., 1977). As a consequence, the final grain weight is much reduced at higher temperatures. Other physical properties of the seed including seed size, seed dimensions, seed coat and the final appearance of the seed, are also affected by high parent growth temperatures (Tashiro and Wardlow, 1990; Keigley and Mullen, 1986; and Khan and Laude, 1969).

In addition to the effects of high temperatures on the physical properties of seed, physiological quality is also strongly influenced. Previous studies have demonstrated that high temperatures during seed development and maturity decrease seed dormancy in several species (Koller, 1962; Fenner, 1992; Gray et al., 1988; Reddy, 1985; Olsson and Mattsson, 1975; and Lalluka, 1976). Several workers have also shown that heat stress may decrease seed germination and/or seedling vigor. Keigley and Mullen (1986) reported that high temperatures during soybean seed fill reduced germination and vigor of mature seeds. Fussel and Pearson (1980) found that the temperature at which pearl millet grain developed did not affect seed viability, but grains developed
at low temperatures produced more vigorous seedlings than grains developed at high temperatures. Similarly, Steiner and Opoku-Boateng (1991) demonstrated that high air temperatures shortly before and after anthesis reduced vigor of mature lettuce seed.

Respiration, or oxygen uptake, is one of the most important metabolic factors necessary for successful seed germination. Impaired respiratory activity and reduced oxygen uptake by either whole seeds, embryos or axes, have been associated with seed deterioration in several species including soybean (Ferguson et al., 1990; Woodstock et al., 1984; and Leopold and Musgrave, 1980), cotton (Woodstock et al., 1985), and rye (Tluczkiewicz, 1980). However, so far there is little evidence of changes in seed respiratory metabolism that may result from excessive temperatures during seed development and maturity. Recently, Madden (1992) showed that desiccation temperature alters respiration rates in imbibed maize axes, i.e., high desiccation temperature (45 °C) resulted in lower oxygen uptake by imbibed axes.

Environment represents a combination of different factors, e.g., water, temperature, light and day-length. This makes it more difficult to assess the effect of each stress on plants growing in field. Each factor must be considered separately under a controlled environment. In the present study, controlled environment facilities were used to
investigate the effect of different temperature regimes during grain development and maturation on subsequent seed quality. The effect of heat stress on wheat yield and yield components is well documented. However, seed quality was often ignored in these studies. The objectives of this study were to examine the effect of high temperatures during seed development and maturation on seed quality as measured by different parameters including physical properties, seed germination and seedling vigor, excised embryo growth potential and oxygen uptake.
MATERIALS AND METHODS

Plant Material

Two spring wheat (Triticum durum) cultivars from Morocco, "OUM-RABIA" and "MARZAK", were grown in growth chambers at Iowa State University, Ames, Iowa. Initially, 8 to 12 seeds were planted per pot. Two weeks after emergence plants were thinned to 3 plants per pot with the tillers removed to keep only the main stem.

Plants were grown to anthesis on a 20 °C day (12 h) and 15 °C night (12 h) cycle. Temperature treatments were initiated 10 days after anthesis (extrusion of first anthers): LOW temperature treatment = 20/15 °C, MEDIUM temperature treatment = 28/21 °C, and HIGH temperature treatment = 36/29 °C. These temperature treatments were maintained until harvest with a cycle of 8/16 hours (day/night). The photoperiod was, however, maintained as initially set. Throughout their life cycle, plants were well watered to avoid any effect of drought stress. Harvests were staged so that seed from the lower temperature treatments could attain full maturity. At maturity ears were harvested, threshed and weighed individually, then all seeds in each treatment for each cultivar were bulked and stored for subsequent laboratory analyses. To avoid any confounding effects from dormancy all seed lots were put in paper bags
and placed in a cold room (10 °C /45% RH) for at least 6 months prior to starting any analysis.

Physical Traits of Mature Seeds

The following parameters were recorded for each cultivar in each treatment: seed number per ear, ear dry weight, seed dimensions, 100 kernel weight, isolated embryo fresh weight, and any irregularities in grain morphology.

Seed Germination and Seedling Vigor

Germination percentages were based on 4 replications of 25 seeds each per treatment for each harvest. Tests were performed on rolled paper towels at 20 °C for 14 days. After germination percentages, and root number and length measurements for each seedling were taken, the caryopses were removed and the shoot and root dry weights were determined according to AOSA (1983) guidelines (drying 24 h at 103 °C).

Single seed conductivity was measured on 3 replications of 100 seeds after 24 hours soaking at room temperature (25 °C), with ASA 1000 seed analyzer (Agro Science, Ann Arbor, Michigan). Single seeds were soaked in individual cells containing 3 ml of deionized-distilled water.
Embryonic Respiratory Measurement

Oxygen uptake by excised embryo was measured polarographically using a small volume Rank oxygen electrode (Rank Brothers Co., Cambridge, England), connected to a YSI 53 Biological Oxygen Monitor (Yellow Spring Instrument Co., Yellow Spring, Ohio). The oxygen electrode was calibrated with air-saturated distilled water at 25 °C. The $O_2$ concentration in air-saturated water was assumed to be 250 $\mu$M. Eight excised embryos per sample were added to the
sample chamber containing 0.5 ml air-saturated water. Oxygen uptake was recorded for 10 min periods at 0, 2, 4, 6, 8, 10, and 12 hours after imbibition. Between measurement periods embryos were removed from the sample chamber and kept on moist Kimpac.

**Statistical Treatment**

This experiment was treated as a split plot design with the temperature treatments as the main plot factors and cultivars as sub-plot factors. Three growth chambers were used and the experiment was repeated 3 times. Laboratory analyses were carried out on bulked seed for each cultivar in each treatment within each harvest. Unless specified, all laboratory analyses were conducted at least two times and only the means are reported here.
Physical Properties of Mature Seeds

Physical characteristics of mature seed, seed yield and yield components were all affected by the maternal plant temperatures applied during seed development and maturation. Seed number per plant slightly decreased by about 14 to 15% in both cultivars with an increase in maternal temperature (Table 1). Regardless of cultivar differences, seeds developing and maturing under high temperature conditions weighed less than those produced under cooler conditions. However, the effect was less severe on isolated embryos. High temperatures decreased intact seed and isolated embryo weights by approximately 50 and 22%, respectively, in both cultivars. Although, the cultivar Marzak produced smaller seeds than Oum-Rabia, Marzak had larger embryos. Seed dimensions were also affected by maternal plant temperatures. Seed length was not significantly affected by high temperatures, but seed width was reduced by approximately 33% in both cultivars. Also, ear dry weight showed a great decline (26 to 60%) as temperature increased.

High maternal temperatures resulted in lower values for both seed yield and physical characteristics of seed quality, and the differences were slightly greater for Marzak.
Table 1. Effect of temperature during seed development and maturation on seed yield and seed characteristics

<table>
<thead>
<tr>
<th>Variety</th>
<th>Temp.</th>
<th>Ear wt.</th>
<th>Seed number</th>
<th>Seed* wt.</th>
<th>Embryo* wt.</th>
<th>Seed length</th>
<th>Seed width</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td>g</td>
<td>/ear</td>
<td>g</td>
<td>mg</td>
<td>mm</td>
<td>mm</td>
</tr>
<tr>
<td>Marzak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20/15</td>
<td>3.13</td>
<td>54.30</td>
<td>5.58</td>
<td>62.75</td>
<td>6.65</td>
<td>3.40</td>
</tr>
<tr>
<td></td>
<td>28/21</td>
<td>2.40</td>
<td>51.60</td>
<td>4.62</td>
<td>59.50</td>
<td>6.59</td>
<td>2.98</td>
</tr>
<tr>
<td></td>
<td>36/29</td>
<td>1.11</td>
<td>46.60</td>
<td>2.43</td>
<td>49.00</td>
<td>6.24</td>
<td>2.12</td>
</tr>
<tr>
<td>Oum-rabia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20/15</td>
<td>3.04</td>
<td>53.10</td>
<td>5.64</td>
<td>59.25</td>
<td>7.23</td>
<td>3.25</td>
</tr>
<tr>
<td></td>
<td>28/21</td>
<td>2.19</td>
<td>49.90</td>
<td>4.57</td>
<td>50.00</td>
<td>6.95</td>
<td>2.41</td>
</tr>
<tr>
<td></td>
<td>36/29</td>
<td>1.36</td>
<td>44.80</td>
<td>3.05</td>
<td>46.50</td>
<td>6.81</td>
<td>2.29</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>0.42</td>
<td>10.51</td>
<td>0.33</td>
<td>1.88</td>
<td>0.39</td>
<td>0.38</td>
</tr>
</tbody>
</table>

* seed and embryo weight on 100 basis.

b day/night temperature.
Germination and Seedling Vigor

Standard germination tests were conducted on seed lots stored 6 to 18 months under 10 °C, 45% RH. The effect of parent plant temperatures on germination percentages is shown in Table 2. There are marked differences between the two genotypes. The temperature under which the seeds had developed and matured did not influence percentage germination for the cultivar Oum-Rabia, whereas seeds of the cultivar Marzak obtained from warm or high temperature treatments showed significantly lower germination values than the control. In contrast to seedlings of Oum-rabia, which were all evaluated as normal under all temperature conditions, Marzak exhibited abnormal seedlings, and this number increased as growth temperatures increased (Plate 1 and 2).

Seedling shoot and root dry weights decreased with an increase in parent plant temperatures (Table 2). However, root development appeared to be more severely affected by warmer temperature conditions than shoot development. High temperature treatments reduced shoot dry weight by 38 and 41%, and root dry weight by 64 and 71% for Oum-Rabia and Marzak, respectively. In contrast, shoot/root (S/R) increased for both cultivars as temperatures increased from 20/15 °C to 36/29 °C. Also, heat stress during seed development and maturation reduced root number and root
Table 2. Effect of temperature during seed development and maturation on seed germination and seedling vigor

<table>
<thead>
<tr>
<th>Variety</th>
<th>Temp.</th>
<th>Germ.</th>
<th>Shoot</th>
<th>Root</th>
<th>S/R</th>
<th>Number</th>
<th>Length</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>°C</td>
<td>%</td>
<td>mg</td>
<td>mg</td>
<td></td>
<td>mm</td>
<td></td>
</tr>
<tr>
<td>Marzak</td>
<td>20/15</td>
<td>93.00</td>
<td>9.45</td>
<td>8.28</td>
<td>1.14</td>
<td>5.28</td>
<td>30.68</td>
</tr>
<tr>
<td></td>
<td>28/21</td>
<td>85.00</td>
<td>9.42</td>
<td>5.02</td>
<td>1.88</td>
<td>4.26</td>
<td>29.70</td>
</tr>
<tr>
<td></td>
<td>36/29</td>
<td>72.00</td>
<td>5.59</td>
<td>2.41</td>
<td>2.50</td>
<td>3.23</td>
<td>20.68</td>
</tr>
<tr>
<td>Oum-rabia</td>
<td>20/15</td>
<td>99.00</td>
<td>11.20</td>
<td>12.74</td>
<td>0.88</td>
<td>5.56</td>
<td>35.21</td>
</tr>
<tr>
<td></td>
<td>28/21</td>
<td>98.00</td>
<td>10.28</td>
<td>8.52</td>
<td>1.22</td>
<td>4.76</td>
<td>34.01</td>
</tr>
<tr>
<td></td>
<td>36/29</td>
<td>98.00</td>
<td>6.86</td>
<td>4.54</td>
<td>1.53</td>
<td>3.48</td>
<td>30.89</td>
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<tr>
<td>LSD (0.05)</td>
<td></td>
<td></td>
<td>4.50</td>
<td>2.54</td>
<td>1.45</td>
<td>0.32</td>
<td>0.64</td>
</tr>
</tbody>
</table>

* day/night temperature.
Plate 1. Influence of temperature during seed development and maturation on seed germination and seedling vigor, Low: 20/15 °C; Med: 28/21 °C; High: 36/29 °C
Plate 2. Influence of temperature during seed development and maturation on seed germination and seedling vigor. Low: 20/15 °C; Med: 28/21 °C; High: 36/29 °C
length per seedling (Table 2 and Plate 3). Root number was reduced by 14 and 19% in medium temperature treatments and by 37 and 39% in high temperature treatments for Oum-Rabia and Marzak, respectively. Medium temperatures had no significant effect on seedling root length; however, high temperature treatments decreased root length by 12 and 32% for Oum-Rabia and Marzak, respectively.

Seeds from all treatments showed an increase in the conductivity after 24 h soaking. Seeds produced at higher temperatures released more electrolytes than those produced at lower and medium temperatures (Figure 1).

**Embryo Growth Potential and Oxygen Uptake**

The parent plant temperatures had no effect on the germination capacity of the excised embryos in either cultivar. However, a delay in germination was observed in embryos isolated from seeds grown at medium and high temperatures (Plate 4 and 5). Embryos excised from seeds produced at cooler temperatures germinated 24 h and 48 h after incubation, respectively, for Oum-Rabia and Marzak. However, embryos from seeds grown at high temperatures germinated 48 h and 72 h, respectively, for Oum-Rabia and Marzak. Also, the rate of development of both the radicle and coleoptile was affected by the temperature conditions during seed growth (Figure 2). Regardless of cultivar
Plate 3. Influence of temperature during seed development and maturation on root length and root number per seedling, Low: 20/15 °C; Med: 28/21 °C; High: 36/29 °C
Figure 1. Conductivity of seeds grown under three different temperature conditions, 1: Marzak; 2: Oum-rabia
Plate 4. Influence of temperature during seed development and maturation on the excised embryo germination and radicle and shoot development, Low: 20/15 °C; Med: 28/21 °C; high: 36/29 °C
Plate 5. Influence of temperature during seed development and maturation on the excised embryo germination and radicle and shoot development 
Low: 20/15 °C; Med: 28/21 °C; high: 36/29 °C
Figure 2. Effect of temperature during seed development and maturation on excised embryo radicle and shoot length
differences, consistent developmental trends were observed. Embryos from high temperature treatments lagged behind low temperature embryos during the entire period of incubation with respect to radicle and coleoptile development.

Figures 3 and 4 illustrate the effect of temperatures on respiration rates and oxygen uptake by isolated embryos. Within 2 h of imbibition, oxygen uptake was markedly higher in embryos isolated from seeds grown at lower temperatures than in those excised from seeds grown at medium and high temperatures. These differences in oxygen uptake increased over time to reach a maximum at 12 h imbibition. In low temperature treatments, oxygen uptake by Marzak embryos was higher than in Oum-Rabia embryos during the entire period of measurement. In contrast, respiration rates per embryo were higher for Oum-Rabia at medium and high temperature treatments. Trends in oxygen uptake were similar when expressed in either nmol oxygen per 10 min per single embryo or per mg of embryonic tissue. However, in the later expression of the respiration rates (on weight basis) and at a corresponding imbibition time, differences between treatments were reduced by approximately 50%. In fact, no differences were observed between low and medium temperature treatments for the cultivar Oum-Rabia.
Figure 3. Effect of temperature during seed development and maturation on isolated wheat 'Marzak' embryo oxygen uptake
Figure 4. Effect of temperature during seed development and maturation on isolated wheat 'Oum-rabia' embryo oxygen uptake.
DISCUSSION

Environmental conditions, in this case temperature, to which the maternal plants are exposed are known to affect seed yield and yield components (Spiertz, 1977; Tashiro and Wardlaw, 1990). In the present study conducted under controlled environmental conditions, parental plant temperatures commencing 10 days after anthesis had a major effect on seed mass. As expected, plants grown under cooler temperatures produced heavier seeds than those grown under higher temperatures. Seed mass in wheat depends mostly on the current photosynthesis of the flag leaf and ears. Although no measurements were made comparing high to low temperature treatments, flag leaves and ears of the plants in the high temperature treatments lost their green color rapidly, that is plants senesced and ears matured early. This leaf senescence would reduce the grain filling period of those seeds grown at high temperatures. Although the seed weight decreased with increasing temperatures, the effect was relatively slight on the embryo weight. Similar results were reported for white-seed bean by Moss and Mullett (1982), with which high temperatures decreased seed dry weight but had no effect on embryo weight.

A decrease in seed dimensions in response to heat stress has been reported by Tashiro and Wardlaw (1990). In the
present investigation, high temperature treatment adversely affected seed dimensions. But in contrast to their results in which seed length was affected, our results showed seed width as more sensitive to heat stress applied during seed development and maturation. Thus, seeds obtained in this study had similar lengths but were thinner with increased temperatures.

Environmental conditions during seed development and maturation are known to affect seed germination (Fussel and Pearson, 1980; Sawhney and Naylor, 1978; Moss and Mullett, 1982; Sawhney and Quick, 1985; Reddy et al., 1985; Alexander and Wulff, 1985; and Junttila, 1973). However, most of these studies were related to seed dormancy. In our study, to avoid any confounding with seed dormancy, seeds were tested for germination after six months of storage. The effect of heat stress on seed germination after 6 months storage was not consistent among cultivars. The cultivar Oum-Rabia was not affected by high temperatures, whereas Marzak showed lower germination percentages in both warm and high temperature treatments. Reduced germination could result from either dormancy or decreased viability. However, in our study, there is no evidence to suggest that either factor had caused the observed decline in germination in the cultivar Marzak, since high temperature is known to break dormancy and all our seeds were viable according to tetrazolium test
(results not shown). These differences in seed germination for Marzak could be attributed to seed damage by high temperature conditions during seed development and maturation. For the genotypic differences, previous investigations reported that the effect of temperature during development and maturation on seed germination and viability may vary with cultivar (Moss and Mullett, 1982; and Sawhney and Naylor, 1978). They suggested that the resultant effect is an interaction between the genotype and the temperature conditions experienced by the parent plants during seed development and maturation. This could be of practical importance to the seed producer in choosing cultivars and the area of production.

In contrast to germination, seedling vigor as indicated by shoot and root dry weight, seedling root length and seedling root number, was reduced by high temperature treatments in both cultivars. Seeds obtained from plants grown under high temperature conditions produced smaller seedlings with lower dry weight, and shorter and fewer roots than those seeds produced at low temperatures. For all measurements, warm temperatures produced intermediate values. Most previous studies related this decline in vigor to seed size. However, Abdus Siddique and Goodwin (1980) showed that seed size alone does not explain the negative effect of high temperatures. In their study, high temperatures applied only
during maturation, when seeds have already attained their final size, still caused damage to soybean seed. These authors and others (Moss and Mullett, 1982) suggested that the rate of drying and the rapid desiccation of seeds at higher temperatures might affect the maturation process and attainment of high seed vigor. Our results support that hypothesis, since the seeds developed at high temperatures matured earlier and dried faster than the seeds produced at cooler temperatures.

The increase in the conductivity of leachates in response to heat stress also indicates a difference in the potential seed vigor between different treatments. Khan and Laude (1969) in their study on barley, showed that exposing plants at seed maturity to very high temperatures resulted in thinner seed coats and increased permeability as indicated by faster imbibition rate and decreased soluble inhibitor content of the seeds. They postulated that this may result in the germination differences of seeds produced in successive years or at different locations in the same year.

In contrast to intact seed germination, especially for Marzak, embryos from all treatments germinated but at different rates. Embryos from low temperature treatments germinated faster and produced seedlings with more rapid radicle and coleoptile development than those from medium and high temperatures. High germination rates in excised
embryos, as compared to intact seed in Marzak, suggest the possibility of an endosperm factor which reduces germination in the intact seed. The possibility that this is related to heat injury requires further investigation.

It is well established that seed deterioration decreases seed respiration rates (Abdul-Baki, 1980; and Woodstock et al., 1984). The present study is the first to investigate the effect of the parent plant temperature on seed metabolism during early stages of germination. Plants that experience heat stress during their reproductive stage produced seeds which expressed lower respiration rates as shown by isolated embryo oxygen uptake. To eliminate the effect of seed weight which is mostly related to the endosperm mass, our studies were focused only on the embryonic tissue. Abdul-Baki and Anderson (1973) had reported that any decrease in the metabolic activity of the excised embryo will be directly associated with a loss in seed vigor. When oxygen uptake was expressed on the embryo fresh weight basis, differences between treatments declined. This suggests that part of the temperature effect was through a decrease in seed weight. However, the remaining difference should be due to a decrease in seed vigor in response to heat stress. The actual results agree with Abdus Siddique and Goodwin (1980) in that seed size alone could not explain the adverse effect of high temperatures during seed development and maturation. Besides
a decrease in seed mass, heat stress may cause some physiological changes that lead to low vigor seeds. The decreased levels of embryo oxygen uptake from high temperature treatment lets suggest some impairment in the respiratory system.
CONCLUSIONS

High parent temperatures during seed development and maturation resulted in low seed yield and poor seed quality. The effect, however, is genotype related. Our results show an inconsistent effect of temperature on seed germination but a consistent effect on seed vigor as indicated by seedling dry weight, root length, root number, seed conductivity, embryo growth potential and embryo oxygen uptake. Results from isolated embryos were comparable to those of intact seeds. This suggests that the observed temperature effect is not totally due to seed size. In addition to its effect on seed weight, high parent temperature may affect seed metabolism and causes some physiological changes which lead to low vigor seeds. Further studies, such as application of temperature treatment at different stages of seed development and analysis of seed quality at different harvest times, are needed in order to better elucidate the effect of temperature on seed quality.

In general, these results suggest that high seed quality in wheat, at least for these two cultivars, will be produced when plants are grown under cool temperature conditions.
REFERENCES


Moss, G.I., and Mullett. 1982. Potassium release and seed vigor in germinating bean (*Phaseolus vulgaris* L.)
seed as influenced by temperature over the previous generations. J.Exp. Bot. 33:1147-1160.


PAPER 2. MITOCHONDRIAL RESPIRATION AND NUCLEOTIDE POOLS DURING EARLY GERMINATION IN WHEAT EMBRYOS AFFECTED BY HEAT STRESS DURING SEED DEVELOPMENT AND MATURATION
ABSTRACT

Wheat seeds were produced under three temperature regimes (20/15, 28/21, 36/29 °C) starting 10 days after anthesis through harvest. Nucleotide levels and respiratory activity of mitochondria isolated from imbibing embryos were determined. Mitochondrial structure from the radicle meristem region of imbibed embryos was examined under electron microscopy. Embryos from low temperature treatments showed rapid accumulation of ATP, higher energy levels and higher rates of oxygen uptake as opposed to embryos from high temperature treatments. Embryos from medium temperature treatments exhibited intermediate values. Parallel to these metabolic changes during early seed germination, results from electron microscopy revealed visible differences in mitochondrial structure. Mitochondria from the low temperature regime were well developed with visible membranes and cristae; while those from high temperature were degenerating. These results provide clear evidence of the influence of parent temperature conditions on the seed metabolism during early stages of germination.
INTRODUCTION

Seed germination involves initiation of numerous metabolic activities. One of the most important events is the marked increase in respiration rates and energy levels, which are believed to be related to mitochondrial activity. This burst of energy, mainly in the form of adenosine triphosphate (ATP), plays an important role in the metabolism of the seed during the early hours of imbibition. Like many other biological systems, the energy status of the seed can be expressed in the form of a ratio, \( \frac{\text{ATP} + 0.5 \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}} \), known as the adenylate energy charge (AEC) (Atkinson, 1968). Similar to ATP content, this ratio is low in dry seed (<0.5), but rapidly increases as the seed is wetted (>0.8) (Moreland et al., 1974; and Bewley, 1985).

The effect of environmental stress on different metabolic processes of plants has been extensively studied. In fact, several investigations reported that environmental stresses such as high temperature (Mangat, 1982), drought (Turner and Wellburn, 1985) and nutrient deficiency (Mendelssohn and McKee, 1985) reduced ATP levels and/or adenylate energy charge. Recently, Madden (1992) demonstrated that high temperature desiccation reduced the functional nucleotide pools during the early stages of the subsequent maize seed germination. Also, AEC has been used

Although the importance of ATP to seed germination and vigor is well established, its origin has been debated for a long time. Recently, Attucci et al., (1991) suggested that most of the ATP produced during the first hours of imbibition is of mitochondrial origin. Regardless of seed quality, mitochondria in quiescent seeds are functionally and structurally deficient. They are poorly differentiated and exhibit low respiratory control and ADP:O ratios (Harman and Staz, 1986; Pradet, 1982; and Bewley and Black, 1985). As soon as the seed is imbibed, mitochondrial activity and quantity increase rapidly (Pradet, 1982; and Morohashi et al., 1981). Previous investigations reported that mitochondria are one of the major organelles which are susceptible to damage from different environmental stresses, such as heat stress, during plant growth and development (Levitt, 1980; and Beever and Hanson, 1964), high desiccation temperatures (Madden, 1992), and seed ageing (Ferguson et al., 1990; and Woodstock et al., 1984).

Different tests have been developed and used to measure seed vigor (AOSA, 1983; and ISTA, 1987). Lately, there have been many attempts to correlate different physiological and biochemical parameters to seed and seedling vigor.
Significant relationships were found between seed vigor and oxygen uptake by the embryo and/or intact seed, ATP levels, mitochondrial activity and quantity of mitochondria (McDaniel, 1969, 1973; Woodstock, 1973; Woodstock et al., 1984; Tluczkiewicz, 1980; Madden, 1992; and Standard et al., 1983). These previous studies suggested that severe impairment in seed metabolism may take place before it is possible to detect any decrease in viability as measured by the conventional germination test.

Evidence of the influence of the environment on nucleotide levels, AEC, and mitochondria is quite strong. However, most of these studies have focused only on plant growth and development and little has been done on the plant progeny. Furthermore, most studies carried out on the seed were mainly interested in the after-harvest stages, especially storage. In fact, it is now well documented that high temperatures, associated with high moisture during storage, strongly affects seed viability, but its effect during pre-harvest stages, while seed is still on the mother plant, remains unknown. So in most of these studies the initial seed quality was ignored, and it is believed that the history of the seed, in other words the environmental conditions under which the seed has been formed, developed and matured, is a very important criterion in seed quality management.
The objectives of the present study was to investigate the effects of the parental environment during seed development and maturation on the biochemical metabolism of the seed during its early stages of germination, as assessed by mitochondrial activity and adenine nucleotide levels.
MATERIALS AND METHODS

Plant Material

Seeds for both cultivars, Marzak and Oum-rabia, were obtained (see paper 1). Seeds were stored in a cold room (10 °C, 47% RH) during the duration of this study. Analyses were performed on isolated embryos and all operations were done on ice.

Nucleotide Extraction and Determination by HPLC

The nucleotide extraction and determination procedures were similar to those used by Standard et al., (1983) and Madden (1992). Samples of 40 excised embryos were used for each cultivar and treatment. Before extraction, embryos were weighed and imbibed in deionized-distilled water on blotter paper for 0, 2, 4, or 6 hours. Imbibed embryos were ground, using a mortar and pestle, in 0.01 ml extraction buffer (8% (w/v) TCA and 20% (v/v) methanol) for each 1 mg of embryonic tissue. Samples were centrifuged for 5 min at 9,000 x g at 4 °C. The first supernatant was collected and the pellet was resuspended in the same initial volume of extraction buffer and centrifuged again for 5 min at 9,000 x g. The two supernatants were combined and the TCA was removed by washing 6 times with 7 volumes of ether. The final volume was recorded and the sample was filtered using a 0.2 μm filter.
into a sample vial and kept on ice for immediate analysis.

The HPLC analyses were performed (Shimadzu, Kyoto, Japan) using 250 x 4.6 mm ID Supelcosil LC-SAX (5 micron) column (Supelco, Inc., Bellefonte, Pa) and gradient buffer at a flow rate of 1.6 mL min⁻¹. The gradient consisting of buffer A (2 mM KH₂PO₄, 20% Acetonitrile at pH 2.8) and buffer B (0.6 M KH₂PO₄, pH 3.0), was run for 25 min as follows: 100% A and 0% B, increasing B in a concave manner to 100% at 20 min; B was maintained at 100% for 1 min and then dropped to 0% and A was maintained at 100% for 5 min. Buffer A was run for 10 min at 100% between injections to regenerate and clean the column. Various concentrations of standard ATP, ADP, and AMP were chromatographed using the same procedure as described above, and a standard curve was established for each nucleotide. These standards were used to identify the peaks and to calculate nucleotide contents of the sample.

**Mitochondria Extraction and Oxygen Uptake**

A modified method described by Pomeroy (1974) and Stewart (personal communication) was used for mitochondria extraction and oxygen uptake. A 100 mg sample of embryonic tissue for each cultivar and treatment was imbibed in deionized-distilled water for 4 h at 25°C. Using a chilled mortar and pestle, embryos were ground in 1.5 ml of grinding medium (0.5 M sucrose, 1 mM EDTA, 67 mM KH₂PO₄, pH was
adjusted to 7.2 with KOH, and 0.75% (v/v) BSA was added just before use). Following grinding the mortar and pestle was rinsed with 1.5 ml of grinding medium and the homogenate was squeezed through four layers of cheese cloth into a centrifuge tube, then centrifuged for 5 min at 2,000 x g. The supernatant was decanted into a clean tube and centrifuged for 4 min at 20,000 x g. The collected pellet was gently resuspended in 0.5 ml of suspension medium (0.3 M mannitol, and 1 mM EDTA adjusted to pH 7.2 with KOH, and 0.1% (v/v) BSA was added just before use), the volume was adjusted to 2.5 ml. The mixture was centrifuged at 1,500 x g for 5 min and the supernatant was decanted into another clean tube. A 1 ml cushion of 0.6 mannitol was layered beneath the mitochondrial suspension. Without mixing the two layers, the solution was centrifuged at 8,000 x g for 15 min after which the supernatant was carefully siphoned off and the mitochondrial pellet resuspended in 250 μl of aerated reaction mixture (0.3 M mannitol, 10 mM MgCl₂, 10 mM KH₂PO₄, and 10 mM TES adjusted to pH 7.2 with KOH, and 0.75% (v/v) BSA was added just before use) for oxygen uptake.

Oxygen uptake was measured polarographically at 28 °C using a YSI Model 53 Biological Oxygen Monitor System (Yellow Springs Instrument Co., Yellow Springs, Ohio). A YSI 5304 Micro Adaptor was used to measure small sample volumes (1 ml). The electrode was calibrated with saturated air-
distilled water at the temperature of measurement. One ml of reaction mixture, through which air was bubbled, was placed in the reaction chamber and the probe was inserted. Ample time was allowed for equilibration before the following compounds were introduced into the chamber with a syringe: 50 µl of mitochondrial solution, 50 µl from stock solution of 20 mM NADH, 200 mM succinate to give a final concentration of 1 mM for NADH and 10 mM for succinate. Succinate was adjusted to pH 7.2 with KOH. The reaction mixture was maintained uniformly dispersed using a magnetic stir bar. State 3 respiration was initiated by adding 5 µl stock solution of 10 mM ADP to a final concentration of 100 nmoles. Two to five min intervals were allowed between two simultaneous addition of materials to equilibrate the system. State 3 was recorded directly from the tracing, and calculated according to Estabrook (1967).

Mitochondrial protein was determined by the Lowry method using the Sigma Protein Assay Kit. This procedure with protein precipitation was based on Peterson’s modification of the Lowry method (Lowry et al., 1951; and Peterson, 1977).

Electron Microscopy

Three isolated embryos per sample were fixed overnight at 4 °C in 2% glutaraldehyde, 2% freshly prepared paraformaldehyde, and 0.01 M phosphate buffer at pH 7.2. The
samples were washed three times for 15 min each with the buffer. Samples were post-fixed in 1% osmium tetroxide for 4 h, and dehydrated in a graded ethanol series at room temperature for 2 h each (25, 50, 70, 95, and 100% X 3, v/v). Dehydrated samples were transferred to 100% propylene oxide in graded steps at room temperature for 4 h each (3:1, 1:1, 1:3, v/v 100% ethanol and 100% propylene oxide, and 3 X 100% propylene oxide). After an intermediate fluid exchange with 100% propylene oxide to 100% resin overnight in graded steps (3:1, 1:1, 1:3, v/v), samples were infiltrated in pure resin 3 times over a 40 h period. Following the final change of resin, the samples were embedded in pure resin cast in aluminum dishes and polymerized at 60 °C for several days.

Ultrathin sections were cut in the meristematic radicle regions with glass knives on the Reichert Ultracut Ultramicrotome and stained with uranyl acetate followed by lead citrate. The sections were examined and photographed with the JEOL 1200EX electron microscope.
RESULTS

Nucleotide Levels and Energy Charge

Figures 1 and 2 illustrate the HPLC chromatograms for dry (0 h) and imbibed (4 h) wheat embryos. The AMP, ADP, and ATP peaks eluted at approximately 8, 12, and 16 minutes, respectively, for both cultivars. The peaks were detected by their absorbance at 254 nm and identified by corresponding standard nucleotide elution profiles.

In the quiescent state (0 h), ATP profile for both cultivars and all treatments was absent, minor amounts of ADP were present while the AMP was highly abundant. After 4 h imbibition, the levels of ATP and ADP greatly increased. Although the amount of AMP decreased with imbibition time, the profiles were not well enough resolved to show any variation. The AMP peaks displayed interference with other unidentified components present in the extract. The ATP levels increased in embryonic tissues extracted from seeds grown under all three temperature regimes. However, this increase was much greater for seeds grown in the low temperature regime. A similar observation was made for ADP profiles.

Standard curves were used to quantify the nucleotide levels (Table 1 and 2). The ATP concentration in dry embryos was not detectable. However, the AMP concentrations were
Figure 1. HPLC Chromatograms: Effect of parental growing temperatures on nucleotide pools of imbibing wheat 'Marzak' embryos, A: 20/15 °C at 0h; B: 28/21 °C at 0 h; C: 36/29 °C at 0 h; D: 20/15 °C at 4 h; E: 28/21 °C at 4 h; F: 36/29 °C at 4 h
Figure 2. HPLC Chromatograms: Effect of parental growing temperatures on nucleotide pools of imbibing wheat 'Oum-rabia' embryos, A: 20/15 °C at 0h; B: 28/21 °C at 0 h; C: 36/29 °C at 0 h; D: 20/15 °C at 4 h; E: 28/21 °C at 4 h; F: 36/29 °C at 4 h
Table 1. Nucleotide content of wheat 'Marzak' embryos exsised from seeds exposed to three different temperature regimes during development and maturation.

<table>
<thead>
<tr>
<th>Imbibition time</th>
<th>Nucleotide</th>
<th>Temperature °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>h</td>
<td>AMP</td>
<td>20/15*</td>
</tr>
<tr>
<td></td>
<td>ADP</td>
<td>28/21</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>36/29</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>829.45</td>
<td>929.56</td>
</tr>
<tr>
<td></td>
<td>201.21</td>
<td>164.06</td>
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<td>-</td>
</tr>
<tr>
<td></td>
<td>1030.66</td>
<td>1093.62</td>
</tr>
<tr>
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<td></td>
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<td>1226.17</td>
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</tbody>
</table>

* day/night temperature.
Table 2. Nucleotide content of wheat 'Oum-rabia' embryos excised from seeds exposed to three different temperature regimes during development and maturation.

<table>
<thead>
<tr>
<th>Imbibition time</th>
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<tr>
<td>0 h</td>
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</tr>
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<td>-</td>
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<tr>
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<td></td>
<td>ADP</td>
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<td>ATP</td>
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<td></td>
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<tr>
<td>4 h</td>
<td>AMP</td>
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</tr>
<tr>
<td></td>
<td>ADP</td>
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</tr>
<tr>
<td></td>
<td>ATP</td>
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</tr>
<tr>
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</tr>
<tr>
<td></td>
<td>ADP</td>
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</tr>
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<td></td>
<td>ATP</td>
<td>989.84</td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>1594.69</td>
</tr>
</tbody>
</table>

* day/night temperature.
higher and ADP was present but at reduced amount. The amount of ATP increased dramatically with increasing imbibition time, but at different rates for each temperature treatment. During the entire period of analysis embryos isolated from seeds grown at low temperature regime had approximately 2 to 3 times the levels of ATP as those which were grown in high temperature regimes. Embryos from the medium temperature treatment showed intermediate levels, but at 6 h these levels were equivalent to those of low temperature treatments. Corresponding to this increase in the ATP pool size, the concentration of AMP had decreased to a greater extent in low and medium temperature regimes. Although there was an increase after 2 h imbibition, the ADP level remained relatively unchanged after 2 h. Similar to individual nucleotides the total adenine nucleotide level increased with the imbibition time for all treatments. A clear difference between treatments was not detected until after 4 h of imbibition, the total nucleotide content was adjusted by either AMP, ADP or both. At that time, 4 h after imbibition, total nucleotide content was 20 to 30% lower in high temperature treatments compared to the control.

The adenylate energy charge (AEC) also was affected by the temperature conditions during seed development and maturation (Figure 3 and 4). Because of the high AMP and very low ATP contents (not detectable) the AEC values of
Figure 3. Effect of parental growing temperatures on Adenylate Energy Charge of imbibing 'Marzak' wheat embryos
Figure 4. Effect of parental growing temperatures on Adenylate Energy Charge of imbibing 'Oum-rabia' wheat embryos
embryos, before imbibition, were very low. After imbibition, the AEC increased in all treatments but at differing rates. The AEC values of low and medium temperature treatments were almost equivalent. However, the AEC of the high temperature treatment was lower. The values from high temperature treatments were 47, 62, and 74% for Oum-rabia and 81, 67, and 82% for Marzak of those of the low temperature treatments for 2, 4, and 6 h, respectively, after imbibition.

Mitochondrial Respiratory Activities

Respiratory activities of mitochondria isolated from wheat embryos grown at three different temperature regimes are shown in Table 3. For both substrates, NADH and succinate, oxygen uptake by mitochondria was clearly stimulated by ADP additions (Figure A-4 and A-5 in appendix). However, the added ADP was never exhausted, therefore the system did not reach state 4. Consequently, respiratory control and ADP:O ratios could not be calculated. The respiratory activities of the isolated mitochondria will be represented in this study by state 3 respiration rates. As mentioned earlier, ADP stimulated oxygen uptake for all treatments but at differing rates. For both substrates, the state 3 respiration rates of mitochondria isolated from 4 h imbibed embryos of seeds grown at high temperatures had lower rates of oxygen consumption than mitochondria isolated from
Table 3. State 3 respiration rates of mitochondria isolated from wheat embryos

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Temp.</th>
<th>Marzak</th>
<th>Oum-rabia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C</td>
<td>nmoles O$_2$ min$^{-1}$ (100 mg tissue)$^{-1}$</td>
<td></td>
</tr>
<tr>
<td>NADH</td>
<td>20/15$^a$</td>
<td>9.68</td>
<td>8.94</td>
</tr>
<tr>
<td></td>
<td>28/21</td>
<td>6.85</td>
<td>7.15</td>
</tr>
<tr>
<td></td>
<td>36/29</td>
<td>5.25</td>
<td>6.25</td>
</tr>
<tr>
<td>Succinate</td>
<td>20/15</td>
<td>5.83</td>
<td>4.59</td>
</tr>
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<td></td>
<td>28/21</td>
<td>5.11</td>
<td>4.07</td>
</tr>
<tr>
<td></td>
<td>36/29</td>
<td>4.01</td>
<td>3.45</td>
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</table>

$^a$ day/night temperature.
embryos of seeds grown at low and medium temperature regimes. For both cultivars, the average ADP-stimulated respiration was 75.4%, 88.2% for medium temperature and 62.1%, 72.9% for high temperature treatments of that of low temperature treatments using NADH and succinate, respectively, as substrates.

Electron Microscopy

To elucidate the effect of heat stress on the respiratory metabolism of the seed during early hours of germination, an ultrastructural study was conducted on dry (0 h) and imbibed (6 h) excised embryos (Plate 1). At 0 h imbibition, mitochondria from all treatments were spherical in shape with undifferentiated internal structures (Plate 1). Mitochondria from low and medium temperature treatments appeared slightly larger in size than their counterpart from high temperature treatments, and had intact outer membrane and a homogenous, dense matrix (Plate 1A and 1B). In contrast mitochondria from high temperature appeared slightly smaller in size with a transparent matrix (Plate 1C).

After 6 h imbibition mitochondria became hydrated and expanded with a developed bilayer membrane and internal cristae. These features were clearly visible in low and medium temperature treatments (Plate 1D and 1E). Whereas mitochondria from high temperature treatments still presented
poorly developed internal structures (Plate 1F), in fact, cristae were hardly visible.
Plate 1. Electron micrographs of mitochondria (M) from radicle meristem region of dry (0h) embryos A: 20/15 °C, bar= 1µm; B: 28/21 °C, bar= 1µm; C: 36/29 °C, bar= 0.1µm; and imbibing (6 h) embryos D: 20/15 °C, bar= 0.1µm; E: 28/21 °C, bar= 1µm; F: 36/29 °C, bar= 0.1µm
DISCUSSION

The results presented in this study confirm the importance of mother plant growing temperatures on the metabolism of its progeny, the seed. And, they provide some indication of the site of damage. Regardless of temperature treatment, there was an increase in the total nucleotide pool size with imbibition time. This was mainly due to an increase in ATP levels as the embryo imbibed water. Analysis of individual nucleotide levels in embryo extracts revealed marked differences between the three temperature regimes.

The first difference was in the rate of ATP and AMP changes over time. During imbibition, the levels of ATP in the embryo extracts increased in all treatments (Table 1 and 2). However, these levels increased more rapidly in low temperature treatment compared to high temperature treatment. Differences in ATP content were especially visible at 2 and 4 h imbibition time. Compared to low temperature there were only slight accumulations of ATP measured after 4 h in the high temperature regime. The second difference was in the content of individual as well as total nucleotide. During the entire period of analysis, the amount of ATP was higher in the low temperature regime as opposed to high temperature regime. Similarly, the total content of nucleotide was also higher. After 6 h imbibition, embryos of the low temperature
regime had approximately 42 and 52% more ATP and 20 and 26% more total nucleotide for Marzak and Oum-rabia, respectively, than those embryos grown under high temperature regimes. Such responses have been demonstrated in plant parts other than seed exposed to different types of stress. Mangat (1982) reported that the total adenine nucleotide as well as the ATP levels were much higher for seedlings grown in low air temperature regimes than for those grown in high air temperatures. Similarly, Turner and Wellburn (1985) showed that water stress and especially a small change in leaf water potential resulted in significant reductions in sweet pepper leaf ATP content to 70-80% of the control level. Also, severe desiccation had been reported to reduce both ATP and AEC of moss tissue (Bewley and Gwozdz, 1975).

Mangat (1982) reported that the higher the amount of the nucleotide, the higher the metabolic rate and thus the higher the growth potential of the seedlings. In our study, heat stress during seed development and maturation caused pronounced changes in the ATP content of seed embryos. These observations were in accordance with the isolated embryo growth potential experiment (Paper 1). Embryos excised from seeds grown at high temperatures germinated 24 to 48 h later and exhibited lower radicle and shoot tissue growth rate than those of the control. This suggests that the delay in embryo germination and radicle and shoot development observed in our
study could be due to differences in the metabolic activity of the seeds produced under different environmental conditions.

Heat stress during seed development and maturation brought about similar changes in the adenylate energy charge (AEC) as for the nucleotide contents. The average AEC values in embryos excised from seeds grown at low temperature regime were 36, 35, and 22% higher than in those of high temperature treatment after 2, 4, and 6 h imbibition respectively. An active biological system is frequently characterized by an AEC of 0.8 or above (Moreland et al., 1974; and Bewley and Black, 1985). In our study the AEC values were very low, especially during the first 4 h imbibition time. This was mainly due to the high levels of AMP and low levels of ATP during this early period of germination (Table 1 and 2). McKee and Mendelssohn (1984) reported that high AEC values appear to be common in those tissues which are highly metabolically active. This is reflected in our study by the rapid increase of ATP and high seedling vigor (Paper 1) in low temperature treatments as opposed to high temperature treatments. Similar results have been demonstrated by Madden (1992) who found that high temperature desiccation resulted in a noticeable decrease of both nucleotide content and AEC values of maize embryos, and consequent low seedling vigor.

Exposing seeds to high temperatures during storage
and/or accelerated aging treatments have been frequently associated with reductions in respiratory rates (Ram and Weisner, 1988; Ferguson et al., 1990; Edje and Burris, 1970; Amable and Obendorf, 1986). However, the effect of these high temperatures while the seed is still on the mother plant remains unknown. The present study was undertaken to examine this effect under controlled conditions. Although state 4, RCR and ADP:O ratios could not be calculated, the isolated mitochondria appear to be in good condition as indicated by relatively high rates of state 3 respiration. A decrease in the isolated mitochondria respiratory activities, indicated here by state 3 oxygen uptake, with an increasing growth temperature was evident for both cultivars. Similar trends seen in embryo respiration (Paper 1) were observed for the mitochondria, indicating that high temperatures during seed development and maturation in fact influence respiratory metabolism of the seed during early germination. A similar decline has been reported in maize axes exposed to high temperatures during desiccation process (Madden, 1992). Woodstock (1973) reported that low utilization of oxygen by germinating seeds is a good indication of their quality decline.

In addition to differences in mitochondrial oxygen uptake, results from electron microscopy revealed marked differences in the mitochondrial ultrastructure. After 6 h
imbibition the radicle meristem region of embryos from high temperature treatment showed expanded and activated mitochondria with well developed membranes and visible cristae. Whereas embryos from high temperature treatment still presented undifferentiated and/or degenerating mitochondria. This represents an additional explanation for the reduced oxygen uptake observed in seeds grown under high temperature conditions, and consequently for the concept that heat stress causes some damage to the respiratory system.

Germination is an energy requiring process. The amount of available energy during seed germination reflects the metabolic activity of the seed and the vigor of the resulting seedling (Mangat 1982). Recently, Attucci et al. (1991) demonstrated that most of this energy originates from oxidative phosphorylation. In our study low ATP production and low total nucleotide content resulting from high temperature treatments are in accordance with the decline in the isolated mitochondrial activity. This may indicate that heat stress during seed growth causes some disruption in mitochondrial activities and stability which results in low levels of energy (ATP) during early seed germination. Such changes have been demonstrated in the high temperature desiccation process where the decline of ATP content in maize axes was shown to be a result of disturbed oxidative phosphorylation (Madden, 1992).
CONCLUSIONS

It is well documented that high temperature stress during seed storage adversely affects seed metabolism during germination process. However, its effect on the seed while on the plant remains unknown. The present study provides clear evidence for metabolic changes, specifically at the mitochondrial level, in the early seed germination in response to heat stress during seed development and maturation. These observations have been supported by decreased levels of nucleotide, reduced activity of isolated mitochondria and the structural changes in mitochondria from imbibing wheat embryos. Additionally, the results support the positive relationship between mitochondria and the energy produced as already demonstrated by previous studies. This is shown in our study by the accordance in energy decline with the changes that occurred in the respiratory system of excised embryos.
REFERENCES


GENERAL CONCLUSIONS

There is general consensus that growing conditions of the parent plant influence its seed quality. This stimulated our interest in clarifying this general concept using only one factor, temperature, under controlled conditions.

As already demonstrated in previous studies, high temperatures during and after anthesis adversely influenced seed production and physical seed characteristics. Although seed mass was severely reduced by high temperature, embryo weight was less affected.

Tests performed on intact seed showed genotypic differences in seed germination behavior. High temperature had no effect on seed germination for Oum-rabia, whereas it decreased percentage germination for Marzak. This may suggest differences in sensitivity to temperature with Marzak being more sensitive. However, this needs to be confirmed by further studies under different field conditions. Seed vigor as measured by seedling dry weight and conductivity methods was decreased by high temperatures during seed development and maturation. Most of the previous studies had related the decline in seed vigor to seed size. In our study most of the seed weight differences were due to endosperm mass. This factor of seed size was eliminated by performing all physiological tests on embryonic tissue excised from dry
seed. The advantage in using the embryo instead of the entire seed is widely explained in the literature.

For both cultivars, germination tests showed no differences between treatments in the germination capacity of excised embryos. However a delay of 24 to 48 h in germination was observed in embryos excised from seeds grown under high temperature conditions. This delay was also observed in radicle and shoot development. The growth of both radicle and shoot tissue was reduced by high temperature treatment. Embryo oxygen uptake on both embryo and weight bases revealed marked differences between treatments. Embryos from the high temperature regime had less oxygen consumption than those from the low temperature regime. Results from isolated embryos were in accordance with those from intact seeds. This suggests that seed size alone does not explain the negative effect of high growing temperatures on seed vigor. Besides a decrease in seed mass, heat stress have caused some metabolic changes that led to this decline in seed vigor. Results from embryo respiration assay let suggest some impairment in the seed respiratory system.

Results from experiments on nucleotide content, isolated mitochondria respiration and electron microscopy showed marked differences in excised embryo metabolism during early stages of germination. Embryos from high temperature treatment exhibited low energy level, low AEC, less
mitochondrial oxygen uptake and less structurally developed and/or degenerating mitochondria compared to embryos from low and medium temperature treatments. This provides clear evidence that high temperature during seed development and maturation had influenced the respiratory metabolism of the seed during early germination. This impairment of mitochondria associated with a decrease in the available energy during early germination, may be the probable origin in seed vigor differences observed in this study. Further study is needed to confirm this hypothesis.

Through the measurements of different physical and physiological parameters, the present study demonstrates that wheat seed quality is strongly influenced by the parent growing temperature at least under controlled conditions, and mitochondrial damage may represent one of the mechanisms of heat injury during grain growth on seed quality.
LITERATURE CITED


APPENDIX
Table A-1. Physical seed quality mean squares

<table>
<thead>
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<th>Response</th>
<th>Source</th>
<th>Temperature</th>
<th>Variety</th>
<th>Temp * Var</th>
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*, **, *** significant at P = 0.05, 0.01, and 0.001, respectively.
Table A-2. Germination and seedling vigor mean squares

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*, ** significant at $P = 0.05$ and $0.01$, respectively.
Figure A-1. Standard curve for AMP as determined by HPLC
Figure A-2. Standard curve for ADP as determined by HPLC
Figure A-3. Standard curve for ATP as determined by HPLC
Table A-3. State 3 respiration rates and protein content of mitochondria isolated from wheat embryos

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<tr>
<th>Cultivar</th>
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<td>nmoles O₂ min⁻¹ (100 mg tissue)⁻¹</td>
<td>nmoles O₂ min⁻¹ (mg protein)⁻¹</td>
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<td>9.68</td>
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<td>478</td>
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* Day/night temperature.
Figure A-4: Polarographic traces of oxygen uptake by isolated mitochondria from wheat embryo using NADH as substrate. A: 20/15 °C; B: 28/21 °C; C: 36/29 °C
Figure A-5: Polarographic traces of oxygen uptake by isolated mitochondria from wheat embryo using succinate as substrate, A: 20/15 °C; B: 28/21 °C; C: 36/29 °C