EFFECTS OF ELEVATED TEMPERATURE DURING GRAIN DEVELOPMENT ON SEED QUALITY OF BARLEY (*HORDEUM VULGARE* L.).

I.S.K. SYANKWILIMBA

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ABSTRACT

In order to investigate the effects of elevated temperatures during grain development on seed quality, a series of experiments was carried out using both controlled environment and glasshouse conditions. Plants of several cultivars of barley (*Hordeum vulgare* L.) were subjected to diferent temperature regimes during grain development. Temperature treatments ranged from 18°C to 38°C and plants were subjected to elevated temperatures at different stages of grain growth and for different lengths of time. After harvest, the seeds were tested for vigour.

Grains which had experienced elevated temperatures for part or all of their developmental period were lighter and had smaller embryos than grains which experienced 18°C for most or all of their developmental period. There was a significant positive correlation between grain dry weight and embryo dry weight. When eleven genotypes were grown in a glasshouse at approximately 18°C/13°C (day/night) and a day length of 18 h, grain dry weight and embryo dry weight varied according to genotype. Genotypes with heavier grains had larger embryos than those with lighter grains.

The viability of grains harvested from all temperature regimes was > 94 %. Grains which had experienced elevated temperatures during grain development had a higher percentage germination in tests using 8 ml and 10 ml of water per 100 grains than grains which had experienced 18°C throughout grain development. It was observed that grains harvested from ears grown in elevated temperature regimes had a higher proportion of pre-germinated grains than grains harvested from ears grown at 18°C.

Seedlings grown in the dark from grains grown in elevated temperatures had fewer roots, shorter seminal roots and longer plumules than had seedlings from grains which had experienced 18°C throughout development. Root number, length of seminal roots and seedling dry matter showed a strong positive correlation with grain dry weight. There was no statistically significant correlation between plumule length and grain dry weight.

When seeds were sown at a depth of 4 cm in fine sand in pots, seedlings from grains grown at elevated temperatures emerged earlier than those from grains grown at 18°C. However, grains grown at elevated temperatures had a higher proportion of germinated grains whose seedlings failed to emerge to the surface than did grains grown at 18°C.

The mean dry weights of the roots and plumules of seedlings from grains grown at 18°C were higher than those of seedlings from grains grown at elevated temperatures. The root dry weight/shoot dry weight ratios (R/S) for seedlings of cultivars normally grown in sub-tropical/tropical climates were lower than those for seedlings of temperate region cultivars, the grains having been grown under the same conditions.

Genotypic variations in seedling vigour characteristics were observed in glasshouse-grown material.

When grown for five days on 1% agar, embryos extracted from grains which had been subjected to elevated temperature regimes during grain development produced seedlings with longer plumules and longer roots than did embryos extracted from grains which experienced 18°C throughout development. These differences were not observed when the embryos were grown on an agar/glucose medium.

The A-type and B-type starch granules in grains grown in elevated temperature regimes were fewer in number and smaller than those in grains grown at 18°C. Starch from grains which experienced a particular sequence of high temperatures during development had a triphasic size distribution of starch granules.

Estimates of ∝-amylase activity made 48 h after the start of imbibition showed that ∝-amylase production was greater in grains grown at elevated temperatures than in grains grown at 18°C.

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DECLARATION

This thesis has been composed by myself and the work of which reflects the true record of the work carried out by myself. All sources of information have been specifically acknowledged by means of reference.

I. S. K. Syankwilimba.

DEDICATION

I dedicate the sweat of this thesis to my children; Clare, Sweetlenna, Ian Jr.and Carol.

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1. INTRODUCTION

Cereals of temperate climate regions such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are increasingly being grown in countries with tropical and sub-tropical climates. The study of the effects of high temperature becomes important for successful production of these crops outside their normal range of production. The importance of high temperature has already been recognised by CIMMYT in Mexico which has embarked on a selection program for high temperature tolerance in wheat and barley. The projected rise in both the mean temperature and the frequency of episodes of high temperature due to the 'greenhouse effect' may also affect crop production in countries with temperate climates (Reynolds, Balota, Delgado, Amani, and Fischer (1994).

The increasing human population, particularly in the developing countries, requires more food than anything else. By the beginning of the 21st century, many countries in Africa will have widened the gap between food demand and food supply because population is increasing at a faster rate than food production (Kaliangile, 1994). In order to sustain food levels, agricultural production will have to increase to feed the increasing population. Diversification and the introduction of agricultural crops outside the normal range of production could ensure sustainability of food security at both household and national levels.

Although poor crop harvest in the developing countries may be attributed to environmental factors such as drought and outbreaks of pests and diseases, it is also possible that seed sown by farmers is of low quality because high quality seed is not readily available. In order to achieve sustainable levels in agricultural production, adequate supplies of high quality seed should be made readily available to meet farmers' requirements.

1. 1. Seed quality

A definition of seed quality which is appropriate for all crops is neither possible nor useful, as the meaning of quality will depend on the use for which the seed is intended. Seed quality may mean different things to different people. It may mean

homogeneity or uniformity. For a farmer, quality means suitability of the planting material for sowing on his farm at a certain period of the year and for his particular purpose. The components of seed quality include the capacity of the seed to produce normal, healthy and uniform seedlings with good field emergence and establishment. Therefore, quality may be defined as the summation of all the attributes that contribute to seed performance in the field.

There is a diversity of opinion regarding the stage at which seed quality reaches its maximum potential during grain development. Harrington (1972) proposed that maximum seed quality was attained at physiological maturity (the stage of seed development at which the seed attains its maximum dry weight), after which deterioration was initiated and seed germination and vigour declined, with the rate of decline dependent upon the storage environment. This hypothesis was supported by research for two decades, until a recent challenge by Ellis and associates who concluded that maximum seed quality does not occur until some time after physiological maturity (Pieta Filho and Ellis, 1991a, b; Ellis and Pieta Filho, 1992; Sanhewe, Ellis, Hong, Wheeler, Batts, Hadley and Morison, 1996). Results of Tekrony and Hunter (1995) and Tekrony and Egli (1995) on maize and wheat respectively, showed that maximum seed quality occurred at or before physiological maturity. The contrasting conclusions reached by the above authors may be related to differences in the harvesting, drying and handling techniques used on seeds harvested at a high moisture content. Differences could occur depending on the methods used to measure seed quality or whether seed harvests were made on an individual seed or plant community basis, or whether all seeds tested were harvested and evaluated at the same stage of seed development.

Abdul-Baki and Anderson (1972) suggested that the highest seed quality level was the theoretical one attained under the most favourable growing conditions. However, seeds like any other form of life, cannot retain their initial quality indefinitely and eventually have to deteriorate and die. Seed viability, germination and vigour each describe different aspects of the quality of a seed population.

1. 2. Seed viability

Seed viability is the relative proportion of live to dead seeds in a particular seed lot. A seed may be viable but not capable of germination because of the presence of dormancy. Roberts (1972) defined a non-viable seed as one which will not germinate when given near-optimal conditions even when it is non-dormant, and he defined a viable seed as one which will germinate under favourable conditions in the absence of dormancy (*dormancy is described in Section 1.5*).

Germination can only proceed once dormancy has been removed, it begins with water uptake by the seed (imbibition) and ends with the start of elongation by the embryonic axis, usually the radicle (Bewley and Black, 1994). Viability and germination have dual meanings depending on whether the audience is physiologically or technologically oriented. From a physiological perspective, a seed can be said to have germinated once the radicle has protruded through the seed coat, indicating whether or not the seed is alive; and from a technological perspective, germination is "the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions'' (AOSA, 1978). The production of a visible radicle, often used as the physiological criterion of germination is unsatisfactory as a measure of germination in the context of seed testing because the seedling which subsequently develops may be abnormal in structure and incapable of establishing a normal plant in the field. Therefore, under the International Rules for Seed Testing (Anon, 1976), seeds must be allowed to germinate to a point at which evaluation of the seedlings can be made and abnormal seedlings, in which parts are damaged, rotted or missing are excluded from the final estimates of percentage germination.

1. 3. Concept of seed vigour

The germination capacity of a seed lot is determined by the proportion of seeds capable of producing a normal plant under conditions designed to ensure maximum germination provided dormancy is removed from the seed lot being tested. Since optimum conditions provided in the germination tests are rare under the field

conditions, the percentage emergence from seed lots of similar germination capacity may differ. It is largely the absence of a consistent relationship between germination in the laboratory and emergence in the field which has been responsible for the development of the concept for seed vigour. This concept evolved as seed technologists attempted to differentiate between seeds having the potential to produce normal, vigorous, healthy seedlings under less than optimum conditions and those that cannot produce vigorous healthy seedlings because of genetic makeup, seed deterioration, injury or other causes. Seed vigour is a qualitative attribute that denotes rapid germination of a seed and seedling establishment under a wide range of field conditions.

The development of a satisfactory definition of vigour has been difficult to achieve, and as a result, many definitions have emerged. In 1957, Isely defined seed vigour as "the sum of all seed attributes which favour stand establishment under unfavourable conditions,'' whereas Delouche and Caldwell (1960) stated that "seed vigour is the sum of all seed attributes which favour rapid and uniform stand establishment in the field.'' Perry (1973) defined seed vigour as the "physiological property determined by the genotype and modified by the environment which governs the ability of a seed to produce a seedling rapidly in soil and the extent to which the seed tolerates a range of environmental factors." In 1977, the International Seed Testing Association (ISTA, Perry, 1973) defined vigour as "the sum total of those properties of the seed which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence."

Seed vigour is not a single measurable property but a concept describing several characteristics associated with seed performance (Perry, 1980). Thus, several factors may individually or collectively influence the level of vigour. As a concept, vigour cannot be described as either a cause or an effect because each measurable component may have both causes and effects forming a long chain of cause and effect relationships extending from seed development to processes of germination, seedling emergence and establishment.

Characteristics of seed and seedling performance such as storability under diverse conditions, uniform germination and emergence under unfavourable conditions and

the ability to produce a normal plant under a diversity of growing conditions constitute components of the concept of seed vigour. The manifestations of differences in vigour depend on an interaction between the seed and its environment. When conditions are favourable, values for percentage emergence in the field approach those for germination in the laboratory, but when conditions impose a stress on the seed, percentage emergence in the field is poor.

Seeds of low vigour are more sensitive to stress factors than high vigour seeds and prediction of plant population potential from seed sowing rates is uncertain. When seed lots of a single genetic strain are sown under identical conditions great differences have been observed.

The rate and uniformity of germination and emergence are also included among the vigour characteristics. Low vigour seeds usually have a slower mean rate of germination and also a wider distribution of rate of germination of individuals than high vigour seeds. A rapid rate of early seedling growth is another characteristics of high vigour seeds. It should also be noted that seed dormancy may obscure vigour potential of the seed lot in a laboratory test and under field emergence.

All these performance characteristics are important in modern crop agronomy and seeds of high vigour are highly desirable in the establishment of most crops.

1. 3. 1. Importance of seed vigour

High vigour seeds may be expected to germinate more synchronously than low vigour seeds and the seedlings from high vigour seeds emerge and grow quickly and evenly. Reduction in yield may be indirectly related to low seed vigour if seedlings fail to emerge rapidly and uniformly to attain an optimum plant population. Therefore, high vigour seed gives the best chance of achieving the desired plant population density. This is very important where harvesting is a once-over operation.

1. 3. 2. Factors influencing seed vigour

The development of a seed encompasses a series of important ontogenetic stages from fertilisation to harvest maturity. Each stage represents a change in morphological and physiological ontogeny that can alter seed performance potential.

At physiological maturity (when a seed achieves its maximum dry weight) the seed is unsafe for storage because at this stage the moisture content is still very high. The seed becomes harvestable when it attains harvest maturity. This is the stage of development at which the moisture content has decreased to approximately 14 % (in cereal crops). Between physiological maturity and harvest maturity, the seed is essentially stored on the plant where it may be exposed to severe environmental conditions that can adversely affect seed quality.

There are several factors that influence seed vigour and the commonly known factors are: genetic constitution, environment and nutrition during seed development, storage environment, mechanical integrity and seed size.

1. 3. 3 Genetic constitution

The maximum level of vigour attainable by a seed lot is determined by the genotype but may be modified by the conditions on the mother plant (Perry, 1980). Certain cultivars are more susceptible to adverse environmental conditions than others, and certain cultivars are less capable of growing rapidly than others. On the other hand, the heterosis exhibited by hybrid cultivars results in resistance to adverse conditions, possibly as a result of their ability to grow rapidly due to their high metabolic activity. Hybrid vigour is a component of heterosis and presents the measurable superiority of the hybrid progeny over its inbred parents. McDaniel (1969) reported that seeds of hybrid barley germinated faster and grew more rapidly than their inbred parents.

Susceptibility to mechanical damage, whether induced by harvesting or conditioning equipment, has been shown to be influenced by genotype in legumes. Barriga (1961) demonstrated that 41 strains of navy beans possessed differing tolerance to mechanical abuse.

1. 3. 4. Environmental conditions

Seed vigour can be affected by a wide range of environmental factors throughout the seed production process from flower initiation through anthesis, development, maturation and harvesting. The concentration of seed production for some crops in

specific areas is persuasive evidence of the environmental influence on seed development and quality (Delouche, 1980). Seed germination potential may be affected by the environment experienced by the seeds while they are still developing on the mother plant. High temperatures during drying, rapid desiccation and poor storage can all reduce seed vigour.

1. 3. 5 Soil moisture and fertility

Moisture stress during seed development often results in light, shrivelled seed which, in turn results in poor-vigour seed (Copeland and McDonald, 1995). Brocklehurst, Moss and Williams (1978) reported that grains of wheat from plants grown under reduced irradiance and under water stress were small, shrivelled and weighed less than grains from unshaded and watered plants. Aspinall, Nicholls and May (1964) and Aspinall (1965) obtained small and shrivelled grains from pot-grown barley plants in lysimeters under conditions of intermittent water stress and for longer periods of stress.

Fernandez and Laird (1959), McNeal, Berg, Brauig, and McGuire (1971) found a direct relationship between soil fertility and seed vigour in wheat. They reported that high levels of nitrogen fertilisers increased protein levels in seeds of wheat. Other results have shown that high-protein wheat seed results in increased germination and seed vigour (Lowe, Ayers, and Ries, 1972, Lowe and Ries, 1973).

1.3. 6. Mechanical integrity

Seed production practices such as harvesting, cleaning, and handling inevitably lead to mechanical damage. Mechanical damage inflicted during harvesting can reduce seed vigour severely. Jahufer and Borovoi (1992) observed decreased seed quality of maize grains harvested mechanically from maize field trials. Injured or deeply bruised areas of a seed may serve as centres for infection or may result in accelerated seed deterioration. Such injuries which may be sustained during mechanical harvesting or during processing and storage can lead to increased seed sensitivity to environmental stress and ultimately to decreased vigour.

1. 3. 7. Seed size

Several workers have observed a positive relationship between seed size and seedling vigour (Radwan, Shiltawi and Mahdi, 1972, Gelmond, 1972; Mathur, Sinha and Singh, 1982 ; Paul and Ramaswamy, 1979). Similarly, Shieh and MacDonald (1982) reported that seed size of two corn inbreds had no effect on seed quality. However, other studies have shown no difference in the performance of large and small seeds in barley (Carver, 1980). The effect of seed size becomes very pronounced in case of deeper planting (Mian and Nafziger, 1994).

1. 3. 8. Seed storage

Seldom are seeds harvested and immediately planted without undergoing at least a brief storage period. The duration of storage, the type of storage facilities used and storage environment (temperature and humidity) may all influence seed vigour.

1. 4. Seed vigour tests

Seed vigour tests are used to provide an indication of whether a seed lot should be sown in the current season or whether it would still be vigorous if it were carried over until the next year. Vigour tests can also be used to determine whether a seed lot could be sown early when the stress is likely to occur, or whether it should be sown only later in the season when conditions are more favourable for germination and seedling growth.

Growers require seeds with good germination and vigour particularly when soil conditions during sowing are not optimal. For this reason, the germination test does not always relate well to seed performance under adverse environmental conditions. Yaklich and Kulik (1979) showed that high percentage germination was not correlated with vigorous seedling growth and Caldwell (1960) and Moore (1969) found that the standard germination test as a measure of seed quality was insensitive, inefficient and inadequate for judging the performance of a seed lot under a range of less than optimum field conditions.

Seed vigour tests are used to provide additional information about the physiological quality of seed lots and their potential to produce healthy seedlings under adverse conditions.

There are several vigour tests , all of which are evaluated according to their ability to predict some aspects of performance of seeds under field conditions and as such, they are grouped into categories based on the parameters monitored. Isely (1957) divided vigour tests into direct and indirect tests. Direct tests imitate the field environment and measure the ability of the seeds to emerge under simulated field stress conditions whereas the indirect tests measure specific physiological aspects of seeds. Vigour tests may also be classified on the basis of the component of seed vigour. Woodstock (1973) separated vigour tests into physiological and biochemical tests. Physiological tests measure some aspect of germination and seedling growth, while biochemical tests evaluate a specific chemical reaction related to the expression of germination. Pollock and Roos (1972) classified vigour tests into stress and quick test categories. The stress test is the subjection of seeds to one or more of the environmental stresses that might be encountered under field conditions. Stress conditions may include low temperatures with or without soil as in the cold test or cool germination test, or osmotic stress imposed by using solutions such as polyethylene glycol (PEG). Quick tests are those tests in which some chemical reaction associated with seed vigour is monitored. The tetrazolium test (see **Section 1. 4. 4.**) and the electrical conductivity test are two of the rapid biochemical method used to determine seed vigour. The electrical conductivity test is used to detect low-vigour seeds with decreased membrane integrity due to mechanical injury or storage deterioration. During imbibition, seeds having poor membrane structure release into the imbibing medium cytoplasmic solutes which carry electrical charges that can be detected by a conductivity meter (Copeland and McDonald, 1995). However, the test has one major limitation in that it expresses results as an average conductivity evaluation of a number of seeds as though all seeds were equally deteriorated. A seed lot is composed of a population of individual seeds and each seed has its own unique potential to perform in the field. The electrical conductivity test results, therefore,

would better reflect the vigour capability of a seed lot if they were presented on an individual seed basis.

However, the method or test used must depend on the purpose of the investigation. Vigour tests may not all be appropriate to evaluate the performance of seeds of all crops under a wide range of field conditions. DasGupta and Austenson (1973a, b) suggested that a combination of the standard germination test with one or more vigour tests could provide useful information for evaluation of vigour.

In the current study, the evaluation of seed quality of grains grown in different temperature regimes during grain development was carried out using the standard germination test and two vigour tests.

1. 4. 1. Seedling growth test

This test is related to the evaluation of the seed's ability to use its food reserves for growth during the heterotrophic phase of seedling development. In the rolled paper towel test, the seed is allowed to germinate in darkness until its food reserves are exhausted. Perry (1977) reported that the seedling growth test on barley seedlings grown in darkness showed a potential to differentiate seed performance under field conditions. This test evaluates both the amount of food reserves stored in the seed and the quality of the enzymatic apparatus which uses the reserves during the heterotrophic phase of seedling growth. Perry (1977) used the seedling growth test to compare the assessment of the performance of seed lots of barley and wheat obtained from the standard germination test with estimation of field emergence of the same seed lots. He observed that when field conditions were favourable, emergence approximated to, and correlated well with germination capacity, and no advantage could be obtained from a vigour test, but that when field conditions were poor, correlation with the germination test was poor and the superiority of the vigour test became apparent. Similarly, Steiner, Grabe and Tulo (1989) using wheat seeds in their test, reported that the results of that seedling growth test were a better indicator of vigour under the field situation than was the germination test.

The seedling growth test has been used successfully by some members of the seed trade in the United Kingdom to classify seed lots of barley and wheat (Perry, 1980).

The test was included among the collaborative tests on wheat by the Vigour Test Committee. Accurate measurements can be made with this test. The test uses simple materials and equipment which occupies little space, and all the materials are generally available in most seed laboratories. However, the test is time-consuming to prepare and is subject to variations due to slight changes in the environment.

1. 4. 2. Rate of germination test

Copeland and McDonald (1995) reported that the percentage of germinated seeds recorded in the first count represents fast-germinating seeds and can be used as an index of vigour. The rate of germination is frequently used to refer to the proportion of a sample of seed which chits on each day of a germination test. However, variations in temperature or moisture and presence of dormancy in seed lots may affect test results.

1. 4. 3. Brick grit test

In this test, seeds are sown to a certain depth in damp brick grit or in a container of sand and are allowed to grow for a specific period of time before evaluation. Fussell and Pearson (1980) used sand to evaluate seed vigour in grains of pearl millet (*Pennisetum americanum* L.) grown in different temperature regimes. Woodstock (1976) also supported the use of sand instead of brick grit in this type of vigour tests. This test has also been accepted and used to evaluate cereal seeds in some of the European countries (Perry, 1980). In comparative tests it has been shown that the method provides more severe conditions for emergence of cereal seeds and the reports showed that the test can detect seed samples with low vigour more reliably than can the germination test (Heydecker, 1969).

1. 4. 4. Tetrazolium staining

The tetrazolium test (TZ test) is a biochemical test which utilizes the activity of dehydrogenase enzymes. Dehydrogenase enzymes react with substrates and release hydrogen ions to the oxidised, colourless, tetrazolium salt solution, which is changed into red formazan as it is reduced by hydrogen ions. The absence or presence and

nature of colour characteristics within tissues permit recognition and location of functional and non-functional tissues within embryo structures (ISTA, 1985).The test is widely used to assess viability but it is not entirely reliable in that it may provide false scores of seed viability. Parker and Proudlove (1993) reported that the tetrazolium test failed to detect heat-damaged barley grains which stained positive with tetrazolium despite being non-viable. The colour reaction on which the TZ test is based, depends not only on the activity of dehydrogenase enzymes, but also on a number of other factors within a seed which can affect the reducing capacity of the tissue. Tetrazolium staining has been used to assess vigour but the results of such tests are very difficult for the less experienced to interpret.

1. 4. 5. Vigour tests relating to seed storage and to performance in drought conditions

The accelerated ageing test and the controlled deterioration test developed by Matthews (1980) are important vigour tests used to predict a seed lot's storage life. An osmotic stress test using polyethylene glycol (PEG) is also an important vigour test used to relate seed emergence under drought stress conditions.

1. 5. Effects of Temperature during Grain Development

Investigations of the effects of temperature on grain growth and development have been reported in cereals, particularly in wheat (Tashiro and Wardlaw, 1990; Wardlaw, 1994; Wrigley, Blumenthal, Grass and Barlow, 1994). Studies have been carried out on grains grown in the field and in controlled environments using different ranges of temperature. In some experiments, treatments commenced from anthesis or shortly after anthesis whereas in others, treatment started before anthesis. Short episodes of temperatures above 30°C have also been investigated. However, differences in the range of temperatures investigated and in the cultivars used make it difficult to compare the results of different experiments. The use of the terms "high'' and "elevated'' in reference to temperature is yet another difficulty in the interpretation of results of temperature-related experiments. Al-Khatib and Paulsen (1984) referred to temperatures above 30°C as "high'' whereas Dawson and

Wardlaw (1989) used the term "elevated'' in reference to temperatures 24-30°C, and Langer and Olugbemi (1970) referred to temperatures above 38°C as "stressful''. In order to make results of different experiments comparable, Jenner (1994) classified temperature into two categories thus, 15-30°C corresponds to the low temperature range (LTR) and 30-40°C corresponds to the high temperature range (HTR). This classification seems to offer a great chance of minimising errors in the interpretation of results. For the purpose of identifying responses of grains to elevated temperatures (≥ 30°C), 18°C was used in the current study as the 'low' or control temperature. Sofield, Evans, Cook and Wardlaw (1977) Dawson and Wardlaw (1989) used 18/13°C as the 'low' or control temperature in their experiments on wheat carried out in the glasshouse and phytotron glasshouse conditions in Canberra, Australia.

Temperature is one of the most important environmental factors affecting not only plant growth and development but also the quality characteristics of the progeny. Grass and Burris (1995) reported that exposure of the parent plant to high temperature affected the quality of the seed produced. Physical properties of the seed including seed size, seed dimensions and the final appearance of the seed are affected by high temperatures experienced by parent plants during their growth, and these are some of the attributes of seed quality. The effects of temperature on the physical properties of seed were demonstrated by Tashiro and Wardlaw (1990) in an experiment on wheat grown in growth cabinets at 36/31°C . They observed that grains from plants transferred from a low temperature (21/16°C) to a high temperatures (36/31°C) were shrivelled and very small. Siddique and Doodwin (1980) showed that the bean seeds from plants grown in a controlled temperature glasshouse at 33/28°C were of lower vigour than seeds grown at 18/13°C.

High temperatures during grain development may affect seedling growth characteristics. Fussell and Pearson (1980) found that grains of pearl millet (*Pennisetum americanum* L.) which developed at low temperatures produced more vigorous seedlings than grains which developed at 33/28°C whereas Steiner and Opoku-Boateng (1991) reported that high temperatures shortly before and after anthesis affected the vigour of lettuce seed.

Several workers have also shown that grain grown at high temperatures were less dormant than grains grown at a low temperature. Khan and Laude (1969) reported that grains of barley grown in greenhouse at 38°C after awn emergence and then exposed to 54°C for 2h, emerged from moist sand more quickly than grains grown at 21/15°C.

Seeds obtained from different seasons or different geographical areas often vary in their germination capacity. Much of this variation has been attributed to differences in environmental conditions prevailing during the formation, development and maturation of the seed while still attached to the mother plant. These variations in environment during grain development can induce seed dormancy. "Dormancy is an arrest in development of seed embryos, buds or spores under conditions otherwise suited for growth'' (Taylorson & Hendricks, 1977). Cochrane (1993) described the extent of dormancy as "the percentage of a sample of viable grains which has failed to germinate in a given set of conditions by the end of a given period of time.''

The intensity of seed dormancy is strongly influenced by environmental factors during both the ripening period and in storage (Strand, 1991). Belderok (1968) observed that grains of cultivars of wheat grown in a warm, dry summer, were less dormant than grains grown in a very cool, wet summer. In a series of observations carried out by Cochrane (1993) on several cultivars of spring barley grown at four sites in Scotland and in controlled environments in the growth rooms, differences were found in the rate of recovery from dormancy in grains grown at the four sites, and in grains which experienced different temperatures in the growth rooms during grain development. Schuurink, van Beckum and Hendkemp (1992) grew barley plants in a range of different light and temperature regimes, and found that low temperatures (14/10°C) and short days during grain growth produced dormant grains, but it is not possible from their results to draw conclusions about the separate effects of photoperiod and thermoperiod on the induction of dormancy. Variation in dormancy may also be caused by differences between grains from the main stem ears and those from tiller ears. Strand (1991) and Cochrane (1994c) showed that grains of barley from the main stem ears were less dormant than grains from tiller ears and that

variation in the depth of dormancy existed between grains from apical parts of the ear and those from the basal parts of the ear.

1 5. 1. Grain weight in relation to the rate and duration of grain development

One environmental factor known to affect the rate of dry matter deposition is temperature. Grain dry weight can be quantified as the product of the duration and the rate of grain-filling (Gallagher, Biscoe and Hunter, 1976) and the effect of the environment on each of these components may then be determined. Sofield, Evans, Cook and Wardlaw (1977) grew wheat plants in controlled environment cabinets from anthesis to harvest-ripeness in a range of temperature regimes and found that the duration of grain growth was less in grains grown at high temperature (30/25°C) than in grains grown at low temperature (15/10°C). Grain weight at maturity of the wheat cultivar Banks was reduced by approximately 5% for each 1 °C rise in daily mean post-anthesis temperature in the range from 17.7°C to 32.7°C (Tashiro and Wardlaw, 1989). The rate of increase in grain dry weight is determined by temperature, but effects of differences in irradiance have also been found (Sofield *et al.*, 1977). High temperatures result in low grain weights at harvest maturity when the higher growth rate at higher temperatures is more than offset by the negative effects of a shorter grain-filling durations (Sofield *et al.*, 1977; Al-Khatib and Paulsen, 1984).

The effect of high temperature on grain weight may depend on the duration of the exposure of the grains to high temperature. Tashiro and Wardlaw (1990) observed that grains of wheat exposed to 30°C from ten days after anthesis onwards were lighter than grains exposed to high temperature (30°C) later in grain development. The above authors also found that the stage

7-12 days after anthesis was the most sensitive to high temperature (> 30°C) in relation to grain dry weight. This is the stage of rapid cell division and cell enlargement in the endosperm. Similarly, Tashiro and Wardlaw (1991) showed that grains of rice exposed to 36/31°C (day/night) twelve days after heading were more affected by high temperature than grains exposed to 36/31°C twenty six days later in grain development, and that there was little effect on grain weight when grains were

exposed to the same temperature twenty days after anthesis. Langer and Olugbemi (1970) found that the period from anthesis to grain-filling was the most sensitive to high temperature (38°C) in wheat grown in a glasshouse. In barley, a brief exposure to 30°C for 7 days after anthesis did not affect the rate of dry matter accumulation during grain growth but affected the final grain weight (MacLeod and Duffus, 1988). The final grain weight was less in plants which had been exposed to 30°C temperature than in control (15°C) plants because dry matter accumulation stopped earlier in the former than in the latter. However, several other factors may operate within the grain itself in relation to high temperatures. Some consideration has been given to the possibility that increased respiratory losses of carbon, that occur with a rise in temperature may affect grain dry weight (Wardlaw, Sofield and Cartwright, 1980).

Difference among varieties in the response of grains to high temperatures are thought to occur because more temperature-tolerant varieties maintain higher rates of grain growth than less-sensitive varieties (Wardlaw and Moncur, 1995). In an experiment designed to determine genetic variation in the response of wheat to high temperature (30°C) in the period from booting to grain maturity, Wardlaw, Dawson, Munibi and Fewster (1989), Wardlaw, Dawson and Munibi (1989) and Dawson and Wardlaw (1989) reported that grains of different wheat cultivars responded differently to high temperature at booting and during grain development. Some cultivars were more sensitive to high temperatures during grain development than at booting and others were more sensitive to high temperature at booting than during grain development. Temperature insensitivity at booting may be required when the normal growing season is extended into warmer periods or in warm conditions associated with the greenhouse effect.

Sensitivity of the grains to high temperatures during grain development may depend on the species. Rice, a sub-tropical cereal is better adapted to high temperature during grain development than is wheat (Chowdhury and Wardlaw, 1978). The tolerance of rice grain to high temperature (20-30°C) is associated with the ability of the grain to increase the rate of grain filling with increasing temperature (Tashiro and Wardlaw, 1989).

In wheat, in the low temperature from range (10-15°C) the shorter duration of grain filling with increased temperature is compensated for by an increased rate of grain filling, resulting in little change in grain weight at maturity (Wardlaw and Wrigley, 1994), whereas at temperatures >21°C, the shorter duration of grain filling is not fully compensated for by the increased rate of grain filling and so grain weight at maturity is less in grains grown at higher temperatures than grains grown at lower temperatures (Sofield, Evans, Cook and Wardlaw, 1977; Tashiro and Wardlaw, 1989).

Apart from temperature, light intensity also may affect grain growth. At high temperatures grains may require more assimilates than the amount produced under conditions of low light intensity. Spiertz (1977) reported that at low temperatures there were ample stem reserves to meet grain demands, nevertheless the rate of grain growth increased with increased light intensities.

Analysis by Al-Khatib and Paulsen (1984, 1990) on the effect of high temperature on photosynthesis and the photosynthetic apparatus and the advantages that derive from a high level of stem reserves (Blum, Sinmena, Mayer, Golan and Shpiler, 1994), suggest that the availability of assimilates may play a role in the response of the grain to high temperature. This is supported by the interaction that has been observed between irradiance and the response to high temperature during grain filling (Wardlaw, Dawson and Munibi, 1989). However, this relationship needs further study given that sugar levels in the grain endosperm have not been shown to limit the rate of starch synthesis under high temperature conditions in wheat (Bhullar and Jenner, 1985) and in maize (Singletary, Banisadr and Keeling, 1994), and changes in the source/sink balance do not greatly alter the response to temperature (Wardlaw, Sofield and Cartwright, 1980). Cause and effect are not easily determined and it is possible that several other factors could be responsible for the degeneration of catalytic activity at high temperatures. For example, Duke, Doehlert and Pratt (1993) found that the expression of genes in maize was affected at 35°C.

1. 5. 2. Starch granules

Ambient temperature affects the of starch deposition rate in cereal grains (Chowdhury and Wardlaw, 1978). Because temperature changes affect starch biosynthesis, the structure of starch from grains grown at different temperatures during development may differ and such changes may affect starch structural properties.

Starch is the major component of mature barley grains and it is deposited as granules within the amyloplasts in the cells of the starchy endosperm. Starch occurs in barley and wheat endosperms as two distinct types of granules, the large (A-type) granules and the smaller (B-type) granules. The large granules tend to be lenticular (oblate spheroids), whereas the smaller granules are approximately spherical. The A-type granules are initiated in wheat early during the phase of endosperm cell division and most of their growth in diameter occurs during this phase. The number per endosperm then remains constant although the granules continue to increase in size. The B-type granules are initiated in the middle stage of grain filling (phase of cell enlargement) and increase in number throughout the remainder of the grain-filling period (Brocklehurst and Evers 1977; Brooks, Jenner and Aspinall, 1982). Thus variations in ambient temperatures during each of these phases can affect the respective starch types to different extents. The final granule size in wheat depends upon temperature during grain filling (Shi, Seib and Bernardin, 1994).

In the analyses of starch granule size distribution, the designation of large (A-type) and small (B-type) starch granules is somewhat arbitrary and depends on the separation techniques used (MacDonald, Stark, Morrison and Ellis, 1991). Different diameter sizes have been used in barley and wheat to distinguish A-type granules from B-types starch granules. For example Kang, Sugimoto and Kato (1985) and Savin, Stone and Nicolas (1996) classified granules with a diameter greater than 10 μm as A-type granules in wheat, and MacLeod and Duffus (1988) classified granules with diameter greater than 8 μm as A-type granules in barley, whereas Bechtel, Zayas, Kaleikau and Pomaranz, (1990) considered that the A-type granules in wheat were those having a diameter greater than 16 μm.

Environmental conditions during grain development strongly influence the number and size of the two types of granules. Hoshikawa (1962) reported that grains from wheat plants grown at 30°C had smaller A-type and fewer B-type starch granules than grain grown at 20°C. Tester, South, Morrison and Ellis (1991) reported that grains from barley plants grown at 20°C during the grain-filling period had smaller A-type and fewer B-type starch granules than grains grown at 10°C. Grains from wheat plants grown in growth chambers at 40°C had smaller A-type granules and fewer A-type and B-type starch granules than grains grown at 21/15°C (Shi, Seib and Bernardin, 1994). Similarly, Lu, Jane and Keeling (1996) reported that grains from maize plants grown in an environmentally controlled greenhouse at 35°C had smaller A-type starch granules than grains grown at 25°C, Kiniry and Musser (1988) found that grains of sorghum grown in a greenhouse at 30°C during grain-filling had smaller A-type starch granules than grains grown at 15°C. MacLeod and Duffus (1988) found that grains from barley plants grown in growth rooms at 30°C had fewer A-type granules than grains grown at 20°C. Buttrose (1960) concluded that A-type granule number per endosperm was genetically determined and that the number of B-type granules per endosperm varied according to the prevailing environmental conditions. Studies carried out under field conditions in barley showed that A-type granule numbers per endosperm were more constant than other parameters (Morrison, Scott and Karkalas, 1986). Values quoted for the total starch granule numbers per grain and for granule size distribution from experiments carried out under different temperature regimes have been inconsistent. Some of the causes of such inconsistency may be due to the arbitrary designation of small and large starch granules and to differences in starch extraction techniques. At high temperature, grain development proceeds much faster than at low temperatures. If the current photosynthesis together with stored assimilates cannot provide an adequate supply of raw materials, the initiation of starch granules and growth of starch granules will be affected. Therefore, when the grain growth period is shorter, the A-type granules will be smaller and B-type granules will be fewer in number. It is also possible that granules classified as B-type granules in starch from grains grown at high temperatures may be a mixture of true B-type granules and of A-type

granules which were underdeveloped due to the shortened duration of grain growth at high temperature.

1.6 .Aims and Objectives

There have been comparatively few systematic investigations into the effects of elevated temperatures during grain development on seed quality in barley. With this background it was decided that useful information might be obtained by making a careful study of barley using controlled environment facilities. Environmental variables such as temperature, water availability, light intensity and day length are difficult to control under field conditions as are the timing, duration and magnitude of high temperature episodes, all of which could significantly alter the response of a crop to high temperature, but under controlled environment each factor can be considered separately.

The use of controlled environment facilities offers the advantage of observing the response of plants to elevated temperature at a particular stage of development i.e. the phase of grain development affected by elevated temperature could be better timed than would be the case for field-based experiments. While controlled environment experiments cannot necessarily be extrapolated to interpret variation in the field, they are useful in understanding responses of plants to specific environmental factors. The aim of this project was to get a better understanding of the effects of temperature during grain development on seed quality in barley. It was therefore decided to carry out a series of experiments to produce seeds using different temperature regimes commonly experienced by plants during grain development under field conditions. Seeds were assessed for quality using:

1. Germination tests using 5 ml, 8 ml and 10 ml water per 100 grains.
2. Measurements of seedling growth characteristics.
3. Emergence test.
4. Embryo dry weight.
5. Embryo growth potential on artificial media.

Studies using glasshouse facilities were carried out on a range of genotypes grown in sub-tropical and temperate climates. However, owing to inadequate availability of seeds harvested from elevated temperature regimes, it was not possible to extend the

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Studies using glasshouse facilities were carried out on a range of genotypes grown in sub-tropical and temperate climates. However, owing to inadequate availability of seeds harvested from elevated temperature regimes, it was not possible to extend the range of vigour tests to include osmotic tests using polyethylene glycol (PEG) which is related to the performance of seeds in drought conditions.

Further investigations were carried out in order to try to develop a better understanding of physiological factors related to aspects of vigour in seeds from grains grown at elevated temperatures. Starch and its structural composition as influenced by elevated temperatures during grain development was felt to be an important feature to study in relation to its accessibility to hydrolytic enzymes during germination and the efficiency of enzymes to mobilise food reserves.

From the practical point of view, results from this study could be used in conjunction with meteorological data to predict how changes in temperature conditions which are likely to occur during plant growth in the field would affect seed quality.

1.7. Experimental Approach

To evaluate effects of elevated temperature on seed quality, plants were subjected to different temperatures during grain development. Temperature treatments ranged from 18°C (control) to temperature regimes in which plants were exposed to step-wise increases in temperature from 18°C to 38°C at different stages of grain growth and for different lengths of time.

Experiment I was a preliminary one in which plants of cv. Blenheim experienced constant day/night temperatures of 18°C or 30°C, during the period from anthesis to harvest-ripeness. Experiment II was a repeat of Experiment I but with modifications in the timing of the temperature treatments. Grains from Experiments I & II grown at 30°C were viable and so in Experiment III, two cultivars, Stirling and Schooner

2. MATERIALS AND METHODS

The experiments were carried out in the glasshouses, growth rooms, growth cabinets and the laboratories in the t Crop Science and Technology Departmen, The Scottish Agricultural College, Edinburgh, Scotland.

2. 1. Source of seed

Six-rowed and two-rowed barley (*Hordeum vulgare* L.) cultivars were used in the experiments. The five six-rowed cultivars were obtained from the CIMMYT Mexico barley germplasm pool and were supplied by Dr Paul Fox, whereas the six two-rowed cultivars were supplied by Dr M.P. Cochrane, Scottish Agricultural College (SAC). The two-rowed barley cvs were Blenheim and Tyne (UK), Clipper, Schooner and Stirling (Australian) and Harrington (Canada). All these are malting cultivars. The six-rowed barley cvs (BRB2, Esperanza, Esmeranda, Centinela and Puebla) were among the breeding lines or cultivars in the Barley Programme at CIMMYT.

There were eleven cultivars in total and all these were used in Experiment V. Three malting barley cultivars, Schooner, Stirling and Blenheim were used in the controlled environment experiments in which plants were exposed to different temperature regimes during grain development. Schooner is a cultivar grown predominantly in the South-Eastern and Victoria growing areas of Australia which have a comparatively cool and long growing season (Savin and Nicolas, 1996). Stirling is grown in Western Australia in areas which are hotter and drier, and which have a shorter growing season than Southern Australia (D Sparrow, personal communication). Blenheim is one of the malting barley cultivars which was recently grown for some seasons in Scotland.

The criterion used was to include cultivars varying in their genetic background and originating from both temperate, subtropical or tropical environments. Cultivars grown under growth room conditions represented both warm and temperate environments.

2. 2. Plant growth conditions

Barley seeds were sown in a glasshouse in 20 cm diameter pots in peat-based compost. At about two weeks after emergence, plants were thinned to six plants per pot. Attempts were made to leave uniform and evenly spaced seedlings which were

allowed to grow naturally without removing tillers. In the glasshouse, day/night temperatures were maintained at approximately 20°C /15°C for an 18 h day. Plants were watered with tap water daily to ensure that they were not subject to water stress during the growing period and they were fed regularly with commercial nutrient solution. Natural day light was supplemented with sodium vapour lamps for plants grown in an SAC glasshouse at Bush Estate Midlothian. Fungicide and pesticide treatments were applied when necessary to prevent biotic stress. The plants destined for the controlled environment conditions were transferred prior to anthesis, to two identical growth rooms in which a constant temperature of 18°C was maintained. In the growth rooms, lighting was supplied by mercury vapour lamps. Day length was 16 h and light intensity at ear level was 140 μmol m^{-2} s^{-1}. Plants grown in SAC's Fisons controlled environment cabinets (Fison, Fi-totron 600H) had a day length of 16 h and light intensity at ear level was 130 μmol m^{-2} s^{-1}. Ears of main stems and tillers were tagged at anthesis. The date on which first anthesis was observed was recorded. 'Day 1' was the date by which anthesis had taken place in 50% of the ears. Relative humidity was not controlled in the growth rooms for Experiments I, II & III. In the growth rooms, plants were not supplemented with any commercial nutrient solution. In Experiment V plants were grown in a glasshouse at SAC (KB) in which day length was supplied with mercury vapour lamps. Records of dates of anthesis were not taken.

2 .3. Harvesting

Harvesting was carried out at four-day intervals starting 960°C days after 'day 1' thus plants grown at elevated temperatures were harvested first. Ears were cut from the ear node and then placed in paper bags. In Experiments I, II & IV, five samples of grains were taken from each temperature regime treatment to determine moisture content. After harvesting, ears were separated into two groups: date 1: ears that anthesed before commencement of treatment. The grains from all the main stem ears and some of the primary tillers were in this seed lot, date 2: the ears of the tillers which anthesed after 50 % of ears in each pot had anthesed and treatment had started. In Experiment 1, ears were divided into four groups according to anthesis dates.

Harvesting was followed by hand-threshing the grains to minimise mechanical damage. The number of grains per ear was recorded and then grains were divided into seed lots according to anthesis dates and dried to approximately 10% moisture content (M. c.) at 10°C using silica gel, and then stored in an incubator set at 10°C.

2. 4 Seed Quality Assessment

2. 4. 1 Grain size distribution

After harvest, the distribution of grain size of barley samples was measured by passing grains through stacked slotted sieves consisting of four grading dimensions; >2.8 mm, <2.8 >2.5 mm, < 2.5 > 2.2 mm and < 2.2 mm. Grains retained on each sieve were counted, weighed and recorded. Finally, all the grains were amalgamated according to seed lots and then stored at 10°C for subsequent laboratory analysis. The grain size distribution was calculated either by counting number of grains in each size class or weighing grains in each size class and then expressing this value as a percentage of the total number of grains in the seed lot or of the total seed weight in each seed lot.

2. 4. 2. Germination test

Germination tests were carried out on triplicate sets of 100 grains in each 90 mm diameter Petri dish, containing two Whatman No. 1 filter papers to which 5 ml distilled water (Experiment I) and 5 ml, 8 ml & 10 ml distilled water (Experiment II) and 5 ml and 10 ml distilled water (Experiment III) was added. The Petri dishes were incubated at 20°C in the dark. Chitted grains were removed each day and the percentage germination was determined after 72 h. Grains which had not chitted by the 3rd day were germinated using hydrogen peroxide to assess their viability level. Viability was assessed by immersing 50 grains in 50 ml hydrogen peroxide (7.5 g/ litre) for three days at 20°C. Grains were transferred to a freshly prepared solution after the second day, and on the third day grains which had chitted were counted. Grains which had not chitted by the fourth day were carefully peeled to remove layers of the tissue covering the embryo and were kept on a moist filter paper in a Petri dish at 20°C. Grains that failed to germinate were then transferred to 5°C

and kept at this temperature for five days to break dormancy and were alternated between 20°C and 5°C every five days until germination occurred or the embryo rotted. The sum of the number of grains which germinated using hydrogen peroxide and the number that germinated after removing the tissue covering the embryo and the number of grains which germinated following exposure to alternating temperatures, was used to determine the percentage of viable grains.

2 . 4. 3. Seedling growth characteristics

Seedling growth analyses were carried out using paper towelling. A line was drawn at the centre of the long axis of the paper and five parallel lines at 2 cm interval to one side of the centre line. The centre line was marked with 25 points at 1 cm intervals to locate seeds. Twenty-five seeds were glued to the paper towels with a non-toxic adhesive (Cow Gum, Lilo Ltd, Woking, Surrey) (**Plate 2. 4. 3a.).** The seeds were placed ventral surface down with the embryo end towards the bottom edge of the paper. Four replicates of 25 seeds from each seed lot were used and these were left at room temperature (20°C) for a day to allow the glue to dry. The papers were sprayed with distilled water, rolled loosely and then placed in a slanting position in a wire basket. The baskets were enclosed in black polythene bags to minimise water loss and to prevent any light reaching the grains. They were incubated at 5°C for 5 days to break seed dormancy, and then grains were transferred to an incubator at 20°C with relative humidity of 90%. Seedling measurements were taken after 7 days (**Plate 2. 4. 3b**). The number of plumule tips lying within each pair of lines was multiplied by the corresponding mid-point length and summed up to provide a mean measure. The total length was divided by the number of germinated seeds.

In Experiments II, III , IV and V grain weight was adjusted to 0 % M.c. by weighing 20 grains from each comparable sample from storage and drying them to constant weight in an oven at 70°C. This was designed to relate seedling dry weight to the amount of stored food reserves available for the seedlings in the Seedling growth and Emergence tests.

Grains used in Experiments II, III and IV were weighed individually before they were glued to the paper towels with a non-toxic adhesive. There were four replicates of 25 grains each. The positions of seeds in each replication were numbered from 1 to 25 to identify the individual seed. Root and plumule lengths were measured using a ruler after seven days seedling growth. Roots and plumules were cut off at grain level. The germinated grains, roots and plumules were dried to constant weight in sets of 25 in beakers in an oven at 70°C.

2. 4. 4. Seedling emergence

Twenty five grains were weighed and were glued to a paper towel with non-toxic adhesive as in (**Section 2.4.3**) and kept at room temperature for 24 h to allow the glue to dry. Four replications were prepared for each seed sample. The paper towels were sprayed with distilled water, rolled loosely and placed in an upright position in baskets. The baskets were enclosed in black polythene bags and then incubated at 5°C for 5 days. 11.4 kg of fully wetted fine sand was added to 30 cm-diameter pots (**Plate 2. 4. 4a**), and then the surface was levelled and the sand was compacted using another pot containing 11.4 kg wet sand. The paper towel was cut on either side of the rows of seeds leaving a narrow strip of paper bearing the seeds which was placed on the surface of the sand across the diameter of the pot. The level of the sand was marked on the pot, another mark was made 4 cm above the surface and then 3.8 kg wet sand was added covering the strips of seeds to the depth of 4 cm. This ensured that all seeds were 1 cm apart and were placed at the same depth in the sand. The surface of the sand was finally levelled using the base of one of the pots of the same weight. Pots were randomly placed in the growth room. The temperature in the growth room was set at 20/15°C day/night for a 16 h day. The relative humidity was maintained at 85%. Emerged seedlings were recorded daily and tags with dates of emergence were placed around each seedling (Plate 2. 4. 4b). Seedlings were counted as emerged when the coleoptiles were visible above the soil surface. The final count was made after 7 days from planting. Seedlings were washed thoroughly with tap water to remove particles of sand. Measurements of plumule length, number of roots and length of the longest root were made using a ruler. Measurements of emerged

seedlings, of seedlings which germinated but did not emerge above the soil surface, and of abnormal (coiled) seedlings were recorded. The roots were removed and discarded due to possible presence of fine sand particles. Plumules and grains (grains after 7 days germination) were placed in beakers, and dried to constant weight at 70°C in an oven.

2. 4. 5. Embryo dry weight

Three replicates of 20 grains were weighed and then soaked in distilled water at 4°C overnight. Embryos were extracted using a dissecting microscope and scalpel and then weighed and dried to constant weight at 70°C.

2. 4. 6. Embryo growth on artificial media

The artificial media used in the experiment were:

(i) 1% w/v agar. (ii) 1 % w/v agar, 0.9 % w/v glucose (iii) 1 % w/v agar, 0.9 % w /v mannitol. Mannitol was in this experiment to act a control. Seedlings cannot use as a nutrient for growth but it has approximately the same osmotic potential as solution of the same concentration. Using a sterilised pipette, 40 ml of medium was placed in each 90 mm diameter Petri dish. Triplicate samples of ten dry grains were soaked in distilled water for 5.5 h at room temperature and surface sterilised in 1 % (w / v) sodium hypochlorite solution for a minute and then rinsed in sterile distilled water. Embryos were extracted using a dissecting microscope, sterilised scalpels and forceps and then dipped in 1 % (w/v) sodium hypochlorite solution one minute, rinsed in 0.05 % (w/v) thiosulphate solution and in sterile distilled water. Ten embryos were placed in a line across the diameter of each 9 cm diameter sterile Petri dish. The Petri dishes were sealed with Parafilm and a line was drawn on the top cover of Petri dishes with a marker pen, showing the orientation of radicle. The Petri dishes were then mounted in a rack so that the longitudinal axis of each embryo was almost vertical. The Petri dishes were then incubated in the dark at 20°C ± 1°C for 5 days. Seedling growth was assessed by measuring roots and plumules using Vernier callipers. Seedlings (roots and plumules) were dried to constant weight in an oven at 70°C.

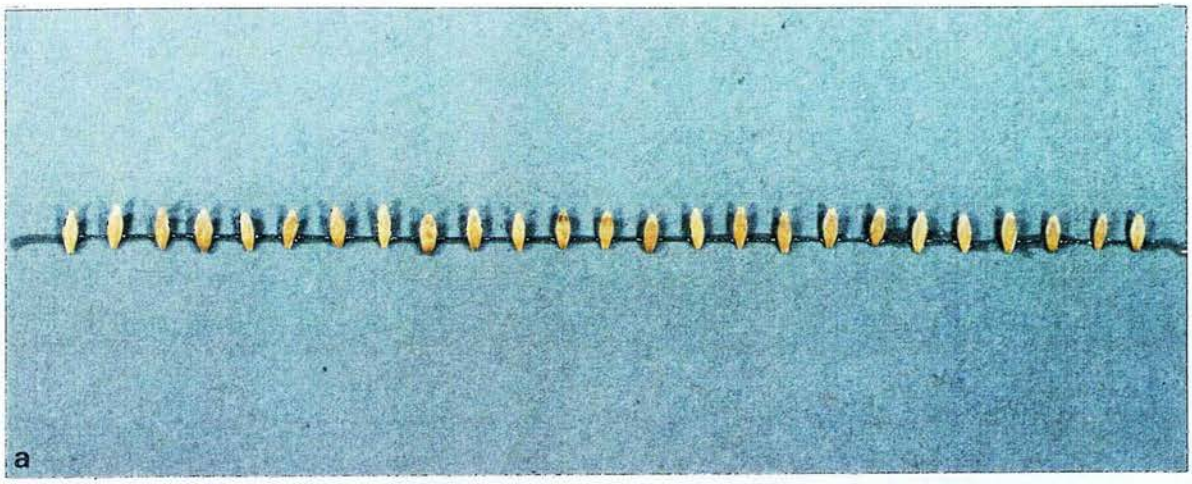


Plate 2. 4. 3. (a). Grains of barley glued to paper towels with non-toxic adhesive for seedling growth test. (b). Seedlings in paper towels ready for measurement after 7 days incubation in the dark.

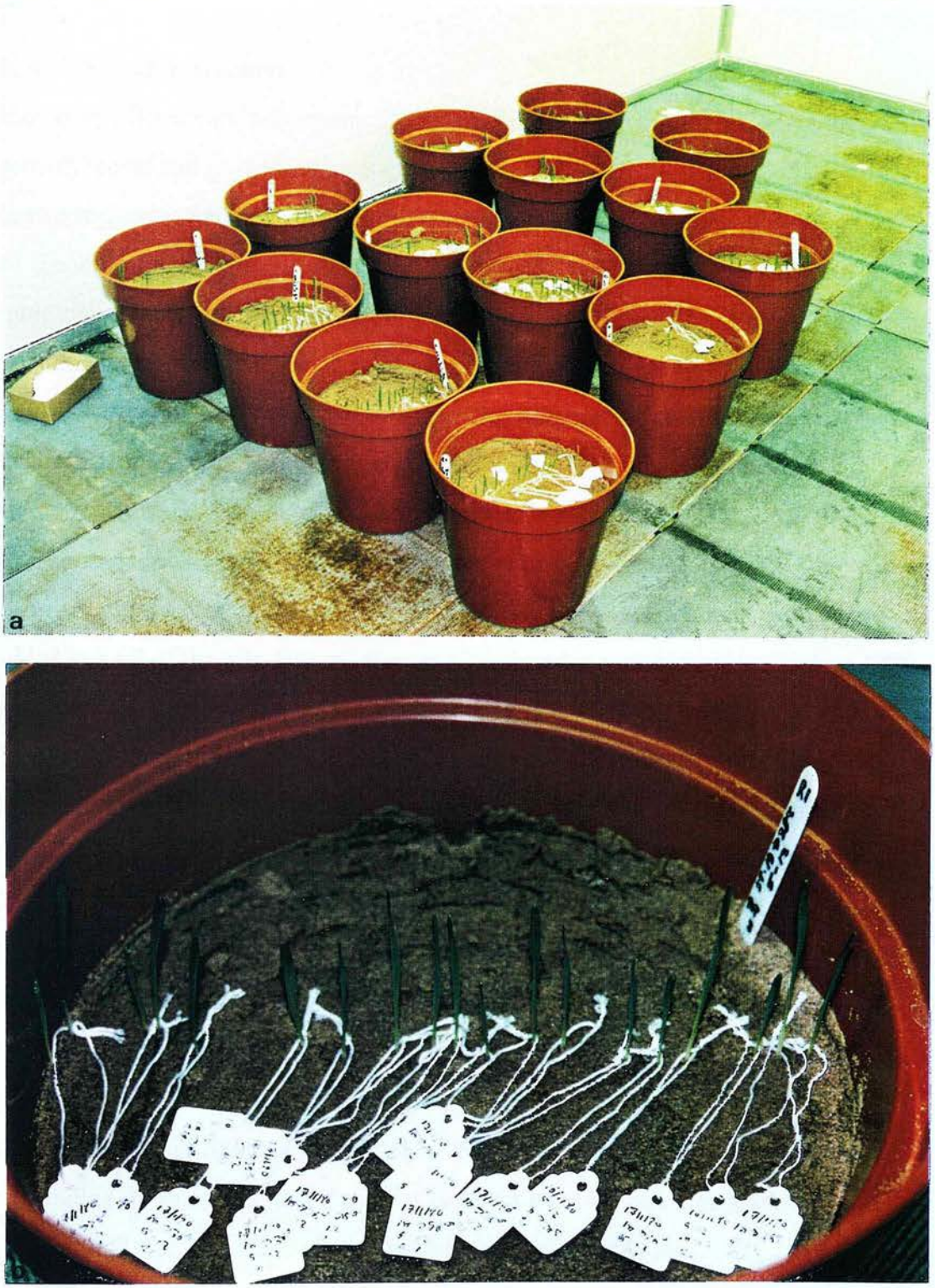


Plate. 2. 4. 4. (a). Emergence test using fine sand. (b). Emerged seedlings grown in the growth room at 18/13°C tagged with dates of emergence.

2. 4. 7. Starch extraction

Ears of cvs Blenheim, Schooner and Stirling with same anthesis date grown in both growth rooms and growth cabinets (Experiments III & IV), were sampled randomly in two replicates for starch extraction. A solution of cellulase (1 mg ml^{-1}) in a 0.025 M citrate/ sodium phosphate buffer pH 5.0, containing 1.7 mg ml^{-1} acarbose to inhibit amylase activity, and 25 µg ml^{-1} amphotericin, 10 mg ml^{-1} streptomycin and 10000 units ml^{-1} of penicillin to inhibit microbial activity, was incubated at 30°C for one hour. Acarbose was supplied by Bayer Ag, Pharma Research Centre, PH-FE Scientific Information and Documentam Building 459, D-42096 Wuppertal, Germany, and cellulase, ampotericin, streptomycin and penicillin were all supplied by Sigma-Aldrich Company Ltd, Fancy Road, Poole, Dorset BH12 4QH, England. Ten barley grains from each sample, cut longitudinally into four parts were then added to 5 ml of the cellulase solution and incubated in a shaking water bath at 30°C for 24 h. The resulting suspension was centrifuged at 20,000 x g for 10 min at 4°C. The pellet was re-suspended in 5 ml of a 0.1 mg ml^{-1} solution of proteinase K (supplied by BDH Laboratory Suppliers, Poole, England) in 0.02M (sodium) phosphate buffer, pH 7.5, containing 0.02 % w/v sodium azide and incubated at 30°C for 24 h in a shaking water bath. The suspension was then filtered through muslin. Fragments remaining on the muslin were suspended in water and sonicated for two minutes in order to remove all starch still attached to the husk. The combined suspensions of starch granules were centrifuged at 20,000 x *g* for 10 min at 4°C. The pellet was incubated for 24 h in 5 ml of the proteinase K solution. The suspension was centrifuged at 20,000 x *g* for 10 min. The pellet was washed three times by re-suspending in water and centrifuging at 20,000 x *g* for 10 min. The granules were dried at 30°C for 24 h. Extractions were carried out in duplicate.

2. 4. 8. Starch granule size distribution

Granules extracted as described above were incubated at 25 °C overnight in 0.05M borate buffer, pH 10, containing 0.4 % v/v dithiothreitol. The suspension of granules was centrifuged at 20,000 x g for 10 min and the pellet was then washed three times by suspension in water followed by centrifugation and the granules were dried for 24 h and weighed. The size distribution of the starch granule population was determined using a Coulter Counter Multisizer II which was fitted with a 100 μm diameter orifice.

2. 4. 9. Alpha-amylase activity

The method used was a modification of the methods described by Tillett and Bryce (1993). Ceralpha was supplied by Megazyme. Four replications of 20 grains were germinated in 90 mm diameter Petri dishes. The seeds were spread on two layers of Whatman No. 1, filter papers to which 5 ml water was added. The Petri dishes were incubated at 20°C ± 1°C in the dark. After 48 h incubation, ten uniform seedlings at the same stage of growth were cut longitudinally in four parts and then homogenised for 5 minutes in 2.5 ml extraction buffer at a room temperature using an Ultra-Turner electrical homogeniser T25. The extraction buffer used in this experiment for the extraction of α-amylase from 48 h-germinated seeds contained 0.05 M sodium malate, 0.05 M sodium chloride, 2 mM calcium chloride and 0.05 % w/v sodium azide adjusted to pH 5.2. After homogenisation, a further 2.5 ml of extraction buffer was used to rinse the homogeniser and the combined extracts were then centrifuged at 7,200 x *g* for 10 minutes. Aliquots (0.1ml) of the supernatant were diluted to 1 ml with extraction buffer and incubated at 40°C for 2 min. A Ceralpha solution was prepared in accordance to the manufacturer's instructions, and incubated in a water bath at 40°C for 1 minute. To each test tube containing pre-incubated extract, 0.1 ml Ceralpha substrate was added. The mixture was then placed in a water bath at 40°C. The enzyme reaction was stopped by adding 1.5 ml of 1 % w / v Trizma base at the end of exactly 10 minutes. The solution was centrifuged at 7,200 x *g* for 3 min. The absorbance readings of the resultant supernatant and an enzyme /Trizma blank, and a

Ceralpha/Trizma blank were read at 410 nm on a spectrophotometer. The difference in absorbance between a sample and the sum of the two blanks was calculated.

2. 5. Experiments

Four experiments were carried out from 1993-1995 to investigate the effects of elevated temperatures during grain development on seed quality of cultivars of barley grown in a controlled environment under different temperature regimes.
Comparisons of seed quality in eleven genotypes were carried out on grains grown in a glasshouse at approximately 18/13°C.

2. 5. 1. Experiment I (November 1993-February 1994)

2. 5. 1. 1. Temperature regimes

Temperatures in this Experiment were constant day and night.

(A) 18°C from anthesis to harvest-ripeness

(B) 18°C from 'day 1' for 35 days and then 30°C to harvest-ripeness

(C) 30°C from 'day 1' for 7 days and then 18°C to harvest-ripeness.

(D) 30°C from anthesis to harvest-ripeness

Seeds of cv. Blenheim were sown on 11 November, 1993 in the glasshouse at Bush Estate. The procedure was as described in **Section 2. 2**. There were 18 pots for each temperature regime. Eight days prior to anthesis, plants were transferred from Bush Estate Midlothian to two growth rooms at King's Buildings (KB) in which a constant temperature of 18°C was maintained. Anthesis was first observed on 4 January, 1994. 24 January was taken as 'day 1' the date by which anthesis had taken place in 50 % of the ears. The timing of all subsequent operations was related to this date. Plants from treatments D (30°C) and C (30-18°C) that were destined to receive a 30°C temperature were given a conditioning temperature of 25°C for a day before the temperature was raised to 30°C. This was to minimise the possible temperature shock effect of transfer from 18°C to 30°C. After seven days at 30°C, plants in temperature regime C (30-18°C) were returned to 18°C (control temperature) to complete the remainder of grain development period whilst plants in temperature regime D (30°C) were retained at this temperature (30°C) to harvest-ripeness. Plants in temperature

regime B (18-30°C) were kept at 18°C for thirty-five days from 'day 1' and then transferred to 30°C to complete the remainder of grain development. Temperature regime A (18°C) was a control, plants being maintained continuously at this temperature from anthesis to harvest-ripeness.

2. 5.1.2. Harvesting

Harvesting was commenced 960°C days after 'day 1'. Harvesting was carried out in the order of earliness of ripening.

(D) 32 days from 'day 1 'to harvest-ripeness.

(B) 46 days from 'day 1' to harvest-ripeness.

(C) 48 days from 'day 1' to harvest-ripeness.

(A) 53 days from 'day 1' to harvest-ripeness.

Ears were then harvested at 4 day intervals and amalgamated to form seed lots 1 and 2. After hand-threshings, seed lots were analysed.

2. 5. 1. 3. Seed lots

(Aa) Ears anthesed 4 to 23 January (all main stems and some tillers).

(Ab) Ears anthesed 24 to 31 January (tillers).

(Ac) Ears anthesed 1 to 12 February (tillers).

(Ad) Ears anthesed 13 to 28 February (tillers).

(Ba) Ears anthesed on 4 to 23 January (all main stems and some tillers).

(Bb) Ears anthesed 24 to 31 January (tillers).

(Bc) Ears anthesed 1 to 12 February (tillers).

(Bd) Ears anthesed 13 to 21 February (tillers).

(C1) Ears anthesed 4 to 23 January (all main stems and some tillers).

(C2) Ears anthesed 24 January to 20 February (tillers).

(D1) Ears anthesed 4 to 23 January (all main stems and some tillers).

(D2) Ears anthesed 24 January to 14 February (tillers).

Aa, Ab, Ac and Ad and Ba, Bb, Bc and Bd were analysed separately in Germination and Seedling Growth tests. In the presentation of results, values obtained for Aa and Ab were combined and labelled A1 (seed lot 1), and values obtained for Ac and Ad

were combined and labelled A2 (seed lot 2). Similarly Ba and Bb were combined and labelled B1 (seed lot 1), and values obtained for Bc and Bd were combined and labelled B2 (seed lot 2).

2. 5. 1. 4. Seed quality assessment

The quality of these seed lots was assessed using: (1). A germination test with 5 ml water per 100 grains. (2) Measurements of seedling growth characteristics.

2. 5. 2. Experiment II (March-June 1994)

2. 5. 2. 1. Temperature regimes

Day and night temperature was kept constant as in (**Section 2. 5. 1. 1**)

(A) 18°C from anthesis to harvest-ripeness.

(B) 18°C from 'day 1' for 25 days and then 30°C to harvest-ripeness.

(C) 30°C from 'day 1' for 10 days and then 18°C to harvest-ripeness.

(D) 30°C from anthesis to harvest-ripeness.

Seeds of cv. Blenheim were sown on 3 March, 1994. Plant culture was the same as in Experiment I. There were 18 pots for each temperature regime. Five days prior to anthesis plants were transferred from the glasshouse at Bush to two growth rooms at KB in which a constant temperature of 18°C was maintained before commencement of treatment. Anthesis was first observed on 23 April, and 5 May was taken as 'day 1' the date by which anthesis had taken place in 50 % of the ears. This experiment differed from Experiment I in that plants in temperature regime B (18-30°C) which were kept at 18°C for 35 days in Experiment I, were kept at 18°C for 25 days and then maintained at 30°C until harvest-ripeness. Plants in temperature regime C (30-18°C) were maintained at 30°C for 10 days instead of 7 days as in Experiment 1. Temperature regimes A and D were the same as in Experiment I.

2. 5. 2. 2. Harvesting

Harvesting of (D) commenced on 5 June, 32 days after' day 1' after, harvesting of (B) commenced 15 June, 42 days after 'day 1' harvesting of (C) commenced on 20 June 46 days after 'day 1' and harvesting of (A) commenced on 25 June, 53 days

after 'day 1'. Grains from each seed lot were hand-threshed and stored in the incubator at 10°C. Seed lot 1 in each of plants was from ears in which anthesis occurred between 23 April and 5 May. Seed lot 2 was from ears in which anthesis occurred after 5 May.

2. 5. 2 3. Seed quality assessment

The quality of these seeds was assessed using:

(1) Germination tests with 5 ml, 8 ml and 10 ml water per 100 grains.

(2)Measurements of seedling growth characteristics.

(3) Embryo dry weight determinations.

2. 5. 3. Experiment III (August-December, 1994)

2. 5. 3 1. Temperature regimes

(1 a) cv. Stirling grown in a growth room at 18°C from anthesis to harvest-ripeness.

(1 b) cv. Stirling grown in a growth room at 30°C. The temperature was raised from 18°C to 30°C 8 days after 'day 1' and maintained at 30°C until harvest-ripeness.

(2 a) cv. Schooner grown in a growth room at 18°C from anthesis to harvest-ripeness.

(2 b) cv. Schooner grown in a growth room at 30°C. The temperature was raised from 18°C to 30°C 8 days after 'day 1' and maintained at 30°C until harvest-ripeness.

(3 a) cv. Schooner growth in a growth cabinet at 18/13°C from anthesis until harvest-ripeness (3 b) cv. Schooner grown in a growth cabinet at 18/13°C for a day, 21/16°C for 2 days; 24/19°C for 1 day; 30/25°C for 5 days; 35/30°C for 3 days, 38/33°C for 1 day and then 30/25°C to harvest-ripeness.

Cultivars Stirling and Schooner grown in growth rooms were grown at a constant temperature. Seeds of cvs Schooner and Stirling were sown on 30 August 1994, in the glasshouse at Bush. Three days prior to anthesis, plants were transferred from glasshouse conditions to the growth rooms set at 18°C. Plants of cv. Stirling were divided into two batches, 18 pots to be grown in at 18°C and 22 pots to be grown at 30°C. In cv. Schooner, plants were divided into four batches, 15 pots to be grown in a growth cabinet in at 18°C, 15 pots to be grown in a growth cabinet in an elevated

temperature regime of 18-38°C, 18 pots to be grown in a growth room at 18°C and 22 pots to be grown in a growth room at 30°C. All plants of cv. Schooner were kept in the growth rooms at 18°C, until 'day 1' (31/11/94) when a batch of plants was transferred to each of two identical growth cabinets set at 18/13°C day and night. In one of the growth cabinets the temperature was increased gradually until a maximum temperature of 38/33°C day/night was attained. Anthesis was first observed in cv. Stirling on 15 October, and 20 October was taken as 'day 1' the date by which anthesis had taken place in 50% of the ears. In cv. Schooner, anthesis was first observed on 22 October and 31 October was taken as 'day 1'. Plants of cvs Stirling and Schooner were transferred directly from 18°C to 30°C i e they had no conditioning temperature treatment.

The relative humidity was between 90% and 100% in plants grown in a growth cabinet at 18-38°C. Frequent watering of the relatively crowded plants in the confined growth cabinets kept the relative humidity high and difficult to control.

2. 5. 3. 2. Harvesting

cv. Stirling grown at 30°C was harvested from 24 November onwards whereas cv. Schooner grown in the growth cabinet at 18-38°C was harvested from 6 December onwards and Schooner grown in the growth room at 30°C was harvested from 5 December onwards. Plants grown at 18°C were harvested from 12 December onwards (Stirling) and from 22 December onwards (Schooner). Grains were processed as in **Section 2. 3.**

2. 5. 3. 3. Seed quality assessment

The quality of seeds was assessed using (1) Germination tests with 5 ml and 10 ml water per 100 grains. (2) Measurements of seedling growth characteristics. (3) Seedling emergence test.

(4) Embryo dry weight determinations. (5) Embryo growth on artificial media (cv. Schooner Gc).

2. 5. 4. Experiment IV (February-June 1995)

2. 5. 4. 1. Temperature regimes

Plants were grown in the growth rooms (Gr) and growth cabinets (Gc) at 18/13 °C control (C) and at high temperatures (HT), regimes detailed in Table 2.1

Table 2. 1. Number of days in each temperature regime that plants of cvs Blenheim, Stirling and Schooner were subjected to during grain development after 'day 1' the date by which anthesis had taken place in 50% of the ears

Temperature regime °C day/night	18/13	21/16	24/19	27/22	30/25	33/28	35/30	38/33
Commencement of (HT) treatment		**13/4/95**						
cv. Blenheim (C)	53	-	-	-	-	-	-	-
cv. Blenheim (HT)	5	1	3	1	5	1	3	4
cv. Stirling (C)	53	-	-	-	-	-	-	-
cv. Stirling (HT)	5	1	3	1	5	1	3	4
cv. Schooner Gr (C)	53	-	-	-	-	-	-	-
cv. Schooner Gr (HT)	-	-	-	-	5	1	3	4
cv. Schooner Gc (C)	53	-	-	-	-	-	-	-
cv. Schooner Gc (HT)	6	1	3	1	4	1	1	3

(C), control (HT), high temperature

In this experiment three cultivars of barley, Blenheim, Schooner and Stirling, were grown. Blenheim is one week later in maturity than Stirling and Schooner. Sowing was therefore staggered to ensure that anthesis occurred at about the same time. 52 pots were sown with seeds of cv. Blenheim on 13 February 1995 while 52 pots were sown with seeds of cvs Stirling and 72 pots were sown with seeds of cv Schooner. Both cvs Stirling and Schooner were sown on 27 February. Plants of all cultivars were transferred from the glasshouse at Bush to controlled environment conditions 4 days prior to anthesis. In the growth rooms and growth cabinets, temperatures were set at 18°C/13°C (day/night), 16 h day. 10 pots of cv. Schooner were allocated to each growth cabinet and 24 pots were allocated to the plants grown at 18°C and 28 pots to the plants grown at 30-38°C in the two identical growth rooms. In cvs Blenheim and Stirling, 24 pots of each were allocated for plants grown in a growth room at 18°C and 28 pots were allocated for plants grown in an elevated temperature regime of 18-38°C.

In cvs Blenheim and Stirling, anthesis was first observed on 4 April and 'day 1' the date by which anthesis had taken place in 50% of the ears was 8 April. Five days after 'day 1' plants of cultivars Blenheim and Stirling destined for high temperature treatment were transferred to 21°C/16°C (13/4/95) and then the temperature was increased by 3°C as shown in Table 2. 1 until a maximum temperature of 38/33°C was attained. Similarly seven days after 'day 1' (11 April) plants of cv. Schooner grown in growth cabinets were transferred to 21/16°C and the temperature was increased as shown in Table 2. 1. Plants of cv. Schooner in the growth room at 18°C had wide ranges of anthesis dates and so the 26 pots containing these plants were transferred to the elevated temperature regime 11 days later than plants of cvs Blenheim and Stirling, i e when the temperature was 30/25°C. Relative humidity in the growth rooms was approximately 75 % for the control and approximately 55% for high temperature regimes.

2. 5. 4. 2. Grain growth analysis

Ears which anthesed on 11 and 12 April were used to monitor grain development. Treatment commenced on 13 April, i.e anthesis had taken place at 18/13°C, 4 days before the plants were subjected to high temperatures ('day 1' was 8 April). Pots were randomly distributed in the growth rooms and the sampling to monitor grain development began 6 days after anthesis. Due to lack of sufficient number of ears reaching anthesis at the same time, only four ears of the same anthesis date (beginning with ears that anthesed on 11 and followed by ears which anthesed on 12 April) were harvested at 2 or 3 day intervals from the batch of plants grown in each growth room. Frequent sampling was undertaken because grain development at high temperature regimes is fast. Four grains were removed from the central part of each ear, placed in a vial and weighed (fresh weight), and then dried to constant weight at 70°C. Grain dry weight was calculated as the difference between the fresh weight and dry weight, expressed as a percentage of the fresh weight.

2. 5. 4 3. Harvesting

The first ears of cvs Blenheim, Stirling, Schooner (Gc) and Schooner (Gr) grown at 18-38°C were harvested on 13, 17 & 26 May and 8 June (Schooner Gr) and those of cvs Blenheim, Stirling and Schooner grown at 18°C were harvested from 5 June to 23 June, 1995. Grains were processed as in **Section 2. 3.**

2. 5. 4. 4. Seed quality assessment.

The quality of these seed lots was assessed using (1) Measurements of seedling growth characteristics (2) Seedling emergence test (3) Embryo dry weight (4) Embryo growth on artificial media. (cvs Blenheim and Schooner (Gc)

2. 5. 5. Experiment V Comparisons of genotype (1994)

2. 5. 5. 1. Growth conditions

Eleven barley cultivars were sown on 15 February 1994 in a peat-based Levington compost in 20 cm diameter pots. Plants were grown under glasshouse conditions in which the temperature was maintained at approximately 18/13°C (day/night) and a natural day light was supplemented when necessary with mercury vapour lamps to give a photoperiod of 18h. Plants were grown in the SAC glasshouse at King's Buildings, Edinburgh from seedling stage to harvest-ripeness.

2. 5 .5. 2. Harvesting

Ears were harvested when grain moisture had reached approximately 16 % grain moisture and the husks had turned brownish white. Grains were processed as in **Section 2. 3.**

2. 5. 5. 3. Seed quality assessment.

The quality of these seed lots was assessed using: (1) Germination tests with 5 ml water per 100 grains, (2) Measurements of seedling growth characteristics and (3) Embryo dry weight determinations.

2.6. Statistical Approach

At least 3 replicates were used in all tests (except in starch granule extraction).The analyses of variance were carried out using Minitab 5 Program Graphs incorporating statistical analysis were produced using the Biosoft Fig P for Windows Program.

3. RESULTS.

3.1.1. Harvest Data

Ear number, number of grains per ear and total grain number

At harvest-ripeness ears were hand-threshed and the grains from each temperature regime were divided into two seed lots according to whether anthesis had taken place before or after 'day 1'. Grains from ears which anthesed before 'day 1' were designated as seed lot 1 whereas ears which anthesed after 'day 1' were designated as seed lot 2. These designations were used in the four experiments carried out under controlled environment conditions. It should be noted that the term 'grain' has been used interchangeably with the term 'seed' wherever applicable.

Experiments I & II

Table 3. 1a. shows the effects of elevated temperatures during grain development on yield components of cv. Blenheim grown in Experiments I & II, in growth rooms at 18°C (A), 18-30°C (B) 30-18°C (C) and 30°C (D) (Sections 2. 5. 1 and 2. 5 .2.). In temperature regimes (A), (B), (C) and (D), the total number of grains harvested in seed lot 1 was greater in both Experiments I & II than the total number of grains harvested in seed lot 2. The effect of elevated temperature on total grain number was more severe in ears grown in regimes (C) and (D) which experienced 30°C early in grain development than in ears which experienced 30°C later in grain development (B). More ears were harvested in Experiment I than in Experiment II. Ears which experienced 30°C [(C) and (D)] early in development exhibited a higher degree of sterility than ears which experienced 30°C later in grain development

Experiments III & IV.

The amount of seed harvested in the two experiments is shown in Table 3.1b. More grains were harvested in ears grown at elevated temperatures (30°C and 18-38°) than from ears grown at 18°C because more pots were allocated to plant batches destined for elevated temperature regimes than to plant batches to be grown at 18°C. In

addition, a post-anthesis waiting period of 5-8 days was applied to minimise adverse effects of elevated temperatures on grain set. In both experiments, the number of grains in seed lot 2 of plants which experienced 30°C and 18-38°C was less than that in seed lot 2 of plants grown at 18°C. However, in Experiment IV the total number of grains of cv. Schooner harvested from ears grown in growth rooms and growth cabinets was greater in ears grown at 18°C than in ears grown at 18-38°C and at 30-38°C respectively. Although the number of pots of cv. Schooner grown in a growth room at elevated temperatures was greater than the number of pots grown at at 18°C, fewer grains were harvested from plants grown at 30-38°C than from plants grown at 18°C. Plants of cv. Schooner (Gr) were transferred from 18°C to 30/25°C without conditioning temperatures. Due to the limited number of seeds of cv. Schooner grown in Experiment IV in the growth rooms and growth cabinets, seeds were combined to form a single seed lot.. Grains of cv. Schooner (Experiment IV) grown in a growth room at 18°C and transferred to another growth set at 30/25°C had black discoloration of the husk at the embryo end (**Plate 3. 1. 1**).

3. 1. 2 Grain Weights

Experiments I & II

The grain weight values in Experiments I and II were obtained by dividing the total weight in each seed lot by the total number of grains at approximately 10% storage moisture content (M. c.). In Experiment I, the mean weight per grain of seed lots 1 & 2 grown in temperature regime (A) was greater than that of grains grown in temperature regimes (B) seed lot 2, (C) and (D) temperature regimes and that of grains grown in the same temperature regime (A) in Experiment II. Mean grain weights of grains of seed lots (C) and (D) in both Experiments, were greater than those of grains of seed lot 1 grown in the same temperature regimes (Table 3.1a). However, in Experiment I, grain weight in seed lot 2 was less than that in seed lot 1 (A).

Experiments III & IV

When grains of cv. Stirling in Experiment III were grown at 30°C the weight per grain was less than when grains were grown at 18°C (Table 3. 1c). Seed lot 1 of cv. Stirling grown at 18°C and 30°C had heavier grains than seed lots 2 grown in corresponding temperature regimes. Grains from seed lots 1 & 2 of cv. Schooner grown in a growth room at 18°C were heavier than grains grown at 30°C but grains from seed lots 1 & 2 of cv. Schooner grown at 30°C did not differ from each other (Table 3. 1c). Grains of seed lot 1 of cv. Schooner grown in the growth cabinet at 18°C (Experiment III) did not differ in weight from grains of seed lot 1 of cv. Schooner grown in the growth room at 18°C (Table 3.1c). When plants of cv. Schooner were grown in growth cabinet at 18-38°C the grain weight was less than when grains were grown in a growth cabinet at 18°C. The effect of temperature on grain weight was more severe in seed lot 2 than in seed lot 1 of cv. Schooner grown in growth cabinets (Gc).

In Experiment IV, grains of cv. Blenheim grown at 18°C were heavier than grains grown at 18-38°C (Table 3. 1c.). Grain weight of seed lot 2 of cv. Blenheim grown at 18°C was less than that of seed lot 1 grown at the same temperature. The weight of grains of cv. Stirling from seed lots 1 grown at 18°C and 18-38°C did not differ substantially from those of seed lots 2 in each corresponding temperature regime (Table 3. 1c). Grains of cvs Stirling and Schooner grown in the growth rooms at 18°C were heavier than grains grown at 18-38°C (Experiment IV). A similar pattern was observed in grains of cv. Schooner grown in growth cabinets (Table 3. 1c).

Table 3. 1a. The effect of elevated temperatures during grain development on grain characteristics of cv. Blenheim grown under growth room conditions.

Temperature regime Expts I & II	Seed lot	Number of ears harvested	Number of grains harvested	Number of grains per ear	Reduction in grain number per ear as % of (A) seed lot 1	Weight per grain (mg)	Reduction in grain wt as (%) of (A) seed lot 1
Experiment I							
18°C (A)	1	227	3348	14.8		46.5	
18°C (A)	2	138	1924	13.9	6.1	44.4	4.5
18-30°C (B)	1	222	3108	14.0	5.4	45.6	1.9
18-30°C (B)	2	156	2137	13.7	7.4	32.0	31.2
30-18°C (C)	1	124	1004	8.1	45.3	38.0	18.3
30-18°C (C)	2	77	416	5.4	63.5	40.0	14.0
30°C (D)	1	151	1621	10.7	27.7	32.7	29.7
30°C (D)	2	126	430	3.4	77.0	33.0	29.0
Experiment II							
18°C (A)	1	185	2484	13.4		40.5	
18°C (A)	2	121	895	7.4	44.8	40.3	0.5
18-30°C (B)	1	201	2558	12.7	5.2	37.8	6.7
18-30°C (B)	2	141	1042	7.4	44.8	38.0	6.2
30-18°C (C)	1	187	2295	12.3	8.2	30.0	25.9
30-18°C (C)	2	78	354	4.5	66.4	35.0	13.6
30°C (D)	1	180	2018	11.2	16.4	27.8	31.4
30°C (D)	2	78	333	4.3	67.9	33.5	17.3

Table 3.1b. Total number of grains harvested from ears grown in growth rooms (Gr) and growth cabinets (Gc) under different temperature regimes during grain development.

Experiment	Cultivar	Temperature regime	Number of grains harvested	
			seed lot 1	Seed lot 2
III	Stirling	18°C	2584	1707
III	Stirling	30°C	3548	988
III	Schooner (Gr)	18°C	2214	1908
III	Schooner (Gr)	30°C	3235	1835
III	Schooner (Gc)	18°C	785	616
III	Schooner (Gc)	18-38°C	1049	544
IV	Blenheim	18°C	1950	1599
IV	Blenheim	18-38°C	3128	2141
IV	Stirling	18°C	727	853
IV	Stirling	18-38°C	554	480
IV	Schooner (Gr)	18°C	2114	
IV	Schooner (Gr)	30-38°C	975	
IV	Schooner (Gc)	18°C	1658	
IV	Schooner (Gc)	18-38°C	1583	

Table 3.1c. Effect of elevated temperatures during grain development on the mean weight of grains grown in growth rooms (Gr) or growth cabinets(Gc)

Experiment	Cultivar	Temperature regime	Dry wt per grain (mg)	
			Seed lot 1	Seed lot 2
III	Stirling	18°C	34.6 ± 1.38	31.7 ± 0.62
III	Stirling	30°C	25.0 ± 0.48	23.0 ± 0.54
III	Schooner (Gr)	18°C	38.9 ± 0.28	37.8 ± 0.13
III	Schooner (Gr)	30°C	25.8 ± 0.75	25.1 ± 0.82
III	Schooner (Gc)	18°C	38.0 ± 0.	37.8 ± 0.49
III	Schooner (Gc)	18-38°C	26.1 ± 0.55	17.4 ± 0.94
IV	Blenheim	18°C	57.4 ± 0.74	49.4 ± 1.09
IV	Blenheim	18-38°C	36.8 ± 0.73	31.0 ± 0.96
IV	Stirling	18°C	45.3 ± 0.90	44. 3 ± 0.27
IV	Stirling	18-38°C	29.8 ± 0.65	28.5 ± 0.63
IV	Schooner (Gr)	18°C	50.70 ± 0.51	
IV	Schooner (Gr)	30-38°C	31.90 ± 0.56	
IV	Schooner (Gc)	18°C	48.00 ± 0.55	
IV	Schooner (Gc)	18-38°C	20.40 ± 0.44	

Values are means ± SEM, n = 4.

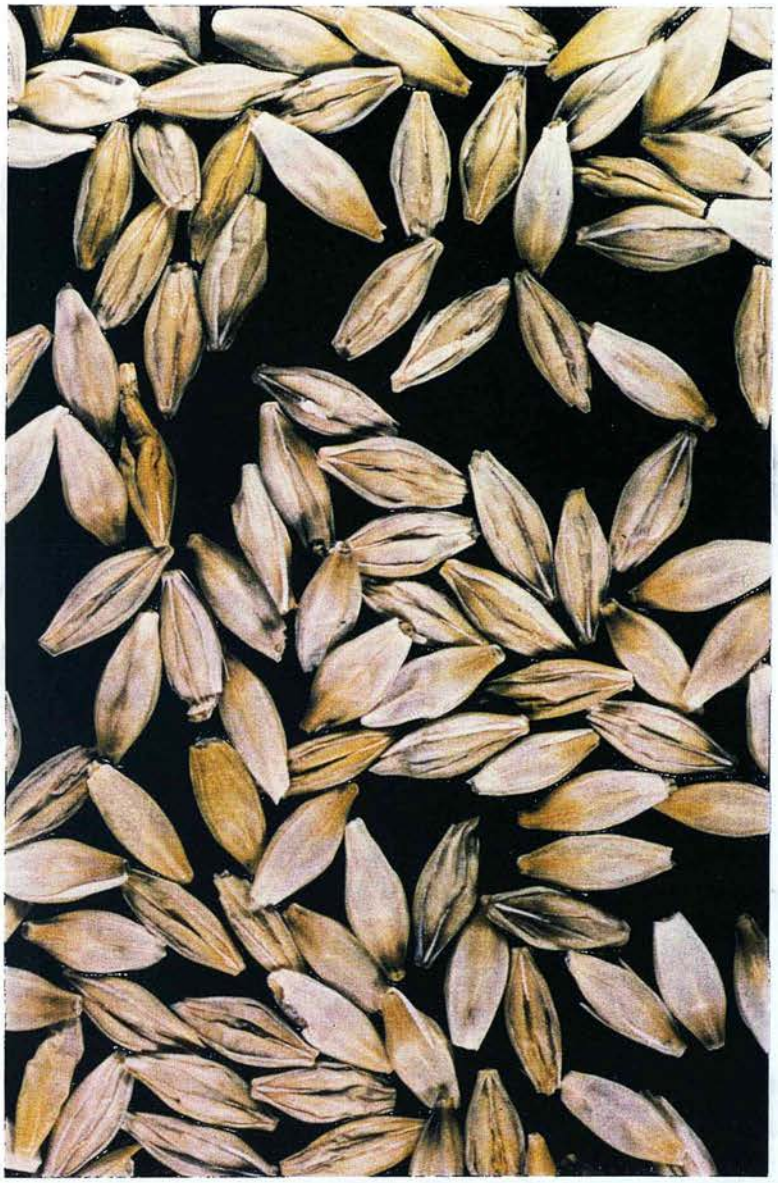


Plate 3. 1. 1. Grains of cv. Schooner from plants transferred from a growth room at 18°C to a growth room at 30/25°C. Note black /brown discoloration on dorsal and ventral sides at the embryo end of many of the grains.

3. 1.3. Grain size distribution

Experiment I

Seed lot 1 grown in temperature regime (A) had a higher proportion of grains in the > 2.8 mm and < 2.8 > 2.5 mm classes than seed lot 1 grown in temperature regime (D) (Figure 3.1a). Seed lot 2 from regime B had a smaller proportion of > 2.8 mm and < 2.8 >2.5 mm grains than had seed lot 1 which contained grains which developed at 18°C up for 35 days before they were transferred to 30°C. In contrast to this, grains grown in temperature regime 30-18°C (C) seed lot 2, had a higher proportion of grains in the > 2.8 mm class than grains in seed lot 1 of (C) (Figure 3. 1b). Seed lot 2 of grains grown at 30°C (D) had fewer grains in the < 2.2 mm class and more grains in the < 2.8 >2.5 mm class than seed lot 1, probably due to sterility in the ears from which seed lot 2 was harvested. The number of grains per ear was lower in C & D than in A & B (Table 3. 1a).

Experiment II

39 % of the grains harvested in seed lot 1 from plants grown in regime (A) were in the size categories >2.8 mm whereas 49% of the grains in seed lot 2 from plants grown in regime (A) were in the > 2.8 mm class (Figure 3.2a). In contrast, seed lot 1 harvested from plants grown in regime (B) had 42 % of its grains > 2.8 mm whereas seed lot 2 had only 18 % of its grains > 2.5 mm, the grains being more or less evenly distributed between the 4 size classes (Figure 3. 2 b). In grains from plants grown in the temperature regimes (C) and (D) (seed lots 1 & 2), the size distribution was shifted towards the <2.5 >2.2 mm and <2 2 mm classes with only a very small proportion of the grains in the > 2.8 mm class (Figures 3 2a, b). Grain size distribution in (A) & (B) was similar in Experiments I & II but grain size distribution in C & D Experiment II was very different from that in C & D in Experiment I.

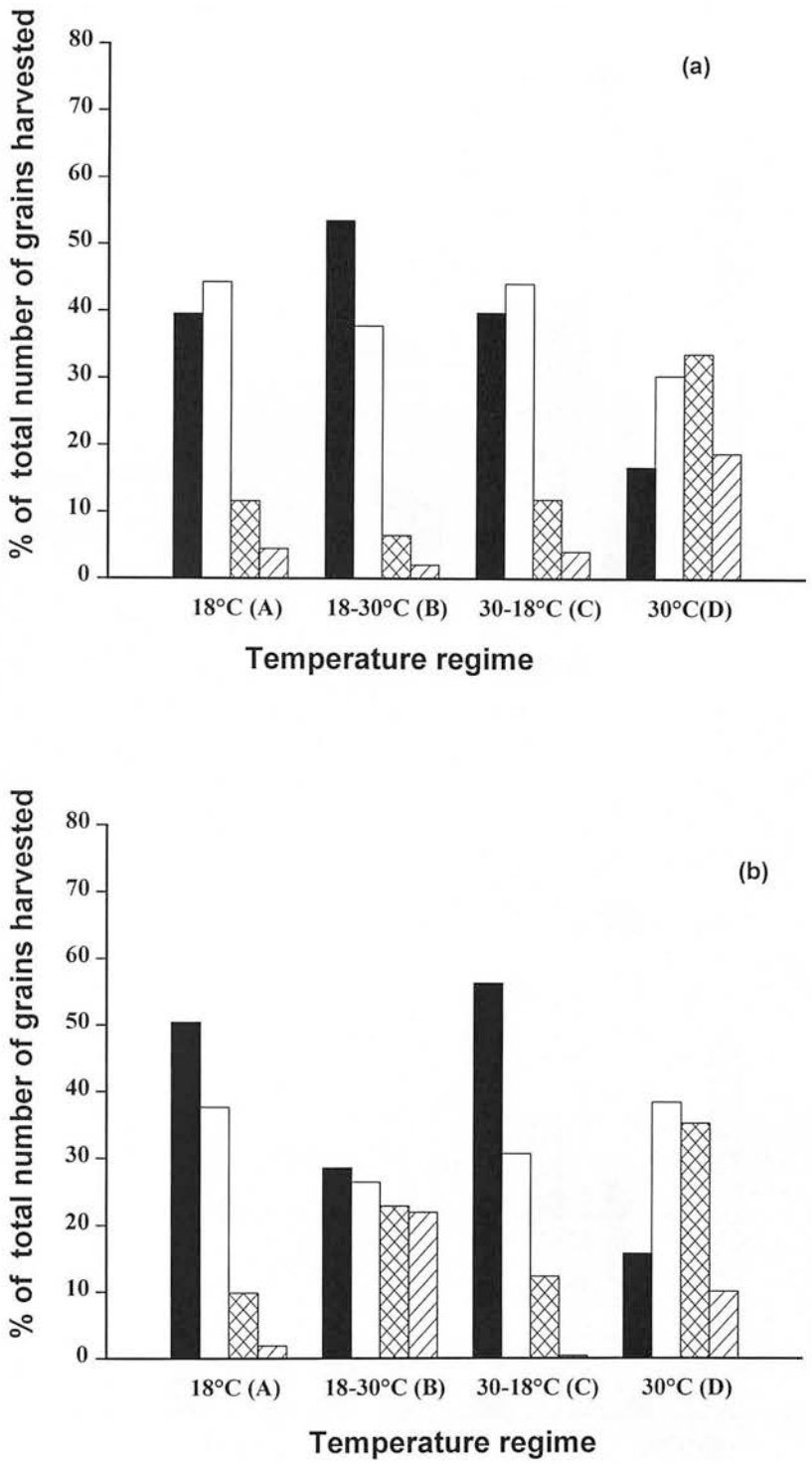


Fig. 3 .1. Experiment I. Size distribution in the total population of grains harvested from plants grown in temperature regimes (A), (B), (C) and (D) in the growth rooms.(a) seed lot 1 (b) seed lot 2: solid histograms grain size > 2.8 mm, open histograms < 2.8 > 2.5 mm, crosshatched histograms < 2.5 > 2.2 mm, and hatched histograms < 2.2 mm.

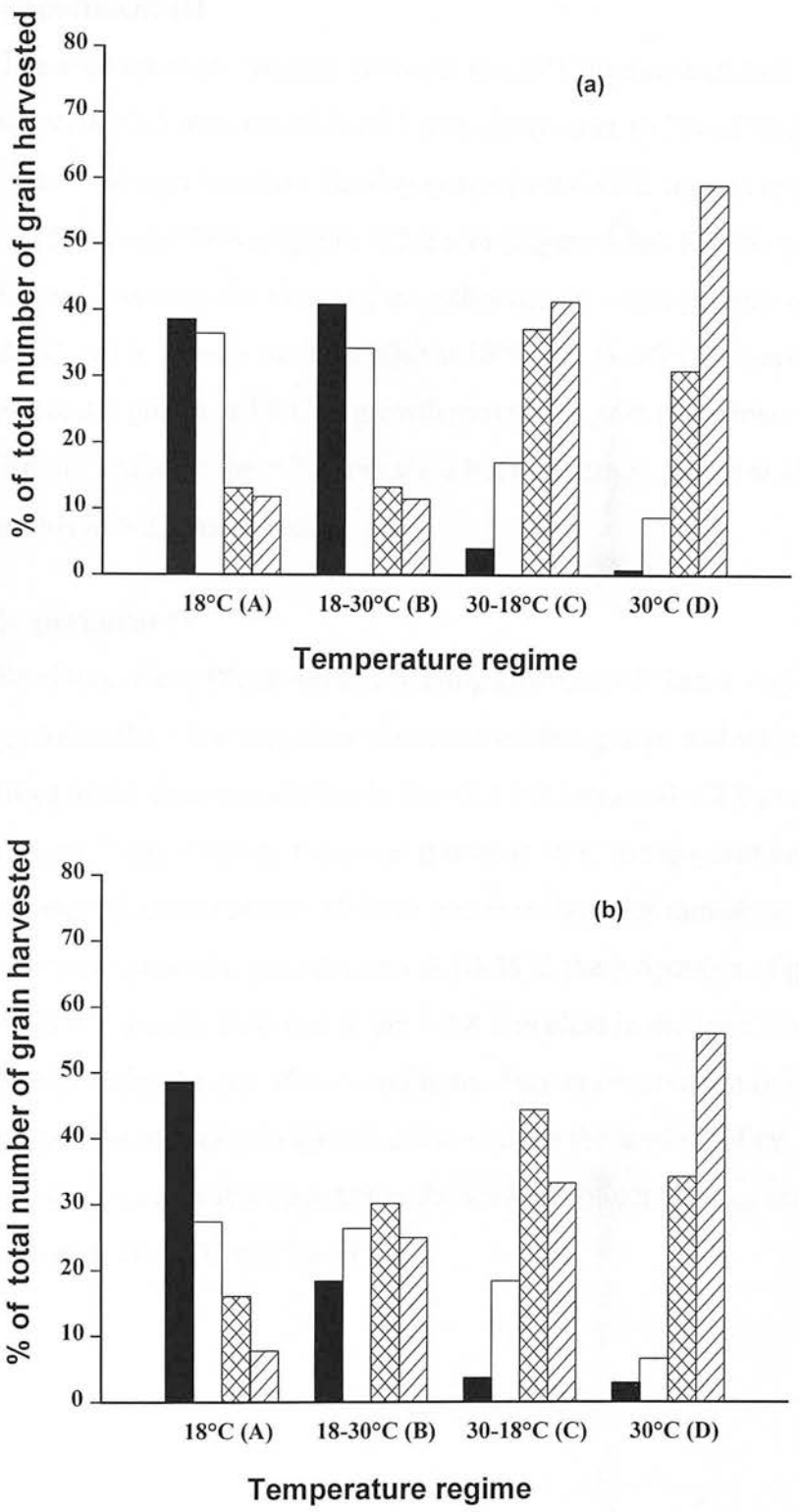


Figure. 3. 2. Experiment II. Size distribution in the total population of grains harvested in from plants of cv. Blenheim grown in temperature regime (A), (B), (C) and (D). (a) Seed lot 1, (b) Seed lot 2. Key to shading as in Figure 3. 1.

Experiment III

The seed lot of cv. Stirling grown in the 18°C regime had 28% and 48% of grains in the <2.8 >2.5 mm and <2.5 >2.2 mm classes and 16.7% of its grains in the < 2.2 mm class. The seed lot of cv. Stirling grown in the 30°C regime had only 3.4% of grains > 2.5 mm and 75% of grains < 2.2 mm (Figure 3.3a). Similar patterns were observed in seed lots from the plants of cv. Schooner grown in growth rooms (Gr) at 18°C and 30°C and in growth cabinets (Gc) at 18°C and 18-38°C (Figure 3. 3 b). Cultivar Schooner grown at 18°C in growth rooms and growth cabinets had approximately 20% of grains in the > 2.8 mm class but cv. Stirling grown at 18°C had very few grains in > 2.8 mm class.

Experiment IV

Seed lots of cvs Blenheim and Stirling grown at 18° had a very high proportion of grains in the > 2.8 mm class whereas seed lots grown under the 18-38°C regime had most of the grain population in the <2.5 > 2.2mm and < 2.2 mm classes (Figure 3.4a). Cultivar Schooner grown at 18°C in the growth rooms (Gr) and growth cabinets (Gc) had almost 80 % of grains in the > 2.8 mm class. In the seed lot of cv. Schooner grown in growth room at 30-38°C the proportion of grains in the > 2.8 mm class was greater than that in the > 2.8 mm class in the seed lots of other cultivars grown under the (18-38°C) conditions. Further observation indicated that the proportion of grains in the < 2.2 mm class in the seed lot of cv. Blenheim grown at 18-38°C was greater than that in the seed lots of cvs Stirling and Schooner (Gr, Gc) grown at 18-38°C and 30-38°C.

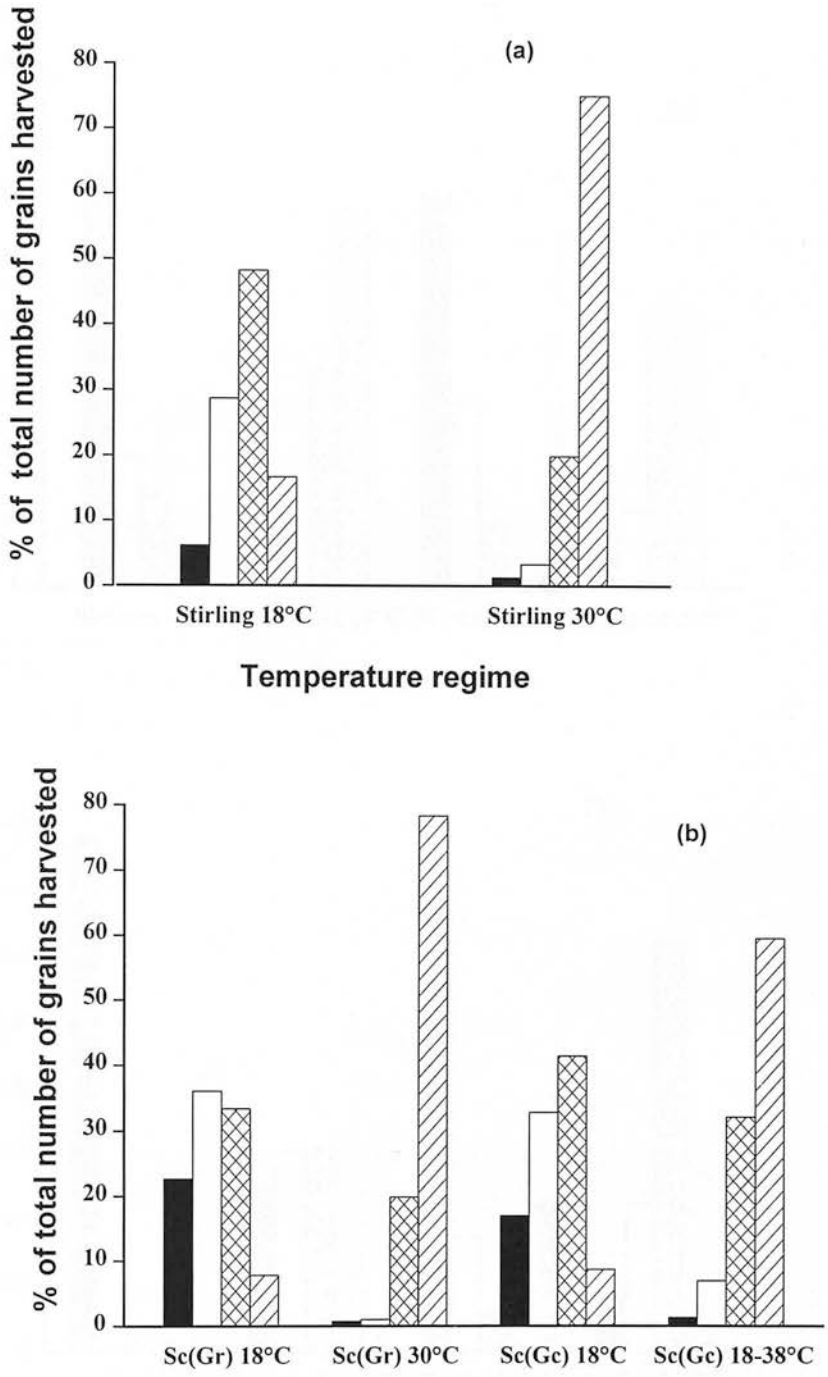
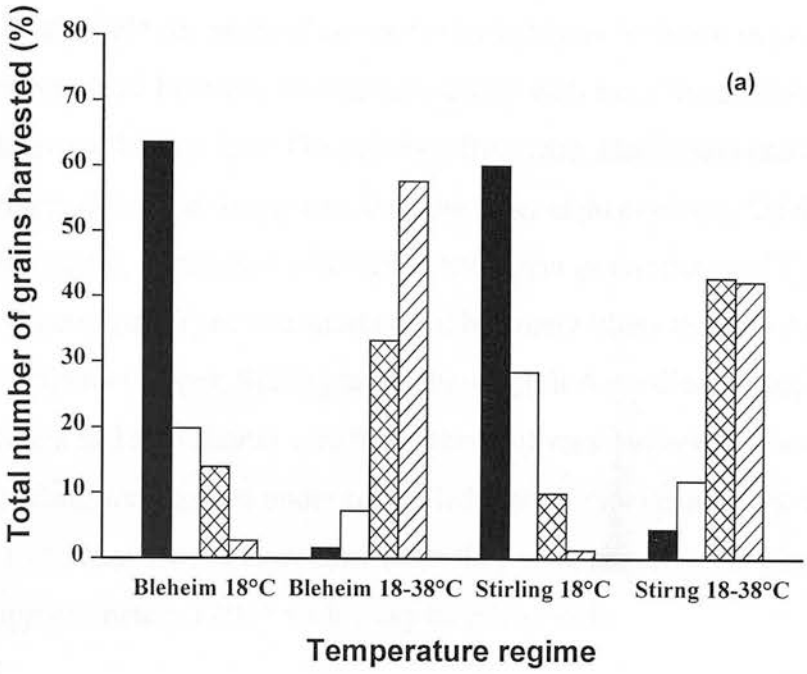


Figure. 3. 3. Experiment III. Size distribution in the total population of grains harvested from plants grown in growth rooms at constant temperatures of 18°C and 30°C and growth cabinets at 18°C or at a temperature which was increased in steps from 18°C to 38°C. (a) cv. Stirling grown in growth rooms at 18°C and 30°C. (b) cv. Schooner grown at 18°C and 30°C in growth rooms Sc (Gr) and at 18°C and 18-38°C in growth cabinets Sc (Gc). Key to shading as in Figure 3. 1.



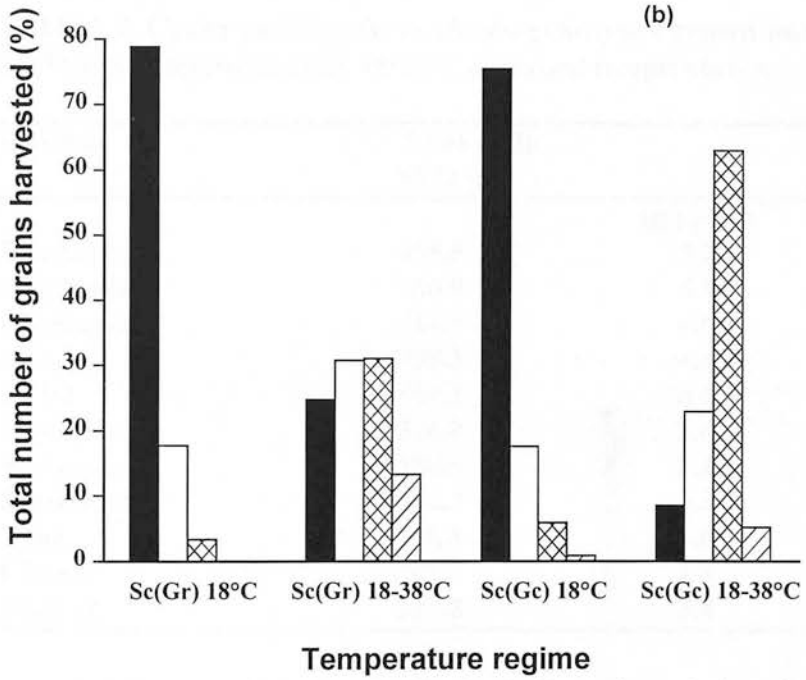


Figure 3.4. Experiment IV. Size distribution in the total population of grains harvested in from plants grown in growth rooms and growth cabinets at constant temperature of 18/13°C and temperature which was increased gradually from 18-38°C and then decreased to 30/25°C. (a) cvs Blenheim and Stirling (Stirng) grown at 18/13°C and 18-38°C. (b) cv. Schooner grown at 18/13°C and 30/25-38°C in growth room, Sc (Gr), and 18°C and 18-38°C in growth cabinets, Sc (Gc). Key to shading as in Figure 3.1.

3. 2. Genotype comparisons Experiment V

Grain yield per plant of eleven barley cultivars is shown in (Table 3.2). Seeds were sown on 15 February and harvest started with cvs Clipper, Schooner, Stirling and Esmeranda on 3 June.The cultivars Blenheim, Harrington and Centinella had more tillers as well as longer ears than the other eight cultivars. Cultivars Clipper, BRB2, Blenheim, Harrington and Puebla had larger grains than cvs Tyne and Esperanza. Although cv. Tyne had short ears it had more tillers than the Australian cultivars. Cultivars Clipper, Stirling and Schooner (all Australian) apart from having fewer tillers had also shorter ears than other cultivars. However, when cvs Schooner and Stirling were grown under controlled growth room conditions with a day length of 16 h, they tillered more than when they were grown under glasshouse conditions at approximately 18/13° with a day length of 18 h.

Table 3.2. Grain yield (g) from eleven genotypes grown under glasshouse conditions at approximately 18/13°C day/night temperatures.

Cultivar	Total grain yield (g)	
		(g) plant $^{-1}$
Blenheim	**465.8**	**5.2**
Centinella	**460.9**	**5.1**
Harrington	**414.6**	**4.6**
Puebla	**398.3**	**4.4**
BRB2	**339.2**	**3.8**
Esperanza	**326.2**	**3.6**
Schooner	**286.6**	**3.2**
Esmeranda	**286.3**	**3.2**
Tyne	**270.8**	**3.0**
Clipper	**247.3**	**2.8**
Stirling	**217.8**	**2.4**

Grain size distribution

Grain size distribution expressed as a percentage of the total number of grains harvested from the eleven cultivars of barley grown under glasshouse conditions is shown in Table 3.3. In five cultivars, BRB2, Harrington, Clipper, Blenheim and Schooner > 90 % of the total number of grains harvested was in the > 2.8 mm class. Grains of cv. Tyne were smallest having < 70% in the > 2.8 mm class and 11% in the < 2.5 > 2.2 mm class.

Table 3.3 Grain size distribution (%) in the total population of grains harvested from plants of eleven genotypes grown under glasshouse conditions at 18/13°C.

	> 2.8 mm	<2.8 > 2.5 mm	< 2.5 >2.2mm	< 2.2 mm
BRB2	99.1	0.8	0.1	0.0
Harrington	97.5	2	0.4	0.1
Clipper	96.2	3.4	0.3	0.1
Blenheim	92.7	4.9	1.9	0.5
Schooner	92.2	5.5	2.1	0.2
Puebla	89.4	4.6	3.7	2.3
Centinela	89.3	6.9	3.6	0.2
Stirling	86.9	8.5	4.2	0.4
Esmeranda	86.3	6.9	5.5	1.3
Esperanza	83.2	11	5.3	0.5
Tyne	68.2	18.6	11.2	2.0

3. 3. Germination test

Experiment I

Germination tests were carried out on grains of cv. Blenheim grown in four temperature regimes during grain development. The grains having been stored at 10°C for 112 days after harvest, were used in germination tests using 5 ml water per 100 grains. Figure 3.5 shows the percentage germination of grains grown in temperature regimes (A), (B), (C) and (D) in Experiment I. The results of the germination test showed that the seed lot 1 of grains grown in regime (A) had a slightly higher percentage germination than that of seed lot 2 grains grown in the same temperature regime. By contrast, seed lot 2 of the grains grown in regime (B) had higher percentage germination ($p < 0.05$) than that of grains from seed lot 1, whereas seed lot 1 of grains grown in regime (C) had higher percentage germination than that of seed lot 2 of grains grown in the same temperature regime. It appears germinability depended on temperature and stage of grain development. Grains of seed lots 1 & 2 grown in regime (D) which experienced 30°C throughout grain development had almost 100% germination.

Grains grown in all the temperature regimes had viability of > 98 % when hydrogen peroxide was used to treat grains which failed to germinate at the end of the germination test. In grains grown in regime (A), 42.7 % & 50.3% of grains were dormant, (B) 48.3% & 19.7%, (C) 18.7% & 38% were dormant in seed lots 1 and 2 respectively.

Experiment II

Only seed lot 1 was used in this test because of the limited amount of seed obtained from ears grown at 30-18 °C (C) and 30°C (D) temperature regimes. The tests were carried out 138 days after harvest, the grains having been stored at 10°C and a moisture content of approximately 10%. Germination tests were carried out using 5 ml, 8 ml and 10 ml water per 100 grains to assess germinability of grains of cv. Blenheim exposed to different temperature regimes during grain development. Figure 3.6 shows the percentage germination of grains of cv. Blenheim germinated in 3 water levels. The results of tests on grains grown in temperature (A) showed that in

10 ml water, the percentage germination was lower ($p < 0.05$) than that in 8 ml and in 5 ml water. In grains grown in regime (B) showed no significant difference in the percentage germination between the 8ml and 10 ml water levels, whilst a significant difference ($p < 0.001$) was detected between 5 ml and 8 ml water levels, and between the 5ml and 10 ml water levels (Figure 3.6). By contrast, the percentage germination of grains in regime (C) was similar at all the 3 water levels. The percentage germination in the test using 5 ml water was the same as that in the test using 8 ml water in grains from regime (D). However, the percentage germination was lower ($p < 0.05$) in grains germinated in the test using 10 ml than in the test using 5 ml and 8 ml water.

Experiment III

Germination tests were carried out on grains of cvs Stirling and Schooner grown in growth rooms at 18°C and 30°C using 5 ml and 10 ml water per 100 grains. The tests were carried out 182 days after harvest, the grains having been stored at 10°C and a moisture content of approximately 10%. The percentage germination of grains of seed lot 1 of cv. Stirling grown at 18°C was greater in the 5 ml test ($p < 0.05$) than in the 10 ml test, but no significant difference was detected between the percentage germination in the 5 ml test and that in the 10 ml test carried out on seed lot 1 of grains grown at 30°C (Figure 3.7a). The percentage germination using 5 ml water per 100 grains was lower in seed lot 1 of cv. Stirling grown at 30°C than in grains of seed lot 1 of cv. Stirling grown at 18°C. The percentage germination of seed lot 2 of cv. Stirling grown at 18°C in the 5ml water test was the same as that of seed lot 2 of cv. Stirling grown at 30°C but the percentage germination at 10ml water was greater in seed lot 1 of grains grown at 30°C than in seed lot 2 of grains grown at 18°C (Figure 3.7b). In cv. Schooner (Gr), grains of seed lot 1 grown at 18°C had a higher ($p < 0.001$) percentage germination in the 5ml test than in the 10ml test, but in grains grown at 30°C the percentage germination in the 5 ml water test was the same as that in the 10 ml water test (Figure 3.7a). The pattern was repeated in seed lot 2 of cv. Schooner (Figure 3.7b). Grains grown in growth cabinets were not tested because of the limited amount of seed produced.

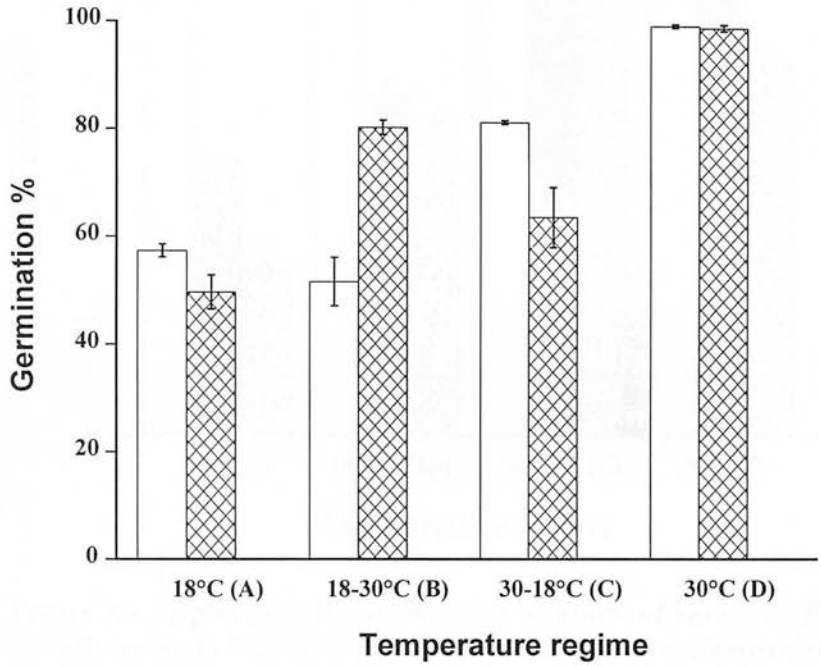


Figure 3. 5. Experiment I. Germination of grains of barley cv. Blenheim grown in growth rooms in four temperature regimes during grain development. Grains were tested using 5 ml water per 100 grains. Grains were divided into seed lots 1 & 2 according to whether anthesis in the ear had taken place before or after 'day 1'.Seed lot 1 (open histograms) and seed lot 2 (crosshatched histograms).Error bars represent ± SEM (n = 3).

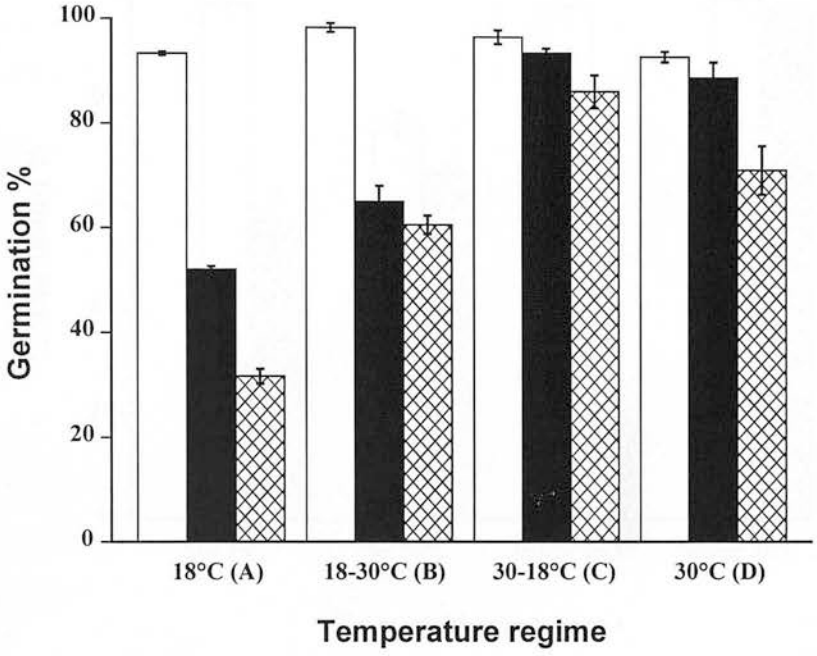


Figure 3.6. Experiment II. Germination of grains of barley cv. Blenheim grown in growth rooms in four different temperature regimes. Grains were tested using 5 ml, 8 ml & 10 ml water per 100 grains. Seeds used in germination tests were obtained from seed lot 1. 5ml water (open histograms), 8 ml water (solid histograms) 10 ml water (crosshatchedhistograms). Error bars ± SEM, (n = 3).

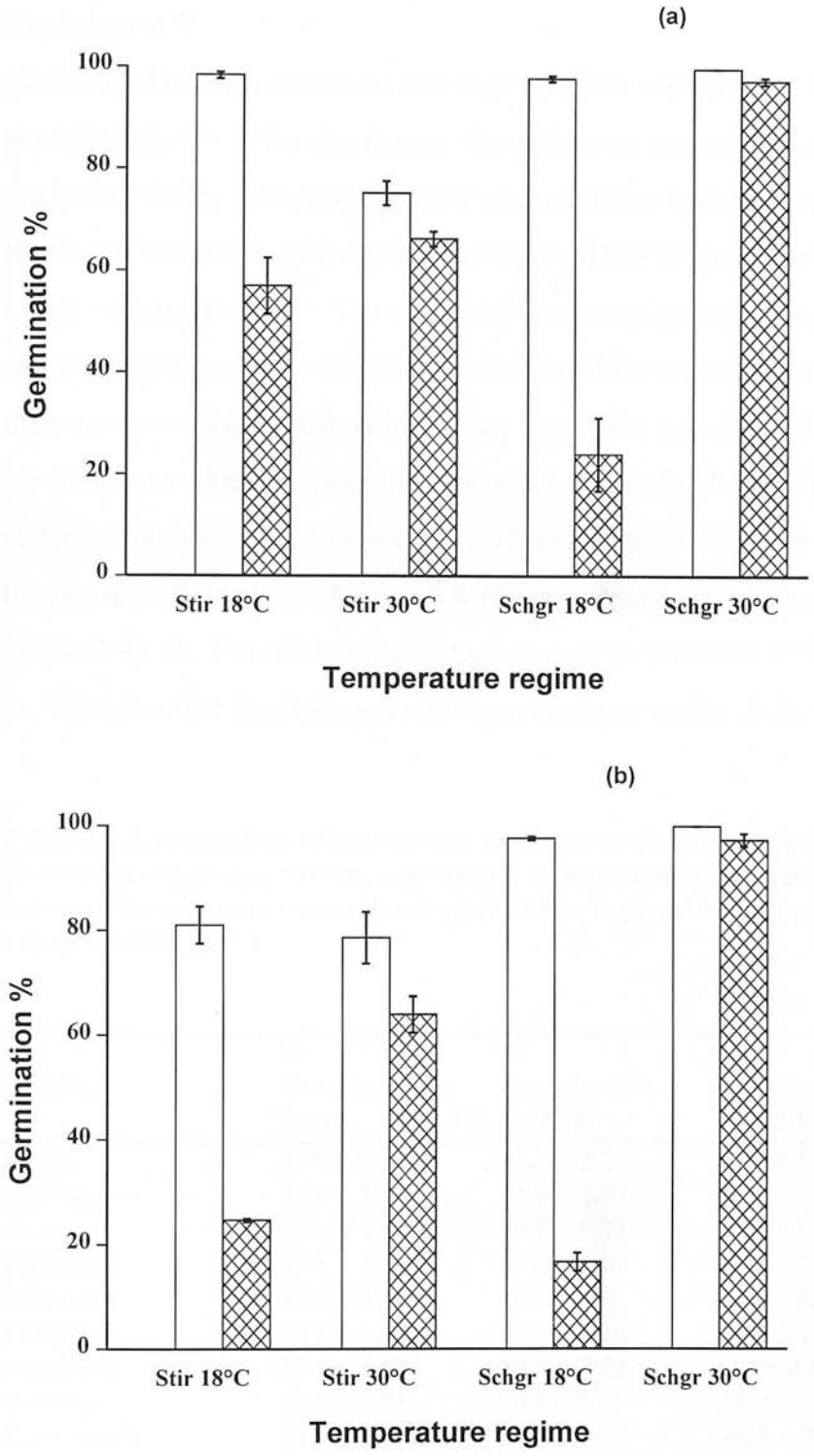


Figure 3. 7. Experiment III. Germination of grains cvs Stirling (Stir) and Schooner (Schgr) grown in growth rooms at 18°C and 30°C from anthesis to harvest-ripeness. Grains were tested using 5 ml and 10 ml water per 100 grains. (a) seed lot 1 (open histograms 5 ml, crosshatched histograms, 10 ml) (b) seed lot 2 (open histograms, 5 ml and crosshatched bars, 10 ml). Error bars represent ± SEM, (n = 3).

Experiment V.

Grains used in the germination test were obtained from six-row and two-row barley genotypes grown in the glasshouse. The tests were carried out 153 days after harvest, the grains having been stored at 10°C and a mosture content of approximately 10%. Results of viability tests indicated that the seed lots produced in this experiment had a high viability (> 97 %). The values for percentage germination after 72 h were analysed using one-way analysis of variance. There was no significant difference in the mean percentage germination values among the genotypes. The variability in germination was much wider in genotypes Esmeranda, Puebla, Blenheim and Stirling (after 48 h and 72 h) and Tyne (after 24 h and 48 h) as shown by the standard error of the mean (SEM) values . Table 3.4. Germination rate varied among the genotypes (Table 3 4). cv. Tyne had the lowest percentage germination after 24 h whilst cv. Schooner had the highest percentage germination after 24 h.

Table 3.4. Germination of four-rowed and two-rowed barley genotypes grown in glasshouse conditions. Grains were stored at 10°C and tested four months after harvest. Germination was assessed after 72 h using 5 ml water per 100 grains. Mean values ± SEM, (n = 4)

Cultivar	% Germination 0-24 h	% Germination 24-48 h	% Germination 48-72 h	% Germination after 72 h
BRB2	14.0± 1.47	62.3 ± 2.29	23.0± 2.16	99.3 ± 0.48
Harrington	14.0 ± 2.27	61.0 ± 2.71	23.3 ± 3.59	98.3 ± 0.75
Clipper	32.0 ± 1.68	39.0 ± 0.71	26 3± 1.32	97.3 ± 0.91
Blenheim	17.8 ± 1.70	49.8 ± 3.01	30.0 ± 3.76	97.6 ± 0.65
Schooner	52.8 ± 3.12	40.0± 1.08	6.8 ± 2.72	99.6 ± 0.29
Puebla	29.5 ± 1.89	52.8 ± 3.28	14.8 ± 2.25	97.1 ± 0.91
Centinela	42.8 ± 2.53	49.5 ± 2.22	7.0± 0.82	99.3 ± 0.25
Stirling	10.8 ± 0.75	58.0± 3.76	29.3 ± 4.4	98.1 ± 1.15
Esmeranda	32 3 ± 4.27	51.3 ± 5.27	14.8 ± 3.82	98.4 ± 0.25
Esperanza	23.3 ±1.93	57.3 ± 2.32	18.3 ± 1.65	98.9 ± 0.75
Tyne	7.3 ± 2.81	87.5 ± 3.07	5.0 ± 1.63	99.8 ± 0.29

3. 4. Seedling growth characteristics

3. 4. 1. Dry weights

Grains were weighed individually before being attached to a paper towel for seedling growth. The percentage moisture content of a sample of seeds was obtained and grain weights were expressed as dry weights (**Section 2.4. 3.**)

Experiment II

The data presented in Figure 3.8 show the effects of elevated temperatures on the mean dry weight of cv. Blenheim grains grown in growth rooms in different temperature regimes. The grain dry weight of seed lot 1 grown in regime (A) was not significantly different from that of seed lot 2 grown in the same temperature regime. However, grains grown in regime (A) differed in the grain dry weight ($p < 0.05$) from grains grown in regimes (C)and (D) while no significant difference was found between grains grown in regime (B) and (A). Plants grown in (B) experienced the 18°C temperature as in (A) for 25 days before they were transfered to 30°C to complete the remainder of grain development. Comparison between seed lots 2 and 1 of grains grown in regimes (C) and (D) showed that seed lot 2 had greater grain dry weight than of seed lot 1 grown in the corresponding temperature regimes (Figure 3.8.). Ears from which grains (C) and (D) of seed lot 2 were harvested had a high degree of sterility (Table 3. 1a) presumably due to elevated temperature experienced at anthesis , while seed lots 1 of (C) and (D) were harvested from ears which did not experience 30°C until later after anthesis.

Experiment III

Results presented in Figure 3.9a indicate that dry the weight of grains from ears of cv. Stirling grown in the growth room at 18°C was greater ($p <0.05$) than that of grains grown at 30°C. There was no significant difference in dry weights between seed lots 1 & 2 of grains grown at either temperature. The dry weight of grains from ears of cv. Schooner grown in growth rooms at 18°C was greater ($p < 0.05$) than that of grains grown at 30°C (Figure 3.8b). Seed lots 1 & 2 of cv. Schooner grown in a

growth room at 30°C had similar dry weights. Due to sterility grain number per ear was much less in ears which made up seed lot 2 than in ears which made up seed lot 1. Dry weight of grains of cv Schooner grown in growth cabinets (Gc) at 18°C was greater ($p < 0.05$) than that of grains grown at 18-38°C (Figure 3.8c). Grains of seed lot 1 of cv. Schooner (Gc) grown at 18-38°C had greater ($p > 0.05$) grain dry weight than seed lot 2 grown in the same temperature regime.

Experiment IV

The mean dry weight of grains of cv. Blenheim grown at 18°C was greater ($p < 0.05$) than that of grains grown at 18-38°C (Figure 3.10a). Grains of seed lot 1 from ears grown in both temperature regimes were heavier ($p < 0.05$) than those in seed lot 2 from ears grown in the same temperature regimes. Similarly, the dry weight of grains of Stirling from ears grown at 18°C was greater ($p < 0.05$) than that of grains from ears grown at 18-38°C. However, grains of seed lots 1 had the same dry weight as grains of seed lot 2 from ears grown at 18°C (Figure 3.10b). The dry weight of grains of cv. Schooner grown in a growth room at 18°C was greater ($p < 0.001$) than of grains grown at 30-38°C. Similarly, the dry weight of grains of cv. Schooner grown in a growth cabinet at 18°C was greater than that of grains grown in a growth cabinet at 18-38°C (Figure 3.10c). Ears of cv. Schooner grown in the growth room at 30-38°C had a high degree of sterility and very few grains were harvested. There was less sterility in the growth cabinet but since comparatively few plants were grown in the growth cabinets and the number of grains harvested was small, it was possible to have only one seed lot from each set of environments.

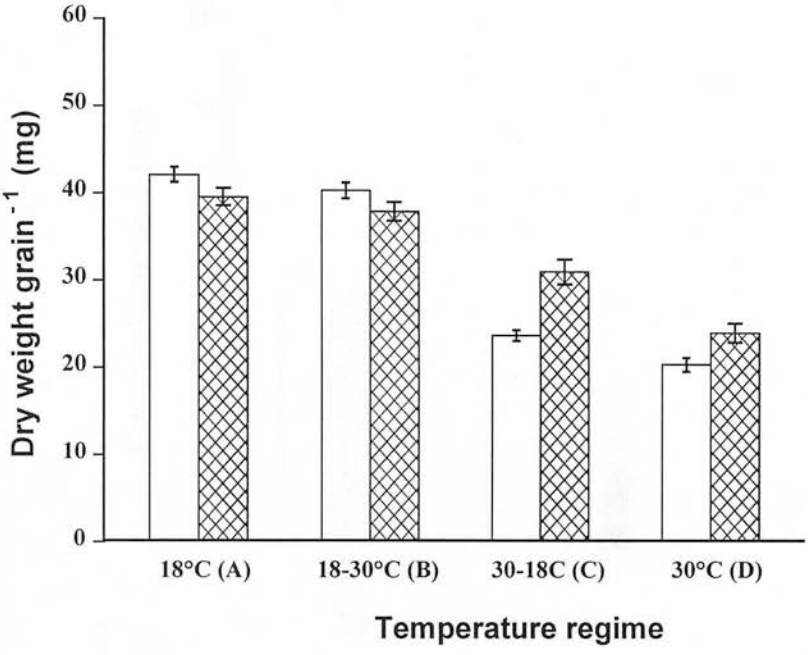
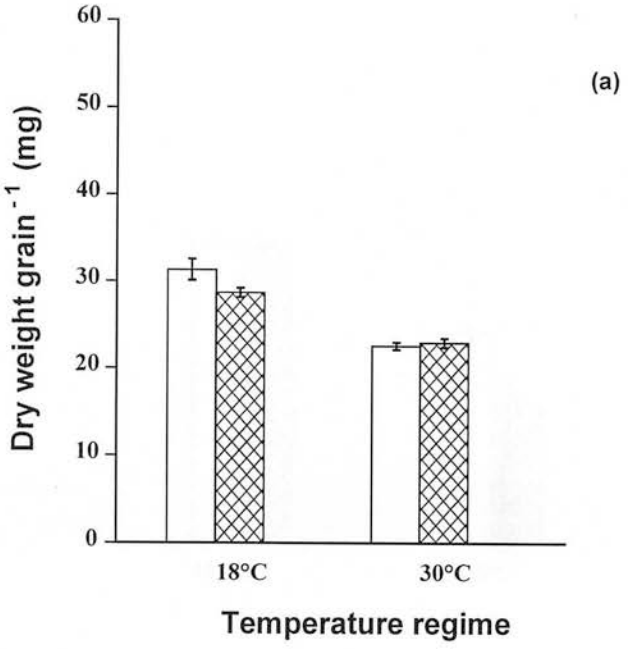


Fig. 3.8. Experiment II Grain dry weights of cvs Blenheim grown in different temperature regimes during grain development. Temperature treatment started on 'day 1'. Seed lot 1 was made up of grains from ears which anthesed prior to 'day 1' (open histograms) and seed lot 2 was made up of grains from ears which anthesed after 'day 1' (crosshatched histograms). Grains were weighed prior to the seedling growth test. Values were expressed as dry weight on the basis of 0% moisture content. Error bars represent ± SEM; (n = 4).



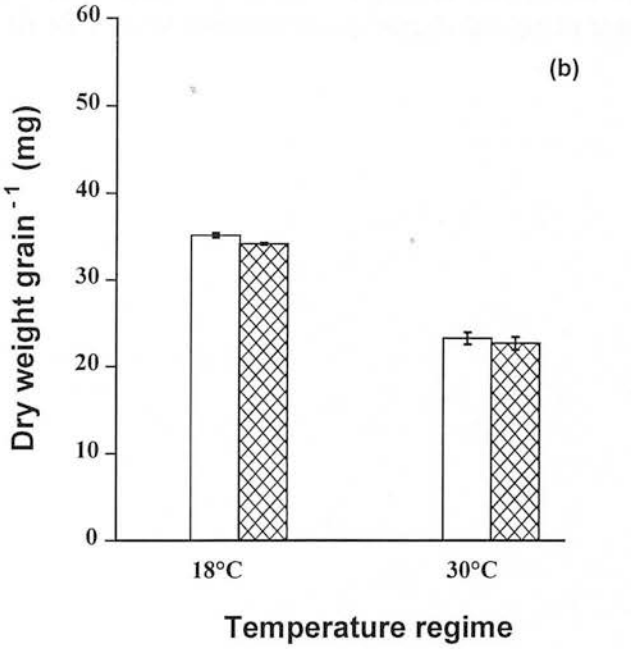


Figure 3.9. Experiment III. Grain dry weights of cvs Stirling and Schooner grown in growth rooms at 18°C and 30°C. Grains from ears grown at 30°C experienced a sudden temperature increase on 'day 1 of treatment. (a) cv. Stirling (b) cv. Schooner grown in growth rooms. Key to shading as in Figure 3.8. Error bars represent ±SEM ; (n = 4)

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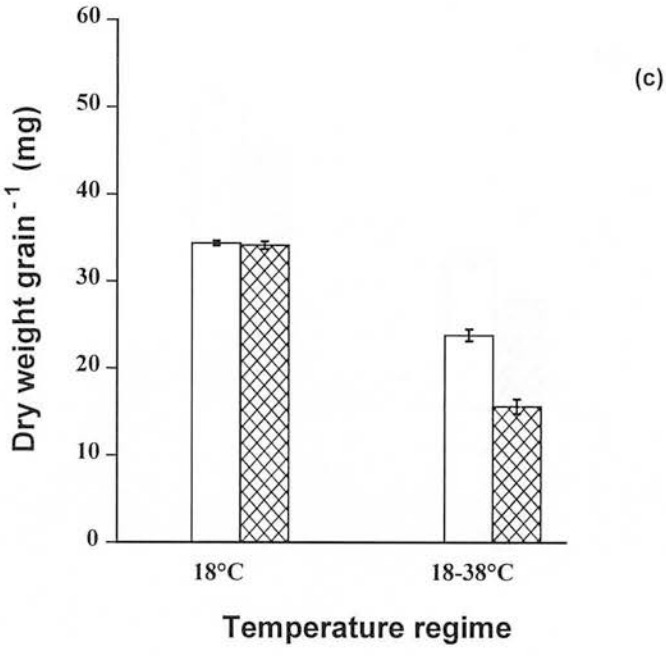


Figure 3. 9. (c) cv. Schooner grown in growth cabinets at 18°C and 18-38°C. Grains grown at 18-38°C were subjected to gradual changes in temperature.

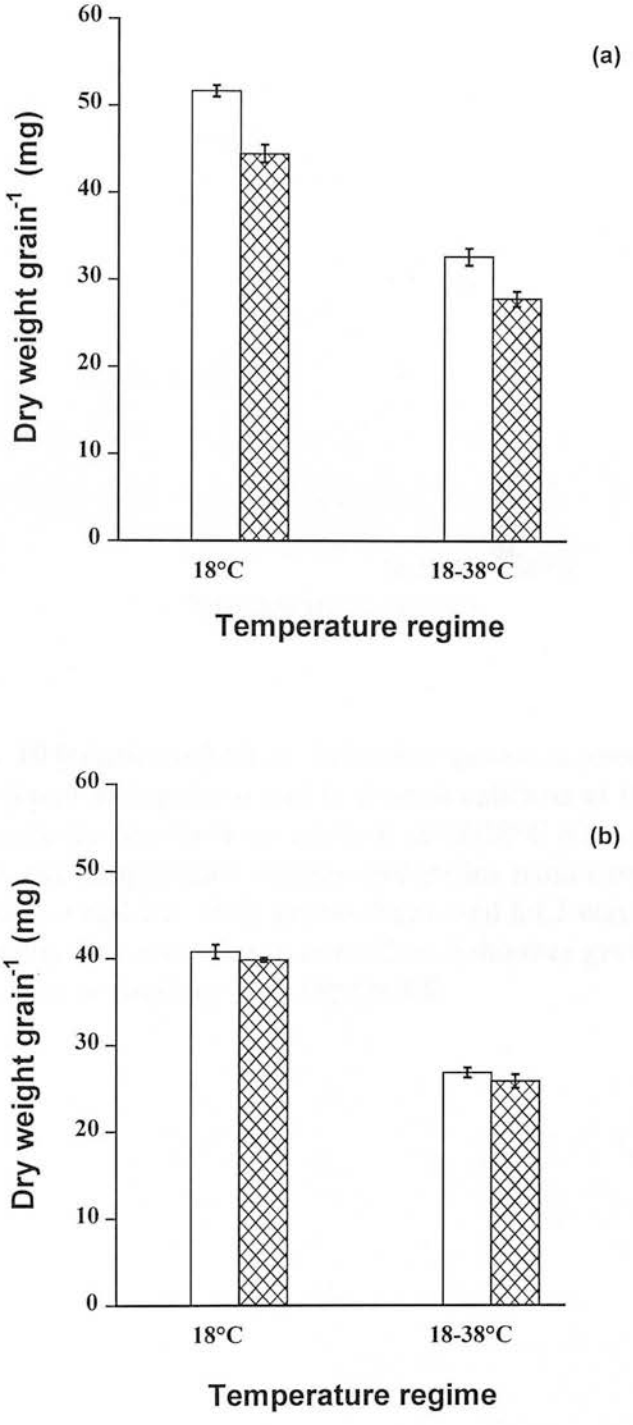


Figure 3.10 Experiment IV. Grain dry weights of grains from ears of cvs Blenheim and Stirling grown in growth rooms at 18°C and 18-38°C. Grains from ears grown at 18-38°C were subjected to gradual change in temperature regime. Error bars represent . ± SEM; (n = 4). (a) cv. Blenheim (b) cv. Stirling. Keys to shading as in Figures 3.8

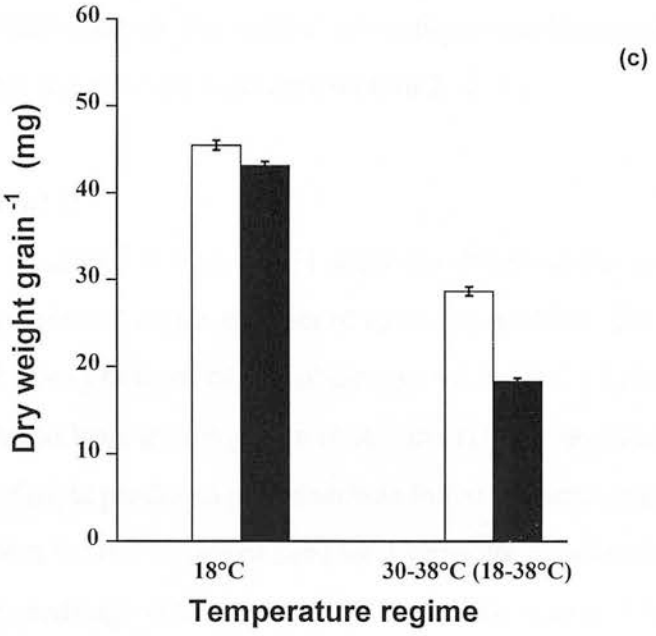


Figure 3. 10 (continued) (c) cv. Schooner grown in growth rooms at 18°C and 30-38°C (open histograms) and in growth cabinets at 18°C and 38°C (solid histograms). Grains from ears grown at 18-38°C were subjected to gradual change in the temperature regime and grains from ears grown at 30-38°C were subjected to a sudden. Only grains from seed lot 1 was used in this experiment. High sterility was recorded in ears of cv. Schooner grown in a growth room at 30-38°C. Key to shading as in Figure 3.8.

3. 4. 2. Number of roots

Counts were made on the number of roots per seedling produced from grains grown in different temperature regimes (**Section 2. 4. 3.**)

Experiment II

The data presented in Figure 3.11 show the effects of elevated temperature during grain development on the number of roots per seedling. The number of roots produced from grains of cv. Blenheim grown at 18°C (A) was greater ($p < 0.05$) than that produced from grains grown in (C) and (D) but no significant difference in the number of roots produced per grain was found between grains grown in (A) and those grown in (B). Grains of seed lot 2 grown in temperature regimes (C) and (D) produced seedlings with more roots per seedling than ($p < 0.05$) than did grains of seed lot 1 grown in the corresponding temperature regimes. Observations showed that roots produced by grains grown in seed lot 1 in (C) and (D) were thinner and weaker than those produced by seed lot 2 grains grown in the same temperature regimes, whilst seedlings with strong and vigorous roots were produced from grains grown in (A) and (B).

Experiment III

Plants of cvs Stirling and Schooner were grown in the growth rooms at constant temperatures of 18°C and 30°C and plants of Schooner (Gc) were grown initially at 18/13°C and then the temperature was raised gradually to 38/33°C.

Grains of cv. Stirling grown in a growth room at 18°C produced seedlings with more roots per seedling ($p < 0.05$) than seedlings from grains grown at 30°C (Figure 3.12a.) The number of roots per seedling from grains in seed lot 1 grown at 30°C was the same as that of seedlings from grains of seed lot 2 grown at the same temperature. The number of roots per seedling produced from grains of cv. Schooner grown in a growth room at 18°C was greater than that from grains grown at 30°C (Figure 3.12b.). Similarly, the number of roots per seedling produced from grains of

cv. Schooner grown in a growth cabinet at 18°C was greater ($p < 0.05$) than that from grains grown at 18-38°C (Figure 3.12c.)

Experiment IV

The number of roots per seedling produced from grains of seed lot 1 of cv. Blenheim grown at 18°C was greater than that produced from grains of seed lot 2 grown at the same temperature (Figure 3.13a). Seedlings from grains grown at 18-38°C had fewer roots ($p < 0.05$) than seedlings from grains grown at 18°C.

Grains of seed lot 1 of cv. Stirling grown at 18°C produced more roots per seedling ($p < 0.05$) than did grains of seed lot 2 grown in the same temperature regime (Figure 3.13b), and approximately the same number of roots per seedling as grains of seed lot 1 grown at 18-38°C.

Grains of cv. Schooner grown in a growth room at 18°C produced fewer number of roots per seedling than did grains grown at 30°C-38°C but the difference was not significant Figure 3.13c

However, the number of roots per seedling produced from grains of cv. Schooner grown in a growth cabinet at 18° was greater ($p < 0.001$) than that in seedlings produced from grains grown at 18°C-38°C. Temperature during grain growth affected seedling root number in cv. Blenheim but not in cv. Stirling or cv. Schooner under growth room conditions, but the effect of temperature was apparent in cv. Schooner grown in growth cabinets. Consistent differences between seed lot 1 & 2 were observed in grains grown at 18°C. The values for cv. Schooner grown in growth cabinets in Experiment III were very similar to those of cv. Schooner grown in growth cabinets in Experiment IV.

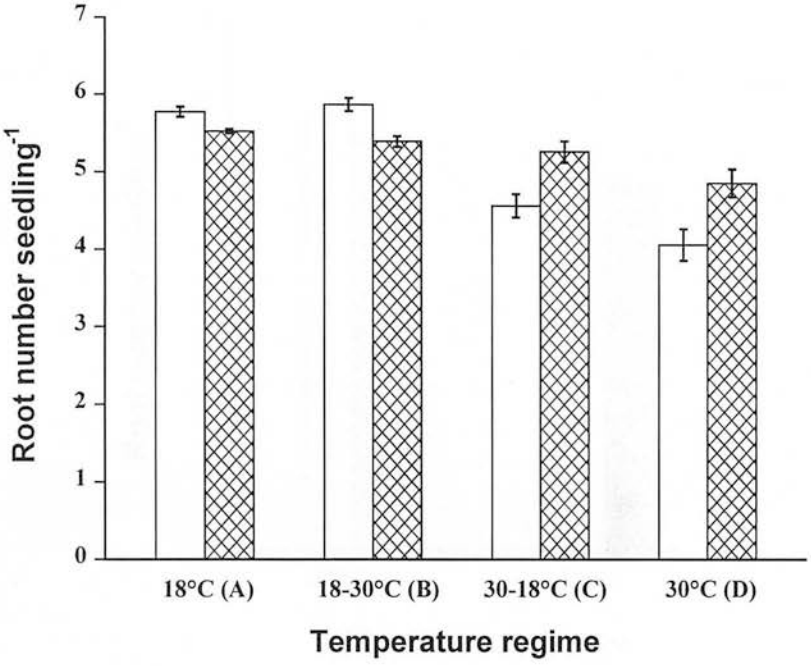


Figure 3.11 Experiment II. Effects of elevated temperatures during grain development on the number of roots per seedling from grains of cv. Blenheim grown in grains of ears grown in growth rooms at 18°C, 18-30°C, 30-18°C and 30°C. Keys to shading as in Figure 3.8. Error bars represent ± SEM; (n = 4).

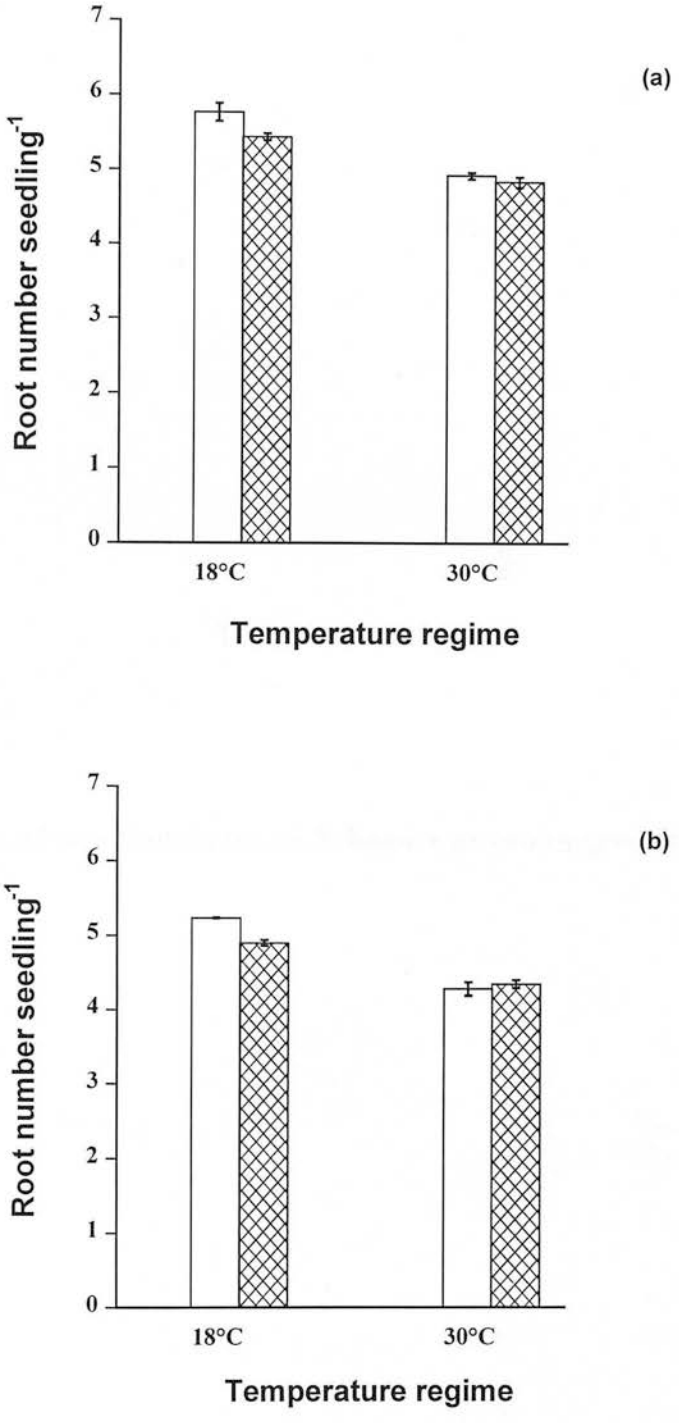


Figure 3.12. Experiment III. Number of roots per seedling produced from grains of cvs Stirling and Schooner grown in growth rooms at 18°C and 30°C. (a) cv. Stirling (b) cv. Schooner grown in growth rooms.Key to shading as in Figure 3.8. Error bars represent ± SEM; (n = 4).

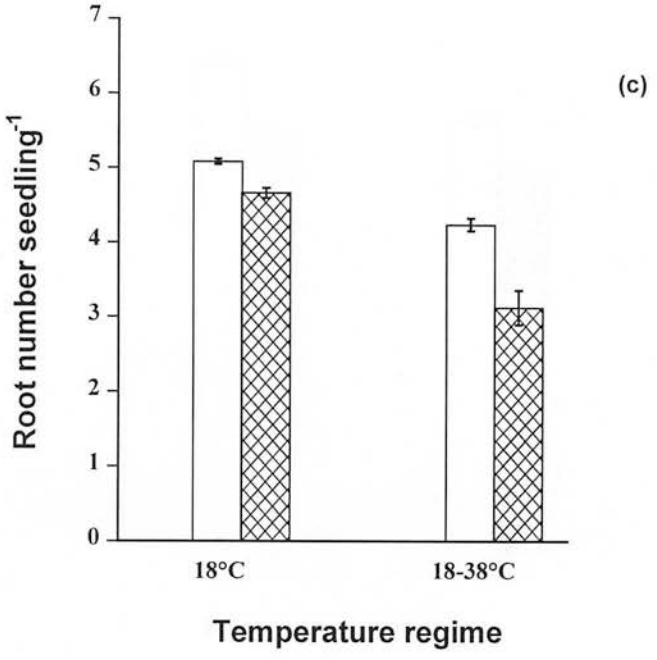
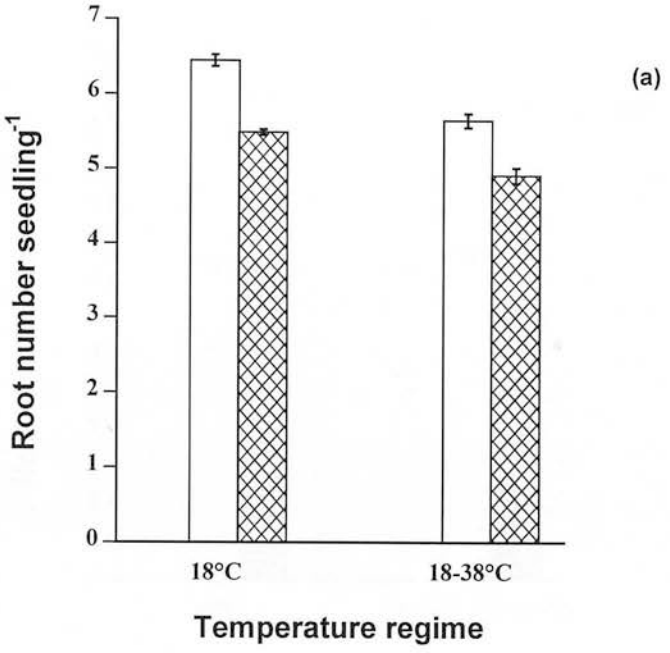


Figure 3. 12 (continued) (c) cv. Schooner grown in growth cabinets at 18°C and 18-38°C.



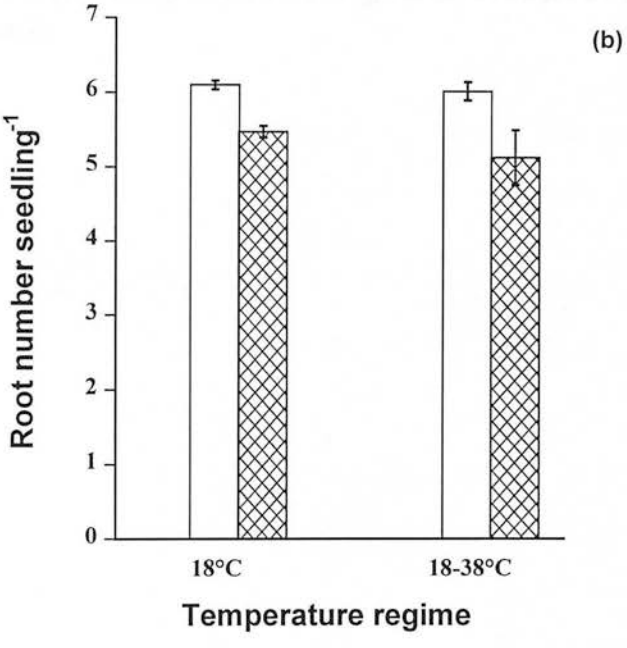


Figure 3. 13. Experiment IV. Number of roots per seedling produced from grains ears of cvs Blenheim and Stirling grown in growth rooms at 18°C and 18-38°C. (a) cv. Blenheim (b) cv. Stirling. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

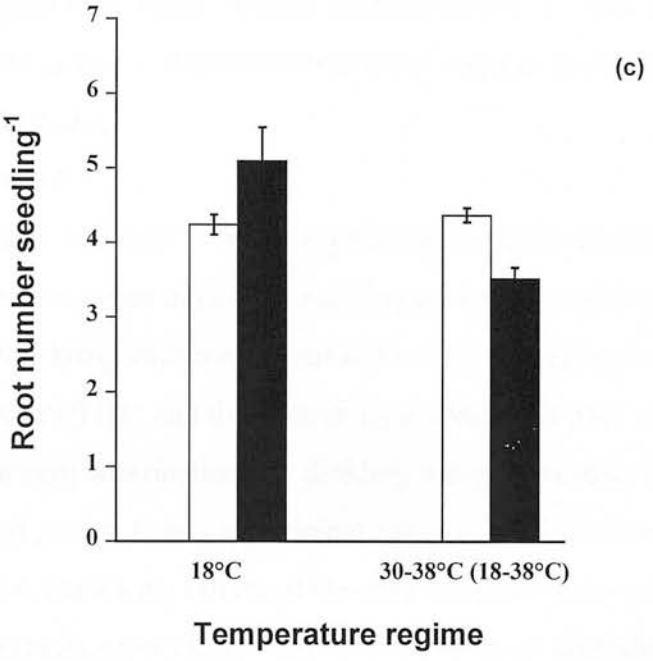


Figure 3. 13 (continued) (c) cv. Schooner grown in growth rooms at 18°C and 30-38°C (open histograms) and in growth cabinets at 18°C and 18-38°C (solid histograms).

3. 4. 3. Plumule length

Measurement were made of mean plumule length of 7-day old seedlings produced from grains grown in different temperature regimes in five experiments **(Section 2. 4. 3.).**

Experiment I

Experiment I was a preliminary experiment in which seedling plumule lengths were measured after seven days (7 d) seedling growth by multiplying the number of plumule tips lying within each pair of lines by the corresponding mid-point distance from the central line and then summing to obtain the total plumule length. The mean plumule length was obtained by dividing the total plumule length by the number of germinated grains. In this experiment only one cultivar (Blenheim) was used. Figure 3.14. shows the effects of elevated temperature on plumule length from grains of cv. Blenheim grown in growth rooms. The mean plumule length values of seed lot 1 in temperature regimes (A), (B), (C) and (D) did not differ significantly from plumule length values of seed lot 2 of the corresponding temperature regimes. The plumule length of seedlings from grains of seed lot 1 grown in temperature regime (A) did not differ significantly from that of seedlings of grains of seed lot 1 grown in regime (B). Similarly, seedling plumule length values from grains grown in regime (C) did not differ significantly from those from grains grown in regime (D) (Figure 3.14).

Experiment II

Experiment II differed from Experiment I only in the timing and duration of exposure of the ears to elevated temperatures (**Section. 2. 5. 2**). Seedling characteristics in this experiment were measured individually (**Section 2. 4. 3**). The plumule lengths of seedlings from seed lot 1 grown in regime (A) were longer ($p < 0.05$) than those of seedlings from seed lot 1 grown in regimes (B) and (C) but not significantly different from those of seedlings from grains grown in regime (D). These seedlings from seed lot 1 did not differ in plumule length from those from seed lot 2 grown in regime (A).

A similar pattern was observed in seedlings from grains grown in regimes (B) and (D) while seedling plumule length values from seed lot 1 grown in regime (C) were significantly different ($p < 0.05$) from seedling plumule length from seed lot 2 grown under the same temperature regime (Figure 3.15).

Experiment III

The mean plumule length of seedlings from seed lot1 of cv. Stirling grown at 18°C was the same as that of seedlings of seed lot 1 from grains grown at 30°C. Plumules of seedlings from seed lot 2 of grains grown at 18°C were shorter ($p < 0.05$) than those in seedlings from grains grown at 30°C (Figure 3.16a).

The plumules of seedlings of cv. Schooner from grains of seed lot 1 grown in growth rooms at 30°C were longer than those of seedlings from grains grown at 18°C, but the difference was statistically not significant (Figure 3.16b) The plumule lengths of seedlings from grains of seed lot 1 from ears grown at 18°C were similar to those from grains of seed lot 2 grown at the same temperature regime. The pattern was repeated in seed lots 1 & 2 grown at 30°C.

Grains of seed lot 1 of cv. Schooner grown at 18°C in a growth cabinet produced seedlings with longer ($p < 0.05$) plumules than the seedlings produced from grains grown at 18°-38°C and those produced from grains of seed lot 2 grown at the same temperature (18°C). Seed lot 1 of grains of cv. Schooner (Gc) grown at 18°C-38°C produced seedlings with longer plumules than those of seedlings produced from grains of seed lot 2 grown at the same temperature (Figure 3. 16c).

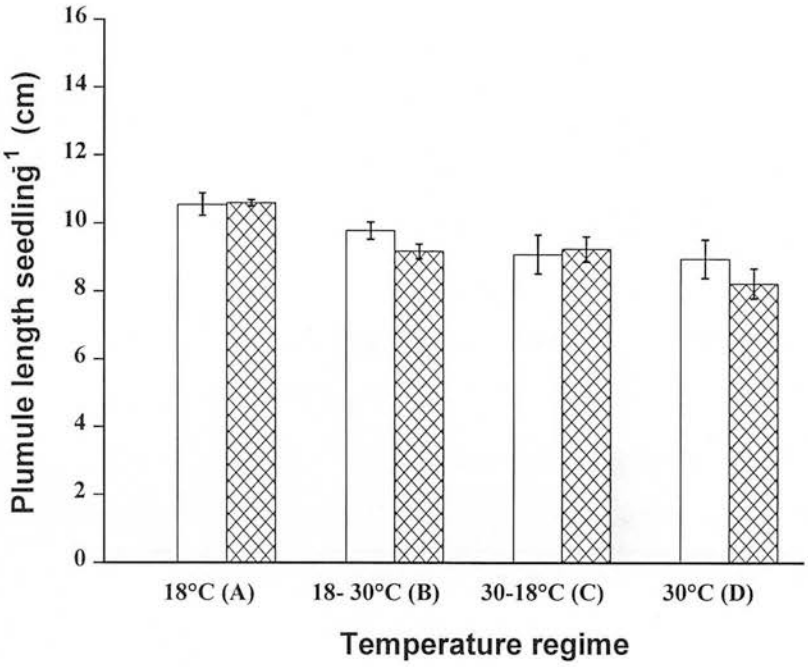


Figure 3.14. Experiment I. Plumule length of 7 d seedlings of grains from ears of cv. Blenheim grown in growth rooms at 18°C from anthesis to harvest-ripeness (A), grown at 18°C for 35 days after anthesis and transferred to 30°C to complete the remainder of grain development (B), grown at 30°C for 7 days before being transferred to 18°C to complete the remainder of grain development (C), grown at 30°C from anthesis to harvest- ripeness (D). Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8

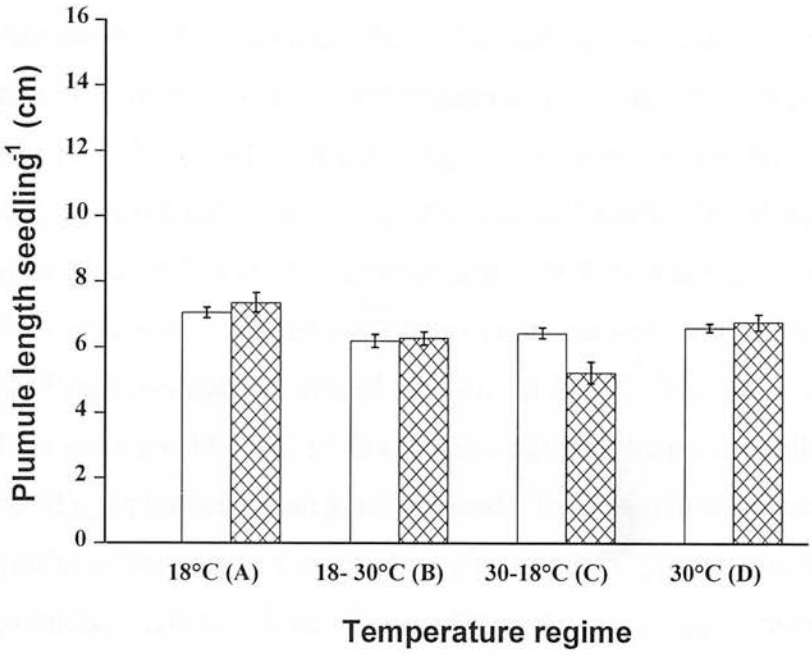


Figure 3.15. Experiment II. Plumule length of 7 d seedlings grown from grains of cv. Blenheim grown at 18°C from anthesis until harvest-ripeness (A), grown at 18°C for 25 days after anthesis and transferred to 30°C to complete the remainder of grain development (B), grown at 30°C for 10 days and then transferred to 18°C to complete the remainder of grain development (C) and grown at 30°C from anthesis to harvest-ripeness (D). Error bars represent SEM: (n = 4). Key to shading as in Figure 3.8

Experiment IV

Assessment of plumule lengths of 7 d seedlings was carried out on seedlings produced from grains of cvs Blenheim and Stirling grown in growth rooms at 18°C and 18°C-38°C and cv. Schooner grown in growth rooms (Gr) at 18°C and 30-38°C and in growth cabinets (Gc) at 18°C and 18°C-38°C. The plumule length of seedlings from seed lot 1 of cv. Blenheim grown at 18°C was the same as that of seed lot 2 from grains grown at the same temperature but shorter ($p < 0.05$) than that of seedlings produced from seed lot 1 grown at 18-38°C (Figure 3.17a). Grains of seed lot 1 grown at 18-38°C produced seedlings with longer plumules than those of seedlings produced from grains of seed lot 2 grown in the same temperature regime. Grains of seed lot 1 of cv. Stirling grown at 18°C produced seedlings with a mean plumule length which was the same as that of seedlings from grains of seed lot 2 grown at the same temperature, but statistically different ($p < 0.05$) from that of seedlings from grains grown at 18-38°C (Figure 3.17b).

The seedlings produced from grains of cv. Schooner grown in a growth room at 30-38°C had longer plumules than seedlings from grains grown at 18°C, however, the differences were non-significant. In contrast, seedlings from grains grown in the growth cabinet at 18°C had a mean plumule length greater ($p < 0.05$) than that of seedlings from grains grown at 18-38° (Figure 3.17c). It was thus observed that plumules in seedlings from grains of cv. Schooner grown in growth cabinets at 18°C were longer than those in seedlings from grains grown in growth cabinets at 18-38°C in both Experiments III & IV.

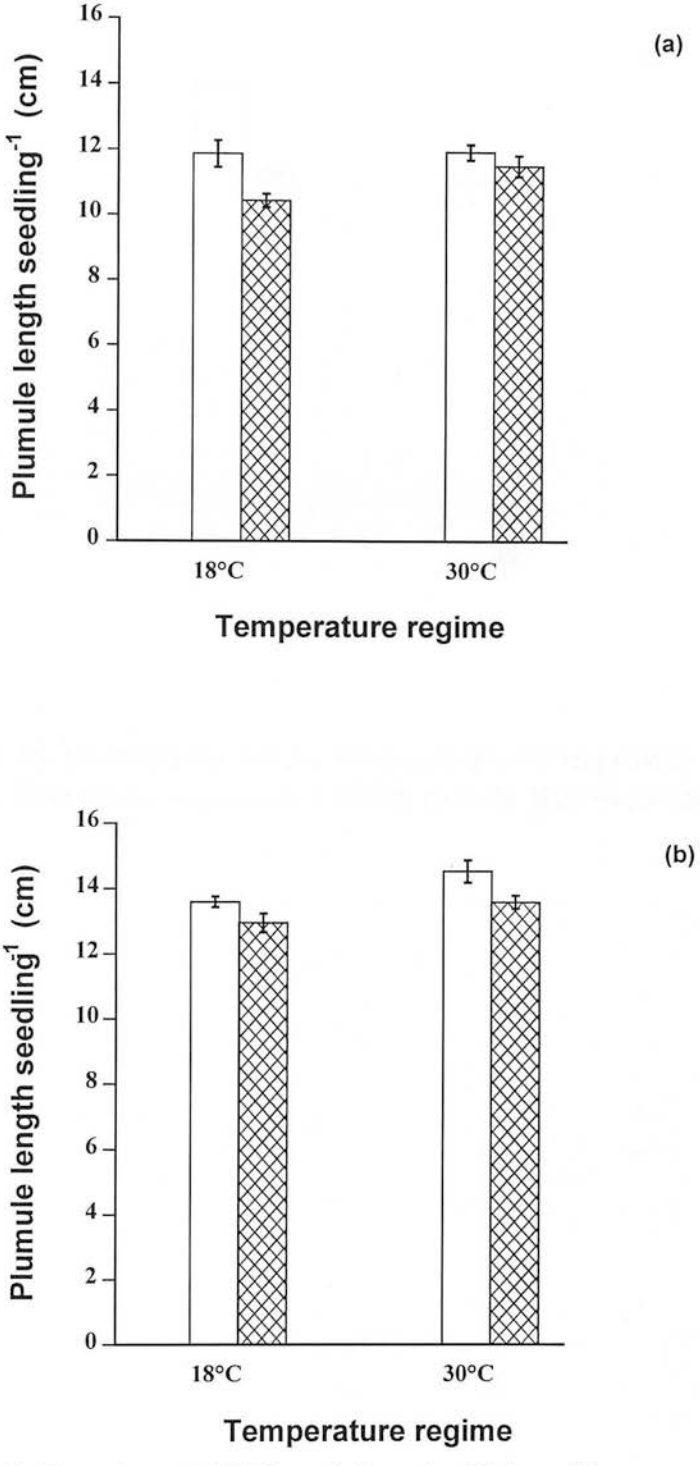


Figure 3.16. Experiment III. Plumule length of 7 d seedlings grown from grains of cvs Stirling and Schooner grown in growth room at 18°C and 30°C. (a) cv. Stirling (b) cv. Schooner. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

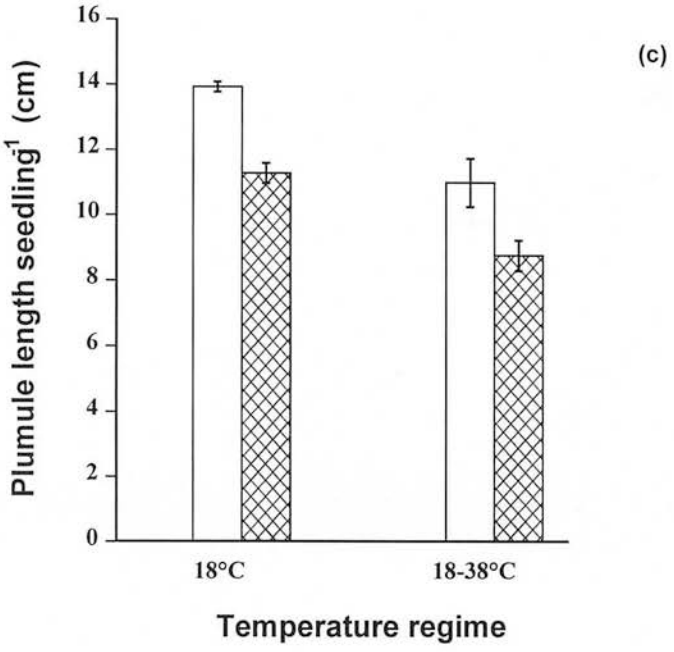


Figure 3. 16. (continued) (c) cv. Schooner grown in growth cabinets at 18°C and 18-38°C. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

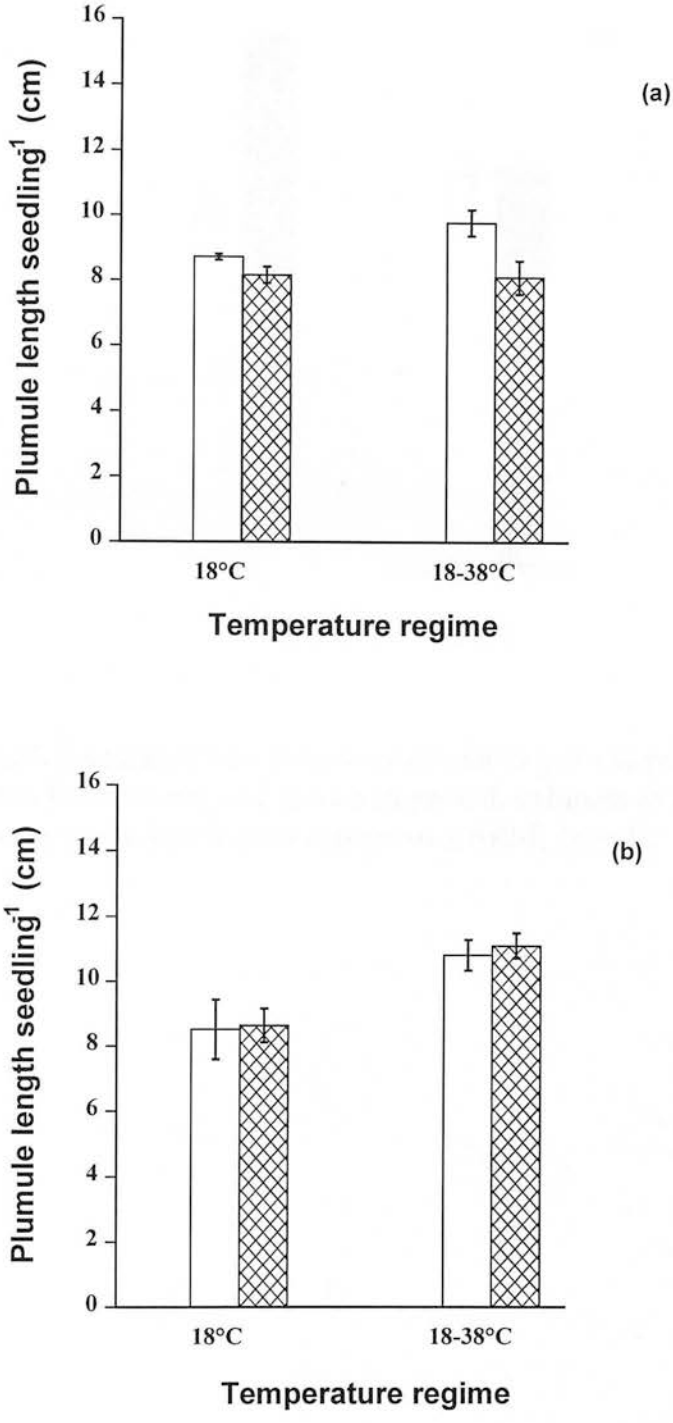


Figure 3.17. Experiment IV. Plumule length of 7d seedlings produced from grains of cvs Blenheim and Stirling from ears grown in growth rooms at 18°C and 18-38°C. (a) cv. Blenheim (b) cv. Stirling. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

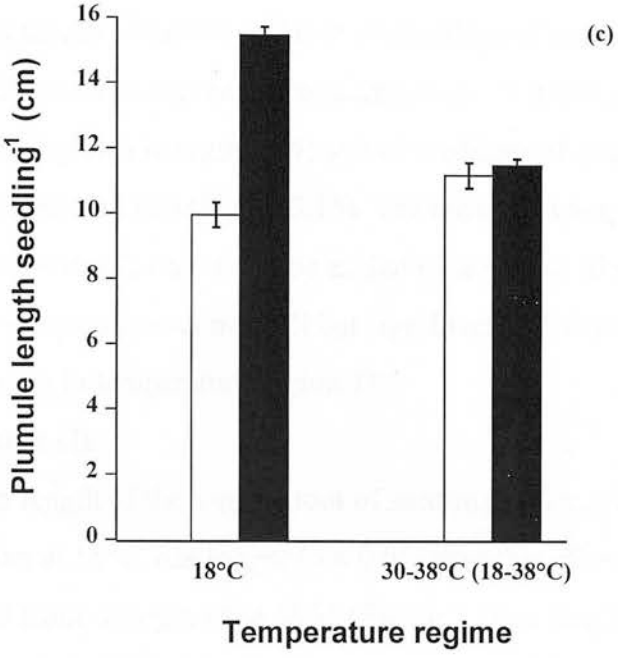


Figure 3.17. (continued) cv. Schooner grown in growth rooms at 18°C and 30-38°C (open histograms) and grown in growth cabinets at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 4. 4. Root length

Experiment II

The mean length of the longest root of seedlings of cv. Blenheim from grains of seed lots 1 & 2 grown in regime (A) was longer ($p < 0.05$) than that of seedlings of grains of seed lot 2 grown in regime (B) and of seedlings of grains of seed lots 1 & 2 grown in regimes (C) and (D) (Figure 3.18). The mean root length of seedlings of seed lot 1 grains grown in (C) was the same as that of seedlings of seed lot 2 of grains grown in the same temperature regime (C) but significantly different from that of seedlings of grains grown in temperature regime (D).

Experiment III

The mean length of the longest root of seedlings of cv. Stirling from grains of seed lot 1 grown at 18°C was longer ($p < 0.05$) than that of seedlings from grains of seed lots 1 & 2 from ears grown at 30°C (Figure 3 19a). Seedlings from seed lot 1 of grains grown at 30°C did not differ in root length from seed lot 2 grains grown in the same temperature regime. In cv. Schooner grains grown in growth rooms at 18°C produced seedlings with longer roots ($p < 0.05$) than those in seedlings produced by grains grown at 30°C (Figure 3.19b). Seedlings of cv. Schooner from grains grown in a growth cabinet at 18-38°C had shorter roots ($p < 0.001$) than seedlings from grains grown at 18°C (Figure 3.19c). Grains of seed lot 1 grown at 18-38°C produced seedlings with longer roots than those of the seedlings produced from grains of seed lot 2 grown at the same temperature.

Experiment IV

Seedlings of cv. Blenheim from grains grown at 18°C had the same mean length of the longest root as seedlings from grains grown at 18-38°C (Figure 3. 20a). However, the longest roots of seedlings of cv. Stirling from grains grown at 18° were slightly shorter than those of seedlings from grains grown at 18-38°C (Figure 3.20b). The mean length of longest roots of seedlings of cv. Schooner from grains grown in a growth room at 30-38°C was the same as that of seedlings from grains grown at 18°C (Figure 3. 20c). By constrast, grains of cv. Schooner grown in a growth cabinet at 18°C produced seedlings with longer roots ($p < 0.05$) than seedlings from grains grown in a growth cabinet at 18-38°C (Figure 3.20c).

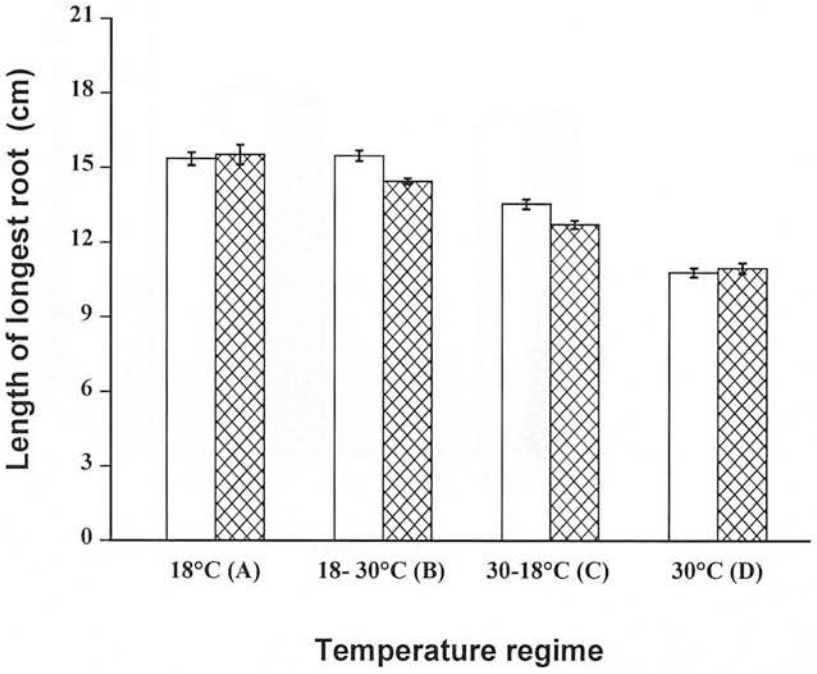


Figure 3. 18. Experiment II. The mean length of the longest roots of 7d seedlings from grains of cv. Blenheim grown in growth rooms at 18°C from anthesis until harvest-ripeness (A), grown at 18°C for 25 days after anthesis and transferred to 30°C to complete the remainder of grain development (B), grown at 30°C for 10 days before being transferred to 18°C to complete the remainder of grain development (C) and grown at 30°C from anthesis to harvest-ripeness (D). Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

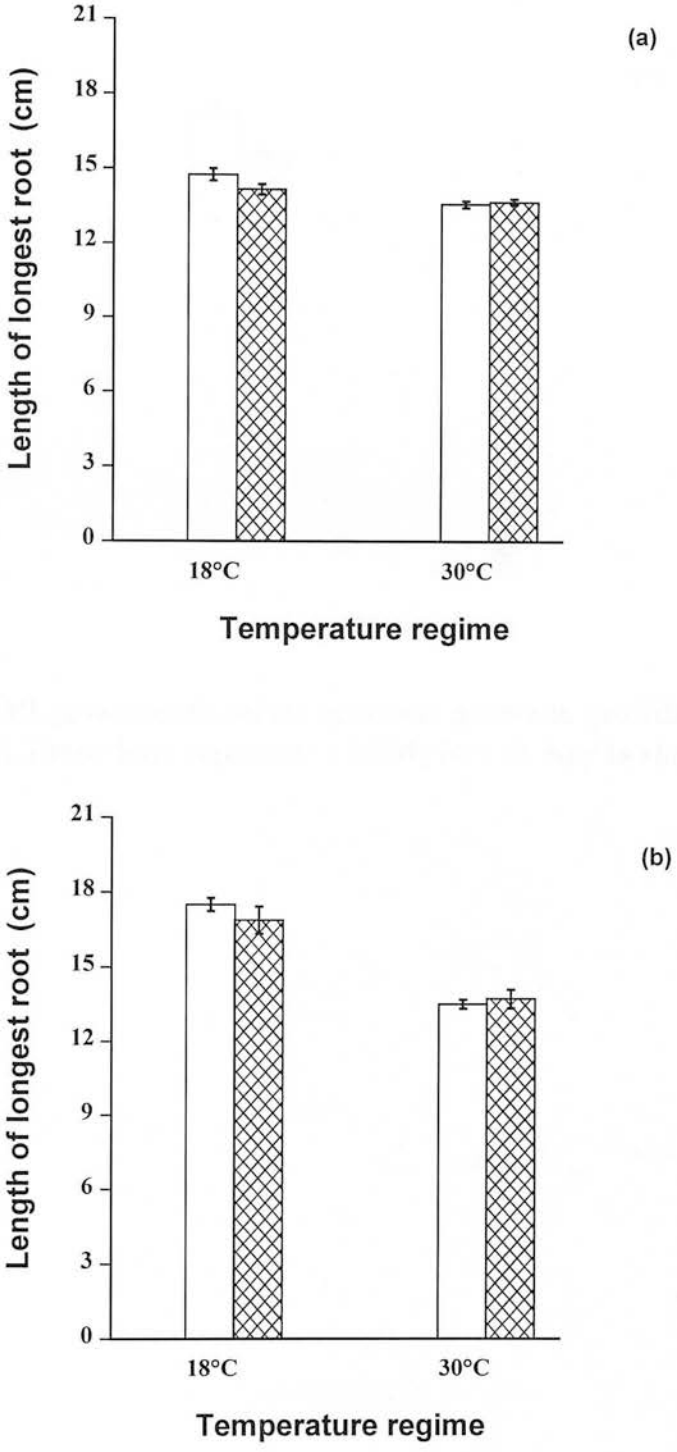


Figure 3. 19 Experiment III. Mean length of the longest roots of 7 d seedlings from grains of cvs Stirling and Schooner grown in growth rooms at 18°C and 30°C. (a) cv. Stirling (b) cv.Schooner. Error bars represent ± SEM; (n= 4). Key to shading as in Figure 3.8.

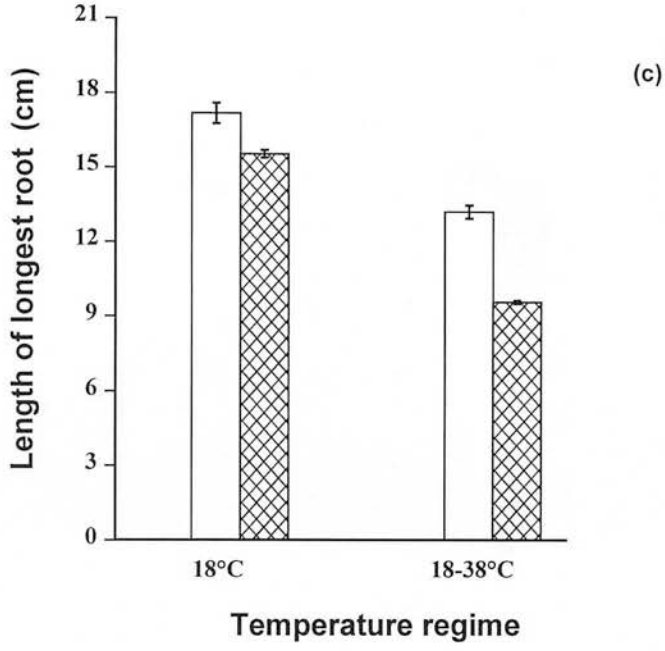


Figure 3.19. (continued). (c) cv. Schooner grown in growth cabinets at 18°C and 18-38°C.). Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

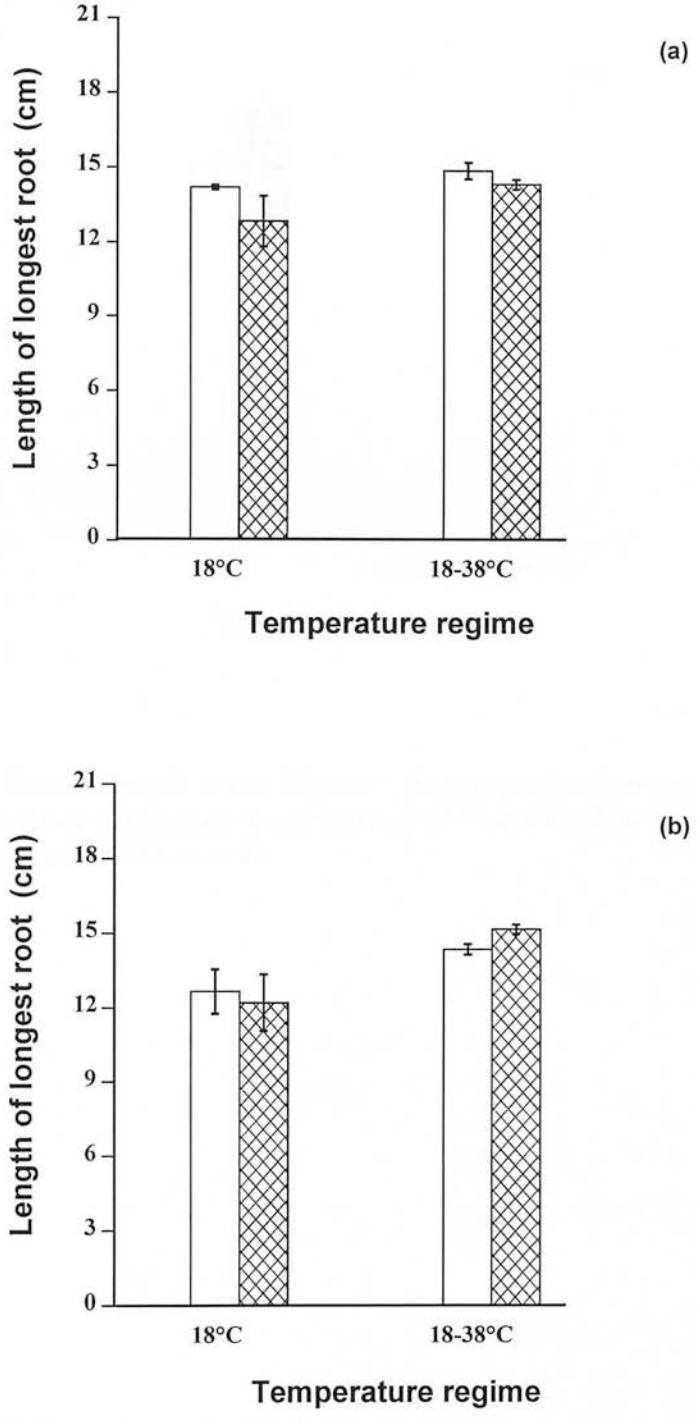


Figure 3.20. Experiment IV. Mean length of the longest roots of 7 d seedlings from grains of cvs Blenheim and Stirling grown in growth rooms at 18°C and 18-38°. (a) cv. Blenheim. (b) cv. Stirling. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

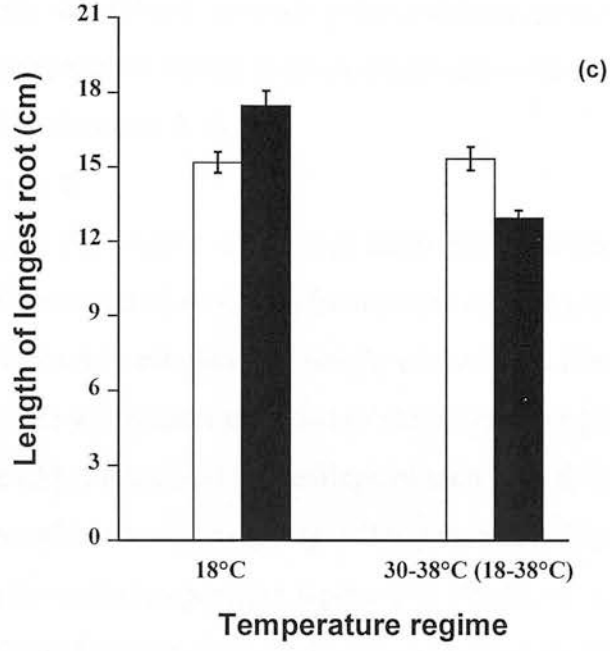


Figure 3. 20 (continued) (c) cv.Schooner grown in growth rooms at 18°C and 30-38°C (open histograms) and growth cabinets at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM, (n = 4).

3. 4. 5. Plumule dry weight

Plumule dry weights of seedlings produced from grains grown at different temperature regimes during grain development were measured and expressed in mg per seedling (**Section 2. 4. 3.).**

Experiment II

The plumule dry weight of seedlings from grains grown in regime (A) was greater ($p < 0.05$) than that of seedlings from grains of seed lots 1 & 2 grown in regimes (C) and (D). However, plumule dry weight of seedlings from grains of seed lot 1 grown in regime (B) was greater than that of seedlings from grains of seed lots 1 & 2 grown in regime (A) (Figure 3.21). Seedlings of seed lot 1 from grains grown in regime (B) had greater plumule dry weight ($p < 0.05$) than seedlings of grains of seed lot 2 grown in the same temperature regime (B). Seedlings of grains of seed lot 1 grown in regime (C) had greater plumule dry weight values ($p < 0.05$) than the seedlings of grains of seed lot 1 grown in regime (D) while seed lot 2 from grains grown in regime (C) produced seedlings with the same plumule dry weight as those of seed lot 2 grown in regime (D).

Experiment III

Grains of cv. Stirling grown at 18°C produced seedlings with greater plumule dry weights ($p < 0.05$) than seedling of grains grown at 30°C (Figure 3. 22a). Plumule dry weights of seedlings of seed lots 1 & 2 from grains grown at 30°C did not differ significantly from each other. Grains of cv. Schooner grown at 18°C in both growth room and growth cabinet produced seedlings with greater plumule dry weights than seedlings from grains grown at 30°C and 18-38°C (Figures 3.22b & c). However, mean plumule dry weight of seedlings from grains of seed lot 2 of cv. Schooner grown in a growth cabinet at 18°C did not differ significantly from that of seedlings from grains of seed lot 1 grown at 18-38°C. Plumule dry weights of seedlings from grains of cv. Schooner grown in a growth room at 18°C did not differ significantly from those of seedlings from grains grown in a growth cabinet at 18°C.

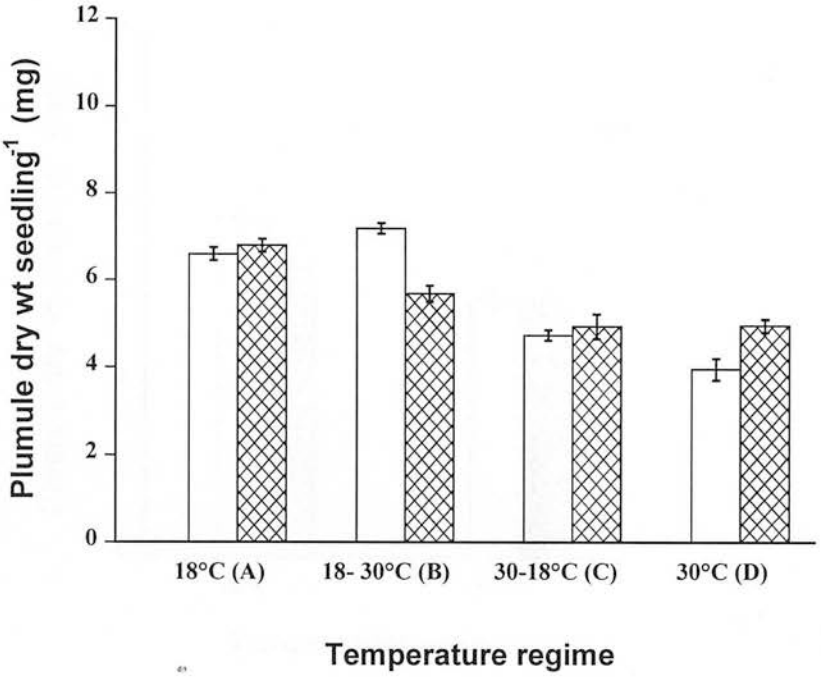


Figure 3.21 Experiment II. Mean plumule dry weight of 7 d seedlings from grains of cv. Blenheim grown in growth rooms in different temperature regimes. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

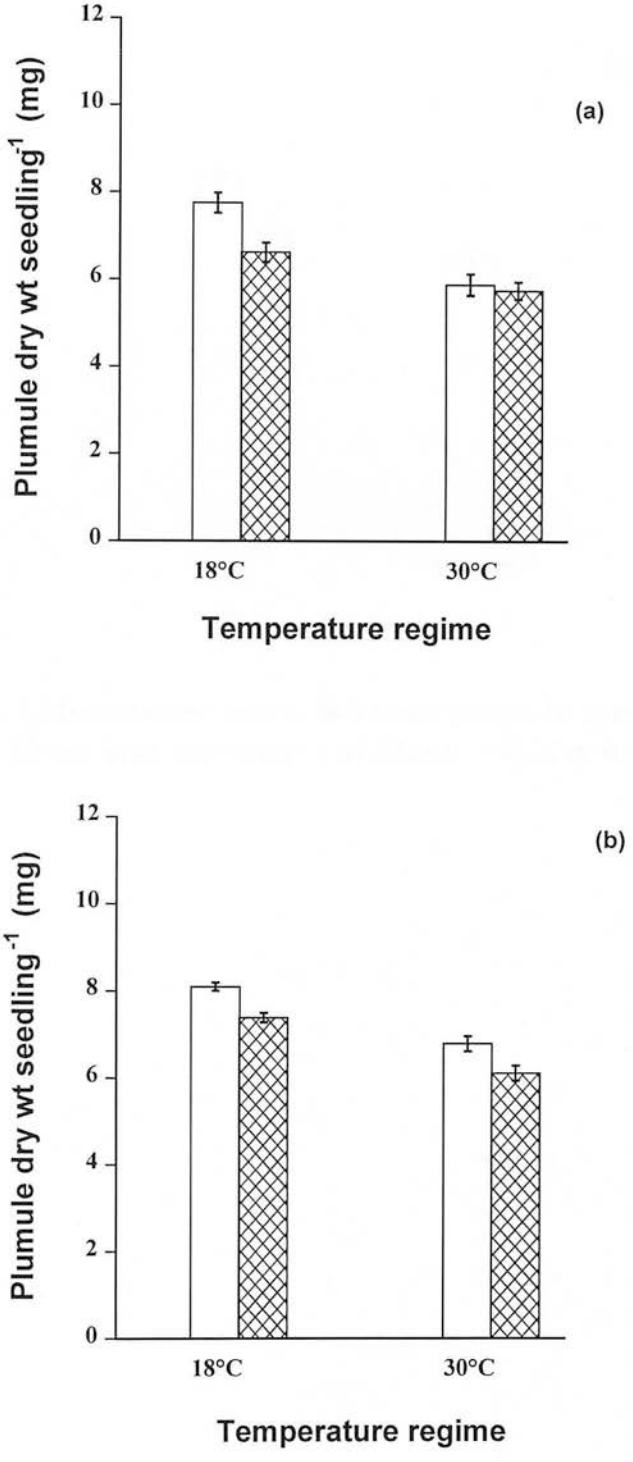


Figure 3.22. Experiment III. Mean plumule dry weight of 7 d seedlings from grains of cvs Stirling and Schooner grown in growth rooms at 18°C and 30°C. (a) cv. Stirling (b) cv. Schooner. Error bars represent ± SEM; (n= 4). Key to shading as in Figure 3.8.

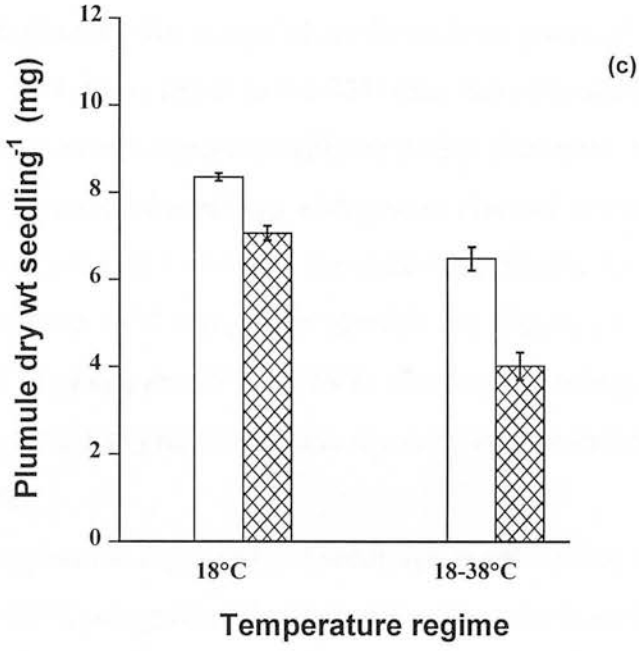


Figure 3. 22 (continued) (c) cv. Schooner grown in growth cabinets at 18°C and 18-38°C. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

Experiment IV

The mean plumule dry weight of seedlings from grains of seed lot 1 of cv. Blenheim grown at 18°C was greater ($p < 0.001$) than that of seedlings from grains of seed lot 2 grown at the same temperature (Figure 3.23a). Similarly, seed lot 1 of grains grown at 18-38°C produced seedlings with greater plumule dry weights ($p < 0.001$) than seedlings of seed lot 2 grown at the same temperature. Seedlings from seed lot 1 of grains grown at 18°C had greater plumule dry weights ($p < 0.05$) than seedlings from seed lot 1 of grains grown at 18-38°C. Similarly, seedlings of seed lot 2 from grains grown at 18°C had greater plumule dry weights than those of seed lot 2 grains grown at 18-38°C.

The mean plumule dry weight of seedlings of cv. Stirling from seed lot 1 grains grown at 18°C was greater that that of seedlings from seed lot 1 grains grown at 18-38°C (Figure 3.23b). However, plumule dry weight of seedlings of seed lot 2 from grains grown at 18°C was the same as the plumule dry weight of seedlings of seed lot 2 from grains grown at 18-38°C. Plumule dry weights of seedlings of cv. Schooner grown from grains grown in a growth room at 18°C were not significantly different from those of seedlings grown from grains grown at 30-38°C whereas seedlings from grains of cv. Schooner grown in growth cabinet at 18°C had greater plumule dry weights ($p < 0.05$) than seedlings from grains grown at 18-38°C (Figure 3.23c).

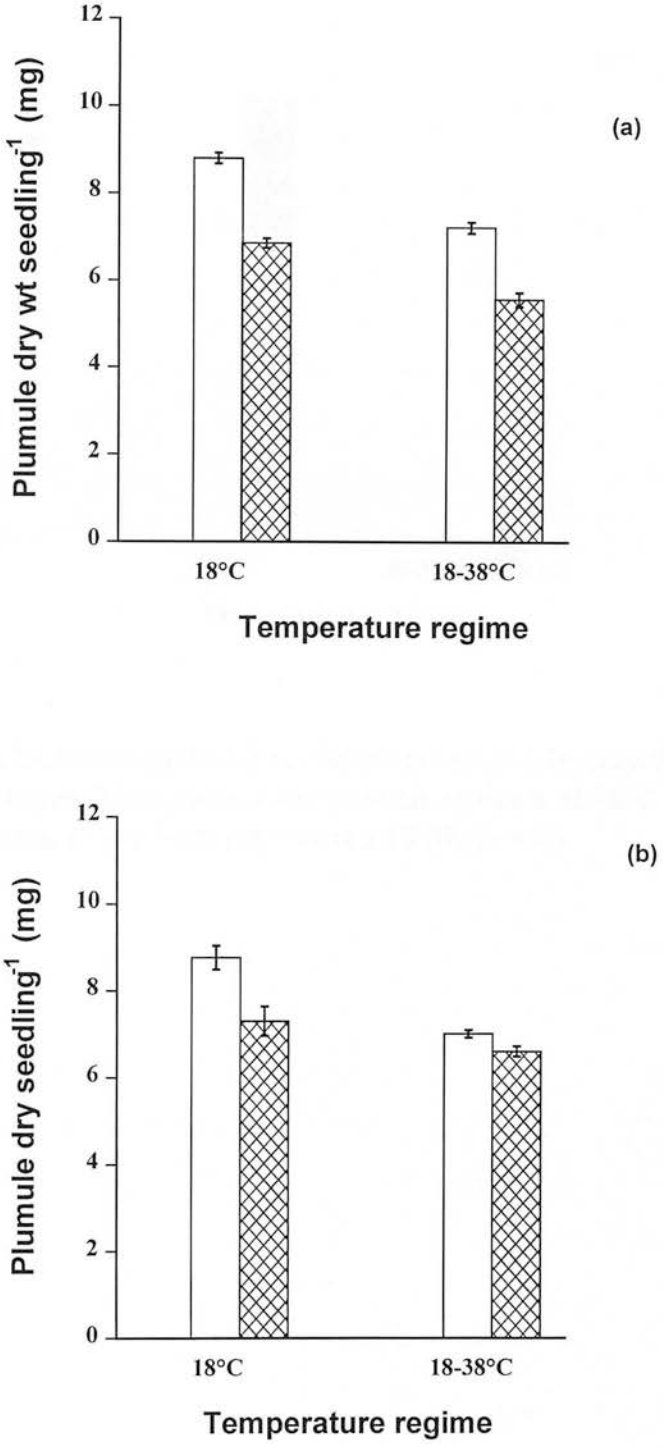


Figure 3.23. Experiment IV. Mean plumule dry weight of 7d seedlings from grains of cvs Blenheim and Stirling grown in growth rooms at 18°C and 18-38°C (a) cv. Blenheim (b) cv. Stirling. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

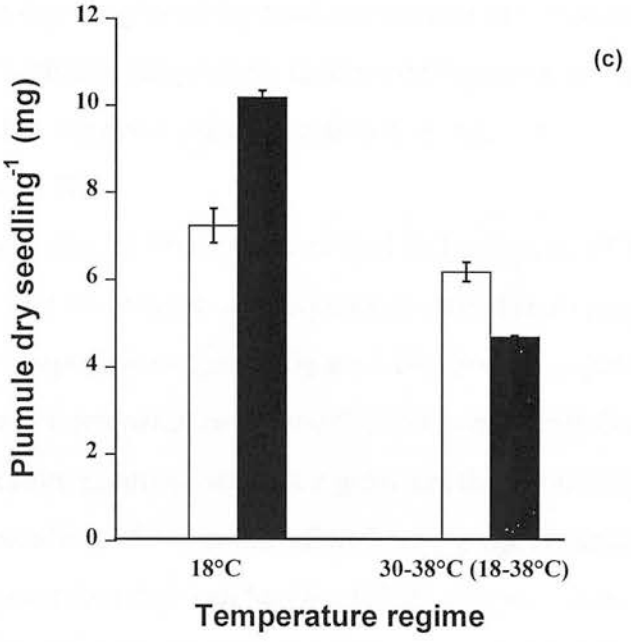


Figure 3.23. (continued) (c) cv. Schooner grown in growth rooms at 18°C and 30-38°C (open histograms) and growth cabinets at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 4. 6. Root dry weight

The mean dry weights of the total root system of 7 d seedlings produced from grains grown in different temperature regimes during grain development were measured and expressed in mg per seedling (**Section 2. 4. 3.).**

Experiment II

Seedlings produced from grains of seed lot1 grown at 18°C (A) had approximately the same root dry weights as seedlings produced from grains of seed lot 2 grown in the same temperature regime (Figure 3.24). Seedlings produced from grains of seed lot 1 grown in temperature regime (B) had greater root dry weights than seedlings produced from grains of seed lot 2 grown in the same temperature regime. In contrast, seedlings from grains of seed lot 1 grown in temperature regimes (C) and (D) had lower root dry weights ($p < 0.001$) than seedlings from grains of seed lot 2 from grains grown in the corresponding temperature regimes. Mean root dry weight of seedlings of seed lot 1 from grains grown in regime (A) did not differ from that of the seedlings grown from grains of seed lot 1 grown in temperature regime (B) but did differ significantly from those of seedlings from grains of seed lot 1 grown in regimes (C) and (D).

Experiment III

Grains from seed lots 1 & 2 of cv. Stirling grown at 18°C produced seedlings with greater mean root dry weight ($p < 0.05$) than that of the seedlings produced from grains of seed lots 1 & 2 grown at 30°C (Figure 3. 25a). Seedling root dry weight values in cv. Schooner (growth rooms) and in cv. Schooner (growth cabinet) followed a similar pattern to that observed in cv. Stirling (Figure 3.25b, c).

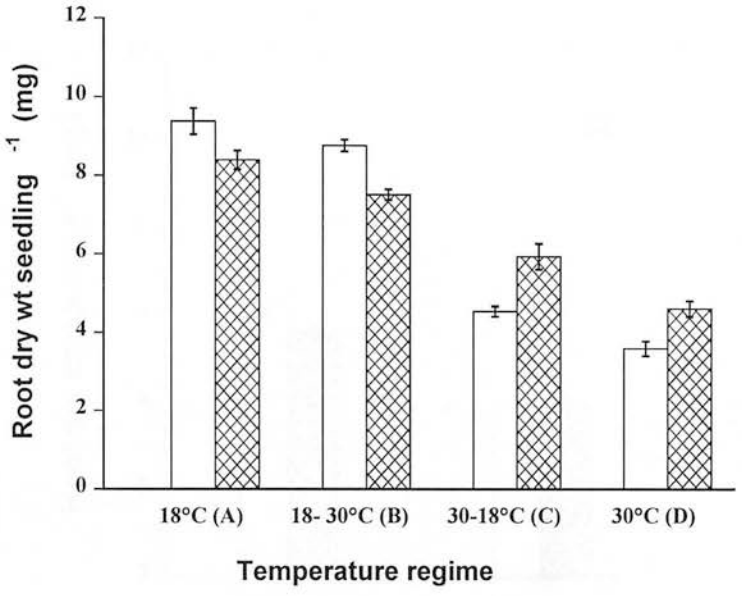


Figure 3.24. Experiment II. Mean root dry weights of 7d seedlings from grains of cv. Blenheim grown in different temperature regimes. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

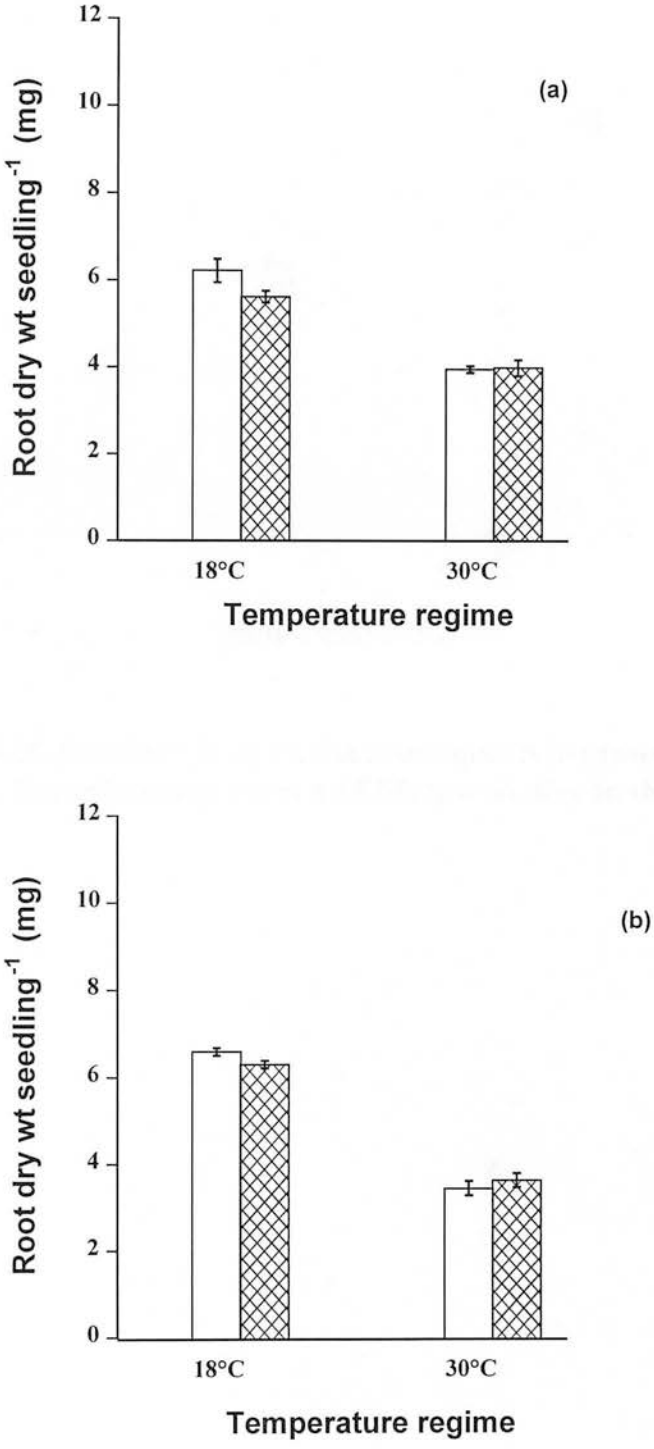


Figure 3.25. Experiment III. Mean root dry weights of 7 d seedlings from grains of cvs Stirling and Schooner grown in growth rooms at 18°C and 30°C (a) cv. Stirling (b) cv. Schooner. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

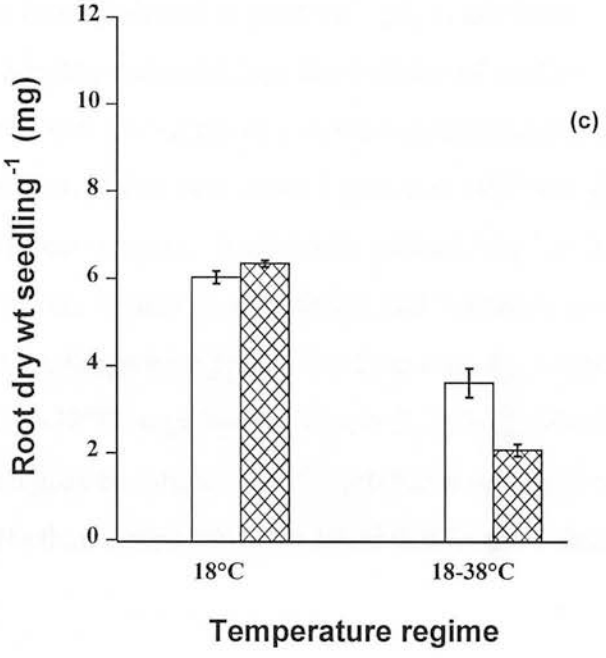


Figure 3.25. (continued) (c) cv. Schooner grown in growth cabinets at 18°C and 18-38°C. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

Experiment IV

Seedlings from grains of of seed lot 1 of cv. Blenheim grown at 18°C had greater root dry weights than seedlings from grains of seed lot 2 grown at the same temperature (18°C) but the difference was statistically not significant. However, seedlings from grains of seed lot 1 grown at 18°C had greater root dry weights ($p < 0.05$) than seedlings from grains of seed lots 1 & 2 grown at 18-38°C (Figure 3.26a). Grains of cvs Stirling and Schooner grown in growth room at 18°C produced seedlings with greater seedling root dry weights ($p < 0.05$) than grains grown at 30-38°C respectively (Figure 3. 25b, c). Similarly seedlings of cv. Schooner grown in a growth cabinet at 18°C produced seedlings with greater root dry weights ($p < 0.001$) than grains grown at 18-38°C (Figure 3.26c).

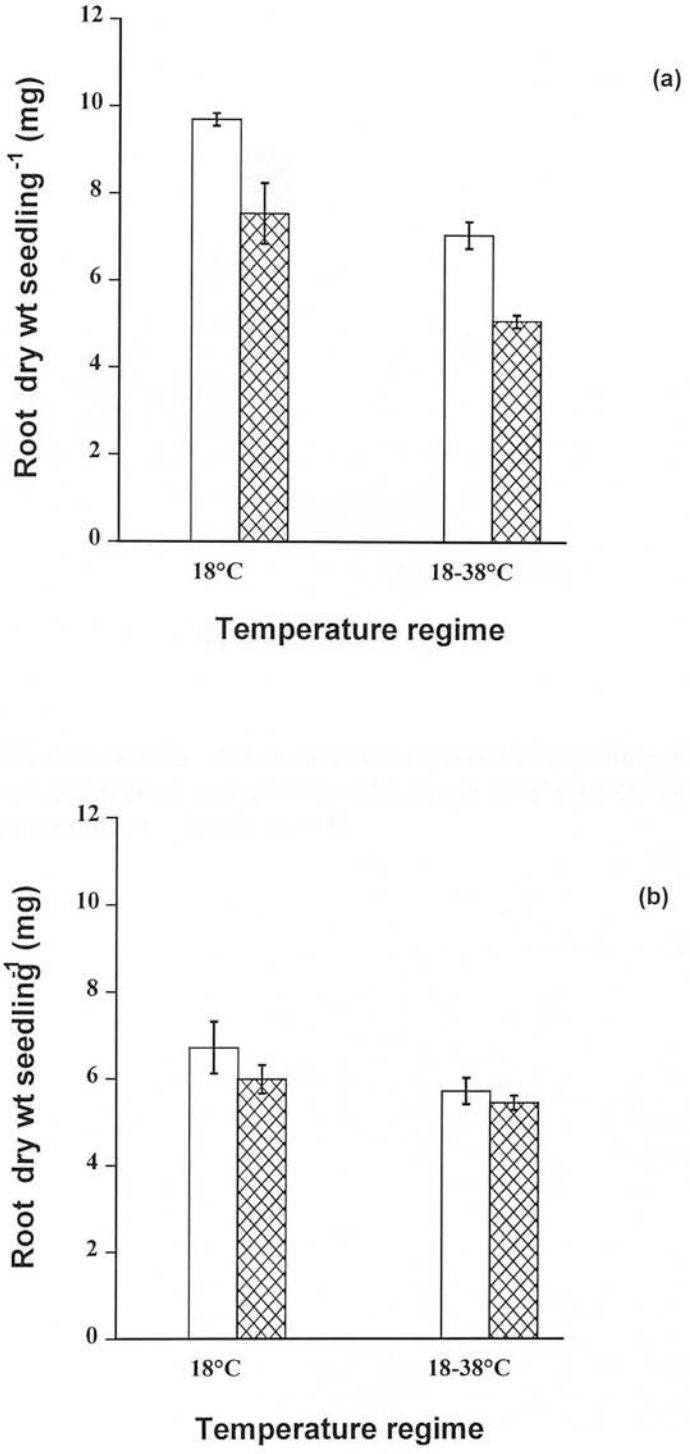


Figure 3. 26. Experiment IV. Mean root dry weight of 7 d seedlings from grains of cvs. Blenheim and Stirling grown in growth rooms at 18°C and 18-38°C (a) cv. Blenheim (b) cv. Stirling. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

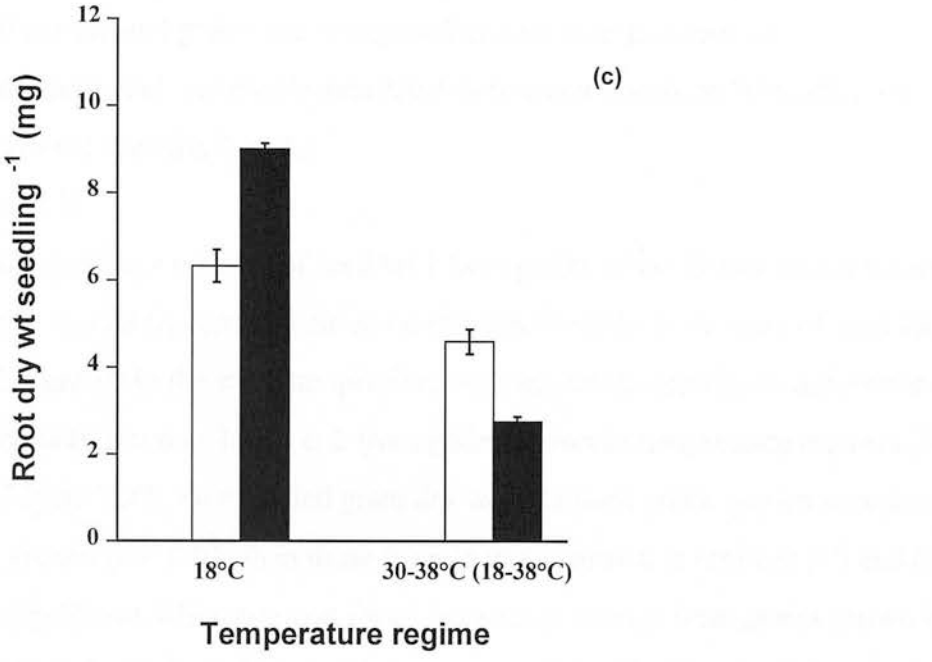


Figure 3.26. (continued) (c) cv. Schooner grown in growth rooms at 18°C and 30-38°C (open histograms) and growth cabinets at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 4. 7. Germinated grain dry weight

Weight of germinated grains was composed of husk and the remnants of the endosperm and embryonic axis dried to constant weight at 70°C after 7 d seedling growth (Section 2. 4. 3.)

Experiment II

Germinated grain dry weights of seed lot 1 from grains of cv. Blenheim grown in temperature regime (A) and (B) did not differ significantly from those of seed lot 2 from grains grown in the same temperature regimes, while significant differences were found between seed lots 1 & 2 from grains grown in temperature regimes (C) and (D) (Figure 3.27). Germinated grain dry weights from grains grown in regime (A) were greater ($p < 0.05$) than those from in grains grown in regimes (C) and (D), while no significant difference was found between seedlings from grains grown in regimes (A) and and those from grains grown in regime (B). Germinated grain dry weight values of grains from seed lots 1 & 2 grown in temperature regime (C) did not differ signicantly from those of grains grown in regime (D).

Experiment III

Germinated grain dry weight values of cvs Stirling and Schooner (gr) and cv. Schooner (gc) from grains grown at 18°C were greater ($p < 0.05$) than in grains grown at 30°C and 18-38°C (Figure 3.28a-c). Mean germinated grain dry weights of seed lot 1 of cv. Schooner from grains grown in a growth cabinet at 18°C had less germinated grain dry weights ($p < 0.05$) than seed lot 2 of grains grown at the same temperature and did not differ significantly from germinated grain dry weight of seed lot 1 from grains grown at 18-38°C. However, seed lot 1 from grains grown at 18-38°C had greater germinated grain dry weight ($p < 0.05$) than seed lot 2 from grains grown at the same temperature (18-38°C).

Experiment IV

Mean germinated grain dry weights of cvs Blenheim, Stirling, Schooner (gr) and cv. Schooner (gc) from grains grown at 18°C were greater ($p < 0.05$) than those from grains grown in temperature regimes 18-38°C and 30-38°C (Figure 3.29a-c).

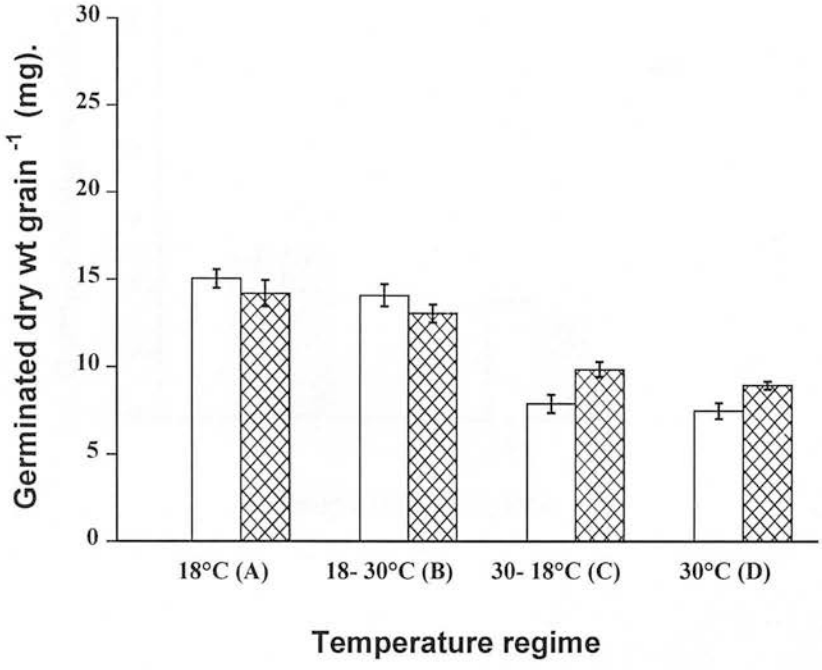
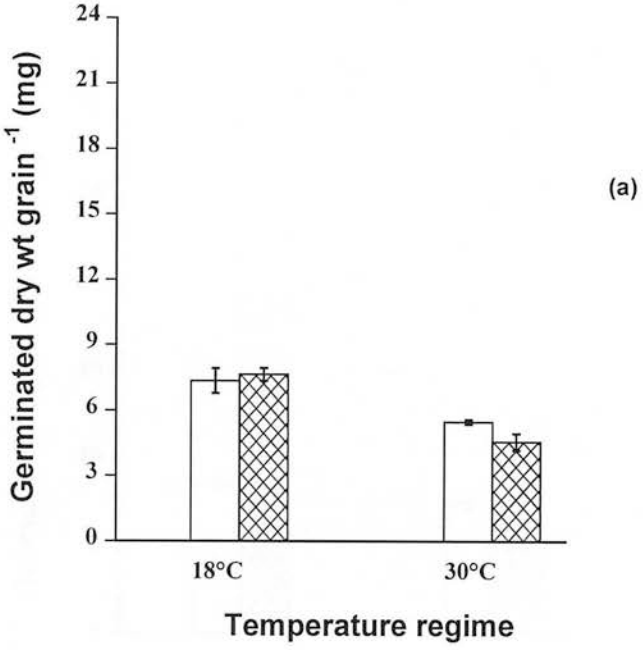


Figure 3. 27. Experiment II. Mean germinated grain dry weights after 7 d seedling growth of seedlings of cv. Blenheim from grains grown in growth rooms at 18°C from anthesis to harvest-ripeness (A), at 18°C for 25 days after anthesis and transferred to 30°C to complete the remainder of grain development (B), grown at 30°C for 10 days before being transferred to 18°C to complete the remainder of grain development (C) and grown at 30°C from anthesis until harvest ripeness (D). Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.



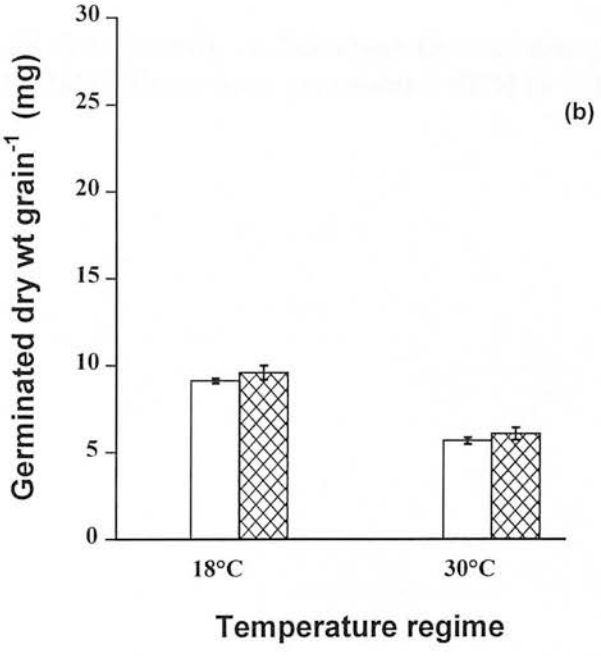


Figure 3.28. Experiment III. Mean germinated grain dry weight after 7d seedling growth of seedlings of cvs Stirling and Schooner produced from grains grown in growth rooms at 18°C and 30°C. (a) cv. Stirling (b) cv. Schooner. Error bars represent ± SEM (n = 4). Key to shading as in Figure 3.8.

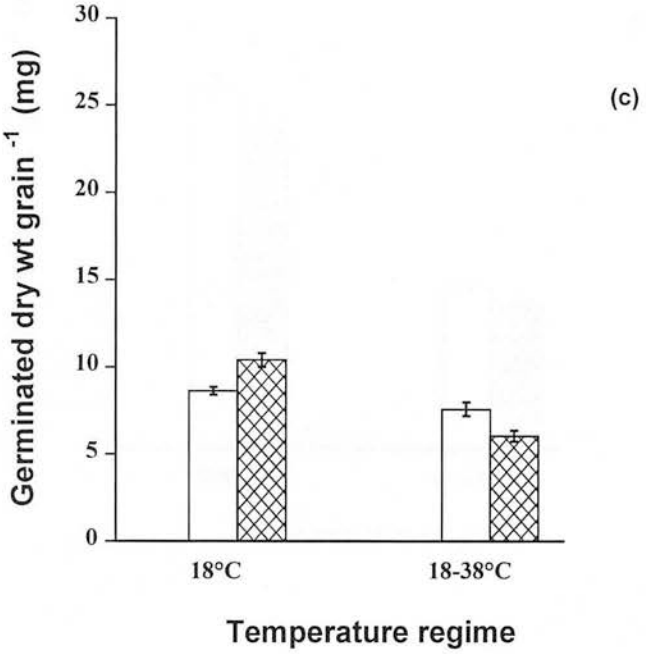


Figure 3.28. (continued), cv. Schooner from grains grown in growth cabinets at 18° and 18-38°C. Error bars represent ± SEM (n = 4). Key to shading as in Figure 3.8.

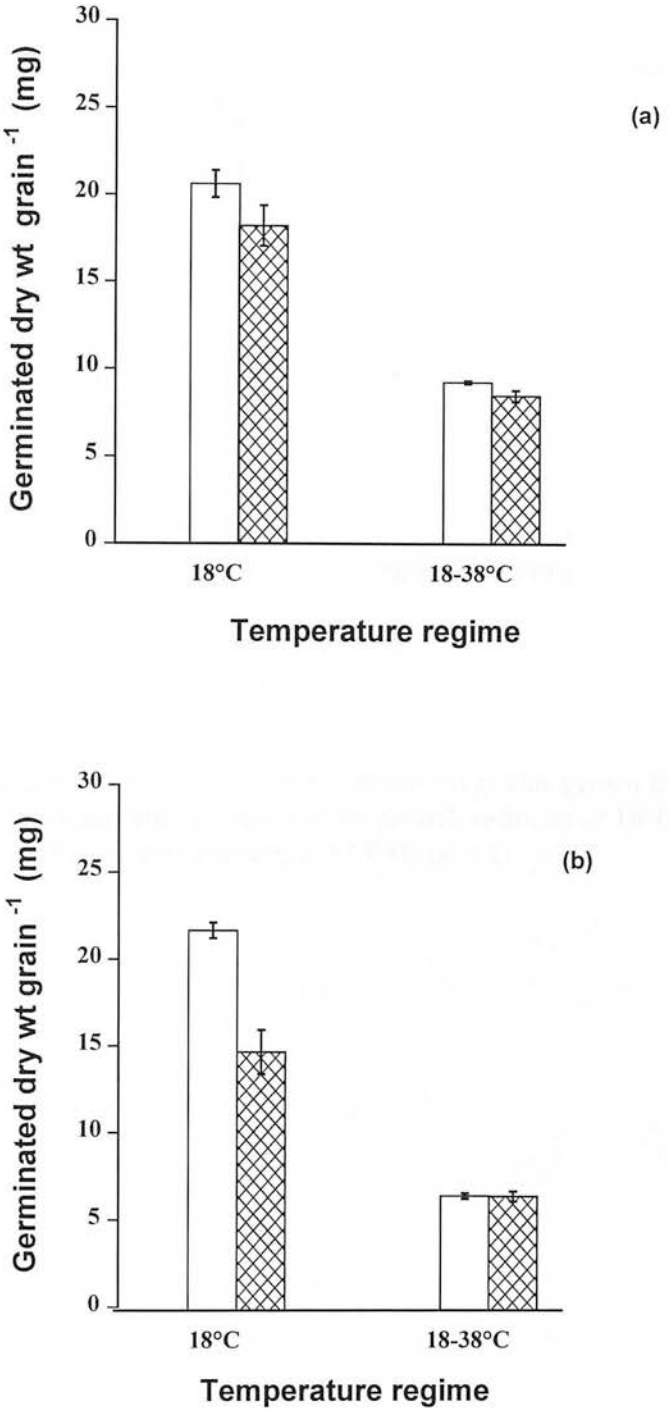


Figure 3. 29. Experiment IV. Mean germinated grain dry weight after 7 d seedling growth of seedlings of cvs Blenheim and Stirling produced from grains grown in growth rooms. at 18°C and 18-38°C (a) cv. Blenheim (b) cv. Stirling. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.8.

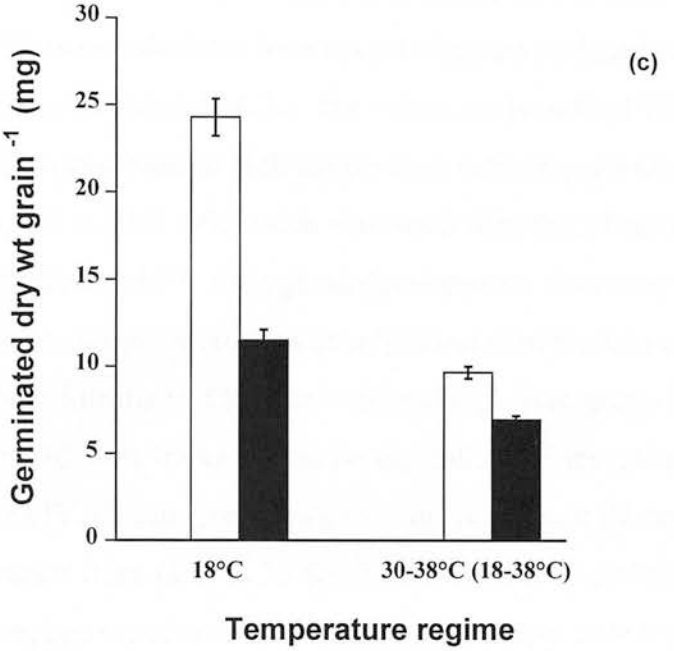


Figure 3.29. (continued) (c) cv. Schooner from grains grown in growth rooms at 18°C and 30-38°C (open histograms) and in growth cabinets at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 4. 8. Root dry weight: shoot (plumule) dry weight ratios (R/S)

The ratios of mean root dry weight per seedling (R) to mean plumule dry weight per seedling (S) were calculated for each set of grains analysed for seedling growth characteristics (**Section 2. 4. 3.**). The values are presented Table 3.5.

Grains which experienced high temperature early in grain development produced seedlings with an R/S ratio which was lower than that of seedlings grown from grains which experienced 18°C throughout development. However, the R/S values in cvs Stirling and Schooner were consistently lower than those in cv. Blenheim. The R/S values of cvs Stirling and Schooner in seedlings from grains grown at 30°C in Experiment III were lower than those in seedlings from grains grown at 18-38°C in Experiment IV. Grains from ears grown in Experiment III experienced a sudden rise in temperature from 18°C to 30°C whereas those in Experiment IV grown in the 18-38°C regime experienced a gradual rise in temperature from 18°C to 38°C. The differences between cultivars of tropical climate and temperate climate in the partioning of assimilates between the roots and shoots indicated by differences in R/S values, were apparent in seedling morphology. Cultivars of tropical origin appeared to channel more assimilates to the shoots than to the roots resulting in greater growth of the shoots than the roots (**Plate 3. 4a**). However, cultivars of temperate climate origin appeared to partition more assimilates to the roots than to the shoots as shown (**Plate 3. 4b).**

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Table 3.5. Mean values for root dry weight (R), plumule dry weight (S) and the ratios R/S for 7d seedlings grown from grains of three cultivars of barley grown in growth rooms (Gr) and growth cabinets (Gc) under different tempearture regimes. Seed lot 1 was made up of grains from ears which anthesed prior to 'day 1' of temperature treatment and seed lot 2 was made up of grains from ears which anthesed after 'day 1' of temperature reatment.

Expt No	Cultivar	Seed lot	Temp. regime	Root dry wt (mg) (R)	Plumule dry wt (mg) (S)	R/S
II	Blenheim	1	(A) 18°C	9.38	6.60	1.42
	Blenheim	1	(B) 18-30°C	8.78	7.20	1.22
	Blenheim	1	(C) 30-18°C	4.55	4.75	0.96
	Blenheim	1	(D) 30°C	4.00	3.98	1.00
II	Blenheim	2	(A) 18°C	8.40	7.30	1.20
	Blenheim	2	(B) 18-30°C	7.53	5.70	1.32
	Blenheim	2	(C) 30-18°C	5.95	4.95	1.20
	Blenheim	2	(D) 30°C	4.63	4.98	0.93
IV	Blenheim	1	18°C	9.70	8.65	1.12
	Blenheim	1	18-38°C	7.07	7.23	0.98
	Blenheim	2	18°C	7.55	6.86	1.10
	Blenheim	2	18-38°C	5.09	5.58	0.91
III	Stirling	1	18°C	6.22	7.95	0.80
	Stirling	1	30°C	3.96	5.90	0.67
	Stirling	2	18°C	5.62	6.62	0.85
	Stirling	2	30°C	4.00	5.76	0.69
IV	Stirling	1	18°C	6.72	8.77	0.77
	Stirling	1	18-30°C	5.73	7.02	0.82
	Stirling	2	18°C	6.00	7.31	0.82
	Stirling	2	18-38°C	5.46	6.74	0.81
III	Schooner (Gr)	1	18°C	6.59	8.10	0.81
	Schooner (Gr)	1	30°C	3.34	6.80	0.49
	Schooner (Gr)	2	18°C	6.29	7.39	0.85
	Schooner (Gr)	2	30°C	3.63	6.12	0.59
IV	Schooner (Gr)	1	18°C	6.33	7.25	0.87
	Schooner (Gr)	1	30-38°C	4.73	6.2	0.76
III	Schooner (Gc)	1	18°C	6.02	8.36	0.72
	Schooner (Gc)	1	18-38°C	3.59	6.50	0.55
	Schooner (Gc)	2	18°C	6.34	7.07	0.90
	Schooner (Gc)	2	18-38°C	2.06	4.03	0.51
IV	Schooner (Gc)	1	18°C	9.03	10.19	0.83
	Schooner (Gc)	1	18-38°C	2.76	4.71	0.59

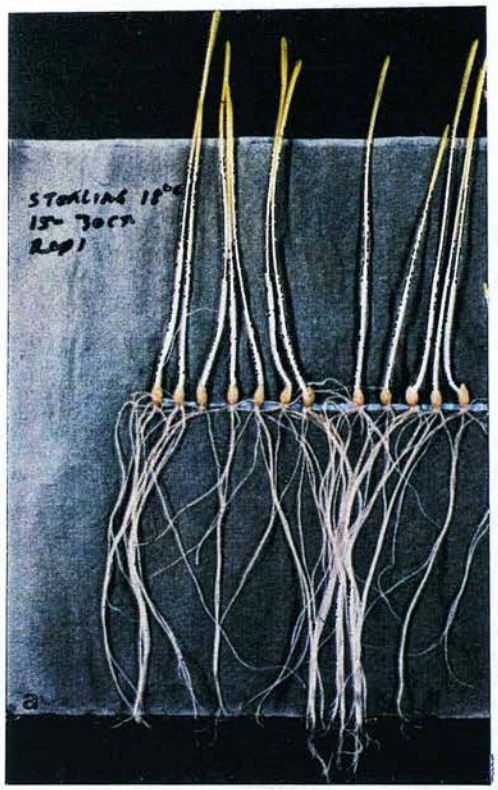


Plate 3. 4 (a). Seedlings of cv. Stirling from grains grown in a growth room at 18°C showing greater growth of plumules than of roots.

(b) Seedlings of cv. Blenheim from grains grown in a growth room at 18°C showing greater growth of roots than of plumules.

3. 4. 9. Relationships between of seedling growth characteristics and grain weights

Table 3.6a-c shows correlations between seedling growth characteristics and grain weight of cvs Blenheim, Stirling and Schooner grown in different tempearature regimes. Root numbers, length of longest roots and root and plumule dry matter showed strong positive correlations with grain weight. There was no statistically significant correlation between plumule length and other seedling growth characteristics in any of the three cultivars (Table 3.6a-c). The relationship was negative in cvs Stirling and Schooner grown in the growth rooms while it was positive but very weak in cv. Blenheim. The relationship between grain dry weight and root number and also between grain dry weight and root dry weight was very close and consistent in all three experiments.

Table 3.6. (a). Correlation coefficients (r) between grain weight and growth parameters of seedlings of cvs Blenheim, Stirling and Schooner produced from grains grown in growth rooms at different temperature regimes during grain development (a) cv. Blenheim grown at 18°C, 18-30°C, 30-18°C and 30°C (Experiment II).

	Grain dry wt	Root number	Plumule length	Root length	Plumule dry wt	Root dry wt
Grain dry wt						
Root number	**0.926** **					
Plumule length	**0.163** ns	**0.013** ns				
Root length	**0.869** **	**0.794** *	**0.209** ns			
Plumule dry wt	**0.937** **	**0.881** **	**0.277** ns	**0.848** **		
Root dry wt	**0.986** ***	**0.923** **	**0.195** ns	**0.897** **	**0.929** **	
Germinated grain dry wt	**0.954** ***	**0.855** **	**0.226** ns	**0.803** ns	**0.900** **	**0.950** ***

* = significant at $p < 0.05$, ** = significant at $p < 0.01$, *** = significant at $p < 0.001$, ns = not significant.

b) cv. Stirling grown in growth room at 18°C and 30°C (Experiment III).

	Grain dry weight	Root number	Plumule length	Root length	Plumule dry weight	Root dry weight
Grain wt						
Root number	0.935 **					
Plumule length	-0.154 ns	-0.022 ns				
Root length	0.794 *	0.829 ns	0.119 ns			
Plumule dry wt	0.898 **	0.923 **	0.158 ns	0.826 *		
Root dry wt	0.984 ***	0.952 ***	-0.214 ns	0.808 *	0.898 **	
Germinated grain dry wt	0.869 **	0.829 *	-0.403 ns	0.560 ns	0.754 ns	0.906 **

(c) cv. Schooner grown in growth room at 18°C and 30°C (Experiment III).

	Grain dry weight	Root number	Plumule length	Root length	Plumule dry weight	Root dry weight
Grain wt.						
Root number	0.971 ***					
Plumule length	-0.497 ns	-0.450 ns				
Root length	0.942 ***	0.933 **	-0.473 ns			
Plumule dry wt	0.893 **	0.876 *	-0.107 ns	0.839 *		
Root dry wt	0.995 ***	0.979 ***	-0.517 ns	0.954 ***	0.871 *	
Germinated grain dry wt	0.950 ***	0.897 **	-0.684 ns	0.858 *	0.741 ns	0.939 **

Levels of significance as in Table 3.6a.

3. 4. 10. Comparison of seedling growth characteristics of 11 genotypes of barley

Experiment V

Mean grain dry weight

The analysis of the mean grain dry weights of the eleven genotypes grown under glasshouse conditions is presented in Figure 3.30 with values arranged in descending order. Cv. Clipper had heavier grains ($p < 0.05$) than any of the other cultivars used in the analysis. cv. Tyne had the lowest grain dry weight of all the cultivars. In this experiment the grain dry weight of cv. Blenheim was less than that of cvs Stirling and Schooner but when these cultivars were grown in growth room conditions, grains of cv. Blenheim were larger and heavier than those of cvs Stirling and Schooner. In the glasshouse, seeds were sown on 13 February and grown under 18 h day length whereas in growth rooms sowing was carried out on 3 March (Experiment III) and 15 February (Experiment IV) with a day length of 16 h. Plants of cvs Stirling and Schooner when grown under the glasshouse conditions had fewer tillers than cv. Blenheim.

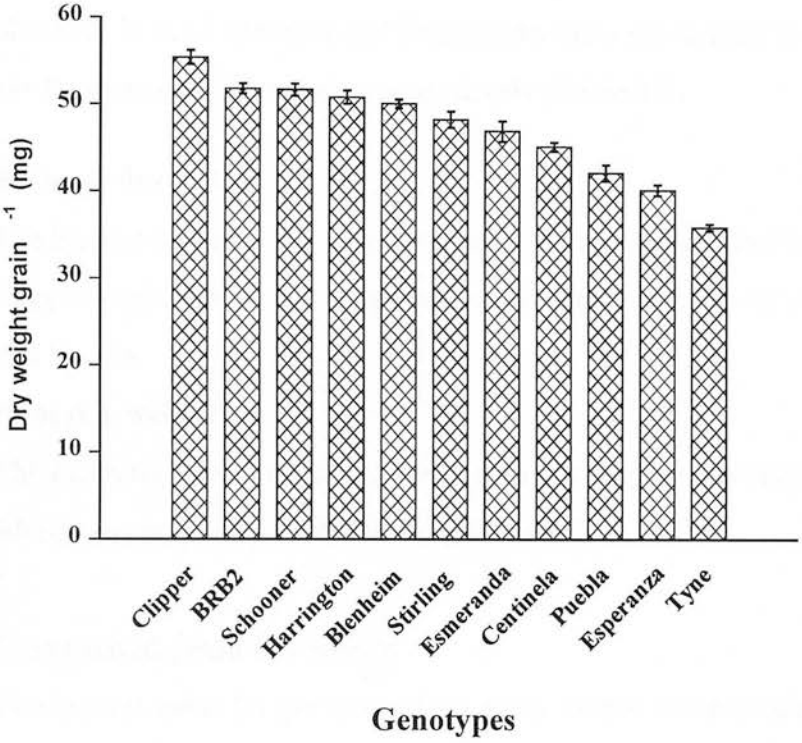


Figure 3.30. Grain dry weights of eleven genotypes grown under glasshouse conditions at approximately 18/13°C and 18 h day length.(Dry weight see Section 3.2).

Root numbers

Seedling growth tests were carried out using grains from eleven barley genotypes grown in the glasshouse conditions at approximately 18/13°C with a day length of 18 h (**Section 2. 4. 3.**). Three cvs Tyne, Esperanza and Emeranda had considerably fewer roots than the other cultivars (Table 3.7).

Plumule length

Plumule length values of eleven genotypes are shown in Table 3.7. The shortest plumule lengths were observed in cultivars BRB2, Puebla and Tyne while the longest plumule lengths were observed in cvs Schooner and Clipper (Table 3.7).

Root length

Table 3.8 shows the mean length of the longest roots. The shortest root lengths were observed in cvs Esperanza and Esmeranda while the longest roots were observed in cvs Schooner and Harrington respectively (Table 3.7).

Plumule dry weight

The highest plumule dry weight values were observed in cvs Schooner, Stirling and Clipper while the lowest values of plumule dry weights were observed in cvs Tyne and Puebla.

Root dry weight

The mean root dry weight was greatest in cvs Clipper, Harrington, Schooner and Blenheim and lowest in Puebla and Esmeranda.

Germinated grain dry weight

The highest value for germinated dry grain weight was recorded in cv. BRB2 and the least value was in cv. Tyne. It was generally observed that the six-row barley cultivars had greater germinated dry weights but lower seedling dry weights than the two-row barley (Table 3.7).

Root dry weight: shoot dry weight ratios (R/S)

cvs Harrington, Tyne and Blenheim had the highest R/S values and Esmaranda, Stirling and Schooner had the lowest R/S values. However, all the genotypes had R/S values > 1.

Table 3.7. The mean values for seedlings growth characteristics including root dry weight (R), plumule dry weight (S) and the ratios (R/S) for 7d seedlings produced from grains of 11 genotypes of barley grown under glasshouse conditions at approximately 18/13°C and 18 h day length from sowing to harvest -ripeness.

Cultivar	Root number	Plumule length (cm)	Root length (cm)	Plumule dry wt (mg)	Root dry wt (mg)	Germ grain wt (mg)	R/S	Type
BRB2	4.9 ± 0.1	4..9 ± 0.10	16.5 ± 0.22	6.3 ± 0.16	9.1 ± 0.14	27.2 ± 0.51	1.45	6-row
Harrington	6.3 ± 0.1	7.7 ± 0.13	17.5 ± 0.17	6.1 ± 0.04	10.8 ± 0.13	18.2 ± 0.18	1.77	2-row
Clipper	5.5 ± 0.1	9.2 ± 0.39	16.9 ± 0.21	8.4 ± 0.14	11.0 ± 0.23	20.9 ± 0.49	1.32	2-row
Blenheim	5.7 ± 0.04	7.2 ± 0.32	15.5 ± 0.28	6.4 ± 0.21	10.4 ± 0.25	18.1 ± 0.65	1.62	2-row
Schooner	5.0 ± 0.03	10.3 ± 0.26	17.6 ± 0.24	8.9 ± 0.10	10.2 ± 0.23	18.4 ± 0.28	1.16	2-row
Puebla	5.2± 0.06	5.2 ± 0.11	15.7 ± 0.14	5.4 ± 0.05	5.4 ± 0.05	21.8 ± 0.94	1.39	6-row
Centinela	5.2 ± 0.03	7.6 ± 0.10	15.6 ± 0.17	6.7 ± 0.16	8.0 ± 0.09	22.7 ± 0.50	1.19	6-row
Stirling	5.8 ± 0.16	8.8 ± 0.23	16.0 ± 0.07	8.6 ± 0.09	9.6 ± 0.17	16.1 ± 0.37	1.12	2-row
Esmeranda	4.2 ± 0.20	6.3 ± 0.03	14.8 ± 0.41	7.3 ± 0.11	7.9 ± 0.10	24.8 ± 1.77	1.08	6-row
Esperanza	4.2 ± 0.25	7.1 ± 0.31	14.6 ± 0.57	6.8 ± 0.23	8.2 ± 0.21	19.3± 0.77	1.22	6-row
Tyne	4.0 ± 0.06	5.9 ± 0.07	15.9 ± 0.30	4.9 ± 0.03	8.1 ± 0.09	11.5 ± 9.23	1.64	2-row

3. 5. Seedling emergence test

In the plumule growth test, seeds were grown in the dark for seven days using paper towels whereas in the present test, seeds were sown to a depth of 4 cm in fine sand in 30 cm pots in a growth room at 18/13°C with a 16h day and 85% relative humidity (**Section 2. 4. 4.).** In both tests, grains were incubated in the imbibed state for 5 days at 4°C in an incubator to break dormancy. The tests were carried out 10.5 months after harvest in Experiment III and 154 days after harvest in Experiment IV, the grains having been stored at 10°C and a moisture content of 10%. Seedling growth characteristics were assessed 7 days from the start of the sowing period. Seedling emergence was recorded as the number of seedlings emerged each day expressed as a percentage of the total number of seeds sown. A seedling was considered emerged when the coleoptile was above the surface of the fine sand used in the experiment. The final count of seedling which emerged was carried out on the 7th day from sowing. Seedling characteristics were measured as in **Section 2. 4. 3**. Root dry weights were not measured because of the difficulty of removing all traces of sand from the roots of the seedlings. The seedlings which failed to emerge through to the surface of the sand, and seeds that did not germinate because they were dormant or dead, were all expressed as percentages of the total number of seeds used in each test. Seeds which had failed to germinate by the end of test (7d) in both Experiments III and IV were tested for viability using the hydrogen peroxide test.

3. 5. 1. Total Percentage emergence

Experiment III

Grains of cvs Stirling and Schooner grown in the growth rooms at 18°C and 30°C were tested. More seedling emerged from seed lot 1 of cv. Stirling grown at 18°C than from seed lot 2 grown at the same temperature ($p < 0.05$) (Table 3.8). Similarly, seed lot 1 of grains grown at 30°C had more seedling emergence than seed lot 2 grown in the same temperature regime. There was no significant difference detected in seedling emergence between seed lot 1 grown at 18°C and seed lot 1 grown at 30°C. Grains which failed to germinate at the end of test were tested for viability

using hydrogen peroxide and germination was observed in these seeds. Grains of seed lot 2 grown at 18°C had a viability of 100 % in which 38 % ± 8.33 % of seeds were dormant. Grains of cv. Schooner grown in growth rooms at 18°C showed no significant differences in seedling emergence from grains grown at 30°C (Table 3.8).

Experiment IV

Figure 3.31a shows seedling emergence of grains of cv. Blenheim grown in growth rooms at 18°C and 18-38°C. Seed lot 1 grown at 18°C did not differ significantly in seedling emergence from seed lot 2 grown at 18°C. Similarly, seed lot 1 grown at 18-38°C had the same seedling emergence as seed lot 2 grown in the 18-38°C temperature regime (Figure 3.31a). Fewer seedlings emerged through to the surface of the sand from grains of cv. Blenheim grown at 18-38°C than grains grown at 18°C. There was no dormancy in grains of seed lot 1 grown at 18-38°C (100 % viability) but a high degree of variability between replicate pots was observed. Seed lot 1 of cv. Stirling grown at 18°C had greater seedling emergence than seed lot 2 grown at 18°C whereas grains of seed lot 1 grown at 18-38°C° did not differ significantly in seedling emergence from grains of seed lot 2 grown in the temperature regime 18-38°C (Figure 3.31b). A viability of 100 % was recorded in grains of seed lot 2 of cv. Stirling grown at 18°C and 48 % of seeds were dormant (**Plate 3.5. 1a, b**). The percentage emergence of seedlings from grains of cv. Schooner (gr) grown at 18°C did not differ significantly from that of seedlings from grains grown at 30-38°C. The viability in grains grown at 18°C and 30-38°C was 100 % (14 % & 22% of seeds being dormant respectively. Grains of Schooner (Gc) grown at 18°C had higher seedling emergence ($p < 0.05$) than grains grown at 18-38°C (Figure 3.31c). There was also a large variability between replicates pots of cv. Schooner (Gc) from grains grown at 18-38°C, and 9 % of the seeds grown in a growth cabinet at 18-38°C were dead.

Table 3.8. Experiment III. Seedling emergence from grains of cv. Schooner and Stirling grown in the growth rooms at 18°C and 30°C temperature regimes. Mean values ± SEM; n = 4

Cultivar	Temperature regime	Emergence %	Germinated (not emerged) %	Dormant %
Stirling	18°C (seed lot 1)	98 ± 2.31	2 ± 2.3	0
Stirling	30°C (seed lot 1)	96 ± 3.26	3 ± 2	1 ± 2
Stirling	18°C (seed lot 2)	54 ± 12	8 ± 5.6	38 ± 8.32
Stirling	30°C (seed lot 2)	81 ± 9.45	3 ± 3.8	16 ± 8
Schooner	18°C (seed lot 1)	100	0.0	0.0
Schooner	30°C (seed lot 1)	100	0.0	0.0
Schooner	18°C (seed lot 2)	94 ± 1.3	4 ± 1.6	2 ± 1.2
Schooner	30°C (seed lot 2)	98 ± 2.0	1 ± 1.0	1 ± 1.0

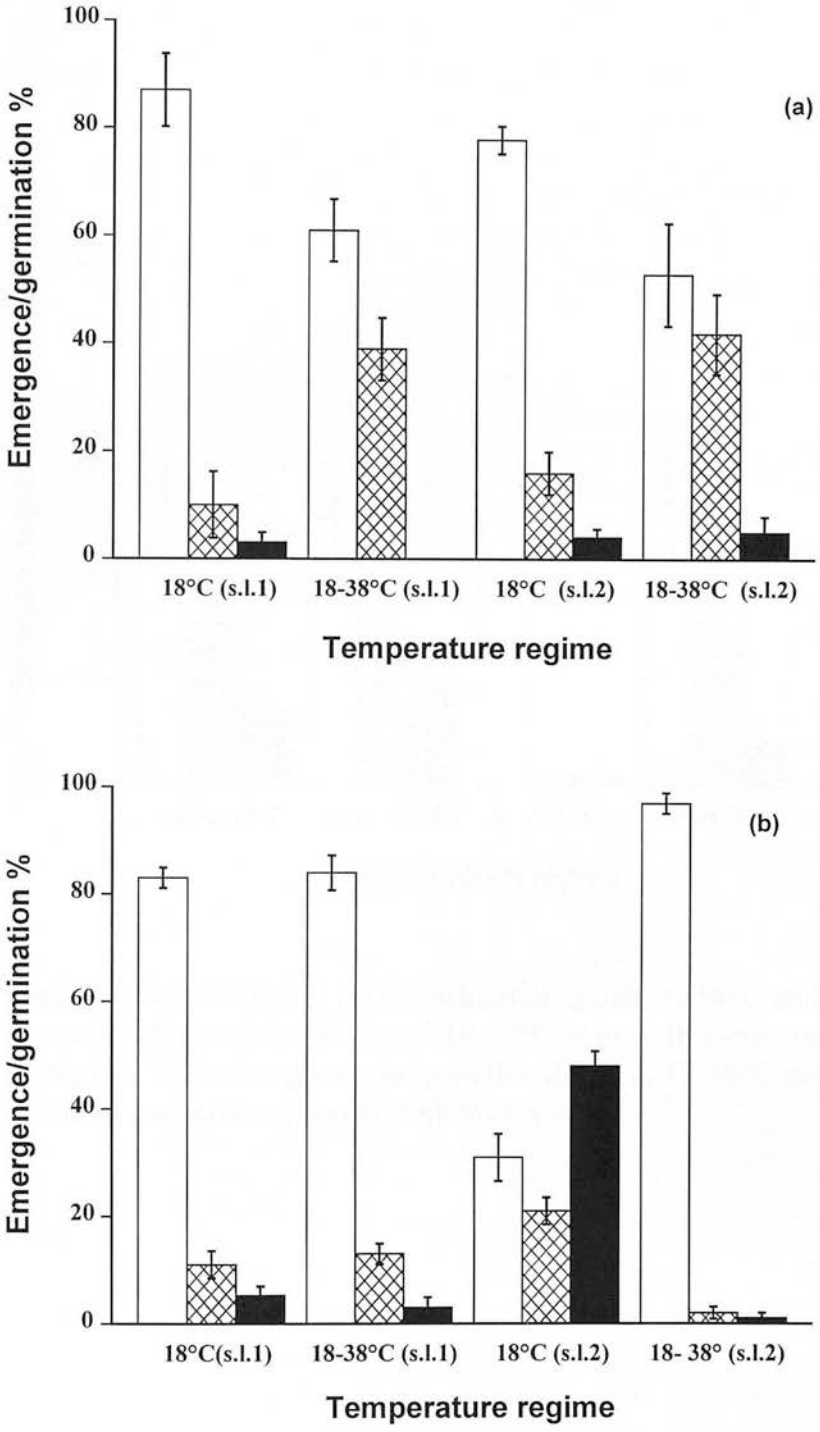


Figure. 3.31. Experiment IV. Seedling emergence from seed lot 1 (s.l.1) and seed lot 2 (s.l.2) of grains grown at 18°C and 18-38°C during grain development. Seedling emergence was evaluated 7 days after sowing. Open histograms represent percentage of emerged seedlings; crosshatched histograms represent percentage of germinated but not emerged seedlings; solid histograms represent percentage of dormant seeds.(a) cv. Blenheim (b) cv. Stirling. Error bars represent SEM ± ;(n = 4).

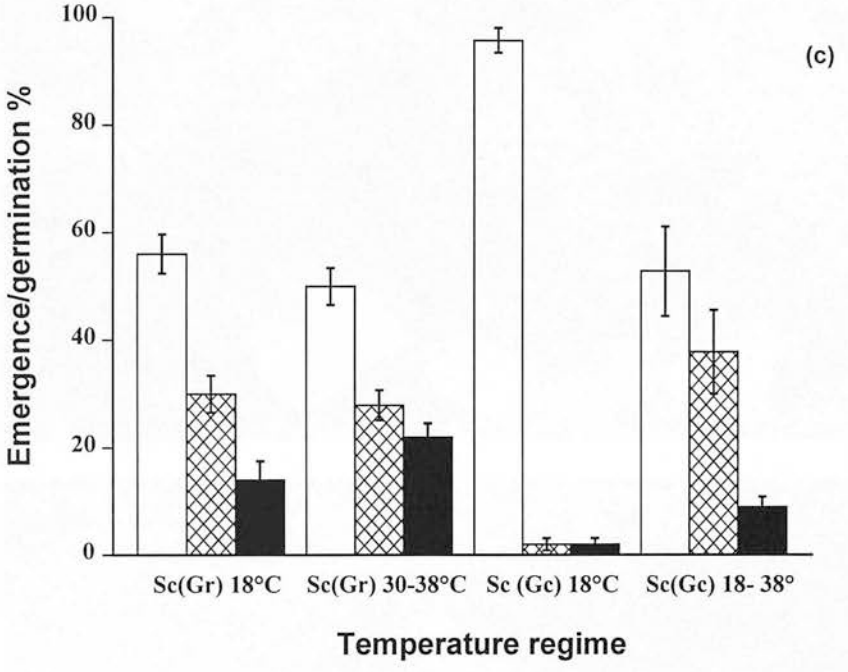


Figure 3.31 (continued) (c) cv. Schooner grown at 18°C and 30-38°C in growth rooms (Sc Gr) and at 18°C and 18-38°C in growth cabinets (ScGc). Solid histogram in grains grown in growth cabinet at 18-38°C represent % of dead seeds. Error bars represent ± SEM; (n = 4).

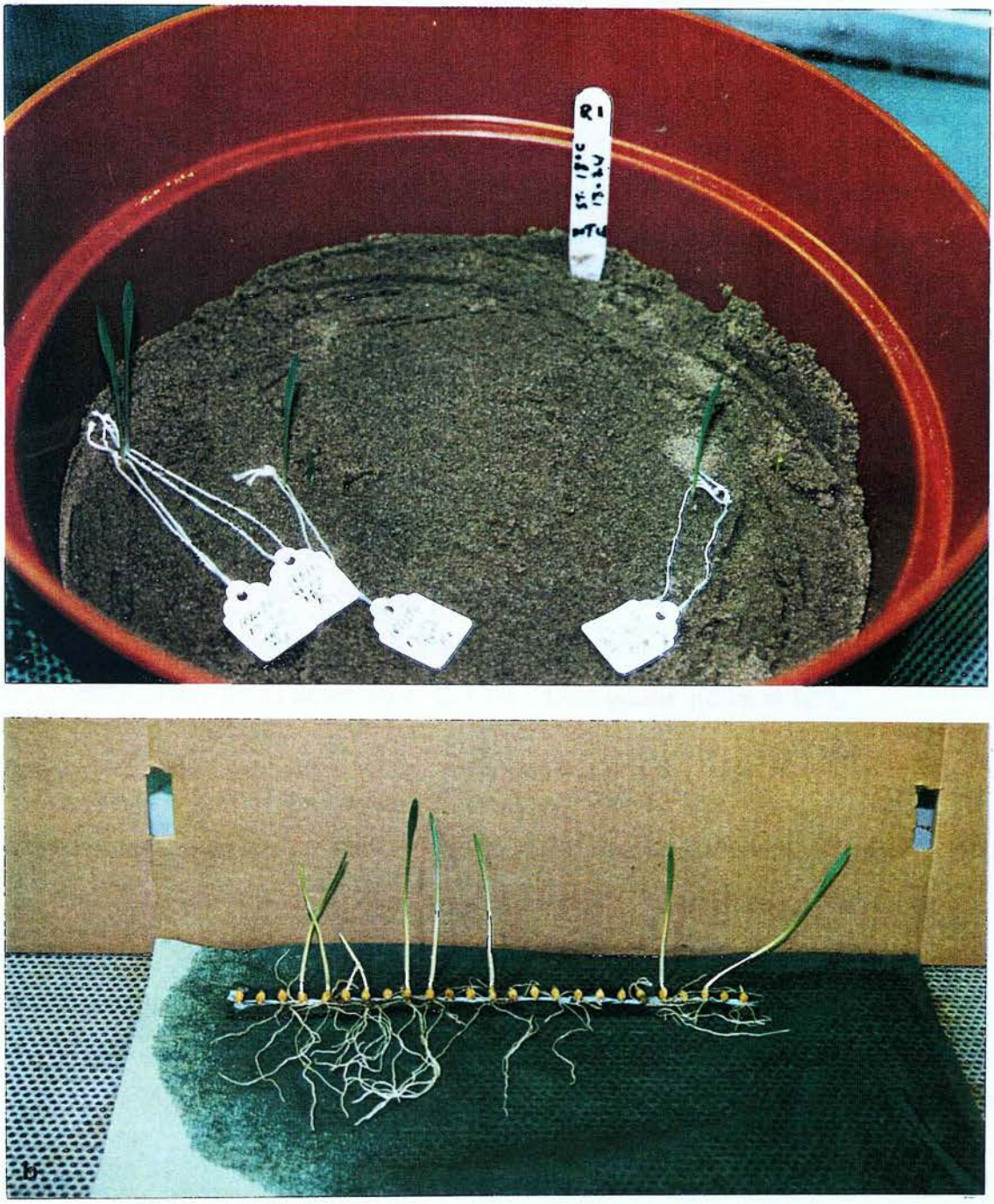


Plate 3.5. 1 (a) Poor seedling emergence due to dormancy of seeds of cv. Stirling from tiller ears grown at 18°C. (b) Seedlings and intact dormant seeds of cv. Stirling from grains of tiller ears grown at 18°C attached to paper strip after careful washing to remove sand particles.

3. 5. 2. Rate of seedling emergence

Experiment IV

The grains of cvs Blenheim, Stirling and Schooner (Gr & Gc) from plants grown in the growth rooms (Gr) and growth cabinets (Gc) at 18°C and 18-38°C were sown at 4 cm depth in fine sand in 30 cm pots to assess the effects of elevated temperatures during grain development on seedling emergence. Assessment of seedling emergence was carried out on the 5th, 6th and 7th days after sowing.

Seed lots 1 of cv. Blenheim from grains grown at 18°C and 18-38°C had greater seedling emergence on day 5 (i.e. day 1 of emergence) than seed lot 2 from grains grown in the corresponding temperature regimes (Figure 3.32a-c).

Seed lot 2 from grains of cv. Stirling grown at 18-38°C had greater seedling emergence on day 5 than seed lot 1 from grains grown at 18-38°C. The least seedling emergence on day 5 was observed in seed lot 2 from grains grown at 18°C. Approximately 50% of the grains in this seed lot were dormant. The rate of emergence of seedlings from grains of

cv. Schooner grown in a growth room (Gr) at 18°C was similar to that of grains of the same cultivar grown in a growth room at 30-38°C. The emergence of seedlings from grains of cv. Schooner grown in a growth cabinet at 18-38°C was considerably slower than that from grains of the same cultivar grown in a growth cabinet at 18°C.

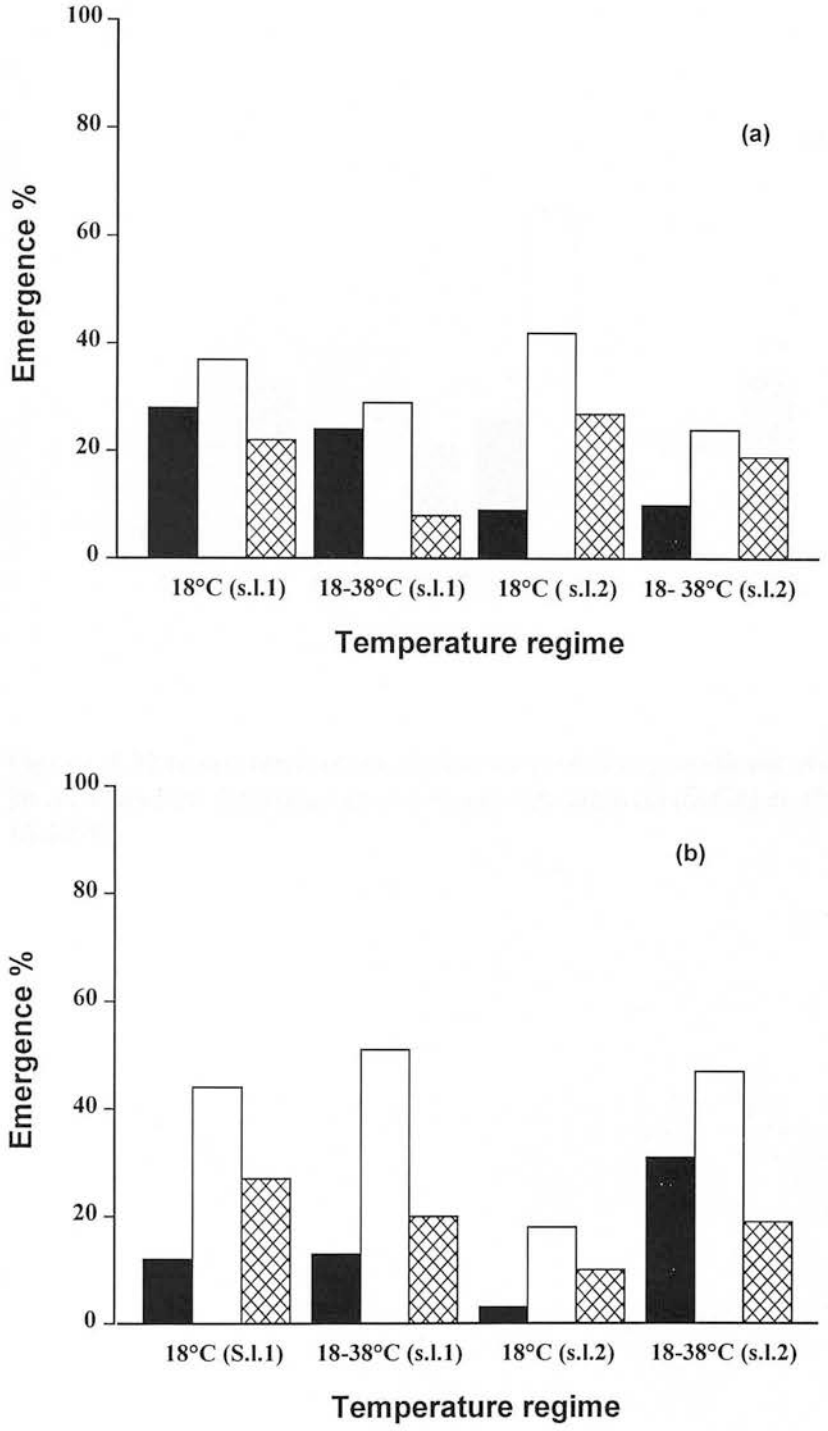


Figure. 3.32. Experiment IV. Rate of seedling emergence from seed lot 1 (s. l. 1) and seed lot 2 (s . l. 2) of grains grown at 18°C and 18-38°C during grain development. The numbers of seedlings emerged were counted on the 5th, 6th and 7th day after sowing. Solid histograms, day 5; open histograms, day 6; crosshatched histograms, day 7. (a) cv. Blenheim (b) cv. Stirling. % emergence was calculated on the basis of total number of seedlings which emerged/total number of seeds sown.

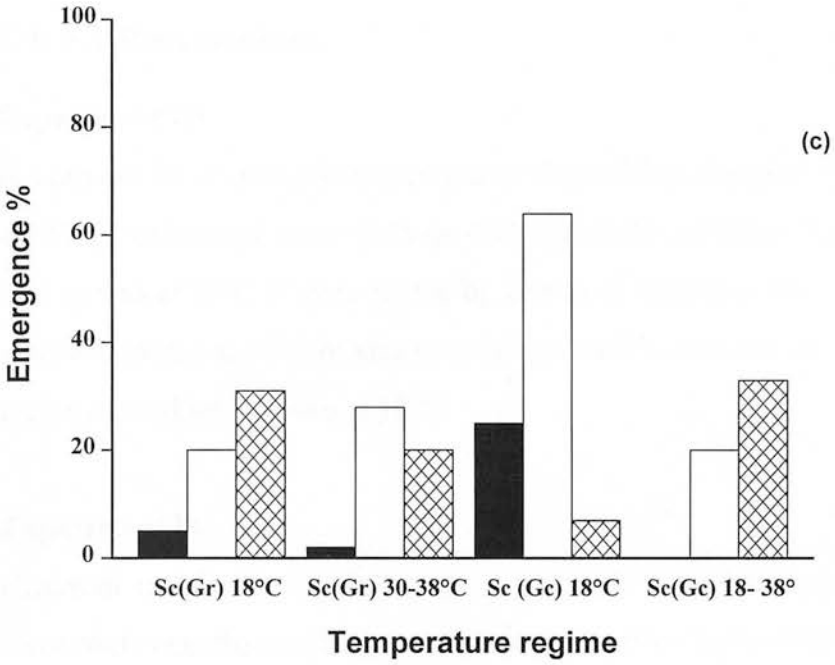


Figure. 3.32 (continued) (c) cv. Schooner grown in growth rooms (ScGr) at 18°C and 30-38°C and cv. Schooner grown in growth cabinets (ScGc) at 18°C and 18-38°C.

3. 5. 3.Seedling growth characteristics of emerged seedlings

3. 5. 3. 1.Root numbers.

Experiment III

In both cvs Stirling and Schooner grains of seed lot 1 grown at 18°C produced seedlings which had more roots ($p < 0.05$) than the seedlings from grains of seed lot 1 grown at 30°C (Figure 3.33 a, b). Grains of seed lot 1 of cv Schooner grown in a growth rooms at 18°C produced seedlings with more roots ($p < 0.05$) than did grains of seed lot 2 grown at 18°C.

Experiment IV

Grains of seed lot 1 of cv. Blenheim grown at 18°C produced seedlings which had more roots than the seedlings from grains of seed lot 2 grown at 18°C (Figure 3.33c). Similarly, seedlings of seed lot 1 of grains grown at 18-38°C had more roots per seedling than those of seed lot 2 grown at 18-38°C. Grains of seed lot 1 of cv. Stirling grown at 18°C produced seedlings which had more roots ($p < 0.05$) than the seedlings of seed lot 2 grown at 18°C but there was no significant difference in number of roots per seedling between seedlings from grains of seed lot 1 grown at 18-38°C and seedlings from grains of seed lot 2 grown at 18-38°C (Figure 3.33d). There were fewer roots in seedlings from grains of cv. Schooner grown at 30-38°C in a growth room and those grown in a growth cabinet at 18-38°C than in seedlings from grains grown at 18°C (Figure 3.33e).

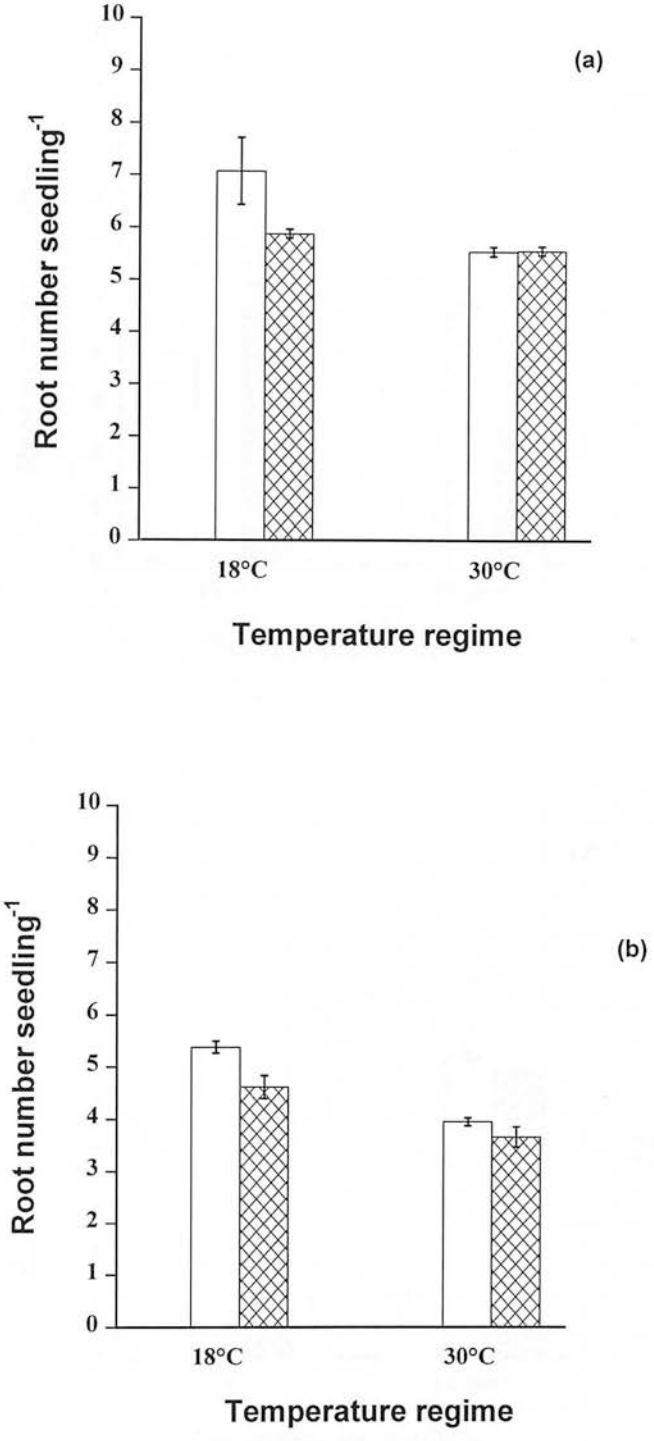


Figure 3.33. Experiment III. The number of roots per seedling from seed lot 1 (open histograms) and seed lot 2 (crosshatched histograms) grains grown at 18°C and 30°C in growth rooms. Root number was counted 7 d after the sowing of the seed in fine sand. (a) cv. Stirling (b) cv. Schooner (Gr) Error bars represent ± SEM; (n = 4).

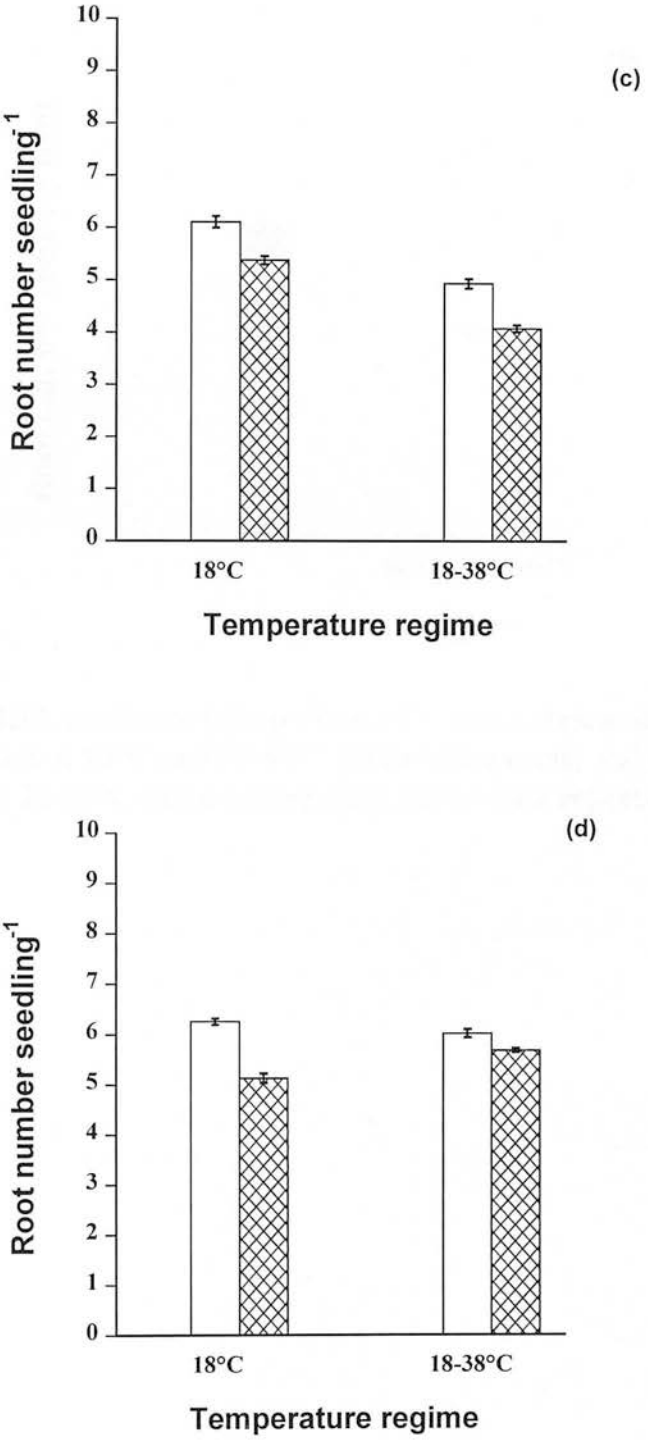


Figure 3.33. (continued) Experiment IV (c) cv. Blenheim (d) cv. Stirling Error bars represent ± SEM; (n = 4).

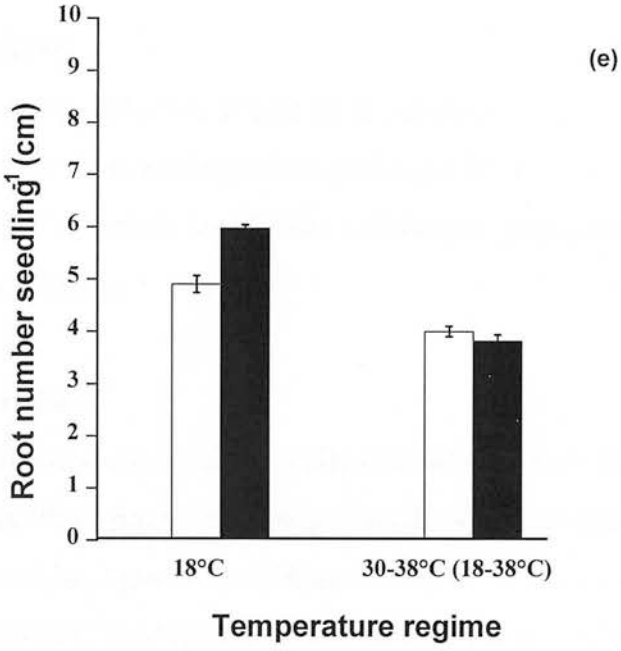


Figure. 3.33. (continued) Experiment IV. (e) cv. Schooner grown in growth rooms (Gr) at 18°C and 30-38°C (open histograms) and growth cabinets (Gc) at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 5. 3. 2. Plumule length

Experiment III

Analyses of mean plumule length measurements consistently showed no significant differences between seedlings from grains grown at 18°C and those from grains grown at 30°C. Plumule lengths in cv. Schooner were greater than those in cv. Stirling (Figures 3.34a, b).

Experiment IV

In cv. Blenheim there were no significant differences in the mean plumule lengths between seedlings produced from grains of seed lots 1 and those produced from grains of seed lot 2 grown at 18°C and 18-38°C (Figure 3.34 c). Grains of cv. Stirling grown at 18-38°C produced seedlings with longer ($p < 0.05$) plumules than those of seedlings from grains grown at 18°C (Figure 3.34d). Seedlings from grains of cv. Schooner grown at 18°C in growth room did not differ significantly in plumule length from seedlings from grains grown in growth room at 30-38°C. Grains of cv. Schooner grown in a growth cabinet at 18°C produced seedlings with much longer plumules than those of seedlings produced from grains grown at 18-38°C (Figure 3.34e).

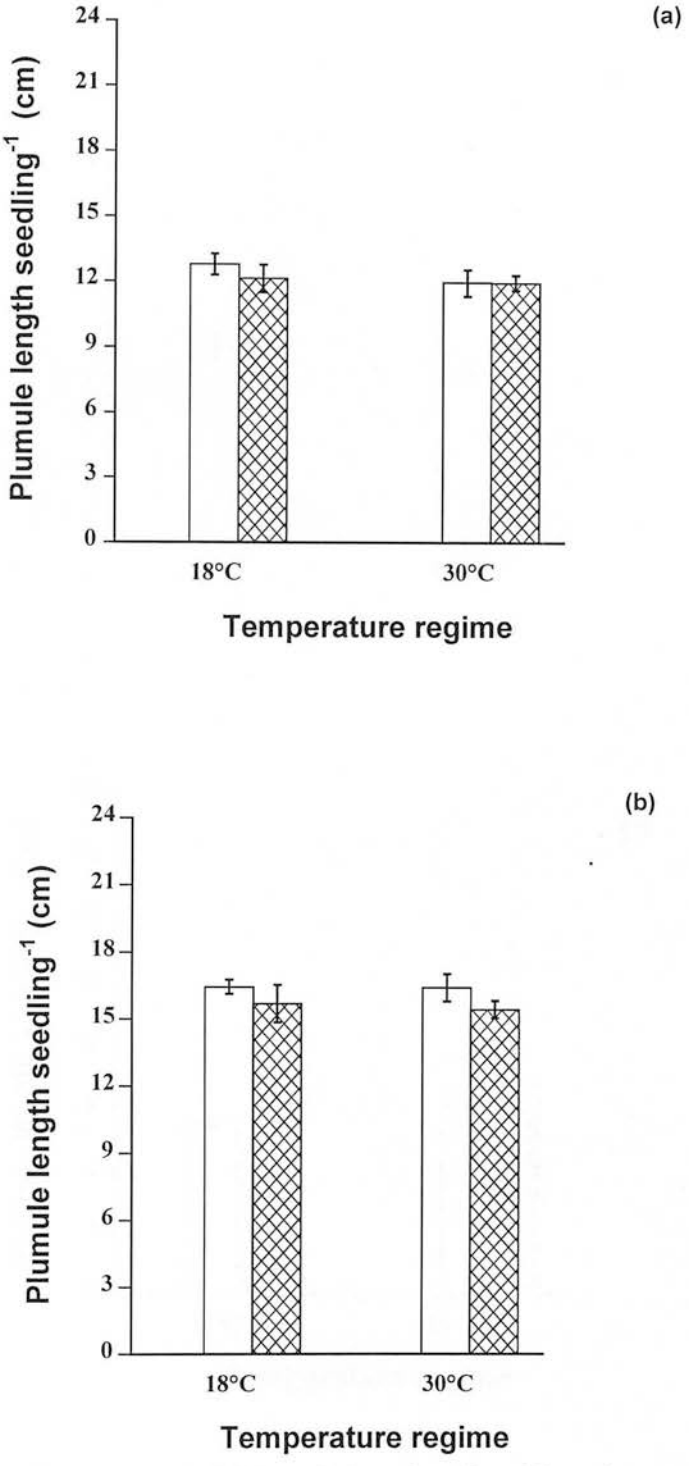


Figure. 3.34 Experiment III Plumule lengths of seedlings from seed lots 1 & 2 of grains grown at 18°C and 30°C during grain development and evaluated 7 days after sowing in sand. (a) cv. Stirling (b) cv. Schooner (Gr). Error bars represent ± SEM (n = 4). Key to shading in Figure 3.33.

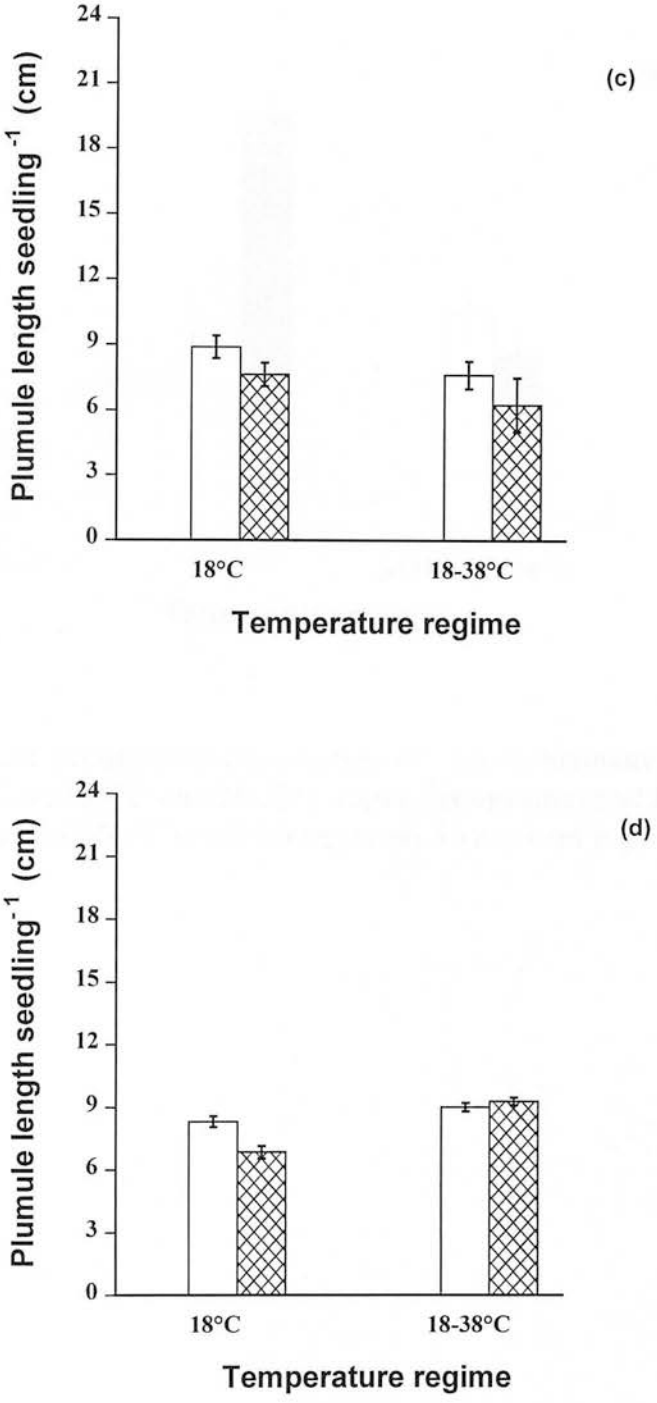


Figure 3.34. (continued) Experiment IV. (c) cv. Blenheim (d) cv. Stirling Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.33.

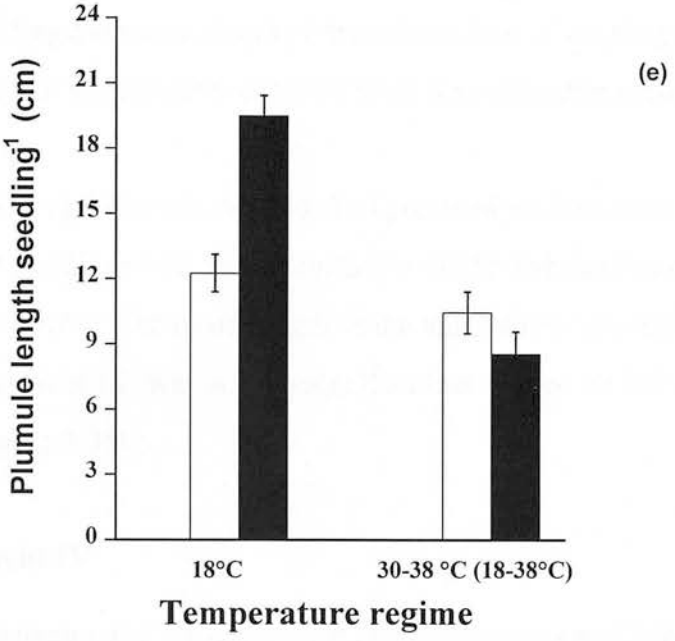


Figure 3.34. (continued) Experiment IV. (e) cv. Schooner grown in growth rooms (Gr) at 18°C and 30-38°C (open histograms) and in growth cabinets (Gc) at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 5. 3. 4. Root length

Experiment III

In cv. Stirling the mean length of the longest root of seedlings from grains grown at 18°C was not significantly different from that of seedlings from grains grown at 30°C

(Figure 3.35a). However, seed lot 1 of grains of cv. Schooner grown at 18°C produced seedlings with longer roots ($p < 0.05$) than seedlings from grains of seed lot 1 grown at 30°C. The mean length of the longest root of seedlings of seed lot 2 from grains grown at 18° was also greater than that of seed lot 2 from grains grown at 30°C (Figure 3.35b).

Experiment IV

In cvs Blenheim and Stirling grown in growth rooms and Schooner grown in a growth cabinet, the lengths of the longest roots of seedlings from grains grown at 18°C were longer ($p < 0.05$) than those of seedlings from grains grown at 18-38°C, whilst no significant difference in root length was detected between seedlings of cv. Schooner (Gc) from grains grown at 18°C and those of cv. Schooner from grains grown at 30-38°C

(Figures 3.35c-e)

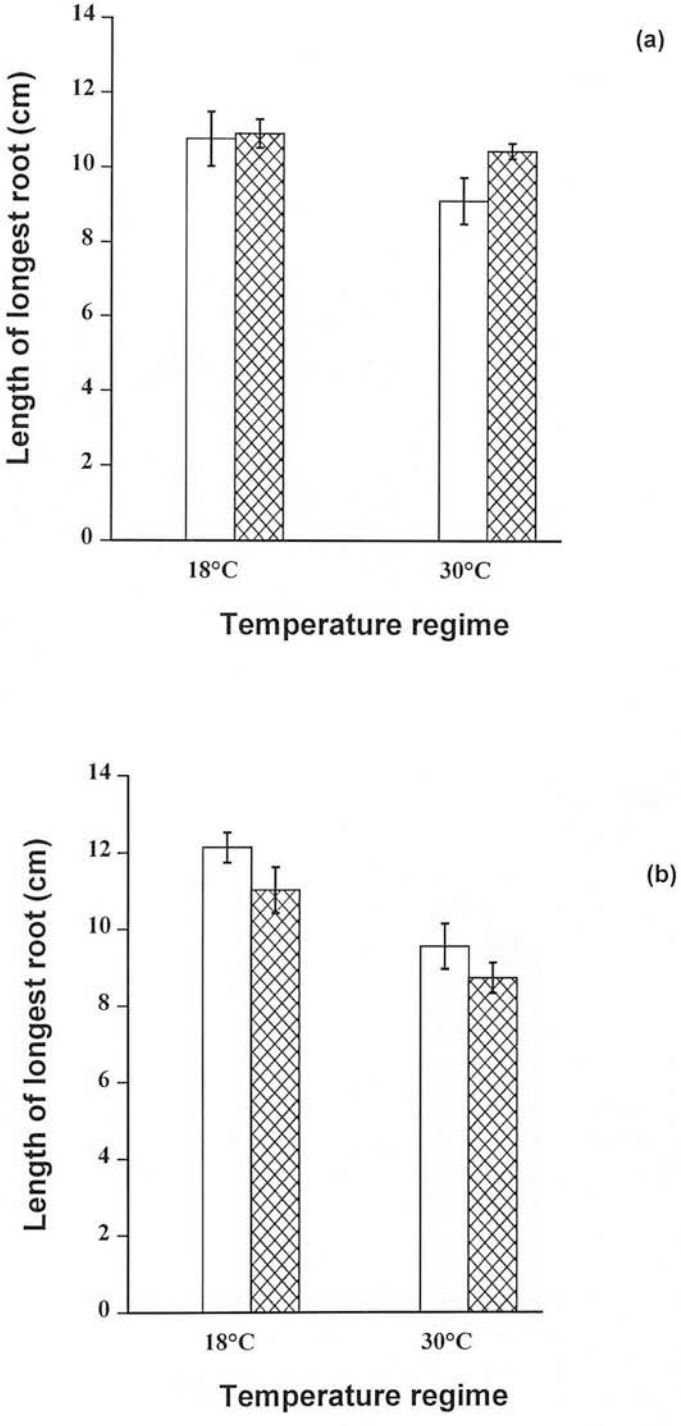


Figure 3.35 Experiment III. The length of the longest roots of seedlings grown from grains grown at 18°C and 30°C. Seedlings were evaluated 7 days after sowing in sand . (a) cv. Stirling (b) cv. Schooner (Gr). Error bars represent SEM ±, (n = 4). Key to shading as in Figure 3. 33

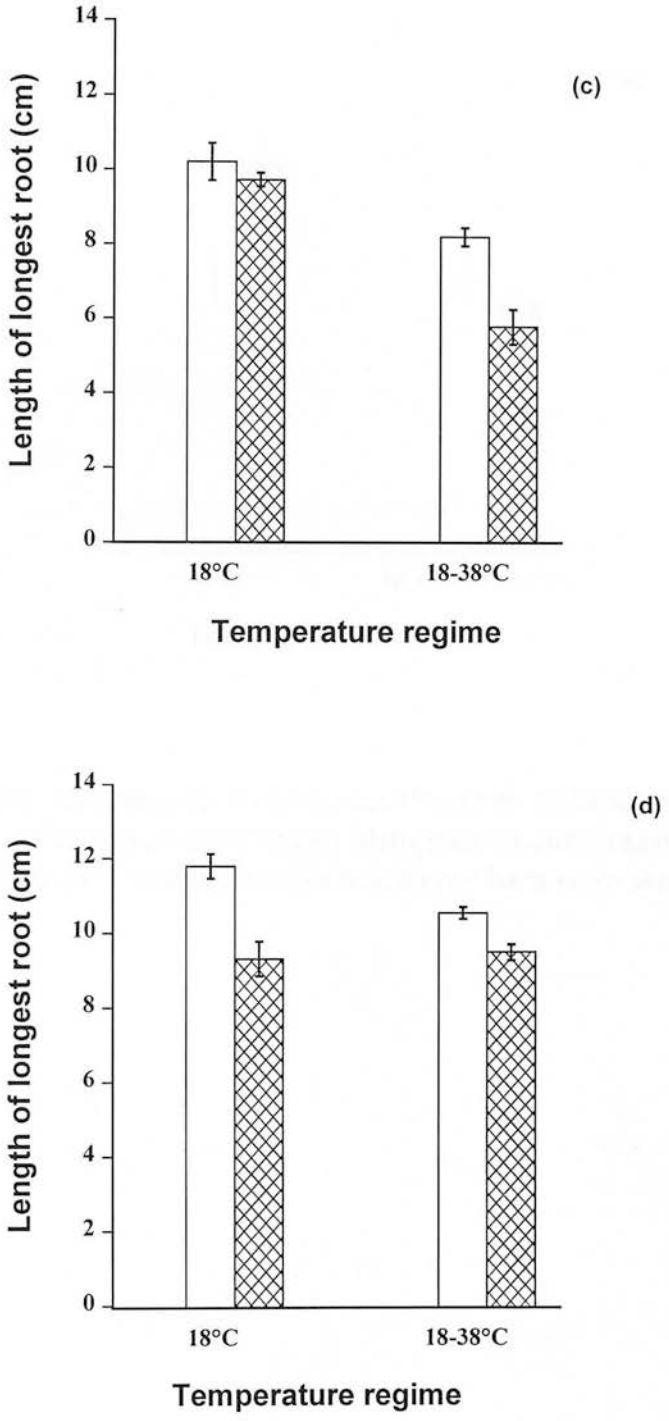


Figure 3.35. (continued). Experiment IV. cvs. Blenheim and Stirling grown in growth rooms at 18°C and 18-38°C (c) cv. Blenheim. (d) cv. Stirling. Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.33.

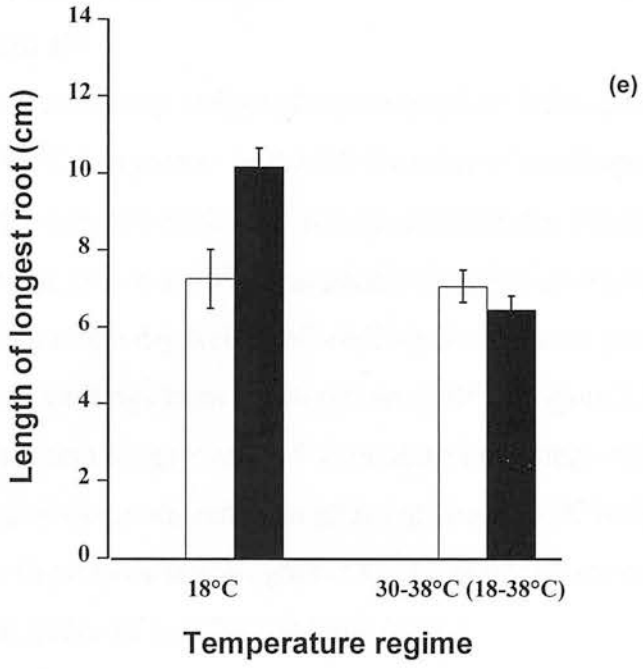


Figure 3.35. (continued). Experiment IV. (e) cv. Schooner grown in growth rooms at 18°C and 30-38°C (open histograms) and in growth cabinets (Gc) at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 5. 3. 5. Plumule dry weight

Experiment III

The mean plumule dry weight of seedlings of cv. Stirling from seed lot 1 of grains grown at 18°C was greater ($p < 0.05$) than that of seedlings from seed lot 2 grown in the same temperature regime. Similarly, plumule dry weight of seedlings from seed lot 1 of grains grown at 30°C was greater than that of seedlings of seed lot 2 grown at 30°C. The plumule dry weight of seedlings from grains grown at 18°C was greater than that of seedlings from grains grown at 30°C (Figure 3.36a). Grains of seed lot 1 of cv. Schooner (Gr) grown at 18°C produced seedlings with greater plumule dry weight than those produced from grains grown at 30°C but there was no significant difference in plumule dry weight between seedlings from grains of seed lot 1 and those from grains of seed lot 2 (Figure 3.36b).

Experiment IV

Grains of seed lot 1 of cv. Blenheim grown at 18°C and 18-38°C produced seedlings with greater ($p < 0.05$) plumule dry weights than seedlings from grains of seed lot 2 grown at 18°C and 18-38°C respectively (Figure 3.36c).

Seedlings of cv. Stirling from grains grown at 18°C did not differ significantly in mean plumule dry weight from seedling grown from grains grown at 18-38°C (Figure 3.36d). Plumule dry weight values in cv. Schooner seedlings grown from grains which were grown in growth rooms at 18°C and 30-38°C (Gr) followed a similar pattern to that observed in cv. Stirling (Expt. IV). In contrast, grains of cv. Schooner grown in a growth cabinet at 18°C produced seedlings which had greater plumule dry weights than seedlings from grains grown at 18-38°C (Figure 3.36e).

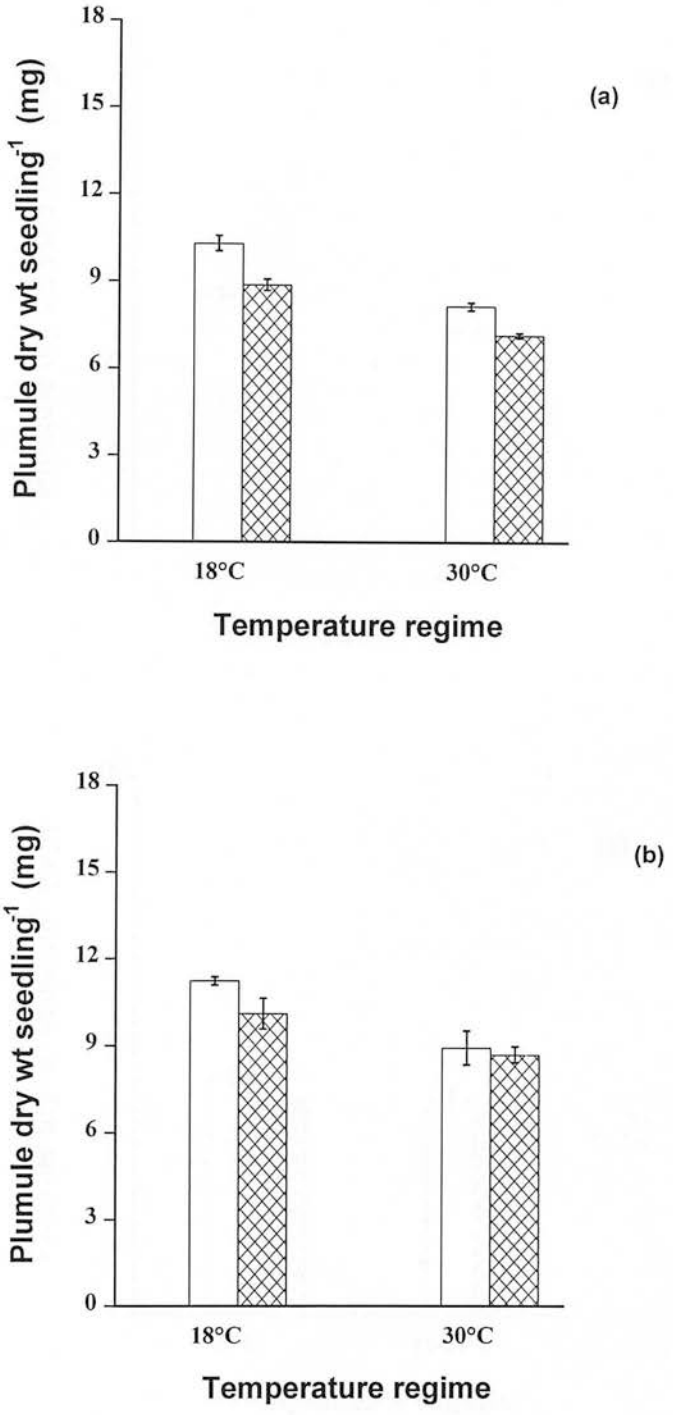


Figure 3.36. Experiment III. Plumule dry weight of seedlings from grains grown in growth rooms at 18°C and 30°C. Seedlings were evaluated 7 days after sowing in sand . (a) cv. Stirling (b) cv. Schooner (Gr). Error bars represent SEM ±; (n = 4). Key to shading as in Figure 3.33.

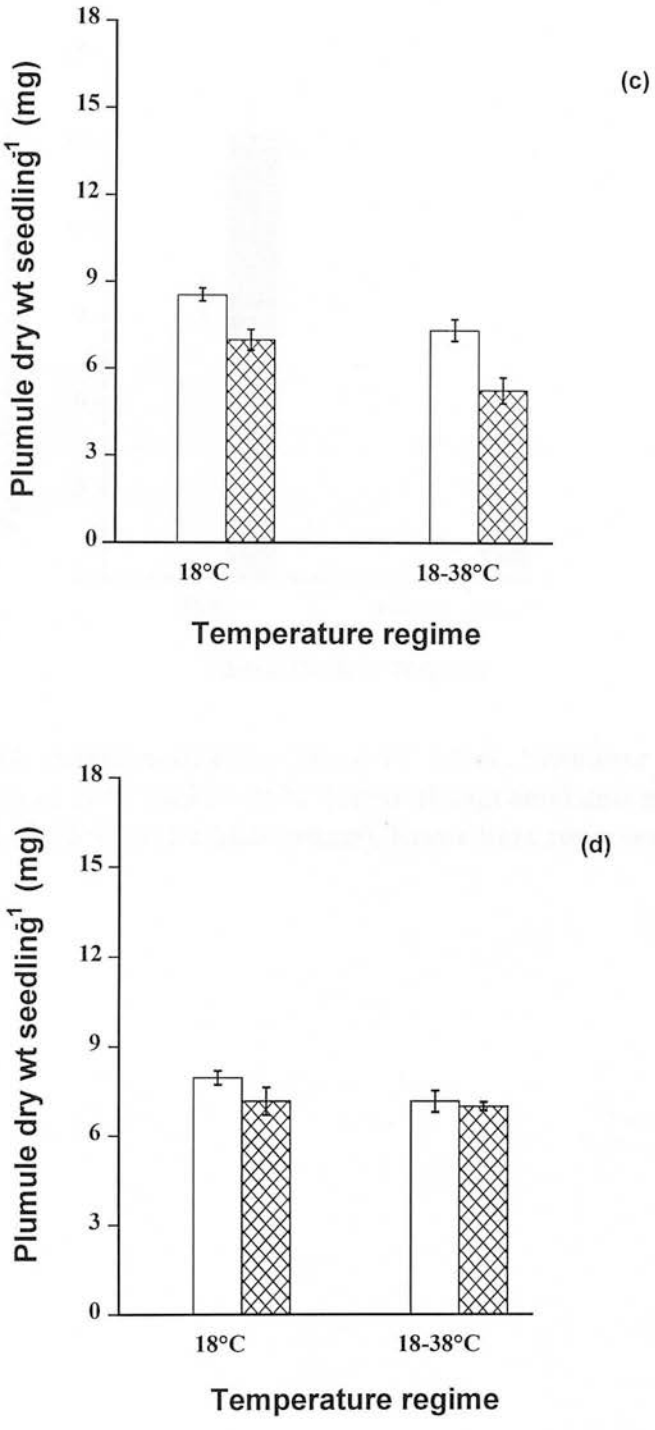


Figure 3.36.. (continued). Experiment IV. cvs Blenheim and Stirling grown in growth rooms at 18°C and 18-38°C. (c) cv. Blenheim (d) cv. Stirling. Error bars represent ± SEM; (n = 4).

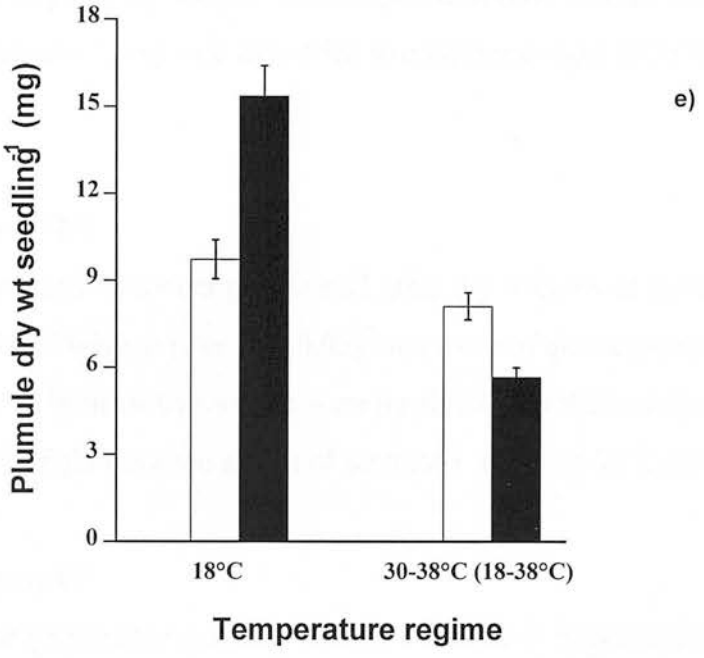


Figure 3.36. (continued) Experiment IV. (e) cv. Schooner grown in growth rooms (Gr) at 18°C and 30-38°C (open histograms) and growth cabinets (Gc) at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 5. 3. 6. Germinated grain dry weight

Germinated grain dry weight was composed of husk and the remnants of the endosperm and embryonic axis dried to constant weight at 70°C after 7 d seedling growth.

Experiment III

cvs Stirling and Schooner germinated grain dry weights of grains grown in a growth room at 18°C were greater ($p < 0.05$) than those of grains grown at 30°C (Figure 3.37a, b). In both cultivars there were no significant differences in mean germinated grain dry weight between grains of seed lot 1 and seed lot 2 grown at 18°C and 30°C.

Experiment IV

Values for germinated grain dry weight obtained in Experiment IV followed a similar pattern to those observed in Experiment III in which germinated grain dry weights were greater in grains grown at 18°C than those grown at 18-38°C or 30-38°C (Schooner Gc) (Figures 3.37c, d, e).

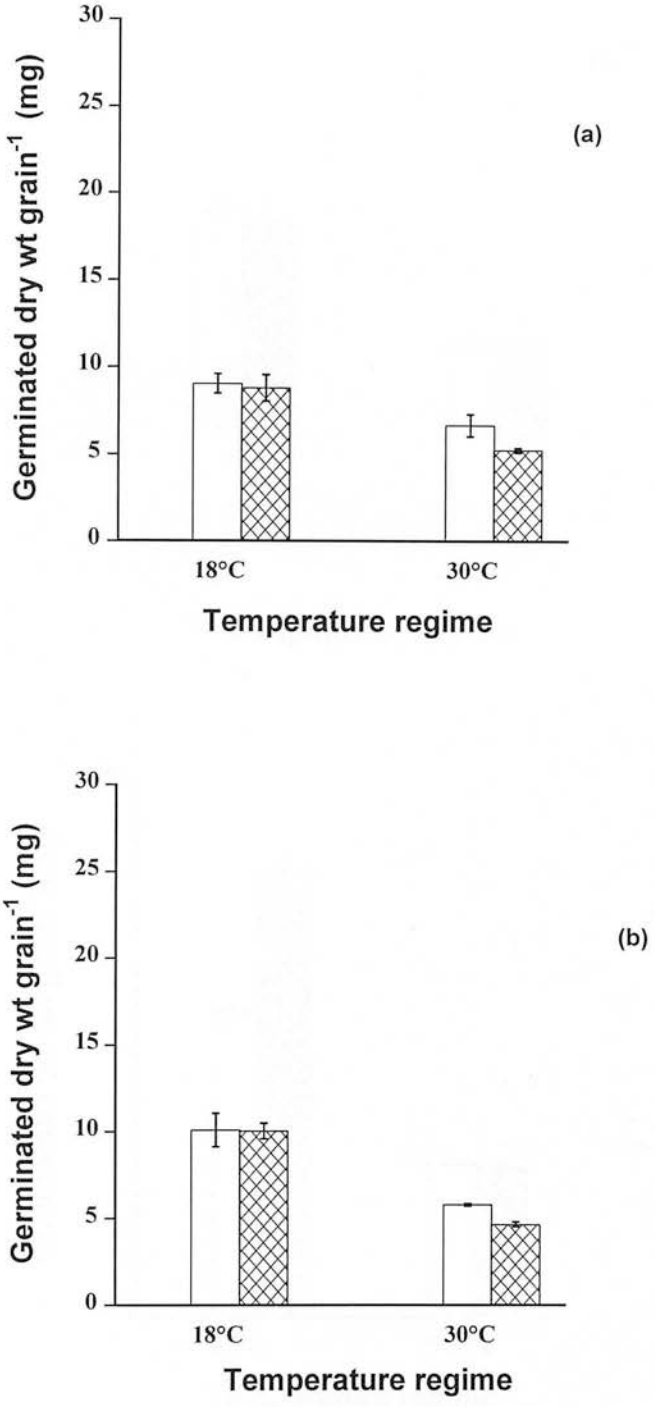


Figure. 3.37. Experiment III. Germinated grain dry weight of seedlings evaluated 7 days after sowing in sand.(a) cv. Stirling (b) cv. Schooner (Gr). Error bars represent ± SEM; (n = 4). Key to shading as in Figure 3.33

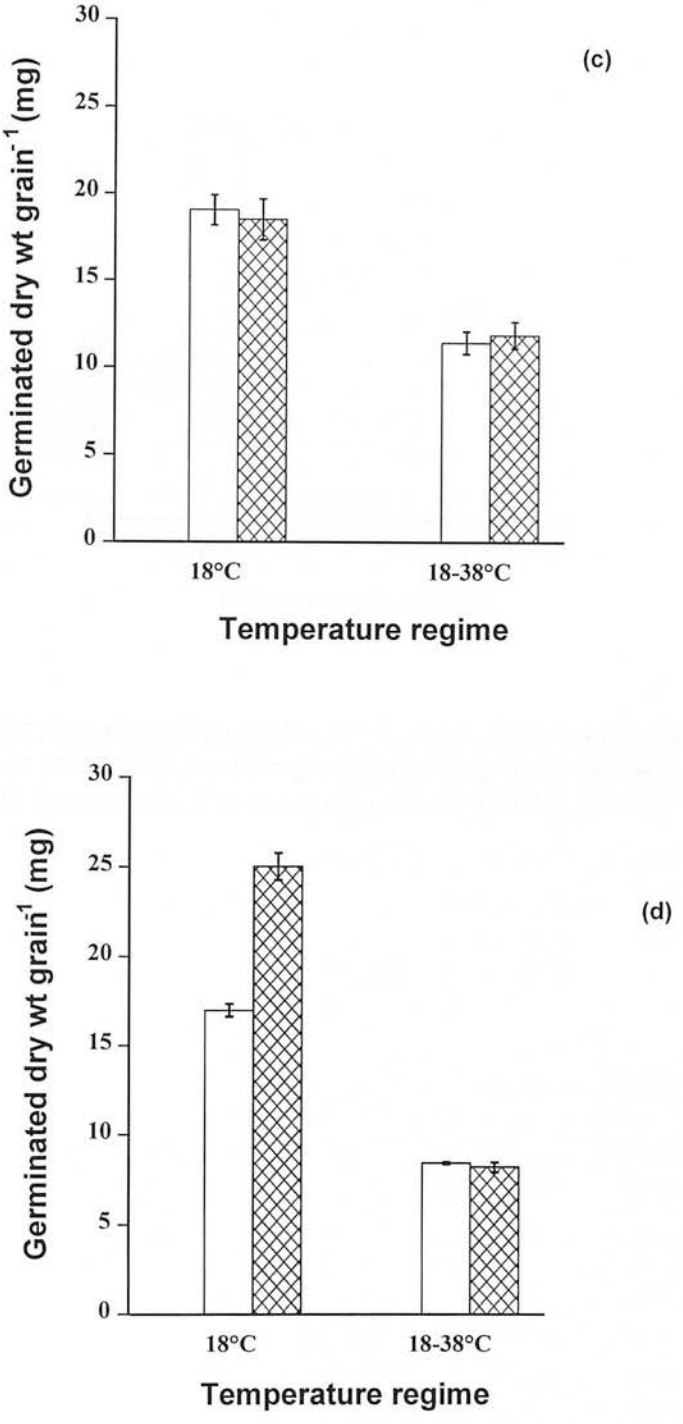


Figure. 3.37. (continued) Experiment IV. cvs. Blenheim and Stirling grown in growth rooms at 18°C and 18-38°C (c) cv. Blenheim (d) cv. Stirlng.

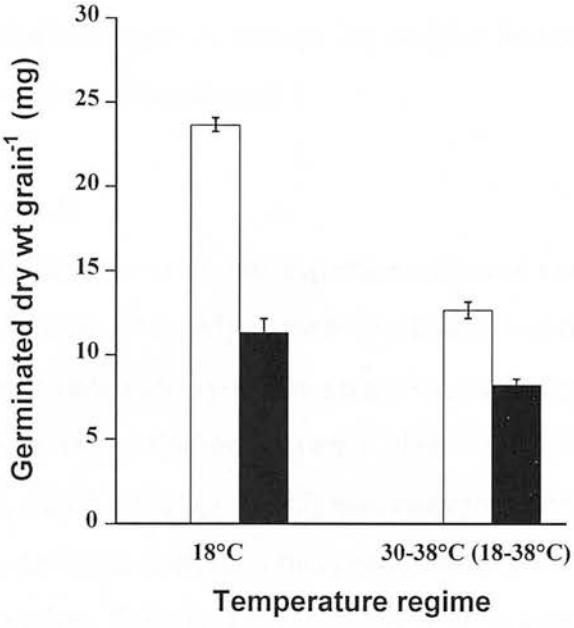


Figure. 3.37. (continued) Experiment IV. (e) cv. Schooner grown in growth rooms (Gr) at 18°C and 30-38°C (open histograms) and in growth cabinets (Gc) at 18°C and 18-38°C (solid histograms). Error bars represent ± SEM; (n = 4).

3. 6. Embroy dry weights

Due to insufficient grains, embryo dry weights assessment was not carried out on grains harvested in Experiment I.

Experiment II

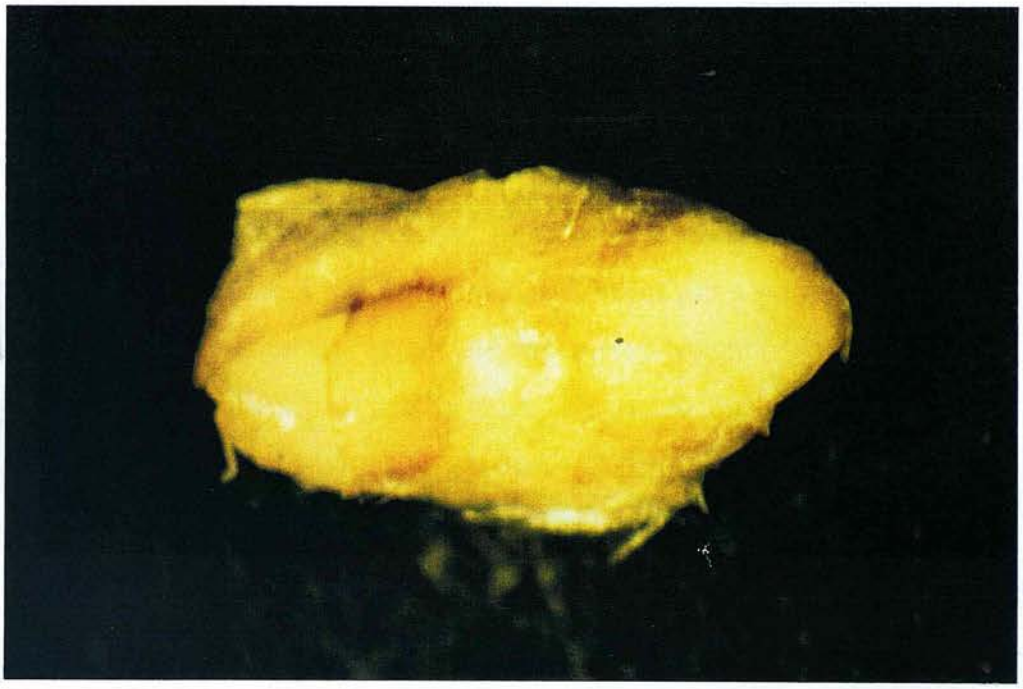
The results obtained from this experiment showed that grains of cv. Blenheim which had experienced 30°C early in their development or for all of their developmental period, had smaller embryos than grains which experienced 18°C from anthesis for most or all their developmental period (Table 3.9a). Embryos from grains grown at 18°C (A), were heavier ($p < 0.05$) than embryos from grains grown at 30-18°C (C). Similarly, embryos from (C) grains had greater ($p < 0.05$) dry weight than embryos from (D) grains. There was a significant positive correlation between grain dry weight and embryo dry weight (r = 0.91).

It was further observed that some of the (D) and (C) grains appeared to have undergone pre-germination. The fact that these grains were pre-germinated was apparant only when the husks were removed during the embryo extraction. The rupture of the germ aleurone by the elongating coleoptile was observed because the torn edge of the germ aleurone/testa had a rim of golden- brown material around the emerging coleoptile of the pre-germinated grains (**Plate 3. 6 a**). In some of the pre-germinated grains an elongation of the coleoptile under the pericarp /husk was observed (**Plate 3.6 b**). Pre-germination was not observed in grains grown at 18°C (A) and 18-30°C (B).

Table 3.9a. Experiment II. Grain and embryo dry weights and % pre-germination of grains (seed lot 1) of cv. Blenheim grown in growth rooms in four temperature regimes Mean values ± standard deviation (s.d.) n = 3.

Temperature regime	(A) 18°C	(B) 18-30°C	(C) 30-18°C	(D) 30°C
Grain dry wt. (mg ± s.d.)	40.97 ± 2.1	41.23 ± 1.6	24.5 ± 0.6	15.78 ± 0.7
Embryo dry wt. (mg ± s.d.)	1.67 ± 0.08	1.56 ± 0.05	1.31 ± 0.07	0.87 ± 0.04
% pre-germination	0	0	29 ± 18	25 ± 13

a



b

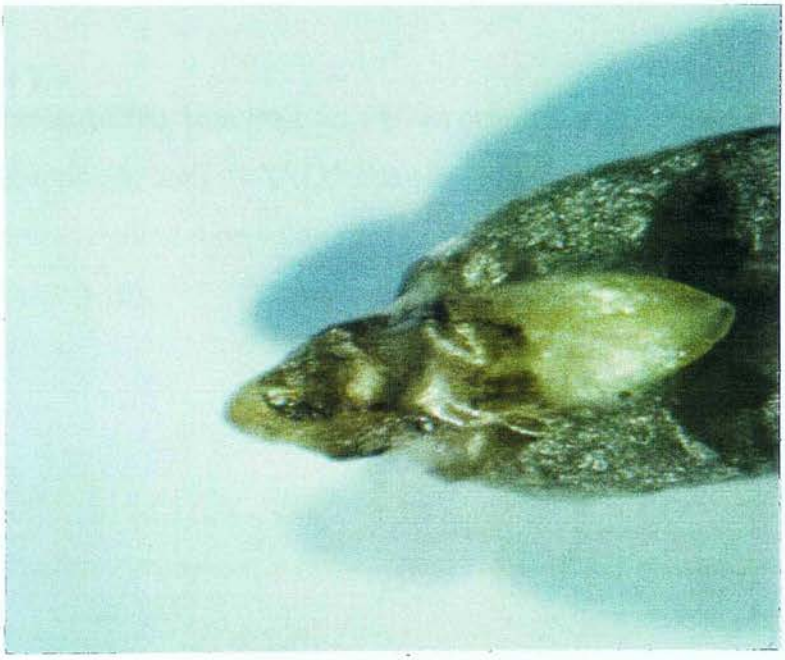


Plate 3. 6 a Embryo from pre-germinated grain showing a golden-brown colour at the end of the ruptured germ aleurone/testa. b Elongation of coleoptile under pericarp/husk of pre-germinated grain.

Experiments III & IV

The mean grain dry weights and mean embryo dry weights of grains grown at 18°C were greater than those of grains grown in elevated temperature regimes (Table 3.9b). The mean grain dry weight was higher in cvs Blenheim and Schooner (Gr) from grains grown at 18-38°C and 30-38°C (Experiment IV) than in grains of cv. Stirling grown at 18-38°C (Experiment IV). There was a significant positive correlation between grain dry weight and embryo dry weight ($r = 0.95$).

Pre-germination was observed only in grains exposed to high temperatures. Grains of cv. Schooner grown in a growth room at 30°C (Experiment III) had a lower percentage of pre-germinated grains than grains of the same cultivar grown in a growth room at 30-38°C (Experiment IV). However, in growth cabinet-grown grains of cv. Schooner, the percentage pre-germination in grains grown at 18-38°C in Experiment IV did not differ significantly from that in grains grown in the same temperature regime in Experiment III.

Experiment V

Embryos were extracted from grains of eleven genotypes grown under the glasshouse conditions at approximately 18°C/13°C day and night temperatures. There was a significant positive correlation between grain dry weight and embryo dry weight ($r = 0.87$) (Table 3.10).

Table 3.9b.Experiments III and IV. Grain and embryo dry weights and % pre-germination of grains grown in growth rooms and growth cabinets in different temperature regimes during grain development. Mean values ± standard deviation (s.d.) n = 3

Cultivar	Expt. number	Temperature regime	Seed lot	Grain dry wt (mg ± s.d)	Embryo dry wt (mg ± s.d.)	Pre-germination %
Schooner(Gr)	III	18°C	1	35.5 ± 0.78	1.44 ± 0.04	0
Schooner(Gr)	III	30°C	1	22 0 ± 2.07	1.06 ± 0.15	10.3 ± 5.5
Schooner(Gr)	IV	18°C	1	46 0 ± 0.45	1.78 ± 0.03	0
Schooner(Gr)	IV	30-38°C	1	31.4 ± 1.09	1.67 ± 0.13	29.0 ± 3.6
Schooner(Gc)	III	18°C	1	34.1 ± 1.99	1.49 ± 0.08	0
Schooner(Gc)	III	18-38°C	1	16 0 ± 0.30	0.95 ± 0.06	16.3 ± 1.16
Schooner(Gc)	IV	18°C	1	45.0 ± 0.93	1.77 ± 0.10	0
Schooner(Gc)	IV	18-38°C	1	18.7 ± 2.31	0.98 ± 0.10	20.3 ± 7.2
Stirling	IV	18°C	1	38.8 ± 2.01	1.49 ± 0.04	0
Stirling	IV	18-38°C	1	27.1 ± 1.26	1.28 ±0.1	28.3 ± 5.8
Blenheim	IV	18°C	1	49.7 ± 2.48	1.92 ± 0.02	0
Blenheim	IV	18-38°C	1	37.2 ± 1.82	1.62 ± 0.11	45.0 ± 5.0

Table 3.10 Grain and embryo dry weights of eleven genotypes grown in the glasshouse at approximately 18°C/13°C. Mean values ± standard deviation (s.d.) n = 4.

Genotypes	Grain dry wt (mg ± s.d.)	Embryo dry wt. (mg ± s.d.)
BRB2	51.0 ± 1.33	1.71 ± 0.05
Harrington	49.3 ± 0.85	1.66 ± 0.05
Clipper	66.8 ± 6.16	2.26 ± 0.06
Blenheim	50.3 ± 1.32	1.75 ± 0.08
Schooner	50.1 ± 2.86	2.12 ± 0.19
Puebla	45.6 ± 1.62	1.66 ± 0.07
Centinella	32.4 ± 0.69	1.24 ± 0.07
Stirling	43.3 ± 3.02	1.81 ± 0.14
Esmeranda	42.4 ± 1.12	1.51 ± 0.15
Esperanza	41.9 ± 1.51	1.52 ± 0.06
Tyne	35.3 ± 2.59	1.24 ± 0.06

3.7. Embryo growth on artificial media.

In order to compare the growth potential of embryos from grains grown in different temperature regimes, embryos were extracted from grains under sterile conditions and incubated on (a) 1% w/v agar, (b) 1 % w/v agar, 0.9% w/v glucose, (c) 1% w/v agar, 0.9% w/v mannitol at 20°C ± 1.5°C in the dark. Measurements of seedling plumule length and roots were made after 5 days. The embryos were extracted from grains of seed lot 1 (see Table 3.9b) except for (D) of Experiment II in which seed lot 2 was used instead of seed lot 1 because of insuficient seeds. The mean grain dry weight of grains used from Experiment II seed lot 2 (D) was 23.8 ± 2.5 mg and mean embryo dry weight was 1.4 ± 0.1 mg (s.d.).

3. 7. 1. Plumule length

Experiment II

Embryos extracted from grains which were grown in high temperature regimes (C and D), produced seedlings with longer plumules (Figure 3.38a) than those of seedlings from embryos of grains grown in temperature regimes (A) and (B). Plumule growth was greater on the medium containing glucose than on the other media. Seed lots (D) and (C) contained a higher proportion of pre-germinated grains than seed lots (A) and (B), and seedlings grown from (D) and (C) embryos had much greater variability in plumule length than seedlings grown from (A) and (B) embryos.

Experiment III

In contrast to Experiment II, on all three media embryos of cv. Schooner grown in the growth cabinets at 18°C produced seedlings in which the plumules were longer than those in seedlings from embryos of grains grown at 18-38°C, however, these differences were not statistically significant (Figure 3 38b).

Experiment IV

When grown on all three media, embryos of cv. Blenheim from grains grown at 18-38°C produced seedlings in which the plumules were longer than those in seedlings from embryos of grains grown at 18°C, however, the differences were not statistically significant (Figure 3.38c). Greater variability in plumule length was observed in seedlings from embryos of grains grown at 18°C than from embryos of grains grown at 18-38°C.

In cv. Schooner grown in growth cabinets, embryos from grains grown at 18-38°C produced seedlings in which the plumules were longer than those in seedlings from embryos of grains grown at 18°C, however, the differences were not statistically significant (Figure 3.38d). Embryos from grains grown at elevated temperatures germinated after a very short period of incubation whereas embryos of grains grown at 18°C took a longer time to germinate.

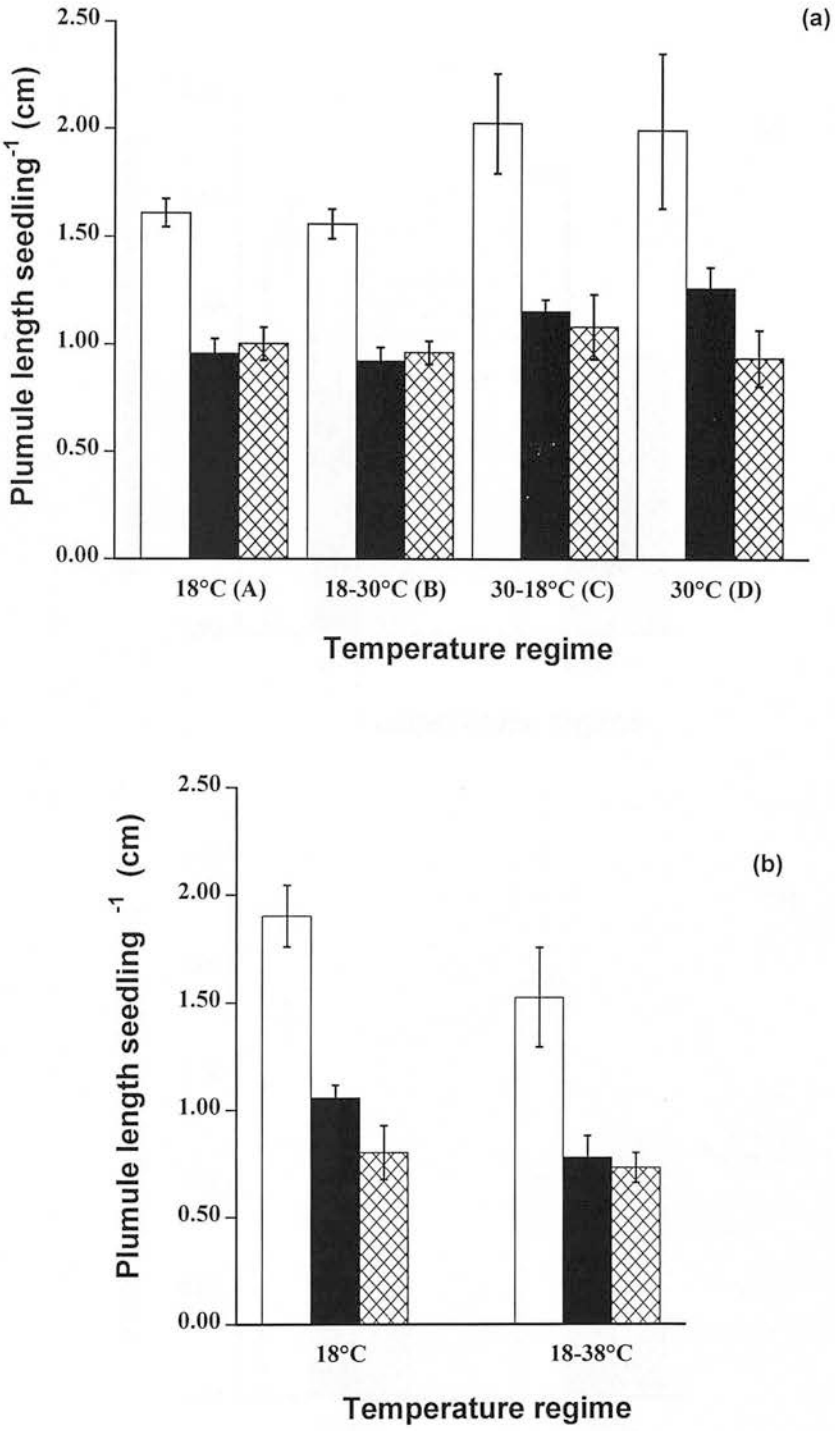


Figure 3.38 Growth of embryos from grains of barley subjected to different temperature regimes during grain development. Plumule lengths of five day old seedlings grown from extracted embryos on artificial media, agar 1% w/v + glucose 0.9% w/v (open histograms); agar 1% w/v (solid histograms); agar 1% w/v+ mannitol 0.9% w/v (crosshatched histograms) (a) cv. Blenheim grown in growth rooms at 18°C, 18-30°C, 30-18°C and 30°C (Expt. II). (b) cv. Schooner grown in growth cabinets at 18°C and 18-38°C (Expt. III). Error bars represent ± SEM; (n=3).

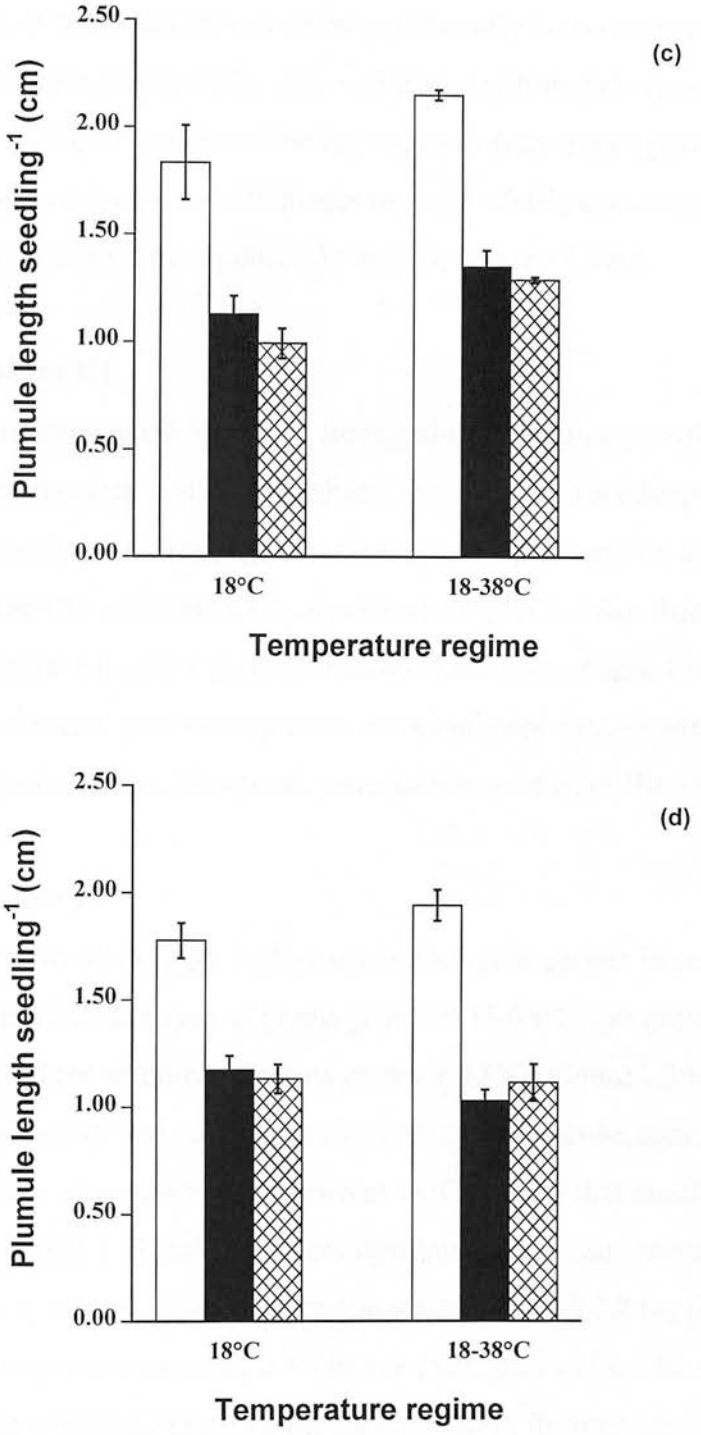


Figure 3.38. (continued) (c) cv. Blenheim (d) cv. Schooner (Gc) (Expt. IV). Error bars represent ± SEM; (n = 3).

3. 7. 2. Root length

Experiment II

Seedlings of cv. Blenheim from embryos of grains grown in temperature regimes (A), (B), (C) and (D) did not differ significantly in root length from each other when embryos were grown on the agar + glucose medium but when embryos were grown on agar and agar + mannitol media, embryos from grains grown in regimes (C) and (D) produced seedlings with longer roots ($p < 0.05$) than those extracted from grains grown in temperature regimes (A) and (B) (Figure 3.39a).

Experiment III

When embryos of cv. Schooner from grains grown in a growth cabinet at 18°C were grown on the agar + glucose medium, they produced seedlings which had longer roots than those of seedlings from embryos of grains grown at 18-38°C but the difference was not statistically significant (Figure 3.39b). Root growth was very much greater on agar + glucose than on either agar or agar + mannitol medium. Root growth was less than shoot growth when embryos were grown on the agar or on agar + mannitol medium. This trend was also observed in cv. Blenheim Experiment II.

Experiment IV

When grown on the agar + glucose medium, root growth in seedlings of cv. Blenheim from embryos of grains grown at 18-38°C was greater than that in seedlings from embryos of grains grown at 18°C (Figure 3.39c). Observation of seedling growth from embryos grown on agar + glucose, agar, and agar + mannitol from grains of cv. Blenheim grown at 18°C showed that seedlings from embryos grown on agar + glucose had more developed roots and shoots than seedlings from embryos grown on agar and agar + mannitol (**Plate 3.7.2 (a) (i, ii, iii)**. Seedlings from embryos grown on agar + glucose from grains of cv. Blenheim grown at 18-38°C had a true leaf much earlier than seedlings from grains grown at 18°C whereas seedlings from grains grown at 18°C had more roots than seedlings from grains grown at 18-38°C **(Plate 3. 7. 2 (b).(i, ii, iii)**. The the true leaf in the seedlings from grains grown at 18-38°C seems to suggest that the embryos were pre-germinated.

The root length of seedlings of cv. Schooner from embryos of grains grown at 18°C was not significantly different from that of seedlings from embryos of grains grown at 18-38°C (Figure 3.39d). Root growth was consistently less than shoot growth when embryos of grains grown at high temperature during grain development were grown on the agar and agar + mannitol media. Seedling growth from embryos from grains of cv. Schooner grown in a growth room at 18°C is illustrated in (**Plate 3. 7. 2.(c) i, ii, iii)**.

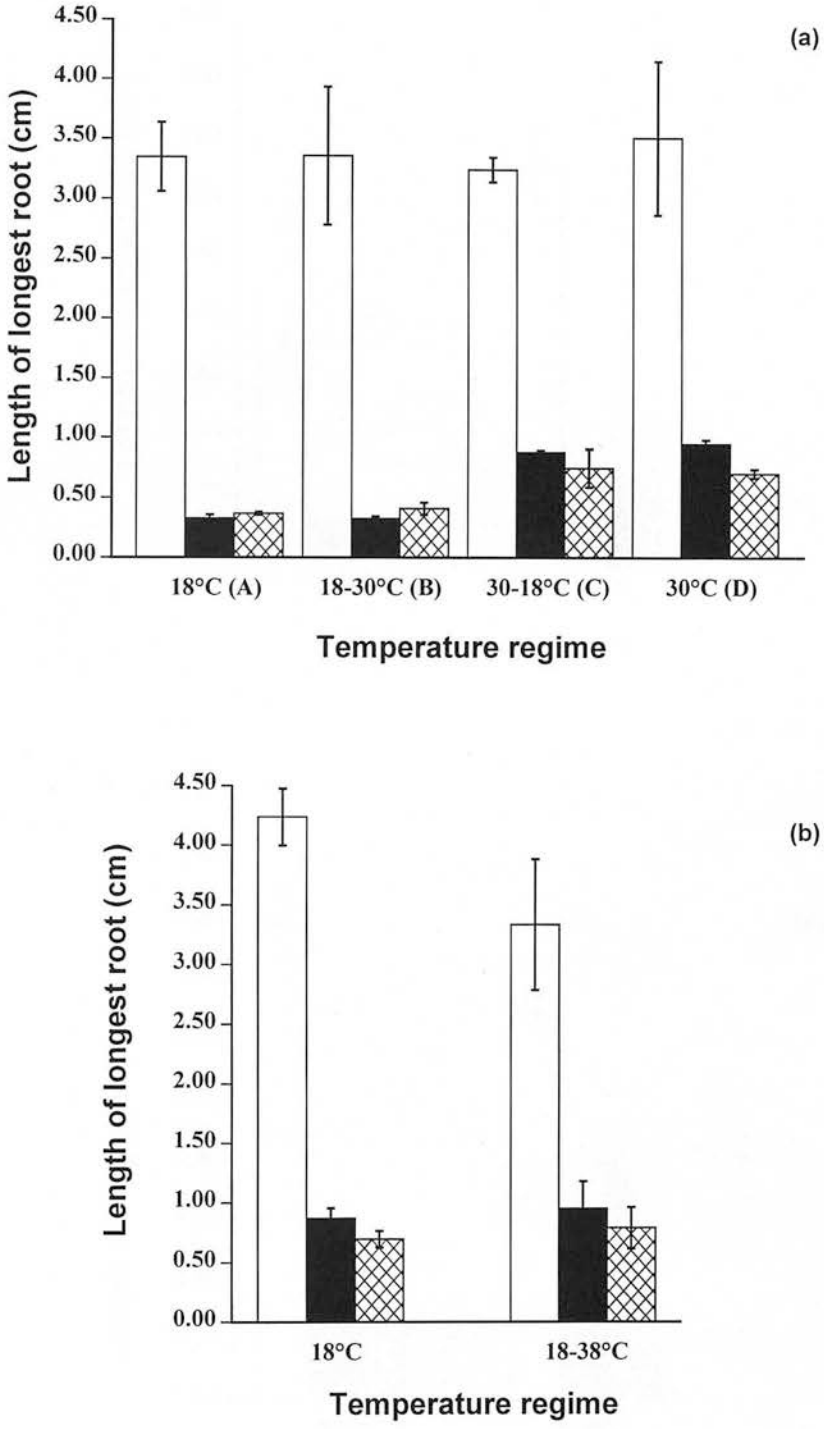
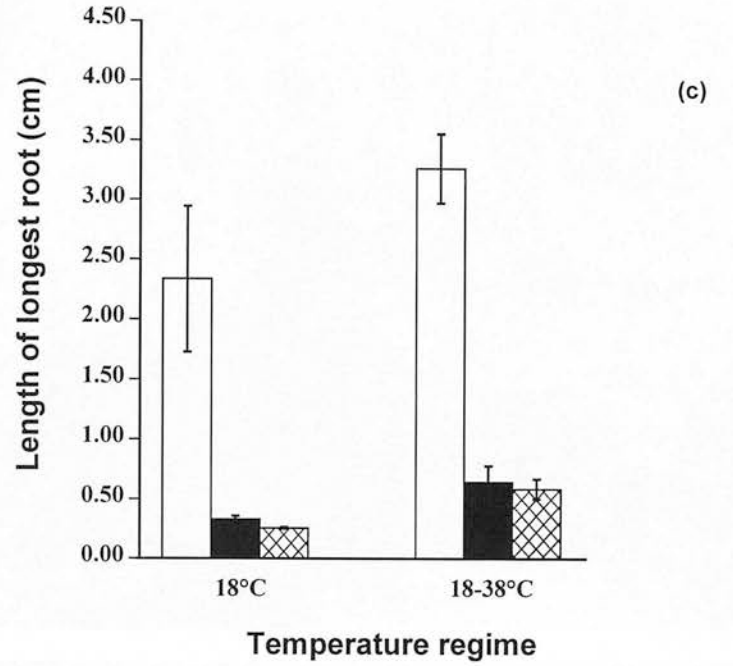


Figure 3.39. Lengths of longest roots of five day old seedlings from embryos of grains of barley subjected to different temperature regimes during grain development. (a) cv. Blenheim grown in growth rooms at 18°C, 18-30°C, 30-18°C and 30°C (Expt. II). (b) cv. Schooner grown in growth cabinets at 18°C and 18-38°C (Expt. III). Key to shading as in Figure 3.38. Error bars represent ± SEM; (n = 3).



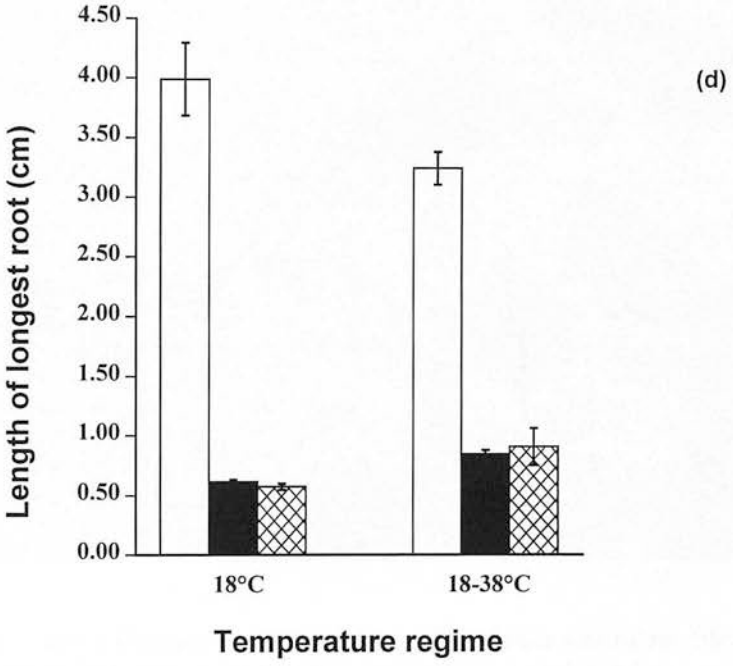
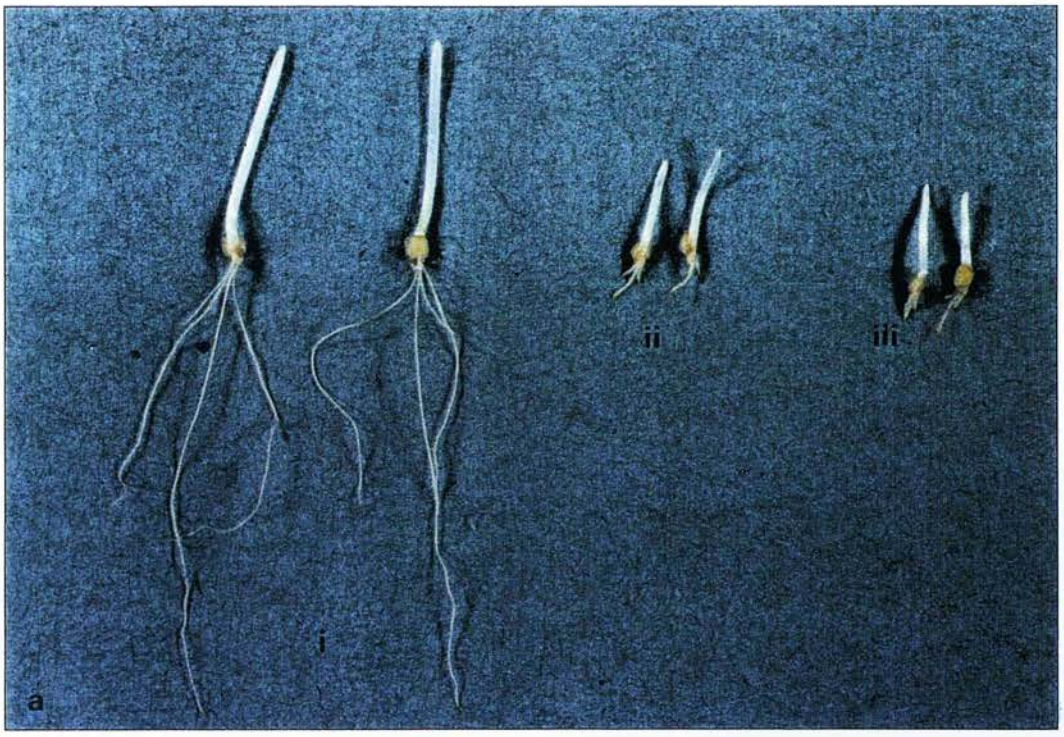


Figure 3.39 (continued) (c) cv. Blenheim grown in growth rooms (d) cv. Schooner grown in growth cabinets (Expt. IV). (Error bars represent ± SEM; (n = 3)



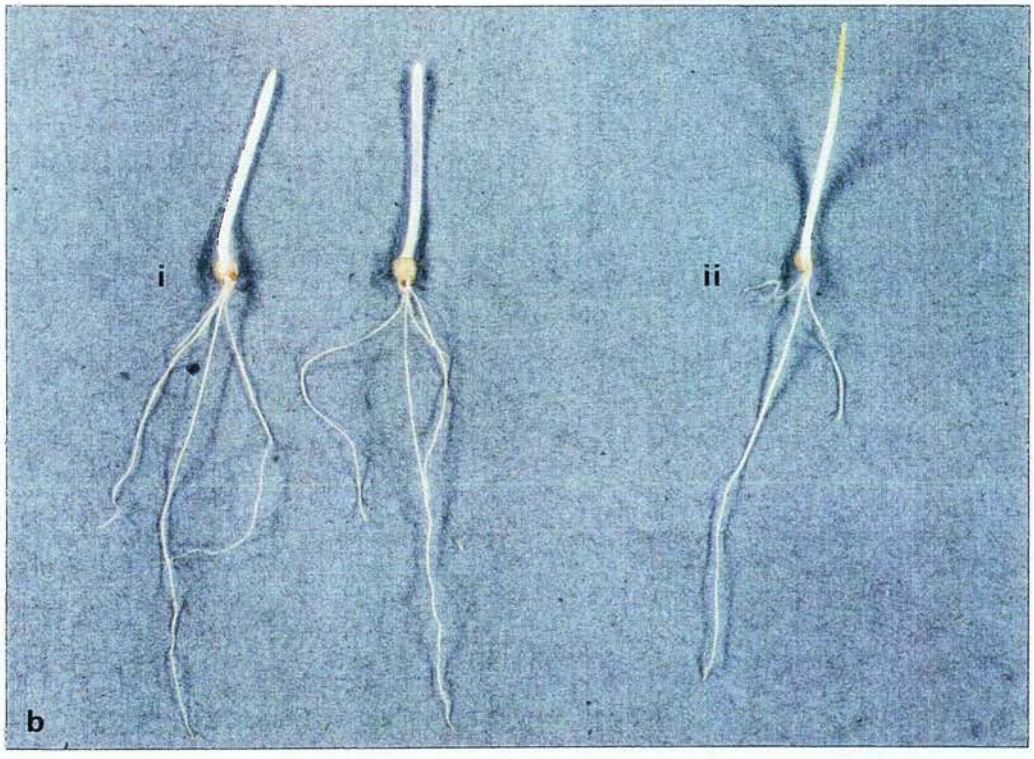
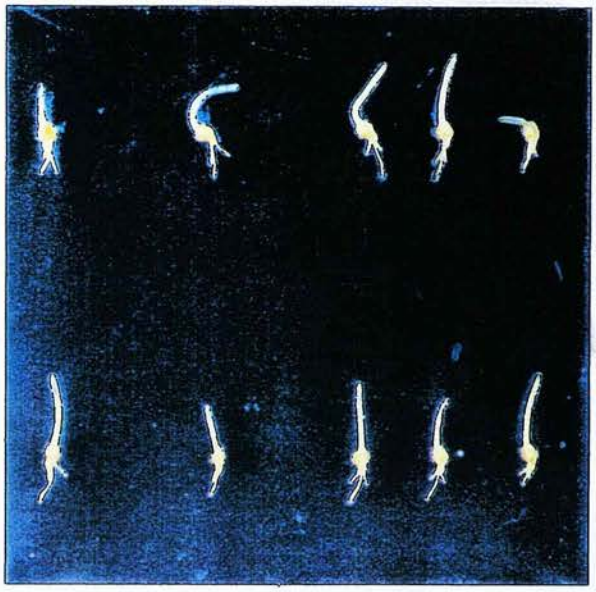
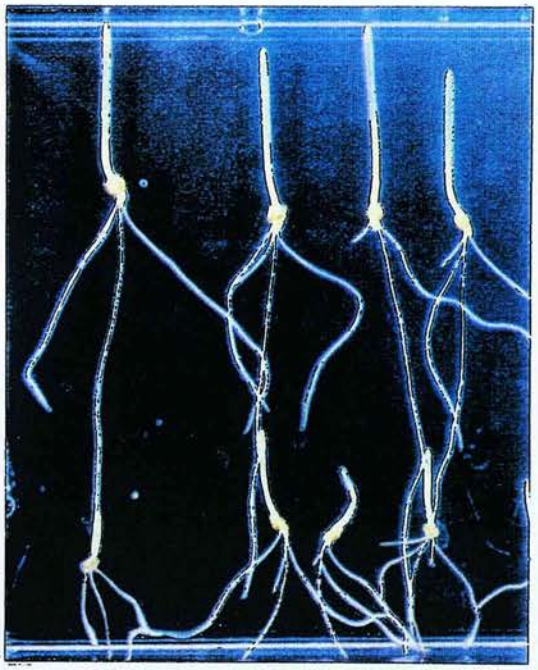


Plate 3. 7. 2.(a) Comparison of seedlings from embryos of cv. Blenheim grown for 5 days on (i) 1% agar + 0.9% glucose (ii) 1% agar (iii) 1% agar +0.9%mannitol.

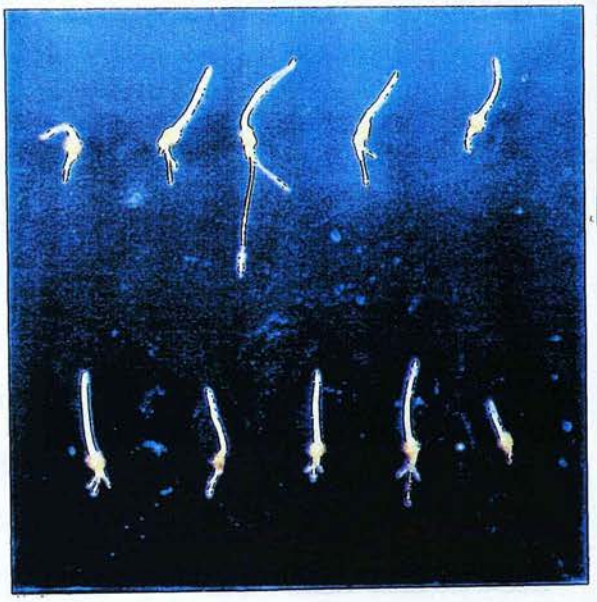
Plate 3. 7. 2.(b) Five day old seedlings grown in the dark on 1% agar + 0.9 % glucose from embryos of grains of cv. Blenheim grown at (i) 18°C and (ii) 18-38°C showing relative sizes of root and shoots.



i



iii



ii

Plate 3. 7. 2.(c) Seedlings grown on (i) 1% agar. (ii) 1% agar + 0.9% mannitol from embryos of grains of cv. Schooner grown in growth cabinets at 18°C.

(iii) Seedlings grown on 1% agar + 0.9% glucose from embryos of grains of cv. Schooner grown in growth cabinets at 18°C.

3. 7. 3. Seedling dry weight

Experiment II

When embryos from grains grown in temperature regimes (A), (B), (C) and (D) were grown on the agar + glucose medium, they produced seedlings with greater ($p < 0.05$) seedling dry weights than those grown on the agar or agar + mannitol. There were no significant differences between seedling dry weights from embryos of grains grown in (A) and those from embryos of grains grown in either (B), (C) or (D) (Figure 3.40a). Dry weight of seedlings grown on the agar and agar + mannitol media did not differ significantlty from each other in (A), (B), (C) or (D).

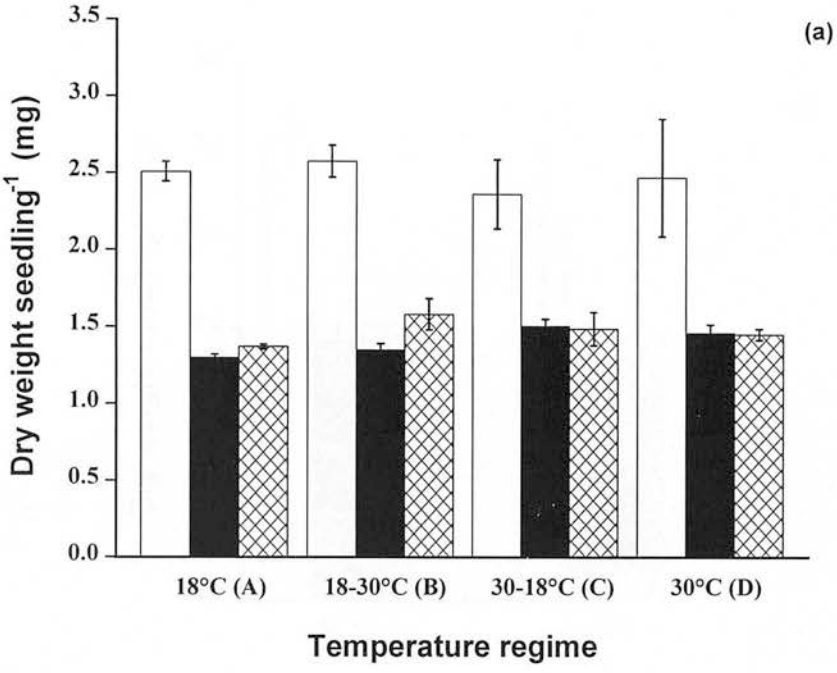
Experiment III

When grown on the agar + glucose medium, seedlings from embryos of grains of cv. Schooner grown in the growth cabinets at 18°C had greater dry weight per seedling ($p < 0.05$) than seedlings from embryos of grains grown at 18-38°C (Figure 3.40b). However, when the seedlings were grown on the agar and the agar + mannitol media, the difference was not observed.

Experiment IV.

In cv. Blenheim , the mean dry weight of seedlings from embryos grown on the agar + glucose medium from grains grown at 18°C was greater than that of seedlings from embryos of grains grown at 18-38°C, but there was high variability in the seedlings from the embryos of grains grown at 18-38°C and the difference in dry weight was not statistically significant (Figure 3. 40c). Dry weights of seedlings of embryos grown on the agar and the agar + mannitol media were similar in both temperature regimes.

As in Experiment III, the embryos of cv. Schooner grown in the growth cabinets at 18°C, when grown on the agra + glucose medium produced seedlings with a greater mean dry weight than that of seedlings from embryos of grains grown at 18-38°C (Figure 3.40d).



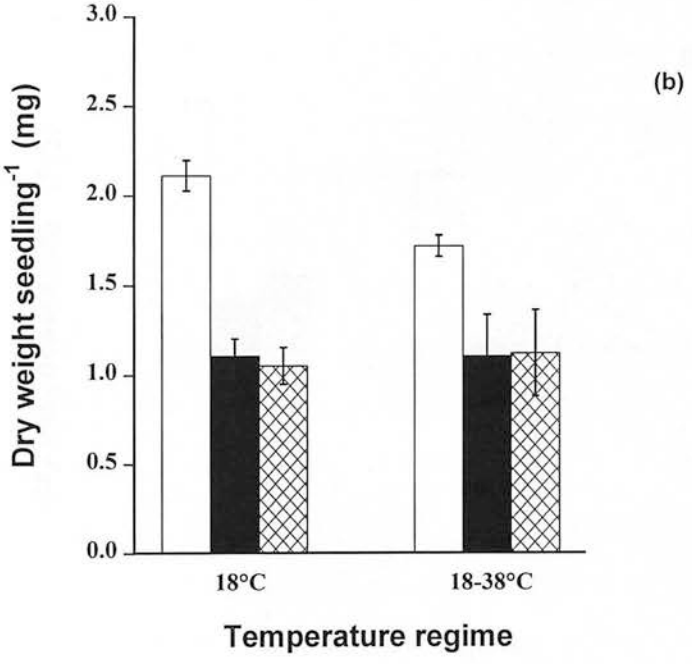


Figure 3.40. Dry weights of seedlings from embryos excised from grains grown under different temperature regimes during grain development (a) cv. Blenheim grown in growth rooms at 18°C, 18-30°C, 30-18°C and 30°C (Expt. II). (b) cv. Schooner grown in growth cabinets at 18°C and 18-38°C (Expt. III). Error bars represent ± SEM; (n = 3). Key to shading as in Figure 3.38.

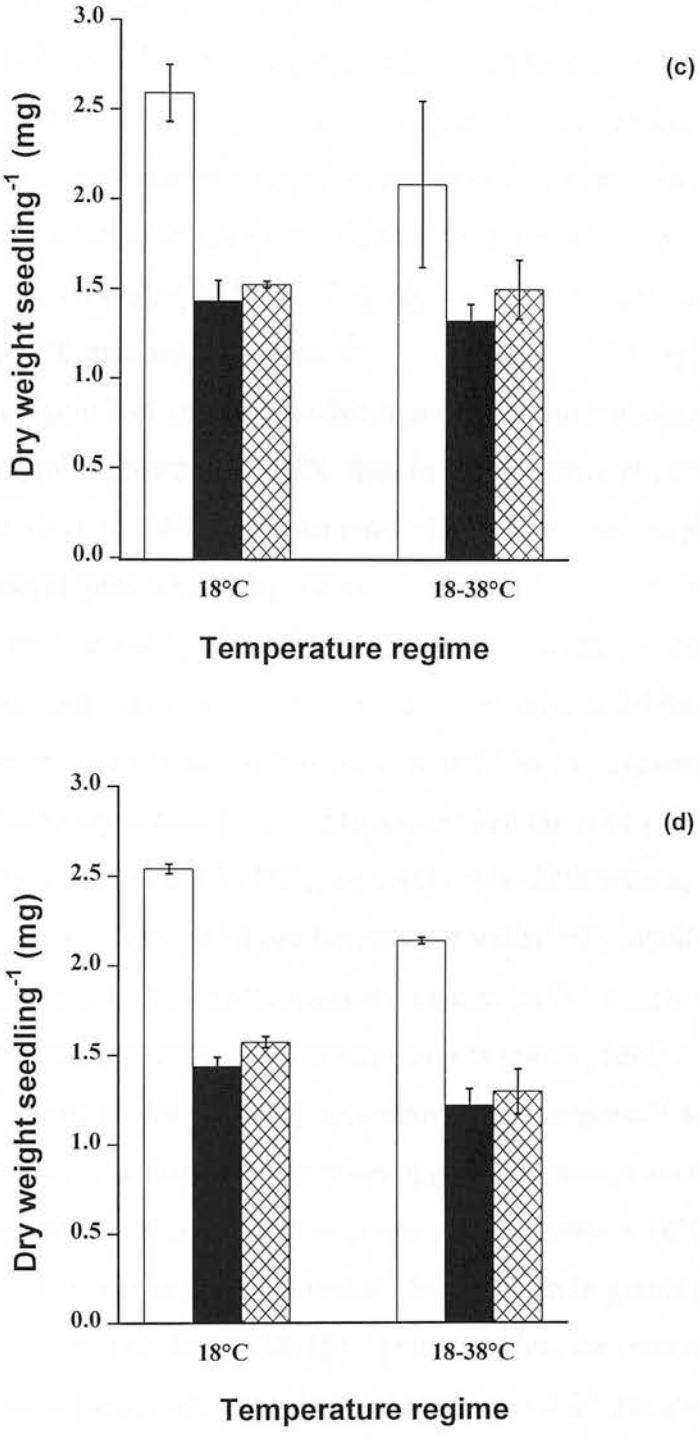


Figure 3.40. (continued) (c) cv. Blenheim grown in growth rooms (d) cv. Schooner grown in growth cabinets (Expts IV). Error bars represent ± SEM; (n = 3).

3. 8. Grain development

For further details on methods (**Section 2. 5. 4. 2.)** and temperature treatment to which ears sampled were subjected to during grain development see Expt. IV. and Table 2. 1. In cv. Blenheim, dry matter appeared to accumulate faster in grains grown at 18-38°C than in grains grown at 18°C. However, the difference in the dry matter accumulation between the two sets of grains (18-38°C and 18°C regimes) was not statistically significant at 14 days after anthesis. Grains grown at 18-38°C reached maximum dry weight (26.1 ± 0.17 mg) at 16 days after anthesis (daa) whereas grains grown at 18°C attained maximum dry weight (50.2 ± 2.55 mg) at 36 days after anthesis (Figure 3.41a). Thus the duration of dry matter accumulation was much shorter in grains grown at 18-38°C than in grains grown at 18°C. In grains from plants grown at 18-38°C the water content per grain rose sharply at the initial stage of grain development reaching a maximum at 11 daa stayed constant until 14 daa then decreased rapidly (Figure 3.41a). The water content in grains grown at 18°C continued to rise and reached a maximum value at 16 daa . After this, grain water content stayed more or less constant until 36 daa and then it decreased sharply. In cv. Stirling dry matter appeared to accumulate faster in grains grown at 18-38°C than in grains grown at 18°C (Figure 3.41b). The difference in dry matter was statistically significant at 13 daa but was not statistically significant at 19 daa or at 21 daa. Grains grown at 18-38°C reached a maximum dry weight (26. 3 ± 2.06 mg) at 13 daa (the end of grain development) whereas grains grown at 18°C accumulated dry matter until 36 daa, attaining a maximum dry weight of (47.0 ± 1.06 mg). As observed in cv. Blenheim, the duration of grain dry matter accumulation was shorter in grains grown at 18-38°C than in grains of ears grown at 18°C. Grain water content was initially greater in grains grown at 18-38°C than in grains grown at 18°C (Figure 3.41b). The water content in 18-38°C grains reached the peak at 11 daa stayed constant until 14 daa, and then dropped rapidly until 20 daa and very rapidly between 20 daa and 24 daa, whereas the grain water content in grains grown at 18°C reached a maximum at 16 daa and stayed constant until 35 daa (Figure 3.41b). Grains from plants of both cvs grown in both temperature regimes appeared to lose dry matter during the dehydration phase of grain maturation.

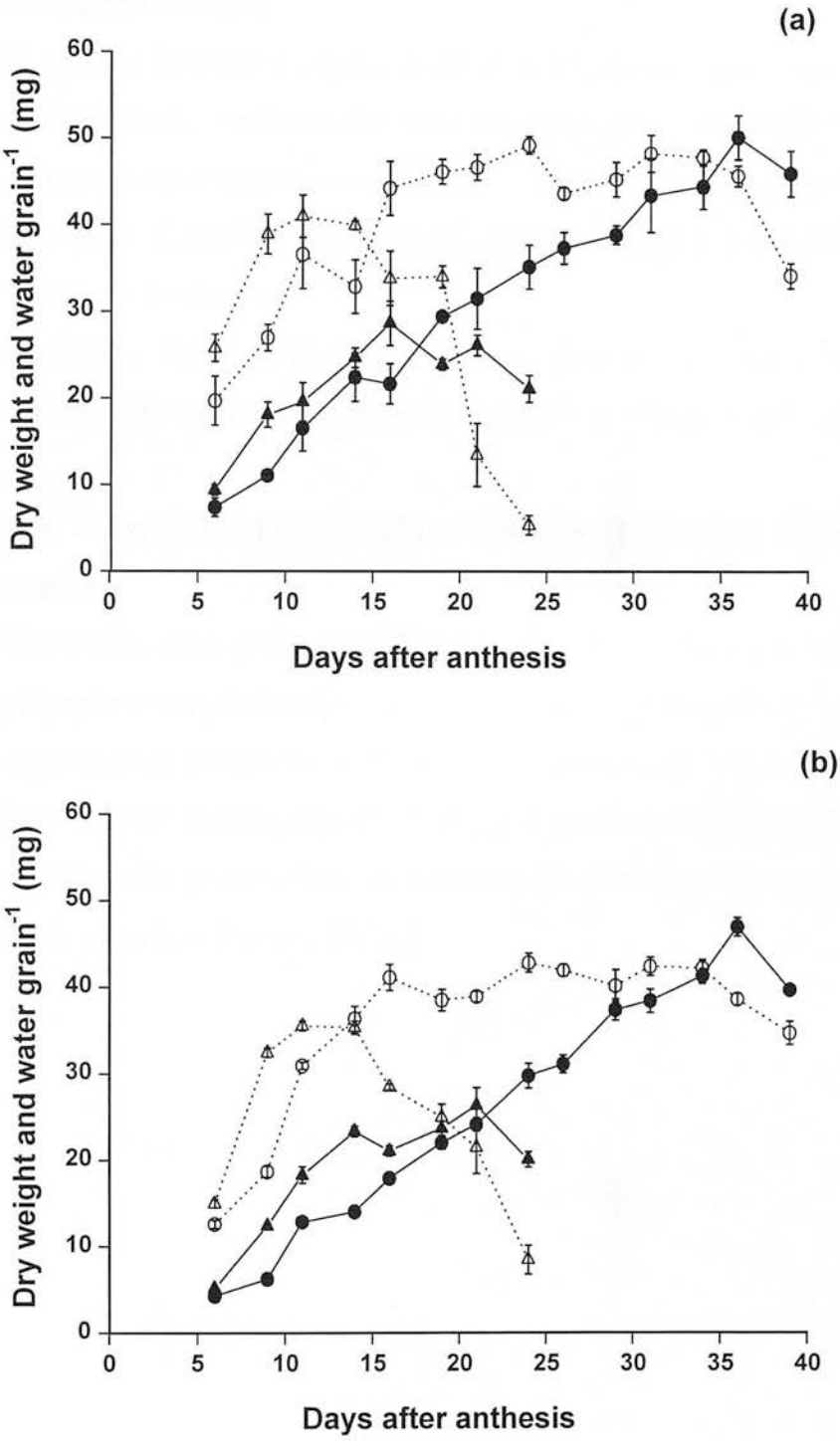


Figure 3.41. Mean dry weight (solid lines solid symbols) and water content (open symbols dotted lines) of grains from plants of barley grown in growth rooms at 18°C (circles) and at 18-38°C (triangles). Error bars represent ± SEM; n = 4. (a) cv. Blenheim (b) cv. Stirling. Ears experienced the following temperature regimes:

3. 8. 1. Comparison of grain development of cvs Blenheim and Stirling in 18°C temperature regime

In order to facilitate comparison between the two cultivars grown under the same environmental conditions, the same data were plotted differently (Figure 3.42a) The pattern of dry matter accumulation in cv Stirling was very similar to that in cv. Blenheim. Variability and grain dry weight throughout development were higher in cv. Blenheim than in

cv. Stirling. Some of the variability in cv. Blenheim was due to missing grains ie sterility. The pattern of grain water content was similar in both cultivars.

3. 8. 2. Grain development of cvs Blenheim and Stirling 18-38°C temperature regime

There were some genotypic differences between cv. Blenheim and cv. Stirling in response to elevated temperatures during grain development (Figure 3.42b). Dry weights of grains grown at 18-38°C were lower by 48.2 % in cv. Blenheim and by 43.3 % in cv. Stirling than those of grains grown at 18°C. It appears therefore, that cv. Blenheim grown in a growth room at 18-38°C was more susceptible to elevated the temperature than cv. Stirling.

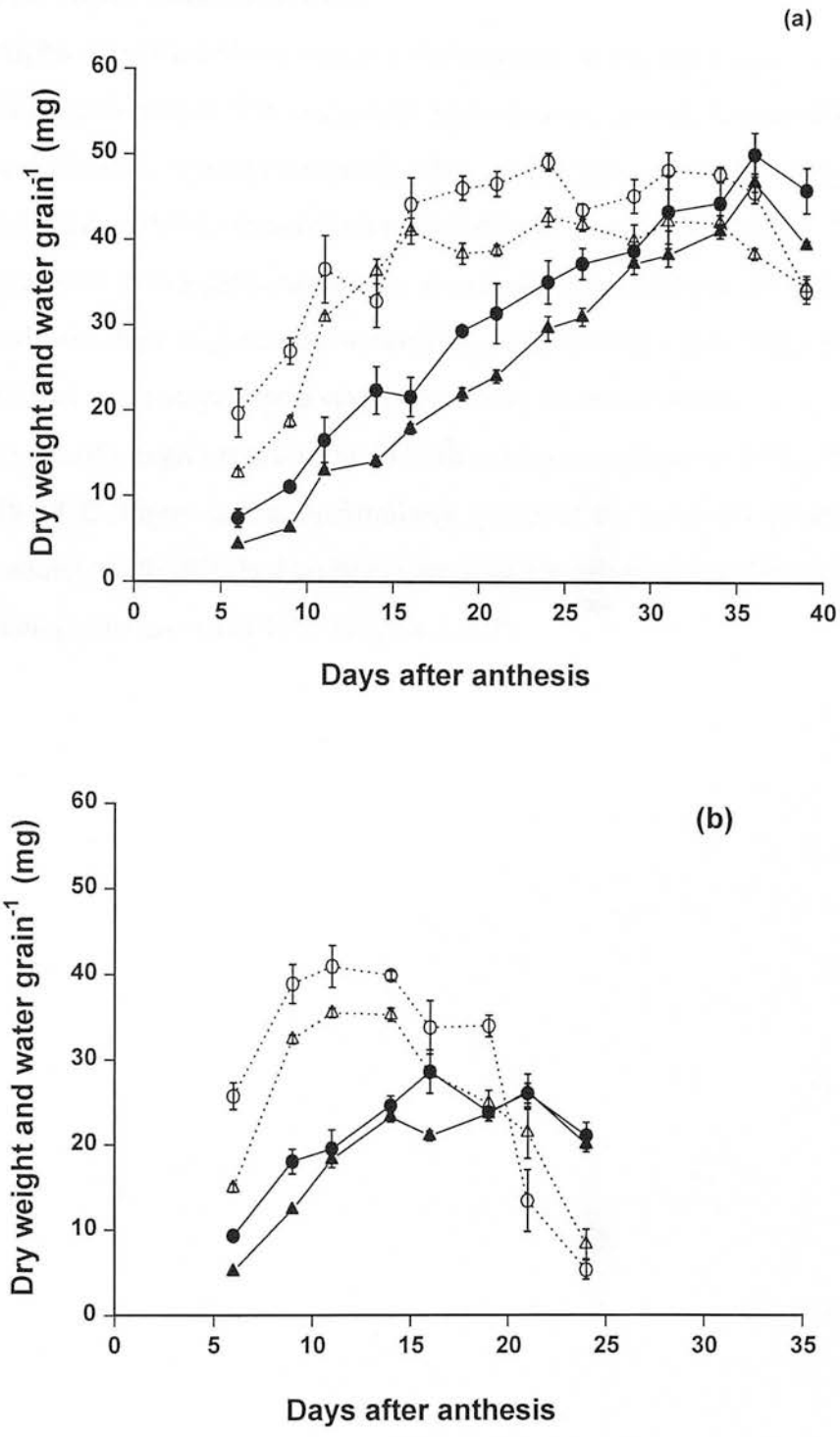


Figure 3.42. Mean grain dry weight (solid lines solid symbols) and grain water contents (open symbols dotted lines) of grains of cvs. Blenhiem (circles) and cv. Stirling (triangles) (a) Grains from plants grown at 18°C (b) Grains from plants grown at 18-38°C. Error bars represent ± SEM; n = 4.

3. 9. Alpha-amylase activity

Alpha-amylase activity was assayed in grains of cvs Blenheim (Experiments II and IV) and Schooner (Experiment IV) grown in the growth rooms and growth cabinets at different temperature regimes. Only grains from seed lots 1 were used in this experiment due to insufficient availability of grains of seed lot 2. The grains were prepared as in a germination test. Grains were incubated at 20°C for 48 h. Grains at a uniform stage of germination/seedling development were homogenised and the crude extract was assayed for α-amylase activity. Alpha-amylase activity was lower ($p < 0.05$) in grains grown at 18°C than in grains grown at 30°C, 18-30°C and 18-38°C (Figure 3.43a, b). Similarly, grains of cv. Schooner grown in a growth cabinet at 18-38°C had higher alpha-amylase activity after 48h incubation ($p < 0.05$) than grains grown at 18°C (Figure 3.43c).

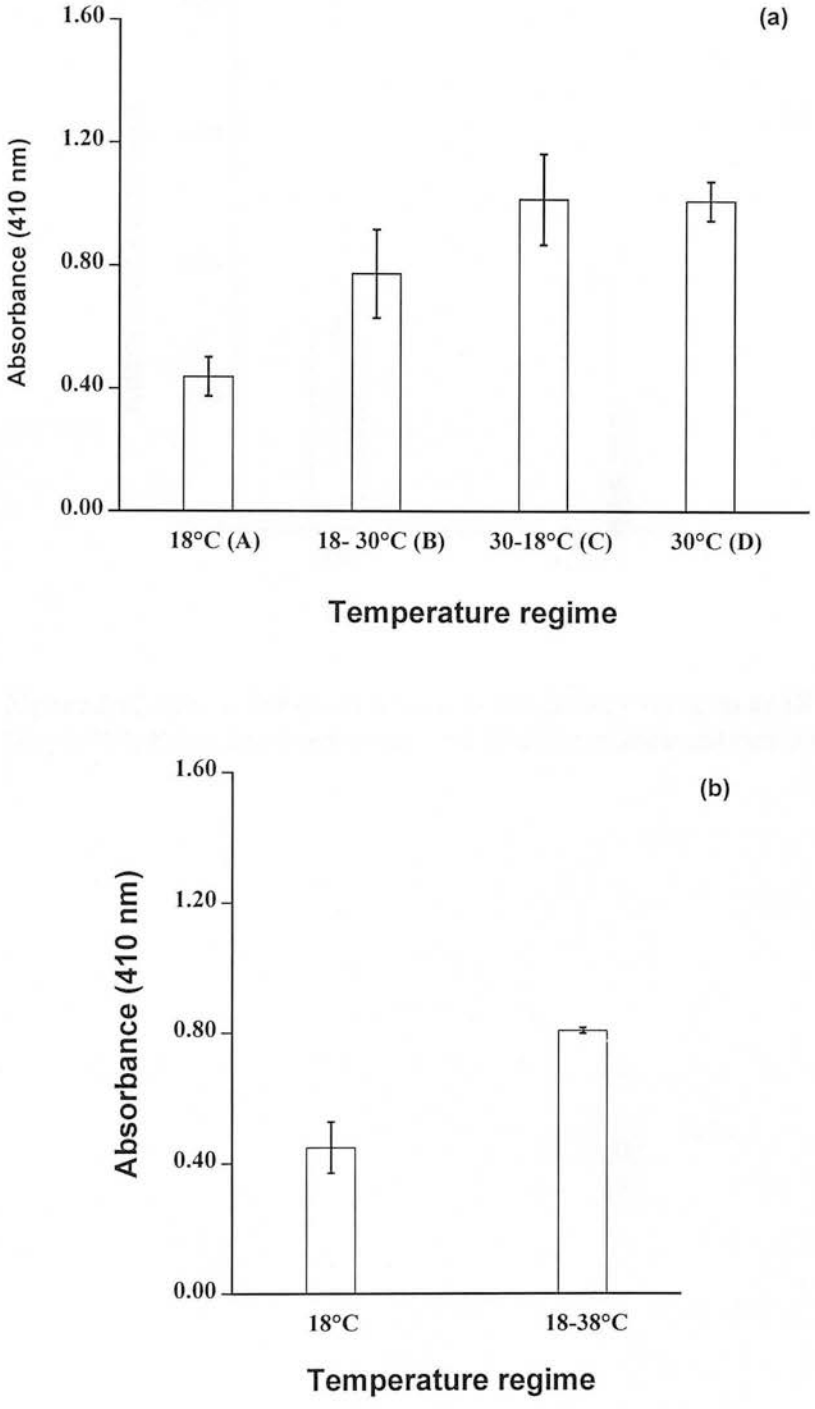


Figure 3.43 α-Amylase activity of grains subjected to different temperature regimes during grain development. α-amylase was extracted from grains grown in growth rooms and growth cabinets and assayed using the Ceralpha substrate (Megazyme) (a) cv. Blenheim grown in the growth rooms at 18°C, 18-30°C, 30-18°C and 30°C (Expt. II). (b) cv. Blenheim grown in the growth rooms at 18°C and 18-38°C (Expt. IV). Error bars represent ± SEM of 3 replicate extractions.

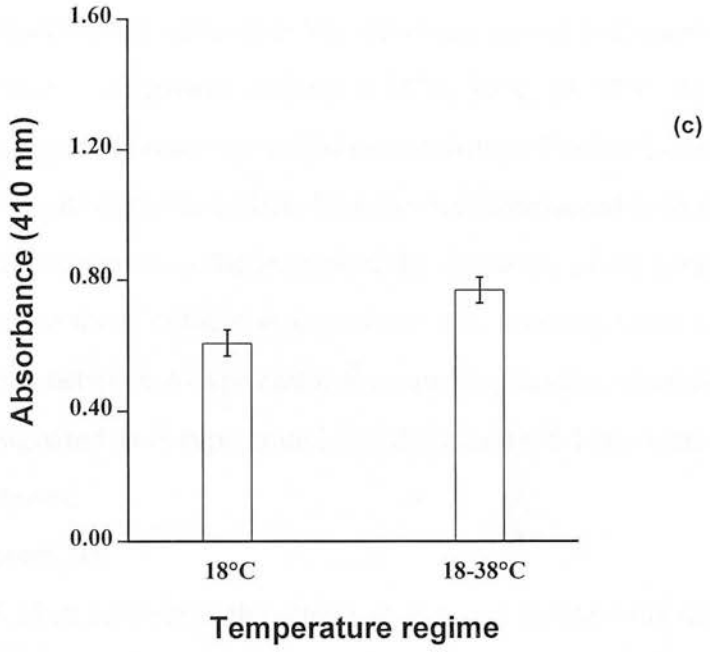


Figure 3.43. (c) cv. Schooner grown in the growth cabinets at 18°C and 18-38°C (Expt. IV). Error bars represent ± SEM of 3 replicate extractions.

3. 10. Starch granule size distribution

Separate extractions of the starch were made from mid-ear grains of two ears which had anthesed on the same date. The ears were grown in Experiments III and IV in growth rooms and growth cabinets at 18°C, 30°C, 18-38°C, 21-38°C and 30-38°C. The starch granule sizes were determined using a Coulter Counter Multizer II fitted with a 100 μm diameter orifice. Samples were measured in triplicate. Starch granule sizes were expressed as the lengths of the diameters of the spheres having volumes equivalent to those of the starch granules. The arbitrary value of 8.1μm was used to distinguish between A-type and B-type starch granules. Granules > 8.1 μm diameter were designated as A-type granules and granule < 8.1 μm were thus designated as B-type granules.

Experiment III.

Tables 3.11 & 3.12 show the effects of elevated temperature during grain development on starch granule size distribution in grains of cvs Stirling and Schooner. The results presented show that grains of cvs Stirling and Schooner (Gr & Gc) grown at 18°C had more starch granules per grain and a greater volume of starch per grain than grains grown at 30°C (Stirling & Schooner Gr) and 18-38°C (Schooner Gc). Both A-type and B-type starch granules were fewer in number in grains grown at 30°C, 18-38°C and 30-38°C than in grains grown at 18°C. Figure3.44a-d shows percentage granule size distribution by number. When the numbers of A-type and B-type starch granules per grain were expressed as percentages of the total number of starch granules, starch from tiller ears of cv. Schooner (Gc) grown at 30-38°C had a higher percentage of A-type starch granules than those from main stem ears grown at 18°C (Table 3.11). The percentage distribution of A-type and B-type starch granules by volume in grains of cvs Stirling and Schooner (Gr) from main stem ears grown at 18°C was similar to that in grains from main stem ears grown at 30°C (Table 3.12 and Figure 3.45a,b). The contribution of granules 8-14μm to the total volume was more in grains from main stem ears of cv. Schooner grown in a growth cabinet at 18-38°C and 30-38°C than in grains from main stem ears grown at 18°C (Figures 3.44c, d).

Table 3.11. Numbers of starch granules of cvs Stirling and Schooner grown in growth rooms at 18°C and 30°C and cv. Schooner grown growth cabinets at 18°C and 18-38°C during grain development. Experiment III. Values for starch extracted from 2 replicate ears.

Cultivar	Temperature regime and anthesis date	Total granules grain $^{-1}$	Number of A-type granules grain $^{-1}$ 8.1-30μm	Number of B-type granules grains $^{-1}$ < 8.1μm
Stirling				
Main stem ear 1 Fig. 3.44a.	18°C 15/10/94	11.3×10^7	0.8×10^7	10.5×10^7
Main stem ear 2	18°C	10.7×10^7	0.73×10^7	9.97×10^7
Main stem ear 1	18-30°C 15/10/94	5.35×10^7	0.27×10^7	5.08×10^7
Main stem ear 2	18-30°C	3.16×10^7	0.24×10^7	2.92×10^7
Schooner Growth room				
Main stem ear 1 Fig.3.44b.	18°C 16/11/94	8.3×10^7	0.60×10^7	7.70×10^7
Main stem ear 2	18°C	18.2×10^7	1.40×10^7	16.8×10^7
Main stem ear 1	30°C 14/11/94	4.07×10^7	0.27×10^7	3.8×10^7
Main stem ear 2	30°C	6.75×10^7	0.41×10^7	6.34×10^7
Schooner Growth cabinets				
Main stem ear 1	18°C 15/11/94	14.10×10^7	1.14×10^7	12.96×10^7
Main stem ear 2 Fig. 3.44c.	18°C	13.48×10^7	1.17×10^7	12.31×10^7
Main stem ear 1	18-38°C 2/11/94	9.40×10^7	0.82×10^7	8.58×10^7
Main stem ear 2	18-38°C	5.63×10^7	0.61×10^7	5.02×10^7
Tiller ear 1 Fig. 3.44d.	30-38°C 20/11/94	2.08×10^7	0.49×10^7	1.60×10^7
Tiller ear 2	30-38°C	3.28×10^7	0.72×10^7	2.55×10^7

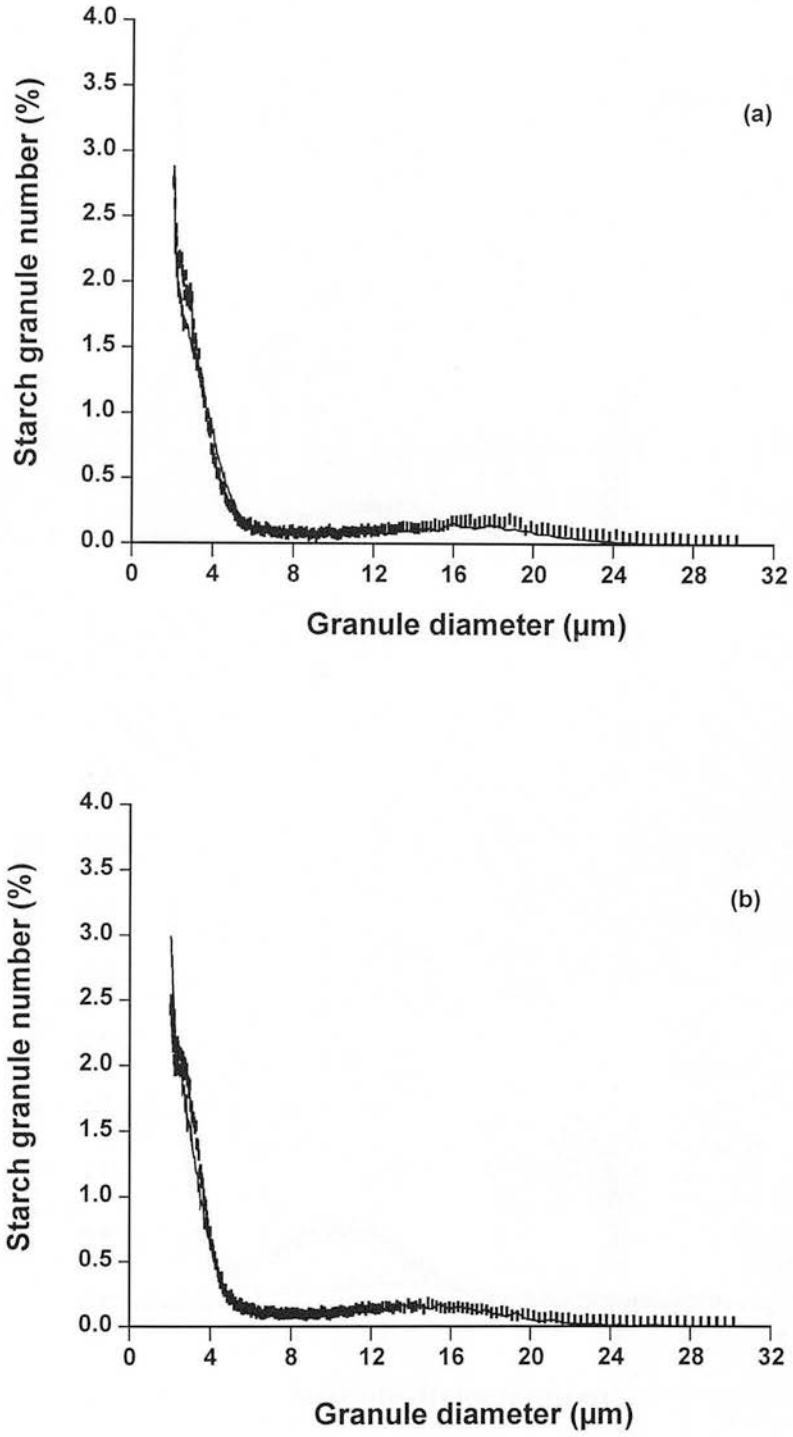


Figure 3.44. Experiment III. Size distribution of starch granules of cvs Stirling and Schooner grown in growth rooms. (a) grains from main stem ears of cv. Stirling grown at 18°C (solid lines) and 30°C (tickmarks) (b) grains from main stem ears of cv. Schooner grown in growth rooms at 18°C (solid lines) and 30°C (tickmarks). Grains of cv. Stirling were transferred to 30°C 13 days after anthesis (daa) and those of Schooner anthesed at 30°C. Values are means of 2 replicate ears.

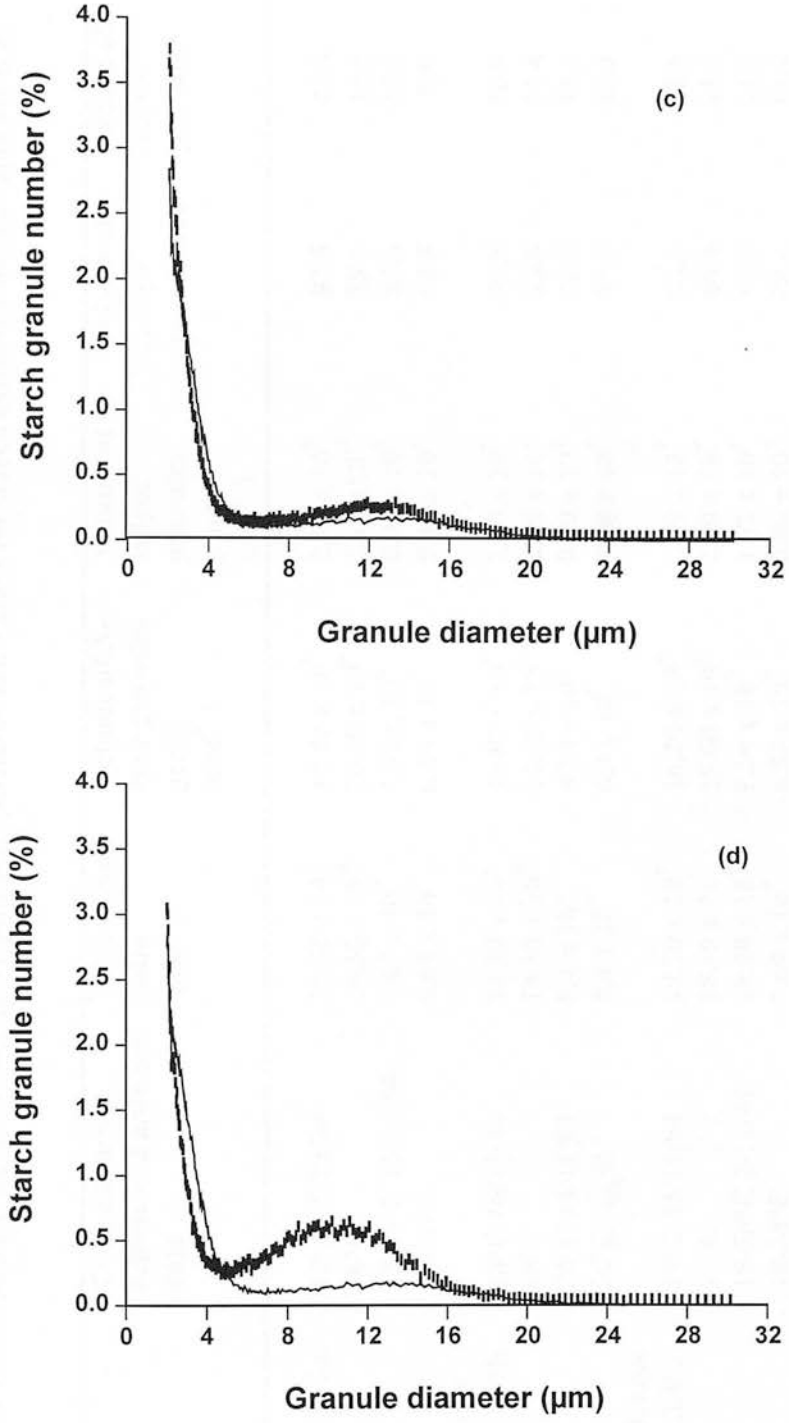


Figure 3.44. (continued) (c) grains of main stems of cv. Schooner grown in growth cabinets at 18°C (solid lines) and 18-38°C (tickmarks). (d) grains of main stem ears of cv. Schooner grown in grown cabinets at 18°C (solid lines) and tiller ears grown at 30-38°C (tickmarks). Grains of main stems grown at 18-38°C had 12 days at 18/13°C, 2d at 21/16°C, 1d at 24/19°C, 5d at 30/25°C, 3d at 35/30° and 1d at 38/33°C and tillers grown at 30-38°C had 2d at 30/25°C, 3d at 35/30°C and 1d at 38°C, before being transferred to 30/25°C to complete development and maturation. Values are means of 2 replicate ears.

Table 3.12. Volumes of starch granules of cvs Stirling and Schooner grown in growth rooms at 18°C and 18-30°C and cv. Schooner grown in growth cabinets at 18°C and 18-38°C during grain development Experiment III. Values for starch extracted from 2 replicate ears.

Cultivar	Tempearture regime and anthesis date	Total volume grain $^{-1}$ (μm^3)	Volume of A-type granules grain $^{-1}$ (μm^3)	Volume of B-type granules grain $^{-1}$ (μm^3)	Volume of A-type granules %	Volume of B-type granules%
Stirling						
Main stem ear 1 Fig. 3.45a.	18°C 15/10/94	16.43×10^9	14.40×10^9	2.03×10^9	87.5	12.5
Main stem ear 2	18°C	18.30×10^9	16.20×10^9	2.10×10^{10}	88.5	11.5
Main stem ear 1	18-30°C 15/10/94	7.67×10^9	6.74×10^9	0.93×10^9	87.5	12.5
Main stem ear 2	18-30°C	6.63×10^9	6.14×10^9	0.49×10^9	92.5	7.5
Schooner Growth rooms						
Main stem ear 1 Fig. 3.45b.	18°C 16/11/94	14.30×10^9	12.80×10^9	1.50×10^9	89.5	10.5
Main stem ear 2	18°C	24.30×10^9	21.50×10^9	2.80×10^9	88.6	11.4
Main stem ear 1	30°C 14/11/94	5.4×10^9	4.70×10^9	0.70×10^9	86.3	13.7
Main stem ear 2	30°C	8.0×10^9	6.9×10^9	1.10×10^9	86.4	13.6
Schooner Growth cabinets						
Main stem ear 1 Fig. 3.45c.	18°C 15/11/94	18.70×10^9	16.20×10^9	2.50×10^9	86.5	13.5
Main stem ear 2	18°C	18.10×10^9	15.60×10^9	2.50×10^9	86.4	13.6
Main stem ear 1	18-38°C 2/11/94	10.90×10^9	9.38×10^9	1.52×10^9	85.7	14.3
Main stem ear 2	18-38°C	7.69×10^9	6.83×10^9	0.86×10^9	88.6	11.4
Tiller ear 1 Fig. 3.45d.	30-38°C 20/11/94	5.25×10^9	4.81×10^9	0.44×10^9	91.6	8.4
Tiller ear 2	30-38°C	6.56×10^9	5.54×10^9	1.02×10^9	84.4	15.6

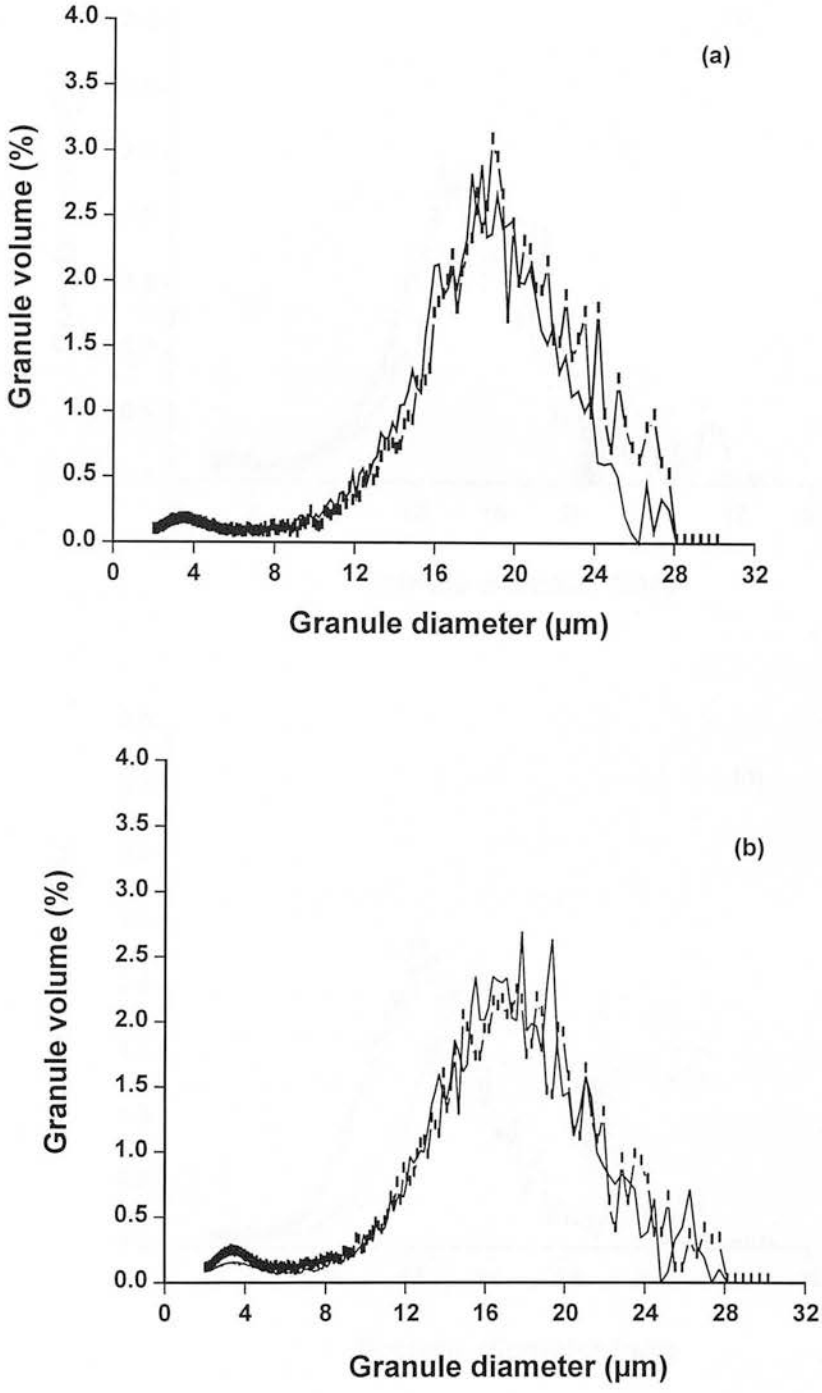


Figure 3.45. Experiment III. Percentage of total starch volume in each granule size category of starch extracted from grains of cvs Stirling and Schooner grown in growth rooms. (a) grains from main stem ears of cv. Stirling grown in growth rooms at 18°C (solid lines) and 30°C (tickmarks). (b) grains from main stem ears of cv. Schooner grown in growth rooms at 18°C (solid lines) and 30°C (tickmarks). Values are means of 2 replicate ears.

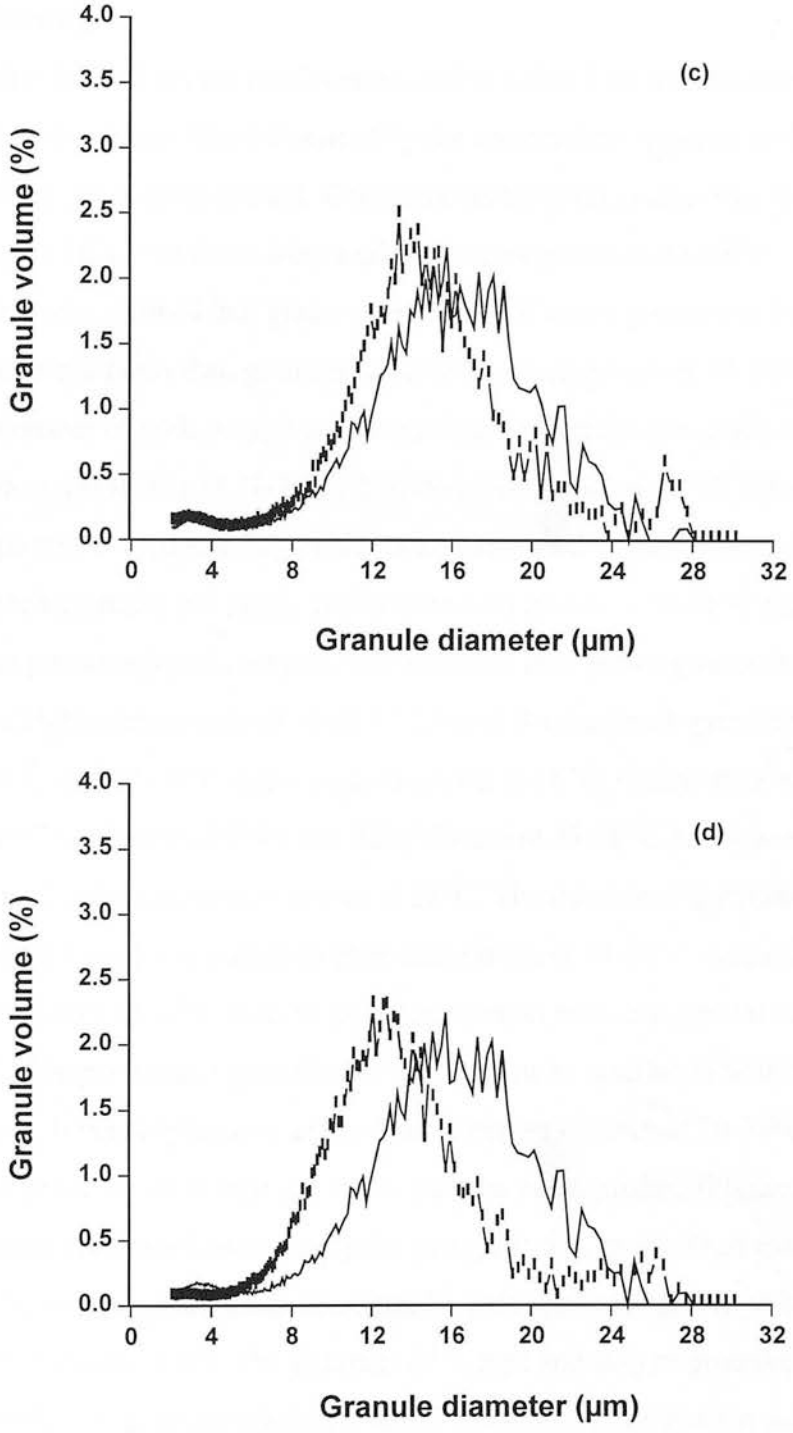


Figure 3.45. (continued) (c) grains of main stem ears of cv. Schooner grown in growth cabinets at 18°C (solid lines) and 18-38°C (tickmarks). (d) grains of main stem ears of cv. Schooner grown in growth cabinets at 18°C (solid lines) and tiller ears of cv. Schooner grown at 30-38°C (tickmarks).

Experiment IV.

cv. Stirling

It was evident from the results presented in Table 3.13 that the total number of starch granules per grain was influenced by the temperature regimes grains were subjected to during grain development. Comparisons between grains from the main stem ears grown at 18°C and those from main stem ears grown at 18-38°C and 21-38°C respectively showed that grains grown at 18°C had a greater total number of starch granules per grain than grains of comparable ears grown at 18-38°C and 21-38°C. The number of both A-type and B-type starch granules per grain was lower in grains grown at 18-38°C and 21-38°C than in grains grown at 18°C. When the numbers of A-type and B-type starch granules were expressed as percentages of the total number of starch granules per grain, grains from ears grown at 18-38°C and 21-38°C had higher percentages of A-type starch granules than grains grown at 18°C. There was also a higher percentage of small (< 2.1μm) B-type starch granules in grains grown at 18-38°C and 21-38°C than in grains grown at 18°C. Grains from tiller ears grown at 30-38°C (anthesed 21/4/95 and 22/4/95) and at 33-38°C had fewer A-type granules than grains from tiller ears grown at 18°C. The number of granules classified as B-type (< 8.1 μm) was higher in tiller ears grown at 30-38°C (anthesed 21/4/95 & 22/4/95) and 33-38°C than in grains from main stem ears grown at 18-38°C and 21-38°C. The percentage granule size distribution by number is illustrated in Figures 3.46a-g. It was triphasic in grains from tiller ears grown at 30-38°C and 33-38°C, ie two size classes of B-type granules could be distinguished (Figures 3.46d-g).
The total volume of starch per grain was greater in grains from main stem ears grown at 18°C than in grains from comparable main stem ears grown at 18-38°C and 21-38°C (Table 3.14). The volumes of A-type and B-type granules per grain were affected by temperature during grain development in both main stem ears and tiller ears. The volume of granules classified as B-type was less in grains from main stem ears grown at 18-38°C and 21-38°C than in grains from tillers grown at 30-38°C and 33-38°C (Table 3.14). The percentage volume of total starch present in the form of B-type granules was less in grains of main stem ears grown at 18-38°C and 21-38°C

than in grains from comparable main stem ears grown at 18°C, and more in grains from tiller ears grown at 30-38°C (anthesed 21/4/95 & 22/4/95) and 33-38°C than in grains from comparable tiller ears grown at 18°C (Table 3.14). The percentage distribution of starch granule by volume is illustrated in Figures 3.47a-g.

Table 3. 13. Numbers of starch granules of cv. Stirling grown in growth rooms at 18°C and 18-38°C. Experiment IV. Values for starch extracted from 2 replicate ears.

Cultivar Stirling Figures 3.46a-g	Temperature regime and anthesis date	Total granules grain $^{-1}$	Number of A-type grain $^{-1}$ 8.1- >30μm	Number of B-type grain $^{-1}$ < 8.1 μm.	% A-type 8.1 > 30μm	% B-type < 8.1μm	B-type < 2.1μm
Main stem ear 1	18°C 9/4/95	1.57×10^8	0.13×10^8	1.44×10^8	8.3	91.7	7.4
Main stem ear2	18°C	2.13×10^8	0.12×10^8	2.01×10^8	5.6	94.0	10
Main stem ear 1	18-38°C 8/4/95	0.70×10^8	0.08×10^8	0.62×10^8	11.4	88.6	13.9
Main stem ear 2	18-38°C	0.64×10^8	0.09×10^8	0.55×10^8	14.1	85.9	11.4
Main stem ear 1	21-38°C 13/4/95	0.49×10^8	0.05×10^8	0.44×10^8	10.2	89.8	14.0
Main stem ear 2	21-38°C	0.45×10^8	0.06×10^8	0.39×10^8	13.3	86.7	15.0
Tiller ear 1	18°C 23/4/95	1.95×10^8	0.09×10^8	1.86×10^8	4.6	95.4	10.1
Tiller ear2	18°C	1.50×10^8	0.07×10^8	1.43×10^8	4.7	95.3	9.2
Tiller ear 1	30-38°C 21/4/95	0.81×10^8	0.04×10^8	0.77×10^8	4.9	95.1	8.0
Tiller ear 2	30-38°C	0.76×10^8	0.04×10^8	0.72×10^8	5.3	94.7	8.5
Tiller ear 1	30-38°C 22/4/95	0.72×10^8	0.04×10^8	0.68×10^8	5.6	94.4	7.2
Tiller ear 2	30-38°C	0.81×10^8	0.04×10^8	0.77×10^8	4.9	95.1	7.4
Tiller ear 1	33-38°C 23/4/95	1.07×10^8	0.04×10^8	1.03×10^8	3.7	96.3	6.5
Tiller ear 2	33-38°C	1.31×10^8	0.05×10^8	1.26×10^8	3.8	96.2	6.4

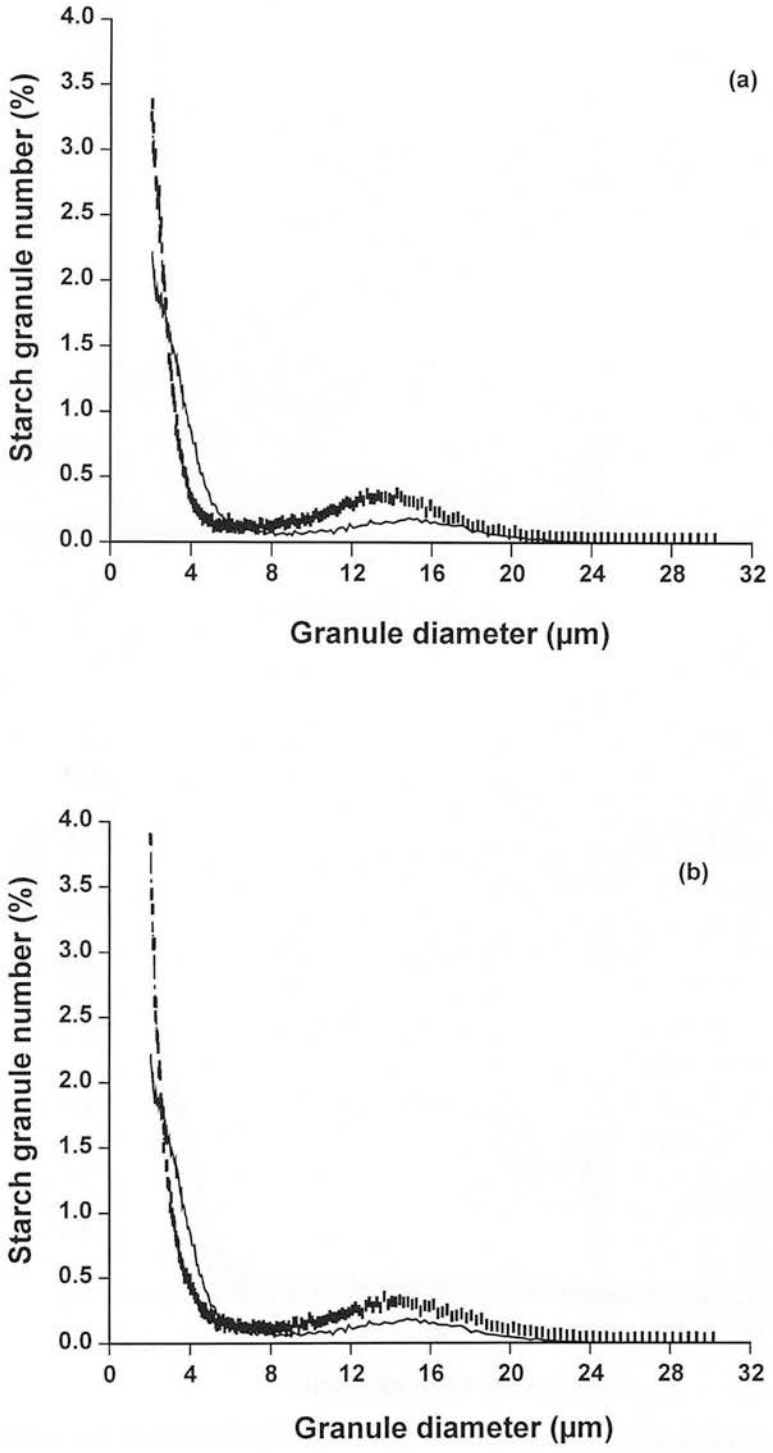


Figure 3.46. Experiment IV. Size distribution of starch granules of cv. Stirling grown in growth rooms. (a) grains of main stem ears grown at 18°C (solid lines) and 18-38°C (tickmarks). (b) grains of main stem ears grown at 18°C (solid lines) and 21-38°C (tickmarks). Values are means of 2 replicate ears.

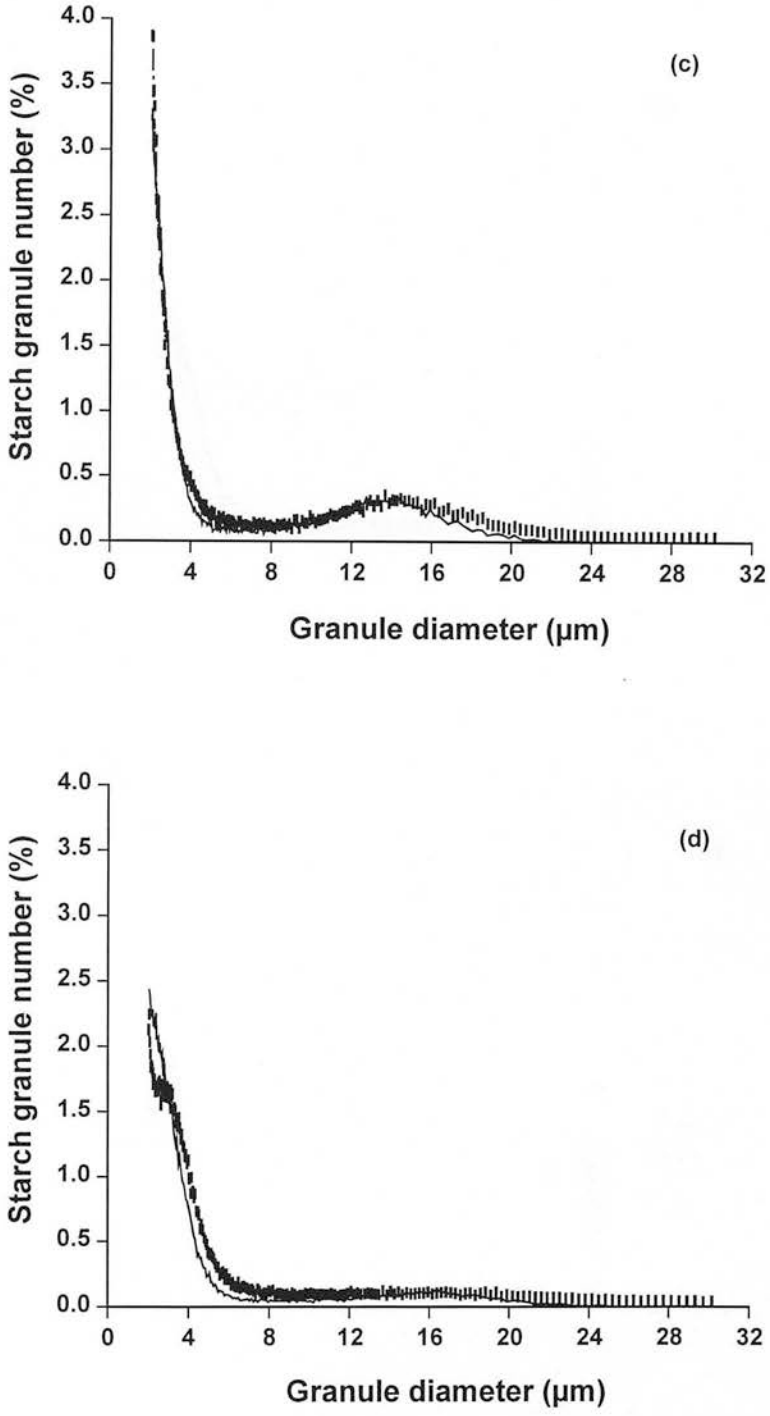


Figure 3.46 (continued) Experiment IV. (c) grains of main stem ears of cv. Stirling grown at 18-38°C (solid lines) and 21-38°C (tickmarks). (d) grains of tiller ears grown at 18°C (solid lines) and 30-38°C (tickmarks). Grains of tiller ears grown at 30-38°C (anthesed 21/4/95) had 2d at 30/25°C, 1d at 33/28°C, 3d at 35/30°C and 4d at 38/33°C before returning to 30/25°C to complete development/maturation. Values are means of 2 replicate ears.

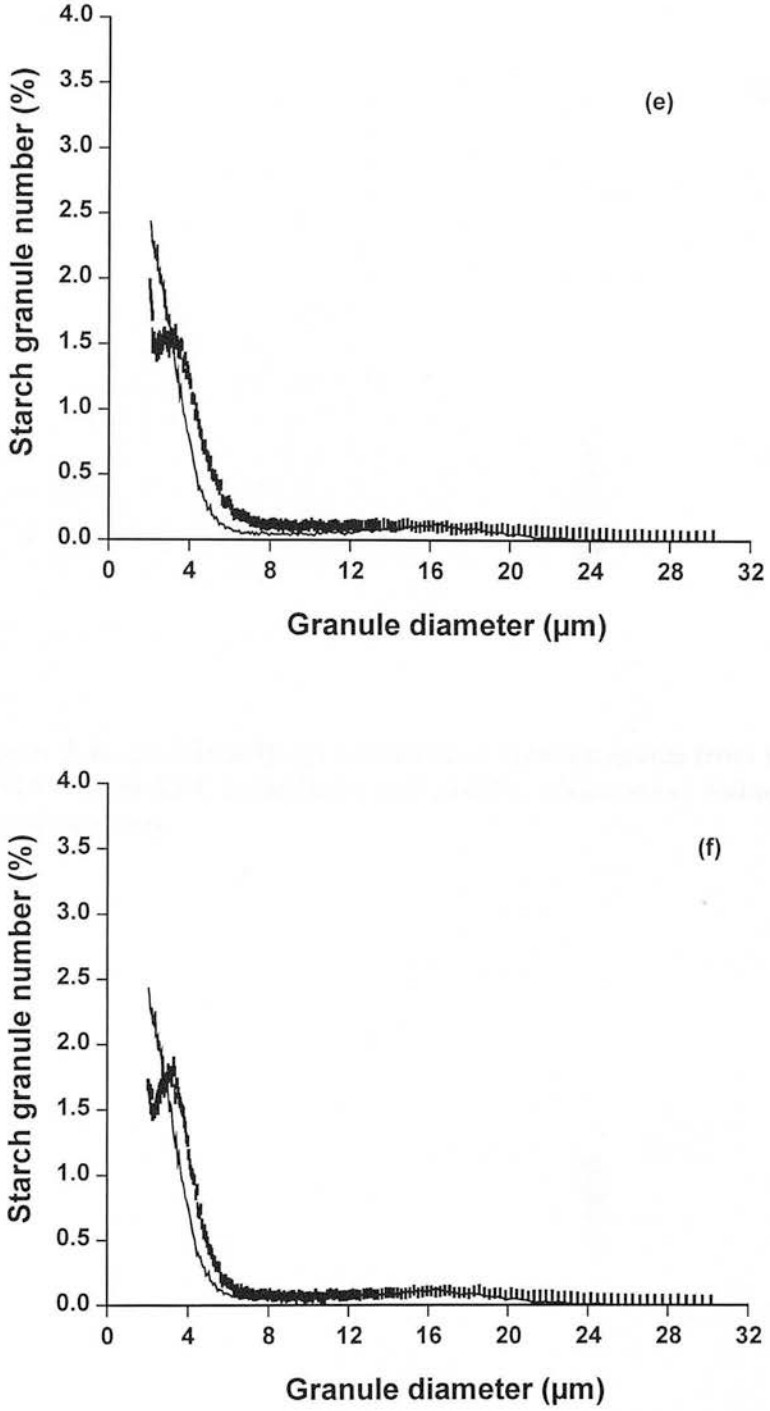


Figure 3.46. (continued) (e) grains of tiller ears of cv. Stirling grown at 18°C (solid line) and 30-38°C (tick marks) (f) grains of tiller ears grown at 18 °C (solid lines) and 33-38°C (tickmarks). Grains grown at 30-38°C (anthesed 22/4/95) had 1d at 30/25°C, 1d at 33/28°C, 3d at 35/30°C and 4d at 38/33°C and grains grown at 33-38°C had 1d at 33/28°C, 3d at 35/30°C and 4d at 38/33°C. Values are means of 2 replicate ears.

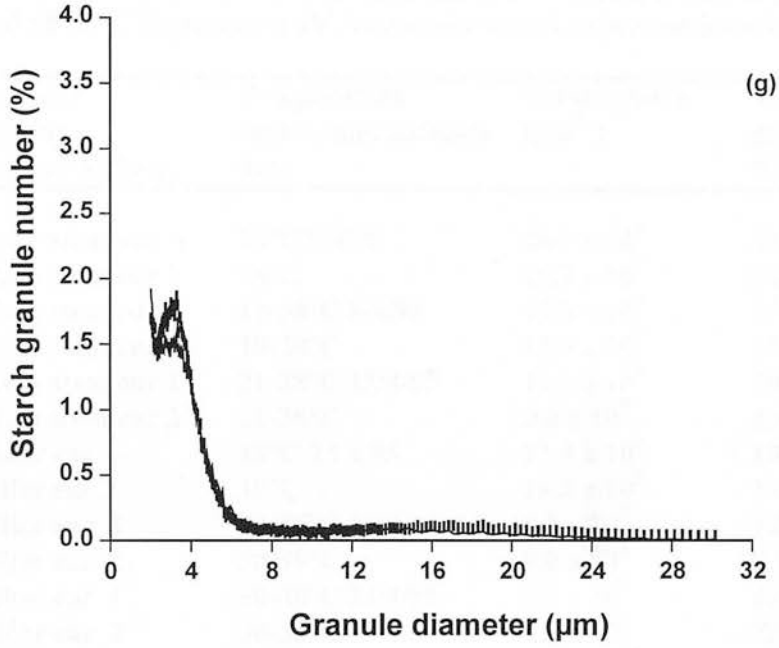


Figure 3.46. (continued). (g) Comparison between grains from tiller ears (anthesed 22/4/95) at 30-38°C (solid lines) and 33-38°C (tickmarks). Values are means of 2 replicate ears.

Table 3.14. Volumes of starch granules of cv. Stirling grown in growth rooms at 18°C and 18-38°C Experiment IV. Values for starch extracted from 2 replicate ears.

Cultivar Stirling Figures 3.47a-g.	Temperature regime and anthesis date	Total volume (μm^3)	Volume of A-type granules $grain^{-1}$ (> 8.1 μm) (μm^3)	Volume of B-t granules grain (< 8.1 μm) (μ
Main stem ear 1	18°C 9/4/95	$26\ 0 \times 10^9$	22.6×10^9	3.4×10^9
Main stem ear 2	18°C	25.2×10^9	21.4×10^9	3.8×10^9
Main stem ear 1	18-38°C 8/4/95	12.5×10^9	11.6×10^9	0.9×10^9
Main stem ear 2	18-38°C	12.8×10^9	11.9×10^9	0.9×10^9
Main stem ear 1	21-38°C 13/4/95	11.0×10^9	10.3×10^9	0.7×10^9
Main stem ear 2	21-38°C	9.6×10^9	8.9×10^9	0.7×10^9
Tiller ear 1	18°C 23/4/95	22.3×10^9	19.0×10^9	3.3×10^9
Tiller ear 2	18°C	16.3×10^9	13.7×10^9	2.6×10^9
Tiller ear 1	30-38°C 21/4/95	9.3×10^9	7.5×10^9	1.8×10^9
Tiller ear 2	30-38°C	9.8×10^9	8.1×10^9	1.7×10^9
Tiller ear 1	30-38°C 22/4/95	9.9×10^9	8.0×10^9	1.9×10^9
Tiller ear 2	30-38°C	$9.8.x\ 10^9$	7.9×10^9	$1.9\ 0 \times 10^9$
Tiller ear 1	33-38°C 23/4/95	12.6×10^9	10.2×10^9	2.4×10^9
Tiller ear 2	33-38°C	13.2×10^9	10.3×10^9	2.9×10^9

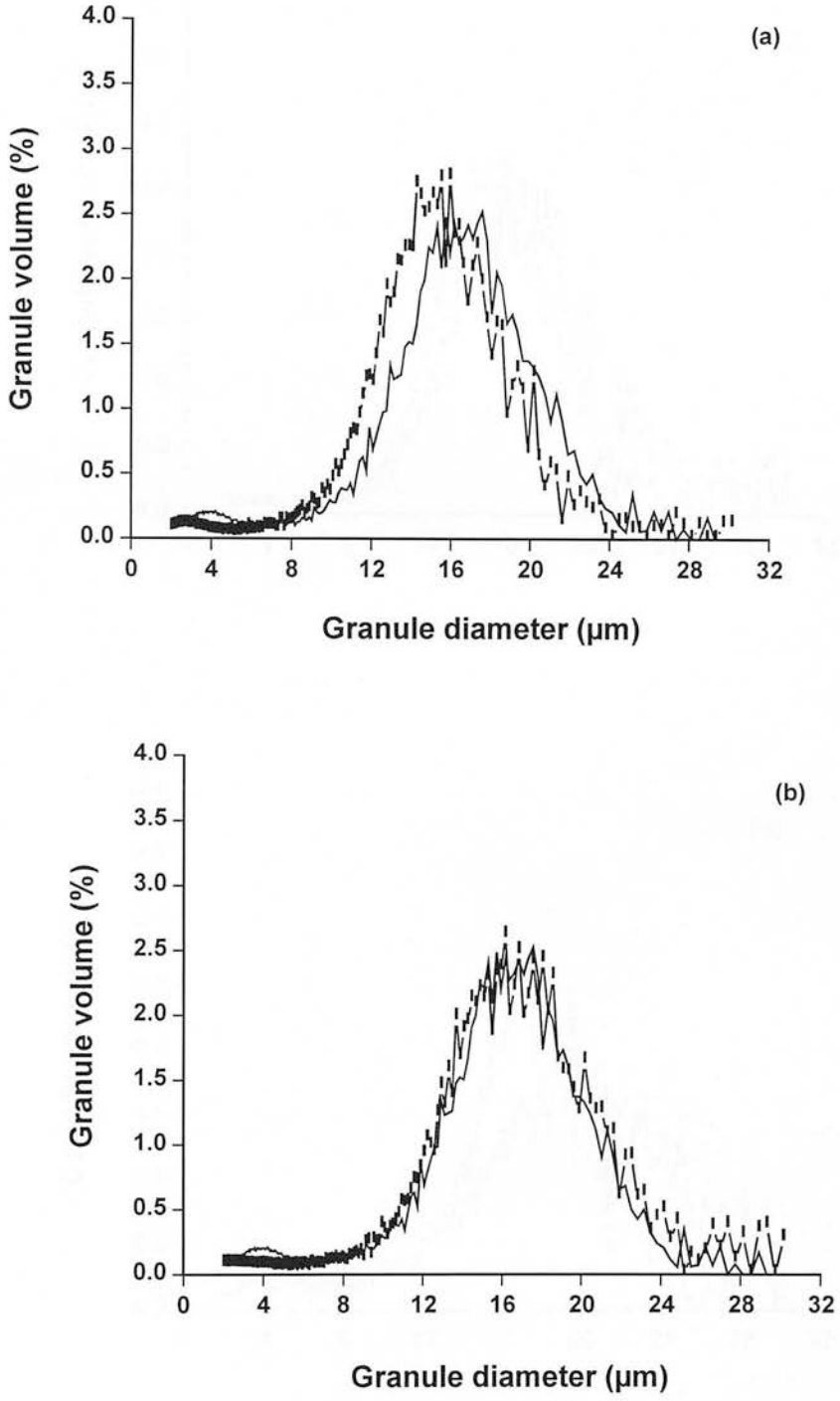


Figure 3.47 .Experiment IV. Percentage of total starch volume in each granule size category of cv. Stirling grown in growth rooms. (a) grains from main stem ears grown at 18°C (solid lines) and 18-38°C (tickmarks).(b) grains of from main stem ears grown at 18°C (solid lines) and 21-38°C (tickmarks). Values are means of 2 replicate ears.

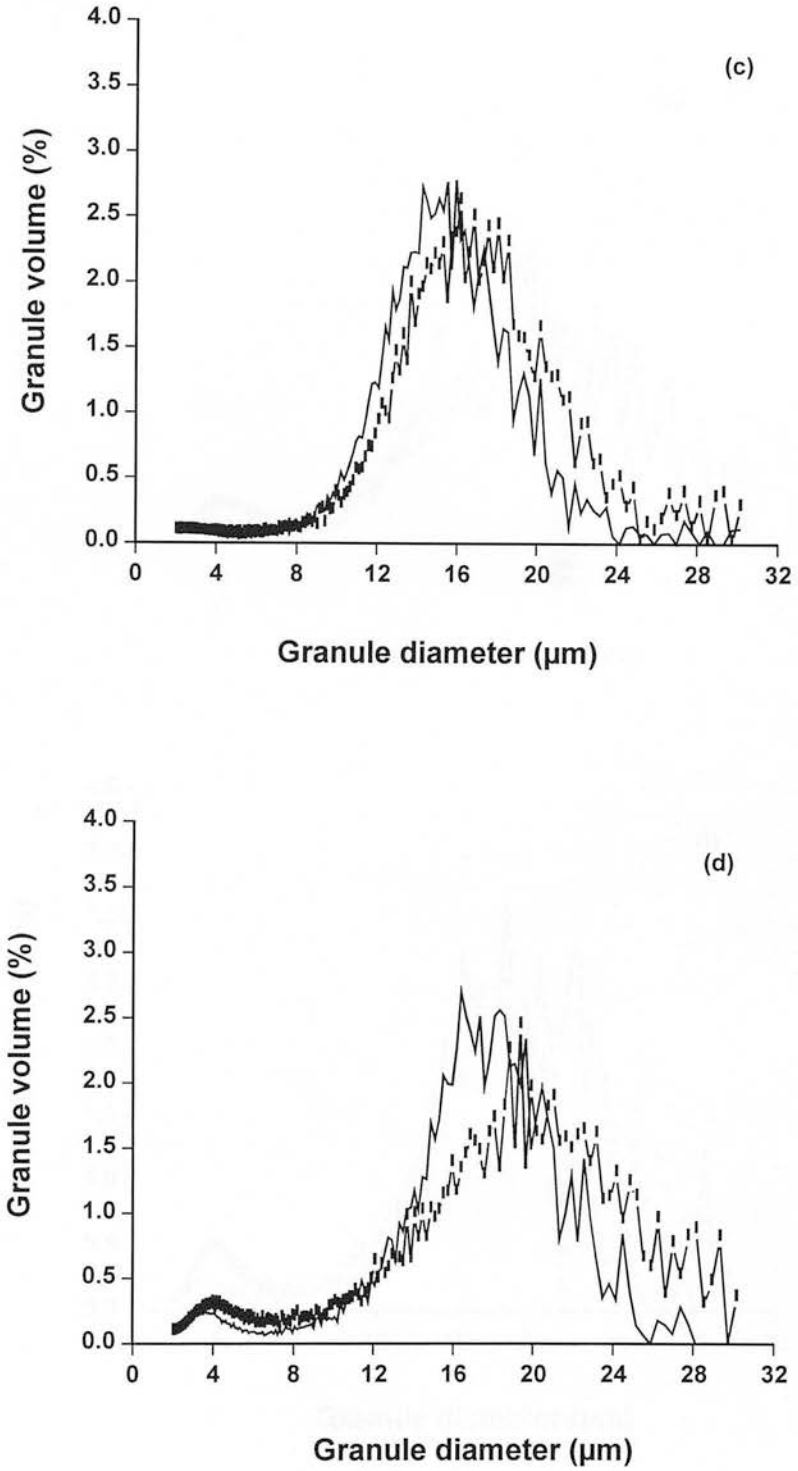


Figure 3.47. (continued) (c) grains of main stem ears (solid lines) and 21-38°C (tickmarks). (d) grains of tiller ears grown at 18°C (solid lines) and 30-38°C (tickmarks.) Values are means of 2 replicate ears.

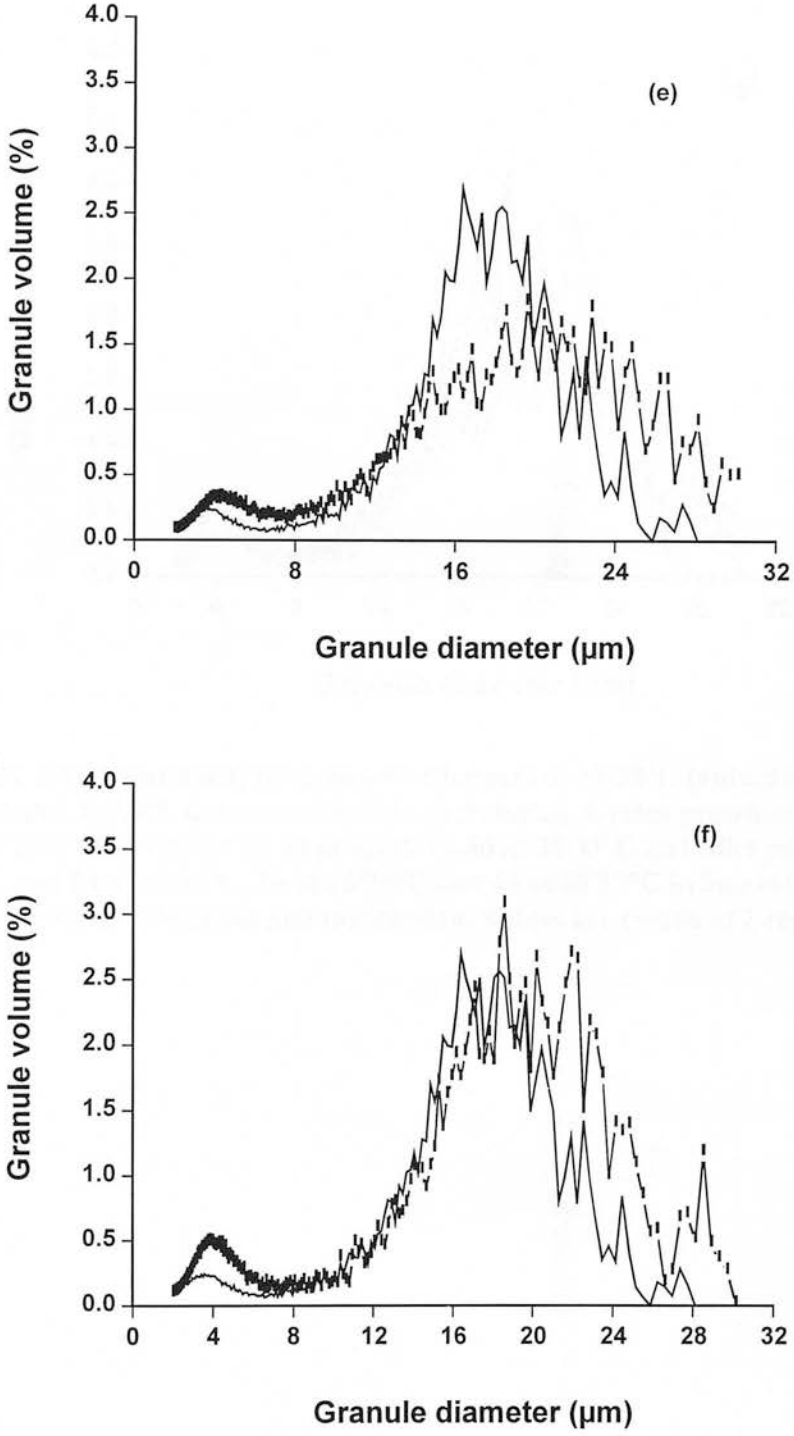


Figure 3.47. (continued) (e) grains of tiller ears at 18°C (solid lines) and 30-38°C (anthesed 22/4/95) (tickmarks). (f) grains of tiller ears at 18°C (solid lines) and 33-38°C (tickmarks). Values are means of 2 replicate ears.

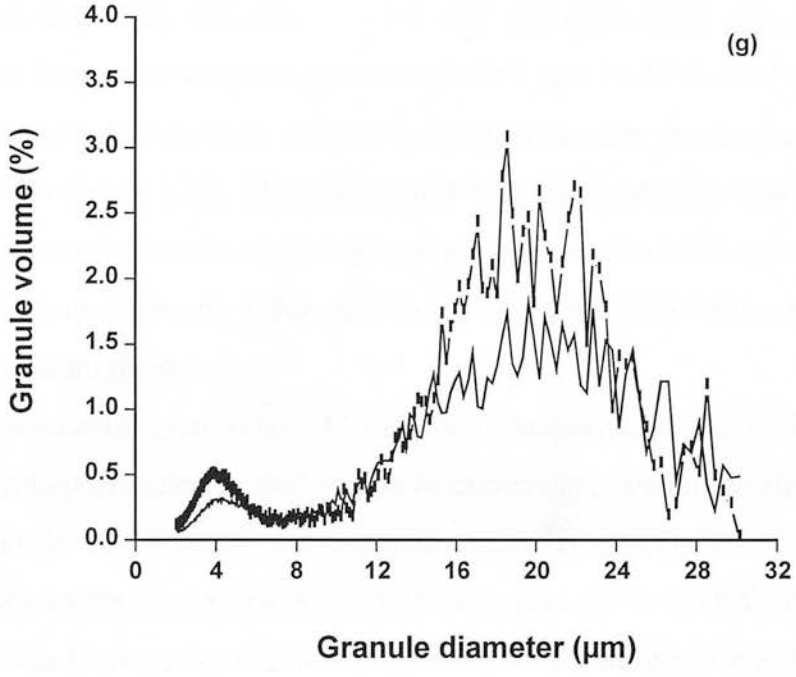


Figure 3.47. (continued) (g) grains of tiller ears at 30-38°C (anthesed 22/4/95) (solid lines) and 33-38°C (anthesed 23/4/95) (tickmarks). Grains grown at 30-38°C had 1 day at 30/25°C, 1d at 33/28°C, 3d at 35/30°C, 4d at 38/33°C and tiller ears grown at 33-38°C had 1d at 33/28°C, 3d at 35/30°C and 4d at 38/33°C before returning to 30/25°C to complete development and maturation. Values are means of 2 replicate ears.

cv. Blenheim

Grains from main stem ears grown at 18°C had more starch granules per grain than grains from main stem ears grown at 18-38°C and 21-38°C. The number of B-type starch granules was more affected by temperature than the number of A-type starch granules (Table 3.15). The number of B-type starch granules was less in grains from tiller ears which anthesed 21/4/95 and grown in the 30-38°C regime than in grains from comparable ears which anthesed one day later (22/4/95) in the same temperature regime.

The percentage granule size distribution by number is shown in Figures 3.48a-f. The percentage granule size distribution by number appeared to be triphasic in grains from tiller ears grown in the high temperature regime (Figures 3.48e, f). The total volume of starch in grains from main stems ears grown at 18°C was greater than that in grains from ears grown in the high temperature regime (Table 3.16). Grains from tiller ears anthesed on 21/4/95 grown at 30-38°C had smaller total volume of B-type granules than comparable ears which anthesed 22/4/95 and grown at 30-38°C. Grains from main stem ears grown at 18°C had a greater percentage volume in the form of B-type granules than grains from comparable main stem ears grown at 18-38°C and 21-38°C. Similarly, grains from tiller ears grown at 18°C had a greater percentage volume in the form of B-type granules than grains from tiller ears which anthesed 21/4/95 (but not in grains from ears which anthesed on 22/4/95) and were grown at 30-38°C (Table 3.16). The percentage distribution of starch granules by volume is illustrated in Figures 3.49a-f).

Table 3.15. Numbers of starch granules of cv. Blenheim grown in growth rooms at 18°C and 18-38°C. Experiment IV. Values for starch extracted from 2 replicate ears.

Cultivar Blenheim Figures 3.48a-f.	Temperature regime and anthesis date	Total granules grain^{-1}	Number of A-type granules grain^{-1} 8.1-> 30μm	Number of B-type granules grain^{-1} < 8.1 μm	% A-type granules 8 > 30μm	% B-type granules < 8.1μm	% B-type granules < 2.1μm
Main stem ear 1	18°C 8/4/95	2.92×10^8	0.092×10^8	2.83×10^8	3.2	96.8	10.0
Main stem ear 2	18°C	2.80×10^8	0.092×10^8	2.71×10^8	3.3	96.7	10.6
Main stem ear 1	18-38°C 5/4/95	1.25×10^8	0.093×10^8	1.16×10^8	7.4	92.6	13.0
Main stem ear 2	18-38°C	1.21×10^8	0.087×10^8	1.12×10^8	7.2	92.8	11.9
Main stem ear 1	21-38°C 13/4/95	1.21×10^8	0.082×10^8	1.13×10^8	6.7	93.3	15.0
Main stem ear 2	21-38°C	0.87×10^8	0.081×10^8	0.80×10^8	9.3	90.7	14.1
Tiller ear 1	18°C 22/4/95	2.80×10^8	0.073×10^8	2.73×10^8	2.6	97.4	9.2
Tiller ear 2	18°C	2.59×10^8	0.092×10^8	2.50×10^8	3.6	96.4	10.0
Tiller ear 1	30-38°C 21/4/95	0.27×10^8	0.044×10^8	0.23×10^8	16.3	83.7	12.7
Tiller ear 2	30-38°C	0.35×10^8	0.038×10^8	0.31×10^8	10.9	89.1	10.3
Tiller ear 1	30-38°C 22/4/95	1.07×10^8	0.046×10^8	1.02×10^8	4.3	95.7	8.7
Tiller ear 2	30-38°C	0.69×10^8	0.038×10^8	0.66×10^8	5.5	94.5	8.6

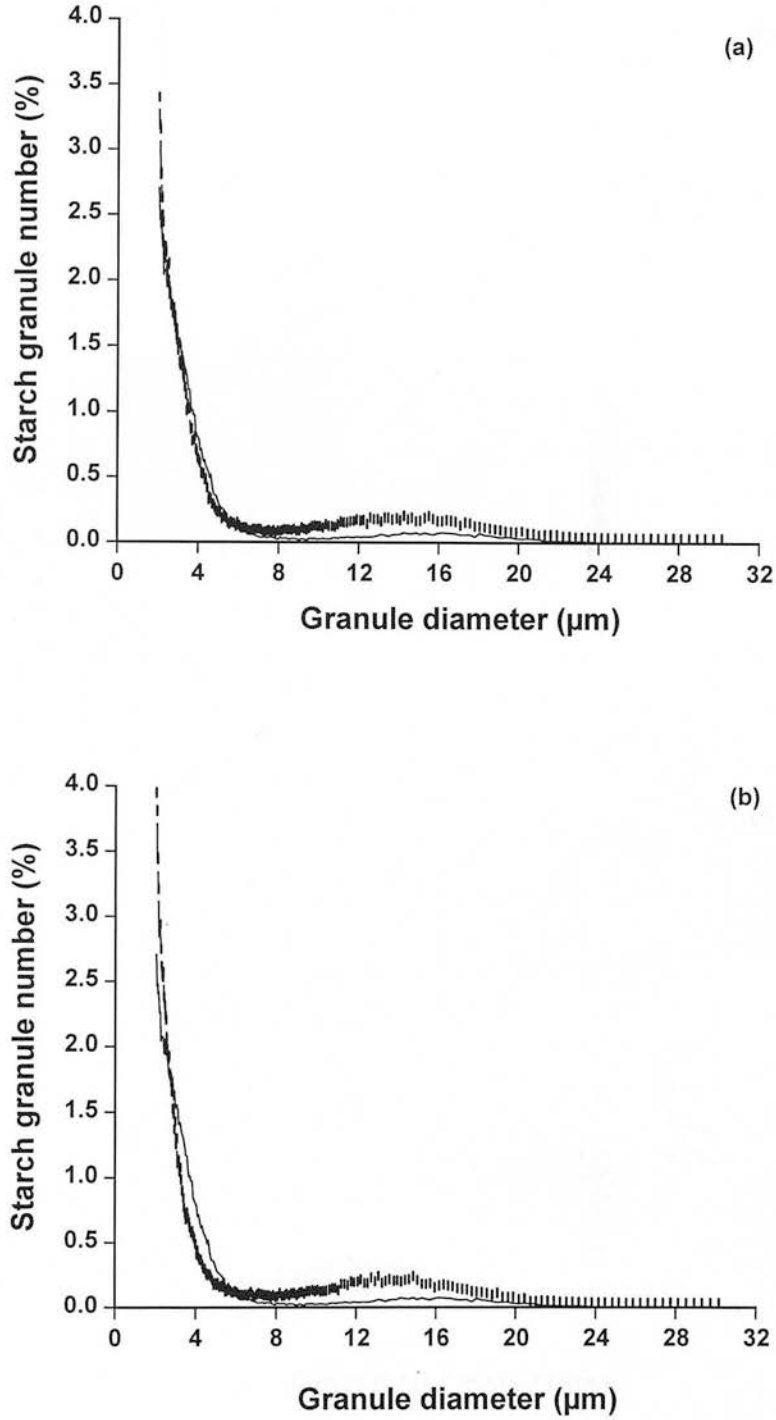


Figure 3.48. Experiment IV. Size distribution of starch granules of cv. Blenheim grown in growth rooms. (a) grains of main stem ears at 18°C (solid lines) and 18-38°C (tickmarks). (b) grains of main stem ears grown at 18°C (solid lines) and 21-38°C (tickmarks). Values are means of 2 replicate ears.

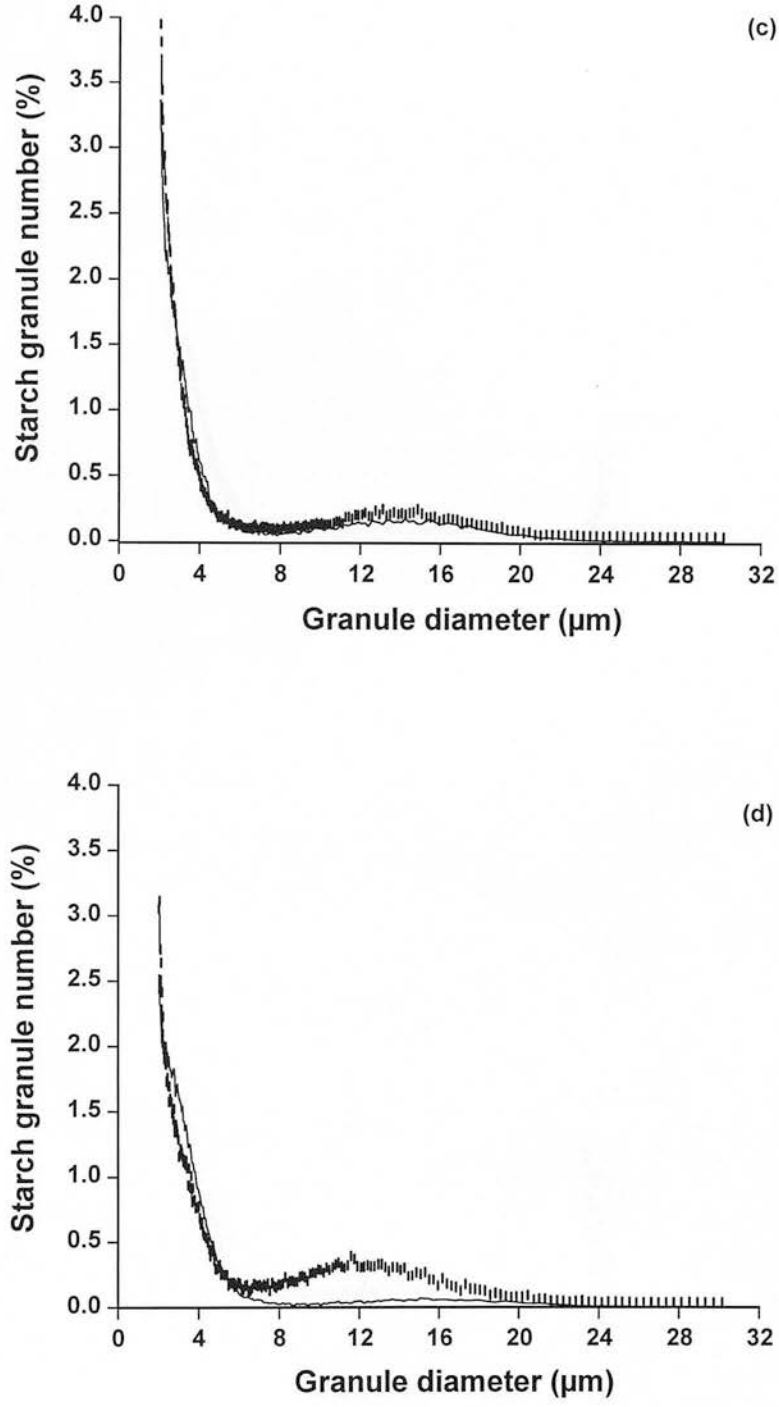


Figure 3.48. (continued) (c) grains of main stem ears at 18-38°C (solid lines) and 21-38°C (tickmarks). (d) grains of tiller ears grown at 18°C (solid lines) and 30-38°C (tickmarks). Grains from tiller ears grown at 30-38°C (anthesed 21/4/95) experienced temperature regimes detailed in Figures 3.47. Values are means of 2 replicate ears.

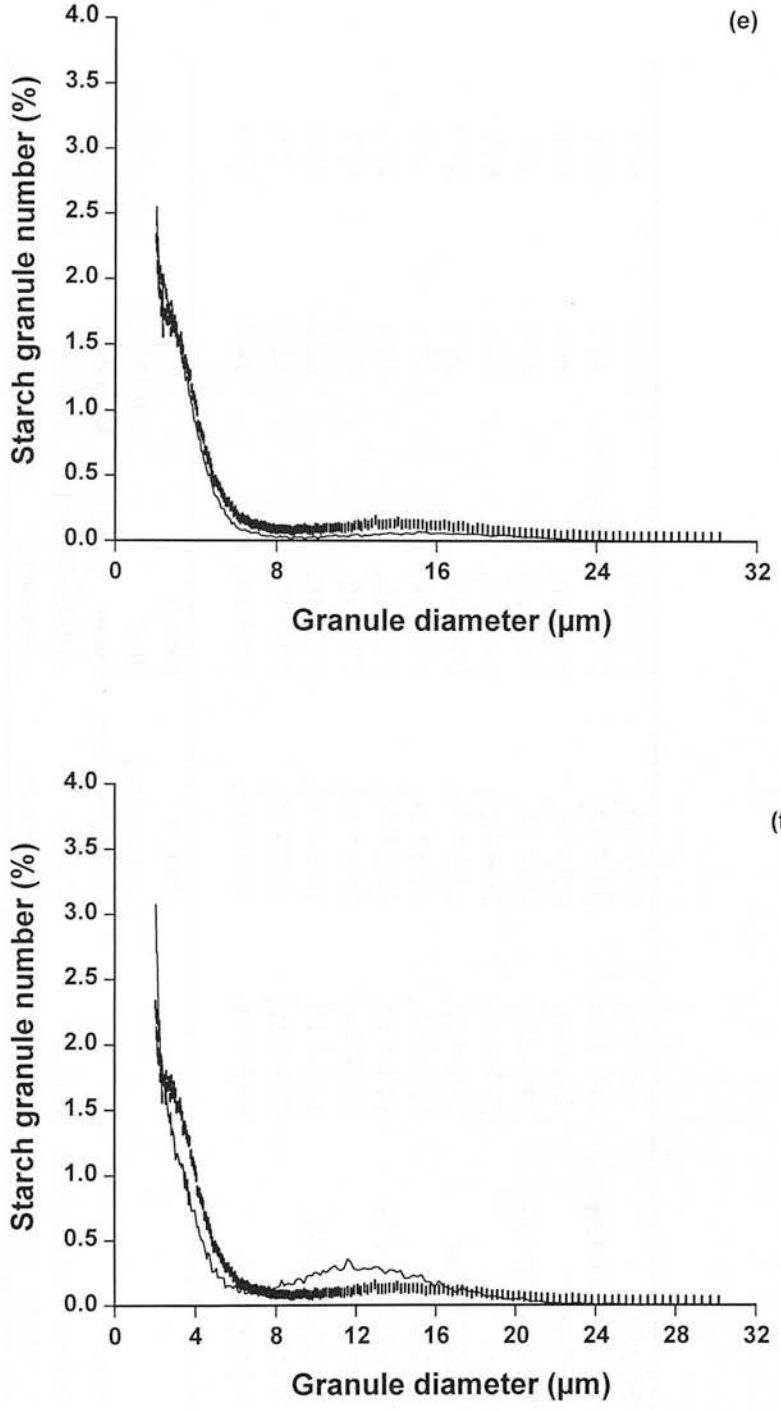


Figure 3.48 (continued) (e) grains of tiller ears grown at 18°C (solid lines) and 30-38°C (anthesed 22/4/95), (tickmarks). (f) grains of tiller ears grown at 30-38°C (anthesed 21/4/95), (solid lines) and 30-38°C (anthesed 22/4/85), (tickmarks). Values are means of 2 replicate ears.

Table 3 16. Volumes of starch granules of cv. Blenheim grown in growth rooms at 18°C and 18-38°C. Experiment IV. Values for starch extracted from 2 replicate ears.

Cultivar Blenheim Figures 3.49a-f.	Temperature regime and anthesis date	Total volume (μm^3)	Volume of A-type granules grain^{-1} (> 8.1 μm) (μm^3.)	Volume of B-type granules grain^{-1} (< 8.1 μm) (μm^3)	Volume of A-type granules > 8.1μm %	Volume of B-type granules < 8.1 μm %
Main stem ear 1	18°C 8/4/95	23.8×10^9	18.6×10^9	5.2×10^9	78.0	22.0
Main stem ear 2	18°C	22.7×10^9	17.6×10^9	5.1×10^9	77.6	22.4
Main stem ear 1	18-38°C 5/4/95	17.1×10^9	15.0×10^9	2.1×10^9	88.0	12.0
Main stem ear 2	18-38°C	16.2×10^9	14.4×10^9	1.8×10^9	88.7	11.3
Main stem ear 1	21-38°C 13/4/95	14.4×10^9	12.7×10^9	1.7×10^9	88.3	11.7
Main stem ear 2	21-38°C	13.3×10^9	12.0×10^9	1.3×10^9	90.4	9.6
Tiller ear 1	18°C 22/4/95	17.9×10^9	12.7×10^9	5.2×10^9	70.7	29.3
Tiller ear 2	18°C	25.9×10^9	20.9×10^9	5.0×10^9	80.6	19.4
Tiller ear 1	30-38°C 21/4/95	5.8×10^9	5.3×10^9	0.5×10^9	91.4	8.6
Tiller ear 2	30-38°C	5.9×10^9	5.2×10^9	0.7×10^9	88.5	11.5
Tiller ear 1	30-38°C 22/4/95	10.9×10^9	8.6×10^9	2.3×10^9	78.5	21.5
Tiller ear 2	30-38°C	7.8×10^9	6.3×10^9	1.5×10^9	80.8	19.2

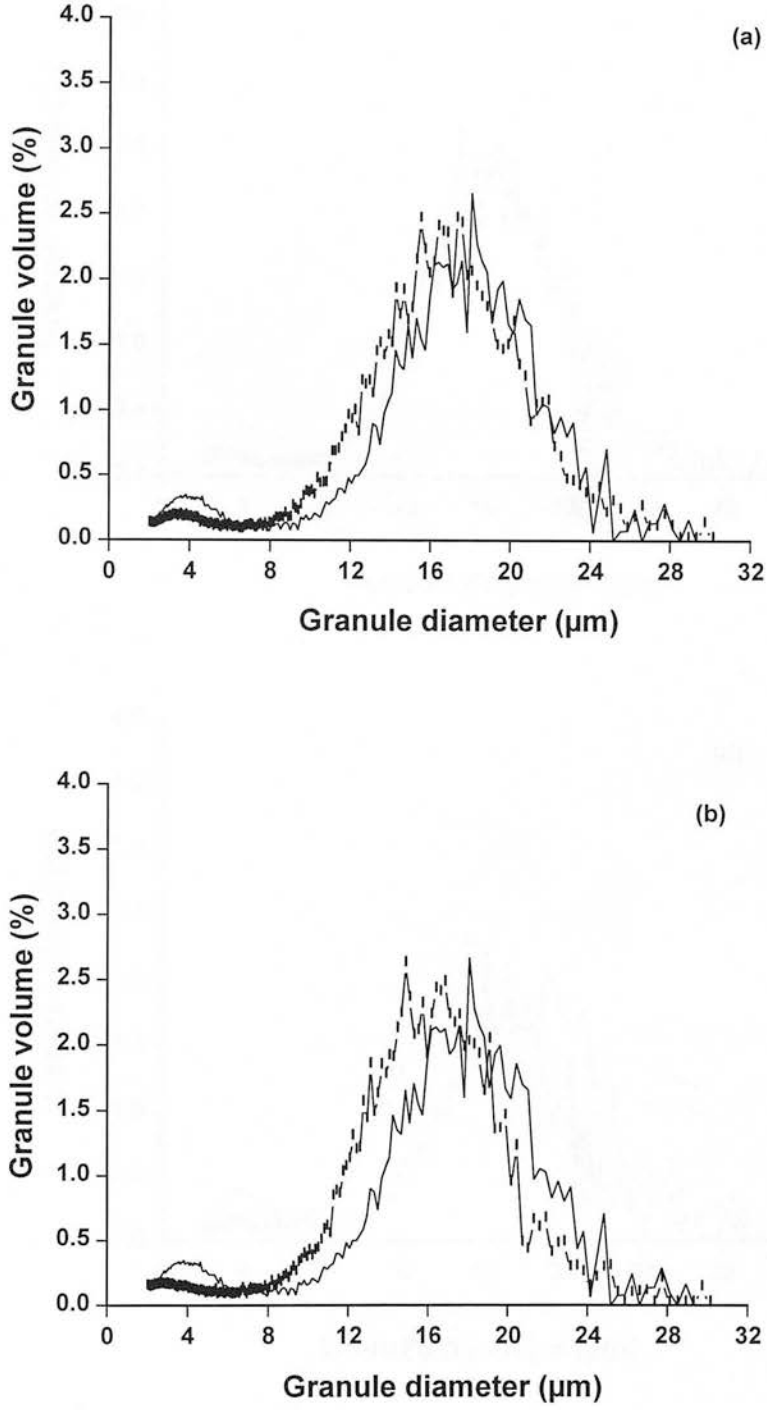


Figure 3.49. Experiment IV. Percentage of total starch volume in each granule size category of starch extracted from grains of cv. Blenheim grown in growth rooms. (a) grains from main stem ears at 18°C (solid lines) and 18-38°C (tickmarks). (b) grains of main stem ears grown at 18°C (solid lines) and 21-38°C (tickmarks). Values are means of 2 replicate ears.

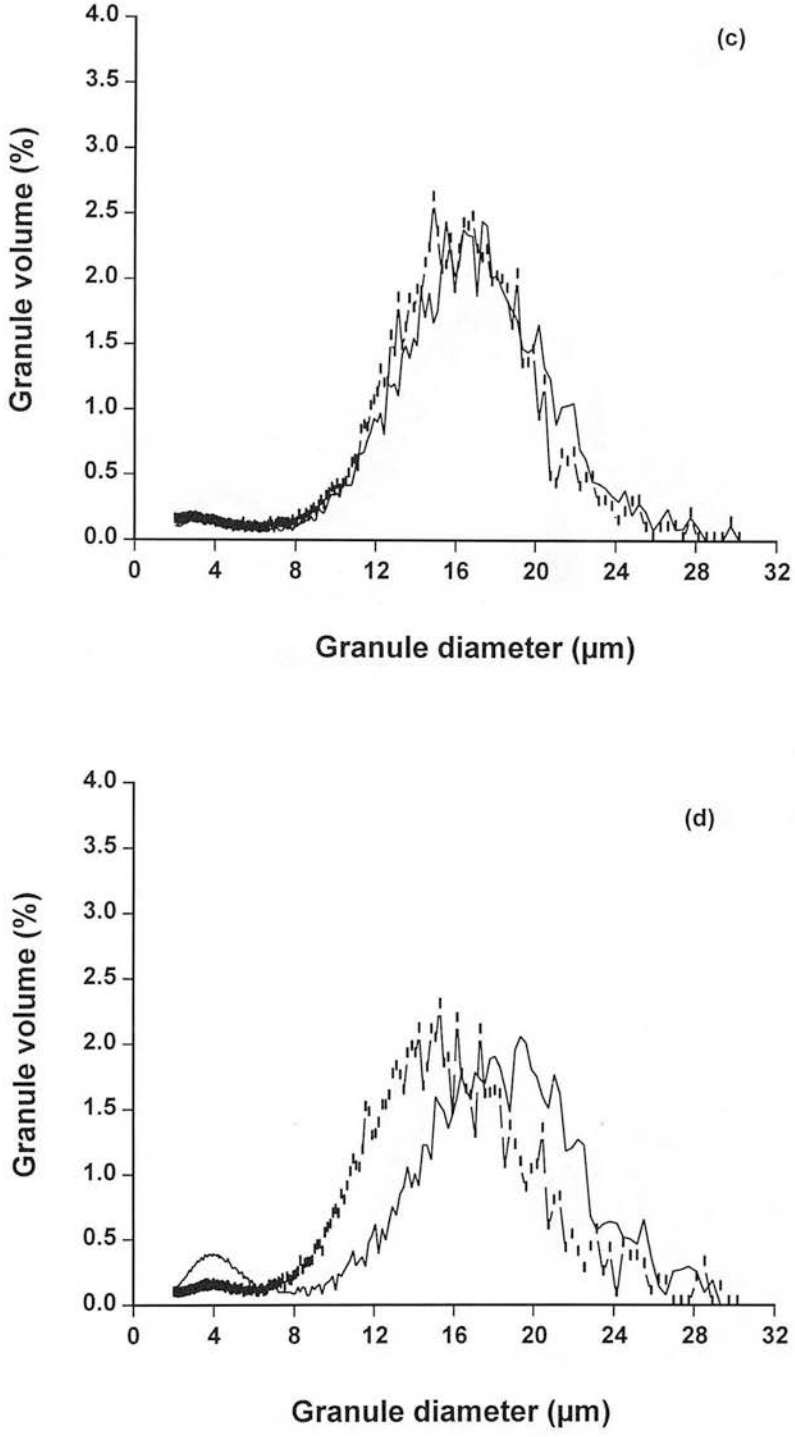
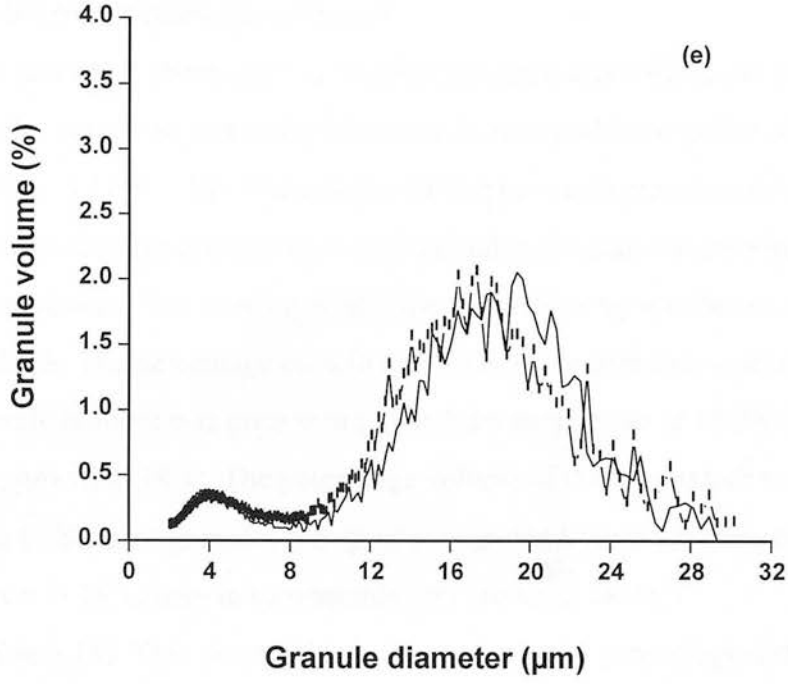


Figure 3.49. (continued) (c) grains of main stems at 18-38°C (solid lines) and 21-38°C (tickmarks). (d) grains of tiller ears at 18°C (solid lines) and 30-38°C (anthesed 21/4/95), (tickmarks). Values are means of 2 replicate ears.



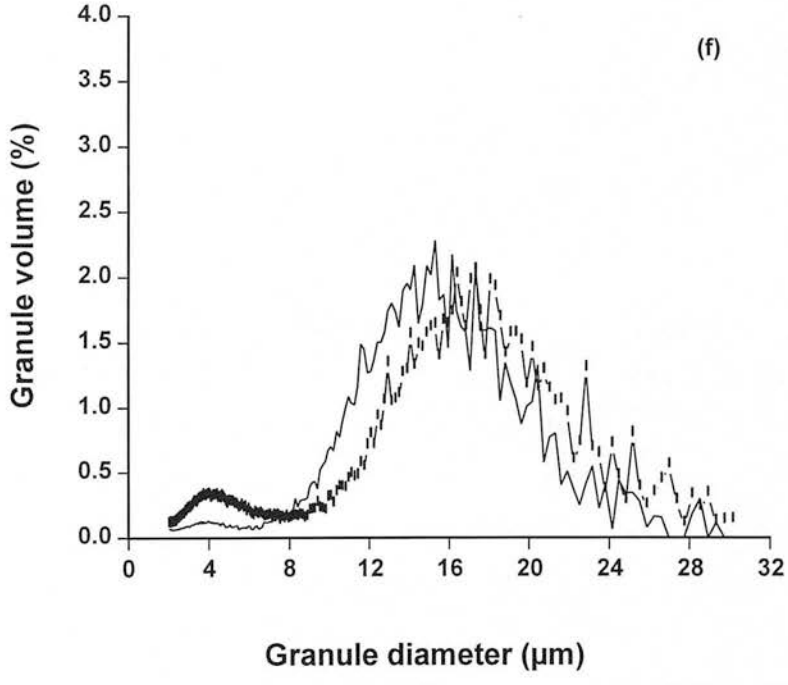


Figure 3.49 (continued) (e) grains of tiller ears grown at 18°C (solid lines) and 30-38°C (anthesed 22/4/95), (tickmarks). (f) grains of tiller ears grown at 30-38°C (anthesed 21/4/95), (solid lines) and 30-38°C (anthesed 22/4/95), (tickmarks). Values are means of 2 replicate ears.

cv. Schooner (Growth cabinets)

The pattern of starch granule number per grain and volume per grain followed a similar pattern to that observed in starch extracted from grains of cv. Blenheim (Tables 3.17 & 3.18). The number of B-type starch granules per grain was more affected than the number of A-type granules per grain by growing the ears at high temperatures. The starch granule size distribution by number is shown in Figure 3.50a, b. The percentage contribution of 6-16μm diameter granules to the total starch granule number was greater in grains from ears grown at 18-38°C than in grains from ears grown at 18°C. The percentage volume of the total starch volume present as B-type (< 8.1 μm) granules was greater in grains from main stem ears and tiller ears grown at 18°C than in comparable ears grown at 18-38°C
(Table 3.18). This observation is illustrated in the percentage distribution by volume graphs in Figure 3.50a, b.

Table 3.17. Numbers of starch granules of cv. Schooner grown in growth cabinets at 18°C and 18-38°C. Experiment IV. Values for starch extracted from 2 replicate ears.

Cultivar Schooner Growth cabinet. Figures 3.50a & b.	Temperature regime and anthesis date	Total granules grain $^{-1}$	Number of A-type granules grain $^{-1}$ 8.1-> 30μm	Number of B-starch granules grain $^{-1}$ < 8.1 μm
Main stem ear 1	18°C 12/4/95	2.08×10^8	0.145×10^8	1.94×10^8
Main stem ear 2	18°C	1.80×10^8	0.124×10^8	1.68×10^8
Main stem ear 1	18-38°C 12/4/95	0.52×10^8	0.086×10^8	0.43×10^8
Main stem ear 2	18-38°C	0.53×10^8	0.093×10^8	0.44×10^8
Tiller ear 1	18°C 22/4/95	1.94×10^8	0.091×10^8	1.85×10^8
Tiller ear 2	18°C	1.85×10^8	0.080×10^8	1.77×10^8
Tiller ear 1	27-38°C 22/4/95	0.26×10^8	0.049×10^8	0.211×10^8
Tiller ear 2	18-38°C	0.36×10^8	0.057×10^8	0.30×10^8

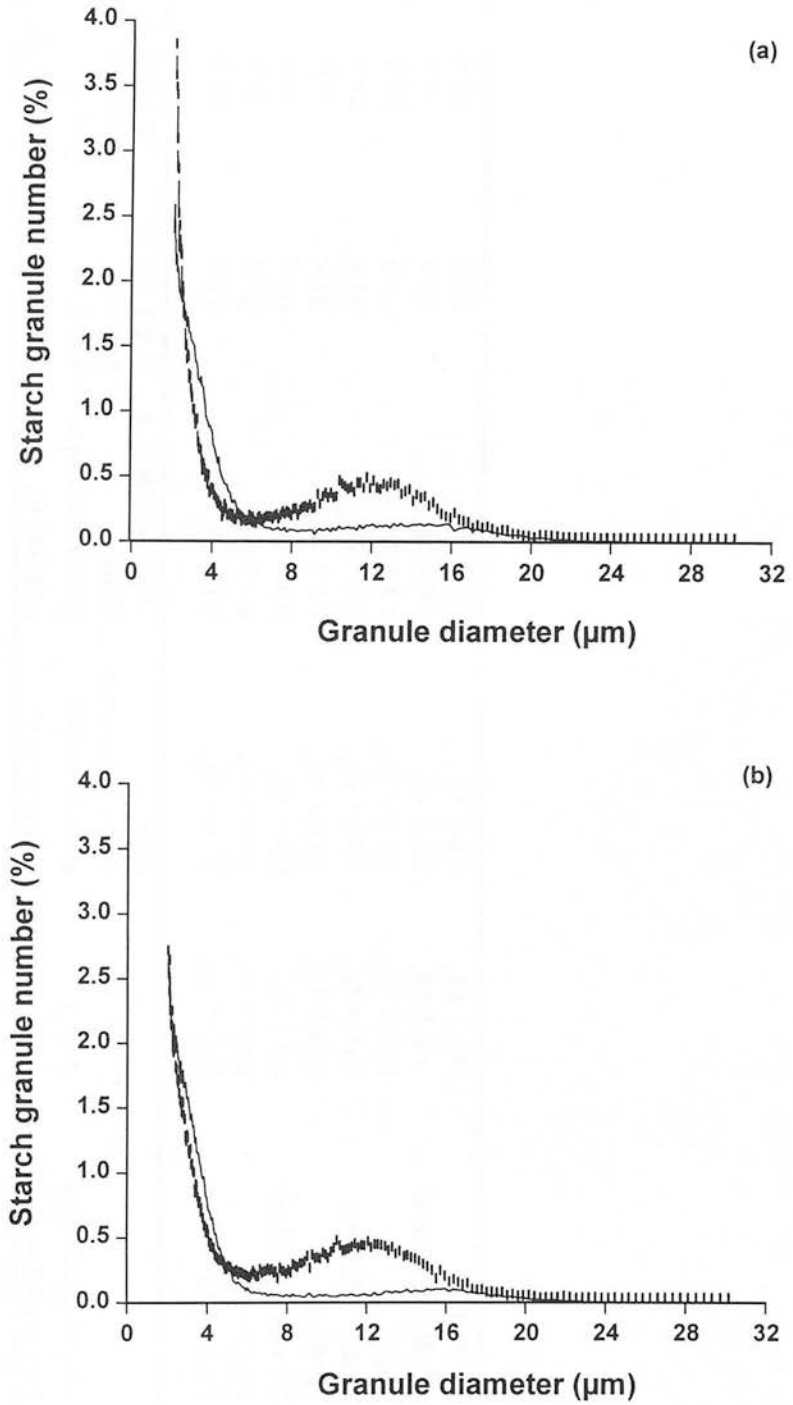


Figure 3.50 Experiment IV. Size distribution of strach granules of cv. Schooner grown in growth cabinets. (a) grains from main stem ears grown at 18°C (solid lines) and 18-38°C (tickmarks). (b) grains of tiller ears grown at 18°C (solid lines) and 18-38°C (tickmarks). Values are means of 2 replicate ears.

Table 3.18. Volumes of starch granules of cv. Schooner grown in growth cabinets at 18°C and 18-38°C. Experiment IV. Values for starch extracted from 2 replicate ears.

Cultivar Schooner Growth cabinets. Figures 3.51a & b.	Temperature regime and anthesis date	Total volume (μm^3)	Volume of A-type granules grain^{-1} >8.1μm (μm^3)	Volume of B-type granules grain^{-1} < 8.1 μm (μm^3)	Volume of A-type granules > 8.1 μm %	Volume of B-type granules < 8.1 μm %
Main stem ear 1	18°C 12/4/95	27.2×10^9	23.1×10^9	4.1×10^9	85.0	15.0
Main stem ear 2	18°C	22.0×10^9	18.3×10^9	3.7×10^9	83.2	16.8
Main stem ear 1	18-38°C 12/4/95	9.8×10^9	8.9×10^9	0.9×10^9	91.0	9.0
Main stem ear 2	18-38°C	11.5×10^9	10.5×10^9	0.9×10^9	91.8	8.2
Tiller ear 1	18°C 22/4/95	19.0×10^9	15.5×10^9	3.5×10^9	81.6	18.4
Tiller ear 2	18°C	16.4×10^9	13.0×10^9	3.4×10^9	79.3	20.7
Tiller ear 1	27-38°C 22/4/95	5.8×10^9	5.2×10^9	0.6×10^9	90.3	9.7
Tiller ear 2	27-38°C	6.7×10^9	6.0×10^9	0.7×10^9	89.2	10.8

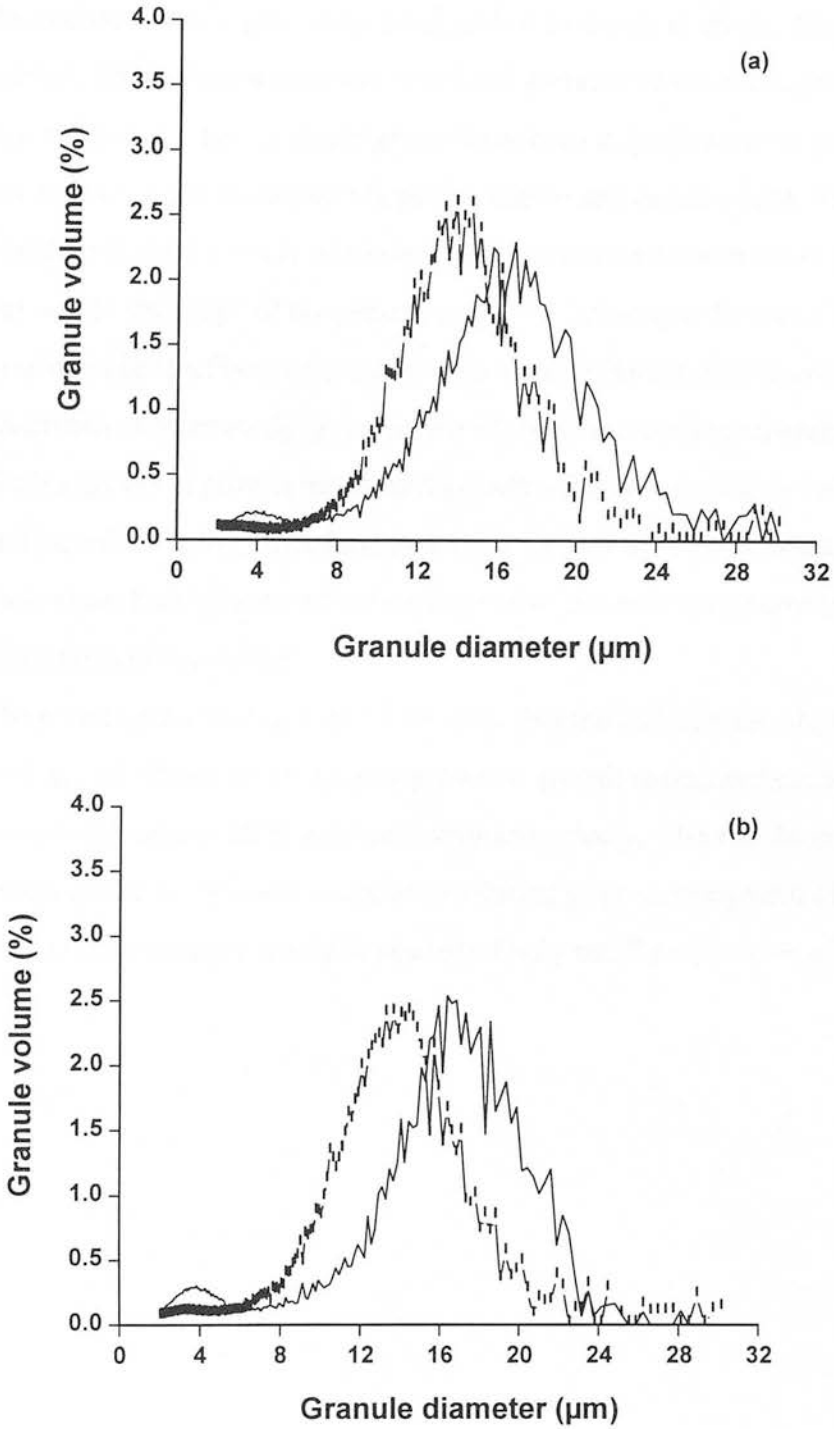


Figure 51. Experiment IV. Percentage of total starch volume in each granule size category of starch extracted from grans of cv. Schooner grown in growth cabinets. (a) grains of main stem ears at 18°C (solid lines) and 18-38°C (tickmarks). (b) grains of tiller ears grown at 18°C (solid lines) and 18-38°C (tickmarks). Values are means of 2 replicate ears.

3. 11. Endosperm morphology

An endosperm of a grain may be classified as mealy or steely. Mealy grains have been defined as those grains in which cut surfaces of the endosperms are white and non-reflecting whereas steely grains have been defined as those grains in which the cut surface of the endosperm is golden brown and reflects light. The conditions which give rise to mealy or steely grains are not well understood. Although, it was not within the scope of the present project to investigate factors responsible for mealiness or steeliness of grains grown under different growth conditions, observations were made in the course of the process of starch granule extraction. Grains grown in growth rooms and growth cabinets were cut in half longitudinally and examined using angled incident light. Grains were classified as mealy or steely if more than three quarters of the endosperm of the endosperm corresponded to the definitions given above.

The results presented in Table 3.19 show that the endosperms of grains of cvs Stirling, Blenheim and Schooner grown in growth rooms and cv. Schooner grown in growth cabinets at 18°C were predominantly steely, whereas the endosperms of grains grown at elevated temperatures during grain development (Table 3.19) were either predominantly mealy or contained only small proportions of steely endosperm.

Table 3.19. Endosperm appearance of cvs Stirling, Blenheim and Schooner grown in growth rooms and growth cabinets at different temperature regimes during grain development.

Cultivar	Temperature regimes and anthesis date	Steely approx. %	Mealy/steely approx. %	Mealy approx. %
Stirling main stems	18°C (9/4/95)	100	0	0
Stirling tillers	18°C (23/4/95)	100	0	0
Stirling main stems	18-38°(C 8/4/95)	0	0	100
Stirling main stems	21-38°C (13/4/95)	0	0	100
Stirling tillers	30-38°C (21/4/95)	0	25/75	0
Stirling tiller	30-38°C (22/4/95)	0	50/50	0
Stirling tillers	33-38°C 23/4/95	0	0	100
Blenheim main stems	18°C (8/4/95)	100	0	0
Blenheim tillers	18°C (22/4/95)	0	50/50	0
Blenheim main stems	18-38°C (5/4/95)	0	0	100
Blenheim main stems	21-38°C (13/4/95)	0	0	100
Blenheim tillers	30-38°C (21/4/95)	0	0	100
Blenheim tillers		0	25/75	0
Schooner (gc) main stems	(30-38°C (22/4/95)	100	0	0
Schooner (gc) tillers	18°C (22/4/95)	100	0	0
Schooner main stems	18-38° (C 12/4/95)	0	50/50	0
Schooner tillers	18-38° (C 22/4/95)	0	25/75	0

4. DISCUSSION

Grain development

The rate of dry matter accumulation in grains of cvs Blenheim and Stirling was higher in the 18-38°C temperature regime than at 18°C, but the duration of grain-filling was shorter in ears grown at 18-38°C than in ears grown at 18°C. These results are in agreement with the findings of Sofield, Evans, Cook & Wardlaw (1977) in an experiment on plants of wheat grown in controlled environment cabinets at 30/25°C. However, results of the current study are at variance with the results of Jenner (1994). He reported that when plants were subjected to temperatures above 30°C i.e. in the high temperature range (HTR) between 30°C and 40°C, even for short periods, the rate of dry matter deposition was slower than that observed at 21°C. However, in the experiment reported by Jenner, ears were exposed to 35/25°C for 7 days, 20 days after anthesis whereas in this experiment the temperature was raised gradually until a maximum temperature of 38/33°C was attained. The effect on the rate of dry matter accumulation at 35/25°C observed by Jenner was possibly due to the stage of grain development at which high temperature was imposed. Imposition of high temperature 20 days after anthesis seems to be rather late.

There were also genotypic differences in the rate of dry matter accumulation at 18-38°C and 18°C. The initial dry matter accumulation was higher in cv. Blenheim than in cv. Stirling. The rate of net deposition of water was greater in grains of both cultivars grown at 18-38°C than in grains grown at 18°C. Grain water content and total dry matter accumulation was affected by elevated temperature to a similar extent in both cultivars. High temperature hastened the rapid water loss leading to early attainment of harvest-ripeness in grains grown at 18-38°C.

Grain number

Temperature, light intensity and day-length are some of the factors which may affect final grain yield. The objective of the research project was not to study grain yield components. Fewer grains were harvested in high temperature regimes than in the control temperature regime therefore the number of grains harvested was determined to relate the data on the number of grains to the number of tests that could be carried out.

In Experiments I & II, the total number of grains harvested and the number of grains per ear was greater in ears grown at 18°C and 18-30°C than in grains grown at 30-18°C and 30°C. The response of ears to temperature varied with the stage of grain development, and the duration of exposure. Grain number was markedly lower in ears which experienced 30°C (C & D) at anthesis than in ears which experienced 30°C after anthesis. This is supported by the finding of Savin and Nicolas (1996) on cvs Schooner and Franklin grown in glasshouse at 20/15°C from anthesis to harvest-ripeness, 40/15°C for 5 days from 15 to 25 days after anthesis and 10 days at 40/15°C in the period 15 to 25 days after anthesis. Their report showed that exposure of ears to high temperatures for 5 or 10 days during the period 15 to 25 days after anthesis did not affect grain number. There was a reduction in the number of grains per ear due to sterility when temperature treatment was commenced at anthesis. This seems to show that in respect of grain number the magnitude of responses to high temperature is usually greater when the temperature treatment is given very early in grain development.

The results obtained in Experiments I & II were confirmed by the results of Experiments III & IV in which fewer grains were harvested from ears which experienced 30°C and 18-38°C (seed lot 2) at anthesis than in ears which experienced the 30°C & 18-38°C after anthesis. Grain number per ear was largely affected by elevated temperatures (30°C & 18-38°C) occurring at anthesis by reducing grain-bearing spikelets. These results are in agreement with the findings of Warrington, Dunstone and Green (1977) and of Tashiro and Wardlaw (1990) in experiments carried out on wheat in controlled environments at 15/10°C, 20/15°C 25/20°C, and 30/25°C. They reported that high temperatures (25/20°C & 30/25°C) imposed at anthesis reduced the number of grains per ear.

The number of grains harvested was lower in seed lot 2 than in grains of seed lot 1 in all four Experiments reported. This trend may not only be due to temperature effects but also to the possibility of competition for assimilates between stem ears and tiller ears. The number of grains in seed lot 2 of ears grown at 18°C was consistently lower than that of seed lot 1 in ears grown at 18°C. Seed lot 2 grains were mostly from tiller ears. Variation in the number of grains on a whole plant basis depends on the relationship between the main stem and tillers. Kirby and Jones (1977) found that the removal of tillers from barley plants grown under field conditions increased the number of grains

per ear of the main stem whereas the removal of the main shoot very early in development encouraged the remaining tillers to produce more grains per ear than there were in main stem ears of plants in which tillers were not removed (control). Kirby and Jones concluded that competition between the main stems and tillers resulted in a low number of grains per ear. However, plants grown under controlled environment conditions tiller more profusely than do field-grown plants (MacNicol, Jacobson, Keys and Stuart, 1993). In a field situation, plants normally produce tillers which die at an early stage without bearing an ear. Competition for assimilates may occur between main stem ears and tiller ears when current photosynthesis is unable to meet the demands for grain-filling. Thus stem-stored assimilate has to be mobilised to meet the shortfall for grain growth. In many experiments carried out in controlled environment conditions, tillers are removed to minimise competition and shading in conditions of low light intensity.

The light intensity in field conditions in Scotland in mid-summer on a sunny day is approximately 460 μmol m^{-2} s^{-1} and on a dull day is approximately 230 μmol m^{-2} s^{-1} (Cochrane and Duffus, 1994) whereas the light intensity supplied by mercury vapour lamps at ear level in growth rooms was 140 μmol $m^{-2}s^{-1}$ and in growth cabinets 131 μmol m^{-2} s^{-1}. It is possible that fewer grains per ear were obtained from tiller ears than from main stems in the current study due to competition and low light intensity caused by shading (in temperature 18°C). Thus tillers produced after the establishment of the main stem competed for assimilates retranslocated from stem stored assimilates in order to support grain growth. Studies on shading or leaf removal have indicated that source limitation during the earlier stage of grain-filling resulted in a decrease in grain number. Spiertz (1977) reported that plants of wheat cv. Orca grown under low light intensity produced fewer grains per ear than plants grown under high light intensity. This evidence was supported by the results of Takahashi and Kanazawa (1996) in a field experiment on wheat using a 95 % cloth shading. They reported that shading initiated during the early stage of grain-filling resulted in fewer grains per ear.

In Experiment V (comparison of genotypes), in which plants were grown to harvest-ripeness under glasshouse conditions at approximately 18/13°C and a day length of 18 h, the total grain yield (g) varied among the 11 genotypes. Plants of cvs Blenheim, Harrington, Centinella and Puebla produced larger ears and a greater number of

productive high order tillers than other cultivars. Although cv. Tyne had a large number of productive tillers the ears were very short and the grains were very small. However, cvs Puebla, BRB2, Esperanza and Esmeranda were of Mexican origin and cvs Clipper, Stirling and Schooner were of Australian origin, and the total grain yield (g) and yield per plant (g) were lower in these cultivars than in cvs Blenheim (UK), Cantinella (Mexican) and Harrington (Canadian). It may be possible that some of these cvs were sensitive to day-length. When cvs Stirling and Schooner were grown in controlled environment conditions with a day length of 16 h, they produced plants with more tillers and longer ears than when they were grown under glasshouse conditions with a day length of 18h. This statement may be supported to a certain extent by the findings of Kernich, Slafer and Halloran (1995) who carried out a field experiment on two Australian barley cvs Bundulla and Gallen using different photoperiods and found that the optimum photoperiod at which there was no further rise was 14-15 h. It would appear that the day length of 18 h used in Experiment V induced the production of fewer tillers and shorter ears in Australian cultivars in comparison to the U.K and Canadian cultivars.

Grain weight

Plants of cvs Blenheim and Stirling grown in growth rooms at 30-18°C, 30°C and 18-38°C and cv. Schooner grown in growth room and growth cabinet at 30 °C, 18-38°C and 30-38°C had a lower mean grain weight than comparable plants grown at 18°C. The effect of elevated temperature on grain weight depended on the timing and duration of the high temperature in relation to the stage of development of the grain. Grain weights of plants of cv. Blenheim (Experiments I & II) which anthesed at 30°C (regimes C & D seed lot 2) were greater than those of comparable plants which experienced 30 °C after anthesis (C & D seed lot 1). Grains in regimes (C & D) which experienced 30°C at anthesis had few grains per ear due to grain sterility. However, grains in temperature regime (C) in which plants were grown at 30°C for 7 days and 10 days at or immediately after anthesis (Experiments I & II) and then returned to 18°C had a lower mean grain weight than grains grown at 18°C. This seems to demonstrate that the residual effects of high temperature applied at anthesis are carried over after transferring the plants from higher temperature to lower temperature conditions. In Experiments III

and IV, mean grain weights of grains grown at 30°C and 18-38°C were less than those of grains grown at 18°C (**Section 3. 1. 2.**).

It is possible that lack of sufficient assimilates in plants grown in the high temperature regimes contributed to the lower mean grain weights observed in ears grown at elevated temperatures (30°C & 18-38°C) than those ears grown at 18°C. Although no measurements were made comparing high and low temperature regimes, flag leaves and ears of the plants in the high temperature regimes lost their green colour rapidly, thus plants senesced early. This leaf senescence would affect the grain filling period and subsequent grain weight in grains grown at elevated temperatures (30°C & 18-38°C). Blum, Sinmena, Mayer, Golan and Shpiler (1995) reported that at high temperatures, current leaf and ear photosynthesis was less than in low temperature conditions and therefore an alternative source of carbon for grain filling was the stored stem reserves. The response thus depends on the pre-anthesis experience of the plants and also on the ability of the enzymes in endosperm cells to convert sucrose to starch at elevated temperatures. The activity of the enzymes involved in starch biosynthesis has been reported to be less in storage organs grown at high temperatures than in those grown at low temperature in barley (MacLeod and Duffus, 1988), wheat (Bhullar and Jenner, 1986; Jenner, 1991; Hawker and Jenner, 1993) and in grape vine (Hawker, 1982). It is possible therefore, that elevated temperatures interfere with the capacity of the endosperm to convert sucrose to starch at high temperature. Grain weight may also be affected by shorter period of grain-filling at elevated temperatures (**Section 3.8.**) which is not compensated for by the increase in the rate of grain filling (Sofield *et al.*, 1977; Bhullar and Jenner, 1986). Thus grain weight at elevated temperatures appears to be affected by a reduction in the amount of starch deposited during grain development as a result of the shortened duration of grain-filling, and of the failure of enzymes involved in starch biosynthesis to function adequately at elevated temperatures.

Grain size/dimensions

Seed quality is influenced by the genetic attributes of the plant and the environment under which the grain is produced. A decrease in seed dimensions in response to high temperature has been reported by Tashiro and Wardlaw (1990). In the present investigation, high temperatures of 30°C and 18-38°C adversely affected grain size.

Grains harvested from plants grown in temperature regime D (30°C) of Experiments I & II had a higher proportion of small grains (**Section 3. 1. 3.**) (< 2.5 > 2.2 mm and < 2.2 mm) than grains grown in temperature regimes (A, B and C). However, the effects of elevated temperatures on grain size and dimensions varied in temperature regimes (B & C) of cv. Blenheim (Experiments I & II) depending on the timing and duration of exposure to elevated temperature relative to the stage of grain development.

Grains grown at 18°C in Experiment III, were smaller than grains grown at the same temperature in other experiments. Pre-anthesis events could have been responsible for the smaller grains observed in this experiment. It is possible that while growing in the glasshouse the plants did not receive adequate nutrients. No supplementary nutrients were given to the plants in controlled environment conditions. The day length was the same in all the experiments. This inconsistency under identical growing conditions indicates variation which may be caused by modifying factors other than temperature. Differences in the results from experiments carried out under similar growth conditions using the same genotypes and the same laboratory facilities have been reported by other research workers.

However, the results of Experiments III & IV clearly demonstrated that grains from plants grown at 30°C and 18-38°C were very small and mostly in < 2.5 > 2.2 mm and < 2.2 mm categories. Circumstantially, grains of cv. Schooner grown in growth room at 30-38°C, transferred from 18°C to 30-38°C had a higher proportion of grain size of > 2.8 mm than grains which experienced gradual change until attaining the maximum temperature of 38°C. Transfer of cv. Schooner from 18-38°C (Gr) resulted in a high degree of sterility.

The results of size analysis of grains of 11 genotypes grown under glasshouse conditions showed that most of the grains were distributed between > 2.8 mm and < 2.8 >2.5 mm categories in the majority of the cultivars apart from cvs Esmeranda, Esperanza and Tyne. Thus grains grown under higher light intensity supplied by natural daylight and supplemented with mercury vapour lamps were bigger than grains grown under controlled environment conditions.

Grain physical appearance /seed quality

Seed quality characteristics such as seed/grain colour and grain size are potentially important market requirements. Grains of cv. Schooner grown in a growth room at 30-38°C and in a growth cabinet at 18-38°C had a dark discoloration of the embryo ends. The grain discoloration of barley can result in reduced seed/grain quality and value in most countries where the crop is grown. Therefore, colour, brightness and general appearance of seed is of considerable importance in commercial transactions. Grains of barley below < 2.5 > 2.2 mm category do not meet the standard requirement for the seed trade and grain industry (Henry, 1990; Duijnhouwer, Grashoff and Angelino, 1993). The small grains of barley may have a high protein content and give low levels of malt extracts, and in wheat, small grains cause a decrease in milling quality due to a reduction in the proportion of endosperm that can be extracted as flour (Randall and Moss, 1990). Small and shrivelled seeds are slow to emerge when placed deeply in the soil, and seedlings from such seeds are less competitive against weeds.

Embryo dry weight

Embryo dry weights were adversely affected by elevated temperatures during grain development. The effect depended on timing and duration of exposure. Grains of cv. Blenheim (Experiment II) which had experienced 30°C for part or all of their developmental period had smaller embryos than grains which experienced 18°C for most or all of their developmental period. These results are supported by those of Experiments III & IV in which grains grown at 18°C had heavier embryos than grains grown at 30°C and 18-38°C. The smallest embryos were obtained from grains of cv. Schooner grown in growth cabinets at 18-38°C.

Grass and Burris (1995) reported that they found no effect of temperature on embryo dry weight in grains of wheat cvs Marzak and Oum-rabia grown at 36/29°C and 20/15°C but the results they published showed that in both cultivars embryo dry weight of grains grown at 36/29°C was 22% less than that of grains grown at 20/15°C. The embryo dry weight in the current study was less in grains grown at elevated temperatures than in grains grown at 18°C and was closely related to grain dry weight giving a significant positive correlation between grain dry weight and embryo dry weight ($r = 0.91$) $p < 0.001$.

During the extraction of embryos to determine embryo dry weights, a ring of golden-brown material around the emerging coleoptile and an elongation of coleoptile under the pericarp/husk were observed in grains grown at 30°C and 18-38°C in both grains grown in growth rooms and growth cabinets. Thus grains grown at elevated temperature showed evidence of pre-germination. This seems to suggest that pre-germination was influenced by the temperature during grain development although the percentage pre-germination was lower in grains grown in a growth cabinet at 18-38°C than in comparable grains grown in growth room at 18-38°C. It should be noted however, that the percentage viability of the seed lots with high percentage pre-germination was > 95 %. Pre-germination was not observed in grains grown at 18°C.

The state of knowledge on pre-germination is scanty as reflected by different definitions given by research workers. Symons, Angold, Black and Chapman (1983) defined pre-germination as abnormal or non-visible sprouting; Sole (1994) defined pre-germination as a premature sprouting of grains while still retained in the ears as a result of prolonged spells of wet weather or alternating spells of frequent heavy rains whereas Gordon (1970) defined it as an arrested germination or premature germination caused by mechanical defects of seed coat. It may be important to make a distinction between pre-harvest sprouting and pre-germination. In pre-harvest sprouting both a root and a shoot (coleoptile) emerge and grains after they have sprouted cannot germinate after being dried whereas pre-germination occurs at a stage of development prior to dehydration and only a coleoptile emerges under the pericarp/husk. Pre-germinated grains can resume normal germination after drying. Pre-germination can be assessed in barley by the extraction of embryos from over night soaked grains or by using microscope-assisted examination of tetrazolium-stained bisected embryos. The rupture of the germ aleurone/testa and the shoot that extends beyond the confines of the embryo area are taken to be positive indicators of pre-germination.

There is insufficient information available on pre-germination. It has not been established when pre-germination sets in during grain development and what triggers it. Gordon (1970) in a field-grown barley cv. Ymer (a Scottish cultivar) reported that pre-germination was caused by a split of pericarp-testa over the embryo. The finding does not give full history of growing environmental conditions of the plants and the type of examinations he carried out to establish the pericarp-testa split.

The effects of pre-germination in cereals can be significant in terms of economics yet no detailed research has been carried out in this area. Pre-germinated grains have short storage longevity and are susceptible to mould attack. In the brewing industry pre-germinated grains produce malt of inferior quality.

Germination tests

The plant physiologist's definition of germination as given by Bewley and Black (1994) states that germination begins with water uptake by the seed and ends with the start of elongation by the embryonic axis, usually the radicle, and on the emergence of the radicle, the seed is considered to be germinated. The term germination is therefore used to describe the initiation of embryo growth. Cochrane (1993) described germination of barley grains as the event known as 'chitting' i.e. the rupture of the pericarp and testa/germ aleurone layers caused by the extension growth of the coleorhiza. In all the germination tests carried out, the above description was used to determine the completion of germination. The results of germination tests using 5 ml water per 100 grains (Experiment I) showed that grains grown at 18°C from anthesis until harvest-ripeness and grains that experienced 18°C during the early stage of grain development and 30°C for the remainder of grain growth (B, seed lot 1) had a lower percentage germination than grains grown at 30°C from anthesis until harvest-ripeness or grains that experienced 30°C for a period after anthesis (**Section 3. 3**.).

Viability tests carried using hydrogen peroxide or alternate periods of incubation at 4°C and 20°C showed that the percentage viability of all seed lots was above 96%. Thus in seed lots grown at 18°C, seeds which failed to germinate were dormant. Other interesting results were obtained in germination tests using 10 ml water. Using 10 ml water, grains grown at 18°C from anthesis until harvested ripeness (Experiments II & III) and grains which experienced 18°C early in grain development (Experiment II) and finished grain development at 30°C had lower percentage germination than grains grown at elevated temperatures (30°C & 18-38°C). Kelly and Briggs (1992) attributed the low percentage germination in tests using 10 ml water per 100 grains to the presence of micro-organisms in and on the grains. Micro-organisms are thought to exert their effect on germination by competing with the embryo for oxygen. This competition is particularly evident when the availability of oxygen is limited as in germination tests

using excess water. However, if the presence of micro-organisms was responsible for low percentage germination in 10 ml water, then the seed lots of temperature regimes A & C (18°C & 30-18°C) were likely to have had similar microflora on the surface since they were harvested from the same growth room, but nevertheless they had different germinabilities. Micro-organisms located inside the pericarp may have influenced germination, but it is perhaps more likely that the low percentage germination in grains grown at 18°C and 18-30°C was probably due to low-temperature induced dormancy which was not expressed when using 5 ml water per 100 grains. From the results presented here, it is clear that grains grown at elevated temperatures throughout grain development were less dormant than grains grown at a lower temperature (18°C). However, the degree of expression of dormancy depends on the temperature at which grains developed and on the genetic potential of a cultivar to express dormancy. Cultivar Stirling grown in both low and high temperature regimes showed a low percentage germination. These results are similar to those obtained by Grass and Burris (1995) in growth chamber-grown plants of wheat cv. Marzak grown at 36/29°C. The low germination in Stirling was due to dormancy because grains which failed to germinate at the end of germination test germinated when hydrogen peroxide was used to determine viability.

The germination tests carried out on eleven genotypes grown in a glashouse showed that the percentage germination of all genotypes was > 97% but that the genotypes differed in the rate at which this was attained. These genotypes included cv. Stirling which showed dormancy when grown in the growth rooms. The level of dormancy expressed by grains of cv. Stirling grown in the growth room could have been due to a shorter period in storage from harvesting to the time germination tests were carried out whereas in the genotypes grown under glasshouse conditions, germination tests were carried out much later after harvesting and temperature was not controlled under glasshouse conditions.

Data on germination have revealed important aspects of dormancy. Both dormancy and lack of dormancy have disadvantages and advantages. When the period between harvest and sowing is short and a significant proportion of the seed is still dormant at the time of sowing, poor seedling emergence and field establishment will occur. Therefore, if highly dormant cultivars are favoured by farmers, then the farmers should be prepared

to plant one-year-old seeds because the period between harvest (mid August) and planting (September-October in Scotland) might be too short to break dormancy during the short storage period. In the case of barley grains used in brewing industry, dormant grains are likely to increase treatment storage and costs. Therefore, breeders in their breeding programmes should aim to develop barley lines with a level of dormancy sufficient to prevent pre-harvest sprouting and yet not high enough to hinder post-harvest utilisation of seed.

Seedling growth characteristics

The seedling growth analyses revealed that elevated temperatures at early stages of grain development may subsequently affect seedling growth characteristics (**Sections 3. 4 and 3. 5**.). Data indicated that grains from ears grown at 18°C from anthesis until harvest-ripeness produced seedlings with more roots, longer seminal roots, more dry matter and fewer abnormal seedlings than grains that were exposed to elevated temperature for part or all of the grain development period. However, results obtained from the plumule growth test in which seedlings were grown in the dark, showed that seedlings from grains grown at low temperature had more plumule dry weight than those from grains grown at elevated temperature. By contrast, in the seedling emergence test in which seedlings were grown in the light, plumule dry weight of seedlings from grains grown at a low temperature did not differ from that of grains grown at elevated temperature with the exception of seedlings from grains grown in a growth cabinet at elevated temperature, because seedlings in the emergence test were able to use light energy for photosynthetic activity. This demonstrates that seeds from grains grown at elevated temperatures when grown under field conditions, provided they were not sown too deep in the soil, could produce seedlings that would not be different from grains grown at 18°C. Therefore, results obtained from the plumule growth test in which seedlings were grown in the dark may not be relevant under the field conditions.

In the plumule growth test and the seedling emergence test, plumule length from grains grown at low temperature did not differ significantly from those grown at elevated temperatures but there was a greater variation in plumule length in seedlings from grains grown at elevated temperatures than in seedlings from grains grown at a low

temperature. This was probably due to the presence of a high proportion of pre-germinated grains in the seed lots grown at elevated temperatures.

There were strong positive correlations between most seedling characteristics and grain dry weight, but there was no statistically significant correlation between grain dry weight and the length of the plumule. Plumule length in a seedling growth test has been used as an indicator of seed vigour (Perry, 1977). In this regard, the number of roots per seedling and the length of the longest seminal roots may be better indicator of vigour than plumule length. In adverse environmental conditions, seedlings that have a long extensive root system may have a better chance of establishment because under drought conditions, long roots would be able to grow into lower soil layer and thus continue drawing water from the soils to sustain seedling growth.

Embryo size and seed vigour

Effects of elevated temperature during grain development were apparent in the embryo size of grains grown in the elevated temperature regimes **(Section 3. 6)**. The extent of the effect depended on the duration of exposure to elevated temperatures and on the stage of grain development at which ears experienced elevated temperature. Embryos from grains grown in the elevated temperature regimes were smaller and produced seedlings which were less vigorous than those from grains grown at 18°C. There was a strong positive correlation between embryo dry weight and grain dry weight. The shortened period of grain development in grains grown at elevated temperature affected the potential for dry matter accumulation during grain-filling which resulted in the production of small embryos. Embryo size may be important during the initial phase of germination when the seedling growth is dependent on embryo reserves. Wood, Longden, and Scott (1977) observed that small seeds with their small embryos and limited endosperm reserves produced small seedlings. Results of Experiment V showed that genotypes which had large embryos (cvs Clipper, Schooner and Stirling) produced seedlings with greater seedling dry weight (root dry weight plus plumule dry weight) than genotypes with small embryos (cv. Tyne). Lopez-Castaneda, Richards, Farquhar and Williamson (1996) found that the size of the embryo was the single most important factor to account for differences in vigour among species. The results of the current study have shown that seedlings produced from grains grown in elevated temperature

regimes were smaller than those produced from grains grown at a low temperature. This was more apparent in grains grown in the growth cabinets than in grains grown in the growth rooms in comparable temperature regimes. The strong relationships observed between embryo size, grain dry weight and seedling growth seem to confirm the importance of an embryo as a starting capital for initial germination and seedling growth.

Root dry weight/shoot dry weight ratios (R/S)

Grains from temperate and tropical cultivars grown in the growth rooms at 18°C produced seedlings which differed in the partitioning of food reserves between roots and shoots (**Section 2. 4. 8**.). The R/S ratio data showed that seedlings of tropical cultivars accumulated greater dry weight in the shoots than in the roots, whereas cv. Blenheim, a cultivar of temperate origin, accumulated more dry weight in the roots than in the shoots. By contrast, the results in Experiment V (**Section 3.4.10**.) demonstrated that in glasshouse grown seeds R/S i.e. was > 1 thus, under these conditions more assimilates were allocated to the roots than to the shoot in both temperate and tropical genotypes although genotypes of temperate origin (cvs Harrington, Blenheim and Tyne) had larger root dry weight/shoot dry weight ratios than genotypes of tropical origin. The lack of consistency may be attributed to differences in grain size. When cultivars of tropical origin were grown in the glasshouse at approximately 18/13°C under high light intensity, they produced larger grains than when they were grown in the growth rooms at a similar temperature but at a lower light intensity. This seems to indicate that in the presence of abundant food reserves, cultivars of tropical origin would allocate more assimilates to the roots than to the shoots. However, seedlings from grains grown at elevated temperatures tended to partition more assimilates to the shoots than to the roots in both sub-tropical and temperate climates.

In seedlings from grains of cv. Blenheim grown in the elevated temperature regimes, plumules grew faster than the roots. The observations of longer plumules in seedlings from grains grown at elevated temperatures could have been due to the presence of a proportion of pre-germinated grains.

The shortened duration of grain growth in the tropics due to high temperatures, may limit the size of grains and therefore seedlings from grains grown under these

conditions. The results presented here showed that more assimilates were partitioned to the shoots than to the roots in seedlings from grains of cultivars of subtropical/tropical origin. This could be seen as an advantageous trait. The tendency of cultivars of tropical origin to allocate more food reserves to the shoots than to the roots seems to indicate that in the presence of sufficient soil moisture such seedlings would form a large leafy canopy quickly in the early stages of seedling growth and this would enable the seedlings to intercept more radiation so that greater quantities of assimilate are available for seedling growth and for effective competition against weeds. In temperate cultivars, the allocation of more assimilates to the roots than to the shoot would be very important in adverse environmental conditions where seedlings that have a well developed root system may have a better chance of establishment in drought conditions or under low nutrient soil status.

Generally, root dry weight/shoot dry weight ratios seem to bear a strong relationship to embryo dry weight and grain size. The root dry weight/shoot dry weight ratios were higher in seedlings from grains grown at a low temperature than in seedlings from grains grown at a high temperature. This appears to reflect the effect of elevated temperature during grain growth on the embryo size and subsequent effect on seedling growth characteristics.

Embryo growth potential

The embryo growth potential experiment was focused on the metabolic potential of embryos from grains grown in different temperature regimes during grain development and was designed to eliminate any contributory effect of endosperm (**Section 3. 7.**). When grown on 1% agar, embryos extracted from grains which had been subjected to elevated temperature regimes in growth rooms produced seedlings with longer plumules and roots than embryos extracted from grains which experienced 18°C throughout development. The presence of a high proportion of pre-germinated grains in the samples of grains grown at elevated temperatures probably contributed to these differences. However, embryos extracted from grains grown in a growth cabinet at elevated temperature produced seedlings which did not differ in plumule length and root length from embryos of grains grown in a growth cabinet at 18°C throughout grain development.

Growing embryos on an agar + glucose medium showed that root growth was much greater than shoot growth. This seems to indicate that since roots emerge earlier than plumules (coleoptiles) they had an advantage of earlier establishment and were able to use available nutrients for growth much earlier than plumules. The seedling growth characteristics from embryos of grains grown in growth room at 18°C did not differ from those of embryos of grains grown at elevated temperature, but differences were evident in seedling growth characteristics between seedlings of embryos of grains grown in a growth cabinet at 18°C and those grown in a growth cabinet in an elevated temperature regime. These differences were therefore due to the effects of elevated temperature during grain development on the embryo. This was demonstrated by the under utilisation of the metabolisable nutrient source by the embryos from grains grown in the growth cabinets at elevated temperature. This inability might have been due to the absence of essential mineral elements or the impairment of physiological mechanisms during grain development which affected storage reserve mobilisation and transportation of metabolites.

Although deleterious changes may not have affected germinabilty (viability was > 95%) of grains grown in the growth cabinets in the elevated temperature regime, they had significant effects on seedling growth. The fact that this test was carried out on metabolisable nutrient source (glucose medium) removes the question of exhaustion of endosperm reserves in grains grown in growth cabinets at elevated temperatures.

Starch granules

The analyses of endosperm starch of Experiments III & IV using three cvs Blenheim, Stirling and Schooner grown in environmentally controlled conditions (**Section 3. 10**.) demonstrated that grains grown at elevated temperatures during grain development had fewer starch granules per grain and lower numbers of both A-type and B-type starch granules per grain than grains grown at 18°C. This reflects the dominance of temperature as a factor affecting the initiation of starch granules. Grains grown in temperature regimes 18-38°C and 21-38°C had a higher percentage of small B-type starch granules (< 2.1 μm) than grains grown at 18°C. These findings confirm the work of Tester *et al.*(1991), MacLeod and Duffus (1988) and Shi *et al.* (1994) which showed that grains of barley and wheat grown at a high temperature had smaller A-type starch

granules and fewer and smaller B-type starch granules than grains grown at a lower temperature. Hoshikawa (1962) also observed that the A-type starch granules of wheat grains grown at 30°C were smaller in size whereas the B-type starch granules were fewer in number than those from grains grown at 20°C. However, the A-type granule number was not determined in the above author's experiment.

Although Buttrose (1960) suggested that the number of A-type starch granules is genetically fixed whereas B-type granule number varies with changes in the environment, the results of this study have shown that the environmental conditions prevailing during grain growth had a modifying influence on the number of A-type starch granules. The relative constancy of A-type starch granule number noted by Buttrose (1960) is likely to be so when the environmental treatment is applied later in grain development after the A-type starch granules have been initiated.

In the current investigation the number of B-type starch granules was higher in grains grown from tiller ears grown in 30-38°C and 33-38°C temperature regimes than in grains from main stem ears grown at 18-38°C and 21-38°C. However, the designation of small and large starch granules of barley endosperms is somewhat arbitrary. The value of the diameter chosen to differentiate between A-type and B-type granules in this analysis was 8.1μm. The higher number of B-type starch granules observed in grains from tiller ears grown at elevated temperature probably included many undeveloped A-type starch granules which could not attain their maximum potential size largely due to the shortened duration of growth resulting from exposure to elevated temperatures. If current photosynthesis together with stored assimilates cannot provide an adequate supply of raw materials, the initiation and growth of starch granules to attain maximum potential will be affected. Therefore grain growth will be curtailed, A-type granules will be small and B-type granules will be fewer in number.

Takahashi and Kanazawa (1996) observed fewer A-type and B-type starch granules initiated as result of shading treatment carried out from 2 days before anthesis to 7 days after anthesis than in shading carried out from 7 days to 14 days after anthesis of field-grown wheat using a 95% shading cloth. Savin *et al.* (1996) reported that grains of barley cvs Schooner and Parwan grown under field conditions in chambers at 40°C for 5 days, 17 days after anthesis, had fewer A-type and B-type granules than grains grown at a low temperature.

Interestingly, grains from tiller ears of cv. Blenheim and Stirling grown in temperature regimes 30-38°C and 33-38°C showed a triphasic size distribution of starch granules. Two classes of B-type starch granule sizes (< 6.9 > 2.0 and <2.0 μm) were observed in the tiller ears grown in these regimes. Bechtel *et al.* (1990) reported the presence of three classes of wheat starch granules (> 16 μm A-type, 5-16 μm B-type, 0-5 μm C-type). However, the values of the diameter chosen to differentiate between A-type and B-type granules in this study was > 8.1 μm. The third class of starch granule could have been initiated during a second wave of granule initiation when the temperature was reduced from 38°C to 30°C. This theory is based on the assumption that the initiation of B-granules ceased when grains were subjected to 38°C and resumed initiation when the temperature was reduced to 30°C. Temperature fluctuation under field conditions is a common experience. It is possible that the triphasic granule size distribution reported by Bechtel *et al.* (1990) was due to temperature fluctuation under field conditions.

The total volume of starch per grain was affected by temperature during grain development. In line with differences in dry weight, the total volume of starch per grain was greater in grains from main stem ears grown at 18°C than in grains from comparable main stem ears grown at 18-38°C and 21-38°C and tiller ears grown at 30-38°C and 33-38°C respectively. Similar results were obtained by MacLeod and Duffus (1988b), Shi *et al.* (1990) and Savin, Stone and Nicolas (1996) in growth room-grown plants of barley and wheat. The volume of A-type and B-type starch granules per grain were also affected by temperature in both main stem ears and tillers. However, in cv. Stirling the percentage volume of granules classified as B-type was lower in grains from main stem ears grown at 18-38°C and 21-38°C than in grains from tiller ears grown at 30-38°C and 33-38°C. This seems to indicate that a number of A-type starch granules whose growth was reduced by elevated temperatures, and thus failed to attain maximum size, were classified as granules B-type together with the true B-granules. These granules therefore occupied a greater volume than in grains from main stem ears grown at 18-38°C and 21-38°C.

Starch granule size distribution is an important aspect of quality in the brewing industry. The A-type starch granules are more degradable during mashing than the B-type starch granules (Tillett and Bryce, 1993). It has also been suggested that malt extract is reduced when a higher proportion of B-type starch granules is present in the endosperm

because they tend to be more embedded in the protein matrix and less accessible to hydrolytic enzymes. The B-type starch granules also have a higher gelatinisation temperature, so that the B-type granules are not as readily solubilised during mashing as A-type granules (Cochrane, Paterson and Duffus, 1996).

Endosperm morphology

Endosperm morphology is an aspect of malting quality which is difficult to define, but it has been recognised for some time that "mealy'' grains those in which the cut surfaces of the endosperms are white and non-reflecting, malt more easily than "steely'' grains, in which the cut surface of the endosperm is golden brown and reflects light (Palmer, 1989). The data on endosperm morphology presented here (**Section 3. 11.)** showed that grains grown in both growth rooms and growth cabinets at 18°C were predominantly steely whereas grains grown at elevated temperatures were either predominantly mealy or contained only small proportions of steely endosperm. These differences were related to differences in the packing of starch granules and the continuity of the matrix in starchy endosperm cells (Tashiro and Wardlaw, 1990; Cochrane and Duffus, 1994).

Alpha-amylase

Data showed that grains of cvs Blenheim and Schooner grown at elevated temperature had higher levels of α-amylase activity during early germination than grains grown at 18°C (**Section 3. 9**.). Henry (1994) reported that pre-germinated grains of wheat had a higher level of α-amylase than normal grains. The results of the current study showed that grains grown at elevated temperatures had a higher proportion of pre-germinated grains than grains grown at 18°C. Therefore, early initiation of α-amylase activity observed in grains grown in elevated temperature regimes may be associated with the presence of pre-germinated grains. The early initiation of α-amylase activity in grains grown at elevated temperature does not necessarily imply that grains grown at 18°C had less potential for the production of α-amylase than grains grown at elevated temperature. These differences were largely due to differences in developmental stages of germination associated with temperature during grain development.

5. CONCLUSIONS

The results obtained from this project have confirmed in sufficient detail that elevated temperatures during grain development affect many of the seed characters that influence the attributes of seed quality. The extent of the effect depended on the duration of exposure to elevated temperatures and the stage of grain development at which elevated temperature occurred. Grains grown at elevated temperature were lighter, and were often discoloured at the embryo end and had a higher proportion of smaller grains with smaller embryos than grains grown at a lower temperature.

A higher proportion of pre-germinated grains was harvested from ears grown at elevated temperatures than from ears grown at a lower temperature. Pre-germinated grains have short storage life and are susceptible to mould attack. In the brewing industry pre-germinated grains produce malt of inferior quality.

Grains grown in the elevated temperature regimes produced seedlings with fewer roots, shorter roots and less dry weight than grains grown at a lower temperature. There were strong positive correlations between seedling characteristics and grain dry weight, but there was no statistically significant correlation between grain dry weight and the length of the plumule.

The results also demonstrated that elevated temperatures and the growth conditions during grain development affected embryo size and the physiological processes involved in the partitioning of assimilates between the roots and shoots in seedlings of cultivars of temperate and sub-tropical origin.

Grains grown in elevated temperature regimes had a higher percentage of small B-type starch granules and fewer A-type starch granules per grain than had grains grown at 18°. Grains from tiller ears which experienced two specific temperature regimes had a triphasic size distribution of starch granules, thus two classes of B-type starch granules existed in grains harvested from tiller ears grown in the elevated temperature regimes.

In relation to vigour characteristics, grains grown at elevated temperatures had positive attributes as well . Data obtained showed that grains grown at elevated temperatures were less dormant than grains grown at a low temperature. The most important attribute of grains grown in the elevated temperatures was their ability to germinate under excess

water which is an essential attribute for seeds sown in water-logged conditions. It was also shown that grains grown at elevated temperatures initiated α-amylase activity much earlier and showed earlier emergence than grains grown at a low temperature.
The results of the current project have shown in detail that elevated temperatures during grain development affected seed vigour of barley. Elevated temperatures during grain development altered seed vigour characteristics. The degree to which seed vigour was affected by elevated temperatures depended on the stage of grain development and the duration of exposure to elevated temperatures. Seeds which experienced elevated temperatures expressed negative and positive attributes whose interpretation in terms of seed vigour depended on targeted field stress conditions. For example, if early germination or early emergence for competition against weeds or if the water-logged conditions were the criteria, then seeds grown at elevated temperatures would be suitable for such conditions and would be considered to be of high vigour. However, under drought conditions, seeds grown at 18°C, because of their ability to produce more roots and longer roots, the seeds would be considered to be of high vigour.

6. SUGGESTIONS FOR FUTURE RESEARCH

Pre-germination

The results of this study have shown that grains grown in the elevated temperature regimes had a higher proportion of pre-germinated grains than grains grown at 18°C. Ears grown in the elevated temperature regimes were amalgamated with other ears which experienced different temperature regimes during grain development because of limitation in the amount of grains obtained from grains grown in the controlled environment conditions. This made it difficult to determine the exact stage of grain development at which pre-germination had taken place. Therefore, further work needs to be carried out to monitor, evaluate and establish the exact stage of grain development at which pre-germination takes place and the environmental conditions which induce it to occur. Plants should be grown under controlled environment conditions so that the phase of grain development could be better timed than would be the case in field experiments. The work should be extended to investigate in detail whether pre-germinated grains can be dormant.

Root length as a better indicator of vigour

Data on seedling growth characteristics demonstrated a weak non-significant positive correlation between grain dry weight and the length of the plumule. Plumule length in a seedling growth test has been used as an indicator of seed vigour in cereals. Root length seems to relate better to seedling establishment in the field than plumule length. The use of root length in seedling growth test as an indicator of vigour could be explored as an alternative to plumule length.

Root dry weight/shoot dry weight ratios

Further work needs to be carried out to investigate root dry weight/shoot dry weight ratios in seedlings from grains grown in different temperature regimes and those grown under different growth conditions. The results of this project have shown that seedlings from grains grown in the elevated temperature regimes partitioned assimilates more or less equally between the roots and shoots but that seedlings from grains of cultivars of tropical origin allocated more assimilates to the shoots than to the roots when ears were grown in the growth rooms at 18°C, and when ears were grown under glasshouse conditions at approximately the same temperature but at a higher light intensity, seedlings allocated more assimilates to the roots than to the shoots.

Utilisation of metabolisable nutrient source by embryos

Further research should be directed to the investigation of changes occurring in the seed structures of the embryos from grains grown at elevated temperatures particularly grains grown in growth cabinets in order to establish causes of decline in embryo vigour. Seeds of barley offer an ideal system in which to study the expression of the effect of elevated temperature on embryo and non-embryo tissues of seeds due to ease of physical separation of the embryo from non-embryonic tissue. The investigation should attempt to establish whether the inability of the embryos to utilise the metabolisable nutrient source are due to the absence of essential mineral element(s) responsible for normal functions of physiological processes or this was due to inadequately formed embryos.

Starch in relation to heat-treatment

Another very important area which may provide a better understanding of physiological processes which govern seedling growth and field establishment would be the exploration of starch quality in relation to starch granules as affected by elevated temperatures during grain development.

The results on starch granules have shown that grains grown at elevated temperatures had fewer A-type granules and a higher proportion of B-type starch granules than grains grown at 18°C. MacDonald *et al.* (1991) found that the starch from the B-starch granules of barley was always different from A-starch granules but little is actually known about the specifics of these granules in relation to seedling growth characteristics and field performance.

It is a routine practice to remove shrunken seeds from seed lots before sowing in preference to plump seeds under the pretext that shrunken seeds contains limited amount of food reserves for seedling growth but very little is known about their physiological properties. The rate of synthesis of hydrolytic enzymes and the extent of release of these enzymes into the starchy endosperm require further investigation. Differences related to the morphological structure of the endosperm (mealiness & steeliness) and the rate of water up take and mobilisation of food reserves during the post-germinative events of grains from plants grown at optimum temperature and those from plants grown under environmental stress need further research.

Grain size assessment

From physiological point of view it would be interesting to evaluate seedling growth characteristics using seeds/grains of the same size in order to establish whether the differences in vigour observed were due to grain size or to the environmental conditions during grain growth.

7. REFERENCES

ABDUL-BAKI, A.A. (1969). Relationship of glucose metabolism to germination and vigour in barley and wheat seeds. *Crop Science*. **9:** 732-736.

ABDUL-BAKI, A.A. and ANDERSON, J.D. (1972). Physiological and biological deterioration of seeds. *In:* Seed Biology, Second Edition, T.T. Kozlowski, Academic Press, New York. pp. 283-309.

AHMED, H.N. (1977). Evaluating differences in vigour amongst sorghum seed lots by various test methods. *Seed Research* **5:** 152-157.

AL-KHATIB, K. and PULSEN, G.M. (1984). Mode of high temperature injury to wheat during development. *Physiologia Plantarum* **61:** 363-368.

AL-KHATIBI, K. and PULSEN, G.M. (1990). Photosynthesis and productivity during high-temperature stress of wheat genotypes from major world regions. *Crop Science* **30:** 1127-1132.

ANON. (1976). International Rules for Seed Testing. *Seed Science and Technology* **4:** 3-177.

AOSA (Association of Official Seed Analysts) (1978). Rules for testing seeds. *Journal of Seed Science and Technology* **3:** 1-126.

ASANA, R.D. and WILLIAMS, R.F. (1965). The effect of temperature stress on grain development in wheat. *Australian Journal of Agricultural Research* **16:** 1-13.

ASPINALL, D. (1965). The effect of soil moisture stress on the growth of barley II: Grain growth. *Australian Journal of Agricultural Research* **16:** 265-275.

ASPINALL, D., NICHOLLS, P. B. and MAY, L. M. (1964). The effects of soil moisture stress on the growth of barley. Vegetative development and grain yield. *Australian Journal of Agricultural Research* **15:** 729-745.

AUSTIN, R.B. (1972). Effects of environment before harvesting on seed viability. *In*: Viability of Seeds, Edition, E. H. Roberts, Chapman and Hall, London, UK. pp. 114-149.

BARRIGA, C. (1961). Effects of mechanical abuse of navy bean seed at various moisture levels. *Agronomy Journal* **53:** 250-251.

BECHTEL, D.B., ZAYAS, I., KALEIKAU, L. and POMERANZ, Y. (1990). Size distribution of wheat starch granules during endosperm development. *Cereal Chemistry* **67:** 59-63.

BELDEROK B. (1968). Seed dormancy problems in cereals. *Field Crops Abstracts* **21:** 203-211.

BEWLEY, J.D. and BLACK. M. (1994). Seed Physiology of Development and Germination, Second Edition, Plenum Press New York.

BHULLAR, S.S. and JENNER, C. F. (1985). Differential response to high temperatures of starch and nitrogen accumulation in the grain of four cultivars of wheat. *Australian Journal of Plant Physiology* **12:** 363-375.

BHULLAR, S.S. and JENNER, C.F. (1986). Effects of temperature on the conversion of sucrose to starch in the developing wheat endosperm. *Australian Journal of Plant Physiology* **13:** 605-615.

BLUM, A., SINMENA, B., MAYER, J., GOLAN, G. and SHPILER, L. (1994). Stem reserve mobilisation supports wheat-grain filling under heat stress. *Australian Journal of Plant Physiology* **21:** 271-281.

BLUMENTHAL, C.S., BARLOW, E.W.R. and WRIGLEY, C.W. (1993). Growth environment and wheat quality: the effect of heat stress on dough properties and gluten proteins. *Journal of Cereal Science* **18:** 3-21.

BLUMENTHAL, C.S., BEKES, F.I.L., WRIGLEY, C.W., MOSS, H.J., MARES, D.J. and BARLOW, E.W. (1991). Interpretation of grain quality results from wheat trials with reference to high temperature stress. *Australian Journal of Agricultural Research* **42:** 325-334.

BRIARTY, L.G., HUGHES, C.E. and EVERS, A.D. (1979). The developing endosperm of wheat - a stereological analysis. *Annals of Botany* **44:** 641-658.

BRIGGS, K.G. and HORAK, A. (1980). Relationship between ATP content, germination and seedling vigour of different types of wheat genotypes. *Canadian Journal of Plant Science* **60:** 1455-1457.

BROCKLEHURST, P.M. and EVERS, A. (1977). The size distribution of starch granules in endosperm of different size kernels of the wheat cultivar Maris Huntsman. *Journal of the Science Food and Agriculture* **28:** 1084-1089.

BROCKLEHURST, P.M., MOSS, J.P and WILLIAMS, W. (1978). Effects of irradiance and water supply on grain development in wheat. *Annals of Applied Biology* **90:** 265-276.

BROOKS, A., JENNER, I.F. and ASPINALL, D. (1982). Effects of water deficit on endosperm starch granules and on grain physiology of wheat and barley. *Australian Journal of Plant Physiology* **9:** 423-436.

BUTTROSE, M.S. (1960). Submicroscopic development and structure of starch granules in cereal endosperm. *Journal of Ultrastructural Research* **4**: 231-257.

CARVER, M. (1980). The production of quality cereal seed. *In:* Seed Production, Edition. P.D. Hebblethwaite, Butterworths, London. pp. 295-306.

CHING, T.M., HEDTKE, S., BOULGER, M.C. and KRONSTAD, W.E. (1977). Correlation of field emergence rate and seed vigour criteria in barley cultivars. *Crop Science* **17:** 312-314.

CHOWDHURY, S.I. and WARDLAW, I.F (1978). The effect of temperature on kernel development in cereals. *Australian Journal of Agricultural Research* **29:** 205-223.

COCHRANE, M.P.. (1993). Effects of temperature during grain development on germinability of barley grains. *Aspects of Applied Biology* **36:** 103-113.

COCHRANE, M.P. (1994c). Factors affecting grain germinability in barley: Plant morphology. *Proceedings of The Fourth Aviemore Conference on Malting, Brewing & Distilling* Editions, Institute of Brewing London, pp. 249-250.

COCHRANE, M.P. and DUFFUS, C.M. (1994). The effect of ambient temperature on grain filling, starch quality and endosperm morphology in barley. *Proceedings of the 44th Royal Australian Chemical Institute Cereal Chemistry Conference,* Editions, J.F. Panozzo and P.G. Downie, Ballarat, Australia, pp. 42-45.

COCHRANE, M.P., PATERSON, L. and DUFFUS, C.M. (1996). The effect of temperature on grain development and starch quality in barley. *Aspects of Applied Biology* **45:** 139-146.

CONROY, J.P., SENEWEERA, S., BASRA, A.S. ROGERS, G. and NISSEN-WOOLER, B. (1994). Influence of rising atmosperic CO_2 concentrations and temperature on growth, yield and grain quality of cereal crops. *Australian Journal of Plant Physiology* **21:** 741-758.

COPELAND, L.D. and McDONALD, M.B. (1995). Soil moisture and fertility. *In:* Seed Science and Technology, Third Edition, Chapman and Hall, New York.

DASGUPTA, P.R. and AUSTENSON, H.M (1973a). Relations between estimates of seed vigour and field performance in wheat. *Canadian Journal of Plant Science* **53:** 4 3-46.

DASGUPTA, P.R. and AUSTENSON, H.M. (1973b). Analyses of interrelationships among seedling vigour, field emergence and yield in wheat. *Agronomy Journal* **65:** 417-422.

DAWSON, I.A and WARDLAW, F.I. (1989). The tolerance of wheat to high temperatures during reproductive growth. III. Booting to anthesis. *Australian Journal of Agricultural Research* **40:** 965-980.

DELOUCHE, J.C. and CALDWELL, W.P. (1960). Seed vigour and vigour tests. *Proceedings of the Association of Official Seed Analysts* **50:** 124-129.

DELOUCHE, J.C. (1980). Environmental effects on seed development and seed quality. *Horticultural Science* **15:** 775-780.

DUIJNHOUWER, I.D.C., GRASHOFF, C. and ANGELINO, S.A.G.F. (1993). Kernel filling and malting barley quality. *Proceedings of the European Brewing Congress* pp.121-129.

DUKE, E.R., DOEHLERT, D.C. and PRATT, R.C. (1993). High temperature stress effects on gene expression and enzymes activity in maize kernels cultured *in vitro*. *Plant Physiology Supplement* **102**: 78.

ELLIS, R. H. and PIETA FILHO, C. (1992). The development of seed quality in spring and winter cultivars of barley and wheat. *Seed Science Research* **2:** 9-15.

FERNANDEZ, R.G. and LAIRD, R.J. (1959). Yield and protein content of wheat in central Mexico as affected by available soil moisture and nitrogen fertilisation. *Agronomy Journal* **51:** 33-36.

FORD, M.A., PEARMAN, I and THORNE, G.N. (1976). Effects of variation in ear temperature on growth and yield of spring wheat. *Annals of Applied Biology* **82:** 317-333.

FUSSELL, L.K. and PEARSON, C.J. (1980). Effects of grain development and thermal history on grain maturation and seed vigour of *Pennistum americanum* L. *Journal of Experimental Botany* **121:** 635-643.

GALLAGHER, J.N., BISCOE, P.N. and HUNTER, B. (1976). Effects of drought on grain growth. *Nature* **264:** 541-542.

GELMOND, H. (1972). Relationship between seed size and seedling vigour in cotton (*Gossypium hirsutum*). *Proceedings Tests Seed Association* **37:** 797-800.

GORDON, A.G. (1970). Premature germination in cereal grains. *Canadian Journal of Plant Science* **50:** 191-194.

GRASS, L. and BURRIS, J.S. (1995). Effects of heat stress during seed development and maturation on wheat seed quality. I. Seed germination and seedling vigour. *Canadian Journal of Plant Science* **75**: 821-829.

HARRINGTON, J.F. (1972). Seed storage and longevity. *In:* Seed Biology, Third Edition, T.T. Kozlowski, Academic Press, New York. pp. 145-245.

HAWKER, J.S. (1982). Effect of temperature on lipid, starch and enzymes of starch metabolism in grape, tomato and broad bean leaves. *Phytochemistry* **21:** 33-36.

HAWKER, J.S. and JENNER, C.F. (1993). High temperature affects the activity of enzymes in the committed pathway of starch synthesis in developing wheat endosperm. *Australian Journal of Plant Physiology* **20:** 197-209.

HENRY, R.J. (1990). Barley quality: an Australian perspective. *Aspects of Applied Biology* **25:** 5-13.

HEYDECKER, W. (1969). Report of the Vigour Test Committee 1965-1968. *Proceedings of the International Seed Testing Association* **27:** 211-222.

HOSHIKAWA, K. (1962). Studies on the ripening of wheat grain 4. Influence of temperature upon the development of the endosperm. *Proceedings of Crop Science Society of Japan* **30**: 228-231.

ISELY, D. (1957). Vigour tests. *Proceedings of the Association of Official Seed Analysts* **47:** 176-182.

ISTA (International Seed Testing Association) (1985). *Hand book on Tetrazolium Testing*, Zurich, Switzerland, pp. 2-18.

JAHUFER, M.Z.Z. and BOROVOI, V.V. (1992). The effects of mechanical damage to maize (*Zea mays* L.) seed on germination, seedling morphology and subsequent grain yield. *Journal of Applied Seed Production* **10:** 67-76.

JENNER, C.F. (1991). Effects of exposure of wheat ears to high temperature on dry matter accumulation and carbohydrate metabolism in the grain of two cultivars. I. Immediate responses. *Australian Journal of Plant Physiology* **18:** 165-177.

JENNER, C.F. (1994). Starch synthesis in the kernel of wheat under high temperature conditions. *Australian Jounal of Plant Physiology* **21:** 791-806.

JOHNSON, R.R. and WAX, L.M. (1978). Relationship of soybean germination and vigour tests to field performance. *Agronomy Journal* **70:** 273-278.

KALIANGILE, I. (1994). Seed quality and seed quality control concept. *Proceedings of 8th Food and Agriculture Organisation/Australia Conference on seed quality control for Southern and Eastern African Countries, Lusaka.* 1-17.

KANG, M.Y., SUGIMOTO, Y., KATO., I., SAKAMOTO, S. and FUWA, H. (1985). Some properties of large and small starch granules of barley endosperm. *Agricultural and Biological Chemistry.* **49:** 1291-1297.

KELLY, L. and BRIGGS, D.E.(1992). The influence of the grain microflora on the germinative physiology of barley. *Journal of the Institute of Brewing* **98:** 395-400.

KERNICH, G. C., SLAFER, G.A. and HALLORAN, G.M. (1995). Barley development as affected by rate of change of photoperiod. *Journal of Agricultural Science* **124:** 379-388.

KHAN, R. A. and LAUDE, H.M. (1969). Influence of heat stress during seed maturation on germinability of barley seed at harvest. *Crop Science* **9:** 55-58.

KINRY, J.R and MUSSER, R.L. (1988). Response of kernel weight of sorghum to environment early and late in grain filling. *Agronomy Journal* **80:** 606-610.

KIRBY, E.J.M. and JONES, H.G. (1977). The relation between the main shoot and tillers in barley plants. *Journal of Agricultural Science* **88:** 381-389.

LANGER, R.H.M. and OLUGBEMI, L.B. (1970). A study of New Zealand wheats IV. Effects of extreme temperatures at different stages of development. *New Zealand Journal of Agricultural Research* **13:** 878-886.

LOPEZ-CASTANEDA, C., RICHARDS, R.A., FARQUHAR, G.D. and WILLIAMSON, R. E. (1996). Seeds and seedling characteristics contributing to variation in early vigour among temperate cereals. *Crop Science* **36:** 1257-1266.

LOWE, L.B., AYERS, G.S. and RIES, S.K. (1972). The relationship of seed protein and amino acid composition to seedling vigour and yields of wheat. *Agronomy Journal* **64:** 603-611.

LOWE. L.B. and RIES, S.K. (1973). Endosperm protein of wheat seed as determinant of seedling growth. *Plant Physiology* **51:** 57-60.

LU, T., JANE, J., KEELING, P.L. and SINGLETARY, G. W. (1996). Maize starch fine structures affected by ear developmental temperature. *Carbohydrate Research* **282:** 157-170.

MacDONALD, M.B. Jr. (1975). A review and evaluation of seed vigour tests. *Proceedings of the Association of Official Seed Analysts (AOSA)* **65:** 109-139.

MacDONALD, A.M.L., STARK, J.R., MORRISON, W.R. and ELLIS, R.P. (1991). The composition of starch granules from developing barley genotypes. *Journal of Cereal Science* **13:** 93-112.

MacLEOD, L.C. and DUFFUS, C.M. (1988). Reduced starch content and sucrose synthase activity in developing endosperm of barley plants grown at elevated temperatures. *Australian Journal of Plant Physiology* **15:** 367-70.

MacLEOD, L.C. and DUFFUS, C.M. (1988). Temperature effects on starch granules in developing barley grains. *Journal of Cereal Science* **8:** 29-37.

MacNICOL, P.K., JACOBSEN, J.V., KEYS, M.M. and STUART, I.M. (1993). Effects of heat and water stress on malting quality and grain parameters of Schooner barley grown in cabinets. *Journal of Cereal Science* **18:** 61-68.

MATHUR, P.N., SINHA, N.C. and SINGH, R.P. (1982). Effect of seed size on germination and seed vigour in oat (*Avena sativa* L.). *Seed Research* **10:**110-113.

MATTHEWS, S. (1980). A new vigour test for crop seeds. *In*: Seed Production, Edition, P. D. Hebblethwaite, Butterworths, London, UK. pp. 605-635.

McDANIEL, R.G. (1969). Relationship of seed weight, seedling vigou and mitochondrial metabolism in barley. *Crop Science* **9:** 823-827.

McNEAL, F.H., BERG, M.A., BRAUIG, P.L. and McGUIRE, C.F. (1971). Productivity and quality response of five spring wheat genotypes (*Triticum aestivum* L.) to nitrogen fertilizer. *Agronomy Journal* **63:** 908-910.

MIAN, M. A.R. and NAFZIGER, E.D. (1994). Seed size and water potential and seedling growth of winter wheat. *Crop Science* **34:** 169-171.

MOORE, R.P. (1969). History supporting tetrazolium seed testing. *Internation Seed Testing Association Proceedings* **34:** 233-242.

MORRISON, W.R. and SCOTT, D.C. (1986). Measurement of the dimensions of wheat starch granule population using a Coulter Counter with 100-channel analyser. *Journal of Cereal Science* **4:** 13-21.

MORRISON, W.R., SCOTT, D.C. and KARKALAS, J. (1986). Variation in the composition and physical properties of barley starches. *Starch* **38:** 374-379.

NICOLAS, M. E., GLEADOW, R.M. and DALLING, M.J. (1984). Effect of drought and high temperature on grain growth in wheat. *Australian Journal of Plant Physiology* **11:** 553-566.

PALMER, G.H. (1989). Cereals in Malting and Brewing. *In*: Cereal Science and Technology, Edition, G.H. Palmer, Aberdeen University Press. pp 61-242.

PARKER, D.K. and PROUDLOVE, M.O. (1993). The relationship between the ATP content of barley and viability. *Journal of the Institute of Brewing* **99:** 327-330.

PARKHE, B.D., SINGH, A.R., CHOULWAR, S.B. and BORIKAR, S.T. (1987). Quality of seed influenced by genotypes and developmental stage in sorghum. *Seed Research* **15:** 128-132.

PAUL, S.R. and RAMASWAMY, K.R. (1979). Relationship between seed size and seed quality attributes in cowpea (*Vigna sinensis* L. Savi). *Seed Research* **7:** 63-70.

PERRY, D.A. (1973). Seed vigour and stand establishment. *Horticultural Abstracts* **42:** 334-342.

PERRY, D.A. (1977). A vigour test for seeds of barley (*Hordeum vulgare* L.) based on measurement of plumule growth. *Seed Science and Technology* **5:** 709-719.

PERRY, D.A. (1980). Deterioration of barley seed and its effects on field performance. *In*: Seed Production, Edition, P. D. Hebblethwaite, Butterworths, London. pp. 327-337.

PIETA FILHO, C. and ELLIS, R.H. (1991a). The development of seed quality in spring barley in four environments. I. Germination and longevity. *Seed Science Research* **1:** 163-177.

PIETA FILHO, C and ELLIS, R. H. (1991b). The development of seed quality in spring barley in four environments. II. Field emergence and seedling size. *Seed Science Research* **1:** 179-185.

POLLOCK, B.M. and ROOS, E.E. (1972). Seed and seedling vigour. *In*: Seed Biology, 1st Edition, T. T. Kozlowski, Academic Press, New York. pp. 314-318.

RADWAN, M.S., SHILTAWI, E.M. and MAHDI, M.T. (1972). The influence of seed size and seed source on germination and seedling vigour of Berseem clover (*Trifolium alexandrinum* L.). *Proceedings of the Seed Test Association* **37:** 763-769.

RANDALL, P.J and MOSS, H.J. (1990). Some effects of temperature regime during grain filling on wheat quality. *Australian Journal of Agricultural Research* **41:** 603-617.

RASYAD, A., van SANFORD, D.A. and TEKRONY, D.M. (1990). Changes in seed viability and vigour during seed maturation. *Seed Science and Technology* **18:** 259-267.

REYNOLDS, M.P., BALOTA, M., DELGADO, M.I.B., AMANI, I. and FISCHER, R.A. (1994). Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. *Australian Journal of Plant Physiology* **21:** 717-730.

ROBERTS, E.H. (1972). Introduction. *In:* Viability of Seeds, Eddtion, E. H. Roberts, Chapman and Hall, London. pp. 1-13.

SANHEWE, A.J., ELLIS, R.H., HONG, T.D., WHEELER, T.R., BATTS, G.R., HADLEY, P. and MORISON, J.I.L. (1996). The effect of temperature and CO_2 on seed quality development in wheat (*Triticum aestivum* L.). *Journal of Experimental Botany* **47:** 631-637.

SAVIN, R. and NICOLAS, M.E. (1996). Effects of short periods of drought and high temperature on grain growth and starch accumulation of two malting barley cultivars. *Australian Journal of Plant Physiology* **23:** 201-210.

SAVIN, R., STONE, P.J. and NICOLAS, M.E. (1996). Responses of grain growth and malting quality of barley to short periods of high temperature in field studies using portable chambers. *Australian Journal of Plant Physiology* **47:** 465-477.

SCH UURINK, R.C., van BECKUM, J.M.M. and HEIDEKAMP, F. (1992). Modulation of grain dormancy in barley by variation of plant growth conditions. *Hereditas* **117:** 137-143.

SHI, Y.C., SEIB, P.A. and BERNARDIN, J.E. (1994). Effects of temperature during grain filling on starches from wheat cultivars. *Cereal Chemistry* **4:** 369-383.

SHIEH, W.J. and MacDONALD, M.B. (1982). The influence of seed size, shape, and treatment on inbred seed corn quality. *Seed Science and Technology* **10:** 307-313.

SIDDIQUE, M.D.A. and GOODWIN, P.B. (1980). Seed vigour in bean (*Phaseolus vulgaris* L. cv. Apollo) as influenced by temperature and water regime during development and maturation. *Journal of Experimental Botany* **31:** 313-323.

SINGLETARY, G.W., BANISADR, R and KEELING, P.L. (1994). Heat stress during grain filling in maize. Effects on carbohydrate storage and metabolism. *Australian Journal of Plant Physiology* **21**: 829-841.

SOFIELD, I., EVANS, L.T., COOK, M.G. and WARDLAW, I.F. (1977). Factors influencing the rate and duration of grain filling in wheat. *Australian Journal of Plant Physiology* **4:** 785-797.

SOLE, S.M. (1994). Effects of pre-germination on germination properties of barley and resultant malt quality. *American Society of Brewing Chemists* **52 :** 76-83.

SPIERTZ, J.H. (1977). The influence of temperature and light intensity on grain growth in relation to carbohydrate and nitrogen economy of the wheat plants. *Netherlands Journal of Agricultural Science* **25:** 182-197.

STEINER, J.J. and OPOKU-BOATENG, K. (1991). Natural season-long and diurnal temperature effects on lettuce seed production and quality. *Journal of American Society of Horticultural Science* **116**: 396-400.

STEINER, J.J., GRABE, D.F. and TULO, M. (1989). Single and multiple vigour tests for predicting seedling emergence of wheat . *Crop Science* **29:** 782-786.

STRAND, E. (1991). Variation in seed dormancy in genetically homogeneous material of small grain species. *Norwegian Journal of Agricultural Science* **5:** 39-43.

SYMONS, S.J., ANGOLD, R.E., BLACK, M. and CHAPMAN, J.M. (1983). Changes in the growth capacity of the developing wheat embryo. *Journal of Experimental Botany* **34**: 1541-1550.

TAKAHASHI, T and KANAZAWA, T. (1996). Grain filling mechanism in spring wheat IV. Effects of shadings on number and size of spikes, grains, endosperm cells and starch granules in wheat. *Japanese Journal of Crop Science* **65 :** 277-281.

TASHIRO, T. and WARDLAW, I.F. (1989). A comparison of the effect of high temperature on grain development in wheat and rice. *Annals of Botany* **64:** 59-65.

TASHIRO, T. and WARDLAW, I.F. (1990). The response to high temperature shock and humidity changes prior to and during the early stages of grain development in wheat. *Australian Journal of Plant Physiology* **17:** 551-561.

TASHIRO, T. and WARDLAW, I.F. (1991). The effect of high temperature on the accumulation of dry matter, carbon and nitrogen in the kernel of rice. *Australian Journal of Plant Physiology* **18:** 259-265.

TAYLORSON, R.B. and HENDRICKS, S.B. (1977). Dormancy in seeds. *Annual Review of Plant Physiology* **28:** 331-354.

TEKRONY, D.M. and HUNTER, J.L. (1995). Effects of seed maturation and genotypes on seed vigour in maize. *Crop Science* **35**: 857-862.

TEKRONY, D.M. and EGLI, D.B. (1995). Accumulation of seed vigour during development and maturation. *In*: Basic and applied aspects of Seed Biology,. Kluwer Academic Publishers, London.pp. 369-384.

TESTER, R.F., SOUTH, J.B., MORRISON, W.R. and ELLIS, R.P. (1991). The effects of ambient temperature during the grain-filling period on the composition and properties of starch from four barley genotypes. *Journal of Cereal Science* **13:** 113-127.

TILLETT, I.J.L.and BRYCE, J.H. (1993). The regulation of starch granule size in endosperm of developing barley grains. *Proceedings of the European Brewing Congress* pp. 45-52.

WALLWORK, M.A., JENNER, C.F. and MacLEOD, L.C. (1994). Effects of heat stress on starch accumulation in the endosperm of barley. *Proceedings of the 44th Royal Australian Chemical Institute Cereal Chemistry Conference*, Editions, J. F. Panozzo and P.G. Downie, Ballarat, Australia, pp. 72-75.

WARDLAW, I.F. (1994). The effect of high temperature on kernel development in wheat: Variability related to pre-heading and post-anthesis conditions. *Australian Journal of Plant Physiology* **21**: 731-739.

WARDLAW. I.F. and WRIGLEY, C.W. (1994). Heat tolerance in temperate cereals: An overview. *Australian Journal of Plant Physiology* **21**: 695-703.

WARDLAW, I.F. and MONCUR, L. (1995). Response of wheat to high temperature following anthesis. I. The rate and duration of kernel filling. *Australian Journal of Plant Physiology* **22:** 391-397.

WARDLAW, I.F., SOFIELD, I. and CARTWRIGHT, P.M. (1980). Factors limiting the rate of dry matter accumulation in the grain of wheat grown at high temperature. *Australian Journal of Plany Physiology* **7:** 387-400.

WARDLAW, I.F., DAWSON, I.A. and MUNIBI, P.(1989). The tolerance of wheat to high temperature during reproductive growth. II. Grain development. *Australian Journal of Agricultural Research* **40:** 15-24.

WARDLAW, I.F., DAWSON, I.A., MUNIBI, P. and FEWSTER, R. (1989). The tolerance of wheat to high temperatures during reproductive growth. Survey procedures and general response patterns. *Australian Journal of Agricultural Research* **40:** 1-13.

WARRINGTON, I.J., DUNSTONE, R.L. and GREEN, L.M. (1977). Temperature effects at three developmental stages on the yield of the wheat ear. *Australian Journal of Agricultural Research* **28:** 11-27.

WILLIAMSON, P.M. (1997). Black point of wheat: *in vitro* production of symptoms, enzymes involved, and association with *Alternaria alternata. Australian Journal of Agricultural Research* **48:** 13-19.

WOOD, D.W., LONGDEN, P.C. and SCOTT, R. K. (1977). Seed size variation, its extent, source and significance in field crops. *Seed Science and Technology* **5:** 337-352.

WOODSTOCK, L.W. (1973). Physiological and biochemical tests for seed vigour. *Seed Science and Technology* **1:** 127-157.

WOODSTOCK. L.W. (1976). Progress report on the seed vigour testing handbook. *Newsletter of the Association of Official Seed Analysts* **50:** 1-78.

WRIGLEY, C.W., BLUMENTAHL, C., GRASS, P.W. and BARLOW, E.W.R. (1994). Temperature variation during grain filling and changes in wheat-grain quality. *Australian Journal of Plant Physiology* **21:** 875-885.

YAKLICH, R.W. and KULIK, M. M. (1979). Evaluation of vigour tests in soybean seeds. Relationship of the standard germination test, seedling length and tetrazolium staining to field performance. *Crop Science* **19:** 247-252.