Effect of storage on seed viability and vigour in different Ethiopian wheat and maize cultivars

by

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DECLARATION

" I declare that the thesis hereby submitted by me for the degree Master of Science in Agriculture at the University of the Orange Free state is my own independent work and has not previously been submitted by me at another University/Faculty. I further cede copy right of the thesis in favour of the University of the Orange Free State."

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Chapter 1

General introduction

Seed deterioration, due to aging, has come to be recognized as a major cause of reduced viability and vigour in many species. Aging involves the accumulation of degenerative changes until eventually the ability to germinate is lost (Naylor and Gurmu, 1990). Maximum seed quality occurs at physiological maturity, after which vigour and viability decline both before and after harvest (Basu, 1995). If the decline in viability and vigour continues seeds eventually die. However, before this catastrophic end point is reached, many sub cellular changes occur, giving rise to slower germination and many other performance symptoms collectively described as poor vigour (Dornbos, 1995). The rate at which this loss of vigour and viability occurs depends on several factors during storage (Aspinall and Paleg, 1971; Basu, 1995).

Within limits, the storage life of seeds of field crops decreases as the temperature increases. Justice and Bass (1979b) stated that the life of the seed is halved for each 5°C increase in seed storage temperature. They also cautioned that this rule should not be used for storage temperatures below 0°C or above 50°C. Cold storage of seeds at 0 - 5°C is generally desirable in order to maintain the viability and vigour of seeds during storage (Roberts, 1972a).

A rise in temperature during storage increases the rate of chemical and biochemical reactions. In orthodox dry-stored seeds (that can be stored in a state of low moisture content), a prolonged quiescent phase, in which enzyme catalyzed metabolic reactions should be at a very low ebb, is ideal (Burton, 1982; Basu, 1995). The dry seed is metabolically active, albeit very feebly. As such, a rise in temperature will also interfere with the rest period. At a very high temperature, beyond the usual physiological range, inactivation of enzymes and disruption of the fine structure of bioorganelles take place not withstanding the high temperature (Burton, 1982; Basu, 1995). The hydrolysis of carbohydrate followed by increased levels of reducing sugars was also reported to be enhanced under high temperature storage conditions (Burton, 1982). Moreover, under such unfavourable storage conditions (high temperature and relative humidity), degradation of structural and enzymatic proteins were also reported by to be enhanced (Kalpana and Madhava Rao, 1997).

Of the various factors influencing the rate of deterioration, moisture content by far seems to be the most important. If the moisture content is maintained at a sufficiently low level, seeds can be stored for many years with little deterioration even under otherwise unfavourable storage conditions (Justice and Bass, 1979b; Pomeranz, 1992). In actual practice, however, seeds as it comes from the farm often have moisture contents near or above the critical value (14% for cereal crops).

Ellis *et al.* (1991) proposed that moisture content in equilibrium with 10-11% relative humidity is optimal for the longevity of orthodox seeds in storage. Moreover, drying increase the longevity of orthodox seeds in a predictable way, and it has long been known that they can survive considerable desiccation. For example, neither the viability nor the vigour of seeds of five graminaceous species was adversely affected by drying to 1% moisture content (Ellis *et al.*, 1991).

Relative humidity of the storage environment is also an important factor affecting maintenance of seed quality during storage period. This is because of its direct relation to seed moisture content (van de Venter, 1999). When seeds are placed into storage it will not remain static unless the seeds are hermetically sealed. Since seeds are hygroscopic, they absorb moisture from the atmosphere or lose moisture to it, until the vapour pressure related to seed moisture content and atmospheric moisture reach equilibrium (Justice and Bass, 1979b; Lin, 1988; Van de Venter, 1999). Therefore, under open storage conditions, the moisture content of the seed will equilibrate with the surrounding air, if given enough time. Obviously, the higher the relative humidity during storage the shorter the life span of the seeds will be (Roberts, 1972a; Ellis *et al.*, 1991; van de Venter, 1999).

There is also considerable variation in the length of time that seeds of different cultivars can survive during storage (Justice and Bass, 1979a). The principles prevailing at the species level are also effective at cultivar level. Several cultivars of the same species that were compared for seed longevity, differed significantly for their ability to withstand poor storage conditions. These differences are relatively small under good storage conditions but, under adverse conditions (elevated temperature and relative humidity), they can be quite large (Bewley and Black, 1994).

The two aspects of seed quality (viability and vigour) affect yield (Ellis, 1992). First, it could reduce potential field emergence so that, even if the subsequent performance of the individual plants were unaffected, yield could be reduced through the establishment of sub optimal plant-population density (Khah *et al.*, 1989). If seed quality only affected percentage emergence, then growers could theoretically overcome such effects by adjusting seed sowing rates. However, in practice, adjustments to seed sowing rates are hampered by difficulties in forecasting the particular seedbed environment (Khah *et al.*, 1989; Ellis, 1992).

Secondly, according to Ellis (1992) and Roberts (1972b), plant yield at a given density may be influenced by seed quality (i.e. independent of the effect of emergence). If seed vigour is defined in terms of the effect of seed quality on crop emergence and establishment, then the term seedling vigour can be applied to describe any subsequent effect of seed quality on crop growth and yield at a given density.

Moreover, the effect of seed quality on final yield has been ascribed to a delay in emergence (Roberts, 1972b). In practice slow emergence may result from delay in germination and/or a reduction in the rate of growth of shoots and roots prior to emergence. If this is the case, emphasis should be placed on prevention rather than the cure, through storing seeds under low temperature, relative humidity and low moisture content which enables the seed to be stored longer without losing their quality attributes (Khah *et al.*, 1989).

It can be argued that the low temperature and relative humidity necessary to achieve very slow seed aging in many crops is only possible by controlled storage in developed countries due to the available infrastructure but that this is not achieved by peasant farmers, such as in Ethiopia, who will store at or near ambient conditions (Anonymous, 1995). Ambient storage conditions would be considered by some to be poor storage conditions (Delouche *et al.*, 1973) resulting in loss of seed qualities due to relatively high temperatures and humidity in many parts of the world. Indeed ambient storage conditions are at the order of the day in Ethiopia, causing inferior seed quality (Anonymous, 1995).

In Ethiopia, among several factors limiting crop yield, poor seed quality caused by unfavourable storage conditions, is of great concern. Therefore, controlled storage conditions are necessary for successful seed storage (Anonymous, 1995). The degree of control needed is determined by ambient conditions and the kind of seed to be stored. Thus, useful data on the responses of seed under ambient and controlled storage temperatures are necessary for proper and economic design of rooms and systems for all levels of seed conditioning (Delouche, *et al.*, 1973).

However, sufficient research data is not currently available in Ethiopia. It is, therefore, important to undertake research of this kind. One of the aims of this study was to determine the effect of ambient temperature (more or less similar to Ethiopian conditions) and low temperature storage conditions (4°C) on viability, vigour and biochemical aspects related to seed viability and vigour of Ethiopian bread and durum wheat as well as maize cultivar seeds. Additionally, the effects of storage in open and closed containers at different temperatures were also investigated.

Moreover, the genetic variations for viability, vigour and biochemical aspects related to seed viability and vigour of different Ethiopian bread and durum wheat as well as maize cultivar seeds should be assessed in order to provide an information base to genetically improve the storage potential of these cultivars. Already in 1973, Delouche *et al.* stated that data of this kind is generally not available in tropical countries (including Ethiopia) and the situation has not dramatically changed since. This supplied the rationale to determine the genetic variations in viability, vigour and biochemical aspects related to seed viability and vigour of various cultivars of Ethiopian bread and durum wheat as well as maize seeds, which was the second aim of this study.

In line with the objectives stated, a literature review, dealing mainly with loss of seed viability and vigour during storage as well as with different tests that have been used in the past to distinguish between poor and good quality seeds, is presented in chapter 2. Chapter 3 describes the material used as well as the methods applied in the study.

Chapter 4 deals with the effect of different storage conditions on viability and vigour of Ethiopian bread and durum wheat as well as maize cultivar seeds. The effect of different storage conditions on biochemical and physiological aspects related to viability and vigour of different cultivars of bread and durum wheat as well as maize seeds is addressed in chapter 5. A general discussion on the tendencies observed during this study as well as final conclusion and recommendations for a future research approach, is presented in chapter 6.

Chapter 2

Literature review

2.1 Seed viability loss

A viable seed is one that is alive and has the capacity to produce enzymes capable of catalyzing the metabolic reactions necessary for germination and seedling growth. Thus, seed viability denotes the degree to which seeds possess these properties. However, seeds may be viable but not capable of germinating because the germination process can be blocked by physical factors, such as dormancy (Roberts, 1972a). Germination can only proceed once these restricting factors have been removed (Hampton, 1995). To the seed industry, from an economic perspective, a seed is either viable or nonviable depending on its ability to germinate and produce a normal seedling (Hampton, 1995).

According to Pomeranz (1992), various theories have been proposed to account for loss of seed viability in storage. Basically, they can be divided into two groups: (a) those that link loss of viability with an intrinsic factor resulting from seed metabolism, and (b) those that postulate extrinsic causes with the emphasis on the effect of microorganisms living in association with the seed. Both external factors such as storage temperature (Roberts, 1972a) and internal factors such as decline in enzyme activity (Ram and Wiesner, 1988) influence the process of aging in seeds at various physiological, cytological and genetic levels. Although external factors such as oxygen tension, temperature, humidity and parasites may accelerate aging, metabolite level changes during storage seems to be the basic cause of the deterioration occurring in old seeds (Pomeranz, 1992).

During germination, aged seeds show significantly lower activity of the hydrolytic enzymes responsible for the mobilization of food reserves to the growing embryo than in non aged seeds (Abdul-Baki, 1969; Ram and Wiesner, 1988). A significant reduction in the synthesis of enzymes necessary for the mobilization of reserves required for new growth, coupled with the inadequate supply of energy for the surge of reactions leading to germination, may determine the extent of germinability of aged seeds (Basu, 1995).

Germination requires active participation of the complex synthetic machinery, consisting of a vast array of enzymes, their factors and cofactors, hormonal regulators, the nucleic acids and perhaps other unidentified factors, along with the apparatus to provide the energy necessary for the various synthetic activities. A major impairment of the functional activity of any one of the components of this complex but closely integrated system, may put a break on the sequence of metabolic events, culminating in the failure of the seed to germinate (Bewley and Black, 1994).

However, equating viability with germination can create confusion, depending upon the definition of germination. Physiologically a seed can be said to have germinated once the radicle has protruded and in this sense viability is germination in the absence of dormancy (Hampton, 1995). During seed testing a seed may germinate (and is therefore viable), but produce a seedling which is classified as abnormal (ISTA, 1985). In this sense viability does not equal the germination percentage as presented on the analysis certificate. However, in practice seed viability is used synonymously with germination capacity (the ability of seeds to germinate and produce normal seedlings) and germination testing is the principal and accepted criterion for seed viability (ISTA, 1985).

2.2 Seed vigour loss

Seed ageing has come to be recognized as a major cause of reduced vigour in many species. Ageing involves the accumulation of degenerative changes until eventually the ability to germinate is lost. Rates of vigour loss vary according to the genetic composition, environmental condition and pathogenic organisms to which the seed is exposed (van de Venter, 1999). Between harvest and planting the following year, however, seed lots must be conditioned, stored, bagged and shipped to be ready for planting. There is a considerable opportunity for seed vigour loss during this period (Ram and Wiesner, 1988). Deterioration of seed vigour can occur through physiological decline and physical damage (Dornbos, 1995).

2.2.1 Physiological deterioration

Just as normal physiological processes during seed development and maturation are required to maximize vigour, impairment of normal physiological processes because of seed deterioration, contributes to non vigorous germination (Ram and Wiesner, 1988). Vigorous germination represents the culmination of normal and active physiological functioning at the cellular level. Proper physiological functioning in seed is correlated with vigorous germination, providing the basis for several seed vigour tests. In deteriorated seeds abnormal physiological functioning prevails, which indicates reduced vigour (Coolbear, 1995).

A progressive highly ordered cascade of events is hypothesized to explain the physiological basis of seed deterioration. The initial phase of seed deterioration is membrane degradation (Pandey, 1989; Pukacka, 1998), which causes and is followed by impairment of ATP synthesis, reduced respiration and biosynthesis rates, poor seed storability and, ultimately, reduced emergence, development of abnormal seedlings and reduced germinability. If membrane degradation is an initial stage of vigour loss, methods that detect membrane deterioration would be preferable to serve as early indicators (Stewart and Bewley, 1980; Dornbos, 1995).

When seed is exposed to high temperature and increased oxygen pressure, the polar lipids, including the phospholipids in the membrane, are susceptible to non enzymatic peroxidative reactions (Priestley, *et al.*, 1985). Membrane lipid peroxidation is also suspected to occur in dry seeds during storage, resulting in the formation of hydroperoxides, oxygenated fatty acids and free radicals. Free radicals from the lipid peroxidation process denature DNA, hinder protein translation and transcription and oxidize certain amino acids, the cumulative effect of which can reduce seed vigour (Francis and Coolbear, 1987; Bewley and Black, 1994).

Because of the fact that membrane degradation contributes to the leakage of cellular constituents, affected cells would be slow to reposition the lipid bilayers during imbibition, which serves as effective barriers to solute loss (Dornbos, 1995). The leaching of nutrients from damaged or aged seed forms the basis for conductivity vigour tests for various crops (Coolbear *et al.*, 1984; Marshall and Naylor, 1985; Lin, 1988; Argerich and Bradford, 1989).

2.2.2 Physical damage

Damage to the seed can be incurred during harvest, conditioning, movement of seeds from bin to bin, improper storage or careless handling of seed bags (Delouche *et al.*, 1973). Physically damaged seedlots are capable of substantially reducing field emergence and therefore yield when planted. Damage to the seed coat, obvious or subtle, is a common form of physical damage. Seed coat damage promotes the rapid leakage of cellular contents upon imbibition (Argerich and Bradford, 1989). Leakage of biochemical substances not only represents a loss of energy and building block resources necessary to drive vigorous germination (Bewley and Black, 1994), but leached compounds may further sustain the growth of soil micro-organisms capable of causing pathogenic infection (Basu, 1995) which may further contribute to the deterioration of seeds.

2.3 Relationship between seed viability and vigour

The property of the seed that enables it to germinate under conditions favourable for germination is termed viability, provided that any dormancy in the seed is removed before the germination test (Basu, 1995). The inability of the seeds to germinate may also be due to loss of viability, a degenerative change which is irreversible and generally considered to represent the death of the seed. A non-viable seed will be considered to be one which could not germinate when given near optimal conditions, even when it is non-dormant (Roberts, 1972a).

Vigour is that quality of the seed which is responsible for rapid and uniform germination, increased storability, good field emergence and the ability to perform well over a wide range of field conditions (Ellis, 1992; Yamauchi and Winn, 1996). Seed vigour refers to both the ability and strength to germinate successfully and establish a normal seedling. Vigour is positively correlated to the ability of a seed population to establish an optimum plant stand in both optimum and sub optimum soil environments, and therefore to maximize yield (Khah *et al.*, 1989).

Because soil conditions during planting are often not optimal, growers require seeds with good germination ability and vigour (Ellis, 1992). Seed vigour is gradually acquired in the seed production environment as the seed develops on the maternal plant. Maximum vigour is achieved at the physiological maturity stage followed by a steady decline thereafter until being planted in the subsequent growing season (Khah *et al.*, 1989).

2.4 Factors affecting seed viability and vigour during storage

2.4.1 Storage temperature

It has long been known that temperature is one of the factors which influences seed viability and vigour during storage (Roberts, 1972a; Delouche *et al.*, 1973; Francis and Coolbear, 1987). With few exceptions, there is no evidence that extremely low temperature is anything but beneficial to the maintenance of viability, providing the moisture content is not high. There is a great deal of evidence for many species to show that very low temperatures, -20°C or less are beneficial for the maintenance of maximum viability, providing the moisture content is not high enough to allow freezing injury (Roberts, 1972a). For example, maize grains were not injured by a sub-freezing temperature at a moisture content less than about 20 % (Roberts, 1972a). On the other hand, if the relative humidity is high, causing the seeds to gain moisture, and if the seeds are then exposed to a higher temperature (e.g., for transport), they might deteriorate because of their high moisture content (Lin, 1988; Bewley and Black, 1994).

At temperatures higher than about 35°C, enzymes are progressively denatured, the more rapidly the higher the temperature, with consequent loss of catalytic activity. This can be manifested in the first few hours after transfer of seeds to the high temperature. The short term effect of high temperature on respiration is thus subject to two opposing influences - an immediately effective increase in the rate of respiration because of the direct influence of temperature, and a progressive decline in the rate because of denaturation of the enzymes (Burton, 1982).

2.4.2 Moisture content

Moisture Content (mc) is defined by the International Seed Testing Association (ISTA, 1985) as:

$$\text{mc} = \frac{\text{Fresh weight of seed - dry weight of seed}}{\text{Fresh weight of seed}} \times 100$$

Justice and Bass (1979b) stated that for each 1% decrease in seed moisture content the storage life of the seed is doubled. However, there is evidence that there are different conditions of moisture content which affect the viability and vigour of seeds (Roberts, 1972a). When moisture content is high (> 30%), providing the temperature is suitable, seeds may germinate but from 18 to 30% moisture content, rapid deterioration through the action of micro-organisms can occur. Seeds stored at a moisture content of >18-20% will respire and in poor ventilation the generated heat will kill them. When oxygen is not readily available, high seed moisture content is still more injurious as the products of anaerobic respiration, such as ethanol and acetaldehyde, are toxic to the seed (Basu, 1995). The catalytic activity of the hydrolyzing enzymes also increase with progressive rise in seed moisture level (Justice and Bass, 1979b).

Seeds with a moisture content below 8-9% are believed to be exposed to little or no insect activity and at a moisture content below 4-5%, seeds are assumed to be immune from attack by insects and storage fungi, but they may still deteriorate faster than those maintained at a slightly higher moisture content (Bewley and Black, 1994). Although it was confirmed by Ellis *et al.* (1991) that the lower the moisture content the better the preservation of the seed, it was also pointed out that there is evidence of damage in some cases if desiccation is too severe (Vertucci and Ross, 1991). According to Basu (1995), excessive seed drying is discouraged not only because of rising fuel and operative costs but also on physiological grounds. At a moisture content well below the critical level of 1% (Ellis *et al.*, 1991), a host of physiological and biochemical reactions, of which the most important is respiration, will be set in motion (Basu, 1995).

2.4.3 Relative humidity

Relative humidity is expressed as a percentage and calculated as follows: the amount of moisture in the air is divided by the amount the air is capable of holding at the same temperature and multiplied by 100. Warm air can hold more water than cool air. Thus, if the amount of water in the air is held constant and if the temperature is increased, the relative humidity will be decreased. Conversely, if the temperature of the air is lowered, the relative humidity will be increased (Justice and Bass, 1979b).

Under all storage conditions the moisture content of the seeds will reach equilibrium with the surrounding air if given enough time. In fact, equilibrium is reached between the seeds and the air in the interstitial space among the seeds. It will have been reached when the net movement of moisture from air to seed, or from seed to air, is zero (Justice and Bass, 1979b). Concerning the relationship between moisture content and relative humidity, Ellis *et al.* (1991) have proposed that moisture content in equilibrium with 10-11% relative humidity (RH) is optimal for the longevity of orthodox seeds in storage.

Furthermore, according to Van de Venter (1999), seeds should be hermetically sealed after equilibration so that the seed moisture remains constant during storage. However, for large amounts of seeds in open storage, it is obviously impossible to equilibrate them at maintained RH (10-11%) as well as to maintain them at corresponding moisture content. In general, the drier the seed the better the storage life will be, but the question is just how dry sould it be, as seeds at

0% moisture may die (Van de Venter, 1999). There are essentially no reports of seed damage caused by drying to 1% moisture content (Ellis *et al.*, 1991).

If control of relative humidity is possible, the following rule stated by Bewley and Black (1994) can be followed. The arithmetic sum of storage temperature in degree °F, and the percentage relative humidity, should not exceed 100 with no more than half the sum contributed by the temperature. This means that the temperature should not exceed 50°F (10°C), and at this temperature, RH should not exceed 50%.

2.4.4 Cultivar differences

Different cultivars of the same species may have different viability and vigour characteristics under the same storage condition (Justice and Bass, 1979a). These differences are mostly evident when moisture content, relative humidity and temperature are very high. For example, the relatively hard flint and dent varieties of maize remained viable longer than starchy or sweet ones in open (unsealed condition) storage. But in closed storage, at fairly constant moisture contents, few differences were evident (Bewley and Black, 1994). In agreement with this, Roberts (1972a) also pointed out that different cultivars of rice showed marked differences in their ability to retain viability. However, when special care was taken to store six different cultivars of rice under identical conditions, the viability was found to be identical.

Thomson (1979) also noted that late cultivars may appear to store less well than early ones, because their seed is more likely to be exposed to inclement weather at harvest time and could therefore deteriorate to some extent before storage. Roberts (1972a) also reported a significant difference between 8 tomato, 8 bean, 5 pea, 15 watermelon, 11 cucumber and 5 sweet corn cultivars. These cultivars were stored under controlled conditions and differences in longevity between cultivars were found within all species. But, the difference between the best and worst cultivars of a species was apparently not very great. The differences in viability periods between genotypes within a species are also hereditary. For example, the inheritance of seed longevity in maize was investigated and it was concluded that the degree of longevity was inherited (but not simply) and reciprocal crosses showed pronounced maternal effects (Roberts, 1972a).

2.5 Seed viability testing

2.5.1 Germination test

Germination is defined by the International Seed Testing Association (ISTA, 1985) as the emergence and development of the seedling to a stage where the aspect of its essential structures indicate whether or not it is able to develop further into a satisfactory plant under favorable conditions. The essential structures are the root and shoot axes, cotyledons, terminal buds, and for the Gramineae, the coleoptile. The germination test reports only on the percentage of normal seedlings, abnormal seedlings (which are probably produced from those seeds close to death; Hampton, 1995) and dead or ungerminated seeds in a seedlot. When maximum viability is reached a seedlot should, in theory, have a germination of nearly 100 percent, providing dormancy is not a factor. Loss of viability from this point results from the deterioration process involved with both pre and post-harvest seed ageing (Delouche *et al.*, 1973; Savino *et al.*, 1979).

The ultimate objective of testing for germination is to gain information with respect to the field planting potential of the seed (Tekrony and Egli, 1977; Matthews, 1981, ISTA, 1985). Field emergence ability is the major aspect of seed quality that is of concern to growers and high germination (>90 %) is obviously a prerequisite for seed to be sown. A further objective of the germination test is to provide information that can be used to decide whether the seedlot is eligible to remain within a certification scheme.

However, the germination test as prescribed by ISTA (1985) has a number of limitations and criticisms. Firstly, it often fails to relate to subsequent seedlot performance in the field or during storage (Marshall and Naylor, 1985). Secondly, it is not yet completely standardized. For example, the rule states that the substrate must at all times contain sufficient moisture, yet do not specify the amount of water to be used (Hampton, 1995). Thirdly, a germination test is largely a subjective assessment where the seed is taken as germinated based on the definition of normal seedling. No account is taken of the strength or weakness of seedlings (Marshall and Naylor, 1985). In practice, only the dead, badly diseased and irrevocably lame seedlings are eliminated while the weak, semi-lame and robust are given equal weight (ISTA, 1885).

Despite these problems, the germination test remains the principal and most accepted criterion for seed viability and, worldwide, several million tests are conducted annually. Providing the limitations are recognized, the germination test is a useful viability index (Thomson, 1979; Matthews, 1981), however, a better understanding of the meaning of the results is required. In particular, the acceptance that a germination result equals field performance must be corrected (Marshall and Naylor, 1985; Steiner *et al.*, 1989).

Germination data should be used for the initial separation of seedlots. A germination percentage less than the accepted standard (e.g., < 90 % for cereals) by themselves indicate that the quality of the lot is suspect and likely to perform poorly in the field (or storage) unless conditions are approaching the optimum for species concerned (Steiner *et al.*, 1989; Hampton, 1995). It is only at a high germination percentage that the standard germination test is not adequate to indicate quality attributes of the seeds. A small difference in percentage germination represents a large difference in the progress of deterioration. It is under these circumstances that more sensitive differentiation of potential seed performance is required and vigour tests are necessary (Powell and Matthews, 1984; Marshall and Naylor, 1985; Steiner *et al.*, 1989).

2.6 Seed vigour tests

2.6.1 Germination index

The properties of vigorous seeds include rapid and uniform germination. Both the rate as well as uniformity of germination generally decline as seeds deteriorate (Heydecker, 1972; Argerich and Bradford, 1989). The theory behind the germination index test, is that radicle protrusion in a relatively short time is a characteristic of vigorous seeds that permits them to germinate and emerge from the soil quicker than less vigorous seeds (Kulick and Yaklich, 1982). According to Ram and Wiesner (1988), the germination index has been found to be positively correlated with seed vigour in wheat (*Triticum aestivum* L.), rice (*Oryza sativum* L.) and barley (*Hordeum vulgare* L.).

In agreement with this, MacKay (1972) reported that vigorous seeds, except when dormant, will normally be expected to germinate rapidly, but seedbed conditions may not permit germination immediately after sowing. In this case a vigorous seed is capable of surviving until conditions improve and then still goes on to produce a vigorous and healthy seedling as well as a good crop. The germination index is calculated as follows: $\Sigma(Dn) / \Sigma n$, where n is the number of seeds which germinate on days D and D is the number of days counted from the beginning of the germination test (Yamauchi and Winn, 1996).

2.6.2 Seedling growth and seedling evaluation test

Seedlings judged to be morphologically abnormal are excluded from the evaluation (germination) test but, providing all the essential structures are present and show a balanced development, no account is taken of the rate of germination or growth nor the strength of the seedling in making this decision (Perry, 1987). Differences in these characteristics between seedlots are frequently observed and they were the basis of the original definition of vigour (Roberts, 1972b; Rodriguez and McDonald, 1989; Yamauchi and Winn, 1996; Guy and Black, 1998).

According to Rodriguez and McDonald (1989) deterioration of field bean (*Phaseolus vulgaris* L.) seeds, after being naturally and artificially aged, culminated in loss of germination ability and vigour. Aged seeds produced less top and root growth as well as poor yield. Steiner *et al.* (1989) stated that, among twenty vigour tests performed on 49 seedlots of soft winter wheat, seedling root length was the best predictor of vigour, followed by seedling dry weight. Similarly, Guy and Black (1998) reported that seeds of *Triticum aestivum* L. aged by storage over saturated ammonium dihydrogen sulphate (RH 80%) at 45°C, showed losses in both vigour and viability.

Guy and Black (1998) measured vigour based on shoot height after 7 days comprising both the rate of seed germination and the subsequent rate of seedling growth. Their finding indicated that seedlings produced from 2 and 3 day aged seeds were healthy and normal in appearance, but were shorter than seedlings from non aged seeds grown for the same time period. Surviving seeds from a 4 day aged seedlot, however, produced a high proportion of abnormal seedlings with stunted root growth, twisted coleoptiles and shoots or failed to produce a coleoptile (Guy and Black, 1998).

It was also suggested that the observed decrease in vigour (as indicated by final shoot length) in fully viable seeds (i.e. up to 3 day of ageing), is in fact because of a decreased rate of germination and not because of a decrease in the rate of seedling growth after germination has taken place (Guy and Black, 1998). The seedling growth and seedling evaluation test is also reported to be used in maize and soybeans (Van de Venter, 1999).

2.6.3 Electrical conductivity of bulk seed exudates

Seed deterioration is associated with deteriorated membranes and cells which leak. When deteriorated seeds are soaked in water they lose more electrolytes (such as amino and organic acids) which increases the conductivity of the water (Coolbear *et al.*, 1984; Marshall and Naylor, 1985; Argerich and Bradford, 1989). Bewley and Black (1994) noticed that, it is not only organic acids and amino acids which leak out, but also sugars, ions and proteins. Bewley and Black (1994), further indicated that damaged legume seeds, e.g. with cracked seed coats which often exhibit poor seedling vigour, may exude starch grains and protein bodies through the coats under pressure when first imbibed. The source of these intracellular substances is the outer layers of the cotyledons, which may blister within minutes of introduction to water.

The electrical conductivity test measures the amount of electrolytes which leach from seeds (Hampton, 1995). The amount of electrolytes leached from an imbibing seed is a function of its soluble mineral ion content, any damage to the seed coat, the proportion of non viable cells and its ability to repair and reorganize cell membranes in living cells. Increased conductivity may indicate the reduction in the ability of seeds to reorganize membranes upon imbibition and indicate reduced viability and vigour (Ram and Wiesner, 1988; Pandey, 1989; Bewley and Black, 1994).

2.6.4 Respiration rate

Given the known predictive limitation and the length of time required for most germinative vigour tests, there has been continued interest over the years in the potential of physiological or biochemical properties of seeds to act as indicators of seed vigour (Coolbear, 1995). One of the most useful of these is the measurement of respiratory capacity. In this test the amount of oxygen uptake by tissue is measured using a Gilson differential respirometer (Steiner *et al.*, 1989).

The work of Ram and Wiesner (1988) showed that a significant positive correlation exists between seedling vigour and respiration rate of different cultivars within a given species. According to Roberts (1972c), only the respiration rates of seeds of the same or closely related lines can be meaningfully compared as indicators of vigour. However, Hampton (1995) pointed out that, while mitochondrial damage in deteriorating seed will be directly related to vigour, oxygen uptake, the most commonly measured parameter of respiratory capacity suggested as a vigour index, may not always be related to the efficiency of ATP production.

2.7 Other biochemical aspects related to seed viability and vigour

2.7.1 Total water soluble protein content

During seed development anabolic processes predominate and bring about a gradual increase in dry matter, including development of the embryo and food reserves. Following maturation, biochemical changes continue and eventually catabolic processes predominate and deterioration becomes apparent. Catabolic changes occur more slowly under low temperature and relative humidity than under opposite conditions (Justice and Bass, 1979b). According to the review of Pomeranz (1992), the change in true protein content of seed stored for 9 months at -1°C was lower (29mg /100g) whereas in seeds stored for 24 months at the same temperature (-1°C) the change in protein content increased to 79mg /100gm. Similarly, the true protein content of seeds stored for 9 months at 24°C was lower (50 mg/100gm) whereas in seeds stored for 24 months at 24°C the change in protein content increased to 163 mg/100gm.

Burton (1982) stated that turnover and changes of proteins in dry grains could be expected to be very slow. There is, however, a decreasing trend, of no known dietary significance, in the proportion of water-soluble nitrogen compounds in wheat seeds during storage up to 16 years. The changes may, however, be important in other respects, particularly in connection with germination and growth. On the other hand, according to Sreeramulu (1983), amino acids and soluble protein of seeds of Bambarra groundnut stored under laboratory conditions (25°C - 35°C) for 24 months, were found to increase.

Apart from storage conditions, a fall in protein content also appears to be related to the degree of tolerance of a cultivar to ageing conditions. Kalpana and Madhava Rao (1997) exposed three cultivars of pigeon pea seeds for 0, 2, 4, 6, 8 days to adverse conditions of 40°C and 100% relative humidity. Of the three cultivars, T21 had the highest protein content throughout the experimental period and the percentage decrease in total protein content was greatest in the most susceptible cultivar ICPLI87 (17.6%) followed by PDM1 (12.1%) and T21 (7.4%).

2.7.2 Carbohydrate content

During storage, α and β amylase attack the starches of seeds, converting them into dextrin and maltose (Burton, 1982). Amylase activity in wheat increases during the early stages of storage. An increase in the dry weight of seed during storage was observed under certain conditions and was explained by the fact that water was consumed in the starch hydrolysis reactions. Thus, the dry weight of the product of starch hydrolysis was greater than that of the original starch (Pomeranz, 1992).

Although this hydrolytic action might be expected to result in a significant increase in the reducing sugar content of the grain (Burton, 1982), conditions that favour starch decomposition usually favour respiration activity also so that the sugars are metabolized and converted into carbon dioxide and water. Under these conditions, which usually occur at moisture levels of 15% or more, the grain looses both starch and sugar and the dry weight decreases (Pomeranz, 1992).

Onigbinde and Akinyele (1988) stored whole seeds and four obtained from two maize (*Zea mays* L.) cultivars, yellow and white, at 0, 20 and 55°C for 7 months at 13-14% moisture content. Significant increase in total soluble sugar was reported in the white maize flour stored at 55°C. The soluble sugar increase could have been the result of amylolytic activity of the endogenous amylases.

High viability and germinabiliy of grain are probably the best and most meaningful index of usefulness, especially in seeds to be used for planting. For seeds several biochemical tests can be used to estimate usefulness for planting. In summary, even under optimal storage conditions, it is impossible to prevent qualitative changes. They can only be slowed down by storage at low temperature. As already discussed, the main changes are best observed in germinability and in quality of starch and protein (Pomeranz, 1992).

Chapter 3

Materials and methods

3.1 Seed source

Fresh seeds of different wheat and maize cultivars of the 1998-99 harvest were obtained from Ethiopia. Wheat and maize cultivars that were included in the experiment are shown in Table 3.1.

Table 3.1: Ethiopian bread and durum as well as maize cultivar seeds that were included in the study.

Bread wheat cultivars (*Triticum aestivum* L.)	Durum wheat cultivars (*Triticum durum* L.)	Maize cultivars (*Zea mays* L.)
HAR - 1685	E – 26	BH- 540
ET – 13	Foka	BH - 660
HAR - 604	Cocorit-71	

3.2 Seed storage treatments

Wheat and maize seeds, from each cultivar, were divided into four lots and were stored under the following storage conditions:

3.2.1 Storage treatment 1: Seeds from all cultivars of wheat and maize, separated in three replicates, were stored under ambient conditions (temperature 18 - 30°C; relative humidity 30 - 50%) in open containers (allowing free flow of air).

3.2.2 Storage treatment 2: Seeds from all wheat and maize cultivars, separated in three replicates, were stored under ambient conditions (temperature 18 - 30°C; relative humidity 30 - 50%) in closed containers (preventing free flow of air).

3.2.3 Storage treatment 3: Seeds from all wheat and maize cultivars, separated in three replicates, were stored at low temperature (4°C; relative humidity 65 - 70%) in open containers.

3.2.4 Storage treatment 4: Seeds from all wheat and maize cultivars, separated in three replications, were stored under low temperature (4°C; relative humidity 65 - 750%) in closed containers.

All of the different storage treatments were carried out for 6 months. Moisture content, viability, vigour and other biochemical aspect tests related to seed viability and vigour were carried out both before and after storage treatments.

3.3 Seed viability test

3.3.1 Standard germination test

Three replicates of 75 seeds each per treatment, were used for the standard germination test. Each replicate was subdivided into three sub replicates (three petri dishes containing 25 seeds each). Two Whatman no. 5 filter papers were placed in each petri dish, moistened with 4 and 6 ml distilled water for wheat and maize seeds, respectively. The petri dishes containing the seeds were kept at 20°C (for wheat) and 25°C (for maize) in a growth chamber for seven days (ISTA, 1985). Normal seedlings were counted, according to the rule formulated by the International Seed Testing Association (ISTA, 1985).

According to the rules of ISTA (1985), wheat seedlings with at least two seminal roots intact or with only slight defects, the mesocotyl (where developed) and the coleptile intact or with only slight defects were counted as normal seedlings. Similarly, maize seedlings with primary root, mesocotyl and coleoptile intact or with only slight defects as well as the leaves intact, emerging through the coleoptile near the tip or at least reaching half-way up, or with only slight defects were taken as normal seedlings.

3.4 Seed vigour tests

3.4.1 Germination index

Three replicates of 75 seeds each per treatment, were placed out for germination as described for the standard germination test. After 24, 48, 72 and 96 hours of incubation, all seeds that had the radicles protruding the testa and had at least grown to a length of 0.5 cm, were considered as germinated (Kalpana and Madhava Rao, 1997). Four days were regarded as sufficient to determine seed vigour according to Kulik and Yaklich (1982). The germination index (X) was calculated as follows (Yamauchi and Winn, 1996):

$$X = \frac{\text{No. of seeds germinated on day 1}}{1} + \ldots + \frac{\text{No. of seeds germinated on day 4}}{4}$$

3.4.2 Shoot dry mass

The seedling growth test was performed by measuring the dry mass (Steiner *et al.*, 1989). Ten seeds from each treatment, in three replicates, were placed in single rows on a single germination paper covered with a second sheet of paper, moistened with distilled water, placed into Erlenmeyr flasks containing 250 ml of distilled water and incubated at 25°C (maize) or 20°C (wheat) in a growth chamber. After 14 days, shoots were separated from their seeds, and the shoot dry mass was determined after drying for three days at 70°C (Argerich and Bradford, 1989) in a drying oven.

3.4.3 Electrical conductivity

Three replicates of 50 seeds each per treatment were placed in a 100 ml Schott Duran glass bottle and 37.5 ml of distilled water was added. Bottles containing the submerged seeds were subsequently placed in an incubator at a constant temperature of 25°C (maize) and 20°C (wheat) for 24 hours. After 24 hours of incubation the contents of the bottle were stirred gently (Ram and Wiesner, 1988). The electrical conductivity was measured with a digital conductivity meter (at a potential of 4V) (Argerich and Bradford, 1989) and expressed as $\mu S\ cm^{-1}\ g^{-1}$ FW.

3.4.4 Respiration rate

Oxygen uptake was measured by means of a Gilson differential respirometer. Three replicates of 25 seeds each per treatment were imbibed in 4 ml (wheat) and 6ml (maize) distilled water on a single filter paper in petri dishes and incubated at 25°C (maize) or 20°C (wheat). After 16, 24, 48 and 72 hours of incubation 1-6 seeds and/or seedlings, per replicate, were taken from the petri dishes and the fresh mass determined. Samples were subsequently placed in Warburg flasks. Three hundred µl 10% (m/v) KOH were placed in the center well of the vials and the absorption surface enlarged by means of a folded piece of filter paper (Steiner *et al.*, 1989). Oxygen consumption was measured 3 - 5 times at different time intervals. The respiration rate was expressed as $\mu mol\ O_2\ h^{-1}\ g^{-1}$ FW at standard pressure and temperature (Steiner *et al.*, 1989).

3.5 Other biochemical and physiological tests related to seed viability and vigour

3.5.1 Total water soluble protein content

Water-soluble proteins were extracted using an extraction buffer (12.5 mM Tris, 2 mM EDTA, 10 mM β-Mercapto-ethanol, 2 mM PMSF; pH = 6.8). One gram of seed from each treatment, in three replications, was homogenized with a mortar and pestle in the extraction buffer in the ratio of 4 ml buffer to one gram of seed. The homogenate was transferred into a clean Eppendorf vial and was centrifuged for 10 minutes at 12000 rpm at room temperature. Finally, 10 µl of the supernatant was transferred into a clean Eppendorf vial and diluted 20 times with distilled water (Pretorius and Small, 1991). Determination of protein content was carried out using the Biorad method and spectrophotometrically determined by means of an Elx808 microplate reader at 595 nm using bovine ϒ-globulin as standard.

3.5.2 Sucrose and D- glucose content

One gram of seed from each treatment, in three replicates, was placed in test tubes containing 8 ml 80% ethanol, preheated to 80°C in a water bath. Test tubes containing the seeds were kept at 80°C in the water bath for 15 minutes to stop all enzyme reactions. As some of the ethanol evaporated during this process, the original volume was restored. After homogenizing the seeds by means of a mortar and pestle, the extracts were centrifuged at 10000 g for 10 minutes. One ml aliquots of each replicate was removed, placed into Eppendorf vials and the ethanol evaporated in an oven at 30°C until dry. The dried supernatant aliquots were dissolved in 1 ml distilled water (Pretorius and Small, 1993). Finally, a 10 µl aliquot of this final solution for each replicate from each treatment, was analyzed for sucrose and D-glucose levels.

For the determination of D-glucose and sucrose content, the method outlined by Boehringer Mannheim (1997) was used. Sucrose and D-glucose levels were determined enzymatically using test kits (Boehringer Mannheim, 1997; cat. No. 716260). Calculation of sucrose and D-glucose levels were carried out according to the method of the suppliers of the test kits (Boehringer Mannheim, 1997).

3.5.3 Starch content

One gram of seeds per replicate, for each treatment, was ground in a mortar with a pestle and passed through a sieve of 0.3 mm pore diameter. Half (0.5 g) of the ground sample was weighed and transferred to a 100 ml Erlenmeyer flask, into which 20 ml dimethylsulfoxide (DMSO) and 5 ml hydrochloric acid (8 M) was added. The Erlenmeyer flask was subsequently sealed with parafilm and incubated for 40 minutes at 60°C on a magnetic stirrer equipped with a hot plate. After cooling to room temperature, 50 ml of distilled water was added and the pH was adjusted to 4 - 5 with sodium hydroxide (5 M) under vigorous stirring. Finally the samples were adjusted to 100 ml with distilled water and filtered with whatman No.1 filter paper. Ten µl aliquots of 10 times diluted samples were immediately used for the starch assay (Boehringer Mannheim, 1997).

Starch levels were determined enzymatically using starch test kits (Boehringer Mannheim, 1997; cat. No. 207748). The calculation of starch content was carried out according to the method of the suppliers of the starch test kits (Boehringer Mannheim, 1997).

3.5.4 Moisture content

The initial moisture content (mc) of seeds was determined using the air oven method at 70°C. The weight of glass petri dishes with their covers was initially determined. Seed samples (2-3 g each) were then placed in the dish, distributed evenly over the bottom of the surface, the cover replaced and weighed again. The petri dishes were subsequently placed on top of their covers in an oven heated beforehand and maintained at 70°C. Seeds were allowed to dry for a period of three days. After termination of the drying period, the mass of the dishes with contents and covers was determined and the moisture content of seeds calculated using the following formula (Roberts and Roberts, 1972) and expressed as a percentage.

$$\frac{(M2-M3)}{M2-M1} \quad X \quad 100$$

M1 is the weight in grams of the dish and its cover,

M2 is the initial weight of the dish, its cover and its contents in grams, and

M3 is the weight in grams of the dish, cover and contents after drying.

All determinations were performed in triplicate and the averages calculated.

3.6 Statistical analysis

All results were analyzed statistically using appropriate routines in agrobase-98. The means of pooled data of different cultivars within each species were used for comparison among the different storage treatments. Similarly, the means of pooled data of different storage treatments within each species were used for comparison among the cultivars. The LSD (Least Significant Difference) procedure was used for comparisons among the means (Mead and Curnow, 1983; Gomez and Gomez, 1984).

Chapter 4

Effects of storage conditions on viability and vigour of Ethiopian wheat and maize cultivar seeds

4.1 Introduction

With time, seeds may ultimately lose their viability and, although this deterioration is sometimes referred to as aging, it is not regarded as senescence as there is no evidence that it is a programmed developmental process (Guy and Black, 1998). Preceding the loss of viability, the effect of aging can be observed in the gradual loss of seed vigour evident as delayed germination, a lower respiration rate, increased amount of leachate upon imbibition as well as lower biomass yield (Argerich and Bradford, 1989; Steiner *et al.*, 1989; Marshall and Naylor, 1985; Pomeranz, 1992).

The loss of seed viability and vigour occurs at a faster rate under stress or unfavourable storage conditions (Roberts, 1972a; Francis and Coolbear, 1987; Bewley and Black, 1994). Relative humidity and temperature of the storage environment are the most important factors affecting seed quality during the storage period. Of these two factors, relative humidity is probably the most important because of its direct relationship with seed moisture content (Delouche *et al.*, 1973). Moreover, understanding the relationship between the storage environment (especially factors such as relative humidity, temperature and moisture content) and the genetic effects thereof, has become very important in determining the appropriate RH and temperature for the safe storage of a seedlot (Basu, 1995).

Ambient temperature and relative humidity in the tropics, including Ethiopia, are not optimal for the storage of seeds. Therefore, the control of an artificial storage environment is of great importance in order to maintain the viability and vigour of seeds during storage. To ultimately control the storage environment, data regarding the effect of ambient conditions on seeds are of particular importance (Delouche *et at.*, 1973).

This study was undertaken to determine the effect of ambient (more or less similar to that applying in Ethiopia) and lower temperature (4°C) storage conditions on the viability and vigour of Ethiopian wheat and maize cultivar seeds. Additionally, the effect of storage in open and closed containers at different temperatures was also investigated.

4.2 Materials and methods

See chapter 3, sections: 3.3.1, 3.4.1– 3.4.4

4.3 Results

4.3.1 Seed viability

The viability of seeds was evaluated on grounds of the ability of seeds to germinate according to Thomson (1979) and Hampton (1995). As illustrated in Figure 4.1, seeds of HAR-1685 and HAR-604 (both bread wheat cultivars)

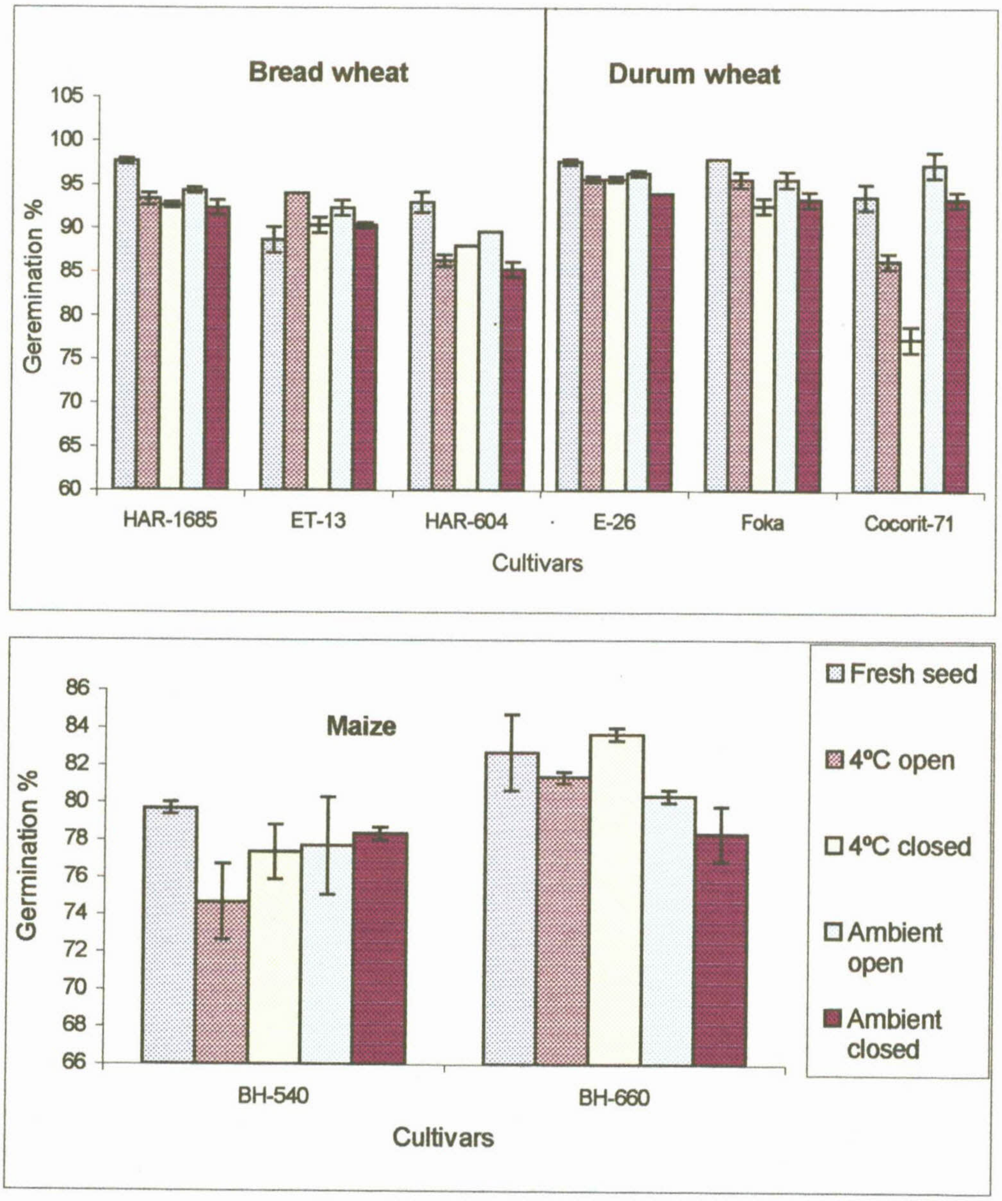

Figure 4.1: Effect of different storage conditions on viability (germination percentage) of different Ethipian wheat and maize cultivar seeds after seven days of incubation.

showed reduced germinability after aging under different storage conditions for 6 months. But, this was not the case for the other bread wheat cultivar (ET-13) where aging under different conditions actually improved seed germinability to different degrees in all cases.

As shown in Table 4.1, the statistical analysis of the mean germination percentages for the different storage treatments (pooled data of the three bread wheat cultivars; see section 3.6), except for those seeds aged in open containers at ambient temperature, showed a significant decline in viability compared to the control seeds (fresh seeds). Seeds aged in closed containers at both 4°C and ambient temperature showed the fastest decline in mean germination percentage (viability) compared to the mean for control seeds and other storage treatments. The mean germination percentage of seeds of different cultivars of bread wheat (pooled data of the different storage treatments within bread species; see section 3.6) also showed significant variation among the three bread wheat cultivars (Table 4.2). Accordingly, HAR-1685 showed the highest viability followed by ET-13 and HAR-604.

The germinability of E-26 and Foka seeds (both durum wheat cultivars) slightly declined under the influence of all the different aging conditions (Figure 4.1). But, this was not the case for seeds of the third durum wheat cultivar (Cocorit-71). Storage in an open container at ambient temperature slightly improved the germinability of these seeds while storage at 4°C in both open and closed containers markedly decreased seed viability in this cultivar. Storage of seeds of Cocorit-71 at ambient temperature in closed containers, on the other hand, did not show a marked effect on the viability.

Table 4.1: Statistical analysis of the effect of different storage conditions on the mean seed viability (germination %) of different Ethiopian wheat and maize cultivars. Seven day old seedlings were used for determination of germination percentage. Data from different cultivars within a species were pooled and means for each storage treatment were calculated separately.

	*Germination percentage (%)		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (before aging)	93.00 a[δ]	96.00 a	81.00 a
4°C in open container	91.00 bc	93.00 b	78.00 a
4°C in closed container	90.00 cd	89.00 c	81.00 a
Ambient in open container	92.00 ab	96.00 a	79.00 a
Ambient in closed container	89.00 d	94.00 b	78.00 a
LSD ($P < 0.05$)	1.27	1.0	

*The averages of germination percentage values were rounded off to the nearest whole number (0.5 was taken to the higher figure) according to the rule of ISTA (1985).

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

Table 4.2: Statistical analysis of cultivar variation in terms of viability (germination percentage) in seeds of different Ethiopian wheat and maize cultivars. Seven day old seedlings were used for determination of germination percentage. Data from different storage treatments were pooled and means for each cultivar (within a species) were calculated separately.

Species and cultivars	[*]Germination percentage (%)
Triticum aestivum (Bread wheat)	Averages
HAR-1685	94.00 a[δ]
ET-13	91.00 b
HAR-604	88.00 c
LSD ($P < 0.05$)	0.98
Triticum durum (Durum wheat)	
E-26	96.00 a
Foka	95.00 b
Cocorit-71	90.00 c
LSD ($P < 0.05$)	0.83
Zea mays (Maize)	
BH-540	78.00 b
BH-660	81.00 a
LSD ($P < 0.05$)	1.53

[*] The averages of germination percentage values were rounded off to the nearest whole number (0.5 was taken to the higher figure) according to the rule of ISTA (1985).

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

The mean germination percentages for most of the different storage treatments (pooled data of the three durum wheat cultivars; see section 3.6) were significantly lower than the mean for the seeds of the control except for those seeds aged in open containers at ambient temperature (Table 4.1). The germinability of the latter treated seeds was similar to that of control seeds. The mean germination percentage of pooled data for seeds from the three durum wheat cultivars also showed significant variation among the cultivars (Table 4.2) where E-26 showed the highest germinability followed by Foka and Cocorit-71.

The germinability of seeds from the two maize cultivars also decreased under the influence of most of the aging conditions, compared to the respective control seeds, except for BH-660 seeds aged in a closed container at 4°C (Figure 4.1) which exhibited a slight improvement in viability. Statistically, the mean germination percentages of maize seeds aged under different storage conditions (pooled data of the two maize cultivars) did not significantly differ from the mean viability of the control seeds. However, significant variations in seed viability between the two cultivars was apparent (Table 4.2) indicating the mean viability of seeds of BH-660 to be slightly higher than that of BH-540.

4.3.2 Seed vigour

4.3.2.1 Germination index

The germination index gives an account of the rate of seed germination (see section 3.4.1). The rate of germination of all the three bread wheat cultivars

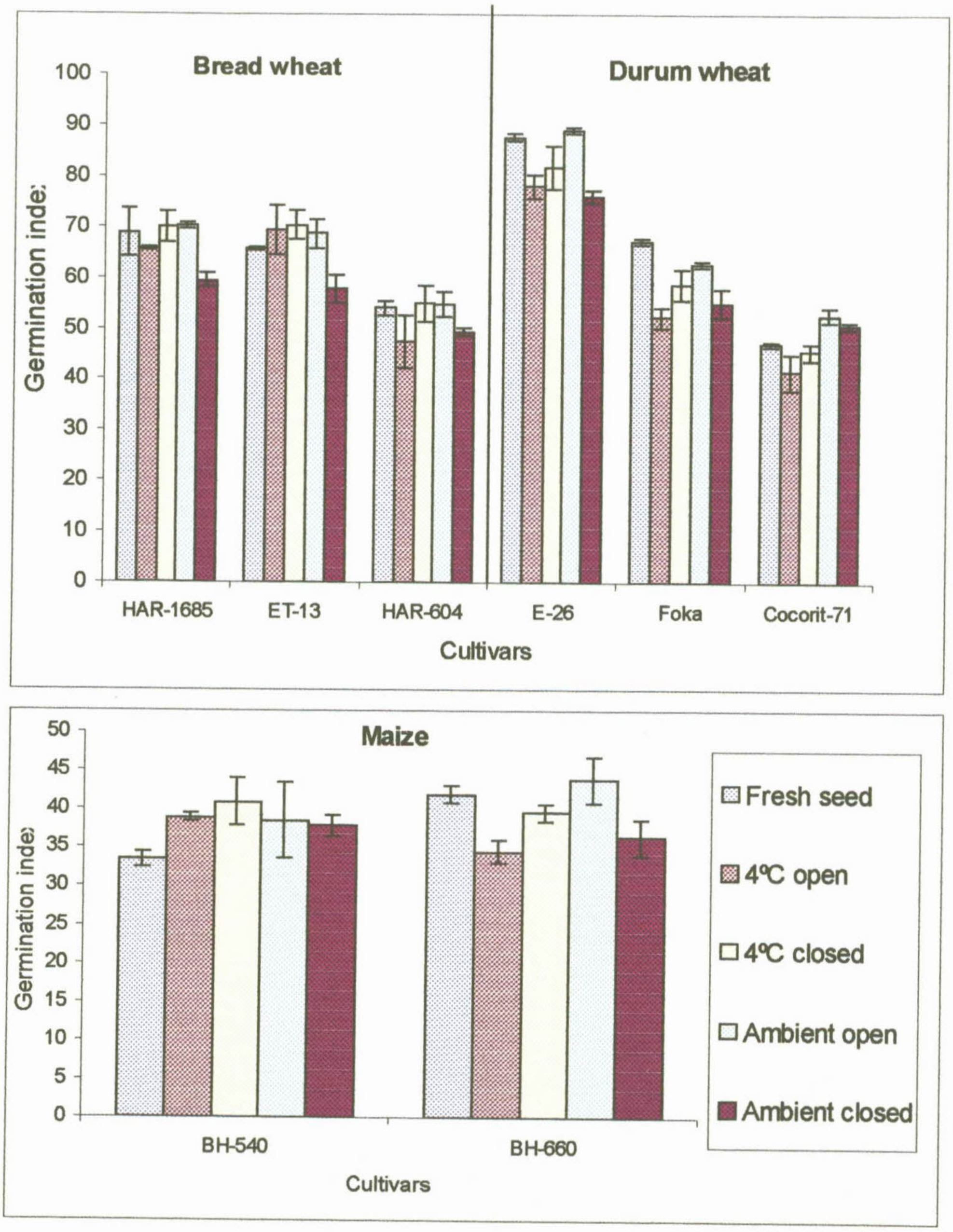

Figure 4.2: Effect of different storage conditions on germination index of different Ethiopian wheat and maize cultivars. The germinated seeds were counted at 24 h intervals until the fourth day of incubation when the index was calculated.

Was not affected or improved slightly when they were aged in closed containers at 4°C or in open containers under ambient conditions (Figure 4.2). The other two storage treatments (open container at 4°C and closed container under ambient conditions), however, caused a slight reduction in the germination indexes of seeds of HAR-1685 and HAR-604. This was not the case for the other bread wheat cultivar (ET-13) where storage in an open container at 4°C rather improved the rate of germination while storage in a closed container under ambient conditions caused a significant reduction in the germination rate of seeds.

The statistical analysis for mean germination indexes of most of the storage treatments (pooled data of the three bread wheat cultivars) did not show significant difference compared to the mean of control seeds except for seeds aged in closed containers under ambient conditions (Table 4.3). These seeds showed a significant reduction in the mean germination index compared to the mean for control seeds. The mean germination indexes of the three bread wheat cultivars also showed significant variations among the cultivars (Table 4.4) where HAR-1685 and ET-13 showed similar mean germination indexes that was significantly higher than the mean for seeds of HAR-604.

Seeds of the two durum cultivars (E-26 and Cocorit-71) showed an increase in the germination index when aged in open containers under ambient conditions (Figure 4.2). Unlike E-26 and Foka, seeds of Cocorit-71 also revealed a significant improvement in the rate of germination when seeds were stored in a closed container under ambient conditions. In contrast, all the different storage conditions reduced the rate of germination of seeds of Foka and a reduced rate was also observed for seeds of E-26 and Cocorit-71 aged at low temperatures in both open and closed containers.

Table 4.3: Statistical analysis of the effect of different storage conditions on the mean germination index of Ethiopian wheat and maize cultivar seeds. The germinated seeds were counted at 24 h intervals until the fourth day of incubation when the germination index was calculated. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Germination index		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (before aging)	62.80 ab[δ]	67.29 a	37.67 a
4°C in open container	60.84 b	57.24 c	36.73 a
4°C in closed container	65.07 a	61.96 b	40.27 a
Ambient in open Container	64.49 ab	68.13 a	41.20 a
Ambient in closed Container	55.51 c	60.58 b	37.13 a
LSD ($P < 0.05$)	4.04	2.81	

[δ]Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

Statistically, the mean germination index for most of the storage treatments under which seeds of durum wheat cutivars were stored showed significantly lower germination indexes compared to control seeds, except for seeds aged in an open container at ambient temperature (Table 4.3). These seeds maintained their rate of germination compared to the control seeds. There also occurred significant variation among the durum wheat cultivars where E-26 showed the highest value followed by Foka and Cocoirt-71 (Table 4.4).

Table 4.4: Statistical analysis of cultivar variation in terms of the germination index in seeds of different Ethiopian wheat and maize cultivars. The germinated seeds were counted at 24 h intervals until the fourth day of incubation when the germination index was calculated. Data from different storage treatments were pooled and means for each cultivar (within a species) were calculated separately.

Species and cultivars	Germination index
Triticum aestivum	
(Bread wheat)	Averages
HAR-1685	66.80 a[δ]
ET-13	66.35 a
HAR-604	52.08 b
LSD ($P < 0.05$)	3.13
Triticum durum	
(Durum wheat)	
E-26	82.51 a
Foka	59.12 b
Cocorit-71	47.49 c
LSD ($P < 0.05$)	2.18
Zea mays	
(Maize)	
BH-540	37.95 a
BH-660	39.25 a
LSD ($P < 0.05$)	

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

The germination index of the maize cultivar, BH-540, increased slightly under the influence of all of the aging conditions under which the seeds were stored. The opposite was, however, true for the other maize cultivar (BH-660) where most of the aging conditions caused a slight reduction in the rate of germination, except for seeds aged in an open container under ambient conditions. The latter seeds showed considerable improvement in the germination index. The statistical analysis of the mean germination index of different storage treatments, on the other hand, did not reveal significant variation among the different storage treatments under which maize seeds were aged (Table 4.3). A similar tendency was also observed for the mean germination index of the two cultivars (pooled data of different storage treatments; Table 4.4) where no significant variation between the two maize cultivars was observed.

4.3.2.2 Dry shoot mass

The dry shoot mass of seedlings obtained from aged seeds of all the three bread wheat cultivars was reduced under the influence of most of the storage treatments (Figure 4.3). However, the decline in dry shoot mass of seedlings of HAR-1685 aged in closed containers at 4°C and under ambient conditions, as well as in open containers at ambient temperature, was less pronounced than in open containers at 4°C. Almost similar results were obtained for HAR-604 but for ET-13 the dry shoot mass of seedlings did not differ much from that of seedlings obtained from control seeds, albeit markedly lower than compared to the other two cultivars.

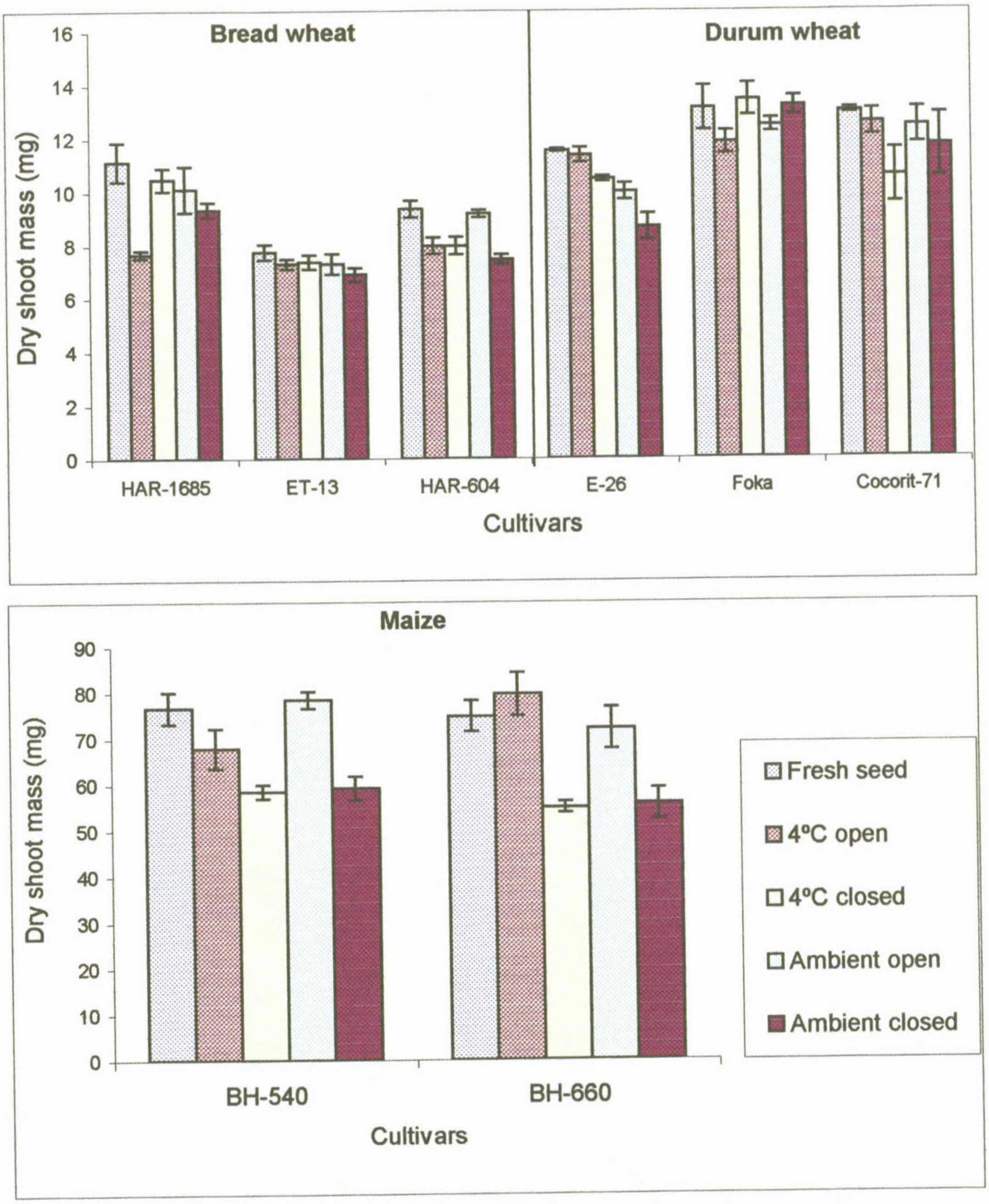

Figure 4.3: Effect of different storage conditions on dry shoot mass of seedlings obtained from seeds of various Ethiopian wheat and maize cultivars. Fourteen day old seedlings were used to compare dry mass accumulation.

Table 4.5: Statistical analysis of the effect of different storage conditions on the mean dry shoot mass accumulation of seedlings obtained from seeds of different Ethiopian wheat and maize cultivars. Fourteen (14) day old seedlings were used to compare mass accumulation. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Dry shoot mass (mg)		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (before aging)	9.41 a$^{\delta}$	12.55 a	75.80 a
4°C in open container	7.64 c	11.92 a	73.68 a
4°C in closed container	8.60 b	11.49 a	56.75 b
Ambient in open container	8.86 ab	11.63 a	75.35 a
Ambient in closed container	7.90 c	11.19 a	57.53 b
LSD ($P < 0.05$)	0.63		6.7

$^{\delta}$Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

Statistically the mean dry shoot mass of seedlings obtained from seeds stored under most of the aging conditions (pooled data of the three bread wheat cultivars) were reduced significantly, compared to the mean for control seeds, except for seeds aged in an open container under ambient conditions (Table 4.5). The mean dry shoot mass of seedlings obtained from bread wheat cultivar seeds (pooled data of the different storage treatments) also exhibited statistically significant variation among the three bread wheat cultivars (Table 4.6) where HAR-1685 had the highest dry shoot mass followed by HAR-604 and ET-13.

Seedlings of the two durum wheat cultivars, E-26 and Cocorit-71, obtained from seeds aged under all of the different storage conditions, showed a decrease in dry shoot mass compared to the respective controls (Figure 4.3). Similar losses were observed in seedlings of Foka aged in open containers at both 4°C and under ambient conditions. However, the dry shoot mass of seedlings of Foka obtained from seeds aged in closed containers at both 4°C and at ambient temperature was more or less similar to that of the control.

Statistically the mean dry shoot mass of seedlings obtained from differently aged seeds of durum wheat (pooled data of the three durum wheat cultivars) did not show significant variation among the different storage treatments (Table 4.5). The mean dry shoot mass of the durum wheat cultivars (pooled data of different storage treatments), on the other hand, revealed statistically significant variation (Table 4.6) between the cultivars where Foka showed the highest value followed by Cocorit-71 and E-26.

As shown in Figure 4.3, seeds of the two maize cultivars aged in open containers under ambient conditions produced seedlings with more or less similar dry shoot mass compared to the respective controls. The closed container at both 4°C and ambient temperature, on the other hand, caused a considerable loss of seedling dry shoot mass in both maize cultivars. BH-660 seeds aged in an open container at 4°C gained slightly higher dry shoot mass while seedlings of BH-540 showed a slight loss in dry mass compared to the control.

Table 4.6: Statistical analysis of cultivar variation in terms of dry shoot mass accumulation of seedlings obtained from seeds of different Ethiopian wheat and maize cultivars. Fourteen (14) day old seedlings were used to compare dry shoot mass accumulation. Data from different storage treatments were pooled and means for each cultivar (within a species) was calculated.

Species and cultivars	Dry soot mass (mg)
Triticum aestivum (Bread wheat)	Averages
HAR-1685	9.74 a[δ]
ET-13	7.32 c
HAR-604	8.38 b
LSD (P < 0.05)	0.48
Triticum durum (Durum wheat)	
E-26	10.41 c
Foka	12.81 a
Cocorit-71	12.05 b
LSD (P < 0.05)	0.73
Zea mays (Maize)	
BH-540	68.19 a
BH-660	67.50 a
LSD (P < 0.05)	

[δ] Averages followed by the same letter were not significantly different (P < 0.05), according to LSD (Least Significance Difference) procedure.

Statistical analysis for the mean dry shoot mass of seedlings obtained from differently aged seeds of maize cultivars (pooled data of the two maize cultivars), revealed that aging of maize seeds in open containers at both 4°C and ambient temperature had no significant effect. However, aging seeds in closed containers at both 4°C and ambient temperature caused a significant loss (Table 4.5). The mean dry shoot mass of seedlings of two maize cultivar seeds (pooled data of different storage treatments), however, did not show a significant difference between the two maize cultivars (Table 4.6).

4.3.2.3 Electrical conductivity

Among bread wheat cultivars, the electrical conductivity resulting from imbibed seeds of HAR-685 showed a remarkable increase under the influence of all the different storage conditions whereas the differently aged seeds of ET-13 showed a considerable decline in electrical conductivity compared to the respective controls (Figure 4.4). The conductivity related to HAR-604 seeds aged at ambient temperature in both open and closed containers was similar to that of the control while the cold (4°C) storage in both open and closed containers caused a decline in the amount of leachate from seeds.

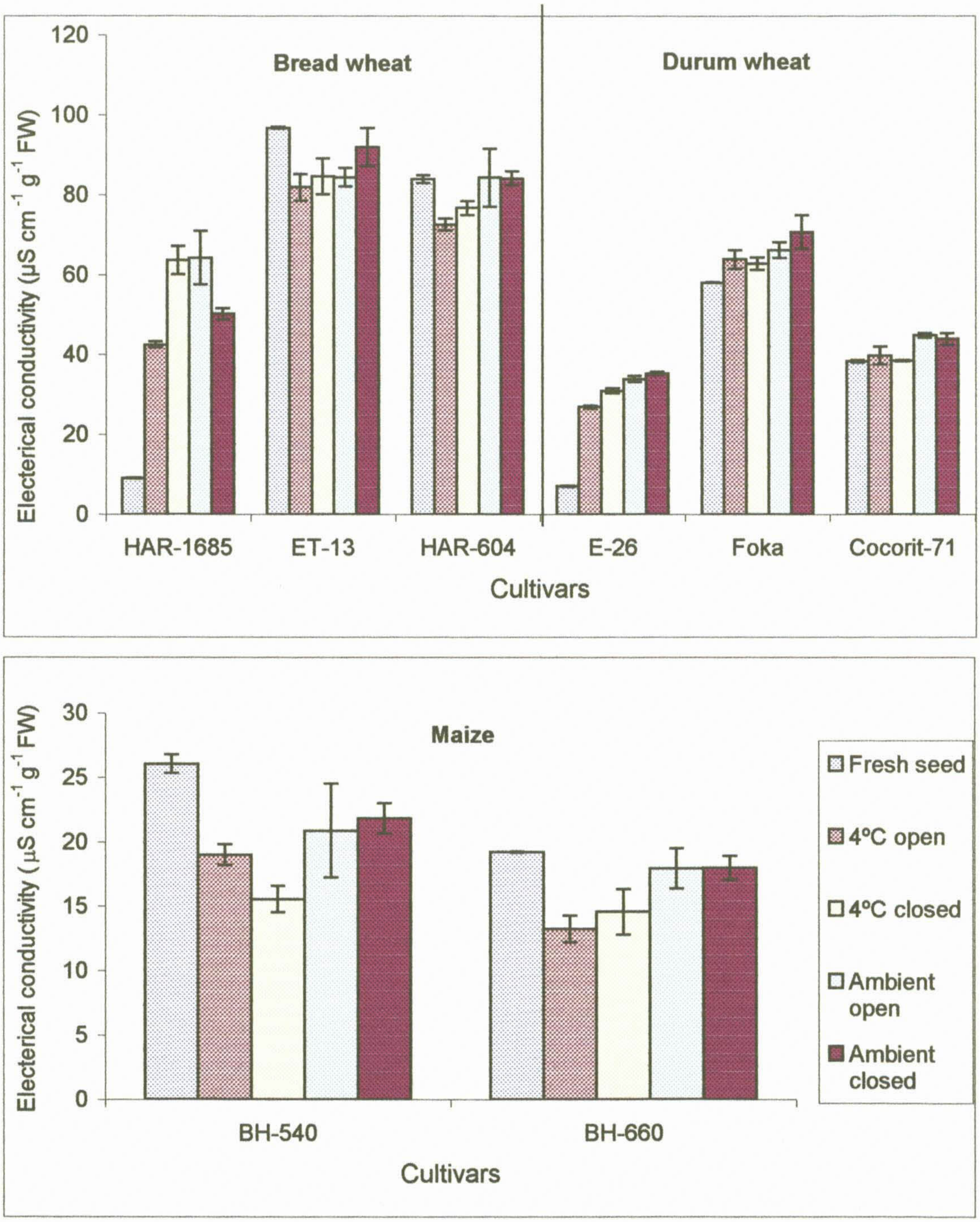

Figure 4.4: Effect of different storage conditions on electrical conductivity of different Ethiopian wheat and maize cultivar seeds. Conductivity measurements were carried out after 24 h of imbibition.

Statistically the mean conductivity values of seeds stored under different storage conditions (pooled data of the three bread wheat cultivars) increased significantly compared to the control seeds except for those seeds aged in open containers at 4°C (Table 4.7). The electrical conductivity of seeds under the latter storage condition remained unchanged. As shown in Table 4.8, statistical analysis of separate mean conductivity for the three bread wheat cultivars (pooled data of all the storage treatments) also showed significant variation among the cultivars. Leaching was more accentuated in ET-13 followed by HAR-604 and HAR-1685.

The durum wheat cultivars, E-26 and Foka, exhibited an increase in conductivity after being subjected to all of the different aging conditions. However, in the case of Cocorit-71 seeds, only storage at ambient temperature in both open and closed containers (Figure 4.4) increased the conductivity slightly. In contrast to the other two durum wheat cultivars, the increase in conductivity was markedly higher in seeds of E-26 and this was true for all of the different storage conditions. As indicated by the statistical analysis, the mean conductivity values for all the different storage treatments (pooled data of the three durum wheat cultivars), were significantly higher than that of the mean value calculated for the control (Table 4.7). Moreover, seeds aged under ambient conditions in both open and closed containers showed significantly higher electrical conductivity compared to those aged at 4°C under the same condition.

Table 4.7: Statistical analysis of the effect of different storage conditions on the mean electrical conductivity of Ethiopian wheat and maize cultivar seeds. Conductivity was measured after 24 h of imbibition. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Electrical conductivity (μS cm^{-1} g^{-1} FW)		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (before aging)	63.25 b[δ]	34.43 c	22.63 a
4°C in open container	65.65 b	43.48 b	16.11 c
4°C in closed container	74.95 a	44.05 b	15.06 c
Ambient in open container	77.63 a	48.29 a	19.40 b
Ambient in closed container	75.42 a	49.97 a	19.90 b
LSD ($P < 0.05$)	4.84	2.20	2.70

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

The separate mean conductivity values for the three durum wheat cultivars (pooled data of all the different storage treatments) also revealed significant variation among the cultivars (Table 4.8). Foka showed the highest leachate followed by Cocorit-71. The electrolytes from imbibed seeds of E-26 was by far lower than the other two durum wheat cultivars.

Table 4.8: Statistical analysis of cultivar variation in terms of electrical conductivity in seeds of different Ethiopian wheat and maize cultivars. Conductivity was measured after 24 h of imbibition. Data from different storage treatments were pooled and means for each cultivar (within a species) were calculated separately.

Species and cultivars	Electrical conductivity ($\mu S\ cm^{-1}\ g^{-1}$ FW)
Triticum aestivum	
(Bread wheat)	Averages
HAR-1685	45.91 c[δ]
ET-13	87.92 a
HAR-604	80.31 b
LSD ($P < 0.05$)	3.65
Triticum durum	
(Durum wheat)	
E-26	26.79 c
Foka	64.27 a
Cocorit-71	41.07 b
LSD ($P < 0.05$)	1.70
Zea mays	
(Maize)	
BH-540	20.66 a
BH-660	16.58 b
LSD ($P < 0.05$)	1.71

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

In imbibed seeds of both maize cultivars, a slight decrease in electrical conductivity was observed under influence of all of the different storage conditions as compared to the respective controls (Figure 4.4). However, in both cases the decline was less marked in maize seeds aged under ambient conditions than those aged at 4°C in both closed and open containers.

Statistical analysis of the mean conductivity for the different storage treatments (pooled data of the two maize cultivars) revealed a significant decline in conductivity of maize seeds aged under different conditions, compared to the mean for the control (Table 4.7). The magnitude of decline was significantly higher in seeds aged at 4°C than those at ambient temperature. The variation in conductivity values between the two cultivars was also statistically significant (Table 4.8) where the mean conductivity value for the imbibed seeds of BH-540 was significantly higher than that of BH-660.

4.3.2.4 Respiration rate

As illustrated in Figure 4.5, the respiration rate of all three bread wheat cultivars aged under different storage conditions showed a slight variation over a 72 h incubation period. Seeds of ET-13 and HAR-604 aged in open containers at ambient temperature showed an elevated respiration rate, especially after 24 h of imbibition, compared to the respective controls and the other storage treatments. In the case of HAR-604 this was also the case for seeds stored at 4°C in open containers. However, seeds stored in closed containers at both 4°C and ambient temperature showed more or less the same respiration rates as that of the control. In the case of HAR-1685, storage in closed containers at both 4°C and ambient temperature tended to be much lower at 24 h imbibition than the control as well as seeds stored in open containers. But at 48 h and 72 h of imbibition, and in all instances, the respiration rate did not differ much from that of the control seeds.

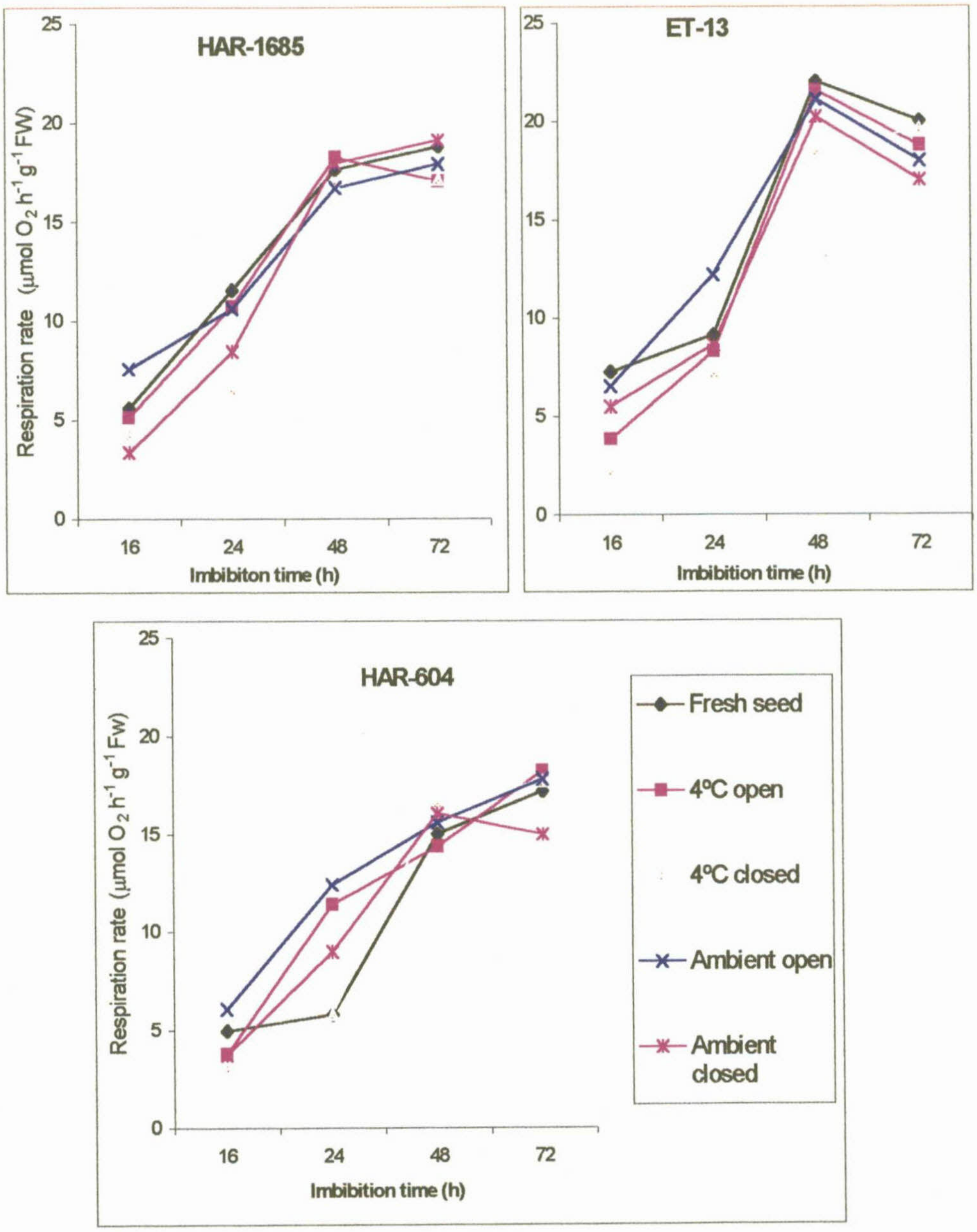

Figure 4.5: Effect of different storage conditions on the respiration rate of seeds from three Ethiopian bread wheat cultivars. Measurements were taken at 16, 24, 48 and 72 h of imbibition.

Statistically, the mean respiration rate at 16 h and 24 h of imbibition for the different storage treatments under which bread wheat seeds were aged (pooled data of the different cultivars within bread wheat species) showed significant differences (Table 4.9). In general, the mean respiration rate of bread wheat seeds aged in open containers at ambient temperature showed an elevated rate of oxygen uptake, especially at 24 h, compared to that of the mean for control seeds while all the other storage treatments tended to decrease the rate of respiration.

Unlike the earlier imbibition intervals (16 h and 24 h), the mean respiration rate of bread wheat seeds aged under all of the different storage conditions did not differ from that of the respective controls at 48 h and 72 h of imbibition (Table 4.9).

Statistical analysis undertaken to determine the cultivar variation in terms of respiration rate revealed no significant variation between the three cultivars during 16 - 24 hours of imbibition period (Table 4.10) even though separate data for the different cultivars showed differences (Figure 4.5). At 48 h of imbibition seeds of ET-13 consumed a higher volume of oxygen compared to the other two bread wheat cultivars (HAR-1685 and HAR-604) while at 72 h of imbibition both HAR-1685 and ET-13 showed higher respiration rates than HAR-604.

Table 4.9: Statistical analysis of the effect of different storage conditions on the mean respiration rate of seeds at 16, 24, 48 and 72 hours of imbibition of different Ethiopian wheat and maize cultivar seeds. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Respiration rate (µmol O_2 h^{-1} g^{-1} FW) at different imbibition intervals			
Storage conditions	At 16 hours	At 24 hours	At 48 hours	At 72 hours
Triticum aestivum (Bread wheat)	Averages	Averages	Averages	Averages
Control (fresh)	5.96 a[δ]	8.85 c	18.25 a	18.68 a
4°C open	4.27 b	10.17 b	18.08 a	18.02 a
4°C closed	3.20 c	6.55 d	17.35 a	18.17 a
Ambient open	6.74 a	11.75 a	17.81 a	17.92 a
Ambient closed	4.22 bc	8.72 c	18.08 a	17.05 a
LSD (P < 0.05)	1.07	0.98		
Triticum durum (Durum wheat)				
Control (fresh)	3.68 c	7.59 b	17.69 c	17.57 b
4°C open	3.67 c	7.28 b	15.83 d	16.28 c
4°C closed	3.36 c	7.91 b	16.43 cd	17.57 b
Ambient open	6.33 a	9.43 a	19.06 b	18.22 ab
Ambient closed	5.11 b	9.28 a	20.69 a	19.12 a
LSD (P < 0.05)	0.69	1.27	1.30	1.28
Zea mays (Maize)				
Control (fresh)	1.25 c	3.64 a	14.45 a	16.86 a
4°C open	1.28 c	3.39 a	8.73 b	12.70 bc
4°C closed	2.04 a	3.11 a	8.44 b	13.65 b
Ambient open	1.40 bc	3.99 a	7.24 b	10.21 c
Ambient closed	1.63 b	2.18 b	8.31 b	13.64 b
LSD (P < 0.05)	0.34	0.91	1.90	2.60

[δ] Averages followed by the same letter were not significantly different (P < 0.05), according to LSD (Least Significance Difference) procedure.

Table 4.10: Statistical analysis of cultivar variation in terms of respiration rates at 16, 24, 48 and 72 hours of imbibition in seeds of different Ethiopian wheat and maize cultivars. Data from different storage treatments within a species were pooled and means for each cultivar were calculated separately.

	Respiration rate (μmol O_2 h^{-1} g^{-1} FW) at different imbibition intervals			
Species and cultivars	At 16 hours	At 24 hours	At 48 hours	At 72 hours
Triticum aestivum (Bread wheat)	Averages	Averages	Averages	Averages
HAR-1685	5.20 a	9.59 a	17.50 b	18.02 a
ET-13	5.10 a	9.12 a	20.70 a	18.71 a
HAR-604	4.33 a	8.90 a	15.54 c	17.18 b
LSD (P < 0.05)			1.14	0.83
Triticum durum (Durum wheat)				
E-26	7.73 a	13.35 a	22.36 a	17.34 b
Foka	3.81 b	7.87 b	20.84 b	21.69 a
Cocorit-71	1.88 c	3.68 c	10.62 c	14.23 c
LSD (P < 0.05)	0.54	0.99	1.00	0.99
Zea mays (Maize)				
BH-540	1.72 a	3.52 a	9.62 a	13.80 a
BH-660	1.32 b	3.04 a	9.25 a	13.06 a
LSD (P < 0.05)	0.26			

[δ] Averages followed by the same letter were not significantly different (P < 0.05), according to LSD (Least Significance Difference) procedure.

The respiration rates of seeds of all the three durum wheat cultivars aged under different conditions showed noticeable variations at 16, 48 and 72 h of imbibition (Figure 4.6). The variations among the different storage treatments were more pronounced in the case of Cocorit-71 where seeds stored at ambient temperature in both open and closed containers tended to respire at an elevated rate over the 72 h incubation period compared to control seeds. A similar tendency was also observed in seeds of E-26 aged at ambient temperature. Storage at 4°C in open containers tended to decrease the respiratory capacity of seeds of Cocorit-71 over a 72 h incubation period while storage in closed containers had no effect (Figure 4.6). However, in the case of the other durum wheat cultivar (Foka) it seemed that none of the storage treatments had any effect on the respiratory capacity of the seeds.

Statistical analysis of the mean oxygen uptake of durum wheat seeds aged in open containers under ambient conditions (pooled data of the three durum wheat cultivars) confirmed significantly higher respiration rates than for storage at 4°C at all the imbibition intervals (Table 4.9). Especially between 48 and 72 h of imbibition, the mean respiration rate of durum wheat seeds aged in open containers at 4°C showed a statistically significant decline compared to the mean for the control and the other two storage treatments (Table 4.9).

The mean oxygen consumption of durum wheat seeds aged in closed containers at ambient temperature was also significantly higher than the mean for the control and the other storage treatments except for seeds aged in an open container under ambient conditions (Table 4.9). At 24 and 72 h of imbibition, seeds aged in a closed container at ambient temperature showed a similar

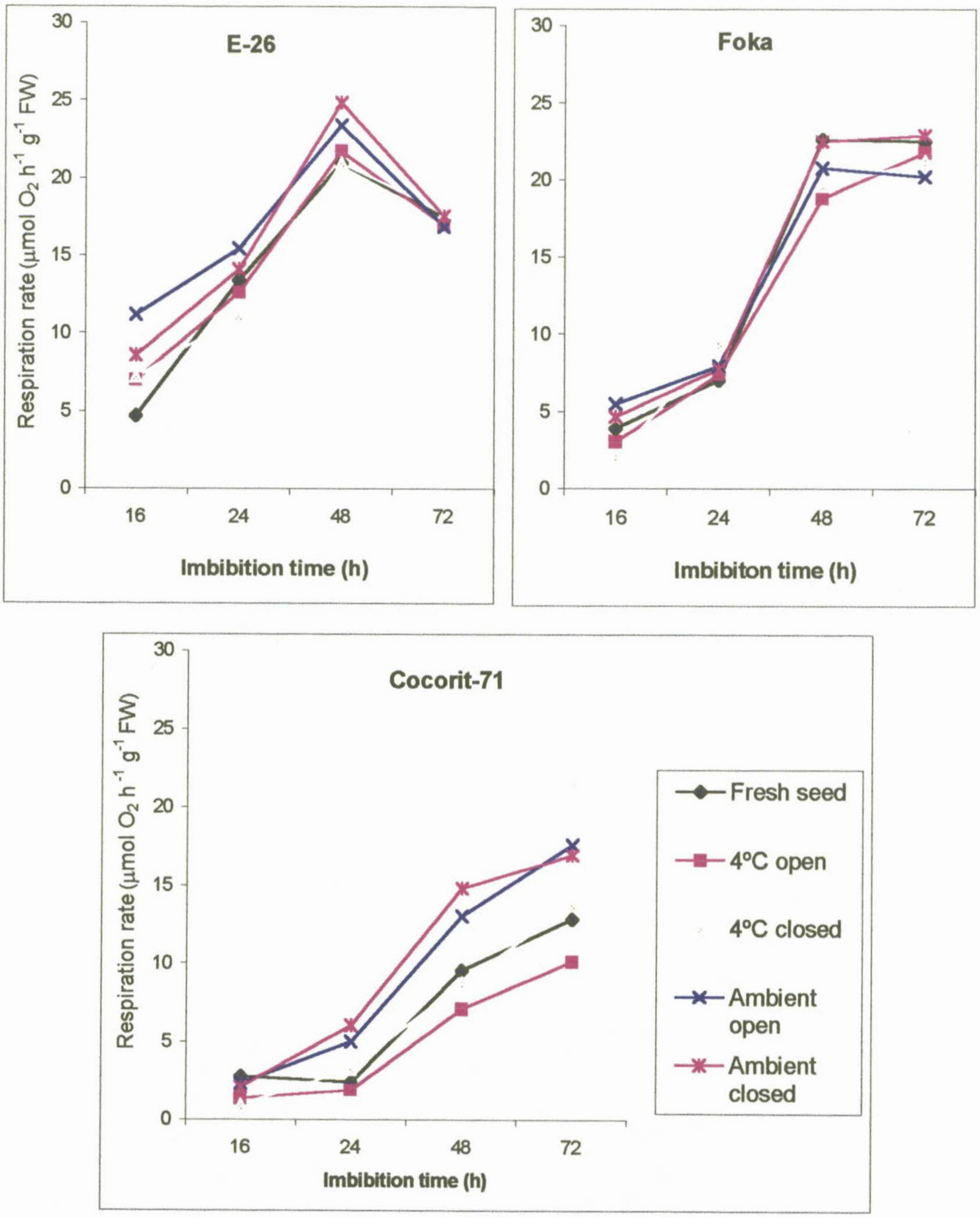

Figure 4.6: Effect of different storage conditions on the respiration rate of seeds from three Ethiopian durum wheat cultivars. Measurements were taken at 16, 24, 48 and 72 h of imbibiton.

respiration rate to that of seeds stored in an open container at ambient temperature, but varied significantly at 16 and 48 h of imbibition. Aging durum seeds in a closed container at 4°C, on the other hand, did not show a significant effect on the rate of respiration during 48 - 72 hours of imbibition (Table 4.9).

Statistical analysis for the durum wheat cultivar variations revealed that the mean respiration rate of E-26 (pooled data of different storage treatments) was significantly higher than that of both Foka and Cocorit-71, especially between 16 - 48 h of imbibition. Seeds of the latter two cultivars showed intermediate to low respiration rates, respectively (Table 4.10). However, at 72 h of imbibition the differences were less marked and the respiration rate of Foka was significantly higher than that of both E-26 and Cocorit-71.

The respiration rate of seeds of the two maize cultivars aged under all of the different conditions markedly decreased during the 72 h imbibition period compared to the control. In BH - 540 seeds stored in an open container at ambient temperature and at 4°C in a closed container the respiration rate decreased by > 50 % between 48 and 72 h of imbibition while the decline was less marked in open storage at 4°C and closed storage at ambient temperature (Figure 4.7). In seeds of BH-600 aged in an open container at ambient temperature the same tendency of decreased respiration as for BH-540 was observed (Figure 4.7). However, storage of seeds of this cultivar at 4°C in an open container had a similar inhibiting effect on the respiration rate as did open storage at ambient temperature while the effect of closed storage at 4°C and ambient temperature had a lesser effect, especially at 72 h of incubation (Figure 4.7).

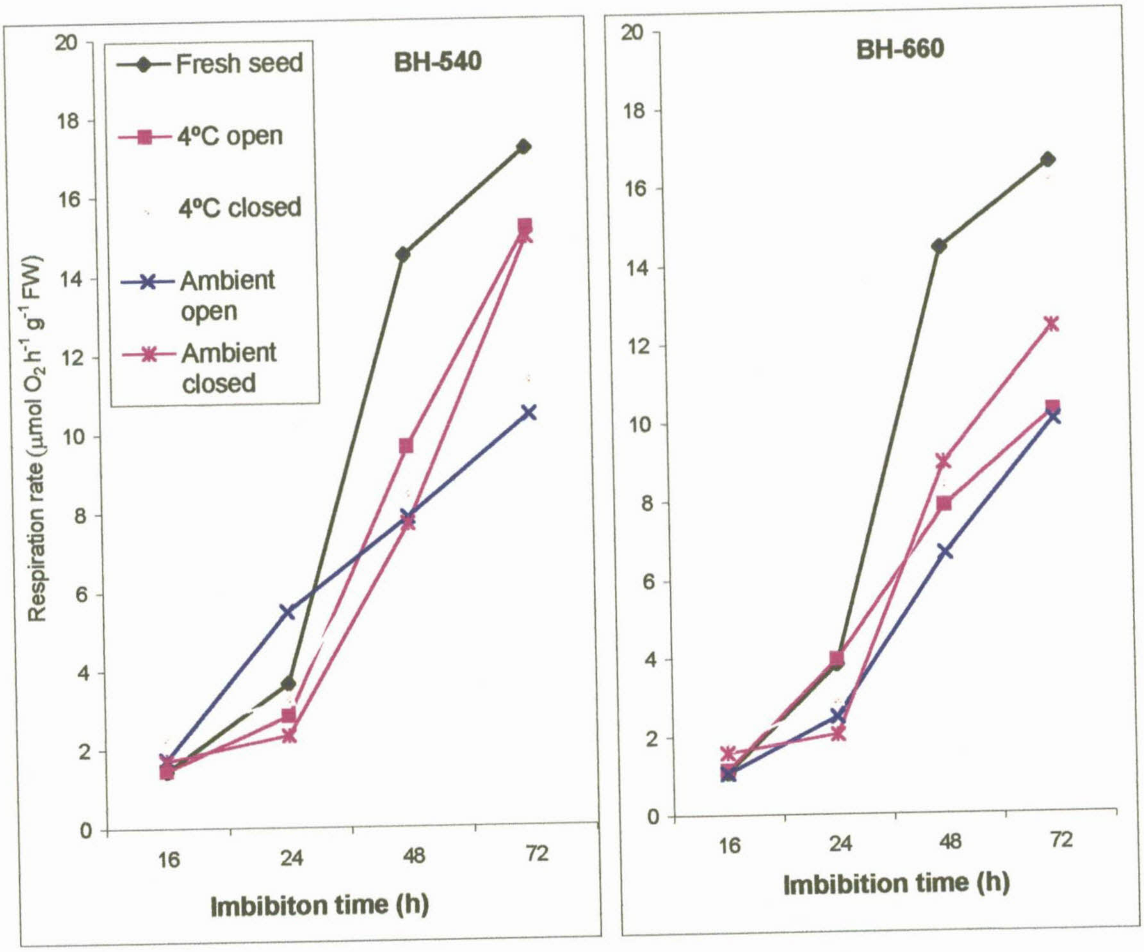

Figure 4.7: Effect of different storage conditions on the respiration rate of seeds from two Ethiopian maize cultivars. Measurements were taken at 16, 24, 48 and 72 h of imbibition.

Statistically the mean respiration rate for different storage treatments (pooled data of the two maize cultivars) also showed significant variation among the storage treatments especially at the 16 h of imbibition (Table 4.9). Moreover, the mean respiration rate of maize seeds aged in open containers at both 4°C and ambient temperature showed similar oxygen uptake as that of the mean for control seeds at 16 h of imbibition. In contrast, the mean oxygen uptake of maize seeds aged in closed containers at both 4°C and under ambient conditions was significantly higher than the mean for seeds of the control and the other storage treatments (Table 4.9).

At 24 h of imbibition, the mean oxygen uptake by maize seeds aged under most of the storage conditions did not differ significantly from the mean for the control seeds except for seeds aged in a closed container at ambient temperature (Table 4.9). The mean oxygen uptake of seeds aged in the latter storage treatment was significantly lower than that of the control and all of the storage treatments. At 48 h and 72 h of imbibition the respiration rates of all differently stored seeds was significantly lower than the respective controls but did not significantly differ from each other (Table 4.9). Only at early (16 h) imbibition the mean respiration rate of seeds from the two maize cultivars (pooled data of the different storage treatments) differed significantly from each other in that the seeds of BH-540 had a higher mean value than that of BH-660. However, during 24 - 72 hours of imbibition, there was no statistical significant variation between the two maize cultivars regarding their respiration rates (Table 4.10).

4. 4 Discussion

The decline in viability of seeds during storage is positively related to the relative humidity of the storage environment and seed moisture content during the storage period (Coolbear *et al.*, 1984; Pukacka, 1998). The genetic composition of the variety (Justice and Bass, 1979a; Thomson, 1979; Bewley and Black, 1994) is also an important factor determining seed viability during storage. There are many reports which show convincingly that significant differences in viability between genotypes within a species exist and that these differences are heritable (Roberts, 1972a; Justice and Bass, 1979a). This was confirmed for the different cultivars of both bread and durum wheat seeds in this study. The germination variability among these cultivars appeared to be related to greater resistance of some cultivars to seed viability loss before and after storage. Justice and Bass (1979a) also speculated that increased longevity of some cultivars might be due to the probability of genetic dominance of longevity.

In this study, the mean viability (germination percentage) of both bread and durum wheat cultivar seeds aged in open containers at ambient temperature was statistically similar to that of the mean for the respective controls (fresh seeds) indicating that this storage condition appeared to be suitable in maintaining the viability of seeds of different cultivars of wheat.

Seed deterioration after storage is normally manifested in the loss of seed vigour (Anderson, 1970; Dornbos, 1995; Guy and Black, 1998) which can be tested by means of several methods. Some of these commonly used methods include determining the rate of seed germination (germination index), the shoot dry mass, the leakage of organic and inorganic constituents upon imbibition and measurement of the respiration rate of imbibibed seeds (Marshall and Naylor, 1985; Argerich and Bradford, 1989; Pandey, 1989; Steiner *et al.*, 1989) which were all applied in this study.

Statistically, the calculated germination index (vigour) of both bread and durum wheat cultivar seeds aged in open containers at ambient temperature did not differ significantly from that of the mean for control seeds. A similar tendency was observed when separate data of wheat and maize cultivars aged in open containers at ambient temperature was considered. From this it can generally be argued that storage of both maize and wheat seeds at ambient temperature in open containers was favourable in maintaining the rate of germination (germination index) of seeds.

The mean germination index of bread and durum wheat cultivars also showed statistically significant variation. In line with the suggestion of Justice and Bass (1979a), the increased germination rate measured for seeds of some cultivars, such as HAR-1685 (bread wheat) and E-26 (durum wheat), is probably due to differences in their genetic constitution. Moreover, the seeds of these two wheat cultivars were on average larger than that of the other cultivars which might have accounted for the higher rate of germination (Basu, 1995).

The mean dry shoot mass of seedlings obtained from seeds of both bread wheat and maize cultivars aged in open containers at ambient temperature was statistically similar to the mean for control seeds. Separate data for different cultivars within a species showed a similar tendency where the bread wheat and the two maize cultivars maintained the dry shoot mass when aged in open containers at ambient temperature. This suggests that this storage treatment was again suitable for maintaining the dry shoot mass of seedlings from both bread wheat and maize cultivar seeds. Even though the dry shoot mass of seedlings of one of the three different durum wheat cultivars (E-26) was slightly reduced under the influence of most of the aging conditions, statistical analysis did not reveal any significant differences. Most of the other storage conditions, especially in the case of E-26 and Cocorit-71, had a reducing effect in terms of the dry shoot mass accumulation in durum wheat seedlings, but this was less pronounced in the case of Foka.

According to the analysis of variance of bread wheat cultivars, HAR-1685 had a higher dry shoot mass than the other two cultivars (ET-13 and HAR-604). The rapid germination rate (germination index) of HAR-1685 as well as the fact that its seeds are larger on average compared to the other two cultivars, might have attributed to the higher dry shoot mass. With regard to the relationship between seed size and dry shoot mass yield, Heydecker (1972) and Basu (1995) reported that the size of seedlings from small seeds are lower than those from larger seeds. However, the reverse was true in the case of the durum wheat cultivar E-26 where, despite of its fast early growth rate as well as larger seed size than Foka and Cocorit-71, it exhibited lower dry shoot mass. This anomaly is difficult to explain but might again be related to genetic variance between species.

As pointed out by Coolbear *et al.* (1984), Marshall and Naylor (1985), as well as Argerich and Bradford (1989), seed vigour loss can also be associated with increased leaching upon imbibition which is measured in terms of increased conductivity. The statistical analysis performed for the mean conductivity of both bread and durum wheat cultivar seeds stored under different storage conditions showed a significant increase in the amount of leachate under influence of most of the storage treatments, suggesting deterioration of seeds during the aging process. However, electrolytes that leached from both bread and durum wheat seeds aged at low temperatures in both closed and open containers were significantly lower than those aged at ambient temperature. The low temperature storage environment could have reduced the deterioration of membranes during the aging period which most probably was responsible for the amount of electrolytes that leaked out upon imbibition. This hypothesis is in agreement with the view of Dornbos (1995).

As opposed to wheat, seeds from both maize cultivars showed reduced conductivity under influence of all the different storage treatments suggesting either a decrease in the rate of membrane deterioration or a reduced rate of solute leakage from these seeds. What is important is the fact that maize seeds from both cultivars showed only very slight reduction in germination capacity and even a slight improvement in germination index (BH-540) which correlated with much lower solute leakage.

The statistical analysis for the mean conductivity of different cultivars of both wheat and maize species also demonstrated significant variation among the cultivars. The lower amount of leachate from imbibed seeds of HAR-1685 (bread wheat) and E-26 (durum wheat) as well as BH-660 (maize) might probably be related to their genetic potential in resisting membrane deterioration during storage. Kalpana and Madhava Rao (1997) speculated that some cultivars are able to withstand membrane deterioration during aging which accounts for the lower amount of electrolyte leakage upon imbibition.

Seed vigour was also evaluated on the basis of oxygen uptake of seeds during different imbibition intervals (Ram and Wiesner, 1988; Steiner *et al.*, 1989 ; Hampton, 1995). Statistical analysis as well as the separate data of both bread and durum wheat cultivars showed the oxygen uptake of aged seeds to be higher than that of the control (fresh) seeds, especially during the early hours (16 - 24) of imbibition. The oxygen uptake of control seeds did not seem to be related to their corresponding high vigour. This agrees with the view of Coolbear (1995), who stated that the mitochondria from aged (lower-vigour seedlot) seeds took up a similar amount of oxygen than that of control seeds, but produced less ATP than the control seeds.

It seems therefore, as suggested by Coolbear (1995), that the measurement of respiration rate alone might not be sufficient to distinguish between the vigour of non aged (fresh) and aged seeds of wheat cultivars. Together with the measurement of respiration rate, determination of ATP production might probably be necessary to distinguish between the vigour of fresh and aged seeds. As opposed to the wheat cultivars, the respiration rate of maize seeds aged under different storage treatments was lower than that of the control seeds, especially during 48 - 72 h of imbibition. However, due to the statistically non significant differences between germination capacities and germination indexes of control and differently aged seeds, this difference in respiration rate did not seem to clearly distinguish between the different storage treatments.

Statistically, the mean oxygen uptake of bread wheat cultivars also showed significant variation with regard to the consumption of oxygen. However, none of the three bread wheat cultivars appeared to show consistently higher respiration rates over the 72 h imbibition period. A similar trend was also observed in the case of the two maize cultivars where the mean oxygen uptake did not show significant variation during the 24 - 72 hours imbibition period. This suggests that the rate of respiration might not be used as a sole criterion in differentiating between low and high vigour of seed from cultivars of both bread wheat and maize species. The opposite tendency was observed in the case of durum wheat cultivars where E-26 showed elevated oxygen consumption compared to Foka and Cocorit-71 over the 48 h imbibition period. E-26 was also the cultivar which showed the highest germination capacity, germination index and lowest electrical conductivity (high vigour). The higher viability and vigour of this cultivar might be also related to its higher rate of respiration.

A number of workers (Ching and Schoolcraft, 1968; Anderson, 1970; Kalpana and Madhava Rao, 1997) assessed the relationship between seed deterioration (loss of viability and vigour) and changes in the levels of metabolites (protein and carbohydrate) in seeds during storage. In this study, seed vigour tests, such as electrical conductivity and respiration rate, did not seem to clearly distinguish between the vigour of control (fresh) and aged seeds, suggesting the importance of these biochemical tests to generate additional information. Moreover, the moisture content change during the six month storage period appeared to be related to the changes in viability and vigour of seeds aged under different conditions. Thus, changes in the levels of protein, carbohydrates and moisture contents during storage needed to be addressed and is discussed in the next chapter (5).

Chapter 5

Effects of storage conditions on physiological and biochemical aspects related to seed viability and vigour in Ethiopian wheat and maize cultivars

5.1 Introduction

As the major portions of most seeds are food reserves, it was natural for earlier researchers to postulate metabolic depletion of reserves as a cause of non-viability and reduced vigour (Basu, 1995). Many physiological and biochemical changes occur during seed aging. Such changes take place at a faster rate under unfavourable storage conditions (increased temperature and relative humidity) and can generally be observed as: increased degradation of enzymatic and structural proteins (Kalpana and Madhava Rao, 1997), increase in total activity of certain enzymes, such as amylase, resulting in depletion of starch and followed by increased levels of reducing sugars (Burton, 1982). Changes in the moisture content of seeds during storage is also an important factor determining the viability and vigour of seeds during storage (Roberts, 1972a; Bewley and Black, 1994; Ellis *et al.*, 1991).

However, little information is available on physiological and biochemical changes in seeds from Ethiopian wheat and maize cultivars. Proper storage conditions are questionable in Ethiopia and are also out of reach of many farmers and seed merchants. Moreover, understanding of the physiological and biochemical differences between various cultivars of the same species is of great importance in order to provide an information base for understanding the deterioration mechanisms and for selecting cultivars with highest storage potential (Coolbear, 1995). Such knowledge is lacking in Ethiopia.

This chapter deals with changes in total water soluble proteins, carbohydrate metabolites and moisture contents of wheat and maize seeds from different Ethiopian wheat and maize cultivars during aging at low temperature (4°C) as well as under ambient conditions (more or less similar to that applying in Ethiopia) where freshly harvested seeds were taken as a control. Additionally, the effects of storage in open and closed containers at different temperatures were also investigated.

5.2 Materials and methods

See chapter 3, sections 3.6.1 - 3.6.4

5.3 Results

5.3.1 Total water soluble protein content

The total water soluble protein content in seeds of all three bread wheat cultivars decreased slightly under the influence of most of the aging treatments, except for HAR-604 seeds aged in an open container at ambient temperature (Figure 5.1). Moreover, this decrease was accentuated in HAR-1685 and ET-13 seeds aged in open containers at 4°C while aging in closed containers at ambient temperature caused the severest decrease in HAR-604 seeds.

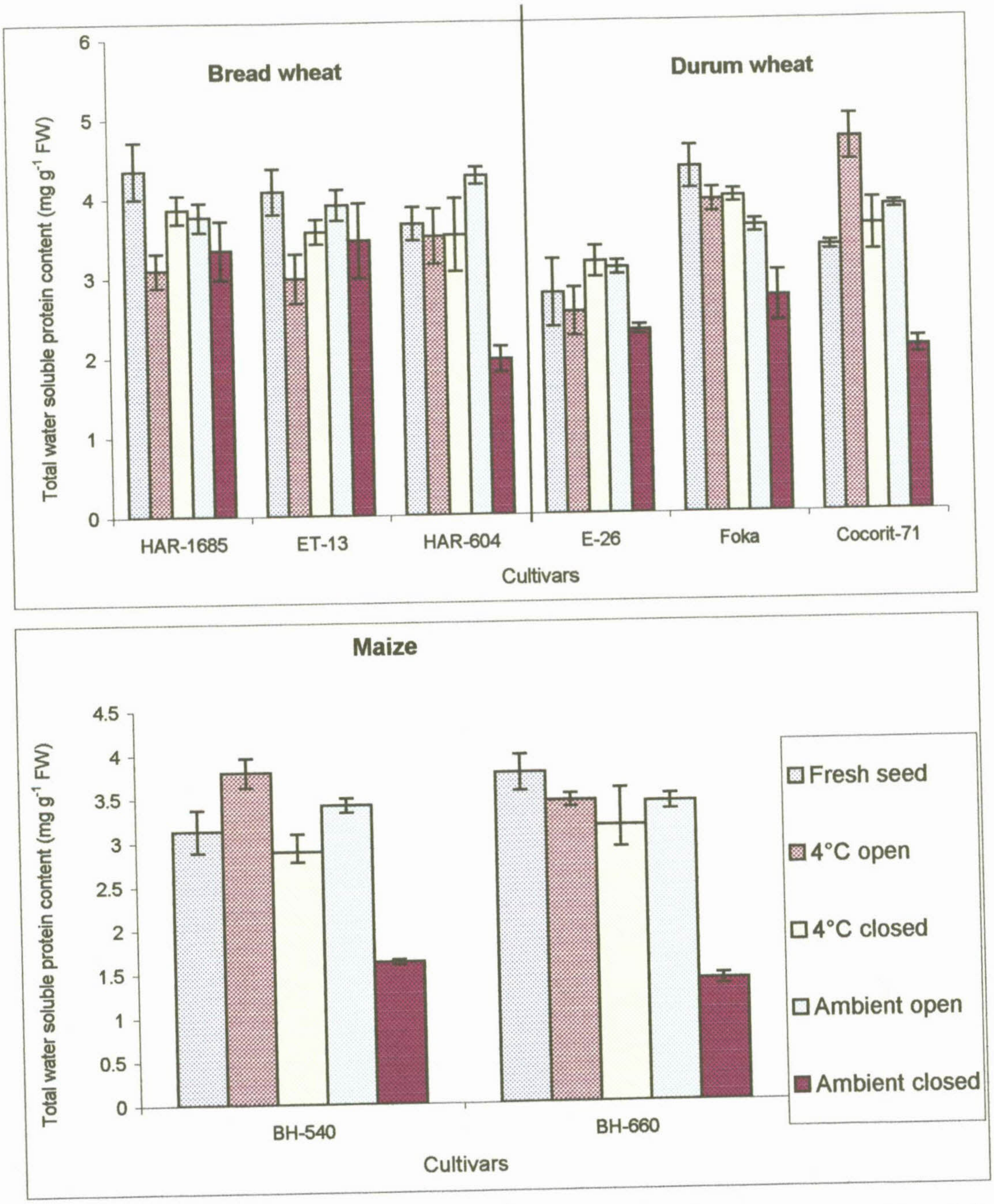

Figure 5.1: Effect of different storage conditions on the total water soluble protein content in seeds of different Ethiopian wheat and maize cultivars.

The analysis of variance of pooled data for the three bread wheat cultivars revealed that the mean total water soluble protein levels of seeds aged in open containers at 4°C, as well as those in closed containers under ambient conditions, reduced significantly compared to the mean for control seeds as well as for other storage treatments (Table 5.1). Storage in closed containers at 4°C as well as in open containers at ambient temperature, on the other hand, tended to maintain the level of protein content of bread wheat seeds. However, statistical analysis (Table 5.2) of the mean water soluble protein content in seeds of three bread wheat cultivars (pooled data of different storage treatments) did not reveal significant variation among the three cultivars.

Among the durum wheat cultivars, the total water soluble protein content of Foka seeds showed a marked decrease under the influence of all the different storage treatments (Figure 5.1). A similar trend was also observed in seeds of E-26 aged in open containers at 4°C as well as in closed containers under ambient conditions. Storage of seeds of both Foka and Cocorit-71 in closed containers under ambient conditions also tended to reduce the level of protein severely. In contrast, seeds of E-26 and Cocorit-71 aged in closed containers at 4°C as well as in open containers under ambient conditions showed a marked increase in the level of the total water soluble protein. Unlike the other two durum wheat cultivars, the total water soluble protein level of seeds of Cocorit-71 aged in open containers at 4°C showed a marked increase.

Statistically, the mean for the total water soluble protein level of most of the storage treatments (pooled data of the three durum wheat cultivars) did not significantly differ from the mean of the control seeds (Table 5.1) except for seeds aged in closed containers under ambient conditions. The latter seeds showed a significant decrease compared to the mean for control seeds.

Statistical analysis of pooled data of the different storage treatments revealed no difference in water soluble protein content between the cultivars Foka and Cocorit-71 (Table 5.2). However, E-26 differed significantly from these two in this regard.

Table 5.1: Statistical analysis of the effect of different storage conditions on the mean total water soluble protein content of different Ethiopian wheat and maize cultivar seeds. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Protein content (mg g^{-1} FW)		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (fresh seeds)	4.03 a[δ]	3.49 a	3.45 a
4°C in open container	3.19 b	3.72 a	3.62 a
4°C in closed container	3.65 a	3.58 a	3.02 b
Ambient in open container	3.98 a	3.50 a	3.42 a
Ambient in closed container	2.92 b	2.36 b	1.50 c
LSD ($P < 0.05$)	0.40	0.30	0.26

[δ]Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

The total water soluble protein content in seeds of the maize cultivar BH-660, decreased under the influence of all the different storage conditions (Figure 5.1), compared to the control. Similar decreases were also observed in seeds of BH-540 aged in closed containers at both ambient and low temperature. This trend was accentuated in seeds of both cultivars aged in closed containers at ambient temperature. In contrast, the total water soluble protein content of BH-540 seeds aged in open containers at both low (4°C) as well as ambient temperature, showed a slight increase.

Table 5.2: Statistical analysis of cultivar variation in terms of total water soluble protein content in seeds of different Ethiopian wheat and maize cultivars. Data from different storage treatments were pooled and means for each cultivar (within a species) were calculated separately.

Species and cultivars	Total water soluble protein content (mg g^{-1} FW)
Triticum aestivum (Bread wheat)	Averages
HAR-1685	3.68 a
ET-13	3.60 a
HAR-604	3.38 a
LSD ($P < 0.05$)	
Triticum durum (Durum wheat)	
E-26	2.77 b
Foka	3.71 a
Cocorit-71	3.50 a
LSD ($P < 0.05$)	0.23
Zea mays (Maize)	
BH-540	2.97 a
BH-660	3.04 a
LSD ($P < 0.05$)	

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

Statistically, analysis of the mean total water soluble protein content of maize seeds aged in closed containers at both low (4°C) and ambient temperature revealed significant decrease compared to the mean for both the control and treatments in open containers, indicating significant treatment variation. Seeds aged in open containers at both 4°C and ambient temperature, on the other hand, did not show significant variation compared to the mean for the control seeds (Table 5.1). No significant variation between cultivars in terms of the mean total water soluble protein content in seeds of the two maize cultivars was apparent (Table 5.2).

5.3.2 Glucose content

The glucose content in seeds from all bread wheat cultivars, aged under all of the different storage conditions increased compared to the respective controls (Figure 5.2) except HAR-1685 stored in open containers at 4°C. This increase was between five and twentyfold in seeds of all the three cultivars aged in open containers at 4°C compared to the respective controls as well as most of the other storage treatments. The significance of these differences was also confirmed by analyzing the pooled data for the three bread wheat cultivars (Table 5.3) statistically. Variation among bread wheat cultivars for the level of glucose in seeds was also significant (Table 5.4). Accordingly, the mean glucose level of HAR-604 was significantly higher than that of both HAR-1685 and ET-13 but the latter two cultivars did not show significant variation.

As was the case in bread wheat cultivars, the glucose level in seeds of all the three durum wheat cultivars increased under the influence of most of the aging conditions (Figure 5.2). However, different storage conditions had different

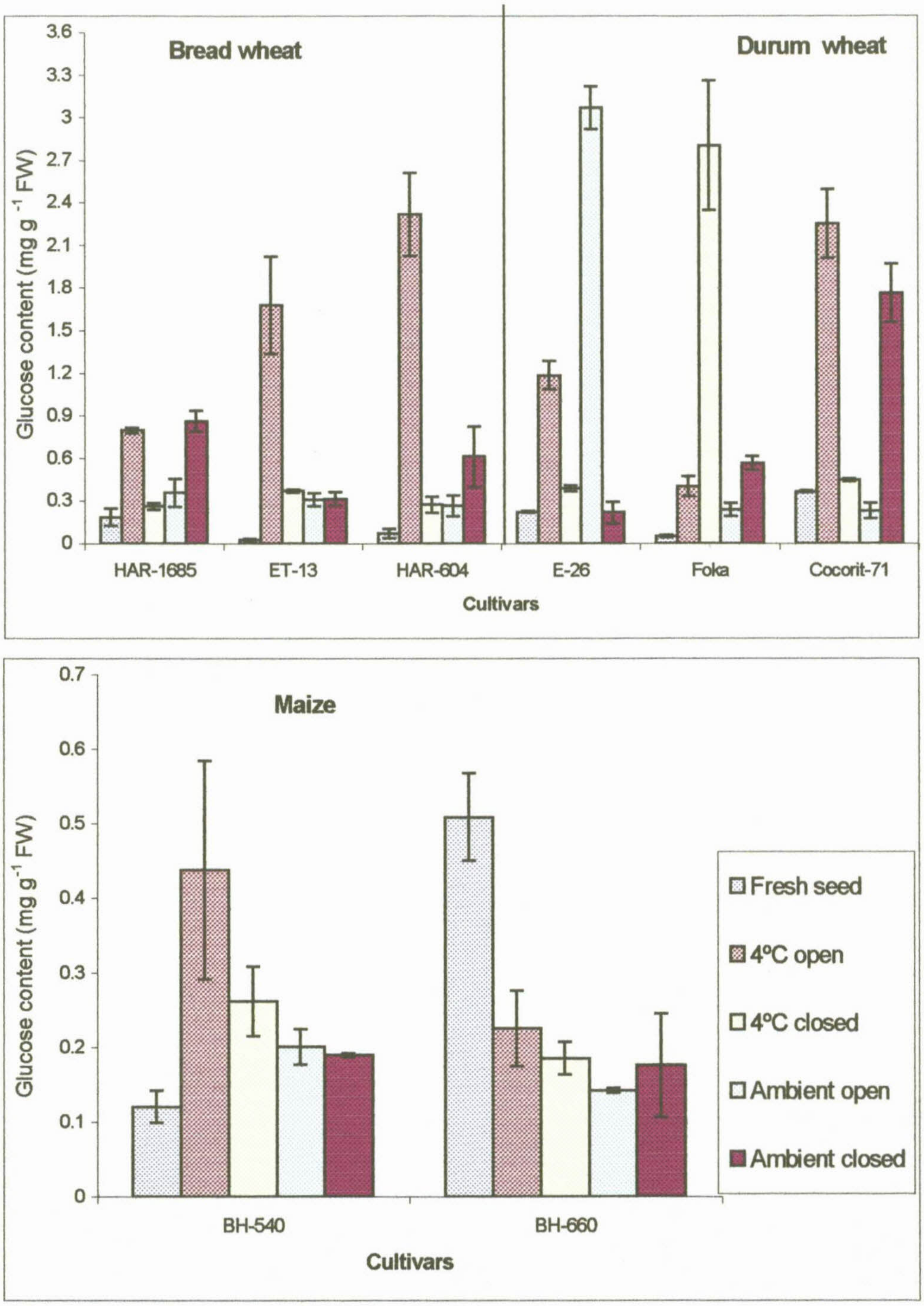

Figure 5.2: Effect of different storage conditions on the glucose content in seeds of different Ethiopian wheat and maize cultivars.

effects on seeds of the three durum wheat cultivars in this regard. Storage in open containers at 4°C caused a fivefold, and at ambient temperature a twelvefold, glucose content increase in seeds of E-26. In Foka seeds, storage in a closed container at 4°C caused more than a twentyfold increase. In Cocorit-71 it was storage at 4°C in open container (sixfold) and ambient temperature in a closed container (fourfold), which caused the most marked increase in glucose content. No universal trend was apparent.

Table 5.3: Statistical analysis of the effect of different storage conditions on the mean glucose content of different Ethiopian wheat and maize cultivar seeds. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Glucose content (mg g^{-1} FW)		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (fresh seeds)	0.09 d	0.21 c	0.31 ab
4°C in open container	1.60 a	1.28 a	0.33 a
4°C in closed container	0.30 c	1.21 a	0.22 bc
Ambient in open container	0.31 c	1.18 a	0.17 c
Ambient in closed container	0.59 b	0.85 b	0.18 c
LSD ($P < 0.05$)	0.18	0.21	0.10

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

Statistical analysis of the pooled data obtained from the mean glucose levels of durum wheat seeds aged under all the different storage conditions were significantly higher than that of the mean for control seeds (Table 5.3), indicating significant variation between treatments. However, no significant variation between cultivars was observed (Table 5.4).

Table 5.4: Statistical analysis of cultivar variation in terms of glucose content in seeds of different Ethiopian wheat and maize cultivars. Data from different storage treatments were pooled and means for each cultivar (within a species) were calculated separately.

Species and cultivars	Glucose content (mg g^{-1} FW)
Triticum aestivum (Bread wheat)	Averages
HAR-1685	0.49 b
ET-13	0.54 b
HAR-604	0.71 a
LSD ($P < 0.05$)	0.14
Triticum durum (Durum wheat)	
E-26	1.02 a
Foka	0.81 a
Cocorit-71	1.01 a
LSD ($P < 0.05$)	
Zea mays (Maize)	
BH-540	0.24 a
BH-660	0.25 a
LSD ($P < 0.05$)	

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

The different storage conditions under which the maize cultivar, BH-540, was aged caused marked glucose level increase in seeds but to varying degrees (Figure 5.2). The increase was accentuated in seeds aged in an open container at 4°C compared to the other storage treatments. The opposite was true in the case of BH-660 seeds where all of the different aging conditions rather caused a marked decrease compared to the control seeds which measured exceptionally high in glucose content.

As indicated by the analysis of variance, the mean glucose level of maize seeds aged at ambient temperature in both open and closed containers was significantly lower than the mean for both the control and seeds aged in open containers at 4°C (Table 5.3) indicating significant variation between treatments. To the contrary, no statistically significant variation between cultivars was apparent (Table 5.4).

5.3.3 Sucrose content

Aging seeds of all the wheat cultivars (both bread and durum) in closed containers at 4°C caused substantial increase in the level of sucrose (Figure 5.3). Similarly, high increases in sucrose were also observed in seeds of HAR-1685 and HAR-604 (both bread wheat cultivars) as well as Foka (durum) aged in open containers at ambient temperature. In contrast, all the bread and durum wheat cultivar seeds aged in open containers at 4°C as well as in closed containers at ambient temperature, showed a decrease in the sucrose content compared to the respective controls and other storage treatments.

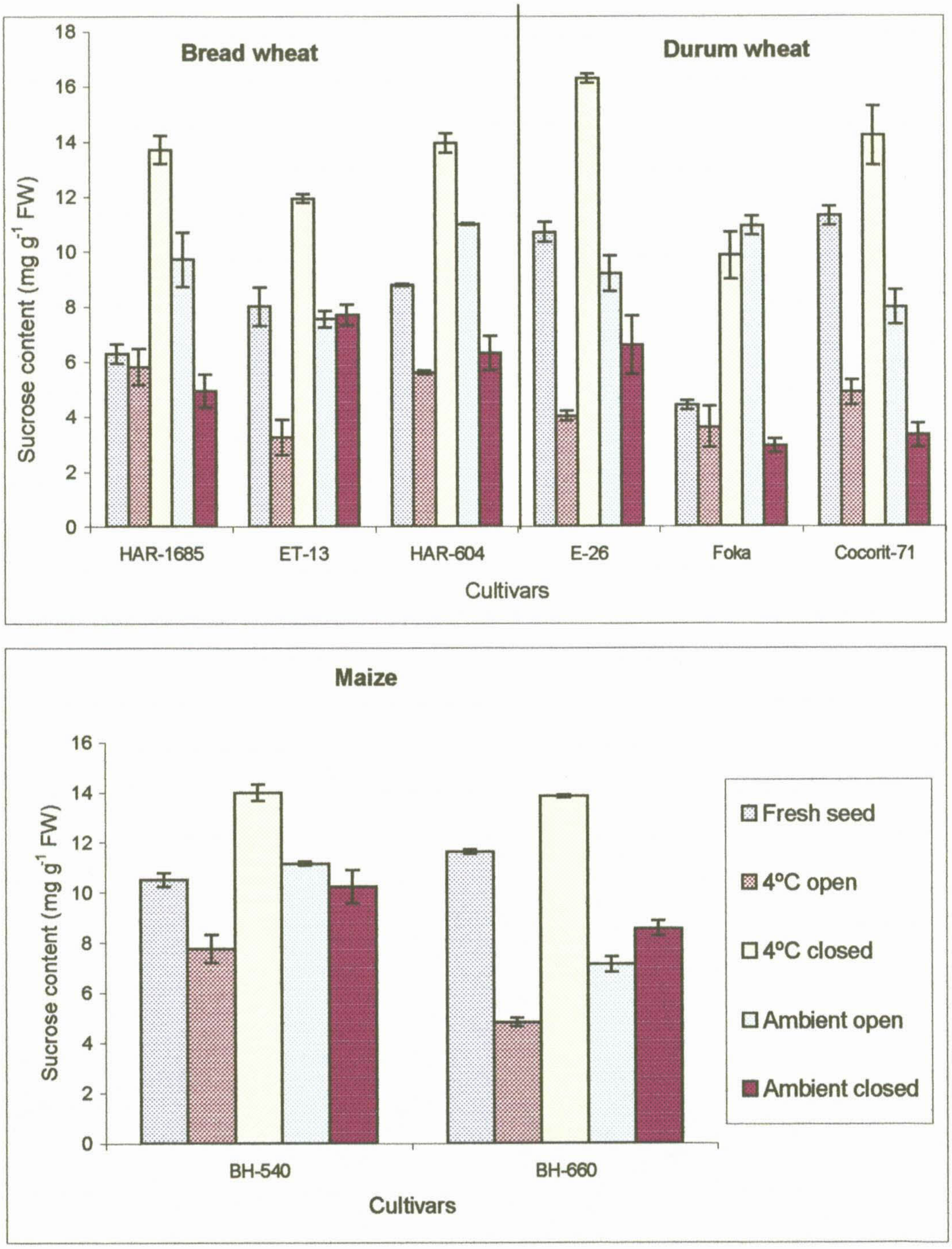

Figure 5.3: Effect of different storage conditions on the sucrose content in seeds of different Ethiopian wheat and maize cultivars.

Statistically, the variation between treatments under which both bread and durum wheat cultivar seeds were aged was significant (Table 5.5) and this was also true for the variation between cultivars (Table 5.6). In the latter instance, HAR-604 revealed a significantly higher sucrose content than the other two bread wheat cultivars (HAR-1685 and ET-13) while the latter two cultivars did not show significant variation. Among the durum wheat cultivars, E-26 showed the highest sucrose content followed by Cocorit-71 and Foka.

Table 5.5: Statistical analysis of the effect of different storage conditions on the mean sucrose content of different Ethiopian wheat and maize cultivar seeds. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Sucrose content (mg g^{-1} FW)		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (fresh seeds)	7.70 c	8.81 b	11.07 b
4°C in open container	4.89 e	4.18 c	6.30 d
4°C in closed container	13.2 a	13.45 a	13.92 a
Ambient in open container	9.42 b	9.37 b	9.14 c
Ambient in closed container	6.32 d	4.29 c	9.40 c
LSD ($P < 0.05$)	0.70	0.81	0.59

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure

Table 5.6: Statistical analysis of cultivar variation in terms of sucrose content in seeds of different Ethiopian wheat and maize cultivars. Data from different storage treatments were pooled and means for each cultivar (within a species) were calculated separately.

Species and cultivars	Sucrose content (mg g^{-1} FW)
Triticum aestivum (Bread wheat)	Averages
HAR-1685	8.09 b
ET-13	7.69 b
HAR-604	9.13 a
LSD ($P < 0.05$)	0.54
Triticum durum (Durum wheat)	
E-26	9.37 a
Foka	6.35 c
Cocorit-71	8.34 b
LSD ($P < 0.05$)	0.63
Zea mays (Maize)	
BH-540	10.73 a
BH-660	9.20 b
LSD ($P < 0.05$)	0.32

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

As was the case for wheat cultivars, the sucrose content in seeds of the two maize cultivars aged in closed containers at 4°C also increased markedly compared to the respective controls and other storage treatments (Figure 5.3). Other storage treatments had either no effect or tended to reduce the sucrose content in maize seeds. Statistically significant variation between both storage treatments (Table 5.5) and cultivars (Table 5.6) was confirmed in maize seeds.

In the latter case, seeds of BH-540 measured significantly higher in sucrose content than that of BH-660.

5.3.4 Starch content

In general, according to raw data presented in Figure 5.4, the starch content in seeds of all three bread wheat cultivars tended to decrease under the influence of the all the different aging treatments. However, only in seeds of the cultivar, HAR-1685, the opposite tendency was observed after storage in closed containers at 4°C. Statistical analysis of the pooled data of the three bread wheat cultivars revealed significant variation between treatments in as much as the mean starch content in seeds aged under all of the storage conditions, except for storage in closed containers at 4°C, tended to be significantly lower than that of the mean for control (Table 5.7). Although statistically accountable, only slight variation between cultivars in terms of starch content of seeds of the three bread wheat cultivars was observed (Table 5.8).

When standard errors are considered, only slight reduction in the starch content of differently stored durum wheat seeds, and in all cultivars, were observed. However, Cocorit-71 seeds, stored at 4°C in both open and closed containers showed a 25% increase (Figure 5.4).

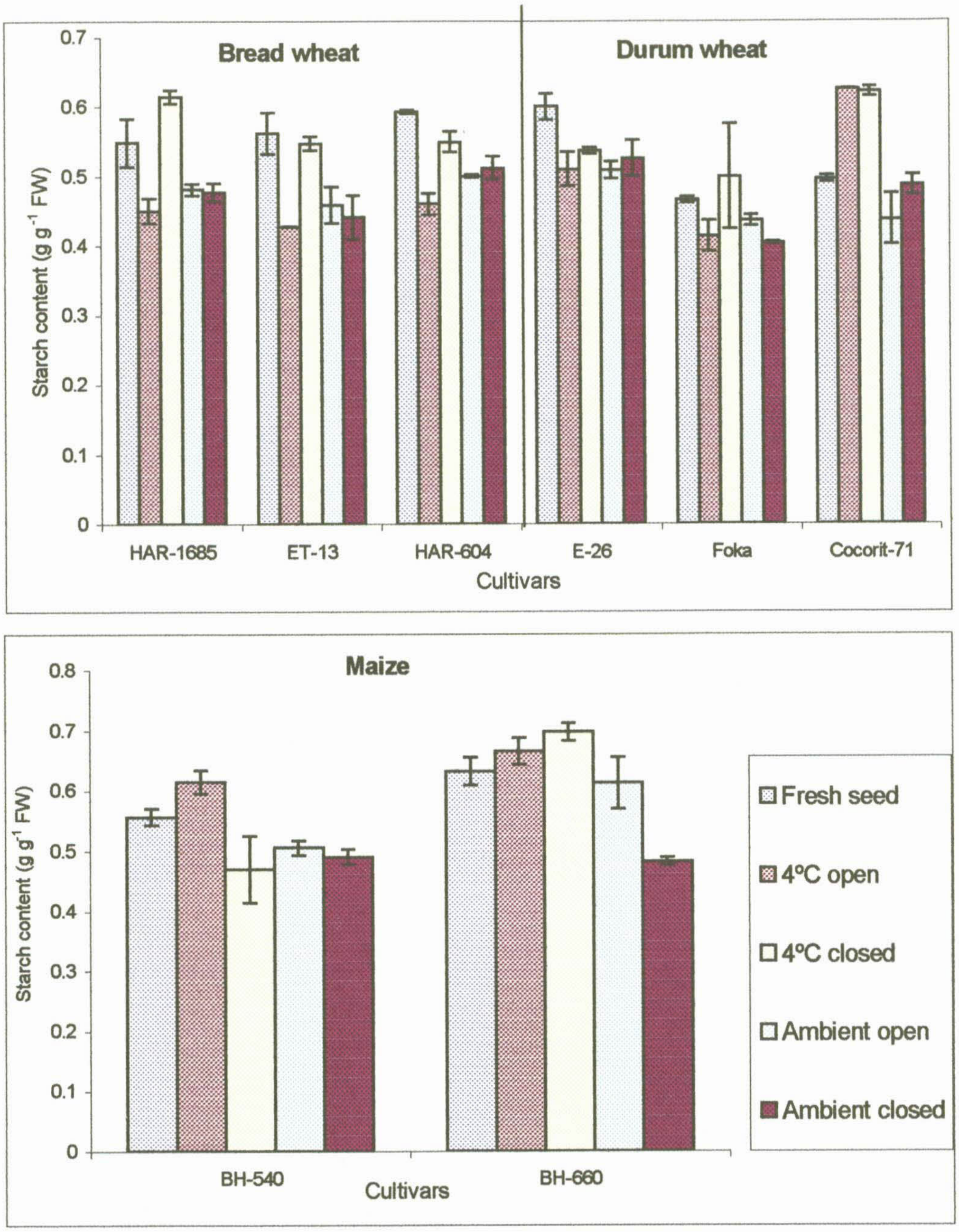

Figure 5.4: Effect of different storage conditions on the starch content in seeds of different Ethiopian wheat and maize cultivars.

Table 5.7: Statistical analysis of the effect of different storage conditions on the mean starch content of different Ethiopian wheat and maize cultivar seeds. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Starch content (g g^{-1} FW)		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (fresh seeds)	0.57 a	0.52 a	0.59 b
4°C in open container	0.45 c	0.52 a	0.64 a
4°C in closed container	0.57 a	0.55 a	0.58 b
Ambient in open container	0.48 b	0.46 b	0.56 b
Ambient in closed container	0.48 b	0.47 b	0.49 c
LSD ($P < 0.05$)	0.02	0.03	0.04

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure

Statistically, variation between treatments was significant for durum wheat seeds stored at ambient temperature in both open and closed containers, measuring lower in terms of starch content compared to the mean for control and other storage treatments (Table 5.7). Moreover, variation among the durum wheat cultivars was also observed where seeds of E-26 and Cocorit-71 showed the same level of starch content which was significantly higher than that of Foka (Table 5.8).

Table 5.8: Statistical analysis of cultivar variation in terms of sucrose content in seeds of different Ethiopian wheat and maize cultivars. Data from different storage treatments were pooled and means for each cultivar (within a species) were calculated separately.

Species and cultivars	Starch content (g g^{-1} FW)
Triticum aestivum	
(Bread wheat)	Averages
HAR-1685	0.51 ab
ET-13	0.49 b
HAR-604	0.52 a
LSD ($P < 0.05$)	0.02
Triticum durum	
(Durum wheat)	
E-26	0.54 a
Foka	0.44 b
Cocorit-71	0.53 a
LSD ($P < 0.05$)	
Zea mays	
(Maize)	
BH-540	0.53 b
BH-660	0.62 a
LSD ($P < 0.05$)	0.02

[δ]Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure.

Except for storage of seeds from the maize cultivar, BH-540, in an open container at 4°C, all the other storage treatments caused slight decreases in the starch content (Figure 5.4). A more pronounced decrease was observed in seeds of BH-660 aged at ambient temperature in a closed container while cold storage (4°C) in both open and closed containers had a slight increasing effect in the starch content of these seeds. Statistically, the mean starch content of maize seeds aged in open containers at 4°C (pooled data of the two maize cultivars) was significantly higher while storage in closed containers at ambient temperature caused a significant decrease compared to the mean for the control and the other storage treatments (Table 5.7), indicating some variation between treatments. Statistically significant variation between cultivars was also observed (Table 5.8) where BH-660 seeds showed significantly higher starch content than that of BH-540.

5.3.5 Moisture content

The same tendency of elevated moisture content was observed in seeds of both durum and bread wheat, as well as maize cultivars, aged at 4°C in both open and closed containers compared to the respective controls and seeds aged at ambient temperature (Figure 5.5). The increase in moisture content was accentuated in seeds of all the wheat cultivars aged in open containers at 4°C. However, seeds from the two maize cultivars responded differently to storage in open and closed containers in terms of changes in the seed moisture content. BH-540 seeds aged in open containers at 4°C showed a more marked increase in moisture content than in closed containers while the opposite was true for BH-660 seeds (Figure 5.5).

Statistical analysis of the mean moisture content in seeds of both bread and durum wheat as well as maize cultivars confirmed the above observation to be significant (Table 5.9), indicating clear variation between treatments. Storage at a low temperature (4°C) caused a moisture content increase, while storage at ambient temperature caused a decrease, in seeds of all crops and all cultivars.

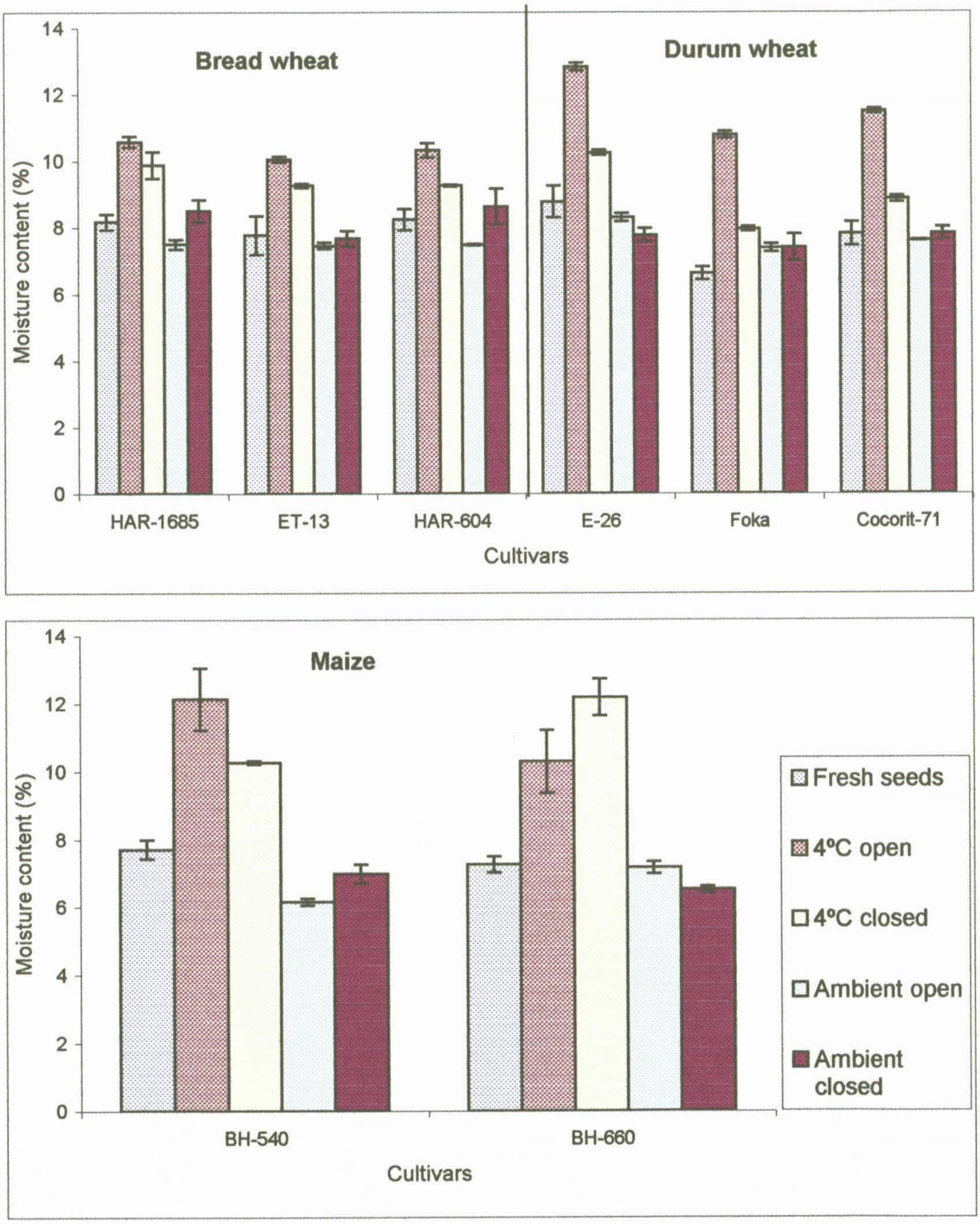

Figure 5.5: Effect of different storage conditions on the moisture content in seeds of different Ethiopian wheat and maize cultivars.

Table 5.9: Statistical analysis of the effect of different storage conditions on the mean moisture content in different Ethiopian wheat and maize cultivar seeds. Data from different cultivars within a species were pooled and means for each storage treatment were calculated.

	Moisture content (%)		
Storage conditions	*Triticum aestivum* (Bread wheat)	*Triticum durum* (Durum wheat)	*Zea mays* (Maize)
Control (fresh seeds)	8.07 c	7.74 c	7.49 b
4°C in open container	10.33 a	11.71 a	11.21 a
4°C in closed container	9.48 b	9.03 b	11.22 a
Ambient in open container	7.48 d	7.77 c	6.67 c
Ambient in closed container	8.28 c	7.66 c	6.76 c
LSD ($P < 0.05$)	0.39	0.29	0.42

[δ] Averages followed by the same letter were not significantly different ($P < 0.05$), according to LSD (Least Significance Difference) procedure

The moisture contents of fresh seeds of different wheat and maize cultivars had slight variations when they were obtained from the source (Ethiopia). As the moisture contents of seeds were not maintained at the same level artificially before the storage period, the analysis of variance for comparison of different cultivars within a species in terms of moisture content changes was not performed. The main reason being that the final moisture content (after the storage treatments) might also have reflected the initial difference in moisture content.

5.4 Discussion

At physiological maturity, seeds are considered to possess maximum germination potential and, during storage, most seeds undergo certain irreversible changes leading to loss of vigour and viability (Anderson, 1970). Changes in protein and amino acid levels are considered the most important factors causing reduced seed vigour and viability (Kalpana and Madhava Rao, 1997), as proteins and amino acids play an important role in the growth and development of seedlings. The degradation of enzymatic and structural proteins, due to unfavourable storage conditions, might play the most pivotal role in this regard (Kalpana and Madhava Rao, 1997).

Even though the raw data indicated the total water soluble protein content in seeds of both bread and durum wheat, as well as maize cultivars, aged under most of the storage conditions to have reduced slightly, most of the storage treatments did not show statistically significant variation compared to the respective controls. This is in agreement with the reviews of Burton (1982), who stated that the turnover and changes of protein in dry wheat seeds to be very slow during a short-term storage period.

However, seeds from both bread and durum wheat, as well as maize cultivars, stored in closed containers at ambient temperature revealed a significant decline in water soluble protein content compared to the respective controls. This anomaly is difficult to explain, and also opposes the findings of Sreeramulu (1983) on Bambarra groundnut, who reported an increase in soluble proteins of seeds during storage. Clearly, differences between crops exist in this regard which emphasizes the need to study them separately.

Seed deterioration during aging was also connected to changes in carbohydrate metabolites in the past (Burton, 1982; Pomeranz, 1992). Storage at ambient temperature, in both open and closed containers, caused a slight depletion of the starch content in seeds of both bread and durum wheat cultivars. This was also true for maize seeds aged in closed containers at ambient temperature. Enhanced activity of amylase could have been an important factor leading to the hydrolysis of the starch, especially at the higher temperature (18 - 30°C) associated with the ambient storage conditions. Similar speculative arguments were presented by Burton (1982) and Pomeranz (1992).

Increases in the glucose content of especially bread and durum wheat seeds, concomitant with a decrease in the starch content, seemed to be in support of the speculation that elevated starch hydrolysis could have been an underlying factor causing these changes under most of the storage conditions studied. This agrees with the findings of Onigbinde and Akinyele (1988), who reported that elevated hydrolytic activity of amylase could have been related to the increase in reducing sugars in maize seeds during storage.

Storage of maize seeds in closed containers at ambient temperature, however, did not reveal the same correlation between starch and glucose as was observed in wheat seeds. Moreover, the two maize cultivars responded differently in this regard. BH-540 seeds followed the same pattern as wheat seeds while BH-660 actually showed a decrease in glucose content with starch content remaining more or less unchanged, especially during cold storage. A decrease in the glucose content of maize seeds was also reported by Pomeranz (1992), who suggested that elevated seed respiration under certain storage conditions could have attributed to this change. In the case of BH-660 seeds, where this tendency was observed, it was difficult to conclude the same as the changes also occurred during cold storage, where one would have expected seed respiration to be lower.

An interesting common trend of seeds from all the wheat and maize cultivars to increase its sucrose content under storage at 4°C, especially in closed containers, was observed. It seemed as if the low temperature favoured an elevated trend to enhance sucrose phosphate synthetase activity. This postulate is supported by the findings of Burton (1982) who reported the activity of this synthesizing enzyme to be enhanced by low temperature.

Seeds from both bread and durum wheat, as well as maize cultivars aged at 4°C in both open and closed containers, showed a substantial increase in moisture content compared to the respective controls and those aged at ambient temperature. Because of the relatively high RH (relative humidity) of the cold storage environment (65 - 75%), seeds stored at 4°C appeared to absorb significant amounts of moisture from the surrounding air. Similar suggestions were also made by Roberts (1972a), Justice and Bass (1979b) and van de Venter (1999).

Since the closed containers must have prevented the movement of air from the seed to the atmosphere and vice versa once an equilibrium state was established, the increase in moisture content of seeds aged under these conditions is difficult to explain. Although precautions were taken, it is possible that the lids of containers (jars) did not seal properly enough to completely restrict the movement of air, and might have to some extent allowed the absorption of moisture by the seeds. However, the moisture content change in seeds of both bread and durum wheat as well as maize cultivar seeds aged in cold storage (4°C) in both open and closed containers, appeared to have no clear effect on metabolite content (total water soluble protein and carbohydrate).

In summary, statistical analysis of the data revealed that most of the metabolites measured were significantly reduced when seeds from both bread and durum, as well as maize cultivars, were aged at ambient temperature in closed containers. Storage in open containers at ambient temperature, on the other hand, did not have a negative effect on the contents of most of the metabolites measured in seeds of both bread and durum wheat cultivars. The observed difference between storage in closed and open containers at ambient temperature is difficult to explain in terms of maintaining metabolite levels. However, the differences in metabolite contents might have been correlated with differences in seed moisture content as well as differences in RH between the ambient open and closed containers. This might have affected the metabolism of stored seeds which in turn must have had an effect on especially carbohydrate metabolism levels.

Chapter 6

General discussion

The deleterious effect of high relative humidity (RH) of seed storage and consequently increased moisture content of the seeds on their viability and vigour have previously been reported by several workers (Roberts, 1972a; Justice and Bass, 1979b; van de Venter, 1999). However, no sufficient information is available under tropical conditions, including Ethiopia (Delouche *et al.*, 1973; Anonymous, 1995). This supplied the rationale to study the effects of different storage conditions on seed viability and vigour as well as biochemical and physiological aspects related to seed viability and vigour of different Ethiopian wheat and maize cultivars. Cultivar variations (within a species) in terms of seed viability and vigour as well as physiological aspects were also determined.

The viability (germination percentage) of seeds from different cultivars of both bread and durum wheat aged at low temperature (4°C) in both open and closed containers, declined significantly compared to the respective controls (fresh seeds). Albeit statistically not significant, a more or less similar tendency was observed in seeds of both cultivars of maize aged in open containers at 4°C. The observed decline in viability of these seeds might probably have been related to increased moisture content of seeds aged at lower temperature, which could be accredited to the relatively high RH (65 - 75%) of the mentioned storage environment, compared to that of the ambient environment (30 - 50%).

Although the reviews of Roberts (1972a), Justice and Bass (1979b) and van de Venter (1999) implicated cold storage (0 – 5°C) to be favourable in maintaining the viability and vigour of seeds during storage, Bewley and Black (1994), on the other hand, reported that cold storage was advisable only if seeds were to be sealed in moisture proof containers or stored in a dehumidified atmosphere. The alternative is a high RH condition, causing the seeds to gain moisture, which further leads to rapid seed deterioration. The results obtained in this study are in support of the finding of Bewley and Black (1994).

Seeds from most of the bread and durum wheat cultivars aged in open containers at ambient temperature tended to retain their viability compared to those aged under other storage conditions. The slower deterioration rate of seeds from these cultivars aged in open containers under ambient conditions could have been related to the lower relative humidity of the ambient condition (Delouche *et al.*, 1973; van de Venter, 1999). However, in contrast to bread and durum wheat cultivar seeds, the mean viability (pooled data of the two maize cultivars) of seeds aged at low temperature, remained unchanged despite the moisture content gain. This might be indicative of differences between stored seeds of wheat and maize regarding its ability to retain viability under different storage conditions.

Moreover, even though the relative humidity of the ambient storage condition was lower (30 - 50%), the germinability of bread wheat seeds stored in closed containers at ambient temperature declined more rapidly compared to durum wheat and maize cultivar seeds. This anomaly appears to be related to differences in the genetic constitution of seeds from different cultivars and/or crops.

As indicated by both the statistical analysis as well as the separate data for the different cultivars, the germination indexes of both wheat and maize cultivar seeds showed more or less a similar response to the different storage treatments as did the viability test (germination percentage). Both viability and germination index test results revealed storage in open containers at ambient temperature to maintain the viability and vigour of both bread and durum wheat cultivar seeds, while storage in closed containers at ambient temperature tended to reduce both seed viability and the germination index. Storage at ambient temperature in open containers seemed to consistently maintain both the viability and germination index of wheat cultivar seeds. Unlike the bread and durum wheat cultivars, neither the viability nor the germination indexes of maize cultivars seeds were influenced by the different storage treatments, which might also be indicative of genetic variations between crops.

As was the case for viability and germination index, storage of seeds from both bread and durum wheat, as well as maize cultivars, at ambient temperature in open containers, did not show a significant effect on dry shoot mass accumulation in seedlings. However, a significant reduction in dry shoot mass was observed in seeds of both bread wheat and maize cultivars aged in closed containers at both 4°C and ambient temperature. This might have been related to the lower total water soluble protein content measured in these seeds under the same storage conditions. This is in accordance with the results presented by Kalpana and Madhava Rao (1997), who subsequently speculated that protein degradation due to aging, influences the degree of dry matter accumulation and possibly play an important role in reducing the vigour of seedlings. They further indicated that the degraded proteins might include the enzymatic proteins, which play a crucial role before and after germination.

As indicated by differences in electrical conductivity measured for imbibed seeds after storage, the rate of deterioration of membranes seemed to be lower when seeds were aged at lower temperature, and this applied for seeds of all bread and durum wheat as well as maize cultivars. However, the other two vigour tests, namely the germination index and dry shoot mass determinations, indicated that seeds aged at ambient temperature in open containers possessed higher vigour than those aged at 4°C. This actually implicates storage at ambient temperature in open containers to be more effective in preventing seed deterioration. According to the report of Steiner *et al.* (1989), seedling dry shoot mass had correlated better with seed vigour while conductivity showed poor correlation. The inconsistency which occurred between conductivity and the other two vigour tests, in correlating with seed vigour, supports the statements of Steiner *et al.* (1989), who expressed doubt towards the inability of the conductivity test to relate to seed vigour.

Additionally, Hampton (1995) suggested that results obtained with the conductivity test for distinguishing between high and low vigour maize seeds, should be treated with caution, as the source of electrolyte leakage can considerably influence the conductivity test results (e.g., cultivar differences in leakage from maize pericarps may interfere with conductivity measurements for vigour). Although the conductivity test did not seem to clearly distinguish between the different storage treatments in maintaining the vigour of both bread and durum wheat as well as maize cultivars seeds, it appeared likely that it confirmed the genetic variation between cultivars as proposed by Hampton (1995).

The deterioration of seeds in storage might also be reflected by reduced oxygen uptake of seeds during germination (Ram and Wiesner, 1988; Steiner *et al.*, 1989 and Hampton, 1995). However, in this study the measurement of respiration rate in terms of O_2 uptake by seeds after storage did not seem to clearly distinguish between the control (non-aged) and aged seeds of bread and durum wheat cultivars. Moreover, the rate of O_2 consumption by seeds of the three bread wheat cultivars did not show clear variations. Similarly, the respiration rate measurements did not distinguish between seeds of the two maize cultivars in terms of vigour, supporting the findings of Coolbear (1995). However, in the case of the durum wheat cultivars, the genetic variation between the three cultivars was clearly distinguished in terms of O_2 consumption. E-26 seeds, which showed the highest viability, germination index as well as the lowest electrolyte leakage, respired at a faster rate than did the other two cultivars.

The observed variation in viability and vigour of seeds from different cultivars within a species might also depend on the inherited difference in metabolite (total water soluble protein and carbohydrate) contents (Coolbear, 1995). This might explain the relatively higher total water soluble protein and starch content of HAR-1685, with its higher viability and vigour, compared to the other two bread wheat cultivars (ET-13 and HAR-604). Among the durum wheat cultivars, E-26 also showed relatively higher content of sucrose than the other two (Foka and Cocorit-71). This might also reflect the higher viability and vigour of this cultivar in comparison to the others.

Apart from the inherent cultivar difference in the content of metabolites, reduced viability and vigour of seed may also occur due to loss of these metabolites during storage. Metabolites are lost because of changes in membrane structure followed by changes in carbohydrate and protein content (Pomeranz, 1992). Already in 1992, Pomeranz stated that biochemical evidence for seed deterioration is lacking and this is currently still the case. Most of the carbohydrate metabolites (glucose, sucrose and starch) tested in this study showed marked changes during the six month storage period under the influence of contrasting storage environments. However, no clear correlation was observed between these changes and changes in viability or vigour. It is possible that the six month storage period might have not been sufficient to bring about changes in the carbohydrate metabolite levels, at least to such an extent that it could have impacted on the viability and vigour of seeds. Pomeranz (1992) presented a similar speculative argument.

Moreover, changes in the total water soluble protein content of seeds from both bread and durum wheat as well as maize cultivars during the six month storage period, was not particularly helpful in predicting changes in either the viability or vigour in these seeds during storage. The only exception in this regard was the positive correlation which existed between dry shoot mass accumulation in both wheat and maize seedlings obtained from seeds aged in closed containers at ambient temperature, and the total water soluble protein content of these seeds.

In summary, seed viability (germination percentage) and vigour (germination index and dry shoot mass) of seeds from both bread and durum wheat cultivars stored in open containers at ambient temperature were not significantly influenced while the other storage treatments affected the viability and vigour to various degrees. A more or less similar tendency was observed in the case of the two maize cultivars, when only the raw data was considered. Open storage under ambient conditions also maintained most of the metabolites measured in the study. The lower relative humidity of the ambient storage environment apparently contributed to maintain the moisture level of seeds at a much lower level compared to those aged at low temperature. This environment must have enabled seeds aged in open containers at ambient temperature, to retain their viability and vigour during the six month storage period indicating the need to dry seeds to a lower moisture content level before storage and/or the need for dehumidifying the storage environment, especially under Ethiopian storage conditions.

Like the other two vigour tests, measurements of electrical conductivity and respiration rate clearly showed cultivar variations. However, they did not seem to clearly distinguish between the vigour of seeds stored under different conditions. It might be unrealistic to expect from a single parameter to be utilized as a complete predictive tool in this regard. This suggests that future work should focus on developing a range of correlating seed vigour tests under prevailing or local conditions. In addition, the results of laboratory vigour tests should be correlated with that obtained under field conditions in order to also assess the quality of seeds with respect to its field performance potential.

Even though the biochemical tests performed in this study did not seem to correlate positively at all times with the viability and vigour of seeds aged under different conditions, the relatively higher metabolite content of some cultivars, such as HAR-1685 (bread wheat) and E-26 (durum wheat) appeared to have contributed to their higher viability and vigour. This indicates the need to focus towards the biochemical and physiological processes related to seed deterioration during storage that can supply the rationale for plant breeders to genetically improve seed viability and vigour as well as its storage potential, especially for commercial seed producing cultivars. The results of this study also indicated that the metabolite level changes in seeds under storage, take longer to impact seed viability and vigour than what was anticipated, suggesting the need for long term storage period for better understanding of the mechanisms of seed deterioration in terms of biochemical processes and metabolite content changes.

Summary and conclusion

Seed viability and vigour loss during storage, is one of the several factors limiting crop yield in Ethiopia. Ambient storage conditions (high temperature and relative humidity) as well as high moisture content during storage are considered to be the most important factors causing inferior quality of seeds (Anonymous, 1995). This supplied the rationale to study the effect of ambient temperature (more or less similar to that applying in Ethiopian conditions) and low temperature (4°C) on seed viability and vigour as well as biochemical and physiological aspects related to seed viability and vigour of Ethiopian bread and durum wheat as well as maize cultivar seeds. Additionally, the effects of storage in open and closed containers at different temperatures were also investigated.

Seeds from three bread and durum wheat as well as two maize cultivars were stored at low (4°C) or ambient temperatures (18-30°C) in open or closed containers for a period of six months. Seed viability and vigour as well as biochemical and physiological aspects related to seed viability and vigour were measured before (fresh seeds) and after different storage treatments. Seed viability (germination percentage) and vigour (germination index and dry shoot mass) of seeds from both bread and durum wheat cultivars stored in open containers at ambient temperature were not significantly influenced while the other storage treatments affected the viability and vigour to various degrees. A more or less similar tendency was observed for seeds of the two maize cultivars, when only the raw data was considered. The mentioned storage condition also maintained most of the seed metabolite levels measured in this study.

The lower relative humidity of the ambient storage environment contributed to maintain the moisture level of seeds at a much lower level compared to those aged at low temperature. This situation contributed to seeds aged in open containers at ambient temperature in retaining their viability and vigour during the six month storage period, indicating the need to dry seeds to a lower moisture content level before storage and/or the need for dehumidifying the storage environment, especially under Ethiopian storage conditions.

Like the other two vigour tests, the measurement of electrical conductivity and the respiration rate clearly showed cultivar variations. However, they did not seem to clearly distinguish between the vigour of seeds stored under different conditions. Future work should focus towards developing a range of correlating seed vigour test methods under local conditions. In addition, the results of laboratory seed vigour tests should be correlated with field emergence in order to assess the quality of seeds with respect to its field performance potential.

Biochemical tests performed in this study did not seem to correlate with the viability and vigour of seeds aged under different conditions. However, the relatively higher metabolite content of some cultivars, such as HAR-1685 (bread wheat) and E-26 (durum wheat) appeared to have contributed to their higher viability and vigour. Future research might focus on elucidating the biochemical and physiological processes underlying seed deterioration and consider developing new and cheaper screening methods for seed viability and vigour. It should also be considered to correlate future findings regarding physiological changes in stored seeds with mechanisms of seed deterioration in order to understand the mechanism of seed deterioration better in terms of biochemical process and metabolite content changes.

Opsomming en gevolgtrekkings

Die verlies van saad kiemkragtigheid ("viability") en vitaliteit ("vigour") tydens berging, is een van die faktore wat oesopbrengste van landbougewasse in Etiopië beperk. Saadberging by heersende omgewingstemperatuur (hoë temperatuur en relatiewe humiditeit) asook die hoë voginhoud van saad word beskou as die belangrikste faktore wat aanleiding gee tot swak saadkwaliteit (Anoniem, 1995). Laasgenoemde het die rasionaal verskaf om die effek van bergingstoestande (kamertemperatuur, wat min of meer ooreenstem met heersende toestande in Etiopië, asook berging by lae temperatuur, 4°C) op saad kiemkragtigheid en vitaliteit in brood en durum koring, asook mielie cultivars, te ondersoek. Daarby is die invloed van berging in oop en geslote houers by verskillende temperature op saad kiemkragtigheid en vitaliteit ook ondersoek. Aandag is ook gegee aan die invloed van berging op biochemiese en fisiologiese aspekte wat met saad kiemkragtigheid en vitaliteit verband hou.

Saad van drie Etiopiese brood- en durum koring, asook twee mielie cultivars, is by lae temperatuur (4°C) of kamertemparatuur (18-30°C) in oop en geslote houers geberg vir ses maande. Saad kiemkragtigheid en vitaliteit, asook biochemiese en fisiologiese aspekte wat hiermee verband hou, is deur middel van verskillende tegnieke voor (vars saad) en na berging bepaal. Saad kiemkragtigheid (persentasie kieming) en vitaliteit (kiemingsindeks en droë biomassa van jong saailinge) van beide brood en durum koringsaad is nie noemenswaardig deur berging in oop houers by kamertemperatuur beïnvloed nie maar wel deur die ander bergingsomgewings. 'n Min of meer soortgelyke tendens is by sade van die twee mielie cultivars waargeneem. Metabolietvlakke het by beide koring en mielies tog tydens die bergingstydperk variasies getoon.

Die laer relatiewe humiditeit van die kamertemperatuur bergingsomgewing het daartoe bygedra dat die voginhoud van sade ook op 'n laer vlak gehou is in vergelyking met die bergingsomgewing by lae temperatuur. Hierdie situasie het waarskynlik daartoe bygedra dat berging in oop houers by kamertemperatuur die saad kiemkragtigheid en vitaliteit nie nadelig beïnvloed het nie. Laasgenoemde dui daarop dat vooraf droging van sade, om die voginhoud op 'n laer vlak te kry voor berging begin, of die verlaging van die relatiewe humiditeit van die beegingsomgewing, moontlikhede is om saadkwaliteit minstens tydens die stoorperiode in Etiopië te behou.

Die tegnieke om saad vitaliteit te meet, en veral elektriese konduktiwiteit en respirasietempo metings, het duidelike cultivar verskille uitgewys maar het nie werklik duidelik tussen die vitaliteit van saad wat op verskillende maniere geberg is onderskei nie. Toekomstige navorsing behoort daarop te fokus om 'n reeks korrelerende tegnieke te ontwikkel wat wel hierdie onderskeiding kan maak. Verder behoort die uitkoms van hierdie tegnieke met saad prestasie onder veldtoestande gekorreleer te word ten einde die werklike potensiaal van saad te evalueer.

Biochemiese toetse wat in hierdie studie uitgevoer is het egter ook nie te alle tye positief met die kiemkragtigheid van saad, wat op verskillende maniere geberg is, gekorreleer nie. Die relatief hoër metaboliet inhoud in sade van sekere cultivars, soos HAR-1685 (brood koring) en E-26 (durum koring), het egter positief met kiemkragtigheid gekorreleer. Toekomstige navorsing behoort daarop te fokus om biochemiese en fisiologiese prosesse onderliggend aan saad kiemkragtigheid en vitaliteit te ondersoek ten einde nuwe, en hopelik vinnige en goedkoop, metodes te ontwikkel. Dit behoort ook oorweeg te word om in die toekoms besondere aandag te gee aan onderliggende fisiologiese prosesse wat met saad agteruitgang tydens storing verband hou, ten einde die meganisme hiervan beter te verstaan. Daar is aanduidings dat koolhidraat metabolietvlakke asook proteïeninhoud as parameters kan dien om saadagteruitgang te meet.

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