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PREDICTION OF FIELD EMERGENCE OF MAIZE (*Zea mays* L.) HYBRIDS EXPOSED TO COLD AND WET CONDITIONS

by

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DECLARATION

I hereby certify that this dissertation is my own work, except where duly acknowledged. I also certify that no plagiarism was committed in writing this dissertation.

Signed ……

Pieter Hermanus Maree

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ABSTRACT

The cold test is one of the oldest and most acceptable vigour tests as it is used to simulate stress conditions commonly occurring in the field. In recent years, some of South Africa's top maize hybrids, with high cold test scores, have shown emergence problems under cold, wet planting conditions. It resulted in major complaints from commercial maize producers with sizable claims involved. Therefore, the need arose to find a more sensitive vigour test that takes into account cold, wet conditions.

In practice, South African maize producers would not plant if it is too cold and wet. However, cold, wet conditions are commonly experienced during planting time in the main maize production regions of South Africa, especially during October and even November. Furthermore, in most of the commercial maize production areas, such as the western Free State, chances of thunder and hailstorms are high during the planting period. These weather conditions are major causes for sudden drops in temperature and flooding which can expose maize seed and emerging maize seedlings to stress conditions.

The effects of cold, wet conditions on germination and emergence of nine maize hybrids were investigated in laboratory, glasshouse and field experiments. Growth chamber and glasshouse experiments were conducted under 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding.

Field experiments were conducted under different climatic conditions, resulting in cool and wet, cold and wet and favourable conditions during planting. The objectives were to investigate the correlations between different laboratory vigour tests and field emergence of maize hybrids under cold, wet conditions in order to identify the most suitable laboratory vigour test for predicting field emergence under cold, wet conditions.

Eight different vigour tests were conducted and each was compared with field emergence under cold, wet conditions. The eight tests conducted, were the cold test, soak test, complex stressing vigour test, electrical conductivity test, accelerated ageing test, tetrazolium test, fast green test and emergence rate test. The soak test was the most sensitive vigour test when considering cold, wet conditions, as it measures seed germination, based on the warm test, after a 48 hour soak in water at 27°C. Correlations found between the soak test and field emergence (53%) under cold, wet conditions was unexpected, since the soak test does not account for low temperatures.

The complex stressing vigour test was conducted to study the effect of fluctuating soaking temperatures on germination of maize seed. Seeds of nine maize hybrids were soaked for 48 hours at a moderate temperature (25°C), followed by another 48 hours soak at a low temperature (5°C), and then planted in sand and grown for 4 days at 25°C, before evaluation. Highly significant correlations were found between the complex stressing vigour test and simulated field emergence under both controlled conditions in a glasshouse (89.9%) and cold, wet conditions in the field (90.0%). The complex stressing vigour test was the best test to predict field performance under a wide range of climatic conditions, especially cold, wet conditions. Implementation of the complex stressing test as a routine vigour test, will be to the advantage of maize seed companies, especially in being proactive in predicting emergence of maize hybrids under cold, wet conditions.

Key words: maize, seed quality, vigour, vigour tests, cold test, soak test, complex stressing vigour test, tetrazolium test, electrical conductivity test, accelerating ageing test, germination, emergence, cold, wet, temperature, flooding

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

In recent years, some of South Africa's top maize (*Zea mays* L.) hybrids have shown emergence problems under very wet and cold conditions, or due to other factors considered to be sub-optimal for emergence. It resulted in major complaints from some commercial maize producers with sizable claims involved.

In the northern U.S.A. Corn Belt and in Europe, maize is generally planted in early spring, into soils that are or may become too cold and wet resulting in reduced field emergence, poor stands, and lower economic yields. For these reasons, improvement and prediction of seedling emergence and early seedling growth are important to the seed industry (Grogan, 1970; Hardacre, 1985). Variables identified as affecting seed germination and field emergence in cold and wet soils are: genotype (Grogan, 1970; Eagles, 1982), seed source or production environment (Burris, 1977), and initial seed moisture content (Cal & Obendorf, 1972).

Burris (1977) reported that growing season differences, such as temperature and moisture, and the interaction of temperature and moisture is the most important factors affecting seed quality. Temperature was the dominant factor affecting germination in wet soil, as well as seedling emergence and root growth. It was also found that temperature plays a dominant role in the absorption of nutrients and water (Burris, 1977).

Exposure of developing maize seed to freezing temperatures decreased seed germination (Fick, 1989). Interaction of three factors influenced the severity of germination loss, namely: seed maturity, temperature to which seeds were exposed, and duration of exposure. Therefore, accurate prediction of germination and emergence responses to unfavourable conditions is of

paramount importance for the seed industry to produce and sell high quality maize seed (Martin *et al*., 1988).

Seed scientists are concerned that breeders do not evaluate seed quality characteristics in breeding programmes. It is essential for producers that the improvement of the nutritional value of maize is not done at the expense of seed quality. Seed quality is an important factors affecting early performance and productivity of most agricultural crops (Burris, 2000).

De Geus *et al.* (2005) stated that conventional farming systems require high levels of inputs. Excessive use of chemicals is a concern to scientists and farmers because of possible long-term impacts on ecosystems. In an effort to achieve sustainability, many farmers have adopted organic and low input farming systems. Plant breeding programmes for organic and low input systems focus on the development of varieties with high nutritional grain quality. Maintaining high seed quality in these genetic materials is crucial for achieving uniform stand establishment under stressful seedbed conditions associated with low input cropping systems. De Geus *et al.* (2005) also reported that seed produced under a conventional cropping system had better seed quality than seed produced in an organic system. They also indicated that seed quality is an important aspect of breeding for sustainable cropping systems as seed production environment affects seed quality and chemical composition of the seeds.

Germination capacity is a crucial aspect of seed quality therefore germination tests are used worldwide to determine the maximum germination potential of a seed batch under optimum conditions. The seed industry uses the standard germination test or warm test (AOSA, 2002) for labeling. Seed quality tests should relate to field emergence. According to Delouche & Baskin (1973), vigour tests have proven to be more useful as predictors of field emergence than the warm test.

Emergence problems in commercial hybrid maize fields under cold, wet conditions with sizable claims involved, motivated this study.

The objectives are:

(i) To study the effect of cold and wet conditions on emergence of maize hybrids

(ii) To compare the different laboratory vigour tests to be able to identify the best test to predict field emergence of South African maize hybrids exposed to cold, wet conditions

1.2 LITERATURE REVIEW

1.2.1 Seed quality

Seed quality includes the ability of seed to germinate and emerge under optimal environmental conditions (Burris, 1977). Seed quality is an important factor affecting early performance and productivity of most agricultural crops, including maize (Burris, 2000). Burris (1977) reported that growing season differences, such as temperature and moisture, and the interaction of temperature and moisture is of utmost importance regarding seed quality. Seed viability and seedling vigour are regarded as the two crucial components of seed quality.

Ellis *et al.* (1993) stated that the primary biological purpose of seeds is to propagate the species by successfully completing germination and resuming plant growth. Native species have innate mechanisms that regulate their potential for germination, often delaying or timing germination to coincide with optimal conditions for growth. Domesticated crops have lost some, but not all, of these mechanisms, and there has generally been strong selection for rapid and uniform germination of crop seeds. They also stated that high quality planting seed is the key to successful crop production, but both biological and environmental factors can reduce seed quality.

1.2.1.1 Seed viability

Although the concept of seed viability is well known, there may sometimes be disagreement and confusion as to its precise meaning. To seed technologists and people in the seed industry, viability means that a seed is capable of germinating and producing a “normal” seedling. Therefore, “seed viability” is used synonymously with “germination capacity”. McDonald &

Copeland (2001) noted that a given seed is either viable or non-viable, depending on its ability to germinate and produce a normal seedling; thus, only seed batches representing populations of seeds may exhibit levels of viability. According to them, viability denotes the degree to which a seed is alive, metabolically active, and possesses enzymes capable of catalyzing metabolic reactions needed for germination and seedling growth. In this context, a given seed may contain both live and dead tissues, and may or may not be capable of germination.

The Association of Official Seed Analysts (AOSA) (2002) reported various definitions of seed germination or viability, and it is important to understand their distinctions. To the seed physiologist, germination is defined as the emergence of the radicle through the seed coat. To the seed analyst, germination is "the emergence and development from the seed embryo to those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions".

McDonald & Copeland (2001) consider germination to be the resumption of active growth by the embryo resulting in the rupture of the seed coat and the emergence of a young plant. This definition presumes that the seed has been in a state of rest, after its formation and development. During this period of rest, the seed is in a relatively inactive state and has a low metabolic rate. It can remain in this state until environmental conditions trigger the resumption of growth.

McDonald & Copeland (2001) mentioned that seed viability is probably highest at the time of physiological maturity reached on the parent plant, although the environment at that stage may not permit germination. After physiological maturity, the viability of seeds gradually declines. They also stated that seed longevity depends on the environmental conditions to which it is exposed.

Stern (1991) suggested that seeds may remain viable for many years if stored under dry conditions. During storage, the cells of mature seeds may have a water content of less than 10%, and when water content becomes this low, respiration does not cease completely but continues at a drastically reduced rate, resulting in only very tiny amounts of heat being released, and of carbon dioxide being given off. Stern (1991) also mentioned that large molecules such as

cellulose and starch develop electrical charges when they are wet, and they attract water molecules that adhere to the internal surfaces of the materials. Due to the asymmetry of water molecules, they have slightly different electrical charges at each end, leading to both ends being highly adhesive to large organic molecules like cellulose or even cohesion to each other. This is known as imbibition. Imbibition results in the swelling of tissues, often to several times their original volume (Figure 1.1). This is the initial step in the germination of seeds (Stern, 1991).

(a) (b)

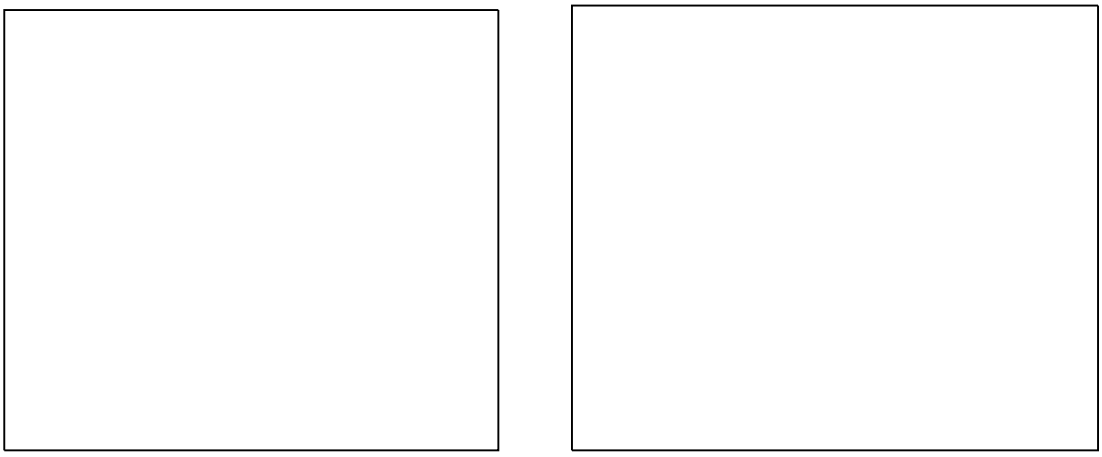
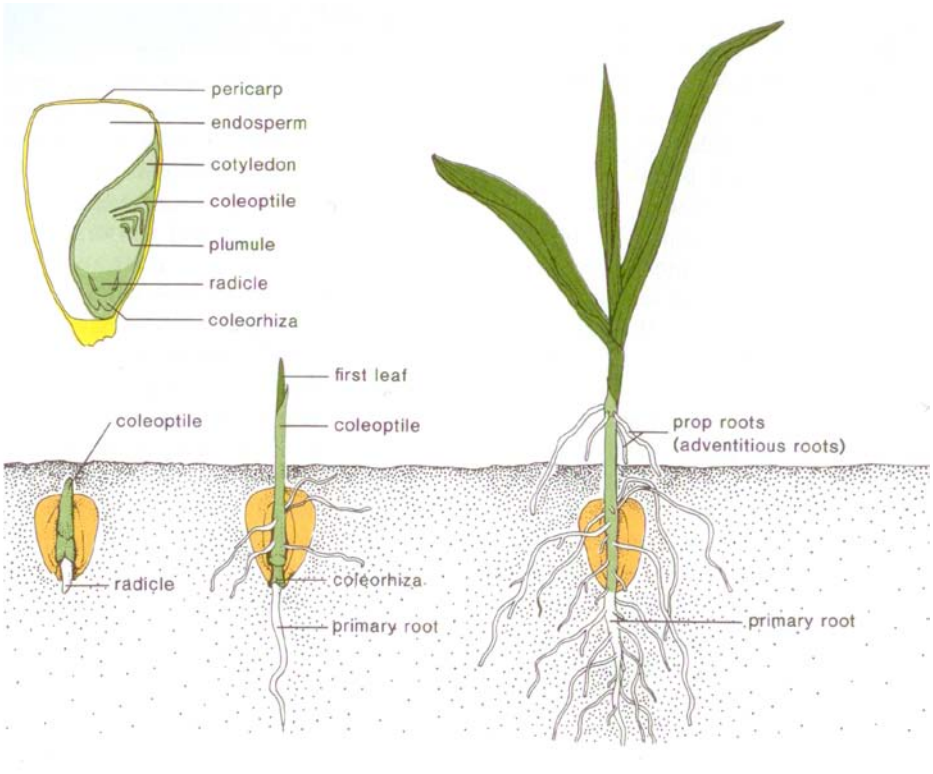


Figure 1.1 Maize seeds (a) before imbibition and (b) after imbibition for 48 hours

The process of imbibition in maize is clearly described by McDonald *et al*. (1994). They suggest that water is absorbed through two separate pathways, the first resulting in near full embryo hydration within 15 hours and the second resulting in endosperm hydration that can take up to 48 hours or more. They found the moisture content of the embryo to be greater than 50% whereas the moisture content of the endosperm was only 25 – 30%. The endosperm can therefore continue to imbibe water after germination. In maize, the embryo is positioned to one side of the seed (Figure 1.2(a)) and imbibition of the embryo is more rapid if that side is in contact with moisture than if the side containing only endosperm is in contact with moisture (Stern, 1991).

After water has been imbibed, enzymes begin to function in the rehydrated protoplasm (Stern, 1991). Respiration releases the energy needed to initiate growth of the embryo, and a new plant begin to develop as mitosis and cell elongation take place. (Figure 1.2(b)).

(a)



(b)

Figure 1.2 Maize (a) seed structure and (b) germination and development of the seedling (Stern, 1991)

The initial energy of the embryo is often supplied through anaerobic respiration, with aerobic respiration furnishing the energy as soon as the splitting of the seed coat admits oxygen (Stern, 1991). If seeds are kept waterlogged after planting, oxygen available to them is greatly reduced and germination then may not commence at all or fail to proceed fully.

Dimsey (1995) studied the effect of temperature on germination. According to this study, most seeds require temperatures within certain ranges to germinate. These usually need to be above freezing but below 45°C. Germination percentages tend to be low approaching either extreme, however. Stern (1991) reported that nearly all summer crop species have an optimum (ideal) germination temperature of between 20°C and 30°C. The temperatures for optimum germination of maize seed range from 16°C to 35°C (Dimsey, 1995).

1.2.1.2 Seedling vigour

The component of seed quality that has a bearing on field emergence potential is seedling vigour. Seedling vigour is defined as "those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions" (AOSA, 1983). Seedling vigour testing has become an increasingly important component of seed testing and more accurately reflects the potential performance of a seed batch if stress would be encountered in the field at planting.

In a seedling vigour study, Burris (1979) found that high vigour seed batches have the capacity for greater emergence and seedling survival than low vigour seed batches. He also suggested seedling vigour as a complex property which is influenced by factors such as the genetic constitution of seed and on events occurring during seed development, harvesting, conditioning and storage. In his study, he also stated that seed ageing is the major cause of reduced viability and vigour. Physiological symptoms of ageing include reduced rate of germination, emergence and seedling growth; production of abnormal seedlings and decreased tolerance to suboptimal conditions (Burris, 1976; Johnson & Wax, 1978). Biochemical changes taking place during deterioration are membrane degradation resulting in solute leakage; enzyme, respiration and hormonal changes; impaired protein and RNA synthesis and accumulation of toxic metabolites (Woodstock & Grabe, 1967; Roos, 1980). Vigour deteriorates faster than germination with age, *i.e.* the loss of vigour precedes the loss of the ability to germinate (Delouche & Caldwell, 1960). Although seed batches may have similar high germination percentages, they can differ in their physiological age, vigour, and thus their ability to emerge in the field.

Harrington (1972) reported that maximum vigour is achieved at the end of seed filling and thereafter declines during further dehydration on the plant. The concept of seed vigour was developed on the basis of the observation that two seed batches or genotypes with similar viability performed differently under stressful field conditions (Delouche & Baskin, 1973). Seed vigour tests have been proposed for detecting differences in potential seed batch performance. The critical requirements of a vigour test include:

(i) It must provide a more sensitive index of seed quality than does the warm test (McDonald, 1980; Perry, 1984)

(ii) It must better predict planting value of high germinating seed batches than does the warm test (Hampton & TeKrony, 1995)

Munamava *et al*. (2004) suggested that, in general, seed vigour measured by the saturated cold test, showed genotypic differences within and among locations. High protein inbred lines had higher vigour than low protein inbred lines. Similar results have been reported for other crops. Schweize & Ries (1969) reported that oats seeds high in protein content tend to show greater vigour. Also, wheat with high protein content germinated faster and developed into larger seedlings (Lopez & Grabe, 1973).

1.2.2 FACTORS AFFECTING SEED QUALITY

In grain seeds the major factors affecting seed quality are imbibition damage, seed ageing and their interaction (Powell, 1998). The International Rice Research Institute (2003) reported that seed quality is determined by a number of genetic and physiological characteristics. The genetic component involves differences between two or more genetic lines, while differences between seed batches of a single genetic line comprise the physiological component. Genetic factors that can influence quality include: genetic make-up, seed size and bulk density, while physical or environmental factors include:

(i) Injury during planting and establishment

(ii) Growing conditions during seed development

(iii) Nutrition of the mother plant

(iv) Physical damage during production or storage by either machine or pest

(v) Moisture and temperature during storage

(vi) Age or maturity of seed

Deterioration in seed quality may begin at any point in the plant's development stage from fertilization onward (International Rice Research Institute, 2003). They also stated that seed quality depends upon the physical conditions that the mother plant is exposed to during its growth stages, as well as harvesting, processing, storage and planting. According to them, temperature, nutrients and other environmental factors also affect seed development and influence seed quality.

High quality seeds are the result of good production practices, which include:

(i) Proper maintenance of genetic purity

(ii) Optimal growing conditions

(iii) Proper timing and methods of harvesting

(iv) Appropriate processing during threshing, cleaning and drying

(v) Appropriate seed storage and seed distribution systems (International Rice Research Institute, 2003)

1.2.2.1 Influence of pre-harvest stress on seed quality

Environmental stress during seed production frequently reduces seed germination and vigour, thereby increasing seed costs and limiting supplies of high quality seed to producers. Common environmental stresses that occur during seed development and maturation include high or low temperature stress and moisture stress (Spears *et al.*, 1997).

It is well known that seed vigour increases until physiological maturity when the seed has acquired its maximum dry weight, although there remains debate about whether vigour continues to increase subsequently during dehydration (TeKrony & Egli, 1997). Temperature extremes during seed development impact seed fill and subsequent seed quality. High temperatures during seed filling reduce germination and vigour of soybeans (Spears *et al.*, 1997). Low temperatures also reduce seed quality. Immature maize is partially protected from low temperature (frost) injury by the ear and husk (Fick, 1989), but loss of viability and cellular damage can occur, and the level of damage is influenced by seed maturity at the time of exposure and the minimum

temperature. Plant exposure to freezing temperatures can also generate cellular water stress. Stress-related dehydrin-like protein and its corresponding transcript were observed in three day-old seedlings from seed exposed to freezing (Hartwigsen & Goggi, 2002), suggesting that the effects of stress during seed development can be exhibited in the seedlings.

1.2.2.2 Effect of molybdenum

Tanner (1979) found in a study that the molybdenum content of maize seed can vary over a wide range depending on the availability of molybdenum from the soil and applications of molybdenum to the parent plants. He also stated that plant population, leaf mass and yield were all significantly depressed when seed molybdenum content was low. Molybdenum seed dressings may partially overcome the effect, but for optimum growth, both adequate seed molybdenum concentrations and seed dressings may be required (Tanner, 1979). Weir & Hudson (1966) showed that the occurrence of molybdenum-deficiency symptoms of maize can be related to the molybdenum concentration in the parent seed. Peterson & Purvis (1961) found that germination of molybdenum-deficient seed was slower than normal and a lower proportion of plants survived. It is possible to treat molybdenum-deficient seed with compounds containing molybdenum and thus prevent the development of deficiency symptoms in the succeeding crop, but quality of growth may still be impaired. Burkin (1971) found that yields of peas grown from seed rich in molybdenum, as a result of treatment of the parent crop, were higher than yield of peas grown with ordinary seed moistened with molybdenum before growth.

1.2.2.3 Seed processing

Field emergence and laboratory germination improved when maize seed were harvested at physiological maturity, then shelled (thrested) and mechanically cleaned using an air-screen cleaner, followed by a gravity separator (Asiedu *et al*. 2003). This significantly increased the field emergence, laboratory germination, pure seed and 1000-seed weight of maize.

Previous research has shown that drying temperatures above 43°C are detrimental to the viability of maize seeds (Muckle & Stirling, 1971). During drying, moisture is initially removed from the

outer layers, creating a moisture gradient from the centre to the periphery. If a high drying temperature is used, this gradient is steep and thus causes stresses between the interior and exterior layers, so that the seed develops stress cracks (Hampton, 1992). Most cracks develop in rough rice within 48 hours after drying (Sharma & Kunze, 1982), when the moisture gradient within the grain is reduced (Kunze, 1996).

However, Thompson & Foster (1963) showed that reducing moisture in maize seeds by 4 to 5% per drying pass with high temperature drying, decreased the development of stress cracks. Watson (1987) confirmed these findings and suggested that stress cracking of maize is further prevented by limiting the rate of drying. On the other hand, Kato & Yamashita (1979) suggested that high storage temperatures after drying rice would help to reduce the development of cracks.

1.2.2.4 Seed storage

The purpose of seed storage is to preserve planting stocks from one season to the next. The objective of seed storage is to maintain seed quality for the longest duration possible. This approach creates a greater diversity in seed inventory and provides a guarantee of seed supply in years when acceptable level of quality seed production is low. In addition to providing seed for the next planting and creation of seed inventory diversity, seed storage enables the maintenance of germplasm over time for improved plant breeding programmes (Modi, 2004). Evidence of seed viability after decades (Priestley, 1986) and even centuries (Porsild & Harrington, 1967) of storage, emphasizes the value of seed storability in germplasm maintenance.

To maintain seed viability during storage, high quality seeds must be selected before they are placed in storage (Modi, 2004). Water content and temperature are the most important factors determining seed storability. According to Modi (2004), some subsistence farmers in South Africa use smoke as an indigenous method of maize seed storage, and the method was found to have merit with respect to seed quality enhancement.

Seed batches that are similar in germination may differ in deterioration level (Delouche & Caldwell, 1960) and they may differ substantially in field or storage performance (Perry, 1980;

Naylor, 1981; Kolasinska *et al*., 2000). Bernal-Lugo & Leopold (1995) suggested that seed sugar content, particularly the ratio of oligosaccharides to sucrose, might be used as an indicator of seed storability. In maize, for example, a ratio higher than 0.2 corresponds to a good seed storability. Oligosaccharides might contribute to the stabilization of intracellular glasses by increasing cytoplasmic viscosity and the glass-to-liquid transition temperature, which are likely to slow down ageing alterations (Bernal-Lugo & Leopold, 1995).

1.2.2.5 Seed ageing

Seed ageing is a well-recognized cause of differences in seed quality in all species, including grain species (Matthews, 1980; Powell *et al*., 1984). Grain legumes, with their large cotyledons, provide an excellent example of how extensive deterioration can occur before the seed fails to germinate. Initially, membrane deterioration results in increased leakage from living cells of the cotyledons (Powell & Matthews, 1977), while further deleterious sub-cellular changes lead to the development of areas of dead tissue and even greater solute leakage. Subsequently, as deleterious changes in enzymes, respiration, synthetic processes and macromolecules accumulate both within the cotyledons and the embryo axis, the seed fails to germinate (Powell *et al*., 1984).

Powell (1998) reported that it is envisaged that the membranes of aged seeds, whose integrity has been reduced by deterioration, are more susceptible to the physical damage resulting from rapid imbibition and hence show greater imbibition damage. Thus seeds that have low vigour as a result of ageing during storage will be more likely to have poor field emergence both due to seed deterioration and, also if soil conditions are wet, due to increased susceptibility to imbibition damage.

Accumulation of Active Oxygen Species (AOS) and free radicals has often been considered as important factors of seed ageing (McDonald, 1999). The ability of seeds to withstand desiccation during maturation and their tolerance to ageing might then be related, at least partly, to their ability to scavenge AOS in order to avoid deleterious events such as lipid peroxidation caused by these compounds (Vertucci & Farrant, 1995). Bailly *et al.* (2000) reported that these mechanisms would involve enzymes such as superoxide dismutase (SOD), catalase (CAT) and enzymes of

the ascorbate-glutathione cycle, or antioxidant compounds such as reduced glutathione or ascorbate. They also mentioned that seed germinability might be related to the efficiency of free radical scavenging because this scavenging may affect merely seed storability and vigour.

The sensitivity of seeds to accelerated ageing is a good indicator of their vigour in various species (McDonald, 1999). According to Demir & Ellis (1992), seed vigour increased slightly after the end of seed filling. Such results contradict the view that maximum seed quality coincides with physiological maturity and thereafter declines (Harrington, 1972).

1.2.3 FACTORS AFFECTING GERMINATION OF SEED AND EMERGENCE OF SEEDLINGS IN THE FIELD

Burris (2000) reported the importance of seed quality as a factor affecting early performance and productivity of most agricultural crops. In a previous study, Burris (1977) mentioned that seed quality includes the ability of seed to germinate and emerge under optimal environmental conditions. According to Burris (1977) growing season differences, such as temperature and moisture, and the interaction of temperature and moisture is important factors affecting germination of seed and emergence of seedlings in the field.

1.2.3.1 Moisture

Waterlogging results from the ponding of water, and affects about 12% of the agricultural soils in the USA (Boyer, 1982). Waterlogging can be caused by inadequate drainage of soils after intense rainfall, excessive irrigation, or from a rising water table (Boyer,1982; Scott *et al.*, 1990). Inadequate oxygen supply for root respiration is the main cause of reduced yield under waterlogged conditions (Grable, 1966; Russel, 1977). Lack of oxygen inhibits nitrogen and mineral uptake, and inhibits root growth and nodulation in soybean (Sallam & Scott, 1987). Crop growth rate was usually affected only when the waterlogging stress was applied for more than two consecutive days (Griffen & Saxton, 1988; Scott *et al.*, 1989).

In South Africa, waterlogging is most often associated with salination (De Villiers *et al*., 2005). Factors involved in potential salination at irrigation areas are soil suitability, poorly designed and operated irrigation systems, and water quantity and quality. In 1990, 54 000 ha of cultivated land was seriously alkaline and waterlogged, while 128 000 ha was moderately affected, that is about 15% of the area under regular irrigation. Waterlogging, mostly associated with irrigation, is an incessant, countrywide problem with salinisation / sodication of both soils and water being a threat to irrigated agriculture.

Waterlogging is largely under control at State controlled irrigation schemes. The fate of mostly privately developed small-scale irrigation schemes, not necessarily conforming to South Africa's well-established selection criteria for irrigated land, could, however, have a devastating effect on the sustainability of such irrigation schemes (De Villiers *et al*., 2005).

Flooding of recently planted fields of maize in the spring can result in poor emergence of some germplasm. Studies from Martin *et al*. (1991) have shown genetic variation for flooding tolerance of maize inbreds and hybrids. Respiration rates of flood tolerant inbreds increased faster and to a higher level after flooding and vending than those of sensitive inbreds (Cerwick *et al*., 1995). The results of these studies and that of Crawford and Zochowski (1984), have led to the hypothesis that the end-products of anaerobic metabolism (ethanol and acetaldehyde) are toxic to seeds, and that flooding leads to increased cellular concentrations of these compounds and to death of the seed (Martin *et al*., 1991).

Wuebker *et al*. (2001) also indicated that if seed are further into the germination process when flooding occurs, the seed are more susceptible to flooding stress. The duration of flooding is an important consideration in anticipating losses due to flooding. They also indicated that flooding for as briefly as 1 hour has the potential to cause significant losses in germinability, however, when the duration of flooding increased to 48 hours, the potential loss in seedling germinability was even greater. The loss in germinability at 1 hour may be associated with physical damage to the seed, while the loss of germinability at 48 hours may be associated with physiological damage to the seed (Woodstock & Taylorson, 1981). The excess water could cause imbibitional

damage to the seed membrane, which the seed is unable to repair (Tully *et al*., 1981; Ladror *et al*., 1986).

Ueno & Takahashi (1997) reported that soaking of seeds for 24 hours to 192 hours in water before sowing, resulted in poor germination of most plants. They also reported varietal differences in flooding tolerance of seeds of maize, soybean and barley. According to them soak-induced inhibition of germination and amounts of ethanol excreted were increased with soaking duration.

1.2.3.2 Temperature

Temperature plays an important role in germination and establishment of seedlings. Research has shown that the rate of hypocotyl elongation increases as temperature increases. Hatfield & Egli (1974) found that at 10°C, soybean (*Glycine max* L.) hypocotyl elongation was extremely slow and that the rate of hypocotyl elongation reached a maximum at 30°C. Alm *et al*. (1993) indicated that as temperature increased from 10 to 25°C, the seedling elongation rate for maize and soybean increased.

Exposure of developing seeds to freezing temperatures decreases seed germination in many species: pea (*Pisum sativum* L.) (Vertucci, 1989), sunflower (*Helianthus annuus* L.) (Zimmerman & Zimmer, 1978), winter rape (*Brassica napus* L.) (Lardon & Triboi-Blondel, 1994) and maize (*Zea mays* L.) (Fick, 1989). The severity of germination loss was influenced by the interaction of three factors: seed maturity, temperature to which seeds were exposed, and duration of exposure.

Regardless of species, immature seeds were susceptible to relatively mild freezing treatments, while seeds that had reached maximum dry seed weight showed little loss of germination. As the freezing treatments increased in severity, either by decreasing air temperature or increasing the duration of exposure, germination loss were also increased (Woltz *et al*., 2006).

Although freezing injury has been shown to reduce germination of immature seeds, few studies have investigated the impact of freeze injury on seed vigour. Fick (1989) reported declines in germination and seed vigour as measured by cold test germination when seeds were frozen at -6 °C at various stages of seed development.

1.2.3.3 Interaction between moisture and temperature

Hou & Thseng (1991) studied the interaction of temperature and flooding. Their research indicated that at 10°C and 15°C, 2 to 8 days of soaking soybean seed prior to planting did not significant affect seed germination. But, at 25°C and 30°C, germination was significantly reduced as the length of soaking increased, and a complete loss in germination occurred when seed were soaked for 4 days at 30°C.

Wuebker *et al*. (2001) indicated that the duration of flooding stress had a significant interaction with germination temperature on germination percentage, total seedling dry weight yield, and average seedling dry weight yield for soybean seed. Flooding for as little as 1 hour at 15°C lowered germination percentage by over 20% compared to the non-flooded control. It took approximately 36 more hours of flooding for seed at 25°C to experience the same loss in germination as 1 hour of flooding at 15°C. Results for total seedling dry weight yield and average seedling dry weight yield had a similar trend.

The genotypic differences in germination due to soaking, indicate that inhibition of germination at <15°C was possibly due to a different mechanism or sensitivity than inhibition at >15°C (Martin *et al*., 1991). The difference in germination at <15°C could be a result of imbibitional chilling injury (Cal & Obendorf, 1972). The inhibition of germination between 15°C and 35°C was of a metabolic nature. The effects of soaking seeds at 35°C to 40°C resulted in complete inhibition of germination (Woodstock & Taylorson, 1981). Common biochemical processes occur in seeds of plants of tropical or subtropical origin during anaerobic soaking and during field imbibition below 10°C. Maize seeds generally do not germinate and grow when imbibed below 10ºC (Eagles, 1982).

1.2.3.4 Light

Light-controlled germination has been associated with phytochrome since the pioneering work of Borthwick (1952). The sensitivity of seeds to the spectral quality of the light mediated by phytochrome is a frequent natural process within species that colonize open areas (Ballaré, 1994).

Godoi & Takaki (2004) reported that many seeds are insensitive to light, but in a number of species germination is stimulated or inhibited by exposure to continuous or short periods of illumination. They also reported that maize, the smaller cereals, and many legumes, such as beans and clover, germinate as well in light as in darkness. Inhibition by light is found in chive, garlic, and several species of the lily family (Godoi & Takaki, 2004). They also indicated that sometimes, imbibed (wet) seeds that do not germinate at all in darkness may be fully promoted by only a few seconds or minutes of white light.

The best studied case of this type, according to Godoi & Takaki (2004), concerns seeds of the Grand Rapids variety of lettuce, which is stimulated to germination by red light (wavelength about 660 nanometres) but inhibited by “far red” light (wavelength about 730 nanometres). Alternations of the two treatments to almost any extent indicate that the last treatment received is the decisive one in determining whether the seeds will germinate (Godoi & Takaki, 2004).

1.2.4 POTENTIAL AND GENERAL TESTS TO DETERMINE SEED QUALITY

Seed quality is one of the primary factors affecting early performance and productivity of most agricultural crops. The fundamental objective of seed testing is to establish the quality level of seed. The most obvious component of seed quality is germination capacity and germination tests are used worldwide to determine the maximum germination potential of a seed batch under optimum conditions. The seed industry uses the standard germination test (AOSA, 2002) for labeling. The standard germination test, also known as the warm test, estimates germination under ideal growing conditions (Munamava *et al*., 2004).

Seed quality tests should relate to field emergence. Many researchers have reported significant correlation coefficients between field emergence and standard laboratory germination tests. However, inconsistencies and difficulties with the prediction of field emergence have also been reported (Kolasinska *et al*., 2000).

Unlike germination testing, most vigour tests do not give a result that provides an absolute vigour value (Hampton & Coolbear, 1990). Vigour test results are not expected to predict an exact value for field emergence for example, because soil and seedbed conditions vary from field to field. What a vigour test should do is consistently rank seed batches in terms of their vigour status (Hampton, 1992), and hence, provide further information as to their performance potential.

Vigour is a complex phenomenon that cannot be quantified or measured directly. However, various tests that are able to rank seed batches according to vigour level have been developed for various crops. Vigour tests can be grouped into three general categories according to Pollock & Roos (1972) and McDonald (1975):

(i) Seedling growth and evaluation tests
(ii) Stress tests (e.g. cold test and accelerated ageing test)
(iii) Biochemical tests (e.g. electrical conductivity test and tetrazolium test)

According to Delouche & Baskin (1973), vigour tests have proven to be more useful as predictors of field emergence than the warm test. The concept of seed vigour was developed on the basis of the observation that two seed batches or genotypes with similar viability performed differently under stressful field conditions (Delouche & Baskin, 1973). Seed vigour tests therefore have been proposed for detecting differences in potential seed lot performance. The critical requirements of a vigour test include:

(i) A more sensitive index of seed quality than the warm test (McDonald, 1980; Perry, 1984)
(ii) A more accurate prediction of planting value of high germinating seed batches than the warm test (Hampton & TeKrony, 1995)

1.2.4.1 Warm test

The warm test describes the percentage of normal seedlings under optimal conditions specified by the International Seed Testing Association (ISTA, 1993). This test, commonly used to evaluate seed quality, is able to predict field emergence provided the conditions for emergence are favourable (Kolasinska *et al*., 2000).

The objective of the warm test is to estimate the germination potential of a seed batch, which can then in turn be used to compare the quality of different batches, and also to estimate the value of field planting (ISTA, 1999). The methodology of the warm test has been refined to a high level of reproducibility and reliability. However, the warm test does not always indicate seed batch potential performance, especially if field conditions are less than optimal (Hampton & TeKrony, 1995). Seed batches that are similar in germination may differ in deterioration level (Delouche & Caldwell, 1960) and they may differ substantially in field or storage performance (Perry, 1980; Naylor, 1981; Kolasinska *et al*., 2000).

Kolasinska *et al*. (2000) mentioned that early planting exposes seed to unfavourable conditions and the commonly used warm test cannot accurately predict field emergence. They also reported that vigour tests have proven to be more useful as predictors of field emergence than the warm test.

1.2.4.2 Cold test

Cold, wet soils have long been associated with poor field performance in many crops, especially in temperate regions where seeds are often planted in early spring. Field conditions at this time can be adverse due to high soil moisture, low temperatures and soil-borne fungi, and these factors can cause poor field emergence. The cold test was developed to simulate these adverse field conditions and measure the ability of maize seeds to emerge (TeKrony, 1983; Ferguson, 1990; Hampton, 1992). There are a large number of reports in the literature showing that seeds of many species, including maize, suffer damage during the early stages of imbibition that is enhanced at low temperatures, especially in seed batches of low vigour (Cal & Obendorf, 1972; Harrison, 1973; Bedi & Basra, 1993). This effect is utilized in the standard ISTA cold test (Hampton & TeKrony, 1995). This test is widely used for assessing the vigour of maize seeds in

Europe and North America where seeds are sown under cool and wet conditions in spring. The cold test is conducted under wet conditions (field capacity) at 10°C, although lower temperatures may be more effective. However, imbibitional damage at higher temperatures, although less severe, can also reduce the percentage of normal seedlings that are produced, an effect which is greatly enhanced by low seed vigour (Bruggink *et al*., 1991).

Munamava *et al*. (2004) reported that, in general, seed vigour measured by the cold test, showed genotypic differences within and among locations. High protein inbred lines had higher vigour than low protein inbred lines. Similar results have been reported for other crops. Schweize & Ries (1969) reported that oats seeds high in protein content tend to show higher vigour. Lopez & Grabe (1973) reported that wheat with high protein content germinated faster and developed into larger seedlings than wheat with lower protein content.

1.2.4.3 Soak test

In the early 1980s, Pioneer Hi-Bred International plant breeders began evaluating an alternative germination test, the soak test, for its ability to predict field emergence. The soak test measures seed germination, according to the warm test, after a 48 hour soak in water at 27°C. The concept of the soak test evolved from unpublished observations made by Reddy of Iowa State College in the late 1940's. Dr. Reddy observed that germination of various batches of maize seed planted in flower pots that were flooded at room temperature for a few days and then drained was similar to cold test of that seed (Martin *et al*., 1988).

The idea of the soak test lay dormant for many years, since correlations between the soak test and field emergence of maize hybrid seed were lower than between the cold test and field emergence. Interest in the soak test was rekindled because of a growing demand for a germination test that could be quickly and inexpensively run on large numbers of individual ears for selection purposes (Martin *et al*., 1988).

Accurate prediction of field emergence is difficult, however, but it is essential if the seed industry is to produce and sell high quality maize seed. The ability of plant breeders to select

vigorous, cold-tolerant inbred lines is an essential step towards this goal because hybrid germination under cold stress is strongly influenced by the maternal parent (Burris, 1977). Although the soak test is not completely accurate, it should improve the chances of developing new inbred lines with good field emergence (Martin *et al*., 1988).

Martin *et al*. (1988) stated that there were highly significant positive correlations between the cold test and field emergence and the soak test and field emergence. There were also significant negative correlations between amounts of ethanol excreted into the soak water (after 24 or 48 hours soaking) and field emergence. The highest negative correlations generally occurred when soil temperatures were low during field germination, but not below freezing. They also found a significant negative correlation between ethanol concentration in the soak water and subsequent germination in the soak test.

Ueno & Takahashi (1997) mentioned that soaking of seeds of most upland plants in water before sowing, resulted in poor germination. They also reported varietal differences in flooding tolerance of seeds in maize, soybean and barley. According to them, soak-induced inhibition of germination and amounts of ethanol excreted were increased with soaking duration.

Cerwick *et al*. (1995) reported that flooding of recently planted fields to maize in the spring results in poor emergence of some germplasm. In the same study, they also found that reduction of germination after soaking was partially reversed by venting the seed with ambient air after soaking and prior to planting. According to them respiration rates of flood tolerant inbreds increased faster and to a higher level after flooding than those of sensitive inbreds.

1.2.4.4 Electrical conductivity test

The electrical conductivity test provides a measurement of electrolyte leakage from plant tissues and was first recognized in seeds of several crop species by Hibbard & Miller (1928). Electrical conductivity measurement of the soak water in which a bulk sample (25 seeds) has been steeped identifies seed batches that have high laboratory germination, but poor field emergence potential. Such seed batches have high electrolyte leakage and are classified as having low vigour, while

those with low leakage are considered to have high vigour (Hepburn *et al*., 1984). Pandey (1994) completed an extensive review of the basis for electrical conductivity as a seed quality test. Pandey (1994) confirmed the findings of Hepburn *et al*. (1984) and stated that the electrical conductivity test can be used as a vigour test.

Changes in the organization of cell membranes occur during the development of seeds prior to physiological maturity, seed desiccation before harvest, and during imbibition prior to germination (Abul-Baki, 1980). The integrity of cell membranes, determined by deteriorative biochemical changes or physical disruption, can be considered the fundamental cause of differences in seed vigour which are indirectly determined as electrolyte leakage during the conductivity test (Powell, 1988). As a seed re-hydrates during early imbibition, the ability of its cellular membranes to reorganize and repair any damage that may have occurred will influence the extend of electrolyte leakage from the seed. The greater the speed with which the seed is able to re-establish its membrane integrity the lower the electrolyte leakage. Higher vigour seeds are able to reorganize their membranes more rapidly, and repair any damage to a greater extend, than low vigour seeds. Consequently, electrolyte leakage measured from high vigour seeds is less than that measured from low vigour seeds.

Matthews & Bradnock (1968) indicated that the leakage from low vigour seed batches also causes secondary effects, in that nutrients exuded from seeds during germination stimulate soil microorganism activity and secondary infection. A positive correlation has been reported by Keeling (1974) between the quantity of carbohydrates exuded from seeds and seedling performance.

1.2.4.5 Accelerated ageing test

Accelerated ageing was initially developed as a test to estimate the longevity of seed in commercial storage (Delouche & Baskin, 1973), and has been used to predict the life span of a number of different species, included maize seed. The test has subsequently been evaluated as an indicator of seedling vigour and has been successfully related to field emergence and stand establishment (TeKrony, 1994). The accelerated ageing test exposes seeds for short periods to

the two environmental variables which cause rapid seed deterioration; high temperature and high relative humidity. High vigour seed batches will withstand these extreme stress conditions and deteriorate at a slower rate than low vigour seed batches.

Early storage studies by Helmer (1962) and Baskin (1970) suggested that accelerated ageing could be utilized as a vigour test to predict field performance. Additional studies have shown that this vigour test functions quite well in forecasting field emergence and stand establishment in a wide range of crop species, included maize. When maize seeds are planted under stressful field conditions, accelerated ageing germination provides higher correlations with field emergence than does the warm test. In the accelerated aging test, the germination of the seed prior to accelerated aging is compared with its germination after accelerating aging and the difference between the two indicates the relative vigour of the sample tested (TeKrony, 1994).

1.2.4.6 Complex stressing vigour test

The complex stressing vigour test was first developed for wheat, and later modified for maize, to provide an indication of the minimum expected range of emergence under the stress conditions imposed by Hungarian soils (Szirtes & Barla-Szabo, 1981). Later Barla-Szabo & Dolinka (1984) modified the method to simulate several different stress conditions which the seed batch may be subjected to in the field, as opposed to other vigour tests which concentrate on a single form of stress. In determining the stress conditions, account was taken on the "worst case" Hungarian spring sowing conditions, an event likely to occur once in ten years. The test has been widely used in Hungary and has consistently identified low and high vigour seed batches, with the result that desired populations of maize can be more frequently achieved (Barla-Szabo & Dolinka, 1988; Barla-Szabo *et al*., 1990).

The test imposes temperature and oxygen deficiency stress by soaking seeds for 48 hours at moderate temperatures (25°C) followed by another 48 hours soak at low temperatures (5°C). The soaking period promotes the initiation of biochemical activity within the seed, but as a result of the permanent oxygen deficiency which soon occurs, these processes slow down and may eventually stop. Cell membranes of seeds in a weaker physiological condition progressively lose

their biochemical control functions (Laudman *et al*., 1979), and leaching of cell contents occurs. The low temperature may cause further physiological damage to seeds suffering from chronic oxygen deficiency, but in the absence of soil borne micro-organisms (Barla-Szabo & Dolinka, 1988).

Barla-Szabo & Dolinka (1988) showed that for 20 maize seed batches, the complex stressing vigour test results were more strongly correlated with field emergence at three spring sowings than warm or cold test results. Barla-Szaba & Dolinka (1988) suggested that under good spring sowing conditions, field emergence would approach the percentage standard germination, but under unfavourable conditions, field emergence would be closer to the complex stressing vigour test results.

The complex stressing vigour test was developed to simulate wet (anaerobic) and cold stress conditions which can occur in the temperate zone of Europe, the Americas and Asia, but probably has little application for other regions of the world. Van de Venter *et al*. (1993) reported that the complex stressing vigour test was no better than the warm test for predicting field emergence of dryland wheat in South Africa.

1.2.4.7 Tetrazolium test

The tetrazolium test is a biochemical test which measures certain metabolic events in seeds associated with germination. This test is essentially a measurement of dehydrogenase enzyme activity. These enzymes reduce the colourless tetrazolium chloride salt to form a water insoluble red compound, formazen, which “stains” living cells a red colour while dead cells remain colourless. Lakon (1942) first developed this technique for seed testing and later the staining patterns of the tissues were used to assess vigour. The advantage of the tetrazolium test is that it requires no elaborate facilities. The test is subjective and reproducible results are difficult to achieve. In the tetrazolium test, seeds are classified as good, medium or poor in vigour (Moore, 1972).

1.2.4.8 Fast green test

According to Koehler (1957), the fast green test reveals physical fractures in the seed coat of light coloured seeds and is often used in maize. Seeds are soaked in a 0.1% solution of fast green stain for 15 – 30 seconds. During this period, the vital stain penetrates any area of the seed which has lost its physical integrity and stains it green. After the soak period, the seeds are washed under running water for 20 seconds and any deformations are clearly delineated by green markings.

1.2.4.9 Emergence rate test

Speed of emergence of seedlings is one of the oldest seed vigour concepts. Seed batches with similar total germination often vary in their rate of emergence and growth. A number of methods for determining emergence rate have been employed. The higher the emergence rate, the higher the seed vigour (Tucker & Wright, 1965; Nichols & Heydecker, 1968). Maguire (1962) suggested the following formula to measure emergence rate:

$$\text{Emergence rate} = \left(\frac{number\ of\ normal\ seedlings}{days\ to\ first\ count} + \ldots + \frac{number\ of\ normal\ seedlings}{days\ to\ final\ count}\right)$$

1.2.4.10 Ethanol excretion during waterlogged conditions

Aerobic imbibition and anaerobic soaking of maize seed results in the excretion of ethanol during the time between imbibition and exsertion of the radicle (Leblova *et al*., 1976; Crawford, 1977). The amounts of ethanol excreted during soaking of rice (*Oryza sativa* L.), lettuce (*Lactuca sativa* L.), maize, broadbean (*Vicia faba* L.), and pea (*Pisum sativum* L.) seeds were shown to be negatively correlated with subsequent laboratory germination after soaking in nonaerated water for 72 hours (Crawford, 1977). High amounts of ethanol excreted by various seed batches of muskmelon (*Cucumis melo* L.) during germination in an enclosed aerobic environment were associated with reduced vigour (Pesis & Ng, 1984).

Martin *et al*. (1988) found significant negative correlations, between amounts of ethanol excreted into the soak water, after 24 or 48 hours and field emergence of different maize inbred lines. The highest negative correlations occurred when soil temperatures were low during field germination but not below freezing. They also found a significant negative correlation between ethanol concentration in the soak water and subsequent germination in the soak test.

Eagles (1982) suggested that maize seeds generally do not germinate and grow when imbibed below 10ºC. Maize seeds imbibed at 10°C perform anaerobic respiration and release ethanol into the environment (O'Neil, 1987). This may occur due to an increase in the activation energy of the mitochondrial oxidative system at low temperatures relative to the constancy of the activation energy of glycolysis (Raison, 1980). Soaking seeds in a cup of water (soak test) results in similar metabolic events. Initially, seeds take up water and oxygen. The oxygen content of the water is depleted within 4 hours. Ethanol synthesis is linear for the entire 48 hours of soaking. The net result of this process is an increase in ethanol content of the soak water resulting in concentrations between 0.02 to 0.04 M ethanol after 48 hours. The similarity between seed germination after imbibition in cold, wet soils and after an anaerobic soak, might then be the ability of a genotype to either limit accumulation of the end products of anaerobic metabolism (ethanol, acetaldehyde, and carbon dioxide) or to withstand the presence of these metabolites (Martin, 1986; O'Neil, 1987).

Martin *et al*. (1991) reported that the end products of anaerobic metabolism (ethanol and acetaldehyde) are toxic to seeds, and that flooding leads to increased cellular concentrations of these compounds and to death of the seed. These studies suggest that seeds may have different biochemical and physiological responses to flooding than do roots or whole plants, and that some interspecific differences in biochemical responses to anaerobic metabolites might exist. For example, in maize, alcohol dehydrogenase has been found to be an inducible enzyme in roots (Sachs *et al*., 1980), while it is constitutive in the seed (VanToai *et al.*, 1985). In this regard, ethanol synthesis and excretion during maize seed germination seems to be an obligatory part of carbohydrate metabolism during this developmental phase (Leblova *et al*., 1974), while in roots it is induced by anaerobic stress (Sachs *et al*., 1980).

Nagodawithana & Steinkraus (1976) suggested that external application of ethanol was not as toxic as internal synthesis. Because the embryo undergoes anaerobic respiration, ethanol concentration around the embryo should be higher than that in the soak water.

Andrews & Pomeroy (1979) reported that during anaerobic stress of ice-encased wheat (*Triticum aestivum* L.) seedlings, cellular death may occur due to a synergistic interaction between carbon dioxide and ethanol, but external application of these metabolites was not as toxic as internal synthesis. Crawford & Zochowski (1984) reported a similar result with chickpea (*Cicer arietinum* L.) seedlings. They indicated that a circulated anaerobic atmosphere was less toxic to seedlings than a static atmosphere.

Martin *et al*. (1991) indicated that maize seeds were less sensitive to imbibition in an anaerobic gaseous environment compared to an aqueous environment. In addition, application of external ethanol under aerobic conditions had little effect on seed germination and had a negative effect only when it was supplied in an anaerobic atmosphere. These different responses may have been due to accumulation of greater amounts of carbon dioxide, a known inhibitor of respiration (Bendall *et al*., 1958), in seeds during soaking than in the anaerobic gas treatment, due to slower rates of diffusion of carbon dioxide into water compared with Argon gas. Improvement of subsequent germination after soaking by continuously purging the soak water with nitrogen gas, which would displace other gases, also indicates that a volatile metabolite may accumulate in the seeds during soaking, resulting in inhibition of germination (Bendall *et al*., 1958).

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CHAPTER 2

EFFECT OF COLD AND WET CONDITIONS ON GERMINATION AND EMERGENCE OF MAIZE (*Zea mays* L.) HYBRIDS

2.1 INTRODUCTION

In recent years, some of South Africa's top maize (*Zea mays* L.) hybrids have shown emergence problems under very wet and cold conditions, or other conditions that is sub-optimal for emergence. The influence of flooding and low temperatures on maize seed germination and emergence has not been widely studied, especially not in South Africa. Due to South Africa's different growing season and the fact that commercial maize was traditionally planted under dry land conditions, there was no need to plant early in the season. As more maize productions are done under irrigation, and the importance of more than one crop per season takes importance due to economic circumstances, maize is planted earlier in the season under more unfavourable climatic conditions. The literature also indicated that limited relevant research on this topic had been done over the last few years. Langan *et al*. (1986) indicated that flooding for three days, starting one day after planting, delayed emergence of maize, soybean and wheat. Temperature plays an important role in germination and the establishment of seedlings. Alm *et al*. (1993) indicated that as temperature increased from 10 to 25°C, the seedling elongation rate for maize and soybean increased.

Waterlogging results from the ponding of water over a poorly drained field after heavy rainfall or excessive irrigation (Boyer, 1982). Waterlogging can also result from a rising water table (Scott *et al*., 1990). Inadequate oxygen supply for root respiration is the main cause of reduced yield under waterlogged conditions (Grable, 1966; Russel, 1977). Crop growth rate was usually only affected when the waterlogging stress was applied for more than 2 consecutive days (Griffen & Saxton, 1988; Scott *et al*., 1989).

Waterlogging associated with high water tables is generally regarded as detrimental to crop production, because prolonged waterlogging reduces soil oxygen concentrations and subsequently root density and depth. Van der Merwe *et al*. (1999) reported the results of Streutker *et al*. (1981) that under low frequency irrigation conditions at the Vaalharts State Irrigation Scheme, with groundwater of fairly low salinity, both cotton and wheat yield increased by almost 50% when grown above a 1.2 m deep water table. A water table of <1.2 m caused the

soil to salinise, sodify and compact with a resultant decrease in seed germination, emergence and growth.

Flooding of recently planted fields of maize in the spring, results in poor emergence of some germplasm. Previous studies have shown genetic variation in flooding tolerance of maize inbreds and hybrids. Respiration rates of flood-tolerant inbreds increased more rapidly and to a higher level after flooding, than those of sensitive inbreds (Cerwick *et al*., 1995).

In the Northern U.S Corn Belt and in Europe, maize is generally planted in early spring, into soils that are, or may become, too cold and wet, resulting in reduced field emergence, poor stands, and lower economic yields. For this reason, improvement and prediction of seedling emergence and early seedling growth is important to the seed industry (Grogan, 1970; Hardacre, 1985). Variables that have been identified to affect seed germination and field emergence in cold and wet soil, are genotype (Grogan, 1970; Eagles, 1982), seed source or production environment (Burris, 1977), and initial seed moisture content (Cal & Obendorf, 1972).

Maize varieties differ in their minimum germination temperature (Segeta, 1964). Pollmer (1969) examined 415 varieties. At 9°C, 89% of the varieties had germinated after 12 days. Only 2.3% had germinated after 12 days at 6°C. Perry (1980) also found several varieties that were able to germinate at 8°C. Earlier varieties exhibit a higher germination rate at lower temperatures than late varieties (Segeta, 1964). Many earlier maturing varieties have since become available, probably with even lower minimum germination temperature requirements. Temperatures below 6°C cause direct damage to the seeds as a result of imbibitional chilling injury (Fuchs, 1983).

In some maize production regions in South Africa unfavourable climatic conditions are sometimes experienced at planting. Although accurate prediction of field emergence is difficult, it is an essential criterium of seed quality if the seed industry is committed to produce and sell high quality maize seed (Martin *et al*., 1988). The standard germination test, commonly known as the “warm test”, measures only germination and seedling performance under ideal planting and climatic conditions. For many years, the cold test has been used as a vigour test to determine seed batch performance under sub-optimal planting conditions. In recent years, some of South

Africa's top maize hybrids, with high cold test scores have shown emergence problems under cold, wet planting conditions. It resulted in major complaints from commercial maize producers with sizable claims involved.

In practice, South African maize producers would not plant if it was too cold and wet. Unexpected cold and wet conditions that appear a day or two after maize planting is not uncommon in South Africa. In most of the commercial maize production areas, like the western Free State, the chance of thunder and hailstorms is high during the planting season. This is the major cause of a sudden drop in temperature and flooding conditions.

Seed companies as well as farmers will benefit from this study. For seed companies to stay competitive in the market, it is critical to sell only seed of the highest quality. Due to high input costs and advanced technology, farming systems are experiencing a change to precision farming, where it is important to know the adaptability of a certain hybrid for a specific area and how it will perform under certain climatic conditions.

The objectives of this study were to investigate the effects of different water saturation and temperature levels on the germination and emergence of maize hybrids.

2.2 MATERIALS AND METHODS

2.2.1 Experimental set-up

Experiments were conducted during 2006 in growth chambers, at the Phytotron facility in the Department of Plant Production and Soil Science, University of Pretoria. Seeds of nine maize hybrids (seven hybrids from Monsanto and one hybrid each from Pannar and PHI), were used in the study. The nine maize hybrids used in the study consist of different genetic sources, such as single crosses and three way hybrids. Different trait versions of the same genetic background, namely: conventional-, Roundup Ready- and Bt.- hybrids were used in the study. The seed was stored in a cold room maintained at 11°C and 38% relative humidity (RH). To investigate the

adaptability of the maize hybrids to cold, wet conditions, an experiment was conducted under controlled temperature and water conditions, representing field conditions.

Since most of the germination problems were encountered on sandy soils, we decided to use a sandy soil as growth medium. Soil was collected from the farm Shokelton in the Viljoenskroon area of the Free State province. Soil was taken from the top 40 cm layer. According to MacVicar *et al*. (1991), the soil can be classified as an Avalon (sandy soil). The total amount of soil was thoroughly mixed and four soil samples were randomly collected before filling of the pots. The samples were combined, air-dried, sieved and analyzed for pH (1:1 water) (McLean, 1982). Soil chemical analyses to determine P, K, Ca, Mg and Na concentrations were done, as described by Warncke & Brown (1998) at the Department of Plant Production and Soil Science, University of Pretoria. Analysis results appear in Table 2.1.

Table 2.1 Soil analysis results for the Viljoenskroon soil used for pot trials

pH (water)	P (Bray I)	Ammonium Acetate Extractable			
		Ca	K	Mg	Na
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
4.8	80.2	257	98	45	11

The soil sample was taken from a maize field. The pH (water) of 4.8 is slightly low for optimum maize production. The availability of nutrients, such as phosphorus and molybdenum is negatively influenced by a pH (water) below 5. The P (Bray 1) analysis of 80.2 is an indication of a high soil phosphorus status. The remaining nutrient analysis (Ca, Mg and Na) also indicated that the soil is suitable for maize production (Johnson, 1991).

Seeds from all nine hybrids were planted in the soil contained in 4-L plastic pots, at a depth of 3.5 cm. Ten seeds were planted per pot and a single pot was considered as one replication.

2.2.2 Treatments

Three growth chambers were used in the study. Each growth chamber was set at a different temperature for the duration of the study. Growth chambers were set at 12 hours day/night intervals at three constant temperatures:

(i) 10°C
(ii) 20°C
(iii) 30°C

The seeded pots were waterlogged for different periods of time. Waterlogging or flooding was simulated by lining the pots with plastic bags that prevented leaching from the soil contained in them. After planting, these pots were watered until the water level was about 2 cm above the soil surface (Figure 2.1). At the end of the simulated flooding duration, drainage holes were made at the bottom of the pots, to allow drainage of excess water. Soil was waterlogged for:

(i) 0 hours (control)
(ii) 24 hours
(iii) 48 hours
(iv) 72 hours

A total of 432 treatment combinations were studied:

(i) 9 hybrids
(ii) 3 temperature levels (10, 20 and 30°C)
(iii) 4 durations of flooding stress (0, 24, 48 and 72 hours)
(iv) 4 replications

Figure 2.1 Simulation of flooding conditions using pots with sandy soil planted with 10 maize seeds per pot

2.2.3 Data collected

Daily emergence counts were taken, with emergence defined as the number of seedlings visible above the soil surface. Emergence counts were done until no further seedlings appeared. Plant height was measured at two weeks after planting and again four weeks after planting. The total dry weight yield of those seedlings showing normal development of roots and shoots were determined by removing the seedlings and drying them for 72 hours in an oven at 75°C. The mean seedling dry weight yield was calculated by dividing the total dry weight yield by the number of normal seedlings. Normal seedlings are defined as all normally developed seedlings, as described in the Seedling Evaluation Handbook (AOSA, 1992). Seedling discussions are based on visual observations from the pot trials.

2.2.4 Statistical analysis

Data were subjected to analysis of variance, using the MINITAB 14.2 software program as well as the JMP7 software program. Differences at the $P < 0.05$ level of significance were reported. The Student's *t* Test was performed to confirm significant differences.

2.3 RESULTS AND DISCUSSION

2.3.1 Warm test results

All varieties showed above 96% germination in the standard germination test, more commonly known as the "warm test". Table 2.2 shows the germination data of the different maize hybrids. There were significant differences between some of the hybrids ($P = 0.039$). The Student's *t* Test results confirmed that Hybrids 4 and 9 had the lowest germination percentages, with that of Hybrid 9 being significantly lower than that of all the hybrids, except Hybrid 4.

Table 2.2 Warm test germination of nine maize hybrids (ANOVA data appear in Appendix, Table1)

Hybrid	Type of hybrid	Mean warm test germination %
1	Conventional *	99.5 (a)
2	Conventional	99.5 (a)
3	Bt *	98.8 (a)
4	Roundup Ready *	97.8 (ab)
5	Conventional **	99.5 (a)

6	Bt **	98.5 (a)
7	Roundup Ready **	98.8 (a)
8	Bt	98.5 (a)
9	Conventional	96.3 (b)
Means followed by the same letter are not significantly different at P = 0.05 * Indication of the same genetic background CV of warm test results = 2.10		

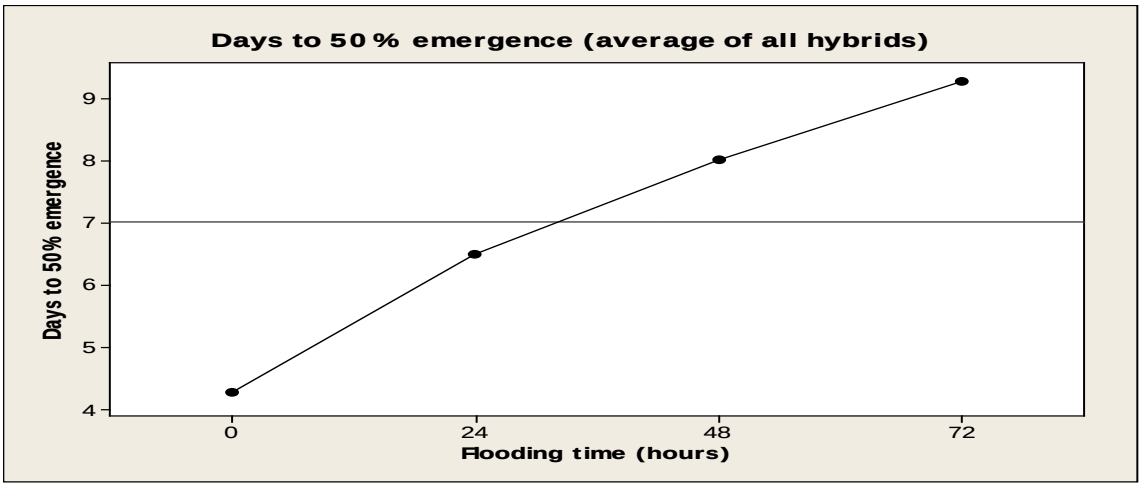
Hybrid 1 (conventional) overall developed the strongest seedlings of all treatment combinations (different temperatures and flooding times). In general, the conventional hybrids developed the strongest seedlings, with the Roundup Ready hybrids the weakest. The mean warm test germinations for the conventional hybrids were slightly higher than the rest. Hybrid 2 was the only three-way hybrid used in the study. Although it's warm test germination was the same as Hybrid 1 (same genetic composition), the development was slightly slower, with slightly weaker seedlings than Hybrid 1. A coefficient of variation of 2.10 is an indication of very little variation in the test. The warm test measures germination under favourable conditions, therefore a low coefficient of variation is expected.

Emergence interacted significantly with flooding duration (Figure 2.2). Figure 2.2 shows the effects of the different flooding times (from left to right), 72 hours, 48 hours, 24 hours and 0 hours, on the emergence of Hybrid 1 at 20°C. All nine hybrids showed similar results, and therefore only one visual example is included.

For all nine hybrids, there were a significant increase in the average days to 50 % emergence (Figure 2.3a), as well as the average days to final emergence (Figure 2.3b) as the time of flooding increased. The control samples (0 hours flooding) emerged first and the 72 hours flooding samples took the longest to emerge. From 0 hours flooding to 72 hours flooding, it took more than double the time to reach 50% emergence.

Figure 2.2 Effects of 72 hours, 48 hours, 24 hours and 0 hours (from left to right) flooding on emergence of Hybrid 1 at 20 °C, 14 days after planting

(a)



(b)

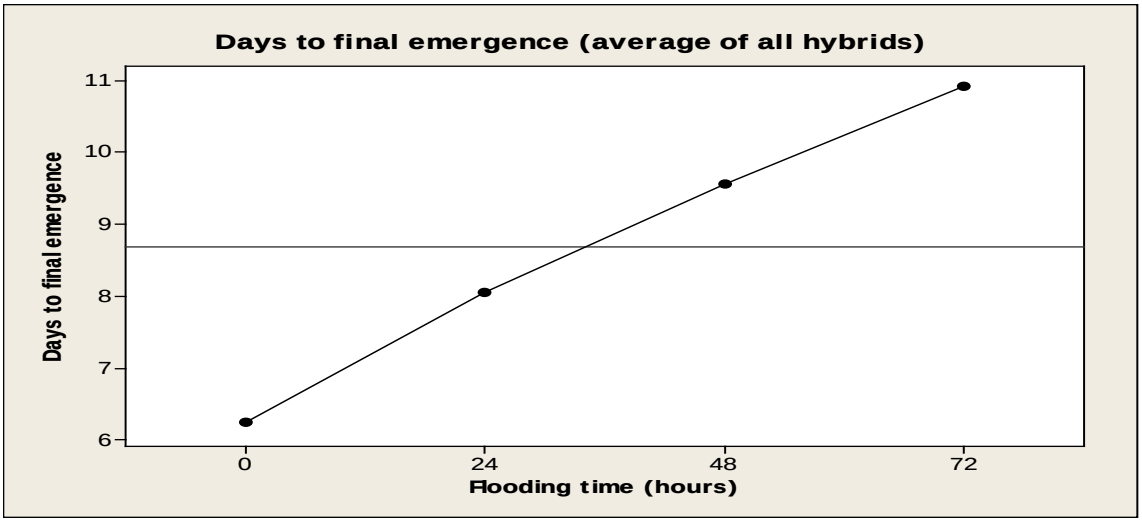


Figure 2.3 Days to (a) 50 % emergence and (b) final emergence for nine maize hybrids as affected by flooding

Table 2.3 shows days to 50 % emergence, as well as days to final emergence of the nine maize hybrids at different temperatures and flooding durations. The interaction of the nine maize hybrids, temperature and flooding was not significant (P = 0.298). No significant differences were found between hybrids and temperature as well as between hybrids and flooding. The interaction between temperature and flooding was highly significant. There was significant differences in both days to 50 % emergence and days to final emergence between 10°C and 30°C. A significant difference was found between days to 50% emergence and the nine hybrids (Figure 2.4). Hybrid 5 took only 6.5 days to 50% emergence. Hybrid 2, the only three way hybrid, took the longest (7.6 days) to 50% emergence.

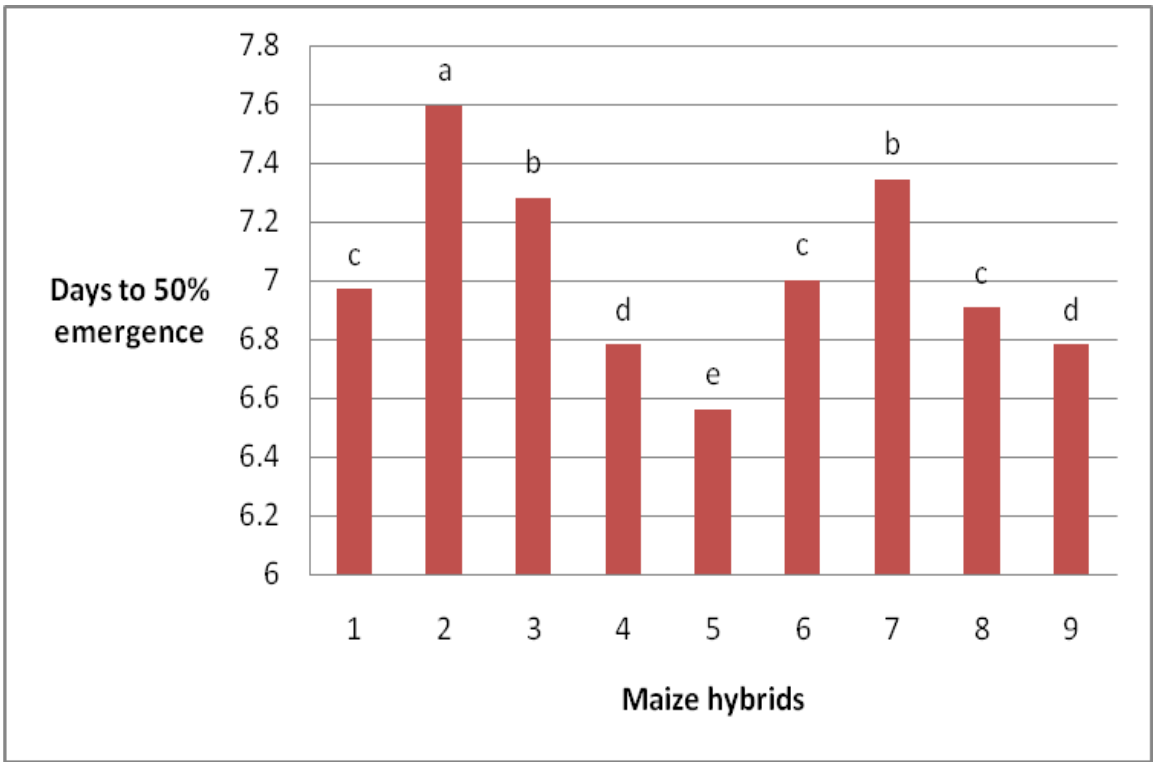


Figure 2.4 Days to 50% emergence of nine maize hybrids (Means followed by the same letter are not significantly different at P = 0.05) (ANOVA data appear in Appendix, Table 2)

Table 2.3 Days to 50 % emergence as well as average days to final emergence of nine maize hybrids at 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding (ANOVA data appear in Appendix, Table 2)

Temperature (°C)	Flooding duration (hours)	Days to 50% emergence	Days to final emergence

10	0	13.0 (a)	13.5 (a)
	24	13.0 (a)	13.5 (a)
	48	0.0 (g)	0.0 (f)
	72	0.0 (g)	0.0 (f)
20	0	5.4 (de)	8.2 (c)
	24	8.0 (c)	10.2 (b)
	48	10.1 (bc)	12.0 (ab)
	72	11.1 (b)	12.9 (a)
30	0	3.2 (f)	4.3 (e)
	24	4.9 (e)	5.9 (d)
	48	6.1 (d)	7.2 (cd)
	72	7.4 (cd)	8.9 (c)

2.3.2 Germination of maize seeds at 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding

Germination data for nine maize hybrids at different temperature and flooding conditions appear in Table 2.4. A significant interaction (P = 0.018) was found between hybrids, temperature and flooding. Significant differences in germination were found between the different hybrids (P = 0.019). There was also, a highly significant difference in germination percentage between the different temperatures (10°C, 20°C and 30°C) (P = 0.000) and the different flooding times (0 hours, 24 hours, 48 hours and 72 hours) (P = 0.000). The interaction effect of temperature and flooding (P = 0.000) and hybrids and temperature (P = 0.005) were also highly significant, that of hybrids and flooding (P = 0.208) was not significant. Results showed that cold conditions

Table 2.4 Germination percentages of nine maize hybrids at 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding (ANOVA data appear in Appendix, Table 3)

Temperature	10˚C	20˚C	30˚C

Flooding duration	0 Hours	24 Hours	48 Hours	72 Hours	0 Hours	24 Hours	48 Hours	72 Hours	0 Hours	24 Hours	48 Hours	72 Hours
Hybrid1	82 cdefghij	80 defghijk	72 ghijklmn	70 ijklmno	98 Ab	98 ab	88 abcdef	98 ab	98 ab	100 a	95 Abc	86 bcdef
Hybrid2	84 bcdefgh	79 defghijk	72 ghijklmn	68 klmnop	85 bcdefg	98 ab	70 ijklmno	90 abcde	98 ab	98 ab	83 cdefghij	78 efghijkl
Hybrid3	83 cdefghi	79 defghijk	72 ghijklmn	59 nopqr	90 Abcde	98 ab	93 abcd	88 abcdef	98 ab	100 a	88 abcdef	82 cdefghij
Hybrid4	83 cdefghi	79 defghijk	69 jklmno	57 opqr	95 Abc	90 abcde	93 abcd	85 bcdefg	100 a	98 ab	88 abcdef	80 defghijk
Hybrid5	83 cdefghi	81 defghijk	69 jklmno	55 pqr	100 A	100 a	95 abc	83 cdefghij	98 ab	100 a	94 Abc	75 fghijklm
Hybrid6	82 cdefghij	80 defghijk	71 hijklmn	65 lmnopq	88 abcdef	95 abc	90 abcde	75 fghijklm	98 ab	95 abc	93 abcd	83 cdefghij
Hybrid7	82 cdefghij	78 efghijkl	70 ijklmno	52 or	93 Abcd	90 abcde	70 ijklmno	83 cdefghij	95 abc	100 a	88 abcdef	75 fghijklm
Hybrd8	81 defghijk	78 efghijkl	70 ijklmno	52 or	85 bcdefg	78 efghijklm	95 abc	83 cdefghij	98 ab	95 abc	82 efghijklm	75 fghijklm
Hybrid9	81 defghijk	78 efghijkl	71 hijklmn	51 r	100 A	95 abc	80 defghijk	64 mnopqr	98 ab	95 abc	80 defghijk	72 ghijklmn
Mean for flooding time	82	79	71	59	93	94	86	83	98	98	88	79
Mean for temperature	73				89				90			
Means followed by the same letter are not significantly different at P = 0.05												

(10°C) and longer flooding periods (72 hours) (Figures 2.5 and 2.6) had a major negative effect on germination of maize seeds. The higher the temperature, and the shorter the duration of flooding, the higher the germination. This clearly indicates that significant lower germination can be expected in cold, wet soils. This verifies what the maize farmers experienced in the fields.

When comparing hybrids at a specific temperature and flood duration, there was no significant difference in germination percentage at 10°C and 0, 24 or 48 hours of flood. But as soon as the flood duration increases to 72 hours, the germination percentage of hybrids 5, 7, 8 and 9 (Hybrid 5>Hybrid 7=Hybrid 8>Hybrid 9) is significantly lower than that of Hybrid 1. After 72 hours flooding the germination percentage of Hybrids 7, 8 and 9 are also significantly lower than that of Hybrids 2 and 6 (Hybrid 2>Hybrid6).

At 10°C the germination percentage of Hybrid 1 did decrease with an increase in flood duration, but the differences were not significant. On the other hand, the germination percentage of Hybrids 4 and 5 was already significantly lower after only 48 hours flood than under control conditions, while the other hybrids did not show the same reaction. After 72 hours flooding it was only Hybrid 1 whose germination percentage was not significantly affected. The germination percentage of Hybrid 2 after 72 hours flooding were only significantly lower than the germination percentage without flooding, while the germination percentage of Hybrids 3, 4 and 6 in addition also differed from seed subjected to 24 hours flooding. Hybrids 5, 7, 8 and 9 were affected the worst by this long period of flooding and the germination percentage after 72 hours differed significantly from that of seeds subjected to 0, 24 and 48 hours flooding.

When the temperature increases to 20°C, the germination percentage of Hybrids 5 and 9 (Hybrid 5=Hybrid 9) was significantly higher that of Hybrids 2 and 8 (Hybrid 2=Hybrid 8). At 20°C, significant differences were thus experienced sooner than at 10°C. After 24 hours flood, the germination percentage of Hybrid 8 was significantly the lowest of all the hybrids. With 48 hours flooding Hybrid 2 is still performing poorly and together with Hybrid 7's germination percentage it is significantly lower than that of Hybrids 1, 3, 4, 5, 6 and 8 (Hybrid 5=Hybrid 8>Hybrid 3=Hybrid 4>Hybrid 6>Hybrid 1). Despite the good germination percentage without flood, Hybrid 9 preformed poorly after 48 and 72 hours flooding. At 48 hours flooding the germination percentage of Hybrid 9 differed significantly from Hybrids 5 and 8, but at 72 hours flooding it preformed significantly poorer than all the hybrids except Hybrid 6.
Hybrids 3, 4 and 8 at 72 hours flooding tended to germinate poorly in comparison to no flooding, but it was not statistically significant. The germination percentage of Hybrid 1 on the other hand were the same with 0 and 72 hours flooding, and thus seems more tolerant to flooding conditions under moderate (20°C) than low (10°C) temperatures. The germination percentage of Hybrid 2, 7 and 9 (Hybrid 9>Hybrid 2=Hybrid 7) were significantly lower after 48 hours flood than with 0 and 24 hours flooding. Unlike with 10°C and 72 hours flooding where eight of the nine hybrids were significantly affected, it was only Hybrids 5, 6 and 9, which were affected under 20°C and 72 hour flooding. Interesting was to note that the germination percentage of some hybrids

(Hybrid 2 at 72 hours and Hybrid 6 at 24 and 48 hours) was stimulated rather than being retarded with flooding.

At 30°C, the germination percentage of none of the hybrids were significantly different with 0 and 24 hours flooding, but with 48 hours flood Hybrids 8 and 9 (Hybrid8>Hybrid9) differed significantly from Hybrids 1, 5 and 6 (Hybrid 1>Hybrid 5> Hybrid6). After 72 hours of flood the only significant difference in germination percentage was between Hybrids 1 and 9 (Hybrid1>Hybrid 9).

At 0 and 24 hours flooding, none of the germinations percentages differed significantly from each other. But with 48 hours flood the germination percentage of Hybrids 2, 8 and 9 was lower than at 0 and 24 hour flooding. With 72 hours flooding significantly difference in germination percentage of Hybrid 6 (0 hours), Hybrids 2, 3, 4, 7, 8 and 9 (0 and 24 hours) and Hybrid 5 (0, 24 and 48 hours) was recorded. Again, some flooding (24 hours) seemed to have stimulated the germination percentage of Hybrid 1, 2, 5 and 7.

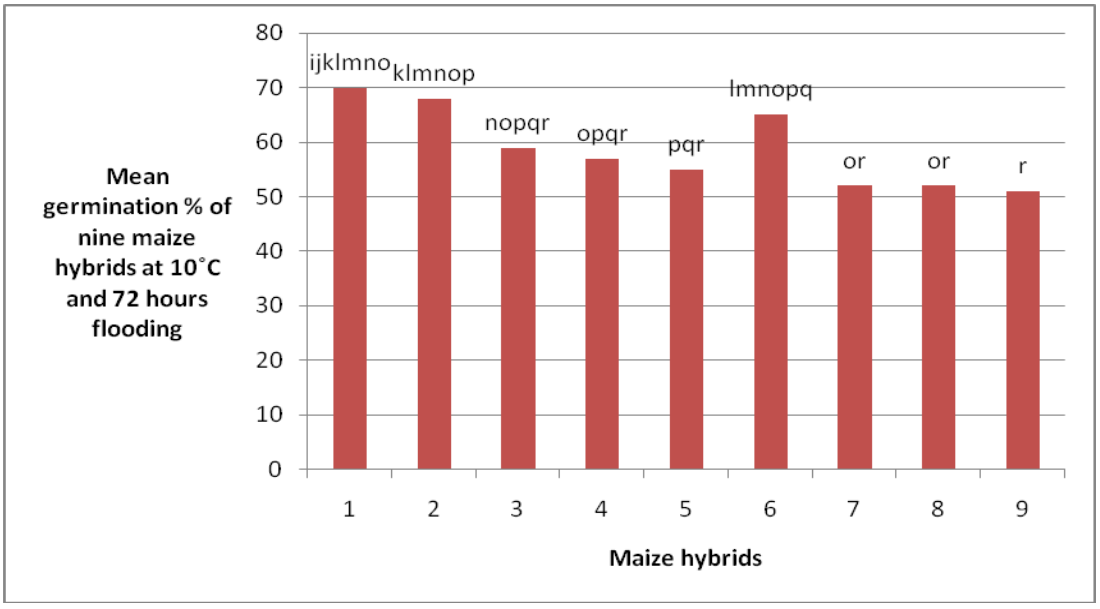


Figure 2.5 Mean germination percentages of nine maize hybrids at 10˚C and

72 hours flooding (Means followed by the same letter are not significantly different at P = 0.05) (ANOVA data appear in Appendix, Table 3)

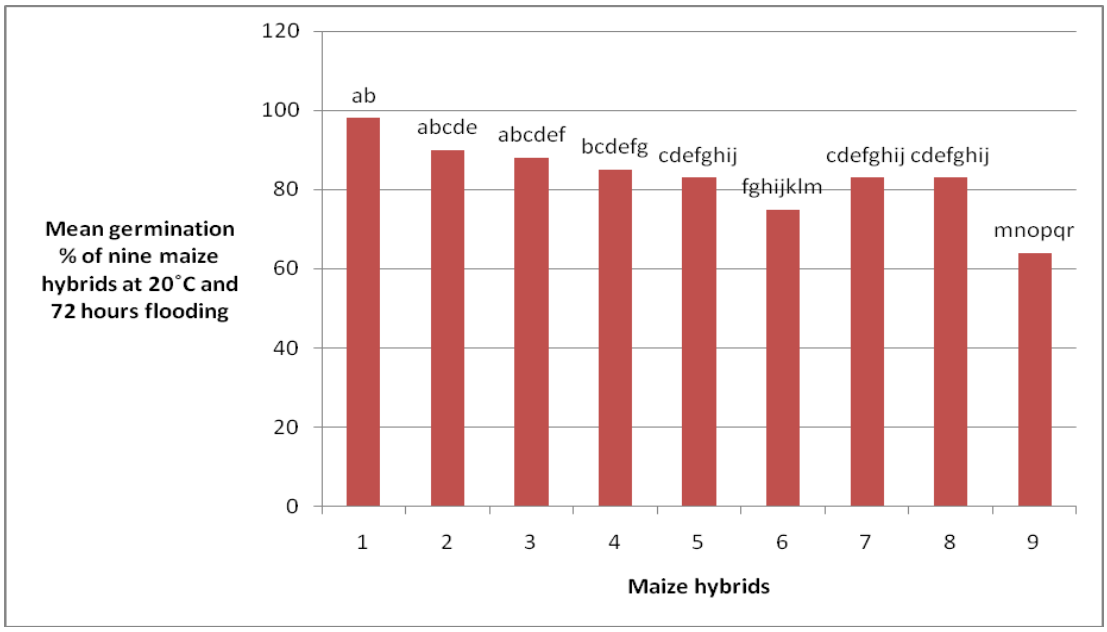


Figure 2.6 Mean germination percentages of nine maize hybrids at 20C and 72 hours flooding (Means followed by the same letter are not significantly different at P = 0.05) (ANOVA data appear in Appendix, Table 3)

In general, the germination of Hybrids 1 and 2 tended to be the best for all flooding treatments at the low temperature (10˚C) . These hybrids also belong to the same genetic group. Hybrids 7, 8 and 9 (with no genetic similarity) germinated the lowest for all flooding treatments at 10 C. At 20˚C, more variation in germination was observed between the different hybrids. With the highest flooding duration (72 Hours), germination of Hybrids 1,2 and 3 was the highest. The same trend has been recorded with the most favourable germination conditions (30˚C with 0 hours flooding).

The germination results correlates well with the visual observations that Hybrid 1 developed the strongest seedlings in the experiment. Hybrid 9, which had the lowest warm test germination, also had the lowest germination percentage at 10 and 20°C and 72 hours flooding.

2.3.3 Emergence percentages and emergence rates of maize seeds at 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding

For the control samples (0 hours flooding) there was a 84.1 % higher emergence at 20°C than at 10 °C, and a 7.3 % higher emergence at 30°C than at 20°C (Table 2.5). At 24 hours flooding, there was a 96.9 % higher emergence at 20°C than at 10°C, and a 7.7 % higher emergence at 30°C than at 20°C.

The interaction effect of temperature and flooding (P = 0.000), hybrid and temperature (P = 0.001), and hybrid and flooding (P = 0.001) as well as hybrid, flooding and temperature (P = 0.000) were all significant. Emergence percentages of the hybrids at different temperature and flooding conditions are shown in Table 2.5. Results indicate that cold conditions (10°C) and longer periods of flooding (48 to 72 hours) had a major negative effect on emergence of maize seeds. Yet again confirming the observations by the farmers.

Table 2.5 Emergence percentages of nine maize hybrids at 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding (ANOVA data appear in Appendix, Table 4)

Temperature	10˚C				20˚C				30˚C			
Flooding duration	0 Hrs	24 Hrs	48 Hrs	72 Hrs	0 Hrs	24 Hrs	48 Hrs	72 Hrs	0 Hrs	24 Hrs	48 Hrs	72 Hrs
Hybrid1	16 (mno)	3 (no)	0 (o)	0 (o)	98 (ab)	98 (ab)	95 (abc)	92 (abcd)	98 (ab)	100 (a)	98 (ab)	95 (abc)
Hybrid2	15 (mno)	2 (no)	0 (o)	0 (o)	83 (abcdefg)	98 (ab)	68 (fghij)	60 (ijkl)	98 (ab)	98 (ab)	80 (bcdefgh)	53 (jkl)
Hybrid3	15 (mno)	4 (no)	0 (o)	0 (o)	90 (abcd)	98 (ab)	75 (defghi)	75 (defghi)	98 (ab)	100 (a)	83 (abcdefg)	78 (cdefghi)
Hybrid4	14 (mno)	3 (no)	0 (o)	0 (o)	95 (abc)	83 (abcdefg)	85 (abcdef)	65 (ghijk)	100 (a)	98 (ab)	85 (abcdef)	78 (cdefghi)
Hybrid5	15	3	0	0	100	100	88	68	98	100	95	75

	(mno)	(no)	(o)	(o)	(a)	(a)	(abcde)	(fghij)	(ab)	(a)	(abc)	(defghi)
Hybrid6	15 (mno)	3 (no)	0 (o)	0 (o)	85 (abcdef)	98 (ab)	70 (efghij)	68 (fghij)	98 (ab)	93 (abcd)	93 (abcd)	83 (abcdefg)
Hybrid7	12 (mno)	2 (no)	0 (o)	0 (o)	90 (abcd)	85 (abcdef)	48 (kl)	45 (l)	95 (abc)	100 (a)	83 (abcdefg)	53 (jkl)
Hybrd8	12 (mno)	3 (no)	0 (o)	0 (o)	78 (cdefghi)	60 (ijkl)	63 (hijkl)	56 (jkl)	98 (ab)	93 (abcd)	75 (defghi)	65 (ghijk)
Hybrid9	14 (mno)	2 (no)	0 (o)	0 (o)	98 (ab)	90 (abcd)	63 (hijkl)	22 (mn)	98 (ab)	93 (abcd)	75 (defghi)	25 (m)
Mean for flooding time	14	3	0	0	90	89	73	62	98	97	85	67
Mean for temperature	4				79				87			
Means followed by the same letter are not significantly different at P = 0.05												

At 10°C and all flood conditions no significant differences between hybrids were recorded. There was also no significant decrease in emergence percentage as the time of flooding increased (Table 2.5). Therefore only emergence at 20 and 30°C will be discussed. At 20°C and 0 hours flooding the emergence percentage of Hybrid 8 was significantly lower than that of Hybrids 1, 5 and 9 (Hybrid 5>Hybrid 1=Hybrid 9). The emergence percentage of Hybrid 8 were also significantly lower than that of all the other hybrids (Hybrid 5>Hybrid 1=Hybrid 2=Hybrid 3=Hybrid 6>Hybrid 9>Hybrid 7>Hybrid 4) after 24 hours flooding. After 48 hours flooding, emergence percentage of Hybrid 1 is significantly higher than that of Hybrids 2, 3, 6, 7, 8 and 9 (Hybrid 3>Hybrid 6>Hybrid 2>Hybrid 8>Hybrid 9>Hybrid 7). The emergence percentage of Hybrid 7 was the lowest, and differed significantly from all the other hybrids except that of Hybrids 8 and 9. With 72 hours flooding the emergence percentage of Hybrid 1 was the highest and apart from Hybrid 3, it was significantly so than that of the rest of the hybrids (Figure 2.7). The emergence percentage of Hybrid 9 was significantly the lowest of all nine hybrids after this long period of flooding.

For Hybrids 2, 3 and 6 is seems as if a 24 hour flooding had a positive effect on emergence percentage, while it had no effect on Hybrids 1 and 5. With 48 hours flood, the emergence percentage of hybrids 2 and 3 was significantly lower than at 0 hours flooding, while the emergence percentage of Hybrids 7 and 9 differed significantly from 0 and 24 h flooding. After 72 hours flooding the emergence percentages differed significantly for Hybrids 4 and 8 at 0 hours, Hybrid 3 and 6 at 24 hours, Hybrids 2 and 7 at 0 and 24 hours and Hybrids 5 and 9 at 0, 24 and 48 hours flooding.

When the temperature increased to 30°C, there were no significant differences among the hybrids at 0 and 24 hours flooding. However, with 48 hours flooding the emergence percentage of Hybrids 8 and 9 (Hybrid 8=Hybrid 9) was the lowest of all the hybrids and significantly so than Hybrids 1 and 5. This correlates will with the warm test germinations. On average the conventional hybrids emerged better than the Bt and Roundup-Ready hybrids.

Apart from Hybrids 8 and 9, which had significantly lower emergence percentage after 48 than 0 hours of flooding, there was no significant decreases in emergence percentage for any of the hybrids from 0 to 48 hours flooding. After 72 hours of flooding, the emergence percentage of Hybrids 1 and 7 was not significantly decrease with an increase in flooding duration. The emergence percentage of Hybrids 3, 4 and 8 at 72 hours flooding was significantly lower than at 0 and 24 hours flooding, while that of Hybrids 2, 5, 7 and 9 was significantly lower than at 0, 24 and 48 hours flooding.

In general, the emergence percentage of Hybrid 1 was not significantly affect by an increase in flooding at a specific temperature. However, the emergence percentage of Hybrids 5 and 9 at 20 and 30°C and 72 hours flooding was significantly lower than at 0, 24 and 48 hours flooding.

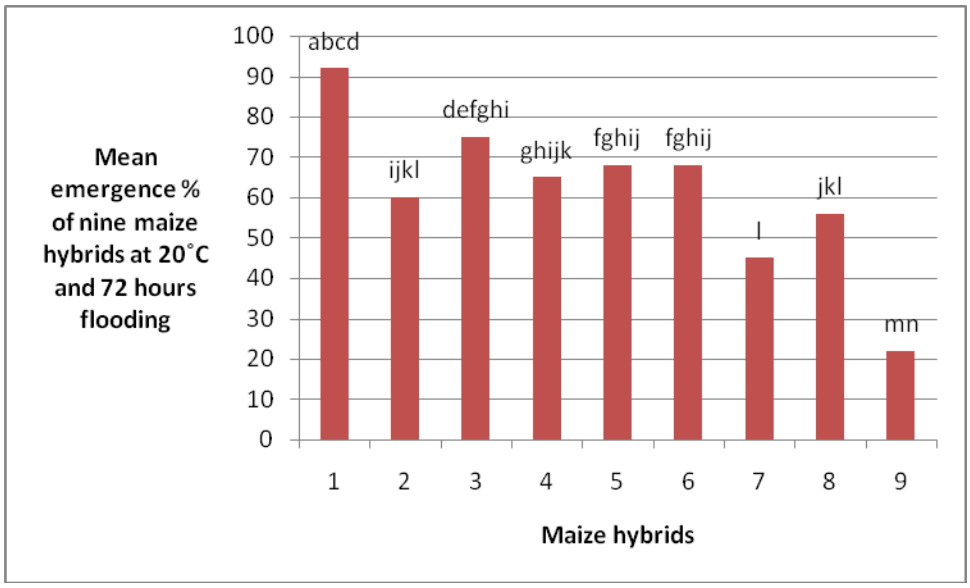


Figure 2.7 Mean emergence percentages of nine maize hybrids at 20°C and 72 hours flooding (Means followed by the same letter are not significantly different at P = 0.05) (ANOVA data appear in Appendix, Table 4)

Emergence rate was calculated for each treatment according to the index of Maguire (1962). Speed of germination and emergence is one of the oldest concepts of seedling vigour. Seed batches with similar total germination often vary in rate of seedling emergence and rate of growth (Maguire, 1962). Emergence rates of the hybrids at different temperature and flooding conditions are shown in Table 2.6. Emergence rate was calculated by dividing the number of normal seedlings per 10 seeds obtained, at each counting by the number of days seeds have been in the growth chamber. The values obtained at each count, were then summed at the end of the germination test to obtain the emergence rate:

$$\text{Emergence rate} = \left(\frac{\textit{number of normal seedlings}}{\textit{days to first count}} + \ldots + \frac{\textit{number of normal seedlings}}{\textit{days to final count}}\right)$$

Emergence rates were significantly influenced by hybrid (P = 0.000), temperature (P=0.000) (Figure 2.8) and flooding (P = 0.000) (Table 2.6). The lower the temperature, the lower the emergence rates, as well as emergence percentages. The longer the duration of flooding, the lower the emergence rates, as well as emergence percentages. The interaction between temperature and flooding was also highly significantly (P=0.000). The interaction between hybrid and temperature (P = 0.177) and hybrid and flooding (P = 0.117) as well as the interaction between hybrid, temperature and flooding (P = 0.352) was not significantly.

According to Table 2.6, the mean emergence rate was significantly lower at 20°C (6.2) than at 30°C (8.9), with 72 hours flooding. At 10°C, with 48 hours as well as 72 hours flooding the mean emergence percentage as well as the mean emergence rate was 0.

Table 2.6 Seedling emergence rates and percentages for nine maize hybrids at 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding (ANOVA data appear in Appendix, Tables 4 & 5)

Temperature (°C)	Flooding (Hours)	Emergence rates	Emergence %
10	0	2.1 (h)	14.4 (d)
	24	0.2 (h)	2.8 (e)
	48	0.0 (h)	0.0 (e)
	72	0.0 (h)	0.0 (e)
20	0	16.6 (c)	90.0 (ab)
	24	11.5 (e)	89.4 (ab)
	48	7.3 (fg)	73.4 (c)
	72	6.2 (g)	62.4 (c)
30	0	31.4 (a)	97.5 (a)
	24	19.4 (b)	96.9 (a)
	48	13.8 (d)	85.0 (b)
	72	8.9 (f)	66.7 (c)
Means followed by the same letter are not significantly different at P = 0.05			

Figure 2.8 Differences between emergence percentages and emergence rates of Hybrid 8 at 0 hours flooding (control) at 30°C (left), 20°C (center) and 10°C (right), 14 days after planting

2.3.4 Plant height

Plant height was measured two weeks after planting and again four weeks after planting – at the end of the experiment. Flooding and low temperatures had a significant negative effect on plant height. The same trend has been recorded on the measurements taken on the two and four week periods. Therefore all results discussed are based on the four week measurements. All interactions were highly significant (P = 0.000). Plant height of all the hybrids was significantly shorter in the flooded plots, than in the control plots across all temperatures (Table 2.7).

Due to very little growth at 10°C, discussions were based on flooding at 20 and 30°C. The mean plant height of Hybrid 1 was the highest, while that of Hybrid 9 was the lowest for both 20 and 30°C with 72 hours flooding. This corresponds well with the germination and emergence findings under these conditions. Under ideal growing conditions, 30°C and 0 hours flooding, no significant differences were found between the different maize hybrids. As conditions become less favourable, more variation and differences appear between the hybrids. At 20°C and 72 hours flooding, plant height range from 28 to 85 mm. At 30°C and 72 hours flooding, plant height range from 159 to 229 mm. According to the plant height results, Hybrids 6 and 9 were the most sensitive hybrids to long periods of flooding at 20 and 30°C, while Hybrid 1 was the most tolerant to these conditions. One can also conclude that none of the nine hybrids are tolerant to cold, wet conditions (10°C and 48/72 hours flooding), since no growth took place under these conditions.

When the plant growth is compared to germination and emergence data, it is clear that the growth of the maize plants were more severely affected by flooding than germination and emergence. For example, there was no significant differences in germination (Table 2.3) and emergence percentage (Table 2.6) at 10°C and 0 and 24 hours flooding, while there was significant differences in plant height (Table 2.7).

Table 2.7 Plant height after four weeks (mm) of nine maize hybrids at 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding (ANOVA data appear in Appendix, Table 6)

Temperature	10˚C				20˚C				30˚C			
Flooding duration	0 Hours	24 Hours	48 Hours	72 Hours	0 hours	24 Hours	48 Hours	72 Hours	0 Hours	24 Hours	48 Hours	72 Hours
Hybrid1	25 (B)	12 (C)	0 (D)	0 (D)	150 (qr)	148 (qrs)	48 (yzA)	85 (vw)	332 (a)	312 (bc)	227 (fgh)	229 (fg)
Hybrid2	23 (B)	10 (C)	0 (D)	0 (D)	162 (op)	163 (o)	51 (yz)	45 (zA)	333 (a)	304 (c)	206 (jk)	219 (hi)
Hybrid3	25 (B)	14 (C)	0 (D)	0 (D)	145 (rs)	163 (o)	79 (wx)	45 (zA)	335 (a)	321 (b)	228 (fgh)	211 (ij)
Hybrid4	23 (B)	10 (C)	0 (D)	0 (D)	142 (s)	141 (s)	95 (tu)	42 (zA)	335 (a)	321 (b)	225 (fgh)	219 (hi)
Hybrid5	25 (B)	10 (C)	0 (D)	0 (D)	147 (qrs)	148 (qrs)	73 (x)	55 (y)	336 (a)	284 (d)	232 (f)	176 (l)
Hybrid6	23 (B)	10 (C)	0 (D)	0 (D)	152 (pqr)	164 (o)	90 (uv)	45 (zA)	336 (a)	269 (e)	209 (jk)	166 (mno)
Hybrid7	25 (B)	8 (CD)	0 (D)	0 (D)	163 (o)	153 (pqr)	44 (zA)	49 (yzA)	333 (a)	275 (de)	201 (k)	174 (lm)
Hybrd8	24 (B)	8 (CD)	0 (D)	0 (D)	163 (o)	94 (uv)	104 (t)	48 (yzA)	333 (a)	281 (d)	209 (jk)	173 (lm)
Hybrid9	24 (B)	8 (CD)	0 (D)	0 (D)	152 (pqr)	98 (tu)	54 (y)	28 (B)	330 (a)	281 (d)	222 (gh)	159 (opq)
Mean for flooding time	24	10	0	0	153	141	71	49	334	294	218	192
Mean for temperature	9				104				260			
Means followed by the same letter are not significantly different at P = 0.05												

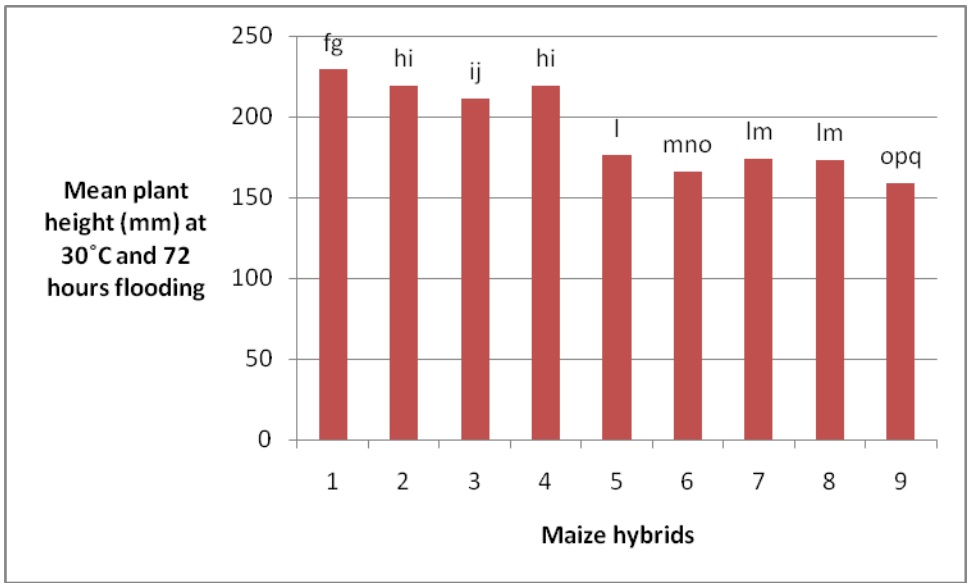


Figure 2.9 Mean plant height (mm) of nine maize hybrids at 30C and 72 hours flooding (Means followed by same letter are not significantly different at P = 0.05) (ANOVA data appear in Appendix, Table 6)

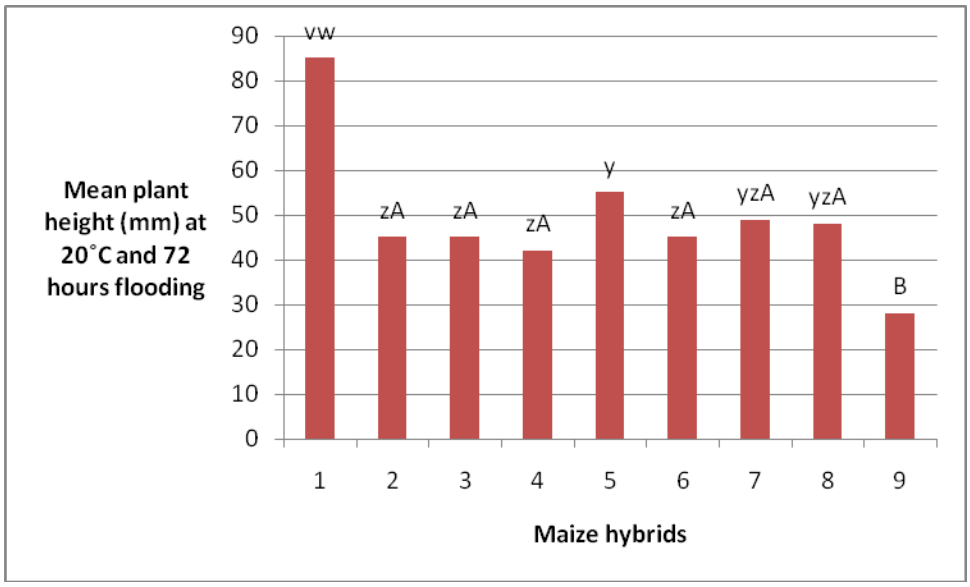


Figure 2.10 Mean plant height (mm) of nine maize hybrids at 20C and 72 hours flooding (Means followed by same letter are not significantly different at P = 0.05) (ANOVA data appear in Appendix, Table 6)

2.3.5 Dry weight

The total dry weight of the normal seedlings, were determined by removing the seedlings and drying it for 72 hours in a 75°C oven. Mean seedling dry weight, was calculated by dividing the total dry weight by the number of normal seedlings. Due to very few abnormal seedlings found during evaluation, abnormal seedlings were handled as normal seedlings. Table 2.8 shows the dry weight of the leaves of the maize hybrids at different temperatures and flooding times. There were highly significant differences between the dry weight of the hybrids (P = 0.000), different temperatures (P = 0,000) and flooding times (P = 0.000). All the interactions were also highly significant (P = 0.000). At 10°C, there was no growth. At 20˚C the dry weight decreased from the control (0 hours flooding) to 72 hours flooding, while at 30°C Hybrids 2, 7 and 9 had a higher dry weight after 72 hours flooding than at 24 hours flooding and even 48 hours flooding (Hybrids 4, 6, 7 and 9).

Figures 2.11 and 2.12 shows mean dry weight of nine maize hybrids at 20°C with 72 hours flooding and 30˚C with 72 hours flooding. Hybrid 1 obtained the highest weight with in all the treatment combinations, while the weight of Hybrid 9 was the lowest for all treatment combinations. Again, this corresponds well with the germination, emergence and plant height findings of Hybrids 1 and 9. No specific trend was obtained between the dry weight of the remaining hybrids. Overall, Hybrid 9 performed the poorest of the hybrids, while Hybrid 1 performed the best. This findings correspond well with the warm test germination, where Hybrid 9 significantly germinate lower than the remaining 8 hybrids.

Table 2.8 Leaf dry weight (g) of nine maize hybrids at 10°C, 20°C and 30°C and 0, 24, 48 and 72 hours flooding (ANOVA data appear in Appendix,

Table 7)

Temperature	10˚C				20˚C				30˚C			
Flooding duration	0 Hrs	24 Hrs	48 Hrs	72 Hrs	0 Hrs	24 Hrs	48 Hrs	72 Hrs	0 Hrs	24 Hrs	48 Hrs	72 Hrs
Hybrid1	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	2.32 (ijklm)	1.83 (pqrst)	1.38 (wxyzABCD)	1.15 (BCDEFGH)	6.34 (a)	4.43 (c)	4.22 (c)	3.43 (d)
Hybrid2	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	2.20 (jklmn)	1.45 (vwxyzAB)	1.15 (BCDEFGH)	0.64 (JKL)	5.87 (b)	2.47 (ghij)	1.68 (rstuvw)	1.63 (stuvwxy)
Hybrid3	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	1.62 (stuvwxy)	1.53 (tuvwxyz)	1.19 (ABCDEF)	1.09 (CDEFGH)	3.23 (d)	3.12 (de)	2.78 (fg)	2.41 (hijkl)
Hybrid4	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	1.71 (qrstuv)	1.42 (vwxyzAB)	1.17 (ABCDEFG)	0.87 (GHIJ)	2.17 (jklmno)	1.48 (uvwxyzA)	1.46 (vwxyzAB)	1.66 (rstuvwx)
Hybrid5	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	1.88 (opqrs)	1.40 (vwxyzABC)	1.07 (DEFGH)	0.85 (HIJ)	2.65 (fgh)	2.44 (hijk)	2.12 (lmnop)	2.00 (mnopq)
Hybrid6	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	2.00 (mnopq)	1.56 (tuvwxyz)	1.33 (yzABCD)	1.00 (EFGHI)	2.61 (fghi)	1.96 (nopqr)	1.35 (xyzABCD)	1.65 (rstuvwx)
Hybrid7	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	2.25 (jklmn)	1.45 (vwxyzAB)	1.25 (zABCDE)	0.48 (KL)	2.28 (jklm)	1.46 (vwxyzAB)	1.64 (stuvwxy)	1.70 (qrstuv)
Hybrd8	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	1.96 (nopqr)	1.63 (stuvwxy)	1.29 (zABCDE)	0.70 (IJK)	2.88 (ef)	2.69 (fgh)	2.18 (jklmno)	2.13 (klmnop)
Hybrid9	0.00 (M)	0.00 (M)	0.00 (M)	0.00 (M)	1.52 (tuvwxyz)	1.35 (xyzABCD)	0.90 (FGHIJ)	0.37 (L)	1.79 (qrstu)	1.15 (BCDEFGH)	1.36 (xyzABCD)	1.51 (uvwxyz)
Mean for flooding time	0.00	0.00	0.00	0.00	1.94	1.51	1.19	0.79	3.31	2.36	2.09	2.01
Mean for temperature	0.00				1.36				2.44			
Means followed by the same letter are not significantly different at P = 0.05												

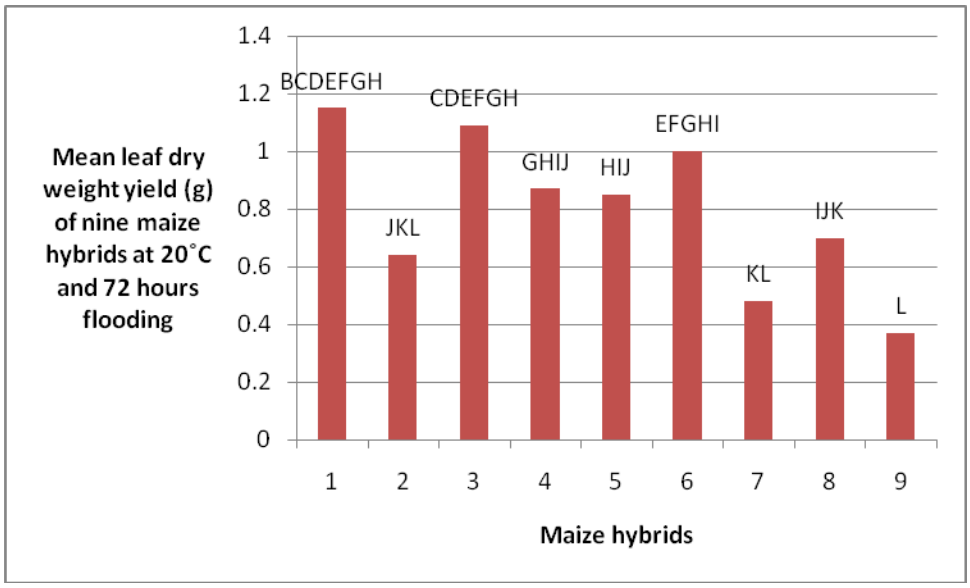


Figure 2.11 Mean plant weight (g) of nine maize hybrids at 20°C and 72 hours flooding (Means followed by same letter are not significantly different at P = 0.05) (ANOVA data appear in Appendix, Table 7)

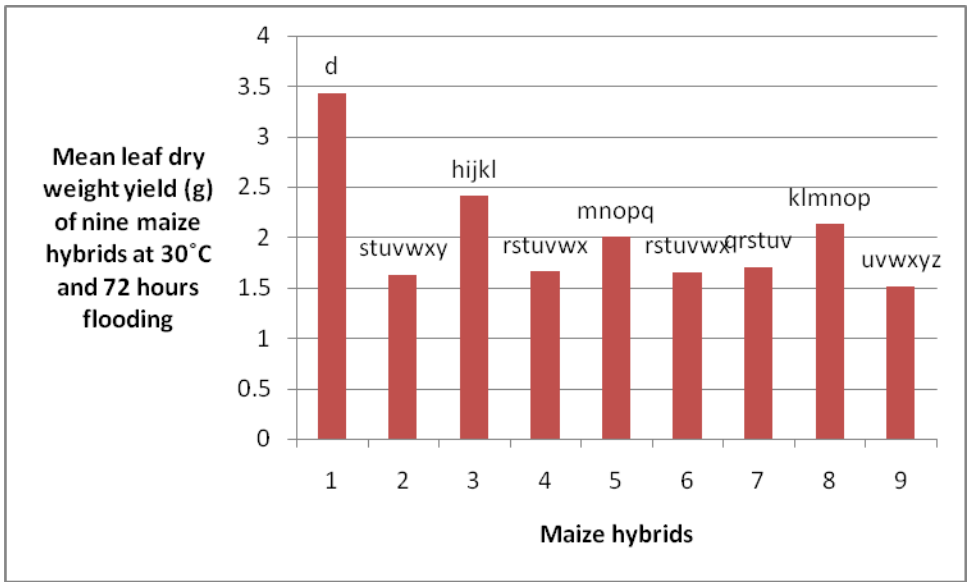


Figure 2.12 Mean plant weight (g) of nine maize hybrids at 30°C and 72 hours flooding (Means followed by same letter are not significantly different at P = 0.05) (ANOVA data appear in Appendix, Table 7)

2.4 CONCLUSION

In practice, the South African maize producers would not plant if it is too cold and wet. The unavoidable cold and wet conditions normally appear a few days after planting. In most of the commercial maize production areas, like the western Free State, the chance of thunder and hailstorms is high during the planting season. That is the major cause for a sudden drop in temperature and flooding conditions.

In the field, uncontrollable environmental factors of temperature, light and moisture may play a critical role in seed responses to flooding. In addition, the variability of soil drainage, microbial populations and disease pressure across a field may have considerable influence on how seeds respond to flooding stress.

Flooding of recently planted fields of maize, results in poor emergence of some germplasm, especially at low temperatures. Studies have shown genetic variation for flooding tolerance of maize inbreds and hybrids (Cerwick *et al.*, 1995). The performance of Hybrid 1, a conventional hybrid, was overall the best under cold, wet conditions. Therefore Hybrid 1 can be classified as the best cold tolerant hybrid of the nine hybrids tested, while the overall performance of Hybrid 9 was the poorest. Therefore Hybrid 9 can be classified as the most sensitive hybrid under cold, wet conditions. Between the hybrids with the same genetic composition used in the study, the conventional hybrids developed stronger seedlings than the Bt-hybrids, with the Roundup Ready hybrids developing the weakest seedlings. The seedling development of the nine maize hybrids correlates well with the germination results obtained in the warm test.

The data for emergence percentages, emergence rates and seedling dry weight yields generally indicated that maize seed exposed to low germination temperature (10°C), was more susceptible to flooding stress than seed exposed to a 20 and 30°C germination temperature. The combination of low temperature (10°C) and flooding duration (48 to 72 hours) had a major negative effect on germination and emergence of maize seed. Flooding conditions at higher temperatures are less detrimental on germination and emergence of maize seed than at lower temperatures. However, even at high temperatures and prolonged flooding farmers can expect seedlings and resulting

plants to be weaker. In practice, when flooding conditions are experienced in conjunction with high temperatures, maize producers can still expect good germinations of seed and emergence of seedlings, while flooding combined with low temperatures, highly improves the chance for a re-plant.

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CHAPTER 3

RELATIONSHIPS BETWEEN ETHANOL EXCRETION UNDER DIFFERENT TEMPERATURE AND FLOODING CONDITIONS AND EMERGENCE OF MAIZE (*Zea mays* L.) HYBRIDS

3.1 INTRODUCTION

The marsh plant (*Acorus calamus* L.) can survive two months under anoxia, but grain seedlings like wheat and barley can survive only a few hours (Menegus, 1989). This difference in flooding tolerance is based on complex anatomical and biochemical adaptations. Different fermentation pathways and products of anaerobic metabolism play essential roles in surviving prolonged periods under anoxia. Ethanolic fermentation is one of the most common pathways that generate NAD+ for the continuation of glycolysis. Ethanolic fermentation is a two step process in which pyruvate is first decarboxylated to acetaldehyde by pyruvate decarboxylase (PDC), and acetaldehyde is subsequently converted to ethanol by alcohol dehydrogenase (ADH), regenerating NAD+. Ethanol is produced to a varying degree by most plants under oxygen stress (Crawford & Brandle, 1996). The results of these studies have led to the hypothesis that the end products of anaerobic metabolism (ethanol and acetaldehyde) are toxic to seeds, and that flooding leads to increased cellular concentrations of these compounds and, thus, to death of the seed (Martin *et al*., 1991).

According to a study done by Martin *et al*. (1988), there were significant negative correlations between amounts of ethanol excreted into the soak water, after 24 or 48 hours and field emergence of different maize inbred lines. The highest negative correlations occurred when soil temperatures were low during field germination but not below freezing. They also found a significant negative correlation between ethanol concentration in the soak water and subsequent germination in the soak test. Maize seeds generally do not germinate and grow when imbibed below 10ºC (Eagles, 1982; Maree, 2006). Maize seeds imbibed at 10°C perform anaerobic respiration and release ethanol into the environment (O'Neil, 1987). This may occur because of

an increase in the activation energy of the mitochondrial oxidative system at low temperatures relative to the constancy of the activation energy of glycolysis (Raison, 1980). Martin (1986) indicated that soaking seeds in water (soak test) results in similar metabolic events. Initially, seeds take up water and oxygen. Martin (1986) reported that the oxygen content of the water is depleted within 4 hours while ethanol synthesis is linear for the entire 48 hours of soaking. The net result of this process is an increase in ethanol content of the soak water resulting in concentrations between 0.02 to 0.04 M ethanol after 48 hours. O'Neil (1987) stated that the similarity between seed germination after imbibition in cold, wet soils and after an anaerobic soak, might then be the ability of a genotype to either limit accumulation of the end products of anaerobic metabolism (ethanol, acetaldehyde, and carbon dioxide) or to withstand the presence of these metabolites.

The objectives of this study were to investigate the relationships between ethanol excretion during soaking of maize seed and simulated field emergence in growth chambers and a glasshouse under different temperature and flooding conditions.

3.2 MATERIALS AND METHODS

3.2.1 Experimental set-up

Experiments were conducted during January 2007 in collaboration with the South African Bureau of Standards (SABS), in Pretoria. Due to the fact that the ethanol analysis are very expensive, it was decided to use only three hybrids in the ethanol study. The three hybrids used in the study were a conventional single cross (hybrid 1) and two Bt- single cross hybrids (hybrids 3 and 6). It was decided on hybrid 1, because this conventional hybrid developed the best seedlings, and it showed the fastest emergence rate between the nine hybrids (Chapter 2). Hybrid 1 and 3 has the same genetic background, for comparison purposes it will be good to have more than one hybrid of the same genetic background. Hybrid 1 and 3 are white hybrids, while hybrid 6 is a yellow hybrid. Due to the high cost involved in ethanol analysis, only two replications were done, and therefore no statistical differences can be indicated. The seed were stored at 11°C at a relative humidity (RH) of 38%.

The seed samples were carefully cleaned and freed from foreign materials. The seed samples were soaked in polystyrene cups in 100 ml distilled water (Figure 3.1). The polystyrene cups were arranged in a completely randomized design with four replicates in three different growth chambers. One milliliter samples were taken from the soak water for ethanol analysis.

Figure 3.1 Soaking of maize seeds in polystyrene cups in 100 ml distilled water

3.2.2 Treatments

Samples of 25 seeds per replicate were soaked in the dark. The maize seeds were soaked at three different temperatures:

(i) 10 °C
(ii) 20 °C
(iii) 30 °C

Each of the above samples were soaked for three different periods:

(i) 24 hours
(ii) 48 hours
(iii) 72 hours

To verify that no ethanol was present in the water, a control sample of the distilled water was analyzed, with all the soak samples. No traces of ethanol was found in the control sample.

3.2.3 Data collection

Quantification of ethanol was accomplished by a modified method similar to that developed with castorbean (*Ricinus communis* L.) seeds (Donaldson *et al*., 1985). Ethanol measurements were made by a gas chromatograph head space equipped with a flame ionization detector and a Poropak Q Column at an oven temperature of 170°C. The helium flow was 40 ml/min, and the flame ionization detector temperature were 250°C. The amount of ethanol in the soak water samples were determined from the peak area relative to the internal standard, isobutanol. Peak areas were quantified using a Nelson data system with an external standard procedure.

3.2.4 Statistical analysis

Due to the high cost involved in ethanol analysis, only two replications were done, and therefore no statistical differences can be indicated.

3.3 RESULTS AND DISCUSSION

For all three maize hybrids used in the study, flooding of seeds resulted in a rapid increase in excreted ethanol from 24 hours flooding to 72 hours flooding. The mean ethanol contents for the flooding periods are shown in Table 3.1. The uncertainty of the measurement is *ca* 5 ppm. Hybrid 3 excreted the highest amount of ethanol under all three flooding periods. The literature indicated that ethanol excretion was negatively correlated with emergence and seedling development of maize seed. According to the literature, it was anticipated that ethanol excretion of hybrid 1 should be the lowest of the three hybrids under flooding conditions, due to best seedling development and emergence rate (Chapter 2). Although the mean ethanol excretion of hybrid 1 was 30.45 ppm lower than that of hybrid 3, it was 9.77 ppm higher than that of hybrid 6. No trends were found between different genetic materials, as well as between different types of hybrids. What was striking about the ethanol results was the fact that most of the complaints

regarding poor emergence of maize seedlings under flooding conditions was from hybrid 3. Hybrid 3 excreted the highest amount of ethanol (117.67 ppm) for all three flooding periods, especially for the longer (72 hours) flooding duration.

Table 3.1 Ethanol excretion of three maize hybrids at 24, 48 and 72 hours flooding

Flooding (hours)	**Ethanol (ppm)**			**Mean**
	Hybrid 1 (Conventional)	**Hybrid 3 (Bt)**	**Hybrid 6 (Bt)**	
24	28.33	45.67	14.67	**29.56**
48	55.00	76.67	33.00	**54.89**
72	65.33	117.67	71.67	**84.89**
Mean	**49.55**	**80.00**	**39.78**	

Temperature also played a very important role in ethanol excretion of maize hybrids under flooding conditions (Table 3.2). Major differences in ethanol excretion were found at all three temperatures. Literature indicated that anaerobic (flooding) conditions are the main reason for developing seedlings to release ethanol in their direct environment (Martin *et al.*, 1988; Crawford & Brandle, 1996). The higher the temperature, the higher the ethanol excretion. Hybrid 3 excreted the highest amount of ethanol for all three temperatures.

Table 3.2 Ethanol excretion of three maize hybrids at 10°C, 20°C and 30°C

Temperature (°C)	**Ethanol (ppm)**			**Mean**
	Hybrid 1 (Conventional)	**Hybrid 3 (Bt)**	**Hybrid 6 (Bt)**	
10	10.00	12.33	3.67	**8.67**
20	41.33	80.33	41.00	**54.22**
30	97.00	147.33	74.67	**106.33**

Mean	**49.44**	**80.00**	**39.78**	

The lower the temperature and the shorter the flooding duration the lower were the average ethanol excretion of the maize hybrids (Table 3.3). A growth chamber experiment (Chapter 2) with different temperatures (10°C, 20°C and 30°C) and flooding periods (24 hours, 48 hours and 72 hours) was run to simulate field emergence of the three maize hybrids. Mean emergence percentages, mean emergence rates and mean ethanol excretion (ppm) are shown in Table 3.3.

Results of a study done by Khosravi & Anderson (1990) indicated that ethanol production of maize seed was negatively correlated with seed viability. The viability of hybrid 1 was the highest at 99.5%, hybrids 3 and 6 were very close at 98.8% and 98.5% respectively. No statistical differences for viability were found for the three hybrids (Chapter 2). Khosravi & Anderson indicated that amounts of ethanol above 200 ppm could be harmful for maize seed emergence. The highest amount of ethanol (213 ppm) was excreted by hybrid 3 at 72 hours flooding and 30°C. Hybrid 6 excreted the lowest amount of ethanol (1 ppm) at 24 hours flooding and 10°C (Table 3.3).

Table 3.3 Emergence percentages, emergence rates and ethanol excretions of three maize hybrids at 10°C, 20°C and 30°C and 24, 48 and 72 hours flooding

Hybrid	**Flooding (Hours)**	**Temperature (°C)**	**Emergence %**	**Emergence rates**	**Ethanol excretion (ppm)**
Hybrid 1	24	10	2.5 (e)	0.0 (f)	4
		20	97.5 (ab)	1.3 (bcd)	12
		30	100.0 (a)	1.98 (a)	68
	48	10	0.0 (e)	0.0 (f)	6
		20	95.0 (ab)	0.7 (e)	48
		30	95.0 (ab)	1.5 (bc)	111

	72	10	0.0	(e)	0.0	(f)	20
		20	92.5	(abc)	0.5	(e)	64
		30	97.5	(ab)	1.4	(bcd)	112
Hybrid 3	24	10	2.5	(e)	0.0	(f)	9
		20	97.5	(ab)	1.2	(cd)	27
		30	100.0	(a)	1.9	(a)	101
	48	10	0.0	(e)	0.0	(f)	13
		20	75.0	(d)	0.7	(e)	89
		30	82.5	(bcd)	1.4	(bcd)	128
	72	10	0.0	(e)	0.0	(f)	15
		20	75.0	(d)	0.7	(e)	125
		30	77.5	(cd)	1.3	(bcd)	213
Hybrid 6	24	10	2.5	(e)	0.0	(f)	1
		20	95.0	(ab)	1.2	(bcd)	25
		30	92.5	(abc)	1.8	(a)	18
	48	10	0.0	(e)	0.0	(f)	3
		20	70.0	(d)	0.7	(e)	36
		30	92.5	(abc)	1.5	(b)	60
	72	10	0.0	(e)	0.0	(f)	7
		20	67.5	(d)	0.6	(e)	62
		30	82.5	(bcd)	1.1	(d)	146

A significant negative correlation of -69.4 % (P = 0.038) was found between emergence percentages and ethanol excretions as well as between emergence rates (-86.2 %) (P=0.003) and ethanol excretions of the three maize hybrids (statistical data appear in Appendix, Table 8).

3.4 CONCLUSION

The results of this study corroborate findings of Martin *et al*. (1991). According to Martin *et al*., (1991), soak-sensitive maize inbreds are less vigorous and have lower field emergence than soak-tolerant inbreds. In addition, the amount of ethanol excreted during soaking were negatively correlated with both laboratory germination after soaking and field emergence (chapter 2) of unsoaked seed sown in cold, wet soil.

According to these results, the period of flooding or waterlogging, was directly correlated with the amounts of ethanol excreted around the developing seeds, and the combined effect of

flooding and temperature had a major negative effect on emergence of maize seeds. There were also differences between genetic material regarding their ability to release ethanol in their direct environment. Temperature also played a role in ethanol excretion of developing seeds. Results indicated that this technique is usable to explain poor germination and emergence of maize seed under flooding conditions at relatively high temperatures, but not under low temperature conditions.

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CHAPTER 4

RELATIONSHIP BETWEEN LABORATORY VIGOUR TESTS AND EMERGENCE OF MAIZE (*Zea mays* L.) HYBRIDS EXPOSED TO COLD AND WET CONDITIONS

4.1 INTRODUCTION

Seed quality is one of the most important factors affecting early performance and productivity of most agricultural crops. The fundamental objective of seed testing is to establish the quality level of seed. The seed industry uses the standard germination test (AOSA, 2002) for labeling. The

standard germination test, also known as the warm test, estimates germination under ideal growing conditions (Munamava, *et al*., 2004).

According to Kolasinska *et al*. (2000), seed quality tests should relate to field emergence. Many researchers have reported significant correlation coefficients between field emergence and standard laboratory germination tests. Early planting exposes seed to unfavourable conditions and the commonly used warm test cannot accurately predict field emergence under these conditions. Kolasinska *et al*. (2000) have proven that vigour tests as predictors of field emergence were more useful than the warm test.

Cold, wet soils have long been associated with poor field performance in many crops, especially in temperate regions where seeds are often planted in early spring. Field conditions at this time can be adverse due to high soil moisture, low temperatures and soil borne fungi, and these factors can cause poor field emergence. The cold test was developed to simulate these adverse field conditions and measure the ability of maize seeds to emerge (TeKrony, 1983; Ferguson, 1990; Hampton, 1992). Martin *et al*. (1988) found highly significant positive correlations between the cold test and field emergence and the soak test and field emergence.
Hepburn *et al*., (1984) reported that electrical conductivity (EC) measurement of the soak water in which a bulk sample (25 seeds) had been steeped, identified seed batches that have high laboratory germination, but poor field emergence potential. Such seed batches have high electrolyte leakage and are classified as having low vigour, while those with low leakage are considered to have high vigour. Pandey (1994) has completed an extensive review of the basis for EC as a seed quality test. Pandey (1994) confirmed the findings of Hepburn *et al*. (1984), and stated that the electrical conductivity test can be used as a vigour test. According to Powell (1988), integrity of cell membranes, determined by deteriorative biochemical changes or physical disruption, can be considered the fundamental cause of differences in seed vigour, which are indirectly determined as electrolyte leakage during the conductivity test. He also found that as a seed re-hydrates during early imbibition, the ability of its cellular membranes to reorganize and repair any damage that may have occurred will influence the extent of electrolyte leakage from the seed. The greater the speed with which the seed is able to re-establish its membrane integrity the lower the electrolyte leakage. He also stated that higher vigour seeds are

able to reorganize their membranes more rapidly, and repair any damage to a greater extent, than low vigour seeds. Consequently, electrolyte leakage from high vigour seeds are less than that from low vigour seeds.

Matthews & Bradnock (1968), as well as Keeling (1974) found that electrolyte leakage from low vigour seed batches also causes secondary effects, in that nutrients exuded from seeds during germination, stimulate soil microorganism activity and secondary infection. Both of them reported a direct correlation between the quantity of carbohydrates exuded from seeds and seedling performance.

Accelerated ageing has subsequently been evaluated as an indicator of seedling vigour and has been successfully related to field emergence and stand establishment (TeKrony, 1994). The accelerated ageing test exposes seeds for short periods to the two environmental variables which cause rapid seed deterioration; high temperature and high relative humidity. According to TeKrony (1994), high vigour seed batches will withstand these extreme stress conditions and deteriorate at a slower rate than low vigour seed batches. Early storage studies by Helmer (1962) and Baskin (1970) suggested that accelerated ageing could be utilized as a vigour test to predict field performance. Additional studies have shown that this vigour test functions quite well in forecasting field emergence and stand establishment for a wide range of crop species, included maize. TeKrony (1994) indicated that when maize seeds are planted under stressful field conditions, accelerated ageing germination provides higher correlations with field emergence than does the warm test.

The complex stressing vigour test was first developed for wheat, and later modified for maize, to provide an indication of the minimum expected range of emergence under stress conditions (Szirtes & Barla-Szabo, 1981). This test has been widely used in Hungary and has consistently identified low and high vigour seed batches, with results that desired populations of maize can be more frequently achieved (Barla-Szabo & Dolinka, 1988; Barla-Szabo *et al*., 1990). Barla-Szabo & Dolinka (1988) showed that for 20 maize seed batches the complex stressing vigour test results were more strongly correlated with field emergence at three spring sowings than warm or cold test results. Barla-Szaba & Dolinka (1988) suggested that under good spring sowing

conditions, field emergence would approach the percentage warm test germination, but under unfavourable conditions, field emergence would be closer to the complex stressing vigour test results.

The tetrazolium test is essentially a measurement of dehydrogenase enzyme activity. Lakon (1942) first developed this technique for seed testing and later staining patterns of the tissues were used to assess vigour. The advantage of the tetrazolium test is that it requires no elaborate facilities. But it is subjective and reproducible results are difficult to achieve. In the tetrazolium test, seeds are classified as good, medium or poor in vigour (Moore, 1972).

The fast green test was developed by Koehler (1957). This test reveals physical fractures in the seed coat of light coloured seeds and is often used in maize. Seeds are soaked in a 0.1% solution of fast green stain for 15–30 seconds. After the soak period, the seeds are washed for 20 seconds under running water and any deformations are clearly delineated by green markings.

Speed of emergence of seedlings is one of the oldest seed vigour concepts. Seed batches with similar total germination often vary in their rate of emergence and growth. A number of methods for determining emergence rate have been employed (Tucker & Wright, 1965; Nichols & Heydecker, 1968).

The objective of this study was to compare different vigour tests to be able to identify the most sensitive vigour test to predict emergence of maize hybrids under cold, wet conditions. According to the literature, the soak test as well as the complex stressing vigour test seems to be more accurate than the cold test in predicting field emergence of maize hybrids under unfavourable climatic conditions, especially cold, wet conditions.

4.2 MATERIALS AND METHODS

4.2.1 Experimental set-up

Different vigour tests were conducted during 2006 at Monsanto's quality laboratory at Endicott and at the Phytotron laboratory at the Department of Plant Production and Soil Science, University of Pretoria. Seed samples were submitted originally as commercial seed batch samples by three different seed companies. Seeds of nine maize hybrids (seven hybrids from Monsanto and one hybrid each from Pannar and PHI) were used in the study. Seed were stored in a room maintained at 11°C and 38% relative humidity (RH). Vigour of the nine maize hybrids was assessed by the cold test, soak test, complex stressing vigour test, electrical conductivity test, accelerated ageing test, tetrazolium test and fast green test.

Results of the vigour tests were compared with seedling emergence in a glasshouse. Seeds from the same batches used in the laboratory tests were used in the glasshouse study. Seeds were planted in plastic pots similar to that of the growth chamber study. The same soil used for the growth chamber study (Chapter 2) was used for the glasshouse study.

4.2.2 Treatments and Data collection

4.2.2.1 Warm test

Seed germination was measured by conducting the standard germination test, commonly known as "warm test". Four replicates of 50 seeds each were planted in 2-L plastic containers filled with wet sand. Lids were placed on the containers and the containers were incubated at 25°C for four days (first count) and seven days (final count). Germination was assessed as the percentage of seeds producing normal seedlings as defined by ISTA rules (ISTA, 1993).

4.2.2.2 Cold test

A cold test was conducted by planting four replications of 50 seeds for each entry. Seeds were planted into 2-L plastic containers, filled with vermiculite and soil from a maize field, 300 ml water was evenly poured over the soil. Lids were placed on the containers and the containers were incubated at 10°C for seven days and then transferred to 25°C, 85 % relative humidity (RH) for four days. All treatments were applied in incubators with +/- 0.1°C temperature precision.

Germinated seeds were counted according to the rules of the Association of Official Seed Analysts (AOSA) (1999). Seeds that did not emerge were regarded as dead.

4.2.2.3 Soak test

A soak test was conducted by submerging four replications of 50 seeds each in 100 ml distilled water in 250 ml polystyrene cups. Seeds were soaked for 24 hours at 25°C in an incubator. After soaking, seeds were planted in sand and incubated for seven days at 25°C, 85% relative humidity (RH). The sand was kept moist for the duration of the test. All treatments were in incubators with +/- 0.1°C temperature precision. On day seven after planting, germinated seeds were counted using germination criteria recommended by the Association of Official Seed Analysts (AOSA) (1999).

4.2.2.4 Complex stressing vigour test

The complex stressing vigour test was conducted to study the effect of fluctuating soaking temperatures on germination of maize seed. Seeds of the maize hybrids were soaked for 48 hours at a moderate temperature (25°C), followed by another 48 hours soak at low temperatures (5°C), where after it was planted in sand and grown for four days at 25°C, before evaluation. Germinated seeds were counted using germination criteria recommended by the Association of Official Seed Analysts (AOSA) (1999).

4.2.2.5 Electrical conductivity test

During initial stages of imbibition most seeds leak ions, amino acids, and sugars. Weaker seeds, i.e. those with poor cell membrane structure or slow restoration of cell membrane function during rehydration, tend to leak more. According to Black & Bewley (2000), seed leakage can be quantified by the increase in the electrical conductivity of the soak water. The higher the electrical conductivity value, the lower the seed quality. Electrical conductivity was measured with an Individual Seed Analyzer. Conductivity of four replicates of 25 seeds each were measured in the study. Seeds were soaked in 100 ml deionized water at three different

temperatures (10°C, 20°C and 30°C). Electrical conductivity of the water was measured after 24 hours, 48 hours and 72 hours. Conductivity was recorded in mS cm^{-1} (AOSA, 1983). To ensure accurate electrical conductivity readings, a control sample of distilled water was included in the study.

4.2.2.6 Accelerated ageing test

According to Delouche & Baskin (1973), accelerated ageing has been used to predict seed storability and has been widely adopted as a vigour test. In the accelerated ageing test, membrane degradation occurs as a consequence of the oxidation of unsaturated fatty acids (Navari-Izzo & Rascio, 1999). Basavarajappa *et al*. (1991) associated lipid peroxidation in aged maize seeds with membrane damage. Seeds were placed on top of the screen inside accelerated aging boxes, each 10 x 10 x 4 cm, and 40 ml of tap water were added. Boxes were covered and placed in an accelerated aging chamber which provided a relative humidity near 100 percent at 51°C for 96 hours. Seeds were weighed before and after being placed in the chamber to calculate seed moisture increase during ageing. Seeds were planted in crepe cellulose paper and covered with sand. Seedlings were evaluated after seven days according to AOSA rules (2002).

4.2.2.7 Tetrazolium test

The tetrazolium test is one of the most widely used and helpful seed viability tests. This test was first developed in Germany in the early 1940's by Professor George Lakon who had been trying to distinguish between live and dead seeds by exposing them to selenium salts. He then tried tetrazolium salts and found them more effective. Today the test is used throughout the world as a highly regarded method of estimating seed viability and is a routine test in many seed testing laboratories. It is often referred to as a "quick test", since it can be completed in 24 to 48 hours (Schultz, 1992).

The tetrazolium test is a biochemical test most commonly used to determine seed viability. According to Schultz (1992), this test relies upon the action of the tetrazolium molecule to react with hydrogen atoms released as a result of the activity of respiration enzymes. As a result, the water insoluble red pigment called formazan is formed in actively respiring cells of living tissues. The staining pattern and intensity may be used as a means of predicting seed vigour. Schultz (1992) also stated that this test is an excellent diagnostic test to help determine why seeds may have performed poorly in germination or vigour tests. Two replicates of 50 seeds each were stained with a 10 g / kg solution of tetrazolium chloride according to ISTA method (ISTA, 1993). Seeds were evaluated and classified as viable or non-viable.

4.2.2.8 Fast green test

Determination of mechanical seed damage has important implications for the seed producer and consumer. This test is used to measure mechanical shocks on the seeds. Two replicates of 100 seeds each per entry were put in a small basket with small holes and submerged in 0.1 % fast green solution for 15 – 30 seconds (Figure 4.1). During this period, the vital stain penetrates any area of the seed which has lost its physical integrity and stains it green. Thereafter it is rinsed under running tap water for 20 seconds, and thereafter air-dried for about 30 minutes. Staining patterns were used to classify seed damage (Koehler, 1957). Seeds were sorted in three categories (Figure 4.2):

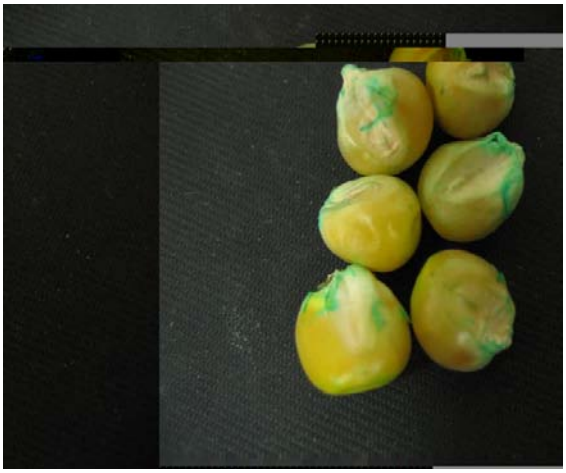
(i) Good quality seeds: few coloured zones and colourings far from embryo

(ii) Intermediate quality seeds: coloured zones near the embryo

(iii) Poor quality seeds: strong coloured zones on the embryo

Figure 4.1 Procedure for submerging maize seeds in fast green solution (Baskets with seeds in the front and container with fast green solution in the back)

(a) (b)



(c)

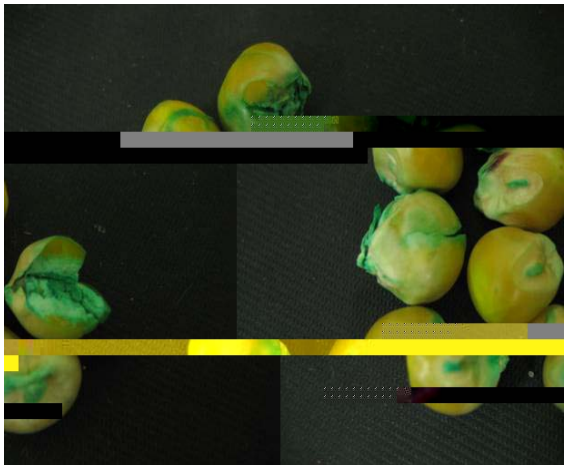


Figure 4.2 Results from the fast green test (a) good quality, (b) intermediate quality and (c) poor quality seeds

4.2.2.9 Glasshouse experiment

All nine maize hybrids were used in the study. Four replicates of 10 seeds each were planted per treatment. The experiment was run during September 2006 under controlled temperatures of 10°C (night) and 25°C (day). The idea was to simulate field emergence under cool temperatures early in the growing season. Plastic pots were flooded for 24 hours, 48 hours and 72 hours similar to that of the growth chamber experiment (Chapter 2). Daily emergence counts were taken and emergence rates were calculated according to the index of Maguire (1962). The higher the emergence rate, the higher the vigour and vice versa.

4.2.3 Statistical analysis

Laboratory and glasshouse tests were conducted in a completely random design. Laboratory tests were repeated at least 3 times. Data were analyzed by analysis of variance, using the MINITAB 14.2 software program as well as the JMP7 software program. Differences at the $P < 0.05$ level of significance were reported. The Student's *t* Test was conducted to confirm significant differences.

4.3 RESULTS AND DISCUSSION

4.3.1 Correlation between the warm test, cold test, soak test, complex stressing vigour test and simulated field emergence under cold, wet conditions

All varieties used in the study showed above 96% germination in the standard germination test or so-called "warm test" (Chapter 2). The warm, cold, soak, and complex stressing vigour test results for the maize hybrids are shown in Table 4.1.

The complex stressing vigour test (Table 4.1) resulted in the highest variation in germination for the nine maize hybrids (standard deviation of 13.76). The lowest standard deviation (1.044) was obtained with the warm test. This was to be expected, since the warm test is conducted under the most favourable growing conditions. The standard deviations of the cold and soak tests were also low and was 1.38 for the cold test and 2.50 for the soak test. The coefficient of variations for the different tests shows the same trends than for standard deviations.

Significant differences were found between the hybrids in all the tests. Differences between the hybrid responses in the warm test and the soak test were minimal, which implies that the soak test seems to be less usable as a vigour test, especially under unfavourable climatic conditions.

Table 4.1 Results for warm test, cold test, soak test, and complex stressing vigour test for nine maize hybrids (ANOVA data appear in Appendix, Tables 8 -11)

Hybrid	Warm test germination (%)	Cold test germination (%)	Soak test germination (%)	Complex stressing vigour test germination (%)
1	99.5 (a)	97.3 (a)	98.5 (a)	95.0 (a)
2	99.5 (a)	94.3 (ab)	98.0 (a)	88.5 (ab)
3	98.8 (a)	96.3 (a)	98.7 (a)	82.5 (b)

4	97.8 (ab)	94.9 (ab)	99.0 (a)	89.8 (ab)
5	99.5 (a)	94.9 (ab)	97.3 (a)	88.5 (ab)
6	98.5 (a)	97.1 (a)	98.5 (a)	87.0 (ab)
7	98.8 (a)	91.3 (b)	98.5 (a)	92.3 (ab)
8	98.5 (a)	92.1 (b)	91.0 (b)	57.8 (c)
9	96.3 (b)	94.1 (ab)	98.3 (a)	59.8 (c)
CV	2.10	3.13	4.96	17.74
Means followed by the same letter are not significantly different at P = 0.05				

Significant differences in emergence percentages as well as in emergence rates were found between some of the hybrids in the glasshouse experiment under cool temperatures (25°C / 10°C) and 72 hours flooding (Table 4.2). Emergence percentage of hybrid 1 and hybrid 6 was the highest (87.5%), while the emergence rate of hybrid 1 was the highest (0.81). The emergence percentage and rate of hybrid 8 was the lowest of the nine hybrids. No correlations were found between the different genetic background as well as between the different trait versions of the same genetic background.

Table 4.2 Emergence percentages and rates of nine maize hybrids from the glasshouse experiment after 72 hours flooding (ANOVA data appear in Appendix, Tables 12 &13)

Hybrid	Type of hybrid	Emergence %	Emergence rate
1	Conventional *	87.5 (a)	0.81 (a)
2	Conventional	72.5 (ab)	0.61 (ab)
3	Bt *	72.5 (ab)	0.56 (ab)
4	Roundup Ready *	82.5 (a)	0.62 (ab)
5	Conventional **	85.0 (a)	0.62 (ab)
6	Bt **	87.5 (a)	0.71 (ab)
7	Roundup Ready **	72.5 (ab)	0.55 (ab)
8	Bt	50.0 (b)	0.41 (b)

9	Conventional	62.5 (ab)	0.47 (b)
CV		21.34	28.04
Means followed by the same letter are not significantly different at P = 0.05 * Indication of the same genetic background			

Significant correlations (Table 4.3) were found between 72 hours flooding in the glasshouse experiment and the cold, soak and complex stressing vigour tests. The best correlation (89.9%) was found between simulated field emergence and the complex stressing vigour test. According to these results, the complex stressing vigour test is the best test to predict field emergence under cold, wet conditions.

Table 4.3 Pearson correlation coefficients for relationships between mean germination of the warm test, cold test, soak test, complex stressing vigour test and simulated field emergence

	Warm test	**Cold test**	**Soak test**	**Complex stressing vigour test**
Cold test	**0.354**			
	P = 0.351			
Soak test	**0.751**	**0.432**		
	P = 0.020	P = 0.245		
Complex stressing vigour test	**0.571**	**0.602**	**0.668**	
	P = 0.109	P = 0.086	P = 0.049	
Simulated field emergence	**0.492**	**0.626**	**0.678**	**0.899**
	P = 0.178	P = 0.031	P = 0.045	P = 0.001

Regression analysis were done (Tables 14-16 Appendix) for the different vigour tests and simulated field emergence to see which test, best predicts field emergence under cold, wet

conditions (Figures 4.3 – 4.5). The adjusted R-square for the cold test as affected by simulated field emergence is 52.2 %. This means that 52.2 % of the variability in simulated field emergence under cold, wet conditions could be explained by the cold test. The adjusted R-square for the soak test as affected by simulated field emergence is 44.5 %, while that for the complex stressing vigour test as affected by simulated field emergence is 69.6%. This means that 44.5 % of the variability in simulated field emergence under cold, wet conditions could be explained by the soak test, while 69.6 % of the variability could be explained by the complex stressing vigour test.

Regression analysis results showed that it is possible to predict field emergence under cold, wet conditions with all three vigour tests. The complex stressing vigour test is the best vigour test to predict field emergence under these extreme climatic conditions. The complex stressing vigour test predicts field emergence 25.1 % better than the soak and 17.4 % better than the cold test under cold, wet conditions.

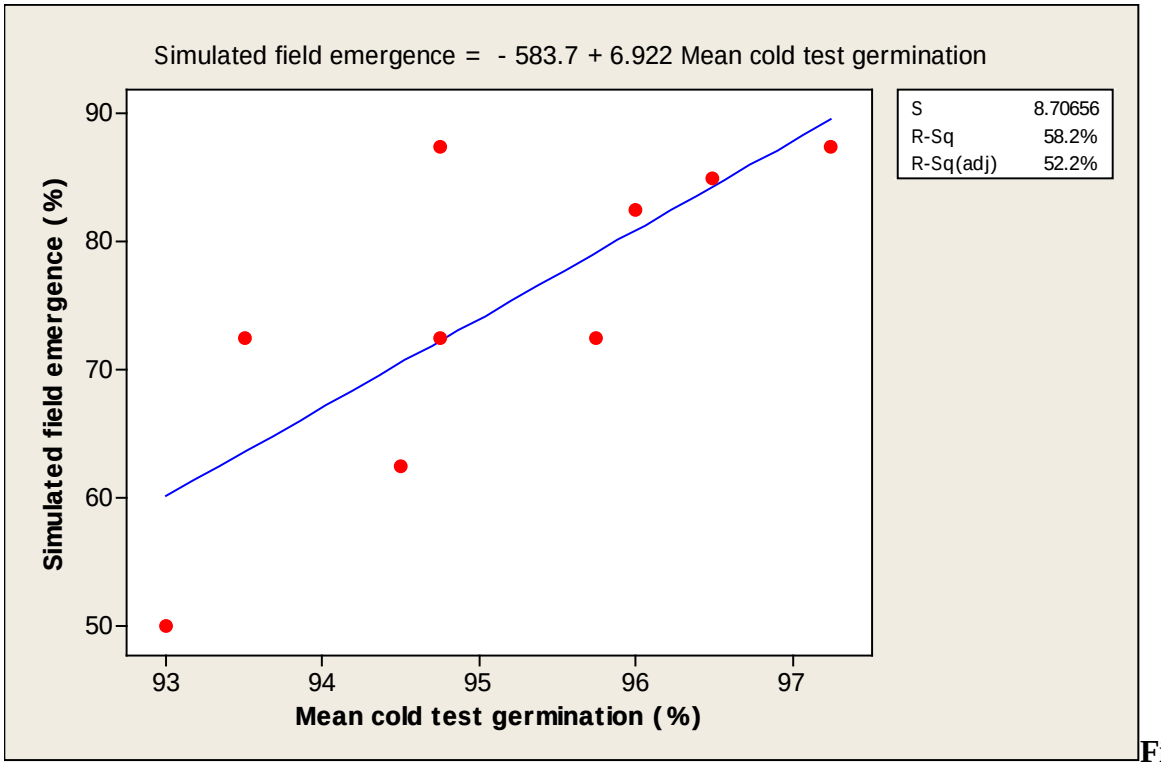


Figure 4.3 Relationship between the cold test and simulated field emergence under

cold, wet conditions

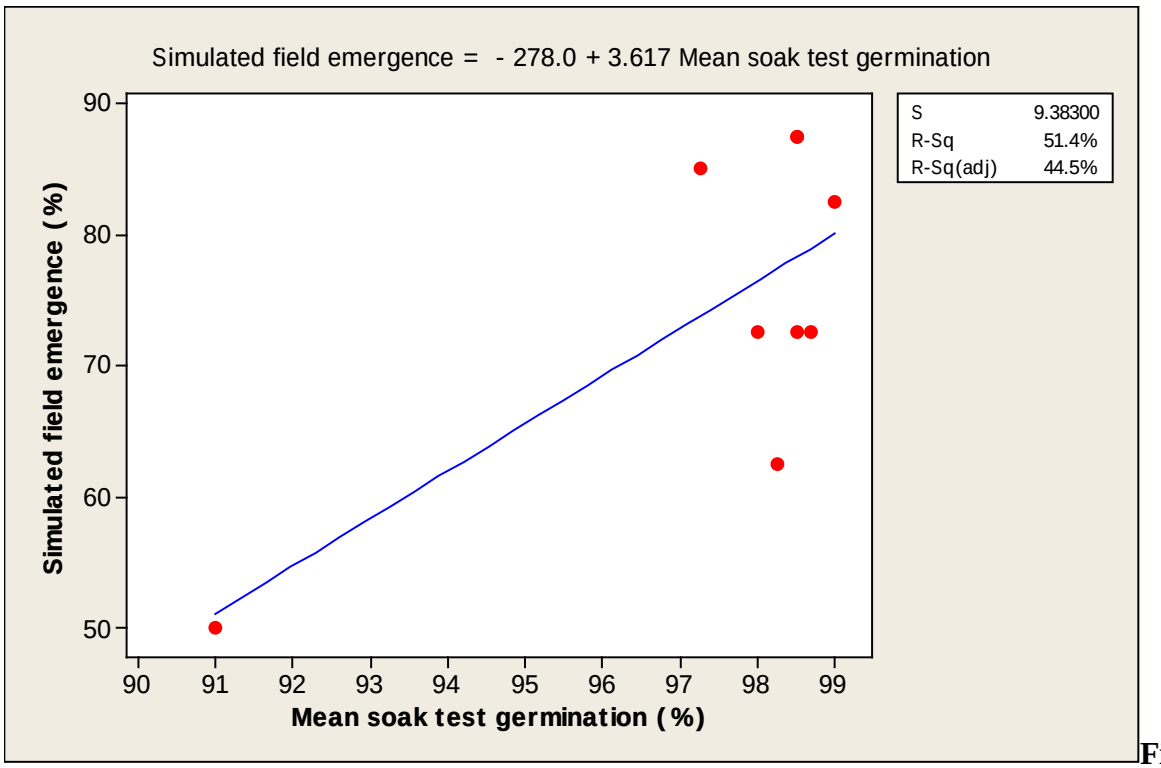


Figure 4.4 Relationship between the soak test and simulated field emergence under cold, wet conditions

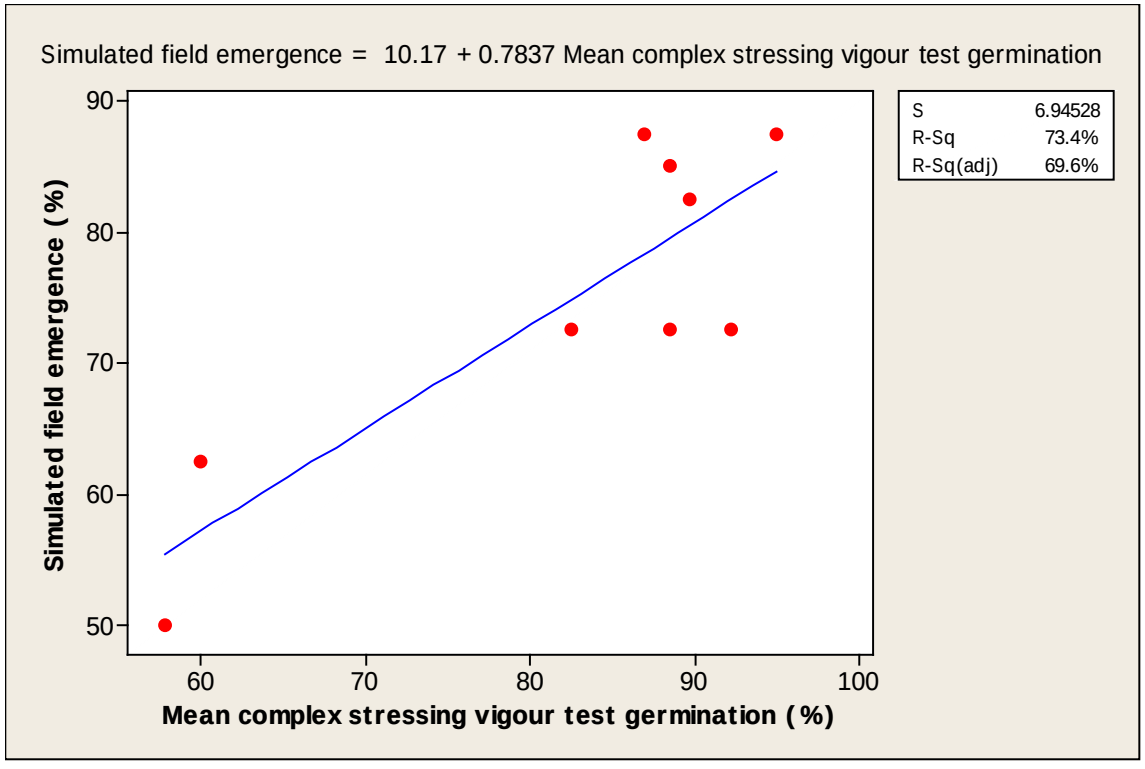


Figure 4.5 Relationship between the complex stressing vigour test and simulated field emergence under cold, wet conditions

4.3.2 Relationship between the electrical conductivity test and emergence of maize hybrids under different temperature and flooding conditions

Black & Bewley (2000) reported that most seeds leak ions, amino acids and sugars during initial stages of imbibition. According to them, weaker seeds, that is, those with poor cell membrane structure or slow restoration of cell membrane function during rehydration, tend to leak more. They also indicated that seed leakage can be quantified by the increase in the electrical conductivity of the soak water. The higher the electrical conductivity value, the lower the seed quality. Fessel *et al*. (2006) found that the electrical conductivity test determines indirectly the integrity of seed membrane systems, and is used for the assessment of seed vigour, because this test detects the seed deterioration process.

Electrical conductivity of the maize hybrids used in the study at different temperatures (10°C, 20°C and 30°C) and soaking times (24 hours, 48 hours and 72 hours) are presented in Table 4.4. The interaction between hybrids, temperatures and soak times was highly significant (P = 0.000). There were also highly significant differences in the electrical conductivity test between the maize hybrids regarding electrolyte leakage into the soak water (P = 0.000). The results (Table 4.4) correlates well with the germination and emergence results obtained in Chapter 2 regarding emergence of the hybrids under cold, wet conditions. Emergence percentage as well as emergence rate of hybrid 1 was the highest of the nine maize hybrids tested. The electrical conductivity measurements of hybrid 7 was on average the highest, however, it does not correlate well with the emergence results obtained in Chapter 2. Furthermore, no specific trends were found between the different genetic material as well as between the different trait versions of the nine hybrids.

The longer the soaking period, and the higher the soaking temperatures, the higher the electrolyte leakage into the soak water. At the lowest temperature (10°C) and regardless the length of

soaking (Table 4.4), no significant differences were found in electrical conductivity of nine maize hybrids. The trend of increase leakage with increased soaking time (24 versus 72 hours) at 10°C was significant for Hybrids 3 and 4 (Hybrid 4>Hybrid3). At higher temperatures (20 and 30°C), some significant differences were found between the nine maize hybrids. At 20°C and 72 hours flooding, Hybrid 5 had a significantly higher EC reading than Hybrids 1, 2, 4, 7 and 9 (Hybrid 4>Hybrid 1>Hybrid 2>Hybrid 7>Hybrid 9). The EC readings of Hybrids 5 and 8 at 20°C and 72 hours soaking were significantly higher than at 24 (Hybrids 5 and 8) and 48 hours (Hybrid 5) flooding.

The EC reading of Hybrid 7 was the highest at 30°C and 72 hours flooding and the EC reading of Hybrids 5, 7, 8 and 9 (Hybrid 7>Hybrid 9>Hybrid 5>Hybrid 8) was significantly higher than that of Hybrid 4, which had the lowest EC reading under these conditions. Unlike at 20°C, some hybrids (Hybrid 1, 8 and 9) had significantly higher EC reading at 30°C and 48 hours soaking than at 24 hour soaking, while the EC reading of all nine hybrids was significantly higher at 72 hour soaking than at 24 hours, while only the EC reading of Hybrid 4 did not differ significantly between 72 and 48 hours soaking.

Higher electrolyte leakage lead to poorer emergence percentages. A significantly negative correlation (-0.406 or -40.6%) was found between mean electrolyte leakage and simulated field emergence of the nine maize hybrids under different temperature and flooding conditions (Table 18 Appendix). According to the results of the study, the electrical conductivity test is, however, not a very good vigour test to predict field emergence of maize hybrids under cold, wet conditions.

Table 4.4 Electrical conductivity (mS cm^{-1}) for seed of nine maize hybrids at 10°C, 20°C and 30°C and 24, 48 and 72 hours flooding (ANOVA data appear in Appendix, Table 17)

Temp	**10°C**			**20°C**			**30°C**		
Soak time	**24 H**	**48 H**	**72 H**	**24 H**	**48 H**	**72 H**	**24 H**	**48 H**	**72 H**
Hybrid1	0.290 w	0.333 qrstuvw	0.350 opqrstuvw	0.313 tuvw	0.393 lmnopqrstuvw	0.433 klmnopqrstuvw	0.353 opqrstuvw	0.523 hijklmn	0.768 def
Hybrid2	0.293 vw	0.310 uvw	0.443 jklmnopqrstuvw	0.308 uvw	0.378 mnopqrstuvw	0.423 klmnopqrstuvw	0.383 lmnopqrstuvw	0.533 hijklm	0.703 defg
Hybrid3	0.323 rstuvw	0.333 qrstuvw	0.480 ijklmnopq	0.383 lmnopqrstuvw	0.395 lmnopqrstuvw	0.470 ijklmnopqrs	0.448 jklmnopqrstuv	0.533 hijklm	0.793 cde
Hybrid4	0.318 rstuvw	0.338 pqrstuvw	0.490 ijklmnop	0.320 rstuvw	0.355 opqrstuvw	0.440 klmnopqrstuvw	0.390 lmnopqrstuvw	0.498 hijklmno	0.648 efgh
Hybrid5	0.325 qrstuvw	0.323 rstuvw	0.458 jklmnopqrstu	0.370 nopqrstuvw	0.365 opqrstuvw	0.618 Fghi	0.413 klmnopqrstuvw	0.503 hijklmno	0.930 c
Hybrid6	0.325 qrstuvw	0.313 tuvw	0.360 opqrstuvw	0.360 opqrstuvw	0.373 nopqrstuvw	0.498 Hijklmno	0.420 klmnopqrstuvw	0.468 ijklmnopqrst	0.705 defg
Hybrid7	0.323 rstuvw	0.315 stuvw	0.355 opqrstuvw	0.358 opqrstuvw	0.370 nopqrstuvw	0.413 klmnopqrstuvw	0.458 jklmnopqrstu	0.598 ghij	1.753 a
Hybrid8	0.323 rstuvw	0.335 pqrstuvw	0.370 nopqrstuvw	0.303 uvw	0.395 lmnopqrstuvw	0.473 ijklmnopqr	0.328 qrstuvw	0.535 hijkl	0.808 cd
Hybrid9	0.288 w	0.328 qrstuvw	0.370 nopqrstuvw	0.303 uvw	0.338 pqrstuvw	0.400 klmnopqrstuvw	0.355 opqrstuvw	0.553 ghijk	1.305 b
Mean for soaking time	0.312	0.325	0.408	0.335	0.373	0.463	0.394	0.527	0.934
Mean for temperature	0.348			0.390			0.618		
Means followed by the same letter are not significantly different at P = 0.05									

4.3.3 Relationship between the accelerated ageing test and emergence of maize hybrids under cold, wet conditions

Accelerated ageing has been used to predict seed storability (Delouche & Baskin, 1973) and has been widely adopted as a vigour test. In the accelerated ageing test, membrane degradation occurs as a consequence of the oxidation of unsaturated fatty acids (Navari-Izzo & Rascio, 1999). Basavarajappa *et al*. (1991) associated lipid peroxidation in aged maize seeds with membrane damage. In the accelerated ageing test, germination of the seed prior to accelerated ageing is compared with its germination after accelerated ageing and the difference between the two indicates the relative vigour of the sample tested. Although the accelerated ageing test is mainly used as a vigour test to predict storability of seed, it has the ability to be used as a vigour test to predict emergence of maize hybrids under cold, wet conditions.

Highly significant differences in accelerated ageing (P = 0.002) among nine maize hybrids were observed (Table 4.5), with a 56% difference in germination between the highest (Hybrid 4) and the lowest (Hybrid 8) germination percentage. This is notably higher than the difference in germination of 4.25% in the cold test, 8% in the soak test and 37.25% in the complex stressing vigour test. No specific trends were observed between Accelerated ageing germination of the hybrids and genetic composition as well as between the different trait versions of the same genetic background.

Table 4.5 Accelerated ageing, cold test, soak test, and complex stressing vigour test results for nine maize hybrids (ANOVA data for accelerated ageing appear in Appendix, Table 19)

Hybrid	Accelerated ageing germination	Cold test germination	Soak test germination	Complex stressing vigour test germination
1	78.0 (abc)	97.3 (a)	98.5 (a)	95.0 (a)
2	69.3 (cd)	94.3 (ab)	98.0 (a)	88.5 (ab)
3	53.0 (de)	96.3 (a)	98.7 (a)	82.5 (b)
4	94.8 (a)	94.9 (ab)	99.0 (a)	89.8 (ab)
5	90.8 (ab)	94.9 (ab)	97.3 (a)	88.5 (ab)
6	84.0 (abc)	97.1 (a)	98.5 (a)	87.0 (ab)
7	88.0 (ab)	91.3 (b)	98.5 (a)	92.3 (ab)
8	38.8 (e)	92.1 (b)	91.0 (b)	57.8 (c)
9	75.5 (bc)	94.1 (ab)	98.3 (a)	59.8 (c)
CV	21.02	3.13	4.96	17.74
Means followed by the same letter are not significantly different at P = 0.05				

Pearson correlation coefficients were determined between mean germination of the cold test, soak test, complex stressing vigour test, accelerated ageing test and simulated field emergence in the glasshouse at 25°C / 10°C and 72 hours flooding (Table 4.6). The best correlation was found between the complex stressing vigour test and simulated field emergence (90%, P = 0.001). A significant positive correlation (P = 0.029) of 71,8% was also found between the accelerated ageing test and simulated field emergence.

Table 4.6 Pearson correlation coefficients for germination relationships between the cold test, soak test, complex stressing vigour test, accelerated ageing test and simulated field emergence under cold, wet conditions

	Accelerated ageing test	Simulated field emergence	Complex stressing vigour test	Soak test
Simulated field emergence	**0.718**			
	P = 0.029			
Complex stressing vigour test	**0.616**	**0.899**		
	P = 0.077	P = 0.001		
Soak test	**0.702**	**0.678**	**0.668**	
	P = 0.035	P = 0.045	P = 0.049	
Cold test	**0.696**	**0.626**	**0.602**	**0.432**
	P = 0.037	P = 0.031	P = 0.086	P = 0.245

Regression analysis was done between the accelerated ageing test and simulated field emergence under cold, wet conditions to see if the test can predict field emergence under cold, wet conditions. Figure 4.6 shows the fitted line plots for the regression analysis of the accelerated ageing test as affected by simulated field emergence under cold, wet conditions. The adjusted R-Squared for the accelerated ageing test as affected by simulated field emergence, is 49.8 %. This means that 49.8 % of the variability in simulated field emergence under cold, wet conditions could be explained by the accelerated ageing test germination. Compared to the results of the complex stressing vigour test (Figure 4.5), the accelerated ageing test is not a good predictor of field emergence under cold, wet conditions.

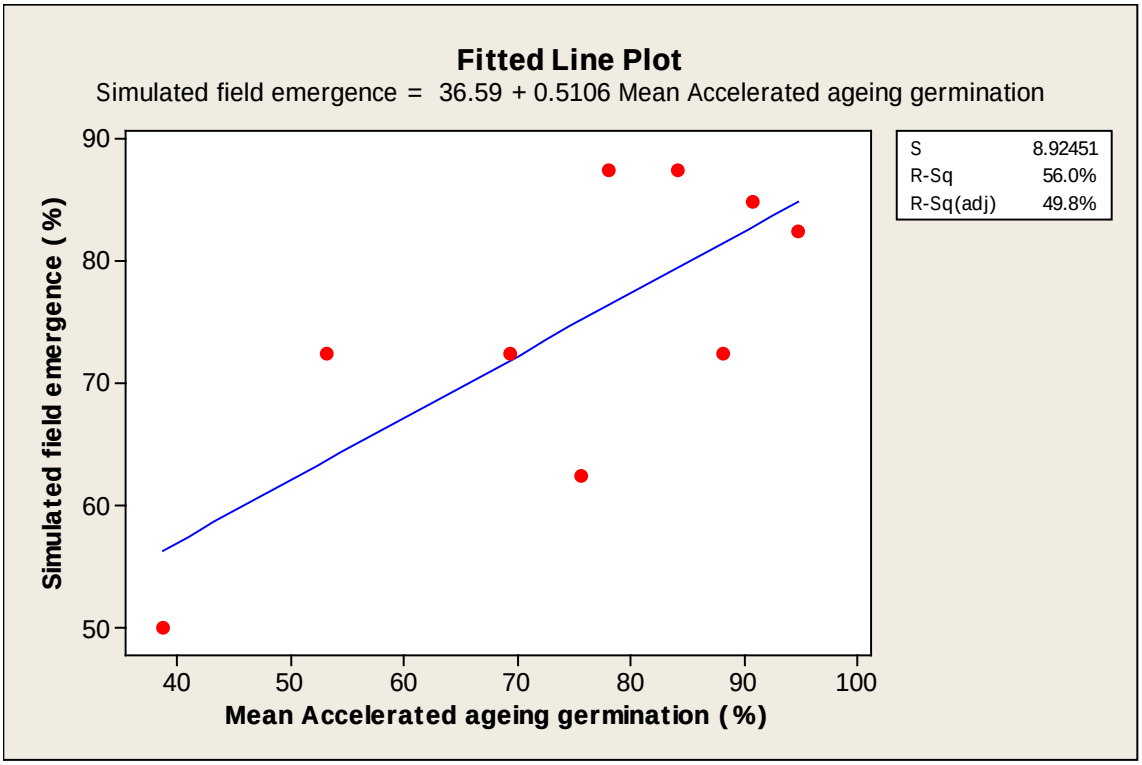


Figure 4.6 Relationship between the accelerated ageing test and simulated field emergence under cold, wet conditions (Regression analysis appear in Appendix, Table 20)

4.3.4 Relationship between the tetrazolium test, fast green test and emergence of maize hybrids under cold, wet conditions

According to the mean tetrazolium results (Table 4.7), the viability of the maize seeds was above 94 % (range 94% - 100%). Hybrid 1 performed the best in all the germination tests. Hybrid 8 performed second best in the tetrazolium test (99 %), while it came second last in the cold test (92.1%) and last in the complex stressing vigour test (57.8 %). On average, hybrid 9 did not perform well in any of the germination tests.

A Pearson correlation study was run between mean germination of the warm test, cold test, complex stressing vigour test, tetrazolium test and simulated field emergence in the glasshouse at 25°C / 10°C with 72 hours flooding and with 0 hours flooding. The best correlation with the tetrazolium test results (27.8 %) was found with field emergence with 0 hours flooding.

According to the different correlations with the tetrazolium test results (Table 4.8), the tetrazolium test is not a good predictor of maize hybrid emergence under cold, wet conditions.

Table 4.7 Results for tetrazolium test, warm test, cold test, and complex stressing vigour test for nine maize hybrids (ANOVA for tetrazolium results appear in Appendix, Table 21)

Hybrid	Tetrazolium test germination (%)	Warm test germination (%)	Cold test germination (%)	Complex stressing vigour test germination (%)
1	100.0 (a)	99.5 (a)	97.3 (a)	95.0 (a)
2	96.3 (ab)	99.5 (a	94.3 (ab)	88.5 (ab)
3	97.5 (ab)	98.8 (a)	96.3 (a)	82.5 (b)
4	97.0 (ab)	97.8 (ab)	94.9 (ab)	89.8 (ab)
5	94.0 (b)	99.5 (a)	94.9 (ab)	88.5 (ab)
6	95.5 (ab)	98.5 (a)	97.1 (a)	87.0 (ab)
7	97.0 (ab)	98.8 (a)	91.3 (b)	92.3 (ab)
8	99.0 (ab)	98.5 (a)	92.1 (b)	57.8 (c)
9	96.3 (ab)	96.3 (b)	94.1 (ab)	59.8 (c)
CV	22.36	2.10	3.13	17.74
Means followed by the same letter are not significantly different at P = 0.05				

Table 4.8 Pearson correlation coefficients for germination relationships between the warm test, cold test, complex stressing vigour test, tetrazolium test and simulated field emergence at 0 hours and 72 hours flooding

	Warm test	**Cold test**	**Complex stressing vigour test**	**Field emergence with 72 hours flooding**	**Field emergence with 0 hours flooding**
Field emergence with 72 hours flooding	**0.492**	**0.626**	**0.899**		
	P = 0.178	P = 0.031	P = 0.001		
Field emergence with 0 hours flooding	**0.575**	**-0.141**	**0.424**	**0.126**	
	P = 0.105	P = 0.717	P = 0.256	P = 0.747	
Tetrazolium test	**-0.157**	**0.159**	**-0.112**	**-0.217**	**0.278**
	P = 0.686	P = 0.683	P = 0.774	P = 0.575	P = 0.468

Regression analysis between the tetrazolium test and simulated field emergence (Figure 4.7) indicates that the tetrazolium test is not a good predictor of field emergence under cold, wet conditions. The adjusted R-Squared value was 0%.

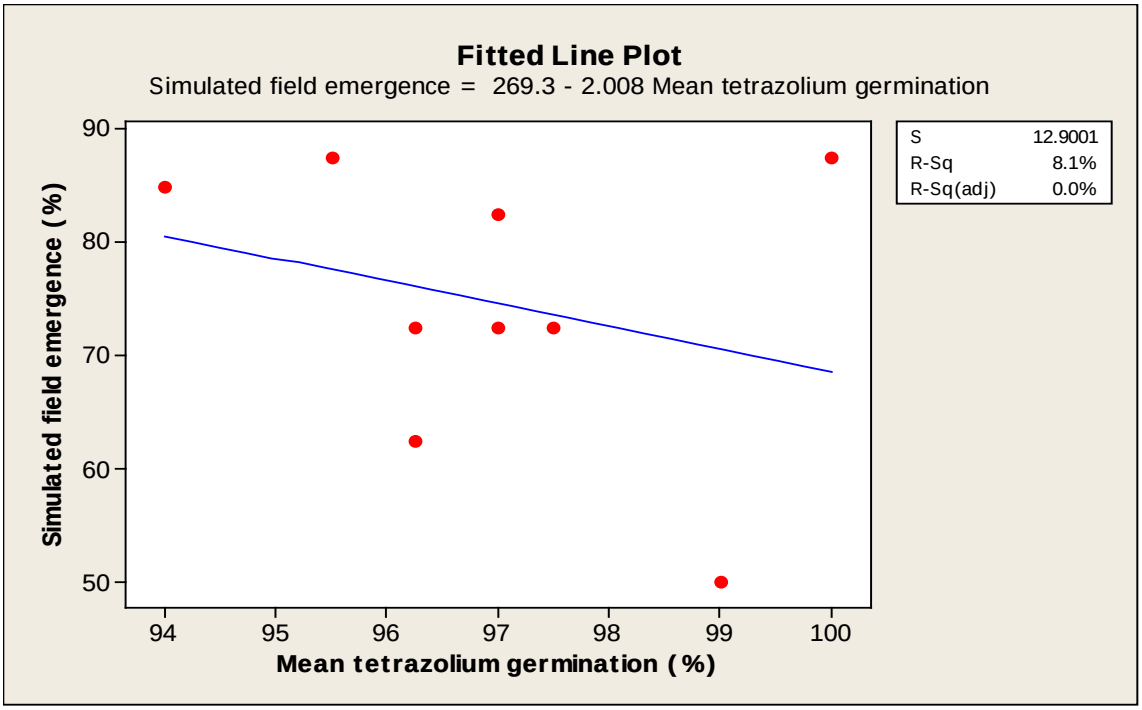


Figure 4.7 Relationship between the tetrazolium test and simulated field emergence under cold, wet conditions (Regression analysis appear in Appendix, Table 22)

Fast green test results for nine maize hybrids are presented in Table 4.9. Hybrid 3 has the highest percentage seed without cracks (68 %) (Significantly more than the remaining 8 Hybrids). Hybrid 1 has only 3 % seed without cracks, while all the seed of hybrid 9 has cracks. Cracks on or near the embryo are signs of lower quality seed. According to the results, hybrid 1, 4, 7 and 9 seems to be lower quality seed, based on nearness of cracks to the embryo, these 4 Hybrids have significantly more cracks near or on the embryo than the remaining 5 Hybrids.

According to the three categories of seed damage (Figure 4.2), hybrid 3 was the only hybrid that was classified as good quality seed. The remaining eight hybrids were all classified as intermediate quality seeds. Table 4.10 gives a comparison of fast green test results with the cold test, soak test, complex stressing vigour test and simulated field emergence under cold, wet conditions (25°C / 10°C and 72 hours flooding). Although the fast green test provides a good indication of seed quality, it is not specific enough to be used as a vigour test to predict field emergence under cold, wet conditions.

Table 4.9 Fast green test results for nine maize hybrids (ANOVA for fast green results appears in Appendix, Table 24)

Hybrid	% Seed without cracks	% Seed with cracks on embryo	% Seed with cracks near embryo	% Seed with cracks far from embryo
1	3.0 (d)	16.0 (b)	71.5 (a)	9.5 (bc)
2	7.0 (cd)	19.0 (ab)	37.5 (c)	36.5 (a)
3	68.0 (a)	8.0 (c)	13.5 (d)	10.5 (bc)
4	9.5 (c)	14.0 (b)	73.5 (a)	4.0 (c)
5	11.5 (c)	27.0 (a)	56.0 (b)	5.5 (c)
6	10.5 (c)	21.0 (ab)	54.0 (b)	14.5 (b)
7	6.5 (cd)	22.0 (ab)	69.5 (a)	2.0 (cd)
8	44.5 (b)	32.5 (a)	14.5 (d)	8.5 (bc)
9	0.0 (d)	21.5 (ab)	77.0 (a)	1.5 (d)
Means followed by the same letter are not significantly different at P = 0.05				

Table 4.10 Comparison of fast green test results with the cold test, soak test, complex stressing vigour test and simulated field emergence under cold, wet conditions (ANOVA data appear in Appendix, Tables 9 – 12)

Hybrid	**Fast green test results**	**Cold test germination (%)**	**Soak test germination (%)**	**Complex stressing vigour test germination (%)**	**Field emergence under cold, wet conditions (%)**
1	Intermediate	97.3 (a)	98.5 (a)	95.0 (a)	87.5 (a)
2	Intermediate	94.3 (ab)	98.0 (a)	88.5 (ab)	72.5 (ab)
3	Good	96.3 (a)	98.7 (a)	82.5 (b)	72.5 (ab)
4	Intermediate	94.9 (ab)	99.0 (a)	89.8 (ab)	82.5 (a)
5	Intermediate	94.9 (ab)	97.3 (a)	88.5 (ab)	85.0 (a)
6	Intermediate	97.1 (a)	98.5 (a)	87.0 (ab)	87.5 (a)
7	Intermediate	91.3 (b)	98.5 (a)	92.3 (ab)	72.5 (ab)
8	Intermediate	92.1 (b)	91.0 (b)	57.8 (c)	50.0 (b)
9	Intermediate	94.1 (ab)	98.3 (a)	59.8 (c)	62.5 (ab)
CV		3.13	4.96	17.74	21.34
Means followed by the same letter are not significantly different at P = 0.05					

4.4 CONCLUSION

Commercial maize hybrids are sometimes planted into soils that are or may become too cold and wet for optimum germination and emergence. For this reason, improvement and prediction of seedling emergence and early seedling growth is important to the seed industry. Accurate prediction of field emergence is difficult, however, it is essential if the seed industry is to produce and sell high quality maize seed.

The cold test is the only vigour test used by most seed companies to access seed vigour. According to the results of this study, the cold test only correlates 62.6% with simulated field emergence under cold, wet conditions, while the soak test correlates 67.8% with simulated field emergence under these unfavourable conditions. The soak test is therefore already 5.2% better in

prediction of simulated field emergence than the cold test. The best correlation with simulated field emergence (89.9%) was found with the complex stressing vigour test.

In practice, the South African maize producers would not plant if it is too cold and wet. The unavoidable cold and wet conditions normally appear a few days after planting. In most of the commercial maize production areas, like the western Free State, the chance of thunder and hailstorms is high during the planting season. That is the major cause for a sudden drop in temperature and flooding conditions. The complex stressing vigour test is a good simulation of these extreme conditions, because the seed is first soak at a moderate temperature (25°C) for 48 hours and then at a low temperature (5°C) for another 48 hours, where after it is planted in sand and grown for four days at 25°C before evaluation. This might be the reason for the good correlation between this test and field emergence under cold, wet conditions.

The electrical conductivity test could not be utilized as a vigour test to predict field emergence of maize hybrids under cold, wet conditions. Neither the tetrazolium nor the fast green tests correlated well with maize hybrid emergence under cold, wet conditions. These three tests would therefore not be recommended for commercial use when evaluating seed for planting under unfavourable growing conditions.

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CHAPTER 5

CORRELATION BETWEEN LABORATORY AND FIELD EMERGENCE UNDER COLD, WET CONDITIONS

5.1 INTRODUCTION

TeKrony *et al*. (1989) stated that vigour test results are not expected to predict an exact value for field emergence because soil and seedbed conditions vary from field to field. However, many studies have evaluated vigour test methods and demonstrated significant correlations between different vigour test results and field emergence of maize (Fiala, 1987; TeKrony *et al*., 1989).

Martin & O'Neil (1987) reported that correlations between vigour test results and field emergence vary due to numerous factors, and may change over years for the same test. According to them, correlations can be influenced by the inherent stress level of the laboratory test, quality levels of the seed batch, and environmental stress in the field. It is impossible to predict emergence percentage in the field correctly, because of the influence of weather conditions on the final percentages. Factors in addition to those discussed above, may also be of importance. Ungerer (2001) reported that storage conditions prior to planting, quality of seed treatment, differences in planting depth, planting date, loss of fungicide after heavy rainfall, salt stress and crust forming, may all lead to differences in field performance of seed batches of the same quality.

The only way in which the usefulness of a seed vigour test can be assessed is to determine whether there is a significant correlation between vigour test results and field emergence. Within a seed company, the level of the vigour test emergence can be related to field emergence and intelligent seed quality decisions made regarding potential seed batch performance. To ascertain the relationship between the different vigour tests and field emergence, a number of field trials were conducted on the seed batches used in Chapter 4.

5.2 MATERIALS AND METHODS

5.2.1 Experimental set-up

Field emergence of nine maize hybrids was determined in eight trials in the 2007 growing season, two on the farm Shokelton near Viljoenskroon in the Free State, two on the experimental farm of Monsanto South Africa at Petit (Highveld area), two on the farm Vlakfontein near Ohrigstad and two on the farm Blyderust near Hoedspruit (Lowveld area). The Viljoenskroon trials were conducted on the same field that was used for soil collection for the glasshouse and growth chamber trials (Chapter 2). As most of the complaints from commercial maize producers regarding poor emergence of maize hybrids under cold, wet conditions were from the Viljoenskroon area, it was important to have at least one location planted in that area. It was decided to plant two trials in the Hoedspruit area, because there would be a better chance of obtaining high temperatures. The Ohrigstad area is normally cool at that time of the year.

Different planting dates were selected for the trials, resulting in cool and wet, cold and wet or favourable conditions during planting. Experimental design in each case was a randomized block with four replicates of 100 kernels each. The kernels were hand-planted in 10-m rows with a spacing of 0.5 m; planting depth of 5 cm, and spacing within the rows was set as 10 cm.

Soil preparation was done as for commercial maize plantings, as was fertilizer applications. No additional chemicals were applied nor were seed treatments done prior to planting. The Viljoenskroon and Hoedspruit areas have sandy soils, while the other two locations have soils with a higher clay content.

5.2.2 Treatments

Air temperatures were recorded hourly with HOBO PRO data loggers located in a Stevenson screen near the trials. Daily minimum and maximum temperatures were calculated for each trial. Two rain-gauges were placed in each trial. No rainfall was, however, recorded for the duration of the trial period. Prior to soil preparation and planting, 500 mm of irrigation was applied to all fields. All the plantings took place in moist soil. A once-off excessive irrigation of 100 mm was

applied approximately 24 hours after planting to simulate flooding conditions. Details of the planting dates and temperature conditions for each trial are supplied in Table 5.1.

Table 5.1 Details of climatic conditions prevailing during the field emergence trials with nine maize hybrids

Field trial	Location	Planting date	Mean air temperature (°C)	
			Minimum	Maximum
1	Viljoenskroon	5 February 2007	12.8	31.7
2	Viljoenskroon	14 April 2007	8.1	23.1
3	Petit	6 February 2007	13.0	28.3
4	Petit	16 April 2007	8.7	22.2
5	Ohrigstad	12 February 2007	12.1	21.5
6	Ohrigstad	13 April 2007	8.4	15.5
7	Hoedspruit	13 February 2007	19.9	33.8
8	Hoedspruit	18 April 2007	14.4	28.6

In each area, two trials were conducted, an early trial under more favourable climatic conditions and a late trial, about two months later, under more unfavourable climatic conditions. In three of the eight trials (trial 2, trial 4 and trial 6), mean minimum temperatures, lower than the minimum for maize production, were obtained. The accepted minimum temperature for maize production is 10°C. In only one trial (trial 7) the mean maximum temperature was higher than the accepted maximum temperature for maize production of 32°C.

5.2.3 Data collection

Each trial was run for about two weeks. Only final emergence counts were taken and used in the analysis. Four replications were planted per entry and used in the analysis.

5.2.4 Statistical analysis

Data were subjected to analysis of variance, using the MINITAB 14.2 software program as well as the JMP7 software program. Differences at the $P < 0.05$ level of significance were reported. The Student's *t* Test was conducted to confirm significant differences.

5.3 RESULTS AND DISCUSSION

Results of emergence trials with nine maize hybrids are shown in Table 5.2. The warm test germination percentages are also included for comparison purposes. With optimum field conditions, (trial 7 and 8), the difference in emergence between the hybrid with the highest and lowest emergence percentage was 4.50 % and 3.25 % for trial 7 and 8, respectively. The difference in emergence for trials 1 to 5 (cool trials) was 6.00 % to 12.25 %.

Trial 6 showed the highest variation in emergence (16.8%) between the different hybrids, that was expexted, because trial 6 was grown under the most unfavourable climatic conditions. A higher variation in emergence percentages were thus observed for the trials with unfavourable climatic conditions. The lowest field emergence counts were also obtained in trial 6. Hybrids 8 (74.00 %) and 9 (76.00 %) were the lowest in the ranking order for this test. Emergence counts of the hybrids in trial 6 indicated considerable differences between emergence values and germination percentages of the warm test. Hybrid 1 was ranked first with an emergence count of 90.75 % (germination

Table 5.2 Field performance and warm test germination of nine maize hybrids.

Hybrid	Emergence %								Warm test germination%
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Trial 8	
Growing conditions	Cool/ Wet	Cool/ Wet	Cool/ Wet	Cool/ Wet	Cool/ Wet	Cold/ Wet	Warm/ Wet	Warm/ Wet	
Hybrid1	**95.0** (a)	**92.3** (ab)	**96.0** (a)	**92.3** (a)	**94.3** (a)	**90.8** (a)	**100.0** (a)	**95.8** (ab)	**99.5** (a)
Hybrid2	**96.3** (a)	**96.3** (a)	**92.3** (abc)	**89.8** (a)	**94.3** (a)	**88.0** (ab)	**98.3** (ab)	**98.3** (a)	**99.5** (a)
Hybrid3	**90.5** (a)	**85.0** (b)	**89.3** (bc)	**86.3** (abc)	**92.5** (a)	**76.8** (c)	**95.5** (b)	**95.0** (ab)	**98.8** (a)

Hybrid4	**90.3** (a)	**88.3** (ab)	**93.8** (ab)	**92.3** (a)	**91.5** (a)	**87.0** (ab)	**98.3** (ab)	**98.0** (a)	**97.8** (ab)
Hybrid5	**90.3** (a)	**88.3** (ab)	**88.3** (c)	**88.3** (ab)	**91.0** (a)	**88.3** (ab)	**98.3** (ab)	**96.8** (ab)	**99.5** (a)
Hybrid6	**92.3** (a)	**90.3** (ab)	**90.3** (bc)	**88.3** (ab)	**89.5** (ab)	**84.2** (b)	**97.8** (ab)	**96.8** (ab)	**98.5** (a)
Hybrid7	**95.3** (a)	**91.3** (ab)	**93.5** (ab)	**92.5** (a)	**94.5** (a)	**89.8** (a)	**98.0** (ab)	**92.8** (b)	**98.8** (a)
Hybrid8	**90.3** (a)	**91.3** (ab)	**91.0** (bc)	**82.0** (bc)	**82.5** (c)	**74.0** (c)	**96.0** (b)	**95.3** (ab)	**98.5** (a)
Hybrid9	**90.3** (a)	**88.3** (ab)	**90.3** (bc)	**80.3** (c)	**84.0** (bc)	**76.0** (c)	**96.3** (b)	**97.0** (a)	**96.3** (b)
Mean trial emergence %	**92.3**	**90.1**	**91.6**	**88.0**	**90.4**	**83.9**	**97.6**	**96.2**	**98.6**
Difference in emergence from highest to lowest	**6.0**	**11.3**	**7.8**	**12.3**	**12.0**	**16.8**	**4.5**	**3.3**	**3.3**
CV	**5.07**	**6.55**	**4.15**	**6.67**	**6.38**	**8.01**	**2.24**	**3.10**	**2.10**

Means followed by the same letter are not significantly different at P = 0.05

from the warm test was 99.50 %) and hybrid 8 was ranked last with an emergence count of 74.00 % (germination from the warm test was 98.50 %). According to these results, it is clear that the trials under favourable climatic conditions (warm conditions) showed smaller differences between field emergence and germination in the warm test than the trial under cold conditions. The variation between the different hybrids was also smaller in the field trial under warm conditions than under cold conditions. The means of the eight field trials also indicate that emergence percentages were the lowest for trial 6 (cold, wet conditions) and the highest for trials 7 and 8 (warm, wet conditions). Emergence percentages for trials 7 and 8 were close to the germination percentages of the warm test. Emergence percentages for trials 1 to 5 (cool, wet conditions) were all somewhere between those of the cold, wet trial and the two warm, wet trials.

The lowest coefficients of variation were obtained with trials 7 and 8, the trials grown under the most favourable climatic conditions. The highest coefficient of variation was obtained with trial 6, the trial grown under the most unfavourable conditions. The most variation is also expected under these unfavourable conditions.

Numerous publications indicate a good correlation between germination and field emergence in near ideal soil seedbed conditions (Delouche, 1973; Ungerer, 2001). Environmental stress will result in varying field performance, depending on the vigour status of the seed batch (Powell,1988; TeKroney & Egli, 1993). High vigour seed batches will perform better under environmentally stressed seedbed conditions than lower vigour seed batches, even though the laboratory germination of the seed batches may not differ.

Correlation coefficients between the warm test, cold test, soak test, complex stressing vigour test and field emergence of the eight field trials under different climatic conditions are shown in Table 5.3. The complex stressing vigour test and field emergence under cold, wet conditions (field trial 6) was highly significantly correlated with a 90% correlation. A significant correlation of 67 % was also found between the cold test results and field emergence in trial 6. No significant correlations were found between the warm test, cold test and soak test and emergence of field trials 1 – 5 (cool, wet conditions) and trials 7 and 8 (warm, wet conditions). Highly significant correlations were found between the complex stressing vigour test and field emergence under cool, wet conditions of trials 4 and 5 as well as with trial 7, (warm, wet conditions). According to these results, the complex stressing vigour test best predicted field emergence under unfavourable field conditions.

Table 5.3 Pearson correlation coefficients for relationships between field emergence under different climatic conditions and germination in the warm test, cold test, soak test and complex stressing vigour test

	Warm test	**Cold test**	**Soak test**	**Complex stressing vigour test**
Field trial 1 (cool/ wet)	**0.52**	**0.10**	**0.29**	**0.56**

	P = 0.15	P = 0.79	P = 0.45	P = 0.12
Field trial 2 (cool/ wet)	**0.41**	**-0.23**	**-0.17**	**0.18**
	P = 0.27	P = 0.54	P = 0.66	P = 0.64
Field trial 3 (cool/ wet)	**0.15**	**0.38**	**0.19**	**0.41**
	P = 0.71	P = 0.32	P = 0.62	P = 0.27
Field trial 4 (cool/ wet)	**0.57**	**0.63**	**0.53**	**0.95**
	P = 0.11	P = 0.07	P = 0.14	P = 0.00
Field trial 5 (cool/ wet)	**0.63**	**0.54**	**0.67**	**0.94**
	P = 0.07	P = 0.13	P = 0.05	P = 0.00
Field trial 6 (cold/ wet)	**0.56**	**0.67**	**0.53**	**0.90**
	P = 0.12	P = 0.05	P = 0.14	P = 0.00
Field trial 7 (warm/ wet)	**0.49**	**0.68**	**0.39**	**0.75**
	P = 0.18	P = 0.04	P = 0.30	P = 0.02
Field trial 8 (warm/ wet)	**-0.15**	**-0.13**	**0.16**	**-0.01**
	P = 0.70	P = 0.75	P = 0.67	P = 0.97

Regression analysis between field emergence under cold, wet conditions (trial 6) and the complex stressing vigour test was done. According to these results, R-square adjusted was 77.4 % (Figure 5.1). This means that 77.4 % of the variability in field emergence under cold, wet conditions could be explained by the complex stressing vigour test germination.

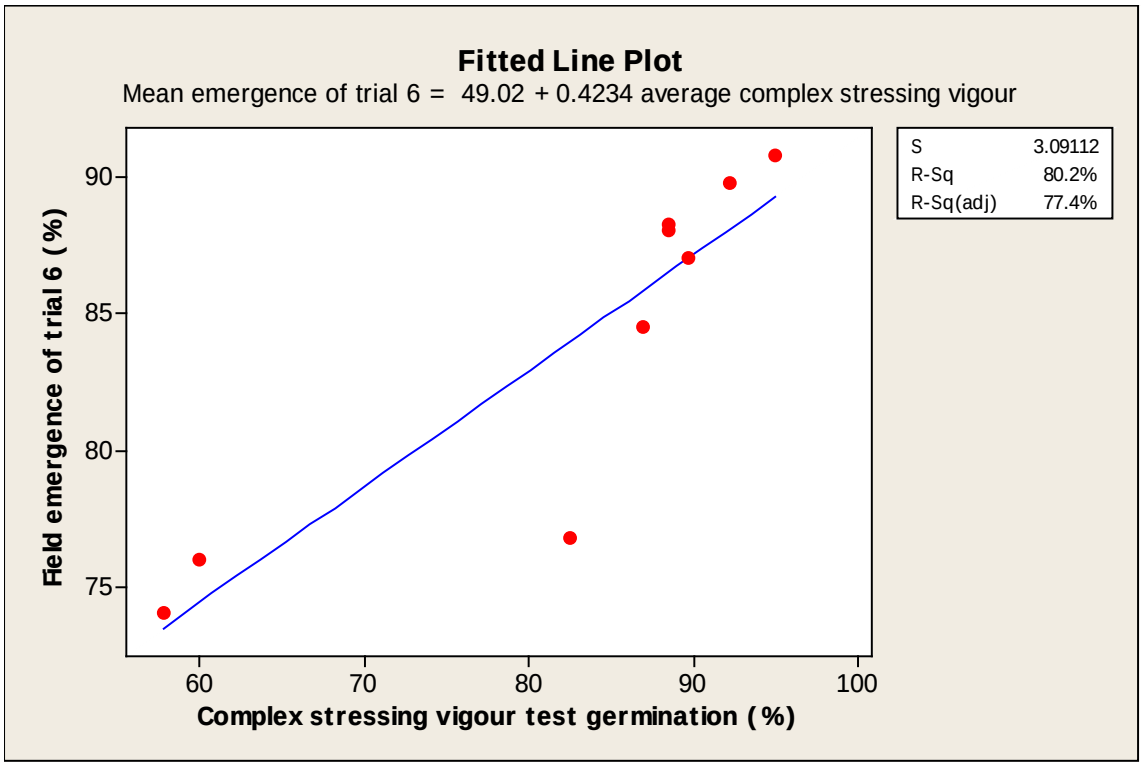

Figure 5.1 Relationship between the complex stressing vigour test and field emergence under cold, wet conditions (Regression analysis appear in Appendix, Table 23)

5.4 CONCLUSION

In the field, uncontrollable environmental factors of temperature, light and moisture may play a critical role in the seed's response to flooding. In addition, the variability of soil drainage, microbial populations and disease pressure across a field may have considerable influence on how seeds respond to flooding stress.

Commercial maize hybrids are sometimes planted into soils that are or may become too cold and wet for optimum germination and emergence. For this reason, improvement and prediction of seedling emergence and early seedling growth is important to the seed industry. Accurate prediction of field emergence is difficult, however, but it is essential if the seed industry is to produce and sell high quality maize seed.

In recent years some of South Africa's top maize hybrids, with high cold test scores, have shown emergence problems under cold, wet planting conditions. It resulted in major complaints from commercial maize producers with sizable claims involved. Therefore, the need to test different vigour tests for the effectiveness in predicting field emergence under cold, wet conditions. According to these results, the complex stressing vigour test best predicts field performance under a wide range of climatic conditions, especially cold, wet conditions.

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CHAPTER 6

GENERAL DISCUSSION

According to Burris (1977), seed quality encompasses the ability of seed to germinate and emerge under optimal environmental conditions. In a later study, Burris (2000) reported that seed quality is one of the most important factors affecting early performance and productivity of most agricultural crops. He also reported that differences in growing season, such as temperature and moisture, and the interaction of temperature and moisture is the most important factors affecting seed quality.

The seed industry uses the standard germination test (AOSA, 2002) for labeling. The standard germination test, also known as the warm test, estimates germination under ideal growing conditions (Munamava, *et al*., 2004). The major limitations of the warm test as an assessment of

potential field performance of a seed batch is its inability to detect quality differences among high germinating seed batches.

The association of official seed analysts (AOSA) (1983) defined seedling vigour as "those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions". Seedling vigour testing has become an increasingly important component of seed testing and more accurately reflects the potential performance of a seed batch if stress is encountered in the field at planting. High vigour seed batches have the capacity for greater emergence and seedling survival than low vigour seed batches.

Delouche & Caldwell (1960) reported that vigour deteriorates faster than germination with age, *i.e.* the loss of vigour precedes the loss of the ability to germinate. They also reported that although seed batches may have similar high germination percentages, they can differ in their physiological age, vigour, and thus their ability to emerge in the field.

Ungerer (2001) stated that high vigour seed batches will perform better under environmentally stressed seedbed conditions than lower vigour seed batches, even though the warm test germination percentages of the batches may not differ. The cold test is one of the oldest and most acceptable vigour tests as it simulates stress conditions commonly occurring in the field. The cold test has been used as a vigour test to determine seed batch performance under sub optimal planting conditions. In recent years, some of South Africa's top maize hybrids, with high cold test scores have shown emergence problems under cold, wet planting conditions. It resulted in major complaints from commercial maize producers with sizable claims involved. Therefore, the need exists to identify a more sensitive vigour test, especially under cold, wet conditions.

In this study, eight different vigour tests were conducted and compared with field emergence under cold, wet conditions. The soak test is a more sensitive vigour test under cold, wet conditions than the cold test, as it measures seed germination, according to the warm test, after a 48 hour soak in water at 27°C. The correlations found between the soak test and field emergence was not what was expected.

The results of the present study correlates well with studies done by Martin *et al*. (1991). According to Martin *et al*. (1991), soak sensitive maize inbreds are less vigorous and have lower field emergence than soak-tolerant inbreds. In addition, the amount of ethanol excreted during soaking were negatively correlated with both laboratory germination after soaking and field emergence of unsoaked seed sown in cold, wet soil. According to own results, the period of flooding or waterlogging, was directly correlated with the amounts of ethanol excreted around the developing seeds, and the combined effect of flooding and temperature had a major effect on emergence of maize seeds. There were also differences between different genetic material regarding their ability to release ethanol in their direct environment. Temperature also played a role in ethanol excretion of developing seeds. Results indicated that this technique is usable to explain poor germination and emergence of maize seed under flooding conditions, but not under low temperature conditions.

During initial stages of imbibition, most seeds leak ions, amino acids, and sugars. Weaker seeds, that is, those with poor cell membrane structure or slow restoration of cell membrane function during rehydration, tend to leak more. Highly significant differences were found between the different treatments (temperatures and soaking times) and electrolyte leakage. The longer the soaking period, and the higher the soaking temperatures, the higher the electrolyte leakage into the soak water. According to Black & Bewley (2000), seed leakage can be quantified by the increase in the electrical conductivity of the soak water. They reported that the higher the electrical conductivity value, the lower the seed quality. Fessel *et al*. (2006) reported that the electrical conductivity test determines indirectly the integrity of seed membrane systems, and is used for the assessment of seed vigour, because this test detects the seed deterioration process. A significantly negative correlation was found between the average electrolyte leakage and simulated field emergence of the maize hybrids under the different temperature and flooding conditions. The correlations was, however very low and therefore the electrical conductivity test is not recommended to predict emergence of maize hybrids under cold, wet conditions.

Accelerated ageing has been used to predict seed storability (Delouche & Baskin, 1973) and has been widely adopted as a vigour test. In the accelerated ageing test, membrane degradation occurs as a consequence of the oxidation of unsaturated fatty acids (Navari-Izzo & Rascio,

1999). Basavarajappa *et al*. (1991) associated lipid peroxidation in aged maize seeds with membrane damage. In the accelerated ageing test, the germination of the seed prior to accelerated ageing is compared with its germination after accelerated ageing and the difference between the two indicates the relative vigour of the sample tested. According to the data of this study, only 49.8 % of the variability in simulated field emergence under cold, wet conditions could be explained by accelerated ageing. A 61.6% non significant correlation was found between the accelerated ageing test results and the complex stressing vigour test results. Compared to the complex stressing vigour test, the accelerated ageing test is not a very good predictor of field emergence under cold, wet conditions.

In practice, the South African maize producers would not plant if it is too cold and wet. The cold and wet conditions normally appear a day or two after planting. In most of the commercial maize production areas, like the Western Free State, the chance of thunder and hailstorms is high during the planting season. That is the major cause for a sudden drop in temperature and flooding conditions. The complex stressing vigour test was conducted, to study the effect of fluctuating soaking temperatures on germination of maize seed. The seeds of the maize hybrids were soaked for 48 hours at a moderate temperature (25°C), followed by another 48 hours soak at low temperatures (5°C), where after it was planted in sand and grown for 4 days at 25°C, before evaluation. Results of the study indicates highly significant correlations (89.9%) between the complex stressing vigour test and simulated field emergence under controlled conditions in a glasshouse as well as with field studies under cold, wet conditions (90.0%). According to these results, the complex stressing vigour test best predict field performance under a wide range of climatic conditions, especially cold, wet conditions.

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CHAPTER 7

SUMMARY

In recent years, some of South Africa's top maize hybrids have shown emergence problems under very wet and cold conditions, or other conditions that is sub-optimal for emergence. It resulted in major complaints from commercial maize producers with sizable claims involved. The main objectives of this study were to determine whether there was differences in germination of maize seed due to cold, wet conditions and to evaluate different vigour tests to identify the most suitable vigour test, to detect a hybrid's ability to germinate and emerge under cold, wet conditions. The different vigour tests were conducted in the laboratories of the University of Pretoria and in the quality laboratory of Monsanto. Growth chamber and glasshouse studies were done at the experimental farm of the University of Pretoria.

If a positive correlation could be found between field emergence of the different maize hybrids under extreme soil saturation conditions (flooding and low temperatures) and a specific vigour test, seed companies will benefit from the study by using this test as a selection tool in the release of new hybrids. If the status of existing hybrids is known, it can be used as a decision making tool for seed distribution. Sensitive hybrids could therefore be recommended for areas where the risk of cold and wet conditions is low during germination. The major findings of the study can be summarized as follows:

1. Results of the growth chamber study indicated significant differences in emergence between the different hybrids. A significant difference in emergence percentage was also found between the different temperatures (10°C, 20°C and 30°C) and the different flooding times (0 hours, 24 hours, 48 hours and 72 hours). All interactions were also significant. It was clear that cold (10°C) and flooding conditions (48 to 72 hours) had a major negative effect on emergence of maize seeds. It was also found that low temperatures as well as long periods of flooding, lead to low emergence rates. Hybrid 1, a conventional hybrid, overall performed the best under cold, wet conditions. Therefore Hybrid 1 can be classified as the best cold tolerant hybrid of the nine hybrids tested, while the overall performance of Hybrid 9 was the poorest. Hybrid 9 can be classified as the most sensitive hybrid under cold, wet conditions. Between the hybrids with the same genetic composition used in the study, the conventional hybrids developed stronger seedlings than the Bt-hybrids, with the Roundup Ready hybrids developing the weakest seedlings. The seedling development of the nine maize hybrids correlates well with the germination results obtained in the warm test.

2. Seed vigour tests are more sensitive in measuring seed quality than the standard germination test. The cold test is one of the most widely used vigour tests for maize, but it is not very effective to predict field emergence under cold and wet conditions.

3. Ethanol is produced to a varying degree by most plants under oxygen stress. The results of these studies have led to the hypothesis that the end products of anaerobic metabolism (ethanol and acetaldehyde) are toxic to seeds, and that flooding leads to increased cellular concentrations of these compounds and, thus, to death of the seed. According to the results of the ethanol study, the period of flooding or waterlogging, was directly correlated with the amounts of ethanol excreted around the developing seeds, and the combined effect of flooding and temperature had a major effect on emergence of maize seeds. There were also differences between different genetic material regarding their ability to release ethanol in their direct environment. Temperature also played a significant role in ethanol excretion of developing seeds. Although the technique of ethanol excretion under anaerobic conditions could be used to provide an indication of

seedling vigour, it is not recommended as a standard vigour test, because it is expensive and time consuming.

4. Strong correlations were found between 72 hours flooding in the glasshouse experiment and the cold, soak and complex stressing vigour tests. The best correlation (89.9%) was found between simulated field emergence under cold, wet conditions and the complex stressing vigour test. The complex stressing vigour test predicted field emergence 25.1 % better than the soak test and 17.4 % better than the cold test under cold, wet conditions.

5. The electrical conductivity test poorly correlated with field emergence of maize hybrids under cold, wet conditions. The cold, soak and complex stressing vigour tests were more accurate in predicting field emergence of maize hybrids under cold, wet conditions than the electrical conductivity test. Although the accelerated ageing test is mainly used as a vigour test to predict storability of seed, it also has the ability to be used as a vigour test to predict emergence of maize hybrids under cold, wet conditions.

6. The best correlation with the tetrazolium test results (27.8 %) was found with field emergence with 0 hours flooding. According to the different correlations with the tetrazolium test results, the tetrazolium test was not a good predictor of maize hybrid emergence under cold, wet conditions. According to the results, the fast green test was also not a good vigour test for the prediction of field emergence under cold, wet conditions.

7. The results of the field experiments indicated highly significant correlations between the complex stressing vigour test and field emergence under cold, wet conditions. Regression analysis indicated that 77.4 % of the variability in field emergence under cold, wet conditions could be predicted by the complex stressing vigour test.

8. Low temperatures and flooding significantly decrease germination of maize seed and emergence of maize seedlings. The interaction of low temperatures and flooding has

a more negative effect on maize seed germination and emergence of seedlings than the two factors on their own. The main purpose of the study was to identify the most suitable vigour test to best predict emergence of maize hybrid seedlings under unfavourable climatic conditions, especially cold, wet conditions. According to the results of the study, the complex stressing vigour test best predict (89.9%) field emergence under cold, wet conditions. Implementation of the complex stressing test as a routine vigour test, will be to the advantage of maize seed companies, especially in being proactive in predicting emergence of maize hybrids under cold, wet conditions.

APPENDIX

Table 1 General Linear Model of warm test results of nine maize hybrids

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Analysis of variance for warmtest germination, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	66.444	66.444	8.306	2.2	0.039
Error	63	237.500	237.500	3.770		
Total	71	303.944				
CV = 2.10 R-Sq = 21.86% R-Sq (adj) = 11.94%						

Table 2 General Linear Model of days to 50% emergence as affected by hybrid, temperature and flooding times

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Temperature	Fixed	2	20,30			
Flooding	Fixed	4	0,24,48,72			
Analysis of variance for days to 50% emergence, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	26.924	26.924	3.365	2.48	0.014
Temperature	1	763.753	763.753	763.753	563.52	0.000
Flooding	3	1003.760	1003.760	334.587	246.87	0.000
Hybrid*Temp	8	16.59	16.59	2.074	1.53	0.148
Hybrid*Flood	24	25.271	25.271	1.053	0.78	0.764
Temp*Flood	3	36.566	36.566	12.189	8.99	0.000
Hybrid*Temp*Flood	24	37.215	37.215	1.551	1.14	0.298
Error	216	292.75	292.75	1.355		
Total	287	2202.83				
CV = 41.68 R-Sq = 86.71% R-Sq (adj) = 82.34%						

Table 3 General Linear Model of germination % as affected by hybrids, temperatures and flooding times

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Flooding	Fixed	4	0,24,48,72			
Temperature	Fixed	3	10,20,30			
Analysis of variance for % germination, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	2643.5	2643.5	330.4	2.33	0.019
Temperature	2	35611.6	35611.6	17805.8	125.69	0.000
Flooding	3	22640.7	22640.7	7546.9	53.27	0.000
Hybrid*Temperature	16	5017.6	5017.6	313.6	2.21	0.005
Hybrid*Flooding	24	4200.9	4200.9	175.0	1.24	0.208
Temperature*Flooding	6	3649.5	3649.5	608.3	4.29	0.000
Hybrid*Temp*Flood	48	10421.3	10421.3	217.1	1.53	0.018
Error	324	45900.0	45900.0	141.7		
Total	431	130085.2				
CV = 21.01 R-Sq = 64.72% R-Sq (adj) = 53.06%						

Table 4 General Linear Model of emergence % as affected by hybrids, temperatures and flooding times

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Flooding	Fixed	4	0,24,48,72			
Temperature	Fixed	3	10,20,30			
Analysis of variance for % emergence, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	8669.9	8669.9	1083.7	3.62	0.000
Temperature	2	553658.8	553658.8	276829.4	923.95	0.000
Flooding	3	46178.5	46178.5	15392.8	51.38	0.000
Hybrid*Temperature	16	11974.5	11974.5	748.4	2.5	0.001
Hybrid*Flooding	24	16665.3	16665.3	694.4	2.32	0.001
Temperature*Flooding	6	11543.1	11543.1	1923.8	6.42	0.000
Hybrid*Temp*Flood	48	31556.9	31556.9	657.4	2.19	0.000
Error	324	97075.0	97075.0	299.6		
Total	431	777322.0				
CV = 37.53 R-Sq = 87.51% R-Sq (adj) = 83.39%						

Table 5 General Linear Model of emergence rates as affected by hybrids, temperatures and flooding times

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Temperature	Fixed	3	10,20,30			
Flooding	Fixed	4	0,24,48,72			
Analysis of variance for emergence rates, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	3.3776	3.3776	0.4222	3.67	0.000
Temperature	2	229.0130	229.0130	114.5065	996.20	0.000
Flooding	3	84.9899	84.9899	28.3300	246.47	0.000
Hybrid*Temperature	16	2.4436	2.4436	0.1527	1.33	0.177
Hybrid*Flooding	24	3.7882	3.7882	0.1578	1.37	0.117
Temperature*Flooding	6	41.8419	41.8419	6.9736	60.67	0.000
Hybrid*Temp*Flooding	48	5.9207	5.9207	0.1233	1.07	0.352
Error	324	37.2415	37.2415	0.1149		
Total	431	408.6163				
CV = 42.32 R-Sq = 90.89% R-Sq (adj) = 87.88%						

Table 6 General Linear Model of plant height after four weeks as affected by hybrids, temperatures and flooding times

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Temperature	Fixed	2	20,30			
Flooding	Fixed	4	0,24,48,72			
Analysis of variance for plant height after four weeks, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	28822	29053	3632	20.78	0.000
Temperature	1	1725275	1725070	1725070	9870.20	0.000
Flooding	3	763336	760933	253644	1451.26	0.000
Hybrid*Temperature	8	16062	16391	2049	11.72	0.000
Hybrid*Flooding	24	37834	37924	1580	9.04	0.000
Temperature*Flooding	3	14511	14527	4842	27.71	0.000
Hybrid*Temp*Flood	24	34313	34313	1430	8.18	0.000
Error	215	37577	37577	175		
Total	286	2657730				
CV = 35.48 R-Sq = 98.59% R-Sq (adj) = 98.12%						

Table 7 General Linear Model of dry weight yield of nine maize hybrids under different temperatures and flooding times

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Temperature	Fixed	3	10,20,30			
Flooding	Fixed	4	0,24,48,72			
Analysis of variance for dry weight yield, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	1.0124	1.0124	0.1266	3.61	0.000
Temperature	2	132.4787	132.4787	66.2394	1888.85	0.000
Flooding	3	27.4497	27.4497	9.1499	260.91	0.000
Hybrid*Temperature	16	1.5668	1.5668	0.0979	2.79	0.000
Hybrid*Flooding	24	2.3066	2.3066	0.0961	2.74	0.000
Temperature*Flooding	6	14.7638	14.7638	2.4606	70.17	0.000
Hybrid*Temp*Flood	48	5.2415	5.2415	0.1092	3.11	0.000
Error	324	11.3622	11.3622	0.0351		
Total	431	196.1818				
CV = 45.78 R-Sq = 94.21% R-Sq (adj) = 92.30%						

Table 8 Analysis of variance of nine maize hybrids for warm test germinations

Source	**DF**	**Sum of squares**	**Mean square**	**F**	**P**
Hybrid	8	66.44	8.31	2.20	0.039
Error	63	237.50	3.77		
Total	71	303.94			
CV = 2.10					

Table 9 Analysis of variance of nine maize hybrids for cold test germinations

Source	DF	Sum of squares	Mean square	F	P
Hybrid	8	271.28	33.91	6.93	0.000
Error	63	308.38	4.89		
Total	71	579.65			
CV = 3.02					

Table 10 Analysis of variance of nine maize hybrids for soak test germinations

Source	DF	Sum of squares	Mean square	F	P
Hybrid	8	286.00	35.75	5.67	0.000
Error	63	397.50	6.31		
Total	71	683.50			
CV = 4.96					

Table 11 Analysis of variance of nine maize hybrids for complex stressing vigour test germinations

Source	DF	Sum of squares	Mean square	F	P
Hybrid	8	12990.40	1623.80	33.70	0.000
Error	63	3035.50	48.20		
Total	71	16025.90			
CV = 17.95					

Table 12 Analysis of variance of nine maize hybrids for emergence percentage in the glasshouse experiment under cool temperatures and 72 hours flooding

Source	DF	Sum of squares	Mean square	F	P
Hybrid	8	5072	634	4.48	0.002
Error	27	3825	142		
Total	35	8897			
CV = 21.34					

Table 13 Analysis of variance of nine maize hybrids for emergence rates in the glasshouse experiment under cool temperatures and 72 hours flooding

Source	DF	Sum of squares	Mean square	F	P
Hybrid	8	0.4516	0.0565	2.85	0.020
Error	27	0.5343	0.0198		
Total	35	0.9859			
CV = 28.04					

Table 14 Regression analysis of simulated field emergence under cold, wet conditions as affected by cold test germination

The regression equation is: Simulated field emergence at 72 hours flooding = -584 + 6.92 average cold test germination					
R-Sq = 58.2 %			R-Sq(adj) = 52.2 %		
Analysis of variance:					
Source	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	737.43	737.43	9.73	0.017
Error	7	530.63	75.80		
Total	8	1268.06			

Table 15 Regression analysis of simulated field emergence under cold, wet conditions as affected by soak test germination

The regression equation is: Simulated field emergence at 72 hours flooding = -278.0 + 3.617 average soak test germination					
R-Sq = 51.4 %			R-Sq(adj) = 44.5 %		
Analysis of variance:					
Source	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	651.77	651.770	7.40	0.030
Error	7	616.29	88.041		
Total	8	1268.06			

Table 16 Regression analysis of simulated field emergence under cold, wet conditions as affected by complex stressing vigour test germination

The regression equation is:					
Simulated field emergence at 72 hours flooding = 10.17 + 0.7837 average complex stress vigor test germination					
R-Sq = 73.4 %			R-Sq(adj) = 69.6 %		
Analysis of variance:					
Source	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	930.40	930.397	19.29	0.003
Error	7	337.66	48.237		
Total	8	1268.06			

Table 17 General Linear model for electrical conductivity as affected by hybrids, temperatures and soaking times

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Temperatures	Fixed	3	10, 20, 30			
Soak time	Fixed	3	24, 48, 72			
Analysis of variance for electrical conductivity, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	0.54052	0.54052	0.06756	5.33	0.000
Temperature	2	4.55883	4.55883	2.27942	179.72	0.000
Soak time	2	3.82516	3.82516	1.91258	150.79	0.000
Temperature*Soak time	4	2.39536	2.39536	0.59884	47.21	0.000
Hybrid*Temperature	16	1.39765	1.39765	0.08735	6.89	0.000
Hybrid*Soak time	16	0.73519	0.73519	0.04595	3.62	0.000
Hybrid*Temp*Soak	32	1.97446	1.97446	0.06170	4.86	0.000
Error	243	3.08205	3.08205	0.01268		
Total	323	18.50922				
R-Sq = 83.35% R-Sq (adj) = 77.87% CV = 19.93						

Table 18 Pearson correlation coefficients of average electrolyte leakage and simulated field emergence

	Electrical conductivity
Simulated field emergence	**-0.406**
	P = 0.002

Table 19 General linear model of accelerated ageing germination as affected by hybrids

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Analysis of variance for accelerated ageing, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**

Hybrid	8	7824.00	7824.00	978.00	16.85	0.000
Error	27	1566.75	1566.75	58.03		
Total	35	9390.75				
R-Sq = 83.32% R-Sq (adj) = 78.37% CV = 21.02						

Table 20 Regression analysis of simulated field emergence under cold, wet conditions as affected by accelerated ageing test germinations

The regression equation is: Simulated field emergence at 72 hours flooding = 36.6 + 0.511 average accelerated ageing germination					
R-Sq = 56.0 %			R-Sq(adj) = 49.8 %		
Analysis of variance:					
Source	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	710.53	710.53	8.92	0.020
Error	7	557.53	79.65		
Total	8	1268.06			

Table 21 General linear model of tetrazolium germination as affected by hybrids

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Analysis of variance for tetrazolium germination, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	7601.5	7601.5	950.2	3.72	0.005
Error	27	6901.5	6901.5	255.6		
Total	35	14503.0				
S = 15.98 R-Sq = 52.41% R-Sq (adj) = 38.31% CV = 22.36						

Table 22 Regression analysis of simulated field emergence under cold, wet conditions as affected by tetrazolium test results

The regression equation is: Simulated field emergence at 72 hours flooding = 269 – 2.01 average tetrazolium test germination

R-Sq = 8.1 %			R-Sq(adj) = 0.0 %		
Analysis of variance:					
Source	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	103.2	103.2	0.62	0.457
Error	7	1164.9	166.4		
Total	8	1268.1			

Table 23 Regression analysis of field emergence under cold, wet conditions as affected by complex stressing vigour test germination

The regression equation is: Field emergence = 36.6 + 0.511 average complex stressing vigour					
R-Sq = 80.2 %			R-Sq(adj) = 77.4 %		
Analysis of variance:					
Source	**DF**	**SS**	**MS**	**F**	**P**
Regression	1	271.504	271.504	28.41	0.001
Error	7	66.885	9.555		
Total	8	338.389			

Table 24 General linear model of fast green test results (cracks near or on embryo) as affected by hybrids

Factor	**Type**	**Levels**	**Values**			
Hybrid	Fixed	9	1,2,3,4,5,6,7,8,9			
Analysis of variance for fast green results, using adjusted SS for tests						
Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Hybrid	8	5081.5	5081.5	2540.7	16.11	0.008
Error	27	16563.3	16563.3	157.7		
Total	35	21644.8				
S = 17.98 R-Sq = 55.41% R-Sq (adj) = 44.31% CV =13.11						