UNIVERSITY OF NATAL

ALLEVIATION OF COTYLEDONAL CRACKING IN GREEN BEANS (*Phaseolus vulgaris* L.) BY CALCIUM SEED TREATMENT

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ABSTRACT

Cotyledonal cracking is a physiological disorder of common beans, and rarely, soybeans that occurs as transverse fissures across the cotyledons. The phenomenon is generally referred to as transverse cotyledonal cracking (TVC). Although TVC has been known for decades now, factors contributing to its occurrence, and how the disorder can be alleviated, are still not well understood. The objective of this study was to investigate the effect calcium seed treatment on cotyledonal cracking in green bean (*Phaseolus vulgaris* L.) seeds. Six cultivars (Imbali, Sodwana, Outeniqua, Elangeni, Tokai and Tongati) were examined for water absorption patterns during a 6-h imbibition in distilled water. Cultivars were categorised according to the rates of water absorption, in the presence of seed coat, and there was a significant correlation between seed size and water absorption rate. To examine seed predisposition to TVC, seeds were imbibed with and without seed coats and TVC was scored every hour for the 6-h duration of imbibition. There was a significant positive correlation between water absorption rate and TVC. Genetic analysis of the cultivars using SDS-PAGE revealed that there are possible differences between the resistant cultivars and sensitive cultivars, with respect to protein patterns. Imbali, one of the small cultivars (~1.5 g $seed^{-1}$) that imbibed water uniformly, was resistant to cotyledonal cracking compared to the largest cultivar (Sodwana ~2.5 g $seed^{-1}$), which also had a high rate of water absorption. Priming seeds with calcium ($CaSO_4$, $Ca(NO_3)_2$ and $CaCl_2$) osmolarities (0, 1, 10, 50, 100, and 100 mM) increased seed calcium content and reduced susceptibility to TVC. Comparison of priming and seed coating with respect to field emergence, TVC, stand establishment and seed yield showed that coating was better than priming. However, greenhouse studies showed that the effect of priming in the progeny of treated seeds was significantly better than that of coating, with respect to TVC reduction. In both laboratory and field studies, it was clear that applying calcium concentrations greater than 50 mM was not necessary to alleviate TVC and improve seed performance. Seed germination and emergence were reduced at calcium concentrations greater than 50 mM. It is concluded that calcium is effective in controlling TVC under both laboratory and field conditions. The effect of calcium is associated with regulation of imbibition and improvement of seed calcium content. Enhanced seed calcium content likely improved cell wall integrity.

DECLARATION

I, Tholakele Gladness Mazibuko, hereby certify that the research work presented in this dissertation, unless specified otherwise, is my own original investigation and has not been submitted in part, or in whole to any other University. The research was carried out at the University of Natal, Pietermaritzburg, South Africa.

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Approved by

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Dr. Albert T. Modi (Advisor)

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Food production is one of the major problems facing the developing countries in the world. Most of the developing countries are also faced with a problem of malnutrition. Green beans (*Phaseolus vulgaris* L.) are among the most important vegetables in the world for human consumption, providing an excellent source of proteins for the growing population (Coertze, 1975), minerals (calcium, phosphorus and iron) and vitamins A and B (McCollum, 1992). Common bean is consumed as an edible young pod (snap bean) and as a dry seed. It has been known to ancient Indian tribes for more than 7000 years (Nonneck, 1989). Beans are used together with maize providing a combined diet rich in proteins and starch, which is essential for people eating little or no meat (Nonneck, 1989). In South Africa, bean consumption goes back to the early years of the Dutch settlement at the Cape (Strydom, 1971). There is evidence that there is a reduction in the production of beans [e.g. 671 tons of dry beans were produced in KwaZulu-Natal in 1997 compared to 796 tons produced in 1993 (Allemann and Young, 1998)]. Encouraging consumption of green beans by young people might help alleviate the problem of malnutrition since proteins play an important role in a growing individual.

Agronomically, beans are important for their short growing season (Rubatzky and Yamaguchi, 1996). This attribute makes beans to be also suitable for relatively dry areas where the amount of seasonal rainfall is not optimal for long season crops such as maize. Although their nitrogen-fixing properties are lower than soybeans, lucerne and

lupins, beans play an important role in crop rotation systems as a nitrogen-fixing crop (Strydom, 1971; Peirce, 1987).

1.2 Effect of seed quality on crop yield

Poor seed quality contributes immensely to the reduction in crop yield by impairing germination, resulting in poor seedlings and emergence that is not uniform. The quality of a seed lot determines whether the seedlings produced from it will withstand harsh field conditions. There are many factors that contribute to the quality of the seed lot and these include environmental conditions during crop growth, seed development and maturation, storage conditions and seed composition (mineral and chemical content). Copeland and McDonald (1995) proposed a scheme (Figure 1.1) to explain the combination of factors influencing seed quality. It is clear from figure 1 that there is a strong relationship between seed quality and crop yield.

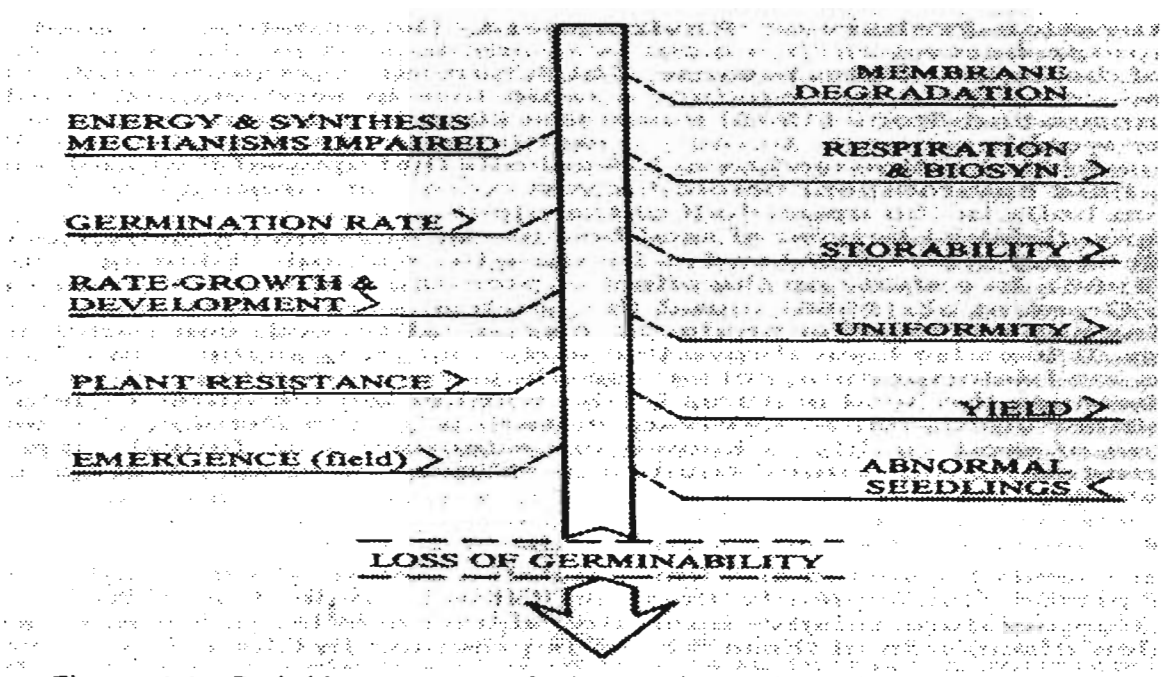


Figure 1.1. Probable sequence of changes in seed quality during deterioration (Copeland and McDonald, 1995).

1.2.1 The phenomenon of transverse cotyledonal cracking

There are many factors that can lead to the reduction of green bean yield, including diseases, pest attacks and physiological disorders. Most of the diseases and pests that affect green beans have been studied and some effective solutions established, but not much is known about how to alleviate a physiological disorder called transverse cotyledonal cracking (TVC). Transverse cotyledonal cracking was first observed more than half a century ago and it affects green beans and other leguminous crops. It is characterized by the occurrence of one or more cracks across the cotyledon during or after germination (McCollum, 1953; Morris, 1971; Dickson *et al.*, 1973; Bradford and Eisinger, 1986). The size of the cracks ranges from minute (hair line) to deep cracks (Figure 1.2), which may cause complete severing of the cotyledon (Morris, 1971).

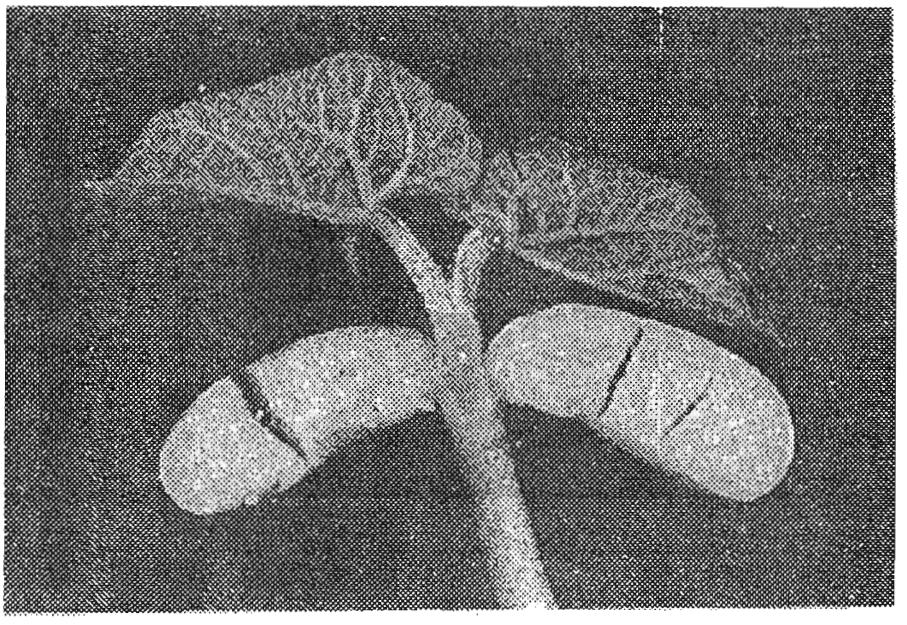


Figure 1.2. Green bean seedling showing cracks in cotyledons (McCollum, 1953).

Green bean cotyledons act as the major nutrient reserve of the seed. During germination and early stages of seedling growth reserves (carbohydrates, proteins and lipids) are mobilised from the cotyledons supplying the growing seedling with essential nutrients

mainly in the form of sucrose (Simon and Mills, 1983; McCollum, 1992). It is because of this that the seedling requires intact cotyledons. The cracks on TVC-affected seedlings impair translocation of these essential nutrients from the cotyledons to the growing seedling (McCollum, 1992), leading to the production of a stunted seedling or no seedling development. Transverse cotyledonal cracking also reduces seed germination, seedling stand establishment and can cause up to 90% yield reduction (Bradford and Eisinger, 1986). In some cases the cracks prevent or delay the shedding of the seed coat (Figure 1.3). Previous studies suggested that TVC might have been inherited with resistance to common bean mosaic in the early 1950's (Morris, 1971) when white-seeded cultivars were developed through breeding. White-seeded cultivars have been found to be more susceptible to TVC (Dickson *et al.*, 1973) and resistant cultivars have dominant genes for TVC (Van Schoonhoven and Voysest, 1990). The bean industry uses white-seeded cultivars for food processing, avoiding the use of dark-coloured cultivars because the latter are less attractive in processed products (Nonneck, 1989). Use of white-seeded cultivars may be one of the reasons why TVC is such a serious problem in seed production of green beans.

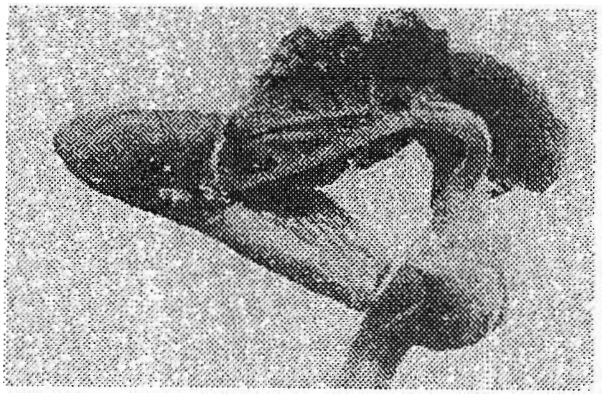


Figure 1.3. Cracks on cotyledons may delay shedding of the seed coat in green bean seeds (McCollum, 1953).

1.2.2 Factors affecting transverse cotyledonal cracking

Although TVC is thought to be genetically inherited, there are different factors that have been implicated to increase its occurrence, these include seed coat permeability and seed moisture and calcium content at sowing (McCollum, 1952; Morris, 1971; Dickson *et al.*, 1973; Aqil and Boe, 1975; Dickson and Boettger, 1976; Bradford and Eisinger, 1986).

1.2.2.1 Possible role of mineral nutrients

Plant growth and development are affected by both environmental and physiological conditions. Plants that are able to take up nutrients from the soil and transport them to different parts where they are required are able to survive better than dysfunctional plants. Different mineral elements have unique roles to play in growth and development of plants. Deficiencies even in small quantities may have detrimental effects on plant growth. Some minerals are required for structural maintenance while others are only needed to maintain the physiology of the plant (e.g. they help in the transportation of other minerals and water throughout the plant) (Marschner, 1995).

Calcium, the mineral element previously implicated in TVC (McCollum, 1953), serves both in structural and messenger roles because of its ability to exist in a wide range of binding states. Calcium plays an essential role in regulating many physiological processes that influence both growth and responses to environmental stress. These processes include water solute movement, influenced through effects on membrane structure and stomatal function; cell division and cell wall synthesis; direct or signalling in systems involved in plant defence and repair of damage from biotic and abiotic stress; rates of respiratory metabolism and translocation and structural chemistry and

function of woody support tissue (McLaughlin and Wimmer, 1999). Calcium is known to play an important role in maintaining cell wall structure and integrity (Cachorro *et al.*, 1994; Fernandez *et al.*, 2000), as it is known to have a very strong texture-increasing effect (Tobias *et al.*, 1993; Moon *et al.*, 2000; Buchanan *et al.*, 2000) and even retard fruit softening during storage (Safner and Conway, 1998). Tobias *et al.*, 1993 observed that the effect of calcium in reducing fruit decay is associated with maintaining cell wall structure by delaying and modifying chemical changes in cell wall composition. A close relationship has been established between fruit calcium levels and numerous physiological and pathological disorders (Safner and Conway, 1998). High or increased calcium content has found to decrease internal breakdown and maintain fruit firmness and quality in apples (Tobias *et al.*, 1993). Lee *et al.*, 2001 found that post-harvest treatment of kiwi fruits with various levels $CaCl_2$ improved flesh firmness and increased calcium content in the treated fruits (Long *et al.*, 1998; Vilas-Boas *et al*, 1998; Moon *et al.*, 2002(a)). They also found that calcium inhibited reduction of the amount of insoluble pectin and total pectin during storage, which ensured fruit firmness.

Studies conducted on post-harvest calcium treatment of different fruits showed a significant degradation of the cell walls of the middle lamella of untreated fruits (Zhang and Guan, 1998; Moon *et al.*, 1999; Shiraishi *et al.*, 1999; Moon *et al.*, 2002(b)). Membrane structure and function and cell wall structure and composition are associated with various effects of calcium on fruit ripening (Safner and Conway, 1998). In studies of the effect of calcium in post-harvest treatment of banana with $CaCl_2$ it was found that calcium did not influence cell wall structure but appeared to influence ripening physiology (Perera and Karunaratne, 2002).

Calcium was found to be associated with cellulose and hemicellulose in the carrot cell walls and is believed to be a factor in cell wall rigidity (Duczmal, 1973). It has also been found that calcium has a protective effect and improves growth of plants growing under saline conditions (Ortiz *et al.*, 1994). Free carboxyl groups on pectin polymers play an important role in stabilizing the cell wall, probably through the co-operative binding of calcium (Marschner, 1995; Buchanan *et al.*, 2000). Calcium accumulation in the cell wall facilitates cross-linking of pectin polymers to a cell wall network that increases wall strength and cell cohesion (Safner and Conway, 1998). Two mechanisms have been proposed to explain the effect of calcium on post-harvest changes in fruit firmness and quality. Calcium binds to cell walls, thus inducing a greater stability in cell wall structure; calcium further interacts with membrane structure to influence physiological functions (Buchanan, *et al.*, 2000).

Dickson (1975) found that calcium increased the occurrence of TVC if it is limiting as a nutrient, but its effect may be less than that of magnesium. Borys and Mamys (1976) found a positive correlation between TVC and leaf calcium content and TVC. Some studies have shown that there is a negative correlation between seed calcium content and TVC (Dickson, 1975; Dickson and Boettger, 1976), and the cracks occur along the cell wall (Morris, 1971). Huang *et al.*, 2001 found that the difference in cracking of susceptible and resistant cultivars of litchi fruit was in the mobilization of calcium to construct the cell walls and that the amount of calcium was lower in the susceptible cultivars. Cracking might be caused by pressure exerted on the dry inner parts of the cotyledon by wet outer parts when they start swelling during imbibition. If the cell

walls are of higher strength they will be able to resist the pressure, leading to less cracking.

1.2.2.2 The role of seed coat

Seed coat colour has been associated with resistance and susceptibility of green beans to TVC in that white seeded cultivars have been found to be more susceptible than cultivars with dark-coloured seed coat (Dickson *et al.*, 1973; Bradford and Eisinger 1986). White-seeded cultivars are desirable for food processing in the bean industry because they do not cause off-colours.

Permeability of the seed coat determines the rate of imbibition and imbibition rate determines the degree of TVC. Seeds with highly permeable seed coats imbibe water rapidly, thus increasing the susceptibility of seeds to TVC and consequently poor germination occurs (Dickson *et al.*, 1973). Removal of the seed coat causes even the resistant cultivars to be prone to cracking. McCollum (1953) confirmed this in his study when using seeds of the resistant and susceptible cultivars. Seed coats were removed from the seeds and the seeds were allowed to imbibe water from a moist filter paper. Pronounced cracking was observed in both cultivars after one hour of imbibition (Figure 1.4). This finding indicates that the rate of imbibition plays a very important role in the occurrence of TVC because removal of the seed coat resulted in the rapid uptake of water by the cotyledons.

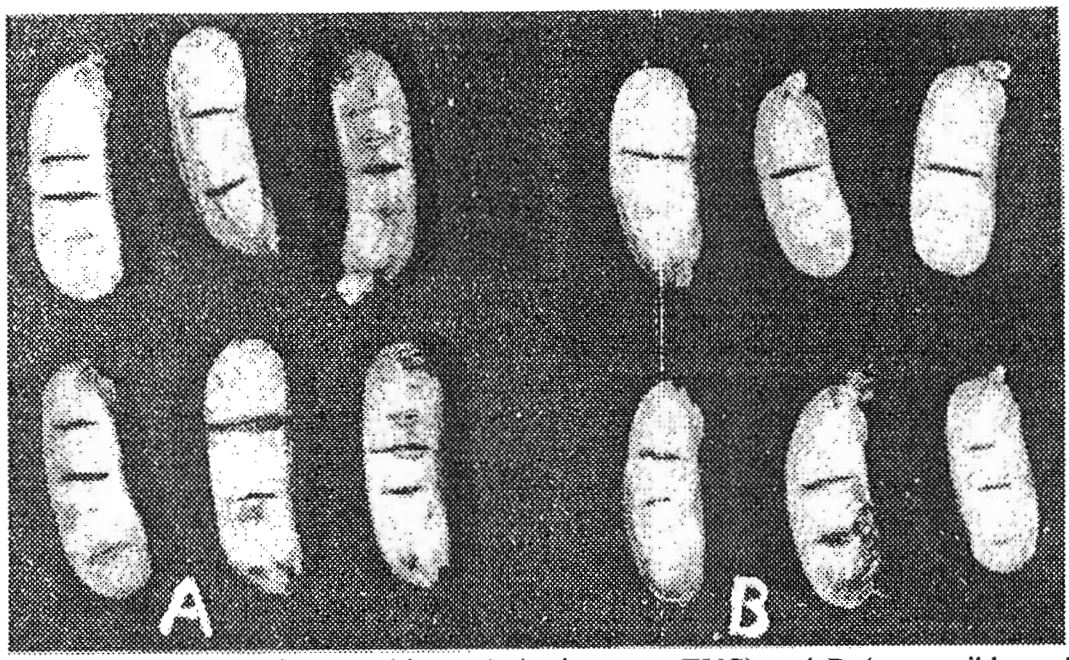


Figure 1.4. Seeds of two cultivars A (resistant to TVC) and B (susceptible to TVC) showing cracks after one hour of imbibition on a moist filter paper. (McCollum, 1953). Note that there is no visual difference between the resistant and the susceptible cultivar with the respect to TVC, because seeds were imbibed without seed coats, which exacerbated water absorption and cracking.

1.2.2.3 Effect of seed moisture content

Previous studies have shown that the seed moisture content also determines the severity of TVC. Seeds of low moisture content (less than 10%) imbibe water more rapidly, resulting in increased cracking and poor seedling vigour (Dickson, 1975; Dickson and Boettger, 1976). Jones (1971) suggested that rapid drying, like rapid imbibition may contribute to a greater moisture differential within the cotyledon thus increasing the amount of cracking. As seeds imbibe water, the outer parts of the cotyledon get wet first and start swelling while the inner parts are still dry. This causes increased pressure on the inner parts resulting in cracking (Bradford and Eisinger, 1986). Because dry seeds are more susceptible to cracking, more essential substances such as ionisable constituents and soluble carbohydrates are leached out during germination in the seed of low moisture content (Simon and Mills, 1983). This affects the growth rate of seedlings since these substances are required for seedling growth and establishment

(Splittstoesser, 1990). Lipid, oil and protein seed content have also been implicated to affect the imbibition rate in different crops (Jones, 1971). Using 15 snap bean cultivars, Aqil and Boe (1975) found no correlation between the protein profile and cracking susceptibility. Jones (1971) reported that there was a positive correlation between seed lipid content and susceptibility to TVC.

1.3 Seed enhancement

Seed quality plays an important role in crop production in determining the yield and performance of a seed lot. Uniformity in seed germination and emergence is desirable as it ensures or increases chances of higher yields. Many factors (storage conditions, seed moisture content, seed mineral content, etc.) contribute to low seed vigour or low emergence and these can be improved by treating seeds prior to sowing. Some of the techniques used in seed enhancement do not necessary improve or affect the physiology of the seeds but merely make the seed easy to handle during planting (Halmer, 1999). Seed hydration, biological seed treatment and seed coating are among the techniques used in seed enhancement. Seed hydration changes the physiology of the seed, by causing an onset of certain processes which are essential for germination but do not allow germination to take place, whereas seed coating is only external (Halmer, 1999; McDonald, 1999). Biological seed coating treatment (biological control) provides effective control of soil and seed borne pathogens (Halmer, 1999) and is applied using seed coating. Pre-hydration, priming and solid matrix priming are the three methods of seed hydration used in seed enhancement.

1.3.1 Priming

Priming is a conclusive term that refers to any technique that is associated with increasing seed hydration, to improve seed performance, followed by seed dehydration (Copeland and McDonald, 1995). Osmoconditioning (priming) refers to conditioning in media with high solute content while matriconditioning uses moist solid carriers with high adsorptive capillary forces (Khan, 1993). Primed seeds have been found to perform better than unprimed seeds with respect to germination percentage (Figure 1.5) and uniformity in emergence.

During priming seeds are soaked in an aerated solution of low water potential, using salts or water-soluble polymers such as polyethylene glycol (PEG) (Corbineau *et al.*, 1999). The low water potential of the solution reduces the rate of water uptake by the seed, which in turn reduces the imbibitional damage (Copeland and McDonald, 1995). Increasing PEG concentration results in a decrease in the osmotic potential of the solution, which in turn leads to slow imbibition rate and reduced physiological damage.

In some crops, e.g., green beans, it has been found that increasing levels of PEG- 6000 results in an increase in number of abnormal seedlings due to a decrease in oxygen availability affecting certain physiological processes (Queiroz *et al.*, 2000). This negative effect was however not observed in low PEG concentrations. Osmotic preconditioning using PEG was found to decrease the percentage germination and emergence of bean seeds both in the field and in the laboratory but increased the insertion height of the first pod, number of pods per plant and number of seeds per pod, thus increasing yield (Sperotto *et al.*, 1999).

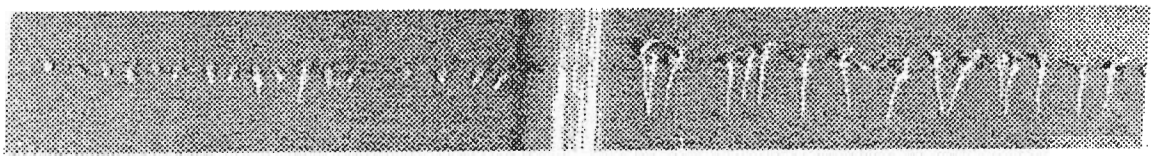


Figure 1.5. Primed lettuce seeds (right) germinate much faster and uniformly that seeds that were not primed (left) (McDonald, 1999).

Inert compounds of high molecular weight *e.g.* PEG, are preferred because they do not penetrate the seeds thus possess no threat to the seed as some salts do (Copeland and McDonald, 1995). Some salts have been found to be toxic to seeds but some have been found to contribute certain micronutrients that are needed by the seed (McDonald, 1999). The choice of salt to be used is made according to what is needed for the seeds, e.g. if seeds leach large quantities of micronutrients during imbibition, salts of low molecular weight will be used so that they can penetrate the seed and supply the needed micronutrient. Some salts may prove useful to certain crops and harmful to others. Some studies show that the type of salt used is not very important as long as the water potential of the salt solution is in the range of –1.0 and –2.0 MPa (Corbineau *et al.*, 1999).

Most of the work on seed conditioning is done using (Polyethylene glycol) PEG and small-seeded species. Although PEG has been very successful in increasing seed performance it has a disadvantage of reducing oxygen availability (McDonald, 1999). Oxygen availability, light and temperature are among the factors that affect the success of seed priming (McDonald, 1999). Amount of oxygen required depends on the type of priming material used. Solid matrix priming provides large amounts of oxygen to the seed since they are not fully saturated.

In certain crops, dormancy can be minimised by providing light during priming of the seeds that require light for germination (McDonald, 1999). It is desirable to use low temperatures during priming as this delays physiological processes of germination and reduces the possibilities of microbial contamination (Bradford, 1986). However in soybean (*Glycine max*) it has been found that physiological repair occurs better when priming is done at high temperatures e.g. 25°C (Armstrong and McDonald, 1992). Light and temperature interactions can also affect the success of priming by reducing percentage germination of the treated seeds (Rufaro *et al.*, 1993).

Initial seed moisture content has been found to play an important role in the success of priming as some studies show an increase in seed performance of seeds that had higher seed moisture content prior to priming (McDonald, 1999). The rate and method of drying seeds after priming also determines the success of priming. Some methods of drying (ambient air drying and drying using saturated salts of different relative humidity) are slow but reduce the occurrence of mechanical damage. Some studies have shown that protein bodies leach from imbibing soybean by separation of the cell walls of the middle lamella if seeds are imbibed in solutions of high water potential at high temperatures (Speath, 1989). Osmoconditioning avoids imbibitional injury by reducing the hydration rate leading to reduced leaching of seed electrolytes and solutions of low water potential minimise the negative effect of drying after priming (Armstrong and McDonald, 1992).

Addition of promotive substance such as biological control agents, chemicals to eliminate seed borne pathogens and plant hormones have been found to improve seed performance by increasing percentage germination and seedling emergence

(McDonald, 1999). Storage conditions play a role in determining seed vigour and this is also true for primed seeds. The physiological advantages gained during priming may be lost during storage conditions are if they are not optimal. Primed seeds have been found to have shorter shelf life with viability decreasing as storage period increased. Seed priming reduces imbibitional damage and leaching of seed electrolyte, enhances embryo growth, increases cell wall elasticity and initiates seed repair by allowing early replication of DNA, increasing RNA elasticity and protein synthesis (McDonald, 1999).

For large-seeded species e.g. beans and soybean, which are highly susceptible to imbibitional damage due to excess leakage of electrolytes from cracked cotyledons (Armstrong and McDonald, 1992), seed humidification has been proposed as a physiological enhancement technique. This method aids by raising seed moisture content by a relatively small degree, reaching hydration levels that are much lower than in priming. However, this method cannot be used if micronutrients need to be applied or taken up by the seed. Seed priming using PEG at low concentrations and seed humidification can be used to improve germination in cultivars susceptible to TVC since imbibition rate is reduced. Manipulation of imbibition rates could also be achieved by using salt-solutions at various osmotic potential levels to imbibe seeds.

1.3.2 Seed enhancement using seed coatings

Unlike seed hydration, which affects the seed physiology only, seed coating can also change seed shape and size. Seed coating improves seed shape and placement (during planting), and seed performance by placing fungicides, safeners, insecticides, micronutrients and other compounds, on the seed coat, which improves seed

performance (Copeland and McDonald, 1995; McDonald, 1999). Commercially, seed coating and seed pelleting are the two major types of coatings that are used (Figure 1.6). Seed coating is more appropriate for large-seeded species which do not need pelleting, as they are easy to handle. Seed pelleting on the other hand is more useful for small seeds.

1.3.2.1 Seed pelleting

Seed shape and size is changed so that it will be easy to handle seeds during planting especially if planters are being used. Small seeds are very difficult to plant using planters since they can be blown away by wind leading to a decrease in germination percentage and emergence (Copeland and McDonald, 1995). Seed pelleting does not exclude the possibility of improving seed performance since chemicals and micronutrients can be added into the pelleting material. Problems associated with pelleting include formation of a pellet around debris leading to a decrease in percentage germination and emergence since the pellet did not contain any seed. A seed pellet can also form around multiple seeds. Seed pelleting can impair germination if the pelleting material is too hard thus preventing radicle protrusion during germination (Copeland and McDonald, 1995).

1.3.2.2 Seed coating

Seed coating improves seed placement (during planting), seed performance and germinability without changing seed shape. Unlike seed pelleting, seed coating is usually used on big seeded crops and improves seed performance by placing certain chemicals or biological control agents on the seed coat (Halmer, 1999). However this does not mean that seed coating cannot be used on small seeded crops since it can be combined with seed pelleting. The coating material should be non-toxic to plants and

humans, a water-based polymer, have low viscosity and a high concentration of solids, an adjustable hydrophilic-hydrophobic balance and form a hard film after drying (Copeland and McDonald, 1995). The advantage of seed coating is that it is cost effective as less chemicals are used since they are placed directly on the seed and are in the vicinity of the germinating seedling, thus reducing environmental damage from excessive pesticide use. Seed coating can also be used to delay seed germination by using plastic film coating, which allows seeds to be planted in autumn but only germinate in the spring, and for seed defence against microorganisms. Shigehiro *et al.* (2000) found that coating soybean seeds with depolymerised chitins increased the chitinase activity, leading to an increase in seed defence against diseases.

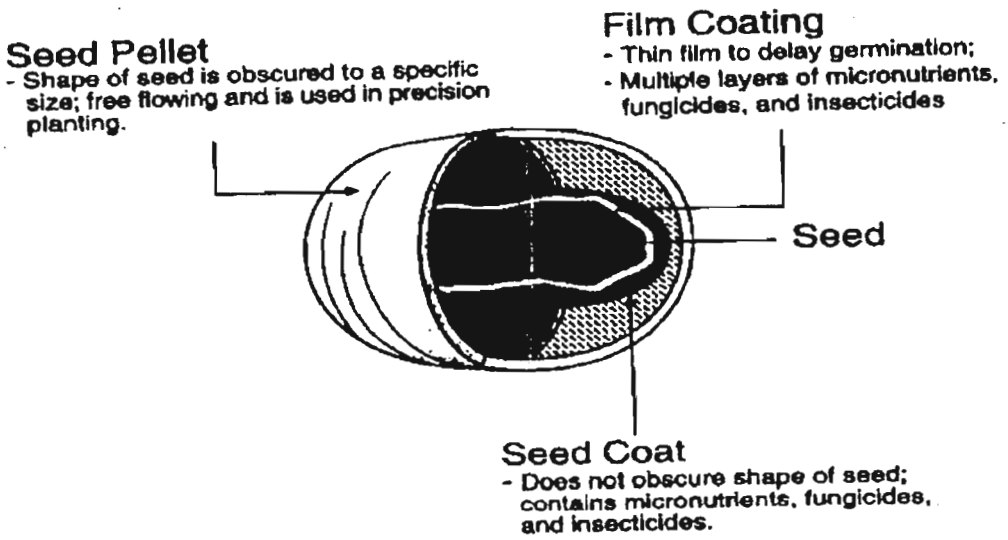


Figure 1.6. Types of seed coatings commercially used for seed enhancement (Copeland and McDonald, 1995).

Different seed enhancement methods can be used together to provide improved seed performance. Seed coating can be used together with seed pelleting to provide chemicals, nutrients or beneficial microorganisms to small seeded crops. Primed seeds can be pelleted to improve seed performance or pesticides, nutrients or growth

regulators can be included in the osmoticum or solid matrix carrier soak water. Even though seed enhancements do not reverse the existing damages they do improve seed quality and present a very useful tool in improving emergence. Unavailability of mineral nutrients can highly retard plant growth and development, and applying the required element using seed enhancements can rectify this. Good knowledge of how different seed enhancements work and which techniques are suitable for a certain crop prior to application is important in this regard.

1.4 Justification and study objectives

Since seeds of cultivars that are susceptible to TVC have cracks, this leads to the leaching of essential nutrients during imbibition. Leaching reduces the amount of nutrients available for germination and emergence. Hence stand establishment may be delayed and uneven or characterized by patches where no emergence occurred. It is expected that seedlings that are produced from these seeds will have cracks on their cotyledons. Cracks may act as sites of infection by pathogens leading to poor germination and emergence. That cracks impair transportation of essential nutrients (section 1.2.1) from the cotyledons to the growing seedlings also leads to poor seedling stand establishment. Poor germination and crop establishment cause yield reduction because 1) fewer plants are produced, 2) produced plants may take too long to develop because of stuntedness and 3) produced seeds may be of poor quality. A hypothetical model to explain how cotyledonal cracking, crop establishment and yield may be related is presented in Figure 1.7.

Transverse cotyledonal cracking is an important problem in the production of green beans and other leguminous crops. Previous studies have shown that seed moisture content and amount of calcium in the seed play an important role in the occurrence of TVC. Cracking occurs even to resistant cultivars if their seed moisture content is low. The rate of imbibition also plays an important role and is also affected by the seed moisture content. The amount of calcium in the seed is very important since the cracks occur along the cell wall. Applying calcium treatment in the form of sprays at seedling stage has not been successful because cracks are formed during germination. It is because of this reason and because of the well-established role of calcium in cell wall integrity that calcium was chosen for an investigation of its effect on cotyledonal cracking. The objectives of this study were to 1) examine the effects of different calcium salts on the regulation of water uptake by green bean seeds, 2) examine the effect of each salt on seed germination, seedling emergence, cotyledonal cracking and crop establishment under field and controlled environmental conditions, and 3) compare the effect of seed priming and coating as a means of applying a calcium treatment. Seed chemical composition and protein expression in response to calcium treatment were also examined.

References

Allemann, L. and B. W. Young. 1998. Vegetable production. Department of Agriculture. (Pamphlets), South Africa, 1- 4.

Aqil, B. A. and A.A. Boe. 1975. Occurrence of cotyledonal cracking in snap beans and its relation to nutritional status in the seed. *HortScience* 10: 509 – 510.

Armstrong, H. and M. B. McDonald. 1992. Effect of osmoconditioning on water uptake and electrical conductivity in soybean seeds. *Seed Science and Technology* 20: 391 – 400.

Borys, M. W. and I. Mamys. 1976. Effect of nutrient level of culture solution on transverse cracking of the cotyledons of *Phaseolus vulgaris* L. during germination. *Roczniki Nauk Rolniczych E* 6: 37- 43.

Bradford, J. K.1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. *Hortscience* 21: 1105 – 1112.

Bradford, K. J. and B. A. Eisinger. 1986. Role of seed mineral in the occurrence of transverse cotyledonal cracking of snap bean. *Journal of the American Society of Horticultural Science* 111: 110 – 114.

Buchanan, B. B., W. Gruissem and R. L. Jones. 2000. Biochemistry and molecular biology of plants. American Society of Plant Physiologists. Rockville, Maryland, 52 - 108.

Cachorro P., A. Ortiz and A. Cerda. 1994. Implication of Calcium nutrition on the response of *Phaseolus vulgaris* L. to salinity. *Plant and Soil* 159: 205-212.

Coertze, A. F. 1975. Green bean production in South Africa. Horticultural Research Institute. Pretoria, South Africa.

Copeland L. O. and M. B. McDonald. 1995. Principles of seed science and technology 3rd edn. Chapman and Hall, 258 - 276.

Corbineau F., N. Ozbingol, D. Vinel and D. Come, 1999. Improvement of tomato seed germination by osmopriming as related to energy metabolism. In Seed biology Advances and applications, ed. Black M. K. J. Bradford and J. Vazquez-Ramos. CABI publishing. 467 - 476.

Dickson, M. H. 1975. Inheritance of transverse cotyledonal cracking resistance to mechanical damage in snap beans (*Phaseolus vulgaris* L.). *Journal of the American Society of Horticultural Science* 100: 231 – 233.

Dickson, M. H. and M. A. Boettger, 1976. Factors associated with resistance to mechanical damage in snap beans (*Phaseolus vulgaris* L.). *Journal of the American Society of Horticultural Science* 101: 541 – 544.

Dickson, M. H., K. Duczmal and S. Shannon. 1973. Imbibition rate and seed composition as factors affecting transverse cotyledonal cracking in bean (*Phaseolus vulgaris* L.) seeds. *Journal of American Society of Horticultural Science* 73: 509 – 513.

Duczmal, K. W.1973. Uptake and transport of nutrients and transverse cracking of bean cotyledons. *Acta Societatis Botanicorum Poloniae* 14: 401 – 410.

Fernandez E. M., C.A. Rosolem, and D.M.T. Oliviera. 2000. Peanut seed tegument is affected by liming and drying method. *Seed Science and Technology* 28: 185 – 192.

Halmer P. 1999. In: Black M and J.D Bewley(Eds) Seed Technology and its Biological Basis. Sheffield Academic press, USA, 257- 283.

Huang X., Li J., Wang H., Huang H., and F Gao 2001. The relationship between fruit cracking and calcium in litchi per carp. *Acta Horticulture* 558: 209 – 215.

Jones, T. L. 1971. Injury to beans (*Phaseolus vulgaris* L.) in relation to imbibition. Agriculture, Plant Culture. University of Illions.

Khan, A. A. 1993. Pre-plant physiological seed conditioning. Horticultural Reviews 13: 131 – 181.

Lee C., S. Kim, S. Kang, J. Ko, C. Kim, and D. Han. 2001. Changes in cell wall metabolism of kiwi fruits during low temperature storage by post-harvest calcium application. *Journal of the Korean Society for Horticultural Science* 42: 91 – 94.

Long C., Z. Zhang, Z. Shen, and J. Yan. 1998. Role of cell wall calcium in red light-inhibited elongation of hypocotyls of etiolated *Phaseolus radiatus*. *Acta Botanica Sinica* 40: 132 – 137.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd edn. Academic Press. New York, 285-299.

McCollum, J. P.1953. Factors affecting cotyledonal cracking during germination of beans (*Phaseolus vulgaris*). *Plant Physiology* 27: 267 – 274.

McCollum, J.P. 1992. Producing vegetable crops. 4th edn. Interstate publishers Inc. Danville, 233 - 254.

McDonald M. B. 1999. In: Black M. and J. D. Bewley (Eds) Seed Technology and its Biological Basis, Sheffield Academic press, USA, 287 - 316.

McLaughlin S. B. and R. Wimmer. 1999. Calcium physiology and terrestrial ecosystem processes. *New Phytopathologist* 142: 373 – 414.

Moon B., J. Choi, and K. Kim. 1999. Effect of pre or post-harvest application of calcium compound extracted from oyster shell on the changes in cell wall calcium content, enzyme activity and cell structure during storage of apple fruits. *Journal of the Korean Society for Horticultural Science* 40:345 – 348.

Moon B., S. Lim, J. Choi and Y. Suh. 2000. Effect of pre or post-harvest application of liquid calcium fertilizer manufactured from oyster shell on the calcium concentration and quality in stored "Niitaka" pear fruits. *Journal of the Korean Society for Horticultural Science* 41: 61 – 64.

Moon B., I. Kang., Y., Lee, K. Nam, and J. Choi. 2002 (a).Effect of tree-spray of liquid calcium compound on the changes in cell wall components, cell wall hydrolases, and cell wall structure during cold storage of non-astringent persimmon fruits. *Journal of the Korean Society for Horticultural Science* 43: 443 – 446.

Moon B., W. Lu, H. Zheng, and J. Choi. 2002 (b). Effect of tree-spray of liquid calcium compounds on the calcium contents, quality and cell wall structure change of "Jingfen" pear fruits. *Journal of the Korean Society for Horticultural Science* 41: 51 – 53.

Morris J. L. 1971. The breeding aspects of vegetable seed quality. *HortScience* 6:553 – 555.

Nonneck, L. 1989. Vegetable production. Van Nonstrand Reinhold Publishers, New York, 268 - 293.

Ortiz, A., V. Martinez, and A. Cerda, 1994. Effect of osmotic shock and calcium on growth and solute composition of *Phaseolus vulgaris* plant. *Physiologia plantarum* 91: 468 – 476.

Peirce, L. C. 1987. Vegetables: Characteristics, Production and Marketing. John Wiley and Sons. New York, 333 - 348.

Perera A. N., and A. M. Karunaratne. 2002. Post – harvest calcium chloride treatment do not help increase shelf life of bananas. *Fruits (Paris)* 57: 87 - 94.

Queiroz M.F., P.D. Fernandez and F. Almeida. 2000. Seed infection and seedling abnormalities of beans under osmotic conditioning induced by polyethylene Glycol 6000. *Revista Brasileira de Engenharia Agricola e Ambiental* 4: 409 - 415.

Rubatzky, V. E. and M. Yamaguchi. 1996. World vegetables: Principles, Production and Nutritive value 2nd edn. Chapman and Hall. New York, 489 - 530.

Rufaro M., E. M., Chirco, and A. A. Khan. 1993. Seed germination of three flower species following matriconditioning under various environments. *Journal of the American Society of Horticultural Science* 118: 330 – 334.

Safner, R. A., and W. S. Conway. 1998. Effect of post-harvest calcium chloride treatment on tissue water relations, cell wall calcium levels and post-harvest life of "Golden Delicious" apples. Journal of the American Society of Horticultural Science 123: 893 – 897.

Shigehiro, H., M. Hayashi, and S. Okuno. 2000. Soybean seed surface- coated with depolymerised chitins: chitinase activity as a predictive index for the harvest of beans in field culture. *Journal of Science Food and Agriculture* 81: 205 – 209.

Shiraishi M., P. Mohammad, Y. Makita, M. Fujibuchi and T. Manabe. 1999. Effect of calcium compound on puffing and the ultrastructural characteristics of the sub-epidermal cell walls of puffy and calcium induced non-puffy Satsuma mandarin fruits. *Journal of the Japanese Society for Horticultural Science* 68: 919 – 926.

Simon, E. W., and L. K. Mills. 1983. In: Nozzoliilo, C., P. J. Lea, and F. A. Loewus, (Eds) Mobilization of Reserves during Germination. Plenum press, New York, 9 - 27.

Speath, S. C. 1989. Extrusion of protoplasm and protein bodies through pores in cell walls of pea, bean and faba bean cotyledons during imbibitions. *Crop Science* 29: 452 – 459.

Sperotto C. C. I., N. L. Menezes and L. Storck. 1999. Performance of bean seeds and plants as affected by osmotic conditioning and zinc application. *Ciencia Rural* 29: 253 - 257.

Splittstoesser, W. E. 1990. Vegetable Growing Handbook: Organic and Traditional Methods. Third edn. Van Nonstrand Reinhold publishers. New York, 198 - 204.

Strydom, E. 1971. The production of green beans. Department of Agriculture Technical Services (Leaflet). South Africa, 1- 9.

Tobias, R. B., W. S. Conway, C. E. Sams, K. C. Gross and B. D. Whitaker. Cell wall composition of calcium treated apples inoculated with Botrytis cinerea. Phytochemistry 32: 35 – 39.

Van Schoonhoven, A., and O. Voysest. 1990. Common beans: Research for crop improvement. Wallingford, Oxon Common Wealth Agricultural Bureaux, 199 - 286.

Vilas- Boas E., A. B. Chitarra and J.B Menezes. 1998. Modification of cell wall components in orange flesh melons subjected to post harvest calcium treatment. *Brazillian archives of Biology and Technology* 41: 467 – 473.

Zhang J., and J. Guan. 1998. Effect of calcium infiltration on quality, physical-biochemistry and ultrastructure of " Starkrimson" apples. *Acta Agriculturae Boreali-Sinica* 13: 76 – 80.

CHAPTER 2

THE INFLUENCE OF CALCIUM OSMOTICA ON SEED IMBIBITION, SEED CALCIUM CONTENT, SEED GERMINATION AND TRANSVERSE COTYLEDONAL CRACKING PRIOR TO SEEDLING EMERGENCE

2.1 Introduction

Many factors have been implicated in the occurrence of transverse cotyledonal cracking (TVC) in beans. Important factors are those that influence imbibition rate, seed coat permeability and seed moisture content at the start of imbibition (Aqil and Boe, 1975; Dickson and Boettger, 1976; Bradford and Eisinger, 1986). The role of seed calcium content was associated with cell wall integrity (Dickson *et al*, 1973) but its role as an osmoticum has, however, been overlooked in literature.

Imbibition is the movement of water along a diffusion gradient into a body, for example a seed. In order for imbibition to take place there has to be water potential gradient between the surface of adsorbent and the liquid imbibed. The component of the adsorbent should have a certain affinity to the imbibed substance (Devlin and Witham, 1983). Seeds of very negative water potential will absorb water rapidly due to the high water potential gradient between them and the imbibing solution or water. Aside from the negative water potential of a seed, there are other factors that affect the rate of imbibition: Seed physiology (metabolic activities influencing water absorption as a result of enzyme activation during imbibition, including changes in solute concentrations), seed coat characteristics (thickness and chemical composition), soil moisture content (osmotic potential of the medium from which water is absorbed) and the temperature (increase in temperature results in an increase in the rate of imbibition and seed temperature may influence water uptake rate) (Copeland and McDonald, 1995; Custodio and Marcos-Filho, 1997; Devlin and Witham,1983). Seed composition

is one of the factors that play a role in influencing imbibition rate. Cultivars susceptible to TVC were reported to have a low lipid content and imbibition rate (Jones, 1971). Seeds that have a high protein content imbibed water at a higher rate than seeds with greater carbohydrate content (Copeland and McDonald, 1995).

Imbibition rate increases susceptibility of seeds to cracking by causing cell wall rupture as the water rushes into the dry cells (Simon and Mills, 1983). Seed moisture content prior to imbibition also influences the magnitude of the osmotic gradient between the seeds and the solution they are imbibing (Bradford and Eisinger, 1986), therefore it controls water absorption. Because calcium plays an important role in maintaining the cell wall structure and integrity, seeds with cell walls that are deficient in calcium are expected to leak more substances during imbibition. This leakage could reduce seed vigour since some of the leaked substances are essential for seed germination and seedling development (Splittstoesser, 1990). In the soil, leachates attract pathogenic microorganisms leading to increased occurrence of diseases because sugars and other organic compounds serve as substrates for the pathogens, and stimulate their pathogenic activity (Ladror *et al*, 1995). The amount of substances present in the leachate can be determined by measuring the electrical conductivity and water potential of seed steep water. The electrical conductivity test is a measure of seed vigour and determines the amount of electrolytes leaked into the steep water during imbibition. It can be used together with other tests such as the standard germination test to predict seed lot performance in the field (Hamman *et al*, 2001). Ions, amino acids and sugars leak from most seeds when placed in excess water during imbibition, and weaker seeds generally leak more substances (Halmer, 1999). Seeds that lose large amounts of electrolytes perform poorly during germination, emergence and stand establishment.

Many factors contribute to the amount of electrolytes that leak out. These include seed coat permeability, seed size, initial seed moisture content and seed physiological conditions (Vieira *et al*, 2001). Seed coat pore size plays an important role in determining the amount of electrolytes leached out (Modi and McDonald, 1999). Water permeability is greatest at the micropylar area where seed coat is ordinarily quite thin (Copeland and McDonald, 1995), and removal of the seed coat results in profuse leakage of electrolytes indicating the importance of seed coat permeability in seed vigour (Simon and Mills, 1983).

Seed age has been found to contribute to the amount of electrolytes leached. Meintis and Smith (2003) observed that kenafu seeds that have been stored for about four years leached 38–50% more electrolytes than freshly harvested seeds. Electrolytes leakage is also dependent on the genetic make-up of the crop. Andreoli and Andrande (2002) found that maize seeds carrying the gene bt_2 leached more electrolytes than the sugary type. Imbibition is also dependent on the seed size since small seeded cultivars absorb greater volumes of water than big seeded cultivars because of their bigger surface area to volume ratio (Copeland and McDonald, 1995).

The amount of substances that leaches into steep water during imbibition may be used as an indication of the cell wall integrity of seeds. Seeds that imbibe water rapidly are prone to cell wall rapture leading to loss of more electrolytes (Simon and Mills, 1983; Custodio and Marcos-Filho, 1997). Besides the amount of substances leached, the nature of substances leached also play an important role in seed and seedling vigour.

In this study the six green bean cultivars were examined for their transverse cotyledonal cracking prior to field planting. Seeds were imbibed in three calcium salt solutions and water absorption rate, cotyledonal crack appearance during imbibition and retention of Ca^{2+} by seed parts after imbibition were determined and correlated with transverse cotyledonal cracking.

2.2 Materials and Methods

2.2.1 Green bean cultivars

Six green bean cultivars were obtained from a seed company (Pro-seed cc) in Pietermaritzburg, KwaZulu-Natal, South Africa. The cultivars are commercially available cultivars whose susceptibility to transverse cotyledonal cracking was unknown. Seeds produced in the 2000 and 2001 seasons were donated for this study in 2001 and 2002, respectively. The cultivars that were used, and their seed sizes (1000 grain mass) were Imbali (150 g) Tokai (200 g), Elangeni (310 g), Outeniqua (350 g), Tongati (290 g) and Sodwana (380 g). Randomly selected individual seeds representing each cultivar are shown in Figure 2.1.

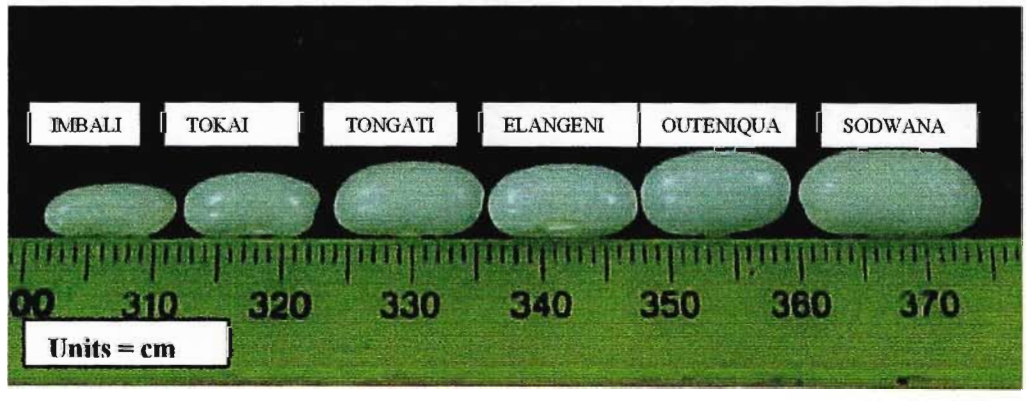


Figure 2.1 Representative seeds depicting cultivar differences in seed size.

2.2.2 Calcium osmotica and seed water potential

Three calcium salts, $CaCl_2$, $Ca(NO_3)_2$, and $CaSO_4$ were used to prepare five molarities each (1,10,50,100 and 1000 mM per salt) in distilled water. Salt electrical conductivity was determined using the YSI 3200 Conductivity meter. The osmotic potential of each solution was determined at approximately 25°C using the WP4 Dew Point Potentiometer (Decagon, Washington). Seed water potential during imbibition in calcium osmotica was also determined using the WP4 Dew Point Potentiometer. Distilled water was used as a control in all experiments.

2.2.3 Seed moisture content conditioning prior to imbibition

Before imbibition studies in calcium salts, seeds were stored overnight (~16 h) over a saturated solution of $Ca(NO_3)_2$ at 50% RH (23°C), and seeds equilibrated to a moisture content of about 10% (fresh mass basis). The apparatus used for seed moisture content conditioning is shown in Figure 2.2.

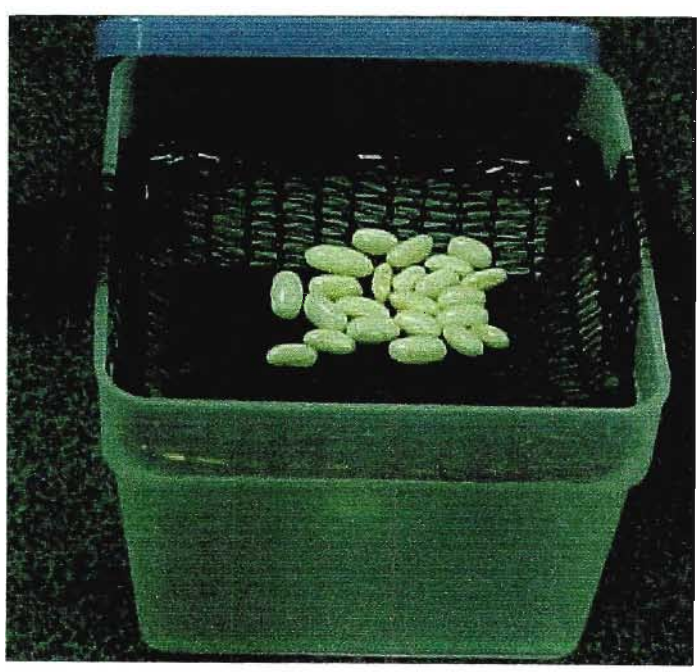


Figure 2.2. Seed conditioning apparatus. Opened plastic container containing saturated solution of $Ca(NO_3)_2$ which at 23°C creates a relative humidity of 50% and maintains seed moisture content (suspended on a nylon gauze) at 10% (fresh mass basis).

2.2.4 Determination of water absorption rate and crack appearance rate during imbibition

To determine the effect of calcium salts and salt concentrations on water absorption rate (WAR) and crack appearance rate (CAR) during imbibition, seeds of three cultivars Imbali, Outeniqua and Sodwana [representing the small, medium and large seeded cultivars respectively (Figure 2.1)] were used following conditioning. Seeds were imbibed in filter papers moistened (5 ml for 4 seeds; replicated four times) with each salt concentration. The experiment was divided into two treatments (seeds with seed coats and seeds without seed coats). Seed mass was recorded for each individual seed before imbibition and at 1h intervals for 6h of imbibition. Seeds without seed coats, with embryonic axes intact, were used to determine CAR by recording the number of cracks appearing after every hour of imbibition, and calculating the slope of crack appearance during the course of imbibition. The slope of the change in seed mass due to absorption of water during imbibition was calculated to determine WAR for each cultivar and salt concentration.

2.2.5 Seed osmopriming

Seeds at 10% (fresh mass) moisture content were imbibed in each salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) solution (1 ml $seed^{-1}$) at different molarities (1,10,50,100 and 1000 mM) for six hours to prevent radicle protrusion. Distilled water was used as a control. Seeds were then blotted dry and allowed to equilibrate to ~10% moisture content at 50% RH as explained previously (Figure 2.2).

2.2.6. Seed calcium content determination

To determine the amount of seed Ca^{2+} prior to germination, dry primed (section 2.2.5.1) seeds were separated into seed parts (coat, cotyledons and embryonic axis) (Figure 2.3).

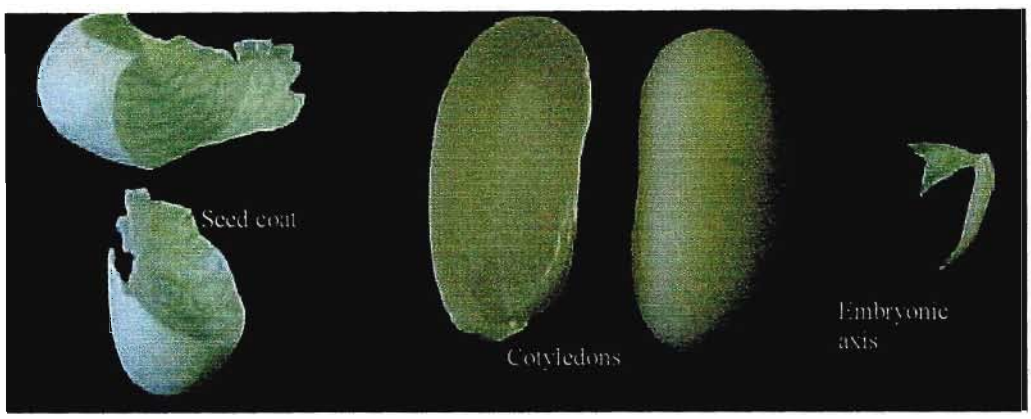


Figure 2.3 Seed parts which were analyzed for calcium content after 6 h of whole seed priming in different calcium salt molarities.

Each tissue (seed part) was ground to pass through a 0.5 mm sieve before 0.5 g was digested in 18 mL of acid mix of nitric: perchloric (4:1 v/v). The digested sample was diluted in distilled water (1:50 to 200 depending on calcium concentration), and 0.2 mL of the diluted sample was mixed with 2.8 mL strontium perchloric acid before Ca^{2+} was determined using the Unicham Solar M6.

2.2.7 Seed germination

Seeds that had been primed (section 2.6) and dried to approximately 10% moisture content (section 2.2.3) were germinated at 25°C using the petri dish method according to the rules for testing seeds (AOSA, 1993). Germination percentage was determined by display of radicle protrusion.

2.2.8 Statistical analysis

Raw data were analysed using generalised statistical analysis (GenStat, Release 6.1, Rothamsted Experimental Station, UK) to produce analysis of variance (ANOVA). Means were presented either using graphs or tables (for high order interactions), and the differences between means were determined using the LSD (least significant difference) or standard error of the mean (where laboratory data were few to justify ANOVA). ANOVA tables are presented in Appendix 3.

2.3 Results

2.3.1 The effect of Ca^{2+} osmolarity on water uptake and occurrence of cotyledonal cracking

2.3.1.1 General evaluation of imbibition by six cultivars in water to relate seed size to imbibition

Since the six cultivars used in this study were genotypically characterised by a wide range of seed sizes (Figure 2.1), it was necessary to evaluate whether they also differed in imbibition rates. Subsequently a fewer cultivars were used to undertake a detailed cultivar response to calcium salt molarities. When all six cultivars were imbibed in water for 4 h, and change in seed mass (water absorption) was determined at one hour intervals, there was a significant difference ($P = 0.01$) between cultivars (Figure 2.4). Sodwana, Outeniqua, Tongati and Elangeni showed similar imbibition levels with respect to the rate at which they imbibed water, and they were higher than those of Tokai and Imbali rate. Imbali displayed the lowest total imbibition. However, Imbali and Tokai also displayed uniform rates of water uptake throughout the imbibition period. Cultivars (large) that imbibed more water than Imbali and Tokai (small) seemed to have a high initial water uptake, which slowed after about 2 h of imbibition (Figure 2.4). Three cultivars (Sodwana, Outeniqua (both large) and Imbali (small) were selected for further examination of water absorption and crack appearance as

influenced by calcium osmotica. There was no clear middle group with respect to imbibition course, but Sodwana and Outeniqua were expected to behave slightly differently in osmotic solutions so that a third category of imbibition could be created.

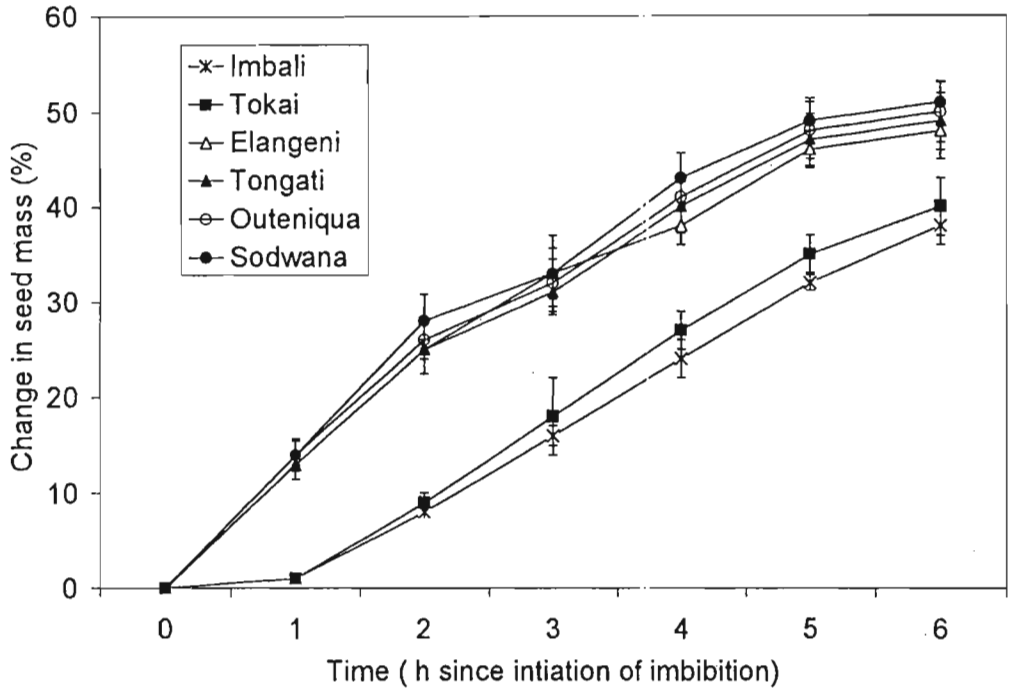


Figure 2.4. Imbibition of six green bean (*Phaseolus vulgaris* L.) cultivars at room temperature.

2.3.1.2 Influence of calcium osmotica on seed water potential during priming

Determination of the chemical factors influencing water movement in liquid systems, electrical conductivity and water potential, showed that there were significant differences between salts and salt molarities, with respect to both properties (Table 2.1 and 2.2). However, $CaCl_2$ and $Ca(NO_3)_2$ did not differ significantly with respect to water potential (Table 2.1)

Table 2.1 Water potential of salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) solutions used for water absorption rate and crack appearance rate studies.

Molarity (mM)	Ca-chloride	Ca-nitrate	Ca-sulphate
1	-0.0074	-0.0074	-0.0074
10	-0.0074	-0.0074	-0.0496
50	-0.37	-0.37	-0.248
100	-0.74	-0.74	-0.496
100	-7.4	-7.4	-4.96

Table 2.2 Conductivity of salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) solutions used for water absorption rate and crack appearance rate studies.

Molarity (mM)	Ca-chloride	Ca-nitrate	Ca-sulphate
1	0.27	0.21	0.09
10	1.95	1.32	0.45
50	6.96	6.28	1.28
100	15.19	14.44	1.74
100	74.5	60.75	1.91

When seeds of Imbali, Outeniqua and Sodwana were examined for changes in water potential during priming (6h of imbibition) in the different calcium salts, no significant difference between control (distilled water) and 1 mM salt molarity was found (data not shown). There were significant differences between cultivars ($P = 0.04$) and salts ($P = 0.01$) (Figures 2.5A to 2.5D). Cultivar Imbali consistently possessed higher water potential than Outeniqua and Sodwana at all molarities. There were no significant differences between $CaCl_2$ and $Ca(NO_3)_2$ in terms of their effects on seed water potential, but $CaSO_4$ imbibition caused a higher seed water potential at all molarities and in all cultivars (Figures 2.5A –D).

2.3.1.3 Determination of water absorption and crack appearance rates during seed priming in calcium osmotica

Water absorption rate (WAR) was significantly ($P < 0.001$) enhanced by the removal of seed coats across all cultivars and salt molarities (Figures 2.6). The significant difference ($P = 0.05$) between cultivars was due to water absorption rates for Imbali in distilled water being greater (without a seed coat) than that for Outeniqua and Sodwana, which were not significantly different. In the presence of seed coats, WAR data concurred with imbibition data shown in Figure 2.4. Removal of the seed coat, however, caused Imbali to absorb more water than Outeniqua and Sodwana. There were significant differences ($P = 0.01$) between salts with respect to WAR, because $CaSO_4$ resulted in ~ 15% more WAR values than $CaCl_2$ and $Ca(NO_3)_2$, which were not significantly different from one another. The pattern of WAR was, however, similar across salts. Data presented in Figure 2.6 represent mean responses to all three salts for each cultivar. It is important to note that although there was a significant difference ($P < 0.01$) between the control (0 mM) and salt molarities, the effect of salt molarity was slow, in respect of WAR response to increasing concentrations. For Imbali, there was no decrease in WAR below 50 mM molarity (in the presence and absence of the seed coat), and for Outeniqua and Sodwana a significant decrease in the rate of water absorption occurred at 100 mM and 1000 mM (Figures 2.6).

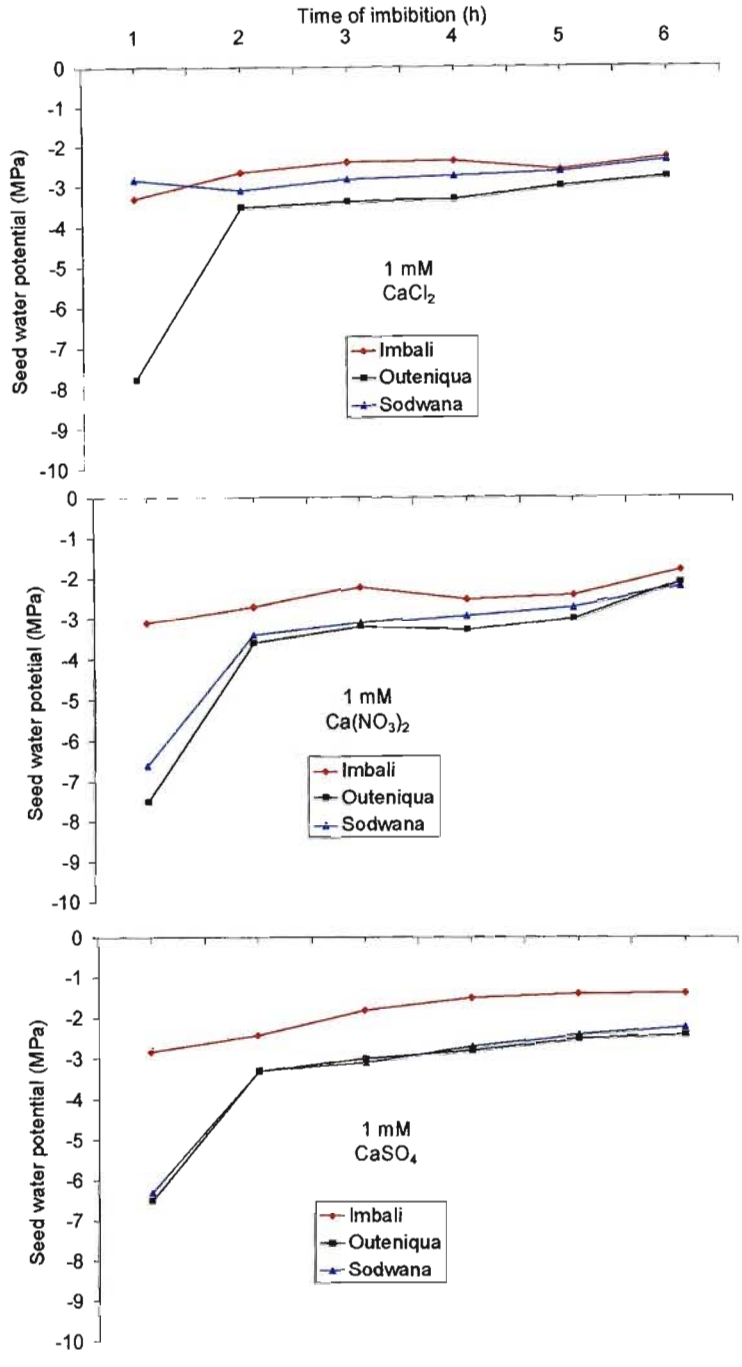


Figure 2.5 A. Changes in seed water potential of three cultivars (Imbali, Outeniqua and Sodwana) during imbibition in 1mM salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) concentration. Data points are means of three replications (± S.E). Note: error bars are covered by data points.

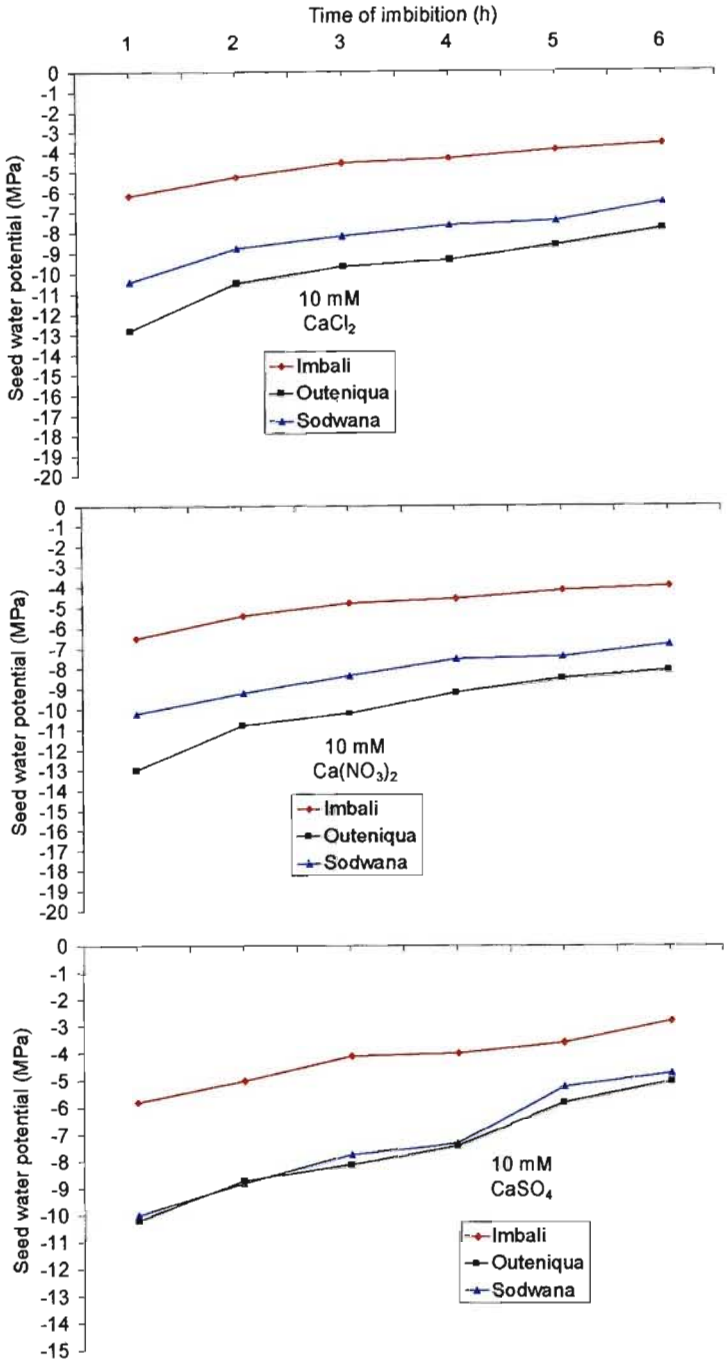


Figure 2.5 B. Changes in seed water potential of three cultivars (Imbali, Outeniqua and Sodwana) during imbibition in 10 mM salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) concentration. Data points are means of three replications (± S.E). Note: error bars are covered by data points.

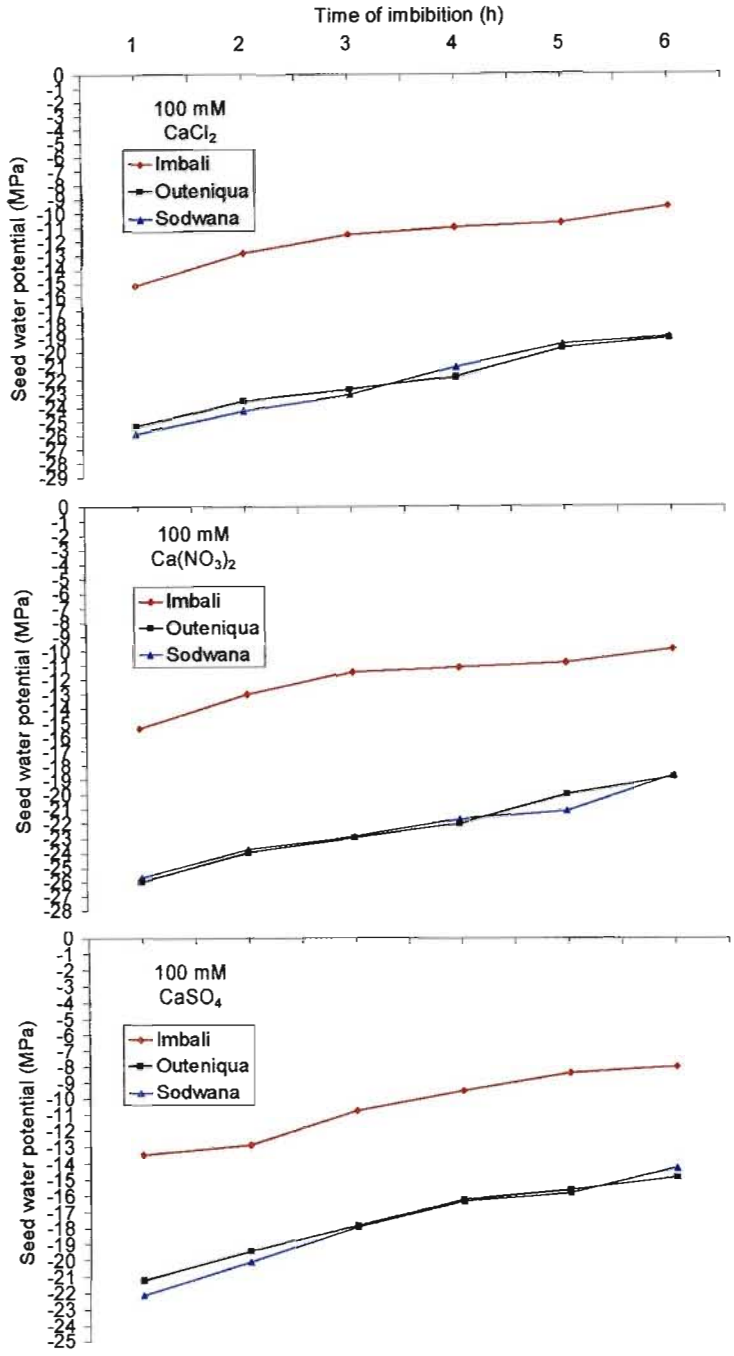


Figure 2.5 C. Changes in seed water potential of three cultivars (Imbali, Outeniqua and Sodwana) during imbibition in 100 mM salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) concentration. Data points are means of three replications (± S.E). Note: error bars are covered by data points.

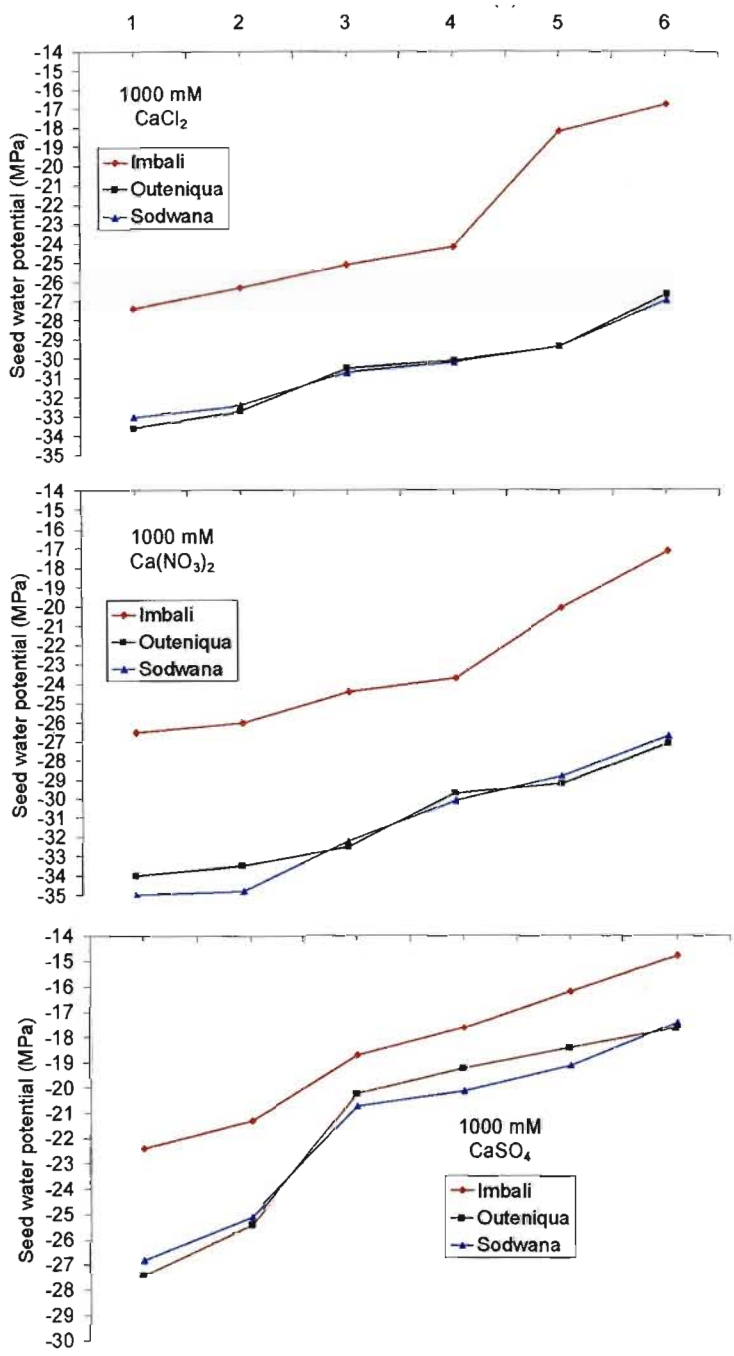


Figure 2.5 D. Changes in seed water potential of three cultivars (Imbali, Outeniqua and Sodwana) during imbibition in 1000 mM salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) concentration. Data points are means of three replications (± S.E). Note: error bars are covered by data points.

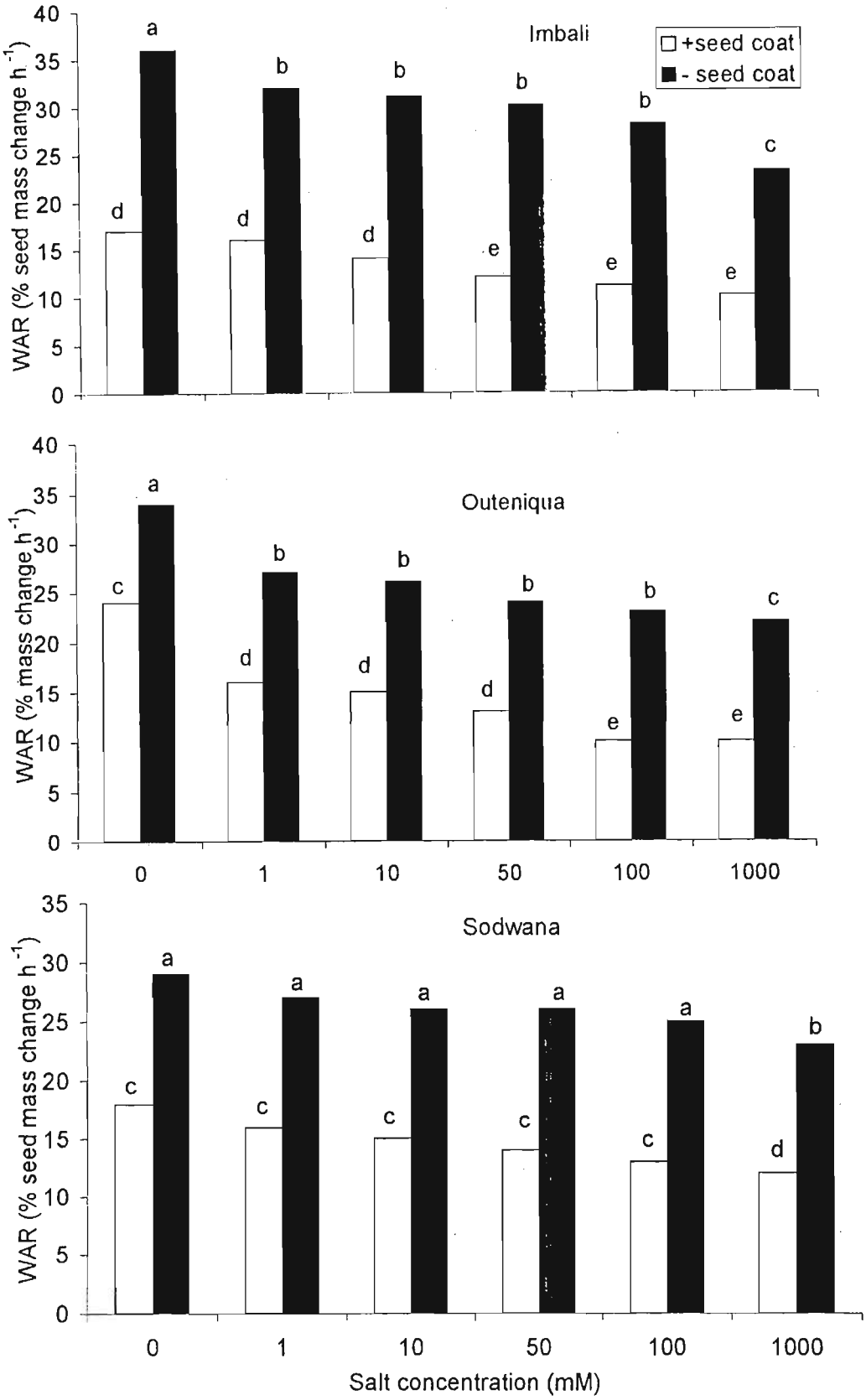


Figure 2.6. Water absorption rate (WAR) for Imbali, Outeniqua and Sodwana cultivars, expressed as a slope of the change in seed mass due to water absorption over a period of six hours during priming in different molarities of calcium salts. Data points are means of responses to $CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$ during a 6 h imbibition. Values sharing the same letter are not significantly different (P = 0.05). Note: + and – denote the presence and absence of seed coat, respectively.

Crack appearance rate (CAR), which was only examined in seeds without seed coats, showed significant differences between cultivars ($P < 0.01$) and salt molarities ($P < 0.01$) (Figure 2.7). Resistance to cotyledonal cracking increased as salt molarity increased (Figure 2.7), however, there was also a significant interaction between cultivars and molarities. Whereas Imbali showed cracks in the absence of salt osmotica only, Sodwana did not show a continuing decline in CAR above 50 mM, and for Outeniqua CAR decreased in response to increasing molarity (Figure 2.7). The mean number of cracks, which occurred in each cultivar during the duration (6 h) of imbibition across all salts and molarities is presented in Figure 2.8. Data showed that Imbali was more resistant to cracking than Outeniqua and Sodwana (Figures 2.7 and 2.8). There was no significant difference between the presence and absence of seed coat, with respect to cotyledonal cracking in Imbali (Figure 2.8). However this was not true for Sodwana and Outeniqua where removal of the seed coats caused a significant increase in the number of cracks observed (Figure 2.8).

The progressive occurrence of cracks on the seeds was examined. Visual inspection of the seeds without a seed coat revealed that during imbibition cracks begin as minor fissures, which enlarge as the seed imbibes water (Figure 2.9).

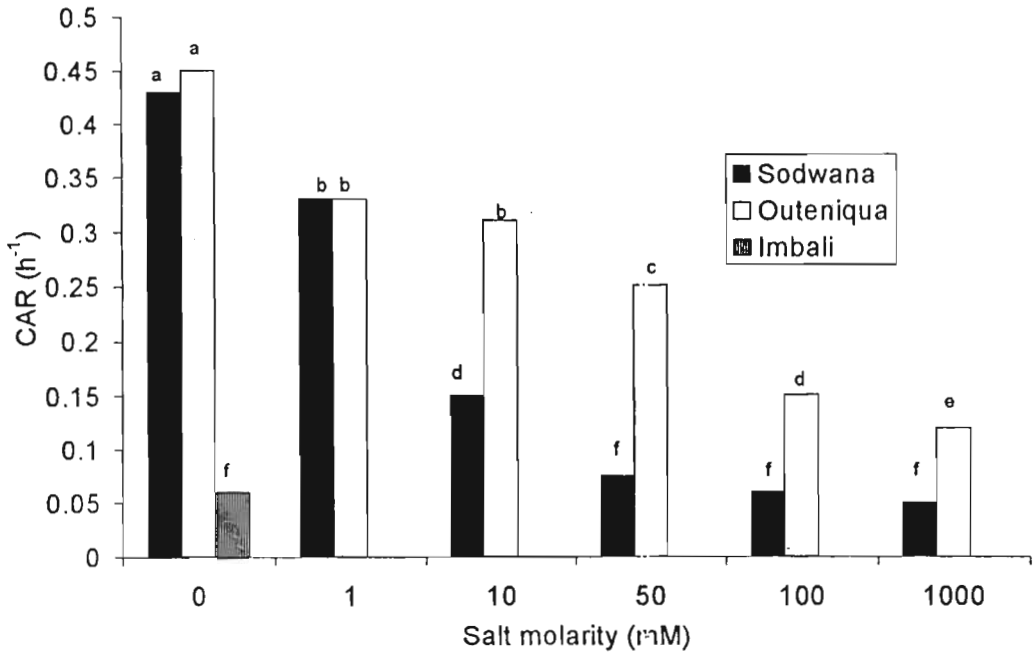


Figure 2.7. Crack appearance rate (CAR) for three cultivars (Imbali, Sodwana and Outeniqua). Data represent means in response to three calcium salts ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) during a 6-h priming period. Means sharing the same letter are not significantly different (P = 0.05).

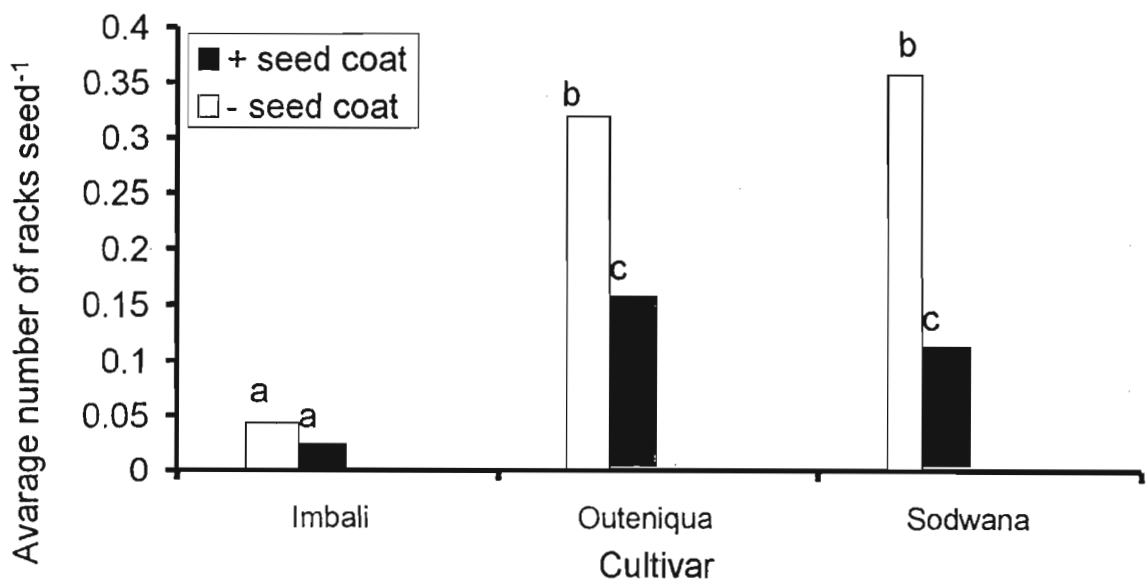


Figure 2.8. Mean number of cracks found in three cultivars at the end of a 6-h imbibition period in different molarities (0, 1, 10, 50, 100 and 1000) of calcium salts ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$). Means sharing the same letter are not significantly different (P = 0.05). Note: + and – denote the presence and absence of seed coat, respectively.

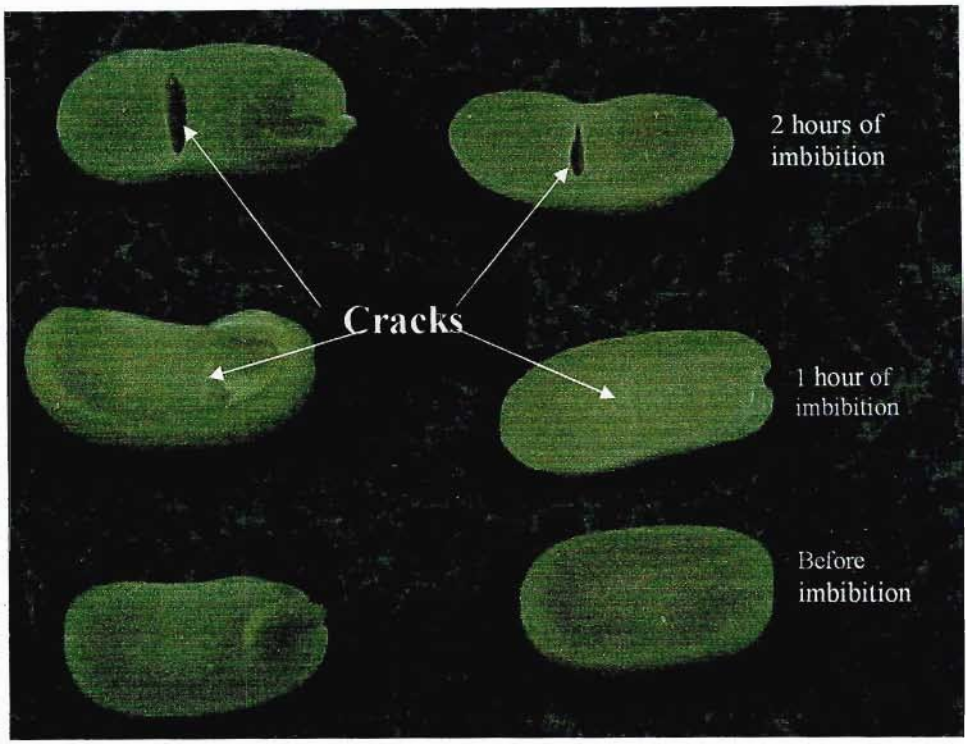


Figure 2.9. Progressive appearance of cracks during imbibition of a seed without seed coat, cultivar (Outeniqua) in distilled water.

2.3.2 Germination tests

Germination tests showed a significant ($P < 0.001$) difference between cultivars, with Imbali and Elangeni having the highest germination percentage. Outeniqua, Sodwana and Tongati were not significantly different from each other. Tokai had the lowest germination percentage (Figure 2.10). A decrease in the germination percentage was observed with an increase in salt concentration (Figure 2.11). No significant difference was observed between the low salt concentrations (1 and 10 mM) and control seeds. However 50 and 100 mM were significantly ($P = 0.01$) different from these three treatments and 1000 mM had the lowest germination percentage. Salts showed a significant ($P < 0.001$) difference, with $CaSO_4$ having the highest germination percentage (Figure 2.12).

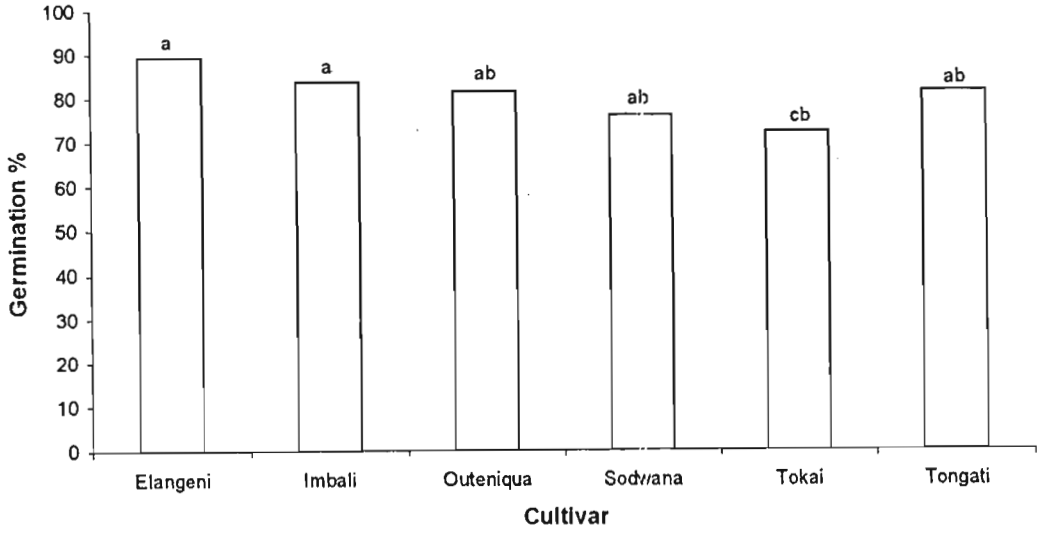


Figure 2.10. Germination of green bean (*Phaseolus vulgaris* L.) cultivars after six hours of seed priming in calcium salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) solutions. Data are means in response to 0, 1, 10, 50 and 1000 mM calcium salts. Means sharing the same letters are not significantly different (P = 0.05).

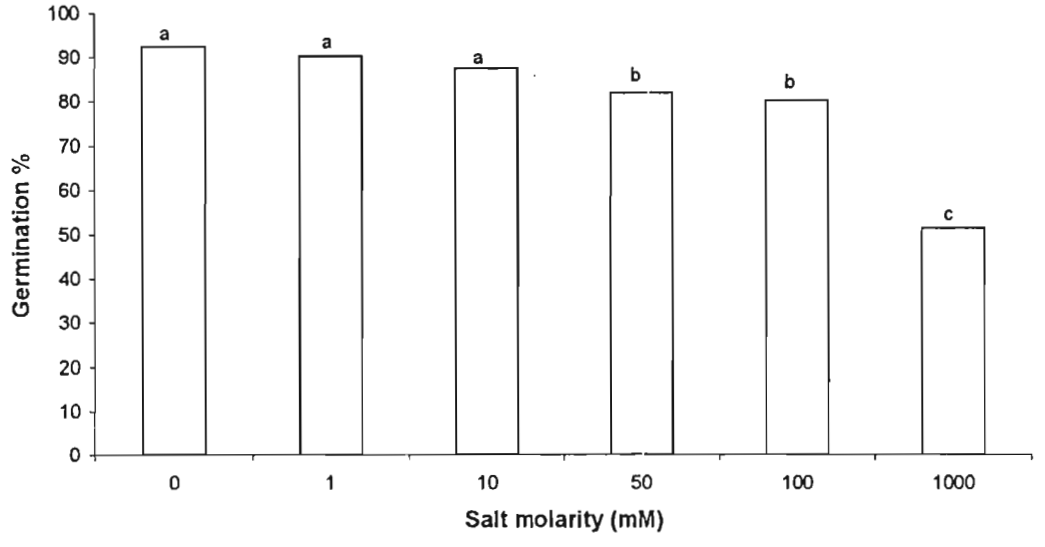


Figure 2.11. Germination of green bean (*Phaseolus vulgaris* L.) cultivars in response to six hours of imbibition in different calcium molarities. Data represent means of three calcium salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) solutions six cultivars (Imbali, Sodwana, Outeniqua, Elangeni, Tokai and Tongati). Means sharing the same letters are not significantly different (P = 0.05).

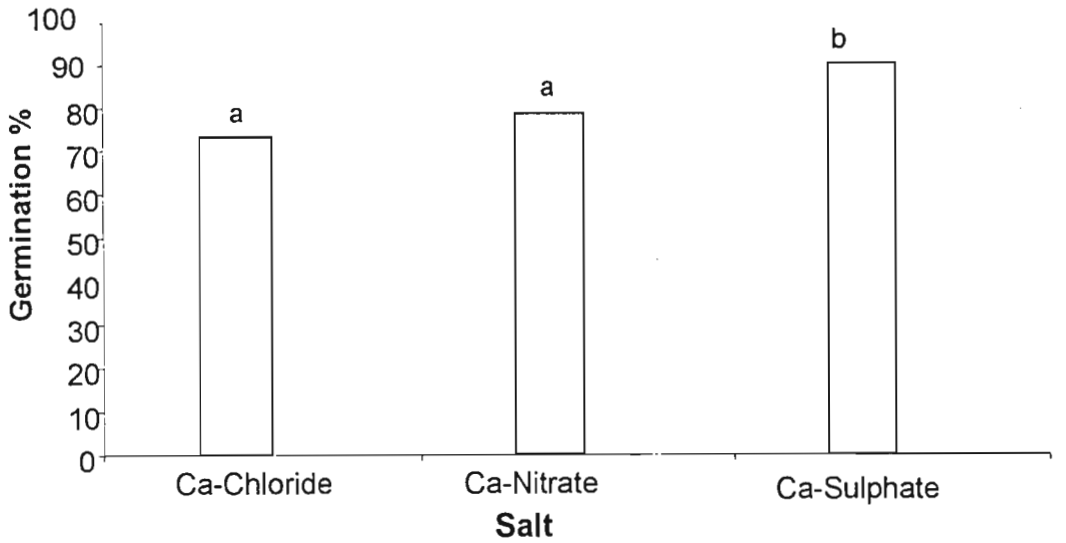


Figure 2.12. Green bean (*Phaseolus vulgaris* L..) seed germination in response to imbibition in calcium salts for six hours. Data represent means of six cultivars (Imbali, Sodwana, Outeniqua, Elangeni, Tokai and Tongati) germinated in 6 molarity levels (see Figure 2.12). Means sharing the same letter are not significantly different (P = 0.05).

2.3.3 Seed calcium content

Calcium analysis using Atomic Absorption spectrophotometer (AA) showed that cultivars absorb different ($P < 0.001$) amounts of calcium after 6 hours of imbibition in different calcium salts. Imbali and Elangeni contained the highest amounts of calcium and Tongati had the lowest (Figure 2.13). Increasing salt concentration resulted in an increase in the amount of calcium taken up by the seeds (Figure 2.14) and a significant ($P < 0.001$) difference was observed among the three salts used. Seeds absorbed the least amount of calcium from $CaSO_4$ whereas there was no significant difference between seeds imbibed in $Ca(NO_3)_2$ and $CaCl_2$ (Figure 2.15). There was also a significant ($P < 0.001$) difference between seed parts with regards to the amount of calcium absorbed, with the least amount found in cotyledons and the highest amount in the seed coat (Figure 2.16). The significant differences among cultivars ($P < 0.001$) and salt molarities ($P < 0.001$) are presented in APPENDIX 3.2.

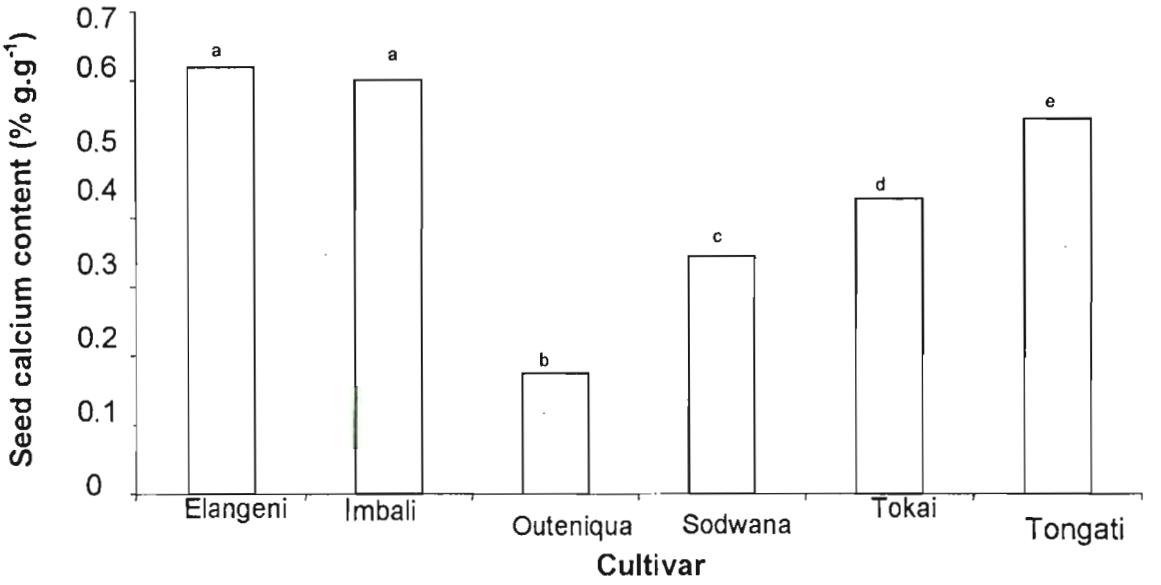


Figure 2.13 Amount of calcium absorbed by cultivars after 6 hours of imbibition in different calcium salt solutions. Means sharing the same letters are not significantly different (P = 0.05).

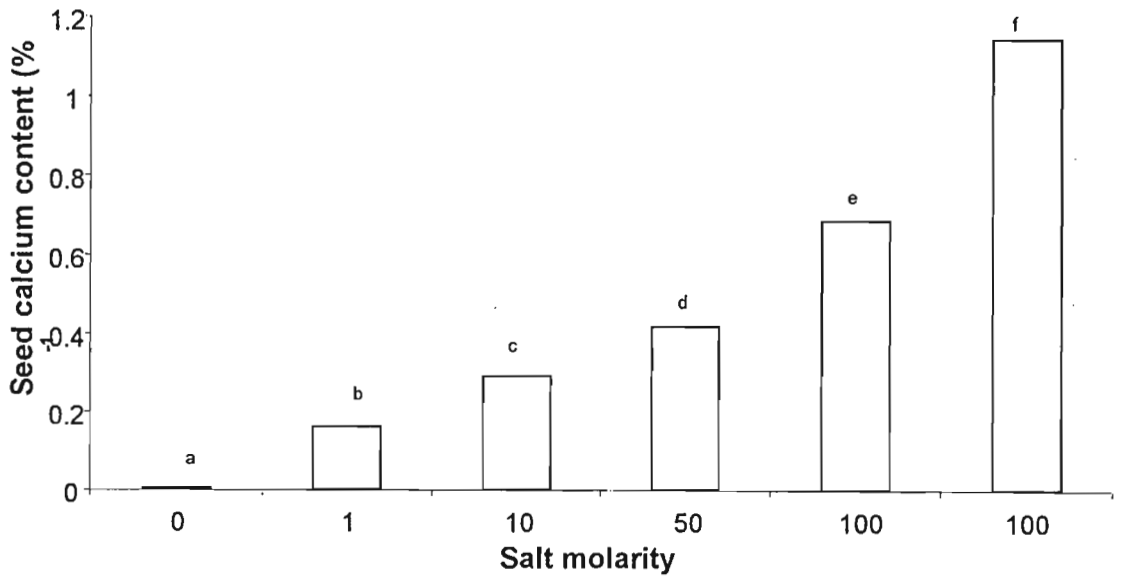


Figure 2.14. Amount of calcium in seeds after imbibition in different calcium osmolarities for six hours. Data represent means of six cultivars (Imbali, Sodwana, Outeniqua, Elangeni, Tokai and Tongati). Means sharing the same letters are not significantly different (P = 0.05).

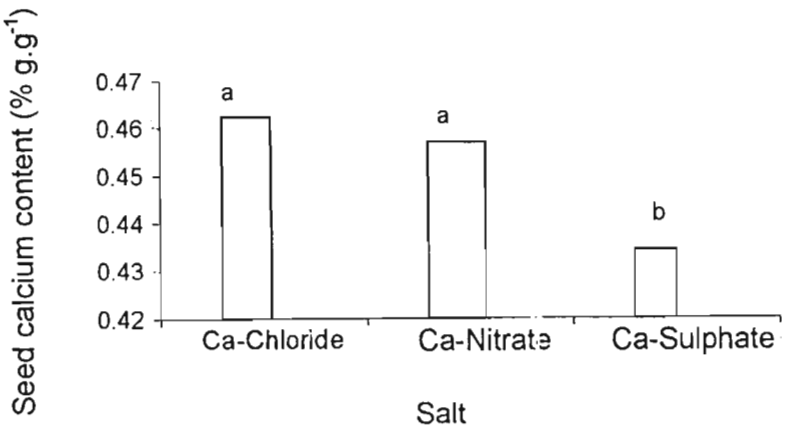


Figure 2.15. Effect of calcium salt used on the amount of calcium taken up by seeds after 6 hours of imbibition. Means sharing the same letters are not significantly different (P = 0.05).

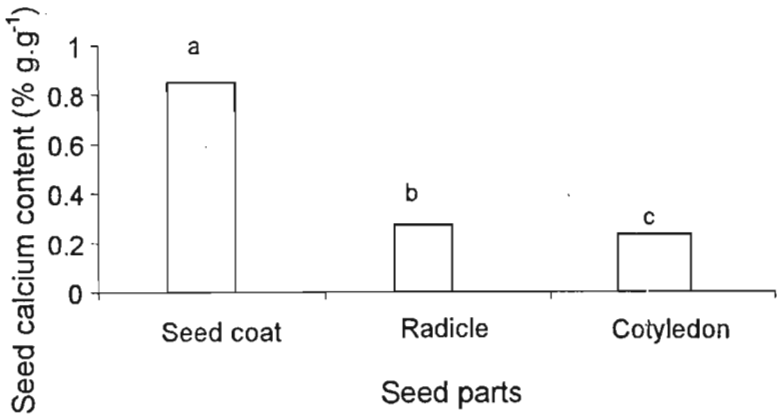


Figure 2.16. Amount of calcium taken up by different parts of the seeds during 6 hours of imbibition in different calcium salt solutions. Means sharing the same letters are not significantly different (P = 0.05).

2.4 Discussion

There was a positive correlation (r^2 = 0.86) between seed size and water absorption rate. The higher water absorption rate shown by the large seeded cultivars (Sodwana, Outeniqua, Elangeni and Tongati) compared to small seeded cultivars (Imbali and

Tokai) during priming in water and calcium osmotica is a confirmation of this correlation (Figures 2.1 and 2.6). Water uptake by large seeds also tends to be fast at the early stages of imbibition, and it tapers off in about 2 h. In smaller seeds water absorption occurs at a uniform linear rate for a longer period of imbibition (Figure 2.1). Rapid inrush of water into the seeds at the early stages of imbibition may have detrimental effects on seed quality. In this study seeds that absorbed water rapidly early in imbibition and shortly slowed down also exhibited more transverse cotyledonal cracking than those that showed a uniform water absorption rate over a longer period (Figures 2.7 and 2.8). Rapid inrush of water that is followed by a slowed water absorption rate may cause cracking due to the outer wetter cotyledonal area stretching faster than the inner drier part. Since the dry part has less extensibility than the wet region, the inner portion breaks (Figure 2.9). Hence, transverse cotyledonal cracking (TVC) occurs within an hour to 2 h of imbibition in a high water potential medium in susceptible green bean cultivars. Cell wall rapture (TVC) is decreased by seed priming in calcium osmotica. The response to osmoticum is more rapid in smaller, resistant cultivars compared with large, susceptible cultivars (Figures 2.7 and 2.8). Presumably osmotica control TVC by regulating water absorption rate in seeds. This was also evidenced by the linear decline in seed water potential with increasing osmotic potential of priming solution (Figure 2.5). High osmolarity reduces the water potential difference between a dry seed and the priming solution, which causes water absorption rate to be slower and more uniform. That way seed tissue wetting occurs slowly and uniformly and there is less stretching caused by within tissue water potential differential.

The role of the seed coat in regulation of water absorption rate and, consequently, crack appearance rate, was clearly demonstrated by the significant increase of TVC in the absence of seed coats compared to when the seed coat was present. Small grain legume seeds generally have thicker seed coots than larger seeds. Perhaps the resistance of small-seeded green bean cultivars observed in this study was associated with seed coat regulation of WAR and CAR.

A secondary effect of priming with salt osmotica is the accumulation of mineral elements in seed tissues. In this study it was shown that seed part Ca^{2+} levels can be increased by priming with Ca^{2+} salts (Figure 2.14). The effectiveness of salts was also shown to be related to osmotic potential since $CaSO_4$ caused less seed part Ca^{2+} content after 6 h of priming than $CaCl_2$ and $Ca(NO_3)_2$ (Figure 2.15). The differences shown by seed parts, with respect to Ca^{2+} content confirmed the water absorption route that was explained for soybeans (Copeland and McDonald, 1995). These authors reported that the micropylar region of soybean seeds has a thinner seed coat and allows more water uptake. This could be the reason why there was more Ca^{2+} content in the embryonic axes than in the cotyledons (Figure 2.16). Cotyledons form the largest part of the seed, the seed coat forms the smallest portion (Modi *et al*, 2002). Hence salt diffusion and accumulation through the cotyledon is expected to be much slower than through the embryonic axis and the seed coat. The amount of Ca^{2+} may have influenced cell wall integrity. However, this was not expected to occur in the 6 h of imbibition. Its evidence was indirectly determined in the cotyledons of emerged seedlings, under field conditions (Chapter 3) and in the first generation seeds (Chapter 4).

Judging by the decrease of seed germination in response to high calcium osmolarities (Figure 2.11), it is likely that Ca^{2+}- salts had some toxic effects on seeds. The toxicity may have been more significant in the embryonic axis than in the cotyledons. Note that there was more germination of seeds primed with $CaSO_4$, which had the lowest osmotic potential and electrical conductivity (Tables 2.1 and 2.2). Moreover, $CaSO_4$ was absorbed more than $CaCl_2$ and $Ca(NO_3)_2$. Small-seeded cultivars, which absorbed less water per unit time, in the presence of a seed coat, also germinated better than large-seeded cultivars, which were characterized by early water inrush and more total water uptake after 6 h of imbibition. Clearly, these findings show that the beneficial effects of Ca^{2+} in water uptake regulation were related more to osmotic potential than to the mineral element *per se*. At high salt molarity the osmotic potential of a solution causes a delay in the imbibition rate of water and this in turn causes a delay in the initiation of metabolic activities required for germination (e.g. enzyme activation which leads to metabolite break down) processes. This argument could be advanced in explanation of lower germination where more osmolarity was concerned.

In conclusion, this study showed that water absorption rate has a major influence on the susceptibility of green bean seeds to transverse cotyledonal cracking. The influence water absorption rate correlates positively with seed size, but seed size may not be a reliable determinant of TVC susceptibility where large seeded cultivars are concerned. Priming seeds in calcium salts reduces TVC by regulating water absorption rate. However, high (> 10 to 50 mM) calcium concentrations decrease seed germination probably due to a low osmotic potential and (or) toxicity.

References

Andreoli, C and R. V. Andrade. 2002. Integrating matriconditioning with chemical and biological seed treatments to improve vegetable crop stand establishment and yield under tropical conditions. *Seed Technology* 24: 89 - 99.

Association of Official Seed Analysts. 1993. *Journal of Seed Technology* 16: 3.

Aqil, B. A. and A.A. Boe. 1975. Occurrence of cotyledonal cracking in snap beans and its relation to nutritional status in the seed. *HortScience* 10: 509 – 510.

Bradford, K. J. and B. A. Eisinger. 1986. Role of seed mineral in the occurrence of transverse cotyledonal cracking of snap bean. *Journal of the American Society of Horticultural Science* 111: 110 – 114.

Copeland L. O. and M. B. McDonald. 1995. Principles of seed science and technology 3rd edn. Chapman and Hall 258 - 276.

Custodio, C.C. and J. Marcos-Filho. 1997. Potassium leachate test for evaluation of soybean seed physiological quality. *Seed Science and Technology* 25: 549 - 564.

Devlin, R. M. and F. H. Witham. 1983. Plant physiology 4th edn. Willard Grant Publishers, Boston. 25 – 52.

Dickson, M. H. and M. A. Boettger, 1976. Factors associated with resistance to mechanical damage in snap beans (*Phaseolus vulgaris* L.). *Journal of the American Society of Horticultural Science* 101: 541 – 544.

Dickson, M. H., K. Duczmal and S. Shannon. 1973. Imbibition rate and seed composition as factors affecting transverse cotyledonal cracking in bean (*Phaseolus vulgaris* L.) seeds. *Journal of American Society of Horticultural Science* 73: 509 – 513.

Halmer P. 1999. In: Black, M. and J. D. Bewley (Eds). Seed Technology and its Biological Basis, Sheffield Academic press, USA, 257 - 283.

Hamman, B., H. Halmajan and D.B. Egli. 2001. Single seed conductivity and seedling emergence in soybean. *Seed Science and Technology* 29: 575 - 586.

Jones, T. L. 1971. Injury to beans (*Phaseolus vulgaris* L.) in relation to imbibition. PhD Thesis, Agriculture, Plant Culture, University of Illions.

Ladror, U., M. J. Silbernagel and R. L. Dyck. 1995. Cold-wet imbibition injury in beans: Oxygen and temperature effect on germination of two bean genotypes at two seed moisture level. PhD Thesis, University of Saskatchewan, Saskatoon.

Meintis, P. D and C.A. Smith. 2003. Kenaf seed duration in germination, emergence and yield. *Industrial Crops and Products* 17: 9 - 14.

Modi, A.T. and M.B. McDonald. (1999). Differe tial leakage of substances from two soybean genotypes is influenced by seed coat pore characteristics. *Acta Horticulturae* 504: 161 - 176.

Modi, A.T., M.B. McDonald, and J.G. Streeter. 2002. Water status influences common events of soluble carbohydrate accumulation during soybean seed development and germination. *Canadian Journal of Botany* 80: 26 - 270.

Simon, E. W., and L. K. Mills. 1983.In: Nozzolillo, C., P. J. Lea, and F. A. Loewus (Eds). Mobilization of Reserves during Germination, Plenum press, New York, 9 - 27.

Splittstoesser, W. E. 1990. Vegetable Growing Handbook: Organic and Traditional Methods. Third edn. Van Nonstrand Reinhold . New York, 198 - 204.

Vieira, R. D., D. M. Tekrony, D. B. Egli and M. Rucker. 2001. Electrical conductivity of soybean seeds after storage in several environments. *Seed Science and Technology* 29: 599 - 608.

CHAPTER 3

EFFECT OF SEED COATING AND PRIMING WITH CALCIUM SALTS ON SEEDLING EMERGENCE, TRANSVERSE COTYLEDONAL CRACKING, STAND ESTABLISHMENT AND SEED YIELD UNDER FIELD CONDITIONS

3.1 Introduction

Crop yield is determined by many factors including environmental conditions, seed physiological conditions prior and during germination, pests and diseases. Seeds of high vigour tend to increase crop yield. That is why vigour and germination tests are essential to determine or predict the performance of a seed lot under field conditions in terms of emergence. Seed enhancement treatments may be used to improve seed performance and yield of seeds with a physiological problem such as transverse cotyledonal cracking (TVC) in leguminous crops. Osmoconditioning, using inert substances such as polyethylene glycol (PEG), can reduce the imbibition injury by regulating water uptake. This in turn can reduce the occurrence of TVC in pulses such as green beans. Transverse cotyledonal cracking may have detrimental effects in the production of green beans by impairing seed germination, delaying seedling emergence and reducing yield (Morris, 1971; Aqil and Boe, 1975).

Halopriming or osmopriming (conditioning of seeds using salt solutions) on the other hand have the added advantage of supplying the seeds with minerals or other beneficial substances that might be lacking or insufficient in seeds (Copeland and McDonald, 1995). Different salts may behave differently when absorbed by seeds because of their different characteristics. Guirdice *et al* (1998), found that osmoconditioning soybean seeds using PEG improved seed germination, reduced leaking of electrolytes, promoted vigorous seedling growth and biomass. In another study, soybean seeds imbibed in

water had reduced seed germination, vigour and seedling emergence (Lucca *et al*, 1997a). The benefits of osmoconditioning (increased germination and seed physiological quality) were maintained in the soybean seeds after up to six months of storage, but soaking in water followed by drying was deleterious to seed quality (Lucca *et al*, 1997b).

Seed coating can also be used to supply seeds with fungicides and (or) minerals to minimize pathogen effect or improve vigour (Li *et al*, 1999). Seed film coating using SB2000 was found to reduce imbibition during early stages of hydration leading to reduction of membrane damage and less leaking of electrolytes (Ni, 2001; Posmyk, 2001). Reduction in the leakage of electrolytes improved yield since seeds that leaked large amounts of electrolytes performed poorly in field emergence and seedling stand establishment (Min, 2001).

In the previous study (chapter 2) it was shown that seed priming in calcium osmotica allows enough time for water and calcium uptake. This effect led to a measurable minimisation of cell wall cracking or disruption. Calcium treatments using foliar sprays during flowering (on green bean cultivars affected by TVC) were not successful in alleviating TVC in a study conducted by Bradford and Eisinger, (1986). Seed priming in calcium salts, and immediate re-drying for planting, may be a better option for alleviating TVC. The present study therefore examined the effect of the three calcium salts ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) on the performance of six green bean (*Phaseolus vulgaris* L.) cultivars (Imbali, Sodwana, Outeniqua, Elangeni, Tokai and Tongati), with respect to TVC, emergence and stand establishment under field conditions.

3.2 Materials and methods

3.2.1 Seed treatment

Six green bean cultivars were obtained from a seed company (Pro-seed cc) in Pietermaritzburg, KwaZulu-Natal, South Africa. The cultivars are commercially available cultivars whose susceptibility to TVC was not known. Three calcium salts ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) were used to treat 180 seeds each from each cultivar at different salt concentrations using osmopriming and seed coating as methods of application. For osmopriming 30 seeds were imbibed in 30 ml of the salt solution (0,1,10,50,100 and 1000Mm) using $CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$ for 4 hours. The seeds were then blot dried and allowed to air dry to 10% moisture content at room temperature for 48 hours. The same number of seeds was used for seed coating and the seeds were coated with 0.2 ml of calcium salt ($CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$) solution containing distilled water, captan [3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione], CMC (carboxyl methyl cellulose) glue (2:2:1) and 0.06% dye. The dye was used to differentiate between osmoprimed and coated seeds during planting. The seeds were then allowed to air dry at room temperature for 48 hours. The treated seeds were then packaged in small paper envelopes, stored at room temperature until sowing.

3.2.2 Study locations

The study was conducted in three locations in KwaZulu-Natal: Pietermaritzburg (University of Natal research farm, Ukulinga: 29°35′S30°25′E), Inchanga (Institute of Natural Resources research farm, Nansindlela: 29°46′S30°44′E) and Harding (Bowles and Northad, neighbouring farms: (30°54′S29°35′E), KwaZulu-Natal, South Africa. Each year, in 2001 and 2002 a site that, in the previous season, had no green beans or a

legume crop was selected for the field experiments conducted in this study. At each site, field experiments were planted on a different field every year in 2001 and 2002 to avoid serious occurrence of diseases and pests that are associated with green beans. A map showing details of site locations in KwaZulu-Natal is presented in Appendix 1. The bioclimatic regions (Department of Agricultural Development, 1992) where the sites are located and some chemical properties of the soil in each experimental field are presented in Appendices 4.2 and 4.3.

As a consequence of the significant differences (no statistical analysis was performed, however) among the soils at different locations, and general similarities within sites, with respect to soil chemical properties, especially Ca^{2+}, it was decided that soil fertility be changed only according to the recommendations of the fertiliser advisory service for green bean production (data not shown). Therefore, the concentrations of soil Ca^{2+} were not changed, and the effect of Ca^{2+} treatments were assumed to be influenced only by sites, apart from seed treatments prior to planting (see section 2.2).

3.2.3 Field experiment design and statistical analysis of field data

The experiment was designed as a split plot with cultivars as main plots, subdivided into subplots of methods of Ca^{2+} application (coating or priming), salts and salt levels, respectively (Appendix 2). The experimental unit was formed by one row occurring between two border rows in a 2-m^{-2} plot. The experimental unit was, therefore, also a sampling unit. Since a large number of plots (108) were created as a result of the large number of treatment combinations in a factorial experiment, the experiment was not replicated at the different locations or sites. This was also done to allow efficient data collection. Locations were used as replications. Treating locations as replications was

justified for two reasons: 1) significant differences in soil fertility among locations associated with relatively close concentrations of calcium concentrations within locations (although Harding and Nansindlela were not very different in this respect) and 2) The environmental effect that could cause differences between locations was regarded as being of less interest than that between seasons, which can be modified by irrigation and planting date, *inter alia*. Repeating the experiment for the second season in 2002 was, therefore, a significant way of testing the validity of observations. The experiment was randomised differently for each location or site, and each season (2001 and 2002). Seeds that were coated and primed as explained in section 3.2.1 were planted (ten seeds per plot) after allowing moisture content to reach about 10% (fresh mass basis) under controlled relative humidity and temperature conditions. Planting was performed by hand-placing (pushing) seeds about 2.5 cm into a ploughed, disked and rotovated seed-bed. Weeds were controlled physically by hand hoeing or hand-pulling twice during the growing season, on the 10^{th} and 20^{th} days after planting. All experiments were conducted under dry land condition in both seasons.

Raw data were analysed using generalised statistical analysis (GenStat, Release 6.1, Rothamsted Experimental Station, UK) to produce analysis of variance (ANOVA). Means were presented either using graphs or tables (for high order interactions), and the differences between means were determined using LSD (least significant difference) generated simultaneously as the ANOVA tables (Appendix 4.1).

3.2.4 Field data collection and determination of variates

3.2.4.1 Seedling emergence and cotyledonal cracking

On the tenth day after planting, a survey of field emergence was undertaken by counting the number of emerged seedlings per plot. The tenth day after planting was selected based on previous preliminary studies, which showed that it was the optimum time to allow complete emergence and retention of unwilted cotyledons. Cotyledons were found to begin to drop by the 12^{th} to the 14^{th} day after planting. Hence, at the same time as seedling emergence was assessed, occurrence of cotyledonal cracking was examined. Cotyledonal cracking occurrence was determined by counting the number of visible transverse fissures across both cotyledons in all emerged seedlings. Data for both seedling emergence and cotyledonal cracking were recorded as counts per plot.

3.2.4.2 Stand establishment and plant height

The number of emerged seedlings was determined firstly on the tenth day after planting, when cotyledonal cracking was also examined. Twenty days after planting the number of surviving seedlings was counted again to assess existing plant stand. Plant stand was anticipated to be affected by pathogens and pests (mainly cutworm) attack on slowly developing seedlings in lack of response to Ca^{2+}-treatment. Calcium was expected to increase plant growth and allow the stronger seedlings to escape cutworm attack. Data on plant height, which were collected to test plant development rate in response to Ca^{2+} showed significant differences between cultivars, and not between treatments. Since the genotype differences were expected with respect to plant height, these data were disregarded for further explanation of treatment effects in this study. The number of plants at harvest was compared with the number of seedlings counted on days 10 and 20 after planting to determine the final, yield influencing, stand

establishment. No significant differences were found between the different dates when stand establishment was determined, at all sites and in both 2001 and 2002 seasons. Hence, stand establishment was reported using the final number of plants per plot at harvest.

3.2.4.3 Photosynthetic efficiency

The effect of Ca^{2+}on plant growth was also determined indirectly by measuring the photosynthetic efficiency using the Plant Efficiency Analyser (Hansatech, Norflok, England) on seedlings for the first 20 days after planting, starting at emergence on day 10 after planting. This study showed no significant differences between cultivars, nor treatments, and there were also no significant interactions of treatments. Hence, as with plant height data, the data on photosynthetic efficiency were also disregarded for further explanations of the effect of Ca^{2+} on cotyledonal cracking.

3.2.4.4 Yield determination

Seed yield was determined 150 days after sowing (February) and dry mass was expressed on a 10% moisture content, fresh mass basis.

3.3 Results

3.3.1 Field assessment of transverse cotyledonal cracking

There were no significant differences between sites with respect to TVC. Means of cracks per plot across all three sites are presented for all variables determined in 2001 and 2002 separately. Osmoprimed seeds had significantly ($P < 0.001$) more cracks than coated seeds (Figure 3.1) in 2001 and 2002 field experiments. A decrease in the number of cracks per plot was observed with an increase in salt concentration (Figure 3.2). No

significant difference was observed between the control seeds and seeds that were treated with the lowest salt concentration (1 mM). However, concentrations of 10 mM and greater gave significantly different results compared to untreated seeds or control seeds (Figure 3.2). Different cultivars responded differently to TVC with Imbali being the most resistant as it had the smallest number of cracks and no significant difference was observed between the other five cultivars (Figure 3. 3). Type of salt used did not show any significant effect on the reduction of the number of cracks in 2001, however $CaSO_4$ had a slightly higher number of cracks than the other two salts in 2002 (Figure3.4).

3.3.2 Seedling emergence and stand establishment

There were no significant differences between sites and means across all three sites are presented. No significant difference was observed between $Ca(NO_3)_2$ and $CaCl_2$ with respect to emergence (Figure 3. 5), but $CaSO_4$ resulted in a slightly higher ($P = 0.01$) number of emerging seedlings than the other two salts. There was a decrease ($P < 0.001$) in the number of seedlings that emerged at the two highest salt concentrations (100 and 1000mM) (Figure 3.6). Seed coating resulted in a higher number of seedlings at emergence than osmopriming (Figure 3.7). Stand establishment 20 days after sowing was not significantly different from the final plant stand at harvest. Hence stand establishment was determined by the final plant stand (Figure 3.8). There were significant ($P < 0.001$) differences between cultivars, with respect to stand establishment. Seed enhancements (Figure 3.9) and salt types (Figure 3.10) also had significant effects (regarding stand establishment. Coating produced a better ($P < 0.001$) stand than priming. Among salts, $CaSO_4$, produced a better stand than

$Ca(NO_3)_2$, but it was not significantly better than $CaCl_2$. There was also no difference between $CaCl_2$ and $Ca(NO_3)_2$.

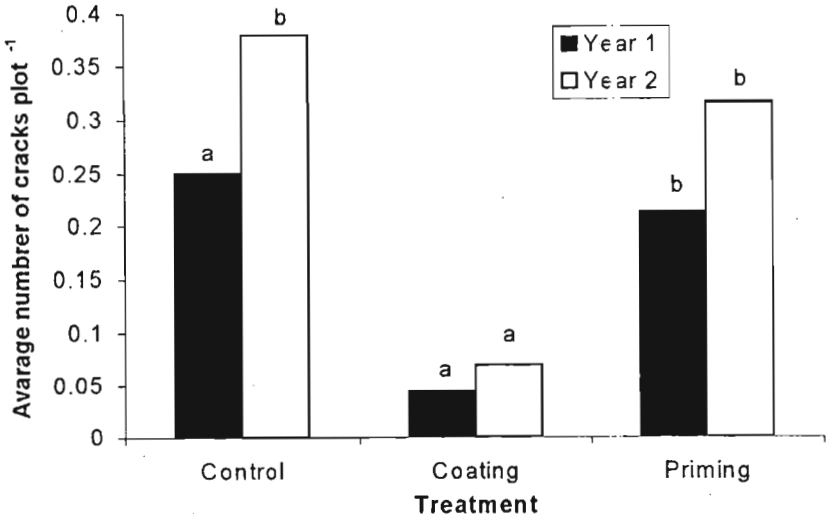


Figure 3.1. Field occurrence of TVC in osmoprimed and coated seeds observed 10 days after sowing in 2001 (year 1) and 2002 (year 2). Data points are means of three sites. Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

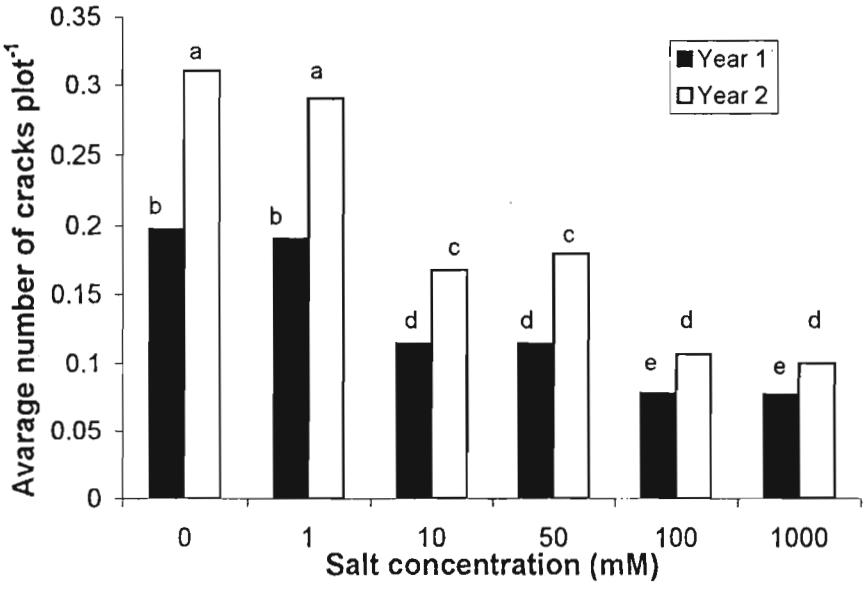


Figure 3.2. Cracks observed in 2001 (year 1) and 2002 (year 2) as influenced by salt concentration during seed treatment. Data represent means of six cultivars (primed and coated seeds). Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

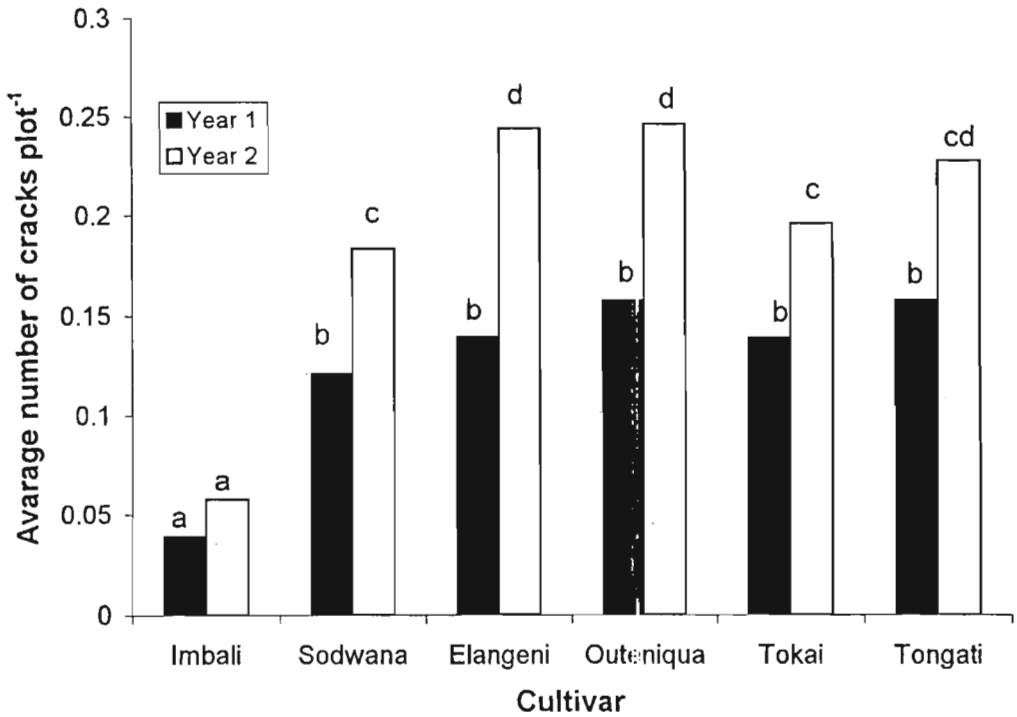


Figure 3.3. Cultivar performance with respect to the number of cracks observed on seedlings in the field in 2001 (year 1) and 2002 (year 2). Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

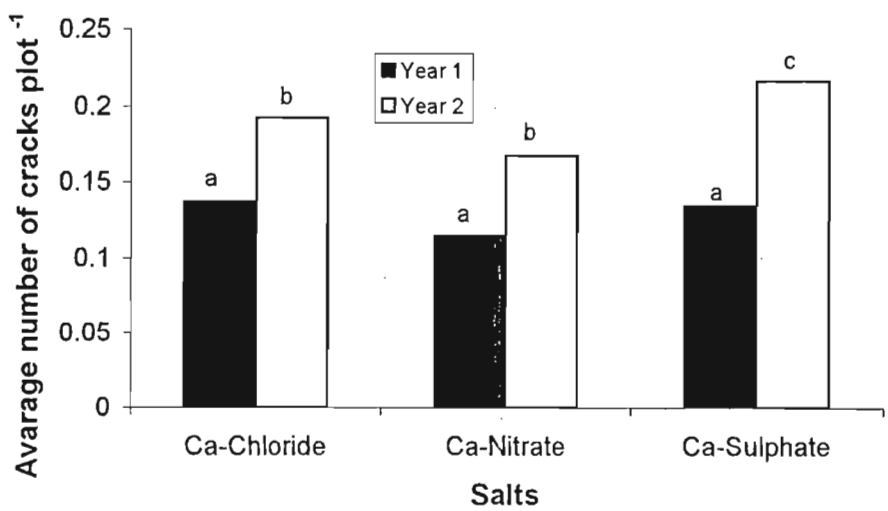


Figure 3.4.Effect of different salts on the occurrence observed on seedlings in the field of cracks 2001 (year 1) and 2002 (year 2). Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

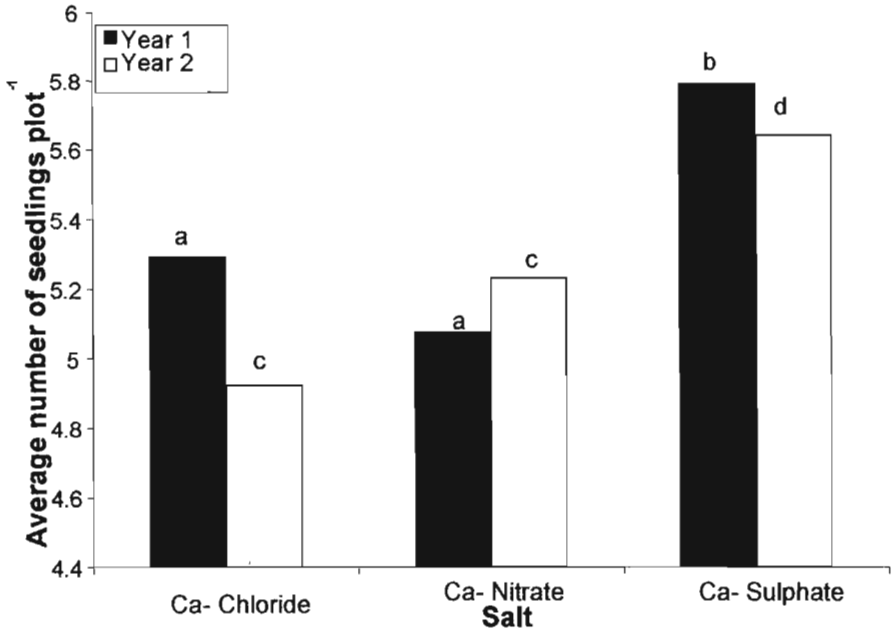


Figure 3.5. Effect of calcium salts on seedling emergence of green beans. Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

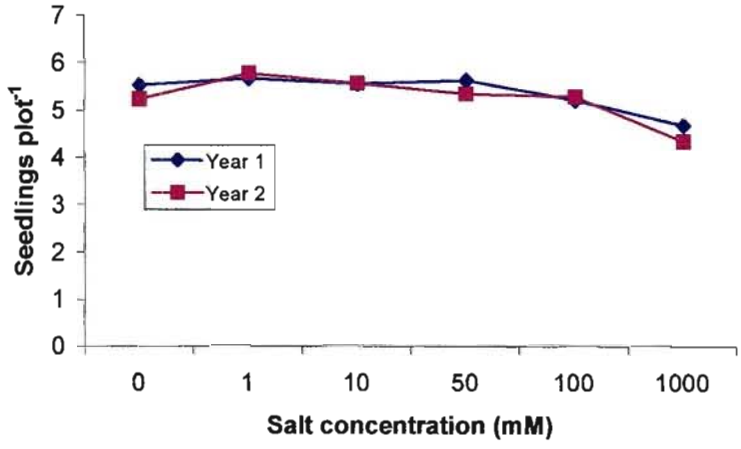


Figure 3.6. Effect of priming salt concentration on the number of seedlings in 2001 (year 1) and 2002 (year 2). Data points are means of three sites. Note: a single row plot was used as a sampling unit.

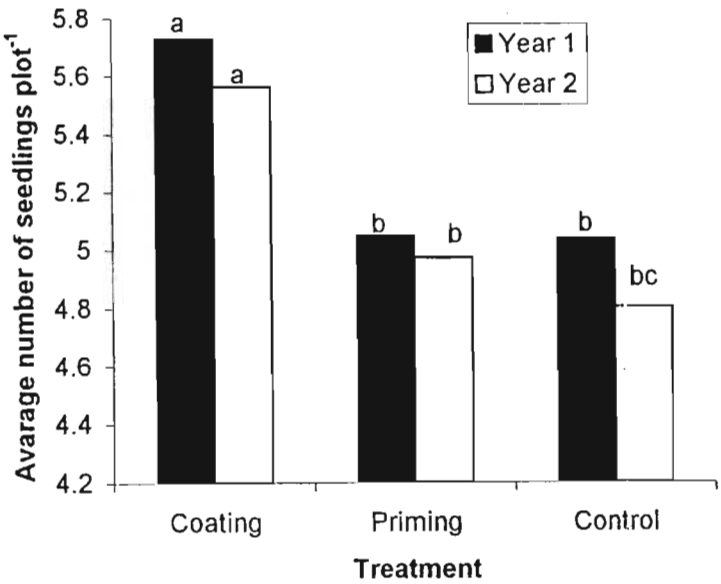


Figure 3.7. Treatment effect on seedling emergence of green beans. Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

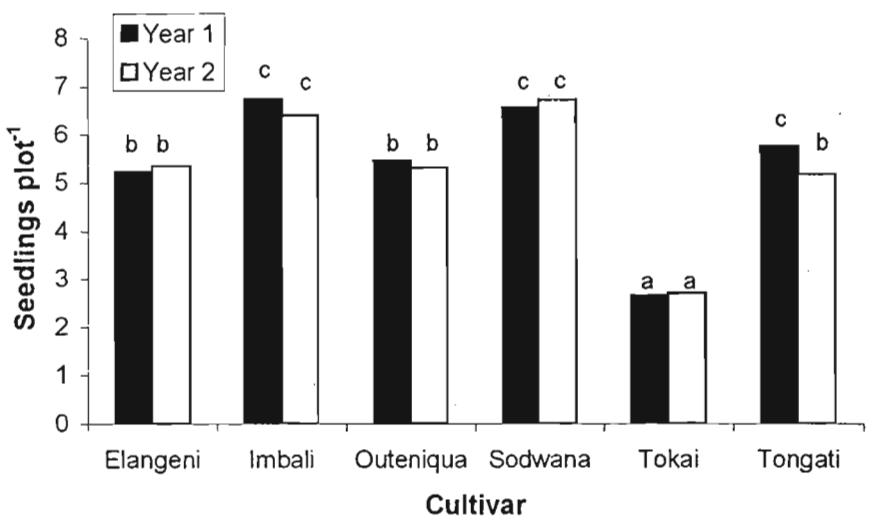


Figure 3.8. Stand establishment of six green bean (*Phaseolus vulgaris* L.) cultivars at harvest 2001 (year 1) and 2002 (year 2). Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

Table 3.1 Seedling stand establishment (total number of seedlings in a plot) as affected by salt (Ca- chloride, Ca-nitrate and Ca-sulphate) and treatment (coating and priming) averaged across all sites, cultivars and salt levels. Means sharing the same letter are not significant different from each other (P< 0.05).

Treatments	Total number of plants per plot (%)
Coating	58.6 a
Priming	50 b
Ca-Chloride	55 cd
Ca-Nitrate	51.8 c
Ca-Sulphate	57 d

3.3.3 Seedling height

There were significant differences between cultivars with respect to seedling height (P < 0.001), but there were no differences between sites. Tokai was found to have the shortest seedlings when compared to the other cultivars, with Sodwana (large-seeded) having the tallest seedlings followed by Imbali (small-seeded). Outeniqua and Tongati were not significantly different from each other (Figure 3.9). Salts showed a significant (P = 0.05) effect on seedling height with $CaSO_4$ resulting in the tallest seedlings (Figure 3.10). Primed seeds performed poorly with respect to seedling height when compared to coated seeds (Figure 3.11).

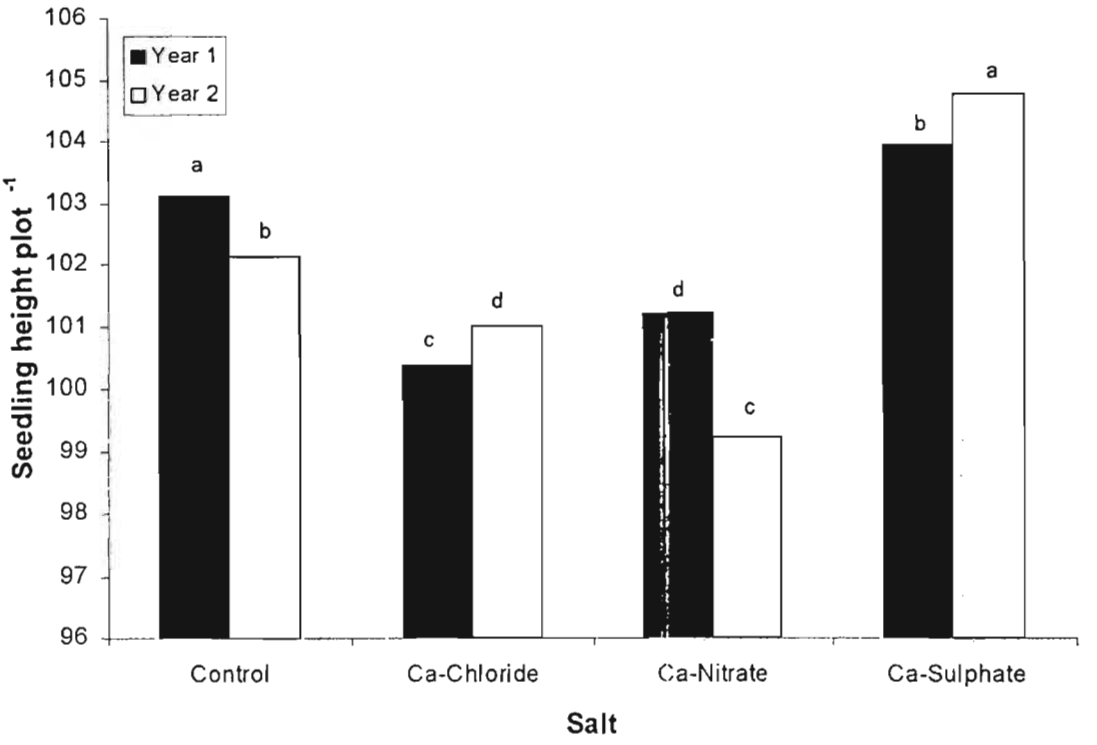


Figure 3.9. Seedling height of different green bean cultivars (recorded 20 days after planting) in 2001 (year 1) and 2002 (year 2). Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

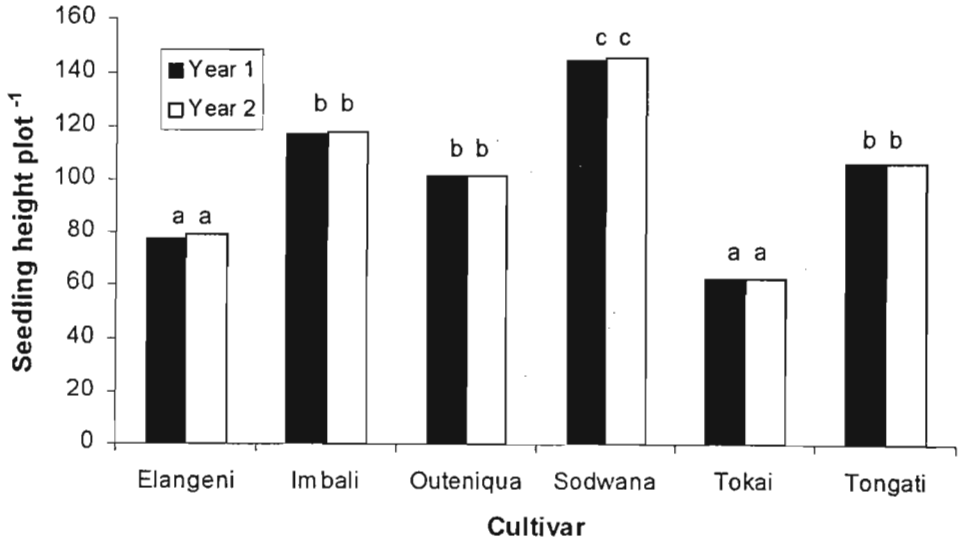


Figure 3.10. Effect of different calcium salts on green bean seedling height in 2001 (year 1) and 2002 (year 2). Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

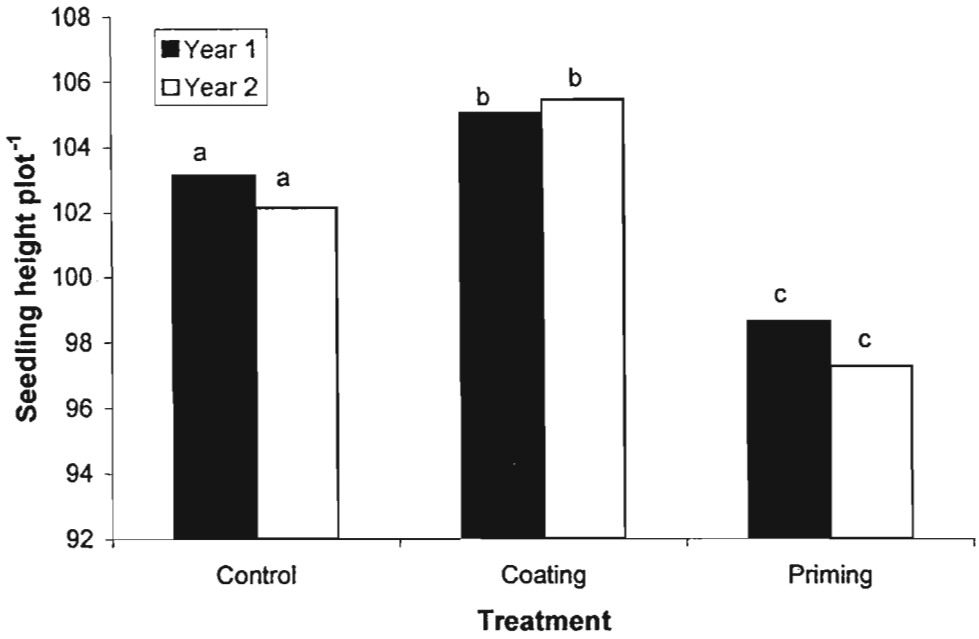


Figure 3.11. Green bean seedling height in response to coating and priming in 2001 (year 1) and 2002 (year 2). Means sharing the same letter are not significantly different (P = 0.05). Note: a single row plot was used as a sampling unit.

3.3.4 Seed yield

Significant ($P < 0.001$) differences were observed between cultivars (Figure 3.12) with respect to the yield. There were, however, no significant site, salt and salt level effects. Most cultivars showed a significant decrease in the yield in the second year except for cultivar Imbali.

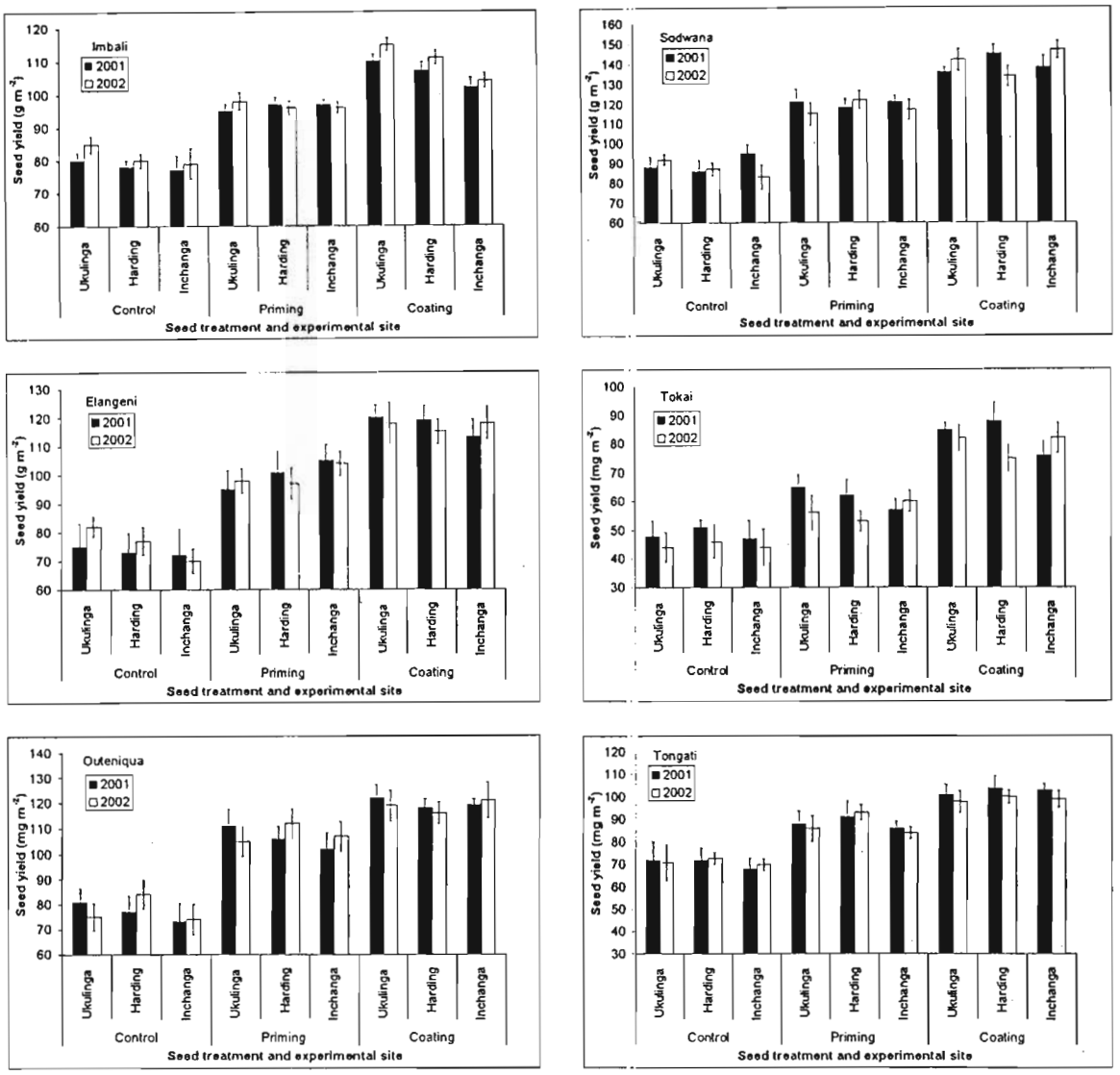


Figure 3. 12. Green bean cultivar performance with respect to total yield (2001 and 2002). Means sharing the same letter are not significantly different (P = 0.05).

3.4 Discussion

Seed coating was better than priming in alleviating cotyledonal cracking irrespective of the cultivar and site. Previous studies showed that imbibition damage can contribute to the occurrence and intensity of TVC (Jones, 1971; Dickson *et al.*, 1973). One of the possible explanations why coated seeds had fewer cracks in this study may be that they were not subjected to imbibitional damage. During imbibition there is a drastic in- rush of water into the seed depending on the seed moisture content prior to imbibition. This

in-rush may lead to cell wall rupture or disruption, especially when the magnitude of water potential gradient between the seeds and the solution being imbibed is great. High salt concentrations decrease the osmotic potential gradient between the seeds and salt solution and minimise the effect of imbibitional damage. This may, however, delay emergence through delayed germination, because green bean seeds need to imbibe a certain amount (~50%) of water before germination can be initiated. Hence the decrease in the emergence at high salt concentrations. The effect of high salt concentration, however, did not compromise seedling vigour since no significant differences were observed between salt concentrations with respect to seedling height. Seeds that imbibed water rapidly in the laboratory study (chapter 2), Sodwana, Outeniqua, Elangeni and Tongati, had a high number of cracks in the field. In contrast, Imbali, which also had a slower water absorption rate, had fewer cracks.

Imbibition injury is accompanied by electrolyte leakage, which in turn reduces germination and emergence. Hence osmoprimed seeds had lower emergence than coated seeds. Transverse cotyledonal cracking impairs seed germination and seedling emergence. Seedlings produced from TVC affected seeds are therefore expected to develop poorly in the field. This was observed in this field study when osmoprimed seeds showed more cracks, poorer emergence and smaller size than coated seeds. The poor seedling performance can predispose weak ones to pathogen attacks and other harsh environmental conditions. In one of the sites (Harding) it was observed that the slow growing seedlings were affected by cut -worms. This can seriously reduce final stand and yield.

Osmopriming was chosen because it can supply the seeds with calcium, which has been implicated to play an important role in the occurrence and degree of TVC. Uptake of calcium by seeds was found to increase with an increase in salt concentration (chapter2). Results showed that seeds that have taken up larger amounts of calcium (seeds treated with salt solutions of high concentrations) had fewer cracks. This indicated the important role played by calcium in minimising TVC occurrence and suggests that calcium seed treatment can be used as one of the corrective measures for this disorder. This was also supported by the fact that Imbali, which has the higher amounts of calcium, consistently had fewer cracks.

When comparing yield of the different cultivars, it was observed that Imbali had the highest yield. This showed that yield is determined by factors affecting the seedling at early stages of development. Seedlings that had fewer cracks grew quickly in height and produced a better stand. Fast growth may have also assisted in the ability to resist the effect of pathogens and improved yield.

In conclusion, findings from this study suggest that alleviation of TVC by osmopriming seeds using different calcium salt concentrations is possible but not as effective as using seed coating.. This leaking may compromise seedling performance from emergence to seed production. Salt concentrations of up to 50 mM may be more effective in reducing TVC together with good emergence under field conditions. Concentrations above this cause a reduction of emergence. Combining a small-seeded cultivar with low salt concentration may produce even better results in trying to alleviate TVC.

References

Aqil, B. A. and A. A. Boe.1975. Occurrence of cotyledonal cracking in snap beans and its relation to nutritional status in the seed. *HortScience* 10: 509 – 510.

Bradford, J.K. and B.A. Eisinger.1986. Role of seed mineral in the occurrence of transverse cotyledonal cracking of snap bean. *Journal of the American Society of Horticultural Science* 111: 110 – 114.

Copeland, L.O. and M.B. McDonald. 1995. Principles of seed science and technology 3rd edn. Chapman and Hall, New York. 258 – 276.

Dickson, M.H., K. Duczmal and S. Shannon. 1973. Imbibition rate and seed composition as factors affecting transverse cotyledonal cracking in bean (*Phaseolus vulgaris* L.) seeds. *Journal of the American Society of Horticultural Science* 73: 509 – 513.

Guirdice, M.P., M.S. Reis, C.S. Sediyama, T. Sediyama, and P.R. Mosquim. 1998. Evaluation of soybean seed quality submitted to osmoconditioning at different temperatures. *Revista Brasileira de Sementes* 20: 254 – 262.

Jones, T. L. 1971. Injury to beans (*Phaseolus vulgaris* L.) in relation to imbibition. Agriculture, Plant Culture. University of Illions.

Li, J., H. Li, H. Yuan, and S. Zhang. 1999. Effect of seed coating on the control of seedling disease, growth and yield of corn. *Journal of China Agricultural University* 4: 82 – 86.

Lucca, B.A., M.S. Reis, C.S. Sediyama, V.S. Rocha, and T. Sediyama. 1997a. Osmoconditioning effect on soybean seed germination and vigour. *Revista Brasileira de Sementes* 19: 71 – 79.

Lucca, B.A., M.S. Reis, C.S. Sediyama, V.S. Rocha, and T. Sediyama. 1997b. Influence of hydration-dehydration process upon soybean seeds physiological quality during storage. *Revista Brasileira de Sementes* 19: 80 – 87.

Min, T. 2001. Field emergence of radish and Chinese cabbage seeds sorted by amino acid leakage. *Journal of Korean Society for Horticultural Science* 42: 57 – 59.

Morris J. L. 1971. The breeding aspects of vegetable seed quality. *HortScience* 6: 553 – 555.

Ni, B.R. 2001. Seed treatment: challenges and opportunities. British Crop Protection Council, United Kingdom 73 – 80.

Posmyk, M. M., F. Corbineau, D. Vinel, C. Bailly, and D. Come. 2001. Osmoconditioning reduces physiological and biochemical damage induced by chilling in soybean seeds. *Physiologia Plantarum* 111: 473 – 482.

CHAPTER 4

EFFECT OF SHORT-TERM STORAGE ON THE PERFORMANCE OF FIRST GENERATION SEEDS DERIVED FROM CALCIUM-TREATED PARENT SEED

4.1 Introduction

Seed performance and longevity are highly affected by the storage conditions prior to planting. Seed stored at favourable storage conditions (optimum relative humidity, favourable temperature and freeness from pathogens) will have a longer shelf life and good field performance in terms of emergence and stand establishment (Bryant, 1985). Seed conditioning has been found to improve longevity of certain crops but this success is crop dependent. Seed deterioration is one of the important factors that contribute to agricultural losses. In the USA alone, 25% of the agricultural revenue losses are due to poor seed quality (Copeland and McDonald, 1995). In areas where temperatures and relative humidity are high, large losses are experienced due to deterioration as a consequence of breakage and microbial spoilage during production, storage and shipping. Seed deterioration is inevitable and irreversible, but it can be slowed down. Some seed treatments can be used to improve the performance of seeds of poor quality but this does not mean that the damage to seeds is reversible (Copeland and McDonald, 1995). These treatments only allow seeds to optimally express their potential.

Storage conditions have a direct effect on seed quality and vigour and the two most important factors in storage conditions are temperature and moisture content as affected by the relative humidity of the environment. Seeds stored at low relative humidity (desiccating environment) tend to have reduced vigour and seed moisture content below 5% has a negative effect on storage life and seed vigour (Bryant, 1985), whereas

seed moisture content above 14% results in seed deterioration. For most crops, relative humidity of 20 to 40% and moisture content of 5 to 8% is the safe range of storage because moisture content above 14% promotes insects and fungal growth (Black and Bewley, 1982). Even though both temperature and relative humidity are important factors to consider during storage of seeds, relative humidity changes with the change in temperature. High seed moisture content results in the formation of ice crystals if storage temperature is low (≤ 0°C) and it promotes fungal growth at high temperatures (Copeland and McDonald, 1995). High temperatures increase the rate of seed deterioration because they increase the biochemical processes that lead to metabolic degradation and energy utilisation. Storage fungi are more important in seed deterioration than fungi found in the field because they can survive without water at low relative humidity, which is ideal for seed storage.

Besides storage conditions, internal factors and genetic make up of the seed also play an important role in the deterioration of a seed lot. Seeds that are physiologically impaired due to lack of minerals, and seeds that are physically damaged, deteriorate faster than sound, undamaged seeds. Because seed deterioration is irreversible, mechanical damage that may be minor during storage can become worse during germination, greatly reducing the seedling quality and vigour. Seeds may have similar chemical make up but have different rates of deterioration under the same storage conditions if physical factors vary (Copeland and McDonald, 1995)

In this study performance of first generation seeds, obtained from field experiments, with respect to cotyledonal cracking, cotyledonal persistence and seedling stand establishment (emergence and seedling dry weight) was evaluated using two green

bean cultivars, Sodwana and Tokai. The effect of treating parent seed (see chapters 2 and 3) with calcium on the first generation of seeds was determined. It was speculated that the effect of the calcium treatment would be persistent in the first generation as determined by reduced cotyledonal cracking and improvement of seed quality.

4.2 Materials and Methods

4.2.1 Source of seed

Two cultivars, Sodwana and Tokai, which showed significant differences with respect to seed and seedling quality in the laboratory (chapter 2) and field (chapter 3) studies were selected to test persistence of calcium seed treatment in the first generation of seeds and seedlings. Under field conditions, Sodwana performed better than Tokai with respect to TVC and stand establishment. Seeds harvested from the field experiments conducted at Ukulinga site (see chapter 1) were used to conduct experiments as explained in the following sections. Seeds were derived from plots representing all calcium concentrations (0, 1, 10, 50, 100 and 100 mM) and seed treatments (coating and priming).

4.2.2 Storage conditions

Treated (osmoprimed or coated; see chapters 2 and 3) seeds were stored in a cold room at 8 °C at a relative humidity of about 40% obtained by using a saturated solution of lithium chloride. The seed were placed in a petri dish, which was suspended above the lithium chloride solution in a closed plastic container. The seeds were not allowed to come into contact with the lithium chloride solution (Figure 2.2).

4.2.3 Glasshouse experiments to determine emergence, cotyledonal persistence and seedling mass

Emergence tests were performed monthly by planting 10 seeds (replicated three times), sampled from storage. Seeds were planted in pasteurised, acid-washed sand (3 kg) in pots (Figure 4.1). Small plastic bags were placed into the pots to create a closed system. Seeds were planted 2.5 cm deep on the moistened sand at 21/17°C alternating day/night temperature in a glasshouse at 60% RH. Natural light was used. Emergence and number of cotyledon cracks per pot were recorded seven days after planting until harvest time, which was 14 days after planting.

Cotyledonal persistence was recorded during harvesting (14 days after planting) by counting the number of seedlings that still had intact (not completely senesced due to nutrient translocation to the growing seedling) cotyledons. Cotyledonal fresh weight was also recorded at harvest. Seedlings (excluding cotyledons) were then oven-dried at 70°C for 48 h and their dry weight determined. The glasshouse studies were repeated three times by sampling stored seed (section 4.2.3) once every a month to determine effects of parent seed treatments with calcium (chapter 3) on seed quality. The results were statistically analysed using Genstat 6. Analysis of variance tables are presented in Appendix 5.

Figure 4.1 Examples of pots containing seedlings 14 days after planting on pasteurised white sand to test emergence of first generation seeds. Note: A, B, C and D denote 0, 10 100 and 1000 mM $CaCl_2$ applied by osmopriming.

4.2.3.1 Determination of leached substances from the progeny seed

To determine the amount of the substances leached into the steep water of imbibed seeds, conductivity (Model 644 Conductometer, Metrohm, Switzerland), water potential (WP4 Dew Point Potentiometer, Decagon, Washington) and water activity (AquaLab, Decagon, Washington) were recorded. For these studies 10 seeds (replicated four times) of each cultivar, derived from each calcium salt treatment and control (chapter 3) were imbibed in 30 ml of distilled water for 6 h. Seeds were taken out of the imbibing solutions at 1-h intervals, blot-dried and weighed to determine water uptake.

To determine leaching of calcium, seeds were imbibed in distilled water (10 seeds in 20 ml) for 6 hours and steep water was analysed using the Atomic Absorption Spectrophotometer (AA) (Spectra AA – 200, Varian, Australia). Steep water (9 ml) was added to 1 ml of a suppressant (20 000 ppm Cesium solution). The solution was thoroughly mixed and analysed using the AA, according to the manufacture's instructions. High Performance Liquid Chromatography (HPLC) was used to determine sugars in the same seed steep water used for mineral element analysis. The sugar standards (sucrose, glucose and myo-inositol) were prepared by dissolving1g of each sugar standard in 1L of filtered distilled water. The HPLC column was calibrated over

night using distilled water. The samples (steep water) were prepared by adding 1ml of the sample in 4 ml of filtered sterile distilled water. To load the samples 100µl was injected into the column and allowed to run for 30 minutes. The samples were kept at 4°C throughout the duration of the experiment.

4.3 Results

4.3.1 Seed leachates

The water activity of steep water generally increased with increasing salt concentration, but there were no significant differences between cultivars. Salts were significantly different with respect to water activity in the following order $Ca(NO_3)$ > $CaSO_4$ > $CaCl_2$. The increase in water activity as influenced by salt level used to treat parent seed also displayed a correlation in the order: $Ca(NO_3)$ > $CaSO_4$ > $CaCl_2$ (Figure 4.2). There were no significant differences between coating and priming, with respect to water activity of seed steep water.

When all salts were considered the water potential of steep water was also found to generally increase with increasing parent seed calcium treatment (Figure 4.3). However, this pattern was mainly due to the effect of $CaCl_2$. There were no significant differences between cultivars with respect to water potential of seed steep water. Conductivity of coated seeds was generally lower than that of primed seeds in all salt concentrations (Figure 4.4). Cultivars and different salt concentrations did not show any differences with respect to conductivity of seed steep water.

In coated seeds results showed that seeds treated with low salt concentrations leached less amount of calcium into the steep water. Primed seeds also showed the same trend

except for the control seeds, which leached more calcium (Figure 4.5). Results from the HPLC showed that the seeds were leaching soluble carbohydrates sucrose, glucose and small amounts of myo-inositol, among other unidentified substances (Table 4.1). However, the sugar quantities were different with different salt concentrations used to treat parent generation seeds (Table 4.1)

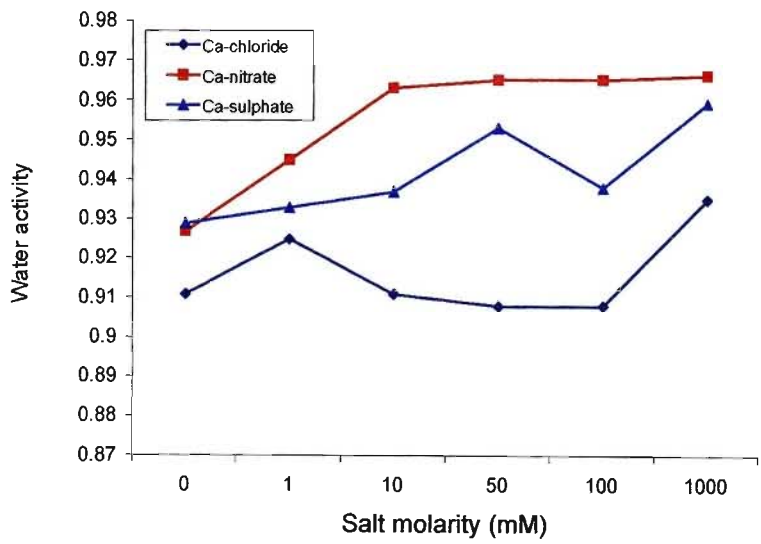


Figure 4.2. Water activity of the steep water of green bean seeds after 6 hours of imbibition in different calcium salts.

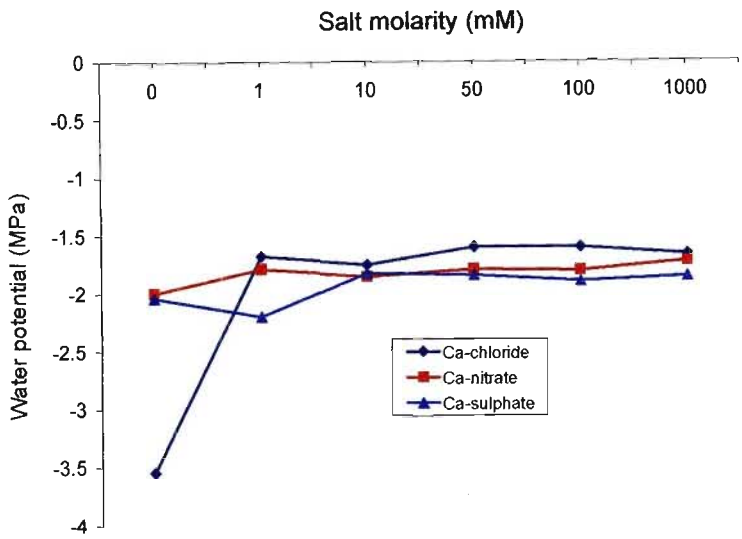


Figure 4.3. Water potential of the steep water after 6 hours of imbibition in different calcium salts.

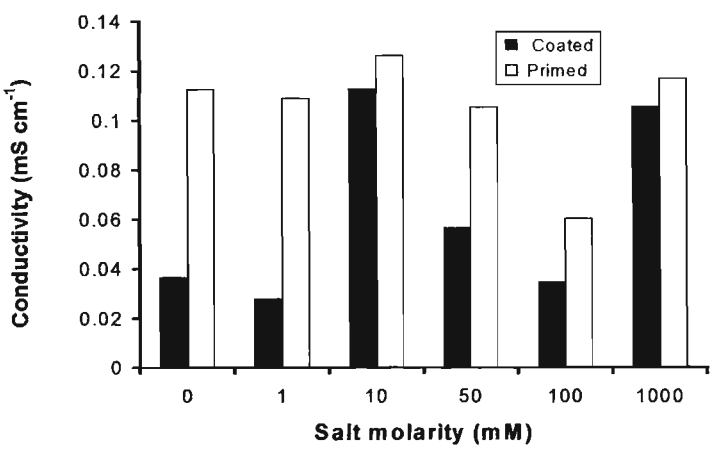


Figure 4.4. Conductivity of first generation seed derived from a parent seed generation coated or primed with different molarities of calcium salts. LSD (priming x coating) = 0.003; $P < 0.05$).

Table 4.1: Amount and types of sugars leached by first progeny seeds into the steep water determined using HPLC. Priming and coating were done in the parent seed generation.

Salt molarity (mM)	Primed seed sucrose ($\mu g\ seed^{-1}$)	Coated seed sucrose ($\mu g\ seed^{-1}$)	Primed seed glucose ($\mu g\ seed^{-1}$)	Coated seed glucose ($\mu g\ seed^{-1}$)	Primed seed myo-inositol ($\mu g\ seed^{-1}$)	Coated seed myo-inositol ($\mu g\ seed^{-1}$)
0	429.65	406.75	0	0	0	0
1	0	183.55	0	0	0	0
10	52.07	312.9	0	0	2.37	0
50	904.8	1286.95	0	0	0	0
100	417.05	238.41	0	0	0	90.74
1000	0	189.41	0	10.55	0	3.81

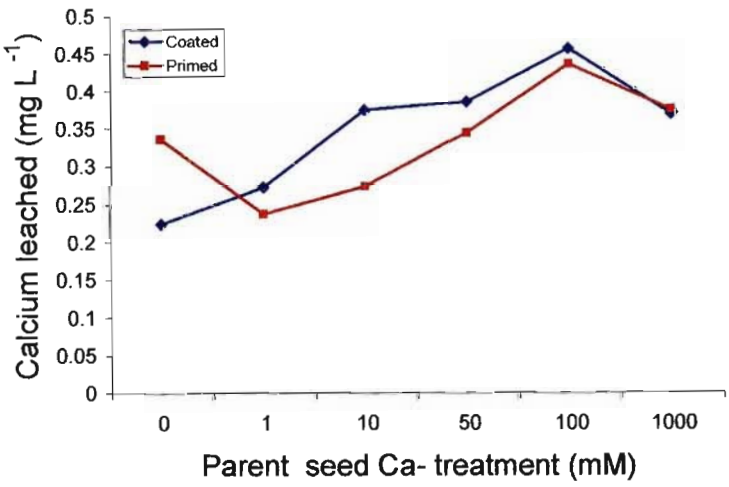


Figure 4.5 Amount of calcium leached into the steep water of the progeny seeds. Parent seeds were coated or primed with different concentrations of calcium salts.

4.4.2 Seedling performance in sand culture

4.4.2.1 Emergence and cotyledonal cracking

No significant differences were observed between the two cultivars (Sodwana and Tokai) with respect to emergence in the progeny seedlings, however differences ($P < .001$) were observed with the different salt concentrations. Salt concentrations did not

show any particular trend with respect to emergence since the control seeds (0 mM), seeds treated with 50mM salt and those treated with 1000 mM were similar and no significant differences were observed in the other three concentrations (Figure 4.6). Cultivars, seed treatments, bio-control agents, salts and salt concentrations showed no significant differences with respect to cotyledonal cracking but Tokai, coating and calcium sulphate had a slightly higher number of cracks (Figure 4.7, 4.8 and 4.9). The number of cracks observed were significantly reduced in the progeny seedlings when compared to the parent seeds (Figure 4.10). This effect was observed for both seed priming and seed coating (Figure 4.11). There were, however, significant differences (P = 0.01) between coating and priming, with respect to persistence of calcium seed treatment in the F1 generation, with priming showing a better effect than coating in the progeny seedlings (Figure 4.11).

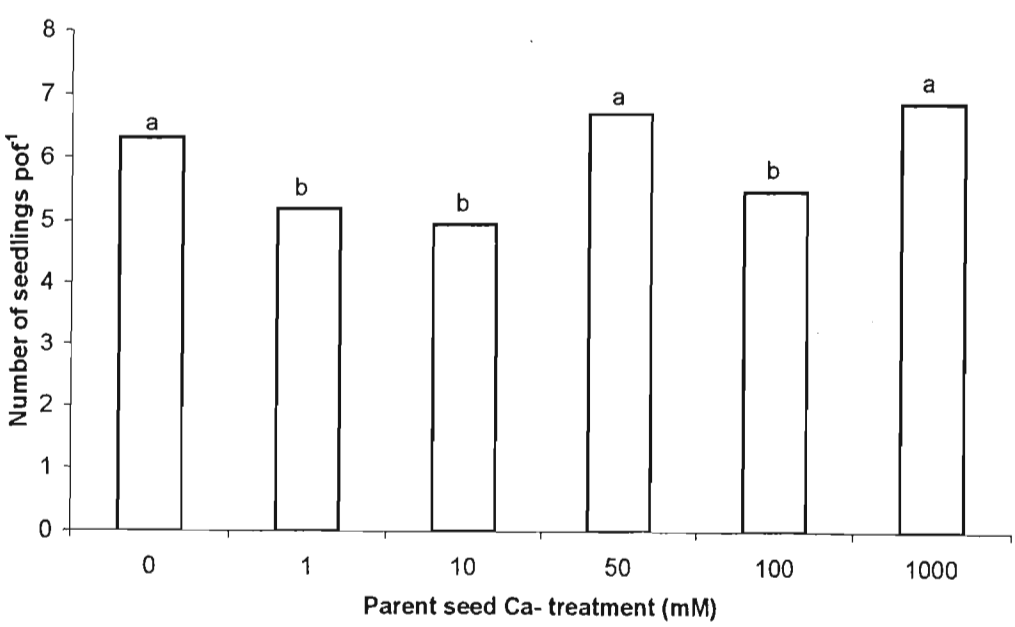


Figure 4.6. Comparison of calcium salt concentrations as applied to parent seeds and number of seedlings emerged using the F1 generation seeds. Means sharing the same letter are not significantly different (P = 0.05).

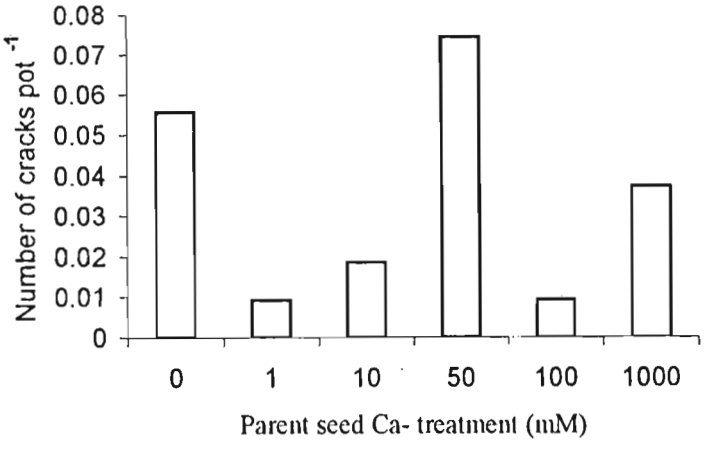


Figure 4.7. Relationship between calcium salt concentrations as applied to parent green bean seeds and number of cracks observed per pot in the first progeny seedlings. . LSD = 0.006; $P<0.05$.

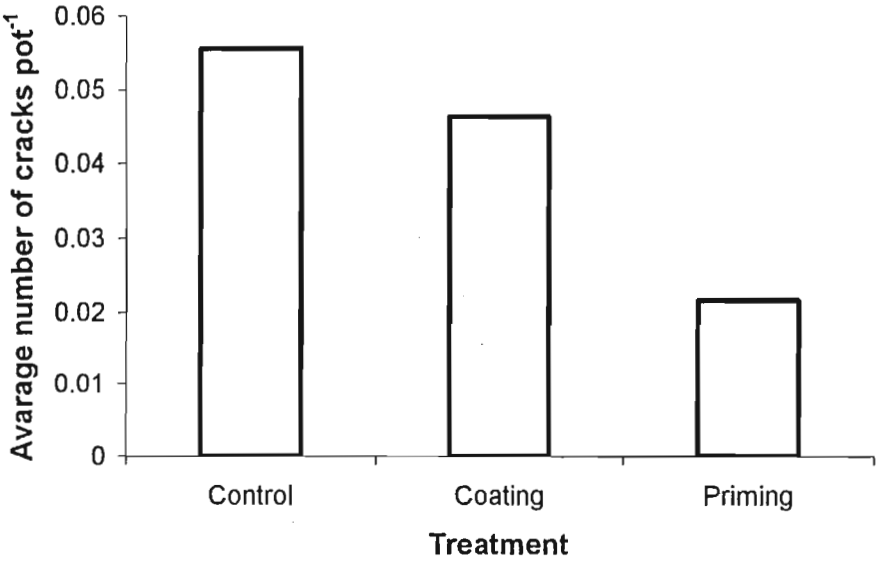


Figure 4.8. Performance of osmoprimed and coated green bean seeds with respect to cracking in the first progeny seedlings. LSD = 0.03; $P<0.05$.

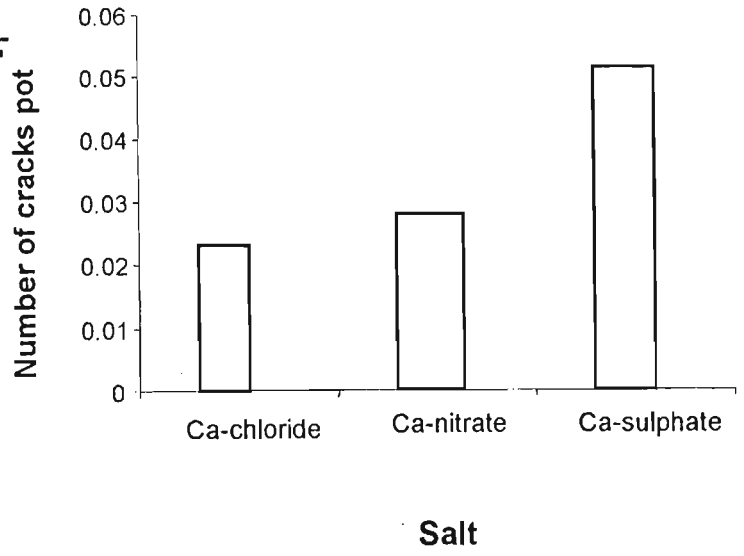


Figure 4.9. Effect of salt on cracking frequency in the first progeny seedlings. LSD = 0.04 ($P < 0.05$).

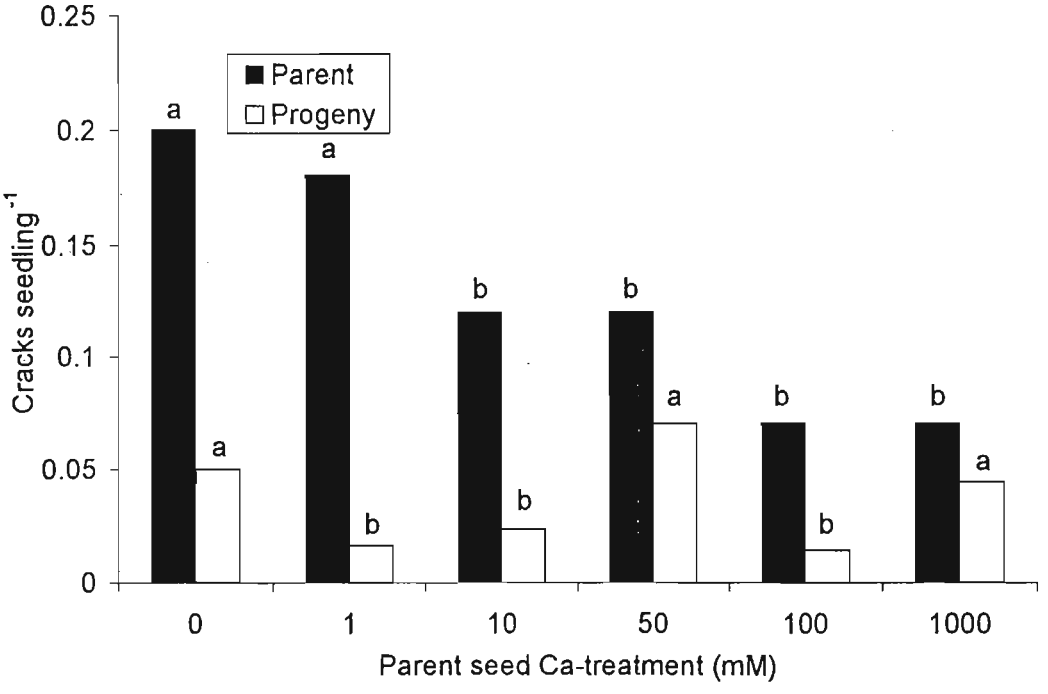


Figure 4.10. Effect of seed calcium treatment on the reduction of cotyledonal cracking in the parent and first progeny seedlings. Means sharing the same letter are not significantly different ($P < 0.05$).

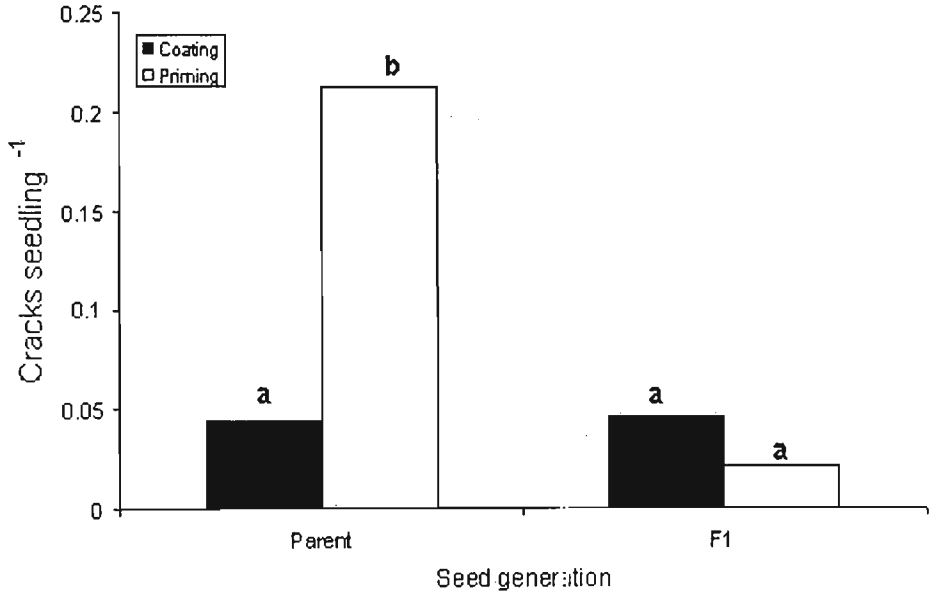


Figure 4.11. Effect of different seed calcium treatments (coating and priming) on reduction of cotyledonal cracking in parent and progeny (F1) generation of green bean seedlings. Means sharing the same letter are not significantly different (P = 0.05).

4.3.2.2 Seedling dry weight

Results showed significant ($P < 0.001$) differences between salt concentrations with respect to the seedling dry weight, which followed a similar trend as emergence with the low, medium, and high concentrations (0,50 and 1000 mM) having similar results (Figure 4.12). Sodwana had significantly bigger seedlings than Tokai and seeds treated with calcium nitrate produced bigger seedlings when compared to the other two salts (Figures 4.12 and 4.13). Cotyledonal persistence followed the same trend as seedling dry weight (Figure 4.12 to 4.15). Storage period did not show any significant differences with respect to TVC and emergence but a significant ($P < 0.001$) increase in seedling dry weight occurred while cotyledon fresh weight decreased (Figure 4.17).

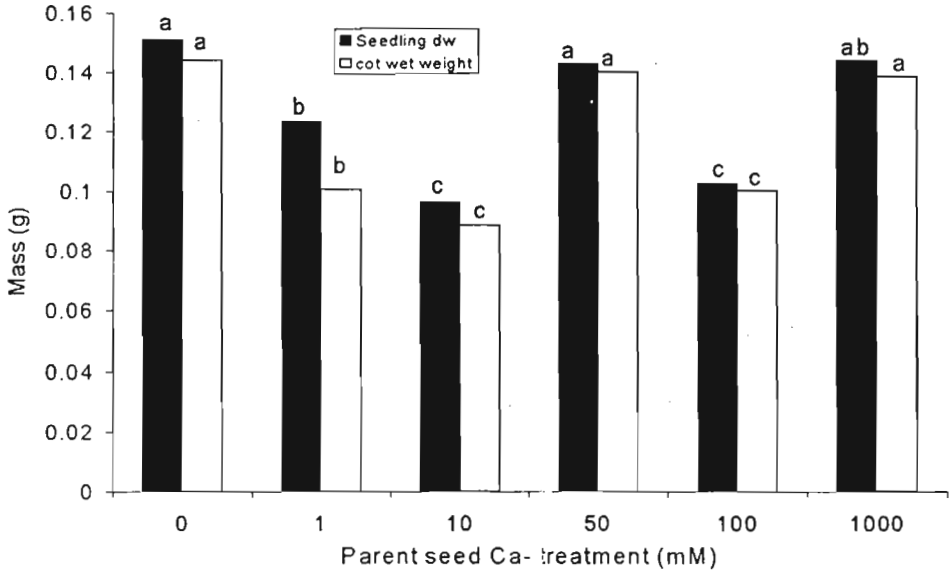


Figure 4.12. Comparison of the effects of salt concentrations as applied to parent seeds on seedling dry weight of F1 generation green bean seedlings. Means sharing the same letter are not significantly different (P = 0.05). Note: Seedling dw = seedling dry weight; cot wet weight = cotyledonal fresh mass).

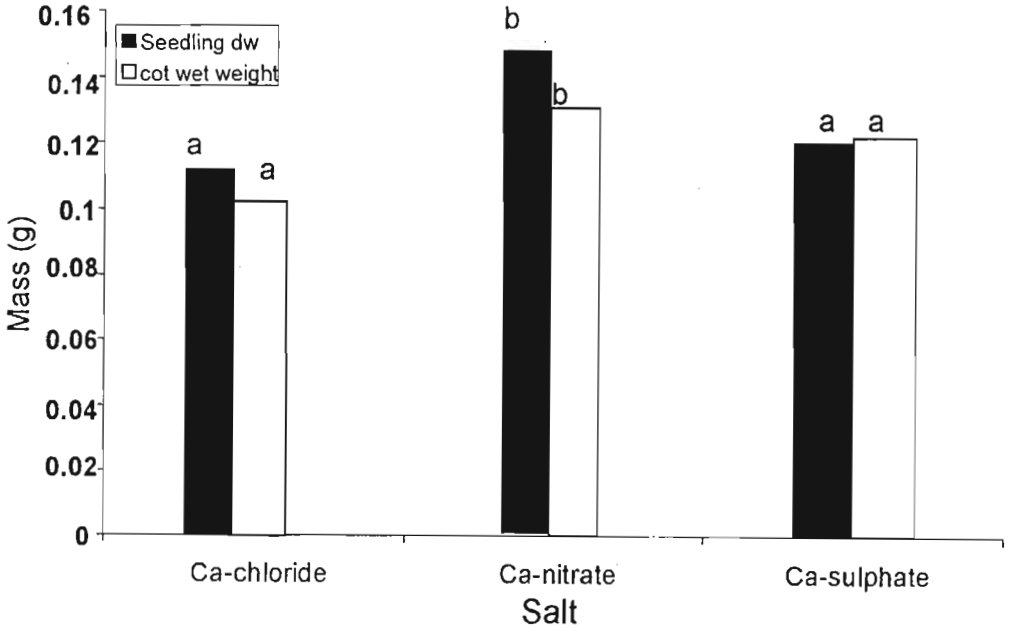


Figure 4.13. Effect of different salts on seedling dry weight (seedling dw) and cotyledonal persistence (cot wet weight) in the F1 generation. Means sharing the same letter are not significantly different (P = 0.05).

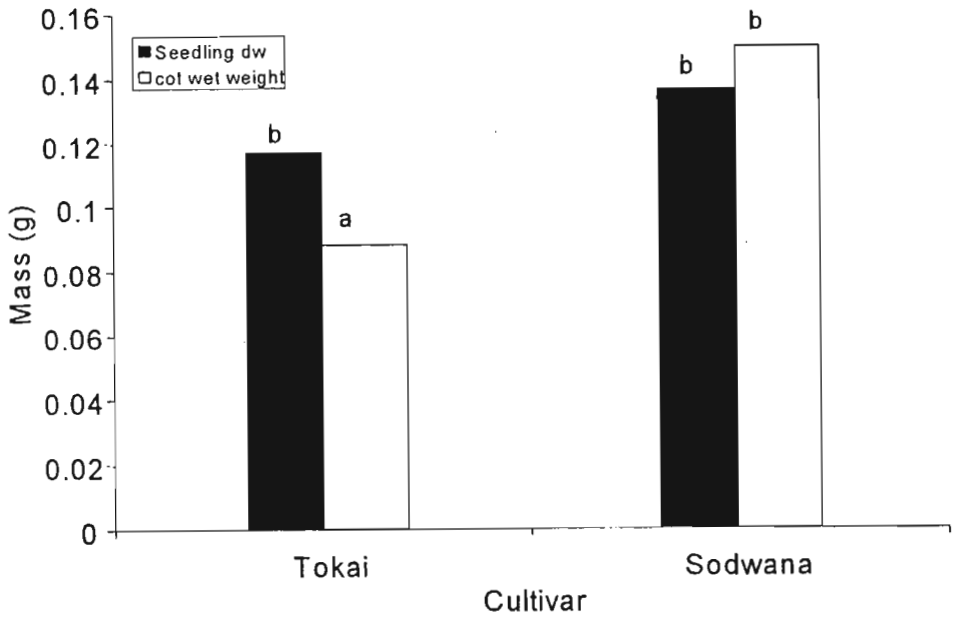


Figure 4.14. Cultivar performance on seedling dry weight (seedling dw) and cotyledonal persistence (cot wet weight) in the F1 generation. Means sharing the same letter are not significantly different (P = 0.05).

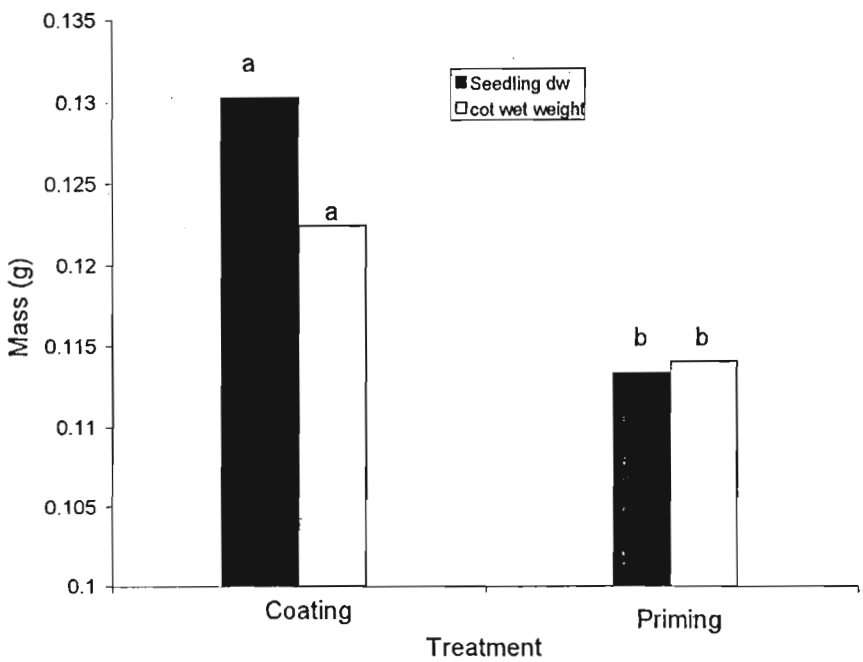


Figure 4.15. Seedling dry weight (seedling dw) and cotyledonal persistence (cot wet weight) of F1 seedlings derived from coated or primed parent seed. Means sharing the same letter are not significantly different (P = 0.05).

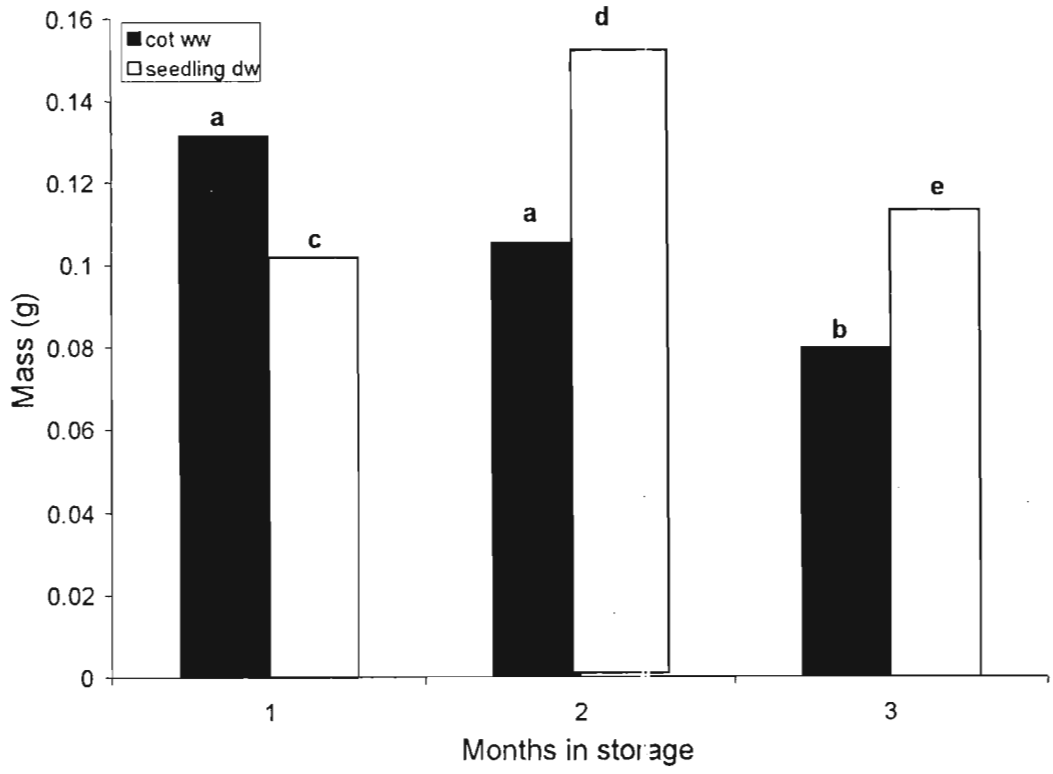


Figure 4.16. Effect of storage period on cotyledon wet weight (cot ww) and seedling dry weight (seedling dw) of the F1 generation of green bean seeds treated with bio-control agents. Means sharing the same letter are not significantly different (P = 0.05).

4.4 Discussion

Transverse cotyledonal cracking (TVC) is easily observed post-emergence, and there is no definite method of detecting this physiological problem before planting, without using a destructive method as explained in chapter 2. It is important that the factor used to control or minimise the occurrence of TVC persists at least to the next generation of seeds. The present study showed that seed calcium treatment (applied to the parent seeds) greatly reduces the occurrence of cracks in the progeny seedlings as well. The occurrence of TVC in progeny seedlings was greatly reduced in response to all salt concentrations (albeit without significant differences among salts) compared to the parental seedlings. This means that the effect of calcium was carried to the next generation of seedlings, possibly by increased calcium salt concentration in the progeny seed. In the field experiments (chapter 3), it was observed that coated seeds performed

better than primed seeds with respect to emergence and cracking, however in the progeny (the present study) priming of the parental seed caused a better reduction of TVC compared to coating. This means that osmopriming allowed more calcium to be absorbed by seeds and transferred to the next generation than coating. Seeds treated with calcium nitrate produced significantly bigger seedlings ($P < 0.001$) than seeds treated with calcium chloride and calcium sulphate. This may be related to the positive effect of nitrate in plant metabolism. Nitrate promotes vegetative growth and is associated with some key enzymes in plants (Marschner, 1995).

Although they had a slightly higher number of progeny cracks than primed seeds, coated seeds had bigger seedlings and their cotyledons lasted longer than those of the primed seeds. This suggests that coated seeds are able to grow and develop much faster and do not depend on the reserves in the cotyledons for a long period of time. Seedlings that had bigger cotyledons at harvest were found to be bigger than those that lost their cotyledons or had shrunken cotyledons. This suggested that cotyledonal persistence can be used as an indicator of good seedling stand establishment but it cannot be used on its own since some of the seedlings that had bigger cotyledons at harvest were not necessarily vigorous seedlings. Some of the seedlings that had shrunken cotyledons were found to be taller but weighed less than the other seedlings. It was hoped that there would be a negative correlation between cotyledonal persistence and seedling growth, because the former would mean efficient transfer of nutrients to the growing seedling. However, the present results were not conclusive in that regard.

Results showed that progeny seeds produced from parent seed treated with calcium salts leached fewer substances than untreated seeds. This was evident in their water

potential, which was more negative for untreated seeds, implying that there are more solutes in the steep water of these seeds. Conductivity readings did not show any specific trend but seeds derived from primed parent seed were found to have higher conductivity values than coated seeds. This may also imply that primed seeds leached more substances than coated seeds. The increasing trend of calcium leaching with increasing calcium concentration applied to the parent seed was possible due to the high seed calcium levels, rather than poor seed integrity (Figure 4.5). It is notable, however, that at 1000 mM, there was a decline in leached calcium.

When seeds start to deteriorate, major reserves are mobilised. In this study starch degradation was indirectly determined by the amount of sucrose that the seeds leached, because sucrose is the major form in which carbohydrates are mobilised in seeds. Seeds were found to leach small amounts of sucrose except for the seeds treated with 50 mM salt solution. This is difficult to explain, but it may indicate that after three months of storage most seeds had not started to deteriorate. Interestingly, primed seeds were found to be better than coated seeds with respect to sucrose leakage. This observation, however, cannot be used to conclusively state that priming improved seed ultrastructural integrity better than coating. That speculation will need to be tested.

In conclusion, the effect of treating parent seed with calcium persists to the F1 generation, but it does not have the same effect as in the parent generation. Judging by measurements of steep water characteristics, the cell wall integrity of the seeds treated with calcium salts was improved even in the progeny. Although priming performed poorly with respect to seed quality under field conditions, its effects have a greater potential to be carried to the progeny generation than those of coating. It is important

to also note that the short-term storage used in this study was insufficient to induce a great response by green beans.

References

Black, M and J. D. Bewley. 1982. Physiology and Biochemistry of seeds: In relation to germination. Vol 2. Springer- Verlag publishers. New York 1 – 56.

Bryant, J. A.1985. Seed physiology: Studies in Biology no. 165. Edward Arnold Publishers Ltd, London, USA. 53 – 71.

Copeland L. O. and M. B. McDonald. 1995. Principles of seed science and technology 3rd edn. Chapman and Hall 258 - 276.

Marschner, H. 1995. Mineral nutrition of higher plants. 2nd edn. Academic Press. New York 285 - 299.

CHAPTER 5

PRELIMINARY INVESTIGATION INTO THE GENETIC EXPLANATION OF COTYLEDONAL CRACKING IN GREEN BEANS

5.1 Introduction

The differences between cultivars with respect to TVC was shown to be associated with the rate if imbibition and also closely related to seed size (chapter 2). The possibility that TVC is a genetic trait, however, could not be ignored. Understanding of genetic relationship between TVC and seed size or cultivar could be important in explaining the response to calcium treatments. That is, could the alleviation of TVC by calcium treatments be associated with gene expression? Reportedly, TVC was inadvertently introduced in green beans (snap beans) decades ago when breeders were selecting for the white seed coat (Morris, 1971). Thus resistance to TVC was lost with seed coat colour. In the present study, white-seeded cultivars were examined and found to vary in the propensity to TVC (Chapters 2-4).

According to Hunter (2002), it is useful to think of genetic diversity at four levels of organisation: among species, among populations, within populations, and within individuals. Hence, it is to be expected that genetic diversity, within populations, exists among the different green bean cultivars examined for cotyledonal cracking in the studies preceding the present one (chapters 2 to 4). Details of how genetic diversity arises through processes such as mutation and natural selection were presented by Hartl and Clark, (1997) and Hartl (2000).

There are many methods of molecular analysis of genetic diversity, including protein electrophoresis, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPDs), microsatellites or simple sequence repeat (SSR) polymorphisms, amplified fragment length polymorphisms (AFLP) and DNA sequencing. Protein electrophoresis, the method selected for this study, is an indirect measure of genetic diversity. It involves determining the rate at which proteins move through a gel when subjected to an electrical field. Different alleles produce different variations of enzymes that move at different rates as a result of differences in size. Variations may represent functionally similar forms of enzymes (isozymes) or variants of enzymes that are different allelic forms of the same gene locus (allozymes). Detection of alleles permits study of variation among populations or similar taxonomic forms.

In this study, a preliminary investigation into the genetic differences between six green bean (*Phaseolus vulgaris* L.) cultivars was undertaken. The objective of the study was to correlate cultivar predisposition to TVC, as explained in chapters 2 3, with protein profiles of the cultivars. It is speculated that cultivars could be categorised into susceptible and resistant groups using crack appearance rate (chapter 3) and imbibition rate (chapter 2), which might be affected by the type of proteins expressed during imbibition. However, it is not known whether the proteins involved are storage or enzyme types.

5.2 Materials and methods

5.2.1 Protein extraction

Seeds of six green bean cultivars (Imbali, Sodwana, Outeniqua, Elangeni, Tokai and Tongati) were used for the extraction of proteins according to Gallardo *et al.* (2002). The seeds were imbibed overnight in petri dishes on 2 Whatman No. 1 filter papers (6 seeds in 12 ml of distilled water). The seeds were then blot-dried and ground (3 seeds) in liquid nitrogen. Proteins were extracted from 1.5 g of seed by adding 1.2 ml of lysis buffer [9.5 M urea, 2% (w/v), Triton X100, 0.8% (w/v), Pharmalyte pH 3-10, 1% (w/v), dithiothreitol (DTT) and 5 mM Pefabloc] and incubating the samples at room temperature for 1 h. This was followed by centrifugation at 4°C for 10 minutes at 16500 rpm. The centrifugation was repeated on the supernatant and the pellet discarded. The samples were then diluted (X100) and added to the "working reagent"[Micro BCA (Reagent A, B and C in a ratio of 25:24:1 (v/v)) in a 1:1 ratio (250 µl sample and 250 µl working reagent), and the BSA standards (1, 5, 10, 20, 40, and 100 µg/ml) (Gorg *et al.*, 2003). The system was then incubated at 60°C for 1 h. After cooling at room temperature, absorbance readings were taken at 562 nm. The absorbance readings of the BSA standards were used to plot a standard curve, which was used to determine the protein concentrations of the samples. Protein profiles of the different cultivars were created using, SDS-PAGE gels, loading 20 µg of sample in each well. The gel was allowed to run for 2 h at 200V (50 A) and stained (45% methanol, 10% acetic acid, 45% distilled H_2O, 0.25% Comassie blue) overnight. Distaining was carried out for 4 h.

5.3 Results

Results showed that there were differences in the protein profiles of the six cultivars (Figure 5.1). The cultivars shared some of the proteins but there were also differences in protein expression among cultivars. Imbali, which was a small-seeded cultivar, had more proteins in common with Tokai (another small-seeded cultivar) (Figure 5.1). These cultivars also showed similar imbibition profiles (Figure 2.4). Outeniqua (a moderate sized cultivar) was found to have a distinct protein (~ 20 KDa) that was also present, but with less expression, in Sodwana (large-seeded cultivar). Outeniqua and Sodwana shared similar imbibition profiles (Figure 2.1).

Comparison of the three cultivars that were selected and studied for susceptibility to cotyledonal cracking in the laboratory (chapter 2) (Imbali, Outeniqua and Sodwana), with respect to protein profiles, showed that Outeniqua and Sodwana (susceptible cultivars, see Figure 2.10) shared a common protein (~ 20 KDa) that was not present in Imbali (TVC-resistant cultivar; see chapters 2 and 3).

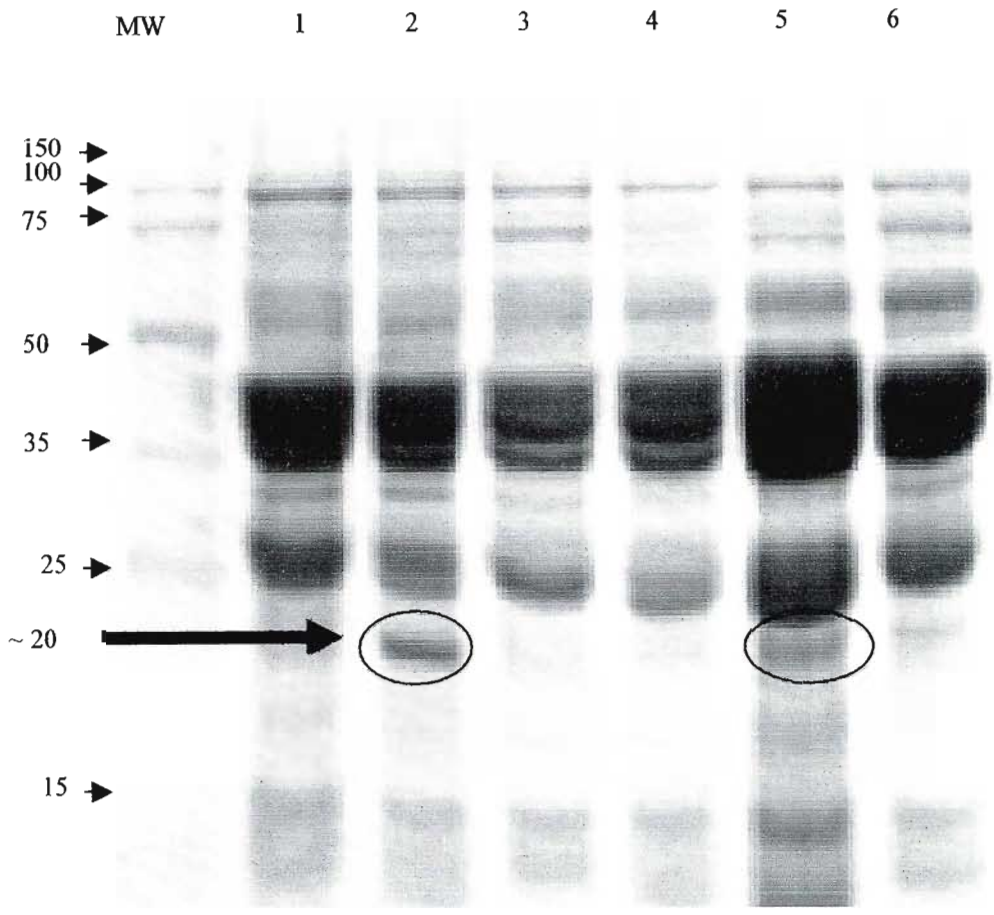


Figure 5.1. SDS-PAGE protein profiles for six cultivars (1= Elangeni, 2 = Outeniqua, 3 = Imbali, 4 = Tokai, 5 = Sodwana and 6. = Elangeni) to determine differences between the cotyledonal cracking-resistant cultivar (Imbali) and cotyledonal-cracking susceptible cultivars (Outeniqua and Sodwana). The susceptible cultivars shared a common protein, ~20 KDa, that was missing from the resistant cultivar. MW = molecular weight marker.

5.4 Discussion

The finding that TVC-susceptible cultivars shared a common protein, expressed in non-germinating seeds, which was absent in TVC-resistant cultivars provided an interesting insight into the molecular level of TVC. While this study did not produce sufficient evidence to suggest that differences in proteins and their expression can be used to determine whether a cultivar is resistant or susceptible to TVC, the protein profiles shown in Figure 5.1 could be used to as a basis to determine whether susceptibility to TVC is genetically controlled. Although these proteins were not identified, it is likely that they influence TVC by regulating factors that affect cell wall integrity and (or) imbibition rate. Seed treatment with calcium may not affect the genetic make up of the seed, but it could influence expression of certain proteins that are involved in imbibition and minimise the occurrence of TVC. Hence, it would be important for future studies to investigate variations in proteins (or protein expression) in response to calcium treatment. The SDS-polyacrylamide protein analysis has previously been used (Kermode and Bewley, 1985; Blackman *et al.*, 1992) to identify genetic changes in legume seeds during development and germination. However, no previous studies have shown patterns of proteins in any legume seeds to explain the genetic basis for transverse cotyledonal cracking.

References

Blackman, S.A., R.L. Obendorf, and A.C. Leopold. 1992. Maturation proteins and sugars in desiccation tolerance of developing soybean seeds. *Plant Physiology* 100: 225-230.

Gallardo, K, C. Job, S.P.C. Groot, M. Puype, H. Demol, J. Vandekerckhove and D. Job. 2002. Importance of methionine biosynthesis for Arabidopsis seed germination and seedling growth. *Physiologia Plantarum* 116:238 - 247.

Gorg, A, G. Boguth, O. Drews, A. Kopf, C. Luck, G. Reil and W. Weiss. 2003. Two-dimensional electrophoresis with immobilised pH gradients for proteome analysis. Technical University of Munich, Munich, Germany.

Kermode, A.R. and J.D. Bewley. 1985. The role of maturation drying in the transition from seed development to germination. Acquisation of desiccation tolerance and germinability during development of *Ricinus communis* L. seeds. *Journal of Experimental Botany* 36:1906 - 1915.

Hartl, D.L. 2000. A primer population genetics, 3rd edition. Sinauer, Suderland, Massachusettes, USA.

Hartl, D. L. and A. G. Clark. 1997. Principles of population genetics., 3rd edition. Sinauer, Suderland, Massachusettes, USA.

Hunter, M.L. 2002. Fundamentals of conservation biology, 2nd edition. Blackwell Science. Massachusetts, USA.

Morris, J. L. 1971. The breeding aspects of vegetable seed quality. *HortScience* 6: 553 – 555.

CHAPTER 6

GENERAL DUSCUSSION AND CONCLUSIONS

Transverse cotyledonal cracking (TVC) is an important problem in the production of green beans and the present information that is available on the study of this disorder does not give conclusive and sound methods for its alleviation. Seed enhancement methods such as priming and seed coating using calcium salts were suggested as means of minimising the effects of TVC in susceptible cultivars.

The effect of seed coat permeability and rate of water absorption was evident in the large-seeded cultivars, which tended to absorb water rapidly at the early stages of imbibition. This rapid in-rush of water into the seeds can cause severe disruption of the cell wall depending on the initial seed moisture content and seed cell wall integrity. For TVC, a susceptible cultivar, which may have tiny cracks in cell walls, rapid water uptake is likely to increase cracking, thus decrease crop stand establishment and possibly yield. Physically, cotyledonal cracking is increased during the inrush of water by stretching of the wet outer part of the cotyledon, which causes the dry inner parts to break. Susceptible cultivars were associated with high water absorption rates. It would be interesting to investigate whether the high rate of water absorption is also associated with permeability of the seed coat. Large legume seeds are generally known to have thin seed coats.

Since cracking compromises cell wall integrity, leaking of electrolytes from the embryo ensues in TVC-susceptible cultivars. Hence, poor stand establishment and yield are also common in these cultivars. Regulating the water absorption rate of the seeds during imbibition can help to minimise the effect or occurrence of TVC. Besides regulating the

water uptake by lowering water potential, calcium salt solutions also supply seeds with calcium, which is probably beneficial in enhancement of cell wall strength and possibly activation of physiological processes during and after germination. This is very important for TVC-susceptible seeds since lack of or insufficient calcium in the cell wall has been implicated as one of the factors that contribute in the occurrence of TVC. It is also important at this point to note that no previous study that showed direct involvement of calcium in TVC was found.

The low water potential gradient between the calcium salt solutions of high concentrations and the seeds during seed priming caused a steady water uptake by the seeds, thus ensuring minimal cell wall disruption. Calcium salt concentrations of 50 mM are ideal to use in the calcium seed treatment when trying to alleviate TVC, because they did not show any negative effect on germination and emergence, which was observed with the higher salt concentrations (100 and 1000 mM). Higher calcium concentrations inhibit sufficient water absorption, which delays germination.

Seed enhancement treatments (priming and coating) improved seed performance under both controlled and field conditions. Calcium seed treatment reduced TVC of the sseedlings derived from direct seed treatment, under field conditions, and in the first progeny seedlings under greenhouse conditions. Osmopriming performed poorly when compared to seed coating, probably because of the damage to seeds during dehydration. Seed coating may not have had the same effect on water absorption as priming. It may, however, have been less effective in supplying the seed with calcium. The main effect of

seed coating may have been in the seed coat permeability. Calcium is mainly deposited in the seed coat during seed coating, which may have reduced the seed coat permeability, thus regulating the rate of water uptake by the seed; which in turn minimised crack occurrence. Once again concentrations of 50 mM significantly reduced the number of cracks and did not have any negative effect on emergence and seedling stand establishment in the field experiments. Reduction in the number of cracks caused reduction in the quantity of electrolytes leaching out of seeds during imbibition, which in turn improved seedling stand establishment in the field. Seedlings that grow vigorously are able to withstand harsh field conditions, such as infection by plant diseases. Because the effect of the calcium salts on the reduction of TVC was found to persist to the progeny of treated seeds, it may not be necessary to treat the F1 generation seeds with calcium again. However, this claim needs to be further investigated.

Attempts to establish a protocol that can be used to identify TVC-susceptible seeds before planting using sucrose density gradient and X-ray studies were not successful. The number of cracks on the seeds that had floated and those that sank during seed separation using sucrose a (2%) sucrose solution was not significantly different and X-ray studies failed to detect the cracks on the cotyledons. This may be because the cracks were too small or absent prior to imbibition. When the same seeds were allowed to imbibe water for 6 hours and the seed coat removed to observe the presence or absence of cracks, it was found that most seeds had cracks. The cracks were observed in seeds that had floated and not those that sank during seed separation and this was in agreement with the germination tests conducted using seeds from the sucrose density gradient studies (data

not shown). In light of these observations, it is recommended concluded that green bean seeds be treated with calcium salts prior to planting, as there is no known method of detecting TVC-susceptible seeds. Conductivity studies on their own cannot be used conclusively and exclusively as indicators of TVC-susceptibility as there are many factors that contribute to the leaking of electrolytes from the seeds.

Laboratory and field results showed that increasing calcium salt concentration resulted in a decrease in the occurrence of cracks. Determination of the protein profiles of the seeds treated with different salt concentrations can be a more convenient way of determining whether there were any differences at the molecular level due to calcium treatment. The current study has already established that there are differences in the protein profiles of the different cultivars, however more investigations into the effects of calcium are required. Identification of the proteins that represent variations will take the elucidation of genetic differences a significant step forward.

More studies need to be conducted to verify the persistence of calcium seed treatment to the first generation using more cultivars than those that were used in the present study. More understanding is required of what is happening to the seed as it takes up calcium during imbibition and seed coating and where the calcium is distributed in the cotyledons. Electron microscopy studies can be of great help in this investigation.

Previous studies on TVC have shown conflicting evidence on the effect of calcium on TVC. Dickson and Boettger (1976) found a negative correlation between calcium in the

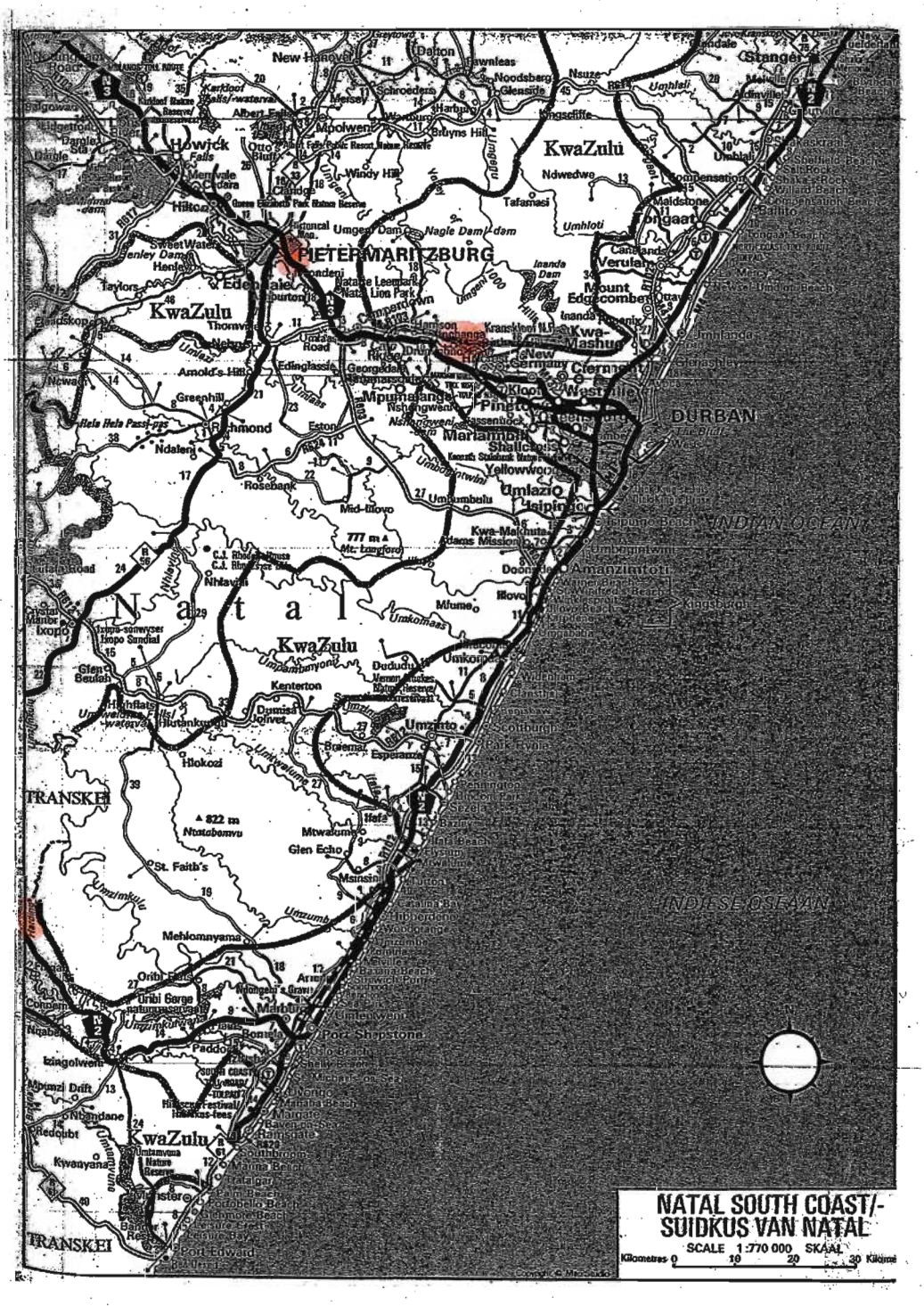
cotyledons and TVC whereas Bradford and Eisinger (1986) found no correlation between the two. These two studies confirmed that supplying calcium to the seedlings in the form of foliar sprays increased the amount of calcium content in the seedlings. The problem with foliar sprays is the lack of certainty that the seeds will be able to take up the supplied calcium, as is the case with the stem and leaves. The present study was the first to use seed treatments to alleviate TVC with calcium. Seed calcium treatments target the seed, and improve the calcium content in the parent seeds reducing the occurrence of cracks in both the parent and F1 generation. Seed calcium treatment can be a more effective way of alleviating cotyledonal cracking in green beans and other leguminous plants because it does not only regulate the rate of water absorption but it also supplements the seeds with calcium. Seed enhancement using calcium and suitable storage conditions prior to planting could also improve the performance of seeds susceptible to TVC.

References

Bradford, K. J. and B. A. Eisinger. 1986. Role of seed mineral in the occurrence of transverse cotyledonal cracking of snap bean. *Journal of the American Society of Horticultural Science* 111: 110 – 114.

Dickson, M. H. and M. A. Boettger, 1976. Factors associated with resistance to mechanical damage in snap beans (*Phaseolus vulgaris* L.). *Journal of the American Society of Horticultural Science* 101: 541 – 544.

APPENDIX 1. Locations of experimental sites (Harding, Inchanga and Pietermaritzburg).



APPENDIX 2. Plan of field plot experiment showing plot numbers (top 1,2,3,...216), salt application methods (P = priming and CT = coating), cultivars (Q, K, G, L, S and I, bottom left), calcium salt and concentration (A1,B1, C1, bottom right). Treatment were allocated randomly according to a split-plot design. Specifications

Plot size: 1 m single row; Seeding rate: 50 cm between rows: 10 cm within rows. Fertilizer: 40 N: 60 P: 40 K kg. ha^{-1};Irrigation: Dr land; Weed control: Mechanical.**Cultivars:** Q = Outeniqua, K = Tokai, G = Tongati, L = Elangeni, S = Sodwana, I = Imbali.**Salts:** A Calcium chloride ($CaCl_2$), B = Calcium nitrate ($Ca(NO_3)_2$), C = Calcium sulphate ($CaSO_4$).

1P Q C50	19 CT Q C50	37P K B1	55CT KB1	73CT GA1000	91P GA1000	109P LB0	127CT LB0	145CT SA100	163P SA100	181P IC10	199C IC10
2CT Q C10	20 P Q C10	38CT K B50	56P K B50	74P GA1	92CT GA1	110CT LB50	128P LB50	146CT SA10	164P SA10	182C IC0	200P IC0
3CT Q C1	21 P Q C1	39P K B0	57CT K B0	75P GA10	93CT GA10	111CT LB1	129P LB1	147P SA50	165C SA50	183C IC100	201P IC100
4C Q C0	22 P Q C0	40CT K B100	58P K B100	76CT GA50	94P GA50	112P LB1000	130CT LB1000	148CT SA1	166P SA1	184P IC50	202C IC50
5CT Q C100	23 P Q C100	41CT K B10	59P K B10	77CT GA100	95P GA100	113P LB10	131CT LB10	149P SA1000	167C SA1000	185C IC1	203P IC1
6C QC1000	24P QC1000	42CT KC1000	60P KC1000	78P GA0	96CT GA0	114CT LB100	132P LB100	150P SA0	168C SA0	186P IC1000	204C IC1000
7P Q A1	25CT Q A1	43P K C1	61CT K C1	79P GB50	97CT GB50	115P LC1	133CT LC1	151C SB50	169P SB50	187C IA0	205P IA0
8CT Q A50	26P Q A50	44CT KC100	62P KC100	80CT GB1000	98P GB1000	116P LC0	134CT LC0	152P SB100	170C SB100	188P IA1	206C IA1
9P QA1000	27CT QA1000	45P K C10	63CT K C10	81P GB1	99CT GB1	117CT LC1000	135P LC1000	153C SB10	171P SB10	189C IA50	207P IA50
10P Q A0	28CT Q A0	46CT K C50	64P K C50	82P GB0	100CT GB0	118CT LC100	136P LC100	154P SB1	172C SB1	190C IA1000	208P IA1000
11CT QA100	29P QA100	47P K C0	65CT K C0	83CT GB100	101P GB100	119P LC10	137CT LC10	155C SB1000	173P SB1000	191P IA10	209C IA10
12CT QA10	30P QA10	48CT K C50	66P K C50	84P GB10	102CT GB10	120CT LC50	138P LC50	156P SB0	174C SB0	192C IA100	210P IA100
13CT Q B1	31P Q B1	49CT K A1	67P K A1	85P GC0	103CT GC0	121P LA1000	139CT LA1000	157C SC1	175P SC1	193P IB50	211C IB50
14P QB50	32CT QB50	50P KA100	68CT KA100	86CT GC1000	104P GC1000	122P LA0	140CT LA0	158P SC50	176C SC50	194C IB1	212P IB1
15CT Q B100	33P Q B100	51P K A50	69CT K A50	87P GC100	105CT GC100	123P LA10	141CT LA10	159C SC1000	177P SC1000	195P IB0	213C IB0
16CT Q B0	34P Q B0	52CT K A10	70P K A10	88CT GC50	106P GC50	124CT LA1	142P LA1	160P SC100	178C SC100	196C IB1000	214P IB1000
17P QB1000	35CT QB1000	53P KA0	71CT K A0	89CT GC1	107P GC1	125P LA50	143CT LA50	161P SC0	179C SC0	197P IB100	215C IB100
18CT Q B10	36P Q B10	54P KA1000	72CT KA1000	90CT GC10	108P GC10	126CT LA100	144P LA100	162C SC10	180P SC10	198C IB10	216P IB10

APPENDIX 3.1. Analysis of variance tables for laboratory experiments

A. Analysis of variance for cracks (TVC) in the presence of the seed coat, under laboratory conditions

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	0.1084	0.0361	0.36	
Rep.*Units* stratum					
cultivar	2	2.1715	1.0858	10.70	<.001
salt	2	3.0115	1.5057	14.84	<.001
salt level	5	5.6096	1.1219	11.06	<.001
time	4	44.5585	11.1396	109.79	<.001
cultivar.salt	4	0.2661	0.0665	0.66	0.623
cultivar.saltlevel	10	4.8178	0.4818	4.75	<.001
salt.saltlevel	10	4.9482	0.4948	4.88	<.001
cultivar.time	8	8.6862	1.0858	10.70	<.001
salt.time	8	12.0460	1.5057	14.84	<.001
saltlevel.time	20	22.4386	1.1219	11.06	<.001
Residual	1002(1)	101.6671	0.1015		
Total	1078(1)	209.9889			

B. Analysis of variance for seed mass change (TVC) in the presence of the seed coat, under laboratory conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	6172.3	2057.4	5.77	
Rep.*Units* stratum					
cultivar	2	26248.3	13124.1	36.83	<.001
salt	2	13408.4	6704.2	18.81	<.001
salt level	5	19829.9	3966.0	11.13	<.001
time	4	252168.6	63042.2	176.89	<.001
cultivar.salt	4	692.1	173.0	0.49	0.746
cultivar.saltlevel	10	16629.9	1663.0	4.67	<.001
salt.saltlevel	10	30369.7	3037.0	8.52	<.001
cultivar.time	8	9970.2	1246.3	3.50	<.001
salt.time	8	4985.4	623.2	1.75	0.084
saltlevel.time	20	14480.6	724.0	2.03	0.005
Residual	1003	357460.4	356.4		
Total	1079	752415.8			

C. Analysis of variance for cracks (TVC) in the absence of the seed coat, under laboratory conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	5.7778	1.9259	6.44	
Rep.*Units* stratum					
cultivar	2	25.3685	12.6843	42.44	<.001
Salt	2	1.8296	0.9148	3.06	0.047
Salt level	5	25.1296	5.0259	16.82	<.001
Time	4	25.9778	6.4944	21.73	<.001
cultivar.salt	4	2.6204	0.6551	2.19	0.068
cultivar.saltlevel	10	17.5537	1.7554	5.87	<.001
salt.saltlevel	10	12.7926	1.2793	4.28	<.001
cultivar.time	8	12.2333	1.5292	5.12	<.001
salt.time	8	1.8556	0.2319	0.78	0.624
saltlevel.time	20	20.1667	1.0083	3.37	<.001
Residual	1003	299.7463	0.2988		
Total	1079	451.0519			

D. Analysis of variance for seed mass change (TVC) in the absence of the seed coat, under laboratory conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4635.9	1545.3	6.88	
Rep.*Units* stratum					
cultivar	2	29130.5	14565.2	64.88	<.001
salt	2	20440.2	10220.1	45.52	<.001
salt level	5	21462.2	4292.4	19.12	<.001
time	4	970655.0	242663.7	1080.90	<.001
cultivar.salt	4	4714.7	1178.7	5.25	<.001
cultivar.saltlevel	10	15832.0	1583.2	7.05	<.001
salt.saltlevel	10	25484.8	2548.5	11.35	<.001
cultivar.time	8	8780.9	1097.6	4.89	<.001
salt.time	8	8523.6	1065.4	4.75	<.001
saltlevel.time	20	15523.0	776.1	3.46	<.001
Residual	1003	225175.8	224.5		
Total	1079	1350358.6			

E. Analysis of variance for cracks (overall ana ysis) under laboratory conditions

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	2.5203	0.8401	3.88	
Rep.*Units* stratum					
cultivar	2	18.5451	9.2726	42.79	<.001
treatment	1	10.4195	10.4195	48.08	<.001
salt	2	4.1790	2.0895	9.64	<.001
salt level	5	23.7903	4.7581	21.96	<.001
time	4	49.0925	12.2731	56.63	<.001
cultivar.treatment	2	8.2549	4.1274	19.05	<.001
cultivar.salt	4	1.5391	0.3848	1.78	0.131
treatment.salt	2	0.4108	0.2054	0.95	0.388
cultivar.saltlevel	10	10.2783	1.0278	4.74	<.001
treatment.saltlevel	5	5.2452	1.0490	4.84	<.001
salt.saltlevel	10	3.4670	0.3467	1.60	0.101
cultivar.time	8	14.1480	1.7685	8.16	<.001
treatment.time	4	12.4409	3.1102	14.35	<.001
salt.time	8	6.0908	0.7614	3.51	<.001
saltlevel.time	20	27.7042	1.3852	6.39	<.001
Residual	2067(2)	447.9370	0.2167		
Total	2157(2)	645.8448			

APPENDIX 3.2. Amount of calcium absorbed by different cultivars at different calcium salt molarities after 6 h of priming. Values are means of response to priming in $CaCl_2$, $Ca(NO_3)_2$ and $CaSO_4$. Note: LSD (cultivars =0.023, molarity = 0.023, molarity x cultivar = 0.056)

Molarity (mM)	Elangeni	Imbali	Outeniqua	Sodwana	Tokai	Tongati	Mean (molarity)
0	0.0059	0.0071	0.0058	0.0038	0.0043	0.0059	0.0055
1	0.2607	0.4261	0.0368	0.0262	0.1028	0.1252	0.1629
10	0.4311	0.5161	0.0565	0.0559	0.3914	0.306	0.293
50	0.6048	0.6581	0.0883	0.2124	0.4754	0.4778	0.420
100	0.7623	0.8041	0.3178	0.6675	0.6448	0.9183	0.686
1000	1.6529	1.191	0.5396	1.0998	0.9419	1.4128	1.14
Mean (cultivar)	0.62	0.60	0.174	0.344	0.427	0.541	

APPENDIX 4.1 Analysis of variance tables for field experiments

A. Analysis of variance for cracks (TVC) under field conditions in 2001

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site stratum	1	2.3882	2.3882	17.46	
Site.*Units* stratum					
cultivar	5	1.7727	0.3545	2.59	0.027
salt	2	0.1756	0.0878	0.64	0.527
salt level	5	2.9197	0.5839	4.27	0.001
treatment	1	6.4680	6.4680	47.28	<.001
cultivar.salt	10	1.4476	0.1448	1.06	0.396
cultivar.saltlev	25	2.4263	0.0971	0.71	0.845
salt.saltlev	10	0.5864	0.0586	0.43	0.932
cultivar.treat	5	1.1296	0.2259	1.65	0.148
salt.treat	2	0.1739	0.0869	0.64	0.531
saltlev.treat	5	0.8218	0.1644	1.20	0.310
cultivar.salt.saltlev	50	6.8685	0.1374	1.00	0.474
cultivar.salt.treat	10	1.6825	0.1682	1.23	0.273
cultivar.saltlev.treat	25	3.2365	0.1295	0.95	0.541
salt.saltlev.treat	10	0.8860	0.0886	0.65	0.772
cultivar.salt.saltlev.treat	50	14.0252	0.2805	2.05	<.001
Residual	215	29.4124	0.1368		
Total	431	76.4207			

B. Analysis of variance for cracks (TVC) under field conditions in 2002

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Site stratum	1	2.3882	2.3882	17.46	
Site.*Units* stratum					
cultivar	5	1.7727	0.3545	2.59	0.027
salt	2	0.1756	0.0878	0.64	0.527
saltlev	5	2.9197	0.5839	4.27	0.001
treat	1	6.4680	6.4680	47.28	<.001
cultivar.salt	10	1.4476	0.1448	1.06	0.396
cultivar.saltlev	25	2.4263	0.0971	0.71	0.845
salt.saltlev	10	0.5864	0.0586	0.43	0.932
cultivar.treat	5	1.1296	0.2259	1.65	0.148
salt.treat	2	0.1739	0.0869	0.64	0.531
saltlev.treat	5	0.8218	0.1644	1.20	0.310
cultivar.salt.saltlev	50	6.8685	0.1374	1.00	0.474
cultivar.salt.treat	10	1.6825	0.1682	1.23	0.273
cultivar.saltlev.treat	25	3.2365	0.1295	0.95	0.541
salt.saltlev.treat	10	0.8860	0.0886	0.65	0.772
cultivar.salt.saltlev.treat	50	14.0252	0.2805	2.05	<.001
Residual	215	29.4124	0.1368		
Total	431	76.4207			

C. Analysis of variance for seedling emergence under field conditions in 2001

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	1	96.333	96.333	24.32	
site.*Units* stratum					
cultivar	5	717.528	143.506	36.23	<.001
salt	2	37.097	18.549	4.68	0.01
salt level	5	86.806	17.361	4.38	<.001
treat	1	37.926	37.926	9.57	0.002
cultivar.salt	10	43.375	4.338	1.09	0.367
cultivar.saltlev	25	68.583	2.743	0.69	0.862
salt.saltlev	10	105.097	10.510	2.65	0.005
cultivar.treat	5	38.796	7.759	1.96	0.086
salt.treat	2	9.949	4.975	1.26	0.287
saltlev.treat	5	29.796	5.959	1.50	0.190
cultivar.salt.saltlev	50	174.431	3.489	0.88	0.698
cultivar.salt.treat	10	28.079	2.808	0.71	0.716
cultivar.saltlev.treat	25	78.815	3.153	0.80	0.745
salt.saltlev.treat	10	18.245	1.825	0.46	0.914
cultivar.salt.saltlev.treat	50	231.394	4.628	1.17	0.225
Residual	215	851.667	3.961		
Total	431	2653.917			

D. Analysis of variance for seedling emergence under field conditions in 2002

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	1	96.333	96.333	24.32	
site.*Units* stratum					
cultivar	5	717.528	143.506	36.23	<.001
salt	2	37.097	18.549	4.68	0.010
Salt level	5	86.806	17.361	4.38	<.001
Treatment	1	37.926	37.926	9.57	0.002
cultivar.salt	10	43.375	4.338	1.09	0.367
cultivar.saltlev	25	68.583	2.743	0.69	0.862
salt.saltlev	10	105.097	10.510	2.65	0.005
cultivar.treat	5	38.796	7.759	1.96	0.086
salt.treat	2	9.949	4.975	1.26	0.287
saltlev.treat	5	29.796	5.959	1.50	0.190
cultivar.salt.saltlev	50	174.431	3.489	0.88	0.698
cultivar.salt.treat	10	28.079	2.808	0.71	0.716
cultivar.saltlev.treat	25	78.815	3.153	0.80	0.745
salt.saltlev.treat	10	18.245	1.825	0.46	0.914
cultivar.salt.saltlev.treat	50	231.394	4.628	1.17	0.225
Residual	215	851.667	3.961		
Total	431	2653.917			

E. Analysis of variance for seedling height under field conditions in 2001

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	2	9445.6	4722.8	7.98	
site.*Units* stratum					
cultivar	5	453710.4	90742.1	153.37	<.001
salt	2	1461.1	730.6	1.23	0.292
salt level	5	3055.4	611.1	1.03	0.398
treatment	1	6674.2	6674.2	11.28	<.001
cultivar.salt	10	6463.3	646.3	1.09	0.366
cultivar.saltlevel	25	23198.6	927.9	1.57	0.041
salt.saltlevel	10	8568.4	856.8	1.45	0.157
cultivar.treatment	5	13688.2	2737.6	4.63	<.001
salt.treatment	2	3422.3	1711.1	2.89	0.057
saltlevel.treatment	5	6361.6	1272.3	2.15	0.059
cultivar.salt.saltlevel	50	24811.3	496.2	0.84	0.775
cultivar.salt.treatment	10	3046.6	304.7	0.51	0.880
cultivar.saltlevel.treatment	25	14268.6	570.7	0.96	0.514
salt.saltlevel.treatment	10	2172.7	217.3	0.37	0.960
cultivar.salt.saltlevel.treat	50	20960.9	419.2	0.71	0.933
Residual	430	254405.1	591.6		
Total	647	855714.1			

F. Analysis of variance for seedling height under field conditions in 2002

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	2	9742.3	4871.2	7.63	
site.*Units* stratum					
cultivar	5	453019.8	90604.0	141.94	<.001
salt	2	3772.5	1886.3	2.96	0.053
salt level	5	2115.4	423.1	0.66	0.652
treatment	1	10871.6	10871.6	17.03	<.001
cultivar.salt	10	9249.6	925.0	1.45	0.156
cultivar.saltlevel	25	24298.6	971.9	1.52	0.052
salt.saltlevel	10	6282.9	628.3	0.98	0.456
cultivar.treatment	5	10161.3	2032.3	3.18	0.008
salt.treatment	2	4455.1	2227.5	3.49	0.031
saltlevel.treatment	5	6912.4	1382.5	2.17	0.057
cultivar.salt.saltlevel	50	21086.4	421.7	0.66	0.964
cultivar.salt.treatment	10	4514.6	451.5	0.71	0.718
cultivar.saltlevel.treatment	25	14336.4	573.5	0.90	0.608
salt.saltlevel.treatment	10	1580.3	158.0	0.25	0.991
cultivar.salt.saltlevel.treat	50	23770.6	475.4	0.74	0.900
Residual	430	274478.9	638.3		
Total	647	880648.6			

G. Analysis of variance for yield (number of seeds/m^2) under field conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
cultivar	5	253845.	50769.	27.94	<.001
salt	2	8188.	4094.	2.25	0.109
salt level	5	7002.	1400.	0.77	0.572
treatment	1	2517.	2517.	1.38	0.241
cultivar.salt	10	23791.	2379.	1.31	0.231
cultivar.saltlevel	25	64182.	2567.	1.41	0.107
salt.saltlevel	10	16704.	1670.	0.92	0.517
cultivar.treatment	5	8757.	1751.	0.96	0.442
salt.treatment	2	1296.	648.	0.36	0.701
saltlevel.treatment	5	5561.	1112.	0.61	0.691
Residual	145	263505.	1817.		
Total	215	655348.			

H. Analysis of variance for plant efficiency (Fo) under field conditions in 2001

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	2	1218948.	609474.	27.48	
site.*Units* stratum					
cultivar	5	1154889.	230978.	10.42	<.001
salt	2	29724.	14862.	0.67	0.512
salt level	5	93312.	18662.	0.84	0.521
treatment	1	339442.	339442.	15.31	<.001
cultivar.salt	10	827574.	82757.	3.73	<.001
cultivar.saltlevel	25	779331.	31173.	1.41	0.094
salt.saltlevel	10	174789.	17479.	0.79	0.640
cultivar.treatment	5	895471.	179094.	8.08	<.001
salt.treatment	2	43578.	21789.	0.98	0.375
saltlevel.treatment	5	85847.	77169.	3.48	0.004
cultivar.salt.saltlevel	50	650507.	13010.	0.59	0.989
cultivar.salt.treatment	10	55192.	5519.	0.25	0.991
cultivar.saltlevel.treatment	25	399587.	15983.	0.72	0.837
salt.saltlevel.treatment	10	235343.	23534.	1.06	0.391
cultivar.salt.saltlevel.treatm	50	971396.	19428.	0.88	0.712
Residual	430	9535908.	22177.		
Total	647	17790838.			

I. Analysis of variance for plant efficiency (Fo) under field conditions in 2002

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	2	1138325.	569162.	26.57	
site.*Units* stratum					
cultivar	5	1177194.	235439.	10.99	<.001
salt	2	23019.	11510.	0.54	0.585
salt level	5	42178.	8436.	0.39	0.853
treatment	1	479438.	479438.	22.39	<.001
cultivar.salt	10	852741.	85274.	3.98	<.001
cultivar.saltlevel	25	739417.	29577.	1.38	0.106
salt.saltlevel	10	155881.	15588.	0.73	0.698
cultivar.treatment	5	775693.	155139.	7.24	<.001
salt.treatment	2	82133.	41066.	1.92	0.148
saltlevel.treatment	5	314477.	62895.	2.94	0.013
cultivar.salt.saltlevel	50	807545.	16151.	0.75	0.891
cultivar.salt.treatment	10	133183.	13318.	0.62	0.795
cultivar.saltlevel.treatment	25	367309.	14692.	0.69	0.872
salt.saltlevel.treatment	10	266580.	26658.	1.24	0.260
cultivar.salt.saltlevel.treat	50	925061.	18501.	0.86	0.733
Residual	430	9209502.	21417.		
Total	647	17489678.			

J. Analysis of variance for Fv/Fo ratio under field conditions in 2001

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	2	1372.4	686.2	1.03	
site.*Units* stratum					
cultivar	5	3156.9	631.4	0.95	0.448
salt	2	1336.9	668.4	1.01	0.367
salt level	5	3354.4	670.9	1.01	0.412
treatment	1	646.9	646.9	0.97	0.324
cultivar.salt	10	6680.7	668.1	1.01	0.438
cultivar.saltlevel	25	16677.9	667.1	1.00	0.461
salt.saltlevel	10	6663.6	666.4	1.00	0.440
cultivar.treatment	5	3230.7	646.1	0.97	0.434
salt.treatment	2	1329.4	664.7	1.00	0.369
saltlevel.treatment	5	3343.4	668.7	1.01	0.414
cultivar.salt.saltlevel	50	33284.1	665.7	1.00	0.475
cultivar.salt.treatment	10	6629.3	662.9	1.00	0.445
cultivar.saltlevel.treatment	25	16728.3	669.1	1.01	0.456
salt.saltlevel.treatment	10	6640.4	664.0	1.00	0.443
cultivar.salt.saltlevel.treat	50	33230.4	664.6	1.00	0.478
Residual	430	285813.4	664.7		
Total	647	430119.2			

K. Analysis of variance for Fv/Fo ratio under field conditions in 2002

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	2	1441.7	720.9	0.99	
site.*Units* stratum					
cultivar	5	3714.9	743.0	1.02	0.404
salt	2	1471.7	735.9	1.01	0.364
salt level	5	3660.1	732.0	1.01	0.413
treatment	1	752.9	752.9	1.04	0.309
cultivar.salt	10	7250.7	725.1	1.00	0.445
cultivar.saltlevel	25	18140.2	725.6	1.00	0.468
salt.saltlevel	10	7282.5	728.3	1.00	0.441
cultivar.treatment	5	3606.0	721.2	0.99	0.422
salt.treatment	2	1452.7	726.4	1.00	0.369
saltlevel.treatment	5	3615.9	723.2	0.99	0.420
cultivar.salt.saltlevel	50	36333.4	726.7	1.00	0.478
cultivar.salt.treatment	10	7280.2	728.0	1.00	0.441
cultivar.saltlevel.treatment	25	18191.7	727.7	1.00	0.464
salt.saltlevel.treatment	10	7269.3	726.9	1.00	0.442
cultivar.salt.saltlevel.treat	50	36329.5	726.6	1.00	0.478
Residual	430	312540.6	726.8		
Total	647	470334.1			

L. Analysis of variance for total number of seedlings at harvest under field conditions in 2002

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
site stratum	2	875.198	437.599	106.46	
site.*Units* stratum					
cultivar	5	930.068	186.014	45.25	<.001
salt	2	33.262	16.631	4.05	0.018
salt level	5	57.512	11.502	2.80	0.017
treatment	1	128.000	128.000	31.14	<.001
cultivar.salt	10	99.404	9.940	2.42	0.008
cultivar.saltlevel	25	60.654	2.426	0.59	0.944
salt.saltlevel	10	94.404	9.440	2.30	0.012
cultivar.treatment	5	109.537	21.907	5.33	<.001
salt.treatment	2	38.065	19.032	4.63	0.010
saltlevel.treatment	5	33.093	6.619	1.61	0.156
cultivar.salt.saltlevel	50	165.151	3.303	0.80	0.829
cultivar.salt.treatment	10	26.454	2.645	0.64	0.776
cultivar.saltlevel.treatment	25	71.037	2.841	0.69	0.867
salt.saltlevel.treatment	10	16.343	1.634	0.40	0.948
cultivar.salt.saltlevel.treat	50	199.139	3.983	0.97	0.537
Residual	430	1767.469	4.110		
Total	647	4704.790			

APPENDIX 4.2. Bioclimatic regions of locations, and soil taxonomic names at field sites in 2001 and 2002 seasons. Note: The bioclimatic groups are also designated by Arabic numerals 1, 2 and 3 according to the Department of Agricultural Development (1992). Soil names were derived from soil profile and soil chemical analysis data interpreted according to Soil Classification Working Group (1991).

Bioclimatic group	Location	Field site	Soil name
1. Coast lowland	Harding	Bowles2001	Inanda highlands
		Northad2002	Lusiki hopewell
2. Coast hinterland	Pietermaritzburg	Ukulinga2001	Bonheim onrus
		Ukulinga2002	Willowbrook ottawa
3. Mistbelt	Inchanga	Nansindlela2001	Tukulu olivedale
		Nansindlela2002	Clovelly laiden

APPENDIX 4.3. Results of chemical analyses of soils at experimental field sites. The analyses were performed by Soil Fertility and Analytical Services, KwaZulu-Natal Department of Agriculture, Pietermaritzburg).

Field site	Soil density and selected chemical concentrations							
	Density	P	K	Ca	Mg	Acid saturation	pH	Clay
	($g\ mL^{-1}$)	..... ($mg\ L^{-1}$)				(%)	(KCl)	(%)
Bowles2001	1.02	58	63	1280	325	5	4.4	46
Northad2002	0.93	64	46	1292	223	5	4.2	44
Ukulinga2001	0.9	35	130	3705	1403	0	4.6	55
Ukulinga2002	0.95	28	138	3519	1395	1	4.4	53
Nansindlela2001	1.11	88	68	1388	160	1	5.4	25
Nansindlela2002	1.27	96	70	1431	178	1	5.0	29

APPENDIX 5. Analysis of variance tables for greenhouse studies.

A. Analysis of variance for cotyledon wet weight under greenhouse conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	0.438672	0.438672	107.00	<.001
Salt	2	0.064297	0.032148	7.84	<.001
Saltlevel	5	0.257352	0.051470	12.55	<.001
Treatment	1	0.006832	0.006832	1.67	0.197
month	2	0.292477	0.146239	35.67	<.001
Cultivar. Salt	2	0.005291	0.002645	0.65	0.525
Cultivar.Saltlevel	5	0.116084	0.023217	5.66	<.001
Salt.Saltlevel	10	0.132638	0.013264	3.24	<.001
Cultivar. Treatment	1	0.085698	0.085698	20.90	<.001
Salt. Treatment	2	0.022641	0.011320	2.76	0.064
Saltlevel.Treatment	5	0.204583	0.040917	9.98	<.001
Cultivar. month	2	0.026552	0.013276	3.24	0.040
Salt. month	4	0.006759	0.001690	0.41	0.800
Saltlevel.month	10	0.021454	0.002145	0.52	0.874
Treatment. month	2	0.001717	0.000859	0.21	0.811
Residual	569	2.332861	0.004100		
Total	647	4.082512			

B. Analysis of variance for seedling dry weight under greenhouse conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	0.074455	0.074455	13.22	<.001
Salt	2	0.129047	0.064523	11.45	<.001
Saltlevel	5	0.246033	0.049207	8.73	<.001
Treatment	1	0.047022	0.047022	8.35	0.004
month	2	0.285123	0.142562	25.30	<.001
Cultivar. Salt	2	0.009825	0.004912	0.87	0.419
Cultivar.Saltlevel	5	0.178207	0.035641	6.33	<.001
Salt.Saltlevel	10	0.335231	0.033523	5.95	<.001
Cultivar. Treatment	1	0.019361	0.019361	3.44	0.064
Salt. Treatment	2	0.075196	0.037598	6.67	0.001
Saltlevel.Treatment	5	0.256219	0.051244	9.10	<.001
Cultivar. month	2	0.008454	0.004227	0.75	0.473
Salt. month	4	0.002320	0.000580	0.10	0.981
Saltlevel.month	10	0.026918	0.002692	0.48	0.905
Treatment. month	2	0.002068	0.001034	0.18	0.832
Residual	569	3.205706	0.005634		
Total	647	5.061262			

C. Analysis of variance for TVC (cracks) under greenhouse conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
cultivar	1	0.02469	0.02469	0.40	0.527
salt	2	0.09568	0.04784	0.78	0.461
salt level	5	0.38272	0.07654	1.24	0.289
treatment	1	0.09877	0.09877	1.60	0.206
month	2	0.31790	0.15895	2.58	0.077
cultivar.salt	2	0.13272	0.06636	1.08	0.342
cultivar.saltlevel	5	0.14198	0.02840	0.46	0.806
salt.saltlevel	10	0.27469	0.02747	0.45	0.924
cultivar.treatment	1	0.05556	0.05556	0.90	0.343
salt.treatment	2	0.22531	0.11265	1.83	0.162
saltlevel.treatment	5	0.47531	0.09506	1.54	0.175
cultivar. month	2	0.15123	0.07562	1.23	0.294
salt. month	4	0.03395	0.00849	0.14	0.968
saltlevel.month	10	0.44136	0.04414	0.72	0.711
treatment. month	2	0.04012	0.02006	0.33	0.723
Residual	569	35.12037	0.06172		
Total	647	39.25309			

D. Analysis of variance for emergence under greenhouse conditions

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
cultivar	1	20.41	20.41	1.71	0.192
salt	2	46.84	23.42	1.96	0.142
salt level	5	354.38	70.88	5.92	<.001
treat	1	0.00	0.00	0.00	0.991
month	2	94.95	47.47	3.97	0.019
cultivar.salt	2	65.78	32.89	2.75	0.065
cultivar.salt_level	5	100.86	20.17	1.69	0.136
salt.salt_level	10	176.13	17.61	1.47	0.146
cultivar.treat	1	13.35	13.35	1.12	0.291
salt.treat	2	166.02	83.01	6.94	0.001
salt_level.treat	5	268.79	53.76	4.49	<.001
cultivar. month	2	23.69	11.84	0.99	0.372
salt. month	4	54.85	13.71	1.15	0.334
salt_level.month	10	187.13	18.71	1.56	0.114
treat. month	2	148.21	74.10	6.19	0.002
Residual	569	6806.66	11.96		
Total	647	8869.26			