Wild watermelon (*Citrullus lanatus L.*) landrace production in response to three seedling growth media and field planting dates

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Declaration

I hereby declare that the research work reported in this thesis is the result of my own original work except where acknowledged. I also declare that the results of this work have not otherwise been submitted in any form for any degree or diploma to any other university. The study was financially supported by the Water Research Commission (Project No. K5/1771//4).

Signature

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I agree with the above statement.

Signature

Professor Albert T. Modi (Supervisor)

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ABSTRACT

The challenge of food security requires that agricultural production is no longer based on a narrow genetic material present in conventional crops. Whereas conventional crops have been genetically improved to suit management practices of the modern farmer, the future farmer requires that there be access to a wide variety of genetic material for economic exploitation and to respond to the challenges of climate change in a sustainable fashion. This study was designed to learn about production of wild water melon [Citrullus lanatus (Thunb.) Matsumura and Nakai] from seed germination, seedling establishment and field crop production. The specific objectives of the study were (a) to determine the effect of water stress on three landraces of watermelon differing in seed colour and provenance during seedling establishment, (b) to determine the effect of planting date on crop growth and yield under field conditions, and (c) to relate proline accumulation to water stress in wild watermelon. Three seedlots, 'B', 'DB' and 'VDB" were derived from seeds collected from subsistence farming communities of the Eastern Cape, and KwaZulu-Natal. Following one season of seed production in Pietermaritzburg, KwaZulu-Natal, seeds were tested for germination capacity, before seedlot response to water stress was determined in three substrates made of pine bark, a 1:1 mixture of fine sand and pine bark and fine sand only. The substrates were kept at 75% FC, 50% F.C and 25% F.C., to create varying levels of water regimes during 12 weeks of seedling growth in a glasshouse (16/21°C (day/night) and 60% RH). Leaf proline content was determined at seedling harvest. Crop production under field conditions occurred at one site with three planting dates late September 2008, November 2008 and January 2009, respectively. There were significant differences among seedlots with respect to seed quality and seedling yield, which consistently showed that B > VDB > DB. The differences in seedlots continued in the same order even in response to field conditions. Wild watermelon was responsive to water stress during seedling growth, but high water regimes compromised water use efficiency. Proline accumulation correlated with water stress. The best plant growth and yield under field conditions was obtained when planting occurred in September, followed by November and January plantings, respectively. Early planting was also associated with high crop growth rate and larger fruit size. It is concluded that despite being a desert crop, wild watermelon responds to water deficits during seedling growth. Results of field studies cannot be conclusively used to determine crop response to water stress, although they gave a good indication of crop response to different conditions of rainfall and temperature at the study site from September to March.

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Wild vegetables played a significant role in the early history of South Africa. It was their abundance at the Cape and their health-giving properties that induced the Dutch and English ships to call there on the way to the East Indies in the early 1600s, and eventually, the establishment of a food garden, at the recommendation of Jan van Riebeeck and the survivors of the wreck of Haarlem, in 1647 (Fox & Norwood Young, 1982). Since the early 1900s, when many (social) studies of the life and customs of the black tribes of southern Africa were undertaken, there have been few records of South African indigenous plant's food properties. However, evidence for the role of wild vegetables in food security has been shown in Africa elsewhere. Agricultural scientists and development communities in South Africa have largely neglected wild vegetables traditionally used by native Africans in South Africa. In a country that is confronted by HIV/Aids, malnutrition and poverty, wild vegetables should be seriously considered as important sources of minerals and vitamins. Agricultural scientists and nutritionists should work together towards a food production system that enhances the value of indigenous fruits and vegetables.

1.1.1 Crop distribution and description

Wild watermelon [*Citrullus lanatus* (Thunb.) Matsumura and Nakai] is indigenous to south-west Asia and throughout Africa (Fox & Norwood Young, 1982; Shosteck, 1974). Other English names for it include bitter melon, colocynth, common wild melon of South Africa and desert melon. The crop is also cultivated or found in a semi-wild state in the

warmer parts of the world. In South Africa, the melon is often cultivated as an intercrop with maize in homestead gardens (Fox & Norwood Young, 1982). Typical features of wild watermelon are shown in Figure 1.1. The crop is a prostrate, spreading annual vine with many herbaceous, longitudinally grooved, straw-coloured, stout stems, up to 3 m long, hairy when young, becoming smooth later (Whitaker & Davis, 1962). Tendrils are bifid, in the axils of the leaves. Leaves are rough to the touch, ovate, rigid, dull green, hairy on the veins and deeply three-lobed. The lobes are obvate, oblong or linear and the margins are finely serrate. Male flowers are campanulate, up to 300 mm in diameter, hairy, greenish on the side, pale yellow inside. Female flowers are solitary and yellow. The fruit is globes to ellipsoid or oblong, smooth, pale green or grayish-green. It is juicy with a pink or reddish pink flesh. Seeds may be black, dark brown, brown, white or mottled (McDonald & Copeland, 1997). Many varieties of the crop occur mainly because of the ability of the cucurbits to cross-pollinate within the same species.



Figure 1.1 Typical vine, flower and fruit of Citrullus lanatus (Kirkbrde, 1993).

Watermelons can be grown almost anywhere, They require warm, dry weather for maximum production and are particularly sensitive to frost (McDonald & Copeland, 1997).

1.1.2 Crop uses

In South Africa, wild watermelon is eaten by various African tribes. The seed can be ground and made into bread. Often, it is the only source of water in the desert in the dry season when no standing water is available (Moradi & Younesi, 2009). The Koi and San tribes are known to use the fruits as their sole source of water for months on end as they consist of 90% water, and can keep for as long as a year in some cases (Bawa & Bains, 1977; Hour et al., 1980; Wani et al., 2006). Bantus in Botswana cut the fruits into slices and dry them on frames in the sun (Hasan, 1993; Godawa & Jalali, 1995). All the tribes in the Eastern Cape and KwaZulu-Natal use the crop as a staple food either fresh or dried. It is peeled, cut into pieces, boiled and mixed with mealie-meal. The young tender leaves and fruits can also be cooked, seasoned with salt and used as a relish for other foods (Whitaker & Davis, 1962). Zulus, use the variety known as *ikhabe* raw, but the variety known as *ibhece* is boiled before eating. Throughout the world, melons have a large variety of uses from fresh salads and deserts to pies, vegetable entrees, snack food, and ornamental decorations (Lazos, 1986; El-Adaway & Taha 2001; Wani et al., 2006). Seeds are a potential source of protein (Oyenga & Fetuga, 1975; Teotia & Ramakrishna, 1984; Kamel et al., 1985; Sharma et al., 1986; Lasztity et al., 1986; Wani et al., 2006) and lipids (Lazos, 1986; El-Adaway & Taha 2001; Wani et al., 2006). Because of its high content of pectin, the fruit is popular as a constituent of jams and jellies (Van Wyk & Gericke 2000). In the Kalahari, the fresh fruits are used as a stock feed in times of drought (Van Wyk & Gericke, 2000). According to Benzioni (1997) fruits are used for making jam and other gelled preserves. Wild and early watermelons were extremely bitter, but this was eliminated quickly under cultivation with the selection of seed and cross-pollination. Existing varieties in KwaZulu-Natal and Eastern Cape have completely lost bitterness (Fox & Noorwood Young, 1982).

1.2 Environmental requirements

That wild watermelon originates in the desert or dry areas of Southern Africa suggests that it may be drought tolerant. However, studies on its drought tolerance are not easily accessible in the literature. Definition of drought is really more subtle and complex according to West (2008). However, Oval Myers *et al.* (1986), defined drought as a sustained period of time without significant rainfall. It is not purely a physical phenomenon that can be defined by the weather. Rather, at its most essential level, drought is defined by the delicate balance between water supply and demand. Whenever human demands for water exceed the natural availability of water, the result is drought. Drought can be caused by too little precipitation over an extended period, and can also be caused by increased demand for the available supply of usable water even during periods of average or above average precipitation (Smith, 2006).

Another factor that can affect water supply is a change in water quality. If some of the available water sources become contaminated, either temporarily or permanently, that decreases the supply of usable water, makes the balance between water supply and demand even more unsafe, and increases the likelihood of drought. (West, 2008)

There are meteorological and agricultural definitions of drought. West (2008) added the hydrological drought. Therefore, there are three conditions that are generally referred to as drought:

Meteorological drought: This type of drought occurs when there is a prolonged period of below average precipitation, which creates a natural shortage of available water.

Agricultural drought: This type of drought occurs when there isn't enough moisture to support average crop production on farms or average grass production on rangelands. Although agricultural drought often occurs during dry, hot periods of low precipitation, it can also occur during periods of average precipitation when soil conditions or agricultural techniques require extra water.

Hydrological drought: This type of drought occurs when water reserves in aquifers, lakes and reservoirs fall below an established statistical average. Again, hydrological drought can happen even during times of average or above average precipitation, if human demand for water is high and increased usage has lowered the water reserves

Although agricultural drought often occurs during dry, hot periods of low precipitation, it can also occur during periods of average precipitation when soil conditions or agricultural techniques require extra water (West 2008). Drought conditions often provide too little water to support food crops, through either natural precipitation or irrigation using reserve water supplies. The same problem affects grass and grain used to feed livestock and poultry. When drought undermines or destroys food sources, people go hungry. When the drought is severe and continues over a long period, famine may occur. All living things must have water to survive. People can live for weeks without food, but only a few days without water (West 2008).

1.2.1 Effects of drought on crop growth

Water deficit is one of the most important environmental factors restricting plant growth and productivity (Boyer, 1982), and the genetic improvement of stress tolerance in plants is an urgent challenge for the future of agriculture (Khush, 1999).Water deficit-induced damage in plants is closely associated with reactive oxygen species (ROS) (Kawasaki et al., 2000). The production of ROS, such as superoxide radicals and hydrogen peroxide, is significantly enhanced under water stress conditions where the light energy captured by the leaves is far in excess of that required for Photosynthetic assimilation. Drought stress induces an ArgE-related polypeptide and causes massive accumulation of the free amino acid citrulline in the leaves (Kawasaki et al., 2000). It would thus be useful to determine the physiological function of the accumulated citrulline under conditions where the plants are subjected to severe drought. Wild watermelons (Citrullus lanatus sp.) from the Kalahari Desert exhibit exceedingly high tolerance to drought and excess light stresses, and have been used as an excellent model system for studying how C3 plants survive severe environmental stresses (Yokota et al., 2002). The uniqueness of this plant is exemplified by its accumulation of a novel compatible solute, citrulline (Kawasaki et al., 2000), which is one of the most potent scavengers of hydroxyl radicals (Akashi et al. 2005). Moreover, a number of unique genes are up-regulated in wild watermelon leaves during stress (Akashi et al. 2005), making this plant an attractive source of useful genetic traits for molecular approaches to the breeding of crop plants. However, in order to analyze these genes further using advanced techniques such as gene knockout by RNA and measurement of gene expression with chimeric promoter-reporter systems, development of an efficient transformation system is needed.

Sugar content and sweetness are the critical factors in determining the quality of the many cultivars of melons. The accumulation of sugars takes place in the later stages of fruit development and can be slowed by excessive rains or severe drought, nutrient stress, or by disease and insect damage to the foliage of the plants.

According to Kawasaki et al (2000), the results from their research showed that in the analysis by two-dimension electrophoresis of leaf proteins, seven spots were newly induced after watering stopped. One with the molecular mass of 40 KDa of the spots was accumulated abundantly. The cDNA encoding for the protein was cloned based on its amino-terminal sequence and the amino acid sequence deduced from the determined nucleotide sequences of the cDNA exhibited homology to the enzymes belong to the ArgE/DapE/Acy1/Cpg2/YscS protein family (including acetylornithine deacetylase, carboxypeptidase and aminoacylase-1). This suggests that the protein is involved in the release of free amino acid by hydrolyzing a peptidic bond. As the drought stress progressed, citrulline became one of the major components in the total free amino acids. Drought-tolerant wild watermelon accumulates high levels of citrulline in the leaves in response to drought conditions. Eight days after withholding watering, although the lower leaves wilted significantly, the upper leaves still maintained their water status and the content of citrulline reached about 50% in the total free amino acids. The accumulation of citrulline during the drought stress in wild watermelon is a unique phenomenon in C3plants. The results suggested that the drought tolerance of wild watermelon is related to (1) the maintenance of the water status and (2) a metabolic change to accumulate citrulline.

Crop species differences in drought resistance depend on the type of economic product of the species. For example, species producing leafy vegetables have little drought resistance compared with tuber crops, which are less resistant to drought compared with grain crops (Condon & Hall, 1997). Where economic yield is a reproductive organ, resistance to drought depends on the stage of reproductive development, the type of economic product, and the determinancy of the plant. Plants are often more drought resistant during the vegetative stage than during early flowering or fruit development stages. Plants producing dry grain are more resistant to late season drought than those producing fleshy fruit, which require high turgor (Condon & Hall, 1997).

1.2.1 Wild watermelon as a possible drought tolerant crop

In the study done by Akashi et al. (2005) there is evidence that wild watermelon plants inhabit the Kalahari desert in Botswana, and exhibits exceedingly high drought tolerance. The plants kept the photosynthetic apparatus intact during prolonged drought in strong light, suggesting that there are mechanisms present which make the plant tolerant to oxidative stress arising from excessive light energy falling on the leaves (Miyake & Yokota, 2000; Kawasaki et al., 2000).

For a period of dry weather to affect a plant community, the rainfall deficit must lead to a soil water deficit and ultimately to a plant water deficit. The degree to which a rainfall deficit is translated into soil water deficit depends on the rate of evaporation during the rain-free period, and on the physical and chemical characteristics of the soil. The degree to which a particular soil water deficit influences the plant again depends on the degree of aridity of the atmosphere (Jones , 1992). However; it also depends on a number of plant

characteristics that influence water uptake by the crop, the rate of transpiration and response of the crop to the water deficit so generated. It is the degree to which the crop can withstand the rainfall deficit that constitutes its drought resistance. Drought resistance is the generic term used to cover a range of mechanism whereby plants withstand periods of dry weather.

Three primary types of drought resistance have been identified (Condon & Hall, 1997). *Drought escape:* The ability of a plant to complete its life cycle before a serious plant water deficit develops.

Drought tolerance at high tissue water potential: The ability of a plant to endure periods of rainfall deficit while maintaining high tissue water potential. Many reviewers (Levitt, 1980; Arnon, 1975; O'Toole & Chang, 1978), for convenience, simply refer to this as drought avoidance.

Drought tolerance at low tissue water potential: The ability of a plant to endure rainfall deficit at low tissue water potential.

Citullus lanatus grows well on well drained soil and seeds require soil temperatures of 21 to 35°C to germinate. Root growth is impeded by compacted soil (Smith, 2006). *Citrullus lanatus* withstands drought better than most melons. The crop has the ability to tolerate severe drought/high light stress conditions despite carrying out normal C3-type photosynthesis. However, in order for a plant to maintain high water content as water is extracted from soil, either a greater volume of soil can be tapped or the water within a particular volume of soil can be extracted to a greater extent. This can be achieved by

roots growing deeper or, where low densities prevail by an increase in root density (Condon & Hall, 1997). Wild watermelon has a good rooting pattern and density and hydraulic conductance to maintain water uptake (Smith, 2006). The deep taproot allows the plants to be extremely drought tolerant once established, with plants rarely dying without fruiting (Condon & Hall, 1997). The root system of the plant is a deep, spreading fibrous semi-taproot system that extends six meters or more below the soil surface (Condon & Hall, 1997).

1.3 Agronomy

Seed germination of vegetables, sown either in the field or in a transplant production system, is a critical step conditioning the economic success of the crop. Seed quality (viability and vigor) can have a profound influence on the establishment and the yield of a crop. The survival and performance of seeds after sowing is affected by physical, mechanical, chemical and biotic factors. Temperature, light, drought, flooding and gaseous environments are physical factors which influence seedling emergence (Khan *et al.*, 1979; Hegarty, 1979; Thomas, 1981). Low temperature after the sowing of many warm-season vegetables can lead to asynchronous seedling emergence (Kotowski, 1962; Thompson, 1974). Poor field emergence and erratic stands lead to increased variation in plant development, which can result in yield reductions. Healthy plants with well developed root systems can better withstand adverse conditions and a vigorous early seedling growth has bean shown to be associated with higher yields (Harris et al., 2000).

The vigor of seeds can be improved by techniques generally known as seed priming, which enhance the speed and uniformity of germination (Moradi & Younesi, 2009).

1.3.1 Planting

Since wild watermelon is a traditional crop of Bantu peasants in sub-Saharan Africa, the agronomic practices for its production have not been determined. However, data are available for curcubits in general. The time of planting is delayed until the danger of frost is over (McDonald and Copeland, 1997). In cool areas, it is best to plantt trailing curcubits in October (possible September to December) in South Africa (Smith, 2006). In warm areas, ideal planting is between September and November, although it is possible to plant from August to January. In hot areas, planting is ideal from August to December (possible July to March). Late crops may be infected with viruses through insect vectors at early growth stages.

Because of the irregular, flat seed shape, the modern day seed industry coats seeds, so that precision planting can be accomplished. Direct seeding using drills into rows that are 91-122 cm apart is practiced (McDonald and Copeland, 1997). Seeding rates of 2 to 3 kg ha⁻¹ for gem squashes and butternuts, and 4 to 6 kg ha⁻¹ for Hubbards and pumpkins are common (Smith, 2006). Direct seeding is usual done at two to three seeds per station, and then thinned to one plant after emergence. Seeds can be grown in seedling trays, although this practice is not common (McDonald and Copeland, 1997). Gems and butternuts are spaced at 300 – 500 mm x 1200 – 1800 mm, and Hubbards and pumpkins at 500 mm X 2000 – 2700 mm. Gems have a growing period of 85 to 90 days, butternuts 90 to 100

days, Hubbards, 100 to 115 days and pumpkins 120 to 130 days (Smith, 2006). Wild melons have an growing period similar to that of pumpkins (Fox & Noorwod Young, 1982).

1.3.2 Fertilisation

Optimum growth requires soils with high organic matter and a pH of 6.5 or above. The organic matter is often supplied by green manure crops that are turned under before they reach maturity and become woody. It is difficult to provide a specific fertilization recommendation, because of the diversity of soils on which the crop successfully grows. However a fertilization regime of 400, and 800 kg ha⁻¹ 2:3:4 (30), for low and high fertility soils, respectively, at planting has been recommended (Smith, 2006). At six weeks after emergence, Smith (2006) recommended 250 and 150 kg ha⁻¹ LAN, for low and high fertility soils, respectively. Acid saturation should not exceed 1% or liming will be necessary to avoid aluminium toxicity.

1.3.3 Weed and pest control

According to Smith (2006), the most common pests are American bollworm, pumpkin flies, ladybird, aphids and nematodes. Diseases include powdery mildew, leaf spot, various fruit rots and mosaic virus. Chemical weed control is used, but land management and mechanical cultivation are less expensive and more certain.

1.3.4 Irrigation

Although wild watermelon is a desert plant, curcubits require high levels of water during vigorous vegetative and reproductive growth. Even in areas where rainfall is plentiful,

periodic droughts substantially reduce yields and irrigation can be necessary (McDonald & Copeland, 1997). Furrow irrigation is preferable, but when overhead irrigation is used, it should be applied early in the day to permit the vegetation to dry out prior to nightfall and thereby minimize fruit rotting and foliar diseases (Smith, 2006).

The crop's total water requirements are modest, but there are certain phases when adequate moisture is more vital than others. A medium water stress can be tolerated during early vegetative growth and fruit yield to same extent. Drought sensitivity increases in the late vegetative period when the vines which will bear flowers and fruits develop. A huge loss in fruit occurs as a result of drought stress at flowering stage. The most drought-sensitive stage is at flowering which is usually spread over 15-20 days, and the ensuing period of three to four weeks when the fruits develop and swell. Water stress during ripening can cause the flesh to become more fibrous and less flavoursome (Whitmore, 2008).

1.4. Proline content in relation to plant water stress

Proline is an amino acid that is found in many proteins (especially collagen). Water deficit in leaf tissue affects many physiological processes, ultimately reducing yield. Drought stress is one of the major factors causing profit loss of the sugar beet crop (Pidgeon *et al.*, 2001; Tognetti *et al.*, 2003). Proline appears to be the most widely distributed metabolite accumulated under stress conditions (Delauney & Verma, 1993). The increase of proline concentration in response to water deficit is a well-documented

fact (Hanson et al, 1977; Hasegawa *et al.*, 1994, Van Rensburg & Kruger, 1994), and a large body of data indicates a positive correlation between proline accumulation and enhanced tolerance to drought and salt stress (Liu & Zhu, 1997). Other experimental evidence suggests that proline accumulation is a symptom of stress injury rather than an indicator of stress tolerance (Liu & Zhu, 1997). Nevertheless, proline accumulation seems to be a useful index of drought stress in plants (Ain-Lhout et al., 2001).

In the study done by Kawasaki et al. (2000), it was suggested that protein is involved in the release of free amino acid by hydrolyzing a peptidic bond. As the drought stress progressed, citrulline became one of the major components in the total free amino acids. Eight days after withholding watering, although the lower leaves wilted significantly, the upper leaves still maintained their water status and the content of citrulline reached about 50% in the total free amino acids. The accumulation of citrulline during the drought stress in wild watermelon is a unique phenomenon in C_3 -plants. These results suggest that the drought tolerance of wild watermelon is related to (1) the maintenance of the water status and (2) a metabolic change to accumulate citrulline.

It is believed that proline accumulation could represent a compensatory mechanism for better plant survival during a period of drought stress, based on the role of proline as an osmotic regulator Proline protects enzymes from being denatured (Paleg *et al.* 1984). Proline act as a reservoir of nitrogen and carbon sources (Fukutaku & Yamada, 1984). Proline can even act as a stabiliser of the machinery for protein synthesis (Kardpal & Rao, 1985). However, some reports indicate no correlation between proline accumulation and drought stress resistance (Tully *et al.*, 1979) and others show higher proline accumulation in varieties which are not resistant to drought (Ilahi & Dorffling, 1982). Furthermore, Ilahi & Dorffling (1982) suggested that proline accumulation is mediated by abscisic acid (ABA), since transient increments of ABA seem to precede proline accumulation in some plant species. Moreover, in many, but not all plants, exogenous application of ABA to turgid leaves causes proline accumulation. On the other hand, experiments with barley plants exposed to salt stress showed an increase in the free proline pool in leaves without increase in ABA.

This study was designed to test the hypothesis that the growth and yield of wild watermelon is responsive to changes in water content, and the response is genotype related. Seed colour is an important morphological character of local genotype (landraces).

1.5 Problem statement, study objectives and structure

In the contemporary era of climate change it is becoming more imperative to look to a wide range of food, fibre and medicine sources. South Africa has a number of indigenous crops about whom there is no sufficient agronomic knowledge to advise farmers. Wild watermelon exists in South Africa in modern forms that are more palatable (not bitter). Although it is likely that these types are genetically similar, maintenance of morphological traits such as seed and rind colour suggests that they may have different responses to environment.

The specific objectives of the study were:

- 1. To determine the effect of water stress on three varieties of watermelon differing in seed colour and provenance during seedling establishment,
- 2. To determine the effect of planting date on crop growth and yield under field conditions, and
- 3. To proline accumulation to water stress in wild watermelon.

The study was conducted during 2008 and 2009 at the University of KwaZulu-Natal, Pietermaritzburg, using material that was collected from three sites in KwaZulu-Natal and the Eastern Cape. The thesis is structured so that the literature review (Chapter 1) is followed by three chapters containing results from a laboratory study to determine seed germination capacity (Chapter 2), and observation of wild melon growth and yield during three planting dates differing in terms of environmental conditions, including water availability (Chapter 3). Each chapter contains a separate discussion section. Consequently, a short overall discussion and conclusion on the findings is presented in Chapter 4.

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CHAPTER 2

SEED GERMINATION CAPACITY AND SEEDLING QUALITY UNDER THREE MEDIA IN SEEDLING TRAYS

2.1 Introduction

As a reproductive unit, a seed must be able to germinate and establish seedlings (McDonald & Copeland, 1997). Germination is a measure of the physiological quality of the seed lot. According to the International Seed Testing Association (ISTA, 1999), there are three aspects of quality that affect a seed lot's performance: viability, germination and vigour. Viable seeds are those that are alive and have the potential to germinate when exposed to favourable germination conditions (Bewley & Black, 1994). When a germination test is conducted, those seeds that fail to germinate must be subjected to a viability test to determine whether they are alive or dead. In some instances, non-germinating seeds are soft, swollen, and decayed, indicating that they are dead and nonviable. In other instances, seeds may not germinate because of any number of dormancy mechanisms and are considered viable. Such seeds are normally firm and physiologically sound. A high incidence of viable seeds indicates the potential for germination and establishment of a seedling, but it does not ensure it (Bewley & Black, 1994).

All germinable seeds are viable. The Association of Official Seed Analysts (AOSA, 1996) defines germination as "the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions". This definitions

supersedes the commonly understood purpose of a germination test, which initially was to provide an indication of field performance of a seed lot. Today, it is more important that germination test results are reproducible among testing laboratories and this objective is more easily accomplished under favourable conditions where even weak seeds are afforded every opportunity to germinate (Bewley, 1997). Such an approach permits the comparison of seed quality for various seed lots and allows the orderly interprovince and international movement of seeds across boundaries, since seed germination results are a product of standardised testing methods.

Cucurbit seeds require high temperatures for successful germination and seedling emergence (Harrington & Minges, 1954; Hegarty, 1973). In the study completed by Simon et al. (1976), cucumber (*Cucumis sativus* L.) seeds germinated rapidly at 20°C, but the time to 50% germination at 14°C decreased substantially and below 11°C only a small percentage of the seeds germinate. Germination of watermelon seeds likely requires similar conditions as those for cucumber (ISTA, 1999).

For the purposes of this study, seed germination test was necessary to compare the quality of seed lots derived from three locations in two provinces of South Africa. In the context of a broader study into drought tolerance of watermelon, it was critical to investigate the physiological aspects associated with young seedlings, in terms of their performance under different media. One of the aspects of early plant establishment physiology is accumulation of proteins that may be responsive to water stress. Protein is the most critical component contributing to the nutritional value of food (Pandey &

Budhathoki, 2007). Its determination allows identification of specific genes or chemicals that may be associated with plant response to environment (Sawhney & Singh, 2000; Young, 1963).

Wild water melon is believed to be water stress tolerant (Yokota et al., 2002). This crop from Kalahari Desert exhibits exceedingly high tolerance to drought and excess light stresses. The uniqueness of the plant is exemplified by its accumulation of a novel compatible solute, citrulline (Kawasaki et al., 2000), which is one of the most potent scaverngers of hydroxyl radicals (Akashi et al., 2001; 2004). Moreover, a number of unique genes are up-regulated in wild watermelon leaves during stress (Akashi et al 2004). Plant responses to water stress involve complex processes which occur at the molecular level. Stomatal closure through the action of abscisic acid (ABA) is an early response to water stress in plants. ABA also causes an increase in hydrogen peroxide (H_2O_2) production, which serves as a signalling intermediate to promote stomatal closure (Zhang et al., 2001). The accumulation of proline is a widespread plant adaptation to water stress (Hanson et al, 1977; Hare et al., 1998). Proline aids in stabilizing sub-cellular structures such as membranes and proteins. Proline accumulation in leaves of rice plants was higher in stress tolerant plants than in stress sensitive plants (Ilahi & Dorffling, 1982). The objectives of this study were to determine seed performance of wild watermelon seed lots derived from various locations in South Africa under laboratory conditions and to assess early establishment of the seedlings grown in seedling trays containing media that vary with respect to water holding capacities.
2.2 Materials and methods

2.2.1 Plant material and its provenance

Seeds of wild watermelon were donated by subsistence farmers from different sites in KwaZulu-Natal and Eastern Cape. Whereas seeds were generally of the same size, they differed distinctly in seed colour. Henceforth, the seed lots will be referred to as 'Red', 'Brown' and 'Dark brown', respectively (Figure 2.1). 'Brown' (B) was collected in June 2008 from one homestead in Centane, Eastern Cape. Centane is located about 50 km east of East London, along the coast, on the cool subtropical coastal zone of South Africa. 'Dark Brown' (DB) was collected in August 2008 from one homestead at Tugela Ferry, a semi arid part of the KwaZulu-Natal midlands. 'Very Dark Brown' (VDB) was collected from one homestead at Umbumbulu, about 40 km north-west of Durban, a warm sub-tropical part of South Africa. All seeds were derived from the 2005/2006 rainfed summer crop. The material colleted from the three locations was used to produce fresh seed lots during the 2006/2007 season at Pietermaritzburg, KwaZulu-Natal (29°35'S 30°25'E). Long term climatic data for the three source locations and the production site are summarised in Table 2.1.



Figure 2. 1 Physical characteristics of seedlots used in this study.

Table 2. 1 Mean climatic data for seedlot provenances compared withPietermaritzburg, the study site, estimated from Smith (2006).

Location	Altitude (m)	Seasonal rainfall	Mean annual precipitation (mm)	Mean annual temperature (°C)	Drought occurrence (%)	Average duration of frost period (days)
Centane	0 - 600	Year round	800 - 1000	17.5 - 20	10	Frost free
Umbumbulu	0 - 600	Summer	> 1000	20 - 22.5	10	Frost free
Tugela Ferry	600-1200	Summer	600 - 800	17.5 - 20	10	Frost free
Pietermaritzburg	600 -1200	Summer	800 - 1000	17.5 - 20	10	1 - 30

2.2.2 Germination test and seed vigour

Seeds were surface-sterilized and germinated according to international seed testing rules (ISTA, 1999), using the paper towel method for cucumber germination test. Four replications of 50 seeds were used. Seedling size and normality were determined eight days after the initiation of germination for seed vigour determination (AOSA, 1996).

2.2.3 Seedling production

To simulate a nursery seedling establishment situation, seeds were planted in seedling trays (200 –celled) containing different growth media. The growth media were: fine sand sand (90.57 cm² g⁻¹), pine bark (typical nursery material; Bark Enterpises) and a 1:1 (v/v) mixture of sand and pine bark. Each medium was watered to 75%, 50% and 25% Field Capacity (F.C.), respectively, throughout the growing period. Water regimes were

determined using a potentiometer (Seung and Pak, 2007). The experiment was designed as a factorial consisting of three seedlots (Brown, Dark Brown and Very dark Brown), three growing media (Sand, Mixture and Pine bark), three water regimes (75%, 50% and 25% F.C.), and replicated three times. The experimental block was a 200-celled seedling tray split into three 60-celled units of one growing medium each, in which 60 seeds of a particular seedlot were planted (Table 2.2).

Table 2. 2Experimental design for the seedling establishment experiment showing nine seedling trays, each one being an experimental unit split into three growing media [Sand, Mixture of sand and pine bark (Mix), and Pine bark (Pine) into which one seedlot (Brown (B) or Dark Brown (DB) or Very Dark Brown (VDB) were planted. Three water regimes (75% F.C., 50% F.C. and 25% F.C.) were separated by tray. The experiment was replicated three times to make 81 units.

Sand _B	Mix _B	Pine _B	75% FC
Sand _B	Mix _B	Pine _B	50% FC
Sand _B	Mix _B	Pine _B	25% FC
Sand _{DB}	Mix _{DB}	Pine _{DB}	75% FC
Sand _{DB}	Mix _{DB}	Pine _{DB}	50% FC
Sand _{DB}	Mix _{DB}	Pine _{DB}	25% FC
Sand _{VDB}	Mix _{VDB}	Pine _{VDB}	75% FC
Sand _{VDB}	Mix _{VDB}	Pine _{VDB}	50% FC
Sand _{VDB}	Mix _{VDB}	Pine _{VDB}	25% FC

Each tray was watered to the same water regime. The nutrient content (mg l⁻¹) of sand and pine bark was determined and found to differ (Sand: N= 800, P = 700, K = 600, Mg = 900 and Ca =1800; Pine bark: N ~0.01 = P; K 0.6 = Mg and Ca = 0.04. Plants were fertilised with Hoagland's solution to supply adequate nutrients through the growing period (Modi & Cairns, 1994). The glasshouse used for seedling establishment was kept at 16/21°C (day/night) and 60% RH. Emergence was determined by counting the number of emerged seedlings on days 9, 10, 11 and 12 after seeding. Thereafter, seedlings were allowed to grow until the sixth week after emergence. On the sixth week, seedlings were harvested to determine seedling height, fresh weight, dry weight, root mass, and leaf area (Modi, 2007). Water use efficiency was determined by: [seedling fresh mass – seedling dry mass]/ seedling dry mass] 100.

2.2.4 Proline determination

Shoots were ground to a fine powder in a pre-chilled mortar under liquid nitrogen (N₂). Samples of 0.5 g were mixed in 5 ml Tris-HCl buffer (pH 7.4) containing 250 mM NaCl, 25 mM EDTA, 0.5% (w/v) SDS 10 mM β -mercaptoethanol and centrifuged (15000 rpm for 15 minutes) at 4^oC. The supernatants were collected and considered as leaf protein extract. Protein concentration was determined by absorbance at 595 nm with bovine serum albumin as standard. Proline accumulation in wild water melon leaves from both stressed and unstressed leaves was determined according to the method of Bates et al., (1973). 0.5 g samples of freeze-dried leaf tissue were homogenised in 10 ml of 3% sulfosalycic acid (w/v) and ultraturaxed for 60 seconds. The homogenate were then centrifuged at 11000 rpm for 10 min at 4°C. Supernatant were added to 2 ml of acid ninhydrin and 2 ml of acetic acid. The mixture was incubated in a hot water bath (100°C) for one hour with constant shaking and the reaction terminated in ice. The reaction mixture was extracted with 4 ml toluene, and vortexed for 15-20 sec. The toluene phase was used to measure the absorbance at 520 nm (Beckman Coulter DU® 800). Toluene was used as a blank. A standard curve was used to determine the concentration of proline by using the formula:

 $[(\mu g \text{ proline/ml x ml toluene})/(115\mu g/\mu mole)]/[(g \text{ sample})/5] = \mu moles \text{ proline/g of dry}$ weight material.

2.2.5 Statistical analysis

Genstat Statistical Package Version 9 was used to perform analysis of variance and to generate values of least significant differences (LSD), which were used to determine differences between treatments (P = 0.05). Analysis of variance table for each variable are presented in Appendix 2. Means of treatments that showed significant differences were summarized in graphs generated using Microsoft Office Excel and tables.

2.3 Results and discussion

2.3.1 Germination test and seed vigour

There were significant differences (Appendix 2 A) between seed types with respect to seed germination capacity, but seed germination was low (Figure 2.1). 'Dark Brown' seeds displayed the lowest maximum germination (~ 40%) compared with the other two seedlots (~ 60%), which were not significantly different from each other (Figure 2.1). On

the last day of the germination test seedling size was used to compare seedlots for seedling size, and it was found that 'Brown' > 'Dark Brown' > 'Very Dark Brown' in terms of seedling length, but 'Brown' had a significantly lower seedling mass that the other two seedlots, which were not significantly different in terms of seedling mass (Table 2.3).



Figure 2. 2 Germination of wild watermelon seeds under laboratory conditions

Seedlot	Length (cm)	Fresh weight (g)	Dry weight (g)	
Brown	21.1	11.1	5.1	
Dark Brown	20.8	14.0	5.3	
Very Dark Brown	19.1	12.9	5.1	
S.E. (Mean)	1.2	1.4	0.1	

Table 2. 3 Seedling size of three wild melon seedlots on the eigth day of germiantion

For the purposes of seed marketing, seed germination capacity is a reliable measure of seed quality, and for most crops, it is generally expected to be at least 80% to ensure

good quality (OECD, 1994). The seedlots used in this study were freshly produced from seeds collected from rural areas. Prior to utilization they had been stored in well aerated brown paper bags at room temperature for about six months. Surface sterilization was performed to ensure that detrimental microorganisms were removed from seed surfaces. During the germination test there was no evidence of seed rot. Non-germinating seeds did show signs of imbibition, suggesting that their dormancy was not physical. No abnormal seedlings were observed among the germinating seeds. The information gained from the germination test was used to gain initial insights about seedlot quality with respect to germination capacity. Seeds the seedlots were not genetically improved, it was expected that their germination capacity would be low and variable. The maximum germination capacity of the seedlots was about 20% to 40% lower than the standard for seed marketing. This finding suggested that the seedlots would not be suitable for commercialization, but their use for crop production would require careful determination of seeding rates and thinning, if necessary. To determine seedlot performance, early in seedling establishment, under media and water conditions that are less favourable than the laboratory, seedling emergence, to mimic nursery conditions, was determined.

2.3.2 Emergence and seedling establishment

There were highly significant effects of seedlot, water regime (FC), interaction between water regime and growing medium (Media) and colour x water regime x media (Appendix 2 A). The best performing seedlot in terms of seedling emergence was



'Brown', followed by 'Very Dark Brown', and 'Dark Brown', respectively (Figure 2.3).

Figure 2. 3 Seedling emergence of different seedlots (B = Brown; DB = Dark Brown; VBD = Very Dark Brown).

Red seeds displayed the highest emergence (~ 51%) followed by the dark seeds (~ 44%) and brown seeds (~34%), respectively (Figure 2.2).

Except that there were significant differences between "Brown" and 'Very Dark Brown', the results of seedling emergence were in agreement with those of the germination test (Figure 2.2). This finding suggested that the favourable conditions of the laboratory test could be used with confidence to predict seed performance under a wide range of nursery conditions.

As expected, 25% FC reduced seedling emergence by ~ 67% compared with 50% FC, which resulted in better emergence than 75% FC. However, there was not significant difference between the latter two water regimes (Figure 2.4).



Figure 2. 4 Seedling emergences at different water regimes (field capacity).

Seedling emergence is preceded by a process of germination, whose critical first step is absorption of water for adequate imbibitions of seed tissues (McDonald and Copeland, 1997). Adequate availability of respiratory air is also important for good germination. Radicle emergence occurs after a process of food reserve degradation by enzymes (Blackman et al., 1992). The germination process and its successful culmination as radical protrusion can be negatively affected by environmental conditions surrounding the seed. One of the well studied aspects of the environment during seed germination is osmotic potential. Xu et al. (1990) found that a low osmotic potential reduced germination capacity in lucerne, but there are osmoticum concentrations that promote protein synthesis of developmental proteins. In the present study the low water content regime (25% FC) is likely to have been associated with delayed imbibitions, and the highest one (75% FC), may have been associated with curtailed respiratory air. Examination of the interaction of growth media and water regimes showed that the average performance shown in Figure 2.4 above, was mostly associated with the mixture of pine bark and sand (Figure 2.5). Although sand displayed a similar trend to that of the mixture of sand and pine bark, there were no significant differences between water regimes, with respect to emergence in sand (Figure 2.5). Data shown in Figure 2.5 suggested that the best seedling emergence was achieved in the mixture of pine bark and sand, with 50% F.C.



Figure 2. 5 Interaction of planting media (Sand, Pine and Mixture of Sand and Pine) and soil water regimes (25% FC, 50% FC and 75% FC) for seedling emergence.

The three-way interaction of colour x water regime x media is shown in Table 2.4. These data distinguished the relationships between treatment factors further by showing that

although 'Dark Brown' generally performed the poorest in terms of seedling emergence, its performance was significantly improved in the media consisting of the pine bark and sand mixture when seeds were subjected to 50% FC (~54% emergence). Interestingly, the poorest performance of 'Dark Brown' was displayed in the same medium, when seeds were supplied with 25% FC (~10% emergence). The best performing seedlot overall, 'Brown' was boosted by the combination of mixed growing medium supplied with 505 FC (~69%), and it performance was not negatively affected on sand supplied with 75% FC (~64%).

		Growing media			
		Mix	Pine	Sand	
'Dark Brown'	25% FC	9.7	29.9	29.9	
	50% FC	54.2	37.5	39.5	
	75% FC	35.8	35.4	38.2	
'Very Dark Brown'	25% FC	19.4	29.2	41	
-	50% FC	69.4	33.3	54.1	
	75% FC	61.1	57.6	33.3	
'Brown'	25% FC	20.8	41	54.1	
	50% FC	68.7	52.8	46.5	
	75% FC	59	52.7	63.8	
LSD (0.5%) = 8.89					

 Table 2. 4 Interaction of growing media x water regime x seedlot for seedling emergence.

2.3.3 Seedling size

Seedlings were harvested six weeks after planting and seedling height, fresh weight, dry weight, root mass, and leaf area were determined. At harvest, leaves were sampled for protein extraction to determine proline content as explained in section 2.2.4. Seedling size was determined to mimic nursery conditions, in case the crop is established using seedling produced by a nursery. Normally, nurseries use pine bark and produce seedlings under conditions of adequate water and nutrients. The hypothesis of this section of the study was that pine bark and 50% F.C. would be ideal conditions or conditions closely mimicking the nursery. This study used controlled temperature conditions, which is not a general practice at nurseries, where a shade cloth is used to cover seedlings growing under ambient temperature conditions. Application of 25% F.C. and sand medium were aimed at introducing stressful conditions, in terms of water availability. Application of 75% F.C. was expected to introduce luxury water consumption, which may have a negative effect on seedling's ability to withstand stress. Proline content would be used for physiological determination of water stress in plant leaves.

2.3.3.1 Seedling height

Media and water regimes were the only ones to have significant effects on plant height (Appendix Appendix 2 B). Seedlings grown on pine bark were about 38% taller than those grown on the mixture of pine bark and sand, and about 70% taller than those grown on sand only (Figure 2.6). These findings suggest that the conditions for growth in pine bark were better than those found in the media containing sand. That is, sand

compromised the quality of pine bark as a media, in the mixture of sand and pine bark, and sand is a poorer medium than pine bark for growing seedlings. Although no study using the same parameters as used in the present study were found in literature, the response shown in Figure 2.6 was in agreement with findings of Ahuja et al. (1985).



Figure 2. 6Seedling height of wild melon in response to growth media (Pine bark only = pine bark, Mix = 1:1 pine bark:sand; Sand = sand only).

The higher the field capacity of the medium, the taller were the seedlings produced, and the tallest seedlings were grown with 75% F.C. (Figure 2.7). There was a difference of 25% between seedling height associated with 75% F.C. compared to that associated with 50% F.C. The seedling produced with 75% F.C. were also 37% taller than those produced with 25% F.C. (Figure 2.7). Interpreted with Figure 2.6, the results shown in Figure 2.7 suggest that there was a positive correlation ($\mathbb{R}^2 = 0.89$) between changes in water content and changes in media type from pine bark to pure sand (Figure 2.8).



Figure 2. 7Seedling height of wild melon in response to media field capacity.



Figure 2. 8 Relationship between growth medium quality from poor (sand) to very good (pine) and field capacity (from 25% to 75% F.C.) with respect to watermelon seedling height.

2.3.3.2 Seedling mass and leaf area

Seedling fresh mass and dry mass were analysed separately (Appendices 2 C and D), and the results were different in some aspects (Appendices 2 C and D). For fresh mass, there were highly significant effects of water regimes, seedlots and media, but there was no significant interaction of these factors. For dry mass, there were highly significant effects of water regimes, seedlots and media, but there was also a highly significant interaction of water regime (FC) and media (Appendix 2 C). Comparison of the effects water regimes on seedling fresh mass are shown in Figure 2.9. The 25% FC produced the smallest seedlings compared to 50% FC and 75% FC, and the latter two were not significantly different.



Figure 2. 9Seedling fresh mass of watermelon grown under three water content regimes in seedling trays.

There was no significant difference between seedlot 'Brown' and 'Very dark Brown', with respect to seedling fresh mass, but these seedlots produced seedlings that were about 18% heavier than those of 'Dark Brown" (Figure 2.10).



Figure 2. 10 Fresh mass of seedlings of three seedlots.

In agreement with seedling height data, pine bark produced the heaviest seedlings, followed by the mixture of pine bark and sand, and lastly sand only (Figure 2.11). Although the difference between pine bark and the mixture was small (~14%), sand-grown seedlings were ~ 50% smaller than those grown on pine bark only (Figure 2.11).



Figure 2. 11 Seedling fresh mass of seedlings grown on three different growth media (Pine bark only = pine bark, Mix = 1:1 pine bark:sand; Sand = sand only).

The comparison of water regimes, media and seedlot, with respect to seedling dry mass, followed the same pattern as that of seedling fresh mass (Figures 2.12 - 2.14). Although seedling fresh mass and dry mass were derived from the same material, it was not a given fact that they should follow the same pattern in terms of their response to treatments. Since plants have a high composition of water and the level of water in a plant will depend on the amount of water in its environment, using dry mass as a measure of plant growth is more reliable than using fresh mass (Salisbury & Ross, 1992).



Figure 2. 12 Seedling dry mass of watermelon grown under three water content regimes in seedling trays.



Figure 2. 13 Dry mass of seedlings of three seedlots.



Figure 2. 14 Seedling dry mass of seedlings grown on three different growth media (Pine bark only = pine bark, Mix = 1:1 pine bark:sand; Sand = sand only).

The interaction data showed that there was consistency in the effect of pine bark, but for the mixture of pine bark and sand, and for pure sand, 50% FC produced equal size or heavier seedlings than both pine bark and sand (Figure 2.15). These results suggest that the performance of growing medium was dependent upon the existing water regime.



Figure 2. 15 Interaction of growth media and water regime for seedling dry mass.

To understand the effectiveness of each treatment (seedlot, water regime and growth medium) in dry mass production, water use efficiency was calculated and shown in Figure 2.16.



Figure 2. 16 Comparison of seedlots (B, VBD and DB), water regimes (25% FC, 50% FC and 75% FC) and growth media for water use efficiency (WUE) to grow wild melon seedling over a period of six weeks in seedling trays.

From Figure 2.16, it is evident that 'there was no significant difference among seedlots with respect to WUE. Comparison of water regimes showed that there was no direct relationship between field capacity and WUE in that 25% FC > 75% = 50%. Sand and the mixture of pine bark and sand had similar efficiencies, which were better than that of pine bark alone (Figure 2.16). The data shown in Figure 2.16 suggest that it would be more efficient to produce seedlings using anyone of the seedlots grown in a mixture of sand or in sand at 25% FC. However, the results shown in the previous sections for seedling height and mass showed that 'Brown' was generally the best performing seedlot,

followed by 'Very Dark Brown', and 'Dark Brown was the worst performer. The best performing water regime was 75% and pine bark was the best medium. The results shown in Figure 2.16 suggest that there is a negative relationship between water use efficiency and yield. That the landrace which had the highest seedling yield is not the one with the best water use efficiency is in agreement with previous findings (Condon & Hall, 1997; Gwathmey & Hall, 1992; Le Roux et al., 1996; Ludlow & Muchow, 1990).

Seedling root dry mass showed highly significant effects of all the treatments and their interactions (Appendix 2 E). The interactions selected for discussion were water regime x growing medium, seedlot x growing medium and seedlot x water regime (Figures 2.17 - 2.19).



Figure 2.17 Seedling root mass response to water regimes (Filed capacity) and growing media [Mixture of pine bark and sand (1:1), Pine bark only and sand only)].



Figure 2. 18 Seedling root mass response of three seedlots (B, VDB and DB) grown on thre media [Mixture of pine bark and sand (1:1), Pine bark only and sand only)].



Figure 2. 19 Seedling root mass response of three seedlots (B, VDB and DB) grown on three water regimes (Filed capacity).

Pine bark displayed the highest seedling root dry mass compared with the other two media, and sand had the lowest root mass, but 75% FC had a tendency to improve rrot mass in pine bark and the mixture of pine bark and sand (Figure 2.17). 'Brown' showed

the highest root dry mass, but there were no significant differences between the other two seedlots (Figure 2.19). The performance of 'Brown' was more improved when grown on pine bark only, and 'Dark Brown' almost surpassed 'Very Dark Brown' when it was grown in pine only. There was no significant difference between 50% FC and 75% FC for the performance of 'Brown' and 'Very Dark Brown'. On average, Very Dark Brown' had the highest root dry mass than the other two seedlots across all water regimes.

Determination of root:shoot ration on a dry mass basis (Figure 2.20) showed that pine bark produced the largest root mass per plant than the other media. The water regime that produced the highest root mass was 75% FC, and 'Brown' showed a significantly higher accumulation of dry mass in the roots than the other two seedlots.



Figure 2. 20 Comparison of media (Pine bark, Mixture of pine bark and sand and Sand), water regimes (25%, 50% and 75% F.C.) and seedlots (B, VDB and DB) for root:shoot ratio in wild melon seedlings.

Media were the only significant main factor with respect to root length, in addition to the water regime x media interaction (Appendix 2 F). Changes in root length in relation with growth media are shown in Figure 2.21.



Figure 2. 21 Seedling root length changes over three water regimes (Field capacity) in three growing media.

It is evident in Figure 2.21 that at 25% F.C., pine bark had the lowest root length, but as the field capacty increased there was no significant difference between pine bark and the mixture of pine bark and sand (at 50% F.C.) and between pine bark and the other two water regimes (at 75% F.C.). The mixture of sand and pine bark did not differ significantly with respect to root length at any given water regime (Figure 2.21).

The last measure of seedling size determined was the leaf area, for which there were highly significant effects of all the factors and their interactions (Appendix 2 G). For the purposes of this discussion, only the seedlot x media and the seedlot x water regime interactions were discussed (Figures 2.22 -2.23). The reason for this choice was based on

the need to avoid explanation of a trend that is repetitive to that of shoot height, which was very similar to that of leaf area.



Figure 2. 22 Changes in wild melon seedling leaf area in response to field capacity and growing media (Pine bark only, 1:1 Mixture of pine bark and sand and sand only). Note: LSD (5%) = 2.8.



Figure 2. 23 Changes in seedling leaf area of three seedlots (B, VDB and DB) in response to growing media (Pine bark only, 1:1 Mixture of pine bark and sand and sand only).



Figure 2. 24 Changes in seedling leaf area of three seedlots (B, VDB and DB) in response to field capacity (25%, 50% and 75% F.C.).

As expected, based on the results of seedling height and mass above, pine bark produced the largest leaves, but its effect was the same as that of the mixture of pine bark and sand at 50% and 75% F.C. (Figure 2.22). Sand showed the lowest seedling leaf area, about 33% less than the other two media across all water regimes (Figure 2.22), but there was a significant positive effect of water regimes even on sand.

Overall, 'Brown' had the largest leaf area than the other two seedlots, but its performance was best pine bark, however the effect of pine bark was reduced by 27% and 34%, respectively with VBD and DB (Figure 2.23). Across all seedlots, there was no significant difference between 50% FC and 75% FC and 25% FC almost consistently showed a 50% lower leaf area than the other two water regimes.

2.3.3.3 Proline determination

There was a significant effect of all treatments and interactions, with respect to proline content in six week old watermelon seedlings (Appendix 2 H). In general, there was a negative correlation between the amount of proline in seedling leaves and the performance of media, water regimes and seedlots, with respect to any measure of seed size discussed in the preceding sections of this chapter (Figures 2.25 - 2.27). At any given water regime, sand was associated with the highest proline content than pine bark and the mixture of sand and pine bark (Figure 2.25).





Whereas the general order of seedlot performance with respect to seedling size had been B > VBD > DB, the amount of proline was found to be DB> VDB> B (Figure 2.26). Seedlot performance, however, was influenced by media in that thre was no significant difference between them when seedlings were grown on sand. In addition, both pine and mixture of pine and sand showed improved proline contents in DB (Figure 2.27).



Figure 2. 26 Comparison of three growth seedlots) across three water regimes (25%, 50% and 75% FC) with respect to proline content in six-week old wild melon seedlings.



Figure 2. 27 Comparison of three growth seedlots) across three seedlots with respect to proline content in six-week old wild melon seedlings grown in different media (Pine bark, Mixture of pine bark and sand and Sand only).

Increased proline accumulation was reported in water-stressed sorghum (Yadav et al., 2005), bell pepper (Nath et al., 2005), wheat (Hamada, 2000) and in salt-stressed *Catharanthus roseus* (Jaleel et al., 2007). Increased proline in the stressed plants may be an adaptation the purpose of which is to overcome the stress conditions. Proline accumulates under stressed conditions supplies energy forgrowth and survival and thereby helps the plant to tolerate stress (Chandrashekar &Sandhyarani, 1996). Under abiotic stress like ultra violet light the proline content showedan increase in wheat (Demir, 2000). NaCl stress showed increased proline content in rice(Lin et al., 2002) and peanut. Proline accumulation in plants might have a scavenger function and act as an osmolyte.

2.5 References

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CHAPTER 3

PLANTING DATE EFFECTS ON GROWTH AND YIELD OF WILD WATERMELON UNDER FIELD CONDITIONS

3.1 Introduction

Many factors interact to determine the number of healthy, autotrophic plants obtained from planting a given sample of seed (Melis, 1991). Germination under laboratory conditions often is a poor simulation of the rigors encountered in the field. Factors influencing seedling establishment under field conditions include the physical, chemical, and biotic properties of the soil; method, date and rate of seeding; and seed treatments. In addition, each of these factors interacts with the environmental factors of water, temperature, oxygen and light that regulate the rate of germination.

Soil moisture conditions exert a dominant influence on stand establishment because of the modifying effects of moisture on soil properties. Soil structure, soil water potential, and seed-soil contact determine the rate of moisture uptake by the seed in soil. Increasing seed surface contact with liquid water decreases germination time and increases emergence percentage. Any factor reducing soil hydraulic conductivity or seed-soil contact reduces the rate of water uptake and delays germination and emergence.

Seedling emergence under field conditions varies with soil moisture content, soil type, and plant species (McDonald & Copeland, 1997). Work with many crops shows that total emergence is affected only slightly by moisture tension from 0.05 to 0.3 MPa (Hunter &

Erickson, 1952; Hegarty, 1976; 1979). The number of emerging seedlings decreases rapidly at moisture tension grater that 0.7 MPa, and the time to maximum emergence increases as moisture stress increases. Many crop seeds are able to imbibe sufficient water at moisture near or slightly below the permanent wilting point to initiate germination, but not elongate. Insufficient aeration presumably limits germination at very high soil moisture conditions, since diffusion of oxygen is air is 10 00 times greater than in water at 20°C.

Emergence strength of individual seedlings varies from a low of 0.15 Newton for lucerne to a high of 2.9 Newtons for corn. Multiple seedlings in a group are able to rupture higher strength soil crusts. For example, studies show that the maximum thrust one, two and three cotton seedlings is 3.8, 5.8 and 8.5 Newtons, respectively. Similarly, subterranean clover, when seeded 2 cm deep in clumps of 1, 2 and 5 seeds has 28, 72 and 85% emergence. Large seeds exhibit greater emergence thrust, but they also encounter more soil resistance due to the greater surface area of their emerging seedling structures. Emergence data for many species and cultivars generally favours the large seeded-types when all other factors are equal. This is particularly true for the smaller-seeded grasses and legumes, where emergence from deeper plantings is promoted by large seed size, even though emergence force per gram of seed weight is slightly greater for small seeds. Large-seeded dicots occasionally fail to establish themselves as well as smaller seeds of the same cultivar. However, this is usually due to differences in seed quality. Each seed quality for comparison of seed size is difficult to obtain from machine-harvested seedlots, because of mechanical injury problems. Large seeds normally exhibit more evidence of
injury than small seeds (Wanjura & Buxton, 1972). Rate of emergence depends more on soil temperature and moisture conditions than on planting depth (Tesar, 1984)

Ellis et al. (1985) provided specific germination information and test recommendations. Poor field emergence and erratic stands lead to increased variation in plant development, which can result in yield reductions. Soil temperature and moisture are the major environmental determinants of seedling establishment with various planting dates. Early plantings in temperate regions usually are associated with favourable moisture supplies, but low soil temperature (Doneen & MacGillivray, 1943). Early-planted watermelons often have difficulty with seed germination and emergence is the soil is cold. Cultivars selected for cold germination ability would provide growers with better stands for crop production. The seeds of cucurbitaceous crops are non-endospermic and germination is epigeal. Cool, moist conditions favour pathogen development on seeds of low vigour by increasing days to emergence due to low temperature and increasing time for pathogen activity and ultimately reduce percentage of emergence. Planting date in non-irrigated warm climates frequently is determined by the onset of rains. In addition, soil temperature above 35°C, as well as the low moisture availability, may restrict germination of cool season species. Early spring soil temperatures in temperate regions decrease rapidly with soil depth. Early plantings should be shallower than later plantings, because temperatures are warmer near the soil surface, and soil moisture conditions are usually better early in the season (Fyfield & Gregory, 1989). Rate of emergence is closely correlated with early season soil temperatures measured at the seed depth. The emergence rate increases linearly from 5 to 25 $^{\circ}$ C for cool season crops to 10 – 35 $^{\circ}$ C for warm season crops (Khan et al., 1979; Hegarty, 1979; Thomas, 1981). Low temperature after the sowing of many warm-season vegetables can lead to asynchronous seedling emergence (Kotowski, 1962; Thompson, 1974). As it has been reported (Fox & Norwood Young, 1982) wild watermelons survive severe droughts of Kalahari desert. Hence they are expected to thrive well under warm temperature conditions and be sensitive to cold.

Plant response to water stress has been the subject of many books and reviews. It has been pointed out that the primary drought-induced strain in crop plants is simply cell dehydration, from which many effects arise. Resulting damage to essential plant processes can be reversible or irreversible depending on the severity of dehydration. As in chilling or high temperature situations, consequencies of membrane damage can be of several types. Damage to membrane bound enzymes usually means accumulation of metabolic intermediates or waste products to the extent that some are toxic. At the same time, photosynthesis can be slowed enough so that the plant literally starves or else the respiratory chain and its associated energy production are sufficiently disrupted to block normal cellular maintenance. If this stress persists long enough, effects are irreversible, and the plant dies. More often, mild to intermediate stress levels are important. In this regard, three excellent reviews by Bradford, 1994; Hall, 2001; Kramer and Boyer, 1995 are available.

Following an investigation into the seed and seedling performance of wild watermelon under laboratory and controlled glasshouse conditions (Chapter 2), it became important to determine how the crop would perform under field conditions where the environmental

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factors are not controlled. Since both temperature and rainfall vary significantly from month to month in Pietermaritzburg (see Chapter 1), the hypothesis was that different planting dates ranging from the early planting in summer to late planting in January, would provide varying soil moisture conditions to allow testing of crop performance. The planting dates were selected to mimic what happens in the small-holder farming areas where the landraces of wild watermelon were collected (Professor A.T. Modi, University of KwaZulu-Natal, Personal communication).

3.2 Materials and methods

Seeds of wild watermelon were sourced as explained in section 2.2.1 above. Seeds were directly seeded in Inhoek Oatlands soil in Pietermaritzburg (Latitude -29.66763; Longitude 30.40599) under dryland conditions on three different dates, staggered: 25 September 2008, 23 November 2008 and 20 January 2009. In anticipation of less than 100% germination (see Figure 2.2 above) three seeds per station were planted and later thinned out to one seedling, one week after emergence. The thinning process was performed to establish uniform seedling size and 100% initial population per plot at a spacing of 50 cm within row and 200 cm between rows (Smith, 2006) in 16-m² plots separated by a distance of 2 m. Before planting, soil samples were randomly taken to determine soil analysis (Table 3.1). Fertiliser application consisted of 50 kg ha⁻¹ N, 20 kg ha⁻¹ P and 0 kg ha⁻¹ K according to the requirements for dryland production of water melon (Smith, 2006).

Parameter	Amount
Density (mg/ml)	1.04
P (mg/l)	9
K (mg/l)	185
Ca (mg/l)	1556
Mg (mg/l)	553
Exchangeable acidity (cmo_{lc}/l)	0.05
Total cations (cmo _{lc} /l)	12.84
Acid saturation (%)	0
pH (KCl)	5.64
Zn (mg/l)	16.9
Mn (mg/l)	7
Cu (mg/l)	6.2
Clay (%)	25
Organic C (%)	1.6
N (%)	0.21

 Table 3. 1 Soil fertility data for the field that was divided to plant from September

 2008 to January 2009.

Each planting date (Main plot), was split into three seedlots (B, DB and VDB – see Figure 2.1) subplots, which were replicated three times. In each subplot, the sampling unit consisted of the three middle plants surrounded by border rows, and these were used to determine leaf number and vine length on the 2^{nd} , 5^{th} and 8^{th} week from the planting date, when flowering began. Final yield was determined 135 days after emergence by fruit number per plot at senescence. Fruits were graded by size into small (< 2 kg),

medium (2 - 5 kg) and large > 5 kg). Gravimetric water content [%w = wet mass – oven dry mass ÷ oven dry mass) × 100] of the soil was determined from the top 30 cm of each sampling unit every week for 12 weeks. Genstat Statistical Package Version 9 was used to perform analysis of variance and to generate values of least significant differences (LSD), which were used to determine differences between treatment().(P). Analysis of variance table for each variable are presented in Appendices 2 and 3.

3.3 Results and discussion

Changes in soil water content during the first 12 weeks of each cropping period are shown in Figure 3.1.



Figure 3. 1 Changes in soil water content during the first 12 weeks of wild watermelon growth in each growing period starting in September, November and December, respectively.

It is evident from Figure 3.1 that there were significant differences among cropping periods, with respect to soil water content. The crop that was planted early (September)

emerged under low soil moisture conditions (< 30%), but there was a steady increase in soil moisture as the rainfall increased from September to January Appendix 1). The November planting occurred under soil moisture conditions better than those of the September planting, but poorer than those of the January planting. The high moisture content at the start of the November and January plantings, was expected to result in faster seedling emergence, however, there were no significant differences between planting dates nor seedlots with respect to field emergence (Appendix 2 A). Despite the lack of differences in emergence, but crop growth for the September planting was sustained better by more rainfall and higher soil water content during the period when plants were growing faster in response to increasing leaf number and plant size (see sections 3.3.1 to 3.3.3 below).

3.3.1 Plant growth

Plant growth was determined using leaf number and vine length on different dates after emergence. Leaf number during plant growth was highly significantly response to planting date and the seedlots also differed significantly. In addition to the main effects of planting date and seedlot, there were also significant interactions of planting among the treatments and sampling times (Appendix 2 B).

Seedlot B displayed the highest leaf number across planting dates (Figure 3.2). On average, the highest leaf number was obtained when the crop was planted in September, but planting in November favoured seedlot VDB the most, and DB displayed no significant differences in leaf number when planted in November and January (Figure 3.2).



Figure 3. 2Comparison of three wild watermelon seedlots for leaf number during the first 8 weeks of growth under field conditions when planted in September, November and Jabuary, respectively.

Although planting in September resulted in the highest leaf number, there was no difference between September and the other two dates two weeks after emergence (Figure 3.3). Both September and November plantings started to show better leaf number than the January planting five weeks after emergence, when they were not significantly different from each other (Figure 3.3). By the eighth week after emergence, there were significant difference among planting dates with September planting giving the highest leaf number, followed by November and January plantings, respectively (Figure 3.3).



Figure 3. 3 Changes in wild watermelon leaf number during the first 8 weeks of growth under field conditions when planted in September, November and Jabuary, respectively.

An examination of the interaction between sampling dates and seedlots showed that there was no significant difference between seedlots B and VDB, with respect to leaf accumulation in the first eight weeks of plant growth, but seedlot DB was lagging behind them throughout the period observed (Figure 3.4).



Figure 3. 4 Changes in wild watermelon seedlot leaf number during the first 8 weeks of growth.

The pattern of growth displayed by wild watermelon in this study (Figures 3.3 and 3.4) was typical of annual crop growth rate during the early exponential phase and the linear growth phase (Salisbury & Ross, 1992, Tesar, 1984). The data shown in Figures 3.3 and 3.4 allowed estimation of leaf accumulation rate for each seedlot and for determination of the effects of planting date (Figure 3.5).



Figure 3. 5 Estimated leaf accumulation rate for planting dates and seedlots during two phases of growth delineated in Figures 3.3 and 3.4, above. Phase 1 = weeks 2 to 5; Phase 2 = weeks 5 to 8.

It is clear from Figure 3.5 that the leaf number accumulation was slow during the first few weeks (Phase 1), but it increased rapidly from week 5 to week 8 (Phase 2). Comparison of planting dates showed that for phase 1 the rate in September was 5% greater than in November, and it declined further by 22% in January (Figure 3.5). During the first phase, seedlot B showed the highest leaf accumulation rate at 5% and 30%

greater than that of VDB and DB, respectively. During phase 2 there was about 12% decline in leaf accumulation rate when planting occurred in November, compared with September, which further declined by 1.5% for the January planting (Figure 3.5). The consequence of the growth pattern shown in Figures 3.3 and 3.4, and explained in Figure 3.5 is expected to be accompanied by a similar pattern for vine growth and to correlate with economic yield data.

There was a significant effect of sampling date on vine number, but there was no other significant main effect. However, there was a significant interaction of planting date and seedlot (Appendix 2 C). The increase in vine number with time was expected (Figure 3.6). An interesting extrapolation of the data on Figure 3.6 was the determination of vine accumulation rate (Figure 3.7).



Figure 3. 6 Average vines per plant accumulating in three wild watermelon landraces planted at different periods from September 2008 to January 2009 under field conditions.



Figure 3. 7 Estimated vine accumulation rate during two phases of wild watermelon growth under field conditions. Phase 1 = weeks 2 to 5; Phase 2 = weeks 5 to 8.

The patter of vine accumulation was similar, but slower that than of leaf accumulation (Figures 3.3 and 3.4). From the perspective of planting dates, the comparison of leaf accumulation with vine accumulation rates during phase 1 showed that leaves accumulated at 81%, 80%, and 76% better than vines for September, November and January plantings, respectively. During phase 2, the differences between leaves and vines, in favour of the former, were 89%, 92% and 92%, for September, November and January plantings, respectively.

The interaction data showed that seedlot B produced plants with 5% to 15% more vines than the other seedlots, when the crop was planted in September. However, the difference between B and other seedlots increased to about 24% to 30% for the November and January plantings (Figure 3.8). Although the November planting was second to the September planting id vine accumulation, for seedlot DB, the November planting was associated with the lowest number of vines per plant (Figure 3.8).



Figure 3. 8 Intearction between seedlots and planting dates for vine accumulation in wild watermelon.

There was a significant difference among all treatments and their interactions with respect to vine length (Appendix 2 D). The interactions are presented in Figures 3.9 - 3.11, below).

From Figure 3.9, it is clear that September planting produced the longest vines. Seedlot performance varied with planting date. For the September planting, seedlot VDB produced about 10% more vines than B, and ~ 50% more than DB (Figure 3.9). For the November and January plantings, there was no significant differences between seedlots B and VDB, which had ~40% more vines than seedlot DB (Figure 3.9).



Figure 3. 9 Interaction between seedlots and planting dates for vine size in wild watermelon.

The best response to planting in September, compared with the other planting dates, was clear from the perspective of weekly growth data (Figure 3.10). Planting in September resulted in ~ 45% more vine length than planting in November and January (Figure 3.10).



Figure 3. 10 Interaction between sampling dates and planting dates for vine size in wild watermelon.

Performance of seedlots with respect to vine size started to differe on week 5 after planting, where B and VDB were significantly better than DB (Figure 3.11). By week 8 there were significant differences among all seedlots, with DB showing the longest vines, followed by VBD and B, respectively (Figure 3.11).



Figure 3. 11 Interaction between sampling date and seedlot (B, VDB and DB) for vine size in wild watermelon.

Taking the data presented in Figures 3.10 and 3.11, vine growth rate was estimated (Figure 3.12). From Figure 3.2, it is evident that during the first three weeks of growth after week 2, there was most rapid growth in response to planting in September, compared with November (~50% less) and January (~68% less), respectively (Figure 3.12). Although the September planting continued to have a significantly better growth rate during phase 2 (weeks 5 to 8). The differences between planting dates were less compared with the phase 1 growth rate (Figure 3.12). Seedlot differences were evident with respect to vine growth rate, where B > VDB > DB (Figure 3.12). It was strange to note that seedlot DB had a better growth rate during weeks 5 to 8 than weeks 2 to 5,

because the second phase would have happened in March, when both temperature and rainfall had started to decline (Appendix 1).



Figure 3. 12 Estimated vine growth rate for planting dates and seedlots during two phases of growth delineated in Figures 3.10 and 3.11, above. Phase 1 = weeks 2 to 5; Phase 2 = weeks 5 to 8.

3.3.2 Crop yield

To determine crop production, both the number of fruits per plant and fruit mass were determined. There was a significant effect of planting date and seedlot, with respect to fruit number (Appendix 2 E). The highest number of fruits was obtained from the September planting, and this was ~32% greater than the fruits from the November planting, and ~64% more than the January planting (Figure 3.13). Grading the fruit revealed that there were fewer large fruit and size distribution differed with planting date (Figure 3.13). Whereas planting in September was associated with a significantly higher number of large fruits compared with the other planting dates, it resulted in the majority of fruits being small (Figure 3.13). The November planting was characterised by a predominance of medium size fruits. The January planting was characterised by the

fewest number of fruit compared with the September and November plantings, and a predominance of small fruits (Figure 3.13).



Figure 3. 13 Fruit size distribution for wild watermelon in response to planting date.

Comparison of seedlots for fruit number showed that B > VDB > DB, but size distribution differed among seedlots (Figure 3.14). For seedlot B, about 60% of the fruits were medium-sized, 32% small and 8% large (Figure 3.14). For VDB, 50% were medium 30% small and 20% were large. For DB, 54% were small, 29% medium and 17% large (Figure 3.14).

Fruit size was the highest when planting occurred in September, and the later it became the less it was (Figure 3.15). From September to November there was a 32% decline in fruit size, and it went down by 50% when planting was delayed to January (Figure 3.14).



Figure 3. 14 Fruit size distribution for three wild watermelon seedlots (B, VDB and DB).



Figure 3. 15 Changes in wild watermelon fruit mass per plant in response to planting date.

The average mass per seedlot is shown in Figure 3.16, and it indicates that DB fruits were

25% greater than VDB fruits and 52% greater than B fruits (Figure 3.16).



Figure 3. 16 Average fruit mass per plant for three wild watermelon seedlots (B, VDB and DB).

Using data shown in Figures 3.15 and 3.16, crop yield was estimated (Figures 3.17 and 3.18). Crop yield decreased from 1368 t ha⁻¹ for September planting to 247 t ha⁻¹ for January planting, suggesting that wild watermelon is a long season crop.



Figure 3. 17 Estimated wild watermelon crop yield in response to planting date.



Figure 3. 18 Estmiated yield of wild watermelon seedlots.

The yield of the highest yielding seedlot, DB, was 14% and 36% greater than that of VDB and B, respectively, suggesting significant seedlot differences.

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CHAPTER 4

GENERAL DISCUSSION AND CONCLUSION

4.1 Introduction

During this study, no data on growth, development and response to varying conditions of water for wild watermelon were found in South Africa. The review of literature relied largely on generalizations and information about curcurbits. The study focused on collecting data about growth and development of wild watermelon under conditions ranging from laboratory seed germination, through to controlled glasshouse conditions and field conditions. The first part of the study, seed germination, was not concerned with crop response to water stress, rather, it was aimed at establishing the quality of seedlots for seedling establishment and field crop production. Under glasshouse conditions (chapter 2) a controlled situation of varying water availability was created comparing planting material ideal for nursery conditions (pine bark), and two other materials designed to have physical characteristics that are different from pine bark (1:1 mixture of pure sand and pine bark, and pure sand only. Under field conditions, the crop production was staggered over a wide range of period encompassing the start of the summer rainfall and autumn, so that the effects of natural conditions differing is soil moisture and temperature would be studied. Climatic data and soil moisture content were determined and used in the presentation of results (Chapter 2). As much as an attempt was made to produce data on the response of wild watermelon to water stress, the goal of collecting agronomic data that will be used in future studies to execute studies that will produce

data for modeling of wild watermelon growth and development under strictly planned irrigation response studies remained important. In this chapter the author provides an overall interpretation of the key findings of chapters 2 and 3, with a view to making recommendations for future research.

4.2 Crop response to germination and water stress under controlled environment conditions

Wild watermelon is not a "major crop". In the context of South African agriculture, it is a minor, underutised crop, whose economic potential is yet to be fully exploited. Smartt and Haq (1997) refer to these crops as new crops, despite the fact that many of these crops have an ancient history of contributing to food security (Wallis, et al., 1989).

The ability of seed to germinate is an important component of seed quality, together with genetic quality, seed purity and seed health. As a reproductive unit, a seed must be able to germinate and establish seedlings. Germination is a measure of the physiological quality of the seedlot. Three important aspects of this ability affect a seedlot's performance: viability, germination and vigour (McDonald & Copeland, 1997). Viable seeds are those that are alive and have the potential to germinate when exposed to favourable germination conditions (McDonald & Copeland, 1997). When a germination test is conducted on healthy, clean seeds, those viable seeds that fail to germinate are considered dormant. It is concluded that the inability of wild watermelon seedlots to reach 100% germination in this study (Figure 2.2) was likely due to physiological dormancy. Physiological dormancy may be due to high levels of inhibitory hormones, eg., Abscisic

acid or embryo immaturity (McDonald & Copeland, 1997. After-ripening of seeds by storing them at ambient temperatures for weeks or months helps to break physiological seed dormancy (AOSA, 1996). Confirmation that the seeds may have had physiological dormancy was confirmed by improved emergence under field conditions, following a further period of storage at ambient conditions before planting.

Sensitivity of curcubits to water and temperature stresses has been reported (Lee et al, 2003; Heuer, 1993; Moon et al., 2007). Blending of media for seedling production is also a common phenomenon in research for the nursery industry (Choi et al., 2007; Warncke, 1986). In the present study, the blending of pine bark and sand improved seedling emergence under moderate soil water availability (Figure 2.5), but seedling growth was best supported in the pine bark only medium with 75% F.C. (e.g., Figures 2.6 and 2.11). The differences in response to media may have been due to the fact that the mixture of sand and pine bark, provided a combination of water availability and temperature that favoured seed germination. Optimum seed germination requires warm temperatures and water availability under well-aerated conditions. Pine bark may have been more suitable for supporting seedling growth than the mixture of pine bark and sand, and sand only, because of its high water holding capacity. When a root substrate is formulated and packed into a plug tray, large and small inter-aggregate pores are formed. The large pores act as air-filled porosity and the small pores contain water (Verdonck & Pennick, 1986). In the present study, the substrates were not analysed for porosity, but it is most likely that blending pine bark with sand resulted in the creation of a larger proportion of larger pores, which were less able to retain water than the smaller pores, which likely occurred in a larger proportion or more balanced ratio in pine bark only. For this reason, it is concluded that using pine bark only provides suitable conditions of water content availability of seedlings and the increase in water status from 50% to 75% is beneficial. The mixture of sand and pine bark might be beneficial in situations where species that are very sensitive to soil water are grown. Being a desert crop, wild watermelon was expected to be less affected by the presence of sand in the media, in terms of growth and final seedling size. The estimation of water use efficiency, however, showed that the sand-based media were more efficient that the pine bark in the amount of water used to produce a unit of seedling dry mass (Figure 2.16). In addition, 25% F.C. was associated with more water use efficiency than 50% and 75% FC. These results suggest that wild watermelon can survive water stress and produce seedlings even under conditions of water stress. This conclusion requires a comparison of wild watermelon with other species.

4.3 Crop response to planting dates of different rainfall content

Climatic information is necessary for successful planning in agriculture. Various indices, such as water efficiency indices, thermal indices, heat units, etc., were developed in early attempts to relate climatic data more closely to factors such as crop growth rate, yield and production as required for land use planning and crop zonation. These usually involved accumulated temperature, some comparison of of rainfall with crop water requirements, and the length of the season with favourable indices (Bidinger and Johansen, 1986). This study relied on naturally occurring climatic conditions to determine the response of wild

watermelon to soil water content over three seasons created by planting at different times during the summer of 2008/2009. The period of crop growth from planting to full flowering of the last crop is depicted in Figure 4.1, using data in Appendix 1. The gravimetric soil water pattern was similar to the rainfall pattern for November and January plantings (Figure 3.1).



Figure 4. 1 Temperature and rainfall during the period of plant growth

For the September planting, the soil water content pattern showed a persistence of high levels until the end of the season (Figure 3.1). The likely reason for the high soil water content for the September planting is that leaf cover reduced evaporation from the soil more effectively than it happened with then later plantings. The later plantings were associated with slow plant growth rate (Figures 3.5 and 3.12). The data in Figure 4.1 could be used to predict plant growth and development that would be optimum between November and February. The results of this study, however, showed that planting in September resulted in the best plant growth and development and final yield.

The theoretical determination of plant growth rate in this study allowed and understanding of biomass accumulation with time (Figures 3.5 and 3.12). Within a season, accumulation generally follows a sigmoidal curve. Sigmoidal curves can be divided into early exponential phase of seedling growth, a grand period during midseason, and a final senescent phase (Cerrato & Blackmer, 1990). In this study, crop growth rate was measured only during the early exponential phase and part of the grand phase (Figures 3.5 and 3.12). It was shown that the highest crop growth rate and final yield occurred when the crop was grown over a long season, planting in September and harvesting in March. This period was associated with long periods of radiation and rainfall precipitation, which resulted in more fruits of larger size and higher final yield than when the crop was planted later in the season. It can be concluded that September is the best time to plant wild watermelon, because the crop is a relatively long season one.

The three landraces used in this study showed a consistent difference in performance, where the Eastern Cape variety (B) produced better plant size and yield than the KwaZulu-Natal varieties (DB and VDB). The good performance of the Eastern Cape landrace could be explained on the basis of the data presented in Figure 2.1, where it is shown that the Eastern Cape provenance had a similar rainfall, temperature and drought occurrence to Pietermaritzburg, the study site. Although landrace B showed better seedling performance under glasshouse and better plant growth and yield under field conditions, it cannot be generalized that it is the best one for growth under drought conditions. The seedling growth study included water stress conditions (25% F.C.), but the field conditions were not droughty, strictly speaking. Instead, the periods of growth differed in the amount of water and temperature availability, with the late planting (January) being associated with drier conditions during fruit development and maturation than the other two seasons. Although the November planting was associated with high temperature and rainfall, it did not produce the best yields. The reason for this anomaly was not investigated in this study. However, it may have to do with the crop being prone to leaf diseases and high respiration losses.

The specific objectives of the study were (a) to determine the effect of water stress on three varieties of watermelon differing in seed colour and provenance during seedling establishment, (b) to determine the effect of planting date on crop growth and yield under field conditions, and (c) to relate proline accumulation to water stress in wild watermelon. It is concluded that wild water melon seedlings were responsive to water stress under controlled environment conditions in that sandy media and 25% F.C. reduced seedling growth, but the crop displayed the ability to improve water use efficiency under water stress. Crop yield improves under long season conditions. Leaf proline accumulation can be used as an indicator of water stress response. Therefore, the ability of a plant to simultaneously accumulate proline and withstand stress, could be used in the strategies to select for drought tolerance.

Future studies should investigate the genetic differences between landraces and simulate drought under field conditions. Soil water content needs to be taken to deeper soil depths that are reached by the crop roots, and leaf area needs to be determined under field conditions. These data, together with climatic data, can be used to develop models for growth and development of wild watermelon under water stress conditions.

4.4 References

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APPENDICES

Appendix 1. Climatic data for the research site, Ukulinga Research Farm. Monthly averages and totals. Source: Agricultural Research Council, South Africa.

	Start	Year		9	Start Mo	nth			End Year			End Month		
	200	08			1			2009				12		
Com	p#			St	ation Na	ame			Latitude	L	ongi	tude	Altitude	
3016	50		PN	1BURG;	UKULIN	GA RES ST	N		-29.66763	:	30.40	599	806	
Compno	Year	Month	Тx	Tn	Т	Rain	RHx	RHn	Rs	U2	ET0	HU	CU	DPCU
30160	2008	1	26.35	16.53	20.44	117.50	91.66	53.56	17.95	0.91	4.15	323.53	-601.50	0.00
30160	2008	2	27.06	16.96	20.72	63.70	91.90	50.96	16.81	0.94	3.98	310.86	-591.00	0.00
30160	2008	3	25.84	15.37	19.53	56.60	90.94	47.61	14.70	0.90	3.52	295.55	-511.50	8.00
30160	2008	4	23.04	12.19	16.61	57.60	88.29	42.64	10.76	0.88	2.67	199.64	-225.00	56.00
30160	2008	5	23.92	11.79	16.94	1.80	86.96	34.60	9.19	0.76	2.44	215.66	-260.00	52.50
30160	2008	6	20.27	9.08	13.80	23.40	85.35	37.71	8.00	0.76	2.03	122.17	41.50	202.50
30160	2008	7	22.04	8.55	14.36	0.30	74.00	23.58	9.73	0.76	2.60	148.06	-3.50	200.00
30160	2008	8	23.85	10.22	15.80	5.20	84.84	28.51	11.64	1.16	3.11	182.76	-115.50	137.50
30160	2008	9	24.23	9.55	16.12	41.60	82.17	27.91	14.72	1.46	3.84	194.79	-103.00	177.50
30160	2008	10	22.85	12.44	16.47	53.30	91.78	52.75	13.61	1.06	3.22	200.64	-190.50	65.00
30160	2008	11	24.17	14.43	18.18	68.30	92.63	55.97	15.29	1.05	2.93	245.26	-386.50	21.00
30160	2008	12	26.16	16.25	20.17	142.20	91.90	54.73	17.16	1.03	3.38	314.97	-535.50	4.50
30160	2009	1	24.72	16.22	19.71	116.40	93.20	65.99	15.24	0.75	2.42	291.34	-516.00	0.00
30160	2009	2	25.95	16.28	20.06	115.10	92.67	58.41	15.53	0.75	3.02	281.61	-497.50	0.00
30160	2009	3	25.54	15.32	19.49	50.70	91.65	52.88	15.60	0.73	3.01	294.08	-525.00	8.50
30160	2009	4	25.10	13.09	18.01	19.10	88.13	42.59	13.08	0.66	2.54	241.36	-347.50	40.00
30160	2009	5	22.81	11.46	15.92	22.10	87.43	39.59	10.01	0.63	1.84	65.18	-76.50	14.50
30160	2009	6	20.88	9.20	12.50	346.80	81.40	31.03	12.84	0.92	2.11	45.06	33.50	99.00
30160	2009	7	20.97	7.70	13.41	1.90	76.29	22.47	12.23	0.75	2.17	121.40	72.00	241.00
30160	2009	8	22.72	9.13	15.13	42.60	86.00	29.00	13.53	1.21	2.63	167.18	-55.50	185.00
30160	2009	9	23.02	10.59	16.57	23.10	87.40	38.10	13.90	1.11	2.89	200.11	-166.00	127.00
30160	2009	10	22.77	12.77	16.81	119.50	91.71	53.32	14.45	0.93	2.76	211.15	-271.50	36.50
30160	2009	11	23.68	13.67	17.40	72.80	88.00	52.11	14.00	1.05	2.84	222.39	-254.50	57.00
30160	2009	12	24.25	14.99	18.83	139.20	92.77	59.98	14.58	0.92	2.94	273.66	-432.50	12.50

Appendix 1 (Continued)

EI EMENT	DESCRIPTION	UNIT	STATION
	DESCRIPTION	UNII	TYPE
Tx	Average Maximum Temperature	°C	AWS
Tn	Average Minimum Temperature	°C	AWS
Т	Average Temperature [Calculated From Hourly Data]	°C	AWS
Rain	Average Total Rainfall [Calculated From Hourly Data]	mm	AWS
RHx	Average Maximum Relative Humidity	%	AWS
RHn	Average Minimum Relative Humidity	%	AWS
Rs	Average Total Radiation [Calculated From Hourly Data]	MJ/m2	AWS
U2	Average Wind Speed [Calculated From Hourly Data]	ms	AWS
ET0	Average Total Relative Evapotranspiration [Calculated From Hourly Data]	mm	AWS
HU	Average Total Heat Units [Calculated From Hourly Data]	Unitless	AWS
CU	Average Total Cold Units [Calculated From Hourly Data]	Unitless	AWS
DPCU	Average Daily Positive Chilling Units [Calculated From Hourly Data]	Unitless	AWS
Тх	Average Maximum Temperature	°C	MWS
Tn	Average Minimum Temperature	°C	MWS
Rain	Total Rainfall	mm	MWS
RHx	Average Maximum Relative Humidity	%	MWS
RHn	Average Minimum Relative Humidity	%	MWS
UTot	Average Windrun	Km/day	MWS
APan	Total Daily Apan Evaporation	mm	MWS
Suns	Daily Wind Run	KM/day	MWS
HU	Average Heat Units [Not yet available]	Unitless	MWS
CU	Average Cold Units [Not yet available]	Unitless	MWS
DPCU	Average Daily Positive Chilling Units [Not Yet Available]	Unitless	MWS

KEY NOTES

Appendix 2 Analysis of variance tables for chapter 2

A. Seedling emergence in nursery seedling trays

Variate: Emergence

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	2597.4	1298.7	10.63	
Rep.*Units* stratum					
Seedlot	2	5461.7	2730.9	22.36	<.001
Day	3	432.4	144.1	1.18	0.318
FC	2	9505.7	4752.9	38.92	<.001
Media	2	287.2	143.6	1.18	0.311
Seedlot.Day	6	377.8	63.0	0.52	0.796
Colour.FC	4	423.5	105.9	0.87	0.485
Day.FC	6	226.4	37.7	0.31	0.932
Seedlot.Media	4	728.1	182.0	1.49	0.206
Day.Media	6	1400.5	233.4	1.91	0.080
FC.Media	4	7909.1	1977.3	16.19	<.001
Seedlot.Day.FC	12	237.0	19.8	0.16	0.999
Seedlot.Day.Media	12	1288.0	107.3	0.88	0.569
Seedlot.FC.Media	8	2638.1	329.8	2.70	0.008
Day.FC.Media	12	114.4	9.5	0.08	1.000
Seedlot.Day.FC.Media	24	240.3	10.0	0.08	1.000
Residual	214	26136.0	122.1		
Total	323	60003.4			

B. Seedling height

Variate: Seedling height (cm)

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Replication stratum	2	0.1667	0.0833	0.08	
Replication.*Units* stratum					
Media	2	20.2500	20.2500	20.40	<.001
FC	2	42.2500	42.2500	42.57	<.001
Seedlot	2	4.5000	2.2500	2.27	0.127
Media.FC	4	0.2500	0.2500	0.25	0.621
Media.Seedlot	4	4.5000	2.2500	2.27	0.127
FC.Seedlot	4	0.5000	0.2500	0.25	0.780
Media.FC.Seedlot	8	0.5000	0.2500	0.25	0.780
Residual	52	21.8333	0.9924		
Total	80	94.7500			

C. Fresh mass

Variate: Fresh Weight (g)

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	16.679	8.339	1.06	
Rep.*Units* stratum					
FC	2	26.339	13.169	1.67	0.017
Seedlot	2	1.629	0.814	0.10	0.002
Media	2	1898.202	949.101	120.69	<.001
FC.Seedlot	4	33.437	8.359	1.06	0.384
FC.Media	4	26.093	6.523	0.83	0.513
Seedlot.Media	4	16.489	4.122	0.52	0.718
FC.Seedlot.Media	8	110.534	13.817	1.76	0.107
Residual	52	408.928	7.864		
Total	80	2538.329			

D. Dry mass

Variate: Dry Weight (g)

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	0.2373	0.1186	0.16	
Rep.*Units* stratum					
FC_%	2	2.3069	1.1535	1.52	0.028
Colour	2	0.5928	0.2964	0.39	0.049
Media	2	101.9573	50.9786	67.24	<.001
FC_%.Colour	4	2.3575	0.5894	0.78	0.545
FC_%.Media	4	14.6264	3.6566	4.82	0.002
Colour.Media	4	1.4205	0.3551	0.47	0.759
FC_%.Colour.Media	8	3.3269	0.4159	0.55	0.814
Residual	52	39.4227	0.7581		

Total 80 166.2484

E. Root mass

Variate: Root mass

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Replication stratum	2	0.0000500	0.0000250	0.10	
Replication.*Units* stratum					
Media	2	0.0142802	0.0142802	56.61	<.001
FC	2	0.0254403	0.0254403	100.84	<.001
Seedlot	2	0.0044955	0.0022477	8.91	0.001
Media.FC	4	0.0080103	0.0080103	31.75	<.001
Media.Seedlot	4	0.0059255	0.0029628	11.74	<.001
%FC.Seedlot	4	0.0232355	0.0116178	46.05	<.001
Media.FC.Seedlot	8	0.0235655	0.0117828	46.71	<.001
Residual	52	0.0055500	0.0002523		

Total 80 0.1105527

F. Root length

Variate: Root length (cm)

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	0.2	0.1	0.00	
Rep.*Units* stratum					
FC	2	157.1	78.5	0.71	0.498
Seedlotr	2	160.9	80.5	0.72	0.490
Media	2	42010.5	21005.2	189.07	<.001
FCSeedlot	4	129.2	32.3	0.29	0.883
FC_Media	4	1864.3	466.1	4.20	0.005
Seedlot.Media	4	92.5	23.1	0.21	0.933
FC.Seedlot.Media	8	988.7	123.6	1.11	0.370
Residual	52	5777.2	111.1		

Total 80 51180.5
G. Leaf area

Variate: Leaf area

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Replication stratum	2	12.892	6.446	0.76	
Replication.*Units* stratum					
Media	2	200.789	200.789	23.82	<.001
FC	2	6561.000	6561.000	778.25	<.001
Seedlot	2	601.841	300.921	35.69	<.001
Media.FC	4	1237.632	1237.632	146.80	<.001
Media.Seedlot	4	212.933	106.467	12.63	<.001
FC.Seedlot	4	214.588	107.294	12.73	<.001
Media.FC.Seedlot	8	296.177	148.088	17.57	<.001
Residual	52	185.470	8.430		
Total 80 9523.324					

H. Proline

Variate: Proline Concentration

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	0.020691	0.010345	2.68	
Rep.*Units* stratum					
FC	2	25.886416	25.886416	6712.11	<.001
Media	2	0.730709	0.730709	189.47	<.001
Seedlot	2	2.076781	1.038391	269.25	<.001
FC.Media	4	0.136052	0.136052	35.28	<.001
FC.Seedlot	4	1.682771	0.841386	218.16	<.001
Media.Seedlot	4	3.235433	1.617716	419.46	<.001
FC.Media.Seedlot					
	8	1.341856	0.670928	173.97	<.001
Residual	52	0.084847	0.003857		

Total 80 35.195555

Appendix 3: List of analysis of variance tables for Chapter 3

A. Seedling emergence

Variate: % Emergence

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	338.4	169.2	0.21	
Rep.*Units* stratum					
Date	2	1600.4	800.2	0.99	0.395
Seedlot	2	1353.5	676.7	0.83	0.453
Date.Seedlot					
	4	1472.3	368.1	0.45	0.769
Residual	16	12995.0	812.2		

Total 26 17759.5

B. Number of leaves

Variate: No of leaves

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	3732.	1866.	1.36	
Rep.*Units* stratum					
Date	2	158659.	79329.	57.65	<.001
Seedlot	2	41367.	20683.	15.03	<.001
Week	2	247732.	123866.	90.02	<.001
Date.Seedlot	4	105399.	26350.	19.15	<.001
Planting_Date.Week	4	84709.	21177.	15.39	<.001
Seed_colour.Week	4	23822.	5956.	4.33	0.004
Date.Seedlot.Week	8	74079.	9260.	6.73	<.001
Residual	52	71550.	1376.		
Total	80	811048.			

C. Number of vines

Variate: No of Vines

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	4.861	2.431	1.27	
Rep.*Units* stratum					
Date	2	9.492	4.746	2.47	0.094
Seedlot	2	6.115	3.058	1.59	0.213
Week	2	22.109	11.054	5.76	0.005
Date.Seedlot	4	37.182	9.295	4.85	0.002
Date.Week	4	7.755	1.939	1.01	0.410
Seed_colour.Week	4	3.264	0.816	0.43	0.790
Date.Seedlot.Week	8	3.903	0.488	0.25	0.977
Residual	52	99.750	1.918		
Total 80 194.433					
D. Vine length					

Variate: Vine length Source of variation d.f. F pr. s.s. m.s. v.r. 2 318.4 159.2 0.21 Rep stratum Rep.*Units* stratum 2 Date 65522.2 32761.1 43.47 <.001 Seedlot 2 19882.4 9941.2 13.19 <.001 Week 2 100249.2 66.51 50124.6 <.001 Date.Seedlot 4 23270.1 5817.5 7.72 <.001 4 Date.Week 9404.0 2351.0 3.12 0.022 Seedlot.Week 4 11170.0 2792.5 3.71 0.010 Date.Seedlot.Week 8 18997.7 2374.7 3.15 0.006 Residual 52 39191.5 753.7 Total 80 288005.5

E. Fruits

Variate: fruits per plant

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	9.85	4.93	0.07	
Rep.*Units* stratum					
Date	2	1302.30	651.15	8.92	0.002
Seedlot	2	19.85	9.93	0.14	0.044
Date.Seedlot	4	218.81	54.70	0.75	0.573
Residual	16	1168.15	73.01		

Total 26 2718.96

F. Large fruits

Variate: No Large Fruits plant

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	0.990	0.495	0.28	
Rep.*Units* stratum					
Date	2	46.113	23.057	13.18	<.001
Seedlot	2	0.706	0.353	0.20	0.819
Date.Seedlot	4	1.844	0.461	0.26	0.897
Residual	16	27.992	1.749		

Total 26 77.644

G. Medium fruits

Variate: Medium fruits plant

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	17.730	8.865	1.23	
Rep.*Units* stratum					
Planting_Date	2	219.607	109.803	15.29	<.001
Seed_colour	2	7.113	3.557	0.50	0.618
Planting_Date.Seed_colour					
-	4	40.745	10.186	1.42	0.273
Residual	16	114.918	7.182		

Total 26 400.113

H. Small fruits

Variate: Small fruits plant

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	32.27	16.13	0.54	
Rep.*Units* stratum					
Planting_Date	2	482.72	241.36	8.10	0.004
Seed_colour	2	1.30	0.65	0.02	0.978
Planting_Date.Seed_colour					
	4	79.33	19.83	0.67	0.625
Residual	16	476.55	29.78		

Total 26 1072.17

I. Fruit mass

Variate: Fruit Mass (kg)

Source of variation	d.f.	S.S.	m.s.	v.r.	F pr.
Rep stratum	2	25.45	12.72	0.88	
Rep.*Units* stratum					
Date	2	723.44	361.72	24.93	<.001
Seedlot	2	4.21	2.11	0.15	0.046
Date.Seedlot	4	45.54	11.38	0.78	0.552
Residual	16	232.17	14.51		
Total	26	1030.80			