Response of local wild mustard (*Brassica* species) landraces to water stress

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Submitted in partial fulfillment of the requirements for the degree of

Master of Science in Agriculture (Crop Science) in the

Crop Science
School of Agricultural Sciences and Agribusiness
Faculty of Science and Agriculture
University of KwaZulu-Natal
Pietermaritzburg

August 2010

Declaration

I hereby declare that the research work reported in this thesis is the result of my own work 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 university. The study was financially supported by the Water Research Commission (Project No. K5/1771//4).

Signature ……..

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I, Albert Thembinkosi Modi, supervised the above candidate in the conduct of her dissertation study.

Signature ……..

Prof. A.T. Modi

Acknowledgements

My special and most sincere gratitude go to the following:

- Prof. A. T. Modi for his patience, guidance and support throughout this study. I know Prof, it was not easy especially last year but thank you and God bless you indeed.
- Water Research Commission for funding the research work.
- Tafadzwa, thank you for your time and support when I was going through a hard time in my life you were there with those trips at Ukulinga thank you! God bless you.
- Matt, Ma Florence and sis Thembi, thank you for your assistance with my field work at Ukulinga.
- Ravin Singh of ARC-CEDARA, thank you for providing with the weather data.
- Mathaba and his mother, I know it was not easy looking after Mfundo and me when you shouldn't have, God bless you.
- Samson thank you for your support to when I was going through hardship, keep on praying my brother!
- The post graduate students in Room 344 thank you guys for your assistance during the course of my study.
- Bab'mncani Thiza, thank you for being a father, mother, brother and sister to me, I love you so much, God bless you.
- Mfundo, my son a blessing to my life, I love you Zibusiso.

Dedication

This work is dedicated to God Almighty the one who gives me strength every day.

ABSTRACT

Wild mustard is an indigenous leafy vegetable. Its use is limited by a lack of knowledge of its agronomy. However, it is a rich source of nutrients and other minerals. Nowadays, the use of indigenous crops has been replaced by exotic crops. Climate change is affecting agricultural productivity. South Africa is a water scarce country with uneven rainfall distribution. Therefore, studies on water stress effects on plant growth were promoted by the Water Research Commission and the University of KwaZulu-Natal to understand plant responses to water stress for commercial and subsistence farming. The objective of the study was to characterise local wild mustard cultivars morphologically and physiologically with respect to production, and for the purposes of identifying their drought tolerance.

Three experiments were conducted at the University of KwaZulu-Natal in order to evaluate the responses of local wild mustard cultivars to water stress. Seeds of wild mustard cultivars were characterised according to seed coat colour. Seed quality was determined by a standard germination test. Vigour was then tested using electrolyte conductivity. Seeds were sown in seedling trays under two water regimes of 25% field capacity (FC) and 75% (FC) on pine bark growing media. The experiment was terminated at 21 days when root and shoot lengths were measured. The effect of water stress on protein content and seedling growth parameters was determined.

Soil was collected from the University of KwaZulu-Natal Research Farm for a pot trial. Seeds of wild mustard were sown in 81 pots, each filled with 2 kg of soil, under three water regimes (25% FC, 50% FC and 75% FC). Pots were maintained at the

corresponding field capacity level by re-weighing the pots, three times a week. Measurements of plant height and leaf number were recorded weekly. The experiment was terminated at the flowering stage. At the end of the experiment, plant growth parameters (plant height, leaf area and number, dry and fresh mass) were measured in order to evaluate the effects of water stress at the vegetative stage.

A field trial was conducted at the University of KwaZulu-Natal Ukulinga Research Farm in Pietermaritzburg. The experiment was conducted during the winter and spring of 2009. A completely randomised design was used for non-irrigated and irrigated (25 mm/week) trials. Emergence was measured as well as plant height and leaf number. Plant growth parameters were also measured at the end of the experiment. Leaf samples were taken for proline determination.

There was a significant interaction ($p<0.05$) between seed colour, landraces and days to germinate with respect to germination capacity. Isaha and Masihlalisane landraces showed higher germination percentages than Kwayimba. There was also a significant interaction ($p<0.05$) between landraces and seed colour with respect to electrolyte conductivity. Lighter seeds of wild mustard landraces showed higher solute leakage. Isaha and Masihlalisane had higher solute leakage than Kwayimba. Significant interactions ($p<0.05$) between landraces and field capacity with respect to emergence, leaf number, root and shoot length and total proteins were also observed. Isaha and Masihlalisane showed higher emergence than Kwayimba. Leaf number was reduced for all landraces under water stress. Total protein content was high in black seeded landraces under water stress. There was a significant interaction ($p<0.05$) between landraces and field capacity with respect to seedling fresh and dry

masses. Under moderate water stress conditions, Isaha and Masihlalisane showed increased biomass accumulation. There were highly significant differences ($P<0.001$) in plant height, leaf area, fresh and dry mass with respect to planting date. Plants performed significantly ($p<0.05$) better in spring than in winter. Isaha and Masihlalisane performed significantly ($p<0.05$) better than Kwayimba. There was a highly significant interaction ($p<0.001$) between landrace and irrigation treatments with respect to proline accumulation. Under water stress, Kwayimba black seeded landrace accumulated more proline.

It is concluded that light-coloured seeds of wild mustard landraces were associated with good seed quality. Masihlalisane brown seeds have good early seedling establishment. Kwayimba black seeds showed tolerance to water stress through accumulation of proteins. Isaha and Masihlalisane showed an increase in biomass accumulation under moderate water stress. Water stress tolerance in some of wild mustard landraces was negatively correlated with proline accumulation. Masihlalisane brown type can grow well, with good yields, under water stress.

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CHAPTER 1

LITERATURE REVIEW

1.1 Introduction

Wild mustard [*Brassica juncea* (L.) Czern & Coss and *Brassica nigra* (L.) W.D.J. Koch] is an indigenous leafy vegetable of South Africa. It is of major importance for the nutrition and livelihoods of rural South Africans. It belongs to the family of *Brassicaceae* or *Crucefereae* (Dixon, 2007). *Brassicas* are believed to have originated in central Asia and later spreading to Europe, India and Africa (Muzishima, 1980). They are commonly used as spices and vegetables. They are also used in oil production and for medicinal purposes (Muzishima, 1980). In South Africa, wild mustard is used as an indigenous leafy vegetable (Jansen van Rensberg *et al*., 2007).

All *Brassicas* have a common characteristic, the presence of glucosinolate compounds. These plants are commonly associated with the occasional bitterness found in some *Brassicas*. The bitterness is as a result of the products of glucosinolate breakdown by the enzyme myrosinase (Chonge & Berard 1983). Products formed during the breakdown of glucosinolate have been reported to have anti-carcinogenic properties (Rosa *et al.,* 1997; Mithen *et al*., 2003).

Nutritional quality of *Brassicas* (especially the micronutrients which increase with different planting seasons) is affected by planting date, environmental conditions

and length of the growing season (Harper & Compton, 1980). Some *Brassicas,* such as rapeseed, are rich in oil content (about 40-42% seed oil content). They are also rich in protein content, with a seed meal of 43.6% protein content (Iqbal *et al*., 2008). *Brassicas* have shown great diversity with regard to their uses. They are widely used for oil production, especially in India (Dixon, 2007). Apart from being used for oil production, they are usually consumed as leafy vegetables which can be eaten either raw or cooked.

Wild mustard is an indigenous leafy vegetable which is able to adapt to different environmental conditions. It is both easy to grow and manage (Schippers, 2002). In South Africa, indigenous leafy vegetables were traditionally widely grown as a dietary supplement (Van Vuuren, 2006). However, nowadays they are looked down upon due to a lack of knowledge and a rural lifestyle less associated with the use of indigenous leafy vegetable crops (Modi, 2003). Indigenous leafy vegetables are important since they provide essential vitamins, trace elements (iron and calcium) and other nutrients that are important for good health (Chweya & Eyzaguirre, 1999). In the wild, *Brassicas* occur either as diploids (*B. nigra*) or amphidiploids (*B. junceae*) and these comprise the species occurring in South Africa, as well as their hybrids.

Brassica nigra (known as Kwayimba in the vernacular Zulu) is a diploid (*2n*) (UN, 1935). *Brassica junceae* is an amphidiploid (*4n*) formed from a crossing between *Brassica nigra* (L.) Koch and *B. rapa* (L.). It has high oil and protein content

(Burton *et al*., 1999) which are environment-dependant; decreasing under unfavourable environments and increasing under favourable environments (Walton, 1999). *Brassica juncea* has been reported to establish fast and achieve optimum ground cover as well as an ability to tolerate different environmental stresses (Woods *et al*., 1991). In South Africa, both *B. juncea* and *B*. *nigra* are commonly used as leafy vegetables (Laker, 2007). Three wild mustard landraces were used in this study Isaha, Masihlalisane and Kwayimba (Fig. 1.1). Seed was collected from Tugela Ferry, KwaZulu-Natal. Seed colour variation within landraces exists (Fig 1.1) and it is hypothesised that seed colour differences within landraces maybe linked to differences in germination, vigour and growth of the crop.

Figure 1.1 Seeds of local wild mustard landraces; (middle) Isaha, (left) Kwayimba and (right) Masihlalisane

South Africa is considered a water-scarce country (DWAF, 2004) and has recently been characterised a water stressed country (Otieno & Ochieng, 2004). Water is the most limiting factor in agriculture. This is as a result of low rainfall and uneven distribution. South Africa is classified by areas of hyper-arid to semi-arid areas (Bennie & Hensley, 2001). Rainfall in South Africa is seasonal and less than 10% of here is humid (Bennie & Hensley, 2001). Except for the Mediterranean climate found in the Western Cape, most of South Africa receives rains in summer. In marginalized environments, water stress is usually the main factor affecting crop production. Scientists working on climate change models have predicted increased evapotranspiration and lower rainfall amounts (Samarakoon & Gifford, 1995; Athar & Ashraf, 2005). This is expected to exacerbate South Africa's water situation. Constraints on water availability can limit normal plant growth and eventually yield itself (Boyer, 1982). *Brassica juncea* and *B*. *nigra* and their hybrids are known for their ability to withstand drought, heat and salt stresses as a result of their natural evolution under inhospitable environments (UN, 1935; Jana, 2007). The use of marginal soils by plants that are drought tolerant may increase the chances of agricultural production in such environments. In these areas, indigenous leafy vegetables have a potential to be used as a source of nutrition.

Limitations of any environmental factor may result in stress, which normally reduces the yield. The accessibility of water in most South African rural communities is still a problem (in cases whereby water is collected from long

distances). Identifying drought tolerant leafy vegetables will be important in fighting poverty and malnutrition within such communities. It is thus important to understand the agronomic requirements, water use and nutritional value of these indigenous leafy vegetables in order to reinstate them to their former status of usefulness in rural communities.

1.2 Environmental effects on seed quality and germination

Seeds are important for both subsistence and commercial farmers as they are the starting point for crop production. Seed quality is important in determining good germination and emergence. Seed vigour and viability are determinants of seed quality. However, seed quality is affected by many environmental factors such as temperature, water, light and soil (Wulf, 1995).

Seed vigour and viability are associated with seed germination and quality. A viable seed can germinate under suitable conditions. However, seed viability can only be measured after seed dormancy has been broken (Bradbeer, 1988). A viable seed should be able to germinate on its own under optimal environmental conditions and produce healthy seedlings. In order to achieve successful field establishment, germination must be vigorous.

Seed vigour has been defined as the sum total of those properties of the seed which determine the potential level of activity and performance of the seed during germination and seedling emergence (Perry, 1978). Seed vigour tells us about the seed's ability to emerge under different environmental conditions and also

gives an idea of seed physiological quality. Bewley and Black (1994) and Powell *et al*. (2005) reported that seed physiological quality was determined by seed genetic constitution, which is dependent on different factors that occur within and outside the seed during seed development on the mother plant, at harvest time and during storage. Loss of seed vigour is normally associated with poor germination. Maximum seed vigour and viability can be attained when seeds attain their maximum physiological maturity (Harrington, 1972). Therefore, different seed physiological responses during growth may have an influence on seed germination and vigour. Physiological responses to seed performance may be associated with membrane damage. Seed vigour can be measured by electrolyte leakage. Most seeds are able to recover from this during the initial phase of soaking (imbibition); however severely damaged seeds do not have the physiological ability to recover, thus affecting germination uniformity and rate (Bewley & Black, 1994).

Seeds are protected from different environmental stresses by the seed coat. The seed coat protects the seed from hydration and electrolyte leakage during germination (Yasseen *et al*., 1987). It thus plays a role in germination. Electrolyte leakage (EC) was used in the study by Matthews and Powell (1981) on dry green pea to select high vigour seed for planting under stressful conditions. A relationship between EC and germination capacity was observed in pea seeds (Vieira *et al*., 1999).

1.2.1 Effects of seed coat colour on germination

Recent studies have shown that seed coat colour has an influence on seed germination and quality (Odindo, 2007). Seed colour is one of the factors that affect seed quality. Seed colour can be linked to different physiological processes that the seed undergoes before it germinates (Odindo, 2007). Asiedo and Powell (1998) and Pimpini *et al*. (2002) reported a correlation between seed colour and seed performance during germination. Electrolyte leakage (EC) was observed in seeds of cowpea, radicchio and *Atriplex cordobensis,* whereby coloured or dark seed performed better than the unpigmented seeds. There was a contradiction when it came to seeds of *Atriplex cordobensis* which showed better performances in light coloured seeds than dark ones (Asiedo & Powell, 1998; Pimpini *et al*., 2002; Aiazzi *et al*., 2006). In a separate study conducted to determine the effect of seed colour on germination of Pansy (*Viola x wittrockiangams),* darker seeds had the highest germination rate compared to brown and yellow seeds (Agnieszka & Hulobowicz, 2008). Dalianis (1980) observed that in *Trifolium alexandrium,* seed colour had an influence on germination capacity, emergence rate and seedling elongation and length.

Seed colour is also associated with seed quality (Pederson & Toy, 2001) which is measured as germination capacity and physiological vigour (Bewley & Black, 1994). In a study done on dry green pea, it was shown that dark green seeds had the highest germination percentage and seed vigour as compared to light-green seeds (Atak *et al.*, 2006). Electrolyte leakage has been associated with loss of

seed viability and germination. During the drying process, seeds lose water resulting in a disruption of the cellular membrane. The more the seeds lose water, the greater the membrane damage (Bewley & Black, 1994). Seeds can recover from membrane damage through different seed treatments that are used to enhance germination (priming).

1.2.2 Environmental effects on seed quality

Seed characteristics are determined by genotype and environment (G x E) interaction (Galloway, 2001). Seed mass, dormancy and germination rate have a high adaptive nature for survival under different environmental conditions such as temperature, photoperiod, nutrient availability and soil water content (Gutterman, 1993).

The environment affects crop growth in a number of ways, one of which is seed quality. Seed quality is defined as the ability of the seed to germinate vigorously under a wide range of environmental conditions. Plant growth parameters can only be measured as a result of a seed being able to germinate and emerge under certain environments. Seed quality is important to achieve uniform emergence in the field.

Temperature affects seed quality in different ways, depending on the conditions under which the seeds are grown (e.g., laboratory or field conditions). Temperature stress also influences seed germination and vigour. Temperature

effects are more pronounced in cereals; low soil water content and high temperatures result in reduction of germination percentages (Al-Karaki, 1998). A reduction in germination percentages and vigour has been shown for crops such as barley (*Hordeum vulgare*. L) and soybean seeds (Dornbos, 1995) under both temperature and water stress.

Germination and seedling establishment are used as the most viable criteria for selecting for salt and drought tolerance in plants (Serrano *et al*., 1999; Boubaker, 1996). Seed germination is affected by salt stress through an osmotic effect (Welbaun *et al*., 1990). Germination velocity decreased in response to water stress in different wheat cultivars but for other cultivars it was high, implying that genotypic differences play a role in plant adaptation to different environments (Vargas *et al.*, 2001).

Generally, plants develop different systems for them to adapt to different environmental conditions that normally affect germination capacity and physiological vigour.

1.3 Plant growth, yield and environmental stress

Biological and economic yield are influenced by abiotic and biotic factors. Abiotic factors include some important climatic conditions that affect crop yield such as water, temperature, solar radiation and flooding. Biotic factors include weeds, pests and diseases that may affect crop growth and yield. If these factors are not controlled, they can be limiting to crop yield. Crop response to the environment is

influenced by interaction between genotype and environment (G x E interaction). Drought stress tolerance is a multigene trait controlled by abiotic and biotic factors (Gazendam & Oelofse, 2006). Asharf & Iqbal (2006) suggested that even short term drought may result in loss of crop yield due to morphological and physiological processes that are affected by temporary and permanent loss of water. Plant growth is inhibited under water stress conditions (Nir, 1970).

Temperature influences seed germination and emergence, root growth, water and nutrient absorption and crop yield. It is considered as a primary determinant of plant development. A study done on canola showed that protein and oil concentration increased in response to high temperatures and water deficits (Blondel & Renard, 1999). Temperature also influences the partitioning of assimilate between the shoot and root. Root temperature affected root extension, root area, length and number in barley and oilseed rape (Macduff & Wild, 1986). Temperature effects on yield depend on the growth stage of the plant and temperature changes that occur at that stage. In maize (*Zea mays* L.), leaf photosynthetic rates increased between 15°C and 31°C in young maize (Duncan & Hesketh, 1968; Tollenaar, 1989). However, information on the effect of drought and temperature on seed oil and protein concentration is not available for wild mustard.

Nutrient stress refers to an absence of essential plant nutrients or elements. These are present in the soil, hence soil is considered to somehow influence

mineral stress (Dudal, 1976). Nutrient stress may be due to poor soil fertility management and soil erosion. Salt stress also reduces nutrient uptake by plants; nitrogen and phosphorus uptake by rice (*Oryza sativa*) and wheat (*Triticum aestivum*) was inhibited under high salt concentrations of root medium (Mahajan & Sonar, 1980). Many studies have been conducted in order to investigate the effect of salinity stress on crop yield and most of these studies were linked to salt and water stress. In maize and melon (*Cucumis melo* L.), significant differences in yield were observed as a result of salinity and water stress (Shani & Dudley, 2001).

1.3.1 Drought tolerance or resistance and yield determinants

The most important factor limiting crop productivity worldwide is drought. Water is the most important component of life. It controls many biological processes; it is the most important component of both the animal and plant cell. It is believed to constitute 80-90% of fresh weight of active plant tissue. It is important for plant growth, metabolism, morphology, physiology and consequently plant productivity.

Studies on maize and sunflower showed that water stress negatively affected leaf expansion and development of the stem. In maize, water stress resulted in a reduction in leaf elongation (Boyer, 1970) while sunflower leaves were found to be even more sensitive to water stress (Boyer, 1968). Husaun & Aspinall (1970) observed a decrease in leaf number while leaf area increased in barley plants that were subjected to water stress. Reduction in leaf number may be as a result

of the effect of water stress on cell division and meristematic tissue enlargement (Simpson, 1981). Drought stress may reduce leaf area and leaf number.

The ability of a plant to grow satisfactorily when exposed to water stress is termed drought resistance. Drought resistance and avoidance are terms used interchangeably in describing a plant's response to water stress. In drought avoidance, plants tend to go through different conservative mechanisms in response to drought stress. These may involve morphological and physiological mechanisms. Roots are normally sensitive to drought stress hence their use as an index for drought stress tolerance. Root to above ground dry matter is said to be generally higher for plants growing in dry than in moist habitats (Simpson, 1981).

Drought tolerance is achieved through the ability of plant tissue to withstand water stress. Another mechanism by which plants adapt to drought stress is through the ability to maintain photosynthesis and plant growth at low cell water potential. This allows for the plant to survive periods of water stress such as mid-season droughts. Increased hydrolysis and reduced rate of protein synthesis are components of drought tolerance in plants. Usually, there is an increase in protein synthesis in response to drought stress as new proteins are synthesized e.g. the late embryogenesis abundant (LEA) proteins. Extreme protein loss can cause plant death (Levitt, 1972). Water stress can affect protein synthesis thus enzyme synthesis.

Plant growth and development is a multifaceted process that is affected by genetic, physiological and morphological processes through their determinants-photosynthesis, respiration, protein synthesis, water and mineral uptake, and cell division and expansion (Boyer, 1970; Egli & Legett, 1973). These processes respond differently to environmental conditions. Morphological, physiological and biochemical responses of plants to water stress differ with severity and duration of stress, thus it is important to know the stage of plant growth at which stress occurs. It is therefore important that there is sufficient soil water to meet plant demands during critical stages of development in order to avoid adverse effects of water stress on physiological processes in plants. The critical growth stage(s) in plants depend on the kind of crop grown and purpose of growing such a crop. In leafy vegetables, the vegetative stage is the most important growth stage since the leaf is the harvestable portion. For other crops were the harvestable portion is seed or fruit, the reproductive stage is the critical growth stage.

Vegetative growth is the most sensitive stage in plant development, after germination and seedling establishment. Effects of water stress on the vegetative stage vary. In some cases, the effects of water stress on the plant may only be seen in the late phenological stages (Simpson, 1981). The effect of water stress occurring during vegetative growth has been shown to have little effect yield compared with water stress occurring during the reproductive stage (Ma *et al*., 2006). However, the occurrence of water stress at the vegetative stage reduces

leaf area and dry matter as a result of reduced leaf expansion (Jamro & Larik, 1991; Hutcheon & Ranie., 1960). Effects of water stress at the vegetative stage inhibit plant growth resulting in reduced leaf area, dry weight and leaf number (Turk & Hall 1980; Hiler *et al*., 1972). Reduced leaf area can be considered as an adaptive mechanism which helps to reduce water loss from plants subjected to water stress (Turk & Hall., 1980). A study done on cowpea grown under water stress showed a reduction in leaf area, leaf number and root dry weight in response to water stress during the vegetative stage (Wien *et al.*, 1979).

Reduced leaf area may also be associated with reduced leaf number or sensitivity of leaf expansion to water stress (Boyer, 1970; Whiteman & Wilson 1965). A study done on sorghum at the early vegetative stage showed that water stress delayed leaf appearance rate and reduced leaf area (Whiteman & Wilson, 1965). Water stress at the vegetative stage results in reduced dry weight (Pandey *et al.,* 1984). Alyemeny (1997) suggested that water stress at the vegetative stage may be as a result of two combined effects, which is reduction in water available in the soil and slow root growth. In cowpea, it was shown that morphological adjustments at the vegetative stage enabled the plant to grow under water stress (Alyemeny, 1997). Most studies done on plant-water relations have involved both physiological and morphological characters of seeds or whole plant. Morphological adjustments in plants subjected to water stress takes place in sequential order, firstly leaf area and number are reduced; this acts as the first line of defence to water stress. Reduction in leaf area aids in lowering the

transpirational losses. The second line of defence is an increase in root length which helps the plant to grow even under drought conditions.

Drought affects plant growth in many ways, one of which is the reduction of dry matter and yield. An effect of water stress in plants is the reduction of water in the whole plant, thus affecting plant growth. Root growth is very sensitive to water stress since they are the main transport of water in the plant. As a result, water uptake in plants may be limited by root growth, thus improved root growth may reduce drought stress (Klepper, 1990; Kage & Ehlers, 1996). In general, roots will grow until the plant's demand for water is met. Alyemeny (1997) showed that severe drought on cowpea plants resulted in increased root length. Crop productivity in plants subjected to water stress relies greatly on dry matter partitioning to the root and shoot distribution. Inhibition of shoot and root growths are well known effects of drought stress and thus an increase in root:shoot ratios may be seen as an adaptation to drought stress. Root:shoot ratio for dry matter increased for different vegetable amaranth genotypes subjected to water stress (Liu & Stützel, 2004). However, this is not always true because root and shoot growth are also controlled by nutrient availability, species and growth stages (Gales, 1968). Growth of roots into deeper soil layers is a function of genotype x environment interactions (Fischer & Maurer 1978). Reduced plant growth under water stress may result in reduced plant yield. However, all these plant growth parameters are controlled by genotype x environment interaction. Planting date or season also influences plant growth and consequently biological yield.

1.3.2 Physiological effects of water stress

Plants growing in stressful environments have evolved and developed different mechanisms for their adaptation to such environments. Such mechanisms may be related to seed morphology and physiology (Venable & Brown, 1988). However, this depends on the availability of nutrients and water. Physiological processes that affect plants may lead to different morphological effects on plants.

Cellular growth is the most sensitive physiological process in plants (Boyer, 1970). Different physiological indices have been studied in order to differentiate genotypic responses to water stress. Physiological processes either take up or store water - most of the metabolic and biochemical processes in plants involve the former. Water stress in plants also affects physiological processes due to metabolic and structural changes such as a decline in photosynthetic rate, increased ethylene and abscisic acid concentrations and reduced protein synthesis and cytokynin levels (Wolfe *et al*., 1988). Under water stress, plants develop regulatory mechanisms in order for them to tolerate drought (Bohnert *et al*., 1995).

Water stress results in changes in the signalling pathway which involves the plant growth hormone abscisic acid (ABA), gene expression and metabolism and cell adjustments. Zeevart (1971) reported that in tobacco ABA was homologous to members of protein inhibitors, thus it was suggested that accumulation of some ABA-inducible mRNAs and proteins that accumulate during seed desiccation are

part of plant responses to water stress (Zeevart, 1971). In maize, pepper (*Capsicum annum* L.) and dayflower (*Commelina communis* L.), it was shown that application of ABA under severe water stress resulted in increased root:shoot ratios (Watts *et al*., 1980).

Another mechanism of physiological adaptation of plants to water stress is the accumulation of osmolytes such as sugars and proline (Ali *et al*., 1999; Rhodes *et al*., 1999; Kavi Kishore *et al*., 2005). Increased sugar levels have been observed as a plant response to water stress. Sesaki *et al*. (1998) reported that water stress resulted in higher levels of sugars in cabbage seedlings when compared to control samples.

Apart from being an osmolyte during water stress, proline can aid in stabilizing sub-cellular structures such as membranes and proteins. It aids in scavenging free radicals under stressful environments. Proline may also act as a protein compatible hydrotrope (Srinivas & Balasubramanian, 1995) in alleviating cytoplasm acidosis and also maintaining the NADP+/NADPH ratios that are necessary for metabolism. Under stress conditions rapid breakdown of proline is required to produce enough reducing agents that are needed for mitochondrial oxidative phosphorylation and ATP for recovery from stress induced damages (Hare *et al*., 1998). Under stress, proline induces the expression of proline responsive elements and their promoters in order to respond to water stress (Chinnusamy *et al*., 2005). Proline normally accumulates in the cytosol in

response to salt and water stress in plants where it aids in cytoplasmic osmotic adjustment (Ketchun *et al*., 1991). Sharp *et al*. (1994) observed that proline accumulation in maize increased with ABA concentration in response to water stress. This study also showed that there were linkages between signalling pathways and changes in metabolism.

Proline accumulation seems to be controlled by both ABA dependant and independent signalling pathways (Hare *et al*., 1998). Xiong & Peng (2001) observed that ABA regulates the expression of the *P5CS* gene which is also involved in proline biosynthesis. Salicylic acid (SA) induces ABA mediated protective reactions in plants under water stress by increasing the accumulation of proline (Yoshiba *et al*., 1995). When *Amaranthus* and tomato (*Lycopersicom esculantum*) leaves were exposed to water stress and treated with SA it was observed that proline accumulation increased two fold at early and late vegetative stage as a result of drought stress (Umebese *et al*., 2008).

The accumulation of free proline is a widespread plant adaption to water stress (Hare *et al*., 1998). Gangopahyay *et al*. (1997) observed that proline accumulation in *Brassica juncea* increased in salt adapted plants as to non-stressed plants. Proline accumulation in leaves of rice plants was higher in stress tolerant plants than in stress sensitive rice plants (Hsu *et al*., 2003). Proline concentration increased in leaves of potato plants subjected to water stress (Knipp & Honermeier, 2005).

Under water stress conditions, protein synthesis is affected by the accumulation of ABA (Zeevart, 1971). Water stress has been reported to inhibit the assimilation of amino acids into proteins (Gales, 1968; Cleland, 1967) resulting in reduced protein content in tissues. A study conducted in *Avena Coleoptiles* showed that water stress reduced the rate and pattern of protein synthesis (Dhindsa & Cleland, 1975). Apart from reduced protein synthesis, plants under water stress undergo an increase in exposure to reactive oxygen species (ROS) or free radicals (Smirnoff, 1993). Accumulation of free radicals is associated with membrane damage and increased lipid peroxidation.

Plants under water stress are protected by antioxidants from free radicals (Elstner, 1982; Winston, 1990). Antioxidants can be divided into three classes of (1) lipid soluble membranes (e.g. Vitamin E and *β*-carotene); (2) Water soluble reductants (e.g. Vitamin C and glutathione) and (3) enzymatic antioxidants (e.g. Superoxide dismutase (SOD), Catalase (CAT) and Peroxidase (POD). Reduced membrane damage in plants subjected to water stress was associated with enzymatic defence against oxygen radicals due to synthesis of antioxidants (Smirnoff, 1993). Badiani *et al*. (1990) studied the relationship between drought stress and enzymatic antioxidants in wheat and observed a reduction in SOD, CAT and POD as a result of water stress. CAT and SOD react directly with H_2O_2 to form water and oxygen (Smirnoff, 1993; Winston 1990). CAT and SOD decline with progression of water stress in most species by favouring the

accumulation of H_2O_2. Reductions of CAT in plants subjected to water stress were observed in maize (Zhang *et al*., 1990), rice (Dwivedi *et al*., 1979) and sunflower (Quartacci & Navari-Izzo, 1992). The process can be explained as shown in equations 1 and 2 below.

$$CAT + 2H_2O_2 \rightarrow 2H_2O + O_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$SOD + 2H_2O_2 \rightarrow 2H2O + O_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

1.4 Aim and objectives

The study aimed to evaluate the drought tolerance of selected wild mustard landraces found in KwaZulu-Natal. Understanding the plant-water relations of wild mustard may help to reinstate the crop within rural communities and as a production option for small-scale farmers in South Africa. The aim of the study was to characterise local wild mustard landraces morphologically and physiologically with respect to production, for the purpose of identifying their response to drought response.

The objectives of this study were:

1. To determine whether seed colour has an effect on seed germination and vigour of wild mustard.
2. To qualify and quantify proteins expressed in seedlings subjected to water stress.
3. To evaluate plant growth in response to water stress under controlled and field conditions.
4. To investigate the accumulation of proline in leaves of wild mustard as a

response to water stress under field conditions.

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CHAPTER 2

GERMINATION CAPACITY, SEED VIGOUR AND SEEDLING ESTABLISHMENT OF WILD MUSTARD (*Brassica* species*)* UNDER TWO WATER REGIMES

2.1 Abstract

Seed quality is related to seed performance during germination and emergence. Seed quality is measured by vigour and viability. Therefore, it is important to understand seed quality for good seedling establishment. The objective of the study was to compare three wild mustard landraces with respect to germination capacity, seed vigour and seedling emergence under two water stress regimes. Three cultivars, Isaha, Masihlalisane (*Brassica juncea* (L.) Czern & Coss) and Kwayimba (*Brassica nigra* (L.) W.D.J.Koch) were characterised according to seed coat colour. Seed germination capacity and vigour were evaluated using the standard germination test and electrolyte leakage, respectively. Seedling emergence was conducted in seedling trays under two water stress regimes, 75% and 25% field capacity (FC), for 21 days. There were highly significant differences ($p<0.001$) between seed colours during germination. Brown, black and grey seeds of Masihlalisane showed high germination capacity (>98%). Brown, greyish-black and reddish-brown seeds of Isaha showed germination capacity of 81%, 75% and 82%, respectively. Seeds of Kwayimba showed the least germination capacity with 0%, 12% and 22% for black, brown and reddish brown, respectively. With respect to seed electrolyte leakage, there were

significant differences ($p<0.001$) between cultivars, as well as a significant interaction ($p<0.05$) between seed colour and cultivar. Under stress conditions (25% FC), Masihlalisane showed only 45% emergence. At 75% FC, Isaha showed 100% emergence while Masihlalisane and Kwayimba both achieved 80% emergence. There was a significant interaction ($p<0.05$) between cultivar and FC with respect to emergence. Isaha and Masihlalisane showed higher emergence than Kwayimba. There was a significant interaction ($p<0.05$) between cultivar and FC with respect to leaf number. All wild mustard landraces showed reduction in leaf number under stress. There were significant differences ($p<0.05$) in terms of root and shoot length among landraces at different water regimes. Masihlalisane and Isaha were of good seed quality. Darker seeds of Kwayimba are associated with poor germination and seedling establishment. Kwayimba landrace showed an increase in root length while shoot length was reduced under water stress. Total protein increased under water stress for all the different landraces and seed colours.

2.2 Introduction

Water stress is a worldwide problem affecting plant growth and yield in many ways and to varying degrees. Global climatic change has made the situation dire for agricultural production (Pan *et al*., 1996). The use of drought tolerant crops may be a solution to this global problem. Indigenous crops have a strong tendency to tolerate drought (Schippers, 2002).

Seed quality is the most important parameter in farming and is measured in different ways. It is indicated by germination capacity and physiological vigour (Salisbury & Ross, 1991; Bewley & Black, 1994). The physiological quality of seed is determined by its genetic makeup (Powell *et al*., 2005). Temperature, water stress, photoperiod and soil fertility are known to affect seed quality (Gutterman, 1992; Wulf, 1995). Seedling emergence is one stage of plant growth that is sensitive to water stress. Therefore, seed germination and vigour are basic requirements for successful stand establishment in crop plants. Electrolyte leakage is associated with the loss of seed viability due to water loss in seeds which damages the membrane (Bewley & Black, 1994).

Another factor that affects seed quality is seed colour (Odindo, 2007). It can limit different physiological processes before germination. In *Trifolium alexxandruim*, seed colour had an influence on germination capacity, emergence rate, seedling elongation and length Dalianis, (1980). Seed vigour and seedling establishment can therefore be used as criterion for selecting for drought tolerance in plants (Serrano *et al*., 1999).

Plants use different mechanisms to avoid stress. Plant height is an apparent growth parameter and an index for stress tolerance; however, plant height alone cannot be used as a measure of stress tolerance. Studies have been conducted to understand the mechanisms by which plants adapt to water stress. Plants under water stress show reductions in leaf area and number as a mechanism to

reduce water loss through transpiration. This is through the inhibition of leaf expansion. Moderate water stress reduced leaf area in African nightshades (*Solanum scabrum*, Mill) (Muthomi & Musyimi, 2009). Leaf area reduction has been reported to be a drought avoidance mechanism in plants subjected to water stress (Muthomi & Musyimi, 2009).

Under low soil water content, the roots will grow deeper in search for water. Roots therefore become the second line of defense after leaf area reduction. Water stress usually changes the source-sink relationship thus altering assimilate partitioning. Under water stress, the roots become the stronger sink. Liu *et al.* (2004) reported that root length increased significantly in wheat (*Triticum aestivum*) cultivars in response to drought stress.

Under water stress, protein synthesis is affected. Plants adapt to stress conditions through the expression of certain proteins (Dhindsa & Cleland, 1975). Under water stress conditions protein synthesis is affected (Wolfe *et al.,* 1988) which result in increased protein content and this aid in normal metabolic processes.

Globally, water is a limiting factor to agricultural production. Water stress is a major problem in low rainfall areas in South Africa. Limitations to water availability, limit agricultural production thus contributing to food insecurity. The objective of this study was to compare three wild mustard landraces in terms of

germination capacity, vigour and seedling emergence under two water regimes.

2.3 Materials and methods

2.3.1Plant material

Seeds were collected from the subsistence farmers of Tugela Ferry in KwaZulu-Natal. Three wild mustard landraces (Zulu names: Isaha (I), Masihlalisane (M) (*Brassica juncea* (L.) Czern & Coss and and Kwayimba (K) (*Brassica nigra* (L.) W.D.J. Koch) were separated according to seed colour in order to create more genotypic variation. Two colours, black and brown, were common among all landraces, however there were five colours shared among landraces with each landraces consisting of three colours Table 2.1.

Table 2. 1: Seed colour separation in wild mustard landraces

Landraces	Colours		
Isaha	Brown(IB)	Greyish-black(IGB)	Reddish-brown(IRB)
Masihlalisane	Black(MBL)	Brown(MBR)	Grey(MG)
Kwayimba	Black(KBL)	Brown(KBR)	Reddish-brown(KRB)

2.3.2 Laboratory germination test

Seed quality was determined using the standard germination test under

laboratory conditions. A completely randomized design was used for the germination experiment (AOSA, 1996). Five replications of 20 seeds from each landrace and colour were rinsed with ethanol for less than 5 minutes and germinated between double layered paper towels, moistened with de-ionized water, and incubated in a growth chamber at 25°C in the presence of light. Germination was assessed by counting seeds with radicle protrusion (2 mm) daily for eight days. Seeds were observed for normality and abnormality (AOSA, 1992). Germination characteristics measured included seedling length and mass (dry and fresh).

2.3.3 Electrical conductivity

Seed electrolyte leakage was done according to ISTA (1995). One hundred (100) seeds from each landrace and seed colour combination were analysed using the CM100 Automatic Single Cell Analyser. Measurements of conductivity were used to determine seed vigour (AOSA, 1996; Copeland & McDonald, 1995).

2.3.4 Seedling emergence

A completely randomized design was used for seedling establishment in a glasshouse (27°C night/ 17°C day, 60% RH) under two water stress regimes of 75% and 25% field capacity (FC). Water content of the growing media in seedling trays was maintained by daily determination of container and plant mass. Seedling emergence was assessed daily for 21 days. Seedling height, leaf

number, leaf surface area, fresh and dry mass and root and shoot length were determined upon termination of the experiment on day 21.

2.3.5 Protein extraction

Shoots were ground to a fine powder in a pre-chilled mortar under liquid nitrogen (N_2) and mixed in 4 ml of 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°C. The supernatants were collected and considered as leaf protein extract. Protein concentration was determined by absorbance at 595 nm (Bradford, 1976) with bovine serum albumin as standard.

2.3.6 Data analysis

Statistical analysis of variance (ANOVA) was done using GenStat® (Version 11). Means were separated using least significant differences (LSD) at the 5% level.

2.4 Results and discussion

2.4.1 Germination test

There were highly significant differences ($p<0.001$) among wild mustard landraces with respect to germination capacity (Fig. 2.1). There was a highly significant interaction ($p<0.001$) between cultivars, seed colour and number of days with respect to germination capacity. Germination percentage increased

from day 1 to day 8. Masihlalisane showed 99% to 100% germination for brown, grey and black seeds. Isaha brown, greyish-black and reddish-brown seeds achieved 87%, 90% and 82% germination, respectively. Kwayimba had the least germination capacity. Black seeds of Kwayimba were the most dormant (<1% germination). Brown and reddish-brown seeds of Kwayimba had 14% and 22% germination, respectively. Masihlalisane and Isaha performed significantly ($p<0.05$) better than Kwayimba with respect to daily germination (Fig. 2.1).

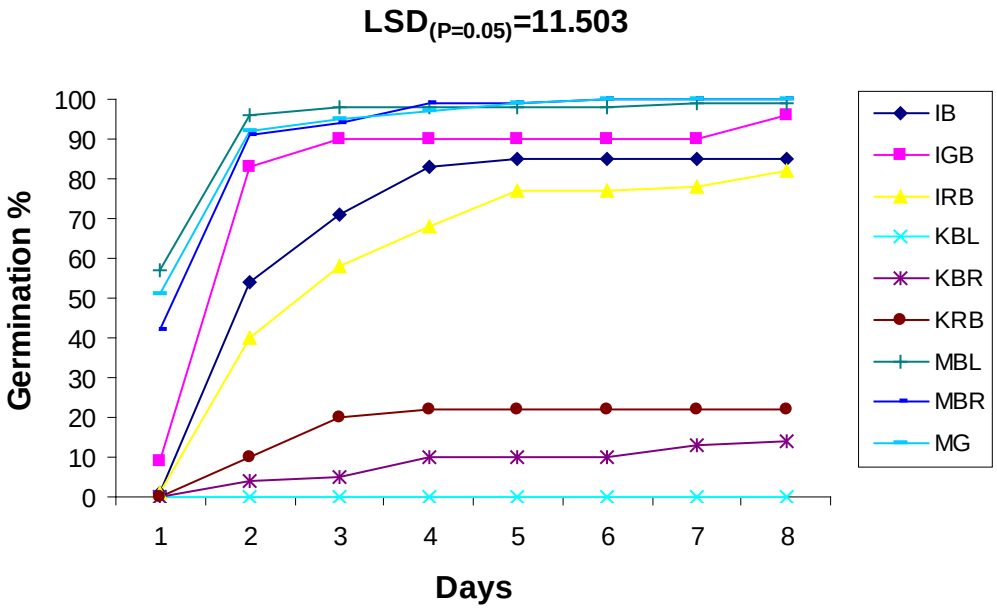

Figure 2. 1: Daily germination for wild mustard landraces**.**

On the final day of germination, seedlings were observed for abnormalities. There were highly significant ($p<0.001$) differences between landraces with respect to final germination capacity (Fig. 2.2). Masihlalisane showed 86%, 83% and 88% for black, brown and grey seeds, respectively. Isaha had 82%, 81% and 75% for brown, greyish black and reddish-brown seeds, respectively. Kwayimba

black seeds had 0% germination capacity with 12% and 21% germination capacity for brown and reddish-brown, respectively. Isaha and Masihlalisane performed significantly better than Kwayimba on the final day of germination (Fig 2.2).

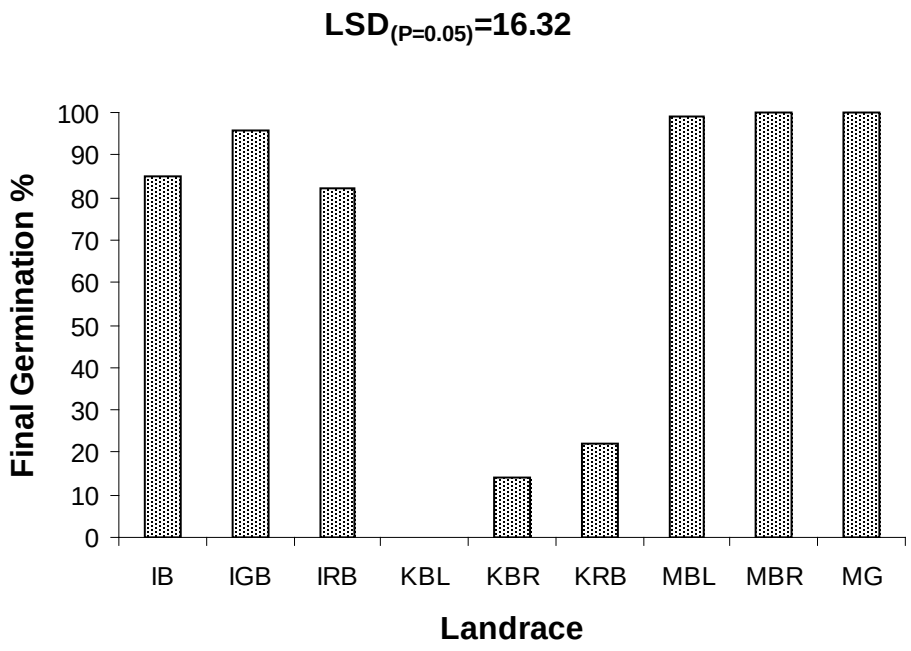

Figure 2. 2: Germination percentage measured on the final day of germination.

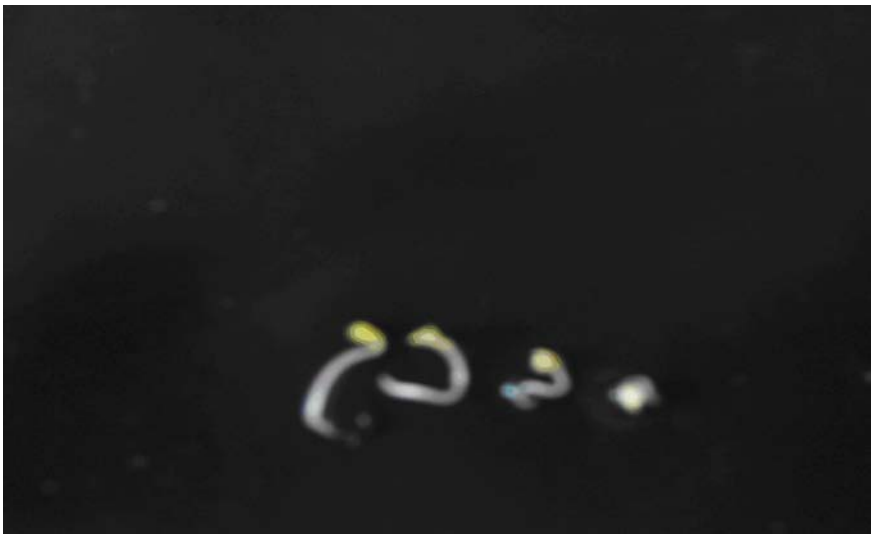

Figure 2. 3: *Coleoptiles* abnormalities observed in wild mustard landraces. Classifications were taken from AOSA (1992).

Few abnormal seedlings were observed (Fig 2.3). Most seedlings abnormalities were associated with poor hypocotyl development and short hypocotyls (AOSA, 1992). Most seeds that did not germinate were hard seeds (data not shown). A hard seed coat may be associated with dormancy and germination failure may have been as a result of seed coat dormancy. In addition, germination failure may be due to seed colour dormancy (Odindo, 2007). Results showed that dark wild mustard seeds of Kwayimba had high germination failure. This observation is similar to reports that seed colour is associated with seed quality (Pederson & Toy, 2001).

Masihlalisane and Isaha seeds performed better than other landraces with respect to germination percentage and electrolyte leakage. Variations between cultivars, with respect to germination, could be due to genotypic variation. Powell *et al.* (2005) observed that seed physiological quality was determined by genetic constitution during seed development on the mother plant and changes that occur from harvest to storage. Genetic makeup of wild mustard landraces might have an effect on their germination capacity.There was a significant interaction ($p<0.05$) between landraces and seed colour with respect to electrolyte leakage (Fig 2.4). Masihlalisane and Isaha light seeds had low electrolyte leakage compared to Kwayimba black type seeds.

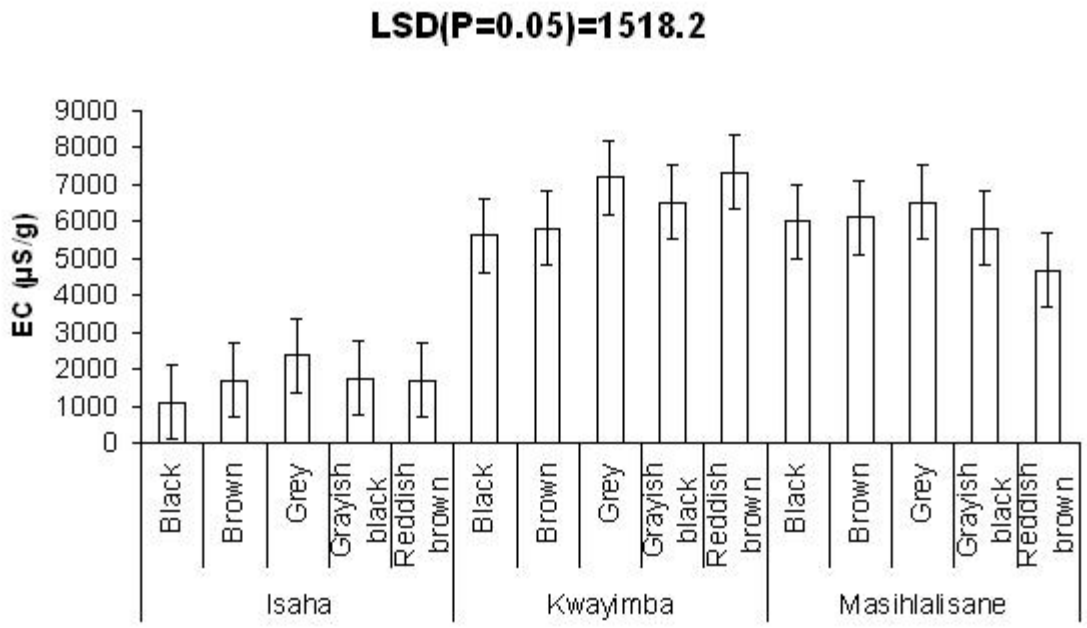

Figure 2. 4: Electrolyte leakage as a measure of seed vigour in wild mustard landraces.

2.4.2 Seedling emergence

There was a highly significant interaction ($p<0.001$) between landraces and field capacity with respect to seedling emergence. Of the nine landrace colour groups, six gave 100% emergence when subjected to 75% F.C. (Table 2.1). The remaining three had emergence rates of 33%, 50% and 60%, respectively. The maximum emergence obtained with 25% F.C. was ~ 78% and the minimum was 10% (Table 2.1). The average emergence for 75% F.C. was 83% and for 25% F.C., it was 43%. Hence seedling emergence was reduced by almost 50%

(48.2%) under water stress (Table 2.1). Across water treatments, Masihlalisane showed highest average emergence (100% at 75% F.C. and 70% at 25% F.C.) than all the other cultivars. The second best landrace was Isaha (100% at 75% F.C. and 39% at 25% F.C.). Kwayimba showed an average of 48% at 75% F.C. and 20% at 25% F.C. (Table 2.1). Within landraces, there were significant seed colour differences (Table 2.1). Whereas all seed colours showed 100% emergence under 75% F.C., black seeds (BL and GB) were generally better than other seed colours for Masihlalisane and Isaha under water stress (Table 2.1). For Kwayimba, black seeds were associated with poor emergence under both water regimes, although there was no statistical difference between seed colours at 25% F.C. (Table 2.1).

Table 2. 2: Seedling characteristics of wild mustard landraces.

Landrace	Emergence %		Leaf number	
	25% FC	75% FC	25% FC	75% FC
IBR	16.7e	100a	2de	3.667a
IGB	73.3b	100a	1.667e	2.667bcde
IRB	26.7de	100a	1.667e	3abcd
KBL	10e	33.3d	1.984de	2.333cde
KBR	26.7de	50c	2de	2.667bcde
KRB	23.3de	60c	2de	3.333ab
MBL	76.7b	100a	2de	2de
MBR	66.7bc	100a	2de	2.667bcde
MG	66.7bc	100a	2de	2de
LSD(P=0.05)	12.51		0.7940	

*Note values in the same column sharing different letters differs at LSD (P=0.05).

Isaha showed highest reductions in leaf number than Masihlalisane and Kwayimba. Leaf number reduction in all the landraces may be associated with

wild mustard's way of avoiding stress. Reduced leaf number helps in reducing the water loss by the plant during the periods of drought. These results are similar to what other researchers have observed in studies relating to drought, that water stress reduces leaf number and area (Alyemeny, 1998). Masihlalisane and Isaha showed better water stress tolerance than Kwayimba. Leaf area depends on leaf appearance rate and expansion (Warrington & Kanemasu, 1983) and can be predicted from leaf number (Muchow & Carberry, 1990). All wild mustard landraces were thus sensitive to water stress while leaf number reduction was a plant mechanism for stress avoidance.

There were significant differences ($p<0.05$) in terms of root length and shoot length among landraces (Fig. 2.5 -2.6).

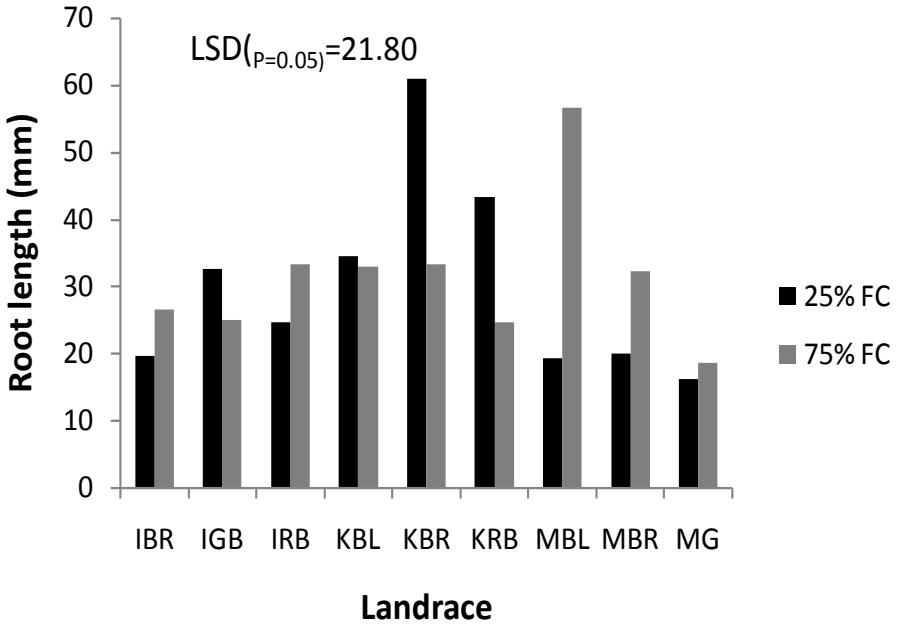

Figure 2. 5: Differences in root length in wild mustard seedlings under two water regimes.

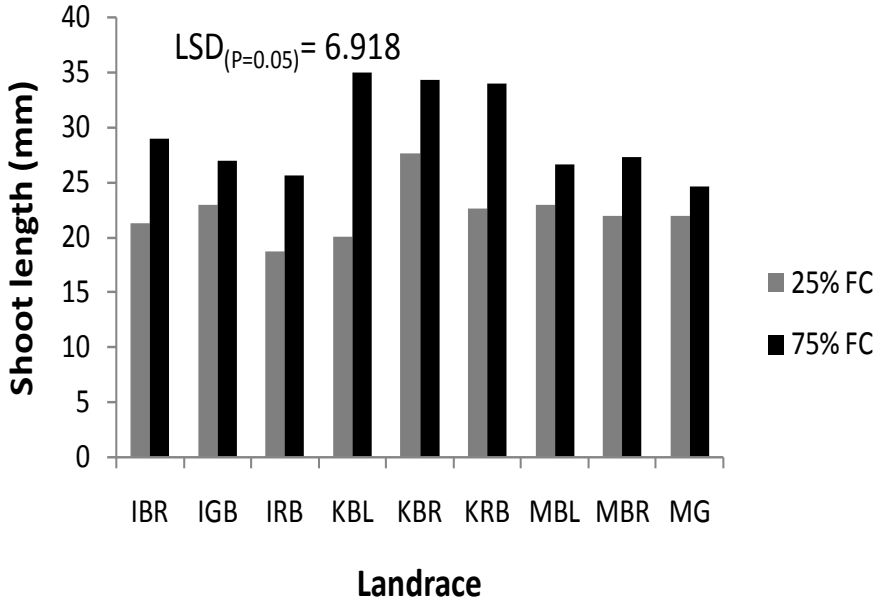

Figure 2. 6: Shoot length of wild mustard landraces under two water regimes.

Root length was high in Kwayimba under severe water stress (25% FC). However, while root length increased under water stress, shoot length was significantly ($p<0.05$) reduced in Kwayimba. Water stress changed the source sink relationship within wild mustard landraces by reducing the shoots rather than roots. Roots became a strong sink than the shoots in Kwayimba's seedlings. Kwayimba and Isaha at (25% FC). Alymeny (1998) showed that in cowpea seedlings (*Vigna ambecensis* L.) root length and root:shoot ratio increased with the severity of water stress.

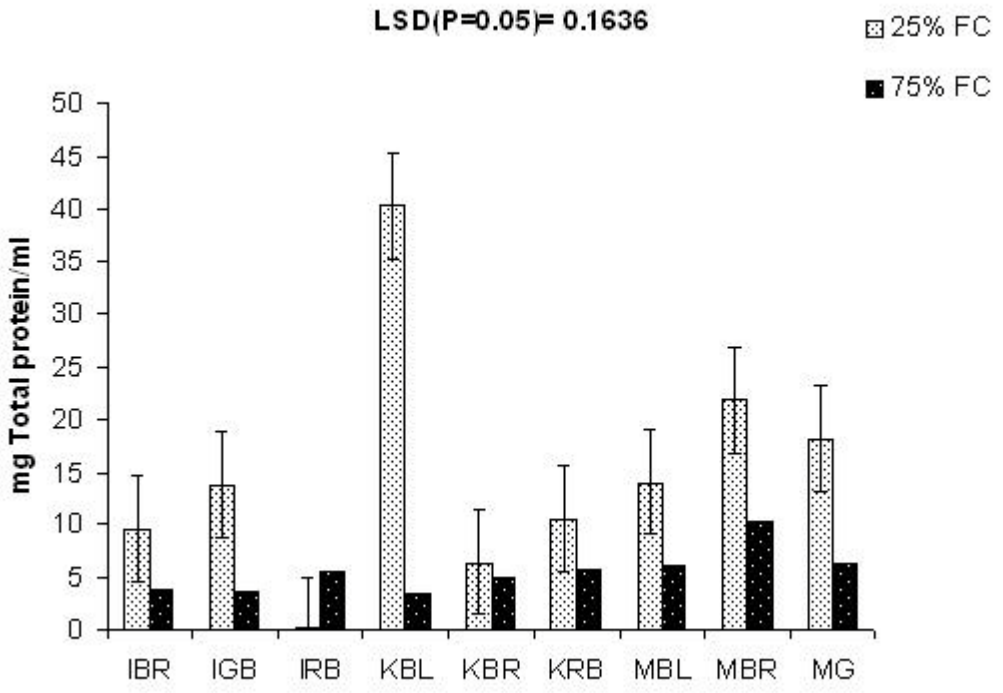

Figure 2. 7: Total protein content for wild mustard landraces under two water regimes.

There was a highly significant interaction ($p<0.001$) between landraces and field capacity with respect to total protein (Figure 2.7). Kwayimba black showed a significant increase in total proteins obtained under water stress. These results suggest that protein content or synthesis will increase under water stress. Under water stress new proteins may be synthesised to avoid the negative effects of water stress in the plant. Kwayimba black seeds under water stress (25% FC) showed less than 1% emergence, and for those seedlings that survived growth, there was a significantly high protein accumulation. However, it cannot be concluded that protein accumulation is correlated with stress survival. However, there are indications that poor emergence can be associated with darker seed

coat colour for Kwayimba. There were highly significant ($p<0.001$) differences among landraces with respect to total proteins (Fig 2.7). Masihlalisane and Kwayimba black seeds showed high total protein contents. There were highly significant differences ($p<0.001$) between field capacities with respect to amount of proteins in seeds. Total proteins increased under severe water stress. Water stress reduced the total protein in wild mustard.

2.5 Conclusions

Black wild mustard was associated with seed colour dormancy and consequently poor seedling establishment. Isaha and Masihlalisane seeds showed more vigour and viability than Kwayimba black type seeds. From the study it can be concluded that water stress affects growth of wild mustard seedlings. Water stress reduced shoot length and leaf number in all wild mustard landraces under stress. However, reduction in leaf number was more pronounced in Kwayimba resulting in reduced biomass. Protein content increased under water stress for all landraces and colour variations. The study showed that seed quality of some wild mustard landraces is associated with poor seedling germination and is responsive to water stress. Kwayimba was sensitive, however, Isaha and Masihlalisane were shown to be tolerant to water stress. Light seeds of Masihlalisane and Isaha may benefit early stand establishment in the field.

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CHAPTER 3

EFFECTS OF WATER STRESS ON THE VEGETATIVE GROWTH OF WILD MUSTARD LANDRACES UNDER THREE WATER REGIMES

3.1 Abstract

The critical growth stage in leafy vegetables is the vegetative stage. There is a need for sufficient soil water to meet plant demand for vegetative growth. The objective of the study was to compare the response of three wild mustard landraces to water stress at the vegetative stage under three water regimes. The experimental design was a completely randomised design with three replications. Seeds of three wild mustard landraces, Isaha, Masihlalisane [*Brassica juncea* (L.) Czern & Coss] and Kwayimba [*Brassica nigra* (L.)W.D.J. Koch] were grown in pots watered to three field capacity levels (25%, 50% & 75%), under controlled glasshouse conditions (27°C day; 17°C night and 60% RH). Daily emergence was measured for 21 days after planting (DAP). Plant growth parameters were measured up to flowering. There were highly significant differences ($p<0.001$) in terms of plant height, leaf area and number, dry and fresh mass with respect to field capacity and landraces seed colour. Black coloured seeds of Isaha and Masihlalisane performed significantly ($p<0.05$) better than Kwayimba with respect to their water stress tolerance. There were no significant differences ($p>0.05$) in plant height, leaf number and area. There was a significant interaction ($p<0.05$) between cultivar and field capacity for plant fresh and dry mass. Isaha and

Masihlalisane showed better tolerance to water stress than Kwayimba under controlled glasshouse conditions.

3.2 Introduction

Water stress studies are mainly limited by duration of stress in plant growth experiments (Gales, 1988). In pot experiments, plants are planted in small soil volumes resulting in plants being severely stressed. However, for field experiments, stress usually develops gradually as a result of large soil volumes found in the field. Begg and Turner (1976) as well as Turner and Stewart (1986) reported that the rate at which water stress develops affects plant metabolic and physiological processes such as growth, photosynthesis and enzyme activity.

Germination and seedling establishment are followed by vegetative growth and later reproductive growth. Vegetative growth overlaps with the period of growth and development of reproductive structures. Therefore, vegetative growth is important to the plant since it supplies assimilates for development of reproductive structures (Simpson, 1981). In leafy vegetables, the harvestable part is the leaf which makes the vegetative stage a critical growth stage.

Plant sensitivity to water stress varies during the vegetative phase; water stress affects plant height, leaf number and area, thus affecting photosynthetic rate and biomass accumulation. Water stress at the vegetative stage affects cell expansion and division, thus affecting leaf expansion. In maize (*Zea Mays* L.) it

was observed that when leaf water potential decreased, leaf area also decreased while photosynthesis was affected later (Boyer, 1968). Water stress affects leaf area at the vegetative stage by reducing leaf appearance rate. In barley (*Hordeum vulgare* L.), leaf number decreased while existing leaves expanded (Husain & Aspinall, 1970). Reduction in leaf area under water stress serves as a drought avoidance mechanism in many plants.

Transport of photosynthetic assimilates from the source (leaves) to sinks and other plant parts is inhibited by water stress (Brown, 1984). Plant yield has been correlated with the source-sink concept. Studies on sugarcane showed that water stress reduced assimilate translocation more than photosynthesis (Hartt, 1967). As water stress increases in the plant, water transport is also affected. Water stress is influenced by genotype and environment interaction of which these factors affect plant yield. Water stress effects were investigated on two cultivars of *C. olitoruis*. Fresh mass and dry mass yields increased in one cultivar as a sign of drought tolerance (Ayodele & Fawusi, 1989) at vegetative stage. Plant fresh and dry mass yield are influenced by assimilation rate. Reduced leaf area and number help the plant in controlling transpirational losses while it maintains dry matter production. *B. juncea* showed a significant increase in dry matter as compared to *B.napus* under water stress (Gunasekera *et al.*, 2005).

Plant growth processes associated with water stress are all sensitive processes. Water stress results in reduced enzyme activity which can limit stomatal opening,

photosynthesis and translocation. Translocation is limited by photosynthate availability and stomatal closing reduces photosynthesis. Reduction in photosynthetic rate affects plant growth and eventually yield. In leafy vegetables, the critical growth stage is the vegetative stage since leaves are the edible part of the plant. Plant adaptations to water stress involve leaf area and plant height reduction without any major effect on fresh and dry mass yields. However, not much work has been done on the effects of water stress in leafy vegetables at vegetative stage. The objective of this study was to determine the effects of water stress at vegetative stage of three wild mustard landraces under three water regimes of controlled conditions.

3.3 Materials and methods

3.3.1 Plant material

Seeds of three wild mustard landraces, Isaha, Masihlalisane [(*Brassica juncea* L.) Czern & Coss] and Kwayimba [*Brassica nigra* (L.)W.D.J. Koch] was collected from Tugela Ferry in KwaZulu-Natal. The seeds were further separated according to seed coat colour in order to increase variation within genotypes.

3.3.2 Experimental layout

A completely randomised design with two treatment factors (See Table 2.1 for description of seed colour abbreviations): landrace [nine levels- Isaha (IB, IRB and IGB), Masihlalisane (MBL, MG and MBR), Kwayimba (KBL, KRB and KBR)

and water stress (25 %, 50% and 75% field capacity, respectively) with three replicates, giving a total of 81 treatment combinations..

3.3.3 Potting procedure

A pot experiment was conducted under controlled conditions (27°C day; 17°C and 60% RH) in a glasshouse at the University of KwaZulu-Natal, Pietermaritzburg. There were two treatment factors, namely, water stress and landrace. There were three levels of water stress, 25%, 50% and 75% field capacity (FC). Clay soil of known field capacity was collected from the University of KwaZulu-Natal Research Farm (Ukulinga) and used as a growing media. 81 pots were each filled with 2 kg of soil, weighed and watered up to the corresponding field capacities. Pots were weighed and watered every two days in order to maintain soil water content. After the establishment stage (21days after planting), measurements of plant height and leaf number were taken weekly until the plants had just flowered (after seven weeks), when the experiment was terminated. Upon termination, measurements of leaf area and fresh and dry weight were taken. Leaf area was measured using the leaf area meter Model LI-3000. Fresh leaves were weighed using a weighing balance METTLER SM-3000. Leaves were than dried in an oven of 90^{o}C for two days and than reweighed on the same balance used for fresh mass.

3.3.4 Description of statistical analysis

The experimental design was a completely randomised design (CRD) with two treatment factors replicated three times. Data were analysed using ANOVA from GenStat® Version 11. Means were separated using least significant differences LSD (P=0.05).

3.4 Results and discussion

3.4.1 Plant height

There was no significant interaction ($p>0.05$) between landrace and water regime (*WR x LR*) with respect to plant height (mm). However there were highly significant differences ($p<0.001$) for both main effects in terms of plant height (Table 3.1 and Figure 3.1). Greyish-black seeds of Isaha had significantly higher plant height than other seed colours in that group, while plant height of all seed colours of Kwayimba was significantly lower than that of Masihlalisane and greyish black seeds of Isaha. None of the seed colours of Masihlalisane differed significantly from each other. Means in plant height over all landraces increased significantly from 61.5 mm to 134.0mm when irrigation levels increased from 25% FC to 50% FC. However, plant height at 75% FC was less than at 50% FC, possibly due to waterlogging. The differences and possible effects of waterloging (yellowing leaves) can be clearly seen in Figure 3.1, five weeks after planting.

Table 3. 1: Main effects for plant height (mm) for different wild mustard landraces and seed colours at water regimes of 25, 50 and 75% field capacity.

Landraces and seed colours	**Water regime**			
	25%FC	**50%FC**	**75%FC**	**Mean**
Isaha				
Black	68.9	146.0	129.3	**114.7ab**
Greyish-black	53.0	191.5	132.7	**125.7a**
Reddish-brown	60.1	127.7	112.4	**100b**
Kwayimba				
Black	47.7	112.3	108	**89.3b**
Brown	52.0	78.4	80.3	**70.2b**
Reddish-brown	41.7	79.5	99.8	**73.6b**
Masihlalisane				
Black	102.5	164.5	149.3	**138.7a**
Brown	73.3	157.8	140.0	**123.7a**
Grey	114.4	148.5	163.5	**142.1a**
Mean	**68.2c**	**134.0a**	**123.9a**	**106.5ab**

LSD Water regimes (WR) =22.8
LSD Landraces (LR) = 13.2
LSD WR x LR= 39.59^{ns}
CV %= 64.2
Means followed by the same letter are not significant at p=0.05

Figure 3. 1: Illustration of Isaha landrace seedlings response to (from left to right) 25%, 70% and 50% field capacity, respectively, five weeks after planting.

3.4.2 Leaf number

The was no significant interaction ($p>0.05$) between landraces and water regime (LR x WR) for leaf number, but highly significant ($p<0.001$) were observed with the main effects (Table 3.2). Masihlalisane black seeds had significantly higher leaf number than other seed colours, while leaf number of Kwayimba black and reddish-brown seed was significantly lower than that of Isaha and Masihlalisane. Means in leaf number over all landraces increased significantly from 4.76 to 6.17 when irrigation levels increased from 25% FC to 50% FC. Leaf number did not increase further at 75% FC.

Table 3. 2: Leaf number interaction for different wild mustard landraces and seed colours under three water regimes 25, 50 and 75% field capacity.

Landraces and seed colour	**Water regimes**			
	25%	**50%**	**75% FC**	**Mean**
Isaha				
Black	5.04	5.67	5.29	**5.33b**
Greyish-black	4.17	5.89	5.10	**5.05b**
Reddish-brown	4.29	5.61	4.76	**4.89b**
Kwayimba				
Black	3.84	5.45	6.10	**5.13b**
Brown	4.67	5.34	4.90	**4.97b**
Reddish-brown	3.26	5.23	5.78	**4.76b**
Masihlalisane				
Black	6.12	7.81	6.76	**6.89a**
Brown	4.96	7.12	6.25	**6.11a**
Grey	6.50	7.37	7.21	**7.03a**
Mean	**4.76c**	**6.17a**	**5.79ab**	**5.57ab**

LSD Water regime (WR) = 0.485
LSD Landraces (LR) = 0.839
LSD WR x LR =1.454 ^{ns}
CV % = 46
Means followed by the same letter are not significant at p=0.05

3.4.3 Leaf area

There was no significant interaction ($p>0.05$) between (WR x LR) in terms of leaf area, however there were highly significant differences ($p<0.001$) among landraces seed colours (Table 3.3). Black seeds of Isaha responded positively to an increase in irrigation with leaf area increasing at 50% FC, while the response of greyish-black seeds was similar but not significant. The opposite response was found with reddish-brown seeds as leaf area decreased significantly when 50% FC was exceeded. Leaf area of all seed colours of Kwayimba was very low and showed no significant response to increasing water levels, except for the black seeds which increased significantly between 25% FC and 75% FC. No significant response was found for Masihlalisane except for brown seeds which showed a decreasing response to increasing irrigation levels. The response was similar to that of reddish-brown seeds of Isaha.

Table 3. 3: Leaf area (cm^2) interaction for different wild mustard landraces and seed colours at water regimes of 25, 50 and 75% field capacity.

Landraces and seed colours	**Water regime**			
	25% FC	**50% FC**	**75% FC**	**Mean**
Isaha				
Black	26.7d	43.8abc	47.7ab	**39.4**
Greyish-black	34.4bc	48.5ab	44.6abc	**42.5**
Reddish-brown	41.0abc	45.8ab	29.5cd	**38.8**
Kwayimba				
Black	10.0e	18.6de	33.4d	**20.6**
Brown	11.0e	20.8de	20.7de	**17.5**
Reddish-brown	8.5e	17.6de	19.0de	**14.8**
Masihlalisane				
Black	53.3a	53.5a	42.7abc	**49.8**
Brown	38.9ab	49.6ab	31.7b	**45.0**
Grey	39.8ab	55.7a	49.0a	**48.2**
Mean	**29.2**	**39.3**	**35.4**	**34.6**

LSD Water regime (WR) = 6.06
LSD Landraces (LR) = 10.49
LSD WR x LR =18.17
CV % = 32
Means followed by the same letter are not significant at p=0.05

3.4.4 Fresh mass and dry mass

There was a significant interaction ($p<0.05$) between landrace and field capacity with respect to plants dry mass (Figure 3.2) and fresh mass (Figure 3.3) and highly significant differences ($p<0.001$) for both the main effects with respect to fresh and dry mass. Masihlalisane brown seeds showed significantly higher fresh and dry mass than Isaha and Kwayimba. Plant fresh mass decreased in response to water stress (25% FC). However, it was higher at 50% FC than at 75% FC. Fresh mass was reduced at 25% FC for all the landraces when compared to 50% FC.

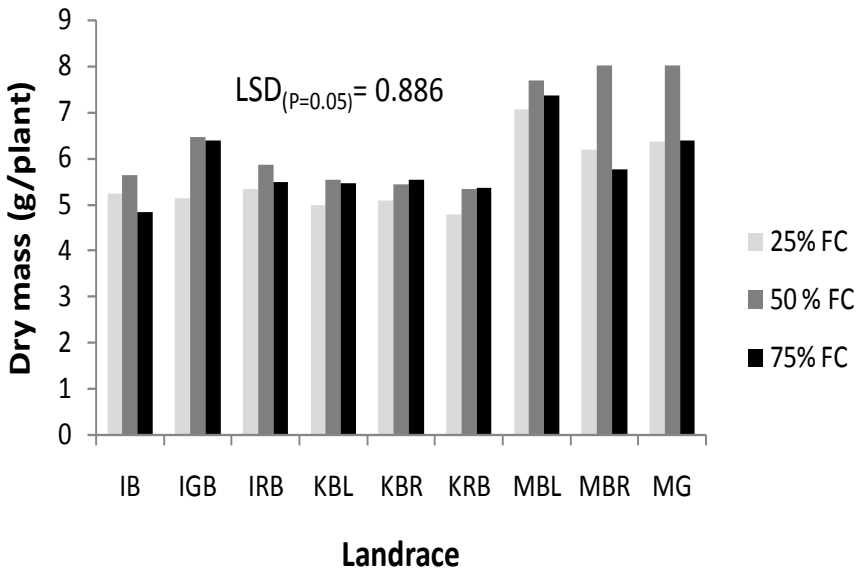

Figure 3. 2: Wild mustard landraces dry mass at three different field capacities.

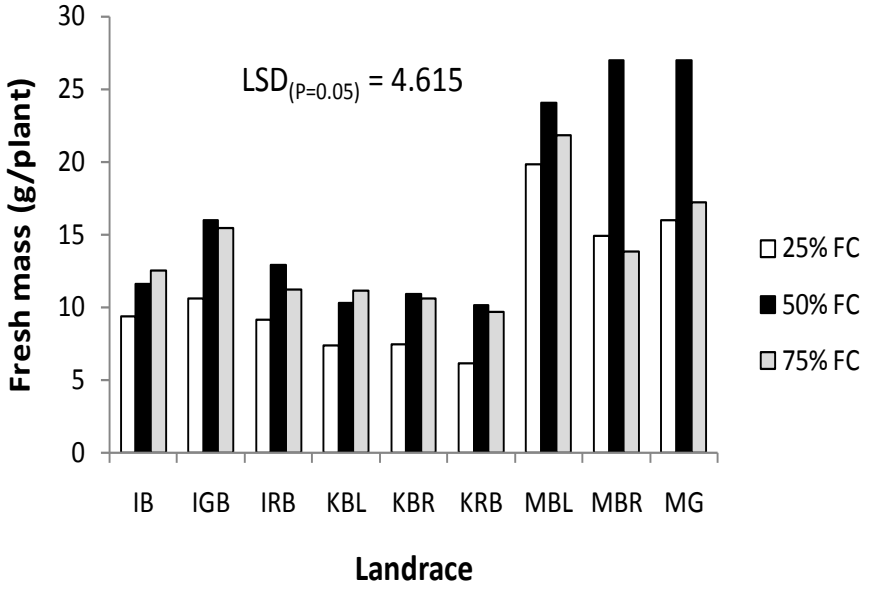

Figure 3. 3: Wild mustard landraces fresh mass at three different field capacities

3.5 Conclusions

Plant height is an apparent growth parameter in plants. Normally, plants subjected to water stress show a reduction in plant height (Brown, 1984). Drought stress reduced plant height in potato cultivars (*Solamum tuberosum* L.) (Deblonde & Ledent, 2001). A similar study on aman rice (*Oryza sativa*) genotypes showed reduction in plant height in response to increasing water stress (Zubaer *et al*., 2007). Reduction in plant height under water stress can be correlated to leaf area reduction which is a result of physiological changes that occur under water stress. Physiologically, plant height reduction is due to inhibition of cell enlargement (Hsiao, 1973) while leaf area reduction is due to a

decrease in the rate of cell expansion (Sharp *et al*., 1979). Reduction in leaf area in response to plant water stress at 25% FC during the vegetative stage is as a result of decreased turgor pressure which is necessary for cell enlargement. Reduction of leaf area in response to drought is said to be associated with leaf senescence and abscission (Acevedo *et al.*, 1971). However, leaf area may also be associated with reduced leaf number (Constable *et al.*, 1978) or sensitivity of leaf expansion to water stress (Boyer, 1970; Whiteman & Wilson 1965). Similar observations were made in this study with regard to leaf number and area. A study done on sorghum at early vegetative stage showed that water stress delayed the rate of leaf appearance and reduced area of individual leaves (Whiteman & Wilson, 1965). Acevedo *et al*. 1971 also reported that reduced leaf area in maize leaves was associated with low leaf water potential. In *Amaranthus*, drought significantly reduced plant total dry mass (Liu & Stutzel, 2004). Under water stress conditions, biomass accumulation decreases with reduction in stomatal closure and photosynthesis, as measured by leaf area, is reduced (Hsiao, 1993).

The study showed that responses of wild mustard water stress at the vegetative stage are associated with morphological and physiological parameters. Genotypic variations were shown to influence wild mustard responses to water stress. Isaha and Masihlalisane showed better tolerance to water stress than Kwayimba. They were able to grow under water stress with minor reductions in leaf area and biomass accumulation. The highest yields, for all wild mustard

landraces, were obtained in response to moderate stress. Isaha and Masihlalisane may be used as drought tolerant crops.

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CHAPTER 4

PLANT GROWTH AND PROLINE ACCUMULATION IN WILD MUSTARD LANDRACES SUBJECTED TO WATER STRESS

4.1 Abstract

Wild mustard (*Brassica spp.*) is an indigenous plant that is consumed as a wild leafy vegetable in many parts of South Africa. Limitations to its use are likely due to a lack of knowledge about its agronomy and water use. The study aimed at identifying wild mustard response to drought tolerance. The objective of the study was to determine the effect of water stress on plant growth and proline accumulation in wild mustard under irrigated and non-irrigated conditions. A field study was conducted at the University of Kwa-Zulu Natal's Research Farm in Pietermaritzburg. Seeds of three wild mustard landraces, Isaha, Masihlalisane (*Brassica juncea* L. (Czern & Coss)) and Kwayimba (*Brassica nigra* L. (W.D.J. Koch)), were separated into black and brown types in order to create more genotypic variation. A completely randomised design was used for non-irrigated and irrigated (25 mm/week) trials. Water stress was imposed on the non-irrigated trial by withdrawing irrigation 14 days after planting (DAP). Emergence was measured up to 21 days. Plant height, leaf area, leaf number and leaf dry mass were measured every 7 days. The experiment was terminated at the flowering stage and leaf samples were taken for proline determination. There were highly significant differences ($P<0.001$) in plant height, leaf area, number, fresh and dry mass with respect to planting date. Plants performed significantly ($p<0.05$) better in spring than in winter. Brown coloured seeds of Isaha and Masihlalisane performed significantly ($P<0.05$) better than Kwayimba. There

was a highly significant interaction ($p<0.001$) between landrace and irrigation treatments with respect to proline accumulation. All wild mustard landraces showed tolerance to water stress; however, their tolerance was not correlated to proline accumulation.

4.2 Introduction

Leafy vegetables are plants that are grown or harvested for their edible leaves. They are highly recommended due to their high nutritional quality (Modi, 2006). They are rich sources of vitamins, mineral trace elements, dietary fibre and proteins (Humphrey *et al.*, 1983; Fafunso & Bassir, 1976). Leafy vegetables are also known for their medicinal properties which include anti-diabetic, anti-carcinogenic and anti-bacterial properties (Kesari *et al.*, 2005; Kubo *et al*., 2004; Khana *et al*., 2002). The use of leafy vegetables by many South Africans is highly dependent on factors such as poverty, urbanisation, accessibility of fresh produce markets and seasonality of production. (Voorster *et al*., 2002). They are important for food and nutrition security during periods of drought and poor harvests as well as for income generation.

Wild mustard is an indigenous leafy vegetable in South Africa. It is one of the crops believed to have been consumed by the Khoisan people in Southern Africa. Wild mustard plants are able to grow under drought conditions since they evolved naturally in the wild. *Brassicas* can be planted throughout the year with variations in yield which is affected by planting date and poor crop management. However, not much is known about its agronomy and mechanisms of adapting to water stress. It is thus important to gain such knowledge (Geissler *et al*., 2002) in order to reinstate them within the rural communities in

South Africa.

Drought is one of the limiting factors to agricultural productivity. The identification of wild and indigenous species as drought tolerant crops will also alleviate pressure on South Africa's water resources. Climate change is expected to result in an increased frequency of drought accompanied by an increase in temperatures. Wild mustard is one crop that may have developed tolerance due to its wild origins. Otieno and Ochieng (2004) recently reported that South Africa is now considered a water scarce country with average annual rainfall of 500 mm. The identification of drought tolerant crops of high nutritional value will improve South Africa's food and nutrition security.

Water stress in plants is said to be controlled by genotypic x environment interactions (Fischer *et al.*, 1978). Water is important for plant growth and development. Water stress affects plants at different levels of growth. Water stress can have major impacts on plant performance and survival which can lead to changes in plant morphology, physiology and metabolism (Ludlow & Muchow, 1990). In leafy vegetables, leaves are the most vulnerable part of the plant as they are the edible part of the plant. Leaf area is an important parameter in plants and is associated with many agronomic and ecological processes such as photosynthesis, transpiration, energy balance and water and nutrient use during plant growth and eventually biological yield (Gardner *et al.*, 1990).

Leaf adjustments are important for plant adaptation to water stress. Detaching of old leaves for the formation of new leaves with smaller leaf area is another way of stress

avoidance, aimed at reducing plant water consumption and hence conserving water during periods of drought. Leaf area depends on leaf appearance rate and expansion (Warrington & Kanemasu, 1983). Leaf area can be predicted from leaf number (Muchow & Carberry, 1990). Water stress significantly reduced leaf number in sunflower (*Helianthus annuus* L.) (Yegappan *et al.*, 1980). Water stress in maize plants significantly reduced leaf area and number (Sah & Zamora, 2005). Reduced leaf area in plants under water stress reduces light interception by a plant and eventually reduces biomass production (Masinde *et al*., 2005). Leaves constitute plant dry weight which is equivalent to plant biomass accumulation. Under water stress, plant dry matter may be affected as a result of failure of the plant to adjust to different environmental conditions.

High yields are important for commercial and small-scale farming. Yield can be expressed in terms of biomass or dry matter production, depending on the harvestable part of the plant. Therefore, plant biomass can be considered to be equivalent to plant yield. Yield is related to soil water content. Soil water content tends to decrease with increasing water stress. In water stress studies, it is also important to maintain soil water content at the required level.

In leafy vegetables, leaves are harvested and used for human consumption. Therefore, in this study the vegetative stage was important since it is the critical growth stage. Not much has been done on the effect of water stress on leafy vegetables at the vegetative stage. A study on *Brassica napus* L. showed a reduction in plant yield in response to water stress imposed at different plant growth stages and seasons. Yield losses were

higher in autumn than in spring (Dembinska, 1970). In winter, days are shorter and plants tend to flower early resulting in reduced biomass accumulation as compared to the warm and rainy season.

The effects of water stress on plants differ with severity of water stress, genotype and growth stage of the plant .Adejare & Umebese (2008) reported that water stress applied for 7 days on two cultivars (A and B) of soybean (*Glycine max* L. Merril) during the vegetative stage resulted in high osmotic adjustments in cultivar B as a result of accumulation of sugars. Gunesekera *et al*. (2005) reported that under low rainfall conditions, mustard produced more dry matter than canola at the vegetative stage. Zubaer *et al*. (2007) reported a reduction in dry mass of aman rice (*Oryza sativa*) in response to increasing water stress. Water stress reduced leaf area, leaf number and dry mass in grape vines (Ussahatanonta *et al*., 1996). Dry mass and leaf number decreased in different potato (*Solanum tuberosum* L.) cultivars subjected to water stress (Deblonde & Ledent, 2001).

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). Plants under water stress adapt through the accumulation of the osmolyte proline. The accumulation of proline is a widespread plant adaptation to water stress (Hare *et al*., 1998). Proline accumulation seems to be controlled by both ABA

dependant and independent signalling pathways (Hare *et al*., 1999). Apart from being an osmolyte, proline aids in stabilizing sub-cellular structures such as membranes and proteins. Gangopahyay *et al*. (1997) observed that proline accumulation in *Brassica juncea* increased in salt adapted plants as to non-stressed plants. Proline accumulation in leaves of rice plants was higher in stress tolerant plants than in stress sensitive plants (Hsu *et al*., 2003). Proline concentration increased in leaves of potato plants subjected to water stress (Knipp & Honermeier, 2005).

Water stress is a major problem in low rainfall areas of South Africa and has contributed to high food insecurity and malnutrition in rural areas. Although wild mustard is an indigenous leafy vegetable in South Africa, literature on its growth response to water stress and its adaptation is lacking. Therefore, there is need to grow crops that are water-stress tolerant to ensure constant food supply and proper use of water. Thus, the objective of this study was to evaluate the responses of wild mustard landraces to water stress under field conditions.

4.3 Material and methods

4.3.1 Plant materials and experimental design

Seeds of three wild mustard landraces, Isaha, Masihlalisane (*Brassica juncea* L. Czern & Coss) and Kwayimba (*Brassica nigra* L. W.D.J. Koch), were produced and used for a field experiment at Ukulinga Research Farm, University of KwaZulu-Natal, Pietermaritzburg (29°16’S 30°33’E). To create more variation within genotypes, seeds of each landrace were separated into black and brown seed colour types. The experiment was conducted

in two seasons winter (May, 2009) and spring (September, 2009). A completely randomized design with three replications was used for non-irrigated and irrigated (25 mm week $^{-1}$) trials. Water stress was imposed in the non-irrigated trial by withdrawing irrigation 14 days after planting (DAP). Soil samples were collected three times a week to measure soil water content at 5 cm, 15cm and 30cm depth. Tensiometers were used to monitor soil water content in both trials. Emergence was measured up to 21 DAP. Determination of plant height and leaf number was done every 7 days. The experiment was terminated at the flowering stage. Thereafter, leaf area, fresh mass and dry mass were measured. The second trial was treated the same way as the first trial. Leaf samples were taken for proline determination at harvesting.

4.3.2 Proline determination

Proline accumulation in wild mustard leaves from both stressed and unstressed leaves was determined according to the method of Bates *et al*. (1973) at harvesting. 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:

[(µg proline/ml x ml toluene)/ (115µg/µmole)]/ [(g sample)/5] = µmoles proline/g of dry weight material.

4.3.3 Statistical analysis

Data were analysed using GenStat® Version 11 and means were separated using LSD (P=0.05).

4.4 Results and discussion

There were significant differences among cropping seasons with respect to soil water content. Crop planted in winter was able to emerge under low soil water content (<20%) under non-irrigated conditions of high rainfall and low temperatures (Figure 4.1). However wild mustard crop emerged very well under moderate rainfall and high temperatures with high soil water content (see other results below).

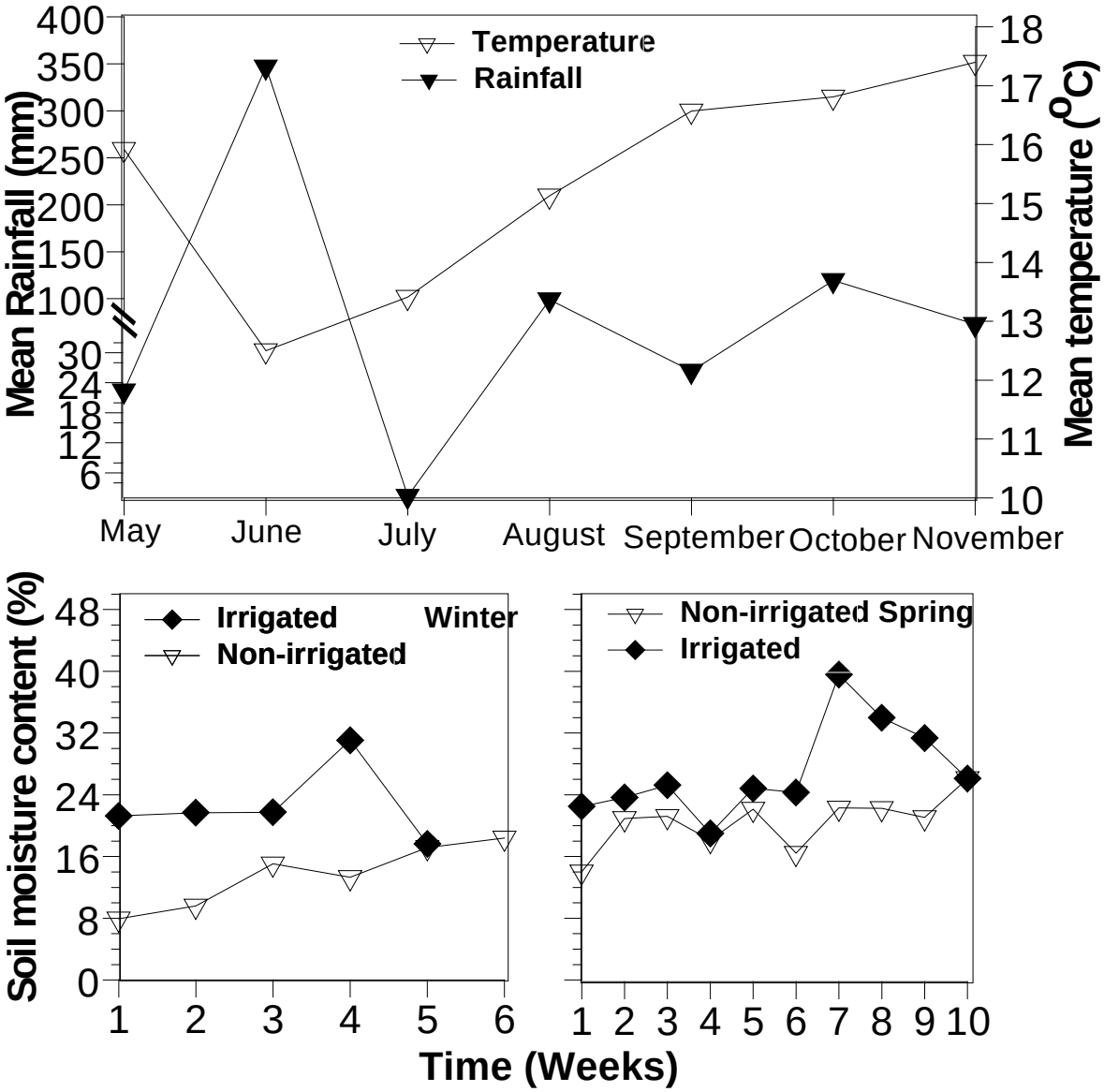

Figure 4. 1: Amount of rainfall received in Pietermaritzburg during the planting season (May-November year) and percentage soil water content.

4.4.1 Plant height

The interaction between planting date, irrigation treatment and landrace (PD x IT x LR) was not significant ($p>0.05$) for plant height (mm), but there were significant differences between (PD x LR) and (PD x IT) (Figure 4.2) and for both the main effects planting date

and landraces. In winter plant height did not differ between IR and NIR for Isaha and Kwayimba landraces however for Masihlalisane, plant height was significantly reduced for Masihlalisane grey seed colour when plots were not irrigated. No significant differences occurred for black seed colour of Masihlalisane (Table 4.1).

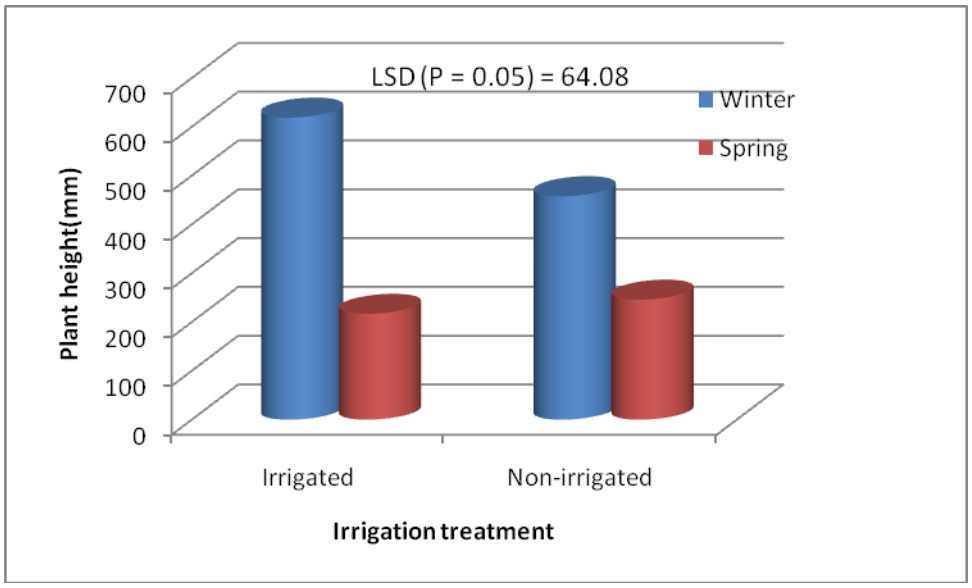

Figure 4. 2: Response of wild mustard landrace in terms of plant height with respect to planting date and irrigation treatment.

Table 4. 1: Plant height (mm) for wild mustard landraces under irrigated and non-irrigated trials in winter and spring.

Landrace and seed colours	Planting date		
	Spring	**Winter**	**Mean**
Isaha			
Brown	567ab	264c	**414.5**
Greyish-black	601a	215c	**408.0**
Reddish-brown	619a	248c	**433.5**
Kwayimba			
Black	455b	210c	**332.5**
Brown	500ab	191c	**345.5**
Reddish-brown	511ab	224c	**367.5**
Masihlalisane			
Black	431b	229c	**330.0**
Brown	439b	287c	**363.0**
Grey	722a	217c	**469.5**
Mean	**538.3**	**231.4**	**384.8**

LSD Planting date (PD) =45.8
LSD Landraces (LR) = 97.2
LSD PD x LR= 137.5
CV %= 30.8
Means followed by the same letter are not significant at p=0.05

4.4.2 Leaf area

Kwayimba black and reddish brown seeds responded positively in spring with an increase in leaf area, with brown seeds showing no significant difference. The similar response was found with brown seeds of Masihlalisane which showed an increase in leaf area in spring while the other grey and black seed colours showed an increase in leaf area of which they were highly significant differences (Table 4.2). The leaf area of all the seed colours of Kwayimba were low in winter, however there was an increase in spring but it was not significant. Leaf area and number are plant mechanisms of stress avoidance. They are important for estimation of photosynthetic rate, light interception, water and nutrient use by the plant during growth. Plants will reduce their leaf area under water stress in order to compensate for transpirational losses. Leaf area and number were sensitive to water stress in some wild mustard landraces. Masihlalisane brown and Kwayimba black landraces did not show their sensitiveness to water stress through leaf area and number reductions.

Table 4. 2: Leaf area (cm^2) interaction for wild mustard landraces at different planting dates (winter and spring).

Landraces and seed colours	Planting date		
	Winter	**Spring**	**Mean**
Isaha			
Brown	125c	557b	**341.0**
Greyish-black	83c	645b	**364.0**
Reddish-brown	83c	645b	**364.0**
Kwayimba			
Black	52c	1145a	**598.5**
Brown	57c	545b	**301.0**
Reddish-brown	54c	1325a	**689.5**
Masihlalisane			
Black	129c	498bc	**313.5**
Brown	131c	1237a	**684.0**
Grey	122c	616b	**369.0**
Mean	**92.8**	**801.4**	**447.1**

LSD Planting Date (PD) =120.4
LSD Landraces (LR) = 255.3
LSD WR x LR= 361.1
CV %= 69.3
Means followed by the same letter are not significant at p=0.05

4.4.3 Fresh mass

The interaction between landrace, irrigation treatment and planting date (LR x IT x PD) was not significant ($p>0.05$) for fresh mass, but significant differences were found for (PD x IT) and (PD x LR) interaction (Table 4.3). Masihlalisane brown seed colour had significantly high biomass accumulation in spring whereas in winter all Masihlalisane seed colours landraces brown seed colour was the lowest but there were no significant difference within the seed colours landrace. Black seeds of Kwayimba showed a significant increase in fresh mass on different planting dates. Reddish-brown seed of Kwayimba had significantly high biomass accumulation in spring. Brown seeds of Isaha showed a similar pattern in terms of fresh mass which showed a significant increase in biomass during spring. Means in biomass over all landraces seed colour increased significantly from 24.0 g to 106.2 g when planting date was changed from winter to spring.

Table 4. 3: Fresh mass interaction for different wild mustard landrace seed colours at two different planting dates winter and spring.

Landraces and seed colours	Planting date		
	Winter	**Spring**	**Mean**
Isaha			
Brown	26.8cd	91.4bc	**59.1**
Greyish-black	13.5d	80.1cd	**46.8**
Reddish-brown	30.4cd	73.8cd	**52.1**
Kwayimba			
Black	12.8d	141.1a	**77.0**
Brown	18.4d	88.8bc	**53.6**
Reddish-brown	20.5d	184.2a	**102.4**
Masihlalisane			
Black	35.2cd	54.2cd	**44.7**
Brown	26.6cd	155.5a	**91.1**
Grey	32.1cd	86.3bcd	**59.2**
Mean	**24.0**	**106.2**	**65.0**

LSD Planting date (PD) =19.86
LSD Landraces (LR) = 42.14
LSD WR x LR= 59.59
CV %= 79.0
Means followed by the same letter are not significant at p=0.05

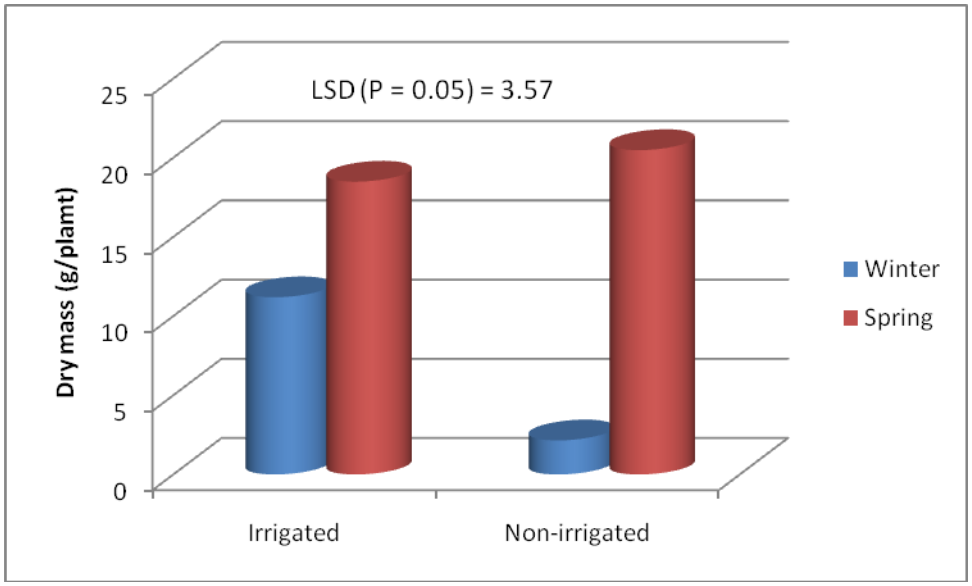

Figure 4. 3: Main effects for dry mass for different planting dates and irrigation treatments of irrigated and non-irrigated.

4.4.4 Dry mass

There was a significant interaction ($p<0.05$) between planting date, irrigation treatment and landraces with respect to dry mass (Table 4.4). Wild mustard landraces showed significantly high dry mass in the irrigated for both winter and spring planting date. However, dry mass was significantly reduced in the non-irrigated plots in winter. Wild mustard plants showed a significant difference in the non-irrigated treatment (Figure 4.3). In the non-irrigated treatment dry mass increased significantly from 2.15 g in winter to 20.48 g spring. Kwayimba reddish-brown of all the landraces had the higher (48.33g) dry mass in the irrigated treatment in winter than all the landraces. Means in dry mass over all landraces was reduced from 11.19 g to 2.15 g in winter in both the irrigated and non-irrigated treatment. However, over all wild mustard landraces dry mass was slightly

reduced from 14.84 g to 11.32 g for both planting date and irrigation treatment. Dry mass was not significantly reduced.

Table 4. 4: Dry mass interaction for planting date, landraces and irrigation treatment.

Planting date	Landraces	Irrigation Treatment		
		Irrigated	Non-irrigated(NIR)	Mean
Winter	IB	4.6d	1.49c	**3.79**
	IGB	2.93d	0.87c	**1.90**
	IRB	16.27cd	1.54c	**8.91**
	KBL	0.99d	3.69c	**2.34**
	KBR	3.98d	3.83c	**3.91**
	KRB	48.33a	2.85c	**25.59**
	MBL	6.63d	1.11c	**3.87**
	MBR	11.79d	1.5c	**6.65**
	MG	5.16d	2.45c	**3.81**
Spring	IB	13.29cd	19.68ab	**16.49**
	IGB	18.8bcd	15.3b	**17.05**
	IRB	13.12cd	17.33b	**15.23**
	KBL	22.63bcd	25.67ab	**26.15**
	KBR	16.65cd	17.37b	**17.01**
	KRB	22.33bcd	24.99ab	**23.66**
	MBL	14.41cd	15.96b	**15.19**
	MBR	29.42b	29.42a	**29.42**
	MG	15.74cd	18.63b	**17.19**
Mean		**14.84**	**11.32**	**13.23**

LSD Planting Date (PD) =2.526
LSD Landraces (LR) = 5.358
LSD Irrigation Treatment (IT) = 2.526
LSD PD x LR x IT= 10.716
CV %= 50
Means followed by the same letter are not significant at p=0.05

4.4.5 Proline

There was a highly significant interaction ($p<0.001$) between landrace and treatment (LR x T) with respect to proline accumulation (Figure 5.2a, b). In winter Kwayimba black seeds accumulated more proline than all landraces seed colour. However, in spring under non-irrigated conditions Masihlalisane black and grey seed colour showed higher proline content than Kwayimba and Isaha. Plants in the non-irrigated trial accumulated more proline than plants in the irrigated trial.

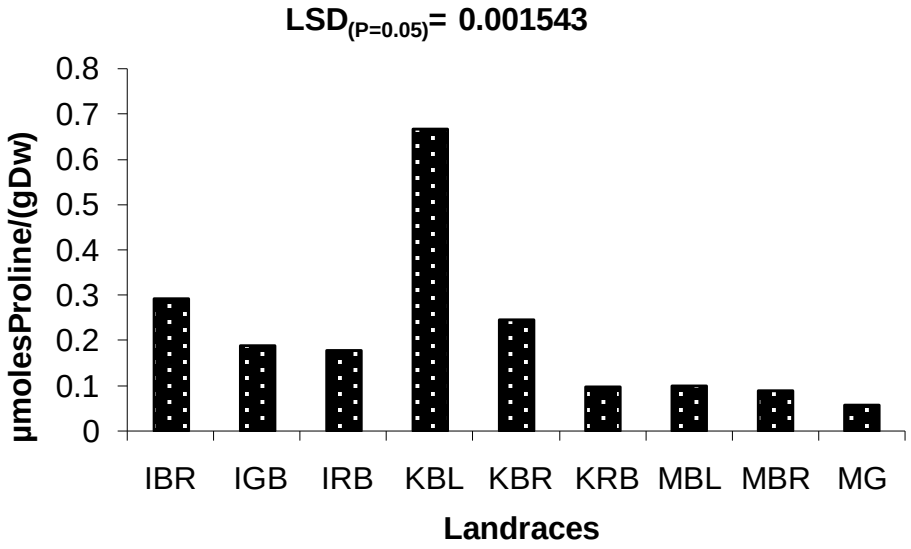

Figure 4. 4: Changes in proline content of plants harvested from a winter planted trial (non-irrigated only).

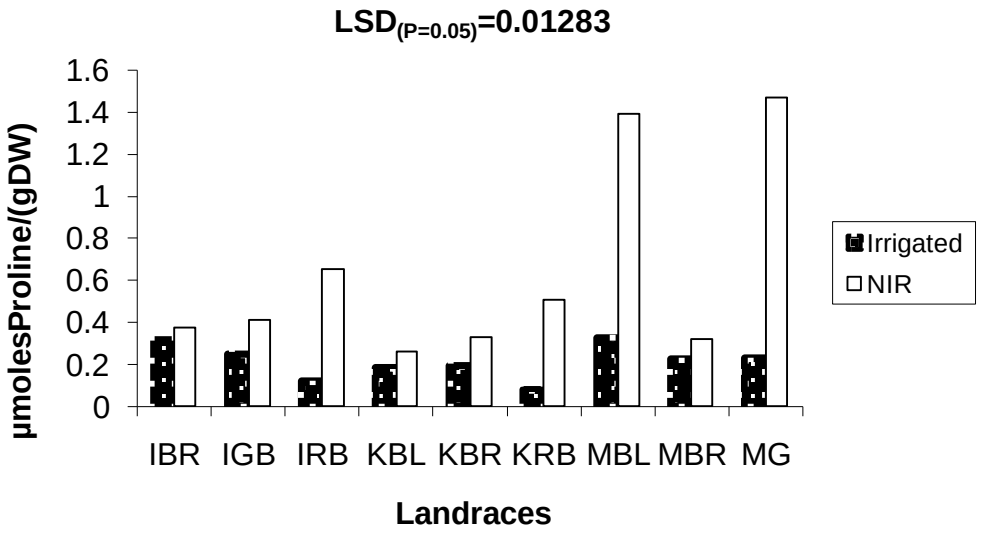

Figure 4. 5: Changes in proline content of plants harvested from a spring planted trial (both irrigated and non-irrigated (NIR). Note: I = Isaha, M = Masihlalisane, K = Kwayimba; BL= black seed, BR = brown seed, G = grey seed, GB = greyish-black, RB = reddish-brown.

Wild mustard landraces showed that the high yield obtained in Kwayimba black during winter was correlated to proline accumulation. Kwayimba avoided stress through accumulation of proline. However, plant growth in Isaha and Masihlalisane under low soil water content during winter was negatively correlated to proline accumulation. Similar to reports in the literature, proline accumulation in Masihlalisane (black and grey) and Isaha was high under low soil water content (Fig 4.1). However, the results agreed with (Lutts *et al*., 1996) results which say proline was involved in the osmotic adjustments under water stress. Proline involvement in osmotic adjustment (under stress) is still debated however, it is believed that it varies according to the species which agrees with the results obtained in this study (Lutts, et al., 1996; Rhodes and Hanson,1993) that proline accumulation in wild mustard varies with cultivars.

4.5 Conclusions

It was shown that wild mustard can grow under low soil water contents (< 40%). It grows well in spring. Water stress tolerance of wild mustard is physiologically negatively correlated with proline accumulation. Masihlalisane brown was shown to be tolerant to water stress. Results of the study may be used as an initial step towards genetic selection for water stress tolerance in wild mustard in an attempt to identify, select and develop wild mustard as a horticultural crop.

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CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

Plant growth and productivity are usually affected by environmental factors such as temperature, photoperiod and water. However, shortage of water in arid areas affects plant growth and productivity more than other environmental factors (Boyer, 1982). Climate change may have major impacts on plant productivity if it is not well understood and controlled. Scientists need to understand the implications of climatic change on plant growth. Under-developed countries have aimed at increasing agricultural productivity by 60% under these changes.

South Africa is now classified a water stressed country (Otieno & Ochieng (2004). A recent report by SASRI showed that rainfall distribution in South Africa is uneven (Singels, 2009) which therefore limits agriculture. Studies have been conducted all over South Africa to come up with a solution that will evaluate plant responses under water stress. Wild plants are believed to be a solution to this problem since they have evolved under natural and often hostile conditions.

Wild mustard (*Brassica spp.)* is an indigenous vegetables consumed by many rural communities in South Africa. The work in this thesis was based on the hypothesis that water stress tolerance in wild mustard involves morphological and physiological changes (Turner, 1991).

The results on seed quality showed that germination and solute leakage may be used as measure of seed vigour and viability in wild mustard cultivars. High vigour and viability observed for Isaha and Masihlalisane may be associated with high germination percentages. Dormancy was, however, not broken in Kwayimba seeds in the germination test since there were many seeds of Kwayimba that were still hard after the experiment. In this study we concluded that using light coloured seeds of Kwayimba, Masihlalisane and Isaha may be recommended for early seedling establishment.

Root and shoot length, leaf number and emergence were used as criteria to select for cultivars that were tolerant to water stress. Water stress in plants is believed to reduce leaf water potentials through osmotic adjustments thereby reducing leaf area and number (Hsiao, 1973). Reduction in leaf water potential results in reduced turgor and stomatal conductance which usually results in affecting the photosynthesis. Under water stress, wild mustard showed tolerance to water stress by increasing the total protein content, especially for Kwayimba black. However, the study did not explore the expression of proteins involved under water stress.

Significant reductions in leaf area and number under water stress were wild mustard ways of adapting to water stress. Leaf area reduction at the vegetative stage is due to decreased cell turgor pressure which is necessary for cell

enlargement (Acevedo *et al.,* 1971). Cell enlargement is a turgor driven process, it reduces cell expansion under water stress through leaf area reduction.

In the pot trial, wild mustard landraces showed better tolerance to moderate water stress as compared to severe and non-stress conditions. Masihlalisane brown showed an adaptation to water stress through leaf area reduction and high biomass accumulation. We concluded that wild mustard landraces are able to grow under moderate stress with high biomass production.

Under field conditions, wild mustard landraces showed a similar pattern as in previous experiments. However, dormancy of Kwayimba black was broken as it was able to emerge well. We observed that its tolerance to water stress was physiologically correlated to protein synthesis and proline accumulation. Understanding the mechanisms by which plants adapt to water stress will aid in the selection of genotypes that are water stress tolerant and can thus be used for crop improvement.

Planting wild mustard landraces during winter resulted in a reduction in biomass accumulation. However, it will be advantageous to grow the crop in winter when it demand is high. It can grow without irrigation, with modest yield, during winter. Planting Masihlalisane brown landrace at soil water contents ≤40% will result in significantly high yield. Soil water availability is important since it affects plant growth (Kramer, 1988).

Wild mustard tolerance to water stress was associated with genotypic variation and physiological changes. Water stress tolerance of Kwayimba black was positively correlated to protein and proline accumulation. Isaha and Masihlalisane showed better water stress tolerance than Kwayimba. It was concluded that wild mustard landraces can grow well under low soil water content (≤ 40%). Masihlalisane brown can be planted in winter under low soil water content with slight reductions in yield while maximum yields are obtainable in spring.

Wild mustard darker seeds are associated with poor dormancy. Isaha and Masihlalisane landraces light seeds are of high vigour and viability. It was also concluded that water stress tolerance in Isaha and Masihlalisane was not associated with protein accumulation. Roots became a stronger sink for Kwayimba cultivars. However, Kwayimba black, it adaptation under water stress was physiologically associated with high accumulation of proteins. Isaha and Masihlalisane can tolerate moderate water stress at vegetative stage with maximum biomass accumulation. We conclude that for Isaha and Masihlalisane landraces tolerance to water stress was physiologically negatively correlated to proline accumulation. However, they were correlated to morphological adjustments that allowed for it growth through leaf area reduction and lowering transpirational losses and increasing root length which helps to balance the demand for water uptake. Kwayimba black tolerance to water stress is associated with proline accumulation.

5.1 Recommendations

For future studies it is recommended that studies should be done on:

- Selecting genes involved in mechanisms for adaption of wild mustard landraces water stress (Proteins expressed under water stress).
- Use of fertilizers for improving wild mustard yield in commercial farming.
- Water use efficiency of the plant should also be well investigated.
- Relationship between ABA and proline accumulation in wild mustard landraces.
- Study the effects of enzymatic involvement of antioxidants under as an adaptation of plants to water.

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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		Start Month		End Year		End Month								
2008		1		2009		12								
Comp#		**Station Name**							**Latitude**	**Longitude**		**Altitude**		
30160		PMBURG; UKULINGA RES STN							-29.66763	30.40599		806		
Compno	Year	Month	Tx	Tn	T	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)

KEY NOTES

ELEMENT	DESCRIPTION	UNIT	STATION TYPE
Tx	Average Maximum Temperature	°C	AWS
Tn	Average Minimum Temperature	°C	AWS
T	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
Tx	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

Appendix 2: ANOVA-Germination test and EC

Variate: %Germination

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Replication stratum						
Landrace.Colour	3	(5)	179.91	59.97	0.01	0.999
Residual	1		8884.32	8884.32	104.95	
Replication.*Units* stratum						
Landrace	2		654145.45	327072.73	3863.59	<.001
Colour	4		9344.71	2336.18	27.60	<.001
Days	7		137802.18	19686.03	232.54	<.001
Landrace.Colour	3	(5)	27363.25	9121.08	107.74	<.001
Landrace.Days	14		45442.61	3245.90	38.34	<.001
Colour.Days	28		11227.25	400.97	4.74	<.001
Landrace.Colour.Days	14	(42)	951.91	67.99	0.80	0.666
Residual	283	(193)	23957.39	84.66		
Total	359	(240)	606359.72			

Variate: Electrolye Conductivity

Source of variation	d.f.(m.v.)	s.s.	m.s.	v.r.	F pr.	
Rep stratum	32	9.330E+08	2.915E+09	2.97		
Rep.*Units* stratum						
Landrace	2	20197E+09	1.098E+09	112.01	<.001	
Colour	3	6.851E+07	2.284E+07	2.33	0.075	
Landraces. colour	4 (4)	1.009E+08	2.523E+07	2057	0.038	
Residue	255(193)	2.501E+09	9.806E+06			
Total	296(198)	4.605E+09				

Appendix 3: ANOVA- seedling trays experiment

Variate: Emergence

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		199.29	99.64	1.76	
Rep.*Units* stratum						
Landrace	8		28924.53	3615.57	63.86	<.001
FC_%	1		21197.61	21197.61	374.40	<.001
Landrace.FC_%	8		6149.89	768.74	13.58	<.001
Residual	32	(2)	1811.77	56.62		
Total	51		(2) 52498.08			

Variate: leaf_number

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.0413	0.0206	0.09	
Rep.*Units* stratum						
Landrace	8		3.8293	0.4787	2.10	0.065
FC_%	1		8.2038	8.2038	35.99	<.001
Landrace.FC_%	8		4.3125	0.5391	2.36	0.040
Residual	32	(2)	7.2941	0.2279		
Total	51		(2) 23.4423			

Variate: Root_length

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		1182.9	591.5	3.44	
Rep.*Units* stratum						
Landrace	8		3614.1	451.8	2.63	0.025
FC_%	1		24.7	24.7	0.14	0.707
Landrace.FC_%	8		4250.8	531.4	3.09	0.011
Residual	32	(2)	5500.0	171.9		
Total	51		(2) 14472.7			

Variate: Shoot_length

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.38	0.19	0.01	
Rep.*Units* stratum						
Landrace	8		354.27	44.28	2.56	0.028
FC_%	1		666.63	666.63	38.53	<.001
Landrace.FC_%	8		185.35	23.17	1.34	0.260
Residual	32	(2)	553.65	17.30		
Total	51		(2) 1694.00			

Variate: leaf_area

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		8.349	4.174	1.63	
Rep.*Units* stratum						
Landrace	8		363.435	45.429	17.70	<.001
FC_%	0	(1)				
Landrace.FC_%	0	(8)				
Residual	16	(18)	41.060	2.566		
Total	26		(27) 226.960			

Appendix 4: ANOVA- POT EXPIRIMENT

Variate: Height_mm

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		84.	42.	0.01	
Rep.*Units* stratum						
Landrace	8		412828.	51604.	10.60	<.001
Field_capacity%	2		543132.	271566.	55.78	<.001
Landrace.Field_capacity%	16		119982.	7499.	1.54	0.082
Residual	442	(177)	2151809.	4868.		
Total	470	(177)	2939964.			

Variate: Leaf_number

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		0.282	0.141	0.02	
Rep.*Units* stratum						
Landrace	8		445.798	55.725	8.48	<.001
Field_capacity%	2		229.048	114.524	17.43	<.001
Landrace.Field_capacity%	16		90.071	5.629	0.86	0.620
Residual	460	(159)	3022.040	6.570		
Total	488	(159)	3633.207			

Variate: Leaf_area_cm^2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	199.5	99.8	0.81	
Rep.*Units* stratum					
Landrace	8	12768.5	1596.1	12.97	<.001
Field_capacity%	2	1381.5	690.8	5.61	0.006
Landrace.Field_capacity%	16	2436.5	152.3	1.24	0.273
Residual	52	6398.6	123.0		
Total	80	23184.6			

Variate: Fresh_weight_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	19.522	9.761	1.23	
Rep.*Units* stratum					
Landrace	8	1831.065	228.883	28.85	<.001
Field_capacity%	2	401.950	200.975	25.33	<.001
Landrace.Field_capacity%	16	328.568	20.535	2.59	0.005
Residual	52	412.532	7.933		
Total	80	2993.637			

Variate: Dry_weight_g

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	1.6488	0.8244	2.82	
Rep.*Units* stratum					
Landrace	8	49.7464	6.2183	21.24	<.001
Field_capacity%	2	10.6618	5.3309	18.21	<.001
Landrace.Field_capacity%	16	10.3346	0.6459	2.21	0.016
Residual	52	15.2231	0.2928		
Total	80	87.6147			

Appendix 5: ANOVA- Field Trial

Variate: Dry_weight_g

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		18.30	9.15	0.21	
Rep.*Units* stratum						
Planting_date	1		4436.38	4436.38	103.71	<.001
Treatment	1		334.84	334.84	7.83	0.007
Landrace	8		2508.94	313.62	7.33	<.001
Planting_date.Treatment	1		821.99	821.99	19.22	<.001
Planting_date.Landrace	8		1344.89	168.11	3.93	0.001
Treatment.landrace	8		1270.08	158.76	3.71	0.002
Planting_date.Treatment.Landrace	8		1395.93	174.49	4.08	<.001
Residual	52	(18)	2224.44	42.78		
Total	89	(18)	10072.89			

Variate: Fresh_weight_g

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		5381.	2690.	1.02	
Rep.*Units* stratum						
Planting_date	1		182083.	182083.	68.82	<.001
Treatment	1		1934.	1934.	0.73	0.396
Landrace	8		39898.	4987.	1.88	0.082
Planting_date.Treatment	1		23660.	23660.	8.94	0.004
Planting_date.Landrace	8		53812.	6727.	2.54	0.020
Treatment.Landrace	8		4350.	544.	0.21	0.989
Planting_date.Treatment.Landrace	8		7823.	978.	0.37	0.932
Residual	52	(18)	137585.	2646.		
Total	89	(18)	383964.			

Variate: Height_mm

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		44980.	22490.	1.60	
Rep.*Units* stratum						
Planting_date	1		2543464.	2543464.	180.58	<.001
Treatment	1		118928.	118928.	8.44	0.005
Landrace	8		229134.	28642.	2.03	0.060
Planting_date.Treatment	1		241074.	241074.	17.12	<.001
Planting_date.Landrace	8		266312.	33289.	2.36	0.030
Treatment.Landrace	8		72607.	9076.	0.64	0.737
Planting_date.Treatment.Landrace	8		55583.	6948.	0.49	0.855
Residual	52	(18)	732425.	14085.		
Total	89	(18)	3985938.			

Variate: Leaf_area_cm2

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		58483.	29241.	0.30	
Rep.*Units* stratum						
Planting_date	1		13115621.	13115621.	135.03	<.001
Treatment	1		4531.	4531.	0.05	0.830
Landrace	8		2457830.	307229.	3.16	0.005
Planting_date.Treatment	1		93559.	93559.	0.96	0.331
Planting_date.Landrace	8		2970575.	371322.	3.82	0.001
Treatment.Landrace	8		629330.	78666.	0.81	0.597
Planting_date.Treatment.Landrace	8		784877.	98110.	1.01	0.440
Residual	52	(18)	5050930.	97133.		
Total	89	(18)	21016287.			

Variate: Leaf_number

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Rep stratum	2		5.783	2.892	0.31	
Rep.*Units* stratum						
Planting_date	1		414.876	414.876	44.55	<.001
Treatment	1		1.591	1.591	0.17	0.681
Landrace	8		65.953	8.244	0.89	0.535
Planting_date.Treatment	1		1.873	1.873	0.20	0.656
Planting_date.Landrace	8		33.731	4.216	0.45	0.883
Treatment.Landrace	8		83.358	10.420	1.12	0.366
Planting_date.Treatment.Landrace	8		51.256	6.407	0.69	0.700
Residual	52	(18)	484.267	9.313		
Total	89	(18)	1007.888			

Appendix 6: Proline determination

Variate: Proline

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.00167645	0.00083822	14.02	
Rep.*Units* stratum					
Landrace	8	2.95248802	0.36906100	6172.66	<.001
Treatment	1	2.41044444	2.41044444	40315.41	<.001
Landrace.Treatment	8	2.40670651	0.30083831	5031.61	<.001
Residual	34	0.00203285	0.00005979		
Total	53	7.77334827			