# EFFECT OF ENVIRONMENTAL STRESS AND MANAGEMENT ON GRAIN AND BIOMASS YIELD OF FINGER MILLET [ELEUSINE CORACANA (L.) GAERTN.]

by

### RACHEL ADOYO OPOLE

B.Sc. (Hons.), University of Eastern Africa, Baraton, Kenya, 2001 MPhil., Moi University, Eldoret, Kenya, 2006

### AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

### DOCTOR OF PHILOSOPHY

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2012

### **Abstract**

Productivity of grain crops is highly sensitive to changing climates and crop management practices. Response of finger millet [Eleusine coracana (L.) Gaertn.] to high temperature stress, and intensive management practices such as increased seeding rates and fertilizer application are not clearly understood. The objectives of this research were to determine the effects of (a) season-long, and short episodes of high temperature stress on growth and yield traits of finger millet, (b) seeding rates and nitrogen fertilizer application rates on grain and biomass yield, and (c) to evaluate the finger millet minicore collection for high grain and biomass yield. Controlled environment studies were conducted to determine the effects of high temperature stress on physiological, growth and yield traits. Field studies were conducted in Manhattan and Hays (Kansas) and Alupe (Kenya) to determine the effects of seeding and nitrogen fertilizer rates on growth and yield traits. Finger millet minicore collection was evaluated under field conditions in India, for phenology, growth and yield traits. Season long high temperature stress of 36/26 or 38/28°C compared to 32/22°C decreased panicle emergence, number of seeds per panicle, grain yield and harvest index. Finger millet was most sensitive to short episodes (10 d) of high temperature (40/30°C) during booting, panicle emergence and flowering stages, resulting in lower number of seeds, and grain yield. Finger millet responded to the interaction between environmental (locations) and temporal (years) factors. In general, locations with higher rainfall had greater grain and biomass yield than those with low rainfall. There was no influence of seeding rates (3.2 or 6.0 kg ha<sup>-1</sup>) at Hays and Alupe. However, in one of the two years in Manhattan, higher seeding rate of 6.0 kg ha<sup>-1</sup> increased grain yield compared to 3.2 kg ha<sup>-1</sup>. There was no influence of nitrogen rates (0, 30, 60 or 90 kg ha<sup>-1</sup>) on grain or biomass yield at all three locations. However, higher fertilizer rates had greater percentage lodging. The finger millet minicore collection displayed large ranges for most quantitative traits including days to flowering, plant height, number of fingers panicle<sup>-1</sup>, grain yield, biomass yield, and lodging; and had >60% heritability. Some of the genotypes from the minicore collection have the potential to increase grain and biomass yield and abiotic stress tolerance of finger millet.

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Approved by:

Major Professor **Dr. P. V.Vara Prasad** 

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# **Dedication**

I dedicate this dissertation to my mother, Janet Odindo, for her lifelong passion and dedication to crop production.

## **Chapter 1 - Overview and Review of Literature**

#### Overview

### Crop production challenged by environmental stresses and management

Crop production and subsequent attainment of maximum yields are highly influenced by environmental factors in addition to management practices. According to Boyer (1982), in agricultural systems, crops are limited to approximately 25% of their potential due to environmental stresses. Environmental factors can be abiotic and biotic in nature. Biotic factors are infections or mechanical damage caused by pathogenic organisms, insect pests or animals, as well as effects of symbiosis or parasitism. Abiotic factors include temperature, humidity, light intensity, the supply of water and minerals, and carbon dioxide. These are the parameters and resources that determine the growth of a crop (Shulze et al., 2005). Future crop production is expected to be highly impacted by both biotic and abiotic stresses as a result of unfavorable climatic conditions. Climate change is expected to have an impact on crop production through aggravating both biotic and abiotic stresses (Jellis, 2009). Global temperature is rising by 0.3°C each decade (Jones et al., 1999) reaching approximately 1°C above the present value by 2025 (Wahid et al., 2005). The rise in temperature is expected to continue in the following decades with global temperature increase reaching a range of 1.6°C to 6°C by 2050 (IPCC, 2007; Jarvies et al., 2011).

The change in climate is expected to have enormous influence on productivity of important food, feed, fiber, and fuel crops in various parts of the world, especially in regions of marginal agriculture where crop production is largely dependent on natural weather variables (Jarvies et al., 2011). It is expected that the yield potential of staple foods will decline in most production environments and commodity prices will increase (Reynolds and Ortiz, 2010). Under such scenario, subsistence farmers in developing countries who depend on indigenous crops like finger millet [*Eleusine coracana* (L.) Gaertn.] may be most affected (Rosenzweig and Hillel, 1995; Kurukulasuriya et al., 2006; Benhim, 2008). Heat stress due to increased ambient temperatures is expected to pose serious threat to crop production worldwide (Hall, 2001). In order to counter these imminent threats, crop science research needs to focus on expanding the knowledge base on the effects of climate change and devise sound adaptation strategies.

Several studies have been conducted to understand and quantify the effects of high temperature stress on a number of crops including maize (*Zea mays* L.) (Thompson, 1986), wheat (*Triticum sp.*) (Stone and Nicholás, 1994), cotton (*Gossypium hirsutum*) (Rehman et al., 2004), pearl millet (*Pennisetum glaucum*) (Ashraf and Hafeez, 2004), groundnut (*Arachis hypogaea* L.) (Prasad et al., 2000), rice (*Oryza sativa*) (Morita et al., 2004), and kidney bean (*Phaseolus vulgaris* L.) (Prasad, 2002) among others. However, the effect of high temperature stress on finger millet has not been determined. This research attempts to quantify the effects of high temperature stress on finger millet growth and development, determine the stages of growth which are most susceptible to high temperature stress and identify finger millet accessions with enhanced tolerance to high temperature stress for use in breeding programs.

Besides climate variables, crop management factors such as seeding rate and nutrient supply are important elements in crop production. Crop management practices can be manipulated to make conditions ideal for crop growth such as through proper choice of plant density and arrangement that minimizes competition (Shinggu et al., 2009) and optimizes radiation use efficiency (Magsood and Azam Ali, 2007) to ensure optimum growth and development. Likewise, proper management of nutrients, particularly nitrogen is critical to enhance productivity and improve financial returns, as well as maintain soil quality and reduce damage to the environment. The rate, source, timing and placement of nitrogen fertilizer are important management considerations for maximizing production and farm profitability. Crop management practices have been determined for finger millet growing regions such as eastern and southern Africa (National Research Council, 1996; Tenywa et al., 1999, Oduori, 1998) and south Asia (Apoorva et al., 2010; Kumara et al., 2007; Joshi et al., 2002). However, such practices have not been determined for the highly mechanized crop production systems of the mid-western region of USA. The goal of this study was to determine optimal seeding rate and nitrogen fertilizer limits that ensure maximum nitrogen and radiation use efficiency to enhance growth, development, and yield of finger millet.

#### Importance and uses of finger millet

Finger millet is an important cereal that belongs to the grass Poaceae family, subfamily Chloridoidae (Dida et al., 2008). It has outstanding attributes as a subsistence food crop. It is grown globally on more than 4 million hectares and is the primary food source for millions of people in tropical dryland regions. The grain of finger millet is globular to oval, ranges from 1.0 to 1.5 mm in diameter and varies widely in color. Its grain can be stored safely for several years without severe damage by insect pests (Duke, 1978). Finger millet also has superior nutritional qualities compared to rice and wheat (Latha et al., 2005). The crop is grown as food grain both in Africa and south East Asia (mainly India and Nepal) (Upadhyaya et al., 2007b). It constitutes about 81% of the minor millets produced in India. In Africa it is mainly grown in Uganda, Kenya, Tanzania, Ethiopia, Rwanda, Malawi, Zambia, and Zimbabwe (Mnyenyembe and Gupta, 1998; Obilana et al, 2002). The crop is cultivated in diverse eco-geographical areas worldwide and displays high genetic variability (Hilu and de Wet, 1976), therefore offers opportunity for genetic improvement. Finger millet is one of the few crops caught in the paradox of being one of the most nutritious cereal yet the most neglected both scientifically and internationally (National Research Council, 1996). However, this attitude toward finger millet is now changing with more research being conducted to exploit its production and utilization potential.

Finger millet is believed to have originated and domesticated in eatern Africa, in the region between western Uganda and the Ethiopian highlands (de Wet, 1995). From Africa, the crop was transported to India about 3000 years ago rendering the Indian sub-continent its secondary center of diversity. Hilu et al., (1979) postulated that cultivated finger millet was likely derived from the selection and domestication of a large-grained mutant of the wild *E*.

coracana subsp. africana. Currently, finger millet is cultivated in diverse eco-geographical areas worldwide and displays high genetic and morphological variability and diversity (Hilu and de Wet, 1976; Liu et al., 2011). Its wide adaptability may be attributed to its C<sub>4</sub> nature (Holt, 2000). It can be cultivated in a wide range of soils and climates and because of its short growing season, it is of specific importance in semi arid regions (Mbithi-Mwikya et al. 2000). Finger millet has high nutritional value (National Research Council, 1996) and excellent storage qualities (Duke, 1978); hence it fits well in farmers' risk avoidance strategies in drought-prone areas (Holt, 2000).

Finger millet is high in dietary fiber and calcium (Malleshi and Hadimani, 1993). It also has medicinal attributes and is used by diverse communities for making specialty foods for diabetics, gluten-free food for people suffering from celiac disease and weaning foods for infants (National Research Council, 1996; Tylor et al., 2006). Finger millet contains nutritionally important starch fractions (Sharavathy et al., 2001) which are slowly digested and absorbed and are favorable in the diet pattern for metabolic disorders such as diabetes, hypertension, and obesity (Asp et al., 1983; Jenkins et al, 1985; Wuresh, 1994). It is consumed in several forms of food products similar to those made from sorghum and other millets. Finger millet products include fermented and nonfermented porridges, pancake-like flatbreads, and fermented alcoholic and nonalcoholic beverages (Murty and Kumar 1995; ICRISAT/FAO, 1996). Finger millet malt has good taste, is easily digested, rich in amino acids and is an ideal base food for people of all age categories.

Finger millet is not only a source of cash for farmers, but also has the potential of saving foreign exchange, which would otherwise be required for the importation of other grains such as maize (Tylor et al., 2006). Malted millet is extensively used in weaning food, infant food, and supplementary food formulations (Malleshi, 2005). Among the tropical cereals, finger millet

provides the best quality malt for local brewing and is more preferred than maize or sorghum (National Research Council, 1996). In Africa it is used to make alcohol (local beer) since its amylase enzymes readily convert starch to sugar, which is subsequently converted to alcohol (Takan et al., 2002; Duke, 1983). Finger millet straw is also a valuable livestock feed. It makes good fodder and contains up to 61% of total digestible nutrients (National Research Council 1996; Upadhyaya et al. 2006). In livestock feeding, finger millet has been reported to be suitable for breeding stock. Finger millet husk, a by-product from brewing as spent grain, has been reported to be a source of fiber as well as a good source of protein and is especially used in household poultry feeding (Obilana and Manyasa, 2002).

Finger millet holds a great potential for the production of plant residues and can be used in rotation or in no-tillage production systems (Segatelli et al., 2008). It has been used successfully as a cover crop under minimum tillage due to its ability to produce a high number of tillers (Samarajeewa et al. (2006); Horiuchi and Yasue, 1980). Above all, finger millet has industrial and economic potential as a result of its high nutritional value (Table 1.1) and malting qualities (National Research Council, 1996; Oduori, 2008). For example, finger millet variety Indaf-15 was identified as a potential variety for malting purposes as it develops high levels of amylases during germination and its malt is a rich source of reducing sugars (Nirmala et al., 2000). In an effort to determine the role of finger millet flour in ethanol production, Reddy and Reddy (2006) and Pradeep et al. (2010) found that the use of very high gravity (VHG) sugar fermentation technology enhanced ethanol yield when finger millet is used as a sole substrate. Therefore, it has been demonstrated that finger millet has potential for ethanol production.

#### Current production levels of finger millet and future prospects

It is estimated that finger millet accounts for some 10% of the 30 million tons of millet produced globally. Its yield potential for the crop is in the range of 4 to 5 tons ha<sup>-1</sup> but yields vary greatly depending on the place of origin of the cultivar (Dida et al., 2008). Recently, there has been a steady decline in yields in some areas in Africa where finger millet is grown. However, in India and Nepal, finger millet yields are on the increase. In India, yields are 1 ton ha<sup>-1</sup> in dryland sites and an average of 2 tons ha<sup>-1</sup> under irrigation. In East African countries, yields as low as 0.3 tons ha<sup>-1</sup> (Zimbabwe), 0.4 tons ha<sup>-1</sup> (Kenya), to as high as 1.6 tons ha<sup>-1</sup> (Uganda) have been recorded (Dida et al., 2008). In West Africa (Nigeria), finger millet yields range between 0.6 to 0.8 tons ha<sup>-1</sup> (Shinggu et al., 2009). There is evidence that finger millet production has been on a declining trend over the years. Production constraints responsible for the low yields have been identified as pests and diseases (blast and Striga), drought, low soil fertility, labor intensity, high weed infestation, low yielding varieties, lodging, and poor attitude to the crop (Oduori, 2008). Finger millet blast disease (Pyricularia grisea) is known to cause as much as 50% losses in yields (Sastri, 1989). Declining yields have also been attributed to constraints such as inadequate knowledge about seeding rate and limited use of inputs (Kidoido et al., 2002).

The effects of other abiotic stresses such as high temperature stress on finger millet production have not been determined; therefore, there is an increasing need to determine and quantify these effects in the face of global climate change and climate variability. Finger millet production may be improved by developing varieties which have the potential to resist biotic and abiotic stresses (Oduori, 2008) by employing various approaches such as conventional, molecular, and participatory breeding approaches. This is expected to result in the development of revolutionary finger millet lines that are adapted to local environmental niches and stresses

(Dida and Devos, 2006). Oduori (2008) further emphasized that the development of new, high yielding, biotic and abiotic stress resistant varieties is desired by farmers; however, no such efforts have been attempted. To fill this gap in knowledge, there is need to identify trait specific germplasm with the ability to withstand high temperature stress (Upadhyaya et al., 2006).

### **Dissertation hypotheses**

- Finger millet growth, development, and yield may be adversely affected by high temperature stress.
- Phenological stages of finger millet growth and development are differentially affected by high temperature stress, resulting in impaired reproduction and yield
- Grain and biomass yield from finger millet is influenced by seeding and nitrogen fertilizer application rates
- Finger millet minicore accessions available at ICRISAT (International Crops Research Institute for the Semi-arid Tropics) genebank are highly variable and contain useful traits for high grain and biomass yield

### **Dissertation objectives**

The broad objectives of this dissertation were:

- To understand the effects of high temperature stress on finger millet physiology, growth, and yield.
- To determine the developmental stages of finger millet most sensitive to high temperature stress.
- To determine the optimum plant density and nitrogen fertilizer application rate for maximizing grain and biomass productivity of finger millet
- To screen finger millet minicore accessions for potential high grain and biomass production.

The specific objectives of each chapter were:

- To determine the effects of high temperature stress on physiology, growth, development, and grain yield of finger millet (Chapter II).
- To identify the stages of growth, development, and reproduction of finger millet most vulnerable to high temperature stress (Chapter III).
- To determine the effects of seeding rate and nitrogen fertilizer application on growth, development, and grain and biomass yields of finger millet (IV).
- To identify finger millet accessions with high potentially grain and biomass yield (Chapter V).

### Literature review

### Finger millet as a crop

Finger millet is an allotetraploid (2n = 4x = 36; genome constitution AABB) and belongs to the subfamily Chloridoideae, together with tef [Eragrostis tef (Zucc.) Trotter] (Babu et al., 2007; Dida et al., 2008). It is a tufted annual grass growing from about 40 to 150 cm in height with erect, compressed and glabrous stems. The leaf blades are linear and taper to an acute point, folded and striated and often have ciliated margins (Rachie and Peters, 1997; Dida et al., 2008). The shape of the inflorescence which consists of a variable number of spikelets resemble fingers on a hand, hence its common name "finger millet". The high variability of the inflorescence size and shape may be a consequence of farmers' selection preferences (de Wet, 1995). The crop matures in 3 to 6 months. The spikelets produce seeds which are globose and smooth and may be colored brown, reddish-brown, black, purple, orange, or white (J. Duke, 1983, Handbook of energy crops. Unpublished, Purdue University). It is mainly grown for food both in Africa and south east Asia. Production in Africa is mainly concentrated in the eastern region, including Uganda, Kenya, Tanzania, Ethiopia, Rwanda, Malawi, Sudan, Zambia, and Zimbabwe. In India, it is mainly grown in the states of Uttar Pradesh, Bilhar, Tamil Nadu, Karnataka, and Andhra Pradesh (Dida and Devos, 2006). It is also grown in other Asian countries including Sri Lanka and China (Fakrudin et al., 2004).

Archeological and linguistic evidence shows that around 5,000 years ago, farming communities in eastern Africa were already cultivating finger millet. It is believed to have been domesticated in the highlands of East Africa about 3000 B.C. (Hilu et al., 1979) and in the same period it was introduced into India via sea routes (Upadhyay, 1995), making India a secondary

centre of diversity (Hilu and de Wet, 1976; Hilu et al., 1979; Hilu and Johnson, 1992; FAO 1995; Dida and Devos, 2006). Hilu and de Wet (1976) and Hilu et al. (1978) presented biosystematic, ethnobotanic, and linguistic evidence which substantiated the East African origin of this cereal and its domestication from subspecies *africana*. According to Werth et al. (1994) the centre of origin for *Eleusine* is East Africa where eight species are found in the wild.

Eleusine coracana subsp coracana is an annual tetraploid (n=18) grown extensively through the semi-arid regions of Africa and India (Werth et al., 1994). Further cytogenetic studies suggested that finger millet is an allopolyploid derived directly from the wild tetraploid *E. coracana* subsp. *africana*, an annual weed occurring across much of Africa (Chennaveeraiah and Hiremath, 1974; Hiremath and Chennaveeraiah, 1982). It was then established that *E. indica* was the source of one of the genomes of *E. coracana* (Hilu, 1988; Hilu and Johnson, 1992; Hiremath and Salimath, 1992). Chloroplast DNAs of both species of *E. coracana* and *E. indica* were all found to be identical in restriction sites, but distinct from other species of *Eleusine*, verifying that *E. indica* was indeed one of the progenitors of finger millet (Hilu, 1988). As the chloroplast genome is maternally inherited in the majority of higher plants, *E. indica* is likely to have been the maternal ancestor of *E. coracana* (Kirk and Tilney-Basset, 1978). Speculation remains whether one or both of the *Eleusine* species *E. intermedia* and *E. semisterilis* are ancestors of *E. coracana*. Both hypothetical ancestral species (X and Y) have been presumed extinct until suitable matches are found in the wild (Werth et al., 1994).

In a recent study to investigate the phylogenetic relationships in the genus *Eleusine*, *Eleusine coracana*, and its putative 'A' genome donor, the diploid *E. indica* were confirmed close allies, but sequence data contradicts the hypothesis that *E. floccifolia* is its second genome donor. The 'B' genome donor has remained unidentified and is thought to be extinct (Neves et

al., 2005). The species *E. coracana* consists of two subspecies, *africana* and *coracana*. The subspecies *africana* has two wild races, *africana* and *spontanea*, while subspecies *coracana* has no wild races but four cultivated races: *elongata*, *plana*, *compacta*, and *vulgraris*. Race *elongata* is further subdivided into subraces *laxa*, *reclusa and sparsa*; race *plana* into *seriata*, *confundere*, and *grandigluma*; race *vulgaris* into *liliacea*, *stellata*, *incurvata*, and *digitata*. Race *compacta* has no subraces (Prasada Rao and de Wet, 1997). These races and subraces can be differentiated from one another by inflorescence morphology (Prasada Rao et al., 1993).

#### **Economic importance and uses**

Although it is not traded in the international market, finger millet is a very important cereal in areas of its adaptation (Hittalmani et al., 2005). Its grain tastes good and is nutritionally rich (compared to cassava, plantain, polished rice and maize meal) as it contains high levels of calcium, iron, and manganese (Table 1.1). The straw is also an important livestock feed, building material, and fuel. Finger millet contains methionine, an essential amino acid lacking in the diets of hundreds of millions of the poor who rely mostly on starchy staples (Hein, 2005). Finger millet contains a low glycemic index and has no gluten, which makes it suitable for diabetics and people with digestive problems. Its grains have high biological value and are consumed as thick or thin porridge, unleavened bread, or used as malt in brewing. Finger millet makes the best quality malt used in both brewing industry and for making nutritious foods (Obilana et al., 2002). Its grain is richer in protein (7 to 8%), fat, and minerals than rice and sorghum (Reed 1976; Barbeau and Hilu 1993). Finger millet grains are particularly rich in tryptophan, cystine, methionine, and total aromatic amino acids compared to other cereals. They

are exceptionally rich in calcium containing about 0.34% in whole grain compared with 0.01–0.06% calcium in most cereals (Kurien et al. 1959). The seeds are also rich in iron containing 46 mg kg<sup>-1</sup> (Serna-Saldivar and Rooney 1995), which is much higher than wheat and rice.

Finger millet malt is a good source of  $\alpha$ - and  $\beta$ -amylases (Chandrasekhara & Swaminathan, 1953) and malted millet is extensively used in weaning food, infant food, and supplementary food formulations (Malleshi, 2005). Finger millet is used as a source of amylases for improving the nutrient density and texture and for weaning food formulations. Although there are reports on the inhibitory activity of the polyphenols on the cereal amylases there are no reports on the inhibition of finger millet malt amylases by its polyphenols (Rohn et al., 2002). In fact, some of the health benefits are attributed to its polyphenol contents. Synergy between phenolics may play a role in mediating amylase inhibition and therefore, have the potential to contribute to the management of type 2 *Diabetes mellitus*, which is characterized by high blood glucose (Cheetan and Malleshi, 2007). Apart from its uses in food, finger millet is useful in other ways. The millet straw is an important livestock feed, building material, and fuel (Hien, 2005). Finger millet is an effective cover crop and can be managed with a single mechanical suppression under minimum tillage with no yield reduction to the main crop (Samarajeewa et al., 2006).

### Finger millet production, production constraints and adaptation

Estimates put worldwide finger millet production at 30% of the world's 30 million ton millet produced. Depending on the country or region of production, yields can range from 4 to 5 tons ha<sup>-1</sup> (Bondale, 1993; Mushonga et al., 1993; Odelle, 1993). In Asia, finger millet has been increasing at a steady rate. In India, yields have increased since 1955 while Nepal has been

expanding the area under the crop at the rate of 8% per year (National Research Council, 1996). Finger millet yields in India are estimated to reach 1 ton ha<sup>-1</sup> under rainfed conditions and 2 tons ha<sup>-1</sup> under irrigated conditions (National Research Council, 1996). Finger millet yields in Uganda and Ethiopia have steadily increased over the last 30 years. In Uganda, yields have increased from 0.9 tons ha<sup>-1</sup> in the 1960s to 1.6 tons ha<sup>-1</sup> in 2006. However, in Kenya, yields have been on a declining trend; from 1.6 tons ha<sup>-1</sup> in 1978 to 0.7 tons ha<sup>-1</sup> in 1981 (FAO, 2006).

Factors contributing to the decline in finger millet production include unfavorable environmental conditions including frequent droughts, pests and diseases, low soil fertility, use of unimproved cultivars, and poor management practices (Oduori, 1998). Drought reduces leaf area, dry matter accumulation, seed weight, radiation use efficiency, and yield of finger millet (Maqsood and Azam Ali, 2007). Finger millet blast caused by the fungus *Pyricularia grisea* Sacc. is the most serious disease, particularly in eastern Africa and India. It causes decline in finger millet grain quality and is responsible for yield losses of up to 10% to 80% in Kenya and Uganda (Holt, 2000, Obilana, 2002; Takan, 2002) and more than 50% in India (Sastri, 1989). Low soil fertility compounded with limited use of inputs such as fertilizers resulted in low finger millet yields in Uganda (Tenywa et al., 1999; Kidoido et al., 2002) and Kenya (Oduori, 1998).

Finger millet as a crop had been stigmatized as a food for the poor, and this negative label had contributed to the decline of its production in recent decades (Dida and Devos, 2006). The National Research Council (1996) listed it among the group of "lost" or "minor" crops, reiterating that the crop is too important to be referred to as such. However, this negative attitude towards finger millet as an important food, feed, and fuel crop is changing fast. Consequently, more research effort has been geared towards ensuring that finger millet finds a niche in the international research community as an important food, feed, and a potential fuel crop. Over the

past few years, breeding efforts in finger millet have been enhanced. The construction of a finger millet genetic map has been viewed as an important step towards mapping traits of agronomic importance and will help in trait transfer in breeding programs (Dida et al., 2006). A comparative analysis has also been carried out to determine the relationship of the finger millet genome with that of rice (*Oryza sativa*). Results showed that information and resources available from rice and other grasses could be readily exploited due to the high colinearity between finger millet and rice, with traits such as blast and drought resistance being of immediate interest to finger millet breeders (Srinivasachary et al., 2007). More recently, Oduori (2008) pioneered the hybridization of finger millet with ethrel CHA (chemical hybridizing agent) and partial emasculation.

Transgenic finger millet lines exhibiting high a level of resistance to leaf blast fungus have also been successfully produced in India (Latha et al., 2005, Ignacimuthu and Ceasar, 2012). These transgenic lines are fortified with inbuilt exotic resistance and appear promising as novel genetic resources for varietal improvement as well as commercial cultivation. Genetic transformation is now widely used as a method of choice for transferring exotic genes into commercial crop cultivars for enhancing various agronomic attributes, and finger millet should be no exception. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) finger millet core and minicore collections are being evaluated for agronomic traits and various biotic and abiotic stresses to identify trait specific germplasm. This is expected to result in enhanced use of germplasm by the breeders to develop high yielding cultivars with a broad genetic base. Since the minicore and reference sets will be shared with the global scientific community, it is expected that minicore and reference sets will enhance use of finger millet germplasm in crop improvement programs (Upadhyaya et al., 2006).

### **Crop production under abiotic stresses**

Abiotic factors such as atmospheric CO<sub>2</sub> concentration, temperature, and ultraviolet-B (UV-B) radiation are projected to change in the near future as a result of climate change. Current CO<sub>2</sub> concentration of 360 μmol mol<sup>-1</sup> could reach anywhere between 560 and 700 μmol mol<sup>-1</sup> by the middle or later part of the 21<sup>st</sup> century (Conway et al., 1988). As a consequence of increased CO<sub>2</sub> concentration, the projected increase in global mean air temperature could range from 1.4 °C to 5.8 °C by 2100 (Houghton et al., 2001) These changes in climate will have an effect on crop production. The need to boost crop production, income, and the level of food security has prompted policy makers to take a keen interest in the impact of environmental stresses since there is a point at which production of yields may respond more to the relief of environmental stress than to additional factors such as fertilizers (Huang and Rozelle, 1995). Environmental stresses such as drought, high salinity, or extreme temperatures are responsible for adverse effects on plant growth and seed production.

Drought is an important environmental constraint that limits the productivity of many crops and affects both quality and quantity of yield. Drought stress brings about a reduction in growth rate, stem elongation, leaf expansion, and stomatal movements. Furthermore, it causes changes in a number of physiological and biochemical processes governing plant growth and productivity, limiting photosynthesis and consequently the yield of plants (Alexieva et al., 2001). Salinity, on the other hand, is an ever-present threat to crop yields especially in countries where irrigation is an essential aid to agriculture (Flowers, 2003). Salinity stress affects development processes such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set (Sairam and Tyagi, 2004). High temperatures (>40°C) are associated with cessation of transpirational cooling following stomatal closure in response to drought. Exposure to

temperatures within a relatively narrow range (45 to 55°C) for as little as 30 minutes can cause severe damage to the leaves of plants from most climatic regions (Barnes et al., 2007). Under field conditions plants usually experience several stresses simultaneously. It has been established that crops grown in the drier areas of the tropics and sub tropics usually experience a combination of water stress and thermal stress (Fitter and Hay, 1987). The stresses may cause a variety of plant responses which can be additive, synergistic, or antagonistic (Alexieva et al., 2001). Because of this close association between drought and high temperature, it can be very difficult to disentangle the effects of each stress on plants growing in the field. To do this, it is necessary to consider the stresses separately under controlled conditions for instance, by studying the influence of high temperature on plants which are adequately supplied with water (Fitter and Hay, 1987). Although high temperature, salt stress, and drought are major ecological factors which prevent crop plants from realizing their full genetic potential, temperature stress is more pervasive and economically damaging. High temperature stress causes reduction in shoot dry mass, growth, and net assimilation rates in a number of plants (Wahid et al., 2007).

### **Crop performance under high temperature stress (heat stress)**

High temperature reduces plant growth and can limit crop yields. It is estimated that in an average growing season, up to 17% yield decrease occurs for each degree centigrade increase in temperature. For example, roughly 25% of corn and 32% of soybean yield trends in the US can be explained by temperature (Lobell and Asner, 2003). Heat stress is a complex function of intensity (temperature degrees), duration and rate at which temperature rises, and the extent of its damage increases rapidly as temperature increases above a threshold level specific for a

particular species (Ismail and Hall, 2007). Threshold temperature refers to a value of daily mean temperature at which a detectable reduction in growth begins (Wahid et al, 2007). The upper threshold temperatures differ for different plant species and genotypes within species. It has been difficult to determine the upper threshold temperature because plant behavior differs depending on other environmental conditions (Miller et al., 2001). However, the threshold temperature for onset of high temperature stress in most species is in the range of 35 to 45°C (Barnes et al., 2007). High temperatures are known to have deleterious effects on photosynthesis, respiration and reproduction (Mitra and Bhatia, 2008). The optimal ranges of temperature for photosynthesis are 25 to 30°C in C<sub>3</sub> plants adapted to sunny habitats and 30 to 40°C in C<sub>4</sub> plants in general (Larcher, 1980). Photosynthesis is one component of crop growth that is most sensitive to high temperatures and photosynthetic rates usually peak at about 30°C, with significant declines in assimilation for each additional degree increase in temperatures (Camejo et al., 2005). For C<sub>3</sub> and C<sub>4</sub> plants, the temperature range for optimum photosynthesis is broad, and at temperatures above this range, photosynthesis decreases (Edwards and Walker, 1983).

The effects of high temperatures on photosynthesis have been investigated by many workers. Earlier investigators considered photosystem II (PS II) to be the most temperature sensitive step in photosynthesis (Berry and Bjorkman, 1980), but studies showed that PSII inhibition does not occur until leaf temperatures are quite high; usually above 40°C (Havaux, 1993, Al Khatib and Paulsen, 1999). Other related studies showed that ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) gets deactivated at temperatures that cause no harm to PSII (Feller et al., 1998). This deactivation is thought to be the primary constraint to photosynthesis in the 30 to 40°C temperature range (Crafts-Brandner and Salvucci, 2000). Indeed, Crafts-Brandner and Law (2000) suggested that heat stress inhibits Rubisco activation via a rapid and direct effect

on Rubisco activase, possibly by perturbing Rubisco activase subunit interactions with each other or with Rubisco.

In general, C<sub>4</sub> photosynthesis is known to vary with growth and temperature (Massad et al., 2007). Net photosynthesis is inhibited in C<sub>4</sub> plants when leaf temperature exceeds 38°C (Berry and Bjorkman, 1980; Edwards and Walker, 1983). The rate of CO<sub>2</sub> exchange (CER) in C<sub>4</sub> plants such as finger millet was highest at the highest temperature (33°C). These responses were larger than those of C<sub>3</sub> plants (rice and soybean), which indicate a decline of enzymatic limitation in C<sub>4</sub> plants with elevating temperature (Edwards et al., 1985). In maize net photosynthesis was inhibited at leaf temperatures above 38°C, and the inhibition was much greater when the leaf temperature was increased rapidly rather than gradually (Crafts-Brandner and Salvucci, 2002). The deactivation is presumed to result from loss of activity of Rubisco activase (Salvucci et al., 2001).

There are many reports of moderate heat damage to components of photosynthetic electron transport other than PSII, especially increased thylakoid proton conductance (Schrader et al., 2004). Other reports indicate that exposure of plants to temperatures as high as 35 to 45°C results in production of reactive oxygen species (ROS) as byproducts which damage the cellular components (Nector and Foyer, 1998). However, plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS, and protect cells from oxidative damage (Sairam and Tyagi, 2004). Additionally, the ability to maintain cell membrane integrity and diminish oxidative stress has been proposed as good indicators of thermotolerance in plants (Liu and Huang, 2000). In C<sub>3</sub> crops, however, there is evidence to show that photosynthetic temperature response is enhanced by growth in elevated CO<sub>2</sub>, and that if temperature acclimation and factors such as nutrients or water availability do not modify or negate this enhancement, the

effects of future increases in air  $CO_2$  on photosynthetic electron transport and Rubisco kinetics may improve the photosynthetic response of  $C_3$  crops like wheat to global warming (Alonso et al., 2009). Temperature-induced decreases in photosynthesis in  $C_3$  species are closely associated with inactivation of Rubisco (Law and Crafts-Brandner, 1999).

High temperatures affect reproduction resulting in yield losses from crops. Studies have been done to determine the effect of high temperature stress on reproductive performance of various crops. In legumes, it was determined that high temperatures affect reproduction by reducing the number of flowers produced and the proportion of flowers which set fruits. Reduced fruit-set was also associated with poor pollen viability and reduced anther dehiscence, particularly when high temperatures were experienced at macrosporogenesis (Prasad et al., 1999). Yield losses due to high temperatures (>34/24°C) were likely to occur particularly if high temperatures coincided with sensitive stages of reproductive development (Prasad et al., 2002). In wheat, high temperatures decreased seed filling duration while increasing seed filling rates (Wheeler et al., 1996b), while they reduced seed size by decreasing the duration of seed-filling in grain sorghum (Prasad et al., 2006). Further investigations are needed to provide insights into understanding and evaluating the reproductive performance of plants, so that suitable genotypes and management practices can be developed to adapt them to high temperature stress which is a consequence of climate change (Koti et al., 2005).

Some mitigation strategies may include basic crop management techniques such as supplying additional nutrients to the plants. Upadhyaya et al. (2011) found that additional nitrogen application could improve heat tolerance of spring chickpea (*Cicer arietinum* L.) and help produce near normal yield irrespective of the genotype. They suggested that the effect of

other nutrients deserves attention to completely mitigate the effects of heat stress. However, effective fertilizer recommendation should consider crop needs and nutrients already available in the soil (Hien, 2005). This knowledge may be applied to finger millet; hence the need to determine the appropriate nitrogen application rates for finger millet production. Finger millet is also known to benefit from residual fertility from the previous crop and this has been found to have marked effect in improving the grain, straw yield, and nutrient uptake of succeeding crops (Saravanane et al., 2011). The ultimate mitigation strategy is to exploit the genetic diversity present in the finger millet minicore collection to develop broad-based finger millet cultivars especially in the context of climate change (Upadhyaya et al., 2010).

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Table 1.1 Comparative nutritional quality between finger millet and other food grains

Component	Maize	Rice	Finger millet
Food energy (Kcal)	408.0	406.0	334.0
Protein (g)	10.5	8.1	7.3
Carbohydrate (g)	83.0	90.0	74.0
Fat (g)	5.3	0.7	1.3
Fiber (g)	3.2	0.3	3.2
Ash (g)	1.3	0.7	2.6
Thiamine (mg)	0.43	0.08	0.24
Riboflavin (mg)	0.22	0.06	0.11
Niacin (mg)	4.1	1.8	1.0
Calcium (mg)	8.0	32.0	358.0
Copper (mg)	0.35	0.25	0.5
Iron (mg)	3.0	0.9	9.9
Magnesium (mg)	142.0	130.0	140.0
Manganese (mg)	0.55	1.1	1.9
Phosphorus (mg)	234.0	130.0	250.0
Potassium (mg)	320.0	130.0	314.0
Sodium (mg)	39.0	6.0	49.0
Zinc (mg)	2.5	1.2	1.5
Essential amino acids (grams per 100 g protein)			
Cystine	1.8	2.0	1.7
Isoleusine	3.6	4.3	4.0
Leusine	12.3	8.3	7.8
Lysine	2.8	3.6	2.5
Methionine	2.1	2.4	5.0
Phenylalanine	4.9	5.3	4.1
Threonine	3.8	3.6	3.1
Tryptophan	0.7	1.2	1.3
Tyrosine	4.1	3.3	4.1
Valine	5.1	6.1	6.4

Source: National Research Council, 1996

# Chapter 2 - Effect of high temperature stress on growth, development and yield of finger millet [*Eleusine coracana* (L.) Gaertn.]

#### **Abstract**

Increase in global temperature is expected to impact crop production worldwide. It has been determined that high temperature stress is one of the major abiotic stresses affecting yield and quality of crops, and is considered more pervasive and economically damaging. One option to mitigate the impact of climate change on food security is to assess the potential of neglected indigenous crops such as finger millet and other local crops in regions where limitations such as high temperature stress and drought are likely to increase, and to adapt them to the changing climates. Finger millet tolerates cooler climates than other millets, and is also known to thrive well under hot conditions. It grows best where the average maximum temperature exceeds 27°C and the average minimum does not fall below 18°C. Though it thrives under hot conditions, the effects of above-normal temperatures on finger millet are still unknown. In other C<sub>4</sub> plants such as sorghum, adverse high temperatures are known to affect pollen viability, seed-set, seed yield and harvest index, and high temperature stress during post flowering stages is known to decrease seed yield. The objective of this study was to investigate the effects of different daytime maximum and nighttime minimum temperatures [32/22°C, Optimum temperature (OT), 36/26°C, High temperature (HT) 1 and 38/28°C, HT2] on phenological, physiological processes, growth, development and yield of finger millet. Finger millet genotype 27116701 SD was exposed to OT, HT1, and HT2 under controlled environment conditions from vegetative to physiological maturity stage. Data on physiological and phenological traits, growth and dry matter production were recorded. Results indicated that high temperature stress (HT1 and HT2)

delayed panicle emergence, flowering, and attaining physiological maturity by an average of 18, 24 and 33 days respectively. High temperature stress decreased growth, yield and harvest index. Increase in temperatures above 32/22°C reduced grain yield 79% and harvest index 58%. This

research highlights the threat faced by crops otherwise considered as 'hardy', such as finger

millet and their vulnerability to the effects of climate change.

Abbreviations: OT = Optimum Temperature, HT = High Temperature

Introduction

The effect of climate change on agriculture has been a subject of heightened investigation

(Reynolds and Ortiz, 2010). Increases in global temperature is expected to affect crop production

with some preliminary data indicating that crops will experience substantial damage from high

temperature stress. For example, recent study on grain sorghum indicated that pollen production,

pollen viability, seed-set, seed yield, and harvest index were reduced under elevated

temperatures (Prasad et al., 2006). High temperature stress in wheat shortened the duration of

grain filling, resulting in reduced kernel growth, low kernel density and weight (Guilioni et al.,

2003). Thus high temperature stress is one of the main abiotic stresses that have a major impact

on the yield and quality of crops in many parts of the world (Neilson et al., 2010). In fact, of the

major ecological factors which prevent plants from realizing their full potential, high temperature

is more pervasive and economically damaging (Wahid et al., 2007; Nagesh Babu and Devaraj,

2008). Maqsood and Azam Ali (2007) suggested that one option to decrease the potential

impacts of climate change on food security was to assess the performance of previously

underutilized crops in regions where limitations such as high temperature stress and drought are

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likely to increase. To do this, it is necessary to consider the stresses separately under controlled conditions, for example, by studying the influence of high temperature stress on plants which are adequately supplied with water (Fitter and Hay, 1987).

This brings into focus finger millet [Eleusine coracana (L.) Gaertn.], which has great potential as a food, feed and fuel crop, but has been grossly neglected, underutilized or relegated to the status of minor importance in terms of its global production and market value compared to major staple crops such as corn, wheat and rice (Hammer and Heller, 1998). However, it is an important cereal in east and southern Africa and southern Asia, and is one of the few special crop species that currently supports the world's food supply (National Research Council, 1996). The crop is adapted to a wide range of environments, can withstand significant levels of salinity, is relatively resistant to water logging, and has fewer diseases (Dida et al., 2007). Finger millet is grown mainly by subsistence farmers and serves as a food security crop because of its highnutritional value and excellent storage qualities. Finger millet straw is also a valuable livestock feed. It makes good fodder and contains up to 61% of total digestible nutrients (National Research Council, 1996; Upadhyaya et al., 2006). It holds a great potential for the production of plant residues and it can be used in crop rotations or in no-tillage production systems (Segatelli et al., 2008). Finger millet can be grown in a wide range of soils and climates and because of its short growing season, it has specific importance in the semi-arid regions (Mbithi-Mwikya et al., 2000). It requires moderate rainfall (500 – 1,000 mm) which should be well distributed during the growing season with an absence of prolonged droughts. Dry weather is required for drying the grain at harvest.

The crop tolerates cooler climates better than other millets, and is known to thrive under hot conditions. It can grow where temperatures are as high as 35°C. In Uganda, the crop grows

best where the average maximum temperature exceeds 27°C and the average minimum does not fall below 18°C (National Research Council, 1996). Although it thrives under hot conditions, the effects of above-normal temperatures are still unknown. The response of crops to high temperature stress has been studied in detail for corn (*Zea mays* L.; Schoper et al., 1987), cotton (*Gossypium barbadense* L.; Reddy et al., 1995), wheat (Ferris et al., 1998), cowpea (*Vigna unguiculata* (L.) Walp; Ismail and Hall, 1999), groundnut (*Arachis hypogaea* L.; Prasad et al., 2000), tomato (*Lycopersicon esculentum* Mill.; Sato et al., 2000), rice (*Oryza sativa* L.; Matsui et al., 2001; Prasad et al., 2006), kidney bean (*Phaseolus vulgaris* L.; Prasad et al., 2002) and sorghum (Prasad et al., 2008). However, no research has been conducted to determine the response of finger millet to high temperature stress. It is important to understand the effects of high temperature stress on finger millet in order to develop strategies to mitigate these effects since it is projected that in some regions, even moderate temperature increases (1 to 2°C) are likely to have negative impacts on yields of major cereals (Jarvies et al., 2011).

The term high temperature stress has been used to describe situations where temperatures are above the optimum and is stressful for a particular process, growth stage or plant species (Prasad et al., 2008). High temperature stress occurs when plants experience temperatures above that to which they are adapted and that adaptation depends strongly on the makeup of the proteins and membranes of the plant (Sharkey and Schrader, 2006). Different crops are known to respond differently to the different aspects of heat stress. High temperature stress is a complex function of intensity, duration, and rate at which temperature rises. The extent of its damage increases rapidly as temperature increases above a threshold level specific for a particular species (Ismail and Hall, 2007). The threshold temperature for onset of high temperature stress in most species is in the range of 35 to 45°C (Barnes et al., 2007). High temperatures are known to have

deleterious effects on photosynthesis, respiration, and reproduction (Mitra and Bhatia, 2008); however the two plant processes that are particularly sensitive to high temperature stress are photosynthesis and pollen development (Berry and Raison, 1981).

During the vegetative stage, high temperatures can damage the components of leaf photosynthesis. For many years, photosystem II (PSII) was considered the most temperature sensitive step in photosynthesis (Berry and Björkman, 1980). Fitter and Hay (1987) reported that studies on thermal tolerance had identified membrane-bound systems and photosystem II in particular as the primary sites of heat injury. However, numerous studies conducted after the 1980's indicated that PSII inhibition does not occur until leaf temperatures are 40°C and above (Havaux, 1993; Al-Khatib and Paulsen, 1999). Cui et al. (2006) found that high temperature stress modified PSII functionality and also reduced photosynthesis by inactivation of chloroplast enzymes, mainly induced by oxidative stress. In other studies, it was shown that ribulose-1,5bisphosphate carboxylase/oxygenase (Rubisco) was deactivated at temperatures that cause no harm to PSII (Feller et al., 1998) and this deactivation has been proposed to be the primary constraint to photosynthesis under high temperature (Crafts-Brandner and Salvucci, 2000). The deactivation was presumed to result from loss of activity of Rubisco activase (Salvucci et al., 2001). Indeed, Crafts-Brandner and Law (2000) postulated that heat stress inhibits Rubisco activation via a rapid and direct effect on Rubisco activase, possibly by perturbing the interaction of Rubisco activase subunits with each other or with Rubisco. Consequently, Rubisco deactivation has been proposed as the primary constraint to photosynthesis in the 30 to 40°C temperature range (Crafts-Brandner and Law, 2000). When temperatures exceed 35°C, a decrease in Rubisco activation occurs resulting in inhibition of net photosynthesis (Crafts-Brandner and Salvucci, 2000).

The effect of high temperature stress is known to affect crops in the reproductive stage more than the vegetative stage, resulting in yield losses of crops (Hall, 1992). In cowpea, reproductive development was found to be more sensitive to heat than photosynthesis, and that high night temperatures were more damaging to reproductive development than were high day temperatures (Warrag and Hall, 1984). High temperature stress can negatively impact floral bud development, flower development, pod set, grain filling and even reduce grain quality (Ismail and Hall, 1999). High temperatures reduced the number of flowers produced and the proportion of flowers which set fruits. Reduced fruit set was also associated with poor pollen viability and reduced anther dehiscence (Ahmed et al., 1992), particularly when temperatures were experienced at microsporogenesis (Warig and Hall, 1984). This may be explained by starchdeficiency. Jain et al. (2007) found that microspores from high temperature stress conditions showed starch-deficiency and considerably reduced pollen germination, translating to 27% loss in seed set in sorghum. In tomato, high temperature affected meiosis in both male and female organs, pollen germination and pollen tube growth, ovule viability, stigmatic and style positions, number of pollen grains retained by the stigma, fertilization and post-fertilization processes, growth of the endosperm, pro-embryo and fertilized embryo (Fooland, 2005).

Additionally, high temperatures reduced fruit set (the proportion of flowers producing pegs or pods) in groundnuts (Prasad et al., 1999). Pollen formation is one of the most heat-sensitive developmental stages in cereals (Saini and Aspinal, 1982; Stone, 2001). Prasad et al. (1999) found that the critical day temperatures for pollen production and viability for groundnuts was 34°C and that there was a strong linear negative relationship between pollen production and pollen viability and accumulated temperature (>34°C). Similarly, in kidney bean, Prasad et al. (2002) found that yield losses owing to high temperatures (>34/24°C) were likely to occur if

high temperatures coincided with sensitive stages of reproductive development. In rice, exposure to >33.7°C at anthesis caused sterility and spikelet fertility was reduced by 7% for every °C above 29.6°C (Jagdish et al., 2007). In a related C<sub>4</sub> crop such as sorghum, high temperature stress was found to compromise grain yield (Prasad et al., 2006a). The optimum mean temperature range for seed germination in sorghum is 21 to 35°C, 26 to 34°C for vegetative growth and development and 25 to 28°C for reproductive growth, and temperatures close to or greater than 32/22°C commonly occur during the life cycle of the crop (Maiti, 1996).

As proposed by Sinclair et al. (2004), one approach to increasing the yield potential of crops is to ameliorate the negative consequences of abiotic stresses on plants so as to increase yield. There is no knowledge of the effects of high temperature stress on growth, development and yield of finger millet. This study was conducted under controlled environment conditions to investigate the effects of different day and nighttime temperatures (32/22°C, 36/26°C and 38/28°C) on phenological and physiological processes, growth, and development, yield and yield traits of finger millet. This knowledge will be useful for making recommendations on the production of finger millet for food, feed and crop biomass in view of the effects of adverse environmental conditions as a result of climate change.

#### Materials and methods

This research was conducted under controlled environment conditions at the Department of Agronomy at Kansas State University, Manhattan, Kansas, USA. The experiment was conducted in the spring of 2009 and 2010.

#### **Experimental and treatment conditions**

Several seeds of finger millet genotype 27116701 SD were sown at 2 cm depth in 3.8-L PVC pots (top and bottom diameters were 15 cm and 13 cm, respectively) containing 1.75 kg of Metro Mix 350 (Hummert Int., Topeka, KS, USA). A controlled release fertilizer Osmocote Classic 90551 (19-6-12, N-P-K) (Scotts, Marysville, OH), was incorporated into the rooting medium at the manufacturer's recommended rate of 1.8 kg m<sup>-3</sup>. Three indoor growth chambers (Conviron Model CMP 3244, Winnipeg, Manitoba, Canada) were used for this research to impose various treatments. Each growth chamber was 75 cm wide, 180 cm long and 185 cm high. After emergence, plants were thinned to three plants per pot.

All three growth chambers were maintained at daytime maximum/nighttime minimum temperature regime of 32/22°C from sowing until 10 days after emergence (DAE). Temperatures in the growth chambers were then adjusted as follows: Growth chamber (GC) 1: 32/22°C [optimum temperature (OT)], GC 2: 36/26°C [high temperature 1 (HT1)], GC 3: 40/30°C [high temperature 2 (HT2)] in 2009. In 2009 plants in GC 3 died within 53 days after applying the treatment. Temperatures were therefore decreased in GC 3 to 38/28°C in 2010. Temperatures in the growth chambers were maintained until the plants reached maturity. Daytime and nighttime temperature regimes were held for 12 h with a 6 h transition period between the daytime

maximum and nighttime minimum temperatures. The photoperiod was 12 h, and photon flux density (400 to 700 nm) provided by cool fluorescent lamps was 940 μmol m<sup>-2</sup> s<sup>-1</sup> measured at canopy level. Relative humidity in the chambers was uniformly set at 85%. Air temperature, relative humidity, and light level were continuously monitored at 20-min intervals in all growth chambers throughout the experiments. Pots were watered daily to keep adequate soil moisture to avoid water stress. Pots were randomly transferred within each growth chamber to eliminate any positional bias with reference to treatment effects (temperature). At booting stage, one plant in each pot was tagged for data collection on physiological and yield traits. For measuring dry weights separate plants were used since destructive sampling was carried out.

#### **Data collection**

Data on phenology, growth, and dry matter production were recorded at frequent time intervals. A self-calibrating chlorophyll meter (SPAD, Model 502, Spectrum Technologies, Plainfield, IL) was used for chlorophyll measurements. Leaf-level photosynthesis, stomatal conductance, transpiration, chlorophyll fluorescence (Fv/Fm) and leaf temperature were measured on individual attached leaves using a LI-6400 XT Portable Photosynthesis System (LI-COR, Lincoln, NE, USA). Gas exchange measurements were taken at growth temperature and ambient CO<sub>2</sub> conditions. The internal LED light source in the LI-6400 XT was set at 1600 μmol m<sup>-2</sup> s<sup>-1</sup> to ensure constant, uniform light across all measurements at different stages of growth and development (1=vegetative, 2=booting, 3=50% flowering, 4=50% grain fill, and 5=physiological maturity (PM). Growth traits were recorded from stages 1 to 5 (1=vegetative, 2=booting, 3=50% flowering, 4=50% grain fill, 5=physiological maturity). Data on plant height (base to tip of the plant) and leaf area was measured and number of leaves and tillers determined. Physiological

traits were recorded from stages 1 to 4 (1=vegetative, 2=booting, 3=50% flowering, 4=50% grain fill) and yield and yield traits were recorded at stage 5 (physiological maturity) of finger millet growth and development.

Vegetative, booting, 50% flowering, 50% grain fill and physiological maturity stages for finger millet correspond to 8.0, 10.0, 10.5.2, 11.2 and 11.4 on the Feekes scale, respectively (Miller, 1999). Leaf area (cm<sup>2</sup>) was measured using the LICOR portable leaf area meter (Model LI-3000) (Lambda Instruments Cooperation). At each stage of growth and development, various parameters were recorded. Total number of leaves was determined by counting all the leaves including green and senesced leaves. Internode length was determined by taking an average of 3 internodes in the middle canopy (6<sup>th</sup> through 8<sup>th</sup> internode). Plants were separated into component parts (leaf, stem, panicle, and seed), and dry weights were recorded. Leaves and stems were dried at 65°C for 7 d and dry weights were recorded. At maturity, panicles were dried at 40°C for 10 d and hand threshed, and seed numbers and seed dry weights were measured. Data on panicle numbers, finger numbers and finger length, hundered seed weight (seed size), and grain yield per plant was recorded at maturity. The experimental design was a randomized block design with three replications. Temperature treatment was randomly assigned to the growth chambers. Class variables consisted of block, temperature, and stages of trait measurement; however yield traits were analyzed by including block and temperature as class variables. Random effects were temperatures and stages while the variables were fixed effects. Statistical analysis was performed using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). The PROC MIXED procedures were used and the Tukey-Kramer adjustment was used to separate the treatment means.

#### **Results**

Mean daytime and nighttime temperatures ( $\pm$ SD) in the optimum temperature (OT) treatment were 31.7°C±0.5 and 21.6°C ±0.5 respectively. They were 35.5°C ±0.0 and 25.8°C ±0.5 for high tempearature (HT1), and 37.9°C ±0.5 and 27.6°C ±0.5 for high temperature (HT2) respectively. Relative daytime and nighttime humidity were similar across all temperature regimes at 85%  $\pm$ 5%.

## Changes in physiological and growth traits across various stages of growth and development under varying temperature regimes

Net photosynthesis increased form vegetative to booting stage, was highest at booting stage, but decreased from 50% flowering to 50% grain fill across all temperature treatment. However, differences between temperature treatments were non significant (Fig. 2.1a). Stomatal conductance increased by 45% from vegetative to 50% flowering stage; was highest at 50% flowering and decreased by 23.9% at 50% grain fill stage across all temperature treatments. HT2 recorded higher stomatal conductance at 50% flowering compared to OT and HT1 (Fig 2.1b). Stomatal conductance increased by 26.1% under HT1, and by 69.7% under HT2 compared to OT. Transpiration rate increased by 32.1% from vegetative to booting stage, was highest at 50% flowering and decreased by 39.3% at 50% grain fill (mid-grain fill) stage across all temperature treatments. HT2 recorded higher rate of transpiration at 50% flowering compared to OT and HT1 (Fig. 2.2a). There was 18.6% increase in transpiration rate for HT1 and 68% for HT2 compared to OT. Leaf temperature increased by 6.8% from vegetative to booting, but decreased by 3.3% at 50% flowering, later increasing by 0.9% at 50% grain fill stage across all temperature treatments.

HT1 recorded higher leaf temperature at booting compared to OT and HT1 (Fig. 2.2b). Leaf temperature increased by 7.1% under HT1 and by 13.8% under HT2 compared to OT.

Chlorophyll fluorescence (Fv/Fm) recorded a small increase of 0.69% from vegetative stage to booting, and declined by 0.9% from booting to 50% grain fill. HT2 recorded the lowest chlorophyll fluorescence compared to OT and HT1 (Fig. 2.3a). There was 2% decrease of chlorophyll fluorescence under HT1 and 1.8% under HT2 compared to OT. Leaf chlorophyll (SPAD units) increased by 42.3% from vegetative to 50% grain fill stage. OT recorded the highest SPAD values compared to HT1 and HT2 (Fig. 2.3b). Leaf chlorophyll increased by 1.8% under HT1 but decreased by 11% under HT2 compared to OT.

Significant interactions were observed between temperature and stage of trait measurement for growth traits (Table 2.1). Leaf dry weight increased significantly by 154% between vegetative and 50% flowering stage, but declined by 45% at 50% grain fill stage (Fig. 2.4a). Stem dry weight increased progressively from vegetative to physiological maturity for all temperature treatments (Fig 2.4b). There was an increase of 8.3% stem dry weight form vegetative to booting stage, 175% at flowering stage, 275% at grain fill and 433.3% at physiological maturity. Stem dry weight increased by 38.1% under HT1 and by 21.4% under HT2 compared to OT. Total dry weight increased by 733.3% from vegetative to physiological maturity stage across all stages (Fig. 2.5). However, temperature decreased total dry weight by 47% under HT1 and 51.3% under HT2 compared to OT.

High temperature stress had significant effects on performance of finger millet. High temperature (HT1, 36/26°C) delayed panicle emergence by 16 days, flowering by 21 days and attaining physiological maturity by 28 days. HT2 (38/28°C) delayed panicle emergence, flowering and attaining physiological maturity by 19, 27 and 38 days respectively. Temperature

significantly decreased plant height, internode length, number of tillers plant<sup>-1</sup>, and total number of leaves plant<sup>-1</sup> across all stages of trait measurement. Overall, at physiological maturity, OT produced significantly taller plants (>80cm) than HT1 and HT2. HT2 produced the shortest plants (<40cm) (Fig. 2.6a). Overall, there was a 40% decrease in plant height under HT1 and 61.2% under HT2 compared to OT (Picture 1). Internode lengths decreased by 36.5% under HT1 and by 63.5% under HT2 compared to OT. OT produced plants with longer internodes (>8cm), HT1 (>5cm) and HT2 (<4cm) (Fig 2.6b). HT1 produced higher number of tillers plant<sup>-1</sup> (basal and nodal tillers) at physiological maturity. HT1 produced 73.1% more tillers than OT, but only 7.7% under HT2 (Fig 2.6c). Total number of leaves plant<sup>-1</sup> was increased by 15.3% under HT1 and 35.3% under HT2 compare to OT (Fig. 2.6d).

#### Effect of temperature on yield and yield traits at physiological maturity

Yield and yield traits were significantly (p<0.05) influenced by temperature treatment (Table 2.2). Number of fingers panicle<sup>-1</sup> was not significantly influenced by temperature at all stages of trait measurement (Fig. 2.7a). Finger length was highest under OT and decreased by 20% under HT1 and by 13% under HT2 compared to OT (Fig. 2.7b). Number of seeds panicle<sup>-1</sup> (Fig 2.7c and Picture 2) 100 seed weight (Fig 2.7d) and was lower under HT1 and HT2 compared to OT. Hundred seed weight decreased by 33% under HT1 and by 55.5% under HT2 compared to OT. Grain yield and harvest index was highest under OT and was markedly lower under HT1 and HT2 (Fig. 2.8a and b) respectively. Grain yield decreased by 75% under HT1 and by 83.6% under HT2 compared to OT, while harvest index decreased by 53.8% under HT1 and by 61.5% under HT2 compared to OT.

#### **Discussion**

Photosynthesis is known to be the component of crop growth that is most sensitive to high temperature stress and photosynthetic rates usually peak at about 30 °C with significant declines in assimilation for each additional degree increase (Wise et al., 2004). Results from this study indicated that temperatures higher than optimum (32/22°C) had adverse effects on phenology, physiology, growth and yield of finger millet at all stages. During vegetative stage, leaf gas exchange properties were affected. We observed that leaf stomatal conductance, transpiration rate, chlorophyll fluorescence (Fv/Fm ratio), and net photosynthesis increased during the early stages of growth and development but decreased from the 50% flowering stage onwards. According to Farquhar and Sharkey (1982) stomata are known to impose a large limitation on the rate of CO<sub>2</sub> assimilation, and this is more severely affected when a plant is stressed. This decline is caused by high temperatures which reduce transport capacity and increases the rates of CO<sub>2</sub> evolution from photorespiration and other sources, causing assimilation rates to decline. They also postulated that stomata limit CO<sub>2</sub> assimilation of C<sub>4</sub> species more than that of C<sub>3</sub> species. It is therefore expected that the same limitation may have been imposed in finger millet by high temperature stress. Results from this study are consistent with those reported by Prasad et al. (2006) where a linear increase in stomatal conductance and transpiration rates with increase in temperature from 32/22 to 44/34°C was observed in sorghum.

Chlorophyll fluorescence (the ratio of variable fluorescence to maximum fluorescence) (Fv/Fm) is a physiological parameter that has been shown to correlate with heat tolerance (Yamada et al., 1996). A decrease in PS II photochemistry (Fv/Fm ratio) suggests that photochemical efficiency and carbon fixation by the leaves is limited (Djanaguiraman et al., 2010). In this study, high temperature stress influenced PSII functionality in the leaves as shown

by lower variable chlorophyll fluorescence yield (Fv/Fm). Similar results were reported by Ciu et al. (2006) in tall fescue (Festuca arundinacea) cultivars. In 2009, high temperatures (40/30°C) resulted in death of finger millet plants. This may have been due to severe cellular injury attributed to the collapse of cellular organization (Wahid et al., 2007). In related studies, bentgrass exhibited growth inhibition, leaf senescence, and death of shoots and roots under high temperature stress (Xiaozhong and Huang, 2000) and high daytime temperatures caused firing and necrosis of leaf tips (Hall, 1993). Combined day and nighttime temperature stress delayed panicle emergence, flowering, and attainment of physiological maturity of finger millet. High temperature stress delayed panicle emergence by 16 days, flowering by 21 days and attaining physiological maturity by 28 days when averaged across the HT stress treatments. Similally, in sorghum, an increase in nighttime temperature to 23°C decreased duration to flowering, seed-set, and physiological maturity by 2, 4, and 10 d, respectively in sorghum (Prasad et al., 2008). In this study, high temperature stress caused a decline in chlorophyll content. According to Liu and Huang (2000), decline in chlorophyll content as a result of premature loss of chlorophyll pigment is due to sensitivity to high temperature stress.

Growth traits viz., plant height and total dry weights were adversely affected by high temperature stress. However, total number of leaves increased with increase in growth temperature. Under high temperature stress, plants remained vegetative for 21 and 28 days for HT1 and HT2, respectively, longer than under optimum, before converting to reproductive stage. This higher growth duration under high temperature stress resulted in production of more number of leaves (green and senesced; data not included). Plants grew increasingly shorter with shorter internode lengths under increasing temperatures (Picture 1). At optimum temperature (32/22°C), plants grew 50% taller than at HT1 (36/26°C) and HT2 (38/28°C). Similarly,

internodes of plants grown at OT were 50% longer than at HT1 and HT2. Sixty seven percent more tillers were produced at OT than HT1 and 33% more than HT2. Dry matter production of finger millet was reduced by high temperature stress. This study confirms similar studies reported elsewhere. In wheat, vegetative dry matter was reduced 55 mg per plant for each 1°C increase in mean temperature (Gibson and Paulsen, 1999). High temperature stress has been reported as one of the most important causes of reduction in yield and dry matter (Giaveno and Ferrero, 2003). High temperatures caused significant declines in shoot dry mass, relative growth rate and net assimilation rate in maize (*Zea mays* L.), pearl millet (*Pennisetum glaucum* L.) and sugarcane (*Saccharum officianarum* L.), though leaf expansion was minimally affected (Ashraf and Hafeez, 2004; Wahid, 2007). Porter and Moot (1998) also reported that growth and development of crops were affected by temperature, limiting yields.

High temperature stress had adverse effects on yield and yield traits of finger millet. Under OT 53% more panicles were produced than at HT1 and HT2, 53% longer fingers than HT1 and 58% longer than HT2, 61% more seeds panicle<sup>-1</sup> than HT1 and 72% more than HT2 (Picture 2), and 60% more seed weight than HT1 and 64% more than HT2. Grain yield and harvest index was also affected by high temperature stress. OT produced 80% more grain plant<sup>-1</sup> than HT1 and 85% more than HT2. Harvest index was higher at OT (63%) than HT1 and 71% higher than HT2. These findings are in agreement with several previous studies. Lobell and Asner (2003) estimated that up to 17% decrease in yield is attained for each degree centigrade increase in temperature in an average growing season. According to Porter (2005), staple cereal crops can tolerate only narrow temperature ranges, which if exceeded during the flowering phase can damage fertilization and seed production, resulting in reduced yield. In wheat (*Triticum* 

aestivum L.), high temperature decreases yield by 3 to 5% per 1°C increase above 15°C in plants under controlled conditions (Gibson and Paulsen, 1999).

Guilioni et al. (2003) found that pollen development and fruit set are critical to field pea production. They also found that the effect of heat stress on crop yield will depend upon the timing of heat stress. If the stress is experienced during anthesis, substantial loss in fruit set and, ultimately, crop yield can occur. Similarly, Dolferus et al. (2011) found that cereal grain number can be affected later when abiotic stress coincides with anther dehiscence. In sorghum growth temperatures >36/26°C significantly decreased pollen production, pollen viability, seed set, seed yield and harvest index compared to 32/22°C (Prasad et al., 2006). In grain crops, decreased seed set is caused by decreased pollen viability and/or stigma receptivity (Prasad et al., 2002; Snider et al., 2009). Losses in cereal yields can be attributed to heat stress induced metabolic changes, to a decrease in the duration of the developmental phases of plants and the consequent reduction in light perception over the shortened life cycle, and to the perturbation of processes related to carbon assimilation (transpiration, photosynthesis and respiration), all of which lead to fewer and malformed and/or smaller organs (Takeoka et al., 1991; Stone 2001 and Maestri et al., 2002).

Temperatures above 30°C during floret formation caused complete sterility in wheat (Owen, 1971; Saini and Aspinall, 1982). In this study, high temperatures significantly decreased seed numbers and seed weight. In 2009, finger millet plants were rendered sterile under high temperature stress (40/30°C). According to Porter (2005), fertilization and seed production in cereals is damaged by high temperature stress resulting in reduced yields. Similarly, Ugarte et al. (2007) found that environmental conditions (precisely temperature) before anthesis affected grain weight. Shah and Paulsen (1999) found that high temperature resulted in decline in grain

mass as well as weight, and sugar content of kernels. Previously, Wardlaw, 1994; Calderinini et al., 1999 a, b, reported that pre-anthesis temperature modified final grain weight in wheat. It was also found that high temperatures and humidity prior to and during the early stages of grain development affected grain setting in wheat and produced shriveled grains (Toshiro and Wardlaw, 1990). Grain number on the main and side tillers of wheat declined by 41% and individual grain weight declined by 45% with heat stress applied at anthesis (Wollenweber and Schellberg, 2003). Therefore it is probable that high temperatures negatively affected growing florets of finger millet, resulting in decreased seed set and seed weight. Results from this study indicate that high temperature stress has a significant effect on the overall vegetative and reproductive growth and development of finger millet. Results also indicate that finger millet tolerates temperatures up to 38/28 °C beyond which the plants will die. Finger millet performed best at optimum temperature which was set at 32/22 °C in the present study. There is need to determine ways to mitigate impacts of high temperature stress on finger millet prodcution by investigating strategies for improving heat tolerance for finger millet. One of the strategies is to screen existing finger millet accessions to identify mechanisms of tolerance to high temperature stress which may be used in breeding programs to develop varieties with ability to tolerate high temperature stress conditions particularly the semi-arid tropics.

#### **Conclusions**

High temperature stress decreased physiological function of finger millet, resulting in reduced growth and development. High temperature stress delayed finger millet phenology including panicle emergence, flowering and attaining physiological maturity resulting in reduced physiological functions, impaired growth and decreased yield and harvest index. Temperatures

greater than 32°C daytime maximum and 22°C nighttime minimum decreased panicle emergence of finger millet an average of 33.6% compared to optimum. Flowering was delayed by an average of 37.5% and attaining physiological maturity was delayed by an average of 25.4%. Temperatures above 32/22°C decreased physiological functions, growth traits including plant height and internode lengths which decreased by an average of 50.6% and 50% respectively. Grain yield decreased by an average of 79.3% and harvest index by 57.7% respectively. The study indicated that increase in high temperatures above 32/22°C daytime maximum and nighttime minimum could have adverse effects on finger millet grain yield and harvest index. This research highlights the threat faced by crops otherwise considered as "hardy" and their vulnerability to the effects of climate change, as well as the need to render them more adaptable to such threats. Some of the strategies that have been suggested include development of heat-tolerant genotypes through conventional plant breeding protocols as well as application of advanced molecular and genetic engineering techniques.

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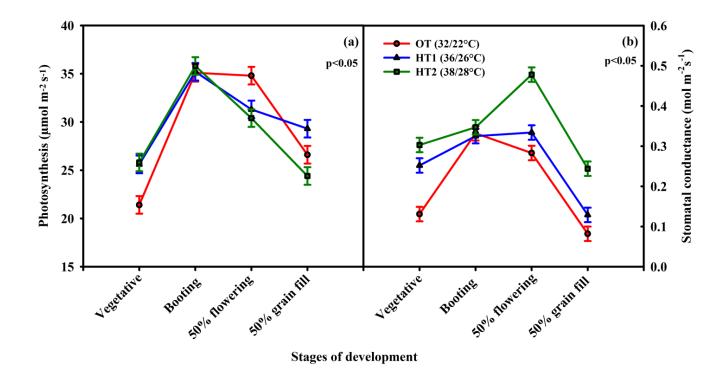


Figure 2.1 Interaction effects of temperature treatments [optimum (OT,  $32/22^{\circ}$ C), high temperature stress (HT1,  $36/26^{\circ}$ C) and (HT2,  $38/28^{\circ}$ C)], and stages of trait measurement on (a) leaf photosynthesis, and (b) stomatal conductance. Vertical bars denote  $\pm$ S.E. of means.

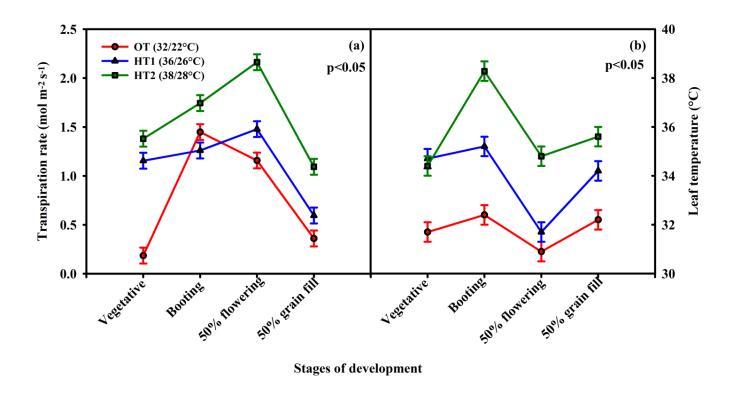


Figure 2.2 Interaction effects of temperature treatments [optimum (OT,  $32/22^{\circ}$ C), high temperature stress (HT1,  $36/26^{\circ}$ C) and (HT2,  $38/28^{\circ}$ C)], and stages of trait measurement on (a) transpiration rate, and (b) leaf temperature. Vertical bars denote  $\pm$ S.E. of means.

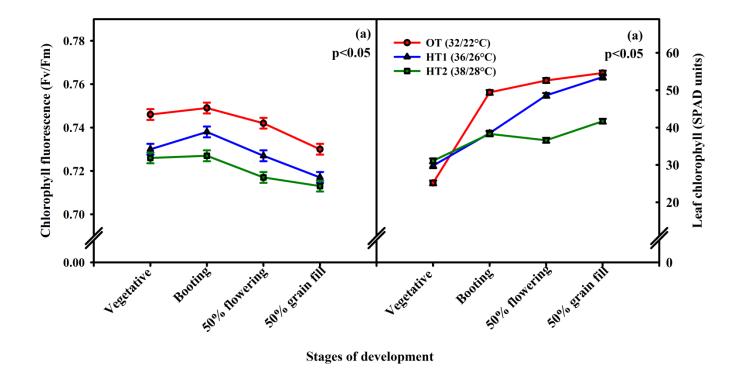
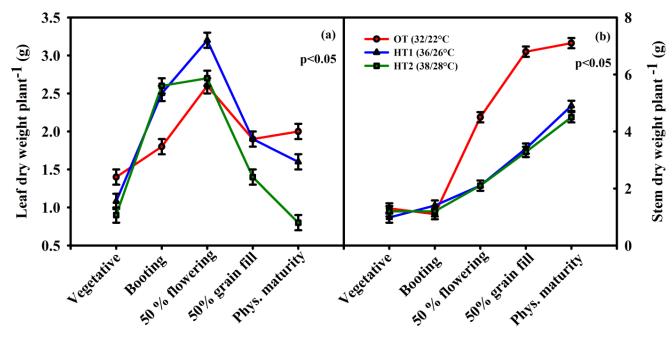


Figure 2.3 Interaction effects of temperature treatments [optimum (OT,  $32/22^{\circ}$ C), high temperature stress (HT1,  $36/26^{\circ}$ C) and (HT2,  $38/28^{\circ}$ C) and stages of trait measurement on (a) chlorophyll fluorescence (Fv/Fm ratio), and (b) leaf chlorophyll content (SPAD units) of finger millet. Vertical bars denote  $\pm$ S.E. of means.



Stages of development

Figure 2.4 Interaction effects of temperature treatments [optimum (OT,  $32/22^{\circ}$ C), high temperature stress (HT1,  $36/26^{\circ}$ C) and (HT2,  $38/28^{\circ}$ C) and stages of trait measurement on (a) leaf dry weight plant<sup>-1</sup> (g) and (b) stem dry weight plant<sup>-1</sup> (g) of finger millet. Vertical bars denote  $\pm$ S.E. of means.

**Phys. Maturity = Physiological maturity** 

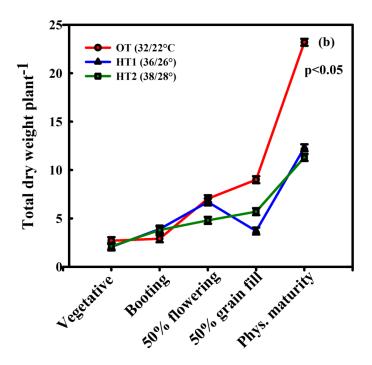


Figure 2.5 Interaction effects of temperature treatments [optimum (OT,  $32/22^{\circ}$ C), high temperature stress (HT1,  $36/26^{\circ}$ C) and (HT2,  $38/28^{\circ}$ C) and stages of trait measurement on total dry weight plant<sup>-1</sup> (g) of finger millet. Vertical bars denote  $\pm$ S.E. of means.

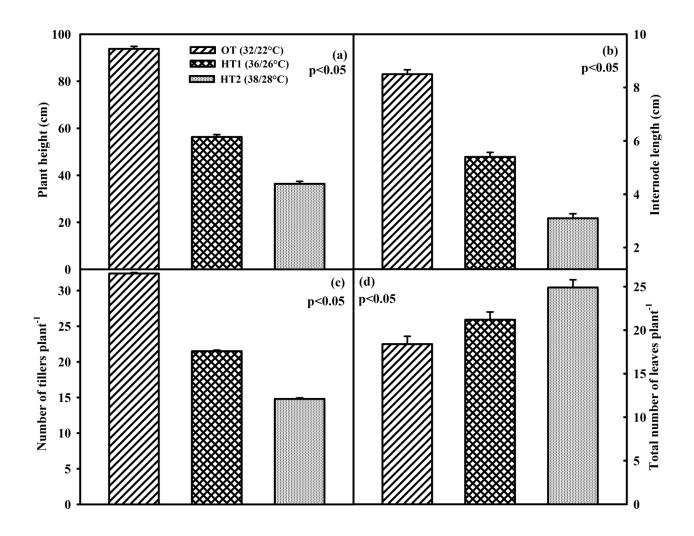


Figure 2.6 Effect of high temperature stress (HT1,  $36/26^{\circ}$ C and HT2,  $38/28^{\circ}$ C) compared to optimum (OT,  $32/22^{\circ}$ C) on growth traits (a) plant height (cm), (b) internode length (cm), (c) number of tillers plant<sup>-1</sup>, and (d) total number of leaves plant<sup>-1</sup> at maturity. Vertical bars denote  $\pm$ S.E. of means.

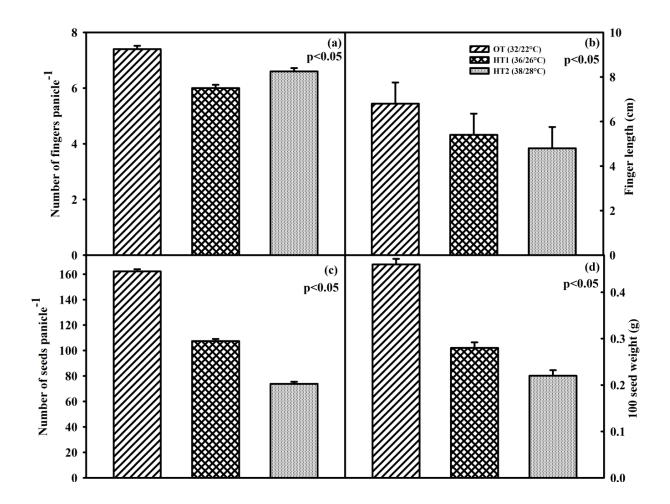


Figure 2.7 Effect of high temperature stress (HT1,  $36/26^{\circ}$ C and HT2,  $38/28^{\circ}$ C) compared to optimum (OT,  $32/22^{\circ}$ C) on yield traits (a) number of fingers panicle<sup>-1</sup> (g), (b) finger length (cm) (c) number of seeds panicle<sup>-1</sup>, and (d) 100 seed weight at maturity. Vertical bars denote  $\pm$ S.E. of means.

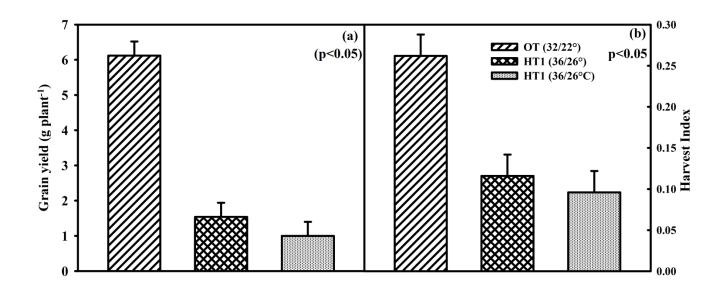


Figure 2.8 Effect of high temperature stress (HT1,  $36/26^{\circ}$ C and HT2,  $38/28^{\circ}$ C) compared to optimum (OT,  $32/22^{\circ}$ C) on (a) grain yield (g plant<sup>-1</sup>) and (b) harvest index at maturity. Vertical bars denote  $\pm$ S.E. of means.

Table 2.1 Analysis of variance (ANOVA) table of main effects (temperature and stages of growth and development) and their interactions on physiological and growth traits of finger millet.

Traits	Temperature (T)	Stages (S)	TxS	
P-value				
Physiological traits				
Photosynthetic rate (µmol m <sup>-2</sup> s <sup>-1</sup> )	$0.2506^{NS}$	<0.0001***	0.0363*	
Stomatal conductance (mol m <sup>-2</sup> s <sup>-1</sup> )	<0.0001****	< 0.0001***	$0.0441^{*}$	
Transpiration rate (mol m <sup>-2</sup> s <sup>-1</sup> )	<0.0001****	<0.0001***	< 0.0001***	
Leaf temperature (°C)	<0.0001****	<0.0001***	$0.0399^*$	
Leaf chlorophyll (SPAD)	<0.0001****	< 0.0001***	< 0.0001***	
PSII photochemistry (Fv/Fm ratio)	<0.0001***	<0.0001***	$0.0035^{*}$	
Growth traits				
Plant height (cm)	< 0.0001	<0.0001***	<0.0001***	
Internode length (cm)	<0.0001***	<0.0001***	<0.0001***	
Number of tillers plant <sup>-1</sup>	<0.0001****	<0.0001***	< 0.0001 ***	
Number of leaves plant <sup>-1</sup>	<0.0001****	<0.0001***	< 0.0001***	
Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	$0.1215^{NS}$	<0.0001***	< 0.0001***	
Leaf dry weight (g plant <sup>-1</sup> )	$0.0007^{**}$	<0.0001***	< 0.0001****	
Stem dry weight (g plant <sup>-1</sup> )	<0.0001***	<0.0001***	<0.0001***	

 $<sup>^*</sup>$ ,  $^{**}$ ,  $^{***}$ , significant at p<0.05, 0.01, 0.001 respectively, NS, non significant at p=0.05

Table 2.2 Analysis of variance (ANOVA) table of main effects (temperature and stages of growth and development) and their interactions on finger millet yield and yield traits at physiological maturity.

Traits	Temperature	
Yield and yield traits	P-value	
Panicle dry weight	<0.0001***	
Panicle numbers	0.0011**	
Finger numbers	$0.0659^{NS}$	
Finger length	0.0003**	
Seeds panicle <sup>-1</sup>	< 0.0001	
100 Seed weight (g)	0.0047**	
Total dry weight (g)	$0.0005^{**}$	
Grain yield plant <sup>-1</sup> (g)	<0.0001***	
Harvest Index	0.0014**	

<sup>\*, \*\*, \*\*\*,</sup> significant at p<0.05, 0.01, 0.001 respectively, NS, non significant at p=0.05

# Chapter 3 - Stage sensitivity of finger millet [*Eleusine coracana* (L.) Gaertn.] to high temperature stress

#### **Abstract**

Gradual temperature changes have a measurable impact on crop yield trends with significant decrease for each degree increase in growing season temperature. However, under climate change, increases in frequent high temperature episodes are likely to occur. Crops suffer from frequent high temperature episodes and, under climate change, and increase in frequency of such episodes is expected. Some plants tend to acclimate when exposed to season long periods of high temperature stress. Therefore, temperature variability may become a major yield-determining factor for some regions in future. Consequently, it is expected that incidences of extreme episodes of high temperature stress would limit productivity of finger millet. This study was conducted to determine the sensitivity of finger millet growth, development and reproduction to short, sudden episodes of high temperature stress. Plants were exposed to a high temperature regime of 40°C daytime maximum and nighttime minimum of 30°C under controlled environment. Results showed that number of seeds panicle<sup>-1</sup> decreased by 69.8% when high temperature stress was imposed at booting stage. Seed weight decreased by 26.1% when high temperature stress was imposed at flowering stage and grain yield decreased by 57% when high temperature stress was imposed at flowering stage. Booting, panicle emergence and flowering were the most sensitive stages of finger millet growth, development reproduction. This study highlights the need to screen finger millet accessions to identify early flowering and maturity, since these traits result in smaller reductions in yield; and to identify those with improved thermotolerance using improved genetic approaches.

# Introduction

Finger millet is an important cereal grown for food in east and southern Africa and southern Asia (mainly India and Nepal) (Upadhyaya et al., 2007b). It is particularly important in the semi-arid regions (Mbithi-Mwikya et al., 2001) and is reported to tolerate cooler weather compared to other millets, while thriving under hot conditions (National Research Council, 1996). The mean temperature range for various stages of growth and development of finger millet has not been determined. The global mean temperature is expected to increase by 1.4 to over 5°C by 2100 (IPCC, 2007; Houghton et al., 2001) with important consequences for crop production (Parry 1990). Both plant growth and development will be affected by high temperature (Prasad et al., 2008). Gradual temperature changes have a measurable impact on crop yield trends with a roughly 17% relative decrease in both corn and soybean yield for each degree Celsius increase in growing season temperature (Lobell and Asner, 2003). In peanut, both short and long-term exposure to air and soil temperatures above optimum can cause significant yield reduction (Golombek and Johansen, 1997; Prasad et al., 1999a, b, 2000a, b). Consequently, it is expected that extreme temperatures would limit growth and productivity of finger millet; therefore it is important to understand the impacts of high-temperature (HT) stress on growth, development and yield of finger millet.

Crops do suffer frequent high temperature episodes (Sharkey, 2005) and under climate change, an increase in frequency of such episodes may occur (IPCC, 2007). The frequency and distribution of heat stress is likely to increase especially if higher air temperatures are accompanied by reduced precipitation (Salvucci and Crafts-Brander, 2004). Therefore, temperature variability will become a major yield-determining factor for some regions in the future (Trnka et al., 2004). Temperature variability is an important determinant of yield, stability

and quality of annual crops, and changes in the frequency of extreme events are important particularly when high temperature episodes coincide with flowering (Wheeler et al., 2000). According to Wollenweber et al. (2003) the developmental stage at which the plant is exposed to the stress may determine the severity of possible damages experienced by the crop.

Prasad et al. (2008) postulated that when plants are exposed to season long periods of high temperatures, they have the opportunity to acclimate and such responses would be different than that of short, sudden episodes of high temperature stress. They further demonstrated that short episodes (10 d) of HT stress (40/30°C) during reproductive development of grain sorghum could be detrimental to yield and yield components. In particular, pre-flowering (10 d before flowering), flowering, and post flowering stages were sensitive to HT stress; however, sensitivity varied by stage and yield losses resulted mainly from decreases in seed number caused by a decrease in the percentage of seed set. In corn (Zea mays L.), Schoper et al. (1987) showed that pollen viability and pollen shed was reduced by high temperature, suggesting that anther dehiscence may also be inhibited by high temperature exposure. In peanut, Prasad et al. (1999a, 2000a) found that day temperatures greater than 34°C decreased fruit-set and resulted in fewer numbers of pods and decreased fruit-set at high temperatures was mainly due to poor pollen viability, reduced pollen production and poor pollen tube growth, all of which lead to poor fertilization of flowers (Prasad et al., 1999b, 2000a, 2001). Additionally, increasing daytime temperature from 26 to 28 to 34 to 36°C significantly reduced the number of subterranean pegs and pods, seed size and seed yield by 30 to 50% (Cox, 1979; Ketring, 1984; Ong, 1984). Similarly, Siddique et al. (1999) found that brief exposure of plants to high temperatures during seed filling can accelerate senescence, diminish seed set and seed weight, and reduce yield

because under such conditions plants divert resources to cope with the heat stress and thus limited photosynthates would be available for reproductive growth and yield.

In an effort to identify the stage(s) during the reproductive development phase of grain sorghum most sensitive to high temperature, Prasad et al. (2008) found that pre-flowering (10 d before flowering), flowering, and post flowering stages were sensitive to high temperature. Reciprocal transfers showed that maximum decreases in yield occurred when high temperature stress was imposed at flowering and 10 d before flowering. Yield losses at these two stages of reproductive development resulted mainly from decreases in seed number caused by a decrease in the percentage of seed set. In most cereals [sorghum, Prasad et al., 2006a; rice, Prasad et al., 2006b; wheat (*Triticum aestivum* L.), Saini et al., 1983] and legumes [peanut, Prasad et al., 2000 a, b, 2001; soybean (*Glycine max* (L.) Merr.), Koti et al., 2005; Salem et al., 2007; cowpea (*Vigna unguiculata*), Ahmed et al., 1992; and common bean (*Phaseolus vulgaris* L.), Gross and Kigel, 1994; Prasad et al., 2002], reproductive processes that occur during flowering, such as pollen production, pollen germination, pollen tube growth, fertilization, and seed set, were found to be highly sensitive to high temperature stress.

High temperature sensitivity is particularly important in tropical and sub tropical climates as it may become a major limiting factor of crop production (Wahid et al., 2005). According to Jarvies et al. (2011) climate change is likely to reduce the length of growing seasons and force large regions of marginal agriculture out of production (Kurukulasuriya et al., 2006; Benhin, 2008) and finger millet is one such crop whose production, mainly by small-scale farmers will be most affected. It is becoming increasingly clear that an improved understanding of the effects of high temperatures on crops and specifically the effect of short episodes of high temperatures will contribute to improved food security (Porch and Jahn, 2001; Prasad et al., 2006a). The adverse

effects of seasonlong high temperature stress on finger millet have been determined (Opole et al., Unpublished); however, the effect of short episodes of high temperature stress at different stages of growth and development and their sensitivity to high temperature stress has not been determined. The hypothesis is that finger millet growth, development and yield are adversely affected by high temperature stress and developmental stages are differentially sensitive to high temperature stress. This study was therefore conducted under controlled environment conditions to (a) determine the impact of short episodes of high temperatures on growth, development and yield, and (b) determine the stages of finger millet growth and development most susceptible to high temperature stress.

## Materials and methods

## **Experimental and treatment conditions**

The research was conducted in the spring of 2009 in the controlled environment facilities at the Department of Agronomy at Kansas State University in Manhattan, Kansas. Several seeds of finger millet genotype 27116701 SD were sown at 2 cm depth in 3L pots and later thinned to 3 plants per pot. Potting soil (Metro Mix 350, Hummert Int., Topeka, KS) was used as the rooting media. Plants were thinned to three plants per pot after emergence until maturity. All plants were maintained in the greenhouse at daytime maximum/nighttime minimum temperature of 27/18°C until the respective developmental stages were attained namely: booting, panicle emergence, flowering, 10 days after flowering (DAF), 20 DAF, 30 DAF, 40 DAF. At these stages, plants were transferred to growth chambers for short periods (10 d) of high temperature treatments and were later transferred to the optimum greenhouse temperature conditions. Plants maintained under greenhouse conditions were used as control while other plants were maintained continuously in the growth chamber at 40/30°C for the continuous HT treatement

Two large indoor growth chambers (Conviron Model CMP 3244, Winnipeg, Manitoba, Canada) were used for this research, each chamber representing a treatment. The growth chambers were 136 cm wide, 246 cm long and 180 cm high. There were 30 pots in each growth chamber. At each respective developmental stage, plants were transferred to the growth chamber (Picture 3) and maintained under high temperature conditions (40/30°C) daytime maximum and nighttime minimum) for 10 days. Daytime and nighttime temperature regimes were held for 12 h with a 6 h transition period between the daytime maximum and nighttime minimum temperatures. The photoperiod was 12 h, and photon flux density (400 to 700 nm) provided by

cool fluorescent lamps was 667µmol m<sup>-2</sup> s<sup>-1</sup> measured at canopy level. Relative humidity in the chambers was set at 85%. Air temperature, relative humidity, and light level were continuously monitored at 20-min intervals in all growth chambers throughout the experiments. Pots were watered daily to keep the soil moisture at field capacity to avoid any water stress. The experiment was a randomized block design with three replications. There were five pots (replications) in each growth chamber that represented a treatment.

#### Data collection

Data on phenology, growth, and dry matter production were collected before and after the developmental stages of heat stress imposition; booting, panicle emergence, flowering, 10 days after flowering (DAF), 20 DAF, 30 DAF, 40 DAF, control and continuous high temperature stress. A self-calibrating chlorophyll meter (SPAD, Model 502, Spectrum Technologies, Plainfield, IL) was used for chlorophyll measurements at the stages of high temperature stress imposition. At maturity, data on plant height (base to tip of the plant), tiller numbers, and leaf area were measured. Plants were separated into component parts (leaf, stem, panicle, and seed), and dry weights were recorded. Leaves and stems were dried at 65°C for 7 d. Panicles were dried at 40°C for 10 d and hand threshed, and seed numbers and seed dry weights were measured. Data on panicle numbers, finger numbers and finger length were estimated. Individual seed weight (seed size) was estimated as the ratio of total seed dry weight and total number of seeds. All data were statistically analyzed using SAS PROC GLM procedures (SAS Institute, 2003) software. Mean separation was accomplished using the LSD test at a probability level of 0.05. Standard error bars are shown as an estimate of variability.

## **Results**

Results indicate that finger millet growth and development was not significantly influenced by high temperature stress imposed at various stages (Table 3.1). Plant height (cm), leaf number, leaf area (cm³), leaf dry weight plant¹ and leaf chlorophyll (SPAD) were not significantly influenced by high temperature stress imposed at vegetative, booting, panicle emergence, flowering, and continuous high temperature stress. However, stem dry weight plant¹ (g) was significantly (P<0.05) influenced by high temperature stress imposed at booting, panicle emergence, flowering, 10 days after flowering (DAF), 20 DAF, 30 DAF, 40 DAF, and continuous high temperature stress (Fig. 3.1a). There was a 26.9% decrease for stem dry weight for booting, panicle emergence, flowering, 10 DAF, 20 DAF, 30 DAF and 40 DAF stages compared to control. At continuous high temperature stress, there was a 50.9% decrease in stem dry weight compared to control.

Yield and yield traits (panicle dry weight, number of seeds panicle<sup>-1</sup>, 100 seed weight (g) and grain yield (g plant<sup>-1</sup>) were significantly influenced by high temperature stress imposed at various stages (Table 3.1). However, there were no significant differences for number of panicles plant<sup>-1</sup>, number of fingers panicle<sup>-1</sup>, and finger length (cm). Significant effects were observed for panicle dry weight (P<0.05) between booting, panicle emergence, flowering, 10 DAF and 20 DAF stages, compared to control, as well as booting, panicle emergence, flowering, 10 and 20 days after flowering, compared to 30 DAF, 40 DAF and continuous high temperature stress (Fig 3.1b). There was a 10.5% decrease between control and booting, panicle emergence, flowering, 10 and 20 days after flowering. There was however, a 25.7% increase in panicle dry weight

when those stages are compared to 30 and 40 days after flowering, and continuous high temperature stress.

Differences were significant (P<0.0001) for number of seeds panicle<sup>-1</sup> (Fig 3.2). Number of seeds decreased at booting (69.8%), panicle emergence (56.9%), flowering (41.4%), 10 days after flowering (11.8%) and continuous high temperature (74.3%) compared to control. The highest percentage yield decrease for number of seeds panicle<sup>-1</sup> was recorded at booting (69.8%), panicle emergence (56.9%) and continuous high temperature (74.3%). There were significant differences (p<0.05) for 100 seed weight at continuous high temperature, but not for other stages of growth and development (Fig. 3.3). At continuous high temperature, seed weight decreased by 52.2% compared to control. Grain yield was significantly (p<0.01) influenced by high temperature stress at booting, panicle emergence, flowering, 40 days after flowering, and continuous high temperature (Fig. 3.4). Grain yield decreased by 35.6% at booting, 71.5% at panicle emergence, 57.0% at flowering, 35.6% at 40 DAF and 78.9% at continuous high temperature. The highest reduction in grain yield was recorded at booting (71.5%) and continuous high temperature (78.9%).

# **Discussion**

These results indicate that different stages of finger millet development respond differently to high temperature stress. This study indicates that short episodes and continuous high temperature stress did not have significant effects on growth traits, but had significant effects on reproductive development of finger millet. Yield and yield components at pre-flowering (booting and panicle emergence), and post-flowering stages (10 DAF, 20 DAF) of finger millet were most sensitive to high temperature stress. Based on most traits (grain numbers and grain yield) the most sensitive

stages were booting, panicle emergence and flowering stages. These results concur with those of Prasad et al. (2008) who reported that maximum decreases in yield of grain sorghum occurred when high temperature stress was imposed at flowering and 10 d before flowering. Similarly, Wheeler et al. (2000) who found that seed yields are particularly sensitive to brief episodes of hot temperatures if these coincide with critical stages of crop development. They found that high temperatures at the time of flowering can reduce the potential number of seeds or grains that subsequently contribute to the crop yield. The adverse effects of HT stress on yield of finger millet could be explained by decrease in seed numbers and seed weight. This could be attributed to injury of microsporogenesis (pollen development) and megasporogenesis (ovule development) under high temperature stress which results in lower seed set (Cross et al., 2003; Young et al., 2004); hence pollen is relatively more sensitive to high temperature stress than ovules. According to Jain et al. (2007), loss of pollen viability is associated with altered carbohydrate metabolism and starch deficiency in developing pollen grains. Previous studies on peanut (Prasad et al., 1999 a, b) and common bean (Gross and Kigel, 1994) also suggested that high temperature stress during pre-flowering stages causes loss in pollen viability that results in lower seed set, seed numbers and seed yield. However, such information is not available on finger millet and needs further investigation.

Prasad et al. (2006a) determined that pollen viability decreased at temperatures >36°C. In most cereals [sorghum, Prasad et al., 2006a; rice, Prasad et al., 2006b and wheat (*Triticum aestivum* L.), Saini et al., 1983], reproductive processes that occur during flowering, such as pollen production, pollen germination, pollen tube growth and fertilization, and seed set, were found to be highly sensitive to high temperature stress. Earlier, Downes (1972) had revealed that high temperature in the later stages of panicle development and at flowering induced floret

abortion and early embryo abortion resulting in lower grain yields. Similarly in peanut, Prasad et al (1999b) reported that reduced pollen viability and pollen number at day temperatures >33°C are indicative of high temperatures during micro and macrosporogenesis, reducing the chances of successful fertilization. When heat is imposed immediately before or during anthesis many plant species become sterile as a result of reduced pollen viability (Siddique et al., 1999). Pulse legumes have been found to be particularly sensitive at flowering stage and only a few days of exposure to high temperatures (30 to 35°C) can cause heavy yield losses through flower drop or pod abortion (Siddique et al., 1999). Consequently, Siddique et al. (1999) determined that under high temperature conditions plants tend to divert resources to cope with the heat stress and hence limited photosynthates would be available for reproductive development. In the present study, seed numbers decreased by 11.8%, seed weight by 8.7% and grain yield by 20.8% when plants were exposed to high temperature stress at 10 days after flowering, and by 74.3%, 52.2% and 78.9% respectively, when finger millet was continuously exposed to high temperature stress (40/30°C) compared to control. This is in agreement with Siddique (1999) who determined that brief exposure of plants to high temperature stress during seed filling can accelerate senescence, diminish seed set and seed weight and reduce yield.

Exposure of finger millet to high temperature stress prior to and during anthesis resulted in reduced growth, development, and yield of finger millet. This could be attributed to loss of pollen viability due to altered carbohydrate metabolism and starch deficiency in developing pollen grains. These stages coincide with pollen development and production. High temperature stress during these stages reduces pollen viability and pollen numbers, reducing the chances of successful fertilization. In case of successful fertilization, grain filling is reduced by diverting photosynthates from reproductive development to cope with stress caused by high temperature

stress (Gross and Kigel, 1994, Prasad, 1999 a, b, Siddique, 1999). These changes affect growth and development and may reduce yields of finger millet drastically. To achieve optimum productivity of finger millet, the most desirable production strategy would be to prevent damage by high temperature stress during the most vulnerable finger millet growth, development and reproductive stages.

# **Conclusions**

The finger millet stages most sensitive to high temperature stress were booting, panicle emergence, and flowering stages. Finger millet exposed continuously to high temperature stress was also negatively affected. The lowest reduction in number of seeds per panicle was observed when high temperature stress was imposed at booting stage (69.8%) compared to control. Seed weight was reduced by 26.1% when high temperature stress was imposed at flowering stage. Grain yield was reduced by 57.0% when high temperature stress was imposed at flowering stage. Booting, panicle emergence, and flowering were the most sensitive stages for finger millet grain yield production. Yield reductions were also recorded when high temperature stress was imposed 10, 20, 30 and 40 days after flowering. It is, therefore, important to screen for and develop finger millet accessions with traits for early flowering and maturity, and for improved thermotolerance using various strategies such as improved genetic approaches.

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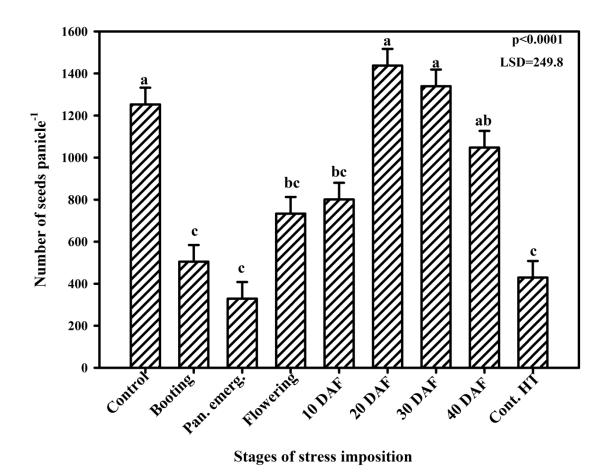


Figure 3.1 Sensitivity of number of seeds panicle<sup>-1</sup> to high temperature exposed at different stages of growth and development of finger millet. Vertical bars denote  $\pm$ S.E. of means. Means with the same letter are not significantly different at p<0.05.

Abbreviations: Pan. emerg. = Panicle emergence, DAF = Days after flowering, Cont. HT = Continuous high temperature

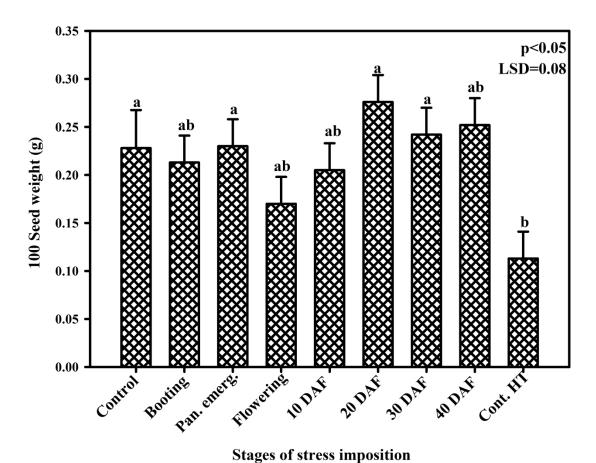


Figure 3.2 Sensitivity of seed weight to high temperature exposed at different stages of growth and development of finger millet. Vertical bars denote  $\pm$ S.E. of means. Means with the same letter are not significantly different at p<0.05.

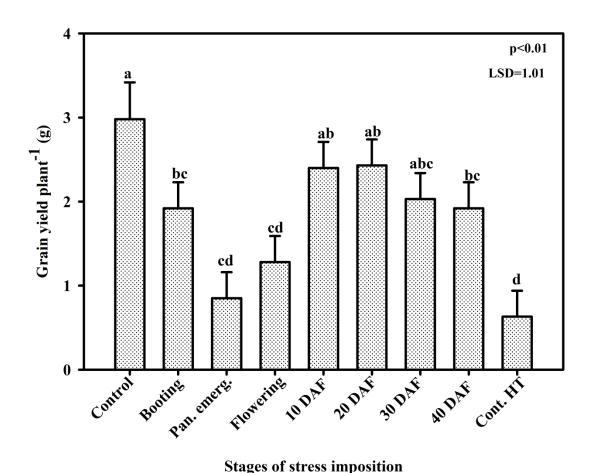


Figure 3.3 Sensitivity of grain yield to high temperature exposed at different stages of growth and development of finger millet. Vertical bars denote  $\pm S.E.$  of means. Means with the same letter are not significantly different at p<0.05.

Table 3.1 Response of finger millet growth and yield traits and leaf chlorophyll content to high temperature stress imposed during different stages of development.

Trait Stages of high temperature stress							ess impos	s imposition		
	<b>1</b> <sup>†</sup>	2	3	4	5	6	7	8	9	-
Plant height (cm)	127.5	129.0	124.5	137.5	125.3	131.0	134.3	138.5	124.3	NS
Leaf number	34.0	47.3	36.8	50.0	64.0	46.8	27.5	27.0	47.3	NS
Leaf area (cm <sup>-2</sup> )	267.0	391.5	178.8	318.0	451.5	437.3	432.0	231.8	194.0	NS
Leaf dry weight (g	24.4	22.3	23.7	22.9	25.5	22.7	19.6	23.2	20.3	NS
plant <sup>-1</sup> )										
Stem dry weight (g	40.5	29.5	30.5	29.6	28.1	29.5	28.1	31.8	19.9	7.4*
plant <sup>-1</sup> )										
Panicle dry weight (g)	38.1	24.6	28.7	27.2	28.8	27.0	34.4	35.5	34.4	$8.7^{*}$
Number of panicles	2.8	1.8	4.0	4.0	6.5	4.5	2.8	3.5	2.8	NS
plant <sup>-1</sup> Number of fingers panicle <sup>-1</sup>	7.8	6.8	5.8	5.5	7.3	7.0	7.5	6.8	7.3	NS
Finger length (cm)	9.8	9.5	7.5	7.0	8.5	8.3	8.5	7.3	11.0	NS
Leaf chlorophyll content (SPAD)	42.8	40.3	43.1	39.0	42.0	43.4	45.0	43.3	47.3	NS

<sup>\*</sup> Significant at p≤0.05, NS, non significant (p>0.05)

<sup>&</sup>lt;sup>†</sup>Developmental stages of high temperature stress imposition: 1 = Control; 2 = booting; 3 = Panicle emergence; 4 = Flowering; 5 = 10 Days after Flowering (DAF); 6 = 20 DAF; 7 = 30 DAF; 8 = 40 DAF; 9 = Continuous high temperature (HT)

# Chapter 4 - Effect of seeding and nitrogen fertilizer application rates on field performance of finger millet [Eleusine coracana (L.) Gaertn.]

#### **Abstract**

Millets are grown in the US at a very limited scale, but may be considered for use as a cover crop especially in rotations with minimum tillage. In Kenya, finger millet is widely grown but faces production challenges such as low soil fertility and inadequate crop management, including seeding rate and plant population. Nitrogen availability is the most limiting crop production factor both in the US and in Kenya. There is need to determine optimal seeding rate and nitrogen fertilizer application rates for the mid western region. In Kenya, there is need to improve current management practices to improve yields. The study was conducted to determine the effect of seeding and nitrogen fertilizer application rates on grain and biomass yield of finger millet. The experimental sites were Manhattan in 2009 and 2010, Hays 2010 and Alupe, Kenya in 2011. Finger millet was sown at a seeding rate of 3.2, 6.0 and 9.0 kg ha<sup>-1</sup> and urea was applied as a topdress at the rate of 0, 30, 60 and 90 kg ha<sup>-1</sup>. Results showed that increase in either seeding rate or nitrogen fertilizer application rate or their interactions did not have an effect on grain and biomass yield. However, grain and biomass yield was significantly influenced by the interaction between spatial and temporal factors (location and years) and seeding rate. In Manhattan, 2009, lower seeding rate of 3.2 kg ha<sup>-1</sup> increased tillering capacity and a higher seeding rate (6.0 kg ha<sup>-1</sup> 1) increased biomass yield. In Manhattan 2010, high seeding rate (6.0 kg ha<sup>-1</sup>) increased leaf dry weight and grain yield. Lack of response of grain and biomass yield to nitrogen fertilizer application may be attributed to prevailing environmental conditions which were unique to each location. Finger millet responded to environmental conditions more readily than the applied treatments. Future studies should, therefore, consider controlling extraneous factors including crop rotation, tillage, fertilizer source and placement to determine the optimum seeding and nitrogen application rates for finger millet in the mid western US and other major finger millet growing areas.

## Introduction

Finger millet [Eleusine coracana (L.) Gaertn.] is among the most cultivated millets and belongs to the genus *Eleusine*, in the *Chloridoidae* subfamily (Clayton and Renvoze, 1986). It is a native African crop which is also extremely important in South Asia (India and Nepal) (National Research Council, 1996). Finger millet is a tufted annual plant which can grow up to about 1.3 m tall but is commonly 1.0 m tall (Taylor and Emmambux, 2008). It is 97 to 99% self pollinating (Hilu and de Wet, 1980) and takes between 2.5 to 6 months to mature (Watson and Dallwitz, 1992). The panicle consists of spikes arranged as digits. The crop is adapted to a wide range of environments and can be grown in a variety of soils with medium or low water holding capacity (National Research Council, 1996), but requires rainfall of at least 800 mm per annum (Van Wyk and Gericke, 2000). It is grown at intermediate elevations between 500 - 2400 m above sea level (Haq, 1989). In Africa, it is grown at altitudes between 1000 - 2000 m above sea level while in Nepal it is grown up to 2400 m above sea level (Kono et al., 1988). It is a short day plant with a photoperiod optimum of 12 h. Daylength types also exist (National Research Council, 1996). Finger millet can grow where temperatures are as high as 35°C, but grow best where the average maximum temperature exceeds 27°C and the average minimum does not fall below 18°C (National Research Council, 1996, Mbithi-Mwikya et al., 2000). It can tolerate water logging (Kono et al., 1988) and can withstand significant levels of salinity (Dida et al., 2007). It was more tolerant to levels of salinity as high as 200 mol m<sup>-3</sup> compared to tef [*Eragrostis tef* (Zucc.) Trotter], and pearl millet [*Pennisetum americanum* (L.) Leeke] (Kebebew and McNeilly, 1995).

In Africa and South Asia, finger millet is a staple food grain upon which millions depend however, finger millet straw also makes good animal fodder, containing up to 60% total digestible nutrients (National Research Council, 1996). Millets are grown in the United States on a very limited scale and are considered minor crops; however, this may change and they may become important as rotational or cover crops (Baker, 2003). Samarajeewa et al. (2006) postulated that since finger millet can produce a higher number of tillers compared to other millets (Horiuchi and Yasue, 1980), it can be considered for use as a cover crop especially for crops such as soybean under minimum tillage. In Brazil, finger millet was introduced to farmers interested in using it as a forage crop and was recommended for hay production in soils with medium to high fertility (Fransisco, 2002). Segatelli et al. (2008) found that finger millet holds a great potential for the production of plant residues and it can be used in rotation culture or in notillage systems. It has been considered as a potential forage crop for cattle in Europe (Northern Ireland). According to Mackay et al. (2007), if the protein in the leaves has the same high methionine content as the grain, it would provide nutritious silage feed for cattle than ensiled maize (Zea mays L.). It would also grow faster than maize, producing a large tonnage per acre, per unit time, and could be harvested much earlier than maize. There is a realization that millets, including finger millet would produce a more dependable harvest compared to other crops especially under marginal and sub marginal conditions of soil fertility and limited moisture

(Seetharam, 1986). Hence there is a potential of finger millet as a forage or cover crop in rotation production systems of the Central Great Plains of US.

Nitrogen (N) availability is a key factor in crop production since it is the nutrient that most often limits crop production (Shukla et al., 2004). Nitrogen is required to synthesize photosynthetic enzymes as well as all other N components of the plant. If N is readily available in the soil, sufficient N will be stored in ribulose 1,5-bisphosphate carboxylase oxygenase (Rubisco) to allow increase in yield. Rubisco is the primary storage site for nitrogen during plant vegetative development. When grains develop, Rubisco is broken down and most of the released N is transformed to the grain. Hence, without additional N accumulation there will be a net decrease in N available for the grain, resulting in grain yield decrease (Sinclair et al., 2004). The efficiency of nitrogen fertilizer is essential for both agricultural production and protection of the environment (Mosier et al., 2004). Consequently, the rate and timing of nitrogen are critical in terms of their effects on yield (Shokri et al., 2009) and optimum use of N can be achieved by matching N supply with crop demand (Shukla et al., 2004). In rice, where the immense role played by nitrogen in increasing productivity is well documented (Kumar and Prasad, 2004), nitrogen increased growth and yield traits such as plant height, panicle number, leaf size, spikelet number, and number of filled spikelets (Doberman and Fairhurst, 2000). In wheat, application of 120 kg N ha<sup>-1</sup> produced higher grain yield (Shukla et al., 2004).

Studies have been conducted to determine seeding and fertilizer rates for finger millet in other parts of the world where finger millet is grown but not in the mid western USA. In some regions of India, finger millet is sown by hand at a seeding rate of 5 to 10 kg ha<sup>-1</sup> (Nagajaran and Smale, 2007). Manipulation of seeding rate and row spacing also contributed in high yields and control of weeds in finger millet in Nigeria. Higher seed rate (10 to 15 kg ha<sup>-1</sup>) and narrow inter-

row spacing (25 to 30 cm) had a positive effect on crop biomass and weed control in finger millet (Shinggu et al., 2004). In Ireland, where finger millet has been considered as a potential forage crop for cattle, a seeding rate of 10 kg ha<sup>-1</sup> gave the best plant growth and tillering (Mackay et al., 2001). In Kenya, finger millet is sown in furrows spaced 30 cm apart using a recommended seed rate of 2.5 kg ha<sup>-1</sup> (Oduori, 1993) while in Uganda, a 10% increase in seeding rate resulted in a 2% increase in finger millet yield (Kidoido et al., 2002).

In Nepal, finger millet grain yields are typically 1160 kg ha<sup>-1</sup> (Katuwal and Tiwari, 1997) and straw yields are 1400 kg ha<sup>-1</sup>, assuming a harvest index of 0.45 (Pilbeam et al., 2000). Fertilizer application is done in a staggered manner; basal application of 20 kg N ha<sup>-1</sup> (Urea) + 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (SSP) + 20 kg K<sub>2</sub>O ha<sup>-1</sup> (Potassium chloride). Topdressing is done at the rate of 20 kg N ha<sup>-1</sup> (Urea) 30 days after sowing (Kumar et al., 2002). In field trials in India, diammonium phosphate (DAP) is applied as a basal dose prior to sowing and urea is applied as a topdress at 100 kg ha<sup>-1</sup> (Upadhyaya and Gowda, 2009). In a trial in Bangalore, application of NPK fertilizer and farm yard manure (FYM) on soil test response basis, along with dual microbial inoculation recorded higher grain yields (3740 kg ha<sup>-1</sup>) and straw yield (9486 kg ha<sup>-1</sup>) of finger millet (Apoorva et al., 2010).

In field trials in East Africa, finger millet grain yields from 2400 to 4100 kg ha<sup>-1</sup> have been obtained with nitrogen rates up of to 150 kg ha<sup>-1</sup> (Stabursvik and Heide, 1974). In Uganda, a combined application of nitrogen and phosphorus significantly increased dry matter and grain yield of finger millet. Soils were found to be of sub-optimal fertility for crop production and phosphorus was found to be a key limiting nutrient in the soil therefore was applied for improved finger millet productivity. A fertilizer application package of 22.5 kg N ha<sup>-1</sup> + 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was recommended for increased yields (Tenywa et al., 1999). In Kenya, where low soil fertility

is one of the production constraints responsible for low finger millet yields farmers planting improved varieties and adopting improved management practices such as use of fertilizers could improve finger millet yields (Oduori, 2000; 2008) and fertilizer is applied at the rate of 20 kg N ha<sup>-1</sup> + 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (Oduori, 1993). In an effort to improve field performance of finger millet, there is need to improve the management of the crop, mainly in terms of plant population and nutrient requirements. There is lack of consistency on the best management practice for optimum finger millet grain and biomass production. The present study was conducted to determine the effect of various seeding and nitrogen fertilizer application rates on field performance of finger millet in Kansas and Kenya. The objective of the study was to determine the effect of seeding and nitrogen fertilizer application rates on growth and yield of finger millet. The hypothesis is that the field performance of finger millet is positively influenced by combined variable seeding and nitrogen application rates.

## Materials and methods

## Plant husbandry and growth conditions

The experiment was conducted in Manhattan, Riley County, at Ashland Bottoms Research farm located south of Manhattan (2009 and 2010) Kansas, located 39° 15′, 15″ N / 90°, 40′ 19″ W under rainfed conditions and at the Western Kansas Agricultural Research Center at Hays, Kansas, located 39° 15′, 15″ N / 90°, 40′ 19″ W in 2010. In Kenya, the experiement was conducted at the Kenya Agricultural Research Center, Alupe sub-center, located 0° 30′ 0″ N, 34° 35′ 0″ E in 2011. Planting was done on June 19, 2009 and June 25, 2010 in Manhattan, June 11,

2010 in Hays and June 7, 2011 in Alupe. Finger millet genotype 27116701 SD was sown in Kansas and Gulu-E in Kenya.

Three seeding rates; 3.2, 6.0 and 9.0 kg ha<sup>-1</sup> and four nitrogen application rates; 0, 30, 60 and 90 kg N ha<sup>-1</sup> were arranged in 3 x 4 factorial experiment run as a randomized complete block design with four replications. Inter-row spacing was 60 cm. Seeding rates were main plots and nitrogen fertilizer application rates were sub-plots. In Manhattan (2009 and 2010), finger millet was sown on a plot where the previous crop was soybean (Glycine max L.). In Hays (2010) the plot had been fallowed for 8 consecutive years. In Alupe, the plot was under pasture for 3 consecutive years. Weather data for the locations are shown in Table 4.1. Soil samples were collected before planting. Soil test results for the plots are shown in Table 4.2. In Kenya, finger millet was sown in a plot which had been fallow for 3 years. A small seed planter was used to sow finger millet in Kansas. In Kenya, hand drilling was done. In Manhattan, Callisto (a.i., mesotrione 40.0%) was applied at the recommended rate of 0.37 L ha<sup>-1</sup> and Bicep (a.i., 2-chloro-N-(2-ethy-6-methylphenyl)-N-(2-metoxy-1-methylethyl) acetamide 26.1% and 2-chloro-4-(ethylamino)-6-(iso-propylamino)-S-triazine 33%) was applied at the recommended rate of 2.75 L ha<sup>-1</sup>. In Hays, Atrazine (a.i., 2-chloro-4-ethylamino)-6-(iso-propylamino)-s-triazine 33%) was applied at the rate of 2.9 L ha<sup>-1</sup> was applied to control weeds. In Kenya hand weeding was done to keep the plots weed free. Urea (46-0-0) was broadcast within the plots 3 weeks after germination (vegetative stage).

#### **Data collection**

Data were collected at 45 days after planting (DAP), 60 DAP, at flowering and at physiological maturity on number of plants plot<sup>-1</sup>, plant height (cm), number of leaves plant<sup>-1</sup>, number of tillers plant<sup>-1</sup>, leaf, stem and panicle dry weights (g), finger numbers panicle<sup>-1</sup> and grain yield (kg ha<sup>-1</sup>). In Manhattan and Hays, plants were hand harvested from 1m<sup>2</sup> and data on aboveground biomass was collected. Stem, leaf, and panicles were separated and oven dried at 60°C for 4 days, and dry weights recorded. Grain yield was measured after oven-drying the seeds. The SPAD chlorophyll meter (Konica, Minolta Inc, Tokyo, Japan) was used to determine leaf greenness at various stages of plant growth and development namely, vegetative, booting, 50% flowering, and 50% grain fill. In Kenya, data on plant population, plant height (cm), number of tillers plant<sup>-1</sup>, grain yield and leaf chlorophyll (SPAD) were collected at the respective growth stages.

#### **Statistical analysis**

Statistical analysis was performed using SAS 9.1.3 (SAS Institute Inc. Cary, USA). The PROC GLM procedure was used. The experimental design was a 3 x 4 factorial with seeding rates randomly assigned to main plots and nitrogen fertilizer rates to sub-plots. There were four replications. Class variables consisted of year/location (Y), stage, block, seeding rates, and fertilizer. Year /location, seeding rates and N fertilizer rates were treated as random effects and the variables as fixed effects. Tukey's Studentized Range Test (HSD) was used to separate the means.

## **Results**

The results from the 2009, 2010, and 2011 studies conducted in Manhattan, Hays (Kansas) and Alupe (Kenya). Analysis of variance indicated significant effects for year/location (year embedded in location) for all the traits measured except number of leaves. There were significant interaction effects for year/location and seeding rates, but not for fertilizer rates. Three-way interactions were also non significant for the traits measured. The effect of seeding and nitrogen fertilizer rates on growth and yield traits and leaf chlorophyll (SPAD) are summarized in Tables 4.3 and 4.4. Therefore, environmental and temporal variability showed significant effects on finger millet growth and yield more than individual effects of the treatments applied. Non-significant data is not discussed in detail.

## Effect of interaction between year/location and seeding rates

Interaction between year/location and seeding rates had significant effects on plant population (Fig. 4.1), number of tillers (Fig 4.2), leaf chlorophyll (Fig. 4.3), leaf dry weight (Fig. 4.4), and biomass yield (Fig. 4.5). There were significant effects of year/location on grain and biomass yield (Fig. 4.6). Plant population was significantly higher across locations/years for Manhattan 2009, 2010, Hays 2010 compared to Alupe 2011. Differences were significant between seeding rates for plant population. Finger millet sown at 6.0 and 9.0 kg ha<sup>-1</sup> produced higher plant population compared to 3.2 kg ha<sup>-1</sup>). There were significant differences between years/location for number of tillers plant<sup>-1</sup>. Alupe 2011 recorded significantly lower tiller numbers compared to Manhattan 2009, 2010 and Hays 2010. In Manhattan 2009 and Hays 2010, more tillers were

produced from the lower seeding rate (3.2 kg ha<sup>-1</sup>) compared to 6.0 kg ha<sup>-1</sup> and 9.0 kg ha<sup>-1</sup>. In Manhattan 2009, a lower seeding rate of 3.2 kg ha<sup>-1</sup> produced 29% more tillers than 6.0 kg ha<sup>-1</sup> and 9.0 kg ha<sup>-1</sup>. In Hays 2010, 3.2 kg ha<sup>-1</sup> produced 11.5% more tillers than 6.0 kg ha<sup>-1</sup> and 9.0 kg ha<sup>-1</sup>.

Significant effects were recorded for leaf chlorophyll across years/locations. Leaf chlorophyll decreased with increase in seeding rate in Manhattan 2009 by 9% from 3.2 kg ha<sup>-1</sup> to 9.0 kg ha<sup>-1</sup>. Leaf dry weight was significantly increased by 10% in Manhattan 2010 compared to Manhattan 2009 and by 22% compared to Hays 2010. Stem dry weight however, increased by 12% from Manhattan 2009 compared to Manhattan 2010 and 52% compared to Hays 2010. Panicle dry weight increased by 10% in Manhattan 2009 compared to Manhattan 2010 and 66% compared to Hays 2019. Grain yield significantly increased by 17% in Manhattan 2010 compared to Hays 2010, by 22% compared to Manhattan 2009 and by 78% compared to Alupe 2011. Biomass yield increased by 6% in Manhattan 2009 compared to Manhattan 2010 and by 41% compared to Hays 2010.

# Effect of fertilizer application rates on lodging (%) of finger millet

Nitrogen fertilizer application rates did not however have any significant effects on finger millet productivity across the locations/years. However, it has significant effects on lodging in Manhattan 2010 (Fig. 4.7). Lodging increased by 6.9%, 53%, and 103% with application of 3.0, 60, and 9.0 kg ha<sup>-1</sup> of urea nitrogen respectively.

## **Discussion**

Seeding rates, among other factors, are known to influence emergence and establishment in crops. High seeding rates depress yield and stand density (Lanini et al., 1990) while low seeding rates have been found to produce significantly high grain yields, with the greatest increases occurring during periods of severe moisture stress (Pelton, 1969). In wheat a reduction in yield was recorded at very high seeding rates (Gooding et al., 2002). In this study, increase in seeding rate did not significantly increase grain and biomass yield of finger millet, despite recording an increase in plant population. In a similar study with wheat, yields did not vary over a wide range of populations (Joseph et al., 1984). This may be attributed by plant survival and tillering. Finger millet is a crop with high tillering ability and this has been found to have a positive effect on crop biomass and yield (Shinggu et al., 2009). Wheat, a crop with similarly high tillering ability, compensated for low population densities by increased production and survival of tillers (Gooding et al., 2002). This study indicated that the lower the seeding rate, the higher the tillering ability and the higher the chlorophyll content of the leaves of finger millet, possibly due to increased radiation capture, hence increased radiation use efficiency.

Leaf chlorophyll concentration estimated through the SPAD meter gives a relative assessment of nitrogen status of the crop. Seeding rates had a significant effect on nitrogen content of finger millet leaves in Manhattan in 2009 as shown by SPAD values; however differences were not significant across the years and locations (Fig. 4.3). In Manhattan 2009, nitrogen content of finger millet leaves was higher at lower seeding rate (3.2 kg ha<sup>-1</sup>) and lower at higher seeding rate (9.0 kg ha<sup>-1</sup>). Nitrogen fertilizer rates had no effect on SPAD values, while higher SPAD values were recorded at lower seeding rate. This is in agreement with Spanner et al. (2005) who found that grain yield increased significantly with increasing seeding rate,

however, seeding rate x nitrogen fertilizer application interaction effects were not significant. They determined that SPAD values may vary among years, locations, varieties and soil characters.

Lodging is considered a major yield limiting in finger millet (Oduori, 2005). It is caused by morphological characteristics, fertilizer application, and prevailing weather conditions. Under optimal fertilizer application and an expected optimum fertilizer application, high incidence of lodging occurs. In finger millet lodging poses problems for mechanical and manual harvesting, as well as yield reduction in terms of total grain yield and quality. In this study, lodging of finger millet plants was significant in Manhattan 2010 with increased N fertilizer application rates. Development of improved tef [*Eragrostis tef* (Zucc.) Trotter] seed with semi-dwarf stature would increase lodging resistance and responsiveness towards fertilizer application, according to Esfeld et al. (2009). Such development would benefit finger millet which is susceptible to lodging under high nitrogen fertilizer application rates.

Finger millet is known to respond positively to N fertilizer application (National Research Council, 1996; Tenywa et al., 1999; Oduori, 2000; Apoorva et al., 2010). In field studies, finger millet grain yield increased from 2400 to 4100 kg ha<sup>-1</sup> with rates of nitrogen (N) up to 150 kg ha<sup>-1</sup> (Stabursvik and Heide, 1979). Finger millet biomass and grain yields also increased with increased level of fertilizer application up to 60 kg ha<sup>-1</sup> N and 60 kg ha<sup>-1</sup> phosphorus (P) in field experiments and NPK (nitrogen: phosphorus: potassium) of up to 160:80:80 kg ha<sup>-1</sup> in pot experiments (Reddy et al., 2004). However, taking soil test results (Table 4.1) showed that soil-N supply may have been adequate for finger millet production in the US experimental sites. According to Al-Kaisi and Yin (2003), N fertilization only increased yield in corn when N supply by the soil was low. However, for Alupe, total available N was too

low to adequately explain lack of response to N application. In this case, it could be argued that soil P, which is extremely low in Kenyan and eastern Africa soils, affected root development at the onset, resulting in inadequate absorption of available N. In this experiment, P was not applied at planting. According to Okalebo et al. (1993), total (Kjedahl) N, soil organic matter (SOM) and available P are extremely low in productivity of these soils in eastern Africa (Tenywa et al., 1999), therefore application of P is a prerequisite for all major cereal crops in Kenya, and hence the use of an NPK fertilizer such as diammonium phosphate (DAP) during planting. Infact, throughout the semi-arid tropics, application of P in finger millet has been demonstrated by several workers (Krishna et al., 1982; Tenywa et al., 1999; Reddy et al., 2003; Apoorva et al., 2010). As a result, growth and phosphorus nutrition on sterile, phosphorus deficient soils have been improved by inoculation with vesicular-arbuscular mycorrhizal fungus (Krishna et al., 1982).

Lack of response to N application has also been recorded in same cereal crops. In a study to evaluate the effects of N fertilization rates on dry matter remobilization among rice cultivars, it was found that the highest total dry matter remobilization was obtained at 0 kg N ha<sup>-1</sup> (Shokri et al., 2009). This lack of response to N fertilization may be attributed to growing conditions (previous crop, soil type, soil fertility status) and environmental conditions, which may have been the case in this study. In grain sorghum, a crop with production requirements are similar to finger millet, long term research has shown that nitrogen (N) fertilizer is usually needed to optimize production. In the United States, improvement in nitrogen fertilizer application, cultural practices, irrigation and tillage are assumed to contribute 60 to 65% of the yield gain in grain sorghum (Duvick and Cassman, 1999). However, this was not demonstrated with finger millet.

The influence of tillage method, crop rotation, and previous crop may also be a factor in soil N status and hence the lack of response to N fertilization in this study. Crop management systems that include rotations with high-residue producing crops and maintenance of surface residue cover with reduced tillage result in greater soil organic carbon and N, which may improve soil productivity (Havlin et al., 1989; Mahli and Lemke, 2007). Finger millet is known to benefit from residual fertility from the previous crop (Saravanane et al., 2011), hence this may explain the lack of response to N application in this study. However, while N fertilization may improve crop production, it also increases the potential for NO<sub>3</sub>-N leaching and N<sub>2</sub>O-N emissions especially when applied in excess of crop requirements (Malhi and Lemke, 2007).

Volatilization of urea could be another reason for lack of response to applied N. In the sites where the experiment was carried out (Manhattan and Hays in Kansas, and Alupe in Kenya), growing conditions could have lead to volatilization caused by moisture conditions at the time of fertilizer application in addition to high amount of surface residue due to zero tillage in Manhattan 2009 and 2010. These conditions were not controlled in this study and could have led to the lack of response of biomass and grain yield to N fertilizer application on finger millet. In studies elsewhere in the US, the absence of yield differences between N applications on small grains may be attributed to cooler temperatures during the growing season (Bendel et al., 1989). It may be useful in future to consider the N fertilizer source, timing and placement method to obtain a positive response of yield of finger millet.

# Conclusion

Increase in seeding rate did not translate into increase in grain and biomass yield; however, lower seeding rate (3.2 kg ha<sup>-1</sup>) recorded higher tillering ability and higher leaf chlorophyll content (based on SPAD readings) in finger millet leaves. Grain and biomass yield of finger millet were significantly influenced by the interaction between spatial and temporal factors, and seeding rates. While lower seeding rate (3.2 kg ha<sup>-1</sup>) increased number of tiller and leaf chlorophyll in Manhattan in 2009, higher seeding rate (6.0 kg ha<sup>-1</sup>) increased leaf dry weight and grain yield in Manhattan in 2010 and biomass yield in Manhattan in 2009. Overall, lodging increased with increase in nitrogen fertilizer application rates. Higher N application (90 kg ha<sup>-1</sup>) increased lodging in Manhattan in 2010 by 103% compared to no application. Lack of response of finger millet to nitrogen fertilizer application rates for grain and biomass yield could be attributed to prevailing environmental conditions including previous cropping, soil type and fertility status (soil nutrient availability), cultural practices and other site-specific effects such as soil moisture conditions and crop residue. Future field experiments should consider controlling crop management practices such as tillage and crop rotation, in addition to fertilizer source and placement to determine optimum seeding and nitrogen fertilizer application rates for finger millet.

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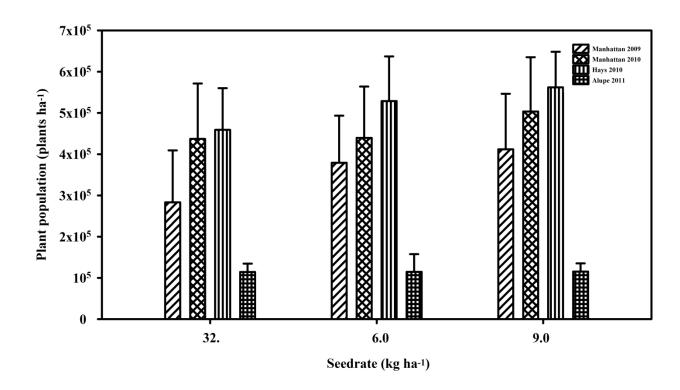


Figure 4.1 Effect of seeding rate on finger millet plant population in Manhattan 2009, 2010, Hays 2010 and Alupe 2011. Vertical bars denote  $\pm S.E.$  of means.

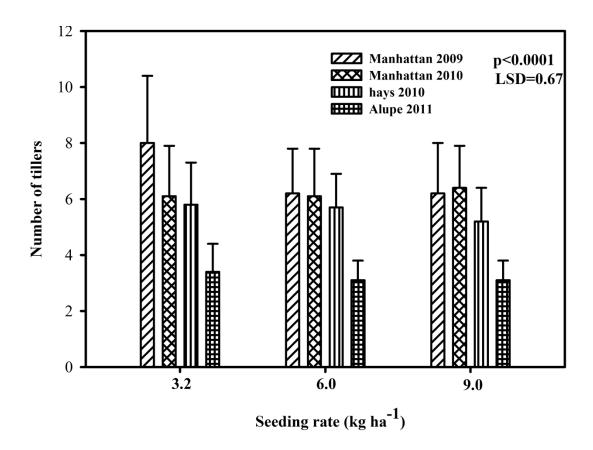


Figure 4.2 Effect of seeding rates on number of tillers of finger millet in Manhattan in 2009, 2010, Hays 2010 and Alupe 2011. Vertical bars denote  $\pm$ S.E. of means.

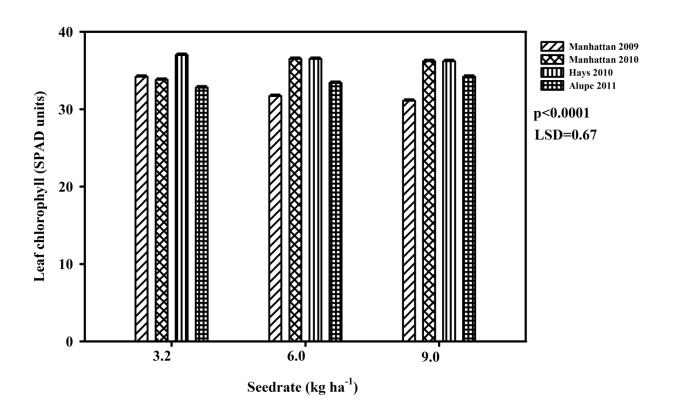


Figure 4.3 Effect of seeding rates on leaf chlorophyll (SPAD units) of finger millet in Manhattan in 2009, 2010, Hays 2010 and Alupe 2011. Vertical bars denote ±S.E. of means.

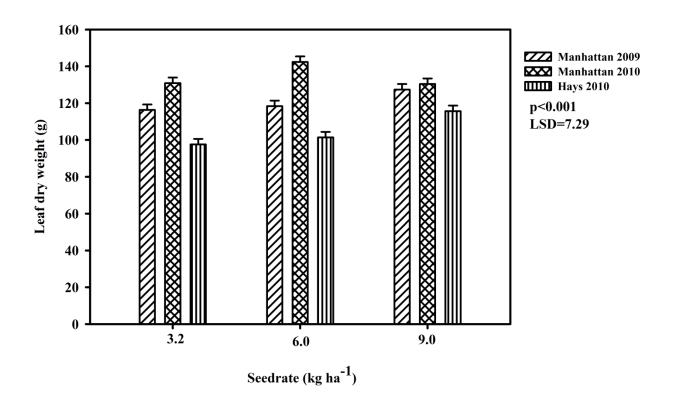


Figure 4.4 Effect of seeding rates on leaf dry weight of finger millet in Manhattan in 2009, 2010 and Hays 2010. Vertical bars denote  $\pm$ S.E. of means.

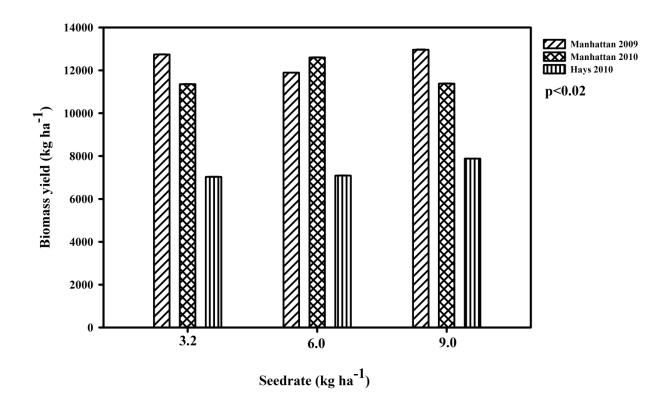


Figure 4.5 Effect of seeding rates on biomass yield of finger millet in Manhattan in 2009, 2010 and Hays 2010. Vertical bars denote  $\pm$ S.E. of means.

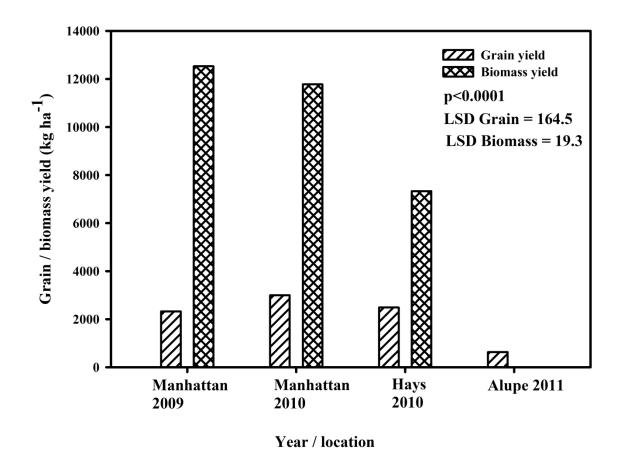


Figure 4.6 Effect of location and year on grain and biomass yield of finger millet in Manhattan in 2009, 2010, Hays 2010 and Alupe 2011.

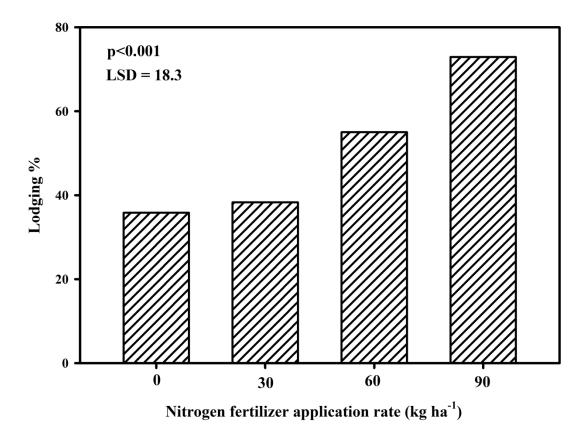


Figure 4.7 Effect of nitrogen fertilizer application rates on lodging (%) of finger millet in Manhattan 2010.

Table 4.1 Monthly precipitation (mm) and temperature (°C) Manhattan, KS, in 2009, 2010, Hays, KS, in 2010 and Alupe, Kenya, in 2011.

Month		Man	hattan		Hays		Alupe	
	Mean Precipitation (mm)	Mean Temp (°C)	Mean Precipitation (mm)	Mean Temp (°C)	Mean Precipitation (mm)	Mean Temp (°C)	Mean Precipitation (mm)	Mean Temp (°C)
	2009		2010		2010		2011	
May	0.4	18.3	3.0	17.0	1.5	16.1	9.1	22.1
June	7.1	23.9	5.6	25.5	3.5	25.1	3.3	23.4
July	4.1	23.3	3.4	27.3	1.3	26.8	6.6	23.2
August	4.4	23.1	2.6	27.2	2.7	26.3	9.3	23.6
September	1.6	18.6	2.5	21.3	1.8	21.0	12.5	23.7
October	1.9	9.6	0.9	15.7	0.2	14.7	5.4	23.2
November	-	-	-	-	-	-	12.9	21.4

Table 4.2 Initial soil test results for Manhattan, KS, and Hays, KS, in 2009, 2010 and Alupe, Kenya, in 2011.

Site	Year	Previous crop	Tillage	pН	OM	Profile NO <sub>3</sub> -N	P	K
		-			%		mg kg <sup>-1</sup> soil	
Manhattan	2009	Soybean	No till	7.2	NA‡	5.3	33.4	374
Manhattan	2010	Soybean	No till	6.9	NA	3.7	48.5	483
Hays	2010	Fallow	Conventional	6.9	1.9	8.7	34.9	593
Alupe	2011	Fallow	Conventional	5.1	2.2	0.21†	13.4	150

†Total N ‡Not available

Table 4.3 P-value and significance of effects of year/location (Y), stage of trait measurement (S), seeding rate (S), fertilizer application rate (F), year/location x seeding rate (F), year/location x fertilizer application rate (F), year/location x seeding rate x fertilizer application rate (F), year/location x s

Effects	Year/location (Y)	Seed (S)	Fertilizer (F)	YxS	YxF	YxSxF
Traits			<i>P</i> -value	s		
Plant population (plants ha <sup>-1</sup> )	<0.0001***	<0.0001 <sup>NS</sup>	0.6192 <sup>NS</sup>	0.0045**	0.9672 <sup>NS</sup>	0.9097 <sup>NS</sup>
Plant height (cm)	<0.0001***	0.0014	< 0.0001	$0.8449^{NS}$	$0.5482^{NS}$	$0.5363^{NS}$
Number of tillers	<0.0001***	<0.0001***	$0.6655^{NS}$	<0.0001***	0.0017**	$0.3165^{NS}$
Number of leaves	$0.0630^{NS}$	$0.0414^{*}$	$0.1912^{NS}$	0.1154 <sup>NS</sup>	$0.1263^{NS}$	$0.8358^{NS}$
Leaf dry weight (g)	<0.0001***	0.0083**	$0.0288^{*}$	0.0070**	$0.9690^{NS}$	$0.2108^{NS}$
Leaf chlorophyll (SPAD units)	<0.0001***	$0.0167^{*}$	$0.0350^{*}$	<0.0001***	$0.4410^{NS}$	$0.7052^{NS}$
Stem dry weight (g)	<0.0001***	$0.0742^{NS}$	$0.0058^{**}$	$0.0742^{NS}$	$0.3392^{NS}$	$0.3067^{NS}$
Panicle dry weight (g)	<0.0001***	0.0015**	$0.1240^{NS}$	<0.0001***	$0.5490^{NS}$	$0.7781^{NS}$
Finger number	<0.0001***	0.9124 <sup>NS</sup>	$0.1649^{NS}$	<0.0001***	$0.5490^{NS}$	$0.7781^{NS}$
Grain yield (kg ha <sup>-1</sup> )	<0.0001***	$0.7786^{NS}$	$0.7873^{NS}$	0.6313 <sup>NS</sup>	$0.5447^{NS}$	$0.8839^{NS}$
Biomass yield (kg ha <sup>-1</sup> )	<0.0001***	0.4067 <sup>NS</sup>	0.0166*	0.0023**	$0.7637^{NS}$	$0.2107^{NS}$

NS, nonsignificant, \*, \*\*, \*\*\*, significant at P<0.05, <0.01, and <0.001 respectively

Table 4.4 Effect of year/location (Y) on growth and yield traits of finger millet.

Year/location (Y)	Plant population (plants ha <sup>-1</sup> )	Plant height (cm)	Tiller number	Leaf dry weight (g)	Stem dry weight (g)	Panicle dry weight (g)	Leaf chlorophyll (SPAD units)	Number of fingers	Grain yield (kg ha <sup>-1</sup> )	Biomass yield (kg ha <sup>-1</sup> )
Manhattan 2009	326,594.0°	89.4 <sup>b</sup>	6.8ª	120.7 <sup>b</sup>	189.8°	209.1ª	32.3 <sup>b</sup>	6.6 <sup>b</sup>	2320.9°	12530.9ª
Manhattan 2010	459,982.6 <sup>b</sup>	103.6 <sup>a</sup>	5.7 <sup>b</sup>	134.6 <sup>a</sup>	166.4 <sup>b</sup>	187.6 <sup>b</sup>	33.7 <sup>b</sup>	7.2 <sup>a</sup>	2998.4ª	11776.7 <sup>b</sup>
Hays 2010	516,493.1 <sup>a</sup>	67.3°	5.8 <sup>b</sup>	104.9°	90.9 <sup>c</sup>	70.1°	36.5 <sup>a</sup>	6.1°	2486.2 <sup>b</sup>	7330.0°
Alupe 2011	114,679.8 <sup>d</sup>	60.8 <sup>d</sup>	3.2°	NA	NA	NA	33.5 <sup>b</sup>	7.3 <sup>a</sup>	631.1 <sup>d</sup>	NA
Mean	419425.1	84.76	5.9	120.1	149.0	155.6	34.0	6.7	2109.1	10545.9
CV%	30.0	8.7	24.4	25.3	27.9	26.2	8.8	9.24	14.7	25.5

Means with the same letter are not significantly different at p<0.05  $\dagger \dagger NA$  = not available

Table 4.5 Effect of seeding and nitrogen fertilizer application rates on growth traits of finger millet in Manhattan, Hays and Alupe in 2009, 2010 and 2011.

Treatment		Plant popul	ation (no)			Plant he	ight (cm)			Tiller nun	nber (no)	
Year/Loc‡	1	2	3	4	1	2	3	4	1	2	3	4
					Seeding	rate (kg ha	ı <sup>-1</sup> )					
3.2	283,072 <sup>b</sup>	436,979 <sup>b</sup>	458,854 <sup>b</sup>	114,352	91.1	101.9	66.0	61.4	8.0ª	6.1	5.8ª	3.4
6.0	379,166 <sup>a</sup>	439,583 <sup>a</sup>	528,646 <sup>a</sup>	114,641	89.5	104.2	67.5	60.7	6.2 <sup>b</sup>	6.1	5.7 <sup>a</sup>	31
9.0	411,718 <sup>a</sup>	503,385 <sup>a</sup>	561,979 <sup>a</sup>	115,046	87.5	104.8	68.3	60.5	6.2 <sup>b</sup>	6.4	5.2 <sup>b</sup>	3.1
$LSD_{0.05}$	44,280	45,726	35,116	NS	NS	NS	NS	NS	0.67	NS	0.45	NS
0	365,972	440,278	515,972	111,497	87.7	101.6	64.4	59.3	7.0	5.9	5.6	3.1
30	348,958	459,028	513,194	120,679	86.4	103.2	66.8	61.7	6.7	5.7	5.6	3.4
60	352,431	468,056	506,250	114,506	91.5	103.8	68.3	62.0	6.4	7.0	5.5	3.2
90	364,583	472,569	530,556	112,037	91.7	105.9	68.6	60.5	7.1	6.0	5.4	3.1
LSD <sub>0.05</sub>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
					And	ova P>F						
Seed (S)	***	**	***	$0.998^{NS}$	$0.602^{NS}$	$0.486^{NS}$	$0.223^{NS}$	0.871 <sup>NS</sup>	***	0.594 <sup>NS</sup>	*	$0.325^{NS}$
Fert (F)	$0.884^{\rm NS}$	$0.635^{NS}$	$0.686^{NS}$	$0.896^{\rm NS}$	$0.463^{NS}$	$0.509^{NS}$	$0.130^{NS}$	$0.523^{NS}$	$0.256^{NS}$	$0.001^{\rm NS}$	$0.829^{\rm NS}$	$0.710^{\rm NS}$
Seed x Fert	$0.759^{NS}$	$0.587^{NS}$	$0.944^{NS}$	$0.992^{NS}$	$0.909^{NS}$	$0.952^{NS}$	$0.962^{NS}$	$0.673^{NS}$	$0.032^{NS}$	$0.494^{NS}$	$0.422^{NS}$	$0.475^{NS}$

<sup>‡</sup>Year/Loc: 1 = Manhattan 2009, 2 = Manhattan 2010, 3 = Hays 2010, 4 = Alupe 2011

Table 4.6 Effect of seeding and nitrogen fertilizer application rates on yield traits of finger millet in Manhattan, Hays and Alupe in 2009, 2010 and 2011.

Within columns, means followed by the same letter are not significantly different at p $\leq$ 0.05 NS, nonsignificant, \*, \*\*, \*\*\*, significant at P<0.05, <0.01, and <0.001 respectively

Treatment		Finger nu	ımber (no)		B	Biomass yie	ld (kg ha <sup>-1</sup> )	)		Grain yie	ld (kg ha <sup>-1</sup> )	
Year/Loc‡	1	2	3	4	1	2	3	4	1	2	3	4
					Seeding	rate (kg ha	a <sup>-1</sup> )					
3.2	6.9 <sup>b</sup>	7.1	6.3	6.9	382.7	340.6	210.8	NA††	2203.7	3018.0	2506.2	639.9
6.0	$6.4a^b$	7.2	6.1	7.6	356.7	378.0	212.6	NA	2355.2	3017.4	2430.9	617.8
9.0	6.7 <sup>b</sup>	6.9	6.1	7.5	386.9	341.4	236.3	NA	2403.8	2959.8	2521.5	635.4
$LSD_{0.05}$	0.32	NS	NS	0.6	NS	NS	NS	NA	NS	NS	NS	NS
0	6.5	7.0	6.1	7.0	358.9	348.5	216.3	NA	2393.8	2983.5	2514.3	625.4
30	6.5	7.0	6.2	7.7	362.9	337.0	218.4	NA	2377.8	3120.2	2352.4	641.7
60	6.9	7.2	6.2	7.3	378.7	358.5	217.1	NA	2286.6	2995.0	2569.6	648.5
90	6.7	7.1	6.2	7.3	403.1	369.1	227.9	NA	2225.3	2895.0	2508.5	608.6
$LSD_{0.05}$	NS	NS	NS	NS	NS	NS	NS	NA	NS	NS	NS	NS
					An	ova P>F						
Seed (S)	**	$0.150^{NS}$	$0.127^{NS}$	$0.054^{NS}$	0.694 <sup>NS</sup>	$0.327^{NS}$	$0.066^{NS}$	NA	0.223 <sup>NS</sup>	$0.724^{NS}$	0.787 <sup>NS</sup>	0.934 <sup>NS</sup>
Fert (F)	$0.093^{NS}$	$0.736^{NS}$	$0.972^{NS}$	$0.307^{NS}$	$0.767^{NS}$	$0.385^{NS}$	$0.083^{NS}$	NA	$0.572^{NS}$	$0.151^{NS}$	$0.575^{NS}$	$0.949^{NS}$
SxF	$0.960^{NS}$	$0.374^{NS}$			$0.988^{NS}$	$0.404^{NS}$	$0.986^{NS}$	NA	$0.934^{NS}$	$0.413^{NS}$	$0.588^{NS}$	0.761 <sup>NS</sup>

<sup>‡</sup>Year/Loc: 1 = Manhattan 2009, 2 = Manhattan 2010, 3 = Hays 2010, 4 = Alupe 2011; ††NA = not available

Within columns, means followed by the same letter are not significantly different at p< 0.05 NS, nonsignificant, \*, \*\*\*, \*\*\*\*, significant at P<0.05, <0.01, and <0.001 respectively

Table 4.7 Effect of seeding and nitrogen fertilizer application rates on leaf chlorophyll (SPAD) and lodging (%) of finger millet in Manhattan, Hays and Alupe in 2009, 2010 and 2011.

Treatment	SPAD	Lodging (%)

Year/Loc‡	1	2	3	4	1	2	3	4							
		Seed	ing rate (kg ha	-1)											
3.2	34.2 <sup>a</sup> †	33.8	37.0	32.8	NA††	45.0	NA	NA							
6.0	31.7 <sup>b</sup>	33.6	36.5	33.4	NA	56.6	NA	NA							
9.0	31.1 <sup>b</sup>	33.6	36.2	34.2	NA	50.0	NA	NA							
$\mathrm{LSD}_{0.05}$	1.25	NS	NS	NS	NA	NS	NA	NA							
	N Fertilizer application rate (kg ha <sup>-1</sup> )														
0	31.5 <sup>b</sup>	33.8	36.1	33.4	NA	35.8°	NA	NA							
30	31.5 <sup>b</sup>	33.8	36.5	33.3	NA	38.3 <sup>bc</sup>	NA	NA							
60	$33.0^{a}$	33.6	37.0	33.6	NA	55.0 <sup>ab</sup>	NA	NA							
90	33.3ª	33.4	36.7	33.6	NA	$72.9^{a}$	NA	NA							
$\mathrm{LSD}_{0.~05}$	1.45	NS	NS	NS	NA	18.3	NA	NA							
			Anova P>F												
Seed (S)	***	$0.956^{NS}$	$0.119^{NS}$	$0.119^{NS}$	NA	$0.342^{NS}$	NA	NA							
Fert (F)	*	$0.918^{\mathrm{NS}}$	$0.977^{\mathrm{NS}}$	$0.977^{\rm NS}$	NA	$0.001^{NS}$	NA	NA							
SxF	$0.803^{NS}$	$0.803^{NS}$	$0.471^{NS}$	$0.471^{NS}$	NA	$0.569^{NS}$	NA	NA							

<sup>‡</sup>Year/Loc: 1 = Manhattan 2009, 2 = Manhattan 2010, 3 = Hays 2010, 4 = Alupe 2011; ††NA = not available Within columns, means followed by the same letter are not significantly different at p< 0.05 NS, nonsignificant, \*, \*\*\*, \*\*\*\*, significant at P<0.05, <0.01, and <0.001 respectively

# Chapter 5 - Evaluation of finger millet [*Eleusine coracana* (L.) Gaertn.] mini core collection for morphological and yield traits

## **Abstract**

Plant genetic resources are the raw materials for development of improved cultivars; however, germplasm collections need to be sampled to achieve a manageable size for meaningful evaluation. In crops with large germplasm collections, minicore collections (10% of core or 1% of entire collection) have been developed. A finger millet minicore collection of 80 accessions has been developed at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) in India. This study was conducted at the ICRISAT research farm evaluate the mini core collection for morphological and yield traits that can contribute to identification of traits for potential use in improving stress tolerance since it has been determined that high yields correlate strongly with the ability to tolerate high temperature stress. Eighty accessions plus 4 high yielding accessions (controls) were grown under field conditions and data on qualitative and quantitative traits were recorded. Results showed that there was high heritability (>60%) among the quantitative traits except for basal tillers which had a 56.5% heritability. The number of days to flowering is a highly heritable trait, and it recorded 98.8% heritability. Panicle length, panicle width, plant height, grain yield, and biomass yield recorded 91.8%, 91.7%, 90.6%, 83.5% and 86.1% heritability respectively. Results also showed high variability among accessions. The range for grain yield was 146 to 3022 kg ha<sup>-1</sup> and biomass yield was 801 to 17564 kg ha<sup>-1</sup>. Three accessions (IE4709, IE 501 and IE 4734) were identified for early flowering. Three accessions (IE 3745, IE 2034 and IE 4057) with consistent high performance were identified for high grain yield and stover yield and IE 3104 for harvest index. Accessions ranked highly for grain yield may be recommended for adoption in finger millet growing areas after undergoing multilocational testing to verify their agronomic and utilization performance.

## Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is an important subsistence cereal in parts of Africa and South Asia. It ranks fourth in importance among millets in the world after sorghum (Sorghum bicolor L. Moench), pearl millet (Pennisetum glaucum), and foxtail millet (Setaria italic) (Upadhyaya et al., 2007a). The species E. coracana consists of two subspecies, africana and coracana. The subspecies africana has two wild races, africana and spontanea, while subspecies coracana has no wild races but four cultivated races: elongata, plana, compacta, and vulgraris. Race elongata is further subdivided into subraces laxa, recluse, and sparsa; race plana into seriata, confundere, and grandigluma; race vulgaris into liliacea, stellata, incurvata, and digitata. Race compacta has no subraces (Prasada Rao and de Wet, 1997). These races and subraces can be differentiated from one another by inflorescence morphology (Prasada Rao et al., 1993). Cultivated finger millet (Eleusine coracana subsp. coracana) has a narrow genetic base, most probably owing to a bottleneck associated with its domestication (Dida et al., 2008). However morphological variation is large. There is a considerable range in flowering time, plant height, number of basal tillers, peduncle length, inflorescence length, and other morphological traits (Dida et al., 2008).

Finger millet is widely cultivated in Africa and South Asia under varied agro-climatic conditions (Dida et al., 2008). In Africa, it is extensively cultivated in Uganda, Kenya, Tanzania, Ethiopia, Rwanda, Burundi, Zambia, and Malawi (Mnyenyembe and Gupta, 1998; Obilana et al., 2002). In South Asia, finger millet is widely cultivated in India and Nepal (Upadhyaya et al., 2007b). It is estimated that finger millet contributes 10 per cent of the total area (34.6 million ha) planted to millets (FAO, 2004). Finger millet is grown mainly by subsistence farmers and serves as a food security crop because of its high-nutritional value and excellent storage qualities.

Under irrigated conditions in field trials, yields of up to 5 to 6 metric tons ha<sup>-1</sup> have been obtained (National Research Council, 1996). Among the millets, finger millet has wider adaptability (Upadhyaya et al, 2007b), higher nutritional quality (National Reseach Council, 1996, Malleshi et al., 1996), higher multiplication rate, and longer shelf life under ambient conditions (Sperling et al., 2004). These qualities make finger millet an ideal crop for use as a staple food and for famine reserve. However, these desirable attributes of finger millet are threatened by the effects of climate change. Even though traditional finger millet varieties are adapted to current environmental conditions, it is predicted that they will be less suitable to the new climates; hence, the challenge will be to accelerate their evolution to adapt to climate change (Jarvies et al., 2011).

The impact of climate change and its effects in parts of the world such as Africa has become a reality with droughts, high temperatures, and floods, out-of-season rain and dry spells affecting the welfare of millions of people. Therefore, the ability of the farming community to become resilient, acclimatize and adapt, will improve their ability to counteract the effects of climate change (Luganda, 2007; Padma, 2010). Climate change is predicted to bring about increased temperatures across the world in the range of 1.6°C to as much as 6°C by 2050. According to IPCC (2007) and other studies, temperature increases of 1 to 2°C will result in an increase in production of some of the world's major staples with increasingly negative impacts (Jarvies et al., 2011). Crop production processes such as seed germination, photosynthesis, membrane stability, nutrient absorption, protoplasmic movement, hormone activity, fertilization and pod set, pod development, seed set and seed quality will be adversely affected by high temperatures (Wahid et al., 2007). Tolerance to high temperature can be achieved by developing early maturing cultivars whose maturity periods match with the available soil moisture and duration of optimum weather conditions available for a crop (Upadhyaya et al., 2011).

Phenological traits such as appropriate early flowering and maturity are major components of crop adaptation, particularly in environments where the growing season is restricted by terminal drought and high temperature (Subbarao et al., 1995). Consequently, climate change experts have called for a paradigm shift in agricultural research to focus on making plants more resilient to changing climate rather than on increasing yields. They emphasize that focus should now be re-oriented towards adaptations such as changing varieties and planting times, which will on average enable avoidance of a 10 to 15% reduction in yield corresponding to 1 to 2°C local temperature increase (Jarvies et al., 2011). In addition, drought proofing crops by developing heat-resistant varieties will be one of the elements of this adaptation strategy where crops such as finger millet adapt to a warming world. This would directly benefit smallholder and subsistence farmers who are expected to suffer complex, localized impacts of climate change as a result of reduction of the length of the growing season (Kurukulasuriya et al., 2006; Benhin, 2008).

With the disappearance of several landraces from their natural habitats, a coherent and efficient system of germplasm development and exchange is needed to address the needs of small scale farmers (Bonham et al., 2010). The need for adapted germplasm requires characterization, evaluation, and the availability of crop materials available in gene banks. Comprehensive assessments of suitable available genetic resources are needed to find new sources of variation to cope with these stresses so as to adapt our agricultural systems to changing environments (Ainsworth et al., 2008); Upadhyaya et al., 2008b; Bonham et al., 2010). This can be achieved by developing efficient and effective screening methods of existing germplasm as well as replacing currently adapted landraces and varieties with new materials, which have the ability to withstand abiotic stress (Jarvies et al., 2011). In future, the key to successful crop improvement will be the ability to identify and access genetic diversity including new or improved variability for target traits by selecting parental germplasm proven to be

resilient under likely climate change, including extreme events such as high temperatures (Newton et al, 2011).

Plant genetic resources are the raw materials for the development of improved cultivars; however, germplasm collections need to be sampled to get the size of the collections to a manageable level for meaningful evaluation (Upadhyaya, et al, 2009). In crops with large germplasm collections, minicore collections (10% of core or 1% of the entire collection) have been suggested. Minicore collections are composed of a smaller number of well characterized accessions which are given priority for use in the improvement of any crop (Upadhyaya and Ortiz, 2001, Upadhyaya et al., 2007). A core subset of finger millet germplasm (622 accessions) based on origin and data on 14 quantitative traits was developed form the entire global collection of 5940 accessions held in the genebank at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India (Upadhyaya et al., 2006c). Subsequently, a finger millet minicore collection of 80 accessions was developed at ICRISAT (Upadhyaya et al., 2010b) (Table 4.1). The reduced size of minicore collections has provided opportunities to breeders due to their efficient and economic multi-environment evaluation, which has led to the identification of several new sources of variation for different traits for utilization in crop improvement programs.

Molecular characterization of the minicore will further enhance its use in plant breeding programs. According to Upadhyaya et al. (2008b) breeders and other crop improvement specialists can evaluate the minicore collection easily and economically for traits of economic importance to identify trait specific germplasm for use. Already, 25 finger millet accessions have been identified for high yield, large seed size and early maturity. The reduction of the number of entries to be evaluated would thus provide a pool of diversity that can be extensively evaluated for economically important traits and fulfill the need to identify new sources of variation for use

in crop improvement programs to counteract the negative effects of climate change which is a threat to the future production of finger millet.

In other crops, when minicore collections were evaluated, researchers were able to identify new sources of variation, for example, drought tolerance in chickpea (Cicer arietinum L.) and groundnut (Arachis hypogaea L.), salinity tolerance in chickpea, groundnut and pigeon pea (Cajanus cajan (L.) Millsp), and low temperature tolerance (at germination) in groundnut. It is expected that the use of minicore approach will lead to greater utilization of diverse germplasm for developing broad-based cultivars, especially in the context of climate change (Upadhyaya et al., 2010a) with special emphasis for accessions exhibiting desirable characteristics for adaptation to high temperature stress. Consequently, successful crop improvement would be supported by the mining of variation associated with heat tolerance in genebank materials (Upadhyaya and Ortiz, 2001). This study was therefore conducted under field conditions in at ICRISAT, Patancheru, India to identify finger millet minicore collection for morphological and yield traits that can contribute to identification of traits for potential use in improving stress tolerance. The hypothesis was that finger millet minicore germplasm available at the ICRISAT genebank contain potentially high yielding accessions which can contribute to high temperature stress tolerance

# **Materials and methods**

# Plant husbandry and growth conditions

Eighty five accessions of finger millet minicore including four controls were sown at ICRISAT research farm, Patancheru, India, on July 20, 2011. The farm is located at 17.3° N and 78.5° E, at an altitude of 545 m, and about 600 km from the sea. Average annual rainfall is about 750 mm most of which occur during the months of June to September. The finger millet lines with desirable attributes such as high yield [VR 708, IE 2043 (PR 202), IE 3618 (RAU 8) and IE 4673 (VL 149)] were used as control. The minicore accessions and controls were sown on red soils (alfisols) on ridges 60 cm apart. There was one row per entry of 4 m length. There were 17 entries per block. Row to row spacing was 60 cm and plant to plant spacing was 10 cm. Experimental design was alpha design with 80 entries and 4 checks replicated 3 times. Diammonium phosphate (DAP) (18-46-0) was applied as a basal fertilizer at the rate of 100 kg ha<sup>-1</sup> and topdressing was done using urea (46-0-0) at a rate of 100 kg ha<sup>-1</sup>. Insecticide was applied on July 19, 2011 and August 11, 2011 to control major pests. Irrigation was done seven times between July and November, 2011 to maintain the water in the soil at field capacity. Hand weeding was done when necessary.

#### **Data collection**

Data were recorded on 14 quantitative and 7 qualitative traits following the "Descriptors of Finger Millet" (IBPGR, 1985). Data for the 14 quantitative traits were basal tiller numbers, culm branching numbers, days to 50% flowering, plant height (mm), flag leaf blade length (mm),

flag leaf blade width (mm), flag leaf sheath length (mm), peduncle length (mm), panicle exertion (mm), longest finger length (mm), longest finger width (mm), number of fingers ear<sup>-1</sup>, inflorescence length and width (mm). The data were recorded on main culms of the five representative plants of the plots. Data on plant height, basal tillers, flag leaf blade length and width, flag leaf sheath length, peduncle length, panicle exertion, inflorescence length and width, length and width of longest finger, fingers per ear, and grain yield per panicle were recorded on five representative plants. Panicle exertion was measured as the length of exposed peduncle from the flag leaf to the base of the panicle. Panicle length and width were measured at maturity as the maximum length from the base to the tip of the panicle and maximum width in the natural position. For quantitative traits, the averages of five plants per plot were used for statistical analysis.

Data on the 8 qualitative traits (growth habit, plant pigmentation, inflorescence compactness and shape, grain color, lodging, overall plant aspect and disease resistance) and one quantitative trait (days to 50% flowering) were recorded on plot basis. Data on plant pigmentation and growth habit were recorded after days to 50% flowering. The number of days to flowering was recorded as the number of days from sowing to the date when 50% of plants in a plot had started flowering. Grain characteristics were recorded at postharvest stage in the laboratory. Grain yield of five plants was added to the plot yield to determine total plot yield in kilograms per hectare. The random model of residual restricted maximum likelihood (REML) (Patterson and Thompson, 1971) was used to analyze data of 17 quantitative traits in GenStat 14.1 (http://www.vsni.co.uk) (Payne et al., 2007). BLUPS (best linear unbiased predictors) were calculated for all the agronomic traits. Variance components due to genotype ( $\alpha^2$ g) and their standard error were calculated and their significance level determined. Broad sense heritability ( $\alpha^2$ ) was estimated using the following model:

Heritability (%) =  $\sigma^2 g/\sigma^2 p*100$ 

 $\sigma^2 p$  was estimated as follows:  $\sigma^2 p = \sigma^2 g + (\sigma^2 e/r)$  where;  $\sigma^2 g =$  genotypic variance,  $\sigma^2 p =$  phenotypic variance,  $\sigma^2 e =$  residual or error, r = no. of replications.

#### **Results**

Table 5.1 shows the distribution of minicore accessions into forty clusters and passport (basic) information of 80 accessions included in the finger millet minicore collection. Some accessions belong to the same cluster, but originate from different countries; however, most of them are from the same country or region. Majority of them are from Africa and Asia (India and Nepal), with 2 accessions from Europe (Germany) and USA (one each). Table 5.2 shows the trait means for 17 quantitative traits of 80 finger millet accessions evaluated and 4 controls. Variance due to genotype and error for the quantitative traits measured are shown in Table 5.3. Although all the traits have relative significance, three major traits of interest for evaluation for high temperature stress were adopted namely, days to flowering (DOF), grain yield (GY), and harvest index (HI). Table 5.4 shows 10 early and late maturing accessions compared to controls. The accessions which recorded early flowering dates were IE 4709 (43 d), IE 501 (49 d), IE 4734 (53 d) and IE 4671 (59 d), compared to 62 d, the average of controls. Accession IE 6537 recorded very late flowering at 105 d after sowing. Table 5.5 shows the 10 most high yielding and low yielding varieties and their harvest indices compared to control. The accessions recording the highest grain yield were accession number IE 3475 (3022 kg ha<sup>-1</sup>), IE 2034 (2937 kg ha<sup>-1</sup>), IE 4057 (2677 kg ha<sup>-1</sup>) and IE 6326 (2549 kg ha<sup>-1</sup>) compared to 2212 kg ha<sup>-1</sup>, the average of controls. Accession number IE 6082 recorded the lowest yield (553 kg ha<sup>-1</sup>). Accession IE 4671 recorded the highest HI of 0.22 while the control IE 4673(VL149) recorded a significantly high HI of 0.32.

Results indicated that all quantitative traits had high heritability (>60%) (Table 5.2) except for basal tillers which had a heritability of 56.5%. The highest heritability was recorded for days to flowering (98.8%), panicle length (91.8%), panicle width (91.7%), and plant height (90.6%). Grain yield had 83.5% heritability while stover yield recorded 86.1% heritability. Basal

tillers had the lowest heritability (56.5%). Results also showed that the genotypic variance for all quantitative traits were significant at p=0.01 (Table 5.3), indicating high variability among the accessions. This is in agreement with those of Dida et al., (2008) that morphological variation of finger millet is large. The substantial variability for the quantitative traits is evident from the estimates of range for a selected few traits: basal tillers (number) (2.7 to 5.5); days to flowering (43 to 105 days); plant height (99 to 150 cm); panicle length (62 to 119 mm); panicle width (60 to 192 mm); panicle weight (3.4 to 11 g), grain yield (146 to 3022 kg ha<sup>-1</sup>), and stover yield (801 17564 kg ha<sup>-1</sup>). A comparison between means of selected agronomic traits and controls revealed that they were comparable to the control cultivars (Table 5.6). The best performing accessions were accession IE 3475 (highest grain yield), accession IE 2034 (highest stover yield), accession IE 4709 (early flowering), and accession IE 4709 (highest number of tillers). This study identified three accessions for consistent high performance, namely, accession IE 4671 for early flowering (59 days) and harvest index (0.22), accession IE 3475 for high grain yield (3022 kg ha<sup>-1</sup>) and harvest index (0.17), and accession IE 2790 for high grain yield (2414 kg ha-1) and harvest index (0.19) (Tables 5.4 and 5.5).

Correlations between quantitative traits on the basis of trait means (Table 5.7) were strongly and positively correlated with each other. Panicle exertion and peduncle length (r=0.88, p<0.0001); panicle weight and plant height (r=0.80, p<0.0001), and flag leaf blade length (r<0.78, p<0.0002). Other correlations between traits measured were moderate to low (r<0.70). Grain yield was positively and strongly correlated to stover yield, but had a positive but weak correlation with panicle weight (Fig. 5.1). Plant height had a strong and positive correlation with lodging (Fig. 5.2).

# **Discussion**

Results showed a high variability in the finger millet minicore collection which would be utilized for screening for stress tolerance. The traits evaluated could potentially shed light into the ability of finger millet to tolerate high temperature stress. However, yield would be a good indicator since potential yield of a genotype can be assessed as its output under ideal crop management and stress-free conditions (Upadhyaya et al., 2011), as observed in this study. In wheat (*Triticum aestivum* L.) both grain weight and grain number were found to be sensitive to heat stress, as the number of grains per ear at maturity declined with increasing temperature (Ferris et al., 1998). It has also been established that brief exposure of plants to high temperatures during seed filling can accelerate senescence, diminish seed set and seed weight, and reduce yield (Siddique et al., 1999); therefore grain yield can be adopted as a trait to evaluate for high temperature stress.

In a study to identify and evaluate chickpea germplasm for tolerance to heat stress, supplemental irrigation was used to estimate the potential yield of the chickpea accessions, so as to make a critical comparison of performance under ideal stress-free and adverse high stress environments. According to Wahid et al. (2007), excess radiation and high temperatures are often the most limiting factors affecting plant growth and final crop yield. Although stress conditions were not imposed in the present study, some accessions outperformed others even under conditions of adequate moisture and moderate temperature. According to Wahid et al. (2007) plants with higher growth potential perform better regardless of the growing conditions. The accessions, whose average performance was below those of control under stress free conditions would be expected to record a poor performance under stressful conditions; therefore would be unsuitable for growing under such conditions. Harvest index would also be a potential trait to be considered for selection for tolerance to high temperature stress. Al-Khatib and Paulsen (1999) suggested that high harvest indices would be among the potential selection

criteria for tolerance to high temperature stress for wheat genotypes. The finger millet accessions recorded relatively low harvest indices; however, those recording high harvest indices such as IE 4671, IE 2790, IE 6154, IE 3475, IE 4057 and IE 5091 would be considered adapted for potential high temperature stress tolerance.

Four accessions displayed a trait for early flowering (<60 days to flowering). This trait has been considered an escape mechanism from damage due to high temperature stress. According to Tewolde (2006), early flowering is advantageous since it results in smaller reductions in yield. These accessions may be evaluated further to determine their ability to escape under stressful field conditions.

Heritability is a reflection of the range of variability for a quantitative trait among a group of genotypes. High heritability (>60%) recorded in this study is an indication of variability between accession as was determined earlier on by Hilu and de Wet (1976) and confirmed by Dida et al., 2008. This attribute of finger millet minicore accessions can be used as a primary set of criteria for selection in stressful environments. According to Holland et al. (2003) and Hallauer (2007), heritability estimates indicate relative importance of genetic variation to the total variation in a population and hence they depend on the absolute size of genetic (type of population) and environmental (experimental conditions) variations. This suggests that the most of the finger millet minicore accessions are potential candidates for screening for high temperature stress; however, further testing under controlled high temperature stress conditions would yield more accurate results.

Consequently, the traits evaluated indicated high adaptive value as shown by the high heritability values except for basal tillering, which had moderate heritability (56%). In studying the sources and extent of quantitative variation under different environmental conditions, Ortiz et al. (1998) recommended measuring many traits of potential functional significance, each group

reflecting a different emphasis on plant processes of interest especially vegetative and reproductive growth under varying moisture and high temperature stress. They also recommend evaluating traits of potential adaptive value with easily interpreted attributes. In this study, vegetative and reproductive traits were evaluated and the results obtained may be adopted for identifying new sources of variation and utilizing them in breeding programs to enhance the genetic potential of finger millet under high temperature stress.

# **Conclusions**

The finger millet minicore collection is highly variable as shown by the significant genotypic variance for all quantitative traits, most of which have a high heritability (>60%). This study is the first step in evaluating a finger millet minicore germplasm for grain yield potential for high temperature stress. Three accessions (IE4709, IE 501 and IE 4734) were identified for early flowering, three accessions (IE 3745, IE 2034 and IE 4057) for high grain yield and stover yield and three (IE 4671, 2790 and 6154) for high harvest index. Overall, accessions IE 4671, IE 2790, IE 6154, IE 3475, IE 4057 and IE 5091 were consistent for both high yield and harvest index. Workers such as Ainsworth et al. (2008) have argued that the need for adapted germplasm is urgent and requires characterization, evaluation, and availability of materials now available in genebanks, since it may take decades to identify germplasm for future growing conditions. This study is one step in the direction of identifying adapted germplasm for future growing conditions. The finger millet accessions will be further evaluated under controlled high temperature stress conditions to determine traits of high functional performance. Those traits would be tagged as an ideal pool for identifying new sources of variation for finger millet germplasm with ability to tolerate high temperature stress and may be used by breeders for finger millet improvement. Accessions ranked highly as having the ability to tolerate high temperature

stress may be recommended for adoption in regions affected by extreme climate conditions after undergoing multi-locational testing to verify their agronomic and utilization performance.

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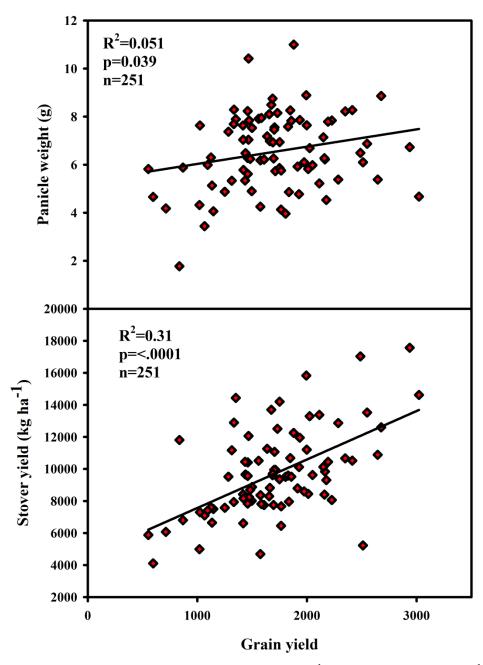


Figure 5.1 Correlation between grain yield (kg ha<sup>-1</sup>), stover yield (kg ha<sup>-1</sup>), and panicle weight (g) of 85 finger millet mini core accessions.

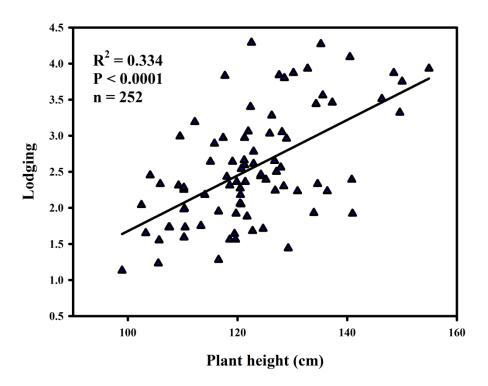


Figure 5.2 Correlation between plant height (cm), and lodging of 85 finger millet mini core accessions

Table 5.1 Passport information of 80 finger millet accessions distributed into 40 clusters.

Cluste	IE no.	Country	Race	Subrace	Cluster	IE no.	Country	Race	Subrace
r					no				
<u>no.</u> 1	18	India	Vulgaris	Incurvata	21	4816	India	Elongata	Reclusa
1	2217	India	Vulgaris	Stellata	22	2572	Kenya	Plana	Grandi- gluma
1	2821	Nepal	Compacta	-	22	2619	Malawi	Vulgaris	Incurvata
2	3104	India	Vulgaris	Incurvata	23	6240	Zimbabwe	Vulgaris	Incurvata
3	501	India	Vulgaris	Stellata	23	3945	Uganda	Plana	Confundere
4	5537	Nepal	Vulgaris	Stellata	24	4622	Zimbabwe	Compacta	-
5	5817	Nepal	Vulgaris	Incurvata	25	2430	Kenya	Vulgaris	Digitata
6	6621	Nepal	Vulgaris	Stellata	25	3614	Unknown	Plana	Confundere
6	2042	India	Vulgaris	Incurvata	25	4073	Uganda	Elongata	Reclusa
7	4491	Zimbabwe	Elongata	Reclusa	25	4795	Zimbabwe	Vulgaris	Digitata
7	2790	Malawi	Elongata	Laxa	26	3721	Uganda	Compacta	Laxa
8	1055	Unknown	Vulgaris	Digitata	26	4057	Uganda	Plana	Seriata
9	4734	India	Vulgaris	Digitata	26	4570	Zimbabwe	Plana	Confundere
9	5201	India	Vulgaris	Digitata	27	5066	Senegal	Vulgaris	Incurvata
10	3392	Zimbabwe	Compacta	-	27	7018	Kenya	Vulgaris	Incurvata
11	5106	Zimbabwe	Vulgaris	Incurvata	28	3475	India	Vulgaris	Incurvata
11	6514	Zimbabwe	Vulgaris	Incurvata	28	4757	India	Vulgaris	Stellata
12	3470	India	Vulgaris	Stellata	28	6165	Nepal	Vulgaris	Incurvata
12	4329	Zimbabwe	Vulgaris	Incurvata	28	6350	Zimbabwe	Vulgaris	Incurvata
12	6294	Zimbabwe	Vulgaris	Incurvata	29	2589	U.S.	Plana	Seriata
13	2710	Malawi	Plana	Confundere	30	4545	Zimbabwe	Compacta	-
13	2872	Zambia	Vulgaris	Digitata	31	4497	Zimbabwe	Vulgaris	Digitata
13	3391	Zimbabwe	Vulgaris	Digitata	32	2457	Kenya	Compacta	-
14	5367	Kenya	Vulgaris	Liliacea	33	2871	Zambia	Compacta	-
14	5870	Nepal	Vulgaris	Digitata	33	6473	Uganda	Plana	Confundere
14	6082	Nepal	Plana	Confundere	34	3973	Uganda	Vulgaris	Stellata
14	7508	Ethiopia	Vulgaris	Incurvata	34	6154	Nepal	Vulgaris	Incurvata
15	2312	India	Vulgaris	Digitata	35	7320	Kenya	Vulgaris	Digitata
16	2957	Germany	Vulgaris	Liliacea	36	4121	Uganda	Plana	Confundere
17	2911	Zambia	Vulgaris	Incurvata	36	5306	Zimbabwe	Vulgaris	Digitata
18	3045	India	Vulgaris	Liliacea	36	6421	Uganda	Vulgaris	Digitata
19	2034	India	Vulgaris	Incurvata	37	4646	Zimbabwe	Plana	Grandi- gluma

19	6337	Zimbabwe	Vulgaris	Incurvata	38	3952	Uganda	Plana	Confundere
20	3077	India	Vulgaris	Incurvata	38	4028	Uganda	Vulgaris	Incurvata
21	2296	India	Vulgaris	Digitata	38	6059	Nepal	Vulgaris	Digitata
21	2606	Malawi	Vulgaris	Incurvata	39	6533	Nigeria	Elongata	Sparsa
21	4671	India	Vulgaris	Digitata	40	7079	Kenya	Vulgaris	Liliacea

Table 5.2 Trait means of 80 finger millet minicore accessions and 4 controls for 5 qualitative and 17 quantitative traits evaluated during the 2011 rainy season at ICRISAT, Patancheru, India.

IE No.	Gro	Pig	Cu	Infl	Seed	Bas	Days	Pla	Fla	Flag	Fla	Pedu	Fin	Fing	Pani	Panicl	Pani	Panicl	Grain	Stover	Over	Lod
	wth	m	lm	sha	color	al	to	nt	g	leaf	g	ncle	ger	er	cle	e	cle	e	yield	yield (kg	all	gin
	habit	ent	br	pe		tille	flowe	ht	leaf	blad	leaf	lengt	wid	num	exert	length	widt	weigh	(kg ha	ha <sup>-1</sup> )	plant	g
		atio	an			rs	r	(cm	bla	e	she	h	th	ber	ion	(mm)	h	t (g)	1)		aspe	
		n	chi					)	de	widt	ath	(mm)	(m		(mm		(mm				ct	
			ng						leng	h	len		m)		)		)					
									th	(m	gth											
									(m	m)	(m											
									m)		m)											
501	Е	G	Н	TC	RB	3.5	49.1	103	352	10.4	109	210.0	9.3	10.5	106	78.2	64.4	4.1	1145.4	7497.8	2.9	1.7
518	E	G	Н	IC	RB	3.7	63.5	121	35	10.4	116	273.2	10.7	7.1	130	88.0	65.6	4.9	1496.2	8010.0	2.7	2.2
1055	E	G	M	TC	RB	3.1	65.5	146	349	10.4	113	253.1	9.8	6.0	114	97.8	74.3	6.5	1438.9	9652.4	2.7	3.5
2034	E	G	Н	TC	RB	3.5	84.9	123	330	10.7	109	198.5	7.4	7.6	110	84.9	63.9	6.7	2936.5	17564.4	1.5	1.7
2042	E	G	Н	TC	W	3.5	60.2	119	317	11.3	95	241.6	9.8	8.2	134	93.5	72.3	5.8	2011.2	8441.2	3.3	2.6
2217	E	G	M	IC	RB	3.1	65.5	114	354	11.8	111	241.6	9.8	8.0	127	92.9	67.8	4.9	1835.6	7963.6	2.0	2.2
2296	E	G	M	TC	LB	3.4	69.3	110	352	10.7	101	227.2	10.5	7.0	116	86.2	64.6	6.2	1575.9	8361.7	2.4	1.7
2312	E	G	Н	LO	RGB	3.5	74.8	125	336	11.1	97	244.5	9.0	6.9	154	110.7	97.5	5.2	2112.5	13381.1	1.7	1.7
2430	E	G	M	IC	LB	3.2	72.8	141	438	12.4	99	278.9	10.2	6.9	161	71.5	60.9	5.7	1710.9	9946.4	3.2	2.4
2437	E	G	M	TC	RGB	3.5	72.8	137	375	12.0	109	256.0	9.5	6.1	154	88.0	71.2	5.9	1748.4	9361.2	3.0	3.5
2457	E	G	Н	TC	RB	3.1	75.2	141	333	11.3	99	233.0	8.8	6.0	147	98.4	69.1	8.5	1674.4	13699.8	3.1	1.9
2572	E	G	M	TC	LB	3.0	103.6	110	341	11.5	89	152.5	12.1	6.8	85	137.9	61.4	10.4	1466.6	12057.6	2.0	2.0
2589	E	G	Н	TC	DB	3.4	81.9	136	365	12.4	97	241.6	8.3	7.1	130	92.3	76.1	7.9	1558.6	10509.2	3.2	3.6
2606	E	G	Н	TC	LB	3.7	84.0	127	338	11.1	87	201.4	6.9	7.0	133	81.9	63.8	6.2	2164.2	9836.2	2.5	2.2
2619	E	G	Н	IC	LB	3.3	76.0	106	344	12.0	99	218.6	7.4	8.2	97	97.2	69.0	7.0	1466.5	7993.1	2.9	1.6
2710	E	G	Н	IC	LB	3.7	88.5	117	336	12.9	104	187.0	8.8	9.1	89	98.4	70.2	8.2	1730.5	12509.5	2.2	1.3
2790	E	G	Н	LO	RB	3.1	76.7	135	352	10.4	97	189.9	10.2	6.5	117	154.2	121.9	8.3	2414.0	10519.7	3.0	4.3
2821	E	G	Н	IC	RB	2.9	67.0	121	312	10.4	138	267.4	8.8	8.0	143	78.8	56.5	4.2	712.7	6071.5	3.9	3.0

Part																							
Part	2871	E	G	Н	IC	LB	3.1	88.9	121	346	12.0	94	178.4	7.4	9.0	91	80.7	64.3	8.9	1993.3	15835.7	2.7	2.1
Part	2872	E	G	Н	IC	W	3.1	84.5	121	317	12.2	100	189.9	7.1	7.0	94	79.4	62.7	7.6	1703.9	11067.8	2.5	2.4
3045         E         G         H         LO         RB         3.3         71.7         131         349         11.5         111         258.8         9.5         6.2         133         116.8         90.2         6.5         2487           3077         E         G         M         IC         LB         3.5         66.2         118         323         10.9         111         241.6         9.8         7.7         126         86.2         64.3         5.4         2285           3104         E         G         H         IC         LB         3.1         54.3         109         294         10.4         109         247.3         9.8         10.1         104         80.7         66.5         4.3         1574           3317         E         G         H         IC         LB         3.7         76.9         117         346         10.4         99         230.1         11.4         6.9         124         66.0         62.2         59.9         1914         66.0         62.2         59.9         1914         66.0         62.2         4.0         1804           3392         E         G         H         IC	2911	E	G	M	TC	LB	3.3	90.2	120	325	12.4	92	181.3	8.8	6.5	95	98.4	70.7	7.9	1350.3	14436.2	2.7	1.9
3077         E         G         M         IC         LB         3.5         66.2         118         323         10.9         111         241.6         9.8         7.7         126         86.2         64.3         5.4         2288           3104         E         G         H         IC         RGB         3.1         54.3         109         294         10.4         109         247.3         9.8         10.1         104         80.7         66.5         4.3         1574           3317         E         G         H         IC         LB         3.4         75.1         110         344         12.6         109         21.29         8.1         7.9         129         83.1         65.4         8.2         1458           3392         E         G         H         IC         LB         3.5         65.3         121         352         104         118         241.6         10.0         69         118         66.0         62.2         5.9         191         434         12.2         89         12.2         89         12.6         6.7         154         80.0         62.2         5.9         191         49         12.2	2957	E	G	Н	TC	DB	3.1	84.1	123	370	12.2	92	161.1	8.8	7.6	77	105.2	76.9	8.3	1847.6	10680.2	2.8	4.3
3104   E	3045	E	G	Н	LO	RB	3.3	71.7	131	349	11.5	111	258.8	9.5	6.2	133	116.8	90.2	6.5	2487.3	17028.8	1.8	2.2
3317         E         G         H         IC         LB         3.4         75.1         110         344         12.6         109         212.9         8.1         7.9         129         8.31         65.4         8.2         1458           3391         E         G         H         TC         LB         3.7         76.9         117         346         10.4         92         189.9         8.1         6.5         135         72.7         61.2         7.2         1636           3392         E         G         H         IC         LB         3.5         65.3         121         352         10.4         118         241.6         10.0         6.9         118         94.7         65.2         4.0         1804           3475         E         G         H         TC         LB         3.5         72.9         122         359         10.7         106         270.3         7.6         6.7         154         80.0         67.2         4.7         3022           3614         E         G         H         TC         LB         3.2         69.7         150         404 <t>12.0         97         250.2         <th< td=""><td>3077</td><td>E</td><td>G</td><td>M</td><td>IC</td><td>LB</td><td>3.5</td><td>66.2</td><td>118</td><td>323</td><td>10.9</td><td>111</td><td>241.6</td><td>9.8</td><td>7.7</td><td>126</td><td>86.2</td><td>64.3</td><td>5.4</td><td>2285.4</td><td>12864.3</td><td>2.5</td><td>2.4</td></th<></t>	3077	E	G	M	IC	LB	3.5	66.2	118	323	10.9	111	241.6	9.8	7.7	126	86.2	64.3	5.4	2285.4	12864.3	2.5	2.4
3391 E G H TC LB 3.7 76.9 117 346 10.4 92 189.9 8.1 6.5 135 72.7 61.2 7.2 1636 3392 E G H C LB 3.1 71.3 123 294 10.4 99 230.1 11.4 6.9 124 66.0 62.2 5.9 1914 3470 E G H TC LB 3.5 65.3 121 352 10.4 118 241.6 10.0 6.9 118 94.7 65.2 4.0 1804 3475 E G H TC LB 3.5 72.9 122 359 10.7 106 270.3 7.6 6.7 154 80.0 67.2 4.7 3022 3614 E G H TC LB 3.2 83.6 128 349 10.7 102 218.6 7.4 6.8 147 61.7 50.5 5.3 1313 3945 E G H TC LB 3.5 77.9 129 338 12.2 99 273.2 7.9 7.4 177 77.0 64.8 5.6 1459 3952 E G H TC RB 3.0 80.4 136 346 12.0 87 192.7 8.1 6.0 138 88.0 64.1 6.9 1750 3973 E G H TC LB 3.1 72.5 134 386 12.6 102 261.7 10.0 7.0 173 77.0 62.7 7.5 1498 4057 E G H TC LB 2.7 78.0 134 444 12.0 109 241.6 10.5 7.4 138 94.7 67.8 6.2 1608 4057 E G H TC LB 3.1 72.3 133 444 12.0 109 241.6 10.5 7.4 138 94.7 67.8 6.2 1608 4057 E G H TC LB 3.7 78.0 134 127 320 12.2 92 175.5 9.5 6.6 116 97.2 78.2 110.3 193.9 68.5 1.1 133 134 141 1 101 247.3 11.7 6.8 130 10.7 19.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 139.5 14.1 14.1 139.5 14.1 139.5 14.1 139.5 14.1 14.1 139.5 14.1 139.5 14.1 14.1 139.5 14.1 139.5 14.1 14.1 139.5 14.1 139.5 14.1 139.5 14.1 14.1 139.5 14.1 139.5 14.1 139.5 14.1 14.1 139.5 14.1 139.5 14.1 139.5 14.1 14.1 139.5 14.1 139.5 14.1 14.1 139.5 14.1 139	3104	E	G	Н	IC	RGB	3.1	54.3	109	294	10.4	109	247.3	9.8	10.1	104	80.7	66.5	4.3	1574.3	4684.8	2.7	2.3
3392         E         G         H         C         LB         3.1         71.3         123         294         10.4         99         230.1         11.4         6.9         124         66.0         62.2         5.9         1944           3470         E         G         H         TC         LB         3.5         65.3         121         352         10.4         118         241.6         10.0         6.9         118         94.7         65.2         4.0         1804           3475         E         G         H         TC         LB         3.5         72.9         122         359         10.7         106         270.3         7.6         6.7         154         80.0         67.2         4.7         302           3614         E         G         H         TC         LB         3.2         83.6         128         349         10.7         102         218.6         7.4         6.8         147         61.7         50.5         5.3         1313           3945         E         G         H         TC         LB         3.5         7.79         129         338         12.2         99         273.2 <th< td=""><td>3317</td><td>E</td><td>G</td><td>Н</td><td>IC</td><td>LB</td><td>3.4</td><td>75.1</td><td>110</td><td>344</td><td>12.6</td><td>109</td><td>212.9</td><td>8.1</td><td>7.9</td><td>129</td><td>83.1</td><td>65.4</td><td>8.2</td><td>1458.8</td><td>7833.1</td><td>2.7</td><td>2.3</td></th<>	3317	E	G	Н	IC	LB	3.4	75.1	110	344	12.6	109	212.9	8.1	7.9	129	83.1	65.4	8.2	1458.8	7833.1	2.7	2.3
3470         E         G         H         IC         LB         3.5         65.3         121         352         10.4         118         241.6         10.0         6.9         118         94.7         65.2         4.0         1804           3475         E         G         H         TC         LB         3.5         72.9         122         359         10.7         106         270.3         7.6         6.7         154         80.0         67.2         4.7         3022           3614         E         G         H         TC         LB         3.2         69.7         150         404         12.0         97         250.2         9.5         6.1         153         103.9         68.5         6.3         1458           3721         E         P         H         IC         LB         3.2         83.6         128         349         10.7         102         218.6         7.4         6.8         147         61.7         50.5         5.3         1313           3952         E         G         H         TC         RB         3.1         72.5         134         386         12.6         102.7         7.4	3391	E	G	Н	TC	LB	3.7	76.9	117	346	10.4	92	189.9	8.1	6.5	135	72.7	61.2	7.2	1636.3	11263.4	2.7	2.0
3475         E         G         H         TC         LB         3.5         72.9         122         359         10.7         106         270.3         7.6         6.7         154         80.0         67.2         4.7         3022           3614         E         G         H         TC         LB         3.2         69.7         150         404         12.0         97         250.2         9.5         6.1         153         103.9         68.5         6.3         1458           3721         E         P         H         IC         LB         3.2         83.6         128         349         10.7         102         218.6         7.4         6.8         147         61.7         50.5         5.3         1313           3945         E         G         H         TC         LB         3.5         77.9         129         338         12.2         99         273.2         7.9         7.4         177         77.0         64.8         5.6         1459           3952         E         G         H         TC         LB         3.0         72.5         134         366         12.0         87         192.7 <th< td=""><td>3392</td><td>E</td><td>G</td><td>Н</td><td>C</td><td>LB</td><td>3.1</td><td>71.3</td><td>123</td><td>294</td><td>10.4</td><td>99</td><td>230.1</td><td>11.4</td><td>6.9</td><td>124</td><td>66.0</td><td>62.2</td><td>5.9</td><td>1914.2</td><td>8788.7</td><td>2.9</td><td>2.8</td></th<>	3392	E	G	Н	C	LB	3.1	71.3	123	294	10.4	99	230.1	11.4	6.9	124	66.0	62.2	5.9	1914.2	8788.7	2.9	2.8
3614         E         G         H         TC         LB         3.2         69.7         150         404         12.0         97         250.2         9.5         6.1         153         103.9         68.5         6.3         1488           3721         E         P         H         IC         LB         3.2         83.6         128         349         10.7         102         218.6         7.4         6.8         147         61.7         50.5         5.3         1313           3945         E         G         H         TC         LB         3.5         77.9         129         338         12.2         99         273.2         7.9         7.4         177         77.0         64.8         5.6         1459           3973         E         G         H         TC         DB         3.1         72.5         134         386         12.6         102         261.7         10.0         7.0         173         77.0         62.7         7.5         1498           4028         E         G         H         TC         LB         3.1         72.3         133         444 <t>12.0         109         241.6         <t< td=""><td>3470</td><td>E</td><td>G</td><td>Н</td><td>IC</td><td>LB</td><td>3.5</td><td>65.3</td><td>121</td><td>352</td><td>10.4</td><td>118</td><td>241.6</td><td>10.0</td><td>6.9</td><td>118</td><td>94.7</td><td>65.2</td><td>4.0</td><td>1804.8</td><td>9508.3</td><td>2.0</td><td>2.6</td></t<></t>	3470	E	G	Н	IC	LB	3.5	65.3	121	352	10.4	118	241.6	10.0	6.9	118	94.7	65.2	4.0	1804.8	9508.3	2.0	2.6
3721         E         P         H         IC         LB         3.2         83.6         128         349         10.7         102         218.6         7.4         6.8         147         61.7         50.5         5.3         1313           3945         E         G         H         TC         LB         3.5         77.9         129         338         12.2         99         273.2         7.9         7.4         177         77.0         64.8         5.6         1459           3952         E         G         H         TC         RB         3.0         80.4         136         346         12.0         87         192.7         8.1         6.0         138         88.0         64.1         6.9         1750           3973         E         G         H         TC         DB         3.1         72.5         134         386         12.6         102         261.7         10.0         7.0         173         77.0         62.7         7.5         1498           4028         E         G         H         TC         LB         3.1         72.3         133         444         12.0         109         241.6 <t< td=""><td>3475</td><td>E</td><td>G</td><td>Н</td><td>TC</td><td>LB</td><td>3.5</td><td>72.9</td><td>122</td><td>359</td><td>10.7</td><td>106</td><td>270.3</td><td>7.6</td><td>6.7</td><td>154</td><td>80.0</td><td>67.2</td><td>4.7</td><td>3022.1</td><td>14619.9</td><td>1.5</td><td>1.9</td></t<>	3475	E	G	Н	TC	LB	3.5	72.9	122	359	10.7	106	270.3	7.6	6.7	154	80.0	67.2	4.7	3022.1	14619.9	1.5	1.9
3945 E G H TC LB 3.5 77.9 129 338 12.2 99 273.2 7.9 7.4 177 77.0 64.8 5.6 1459 3952 E G H TC RB 3.0 80.4 136 346 12.0 87 192.7 8.1 6.0 138 88.0 64.1 6.9 1750 3973 E G H TC DB 3.1 72.5 134 386 12.6 102 261.7 10.0 7.0 173 77.0 62.7 7.5 1498 4028 E G H TC RB 3.1 72.3 133 444 12.0 109 241.6 10.5 7.4 138 94.7 67.8 6.2 1608 4057 E G H TC LB 4.2 89.0 120 386 10.9 104 201.4 9.5 8.4 89 110.7 81.1 8.9 2677 4073 E G H TC LB 3.1 71.3 127 320 12.2 92 175.5 9.5 6.6 116 97.2 78.2 7.4 1283 4329 E G M TC LB 3.1 71.3 127 320 12.2 92 175.5 9.5 6.6 116 97.2 78.2 7.4 1283 4491 E G H TC LB 3.3 68.2 155 354 12.2 116 278.9 10.0 7.1 144 139.5 96.7 6.0 2050 4497 E G H TC LB 3.1 74.0 124 383 11.3 109 238.7 8.8 9.3 113 93.5 76.5 7.6 1997 4565 E G H TC LB 3.1 70.9 126 283 10.4 99 230.1 9.3 5.6 124 103.3 74.0 8.8 1685 4570 E G H TC LB 3.3 76.9 120 323 10.9 94 238.7 7.4 7.0 117 67.2 60.7 7.1 2149 4646 E G H TC LB 3.2 76.2 110 402 12.4 97 184.1 9.3 7.7 90 119.2 76.3 11.0 1879	3614	E	G	H	TC	LB	3.2	69.7	150	404	12.0	97	250.2	9.5	6.1	153	103.9	68.5	6.3	1458.7	9565.6	3.0	3.3
3952 E G H TC RB 3.0 80.4 136 346 12.0 87 192.7 8.1 6.0 138 88.0 64.1 6.9 1750 3973 E G H TC DB 3.1 72.5 134 386 12.6 102 261.7 10.0 7.0 173 77.0 62.7 7.5 1498 4028 E G H TC RB 3.1 72.3 133 444 12.0 109 241.6 10.5 7.4 138 94.7 67.8 6.2 1608 4057 E G H TC LB 4.2 89.0 120 386 10.9 104 201.4 9.5 8.4 89 110.7 81.1 8.9 2677 4073 E G H TC LB 2.7 78.0 134 470 13.7 109 235.8 8.8 6.7 123 111.3 79.5 5.1 1133 4121 E G M TC LB 3.1 71.3 127 320 12.2 92 175.5 9.5 6.6 116 97.2 78.2 7.4 1283 4329 E G M TC LB 3.7 71.6 129 328 11.1 101 247.3 11.7 6.8 130 84.9 72.3 7.6 1827 4491 E G H TC RB 3.3 68.2 155 354 12.2 116 278.9 10.0 7.1 144 139.5 96.7 6.0 2050 4497 E G H TC LB 3.1 74.0 124 383 11.3 109 238.7 8.8 9.3 113 93.5 76.5 7.6 1997 4565 E G H TC LB 3.1 70.9 126 283 10.4 99 230.1 9.3 5.6 124 103.3 74.0 8.8 1685 4570 E G H TC LB 3.3 76.9 120 323 10.9 94 238.7 7.4 7.0 117 67.2 60.7 7.1 2149 4646 E G H TC LB 3.3 76.9 120 323 10.9 94 238.7 7.4 7.0 117 67.2 60.7 7.1 2149 4646 E G H TC LB 3.2 76.2 110 402 12.4 97 184.1 9.3 7.7 90 119.2 76.3 11.0 1879	3721	E	P	Н	IC	LB	3.2	83.6	128	349	10.7	102	218.6	7.4	6.8	147	61.7	50.5	5.3	1313.6	11163.5	3.4	2.3
3973 E G H TC DB 3.1 72.5 134 386 12.6 102 261.7 10.0 7.0 173 77.0 62.7 7.5 1498 4028 E G H TC RB 3.1 72.3 133 444 12.0 109 241.6 10.5 7.4 138 94.7 67.8 6.2 1608 4057 E G H TC LB 4.2 89.0 120 386 10.9 104 201.4 9.5 8.4 89 110.7 81.1 8.9 2677 4073 E G H TC LB 2.7 78.0 134 470 13.7 109 235.8 8.8 6.7 123 111.3 79.5 5.1 1133 4121 E G M TC LB 3.1 71.3 127 320 12.2 92 175.5 9.5 6.6 116 97.2 78.2 7.4 1283 4329 E G M TC LB 3.7 71.6 129 328 11.1 101 247.3 11.7 6.8 130 84.9 72.3 7.6 1827 4491 E G H TC LB 3.3 68.2 155 354 12.2 116 278.9 10.0 7.1 144 139.5 96.7 6.0 2050 4497 E G H TC LB 3.1 74.0 124 383 11.3 109 238.7 8.8 9.3 113 93.5 76.5 7.6 1997 4565 E G H TC LB 3.1 70.9 126 283 10.4 99 230.1 9.3 5.6 124 94.7 65.6 6.1 1974 4622 E G H TC LB 3.3 76.9 120 323 10.9 94 238.7 7.4 7.0 117 67.2 60.7 7.1 2149 4646 E G H TC LB 3.2 76.2 110 402 12.4 97 184.1 9.3 7.7 90 119.2 76.3 11.0 1879	3945	E	G	Н	TC	LB	3.5	77.9	129	338	12.2	99	273.2	7.9	7.4	177	77.0	64.8	5.6	1459.9	10408.7	2.3	1.4
4028         E         G         H         TC         RB         3.1         72.3         133         444         12.0         109         241.6         10.5         7.4         138         94.7         67.8         6.2         1608           4057         E         G         H         TC         LB         4.2         89.0         120         386         10.9         104         201.4         9.5         8.4         89         110.7         81.1         8.9         2677           4073         E         G         H         TC         LB         2.7         78.0         134         470         13.7         109         235.8         8.8         6.7         123         111.3         79.5         5.1         1133           4121         E         G         M         TC         LB         3.1         71.3         127         320         12.2         92         175.5         9.5         6.6         116         97.2         78.2         7.4         1283           4329         E         G         M         TC         LB         3.7         71.6         129         328         11.1         101         247.3	3952	E	G	Н	TC	RB	3.0	80.4	136	346	12.0	87	192.7	8.1	6.0	138	88.0	64.1	6.9	1750.2	14201.9	2.2	2.2
4057         E         G         H         TC         LB         4.2         89.0         120         386         10.9         104         201.4         9.5         8.4         89         110.7         81.1         8.9         2677           4073         E         G         H         TC         LB         2.7         78.0         134         470         13.7         109         235.8         8.8         6.7         123         111.3         79.5         5.1         1133           4121         E         G         M         TC         LB         3.1         71.3         127         320         12.2         92         175.5         9.5         6.6         116         97.2         78.2         7.4         1283           4329         E         G         M         TC         LB         3.7         71.6         129         328         11.1         101         247.3         11.7         6.8         130         84.9         72.3         7.6         1827           4491         E         G         H         TC         LB         3.2         77.2         110         317         11.8         104         221.5	3973	E	G	Н	TC	DB	3.1	72.5	134	386	12.6	102	261.7	10.0	7.0	173	77.0	62.7	7.5	1498.3	8874.0	2.5	1.9
4073       E       G       H       TC       LB       2.7       78.0       134       470       13.7       109       235.8       8.8       6.7       123       111.3       79.5       5.1       1133         4121       E       G       M       TC       LB       3.1       71.3       127       320       12.2       92       175.5       9.5       6.6       116       97.2       78.2       7.4       1283         4329       E       G       M       TC       LB       3.7       71.6       129       328       11.1       101       247.3       11.7       6.8       130       84.9       72.3       7.6       1827         4491       E       G       H       TC       RB       3.3       68.2       155       354       12.2       116       278.9       10.0       7.1       144       139.5       96.7       6.0       2050         4497       E       G       H       TC       LB       3.2       77.2       110       317       11.8       104       221.5       8.1       6.3       117       96.0       76.5       7.6       1997         4565       <	4028	E	G	Н	TC	RB	3.1	72.3	133	444	12.0	109	241.6	10.5	7.4	138	94.7	67.8	6.2	1608.7	7747.4	3.6	3.9
4121       E       G       M       TC       LB       3.1       71.3       127       320       12.2       92       175.5       9.5       6.6       116       97.2       78.2       7.4       1283         4329       E       G       M       TC       LB       3.7       71.6       129       328       11.1       101       247.3       11.7       6.8       130       84.9       72.3       7.6       1827         4491       E       G       H       TC       RB       3.3       68.2       155       354       12.2       116       278.9       10.0       7.1       144       139.5       96.7       6.0       2050         4497       E       G       H       TC       LB       3.2       77.2       110       317       11.8       104       221.5       8.1       6.3       117       96.0       76.3       7.8       1471         4545       E       G       H       TC       LB       3.1       74.0       124       383       11.3       109       238.7       8.8       9.3       113       93.5       76.5       7.6       1997         4565 <t< td=""><td>4057</td><td>E</td><td>G</td><td>Н</td><td>TC</td><td>LB</td><td>4.2</td><td>89.0</td><td>120</td><td>386</td><td>10.9</td><td>104</td><td>201.4</td><td>9.5</td><td>8.4</td><td>89</td><td>110.7</td><td>81.1</td><td>8.9</td><td>2677.1</td><td>12599.1</td><td>2.4</td><td>1.6</td></t<>	4057	E	G	Н	TC	LB	4.2	89.0	120	386	10.9	104	201.4	9.5	8.4	89	110.7	81.1	8.9	2677.1	12599.1	2.4	1.6
4329       E       G       M       TC       LB       3.7       71.6       129       328       11.1       101       247.3       11.7       6.8       130       84.9       72.3       7.6       1827         4491       E       G       H       TC       RB       3.3       68.2       155       354       12.2       116       278.9       10.0       7.1       144       139.5       96.7       6.0       2050         4497       E       G       H       TC       LB       3.2       77.2       110       317       11.8       104       221.5       8.1       6.3       117       96.0       76.3       7.8       1471         4545       E       G       H       TC       LB       3.1       74.0       124       383       11.3       109       238.7       8.8       9.3       113       93.5       76.5       7.6       1997         4565       E       G       H       TC       LB       3.1       70.9       126       283       10.4       99       230.1       9.3       5.6       124       103.3       74.0       8.8       1685         4570       <	4073	E	G	Н	TC	LB	2.7	78.0	134	470	13.7	109	235.8	8.8	6.7	123	111.3	79.5	5.1	1133.3	6648.2	3.8	3.4
4491       E       G       H       TC       RB       3.3       68.2       155       354       12.2       116       278.9       10.0       7.1       144       139.5       96.7       6.0       2050         4497       E       G       H       TC       LB       3.2       77.2       110       317       11.8       104       221.5       8.1       6.3       117       96.0       76.3       7.8       1471         4545       E       G       H       TC       LB       3.1       74.0       124       383       11.3       109       238.7       8.8       9.3       113       93.5       76.5       7.6       1997         4565       E       G       H       TC       LB       3.1       70.9       126       283       10.4       99       230.1       9.3       5.6       124       103.3       74.0       8.8       1685         4570       E       G       H       TC       LB       2.9       70.5       123       325       11.3       107       247.3       10.2       6.5       124       94.7       65.6       6.1       1974         4622       <	4121	E	G	M	TC	LB	3.1	71.3	127	320	12.2	92	175.5	9.5	6.6	116	97.2	78.2	7.4	1283.1	9516.7	2.6	2.7
4497       E       G       H       TC       LB       3.2       77.2       110       317       11.8       104       221.5       8.1       6.3       117       96.0       76.3       7.8       1471         4545       E       G       H       TC       LB       3.1       74.0       124       383       11.3       109       238.7       8.8       9.3       113       93.5       76.5       7.6       1997         4565       E       G       H       TC       LB       3.1       70.9       126       283       10.4       99       230.1       9.3       5.6       124       103.3       74.0       8.8       1685         4570       E       G       H       TC       LB       2.9       70.5       123       325       11.3       107       247.3       10.2       6.5       124       94.7       65.6       6.1       1974         4622       E       G       H       TC       LB       3.2       76.2       110       402       12.4       97       184.1       9.3       7.7       90       119.2       76.3       11.0       1879	4329	E	G	M	TC	LB	3.7	71.6	129	328	11.1	101	247.3	11.7	6.8	130	84.9	72.3	7.6	1827.1	9586.1	2.2	3.0
4545 E G H TC LB 3.1 74.0 124 383 11.3 109 238.7 8.8 9.3 113 93.5 76.5 7.6 1997 4565 E G H TC LB 3.1 70.9 126 283 10.4 99 230.1 9.3 5.6 124 103.3 74.0 8.8 1685 4570 E G H TC LB 2.9 70.5 123 325 11.3 107 247.3 10.2 6.5 124 94.7 65.6 6.1 1974 4622 E G H TC LB 3.3 76.9 120 323 10.9 94 238.7 7.4 7.0 117 67.2 60.7 7.1 2149 4646 E G H TC LB 3.2 76.2 110 402 12.4 97 184.1 9.3 7.7 90 119.2 76.3 11.0 1879	4491	E	G	Н	TC	RB	3.3	68.2	155	354	12.2	116	278.9	10.0	7.1	144	139.5	96.7	6.0	2050.8	9624.8	2.9	3.9
4565 E G H TC LB 3.1 70.9 126 283 10.4 99 230.1 9.3 5.6 124 103.3 74.0 8.8 1685 4570 E G H TC LB 2.9 70.5 123 325 11.3 107 247.3 10.2 6.5 124 94.7 65.6 6.1 1974 4622 E G H IC LB 3.3 76.9 120 323 10.9 94 238.7 7.4 7.0 117 67.2 60.7 7.1 2149 4646 E G H TC LB 3.2 76.2 110 402 12.4 97 184.1 9.3 7.7 90 119.2 76.3 11.0 1879	4497	E	G	Н	TC	LB	3.2	77.2	110	317	11.8	104	221.5	8.1	6.3	117	96.0	76.3	7.8	1471.8	8239.4	3.7	3.0
4570 E G H TC LB 2.9 70.5 123 325 11.3 107 247.3 10.2 6.5 124 94.7 65.6 6.1 1974 4622 E G H IC LB 3.3 76.9 120 323 10.9 94 238.7 7.4 7.0 117 67.2 60.7 7.1 2149 4646 E G H TC LB 3.2 76.2 110 402 12.4 97 184.1 9.3 7.7 90 119.2 76.3 11.0 1879	4545	E	G	Н	TC	LB	3.1	74.0	124	383	11.3	109	238.7	8.8	9.3	113	93.5	76.5	7.6	1997.9	11210.0	2.3	2.4
4622 E G H IC LB 3.3 76.9 120 323 10.9 94 238.7 7.4 7.0 117 67.2 60.7 7.1 2149 4646 E G H TC LB 3.2 76.2 110 402 12.4 97 184.1 9.3 7.7 90 119.2 76.3 11.0 1879	4565	E	G	Н	TC	LB	3.1	70.9	126	283	10.4	99	230.1	9.3	5.6	124	103.3	74.0	8.8	1685.7	9610.4	3.4	3.3
4646 E G H TC LB 3.2 76.2 110 402 12.4 97 184.1 9.3 7.7 90 119.2 76.3 11.0 1879	4570	E	G	Н	TC	LB	2.9	70.5	123	325	11.3	107	247.3	10.2	6.5	124	94.7	65.6	6.1	1974.4	8594.4	3.0	2.6
	4622	E	G	Н	IC	LB	3.3	76.9	120	323	10.9	94	238.7	7.4	7.0	117	67.2	60.7	7.1	2149.1	10124.6	2.0	1.6
4671 E G M IC LB 2.7 59.0 104 354 11.1 97 221.5 10.2 8.6 130 102.1 74.3 7.8 2226	4646	E	G	Н	TC	LB	3.2	76.2	110	402	12.4	97	184.1	9.3	7.7	90	119.2	76.3	11.0	1879.1	12251.6	2.4	2.3
10/1 2 0 12 10 22 2/7 0/10 10/1 00/1 10/2 0/0 0/0 10/2 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0 0/0	4671	E	G	M	IC	LB	2.7	59.0	104	354	11.1	97	221.5	10.2	8.6	130	102.1	74.3	7.8	2226.0	8064.8	2.4	2.5
4709 E G H OP RGB 5.5 42.7 130 323 7.6 133 330.6 5.0 13.0 201 189.1 193.2 1.8 836.	4709	E	G	Н	OP	RGB	5.5	42.7	130	323	7.6	133	330.6	5.0	13.0	201	189.1	193.2	1.8	836.0	11806.7	3.2	3.9

4734	E	G	Н	TC	LB	3.1	52.6	106	259	10.0	108	212.9	8.6	8.8	100	70.9	60.4	3.4	1065.1	7100.1	3.1	1.2
4757	E	G	Н	IC	LB	3.1	68.8	110	352	10.7	119	253.1	9.8	6.8	132	83.1	59.9	4.5	2176.2	9304.3	2.0	2.0
4795	E	G	Н	TC	LB	3.3	74.5	117	375	11.5	102	227.2	8.8	7.6	124	97.2	72.4	6.2	1481.5	8701.7	2.9	3.0
4797	E	G	Н	TC	RGB	3.1	73.4	107	367	11.3	116	261.7	8.8	7.4	129	100.3	78.5	4.3	1018.5	4993.0	2.7	1.7
4816	E	G	Н	TC	LB	3.1	90.6	99	320	11.1	90	161.1	10.2	9.3	74	122.9	85.2	7.0	1661.0	8819.8	1.9	1.1
5066	E	P	Н	TC	LB	3.0	71.0	141	362	10.7	116	235.8	8.8	7.1	107	100.9	75.1	6.3	2158.9	8396.1	2.4	4.1
5091	E	G	Н	TC	LB	2.9	75.0	121	394	11.8	99	238.7	9.0	7.9	126	99.6	77.2	7.8	2191.9	10447.5	2.5	2.7
5106	E	G	Н	TC	LB	3.3	72.7	121	323	10.4	102	230.1	7.9	7.1	119	66.6	59.4	7.5	1700.2	9939.7	1.8	2.1
5201	E	G	Н	TC	DB	3.5	81.9	135	396	11.3	119	233.0	10.7	7.0	127	136.4	115.3	6.7	2024.4	13295.8	2.2	2.3
5306	E	G	Н	TC	LB	3.1	76.9	108	278	10.4	97	161.1	9.3	7.3	119	94.7	70.7	5.9	868.8	6799.7	3.4	1.7
5367	E	G	Н	TC	W	3.5	67.7	115	365	12.6	126	267.4	7.9	7.6	146	109.4	80.1	7.6	1416.4	8423.3	3.2	2.6
5537	E	G	Н	IC	DB	3.5	71.1	124	299	10.4	116	247.3	9.8	8.5	134	66.0	60.3	5.3	1435.2	10444.5	3.1	2.5
5817	E	G	M	TC	DB	2.9	64.3	128	296	10.4	116	253.1	10.2	7.7	127	118.0	93.3	4.9	1249.6	7575.8	3.4	3.1
5870	E	G	Н	IC	LB	2.8	65.1	112	291	10.7	97	261.7	9.3	9.0	111	74.5	61.8	4.7	596.0	4103.6	4.3	3.2
6059	E	G	Н	TC	RB	3.1	74.3	116	359	11.1	107	204.2	8.8	9.0	97	106.4	81.3	7.7	1332.2	7937.9	2.9	2.9
6082	E	G	Н	IC	RB	2.7	63.9	119	325	10.7	116	264.6	9.8	8.2	122	71.5	60.9	5.8	552.9	5878.1	3.6	2.3
6154	E	G	M	TC	RB	3.2	72.9	121	328	11.5	106	250.2	9.5	7.9	117	121.1	88.4	8.2	2348.6	10660.5	2.5	2.5
6165	E	G	Н	IC	RB	3.5	71.5	121	330	10.4	123	241.6	10.2	8.4	127	79.4	70.3	5.8	1420.6	8160.1	2.2	2.3
6221	E	G	M	IC	RB	3.3	69.8	126	320	12.9	101	267.4	9.8	9.0	154	79.4	65.1	7.0	1418.1	6607.6	3.4	3.0
6240	E	G	M	TC	LB	2.9	69.7	127	317	11.1	107	256.0	9.8	6.8	145	86.2	66.0	6.9	1688.1	9705.3	2.7	2.5
6294	E	G	Н	TC	LB	3.1	75.7	122	338	11.1	111	227.2	9.0	8.5	126	96.0	75.3	7.8	1856.8	9513.5	2.9	3.4
6326	E	G	M	TC	LB	3.5	85.0	125	325	10.9	103	189.9	6.9	7.1	109	97.2	65.5	6.9	2548.7	13523.2	2.0	2.4
6337	E	G	Н	TC	RB	3.3	74.6	119	328	11.1	114	247.3	7.6	7.7	133	97.8	69.9	6.3	1124.2	7601.7	3.3	1.6
6350	E	G	Н	TC	LB	3.2	83.8	106	323	13.3	94	235.8	7.9	7.4	128	84.9	63.7	6.0	1094.6	7400.2	3.2	2.3
6421	E	G	M	TC	LB	3.1	70.5	150	388	10.4	113	253.1	9.8	6.4	119	89.8	73.3	5.8	1764.0	7666.8	3.4	3.8
6473	E	G	Н	TC	LB	2.9	75.6	128	370	12.9	118	184.1	8.6	7.9	120	99.0	69.7	7.6	1023.7	7302.0	3.6	3.8
6514	Е	G	Н	TC	LB	3.1	76.2	128	328	11.3	109	238.7	8.6	7.1	136	89.8	76.8	7.9	1584.3	7813.0	2.9	2.6
6537	E	G	M	TC	LB	3.1	104.9	110	330	11.5	104	188.4	9.8	7.1	103	118.0	65.2	8.3	1334.2	12890.5	1.5	1.6
7018	Е	G	Н	TC	DB	3.1	76.0	149	367	11.1	107	215.7	8.3	6.5	139	98.4	67.8	7.9	1934.6	11951.0	3.3	3.9
7079	Е	G	M	TC	RGB	3.5	68.9	122	354	11.1	116	284.7	9.8	6.2	154	108.8	77.5	6.3	1695.7	7753.8	3.4	3.1
7320	E	G	Н	TC	LB	3.1	73.6	129	404	11.5	101	227.2	9.8	7.7	128	101.5	69.8	8.1	1654.9	8290.5	2.7	3.8

Control																						
VR 708	E	G	Н	TC	RB	3.5	53.4	103	344	10.7	104	215.7	9.8	9.0	97	84.9	72.0	4.1	1763.2	6449.3	2.7	2.0
2043(P	E	G	M	TC	RB	3.1	70.4	120	330	10.4	114	250.2	9.5	6.7	110	80.0	62.9	5.4	2645.8	10885.7	1.7	2.4
R 202)																						
3618	E	G	Н	IC	LB	3.1	66.3	113	265	10.9	109	233.0	9.8	7.1	131	98.4	68.6	4.8	1927.0	10120.1	2.2	1.8
(RAU8																						
)																						
4673(V	E	P	Н	TC	LB	3.1	57.7	118	370	10.9	109	247.3	10.5	7.6	121	111.9	88.8	6.1	2511.2	5224.7	3.2	3.8
L 149)																						
		Trial m	nean			3.25	73.3	123	345	11.3	106	230.7	9.12	7.5	125	95.55	73.08	6.5	1700.2	9684.2	2.7	2.6
		SEM	±			0.32	1.14	3.9	18.8	0.66	6.1	13.2	0.75	0.51	8.4	6.078	5.331	0.69	223.3	1108.7	0.40	0.48
	Не	eritabilit	ty (%)			56.5	98.8	91	79.0	65.7	72.8	86.2	71.3	84.0	87.4	91.89	91.71	84.2	83.5	86.1	69.6	73.7
		CV (%	6)			22.6	2.7	5.6	10.6	12.4	11.6	10.7	16.7	12.7	12.5	11.49	13.1	19.7	24.6	20.8	29.9	37.1
		LSD (5	5%)			0.9	3.2	11	52.3	1.82	16.9	36.7	2.07	1.42	23.5	16.93	14.85	1.9	622.1	3088.2	1.1	1.33

Abbreviation: Growth habit: E=Erect; Pigmentation: G=Green, P=Pigmented; Culm branching: M=Medium, H=High; Inflorescence compactness and shape: TC=Top curved, IC=Incurved, C=Curved, OP=Open, LO=Long Open

Table 5.3 Variance due to genotype  $(\sigma^2 g)$  for 17 quantitative traits of traits of finger millet mini-core collection accessions evaluated during the 2008 rainy season at ICRISAT, Patancheru, India.

Traits	Genetic variance	s.e.	Residual (σ²e)	s.e.
Basal tillers number	$(\sigma^2 \mathbf{g})$ 0.23**	0.07	0.54**	0.06
Days to flowering	105.26**	16.54	3.81**	0.43
Plant height (cm)	151.59**	26.19	47.39**	5.40
Flag leaf blade length (mm)	1684**	334.00	1341**	152.00
Flag leaf blade width (mm)	1.25**	0.30	1.96**	0.22
Flag leaf sheath length (mm)	134.6**	29.30	150.9**	17.10
Peduncle length (mm)	1254.3**	227.00	603.2**	68.40
Panicle exertion (mm)	829.5**	154.60	480**	54.40
Longest finger width (mm)	1.94**	0.43	2.33**	0.26
No of fingers ear <sup>-1</sup>	1.59**	0.30	$0.91^{**}$	0.10
Exsertion (mm)	559.1**	99.80	242.6**	27.50
Ear head length (mm)	455.3**	77.10	120.6**	13.70
Ear head width (mm)	338.09**	57.39	91.68**	10.42
Weight of 5 panicles	2.94**	0.55	1.66**	0.19
Grain yield (kg ha <sup>-1</sup> )	293498**	55212	174227**	19785
Stover yield (kg ha <sup>-1</sup> ) Overall plant aspect	8420856 <sup>**</sup> 0.51 <sup>**</sup>	1535687 0.12	4068969** 0.66**	462942 0.08
Lodging	0.83**	0.18	0.89**	0.10

<sup>\*,</sup> significant at p=0.05; \*\*\*, significant at p=0.01

Table 5.4 Top 10 most early and late maturing finger millet accessions compared to control evaluated during the 2008 rainy season at ICRISAT, Patancheru, India.

10 early	flowering acces	10 late flowering accessions					
IE No.	Days to flowering	Grain yield (kg ha <sup>-1</sup> )	IE No.	Days to flowering	Grain yield (kg ha <sup>-1</sup> )		
4709	42.7	836.0	2872	84.5	1703.9		
501	49.1	7497.8	2034	84.9	1710.9		
4734	52.6	1065.1	6326	85.0	2548.7		
3104	54.3	1574.3	2710	88.5	1730.5		
4671	59.0	8064.8	2871	88.9	1993.3		
2042	60.2	8441.2	4057	89.0	2677.1		
518	63.5	1496.2	2911	90.2	1350.3		
6082	63.9	552.9	4816	90.6	1661.0		
5817	64.3	1249.6	2572	103.6	1466.6		
5870	65.1	596.0	6537	104.9	1334.2		
Control							
VR 708	53.4	1763.2					
2043 (PR 202)	70.4	2645.8					
3618 (RAU8)	66.3	1927.0					
4673 (VL 149)	57.7	2511.2					

Table 5.5 The top 10 highest and lowest yielding finger millet accessions compared to control evaluated during the 2008 rainy season at ICRISAT, Patancheru, India.

High yielding acc		Low yielding accessions				
IE No.	Grain yield (kg ha <sup>-1</sup> )	НІ	IE No.	Grain yield (kg ha <sup>-1</sup> )	HI	
3475	3022.1	0.17	6082	552.9	0.09	
2034	2936.5	0.14	5870	569.0	0.13	
4057	2677.1	0.17	2821	742.7	0.11	
6326	2548.7	0.16	4709	836.0	0.07	
3045	2487.3	0.13	5306	868.8	0.11	
2790	2414.0	0.19	4797	1018.5	0.17	
6154	2348.6	0.18	6473	1023.7	0.12	
3077	2285.4	0.15	4734	1065.1	0.13	
4671	2226.0	0.22	6350	1094.6	0.13	
5091	2191.9	0.17	6337	1124.2	0.13	
Control						
VR 708	1763.2	0.21				
2043 (PR 202)	2645.8	0.20				
3618 (RAU8)	1927.0	0.16				
4673 (VL 149)	2511.2	0.32				

Table 5.6 Range and means of selected agronomic traits of 80 finger millet accessions compared to controls evaluated during the 2008 rainy season at ICRISAT, Patancheru, India.

Quantitative traits	Range	Mean	Mean of
			controls
Basal tillers	2.7 - 5.5	3.3	3.2
Days to flowering	43 - 105	73	62.0
Plant height (cm)	99 - 150	123	113.5
Panicle length (mm)	62 - 119	96	94.0
Panicle width (mm)	60 - 192	73	73.0
Panicle weight (g)	3.4 - 11	6.5	5.1
Grain yield (kg ha <sup>-1</sup> )	146 - 3022	1700	2212
Stover yield (kg ha <sup>-1</sup> )	80 - 17564	9648	8170

Table 5.7 Correlation matrix of selected finger millet traits during the 2011 rainy season at ICRISAT, Patancheru, India.

	Basal tillers	Days to flower	Plant height	Flag leaf blade length	Flag leaf blade width	Flag leaf sheath length	Pedunc le length	Longest finger width	No. of fingers ear <sup>-1</sup>	Panicle exertion	Panicle weight	Grain yield	Stover yield	Lodgir g
Basal tillers	1.00					· i caison s	correlatio	ii coemicien	ι (1)					
Days to flower	0.13	1.00												
Plant height	0.03	0.23	1.00											
Flag leaf blade	0.02	0.00	-0.1	1.00										
length Flag leaf blade	0.06	0.38	0.78	-0.23	1.00									
width Flag leaf sheath	0.11	-0.25	0.29	-0.08	0.23	1.00								
length Peduncle length	0.16	-0.54	0.0	0.17	-0.22	0.33	1.00							
Longest finger	-0.22	-0.08	0.02	0.08	0.002	0.07	0.00	1.00						
width No. of fingers	0.24	0.36	-0.36	0.00	0.0	0.13	0.05	-0.10	1.00					
Panicle exertion	0.14	0.14	-0.48	-0.02	0.12	0.06	0.88	-0.01	0.03	1.00				
Panicle weight	-0.03	0.33	0.80	-0.35	-0.96	0.28	-0.24	0.002	0.23	-0.23	1.00			
Grain yield	0.04	0.22	0.42	-0.05	0.4	0.03	-0.13	0.06	-0.23	-0.16	0.44	1.00		
Stover yield	0.18	0.33	0.02	0.11	-0.14	-0.18	-0.15	-0.17	-0.09	-0.18	-0.15	0.39	1.00	
Lodging	-0.02	-0.02	0.35	0.16	0.07	0.19	0.16	0.12	-0.11	0.11	0.07	0.02	-0.13	1.0

#### **General conclusions and future directions**

This research was conducted under controlled environments and field conditions. Two experiments were conducted under controlled environment conditions to understand the effects of high temperature stress (36/26°C, and 38/28°C) on growth, development, and yield of finger millet and to determine the sensitivity of finger millet growth, development, and reproduction to short, sudden episodes of high temperature stress. Field experiments were conducted at various locations to determine the effect of seeding and nitrogen fertilizer application rates on finger millet grain and biomass yield, and to evaluate the finger millet minicore collection for morphological and yield traits. Important conclusions from each experiment are as follows:

Chapter 1 (Experiment I): There were significant negative effects of high temperature stress during reproductive development (from 30 DAS to harvest maturity) on growth, yield, and components of yield on figure millet. Compared to 32/22°C, exposure to 36/26°C decreased grain weight, grain yield and harvest index by 33%, 75% and 54%, respectively. The corresponding decreases of the same parameters at 38/28°C were 56%, 84%, and 62%, respectively. This study highlights the threat faced due to changing climates (particularly high temperature stress) by finger millet which is an important crop for food security in several parts of Asia and Africa.

Chapter 2 (Experiment II): This study determined the sensitivity of finger millet to high temperature stress during reproductive development (booting through maturity). My research showed that three stages (booting, panicle emergence, and flowering) were most sensitive stages

to short periods (10 d) of high temperature stress (40/30°C) resulting in maximum decreases in seed number, seed weight, and grain yield. Post flowering stages were relatively less sensitive in decreasing grain yield compared to those at booting, panicle emergence, or flowering. Maximum reduction in seed numbers occurred during panicle emergence. This study highlights that processes occurring during flowering (pollination, pollen germination, pollen tube growth, and fertilization) are highly sensitive to temperature stress. Further studies are needed to determine if finger millet genotypes vary in their response to high temperature stress and sensitive stages.

Chapter 3 (Experiment III): The study to determine the effect of seeding rates (3.2, 6.0, and 9.0 kg ha<sup>-1</sup>) and nitrogen fertilizer application rates (0,30, 60, and 90 kg ha<sup>-1</sup>) on grain and biomass yield of finger millet revealed that responses varied with location. There was no effect of nitrogen fertilizer application rates on grain yield across all locations. This suggests that the site selected for this study had higher residual N in the soil or environmental conditions helped to release stored N from the soil. However, higher rates of fertilizer application and seeding rates increased lodging due to greater plant height and growth. Lower seeding rate of 3.2 kg ha<sup>-1</sup> produced higher tillering, and medium seeding rate (6.0 kg ha<sup>-1</sup>) increased grain yield in Manhattan, Kansas.

Chapter 4 (Experiment IV): Finger millet minicore accessions were evaluated for morphological and yield traits. Results from this study showed that there was high variability and heritability (>60%) of quantitative traits (e.g., days to flowering, panicle length, panicle width, panicle weight, plant height, grain yield, and stover yield). Overall, two accessions (IE 3104 and IE 3475) showed consistent values for high grain yield, stover yield, and harvest index.

#### **Future research**

This research revealed the vulnerability and sensitivity of finger millet to increasing temperatures especially during early reproductive stages. There is need to develop new screening tools to identify tolerance during reproductive stages of development. In addition, there is also a need to screen large germplasm collections for tolerance during reproductive stages. An initial step will be to evaluate the entire finger millet minicore accessions under controlled environment conditions to determine their performance under high temperature stress during flowering. High temperature tolerant accessions can be used for genetic improvement of already existing finger millet varieties to render them more adaptable to high temperature stress conditions. Field studies on finger millet minicore identified accessions with relatively high performance for grain, biomass yield, and harvest index. Multilocational testing of these accessions would further reveal their performance under different environmental conditions. In addition to quantifying variation in growth and yield traits, grain quality traits (particularly for nutrition) and other uses such as feed value and biofuel production needs investigation. Studies on finger millet management revealed that there was interaction between location/years and seeding rate, and nitrogen fertilizer application rates. Further investigations should be conducted to determine the optimum seeding and nitrogen fertilizer application rates for the mid western USA and other finger millet growing areas in Africa and Asia.

## **Appendices**

# Appendix 1 – Effect of seeding and nitrogen fertilizer application rates on ethanol production of finger millet grain

#### Introduction

Global energy demand is increasing at the rate of 2 to 3% every year. The global daily oil consumption of 86 billion barrels is equivalent to 2.8 L day<sup>-1</sup> person<sup>-1</sup> for a world population of 6.7 billion in 2008 (Lal, 2010). Therefore, there has been considerable interest in developing biorenewable alternatives to petroleum-based commodity chemicals such as transportation fuels. The US, Brazil and several EU member states have the largest programs promoting biofuels in the world (Balat and Balat, 2009). The recent commitment by the United States government to increase bioenergy threefold in ten years has added impetus to the search for viable biofuels (Demirbas and Balat, 2006).

The most prominent example is ethanol, which has emerged as a potentially important alternative transportation fuel. Currently, nearly all bioethanol fuel is produced by fermentation of corn (*Zea mays* L.) glucose in the United States or sucrose in Brazil (Schapouri et al., 2006). In Europe, the feedstock used for bioethanol is predominantly wheat (*Triticm aestivum* L.), sugar beet (*Beta vulgaris*), corn and waste from the wine industry (Kline et al., 2008). Currently, approximately 80% of total world ethanol production is obtained from the fermentation of simple sugars by yeast (Macović et al., 2009, Bennett and Anex, 2008). Starch-rich materials, such as grains, have the advantage of established feedstock and processing infrastructure, and a more homogeneous and reactive form of carbohydrate than that found in cellulosic materials. Plant

materials high in soluble sugars yield the most readily converted form of carbohydrate, requiring lower inputs of chemicals and energy for processing, and the technology for the extraction of sugars is fully mature and highly efficient, reducing processing costs (Bennet and Anex, 2008, Pradeep et al., 2010).

Finger millet is an important cereal in East and Central Africa and Southern Asia and is adapted to a wide range of environments, with outstanding attributes as a food crop (National Research Council, 1996; Dida et al., 2008). Its outstanding properties as a subsistence food crop notwithstanding, its use as a biofuel crop has received little attention. Forty percent of total world ethanol production is from starchy materials (Trinidade, 2005). A lot of studies have been conducted on wheat, corn, barley, oats and recently sorghum, however, very little or neglibible attempts have been made using starches/grains available in tropical regions such as finger millet (Pradeep et al., 2010). Finger millet is a grain with high potential for biofuel production (Sarath et al., 2008; Pradeep et al., 2010). It is important to determine the the effect of production methods on subsequent ethanol production. Studies have suggested that the suitability of the grain of different cereal species for bioethanol production is dependent on cultivars and growing conditions (Aufhammer et al., 1993). Ethanol yields per hectare are known to be influenced by grain yields per hectare and variation of ethanol output within species is known to be as a result of the effects of cultivars and growing conditions (Rosenberger et al., 2002).

Another important aspect of biofuel production is the cost. It has been suggested that decreasing raw material cost is a substantial source of improving the competitiveness of ethanol. In Germany, the grain for production of ethanol is usually derived from stocks originally destined for food or feedstuff. Ethanol grain differs at the quality level; hence grain protein enhanced traits could be ignored in favor of carbohydrate accumulation. Improved grain

carbohydrate improves the bioethanol conversion rate per ton of grain fermented. Implementing different crop production intensity levels would therefore serve the dual purpose of cost saving and reinforcing grain carbohydrate rather than protein accumulation (Rosenberger et al., 2002).

The conditions under which a crop is grown are known to alter protein composition (Borghi et. al., 1995). Timms et al. (1981) suggested that late nitrogen application in the absence of sulfur in wheat may sufficiently alter the balance between these two nutrients so that sulfur levels become inadequate for normal grain protein development. Sulfur deficiency during grain filling has also been associated with alterations in the ratios between groups of storage proteins (Fullington et. al., 1987). Therefore it was concluded that sulfur fertilization may be used to manipulate the protein content of wheat grains, depending on the cultivar (genotypic differences) (Wooding et. al., 2000). This seems to imply that late N application and absences of sulfur will result in less grain protein. It would be interesting to pursue this study to test this hypothesis. Other suggestions for crop management include use of previous legume crops for cost saving by substitute for mineral fertilizer nitrogen in bioethanol grain production (Rosenberger et al., 2002). Appropriate bioethanol grain production is a substantial source of cost savings in bioethanol production. The objective of this study was to determine the effect of crop management on ethanol yield of grain harvested from finger millet grown under varying seeding and nitrogen fertilizer rates.

#### Materials and methods

Finger millet was grown in Manhattan, Kansas in 2009 under three seeding rates and four nitrogen application rates. Starch, glucose and ethanol content of finger millet grain were estimated using the high performance liquid chromatogrophy (HPLC) analysis at the Bioprocessing and Industrial Value Added Program (BIVAP) laboratories of the Grain Science and Industry Department, Kansas State University. About 5 g of clean grain samples were ground using the Perten grinder at zero setting to generate fine fractions. Ground finger millet was sieved through US standard sieves and particles <600 µm were collected. Samples were stored at 4°C until further processing.

Three grams of the samples were mixed with 10 ml of deionized water in 250 ml screw cap flasks. About 50 μl of α-amylase (Liquizyme-XTM, Novozymes, CA, USA) was added into each of the flasks, incubated at 84°C for 75 min in a water bath and colled to room temperature. Flasks were closed tightly to avoid loss of moisture. This treatment was followed by the addition of 400 μl of β-glucoamylase (Spirizyme, Novozymes, CA, USA) into each of the flasks and incubated at 30°C for 24 h at 30°C, followed by the addition of 40 ml of salt solution 0.36% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.06% K<sub>2</sub>HPO<sub>4</sub>, 0.06% ZnSO<sub>4</sub> and 0.006% MnSO<sub>4</sub>). About 500 μl of zero hour and 24 h samples were drawn to analyze the amounts of glucose released and ethanol produced soon after incubation and at the end of fermentation respectively (Picture 4).

The Shimadzu high performance liquid chromatography (HPLC) equipped with RID detector and Lab solution was used for determining levels of glucose and ethanol. For separation, phenomenex Rezex-ROA organic acid column cross linked with 8% hydrogen resin was used. Column oven temperature was adjusted to 82°C. Mili-Q water was used as the mobile phase with a fow rate of 0.6 ml/min. Data was collected on the amount of glucose (mg/g), amount of starch

(%), and amount of ethanol (mg/g) of finger millet grain. The experimental design for grain production was a 3 X 4 factorial with 4 replications. Statistical analysis was performed using SAS 9.1.3 (SAS Institute Inc. Cary, USA). The PROC GLM procedure was used. Seeding and nitrogen fertilizer rates were treated as random effects and starch, glucose and ethanol as fixed effects. Tukey's Studentized Range Test (HSD) was used to separate the means.

#### **Results and discussion**

There were no significant effects of seeding rates on starch, glucose and ethanol production form finger millet grains. Effects were also non significant for nitrogen fertilizer application rates on finger millet glucose and ethanol production. However, significant differences were observed for percentage starch in finger millet grains. Finger millet grown under 0 kg N ha<sup>-1</sup> produced more starch compared to 30, 60 and 90 kg N ha<sup>-1</sup> (Table 6). According to Aufhammer et al. (1993), effects of cultivars and growing conditions affect ethanol outputs and ethanol yields ha-1 are substantially influenced by grain yield ha<sup>-1</sup>. Results from this study, however indicate that growing conditions did not influence ethanol production from finger millet. However, the study showed that finger millet has the capacity to produce ethanol at relatively significant quantities regardless of the growing conditions. Ethanol production from whole grain is essentially ethanol production for starch. Our results showed significant starch yield from finger millet grain. Finger millet produced 56 to 58 % starch. This can be compared to grain sorghum which produced 60 to 80% starch (Wang et al., 2000). The study was conducted during one season (2009), therefore there is need to repeat the experiment to verify the results and to determine the efficiency and capacity of finger millet grain ethanol production compared to existing feedstocks.

### **Conclusion**

Results from this research has provided a foundation for further, more comprehensive research to determine the productivity and efficiency of finger millet as a biofuel feedstock especially in the mid-western region of US. This study has provided the evidence that production of ethanol form finger millet grain is feasible, and since finger millet produces significant amounts of biomass, this could also be considered as a biofuel feedstock to maximize on productivity of the crop. If the results are verified after further testing, finger millet may become an option for the production of grain and biomass without the addition of fertilizer, thereby reducing the cost of production for the biofuel feedstock. Indeed, it would fulfill the need to optimize grain and biomass yield while minimizing inputs of fertilizer. Further studies are needed to verify these hypotheses. Overall, the benefit of finger millet as a biofuel feedstock in the mid-west would be significant.

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Table 6. Effect of seeding and nitrogen fertilizer application rates and their interactions on starch, glucose and ethanol production of finger millet grains harvested from a crop grown in Manhattan in 2009.

Treatment	Starch (%)	Glucose (mg/g)	Ethanol (mg/g)			
	Seeding	grate (kg ha <sup>-1</sup> )				
3.2	57.1	714.9	314.0			
6.0	57.3	633.3	319.0			
9.0	57.7	729.4	315.0			
Mean	57.3	702.5	316.3			
CV%	2.96	28.1	14.3			
LSD <sub>0.05</sub>	1.22 <sup>NS</sup>	$90.5^{NS}$	32.3 <sup>NS</sup>			
	Nitrogen fertilizer	application rate (kg ha <sup>-1</sup> )				
0	58.4 <sup>a†</sup>	742.2	323.4			
30	57.3 <sup>ab</sup>	669.6	314.4			
60	57.3 <sup>ab</sup>	719.5	305.5			
90	56.3 <sup>b</sup>	678.7	321.7			
Mean	57.3	702.5	316.2			
CV%	2.96	28.1	14.3			
LSD <sub>0.05</sub>	1.32*	104.5 <sup>NS</sup>	32.3 <sup>NS</sup>			
	Ar	nova P>F				
Seeding rate (S)	$0.6049^{NS}$	0.6469 <sup>NS</sup>	0.9554 <sup>NS</sup>			
N Fertilizer rate (F)	$0.0340^*$	$0.9427^{NS}$	$0.7598^{NS}$			
SXF	$0.9494^{\rm NS}$	$0.9952^{NS}$	$0.9678^{\mathrm{NS}}$			

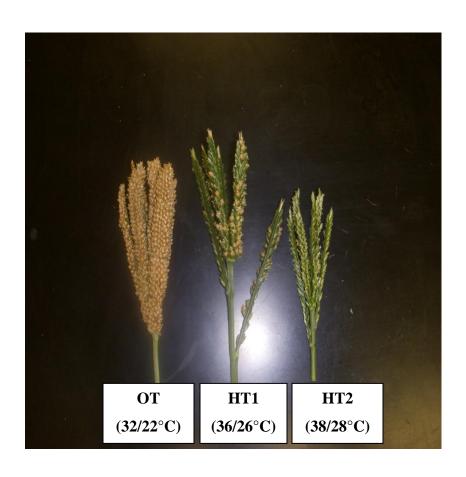
NS, nonsignificant, \*, significant at P<0.05

†Within columns, means followed by the same letter are not significantly different at p< 0.05

# **Appendix 2 - Pictures**



Picture 1. Effect of high temperature stress on height and internode lengths of finger millet plants grown under controlled environment conditions



Picture 2. Effect of high temperature stress on number of seeds panicle<sup>-1</sup> under controlled environment conditions.



Picture 3. Transfer of finger millet plants out of growth chambers to green house to in the experiment to determine sensitivity of finger millet to short episodes of high temperature stress.



Picture 4. Digested finger millet samples in the experiment for determination of the effect of growing conditions on ethanol production from finger millet.