

Development of pigeonpea [*Cajanus cajan* (L.)] hybrids for the semi-arid Kenya

By

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**A thesis submitted in fulfillment of the requirements for the
degree of Doctor of Philosophy (PhD) in Plant Breeding**

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December 2011

Thesis abstract

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is cultivated by many farmers in the semi-arid areas of Kenya as a source of food and cash. However, the yields have remained low, ranging between 500 to 800 kg ha⁻¹. Apart from drought, fusarium wilt is reported to affect yield. Breeding pigeonpea hybrids, using cytoplasmic male sterile (CMS) lines, hybridized with the local improved germplasm, have the potential for increasing yield and improve income for smallholder farmers. The objectives of the study were to: 1) examine the various stakeholders of the pigeonpea value chain and their core functions and identify characteristics of the pigeonpea varieties preferred by the market to be considered in the hybrid breeding programme, 2) evaluate cytoplasmic male sterile lines of Indian origin for stability across several environments in Kenya, 3) screen pigeonpea genotypes for general resistance to *Fusarium udum* Butler and 4) evaluate pigeonpea hybrids for grain yield and earliness across sites and seasons in Kenya.

The stakeholder analysis established that the main players in the pigeonpea value chain were farmers, traders/processors, International Crops Research Institute for the Semi-Arid Tropics, Kenya Agricultural Research Institute, Kenya Plant Health Inspectorate Services, and Ministry of Agriculture. White seed, large seed size and medium maturity were the preferred traits by farmers and processors/exporters for both domestic and export markets. The unavailability of quality seed in sufficient quantities of high yielding varieties was cited as the main factor negatively affecting pigeonpea production. The stakeholder analysis approach, used for the first time in a breeding programme, demonstrated that it can be an important tool that can be used to diagnose crop production constraints, and define opportunities available for setting up a breeding programme that is highly client-oriented.

Two CMS lines, ICPA2043 and ICPA2039 were the most stable across sites with 100% and 99% pollen sterility respectively. Screening for the presence of physiologic races of *F. udum* based on morphological and cultural characteristics on PDA identified three distinct isolate groups named ISO-A, ISO-B, and ISO-C. Studies under controlled conditions using the three isolates identified seven pigeonpea genotypes (ICPB2043, ICP12012, ICP13092, ICPA2039xICP13092, ICPA2043xICP12012, ICPA2043xICP13092, ICPA2043xICP9135) resistant to the three *F. udum* isolates. In the field evaluation, seven genotypes (ICPA2039xICP13092, ICPA2039xAsha, ICPA2043x12012, ICPA2043xICP13092, ICPA2043xICEAP557, ICPB2043 and Maruti) were found to be moderately resistant. The

variances due to GCA and SCA were significant, showing that both additive and non-additive gene actions were important. The resistant hybrid, ICPA2043xICP12012 had the highest negative SCA that was highly significant for all the isolates and in the field indicating general resistance. The CMS (A) line ICPA2043 was found stable across environments and highly resistant to the three *F. udum* isolates. Therefore, it can be evaluated further for commercial hybrid seed production in Kenya.

Evaluation of the pigeonpea genotypes across environments indicated that the highest yielding environment was Kiboko, with average and maximum yield of 2,249 kg ha⁻¹ and 4,234 kg ha⁻¹ respectively. Most hybrids were in the medium duration maturity group with days to maturity ranging from 147 to 186. Overall, the highest yielding hybrids were A2043xTZ26 and ICPA2039xTZ24 with mean yields 2,803 kg ha⁻¹ and 2,527 kg ha⁻¹ respectively. Mean yields for the best performing parents were 2,036 kg ha⁻¹ for ICP12012 and 1,629 kg ha⁻¹ for Asha. For specific sites, the highest yielding hybrids in Kabete, Kiboko and Leldet were A2039xTZ24 (2,057 kg ha⁻¹), A2043xTZ26 (2,803 kg ha⁻¹), and A2043xUG8 (1,708 kg ha⁻¹) respectively. Mean heterosis for yield varied from -35% (A2039xA2043) to 50% (A2043xUG8). In Kenya, the potential for production and commercialization of hybrid pigeonpea is feasible due to high hybrid vigour recorded, and the stability of the CMS lines. Hybrids also have greater uniformity in grain size a factor which is important for the market.

Declaration

I, **Margaret Nafula Makelo**, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original research.
2. The thesis has not been submitted for any degree or examination at any other university.
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As the candidate's supervisors, we agree to the submission of this dissertation for examination:

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Prof. Rob Melis (Principal supervisor)

Signed..... Date.....

Prof. Mwangi Githiri (Co-supervisor)

Acknowledgements

I wish to sincerely thank my supervisors Professor Rob Melis and Professor Githiri Mwangi for their sustained interest, guidance and helpful criticisms from the beginning of this research project through to the final write-up. I am grateful to AGRA for generously funding this study through the African Centre for Crop Improvement (ACCI). Special thanks to the ACCI administration, in particular Professor Mark Laing and Lesley Brown, for facilitating the research project and my stay in South Africa.

I wish to thank the following people and organizations who contributed to the success of the research; Dr Ephraim Mukisira, the Director KARI for giving me study leave to pursue this PhD, and allowing me to use the research facilities at Katumani and Kiboko; Dr Said Silim, my in-country supervisor for the guidance during the research period and ICRISAT for providing germplasm. I also wish to sincerely thank Dr Julia Sibiya, for her contribution to the pathology aspects in this thesis.

I am deeply indebted to my husband Ken, and my children Duncan and Winnie for their love, sacrifice, encouragement and patience throughout the study period. What a wonderful family I have.

I am grateful to God for the good health and grace that enabled me through, may He be praised.

Dedication

To my dearest husband Kenneth Lusaka, beloved son Duncan Aura, and adorable daughter,
Winnie Gloria Makelo

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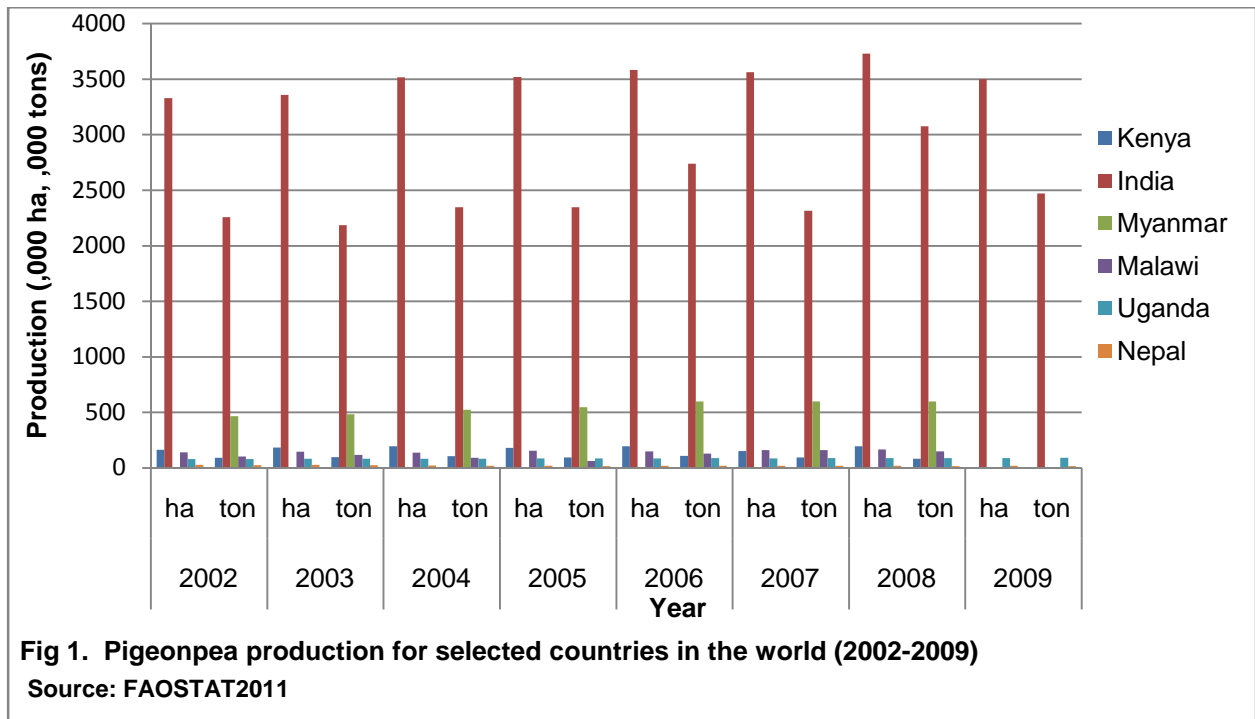
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Introduction to thesis

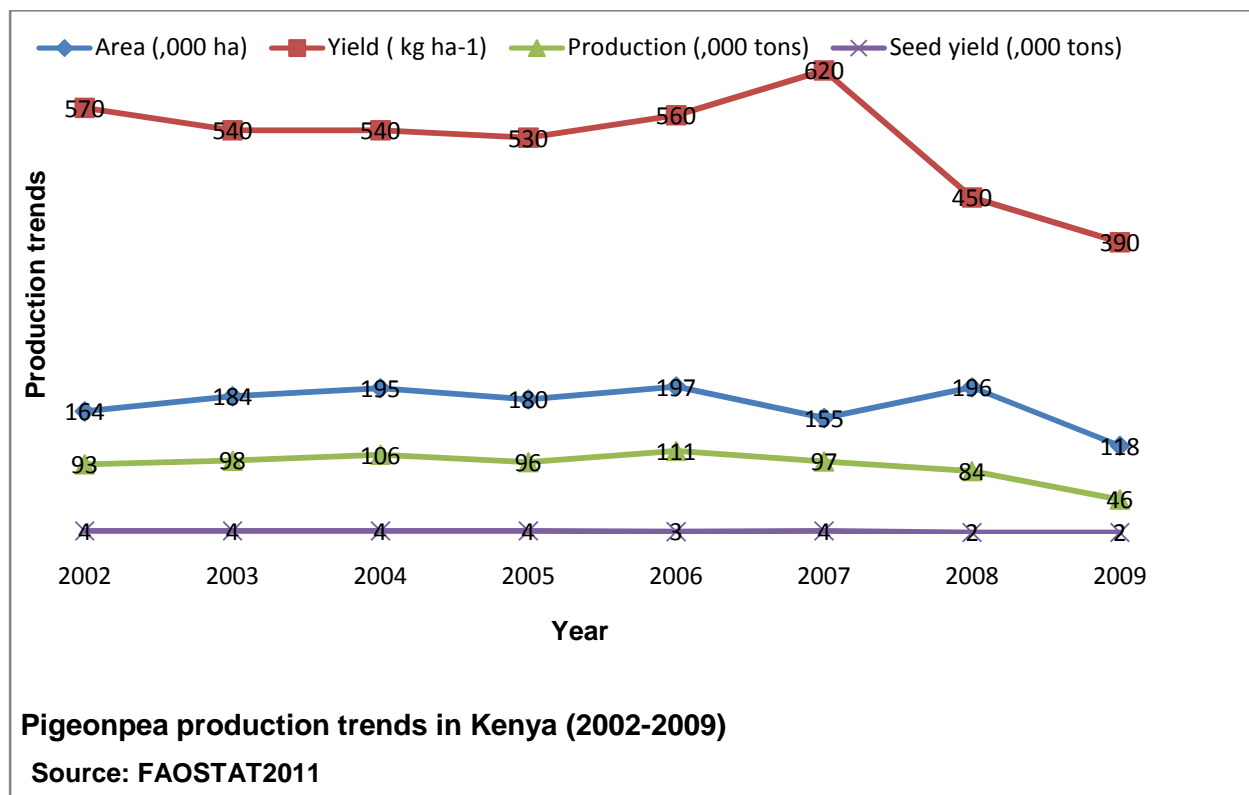
Pigeonpea production

Pigeonpea [*Cajanus cajan* (L) Millsp.] is adapted to a range of environments with varying temperatures, altitudes and latitudes (Silim et al., 2006; Troedson et al., 1990), but is mainly grown in the tropics and sub-tropics. The crop represents about 5% of the world legume production (Hillocks et al., 2000) with India producing 70% and substantial quantities being produced in eastern Africa especially from Kenya, Malawi and Tanzania. Global production is estimated to be about 3.7 million metric tons valued at US\$ 1,600 million (FAOSTAT, 2011). Pigeonpea production statistics for selected countries from 2002 to 2009 are shown in Figure 1. India is the leading producer in both hectarage and production recording 3,729,000 ha in 2008 and 2,738,000 tons in 2006. In Africa, Malawi was the leading producer on 168,000 ha with a production of 160,000 tons in 2007.



In Kenya, pigeonpea is the third most important food grain legume after common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* L.) (Jones et al., 2002; Kimani et al., 1994; Mergeai et al., 2001). Pigeonpea is cultivated by smallholder farmers in the arid and semi-arid eastern Kenya where it is commonly intercropped with other legumes, cereals and

fruit trees. It is becoming one of the most sought after crops in plant introduction trials aimed at bringing new areas under cultivation (ICRISAT, 2006/7; Shiferaw et al., 2008). The expansion is to non-traditional growing areas such as Central Kenya, Rift Valley, and Western Kenya (Freeman et al., 1999; ICRISAT, 2006/7). However, production trends between 2002 and 2009 (Fig 2) show stagnation for yield, area and seed sales with a decline in 2008-2009. The decline was attributed to prolonged drought that led to crop failures and low yield.



Pigeonpea is an important source of income for farmers. The crop also improves long-term soil quality and fertility when used as green manure, cover or alley crop (Onim et al., 1990). The legume also has the ability to reduce the level of root-knot nematodes in the succeeding crop when used as green manure (Daniel and Ong, 1990). It is a valuable source of protein with a content that ranges between 18-26% (Swaminathan and Jain, 1972), while up to 30% has been reported in other closely related *Cajanus* spp (Reddy et al., 1979). Pigeonpea is also widely used as fodder and feed for livestock (Rao et al., 2002). Tall perennial pigeonpea is often used as live fences, windbreaks and in soil conservation.

Pigeonpea yields in Kenya range between 500 to 800 kg ha⁻¹ (ICRISAT, 2006/7), although potential yields of 3,000 kg ha⁻¹ have been recorded on research plots (Omanga et al., 1995). The low yields have been attributed to lack of cultivars with superior agronomic traits, poor production practices such as low plant densities, low soil fertility, insufficient weeding and application of pesticides and herbicides (Gwata et al., 2006; Onim, 1981). In addition, marketing, institutional and policy failures are also constraints to expanded production (Chauhan et al., 1998). However, the most important limiting factors in pigeonpea production in Kenya are; lack of quality seed in sufficient quantities, lack of a structured seed provision and distribution mechanism and fusarium wilt caused by *Fusarium udum* (L.) Millsp. (Freeman et al., 1999; Jones et al., 2001).

Pigeonpea breeding

Over the years, desired traits in pigeonpea have been selected by farmers from landraces to suit their production systems and uses. High stable yield, with acceptable grain quality, are the major breeding objectives of pigeonpea. Through the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), research focused on early maturity with relatively low sensitivity to photoperiod and temperature interactions, and tolerance to biotic and abiotic stress (Omanga et al., 1995; Singh et al., 2003). Other objectives focused on breeding pigeonpea for specific production systems; special traits such as suitability for vegetable products and fodder; high protein content for the animal industry; suitability for processing and canning; the milling quality for split peas (dhal); and market preferences (Singh et al., 1990). Gwata et al. (2006) released pigeonpea variety, ICEAP00040 that is resistant to fusarium wilt.

Breeding programmes in Kenya have focused on high stable yields, resistance to wilt (Odeny et al., 2009) and leaf spot diseases, adaptability to intercropping systems, appropriate maturity period, and market preferences (seed size and colour) (ICRISAT, 2007). Collaborative research work between ICRISAT, Kenya Agricultural Research Institute (KARI) and the University of Nairobi developed and tested a number of short, medium, and long duration pigeonpea varieties, some of which have been released (Silim, 2001). These varieties have been found to be higher yielding and resistant to fusarium wilt as compared to the locally adapted landraces. Other promising varieties, some of which are already being promoted and grown by farmers are ICEAP00068, ICEAP554, ICEAP557 and ICP6927 for medium maturity and ICEAP00020 and ICEAP00053 for late maturity.

Development of hybrids based on cytoplasmic male sterility

Cytoplasmic male sterility (CMS) is a maternally inherited condition and is a phenomenon observed in more than 150 plant species; (Akagi et al., 1995; Murai et al., 2002). It can arise spontaneously in breeding lines, induced by mutagens, or can be the result of interspecific, intraspecific and intergeneric crosses (Hanson and Conde, 1985; Hanson and Bentolila, 2004). Cytoplasmic male sterility is widespread among higher plants and is commonly used by plant breeders for hybrid seeds production (Kaul, 1988). Most extensive use has been in maize, sorghum, pearl millet, sunflower, and sugar beets. Maize and sorghum were the first major field crops in which cytoplasmic male sterility:fertility restorer gene system was used for commercial production of hybrid seed (Kempken and Pring, 1999).

Commercial production of pigeonpea hybrid seeds became possible with the discovery of genetic male sterility (Reddy et al., 1978; Saxena et al., 1983). However, this type of male sterility required roguing 50% of the normal male fertile plants from the female rows in the hybrid seed production blocks, an expense that hindered adoption of the technology. This was overcome after the identification of CMS from crosses between cultivated types as male parents with their wild relatives as female parent (Mallikarjuna and Saxena, 2005). The most stable CMS system was identified in a *C. cajanifolius* x *C. cajan* cross (Saxena et al., 2005). Studies revealed that in both male sterile as well as the fertile floral buds, meiosis proceeded normally till the tetrad stage. However, in the male sterile genotypes the pollen mother cell (PMC) wall did not dissolve to release the tetrads unlike in the fertile genotypes and this major event was found to be responsible for male sterility (Mallikarjuna and Saxena, 2005). In India several male sterile lines have been developed that can be used in production of commercial hybrids (Pandey and Singh, 1998; Saxena et al., 2006). Experimental hybrids have demonstrated a yield advantage of over 25% and 50% hybrid vigour for seed yield (Saxena et al., 2010). The utilization of this technology has a potential in Kenya to improve and increase pigeonpea production.

Research approach

Yields of pigeonpea in Kenya have remained low despite the availability of improved varieties. Jones et al. (2001) reported that in pigeonpea value chain, stakeholder collaboration (farmer, extension officer, researcher, private sector, market player, policymakers) is largely lacking. In this study, the pigeonpea stakeholders and their interrelationships were analysed in order to give direction to a pigeonpea hybrid development programme for Kenya. Involving both the

formal and informal sectors will enable identification of the entry points for introduction of pigeonpea hybrids and setting up of a breeding programme targeting preferred traits.

Pigeonpea is the first food legume where commercial hybrids have been developed (Stakstad, 2007). The first stable CMS system in the crop was derived from a *Cajanus cajanifolius* x *Cajanus cajan* cross and has been used to breed hybrid pigeonpea seed in India (Saxena et al., 2006). The hybrids gave yields ranging from 2.7 tons ha⁻¹ to 3.4 tons ha⁻¹ with 20-60% superiority over the controls recorded (Saxena et al., 2006). In India the CMS system was found to be stable across environments ranging from 15°N to 28°N. India and Kenya have similar climatic conditions and hence the potential exists for adoption of this technology in Kenya. Stability for sterility of the CMS systems sourced from India was tested under Kenyan conditions. The stable CMS lines were crossed with superior local male parents to produce pigeonpea hybrids which were subsequently tested across environments for grain yield. The hybrids were also tested in a fusarium wilt infested soil in order to establish their reaction to this major disease in Kenya.

Thesis structure

The thesis consists of five chapters starting with a literature review that summarizes aspects of origin, taxonomy, uses, constraints, breeding techniques of pigeonpea, and identifies research gaps. Chapter 2 analyses and reviews the pigeonpea value chain in Kenya with special emphasis on stakeholders involved, markets and marketing channels. A brief description of the Indian market is also included as it is a potential market for pigeonpea hybrid products. Chapter 3 discusses evaluation of cytoplasmic male sterile lines in different environments for stability and also characterization of the yield components. Chapter 4 covers hybrid genotype screening for resistance to fusarium wilt isolates under field and screen house conditions. Methodologies used for isolation and identification of the isolates and subsequent screening of genotypes are provided. Chapter 5 discusses performance of hybrids in diverse environments for yield and earliness. Finally an overview on how the objectives were met and implications for research findings are given.

Research objectives

The overall objective of this research was to introduce CMS technology and breed pigeonpea hybrids resistant to fusarium wilt for increased productivity and improved income for the resource poor farmers in Kenya.

Specific research objectives

The specific objectives of the research were to:

- Undertake stakeholder analysis to identify the interrelationships between pigeonpea value chain role players.
- Evaluate CMS systems under Kenyan conditions for stability
- Screen pigeonpea hybrids for resistance to *F. udum* isolates
- Evaluate pigeonpea hybrids in different environments for yield and earliness.

Referencing follows the American Crop Science Journal format. The thesis is structured in such a way that the chapters are in the form of research articles. Therefore, there could be a certain amount of overlap among the chapters.

References

- Akagi, H.A., A. Nakamura, R. Sawada, and T. Fujimura. 1995. Genetic diagnosis of cytoplasmic male-sterile hybrid plants of rice. *Theoretical and Applied Genetics* 90:948-951.
- Chauhan, Y.S., D.H. Wallace, C. Johansen, and L. Singh. 1998. Genotype-by-environment interaction effect on yield and its physiological bases in short-duration pigeonpea. *Field Crops Research* 59:141-150.
- Daniel, J.N., and C.K. Ong. 1990. A multipurpose species for agroforestry systems. *Agroforestry Systems* 10:113-129.
- FAOSTAT. 2011. Pigeonpea agricultural production statistics for Kenya [Online]. Available by www.faostat.org (verified 21 September 2011).
- Freeman, H.A., R.B. Jones, S.N. Silim, and M.A. Ochere. 1999. The pigeonpea subsector in Kenya. ICRISAT (limited distribution), Nairobi.
- Gwata, E.T., S.N. Silim, and M. Mgonja. 2006. Impact of a new source of resistance to fusarium wilt in pigeonpea. *Phytopathology* 154:62-64.
- Hanson, M.R., and M.F. Conde. 1985. Functioning and variation of cytoplasmic genomes: lessons from cytoplasmic-nuclear interactions conferring male sterilities in plants. *International Review of Cytology* 94:213-267.
- Hanson, M.R., and S. Bentolila. 2004. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell* 16:154-169.
- Hillocks, R.J., E.M. Minja, A. Mwaga, M.S. Nahdy, and P. Subrahmanyam. 2000. Diseases and pests in pigeonpea in Eastern Africa: A review. *International Journal of Pest Management* 46:7-18.
- ICRISAT. 2006/7. Pigeonpea improves livelihoods: Diversification of pigeonpea genetics to enhance productivity in Eastern and Southern Africa. ICRISAT, Nairobi.
- ICRISAT. 2007. Improved pigeonpea hybrid parents for increased and stable production. ICRISAT India, Patancheru, 502 324, Andhra Pradesh, India.

- Jones, R., H.A. Freeman, and G. Lo Monaco. 2002. Improving the access of smallholder farmers in Eastern and Southern Africa to global pigeonpea markets. *Agricultural Research and Extension Network* 120:1-11.
- Jones, R.B., P. Audi, and R. Tripp. 2001. The role of informal seed systems in disseminating modern varieties. The example of pigeonpea from a semi-arid area of Kenya. *Experimental Agriculture* 37:539-548.
- Kaul, M.L.H. 1988. Male sterility in higher plants. Springer-Verlag, New York, USA.
- Kempken, F., and D.R. Pring. 1999. Male sterility in higher plants: fundamentals and applications. *Botany* 60:139-166.
- Kimani, P.M., A.B. Nyende, and S.N. Silim. 1994. Development of early maturing fusarium wilt resistant pigeonpea cultivars. *African Crop Science Journal* 2:35-41.
- Mallikarjuna, N., and K.B. Saxena. 2005. A new cytoplasmic nuclear male-sterility system derived from cultivated pigeonpea cytoplasm. *Euphytica* 142:143-148.
- Mergeai, G., P. Kimani, A. Mwang'ombe, F. Olubayo, C. Smith, P. Audi, J.P. Baudoin, and A. Roi. 2001. A survey of pigeonpea production systems, utilization and marketing in semi-arid lands of Kenya. *Biotechnology, Agronomy, Society and Environment* 5:145-153.
- Murai, K., S. Takumi, H. Koga, and Y. Ogihara. 2002. Pistillody, homeotic transformation of stamens into pistil-like structures, caused by nuclear-cytoplasm interaction in wheat. *Plant Journal* 29:169-181.
- Odeny, D.A., S.M. Githiri, and P.M. Kimani. 2009. Inheritance of resistance to fusarium wilt in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Journal of Animal and Plant Sciences* 2:89-95.
- Omanga, P.A., R.J. Summerfield, and A. Qi. 1995. Flowering of pigeonpea (*Cajanus cajan* L.) in Kenya: Responses of early maturing genotypes to location and date of sowing. *Field Crops Research* 41:25-34.
- Onim, J.E.M. 1981. Pigeonpea improvement research in Kenya, pp. 427-436, *In* ICRISAT et al. (Eds.) *Proceedings of the international workshop on pigeonpeas*, Vol. 1. ICRISAT, Patancheru 502 324, Andhra Pradesh, India.

- Onim, J.E.M., M. Mathuva, K. Otieno, and H.A. Fitzhugh. 1990. Soil fertility changes and response of maize and beans to green manures of leucaena, sesbania and pigeonpea. *Agroforestry Systems* 12:197-215.
- Pandey, N., and N.B. Singh. 1998. Stability for seed yield in pigeonpea hybrids. *Legume Research* 21:233-235.
- Rao, S.C., S.W. Coleman, and H.S. Mayeux. 2002. Forage production and nutritive value of selected pigeonpea ecotypes in the southern Great Plains. *Crop Science* 42:1259-1263.
- Reddy, B.V.S., J.M. Green, and S.S. Bisen. 1978. Genetic male sterility in pigeonpea. *Crop Science* 17:362-364.
- Reddy, L.J., J.M. Green, B. S.S., U. Singh, and R. Jambunathan. 1979. Seed protein studies on *Cajanus cajan* (L.), *Atylosia* spp, and some hybrid derivatives. *Seed Protein Improvement in Cereals and Grain Legumes* 2:105-117.
- Saxena, K.B., D.E. Byth, E.S. Wallis, and I.S. Dundas. 1983. Genetic basis of male sterility in pigeonpea. *International Pigeonpea Newsletter* 5:21-23.
- Saxena, K.B., R.V. Kumar, N. Srivastava, and B. Shiyang. 2005. A cytoplasmic-nuclear male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. *Euphytica* 145:289-294.
- Saxena, K.B., R.V. Kumar, K.M. Latha, and V.A. Dalvi. 2006. Commercial pigeonpea hybrids are just a few steps away. *Indian Journal of Pulses Research* 19:7-16.
- Saxena, K.B., R. Sultana, N. Mallikarjuna, R.K. Saxena, R.V. Kumar, S.L. Sawargaonkar, and R.K. Varshney. 2010. Male sterility systems in pigeonpea and their role in enhancing yield. *Plant Breeding* 129:125-134.
- Shiferaw, B., J. Okello, G. Muricho, J. Omiti, S.N. Silim, and R. Jones. 2008. Unlocking the potential of high value legumes in the semi-arid regions: Analyses of the pigeonpea value chains in Kenya. International Crops Research Institute for the Semi-Arid Tropics, Nairobi, Kenya.
- Silim, S.N. 2001. Strategies and experiences in pigeonpea variety and development for Eastern and Southern Africa, pp. 232. *In* S. N. Silim, et al. (Eds.) Status and potential of

- pigeonpea in eastern and southern Africa: Proceedings of a regional workshop, 12-15 Sept 2000. ICRISAT, Nairobi, Kenya.
- Silim, S.N., R. Coe, P.A. Omanga, and E.T. Gwata. 2006. The response of pigeonpea genotypes of different duration types to variation in temperature and photoperiod under field conditions in Kenya. *Journal of Food Agriculture and Environment* 4:209-214.
- Singh, M., R.S. Malhotra, S. Ceccarelli, A. Sarker, S. Grando, and W. Erskine. 2003. Spatial variability models to improve dryland field trials. *Experimental Agriculture* 39:1-10.
- Singh, U., R. Jambunathan, K.B. Saxena, and N.C. Subrahmanyam. 1990. Nutritional quality evaluation of newly developed high-protein genotypes (*Cajanus cajan* L.). *Journal of the Science of Food and Agriculture* 50:201-209.
- Stakstad, E. 2007. The plant breeder and the pea. *Science* 316:196-197.
- Swaminathan, M.S., and H.K. Jain. 1972. Food legumes in Indian agriculture, pp. 69-82, *In* M. M. Milner (Ed.) *Nutritional improvement of food legumes by breeding*. FAO, Rome.
- Troedson, R.J., E.S. Wallis, and L. Singh. 1990. Pigeonpea adaptation, pp. 159-177, *In* Y. L. Nene, et al. (Eds.). *The Pigeonpea*. CABI, Wallington, UK.

Chapter 1: Literature review

1.1 Introduction

This literature review provides information relevant to the research focus of this thesis and aims to lay the theoretical foundation for the research. Consequently, it seeks to a) describe pigeonpea in terms of origin, morphology, distribution, importance and constraints, b) review breeding methods and mating designs that are principally used for pigeonpea breeding and c) provide background information on breeding hybrids using cytoplasmic male sterility. Thus, this review creates a frame of reference for the study of the potential of pigeonpea hybrid technology using cytoplasmic male sterility under Kenyan climatic conditions.

1.2 Taxonomy of pigeonpea

The taxonomy of pigeonpea was described and revised by Van der Maesen (1990). The plant belongs to the sub-tribe Cajaninae of the tribe Phaseoleae under sub-family Papilionoideae of the family Leguminosae. Among the members of Phaseoleae, Cajaninae is distinguished by the presence of vesicular glands on the leaves, calyx and pods. The genus *Atylosia* closely resembles the genus *Cajanus* in vegetative and reproductive characters and was later merged following systematic analysis of morphological, cytological and chemo-taxonomical data which indicated the congenicity of the two genera.

Based on growth habit, leaf shape and hairiness, nature of corolla, pod size, and strophiole characteristics, Van der Maesen (1990) grouped 32 *Cajanus* species into six sections. Three *Cajanus* species have been further sub-divided into botanical varieties; *C. scarabaeoides* into var. *pedunculatus* and var. *scarabaeoides*, *C. reticulatus* into var. *grandifolius*, var. *reticulatus*, and var. *maritimus*, and *C. volubilis* into var. *burmanicus* and var. *volubilis*. Recently, PCR-based marker technologies, such as restriction fragment length polymorphism of both nuclear and mitochondrial DNA, and randomly amplified polymorphic DNA, have been successfully used to detect genetic diversity and determine phylogenetic relationships in the genus *Cajanus* and other related genera (*Dunbaria*, *Eriosema*, and *Rhynchosia*) (Nandimpalli et al., 1994; Choudhury et al., 2008; Malviya and Yadav, 2010). Studies detected sufficient polymorphisms among the species to resolve in-group taxa into distinct clusters.

1.3 Origin and distribution of *Cajanus cajan*

The true origin of pigeonpea is still disputable. Most of the evidence point to India as the place where pigeonpea originated because of the presence of several wild relatives, large diversity of the crop gene pool, ample linguistic evidence, a few archaeological remains, and the wide usage in daily cuisine (Van der Maesen, 1990). According to Van der Maesen (1990), old world distribution of the genus *Cajanus* includes 18 species in Asia, 15 in Australia, and one in Africa. Of these, 13 are endemic to Australia, eight to Indian sub-continent and Myanmar, and one to West Africa. The rest occur in more than one country. Several species such as *C. villosus*, *C. elongatus*, *C. granadiflorus*, and *C. niveus*, which were earlier collected from or known to occur in northeastern India, are either rare or have become extinct.

The greatest diversity of wild species of *Cajanus* is found in Myanmar, Yunnan-China, and northern Australia (Sharma and Green, 1980). Apart from pigeonpea, only one wild species, *C. scarabaeoides*, is common and widespread throughout South and Southeast Asia, the Pacific Islands, and Northern Australia. *Cajanus cajan* is the only domesticated species under Cajaninae. This indicates that the crop was most likely introduced into East Africa from India by immigrants in the 19th century who moved to Africa to become railway workers and storekeepers.

1.4 Uses of pigeonpea

Pigeonpea uses are many and diverse. It offers the benefits of improving long-term soil quality and fertility when used as green manure, cover or alley crop (Onim et al., 1990). Pigeonpea has been used successfully under coffee plantations as a cover crop to improve soil properties, reduce weed competition as well as act as a food source for predators (Venzon et al., 2006). Maize yields have been increased by 32.1% in West Africa by using pigeonpea as a cover crop for rotation (Sogbedji et al., 2006). Pigeonpea is used in alley cropping and, being perennial, can be ratooned (Sharma and Green, 1977) successfully for subsequent crops in no-till production systems (Lal et al., 1978). It also reduces the levels of root-knot nematodes in the succeeding crop when used as green manure (Daniel and Ong, 1990). Other than transferring up to 40 kg N ha⁻¹ fixed nitrogen to the inter-planted crop, pigeonpea has the ability to bring minerals from deeper soil horizons to the surface, as well as improving soil air circulation (Kumar Rao et al., 1983) to the benefit of the accompanying crop. There is also the potential benefit of using pigeonpea in the control of *Striga* (*Striga* spp.) weed, which is a major problem in Africa. Rotation with pigeonpea in *Striga* infested soils of western Kenya showed pigeonpea

as one of the most productive crops, with a remarkable decrease of *Striga* populations in maize planted after pigeonpea (Oswald and Ransom, 2001).

Nutritionally, pigeonpea contains more minerals, ten times more fat, five times more vitamin A and three times more vitamin C than ordinary peas (Faris and Singh, 1990). The protein content of commonly grown pigeonpea has been reported to range between 18-26% (Swaminathan and Jain, 1972), while up to 30% has been reported in other closely related *Cajanus* spp. (Reddy et al., 1979).

Pigeonpea is also widely used as fodder and feed for livestock (Rao et al., 2002). The seeds are used as animal feed (Wallis et al., 1986) and its fodder has been demonstrated to increase the intake of low quality herbage resulting in high animal live mass (Karachi and Zengo, 1998). By-products of split and shriveled seeds are used as livestock feed and as an inexpensive alternative to high cost animal feed sources such as bone meal and fish meal (Phatak et al., 1993). Pigeonpea seed has been recommended as an alternative to maize and soybean meal, or groundnut cake in the diets of broilers, pullet chicks and layers in Nigeria (Onu and Okongwu, 2006).

Ease of establishment and the simultaneous crop production for food makes perennial pigeonpea a special agro-forestry option in several parts of Africa (Kwesiga et al., 2003) such as Zambia (Boehringer and Caldwell, 1989). These tall perennial pigeonpea types are also favoured for use as fuel wood, basket weaving, and roofing.

Pigeonpea is an important source of income for farmers as export markets are reportedly the key outlets for pigeonpea commercialization in Africa (Lo Monaco, 2003). A large market exists regionally and internationally for a range of processed pigeonpea products from Africa (Jones et al., 2002). Pigeonpea from Eastern and Southern Africa has been exported to India for at least three decades (Jones et al., 2002). A study carried out by ICRISAT (2007) reported that the demand for processed pigeonpea products on the local, regional and export markets in Asia, North America and Europe outstrips supply. India is by far dominating the international market but there is also demand in the European Union, North America and the Middle East provided certain quality and quantity requirements are met (Kunde, 2000). For example, Indian millers prefer medium-grained varieties, while their European counterparts choose large-sized grains.

1.5 Constraints to pigeonpea production

Constraints to pigeonpea production include photoperiodic sensitivity, diseases and pests. Other constraints include; lack of cultivars with superior agronomic traits, and marketing, institutional and policy shortcomings (Chauhan et al., 1998). Poor production practices such as low plant densities, low soil fertility, insufficient weeding and lack of application of pesticides and herbicides are other constraints (Onim, 1981).

There are no known pigeonpea cultivars that are truly photoperiodic-insensitive but the degree of sensitivity varies. Duration from sowing to flowering is the most important trait influencing adaptation in pigeonpea. Studies by McPherson et al. (1985) established that the rate of development from sowing to flower bud initiation (FBI) varied among cultivars. The responses to temperature were all strongly curvilinear with optima between 20 and 24°C. They also observed that the effect of daylength on the rate of development was the greatest between sowing and FBI, with the greatest sensitivity between 12 and 14 h. According to Mohamed and Ariyanayagam (1983) both temperature and daylength had substantial effects over the range 16-32°C and 10-14 h respectively. Comparisons within the 12 h daylength treatment showed no consistent photoperiod sensitivity pattern with mean temperature.

Pigeonpea can be attacked by more than 210 pathogens (Kannaiyan et al., 1984) that include fungi, bacteria, viruses, nematodes, and mycoplasma-like organisms. Fusarium wilt (*Fusarium udum* Butler), sterility mosaic disease (SMD), leaf spots and to a lesser extent powdery mildew (*Leveillula taurica* Arnaud.) were identified as the most important diseases (Hillocks et al., 2000) distributed in many pigeonpea growing areas, but more important in India and Eastern Africa (Marley and Hillocks, 2002). In Eastern Africa, (Kannaiyan et al., 1984) noted 16% plant stand reduction, and 60% incidences of fusarium wilt, leading to annual losses of US\$ 5 million. Recently, this pathogen was reported to be spreading to Southern Africa reaching Mozambique (Gwata et al., 2006).

Pigeonpea is attacked by an array of insects but the most damaging are those attacking the flowers, pods and seeds (Shanower et al., 1999). Important field insect pests in this region include the podboring Lepidoptera (*Helicoverpa armigera* Hübner, *Maruca vitrata* Geyer and *Etiella zinkenella* Treitsche), podsucking bugs (*Clavigralla tomentosicollis* Stål and *Clavigralla horrida* Germar) and podfly (*Melanagromyza chalcosoma* Spencer) (Minja et al., 2000). Podborers and pod suckers are the most important pests with damage ranging between 10-98% in some seasons (Reed and Lateef, 1990). Pre-harvest infestation by bruchids (*Callosobruchus*

maculatus F. and *Callosobruchus chinensis* L.) may cause only limited damage in the field, but can have serious implications during storage (Minja et al., 2000). Lack of proper storage facilities and inappropriate dehulling methods (Agona and Muyinza, 2005) worsen the storage pest problem by enhancing cross-contamination.

Most farmers in Kenya grow landraces that have the preferred seed size, taste and tolerance to diseases and pests, but are late maturing, taking up to 11 months to maturity and are about 2 m tall with low yields averaging 0.4 ton ha⁻¹. The late maturing varieties delay field preparation for the next crop, causing farmers to practice wide spacing to allow sowing of other early maturing crops, thus contributing to low yields.

Farmers in Kenya lack access to market information, and their small scattered units of production (Agona and Muyinza, 2005) make it difficult to form valid associations that would help with collective bargaining (Rusike and Dimes, 2006). They therefore end up being price takers in a highly volatile market with the result that they get the least share of the final consumer prices. The Indian market is also becoming increasingly inaccessible for African exporters especially with the increasing exports from Myanmar (Lo Monaco, 2003). The higher transaction costs, inferior quality from some producers, and lack of incentives to the African producers compared to their competitors are to blame (Freeman and Jones, 2001).

Availability of sufficient and pure line quality seed of pigeonpea has been hampered by the out-crossing nature of the crop in the field. During seed multiplication, out-crossing cannot be prevented, which in turn means that the characteristics of the crop are not stable and a pure line will degenerate into a population if seed is not produced in isolation. Study on the adoption of a modern varieties in Kenya indicated that the demand for seed is higher than supply, meaning the deficit could be met by the formal seed sector (Jones et al., 2000). However, there is little interest from seed companies to market open-pollinated pigeonpea seeds.

1.6 Fusarium wilt

Of the diseases attacking pigeonpea, fusarium wilt caused by *F. udum* is the most important in the Indian sub-continent and eastern Africa (Gwata et al., 2006; Reddy et al., 1990). It is a soil-borne fungus that causes wilting, mainly during pod-filling stage and can also lead to seed infection (Haware and Kannaiyan, 1992). The disease causes up to 100% yield loss. The

pathogen survives on crop debris and also in the soil as spores, hyphae or as saprophyte for up to three years.

Physiological races of *F. udum* have been reported by several workers (Kiprop et al., 2002; Reddy and Raju, 1997). Changes in cultural and morphological characteristics and increased virulence have been used to characterize these physiological races. Morphological variability appears as patches in the parent colony, loss of aerial mycelium, increased mycelia production, or increased pigmentation. Pathogenic variability leads to increased virulence (Kiprop et al., 2002) with genotypes responding differently in different sites.

Cultural, chemical and biological control measures exist for control of *F. udum* (Patel et al., 2011). Because it is a soilborne pathogen following, rotation, intercropping, and nitrogen application reduce or suppress soil inoculum. However, the most practical option is use of resistant varieties in an integrated pest management system.

1.7 Pigeonpea breeding techniques

The commonly used breeding methods in any self-pollinated crop are applicable to pigeonpea, even though a considerable amount of out-crossing occurs in the species. Varietal improvement methods based on pedigree, bulk pedigree, back-crossing and multiple-crossing techniques have been useful in recombining simply inherited characters such as disease resistance, seed size and colour, and maturity period (Singh et al., 1990). Pedigree selection has been useful in breeding for highly heritable traits such as disease resistance, seed size, seed colour, growth habit, and seed number per pod (Green et al., 1981). Diallel and Line x Tester mating schemes, using three or more well adapted cultivars as testers, have been used to determine the combining ability of parents (Omanga et al., 1995; Singh et al., 1990). Bulk hybrid advance by single-pod descent, and single-seed descent, have proven successful in breeding for high yielding lines (Kimani et al., 2003).

Stratified mass selection and mass selection with progeny testing have been tried in Kenya for yield gains (Omanga et al., 1995) and in India to estimate heritability and genetic advance (Singh et al., 2003). Mass selection, selfed progeny selection, and half-sib progeny selection, have been used to estimate genotypic and phenotypic variance, heritability, and genetic advance for some yield traits (Singh et al., 2003). Singh et al. (1990) suggested recurrent

selection and population breeding methods as ways to accumulate desirable genes and facilitate the breaking of linkages in pigeonpea and other self-pollinated species.

1.8 Heterosis in pigeonpea

Hybrid vigour in pigeonpea was first reported by Solomon et al. (1957). Exploitation of wild relatives of pigeonpea have had an impact on broadening the genetic base of variation and introduction of useful biotic, abiotic and agronomic traits (Nalini et al., 2011). Saxena et al. (1987) and Sujana et al. (2008) derived lines from *C. sericeus*, *C. albicans* and *C. scarabaeoides* that had high seed weight, and resistant to podborer (*Heliothis armigera* Hub.). Reddy et al. (1997) developed ICPL 87162 from *C. cajan* x *C. scarabaeoides* with protein content ranging from 30 to 34% compared 23% in the control. The line was also resistant to sterility mosaic disease. Saxena et al. (1983) reported some experimental hybrids out-yielding the best control cultivar by 100% with hybrids having more branches and pods as compared to the controls. Work by Saxena and Rajni (2001) concludes that all hybrids exhibited positive heterosis for plant height, seeds pod⁻¹ and seed yield. Further work by Patel et al. (1997) who studied 60 hybrids involving genetic male sterile lines and short, medium duration varieties showed that the hybrids significantly exceeded their parents for harvest index. Srinivas et al. (1999) indicated 24% hybrid vigour in pigeonpea, although the best hybrid did not out-yield the best parent. The study further reported a mean heterosis of 39% for yield. In general early x late and medium x late maturity crosses resulted in high heterosis for seed yield. A high number of branches had more plant spread and more pods per raceme. Hybrid vigour for seed size was reported by several authors (Jag, 1985; Saxena et al., 2006; Sharma and Green, 1980).

1.9 History of pigeonpea hybrid breeding

Commercial pigeonpea hybrid production became possible with the discovery of a genetic male sterile mutant by the International Crops Research Institute for Semi-Arid Tropics (ICRISAT) scientists (Reddy et al., 1978; Saxena et al., 1983). Later Saxena and Sharma (1983) reported another source of stable GMS system, characterized by brown shriveled and arrowhead anthers. The GMS lines of different maturity were used to develop commercial hybrids, releasing the world's first pigeonpea hybrid ICPH 8 in 1991 that exhibited 41% heterosis (Saxena et al., 1992). However, genetic male sterility required roguing of 50% of the normal fertile plants at flowering from the female rows in the hybrid seed production blocks. Lower plant population reduced the amount of seed that could be produced per unit area; labour for roguing were expensive that hindered adoption of the technology. This was overcome after the

identification of cytoplasmic male sterility (CMS). Studies to assess the effectiveness of natural cross-pollination in setting pods on the male-sterile plants, extents of heterosis in different maturity groups and seed production cost of hybrid seeds and their parents, indicated that under field conditions sufficient levels of cross-fertilization occurs, and large quantities of seed could be produced using male-sterile populations (Saxena et al., 2010).

1.10 Cytoplasmic male sterility

Cytoplasmic male sterility is a maternally inherited condition in which a plant is unable to produce functional anthers or pollen (Warmke and Lee, 1978) and is as a result of failure of formation or development of functional stamens, microspores or gametes (Hanson and Bentolila, 2004; Horner and Palmer, 1995; Linke and Borner, 2005). In some instances, CMS results from homeotic changes where stamens are converted to petals, which are non-reproductive, or to carpels. Cytoplasmic male sterility can arise spontaneously, induced by mutagens, or through interspecific, intraspecific and intergeneric crosses (Hanson and Conde, 1985; Hanson and Bentolila, 2004). For example, CMS-WA (wild abortive) rice was developed in indica rice cultivars from a male sterile plant found in a natural population of the wild rice *Oryza rufipogon* Griff (Hayashi et al., 1988). Texas cytoplasm in maize arose spontaneously in a breeding line, and CMS-PET1 cytoplasm of sunflower arose from an interspecific cross between *Helianthus petiolaris* Nutt. and *H. annuus* L. (Roath et al., 1981).

1.11 Genetics of cytoplasmic male sterility

Cytoplasmic male sterility is controlled by factors in the cytoplasm, but may be influenced by the genes in chromosomes. It has been observed in more than 150 plant species, and has been studied in maize, sorghum, petunia, sunflower, radish, rice, beans, brassica, tobacco, pigeonpea, and wheat (Akagi et al., 1995; Andersen, 1963; Bino, 1985). It is based on changes in the mitochondrial (mt) chromosomal DNA structure and gene expression as influenced by nuclear genes (Kempken and Pring, 1999). Alloplasmic male sterility results from interspecific and intergeneric crosses, due to incompatibility between the nuclear genome of the male and the mitochondrial genome of the female parents (Lonsdale, 1987). The combination of alien cytoplasmic and nuclear genomes leads to mutations and disturbances in the mitochondrial genome, and/or reveals the expression of abnormal mitochondrial genes, the detrimental effect of which is not suppressed by nuclear genes present in the original maternal species, thus resulting in CMS (Hanson and Conde, 1985)

The abnormal development of the tapetum is a prominent feature of the CMS phenotype in plants (Bino, 1985; Warmke and Lee, 1978). The most pronounced is the differentiation of the pollen mother cells (PMC) and involves its abnormal vacuolisation, fusion of cells into multinuclear syncytia, and disturbances in the time of the programmed tapetum death (Horner and Palmer, 1995). Since the tapetum provides nutrition and materials for the formation of the complex pollen wall (Schnable and Wise, 1998), its development and function plays a critical role in microsporogenesis, and aberrations in these can lead to male sterility. Tapetal cells undergo developmentally regulated lysis, the timing of which is critical for pollen development and when it occurs too soon causes pollen abortion as in genetically engineered plants (Kapoor et al., 2002). Verulkar and Singh (1997) obtained spontaneously a male sterile UPAS 120 plant, which had white translucent anthers with complete pollen sterility.

Studies at the molecular level revealed the presence of CMS-associated chimeric genes resulting from mtDNA rearrangements in sterile cytoplasm. The CMS-associated loci demonstrate common features that are composed of copies or portions of coding regions of known mt genes and/or of unidentified sequences. However, the genetic structures of the CMS loci studied to date differ (Hanson, 1991). They often have ATP synthase sub-unit sequences and genes located within or near the CMS-associated loci as seen in the cytoplasm of petunia, maize S and T, brassica, rice, sorghum, tobacco, sunflower PET-1 and arabidopsis. The coding regions of the cytochrome oxidase sub-unit gene have also been implicated in CMS-associated loci in wheat, rice, and petunia, while the coding regions of the nad sub-unit gene were found in petunia, *brassica nap* and four sterile cytoplasm.

1.12 Cytoplasmic male sterility in pigeonpea

The first attempt to develop a stable CMS system was made by crossing a wild relative of pigeonpea (*Cajanus scarabaeoides*) with a cultivated type (Reddy and Faris, 1981). The male sterile plants derived from this cross were found to have female sterility also. Ariyanayagam et al. (1995) crossed another wild relative of pigeonpea (*Cajanus sericeus*) with a short duration advanced breeding line of pigeonpea. The F₁ plants were partially male sterile and the backcross populations were found segregating for male sterility. The reversion of some male-sterile plants to male fertility or partial male fertility further complicated the selection and stabilization of this trait. Intensive selection in the five subsequent backcross generations, however, resulted in the identification of a promising CMS line (Saxena and Kumar, 2003). Saxena et al. (2005) developed a stable CMS system using *C. cajanifolius* and a cultivated

variety, ICP11501. Using this genetic material, designated as the A₄ cytoplasm, a number of fertility restorers and maintainers were developed. The male sterile plants in this material showed no morphological deformity and produced plenty of pollen grains in hybrid combinations with fertility restoring lines and the frequency of fertility restorers in this CMS source was higher than those of others.

So far five primary CMS systems derived from various inter-specific crosses have been reported in pigeonpea. These are designated as (i) A₁ cytoplasm, derived from *C. sericeus* (Ariyanayagam et al., 1995); (ii) A₂ cytoplasm, derived from *C. scarabaeoides* (Saxena and Kumar, 2003); (iii) A₃ cytoplasm, derived from *C. volubilis* (Wanjari et al., 2001); (iv) A₄ cytoplasm derived from *C. cajanifolius* (Saxena et al., 2005), and (v) A₅ cytoplasm derived from cultivated pigeonpea (Mallikarjuna and Saxena, 2005). Of these, the CMS systems with A₂ and A₄ cytoplasm have been found to be stable and are being used in the hybrid breeding programmes in India.

1.13 Pollen morphology of male sterile pigeonpea

CMS plants usually appear normal, vigorous, and undistinguishable from the fertile analogue (Hanson and Conde, 1985). The main effect of CMS is revealed in the development of anthers and pollen, and leads to pollen abortion. Other changes may affect floral morphology and color. Premature degeneration of the tapetum at the early to mid uninucleate microspore stage leads to the development of non-viable pollen (Roberts et al., 1995).

The CMS plants of pigeonpea are identifiable by the pollen morphology. There are those with translucent anthers, which result from the non-separation of tetrads (Reddy et al., 1978) and others with brown, shriveled, arrowhead shaped anthers devoid of pollen (Dundas et al., 1981). Five male-sterile variants were reported in pigeonpea (Reddy et al., 1978). Ordinary male steriles were found to have small pale-yellow anthers, apparently empty scale with some plants showing partial pollen fertility. The long-styled types had longer style than stamen and possessed a groove on the bud that did not occur on the normal type. The short-styled type had the stigma enclosed inside the staminal column. The style was shorter than normal without affecting the length of stamens and showed partial pollen sterility. Incomplete short-styled types had both normal and short-styled flowers and pollen sterility ranged from 5-60%. A natural male-sterile mutant found in a population of ICPL 85010, was found to be characterized by light yellow anthers of reduced size that were devoid of pollen grains (Saxena and Kumar, 2003). Progenies of cultivated pigeonpea and its wild relatives (*Cajanus acutifolius*) as the pollen donor

gave pale-white anthers with shrunken growth devoid of pollen grain (Mallikarjuna and Saxena, 2005).

1.14 Induction of male sterility in pigeonpea

Studies have been reported in which chemical mutagens were used to induce male sterility in diverse pigeonpea genotypes that resulted in complete male sterility to varying degree of pollen sterility. Kaul (1988) used coumarin, maleic hydrazide and Mendok (Sodium 2,3 dichloro-2-methyl-propane) to induce male sterility in pigeonpea. In all these cases, complete male sterility was coupled with lower reduction in female fertility. Ravikesavan et al. (1988) observed that when pigeonpea plants were sprayed with 200 ppm concentration of gibberellic acid, it induced only 62.2% sterility. Pandey et al. (1994) induced male sterility in long duration pigeonpea varieties viz., Bahar, DA11 and Pusa 9 using chemical mutagen, streptomycin sulfate (SS) and sodium azide (SA). On a limited scale, gamma rays have been used to induce male sterility.

1.15 Genetics of pigeonpea cytoplasmic male sterility

In pigeonpea cytoplasmic male sterility in most systems has been reported to be controlled by single recessive genes (Saxena et al., 1983). The authors further reported that male sterility represented by translucent anthers is controlled by single recessive gene ms_1 , whereas that with arrow-head shaped anthers is controlled by ms_2 . The two systems are independent of each other. In *C. sericeus* x *C. cajan* hybrids, Wanjari et al. (1999) reported monogenic recessive male sterility in two progenies, whereas another three progenies expressed dominant sterility.

1.16 Pigeonpea hybrid breeding using cytoplasmic male sterility technique

Saxena et al. (2006) reported that the availability of CMS in pigeonpea has opened up the possibilities of developing commercial hybrids. These hybrids demonstrated a yield advantage of over 25% and 50% hybrid vigour for seed yield. The A_2 and A_4 CMS systems are being used in developing pigeonpea hybrids in India. Approximately 100 to 150 tons of hybrid seed, enough to plant about 25,000 hectares, was to be available to farmers in 2008 (Saxena and Nadarajan, 2010). To obtain maintainer and restorer lines, advanced generation (F6, F7) breeding lines of diverse parentage, popular cultivars and elite germplasm were crossed with CMS lines. The diversity has been used to breed for hybrids adapted to different agro-ecological areas, cropping systems and specific disease-prone areas (Saxena, 2008). Commercial seed production required a female line (A), maintainer line (B), restorer line (R), and hybrid combination (A x R). The recommended isolation distance for seed production was

500 m with a A:R and A:B ratio of 4:1. However, determination of ratios for specific maturity groups in different environments should be done for any hybrid developed so as to maximize on seed yield.

1.17 Summary

There is abundant knowledge on pigeonpea to satisfy the objectives set forth in this research. Eastern Africa is a secondary centre of diversity and hybridization of improved and locally adapted genotypes with those from the Asian gene pool would result in considerable heterosis. Studies have also shown that germplasm from Asia and Africa possess different genetic mechanisms for resistance to fusarium wilt. This is to be expected since major character differences have been shown to occur between Asian and African pigeonpea types (Sharma and Green, 1980). An association between the patterns of inheritance and evolutionary relationships has been shown in previous investigations (Van der Maesen, 1990). Such an association would suggest that the lines from India used in this study have evolved separate and different forms of resistance to fusarium wilt. Further studies that will include more diverse pigeonpea lines with resistance to specific virulent isolates should be undertaken in order to confirm or reveal additional resistance genes. The CMS lines are also known to be resistant to fusarium wilt, a constraint that has become elusive to tackle due to the existence of physiological races.

With the availability of hybrid seed, the package will need to be taken to the stakeholders. The farmer is the most vulnerable in this situation because of the likelihood of high seed prices. This will require working closely with both the private and public seed sector so that the small scale farmer benefits. Hybrid seed production is likely to attract private seed companies and this will only be successful if markets will be strengthened by involving both formal and informal sectors. This will foster transparency that is likely to reduce transaction costs and therefore improve the competitive position of farmers and other market intermediaries (Freeman and Jones, 2001).

References

- Agona, J.A., and H. Muyinza. 2005. Promotion of improved handling, processing, utilization and marketing of pigeonpea in Apac district. NARO, Kampala, Uganda.
- Akagi, H.A., A. Nakamura, R. Sawada, and T. Fujimura. 1995. Genetic diagnosis of cytoplasmic male-sterile hybrid plants of rice. *Theoretical and Applied Genetics* 90:948-951.
- Andersen, W.R. 1963. Cytoplasmic male sterility in hybrids of *Lycopersicon esculentum* and *Solanum pennellii*. *Tomato Genetics Co-operative* 13:7-8.
- Ariyanayagam, R.P., A.N. Rao, and P.P. Zaveri. 1995. Cytoplasmic male sterility in interspecific matings of *Cajanus*. *Crop Science* 35:981-985.
- Bino, R.J. 1885. Ultrastructural aspects of cytoplasmic male sterility in *Petunia* hybrid. *Protoplasma* 127:230-240.
- Boehringer, A., and R. Caldwell. 1989. *Cajanus cajan* (L.) Millsp. as a potential agroforestry crop in the eastern province of Zambia. *Agroforestry Systems* 9:127-140.
- Chauhan, Y.S., D.H. Wallace, C. Johansen, and L. Singh. 1998. Genotype-by-environment interaction effect on yield and its physiological bases in short-duration pigeonpea. *Field Crops Research* 59:141-150.
- Choudhury, P.R., I.P. Singh, B. George, A.K. Verma, N.P. Singh (2008). Assessment of genetic diversity of pigeonpea cultivars using RAPD analysis. *Biologia Plantarum* 52:648-653.
- Daniel, J.N., and C.K. Ong. 1990. A multipurpose species for agroforestry systems. *Agroforestry Systems* 10:113-129.
- Dundas, I.S., K.B. Saxena, and D.E. Byth. 1981. Microsporogenesis and anther wall development in male-sterile and fertile lines of pigeonpea (*Cajanus cajan* (L.) Millsp. *Euphytica* 30:341-345.
- Faris, D.G., and U. Singh. 1990. Pigeonpea: Nutrition and products, *In* Y. L. Nene et al. (Eds.) *The Pigeonpea*. CAB International, Wallington, Oxon, UK.
- Freeman, H.A., and R.B. Jones. 2001. Sub-sector analysis as a tool for improving commercialization and market access for pigeonpea producers, *In* S. N. Silim, et al.

- (Eds.) Status and potential of pigeonpea in eastern and southern Africa: Proceedings of a regional workshop, 12-15 Sep 2000, Nairobi, Kenya.
- Green, J.M., D. Sharma, J.L. Reddy, K.B. Saxena, S.C. Gupta, K.C. Jain, B.V.S. Reddy, and M.R. Rao. 1981. Methodology and progress in the ICRISAT pigeonpea breeding programme, pp. 437-449, *In* ICRISAT et al. (Eds.) Proceedings of the international workshop on pigeonpea, International crops research institute for the semi-arid tropics, Patancheru 502 324 Andhra Pradesh, India.
- Gwata, E.T., S.N. Silim, and M. Mgonja. 2006. Impact of a new source of resistance to fusarium wilt in pigeonpea. *Phytopathology* 154:62-64.
- Hanson, M.R. 1991. Plant mitochondrial mutations and male sterility. *Annual Review of Genetics* 25:461-486.
- Hanson, M.R., and S. Bentolila. 2004. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. *Plant Cell* 16:154-169.
- Hanson, M.R., and M.F. Conde. 1985. Functioning and variation of cytoplasmic genomes: lessons from cytoplasmic-nuclear interactions conferring male sterilities in plants. *International Review of Cytology* 94:213-226.
- Haware, M.P., and J. Kannaiyan. 1992. Seed transmission of *Fusarium udum* in pigeonpea and its control by seed treatment fungicides. *Seed Science and Technology* 20:597-601.
- Hayashi, Y., J. Kyojuka, and K. Shimamoto. 1988. Hybrids of rice (*Oryza sativum* L.) and wild *Oryza* species obtained by cell fusion. *Molecular Genetics* 214:6-10.
- Hillocks, R.J., E.M. Minja, A. Mwaga, M.S. Nahdy, and P. Subrahmanyam. 2000. Diseases and pests in pigeonpea in eastern Africa: A review. *International Journal of Pest Management* 46:7-18.
- Horner, H.T., and R.G. Palmer. 1995. Mechanics of genic-male sterility. *Crop Science* 35:1527-1535.
- ICRISAT. 2007. Improved pigeonpea hybrid parents for increased and stable production. ICRISAT, Patancheru, 502 324, Andhra Pradesh, India.

- Jag, S. 1985. Role of plant characters in determining adaptation in pigeonpea. *International Pigeonpea Newsletter* 4:15-18.
- Jones, R., H.A. Freeman, and G. Lo Monaco. 2002. Improving the access of smallholder farmers in eastern and southern Africa to global pigeonpea markets. *Agricultural Research and Extension Network* 120:1-11.
- Jones, R.B., P. Audi, and S.N. Silim. 2000. Seed delivery systems: status, constraints and potential in eastern and southern Africa *In* S. N. Silim, et al. (Eds.) *Status of potential of pigeonpea in eastern and southern Africa: Proceedings of a regional workshop*. ICRISAT Nairobi, Kenya.
- Kannaiyan, J., Y.L. Nene, M.V. Reddy, J.G. Ryan, and T.N. Raju. 1984. Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and the Americas. *Tropical Pest Management* 30:62-71.
- Kapoor, S., A. Kobayashi, and H. Takatsuji. 2002. Silencing of the tapetum-specific zinc finger gene TAZ 1 causes premature degeneration of tapetum and pollen abortion in petunia. *Plant Cell* 14:2353-2367.
- Karachi, M., and M. Zengo. 1998. Legume forages from pigeonpea, leucaena and sesbania as supplements to natural pastures for goat production in western Tanzania. *Agroforestry Systems* 39:13-21.
- Kaul, M.L.H. 1988. Male sterility in higher plants. *In* Frankel, R., M. Grassman, P. Maliga, R. Riley (Eds.) *Monograph 10*. Springer-verlag, Berlin, Heidelberg, Germany.
- Kempken, F., and D.R. Pring. 1999. Male sterility in higher plants, fundamentals and applications. *Botany* 60:139-166.
- Kimani, P.M., G. Mergeai, S.N. Silim, J.P. Baudoin, P.R. Rubaihayo, and M. Jansens. 2003. New regional initiatives in pigeonpea improvement, *In* ICRISAT et al. (Eds.) *Pigeonpea improvement*, Vol. 3. ICRISAT, Nairobi, Kenya.
- Kiprop, E.K., J.P. Baudoin, A. Mwang'ombe, P.M. Kimani, G. Mergeai, and A. Maquet. 2002. Characterization of Kenyan isolates of *Fusarium udum* from pigeonpea [*Cajanus cajan*

- (L.) Millsp.] by cultural characteristics, aggressiveness and ALFP analysis. *Journal of Phytopathology* 150:517-525.
- Kumar Rao, J.V.D.K., P.J. Dart, and P.V.S.S. Sastry. 1983. Residual effect of pigeonpea (*Cajanus cajan* L.) on yield and nitrogen response of maize. *Experimental Agriculture* 19:131-134.
- Kunde, G. 2000. Business principles for pigeonpea market linkages, *In* S. N. Silim and P. M. Kimani (Eds.) Status and potential of pigeonpea in eastern and southern Africa. ICRISAT Nairobi, Kenya.
- Kwesiga, F., F.K. Akinnifesi, M.H. Mafongoya, A. McDermont, and A. Agumya. 2003. Agroforestry research and development in southern Africa during the 1990s: Review of challenges ahead. *Agroforestry Systems* 59:173-186.
- Lal, R., G.F. Wilson, and B.N. Okigbo. 1978. No-till farming after various grasses and leguminous cover crops in tropical alfisols. *Field Crops Research* 1:71-84.
- Linke, B., and T. Borner. 2005. Mitochondrial effects on flower and pollen development. *Mitochondrion* 5:389-402.
- Lo Monaco, G. 2003. Competitiveness of African pigeonpea exports in international markets. Socio-economics and policy working paper series no. 15. International Crops Research Institute for the Semi-Arid Tropics, Bulawayo, Zimbabwe.
- Lonsdale, D.M. 1987. Cytoplasmic male sterility: a molecular perspective. *Plant Physiology and Biochemistry* 25:265-271.
- Mallikarjuna, N., and K.B. Saxena. 2005. A new cytoplasmic nuclear male-sterility system derived from cultivated pigeonpea cytoplasm. *Euphytica* 142:143-148.
- Malviya, N. and D. Yadav. 2010. RAPD analysis among pigeonpea [*Cajanus cajan*] (L.) Mill. Sp] cultivars for their genetic diversity. *Genetic Engineering and Biotechnology Journal*. <http://astonjournals.com/gebj>.
- Marley, P.S., and R.J. Hillocks. 2002. Induction of phytoalexins in pigeonpea (*Cajanus cajan*) in response to inoculation with *Fusarium udum* and other treatments. *Pest Management Science* 58:1068-1072.

- McPherson, H.G., I.J. Warrington, and H.L. Turnbull. 1985. The effects of temperature and daylength on the rate of development of pigeonpea. *Annals of Botany* 56:597-611.
- Minja, E.M., T.G. Shanower, and O. Karuru. 2000. Efficacy of different insecticides for pigeonpea pest management in Kenya. *International Chickpea and Pigeonpea Newsletter* 7:30-43.
- Mohamed, M.S., and R.P. Ariyanayagam. 1983. The effect of photothermal environment on growth and flowering in dwarf pigeonpea (*Cajanus cajan* (L.) Millsp) and *Atylosia sericea* Benth. *Euphytica* 32:777-782.
- Nandimpalli, R G., R.L. Jarret, S.C. Phatak, G. Kochert. 1994. Phylogenetic relationships of the pigeonpea (*Cajanus cajan*) based on nuclear restriction fragment length polymorphism. *Genome* 36: 216-223
- Omanga, P.A., R.J. Summerfield, and A. Qi. 1995. Flowering of pigeonpea (*Cajanus cajan* (L.) in Kenya: Responses of early maturing genotypes to location and date of sowing. *Field Crops Research* 41:25-34.
- Onim, J.E.M. 1981. Pigeonpea improvement research in Kenya, pp. 427-436, *In* ICRISAT et al. (Eds.) *Proceedings of the international workshop on pigeonpeas*, Vol. 1. ICRISAT, Patancheru, 502 324, Andhra Pradesh, India.
- Onim, J.E.M., M. Mathuva, K. Otieno, and H.A. Fitzhugh. 1990. Soil fertility changes and response of maize and beans to green manures of leucaena, sesbania and pigeonpea. *Agroforestry Systems* 12:197-215.
- Onu, P.N., and S.N. Okongwu. 2006. Performance characteristics and nutrient utilization of starter broilers fed on raw and processed pigeonpea (*Cajanus cajan* (L.) seed meal. *International Journal of Poultry Science* 5:693-697.
- Oswald, A., and J.K. Ransom. 2001. Striga control and improved farm productivity using crop rotation. *Crop Protection* 20:113-120.
- Pandey, N., C.B. Ojha, P.N. Narula, and S.K. Chowdhury. 1994. Bi and tri carpels and male-sterility in pigeonpea. *Indian Journal of Pulses Research* 7:62.

- Patel, J.A., D.B. Patel, P.P. Zaveri, and A.R. Pathak. 1997. Combining ability for yield characters in pigeonpea. *Indian Journal of Pulses Research* 10:107-108.
- Patel, S.I., R.L. Patel, A.G. Desai, and P. D.S. 2011. Morphological, cultural and pathogenic variability among *Fusarium udum* and root dip inoculation technique for screening pigeonpea germplasm. *Journal of Mycological and Plant Pathology* 41:57-62.
- Phatak, S.C., R.G. Nadipali, S.C. Tiwari, and H.L. Bhardwaj. 1993. Pigeonpeas: Potential new crop for the southeastern United States, p. 597-599, *In* J. Janick and J. E. Simon (Eds.) *New Crops*. Wiley, New York.
- Rao, S.C., S.W. Coleman, and H.S. Mayeux. 2002. Forage production and nutritive value of selected pigeonpea ecotypes in the southern Great Plains. *Crop Science* 42:1259-1263.
- Ravikesavan, R., T. Kalaimagal, and R. Rathnaswamy. 1988. Chemically induced male sterility in pigeonpea (*Cajanus cajan* L.) Mill. *Advances in Plant Science* 11:275-278.
- Reddy, B.V.S., J.M. Green, and S.S. Bisen. 1978. Genetic male sterility in pigeonpea. *Crop Science* 17:362-364.
- Reddy, L.J., and D.G. Faris. 1981. A cytoplasmic-genetic male sterile line in pigeonpea. *International Chickpea and Pigeonpea Newsletter* 1:16-17.
- Reddy, L.J., J.M. Green, B. S.S., U. Singh, and R. Jambunathan. 1997. Seed protein studies on *Cajanus cajan* (L.), *Atylosia* spp, and some hybrid derivatives. *Seed Protein Improvement in Cereals and Grain Legumes* 2:105-117.
- Reddy, M.V., and T.N. Raju. 1997. Evaluation of pigeonpea varieties for resistance to wilt caused by *Fusarium udum* and sterility mosaic disease in a perennial system. *Indian Journal of Agricultural Sciences* 67:437-439.
- Reddy, M.V., S.B. Sharma, and Y.L. Nene. 1990. Pigeonpea: disease management, p. 303-347, *In* Y. L. Nene, et al. (Eds). *The Pigeonpea*. CABI, Wallington, Oxon, UK.
- Reed, W., and S.S. Lateef. 1990. Pest management, p. 349-374, *In* Y. L. Nene et al. (Eds). *The Pigeonpea*. CAB International, Wallington, Oxon, UK.

- Roath, W.W., J.F. Miller, and T.I. Gulya. 1981. Registration of RHA 801 sunflower germplasm. *Crop Science* 21:479.
- Roberts, M., E. Boyes, and R. Scott. 1995. An investigation of the role of the anther tapetum during microspore development using genetic cell ablation. *Sexual Plant Reproduction* 8:299-307.
- Rusike, J., and J.P. Dimes. 2006. Effecting change through private sector client services for smallholder farmers in Africa 4th International Crop Science Congress, Brisbane. http://www.cropscience.org.au/ics2004/symposia/4/6/997_rusike.htm.
- Saxena, K.B. 2006. Seed production systems in pigeonpea. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 502 324, Andhra Pradesh, India.
- Saxena, K.B. 2008. Genetic improvement of pigeonpea - a review. *Tropical Plant Biology* 1: 159-178.
- Saxena, K.B., D.E. Byth, E.S. Wallis, and I.S. Dundas. 1983. Genetic basis of male sterility in pigeonpea. *International Pigeonpea Newsletter* 5:21-23.
- Saxena, K.B., Y.S. Chauhan, C. Johansen, and L. Singh. 1992. Recent developments in hybrid pigeonpea research. Directorate of research, Kanpur 208024, India.
- Saxena, K.B., D.G. Faris and R.V. Kumar. 1987. Relationship between seed size and protein content in newly developed high protein lines of pigeonpea. *Plant Foods for Human Nutrition* 36:335-340.
- Saxena, K.B., and R.V. Kumar. 2003. Development of a cytoplasmic nuclear male sterility system in pigeonpea using *C. scarabaeoides* (L) Thours. *Indian Journal of Genetics and Plant Breeding* 63:225-229.
- Saxena, K.B., R.V. Kumar, K.M. Latha, and V.A. Dalvi. 2006. Commercial pigeonpea hybrids are just a few steps away. *Indian Journal of Pulses Research* 19:7-16.
- Saxena, K.B., R.V. Kumar, N. Srivastava, and B. Shiyong. 2005. A cytoplasmic-nuclear male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. *Euphytica* 145:289-294.

- Saxena, K.B., and N. Nadarajan. 2010. Prospects of pigeonpea hybrids in Indian agriculture. *Electronic Journal of Plant Breeding* 1:1107-1117.
- Saxena, K.B., and R. Rajni. 2001. Pattern analysis for genotype by environment effects for seed weight and grain yield in pigeonpea hybrids. *Indian Journal of Genetics and Plant Breeding* 61:226-231.
- Saxena, K.B., and D. Sharma. 1983. Early generation testing in pigeonpea (*Cajanus cajan* (L.) Millsp.). *Tropical Plant Science Research* 1:309-313.
- Saxena, K.B., R. Sultana, N. Mallikarjuna, R.K. Saxena, R.V. Kumar, S.L. Sawargaonkar, and R.K. Varshney. 2010. Male sterility systems in pigeonpea and their role in enhancing yield. *Plant Breeding* 129:125-134.
- Schnable, P.S., and R.P. Wise. 1998. The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends in Plant Science* 3:175-180.
- Shanower, T.G., J. Romeis, and E.M. Minja 1999. Insect pests of pigeonpea and their management. *Annual Review of Entomology* 44:77-96.
- Sharma, D., and J. Green, M. 1977. Problems and prospects of upgrading yield levels in pigeonpea. *Sabrao Journal* 2:6-10.
- Sharma, D., and J.M. Green. 1980. Pigeonpea, *In* S. B. S. Tikka (Ed.) *Hybridization of crop plants*. Crop Science Society of America, Wisconsin, USA.
- Singh, M., R.S. Malhotra, S. Ceccarelli, A. Sarker, S. Grando, and W. Erskine. 2003. Spatial variability models to improve dryland field trials. *Experimental Agriculture* 39:1-10.
- Singh U. 1988. Anti-nutritional factors of chickpea and pigeonpea and their removal by processing. *Plant and Foods for Human Nutrition* 38: 251-261
- Singh, U., R. Jambunathan, K.B. Saxena, and N.C. Subrahmanyam. 1990. Nutritional quality evaluation of newly developed high-protein genotypes (*Cajanus cajan* (L.)). *Journal of the Science of Food and Agriculture* 50:201-209.
- Sogbedji, J.M., H.M. van Es, and K.L. Agbeko. 2006. Cover cropping and nutrient management strategies for maize production in Western Africa *Agronomy Journal* 98:883-889.

- Solomon, S., G.P. Argikar, S. M.S., and I.R. Morbad. 1957. A study of heterosis in *Cajanus cajan* (L) Millsp.). Indian Journal of Genetics and Plant Breeding 17:90-95.
- Srinivas, T., K.C. Jain, M.V. Reddy, and M.S.S. Reddy. 1999. Genetic relationships among yield components in pigeonpea. Indian Journal of Pulses Research 12:180-186.
- Sujana, G., H.C. Sharma, D.M. Rao. 2008. Antixenosis and antibiosis components of resistance to pod borer *helicoverpa armigera* in wild relatives of pigeonpea. International Journal of Tropical Insect Sciences 28:191-200.
- Swaminathan, M.S., and H.K. Jain. 1972. Food legumes in Indian agriculture, pp. 69-82, *In* M. M. Milner (Ed.) Nutritional improvement of food legumes by breeding. FAO, Rome, Italy.
- Van der Maesen, L.J.G. 1990. Pigeonpea: origin, history, evolution and taxonomy, *In* Y. L. Nene et al. (Eds.). The Pigeonpea. CABI, Wallingford, Oxon, UK.
- Venzon, M., M.C. Rosado, D.E. Euzebio, B. Souza, and J.H. Shroeder. 2006. Suitability of leguminous cover crop pollens as food for green lanchewing *Chrysoperla externa* (Hagen). Neotropical Entomology 35:371-376.
- Verulkar, S.B., and D.P. Singh. 1997. Inheritance of spontaneous male-sterility in pigeonpea. Theoretical and Applied Genetics 94:1102-1103.
- Wallis, E.S., D.G. Faris, R. Elliot, and D.E. Byth. 1986. Varietal improvement of pigeonpea for smallholder livestock production systems. Proceedings of workshop on crop-livestock systems research, July 7-11 1986, Khon, Kaen, Thailand.
- Wanjari, K.B., A.N. Patil, P. Manapure, J.G. Manjaya, and P. Manish. 1999. Cytoplasmic male-sterility in pigeonpea with cytoplasm from *Cajanus volubilis*. Annals of Plant Physiology 13:170-174.
- Warmke, H.E., and L.J. Lee. 1978. Pollen abortion in T cytoplasmic male sterile corn (*Zea mays*): a suggested mechanism. Science 200:561-563.

Chapter 2: Analysis of stakeholders in the pigeonpea [*Cajanus cajan* (L.) Millsp.] value chain and assessment of opportunities for introduction of pigeonpea hybrids in Kenya

Abstract

Understanding market requirements for crop improvement is a prerequisite for a successful breeding programme. New pigeonpea varieties must possess the desired pre-and post-harvest traits as defined by stakeholders in the value chain. The purpose of this study was to examine the various stakeholders and their core functions in order to identify characteristics of the market preferred pigeonpea varieties to be considered in the hybrid breeding programme, and to understand constraints affecting development of the sector. The study was carried out in Machakos, Makueni, Mbeere and Nairobi districts. Key informant survey, semi-structured questionnaire, analysis of secondary data and literature review were tools used in the study. The key stakeholders identified were farmers, traders/processors, International Crops Research Institute for the Semi-Arid Tropics, Kenya Agricultural Research Institute, Kenya Plant Health Inspectorate Services, and Ministry of Agriculture. Others were KARI Seed Unit, Leldet Ltd and financial intermediaries. Large, white seed and medium maturity varieties were important traits preferred by farmers and processors/exporters for both domestic and export markets. Domestic as well as export markets exist, but exploitation has been hampered by low grain volumes. The unavailability of quality seed in sufficient quantities of high yielding varieties were cited as the main factors negatively affecting pigeonpea production. Introduction and production of hybrids will attract private seed companies that will guarantee quality seed supply in sufficient quantities for commercialization. The stakeholder analysis approach, used for the first time in a breeding programme, demonstrated that it is a vibrant tool that can be used to diagnose crop production constraints, and define opportunities available for setting up a targeted breeding program that is highly client-oriented.

2.1 Introduction

Pigeonpea is the third most important legume in Kenya after beans and cowpea (Mergeai et al., 2001; Shiferaw et al., 2008), cultivated largely by small scale farmers in the arid- and semi-arid areas that are characterized by high rainfall variability. Ryan (1997) and Jones et al. (2002) reported that the importance of pigeonpea in rainfed agriculture is on the increase due to steady changes in various social and climatic factors. Productivity per unit area and seed quantities has been declining despite the crop being an important cash crop for communities in the semi-arid areas.

Pigeonpea is used both as a cash and food crop, and is a cheap source of protein. Its leaves and hulls can be used as animal feeds, it improves soil fertility by fixing nitrogen, and the stems can be used as fuel. Whole and processed grain is exported to India and the Indian diaspora, and vegetable products to Europe and the Carribean (Gwata et al., 2006; Jones et al., 2002). Constraints to pigeonpea production are many and diverse, the most important being lack of high yielding genotypes with superior agronomic traits, organized quality seed production systems, lack of structured marketing institutions, diseases and pests (Chauhan et al., 1998). Jones et al. (2002) noted that lack of collaboration between pigeonpea stakeholders, marketing constraints and seed distribution problem also affect pigeonpea production.

According to a survey by Nagajaran et al. (2007), demand for seed in the marginal areas, is dictated by disasters, poverty, and desire for better seed quality and use of new varieties. During normal seasons, farmers will replace seed of their varieties when the quality deteriorates, or change varieties to increase expected yield. In emergency situations farmers require new seed due to losses caused by drought, civil strife or some other disaster. Private firms are reluctant to produce high volumes, of low-value seeds of dryland cereals and legumes as they are considered as secondary crops of importance in terms of profitability. The survey revealed that the various local seed production groups that were initiated through KARI and ICRISAT were unsustainable. The reason given for the lack of success was limited participation of stakeholders, such as local traders in the activities of the farmer groups. The main factor inhibiting private sector involvement in pigeonpea seed production has been cited as low profit margins due to farmer recycling of own seed (Freeman and Jones, 2001). Farmer investment in quality seed has been hampered by lack of structured markets leading to low prices for dry grain and green vegetables offered by traders.

Farmer participatory techniques when appropriately employed in plant breeding can have an impact by fast and cost-effective development of improved crop varieties and have been identified as ways of addressing the problem of non-adoption of new technologies (Ceccarelli et al., 2003; Lusby and Panlibuton, 2004; Morris and Bellon, 2004). Participation by farmers and other stakeholders has been found to lead to additional outputs such as the development or empowerment of individuals or communities (Okali et al., 1994). Participatory techniques commonly used include participatory rural appraisal (PRA), participatory plant breeding (PPB), and participatory varietal selection (PVS).

The PRA technique has been used extensively in the identification of the production constraints of many crops and provides the information needed to specify the characteristics needed in a new variety. In PVS, farmers test under farmer management an appropriate range of products (e.g., varieties and hybrids) from formal breeding programmes (Witcombe, 1996). Sperling et al. (2001) defined PPB as one that uses PVS without involving farmers in selection during the segregating generations. The use of stakeholder and value chain analysis techniques in plant breeding is not common. Stakeholder analysis has largely been used as a tool for assessing different interest groups around a policy issue or intervention, and their ability to influence the final outcome. The value chain analysis technique has been used to describe a full range of value-adding activities from its design, source of raw materials, inputs, its distribution and support to the final consumer (Kaplinsky, 2000; McCormick and Schmitz, 2001).

Pigeonpea stakeholders fall under different categories (i.e. farmers, government departments, private sector, researchers, extension workers and traders). However, these stakeholders are currently working independently of each other (Mergeai et al., 2001). For example, researchers and extension staff seldom interact with the private sector, leading to a situation where neither producers nor private sector benefit from investments made in development of new pigeonpea technologies. Researchers involve farmers in technology development, but not traders, processors and exporters (Freeman and Jones, 2001).

Besides working with farmers in testing/evaluation and selection of improved varieties, strategic partnerships with traders, processors, and consumers are needed to ensure that identified technologies are market friendly. Such partnerships can be forged by soliciting the participation of traders, processors, and consumers in technology development and by identifying and establishing quality standards. For successful introduction of pigeonpea hybrids in Kenya,

collaborative breeding is highly desirable in order to meet farmer and consumer demands and also empower farmers. Stakeholder analysis of the pigeonpea value chain will be used as a novel participatory tool in support of a plant breeding programme. The objectives of this study were to:

1. Examine the various stakeholders and their core functions in order to identify key elements that may influence their active involvement in the pigeonpea value chain,
2. Identify characteristics of the market preferred pigeonpea varieties to be considered in the hybrid breeding programme,
3. Understand constraints affecting development of the pigeonpea sector.

2.1 Materials and methods

The study used primary data collected in February-June 2009 from a random sample of 120 pigeonpea producers, transporters and processors in Machakos, Wote, Kiboko, Mbeere districts, and Nairobi city. The districts were selected because they are the leading pigeonpea producers and producer marketing groups (PMGs) exist. A total of 80 farmers, 30 traders (rural traders, urban traders), five processors/exporters and five transporters were interviewed. Exporting/processing company representatives from Kenya Millers Limited, Spice World Limited, Superveg Limited, Makindu Growers and Packers Limited and Pisu & Company Limited provided information on market preferences and limitations. Individual and/or group farms were visited and farmers who were also head of households interviewed. Because of the importance of weekly markets, survey dates coincided with main market days. In each district five markets were visited. Farmers, traders as well as transporters come to these markets. A standardized questionnaire served as a guide to obtain consistent information.

To understand the role of various institutions involved in the pigeonpea sub-sector, institute representatives were interviewed and secondary data used. Main institutions were Kenya Agricultural Research Institute (KARI), International Crops Institute for Semi-Arid Tropics (ICRISAT), KARI Seed Unit (KSU), Kenya Plant Health Inspectorate Services (KEPHIS), Leldet Seed Company, and non-governmental organizations operating in the survey areas. Because India is the main international market for the Kenyan pigeonpea exports, an analysis of the Indian market using secondary data was done in order to understand her specific requirements.

2.2 Results

2.2.1 Situation analysis

Table 2.1 summarizes characteristics of pigeonpea farming survey areas. Traditional land under pigeonpea varied between 1.2 ha in Mbeere to 2.6 ha in Kiboko. Leasing of land by individual farmers or farmer groups from other communities for growing of pigeonpea has become a common practice with some farmers leasing up to 5 ha. Planting starts at the on-set of the September/October short rains. Most farmers intercrop with cereals, beans, cowpea and fruit trees and apply farm yard manure only when available. However, in Mbeere, most farmers plant using inorganic fertilizer and regularly apply pesticides. Harvesting as green vegetables takes place from February to April, while long-duration types are usually harvested as dry grain in August and September, with small quantities harvested as vegetable pigeonpea usually during June/July. Grain yields in Mbeere averaged 0.9 tons ha⁻¹ as compared to 0.5 tons ha⁻¹ in Machakos and Wote. The higher yields obtained in Mbeere are due to the willingness of farmers to invest in quality seed purchased from KARI or ICRISAT, use of sufficient quantities of fertilizer and pesticides sprayed regularly to control podborers.

Table 2.1. Characteristics of pigeonpea farming in Machakos, Wote, Kiboko and Mbeere in 2009.

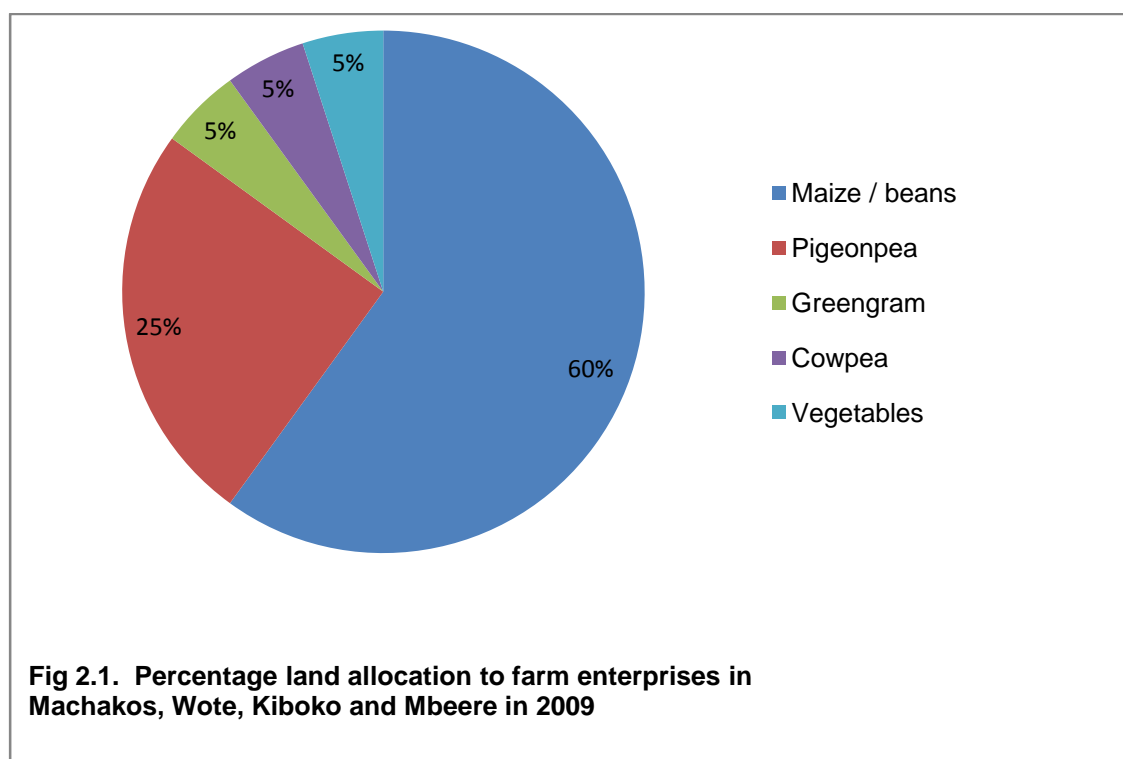
Farm characteristics	Machakos	Wote	Kiboko	Mbeere
Average area under pigeonpea (ha)	1.8	2.3	2.6	1.2
Average yield (tons ha ⁻¹)	0.5	0.5	0.7	0.9
Cropping system	Beans, Maize, pigeonpea	Cowpea, Maize, pigeonpea	Greengrams, Sorghum, pigeonpea	Beans, pigeonpea

Table 2.2 summarizes farmer ranking of crop enterprises. Pigeonpea was ranked second as the most important crop after maize. Traditionally maize is the staple food crop and always given priority in terms of land and inputs. However, this is changing due to frequent maize crop failures, forcing farmers to seek alternative drought tolerant crops. Pigeonpea was ranked as the main source of cash, and for food. Resource allocation was minimal for pigeonpea (Fig 2.1, 2.2, 2.3) with farmers in Machakos, Wote and Kiboko arguing that yield remained low despite application of fertilizer. However, they all agreed that podborers (*Helicoverpa spp.*) control was mandatory with most applying pesticides three to four times required during the crop growing season from flowering stage to physiological maturity. Land allocated to pigeonpea was also used to grow other legumes, mainly cowpea and green grams.

Table 2.2. Farmer ranking of crop enterprises on-farm in Machakos, Wote, Kiboko and Mbeere in 2009.

Farm enterprises	Food security	Income
Maize/beans	2	5
Pigeonpeas	1	1
Greengrams	4	2
Cowpeas	3	2
Vegetables	4	4

1=Most important, 5= Least important



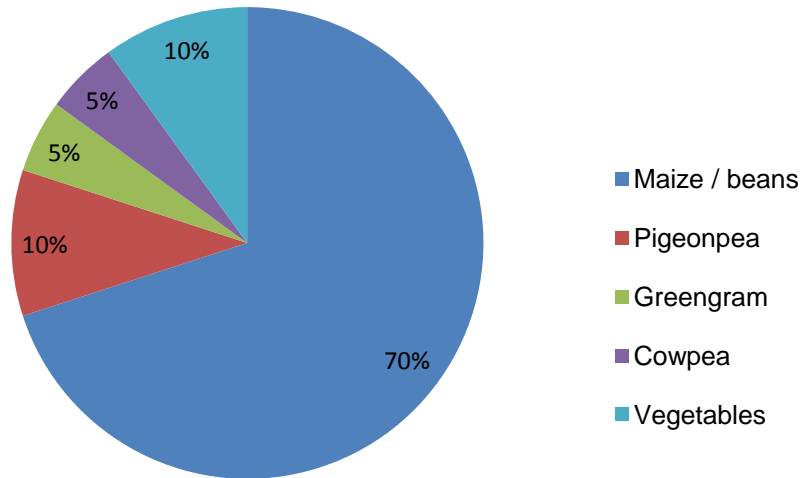


Fig 2.2 Percentage labour allocation to farm enterprises in Machakos, Wote, Kiboko and Mbeere in 2009

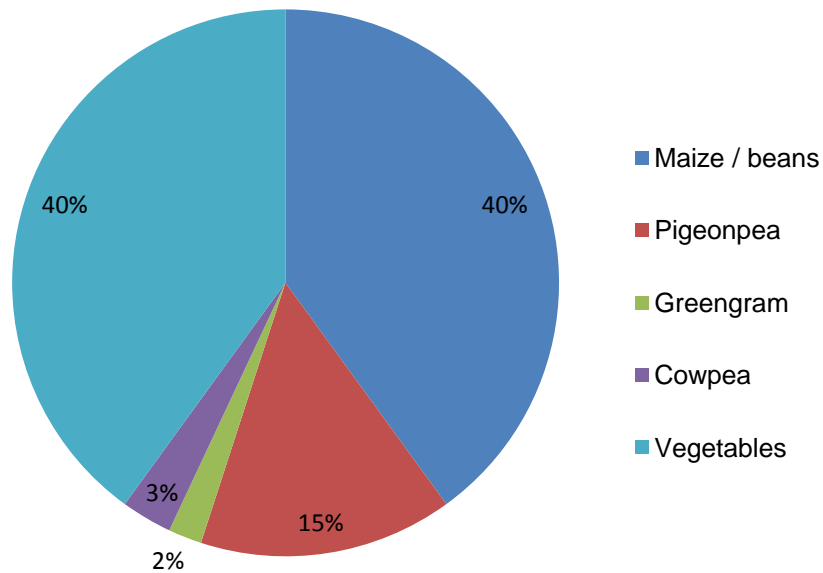
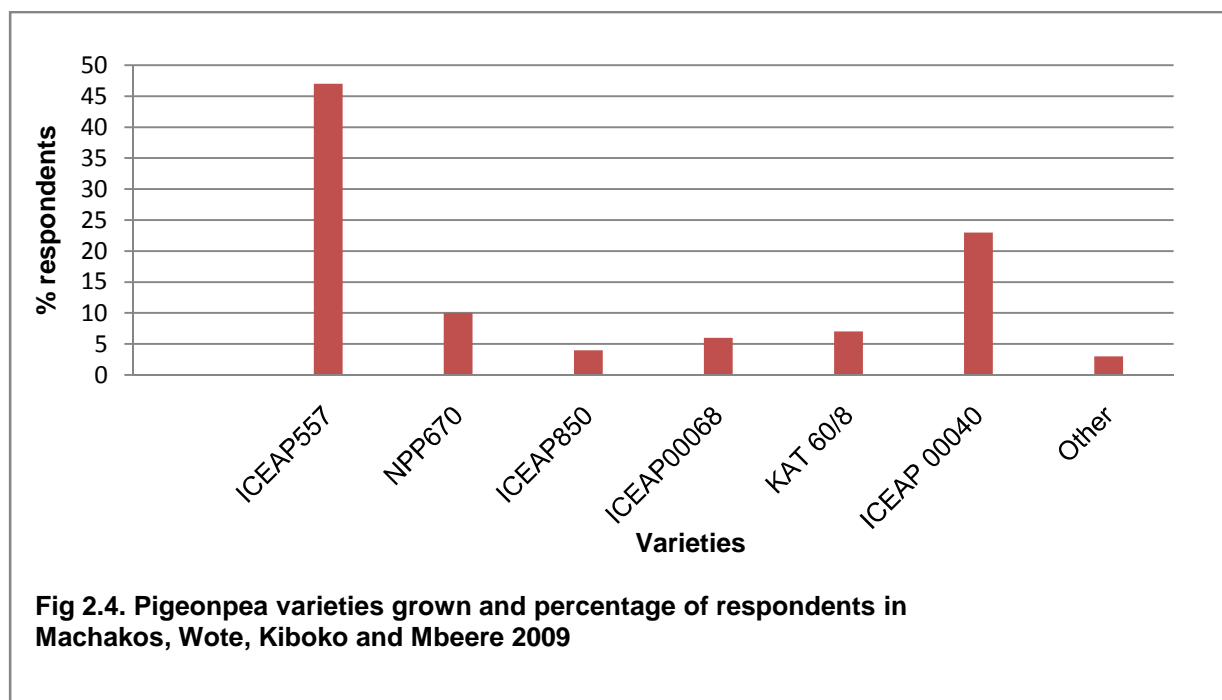


Fig 2.3. Percentage input (Fertilizers, Pesticides) allocation to farm enterprises in Machakos, Wote, Kiboko and Mbeere 2009

There are no formal pigeonpea seed supply mechanisms in the areas surveyed, with farmers depending on the local markets, ICRISAT and KARI for seed. Information from the Ministry of Agriculture revealed that over the last decades, the government, research institutions and non-governmental organizations initiated interventions to improve farmer access to seed and its availability. Some of the initiatives included community-based seed production programmes, producer marketing groups and distribution of small seed packs. Unfortunately they proved unsustainable due to poor marketing linkages and low quality standards adopted by some farmer groups and free delivery of large scale seed quantities through emergency/relief mechanisms. However, farmers still expressed interest in using improved varieties even at a cost.

Farmers grow a range of varieties dictated by availability (Fig 2.4). Commonly grown varieties are medium duration varieties; ICEAP557, ICEAP850, KAT60/8 and the long duration variety ICEAP00040. Variety ICEAP557 is grown by 47% of farmers and ICEAP00040 by 28%. Seeds of these varieties are produced and aggressively marketed by ICRISAT and KARI, the main seed sources in the region. However, some farmers save their own seed or purchased from local stockists.



The variety NPP670 is only grown in Mbeere, after it was introduced in the early 1990s and is still popular as farmers save their own seed. Variety ICEAP00040 is still grown due to its resistance to fusarium wilt and when high yield potential.

2.2.2 Characteristics of important traits for selection of pigeonpea varieties by farmers, traders and processors

Farmer ranking of pigeonpea variety selection criteria is presented in Table 2.3. Yield was ranked as the most important criteria followed by medium maturity. White and white seeded grains were also important attributes. Varieties with these attributes are preferred because of the better economic returns when sold as green pods and while the crop matures in periods of food shortage. Storage pests ranked low, probably because the farmers dispose of the produce immediately after harvest while keeping only small quantities of seed.

Farmers scored criteria for selection of pigeonpea varieties and ranks are shown in Table 2.3. High yield, medium maturity varieties that are large and white seeded were the most important criteria for both score and rank. Varieties with these traits fetched more cash when sold as green vegetable and also served as food security because maturity coincided with when there is food shortage. Fusarium wilt and drought scored as important criteria (4) but ranked low (5) because farmers have observed that some of the medium maturity varieties they grow are less affected and therefore concluded that a medium duration variety was likely to be fusarium wilt resistant and will mature before drought set in. Storage pests ranked low because the farmers dispose of the produce immediately after harvest with some keeping small quantities of seed and others depending on ICRISAT and KARI.

Table 2.3. Pigeonpea variety selection criteria score and ranking by farmers in Kenya in 2009

Criteria	Score	Rank
Yield	5	1
Medium maturity	4	2
Large/medium grain	3	3
White/cream grain	3	3
Drought tolerant	4	5
Fusarium wilt resistance	4	5
Podborer resistance	2	7
Storage pest resistance	1	8

Traders and processors showed preference for varieties that are high yielding (Table 2.4). White grains were preferred for processing of vegetable peas and for sale as dry grain for domestic markets. For processing into dhal, grain colour was not important but large/medium size grain are preferred because the machines used for dehulling give a high recovery rate when large and round grains are used. Small quantities of white grain are also exported to India.

Table 2.4. Pigeonpea variety selection criteria score and ranking by traders and processors in Kenya in 2009

Criteria	Score	Rank
Large/medium size grain	4	2
White grain	3	3
Grain homogeneity	2	4

Farmers sell the produce at the farm gate or take the produce to the local markets of Mombasa and Nairobi with relatively smaller quantities sold in Emali, Wote, Machakos and Embu towns. Some farmers interviewed said they arrange with buyers in Nairobi or Mombasa, who send transporters to collect the produce and payment is done via MPESA (a mobile money transfer system). Otherwise the majority of farmers sell to rural assemblers who are normally agents (middlemen) of urban traders. Other traders also buy to sell on the same market later in the day or transport to other smaller towns. Farmers who are also members of a farmer group in Wote said they were contracted by two boarding schools where they supply dry grain.

Processors/exporters normally obtained the supplies from middlemen except in rare cases where they have contracted farmers directly. For example, Makindu Growers and packers Limited contracted farmers after they identified a foreign market in India. They supply them with seed and chemicals, and packaging materials, collected the produce and paid the farmers two weeks after collection. Otherwise the common practise was traders targeting market days when they anticipate large volumes in order to buy from farmers and/or local assemblers and proceed to sell in Nairobi or Mombasa to processors and exporters. Although information on buying and selling prices was not readily available, the average buying price for green pods on the local markets was KES 25 kg⁻¹ in 2009 and sold for KES 100 in Nairobi and Mombasa. Dry grain was bought for KES 60 kg⁻¹ and sold for KES150 kg⁻¹.

2.2.3 Pigeonpea production constraints

Constraints to pigeonpea production and marketing are summarized in Table 2.5 and 2.6. Farmers ranked drought, lack of credit and fusarium wilt as the main constraints to pigeonpea production. In some areas, there had been three consecutive crop failures due to lack of rainfall. Water sources (dams, boreholes) available in the area are only for home use and livestock. Lending institutions found in the area demand land title deeds and other collaterals that most farmers do not own individually. Some creditors charge high interest rates that most farmers find inhibitive. Other important constraints are low prices and lack of reliable seed sources in sufficient quantities and quality. The farmers indicated that prices of the produce were low and fluctuated as they are determined by the traders and demand and supply. Most farmers deposited the crop immediately after harvest, causing a glut in the market that leads to low prices being offered by traders.

Table 2.5. Pigeonpea production and marketing constraints ranked by farmers in Machakos, Wote, Kiboko and Mbeere 2009.

Constraint	Machakos	Wote	Kiboko	Mbeere	Overall ranking
Drought	1	1	1	3	1
Lack of credit	4	2	2	1	2
Diseases and pests	2	3	3	3	3
Low prices	3	3	2	5	4
Availability of seed	2	2	5	4	5
Lack of market	5	5	4	4	6

Traders, processors and exporters ranked lack of credit, low volumes and poor infrastructure as the limited factors to pigeonpea trade. During the rainy season, most roads are impassable leading to high transport costs. Low and unreliable volumes make sourcing for an export market difficult. Also exporters collect grain from different varieties and have no basis for negotiating higher prices, making Kenyan pigeonpea less competitive in the international markets.

Table 2.6. Ranking of constraints by traders, processors and exporters in Machakos, Wote, Kiboko and Mbeere in 2009

Constraint	Machakos	Wote	Kiboko	Mbeere	Overall ranking
Lack of credit	1	1	1	1	1
Low volumes	2	1	2	2	2
Price variability	5	2	2	4	3
Poor infrastructure	3	3	3	4	3
Lack of buyers	5	3	3	4	5
Low quality	4	4	3	3	5
Storage pests	3	5	4	5	7

2.2.4 Pigeonpea stakeholders and value chain in Kenya

The pigeonpea value chain in Kenya starts with the smallholder farmer, to local and urban traders, supermarkets and finally exporters and consumers, all interlinked through transporters (Fig 2.5). Local traders comprise of rural assemblers, retailers and wholesalers. Urban traders and producer marketing groups set up buying centres within the community, from where they buy pigeonpeas from individual farmers and subsequently sell to supermarkets and processors, some of who are also exporters. Local and urban traders (Fig. 2.7) serve as a link between smallholder farmers and processors/exporters of pigeonpea. The grain is cleaned to remove chaff and shrivelled seeds and packaged for either the local or export market. However, larger quantities are processed into tur dhal, packaged and sold to the urban retailers and supermarkets or exported to India. According to Superveg Limited, the main exporter of vegetable peas, the value chain is similar to that of dry grain, but most of the processed volumes are exported to Europe. Kenya exports an average of 5,623 tons dry grain to India (FAOSTAT, 2011). The survey noted that in Kenya, the structure of the pigeonpea value chain is buyer-driven where importers' prices being offered to the exporters influence the prices along the chain.

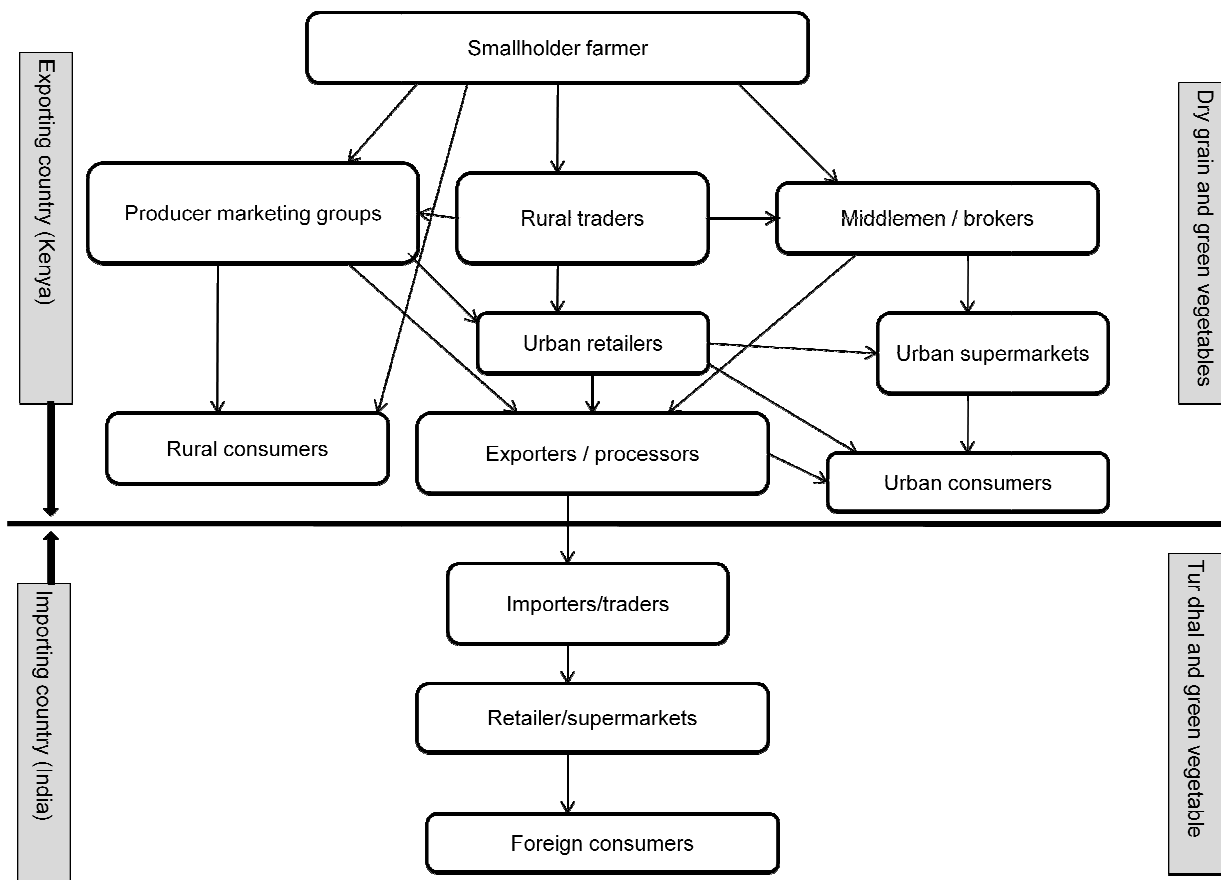


Fig 2.5. Pigeonpea stakeholder value chain in Kenya

Several stakeholders influence the pigeonpea sub-sector in Kenya. The main ones are PMGs, ICRISAT, KARI, Ministry of Agriculture, KEPHIS, local NGOs, seed agents, credit providers, and exporting agencies (Fig 2.6).

Producer marketing groups

In Mbeere and Makueni Districts, pigeonpea farmers were sensitized and assisted to form PMGs by ICRISAT in 2003 with a view to test the potential of these groups to improve market access for smallholder farmers. Members of these groups interviewed explained that they are not as active presently as when ICRISAT was supporting them. Some members had started their own seed production, but did not make the anticipated profits because they were relying on a local market that was not ready to pay a higher premium for the improved seed. When asked about reverting back to seed production, they said they are willing so long as there is guaranteed market and farmers are ready to pay for quality seed.

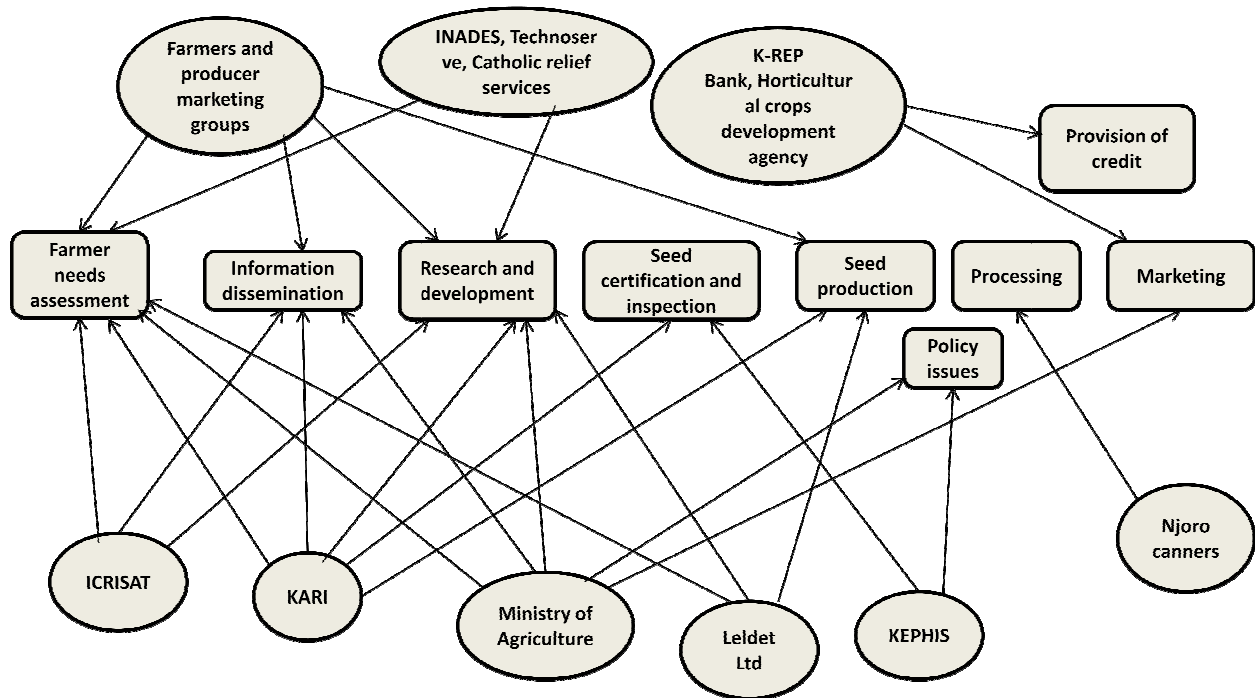


Fig 2.6 Pigeonpea stakeholders and their core functions

Those involved in farming indicated a desire to increase hectareage if market prices of grain improve and demand is more stable. A follow-up survey by Shiferaw et al. (2006) revealed that PMGs have the potential to simplify and shorten the marketing chain by directly connecting small producers to secondary and tertiary markets, better coordinate production and marketing activities, and facilitate farmer access to production inputs at fair prices.



Fig 2.7. Smallscale trader at Wote market selling green pigeonpea and other fresh produce

Research organizations

ICRISAT, KARI and University of Nairobi have been instrumental in undertaking pigeonpea research. The combined efforts led to the release of KARI Mbaazi II, Kat-60/8 and ICEAP 00040 that are higher yielding and more resistant to fusarium wilt (Silim, 2001). ICRISAT has been involved in production of breeder and foundation seed as well as facilitating the commercial production of seed that led to adoption of improved varieties and crop management methods, which sharply increased yields wherever they were adopted. The KARI Seed Unit (KSU) maintains, produces and distributes food crop and fruit tree planting materials on cost recovery basis. The unit is involved in commercialization of pigeonpea seed by contract farmers whose seed crops are inspected by officers from KEPHIS and KARI before it is harvested, packaged and sold to farmers. Varieties under commercial production are KAT 60/8, KARI Mbaazi and ICEAP00040.

Ministry of agriculture

The Ministry of Agriculture objectives includes increasing food production, growth in agricultural employment, and expansion of agricultural exports, resource conservation and poverty alleviation. It has the core function of ensuring food security through appropriate crop production technologies and creates an enabling environment for the players in the industry by developing

effective policies and strategies. Testing and evaluation of pigeonpea varieties is a collaborative activity with KARI (GOK, 2007).

Seed agents and stockists

Due to the underdevelopment of the pigeonpea seed markets, access to improved seed remains a challenge. Seed distribution in the pigeonpea sector is dependent on formal and informal systems. Informal system is the most dominant and involves local grain stores, neighbours, relatives and friends, except during emergency and hardship when farmers source from the formal system. Seed supply initiatives have over the years been promoted through small seed pack programs and PMGs by ICRISAT, and KARI (Shiferaw et al., 2006; Shiferaw et al., 2008). During disaster periods the public institutions, in partnership with NGOs, are involved in direct seed distribution, seed vouchers and fairs. Leldet Seed Company, a registered seed merchant, currently offers its customers certified seed for pigeonpea.

2.2.5 Characteristics of Indian pigeonpea market

In India pigeonpea is grown on 3.5 million hectares under rain fed conditions in the semi-arid tropics an area with growth rate of 0.5% between 1981-2007 (FAOSTAT, 2011). The increase was attributed to availability of short to medium duration varieties that are also resistant to fusarium wilt, sole cropping and increase in prices (Joshi et al., 2001). Yields have stagnated at 715 kg ha⁻¹; a factor attributed to a shift from favourable environments to marginal areas (Bantilan and Joshi, 1996) and frequent drought conditions. India is the largest single producer of pigeonpea in the world, with 2.5 million tons in 2005-7 followed by Myanmar (544,000 tons) (FAOSTAT, 2011). This leaves an average shortfall of 0.15 million tons (Lo Monaco, 2003), although this fluctuates depending on production, with 90% of this covered by Myanmar. Imports of yellow pea (*Pisum sativum* (L.)), a substitute pulse, has affected pigeonpea trade. It is the cheapest and among the most widely traded pulses in international markets.

Domestic pigeonpea production variation has been observed to cause price fluctuations in both domestic and import/export markets. However, increased imports from Myanmar for pigeonpea and Canada/France for yellow pea have stabilized price variations. Studies show that prices are lowest in March-April and peak in November-December, a window that could be exploited by Kenyan exporters where harvesting is between July-September.

2.2.6 Grain quality and market preferences in India

The Indian consumer market prefers large and cream coloured grains, which fetch higher prices. These are used for production of oily dhal, popular in Gujarat, a specific market segment. On the other hand the intermediary (milling industry) market considers grain-to-dhal conversion rates and the overall cost of the operations. Studies by Lo Monaco (2003) revealed that industrial users prefer medium size grains as they suffer less damage on the edges, a factor that is used to determine the price of dhal. The study also revealed that due to technical innovations in milling, millers are less oriented to colour, hence preference is now largely neutral. Other traits considered are cleanliness, homogeneity and content of immature grains. Consumers prefer sweet and easy to cook grains, and less seed coat residues on the outer part of the split halves.

2.2.7 Critical success factors in the Indian market

The Indian market will continue reliance on imports of pulses to meet domestic demand. Pigeonpea and other pulses remain the cheaper substitutes for processed products for the low income and price sensitive consumers. Harvesting of pigeonpea in Kenya coincides with a period of relative shortage and high prices in India. Kenya can therefore exploit this opportunity to enter into the Indian market and establish her own niche competitively. This will be possible if high yielding medium maturity hybrids are available through a viable seed supply system. Around the year supply of large quantities will reduce unit cost of production and therefore achieve economies of scale which in turn will reduce overall marketing costs and hence profitable trade for Kenyan pigeonpea.

2.3 Discussions and conclusions

Several participatory techniques have been used in recent years to engage farmers at different levels of a breeding process. In this study a stakeholder analysis was used as a novel tool to involve all the key stakeholders along the pigeonpea value chain, in order to ensure broadbased input in the breeding process.

Key stakeholders identified in the pigeonpea value chain can be categorized into six groups; production, trade/processing, research, information dissemination, seed quality assessment and policy making. Farmers and producer marketing groups determine the traits of pigeonpea varieties grown as they combine production both for food and market; processors influence the varieties grown and the quality and quantities required for specific markets; research institutions provide improved varieties; Ministry of Agriculture formulate policies and disseminates

information related to production practices and new technologies; KEPHIS and KARI seed unit release new varieties and certified seed. Non-governmental organizations undertake cross-cutting activities such as information dissemination; enable linkages to credit and other inputs. However, most of these stakeholders work independent of each other and this is one of the reasons why pigeonpea productivity and commercialization has remained low.

Improving the linkages between different actors through public-private partnerships has been shown to satisfy market demands while retaining smallholder farmers in the supply chain (Narro et al., 2007). For example, according to Shiferaw et al. (2008) vegetable pigeonpea destined for export is usually collected by the exporters at various designated collection points depending on shipping logistics. Because the peas are perishable, picking, collection, processing, and export need to be carefully synchronized, such that farmers growing pigeonpea for export markets work closely with exporter agents to plan their harvesting, transportation and sale.

Poor scheduling of these activities can result in losses to producers because of perishability of the product. Drought constraint can also be overcome by provision of irrigation water through public-private sector partnerships. In other crop sectors, with reliable water supply for irrigation, farmers have access to credit more easily because they use proceeds from crop sales as collateral (Lo Monaco, 2003). This has already occurred in Asia, Latin America and Europe, and North America (Thorbecke, 1993), where private firms have improved smallholder crop management practices and productivity by supplying farmers with new cultivars, pesticides, farm equipment, information, capital, and other services.

Pigeonpea is gaining in importance over maize due to perennial drought that has led to total maize crop failure over the years and therefore farmers have turned to pigeonpea as a cash crop as well as food crop. Most of the pigeonpea varieties currently grown possess the desired attributes listed by farmers, traders and processors who also represent consumers. The study has shown that white seed colour, large seed size and medium-maturity period are important traits preferred by farmers and processors/exporters for both domestic and export markets. However, limited hectareage under pigeonpea was blamed on untimely and insufficient seed supply and lack of market for the produce. Similar results were reported by Shiferaw et al. (2008) who concluded that increased pigeonpea production will be determined by improved seed production and supply systems that meet customer demand.

The stakeholder analysis has generated important information from a wide spectrum of role-players in the pigeonpea value chain. The results indicate that hybrid pigeonpea could be a viable option to improve pigeonpea production in Kenya, provided that the interests and preferences of all stakeholders in the value chain are taken into account in the breeding process.

References

- Bantilan, M.C.S., and P.K. Joshi. 1996. Returns to research and diffusion investments on wilt resistance to pigeonpea. Impact series (No.1). International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 502 324, Andhra Pradesh, India.
- Ceccarelli, S., S. Grando, M. Singh, M. Michel, A. Shikho, M. Al Issa, A. Al Saeh, G. Kaleonjy, S.M. Al Ghanem, A.L. Al Hasan, H. Dalla, S. Basha, and T. Basha. 2003. A methodological study on participatory barley breeding, II. Response to selection. *Euphytica* 133:185-200.
- Chauhan, Y.S., D.H. Wallace, C. Johansen, and L. Singh. 1998. Genotype-by-environment interaction effect on yield and its physiological bases in short-duration pigeonpea. *Field Crops Research* 59:141-150.
- FAOSTAT. 2011. Pigeonpea agricultural production statistics for Kenya [Online]. Available by www.faostat.org (verified 21 September 2011).
- Freeman, H.A., and R.B. Jones. 2001. Sub-sector analysis as a tool for improving commercialization and market access for pigeonpea producers, *In* S. N. Silim, et al. (Eds.) Status and potential of pigeonpea in eastern and southern Africa: Proceedings of a regional workshop, 12-15 Sep 2000, Nairobi, Kenya.
- GOK. 2007. District agricultural annual report-several districts, *In* Agriculture et al. (Eds.). Government Printers, Nairobi, Kenya.
- Gwata, E.T., S.N. Silim, and M. Mgonja. 2006. Impact of a new source of resistance to fusarium wilt in pigeonpea. *Phytopathology* 154:62-64.
- Jones, R., H.A. Freeman, and G. Lo Monaco. 2002. Improving the access of small farmers in eastern and southern Africa to global pigeonpea markets. *Agricultural Research and Extension Network* 120:1-11.
- Joshi, P.K., R.P. Parthasarathy, C.L.L. Gowda, R. Jones, S.N. Silim, K.B. Saxena, and K. Jagdish. 2001. The world chickpea and pigeonpea economies: facts, trends and outlook. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 502 324, Andhra Pradesh, India.

- Kaplinsky, R. 2000. Spreading the gains from globalisation: What can be learnt from value chain analysis? *Journal of Development Studies* 37:117-146.
- Lo Monaco, G. 2003. Competitiveness of African pigeonpea exports in international markets. Socio-economics and policy working paper series no. 15. International Crops Research Institute for the Semi-Arid Tropics, Bulawayo, Zimbabwe.
- Lusby, F., and H. Panlibuton. 2004. Promotion of commercially viable solutions to sub-sector and business constraints action for enterprise (AFE) USAID, Washington, D.C, USA.
- McCormick, D., and H. Schmitz. 2001. Manual for value chain research on home workers in the garment industry [Online]. www.ids.ac.uk/ids/global/pdfs/wiegomanualendnov01.pdf.
- Mergeai, G., P. Kimani, A. Mwang'ombe, F. Olubayo, C. Smith, P. Audi, J.P. Baudoin, and A. Roi. 2001. A survey of pigeonpea production systems, utilization and marketing in semi-arid lands of Kenya. *Biotechnology, Agronomy, Society and Environment* 5:145-153.
- Morris, L., and R. Bellon. 2004. Participatory plant breeding research: opportunities and challenges for the international crop improvement system. *Euphytica* 136:21-35.
- Nagarajan, L., P. Audi, R. Jones, and M. Smale. 2007. Seed provision and dryland crops in the semi-arid regions of eastern Kenya. IFPRI Discussion paper 00738.
- Narrod, C., D. Roy, J. Okello, B. Avendano, K. Rick, and A. Thorat. 2007. The role of public-private sector partnerships and collective action in ensuring smallholder participation in high value fruit and vegetable supply chains, Cali, Colombia.
- Okali, C., J. Sumberg, and J. Farrington. 1994. Farmer participatory research. Rhetoric and reality, UK.
- Ryan, J.G. 1997. A global perspective on pigeonpea and chickpea sustainable production system: Present status and future potential, *In* A. N. Asthana and A. Masood (Eds.). Recent advances in pulses research. Indian Institute of Pulses Research, Kanpur, India.
- Shiferaw, B., G. Obare, and G. Muricho. 2006. Rural institutions and producer organizations in imperfect markets: Experiences from producer marketing groups in semi-arid eastern Kenya. CAPRI working paper 60. International Crops Institute for the Semi-Arid Tropics, Patancheru 502 324, Andhra Pradesh, India.

- Shiferaw, B., J. Okello, G. Muricho, J. Omiti, S.N. Silim, and R. Jones. 2008. Unlocking the potential of high value legumes in the semi-arid regions: Analyses of the pigeonpea value chains in Kenya. International Crops Research Institute for the Semi-Arid Tropics, Nairobi, Kenya.
- Silim, S.N. 2001. Strategies and experiences in pigeonpea variety and development for Eastern and Southern Africa, pp. 232 pp, *In* S. N. Silim, et al. (Eds.) Status and potential of pigeonpea in eastern and southern Africa: Proceedings of a regional workshop, 12-15 Sept 2000. ICRISAT, Nairobi, Kenya.
- Sperling, L., J.A. Ashby, M.E. Smith, E. Weltzien, and S. McGuire. 2001. A framework for analyzing participatory plant breeding approaches and results. *Euphytica* 122:439-450.
- Thorbecke, E. 1993. Impact of state and civil institutions on the operation of rural markets and non-market configurations. *World Development* 21:591-605.
- Witcombe, J.R. 1996. Participatory approaches to plant breeding and selection. *Biotechnology and Development Monitor* 29:2-6.

Chapter 3: Evaluation of pigeonpea cytoplasmic male sterile A- and B-lines under different environmental conditions in Kenya

Abstract

The use of highly stable cytoplasmic male sterile lines (CMS) can drastically reduce the cost of hybrid seed production by eliminating the task of emasculation. Promising stable CMS lines have been developed in India and the hybrids produced with these lines have high heterosis. The aim of this study was to investigate the stability of pollen sterility and morphological characteristics of several CMS lines under Kenyan conditions. Three sites; Katumani (1°35'S: 37°14'E 1,600 m), Kiboko (2°15'S;37°45'E, 960 m), and Leldet-Nakuru (0°31'E;0°09'S, 1,275 m) were selected for evaluation. Six CMS lines, of proven stable pollen sterility, and their maintainers were sourced from ICRISAT India and evaluated for two seasons in 2009 in a screen house at Katumani and Kiboko and in an isolated field at Leldet Nakuru. Two CMS lines, ICPA2043 and ICPA2039 were the most stable across sites with 100% and 99% pollen sterility respectively. Days to flower showed 2- to 11-day variations between the A- and B-lines, but were not significantly different. Performance of the two promising CMS lines under Kenyan conditions for pollen sterility was comparable to the results obtained in India and can therefore be used in commercial hybrid breeding.

3.1 Introduction

Pigeonpea improvement research in Kenya was initiated in the mid-1970s with breeding activities centred on collection, evaluation and selection. Adoption of improved varieties released by national and international research institutions is evident in many parts of eastern province (Jones et al., 2001; Sutherland et al., 1999). Recently pigeonpea hybrids have been successfully developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) for India and this technology could be of importance for future pigeonpea breeding in Kenya. One of the first steps is to study the stability of the cytoplasmic male sterility (CMS) in pigeonpeas under Kenyan conditions.

In pigeonpea, several stable CMS systems have been developed (Mallikarjuna and Saxena, 2005; Saxena and Kumar, 2003; Saxena et al., 2005). Wanjari et al. (2001) crossed *Cajanus volubilis* (Blanco) (A_3 cytoplasm) with a cultivated type and selected a number of male sterile segregants with maternal inheritance for male sterility. These selections, however, could not be used in any hybrid breeding programme due to lack of fertility restoring genotypes. Studies are underway for another promising source of male sterility found in a naturally out-crossed partial, male sterile plant that was observed in a population of *Cajanus lineatus* Lin (A_6 cytoplasm) (Saxena et al., 2010b). In subsequent evaluations it showed that an effective male sterility and maintainer system was available. Saxena et al. (2005) crossed ICPW29, an accession of *C. cajanifolius* (Haines) van der Maesen) (A_4 cytoplasm), a wild relative of pigeonpea and after backcrossing developed the CMS line ICPA2039. It was found to be a highly stable male sterile line across environments and years (Dalvi et al., 2008; Saxena et al., 2010b) and never showed any morphological deformity. The ICPA2039 system has been used to develop other CMS lines with resistance to diseases, with various maturity periods and with adaptation to diverse environments of India (Saxena, 2008).

Environmental conditions are known to influence the expression of nuclear and cytoplasmic male sterility genes in some crops, whereby sterility and fertility changes depend on daylength and/or temperature (Janska and Mackenzie, 1993; Mcvetty, 1988). Ariyanayagam et al. (1995), using sensitive pigeonpea genotypes, established that short daylength and low temperatures induce male fertility, while high temperatures and longer days maintain male sterility. In CMS cotton (*Gossypium* spp.), wind velocity, air temperature, global radiation, and pan evaporation have been shown to influence expression of male sterility two to three weeks before anthesis (Marshall et al., 1974; Sarvella, 1966; Weider et al., 2009). In rape seed

(*Brassica napus* L.), day-night temperatures of 22-16°C resulted in stability of sterility while day-night temperatures of 30-24°C promoted anther development (Fan and Stefansson, 1986). On the other hand, CMS onion (*Allium cepa* L.) had more mature pollen at low temperature (Peterson and Foskett, 1953).

For successful commercialization of a hybrid, an easy seed production method is a prerequisite, which is dependent on insect behaviour in the particular location, stability of male sterile line, and duration of stigma receptivity (Saxena et al., 2006). In pigeonpea, studies have shown that for optimum pod setting, the stigma is highly receptive a day before and continues for three to five days after flower opening (Prasad et al., 1977). Luo et al. (2009) studied the stigma receptivity for CMS lines ICPA2039 and ICPA2043 and concluded that high receptivity occurred two days before flower opening and continued for four days. The study further revealed that the peak stigma receptivity for ICPA2039 and ICPA2043 was on the day of flower opening with 84 and 86% pod set respectively, after hand pollination. However, ICPA2039 remained receptive for eight days while ICPA2043, five days.

The long time span of stigma receptivity in pigeonpea encourages insect-aided natural out crossing and this also facilitates good seed yield in seed production blocks. Variations in days to flower have been observed between A- and B-lines (Saxena, 2006). Prior knowledge of days to flower for each line will enable synchronized nicking especially. The precise time interval should be determined for individual CMS lines and in each growth environment.

As in any breeding programme for pigeonpea, a desirable CMS line should possess yield components that have a direct positive effect on yield. These include; days 50 % flowering, primary branches, plant height, number of pods per plant and seed mass (Bhadru, 2010; Egbe and Vange, 2008; Lal and Raina, 2002). Favourable yield components will enable availability of sufficient number of flowers for pollination hence high seed yields.

The objective of this study was to evaluate several cytoplasmic male sterile lines of Indian origin for stability across several environments in Kenya.

3.2 Materials and methods

3.2.1 Site description

The study was conducted at three sites; Katumani, Kiboko and Leldet farm-Nakuru. Katumani lies at 1°35'S and 37°14'E at 1600 m above sea level (masl). The centre experiences a semi-arid tropical climate in AEZ IV with a bimodal pattern of rainfall. The first rains come in March with a peak in April followed by a dry period extending from June to mid-October. The short rains occur from mid-October with a peak in November and taper off towards mid-December. Long rains receive 272 mm and short rains 382 mm. Mean maximum temperature is 24.7°C and mean minimum is 13.7°C. Kiboko lies at 37°45'E and 2°15'S with an elevation of 960 masl. It is characterized by high temperatures with a mean minimum and maximum of 16.9°C and 31°C respectively. It has a bimodal pattern of rainfall ranging between 200-400 mm per season. Leldet lies at 0°31'E and 0°09'S with an altitude of 2,275 masl on the outskirts of Nakuru. The area experiences bimodal pattern of rainfall which are erratic with peaks in April and August and annual a mean of 380 mm. The area can be hot with temperatures mean maximum of 26°C and mean minimum 14°C, with warmest months from November to February.

3.2.2 Plant materials

The experimental materials comprised of six CMS lines (ICPA2043, ICPA2039, ICPA2091, ICPA2050, ICPA2042 and ICPA2101) with A₄ cytoplasm and their corresponding maintainers (Table 3.1) The A-lines were obtained from segregating progeny of a cross between ICPW29, an accession of *Cajanus cajanifolius* as a female parent, with a cultivated pigeonpea line, ICPL28. *Cajanus cajanifolius* was collected from central India and differs from the cultivated type (De, 1974). Seeds of the A-lines and their corresponding maintainers were obtained from ICRISAT India where they had been developed by manual hand pollination under cages. The genotypes were of indeterminate and determinate growth habits. Seed colour in the study materials were brown and cream (beige) of small, medium and large sizes.

Table 3.1. Characteristics of pigeonpea CMS (A) and maintainers (B) study materials

Genotypes		Characteristics		
CMS lines (A)	Maintainers (B)	Seed colour	Growth habit	Seed size
ICPA2039	ICPB2039	Brown	Determinate	Small
ICPA2042	ICPB2042	Cream/beige	Indeterminate	Small
ICPA2043	ICPB2043	Brown	Indeterminate	Medium
ICPA2050	ICPB2050	Cream/beige	Indeterminate	Large
ICPA2091	ICPB2091	Brown	Determinate	Small
ICPA2101	ICPB2101	Brown	Determinate	Medium

Small=6-8 g 100; medium=9-12, and large=>12 (100- seed weight¹)

3.2.3 Trial design and crop management

To protect the experimental materials from pollinating insects, all the CMS lines and the maintainers were planted inside a nylon net (0.5 mm size) screen house at KARI Kiboko and KARI Katumani. At Leldet Farm in Nakuru the A- and B-lines were planted in an open field, but at an isolation distance of 500 m to other pigeonpeas as recommended by Saxena et al. (2005). Each line was planted in 9.5 m² plot of two rows spaced at 100 cm x 50 cm inter- and intra-row in a randomized complete block design with two replications. Twenty seeds were planted per plot with one seed per hill. Standard cultural practices were adopted to grow a healthy crop.

3.2.4 Data collection

Individual plants of the CMS lines were examined for male sterility. The B-lines were used as controls. Male sterility/fertility was assessed by sampling 10 fully grown but unopened flower buds from 15 plants. Initial examination was done by rubbing anthers between fingers and a 10X hand lens was used to establish the presence or absence of pollen. Further analysis was done by squashing anthers in 2% aceto-carmin solution (Zhang et al., 2002) and examination done under the microscope using a haemocytometer. In each slide, three microscopic fields were examined and counts were made for male-sterile (shriveled and unstained) and male fertile (round and red colour stained) pollen grains. The counts were converted to percentage sterility. The relative amount of non-viable pollen was used to classify the CMS lines. For morphological characterization, in each set, 10 plants were randomly selected and data was recorded on days to 50% flower, days to 75% maturity, plant height (cm), and number of primary branches, growth habit, seed mass, and seeds pod⁻¹. Days to maturity, seeds pod⁻¹, and seed mass (100-seed weight) were estimated based from the B-lines only.

3.2.5 Data analysis

Analysis of variance (ANOVA) was done for each location separately and combined across sites and seasons. For the combined analysis, variances were partitioned to test for differences among genotypes and the presence of G x E interactions. Stability patterns of the parameters were measured using regression coefficient (b) between the genotypic mean values and the environmental mean values. Genotypes with $b < 1.0$ were considered above average and therefore adapted to unfavourable environments whereas, those with b values > 1.0 were considered below average and specially adapted to favourable environments. If the $b = 1.0$, then the genotypes were considered to be suited or poorly adapted to all environments (Finlay and Wilkinson, 1963).

3.3 Results

3.3.1 Analysis of variance

Table 3.2 presents the combined analysis of variance across sites. The analysis of variance showed significant differences among genotypes, sites, seasons and their interactions for most traits recorded. Genotypes and genotype x site interactions were significantly different for all the traits recorded. Days to flower and plant heights were significant for genotypes, sites, and genotype x site interaction. Table 3.3 presents mean squares for B-lines for days to maturity, seed mass and seeds pod⁻¹. Days to maturity was significantly different between genotypes, sites and site x season interactions. Means for seeds pod⁻¹, seed mass were significantly different for genotypes, season and their interactions.

Table 3.2. Combined analysis of variance for some agronomic traits recorded on CMS (A) and B-lines of pigeonpea at Katumani, Kiboko and Leldet in 2009

Source	d.f.	Mean squares		
		Number of primary branches	Days to flower	Plant height (cm)
Rep	1	16.6	149.5	90.4
Genotype	11	94.7***	6706.4***	13190.7***
Site	2	185.4***	11616.3***	63.1
season	1	12.8*	1.9	1894.4**
Genotype x site	22	3.9	1130***	628.1***
Genotype x season	11	2.3	143.9	341.8
Site x season	2	132.6***	427.6	717.6*
Genotype x site x season	22	13.3***	188.7	409.8**
Error	71	1.6	153.5	187.1

*, **, ***significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Table 3.3. Combined analysis of variance for some agronomic traits recorded on CMS (B)

lines of pigeonpea at Katumani, Kiboko and Leldet in 2009

Source of variation	d.f.	Mean squares		
		Days to maturity	Seed mass (g)	seeds pod ⁻¹
Rep	1	4480.9	0.6	0.5
Genotype	5	24233.4***	166.7***	5.7***
Site	2	10271.3***	3.4	2.5**
season	1	1088.9	19.2***	0.9*
Genotype x site	10	1162.0	12.5*	1.2***
Genotype x season	5	822.9	35.1**	1.7**
Site xseason	2	2996.5**	12.3*	1.9*
Genotype x site x season	10	496.0	12.1***	1.7***
Error	35	426.5	0.7	0.2

*, **, ***significant at P≤0.05, P≤0.01, and P≤0.001, respectively.

Table 3.4. Means of agronomic traits recorded on pigeonpea cytoplasmic male sterile (A) and B-lines across sites and seasons

Genotype	Number of primary branches	Days to flower	Days to maturity	Seed mass (g)	Plant height (cm)	Pollen sterility (%)	Seeds pod ⁻¹
ICPA2039	6.9	80.0	-	-	69.0	99.0	-
ICPA2042	6.1	93.0	-	-	92.0	5.0	-
ICPA2043	9.7	96.0	-	-	119.0	100.0	-
ICPA2050	9.5	98.0	-	-	116.0	53.0	-
ICPA2091	14.2	146.0	-	-	170.0	71.0	-
ICPA2101	6.7	91.0	-	-	77.0	39.0	-
ICPB2039	6.3	77.0	136.0	9.5	72.0	0	4.0
ICPB2042	5.3	90.0	172.0	5.4	90.0	0	2.8
ICPB2043	9.3	94.0	165.0	11.2	120.0	0	4.0
ICPB2050	8.5	100.0	199.0	14.9	111.0	0	4.8
ICPB2091	12.6	152.0	269.0	5.2	159.0	0	3.9
ICPB2101	5.7	88.0	184.0	10.4	75.0	0	4.7
Mean	8.4	100.0	188.0	9.4	106.0	31.0	4.0
LSD _(0.05)	2.5	25.0	46.0	1.6	27.0	5.0	0.9
CV (%)	15.0	12.0	12.0	8.7	13.0	9.0	10.6

When means were compared across sites (Table 3.4), the mean for days to flower was 100 days but the variation ranged from 77 days (ICPA2039) to 152 days to flower (ICPA2091). Pollen sterility for A-lines ranged from 5 to 100%. The A-lines, ICPA2043 and ICPA 2039 recorded 100% and 99% sterility across sites and seasons. Although the mean for plant height

was 106 cm, it showed variation among the genotypes ranging from 69 cm (ICPA2039) to 170 cm (ICPA2091).

3.3.2 Description of agronomic traits

Tables 3.5 and 3.6 present results recorded for some of the agronomic traits recorded on A- and B-lines.

Plant height

The growth habit and environments had a direct influence on plant height with significant differences recorded between genotypes, genotype x site and genotype x season interactions. ICPA2039 and ICPA2101 were determinate and therefore gave the shortest height. The tallest genotype across locations was ICPA2091 (170 cm) and the shortest was ICPA2039 (69 cm). Across environments, Katumani in the second season had the highest mean plant height (114 cm), but lowest (98 cm) in the first season.

Number of primary branches

Mean number of branches per plant varied among the genotypes. The highest number of branches were recorded on ICPA2091 (14.2) and lowest on ICPA2042 (5.3). The A- and B-lines of ICPA2043 produced an average of 10 branches per plant while ICPA2039 produced seven branches.

Days to flower

Days to flower did not vary markedly between A- and B-lines. Most genotypes flowered earlier in long rains than short rains. All genotypes showed delayed flowering at Katumani in the first season as compared to other sites with a mean of 100 days. ICPB2039 flowered earliest (77 days) while ICPA2091 flowered latest (152 days). The difference in days to flower between ICPA2043 and the corresponding B- line was 2 days. A t-test analysis to compare days to flower for A-and B-lines was not significantly different (t-stat of 0.04), with A-lines having a mean of 101 and B-lines, 100 days.

Days to maturity

The mean for days to maturity was 188 days and ranged between 269 days (ICPB2091) and 136 days (ICPB2039). The B-lines at Katumani season 1 took more days to maturity (221) as compared to 162 days at Kiboko. ICPB2039 matured earliest at Katumani (161 days), but ICPB2043 matured at 193 days.

Seed mass

Overall, mean seed mass was 9 g across sites. The highest seed mass was recorded on ICPB2050 (14.9 g) and the lowest was for ICPB2042 (5.4 g) and ICPB2091 (5.2 g). Seed mass was consistent across sites.

3.3.3 Male sterility

The extent of male sterility among the A-lines ranged between 5% and 100%. The highest percentage was recorded for ICPA2043 (100%) and ICPA2039 (99%), which was also significantly different from the other genotypes. The two A-lines recorded the highest sterility across sites and seasons. Pollen sterility for ICPA 2050 and ICPA2091 was between 41 and 81%). ICPA2042 recorded less than 5% pollen sterility, except during the first season at Kiboko where 21% was recorded. ICPA2101 varied between 14% and 80% but without showing any particular trend.



Fig 3.1. A-Sterile anthers, (ICPA2043) B- fertile anthers with viable pollen (ICPB2043)

3.3.4 Regression coefficients for selected morphological characteristics of pigeonpea A- and B-lines

Regression coefficients for some of the morphological characteristics studied are presented in Table 3.7. Regression analysis describes the stability of the response of pigeonpea genotypes grown in several locations. Regression coefficients greater than unity could be characterised as suitable for specific adaptation in favourable environments. Those close to unity could be categorised as well adapted to all environments. Regression coefficients >0.7 for days to flower was recorded for ICPA2039 (1.1), ICPB2039 (1.1), ICPA2043 (0.8), and ICPB2043 (0.8). ICPA2039 and ICPA2043 gave consistently high scores for sterility across sites and seasons. Number of primary branches recorded a regression coefficient of >1.0 for ICPA2039 and ICPA2101.

Table 3.5. Means for pollen sterility, days to flower and days to maturity across sites and seasons

Genotype	Pollen sterility (%)						Days to flower						Plant height (cm)					
	Katumani		Kiboko		Leldet		Katumani		Kiboko		Leldet		Katumani		Kiboko		Leldet	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
ICPA2039	100	100	96	100	100	100	86	100	73	71	72	77	77	82	62	64	61	66
ICPA2042	2	2	21	1	2	3	123	118	83	70	88	79	86	94	97	101	77	95
ICPA2043	100	100	100	100	100	100	107	123	91	77	89	88	123	129	104	156	102	99
ICPA2050	67	56	46	62	47	41	104	122	74	86	112	92	94	131	118	98	112	140
ICPA2091	77	69	45	77	75	81	175	190	123	134	134	121	147	193	189	167	153	168
ICPA2101	14	38	80	17	35	50	77	72	81	88	107	121	69	63	63	64	119	86
ICPB2039	0	0	0	0	0	0	84	97	75	68	74	67	77	84	68	69	63	71
ICPB2042	0	0	0	0	0	0	127	104	65	70	85	88	77	95	101	95	83	94
ICPB2043	0	0	0	0	0	0	119	107	90	79	89	81	119	129	116	150	108	99
ICPB2050	0	0	0	0	0	0	117	114	74	90	116	87	93	119	98	111	113	132
ICPB2091	0	0	0	0	0	0	185	218	121	139	130	119	139	184	172	165	150	143
ICPB2101	0	0	0	0	0	0	78	75	80	86	107	106	71	61	71	69	106	74
Mean	30	31	33	30	30	32	115	120	86	88	100	94	98	114	105	109	104	105
LSD _(0.05)	5						25						27					
CV (%)	9						12						13					

1=2009 growing season, 2=2010 growing season.

Table 3.6. Means for days to maturity, 100-seed weight and seeds per pod across sites and seasons at Katumani, Kiboko and Leldet

Genotype	Days to maturity						Seed mass (100-seed weight)						seeds pod ⁻¹					
	Katumani		Kiboko		Leldet		Katumani		Kiboko		Leldet		Katumani		Kiboko		Leldet	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2
ICPB2039	161	157	104	118	131	147	10	10	7	9	10	10	4	4	4	4	4	4
ICPB2042	202	170	164	160	168	168	10	2	7	7	8	3	3	2	3	3	4	3
ICPB2043	193	181	135	187	144	154	13	12	10	11	9	13	4	4	4	4	4	4
ICPB2050	221	190	173	163	225	226	16	18	13	14	13	16	5	5	5	5	5	5
ICPB2091	309	244	250	216	280	314	7	6	8	8	7	7	5	5	4	5	5	5
ICPB2101	240	195	176	133	172	188	10	10	12	8	9	13	4	5	4	5	5	5
Mean	221	189	167	162	187	199	11	8	9	10	9	9	4	3	4	4	4	4
LSD _(0.05)	42						2						1					
CV (%)	11						9						11					

1=2009 growing season, 2=2010 growing season.

Table 3.7. Regression coefficients of some morphological traits of cytoplasmic male sterile pigeonpea lines and their maintainers across sites and seasons

Genotypes	Morphological traits				
	Days to flower	Days to maturity	Number of branches	Seed mass (g)	Plant height (cm)
ICPA2039	1.1	-	1.1	-	0.1
ICPA2042	0.6	-	0.6	-	0.3
ICPA2043	0.8	-	0.7	-	0.1
ICPA2050	0.7	-	0.4	-	0.2
ICPA2091	0.5	-	0.6	-	0.2
ICPA2101	0.3	-	1.5	-	0.1
ICPB2039	1.1	0.7	0.9	0.2	0.2
ICPB2042	0.6	0.9	0.7	0.2	0.4
ICPB2043	0.8	0.2	0.8	0.1	0.1
ICPB2050	0.7	0.5	0.5	0.1	0.2
ICPB2091	0.3	0.4	0.6	0.2	0.2
ICPB2101	0.4	0.4	1.2	0.2	0.1

3.4 Discussion and conclusions

The study was aimed at determining the most suitable CMS lines for use in the pigeonpea breeding programmes in Kenya. Highly significant differences between the genotypes and sites for most characters revealed that considerable variability exists amongst the CMS lines and the maintainers in different seasons for the traits recorded. This was also reported by Sharma and Green (1977) and Sidhu et al. (1985). There is therefore need to categorize genotypes for their adaptation to varying environmental conditions using their respective regression coefficient values as suggested by Finlay and Wilkinson (1963). ICPA2043 and ICPA2039 recorded the recommended levels of male sterility (>95%) (Saxena et al., 2005) across all sites and seasons. This implies that the two A-lines could be used for seed production in different environments in Kenya.

Days to maturity of the A- and B- lines shows that they belong to the early and medium maturity groups. There is therefore the potential to utilize these A-lines to develop medium maturity hybrids. Similar work has been done in India (Saxena et al., 2010a), where relatively late flowering CMS were used in breeding long- and medium-maturity hybrids for specific environments. Days to flowering for the promising CMS lines and their maintainers were not

significantly different. This indicates that the A- and B-lines can be planted at the same time without affecting seed production.

Traits such as number of primary branches indirectly influences the seed yield through the number of pods plant⁻¹ (Lal and Raina, 2002). On average ICPA2043 and ICPA2039 produced 10 and 7 branches respectively. However, the potential for ICPA2043 to produce 21 branches and ICPA2039 (15) has been recorded (Saxena et al., 2005) and this was observed on individual plants in the trial plots. The differences were expected as ICPA2043 is non-determinate but ICPA2039 is determinate.

Commercial use of CMS requires highly stable male sterility, to ensure genetically pure F₁ hybrid seed. The CMS lines ICPA2043 and ICPA2039 were highly stable and recorded mean pollen sterility of 100% and 99% respectively across the locations and seasons. The stable sterility of these lines was also reported by Dalvi et al. (2008) and Saxena (2008) who found high stability for pollen sterility across environments in India. The two CMS-lines were therefore suitable for use in commercial hybrid breeding programmes in that country (Saxena, 2008; Saxena et al., 2010b).

Performance of the two CMS lines under Kenyan conditions for pollen sterility was comparable to that in India. The most preferred sites for seed production are those with optimal growth conditions for pigeonpea. In this study, Kiboko, where irrigation can be used was the ideal site as plants produced more branches and were taller indicating high vigour that is likely to lead to high seed yields. The study has shown that commercial hybrid seed production in Kenya is feasible using the two A-lines with A₄ cytoplasmic male sterility.

References

- Ariyanayagam, R.P., A.N. Rao, and P.P. Zaveri. 1995. Cytoplasmic male sterility in interspecific matings of *Cajanus*. *Crop Science* 35:981-985.
- Bhadru, D. 2010. Studies on genetic parameters and interrelationships among yield and yield contributing traits in pigeonpea [*Cajanus cajan* (L.) Millsp)]. *Legume Research* 33:23-27.
- Dalvi, V.A., K.B. Saxena, and I.A. Madrap. 2008. Fertility restoration in cytoplasmic-nuclear male sterile lines derived from three wild relatives of pigeonpea. *Journal of Heredity* 99:671-673.
- De, D.N. 1974. Pigeonpea, *In* J. Hutchinson (Ed). *Evolutionary studies in world crops, diversity and change in the Indian sub-continent*. Cambridge University Press, London, UK.
- Egbe, M.O., and T. Vange. 2008. Yield and agronomic characteristics of 30 pigeonpea genotypes at Otobi in Southern Guinea Savanna of Nigeria. *Life Science Journal* 5:70-80.
- Fan, Z.G., and B.R. Stefansson. 1986. Influence of temperature on sterility of two cytoplasmic male sterile systems in rape (*Brassica napus* L.). *Canadian Journal of Plant Sciences* 66:221-227.
- Finlay, K.W., and G.N. Wilkinson. 1963. The analysis of adaptation in a plant breeding program. *Australian Journal of Agricultural Research* 14:742-754.
- Janska, H., and S.A. Mackenzie. 1993. Unusual mitochondrial genome organization in cytoplasmic male sterile common bean and the nature of cytoplasmic reversion of fertility. *Genetics* 135:869-879.
- Jones, R.B., P. Audi, and R. Tripp. 2001. The role of informal seed systems in disseminating modern varieties. The example of pigeonpea from a semi-arid area of Kenya. *Experimental Agriculture* 37:539-548.
- Lal, S.K., and R. Raina. 2002. Inter-relationships between yield and its component traits in long duration hybrid in pigeonpea. 23:101-104.

- Luo, R.H., V.A. Dalvi, Y.R. Li, and K.B. Saxena. 2009. A study on stigma receptivity of cytoplasmic nuclear male sterile lines of pigeonpea *Cajanus cajan* (L.). *Journal of Plant Breeding and Crop Science* 1:254-257.
- Mallikarjuna, N., and K.B. Saxena. 2005. A new cytoplasmic nuclear male-sterility system derived from cultivated pigeonpea cytoplasm. *Euphytica* 142:143-148.
- Marshall, D.R., N.J. Thomson, G.H. Nicholls, and C.M. Patrick. 1974. Effect of temperature and daylength on cytoplasmic male sterility in cotton (*Gossypium*). *Australian Journal of Agricultural Research* 25:443-450.
- Peterson, C.E., and R.L. Foskett. 1953. Occurrence of pollen sterility in seed fields of Scott County Globe onions. *Proceedings of American Society for Horticultural Sciences* 62:443-448.
- Prasad, S., R. Prakash, and F. Hague. 1977. Floral biology of pigeonpea. *Tropical Grain Legume* 7:12.
- Sarvella, P. 1966. Environmental influence on sterility in cytoplasmic male sterile cottons. *Crop Science* 6:361-364.
- Saxena, K.B. 2006. Seed production systems in pigeonpea. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, 502 324, Andhra Pradesh, India.
- Saxena, K.B. 2008. Genetic improvement of pigeonpea. A review. *Tropical Plant Biology* 1:159-178.
- Saxena, K.B., and R.V. Kumar. 2003. Development of a cytoplasmic nuclear male sterility system in pigeonpea using *C. scarabaeoides* (L) Thours. *Indian Journal of Genetics and Plant Breeding* 63:225-229.
- Saxena, K.B., R.V. Kumar, N. Srivastava, and B. Shiyong. 2005. A cytoplasmic-nuclear male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. *Euphytica* 145:289-294.
- Saxena, K.B., R.V. Kumar, K.M. Latha, and V.A. Dalvi. 2006. Commercial pigeonpea hybrids are just a few steps away. *Indian Journal of Pulses Research* 19:7-16.

- Saxena, K.B., R.V. Kumar, A. Vijay, L.B. Dalvi, N. Pandey, and G. Gaddikeri. 2010a. Development of cytoplasmic-nuclear male sterility, its inheritance, and potential use in hybrid pigeonpea breeding. *Journal of Heredity* 101:497-503.
- Saxena, K.B., R. Sultana, N. Mallikarjuna, R.K. Saxena, R.V. Kumar, S.L. Sawargaonkar, and R.K. Varshney. 2010b. Male sterility systems in pigeonpea and their role in enhancing yield. *Plant Breeding* 129:125-134.
- Sharma, D., and J. Green, M. 1977. Problems and prospects of upgrading yield levels in pigeonpea. *Sabrao Journal* 2:6-10.
- Sidhu, P.S., M.M. Verma, H.S. Cheema, and S.S. Sra. 1985. Genetic relationships among yield components in pigeonpea. *Indian Journal of Agricultural Sciences* 55:232-235.
- Sutherland, A.J., W. Irungu, J. Kangara, and J. Muthamia. 1999. Household food security in semi-arid Africa: the contribution of participatory adaptive research and development to rural livelihoods in Eastern Kenya. *Food Policy* 24:363-390.
- Wanjari, K.B., A.N. Patil, P. Manapure, J.G. Manjaya, and P. Manish. 2001. Cytoplasmic male-sterility in pigeonpea with cytoplasm from *Cajanus volubilis*. *Annals of Plant Physiology* 13:170-174.
- Weider, C., P. stamp, N. Christov, Husken, A., X. Foueillassar, and K.H. Camp, M. 2009. Stability of cytoplasmic male sterility in maize under different environmental conditions. *Crop Science* 49:77-84.
- Zhang, B.X., L.H. wang, and J.Z.H. Guo. 2002. Investigation of male fertility of pepper in CMS. *China capsicum* 4:6-9.

Chapter 4: Evaluation of pigeonpea genotypes for general resistance to Fusarium wilt in Kenya

Abstract

To develop high yielding fusarium wilt resistant varieties, it is essential to identify new sources of resistance that can withstand multiple races of the pathogen. The aim of this study was to identify [*Fusarium udum* (L.) Millsp.] races occurring in the study field, determine reaction of pigeonpea hybrids to the different isolates and establish the mode of gene action responsible for fusarium wilt inheritance. Field and pot experiments were conducted during 2009 and 2010. A root-dip inoculation and transplantation technique in pots and sowing in a wilt-sick field were used for studying the reactions of 54 pigeonpea genotypes to *F. udum* isolates. Field evaluation was done in the infested plot in a 9 x 6 alpha lattice design with two replications. Purification of the isolates on potato dextrose agar identified three isolates and were designated as ISO-A, ISO-B, and ISO-C. The isolates produced whitish to light pink or orange mycelia. The purple was predominant on the substrate, but whitish to light pink were also identified. Pot inoculation trials with the three isolates identified seven genotypes (ICPB2043, ICP12012, ICP13092, ICPA2039xICP13092, ICPA2043xICP12012, ICPA2043xICP13092, and ICPA2043xICP9135) resistant to all the isolates. Under field evaluation, seven genotypes (ICPA2039xICP13092, ICPA2039xAsha, ICPA2043xICP12012, ICPA2043xICP13092, ICPA2043xICEAP557, ICPB2043, and Maruti) were moderately resistant. The cytoplasmic male sterile (A) line, A2043, showed resistance to the three isolates. The resistant hybrid, ICPA2943xICP12012 had the highest negative SCA that was highly significant for all the isolates in the field.

4.1 Introduction

Pigeonpea is the third most important legume in Kenya, after beans and cowpea often intercropped with maize, cowpea, greengrams, and fruit trees (Mergeai et al., 2003). It is the most dominant legume in the eastern parts of Kenya. Pigeonpea grain is a source of food and cash, leaves serve as livestock feed, and dry twigs are used as firewood. Some farmers grow it as a green hedge or strips for soil conservation and improvement. However, production is hampered by various biotic and abiotic constraints, notably diseases, pests and drought. Important diseases include fusarium wilt (*Fusarium udum* Butler), pigeonpea sterility mosaic virus and cercospora leaf spot (*Cercospora cajani* Hennings) (Kiprop et al., 2002).

Fusarium udum is a soil borne fungus that can affect the plant at all stages of development resulting in up to 100% yield loss (Reddy et al., 1990). In East Africa, the disease incidence has been estimated to be 60% in Kenya as compared to 36% and 24% in Malawi and Tanzania, respectively (Kannaiyan et al., 1984). The disease is reported to be widespread in pigeonpea growing areas, but severity and yield loss are varied (Kannaiyan et al., 1981; Kiprop et al., 2002).

Several management strategies for fusarium wilt have been developed that include use of resistant varieties, crop rotation, fallow, and green manuring. Use of resistant varieties is the most eco-friendly and sustainable management of the disease. Reddy and Raju (1997) identified various sources of resistance at International Crops Research Institute for Semi-Arid Tropics (ICRISAT) that included ICP7035, ICPL87119, ICPL98063 which have been used in breeding programmes. In Malawi, Changaya (2007) identified AP10, a local landrace, which was resistant to the disease and also high yielding and recommended that it could be utilized in the local breeding programme. Studies by Gwata et al. (2006) identified ICEAP00040 as consistently resistant to *F.udum* in Kenya, Malawi and Tanzania.

Screening of pigeonpea germplasm for resistance to fusarium wilt has been undertaken both under controlled conditions (Joshi, 2001; Kimani et al., 1994; Kiprop et al., 2002) and/or in the field (Gwata et al., 2006; Pawar and Mayee, 1986; Reddy et al., 1990), each with its unique limitations. The disadvantages of field screening are poor distribution of the pathogen and varying concentrations of inoculum in the field (Nene et al., 1981), and variation in environmental conditions such as moisture content and temperature (Burgess et al., 1994). As a result, the wilt-sick plot technique was developed (Kannaiyan et al., 1981; Nene et al., 1981) for

large scale screening of pigeonpea. The technique ensures uniform distribution of the pathogen.

Several artificial inoculation techniques are available and have been used by a number of workers for screening pigeonpea germplasm for resistance to fusarium wilt. The techniques are; root tip method (Hillocks, 1984; Marley and Hillocks, 2002; Okiror, 1998; Patel et al., 2011), water culture technique (Kraft et al., 1994; Nene and Kannaiyan, 1982), infected soil (Kraft et al., 1994; Okiror, 1998), seed inoculation and injection of inoculum (Hillocks, 1984). Changaya (2007) developed large wheat seed medium as an infested-seed inoculation technique, which was found to be a viable and effective option for screening pigeonpea germplasm.

A number of studies have shown the existence of races of *F.udum* and the variation in resistance levels of pigeonpea genotypes to the different isolates of the pathogen. Studies by several authors in India revealed that *F. udum* exists in different races (Baldev and Amin, 1974; Kumar and Sharma, 1989; Shit and Sen Gupta, 1978). Other studies found high variability in cultural characteristics of *F. udum* isolates collected from the same or different sites (Gaur and Sharma, 1989; Jeswani et al., 1977; Kiprop et al., 2002; Reddy and Chaudhary, 1985). In Kenya, Okiror and Kimani (1997) and Kiprop et al. (2002) identified several isolates of the pathogen by analysing samples collected from traditional and new pigeonpea growing areas and all the isolates conformed to the species *F. udum*. Other studies showed differences in the reaction of pigeonpea genotypes to different isolates indicating that they were races (Kimani et al., 1994; Okiror and Kimani, 1997; Pawar and Mayee, 1986).

The presence of pathogenic races of *F. udum* in Kenya is one of the factors affecting adoption and productivity of pigeonpea varieties released. Improved varieties currently grown by farmers are susceptible to the disease (Kannaiyan et al., 1984; Shiferaw et al., 2008) hence the need to develop a breeding programme that will target multiple races. Kenya Agricultural Research Institute (KARI) Kiboko's wilt-sick field is ideal for screening pigeonpea germplasm as it is presumed to contain most of the races of the pathogen found in Kenya and the field conditions are similar to farmers' fields. Inoculation of genotypes with specific races of the wilt pathogen will guide in selection of general resistant hybrids whose parents could be used as sources of resistance in breeding programmes.

Therefore the objectives of the study were to:

1. Characterize *F. udum* isolates found at KARI Kiboko wilt-sick field using morphological and cultural characteristics.
2. Screen pigeonpea genotypes for resistance using different *F. udum* isolates in pots using root-dip inoculation technique.
3. Evaluate pigeonpea genotypes in the wilt-sick field at KARI Kiboko for resistance to fusarium wilt.

4.2 Materials and methods

4.2.1 Description of wilt-sick field

The wilt-sick plot at KARI Kiboko was established in 1990 following the procedure developed by Haware and Nene (1994). An isolated field with wilt infection was planted with a fusarium wilt susceptible pigeonpea variety. When 10% of the crop showed wilt symptoms, tops were cut and after 30 days, the entire crop was cut, chopped and incorporated into the soil. Infected plant parts were also collected from other pigeonpea growing areas of Kitui, Makueni, Machakos, Mbeere and introduced in the plot. This procedure was repeated for several seasons and the field was considered sufficiently infected when 70% mortality of the susceptible varieties was recorded.

4.2.2 Morphological and cultural characterization of *F. udum* isolates

A known susceptible pigeonpea genotype, KAT 60/8 was obtained from KARI Katumani and sown on the wilt-sick plot at KARI Kiboko in January 2009. At flowering, 42 infected plant stem portions showing typical symptoms of wilt were sampled randomly from the entire field using a zigzag transect line to ensure adequate representation of the field (Jones Jr et al., 1971). The stem pieces were labeled, stored in a cooler box and then transported to KARI Katumani for laboratory analysis.

Stem sections of (0.05 cm²) were aseptically placed on freshly prepared Peptone PCNB (PPA or Nash-Snyder Medium) medium, a natural substrate medium that enhances fusarium sporulation (Fisher et al., 1982; Snyder and Hansen, 1947). Nash-Snyder medium is highly inhibitory to most other fungi and bacteria, but allows slow growth of *Fusarium* spp. The plates were incubated at 25°C in 12-h light/dark cycle for 48 hours. Single spores from colonies showing morphology typical of *F. udum* (Booth, 1978; Gerlach and Nirenberg, 1982) were transferred on freshly prepared potato dextrose agar (PDA) and carnations leaf-piece agar

(CLA) (appendix 1). Using a cork borer, 6 mm diameter discs were cut from 7-day old single-spore cultures from PDA and transferred onto the centre of fresh PDA and CLA plates, with one disc on each plate. The plates were arranged in a completely randomized design with six replications. These were incubated at 25°C in a 12-h light/dark cycle for seven days.

Both PDA and CLA cultures were used to record colony diameter, while PDA was also used to determine pigmentation and mycelia texture. CLA is a good medium for studying micro and macroconidia as it ensures abundant production of macro-conidia with least phenotypic variation hence was used to record sporulation and spore characteristics. Colony diameters were measured using a vernier calliper from the same axis throughout the experiment except where there was contamination. For colony diameter, the measurements were done on days 3, 6, 9, and 12 or until fungal growth filled at least one of the plates. Pigmentation of the mycelium and the substrate on PDA cultures were determined after 12 days with the help of a mycological colour chart (Rayner, 1970). The length and breadth of conidia were measured by micrometry. From the morphological and cultural characterization of the 42 isolates, three distinct isolate groups designated as ISO-A, ISO-B and ISO-C were identified and used in screening pigeonpea genotypes using pot inoculation technique.

4.2.3 Experimental site and germplasm

Pathogenic variability among different isolates was studied through the soil inoculation technique (Nene et al., 1981) at KARI Katumani in 2009. Field evaluation of pigeonpea genotypes was done at KARI Kiboko wilt-sick plot in 2009 and 2010. Katumani lies at 1°35'S and 37°14'E at 1600 m above sea level (masl). Mean maximum temperature is 24.7°C and minimum is 13.7°C. Kiboko lies at 37° 45'E and 2° 15'S with an elevation of 960 masl. It is characterized by high temperatures with a mean minimum and maximum of 16.9°C and 31°C respectively.

Experimental materials used in the study are presented in Table 4.1. Two cytoplasmic male sterile lines (female parents) obtained from ICRISAT India were crossed with 21 improved genotypes (male parents) from Kenya, Uganda, Tanzania and India (Fig. 4.1). However, not all crosses generated sufficient F₁ seeds and only those with enough seed for the screen house and field evaluations were included in the trials.



Fig 4.1. Crossing block at KARI Kiboko

Table 4.1. Pigeonpea genotypes used in the development of F₁ hybrids

Genotype	Type	Source	Maturity	Important attributes
ICEAP557	Improved	Kenya	Late / Medium	High yielding
ICEAP554	Improved	Kenya	Late / Medium	High yielding
ICEAP911	Improved	Kenya	Medium	High yielding
ICEAP902	Improved	Kenya	Medium	High yielding
ICEAP00068	Improved	Kenya	Medium	High yielding
KAT60/8	Improved	Kenya	Medium	High yielding
ICEAP850	Improved	Kenya	Medium	High yielding
TZ26	Landrace	Tanzania	Unknown	High yielding
TZ24	Landrace	Tanzania	Unknown	High yielding
UG1	Landrace	Uganda	Unknown	Podborer/bruchid resistant
UG8	Landrace	Uganda	Unknown	Podborer/bruchid resistant
UG18	Landrace	Uganda	Unknown	Podborer/bruchid resistant
ICP12023	Landrace	India genebank	Unknown	White large seeded
ICEAP7035	Landrace	Kenya	Unknown	White large seeded
ICP12091	Landrace	India genebank	Unknown	White large seeded
Kanchan	Improved	India	Medium	White large seeded
Maruti	Improved	India	Medium	Fusarium wilt resistant
Asha	Improved	India	Medium	Fusarium wilt resistant
ICP12012	Landrace	India genebank	Unknown	White large seeded
ICP9135	Landrace	India genebank	Unknown	White seeded
ICP13092	Landrace	India genebank	Unknown	White seeded
ICPA2043(P1)	Improved	India	Medium	A ₄ CMS stable
ICPA2039(P1)	Improved	India	Early	A ₄ CMS stable

4.2.4 Pot inoculation studies

Pathogenic variability among the three isolates; ISO-A, ISO-B and ISO-C and response of pigeonpea genotypes to the three isolates were studied in pots using the root-dip inoculation technique (Haware and Nene, 1994). To obtain sufficient sporulation, single spore cultures were inoculated on freshly prepared CLA, incubated for seven days at 27°C after which the cultures were rinsed with sterile water and filtered through a fine muslin cloth. Spore concentration was adjusted to 1×10^6 spores ml^{-1} using a haemocytometer.

Seeds of each of the pigeonpea genotypes were sown in large pots, watered regularly and sprayed with pesticides to control insect pests and fungicides to control diseases. After one month, the seedlings of each genotype were removed and roots washed under running water. Seedling root tips were aseptically trimmed and separately immersed in the spore suspension for 30 seconds, transplanted into plastic pots filled with autoclaved moistened soil, and regularly watered. For each isolate, six seedlings were transplanted in each pot and replicated three times. Seedlings immersed in distilled water served as controls. Days to wilt were recorded for each entry and wilt incidence was recorded 60 days after transplanting. Disease incidence was determined by counting the wilted seedlings expressing it as a percentage of the total number of plants in the three pots. Disease rating was also done using a scale of 1-5 (Table 4.2), 1=resistant and 5=susceptible.

4.2.5 Wilt-sick field screening

At KARI Kiboko's wilt-sick field, the trials were planted on 4th November 2009 and 2nd November 2010 using a 9 x 6 alpha lattice design with two replications. Each plot had two 4.5 m long rows at spacing of 50 cm between rows and 50 cm within rows. Recommended crop husbandry practices were done during crop growth. Due to differences in maturity dates for the different genotypes, data on wilt incidence (percentage mortality) were recorded two weeks before harvesting for each of the genotypes. Wilt incidence was rated using a scale of 1-5 with corresponding percentages, as developed by Nene and Kannaiyan (1982) (Table 4.2).

Table 4.2. Scale for rating pigeonpea genotype resistance to fusarium wilt

Scale	Incidence (%)	Rating
1	No wilted plants	Resistant
2	1-10 plants wilted	Moderately resistant
3	11-20 wilted	Tolerant
4	21-50 wilted	Moderately susceptible
5	>50 wilted	Susceptible

Source:(Nene and Kannaiyan, 1982)

4.2.6 Data analysis

Data were analyzed with Genstat 14th edition statistical programme (Payne et al., 2011). Test for normality of the data showed it was normally distributed so transformation was not done. Analysis of variance was determined using Residual Maximum Likelihood (REML). The analysis allowed for estimation of general combining ability (GCA) and specific combining ability (SCA) effects.

4.3 Results

4.3.1 Morphological and cultural characterization of *Fusarium udum* isolates

There were significant differences ($p \leq 0.001$) among the 42 isolates, media and isolate x medium interactions for colony diameter (Table 4.3). Colony growth on PDA and CLA revealed a maximum 12 days and 15 days after inoculation respectively (Fig 4.2 and Fig 4.3). On PDA, growth ranged between 49 mm to 90 mm while on CLA it ranged between 22 mm and 90 mm, with the majority of cultures filling the plates (Table 4.4).

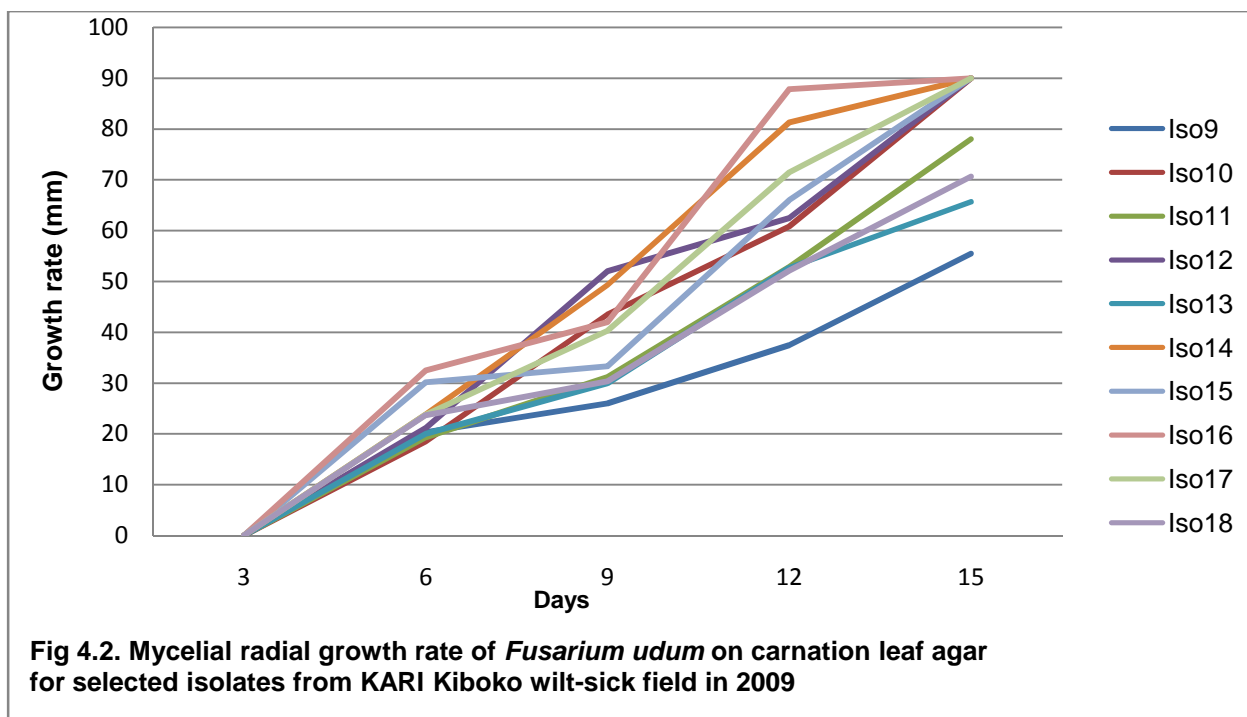
Table 4.3. Analysis of variance for colony diameter under laboratory conditions

Source of variation	d.f	Mean squares			
		<u>D3</u>	<u>D6</u>	<u>D9</u>	<u>D12</u>
Rep	5	4.435	2.57	56.1	2.335
Isolate	41	326.2***	806.9***	1352.8***	1012.8***
Medium	1	12311.7***	13145.8***	2669.8***	3225.4***
Isolate x Medium	41	360.3***	980.0***	1955.1***	1214.6***
Error	415	9.0	17.1	21.24	5.179

*** indicates the term is significant at $P \leq 0.001$. D3, D6, D9, D12= days after inoculation when diameter was recorded.

Cultural characteristics, pigmentation and colony diameter of isolates on PDA and CLA were variable (Table 4.4). Shades of purple, orange and white were recorded on both mycelium and substrate (Fig 4.4). Light pink and purple colours were dominant colour on mycelium, with

33.0% and 23.8% respectively. On the substrate, purple, cream/white and orange recorded 38.0%, 16.7%, and 14.3% respectively. Aerial mycelia were more abundant on PDA than CLA. Mycelial texture was categorized into fluffy, scanty and cottony with the latter dominating (57%). The size of micro- and macro-conidia showed large variability. The size of micro-conidia ranged from 1.5-2.7 x 2.6-9.3 μm and macro-conidia ranged from 9.6-15.9 x 30.2 x 58.5 μm . Macro-conidia were straight to falcate and thin walled, and the majority had three septa. Micro-conidia were fusiform to oval shape with none or one septum borne on monophialides with false heads.



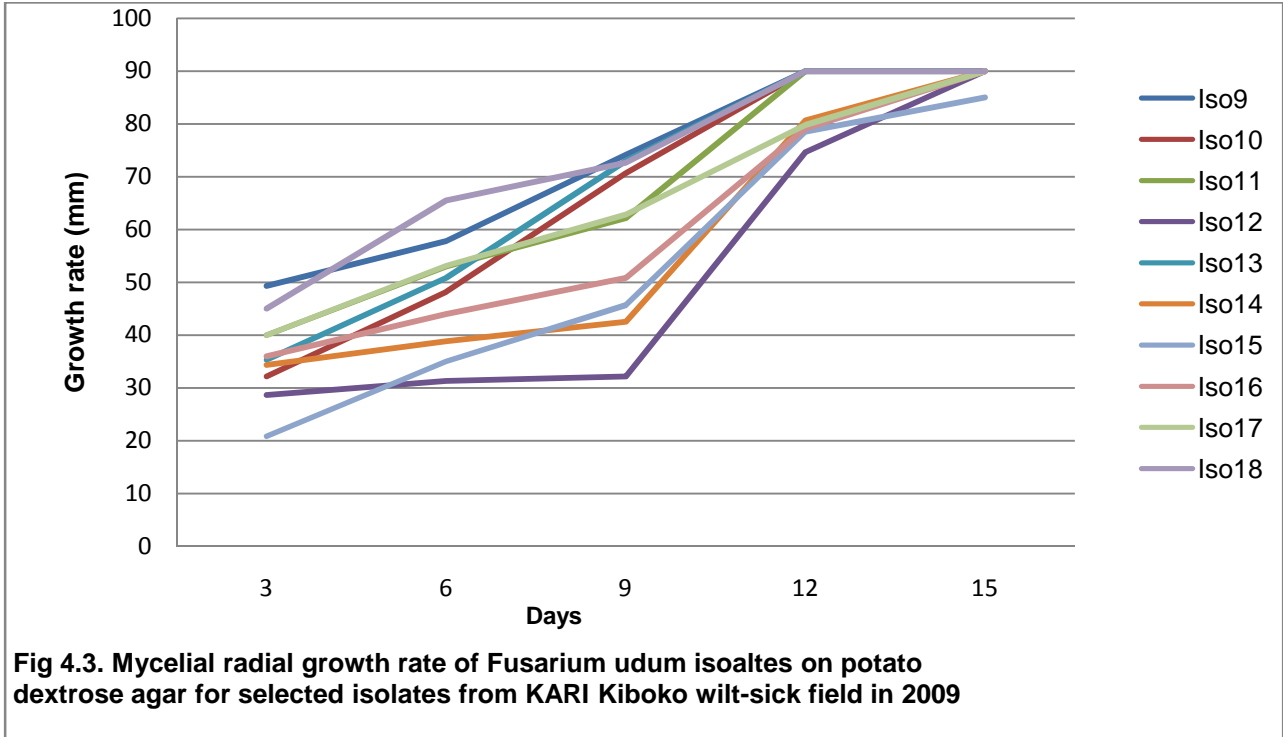


Table 4.4. Cultural characteristics¹ and colony diameter of *Fusarium udum* isolates on potato dextrose agar (PDA) and carnations leaf agar (CLA)

Isolate no.	Mycelial colour	Substrate colour	Mycelial texture	Colony diameter (mm)	
				PDA	CLA
1	Purple	Purple	Moderately fluffy	49	63
2	Purple	Cream	Scanty	90	79
3	White	Plum purple	Scanty	90	90
4	Light pink	Orange	Cottony	90	68
5	Purple	Purple	Cottony	66	90
6	Light pink	Purple	Scanty	82	90
7	Light pink	Purple	Cottony	90	90
8	Light pink	Purple	Cottony	90	90
9	Orange	Plum purple	Scanty	56	90
10	Light pink	Purple	Scanty	90	90
11	White	Plum purple	Moderately fluffy	78	90
12	White	Purple	Moderately fluffy	90	75
13	Purple	Purple	Moderately fluffy	66	90
14	Light pink	Light pink	Cottony	90	81
15	White	Purple	Cottony	90	79
16	Light pink	Light pink	Cottony	90	79
17	White	Plum purple	Scanty	90	80
18	Orange	Light pink	Cottony	71	90
19	White	Light pink	Cottony	90	69
20	Orange	Light pink	Scanty	90	25
21	Purple	Purple	Cottony	90	22
22	Purple	Purple	Scanty	90	53
23	Light pink	Purple	Cottony	90	90
24	white	Plum purple	Moderately fluffy	90	90
25	Light pink	Purple	Cottony	90	90
26	Purple	Purple	Cottony	90	90
27	Light pink	Purple	Cottony	90	90
28	Purple	Orange	Scanty	90	66
29	White	Purple	Scanty	90	90
30	Purple	Purple	Cottony	90	90
31	Light pink	Plum purple	Cottony	90	90
33	Orange	white	Cottony	70	90
34	Purple	Purple	Scanty	90	75
35	Purple	Plum purple	Moderately fluffy	90	82
36	Light pink	Orange	Cottony	90	56
37	White	Light pink	Cottony	87	90

Table 4.4 continued...

Isolate no.	Mycelial colour	Substrate colour	Mycelial texture	Colony diameter (mm)	
				PDA	CLA
38	White	Light pink	Cottony	90	90
39	Light pink	Purple	Moderately fluffy	90	90
40	Purple	Plum purple	Cottony	90	90
41	White	Orange	Cottony	90	90
42	White	Orange	Cottony	90	90
Mean				83	81
LSD (_{0.05})				1.8	2.6
CV (%)				2.7	2.7

¹Cultural characteristics determined on cultures growing on PDA only.

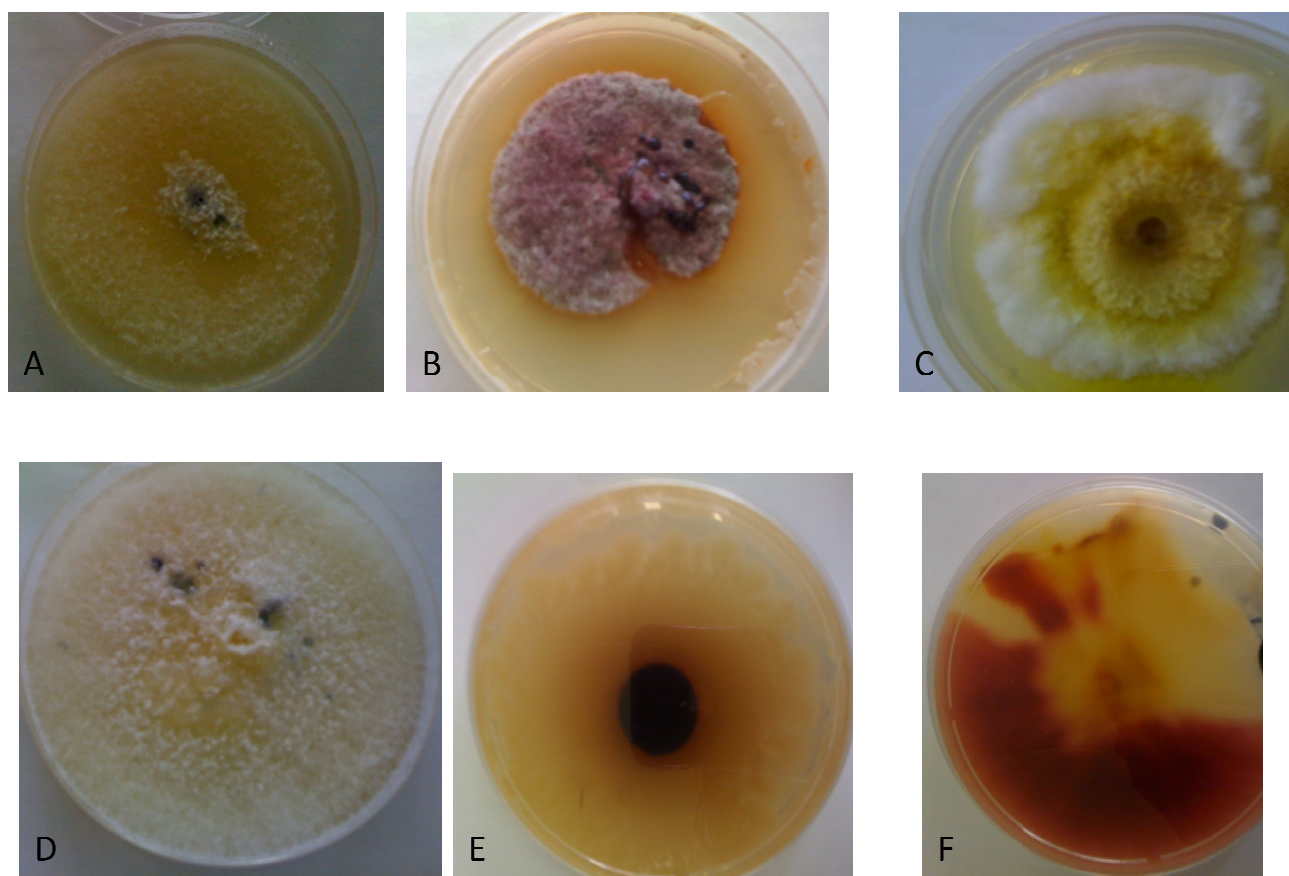


Fig 4.4. *Fusarium udum* morphological characteristics and pigmentation on PDA plates.
 A=Scanty, orange; B= Cottony, purple;; C=Cottony, orange ; D=Fluffy light pink; E=Light pink (reverse side), F= Deep purple (reverse side).

4.3.2 Pot inoculation studies

There were significant differences ($p \leq 0.001$) between genotypes, and genotype x isolate interactions wilt incidence. Significant differences ($P \leq 0.001$) genotypes, isolates, and genotype x isolate interactions days to wilt (Table 4.5).

Table 4.5. Analysis of variance for pot inoculation studies for days to wilt and wilt incidence

Source of variation	d.f.	Mean squares	
		Wilt incidence	Days to wilt
Rep	2	77.8	12.4
Genotype	59	5584.3***	660.8***
Isolate	2	1123.2	397.9***
Genotype x isolate	118	1129.3***	145.6***
Residual	358		24.9

*** indicates the term is significant at $P \leq 0.001$

Wilt incidence and days to wilt varied among isolates, and ranged between 0 to 97% and no wilting to 37 days (Table 4.6). The genotypes that did not record any wilt symptoms to any of the isolates were ICPB2043, ICP12012, ICP13092, ICPA2039xICP13092, ICPA2043xICP12012, ICPA2043xICP13092, and ICPA2043xICP9135 and were classified as resistant (R). Hybrids ICPA2039xICP9135, ICPA2039xKAT, did not show wilt symptoms when inoculated with isolates ISO-A and ISO-B. Inoculation with isolates ISO-A, ISO-B and ISO-C, recorded seven, eleven and eight hybrids with no wilt symptoms. Disease rating classified genotype ICPA2039xICEAP911 to be tolerant to isolate ISO-A, but susceptible to ISO-B and ISO-C. Genotypes A2043x554, ICPA2043xMaruti, ICPA2043xTZ26, and ICPA2043xUG18 were moderately susceptible (MS) to ISO-A. The genotypes that were MS to isolate ISO-B were ICPA2039xTZ26, ICPA2039xUG8, ICPA2043xTZ24 and Maruti.



Fig 4.5. Pot inoculation trial showing resistant and susceptible (wilted/dead) pigeonpea genotypes

4.3.3 Field screening trials

There were significant differences ($p \leq 0.001$) between genotypes and genotype x season interactions for fusarium wilt incidence at KARI Kiboko wilt-sick field in 2009 and 2010 seasons (appendix 2). The highest genotype reaction was MR with the majority of genotypes recording MS or S (Table 4.7). Overall, genotypes that were rated as MR were ICPA2039xICP13092, ICPA2043xICP13092, ICPA2043xICP12012, ICPA2043xICEAP557, ICPA2039xAsha, Maruti and ICPB2043. Tolerant genotypes were ICP12012 and Asha.

Table 4.6. Incidence, reaction to fusarium wilt and days to wilt of pigeonpea hybrids inoculated with *Fusarium udum* isolates in pots at KARI Katumani

Genotype	Wilt incidence and reaction levels						Days to wilt		
	ISO-A	Reaction	ISO-B	Reaction	ISO-C	Reaction	ISO-A	ISO-B	ISO-C
ICPA2039	25	MS	56	S	85	S	18	20	22
ICPA2043	0	R	0	R	0	R	∞	∞	∞
ICPA2039XICEAP00068	96	S	82	S	55	S	22	18	21
ICPA2039XICP12012	83	S	62	S	80	S	27	22	26
ICPA2039XICP12023	56	S	81	S	62	S	25	27	26
ICPA2039XICP13092	0	R	0	R	0	R	∞	∞	∞
ICPA2039XICP2043	68	S	75	S	31	MS	24	29	18
ICPA2039XICEAP554	70	S	72	S	39	MS	22	21	24
ICPA2039XICEAP557	73	S	53	S	41	MS	19	22	24
ICPA2039XICEAP7035	82	S	60	S	46	MS	23	23	22
ICPA2039XICEAP850	84	S	78	S	39	MS	22	21	20
ICPA2039XICEAP902	76	S	75	S	73	S	28	24	25
ICPA2039XICEAP911	17	T	64	S	66	S	25	19	22
ICPA2039XICP9135	0	R	0	R	65	S	∞	∞	25
ICPA2039XASHA	34	S	61	S	25	MS	20	26	21
ICPA2039XKAT	0	R	0	R	49	MS	∞	∞	26
ICPA2039XMARUTI	57	S	86	S	54	S	24	25	31
ICPA2039XTZ24	60	S	72	S	70	S	20	20	23
ICPA2039XTZ26	44	S	48	MS	73	S	19	24	28
ICPA2039XUG1	48	S	65	S	70	S	21	24	22
ICPA2039XUG18	76	S	82	S	57	S	19	16	19
ICPA2039XUG8	48	S	45	MS	38	MS	21	19	17
ICPA2043XICEAP00068	57	S	76	S	66	S	23	29	26
ICPA2043XICP12012	0	R	0	R	0	R	∞	∞	∞
ICPA2043XICP12023	82	S	61	S	65	S	28	23	31

Table 4.6.continued...

Genotype	Wilt incidence and reaction levels						Days to wilt		
	ISO-A	Reaction	ISO-B	Reaction	ISO-C	Reaction	ISO-A	ISO-B	ISO-C
ICPA2043XICP13092	0	R	0	R	0	R	∞	∞	∞
ICPA2043XICP2039	71	S	74	S	73	S	28	28	34
ICPA2043X554	44	MS	77	S	70	S	19	24	36
ICPA2043X557	88	S	82	S	71	S	26	26	27
ICPA2043X7035	93	S	88	S	75	S	24	24	26
ICPA2043X850	68	S	71	S	49	MS	29	28	30
ICPA2043X902	65	S	61	S	89	S	19	30	32
ICPA2043X911	80	S	0	R	53	S	34	∞	37
ICPA2043XICP9135	0	R	0	R	0	R	∞	∞	∞
ICPA2043XASHA	0	R	58	S	0	R	∞	34	∞
ICPA2043XKAT	83	S	59	S	62	S	35	31	35
ICPA2043XMARUTI	28	MS	0	R	59	S	19	∞	35
ICPA2043XTZ24	76	S	43	MS	55	S	23	27	36
ICPA2043XTZ26	45	MS	68	S	0	R	22	27	∞
ICPA2043XUG1	75	S	72	S	68	S	29	29	29
ICPA2043XUG18	37	MS	0	R	44	MS	23	∞	28
ICPA2043XUG8	71	S	63	S	66	S	22	22	31
ASHA	64	S	86	S	0	R	25	32	∞
ICEAP554	96	S	75	S	77	S	22	22	26
ICEAP557	87	S	81	S	57	S	18	18	22
ICEAP7035	89	S	53	S	68	S	27	26	27
ICEAP850	88	S	52	S	47	MS	17	19	17
ICEAP902	60	S	80	S	94	S	22	24	28
ICP12012	0	R	0	R	0	R	∞	∞	∞
ICP12023	61	S	81	S	79	S	20	20	20
ICP13092	0	R	0	R	0	R	∞	∞	∞
ICP9135	51	S	54	S	66	S	25	25	25

Table 4.6 continued....

Genotype	Wilt incidence and reaction levels						Days to wilt		
	ISO-A	Reaction	ISO-B	Reaction	ISO-C	Reaction	ISO-A	ISO-B	ISO-C
KAT	82	S	68	S	80	S	20	22	32
MARUTI	0	R	49	MS	64	S	∞	26	27
TZ24	85	S	62	S	48	MS	20	20	20
TZ26	60	S	89	S	70	S	20	29	23
UG1	82	S	59	S	49	MS	19	19	19
UG18	81	S	45	MS	49	MS	25	21	20
UG8	87	S	36	MS	64	S	22	22	23
Mean	56		54		51		19	19	21
LSD (_{0.05})	33						25		
CV (%)	38						8		

∞= No wilt symptoms were observed throughout the study period for days to wilt. R, MR, T, MS and S = Resistant, moderately resistant, tolerant, moderately susceptible and susceptible, respectively

Table 4.7. Pigeonpea genotype incidence and reaction to fusarium wilt at KARI Kiboko wilt-sick field in 2009 / 2010 growing seasons.

Genotype	2009		2010	
	Incidence	Reaction	Incidence	Reaction
ICPA2039XICEAP00068	71	S	68	S
ICPA2039XICP12012	77	S	76	S
ICPA2039XICP12023	80	S	81	S
ICPA2039XICP12091	56	S	69	S
ICPA2039XICP13092	7	MR	11	T
ICPA2039XICPB2043	29	MS	32	MS
ICPA2039XICEAP554	16	T	54	S
ICPA2039XICEAP557	43	MS	52	S
ICPA2039XICEAP7035	33	MS	77	S
ICPA2039XICEAP850	49	MS	65	S
ICPA2039XICEAP902	28	MS	40	MS
ICPA2039XICEAP911	55	S	67	S
ICPA2039XICP9135	59	S	71	S
ICPA2039XASHA	11	T	9	MR
ICPA2039XKAT	73	S	50	S
ICPA2039XMARUTI	35	MS	41	MS
ICPA2039XTZ24	56	S	66	S
ICPA2039XTZ26	18	T	63	S
ICPA2039XUG1	80	S	67	S
ICPA2039XUG18	57	S	74	S
ICPA2039XUG8	38	MS	40	MS
ICPA2043X ICEAP00068	31	MS	11	T
ICPA2043XICP12012	13	T	3	MR
ICPA2043XICP12023	21	MS	30	MS
ICPA2043XICP13092	6	MR	9	MR
ICPA2043XICPB2039	12	T	20	T
ICPA2043XICEAP554	28	MS	16	T
ICPA2043XICEAP557	8	MR	7	MR
ICPA2043XICEAP7035	6	MR	27	MS
ICPA2043XICEAP850	6	MR	18	T
ICPA2043XICEAP902	11	T	27	MS
ICPA2043XICEAP911	18	T	12	T
ICPA2043XICP9135	73	S	26	MS
ICPA2043XASHA	6	MR	17	T
ICPA2043XKANCHAN	11	T	14	T
ICPA2043XKAT	11	T	13	T
ICPA2043XMARUTI	20	T	14	T
ICPA2043XTZ24	12	T	13	T

Table 4.7 continued...

Genotype	2009		2010	
	Incidence	Reaction	Incidence	Reaction
ICPA2043XTZ26	26	MS	12	T
ICPA2043XUG1	13	T	23	MS
ICPA2043XUG18	41	MS	46	MS
ICPA2043XUG8	46	MS	39	MS
ASHA	13	T	10	MR
B2039	13	MS	69	S
B2043	23	MR	13	T
ICEAP00068	58	S	38	MS
ICEAP554	86	S	28	MS
ICEAP557	68	S	56	S
ICEAP7035	46	MS	35	MS
ICEAP850	63	S	47	MS
ICEAP902	36	MS	33	MS
ICEAP911	58	S	61	S
ICP12012	12	T	10	MR
ICP12023	77	S	67	S
ICP13092	98	S	14	T
ICP9135	28	MS	60	S
KANCHAN	38	MS	36	MS
KAT	77	S	52	S
MARUTI	3	MR	9	MR
TZ24	54	S	72	S
TZ26	24	MS	26	MS
UG1	38	MS	62	S
UG18	49	MS	65	S
UG8	41	MS	73	S
Mean	39		40	
CV (%)	41			
LSD (_{0.05})	29			

4.3.4 Gene action

Mean squares due to GCA and SCA were highly significant ($P \leq 0.001$) for reaction to fusarium wilt both in the pot inoculation trials and under field conditions (Table 4.8). Therefore, both GCA and SCA effects were relevant in controlling wilt resistance.

Table 4. 8. Analysis of variance table for Line x Tester mating design for pigeonpea genotypes' resistance to fusarium wilt under controlled and field conditions.

Source of variation	d.f.	Mean squares			
		ISO-A	ISO-B	ISO-C	Field
Rep	2	1.8246	0.3421	0.1842	2.9829
GCA (Lines)	1	0.0789	6.3947***	10.1404***	95.9211***
GCA (Testers)	18	9.6676***	10.1725***	6.7788***	3.3474***
SCA (Line x Tester)	18	4.1901***	5.0244***	3.8255***	2.1988***
Error	74	0.3561	0.4052	0.3284	0.6882

*** indicates the term is significant at $P \leq 0.001$. ISO-A, ISO-B, and ISO-C = *F. udum* isolates used in the study

The means for genotype ratings to fusarium wilt and SCA effects of the parents are presented in Table 4.9. Negative and highly significant SCA effect was recorded on ICPA2039xICEAP911 (-1.2), ICPA2039xKAT (-2.0) and ICPA2043xICP12012 (-2.0). Hybrids ICPAx2039xKAT (-2.1), ICPA2039xMaruti (-1.8), ICPA2043xMaruti (-1.8), ICPA2043xUG18 (-1.6), ICPA2043xICP9135 (-1.5), ICPA2043xICP12012 (-1.3), ICPA2043xICEAP911 (-1.3) showed negative and highly significant SCA for ISO-B. The highest negative, SCA which was also highly significant were for genotypes, ICPA2043x12012 (-1.7) and ICPA2043xTZ26 (-1.5) for ISO-C. In the field, the highest negative SCA, which was highly significant was -0.9 (ICPA2039xAsha and ICPA2043xICP12012). The high and negative SCA values indicate that that the hybrids are more resistant than average.

Table 4.9. Specific combining ability (SCA) and mean fusarium wilt resistance rating levels for pigeonpea genotypes under pot inoculation and field evaluation trials.

Hybrids	ISO-A		ISO-B		ISO-C		Field	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
ICPA2039XICP12012	2.0***	5.0	1.3***	4.0	1.7***	5.0	0.9**	5.00
ICPA2039XICP12023	-0.4	4.3	-0.1	4.7	-0.3	4.7	0.1	5.00
ICPA2039XICP13092	0.0	1.0	-0.2	1.0	-0.3	1.0	-0.4	2.60
ICPA2039XICEAP554	0.3	5.0	-0.2	4.7	-0.8***	4.0	-0.3	3.80
ICPA2039XICEAP557	-0.2	4.7	-0.7	4.0	-0.5*	4.0	0.5	4.4
ICPA2039XICEAP7035	0.0	5.0	-0.6	4.3	-0.8***	4.0	0	4.6
ICPA2039XICEAP850	0.1	5.0	-0.1	5.0	-0.8***	3.7	0.4	4.6
ICPA2039XICEAP902	-0.2	4.7	-0.1	5.0	-0.3	5.0	0	4.2
ICPA2039XICEAP911	-1.2***	2.7	1.3***	4.0	-0.3	4.7	0.4	5.0
ICPA2039XICP9135	0.0	1.0	-0.2	1.0	1.5***	4.7	-0.2	5.0
ICPA2039XASHA	1.1***	3.3	-0.4	4.3	0.9***	3.3	-0.9**	2.2
ICPA2039XICP00068	0.3	5.0	-0.1	5.0	-0.1	4.7	0.2	4.6
ICPA2039XKAT	-2.0***	1.0	-2.1***	1.0	-0.3	4.7	0.4	4.6
ICPA2039XMARUTI	0.8*	4.7	1.8***	5.0	-0.3	4.7	0	4.4
ICPA2039XTZ24	-0.4	4.3	0.3	5.0	-0.1	4.7	0.5	4.8
ICPA2039XTZ26	0.1	4.3	-0.4	4.3	1.5***	4.7	-0.1	4.0
ICPA2039XUG1	-0.5	4.0	-0.4	4.7	-0.1	4.7	0.1	4.8
ICPA2039XUG18	0.5	5.0	1.6***	4.7	0.0	4.7	-0.6	4.6
ICPA2039XUG8	-0.4	4.3	-0.6	4.3	-0.6***	4.0	-0.8**	4.0
ICPA2043XICP12012	-2.0***	1.0	-1.3***	1.0	-1.7***	1.0	-0.9**	1.8
ICPA2043XICP12023	0.4	5.0	0.1	4.3	0.3	4.7	-0.1	3.4
ICPA2043XICP13092	0.0	1.0	0.2	1.0	0.3	1.0	0.4	2.0
ICPA2043XICEAP554	-0.3	4.3	0.2	4.7	0.8***	5.0	0.3	3.0
ICPA2043XICEAP557	0.2	5.0	0.7	5.0	0.5	4.3	-0.5	2.0
ICPA2043XICEAP7035	0.0	5.0	0.6	5.0	0.8***	5.0	0.0	3.2
ICPA2043XICEAP850	-0.1	4.7	0.1	4.7	0.8***	4.7	-0.4	2.4

Table 4.9. continued.....

Hybrids	ISO-A		ISO-B		ISO-C		Field	
	SCA	Mean	SCA	Mean	SCA	Mean	SCA	Mean
ICPA2043XICEAP911	1.2	5.0	-1.3***	1.0	0.3	4.7	-0.4	2.8
ICPA2043XICP9135	0.0	1.0	0.2	1.0	-1.5***	1.0	0.2	4.0
ICPA2043XASHA	-1.1	1.0	0.4	4.7	-0.9***	1.0	0.9***	2.6
ICPA2043XICP00068	-0.3	4.3	0.1	4.7	0.1	4.3	-0.2	2.8
ICPA2043XKAT	2.0***	5.0	2.1***	4.7	0.3	4.7	-0.4	2.4
ICPA2043XMARUTI	-0.8**	3.0	-1.8***	1.0	0.3	4.7	0	3.0
ICPA2043XTZ24	0.4	5.0	-0.3	4.0	0.1	4.3	-0.5	2.4
ICPA2043XTZ26	-0.1	4.0	0.4	4.7	-1.5***	1.0	0.1	2.8
ICPA2043XUG1	0.5	5.0	0.4	5.0	0.1	4.3	-0.1	3.2
ICPA2043XUG18	-0.5	4.0	-1.6***	1.0	0.0	4.0	0.6	4.4
ICPA2043XUG8	0.4	5.0	0.6	5.0	0.6***	4.7	0.8**	4.2
Mean		3.9						3.6
SE	0.3		0.4			0.2		23.0
LSD _(0.05)	1.0		1.0			0.9		1.1
CV%(Means)	15.4		16.9			14.5	0.4	

*, **, *** indicates the term is significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively. ISO-A, ISO-B, and ISO-C = *F. udum* isolates used in the study. mean=resistance mean. 1=resistant, 2=moderately resistant, 3=tolerant, 4=moderately susceptible, 5=susceptible

4.4 Discussion and conclusions

Fusarium udum isolates varied in morphological and cultural characteristics. The dominant colour range on both mycelia and substrate was light pink to plum purple with cottony mycelia. Orange cottony mycelia and whitish substrate was distinct. Three isolates were identified and were designated as ISO-A, ISO-B, and ISO-C. The isolates produced whitish to light pink or orange mycelia. The Purple was predominant on the substrate, but whitish to light pink were also identified. Similar results were reported by Kiprof et al. (2002), Okiror (1997), Shit and Sen Gupta (1978), Gaur and Sharma (1989) and Patel et al. (2011). Mycelial radial growth rate based on the number of days for mycelia to cover the petri-dish, and colony diameter also varied among the isolates with cultures filling the petri-dish being more predominant on PDA. The results are similar to the studies by Gerlach and Nirenberg (1982) who reported that colonies of *F. udum* are fast growing on PDA. Although there was variation in micro- and macro-conidia size, the range observed of 1.5 - 2.7 x 2.6 - 9.3 μm for micro-conidia and 9.6-15.9 and 30.2 x 58.5 μm for macro-conidia was reported by Rajendra and Patil (1992) and conformed to the description by Butler and Booth (1978). The findings indicated the existence of

considerable morphological, cultural variability among the isolates collected from the KARI Kiboko wilt-sick field.

Wilt incidence and days to wilt varied among isolates, and ranged between 0 to 97% and no wilting to 37 days. Pathogenic variability among different isolates and reaction of genotypes was observed. Several genotypes did not show wilt symptoms to any of the isolates and were therefore rated as resistant (R). These genotypes could be good sources of genes conferring general resistance to the several physiologic races. Other genotypes were moderately resistant, tolerant or susceptible, but to different isolates, confirming that the isolates were different which is an indication of the existence of races. The variation in the reactions of the same genotype to different isolates shows that pigeonpea varieties are resistant/susceptible to different races of the pathogen. In Kenya, Okiror et al. (1986) and Kiprof et al. (2002) using different genotypes and isolates concluded that several aggressive groups of *F. udum* isolates exist. Several authors in India and Nepal reported similar results (Patel et al., 2011; Reddy and Raju, 1997; Shit and Sen Gupta, 1978).

In the field, several genotypes were classified to be moderately resistant and tolerant. However, the high disease pressure present in the wilt-sick field under which these genotypes were evaluated is not expected in most cropping systems, indicating that several would not show symptoms under farmer conditions.

Significant and high negative SCA effects are desirable for resistance. For example ICPA2043xICP12012 and ICPA2039xAsha recorded significant and negative SCA, which implies that they were more resistant than average. A significant proportion of hybrids in this study manifested significant and negative SCA effects for resistance to fusarium wilt among the isolates and even in the field. The additive gene action observed is desirable as selection for resistance is direct. However, studies have shown that genotypes with resistance to one race of *F. udum*, but susceptible to other races, have different resistance genes conferring resistance to different races (Okiror, 2002; Reddy and Raju, 1997). The CMS (A) line A2043, showed good resistance to the three fusarium isolates in the pot inoculation studies. It is therefore a desirable A-line when breeding for general resistance. The results of this study therefore will be valuable to other breeders in developing adapted, wilt resistant varieties of pigeonpea.

References

- Baldev, B., and K.S. Amin. 1974. Studies on the existence of races in *Fusarium udum* causing wilt of *Cajanus cajan*. *Sabrao Journal* 6:201-205.
- Booth, C. 1978. *Fusarium udum*. Commonwealth mycological institute, Kew, UK.
- Burgess, L.W., B.A. Summerell, K.P. Gott, and D. Backhouse. 1994. Laboratory manual for Fusarium research. 3rd ed., University of Sydney and Royal Botanical Gardens, Sydney, Australia.
- Changaya, A.G. 2007. Development of high yielding pigeonpea (*Cajanus cajan* L.) germplasm with resistance to fusarium wilt (*Fusarium udum*) in Malawi. PhD Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa.
- Fisher, N.L., L.W. Burgess, T.A. Toussoun, and P.E. Nelson. 1982. Carnation leaves as a substrate and for preserving cultures of *Fusarium* species. *Phytopathology* 72:151-153.
- Gaur, V.K., and L.C. Sharma. 1989. Variability in single spore isolates of *Fusarium udum* Butler. *Mycopathologia* 107:9-15.
- Gerlach, W., and H. Nirenberg. 1982. The genus *Fusarium*- a pictorial atlas, p. 355-358, *In* W. Gerlach and H. Nirenberg (Eds.) Kommissionsverlag Paul Parey, Berlin and Hamburg.
- Gwata, E.T., S.N. Silim, and M. Mgonja. 2006. Impact of a new source of resistance to fusarium wilt in pigeonpea. *Phytopathology* 154:62-64.
- Haware, M.P., and Y.L. Nene. 1994. A rapid method for pigeonpea wilt resistance screening. *Indian Phytopathology* 47:400-402.
- Hillocks, R.J. 1984. Production of cotton varieties with resistance to fusarium wilt with special reference to Tanzania. *Tropical Pest Management* 30:234-246.
- Jeswani, M.D., N. Prasad, and P.D. Gemawat. 1977. Morphological variability in *Fusarium lateritium* f sp. *cajani*. *Indian Journal of Mycology and Plant Pathology* 5:4-6.
- Jones Jr, J.B., R.L. Large, D.P. Pfeifferederer, and H.S. Klosky. 1971. How to properly sample for plant analysis. *Crop and Soil* 23:15-18.

- Joshi, S. 2001. Pathogenic variability in pigeonpea wilt pathogen *Fusarium udum* Butler in Nepal. Nepal Agricultural Research Journal 5:64-65.
- Kannaiyan, J., M.V. Reddy, and Y.L. Nene. 1981. Survey of pigeonpea diseases with special reference to wilt and sterility mosaic in India, pp. 297-303 Proceedings of International Workshop on Pigeonpea. ICRISAT, Patancheru, 502 324 Andhra Pradesh, India.
- Kannaiyan, J., Y.L. Nene, M.V. Reddy, J.G. Ryan, and T.N. Raju. 1984. Prevalence of pigeonpea diseases and associated crop losses in Asia, Africa and the Americas. Tropical Pest Management 30:62-71.
- Kimani, P.M., A.B. Nyende, and S.N. Silim. 1994. Development of early maturing fusarium wilt resistant pigeonpea cultivars. African Crop Science Journal 2:35-41.
- Kiprop, E.K., J.P. Baudoin, A. Mwang'ombe, P.M. Kimani, G. Mergeai, and A. Maquet. 2002. Characterization of Kenyan isolates of *Fusarium udum* from pigeonpea [*Cajanus cajan* (L.) Millsp.] by cultural characteristics, aggressiveness and ALFP analysis. Journal of Phytopathology 150:517-525.
- Kraft, J.M., M.P. Haware, R.M. Jimenez-Diaz, B. Bayaa, and M. Harrabi. 1994. Screening techniques and sources of resistance to root rots and wilts in cool season food legumes. Euphytica 73:27-39.
- Kumar, V., and L.C. Sharma. 1989. Variability in single spore isolates of *Fusarium udum* Butler. Mycopathologia 107:9-15.
- Leslie, J.F., and B.A. Summerell. 2006. The Fusarium manual. Blackwell Publishing Ames, Iowa, USA.
- Marley, P.S., and R.J. Hillocks. 2002. Induction of phytoalexins in pigeonpea (*Cajanus cajan*) in response to inoculation with *Fusarium udum* and other treatments. Pest Management Science 58:1068-1072.
- Mergeai, G., P. Kimani, A. Mwang'ombe, F. Olubayo, C. Smith, P. Audi, J.P. Baudoin, and A. Roi. 2001. A survey of pigeonpea production systems, utilization and marketing in semi-arid lands of Kenya. Biotechnology, Agronomy, Society and Environment 5:145-153.

- Nene, Y.L., and J. Kannaiyan. 1982. Screening pigeonpea for resistance to fusarium wilt. *Plant Disease* 66:306-307.
- Nene, Y.L., J. Kannaiyan, M.V. Reddy, and P. Remanandan. 1981. Sources of resistance to selected pigeonpea diseases. ICRISAT, Patancheru, 502 324, Andhra Pradesh, India.
- Okiror, M.A. 1986. Breeding for resistance to fusarium wilt. PhD Thesis. University of Nairobi, Nairobi, Kenya.
- Okiror, M.A. 1998. Screening techniques for Fusarium wilt of pigeonpea. *African Crop Science Journal* 6:345-350.
- Okiror, M.A. 2002. Genetics of resistance to *Fusarium udum* in pigeonpea (*Cajanus cajan* (L.) Millsp). *Indian Journal of Genetics* 62:218-220.
- Okiror, M.A., and P.M. Kimani. 1997. Pathogenic variation of *Fusarium udum* of pigeonpea. *Indian Journal of Genetics and Plant Breeding* 57:186-192.
- Patel, S.I., R.L. Patel, A.G. Desai, and Patel, D.S. 2011. Morphological, cultural and pathogenic variability among *Fusarium udum* and root dip inoculation technique for screening pigeonpea germplasm. *Journal of Mycology and Plant Pathology* 41:57-62.
- Pawar, S.E., and C.D. Mayee. 1986. Reaction of pigeonpea genotypes and their crosses to fusarium wilt. *Indian Journal of Phytopathology* 39:70-74.
- Rajendra, M.M., and R.L. Patil. 1992. Morphological, cultural and physiological variation in *Fusarium udum* Butler. *Journal of Maharashtra Agricultural University* 17:465-467.
- Rayner, R.W. 1970. A mycological colour chart. Commonwealth mycological institute, Kew, UK.
- Reddy, M.V., and T.N. Raju. 1997. Evaluation of pigeonpea varieties for resistance to wilt caused by *Fusarium udum* and sterility mosaic disease in a perennial system. *Indian Journal of Agricultural Sciences* 67:437-439.
- Reddy, M.V., S.B. Sharma, and Y.L. Nene. 1990. Pigeonpea: disease management, pp. 303-347, *In* Y. L. Nene, et al. (Eds.) *The Pigeonpea*. CAB International, Wallington, Oxon, UK.

- Reddy, N.P.E., and K.C.B. Chaudhary. 1985. Variation in *Fusarium udum*. Indian Journal of Phytopathology 38:172.
- Saxena, K.B., R.V. Kumar, K.M. Latha, and V.A. Dalvi. 2006. Commercial pigeonpea hybrids are just a few steps away. Indian Journal of Pulses Research 19:7-16.
- Shiferaw, B., J. Okello, G. Muricho, J. Omiti, S.N. Silim, and R. Jones. 2008. Unlocking the potential of high value legumes in the semi-arid regions: Analyses of the pigeonpea value chains in Kenya. International Crops Research Institute for the Semi-Arid Tropics, Nairobi, Kenya.
- Shit, S.K., and P.K. Sen Gupta. 1978. Possible existence of physiological races of *Fusarium oxysporum* f. sp. *udum*, the incitant of the wilt of pigeonpea. Indian Journal of Agricultural Sciences 48:629-632.
- Snyder, W.C., and H.N. Hansen. 1947. Advantages of natural media and environments in the culture of fungi. Phytopathology 37:420-421.

Chapter 5: Multi-locational evaluation of pigeonpea hybrids for grain yield and earliness in Kenya

Abstract

A total of 29 pigeonpea hybrids from crosses between two CMS A-lines from India and 19 improved lines from East Africa and India were evaluated for yield and earliness in three locations in Kenya (KARI Kiboko, University of Nairobi-Kabete and Leldet farm-Nakuru) in 2009 and 2010. Data was recorded from ten randomly selected plants in each plot on days to 50% flowering and days to 75% maturity. Grain yield was recorded from all the plants in each plot and recorded as the mass of harvested seed plot⁻¹ (kg ha⁻¹). Results from the study indicated that the most stable environment for all traits was Kabete. The highest overall mean yield for the best performing hybrids was 1,930 kg ha⁻¹ by A2043xTZ26 followed by A2039xTZ24 with 1,870 kg ha⁻¹. The highest yielding environment was Kiboko in both seasons. Mean yields for the best performing parents were 2,036 kg ha⁻¹ for ICP12012 and 1,629 kg ha⁻¹ for Asha. For specific sites, the highest yielding hybrids in Kabete, Kiboko and Leldet were A2039xTZ24 (2,057 kg ha⁻¹), A2043xTZ26 (2,803 kg ha⁻¹), and A2043xUG8 (1,708 kg ha⁻¹) respectively. Most hybrids were in the medium duration maturity group with days to maturity ranging from 147 to 186. Mean heterosis for yield varied from -35% (A2039xA2043) to 50% (A2043xUG8). In Kenya, the potential for production and commercialization of hybrid pigeonpea is feasible due to high hybrid vigour recorded, and the stability of the CMS lines. Hybrids also guarantee uniformity in grain size, a factor that is important for the market. Further evaluations should be carried out over several seasons and sites to confirm the hybrid yield stability.

5.1 Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the third most important grain legume crop, after common bean and cowpea, in Kenya. It is grown in the arid and semi-arid areas of the country predominantly by small-scale farmers, as a source of food and cash. The main producing counties are Machakos, Meru and Embu. Pigeonpea productivity has remained low and highly variable over environments, with yields ranging between 200 kg ha⁻¹ to 800 kg ha⁻¹ (Mergeai et al., 2001), although high yield of >3000 kg ha⁻¹ has been achieved under research conditions. Factors attributed to the low yields include lack of high yielding cultivars resistant to diseases and pests, low soil fertility and drought, with some of these factors being seasonally significant (Chauhan et al., 1998).

Traditionally pigeonpea production systems have evolved around long-duration types intercropped with other legumes or cereals. The wider spacing and crop competition led to low yields. Due to the importance of pigeonpea as a source of protein and cash, a breeding programme for early maturity, coupled with developing high density production in pure stand for maximum yield, was initiated in India in the mid-1970s, and 20% higher yields were attained (Byth et al., 1981). Saxena (2008) reported that early maturing varieties with uniform growth were found suitable for mechanized harvesting of dry pods and this led to a world wide release of varieties such as ICPL 151, ICPL 2 (in India), ICPL 87091 (in Kenya, Malawi and Uganda), with yields ranging from 1,500 to 3,000 kg ha⁻¹ (Saxena, 2000). A study by Kimani et al. (1994) developed early maturing, fusarium wilt resistant pigeonpea cultivars with a yield potential of more than 2 ton ha⁻¹.

Commercial production of pigeonpea hybrid seeds became possible with the discovery of a genetic male sterility (GMS) system in 1974 (Reddy et al., 1978). Hybrids developed with this GMS system gave 25-30% yield advantage over the control, demonstrating that heterosis in pigeonpea could be exploited. However, due to the genetic nature of this system, where roguing of 50% of fertile parents was necessary, large scale seed production was not feasible. This was overcome with the development of cytoplasmic male sterile (CMS) lines from crosses between cultivated and wild relatives of pigeonpea with A₄ cytoplasm (Mallikarjuna and Saxena, 2005). The A₄ cytoplasmic system was found to be stable across environments and years, and it had perfect fertility restoration in the F₁ hybrid plants. A high level (30-60%) of hybrid vigour observed over the standard cultivars demonstrated its feasibility, and the system is now extensively used by breeders to develop commercial pigeonpea hybrids in India (Saxena, 2008;

Saxena et al., 2005). Notable CMS lines developed and extensively used in hybrid seed production are ICPA2043 and ICPA2039. The profitability of seed production using this system was demonstrated by Saxena et al. (2011), where Rs 70,005 was obtained from planting one hectare of seed. Hybrids have demonstrated a yield advantage of over 25% over the local control, Maruti (Saxena et al., 2005). In India the most outstanding medium duration hybrid, ICPH2671, produced 2,937 kg ha⁻¹ and exhibited 61% heterosis over the better parent and has been commercialized (Saxena et al., 2006). Due to similar climatic conditions in Kenya and India, introduction and utilization of the hybrid technology in Kenya appears feasible and promising.

Genotype x environment (G x E) studies for stability of pigeonpea genotypes have been undertaken by several authors. Saxena and Rajni (2001), Pandey and Singh (1998), and Sreelakshmi et al. (2010) concluded that hybrids and the controls showed specific adaptation to particular environments and emphasized the need to breed for location specific hybrids. The studies also concluded that the environments did not reflect any specific pattern to the performance of hybrids. Holkar et al. (1991) recorded significant differences in yield and yield components and G x E interaction of pigeonpea genotypes grown under dry land conditions.

In Kenya, conditions in pigeonpea production areas differ from season to season in moisture, temperature, disease and pest pressure and agronomic management (Mergeai et al 2003). Due to climate change, superior genotypes for now and the future should be stable across environments and should be able to withstand effects of climate change. The stability of a genotype is depended on G x E effects. Significant G x E interaction leads to crossover effects that hinder selection of superior varieties (Signor et al., 2001). Selection of suitable genotypes does not only depend on average performance, but also on stability (Primomo et al., 2006). Stable genotypes also need to be responsive, so that they utilize resources available in the high yielding environment, while maintaining above average performance in all other environments (Miezan et al., 2004). Such genotypes produce reasonable yields under poor environments and realize bumper harvests in good environments.

With pigeonpea hybrid technology, high, stable yield and desired maturity group can be achieved if male parents are carefully selected and performance of the hybrids across environments evaluated. The objective of this study was to evaluate pigeonpea hybrids for grain yield and earliness across sites and seasons in Kenya.

5.2 Materials and methods

5.2.1 Study locations

The experiments were carried out at three locations; Kiboko, Kabete and Leldet-Nakuru in the 2009 and 2010 growing seasons. Kiboko is located in the semi-arid zone and lies at 37°45'E and 2°15'S with an elevation of 960 m above sea level (masl). It is characterized by high temperatures with a mean minimum and maximum of 16.9°C and 31°C respectively with bimodal rainfall pattern averaging 300 mm per season. The main growing season is in the short rains (October/December), which is more reliable. Soils are sandy clay loam and calcareous.

Kabete lies at 36°45'E and 1°14'S with an altitude of 1960 masl. It is characterized by low temperatures with a mean minimum and maximum of 12.6°C and 23.4°C respectively with the cold months usually in June and July. It has bimodal rainfall pattern with the long rains in March to May and short rains in October to December with annual average of 1046 mm. Soils are extremely deep and friable clay. Leldet lies at 0°31'E and 0°09'S with an altitude of 2,275 masl. It experiences hot climatic conditions with a mean minimum and maximum of 28°C and 14°C, respectively and the warmest months from November to February. Leldet has bimodal pattern of rainfall which is also erratic with peaks in April and August and an annual mean of 180 mm. Soils are mainly sandy clay loams.

5.2.2 Selection of genotypes and hybridization

Two stable CMS A-lines, their B-lines and 19 improved pigeonpea genotypes were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) India, ICRISAT Kenya and KARI Katumani and are presented in Table 1. Some of the criteria for selection of genotypes to be used in the study were based on the list of preferred traits as given by farmers and traders during the stakeholder analysis (Chapter 2, this thesis). Materials from Uganda and Tanzania were sourced through ICRISAT Kenya. All the genotypes collected from Kenya were categorized as medium maturity with large white/cream seeds. The two from Tanzania were large and white/cream seeded, but maturity duration was not known. Three small, cream mottled seeds with known resistance to storage bruchids and podborers were collected from Uganda. Materials collected from ICRISAT India were the two CMS lines and their maintainers, three high yielding commonly grown genotypes resistant to fusarium wilt, and four white-seeded germplasm from the genebank. The genotypes were maintained pure through self-pollination in a screen house.

Table 5.1. Pigeonpea genotypes used to make hybrids

Genotype	Type	Source	maturity	Important attributes
ICEAP557	Improved	Kenya	Late / Medium	High yielding
ICEAP554	Improved	Kenya	Late / Medium	High yielding
ICEAP911	Improved	Kenya	Medium	High yielding
ICEAP902	Improved	Kenya	Medium	High yielding
ICEAP00068	Improved	Kenya	Medium	High yielding
KAT	Improved	Kenya	Medium	High yielding
ICEAP850	Improved	Kenya	Medium	High yielding
TZ26	Landrace	Tanzania	Unknown	High yielding
TZ24	Landrace	Tanzania	Unknown	High yielding
UG1	Landrace	Uganda	Unknown	Podborer/bruchid resistant
UG8	Landrace	Uganda	Unknown	Podborer/bruchid resistant
UG18	Landrace	Uganda	Unknown	Podborer/bruchid resistant
ICP12023	Landrace	India genebank	Unknown	White large seeded
Maruti	Improved	India	Medium	Fusarium wilt resistant
Asha	Improved	India	Medium	Fusarium wilt resistant
Kanchan	Improved	India	Medium	Fusarium wilt resistant
ICP12012	Landrace	India genebank	Unknown	White large seeded
ICP9135	Landrace	India genebank	Unknown	White seeded
ICP13092	Landrace	India genebank	Unknown	White seeded
ICPA2043	Improved	India	Medium	A ₄ CMS stable
ICPA2039	Improved	India	Early	A ₄ CMS stable
ICPB2043	Improved	India	medium	B-line
ICPB2039	Improved	India	Early	B-line

5.2.3 Hybrid development

A total of 19 improved pigeonpea genotypes (male parents) and two CMS A-lines (female parent) were used in this study. Ten plants of each male genotype and 60 plants for each A-line were raised in large plastic pots filled with soil, two plants pot⁻¹ in a screenhouse at KARI Katumani and KARI Kiboko in 2009. Because the A-lines were early maturing, the male parents were planted two weeks earlier in order to synchronize flowering. At flowering each of the 19 improved lines was crossed to each of the CMS lines to give a potential 38 F₁ hybrid seed. Hand-pollination was done during the cool parts of the day (morning or evening hours) as heat is known to affect fertilization (Sharma and Green, 1980). Flowers that showed bright shiny colour in the petals were gently opened using forceps sterilized in 70 % alcohol. Freshly

opened flowers were also pollinated. Because the female parents were known to be sterile, emasculation was not required. Anther heads with mature pollen were harvested from the male parents and immediately gently tapped on the stigma of the female flower. Each pollinated flower was tagged with a label showing parents used; date of crossing and by whom. Not all crosses generated sufficient F_1 seeds and only those hybrids with enough seed for six trials were included in the evaluation trials.

5.2.4 Evaluation of pigeonpea genotypes

The trial was planted at three sites, KARI Kiboko, University of Nairobi field station-Kabete and Leldet-Nakuru in 2009 and 2010. A total of 50 genotypes (29 hybrids and 21 improved fixed lines) were planted in 5 x 10 alpha design with two replications. Each plot comprised of two rows of 4.5 m long, spaced at 1 m apart and 50 cm within the row with one plant hill⁻¹. An extra single row at the beginning and end of each block was added to act as guard rows. In 2009, plantings at Kiboko, Kabete and Leldet were done on 1st October, 5th October and 17th October respectively. In 2010, plantings were done on 3rd October, 26th October and 17th October at Kiboko, Kabete and Leldet respectively. Three manual weedings were done. Dimethoate (Duduthrin) was used as a standard commercial insecticide. The first spray was applied at flower bud expansion stage and subsequent sprays at 14-day intervals.

At Kiboko, regular supplementary sprinkler irrigation was applied during dry spells. At Kabete, only emergency sprinkler irrigation was done because the irrigation facility in the trial field was not well developed. Leldet site was purely rainfed and a minimum tillage system was applied to conserve moisture, whereby a broad-based herbicide spray was used to kill weeds before planting. At planting furrows were opened for seed sowing.

Data were collected on days to 50% flowering (DTF), days to 75% maturity (DTM) and grain yield. Days to flower were recorded as the number of days when 50% of all the plants in a plot showed at least one open flower. Days to maturity was recorded as the number of days when 75% of the plants in a plot showed pods that were physiologically mature. Yield was measured from plants harvested from the entire plot and recorded as the mass of harvested seed plot⁻¹, then converted to kg ha⁻¹ before analysis.

5.2.5 Data analysis

Statistical analyses were done using Genstat 14th edition (GENSTAT, 2011) statistical programme. Additive main effects and multiplicative interactions (AMMI) analysis was done using data recorded on genotypes that were common across environments and seasons. The AMMI analysis uses analysis of variance (ANOVA) followed by principal component analysis (PCA) applied to the sums of squares allocated by the ANOVA to genotype by environment interaction (GEI). The genotype main effects plus genotype by environment interaction effects (GGE) biplots (Yan et al., 2000), using the first two principal components (PC1 and PC2), was used to compare differences between environments. Regression coefficient analysis was computed using environment and genotype means to determine the stability of the genotypes. Percentage heterosis of F₁ hybrids relative to the better parent were calculated according to Hayes and Foster (1976), using the formula:

$$HF_1 = [(F_1 - BP)/BP] \times 100.$$

Where:

HF₁ = Heterosis of F₁ hybrid

F₁ = Performance of F₁ hybrid

BP = Performance of the better parent

5.3 Results

At Leldet 2010, sheep grazed the entire experimental field one month after planting to the extent that the crop could not recover. No further data is presented from this season.

5.3.1 Mean performance of pigeonpea genotypes across environments

Mean performance of genotypes at different sites, seasons and within sites for DTF, DTM and yield are presented in Table 5.5 and Figures 1 to 3. A combined analysis of variance for Kabete, Kiboko and Leldet over two seasons showed that genotype, environment and G x E interaction effects were significant for DTF, DTM, and grain yield (kg ha⁻¹) (Table 5.2).

Table 5. 2. ANOVA for mean days to flower, days to maturity and grain yield across sites

Source	d.f	Days to flower	Days to maturity	Grain yield
		Mean squares		
Total	499	284	703	606800
Treatments	249	439***	1010***	1034217***
Genotypes	49	620***	1371***	956832***
Environments	4	8367***	18470***	33698606***
Block	4	216	1073	492098*
Interactions	196	232***	564*	386943***
IPCA	52	524*	1109***	701321***
IPCA	50	184	716**	418533***
Residuals	94	97	181	196228
Error	245	128	384	174745
Mean		106	160	1371
SE±		0.6	0.9	31.7
CV (%)		11	13	31

*, **, *** indicates the term is significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively.

Analysis of variance for comparison of seasons within Kabete and Kiboko recorded significant differences for all the traits except at Kabete, where interactions of DTF and DTM and genotype DTM were not significantly different (Table 5.3). There were significantly different effects for yield at Kiboko in both seasons. However, at Kabete, the variation was not significantly different.

Table 5.3. ANOVA for mean days to flower, days to maturity and grain yield of pigeonpea genotypes within sites.

Source	d.f	Kabete			Kiboko		
		Days to flower	Days to maturity	Grain yield	Days to flower	Days to maturity	Grain yield
Total	199	170	310	251801	422	642	640413
Treatments	99	275***	413***	333324***	644***	1128*	1063328***
Genotypes	49	287***	244	365515***	658***	1110*	1056142***
Season	1	9238***	21642***	191134	10662***	15107*	28476257***
Block	2	96	1050**	1009071**	63	13	221050
Interactions	49	81	150	304035**	426**	860***	511067**
IPCA 1	49	81	150	304035**	426**	860***	511067**
IPCA 2	47	0	0	0	0	0	0
Error	98	66	191	153992	205	163	221741
Mean		111	170	1233	105	151	1872
SE±		6.8	10.4	32.8	7.3	8.7	37.3
CV (%)		7.4	8.4	33.7	13.6	8.4	23.3

, *indicates the term is significant at $P \leq 0.01$, and $P \leq 0.001$, respectively.

The site/season means were computed to determine the performance of genotypes in the different sites and seasons. The means for DTF, DTM and grain yield for all the sites are shown in Table 5.4. Flowering was early at Kiboko and Leldet (97 days), but at Kabete flowering was within 118 days. However the means for DTM show genotypes at Kiboko in 2010 maturing earliest at 142 days as compared to 180 days at Kabete. Mean grain yield was highest at Kiboko in both seasons (1,494 and 2,249 kg ha⁻¹), but lowest at Leldet (649 kg ha⁻¹).

Table 5.4. Individual site means for days to flower, days to maturity and grain yield in 2009 and 2010

Site	Season	Days to flower	Days to maturity	Yield (kg ha ⁻¹)
Kabete	2009	104	160	1264
Kabete	2010	118	180	1202
Kiboko	2009	112	159	1494
Kiboko	2010	97	142	2249
Leldet	2009	97	160	649

5.3.2 Days to flowering

The results of the combined analysis show that DTF for the majority of hybrids ranged between 95-110 days and that of parents between 98-124 days. The earliest hybrid to flower was ICPA2039xMaruti (95 days), while Maruti was the first male parent to flower at 98 days. Within sites, the earliest genotypes to flower at Kabete, Kiboko and Leldet were A2039x12091 (98 days), A2039x850 (91 days) and A2039x12023 (91 days) respectively. GGE biplot analysis revealed that PC1 and PC2 scores accounted for 79.4% of the total variability (Fig 5.1). Both seasons at Kabete and to some extent Leldet, were close to origin. The 2009 season at Kiboko was furthest from origin. Most genotypes clustered around the origin except 39 (ICP12012) that was located furthest. Based on regression coefficient analysis, A2039xTZ24 was the only hybrid that recorded $b > 1$ ($b = 1.2$) (Table 5.6). The other hybrids scored regression coefficients ranging between 0.3 and 0.8.

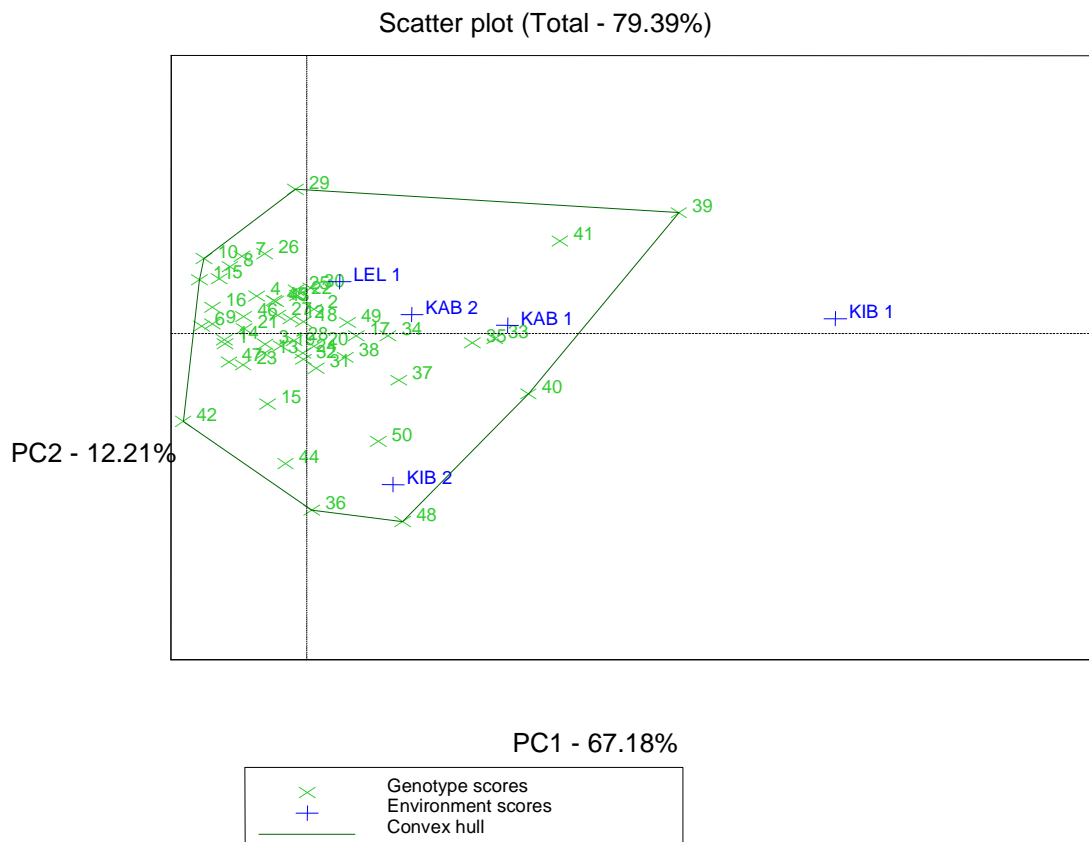


Fig 5.1. GGE biplot showing site stability in 2009 and 2010 for days to flower for pigeonpea genotypes in Kenya

5.3.3 Days to maturity

Genotypes varied in DTM from 146 to 201 days with the majority of hybrids having a DTM ranging from 146 and 173 days. Hybrid A2039xTZ24, and A2043xKAT were the earliest to mature (146 days), whereas ICP12012 was the latest (201 days) to reach maturity. Other early maturity hybrids were A2043x12023, and A2043x557. The medium maturity hybrids were A2043xTZ26, A2043x12012 and A2039x13092. Most parents matured later than hybrids. Within site and season comparisons, genotypes recorded a similar trend as that shown for combined analysis. The GGE biplot (Fig 5.2) shows that both seasons at Kabete and Kiboko 2010 were close to origin. Leldet and Kiboko 2009 were furthest. The biplot analysis shows that the PC1 and PC2 accounted for 85.5% of the variation for days to maturity. Kabete and Kiboko 2010 were closest to origin but Kiboko 2009 and Leldet were furthest. ICP12012 was the furthest. The regression coefficients analysis (Table 5.6), shows that genotype A2039x12091 recorded $b=1$ and the rest ranged between $b=0.2$ and 0.8 .

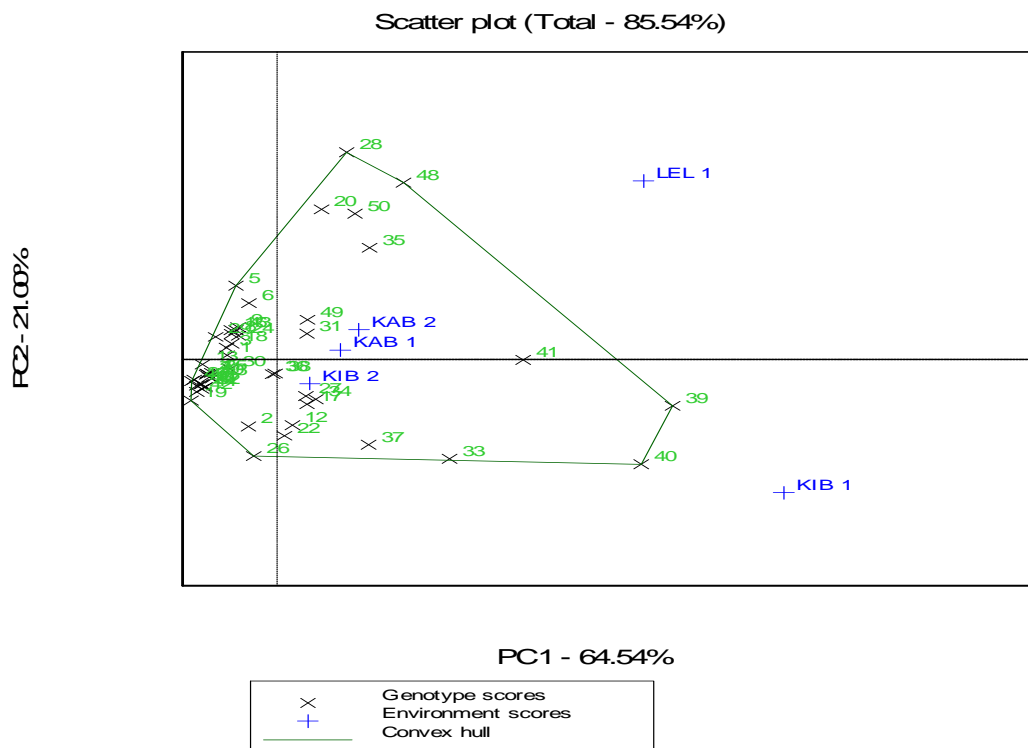


Fig 5.2 GGE biplot showing site stability in 2009 and 2010 for days to maturity for pigeonpea genotypes in Kenya

5.3.4 Grain yield

Across sites, mean yields ranged between 847 kg ha^{-1} (UG1) and $2,036 \text{ kg ha}^{-1}$ (ICP12012). Kiboko in 2010 gave the highest mean yield ($2,249 \text{ kg ha}^{-1}$), while the lowest mean yield was recorded at Leldet (649 kg ha^{-1}). Overall, the highest yielding hybrids was A2039x12091 ($3,146$

kg ha⁻¹) at Kiboko in 2009 and best performing parent was ICP12012 (4,227 kg ha⁻¹) at Kiboko in 2010. The GGE biplot (Fig 5.3) shows Kabete in season 2010 and Leldet were closest to the origin whereas Kiboko 2010 was furthest. Regression coefficients results (Table 5.6) indicate that hybrids A2039x850, A2039x12091, A2039xTZ24 and A2039x2043 gave regression coefficient values greater than unity. Values for the other hybrids ranged between 0.3-0.9.

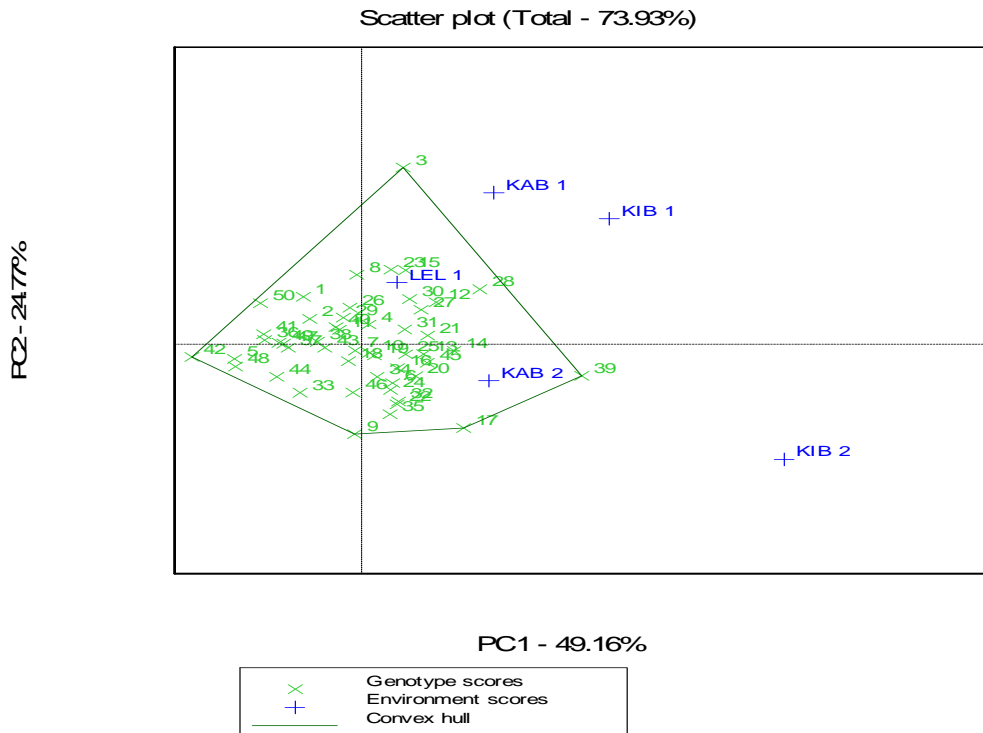


Fig 5.3. GGE biplot showing site stability in 2009 and 2010 for grain yield of pigeonpea genotypes in Kenya

Table 5.5. Mean across and between sites for days to flower, days to maturity and seed yield at Kabete, Kiboko and Leldet 2009/2010

Genotypes	Days to flower								Days to maturity								Grain yield (kg ha ⁻¹)							
	ka1	ka2	μ	ki1	ki2	μ	lel	Gμ	ka1	ka2	μ	ki1	ki2	μ	lel	Gμ	ka1	ka2	μ	ki1	ki2	μ	lel	Gμ
A2039X12012	99	115	104	95	97	97	95	100	160	181	168	145	143	146	151	156	1379	847	1008	1566	1447	1554	569	1162
A2039X12023	101	113	105	118	92	106	91	103	157	176	165	164	143	155	139	156	1246	836	999	1583	1650	1629	413	1146
A2039X12091	97	111	98	107	97	103	89	100	158	179	165	145	140	145	154	155	2375	1088	1687	3146	1424	2317	906	1788
A2039X13092	107	124	118	97	93	94	106	105	164	184	181	143	150	141	142	157	1509	1333	1489	1627	2202	1878	900	1514
A2039X2043	103	120	107	91	90	92	102	101	154	176	163	137	133	137	170	154	942	728	902	692	1308	967	509	836
A2039X554	96	112	106	91	91	90	93	96	153	175	161	144	132	141	169	155	997	1208	1004	1590	2749	2200	318	1373
A2039X557	100	115	107	100	83	92	96	99	157	177	172	144	141	139	143	152	1177	1160	1160	1384	2230	1818	595	1309
A2039X7035	103	120	107	94	88	92	102	101	155	177	168	144	137	139	158	154	1749	1170	1397	1993	1769	1897	886	1514
A2039X850	98	115	109	92	92	91	96	99	157	179	169	144	139	141	161	156	614	1144	754	924	2771	1888	233	1137
A2039XKAT	99	116	108	90	83	87	98	97	157	178	173	145	142	139	143	153	1185	1133	1343	1671	2368	1943	440	1359
A2039XMARUTI	96	112	107	91	83	86	94	95	156	177	171	145	142	140	141	152	1286	1093	1211	1436	1967	1701	653	1287
A2039XTZ24	102	115	107	110	93	102	94	103	157	177	168	175	142	158	148	160	1835	1662	1749	2072	2614	2220	1166	1870
A2039XTZ26	98	112	107	105	96	101	91	100	161	182	173	140	146	142	144	155	1394	1557	1476	1664	2832	2148	863	1662
A2043X00068	95	110	106	97	92	94	90	97	155	175	166	144	140	142	140	151	1357	1528	1399	1961	3041	2527	654	1708
A2043X12012	102	117	108	102	108	105	97	105	167	187	172	144	154	153	141	158	1971	1600	1567	1874	2145	2103	1384	1795
A2043X12023	98	115	105	92	91	93	96	99	151	172	160	144	131	140	160	152	1299	1509	1434	1417	2719	2046	869	1563
A2043X13092	108	120	111	123	100	111	98	110	159	179	169	175	143	159	155	162	1003	1839	1414	1179	3643	2422	834	1700
A2043X554	103	117	113	112	94	103	96	104	158	179	170	145	141	142	155	156	1042	978	970	1590	2243	1920	259	1223
A2043X557	98	110	105	110	95	102	88	100	155	175	165	145	141	142	135	150	1251	1299	1290	1473	2437	1944	691	1430
A2043X7035	103	116	110	115	98	107	94	105	163	186	168	145	137	146	203	167	1272	1616	1370	1347	2915	2157	928	1615
A2043X850	100	116	108	99	95	97	96	101	154	175	164	144	139	143	144	151	1423	1465	1502	1830	2727	2272	763	1641
A2043X902	102	114	101	114	91	104	93	103	159	178	173	174	145	157	143	160	947	1403	1267	1208	2931	2039	557	1409
A2043X911	102	119	113	96	100	97	99	103	160	180	168	141	146	145	139	153	1653	1054	1322	2400	1983	2216	516	1521
A2043X9135	104	118	114	111	99	105	97	106	159	181	175	145	142	140	158	157	963	1186	993	1617	2780	2214	258	1361
A2043XASHA	103	117	110	111	90	101	96	104	157	177	171	145	141	140	144	153	1264	1381	1501	1611	2668	2084	671	1519
A2043XKAT	104	120	104	103	87	97	101	103	138	157	141	174	121	153	137	146	1442	1073	1450	1762	1910	1768	637	1365
A2043XTZ24	106	122	113	103	96	100	103	106	164	184	176	172	149	159	156	165	1611	1518	1629	1799	2508	2155	1004	1688

Table 5.5 continued....

Genotypes	Days to flower								Days to maturity								Grain yield (kg ha ⁻¹)								
	ka1	ka2	μ	ki1	ki2	μ	lel	Gμ	ka1	ka2	μ	ki1	ki2	μ	lel	Gμ	ka1	ka2	μ	ki1	ki2	μ	lel	Gμ	
A2043XTZ26	103	117	114	109	97	102	97	105	170	194	181	140	141	142	220	173	1807	1563	1533	2625	2856	2803	798	1930	
A2043XUG18	105	117	104	113	76	96	98	102	153	174	165	141	135	137	155	152	1405	1152	1574	1485	1926	1607	780	1350	
A2043XUG8	107	120	115	112	91	102	100	106	163	183	184	148	148	139	148	158	1926	1862	1444	1401	2382	2070	1708	1856	
ASHA	104	117	112	114	102	107	95	106	164	185	171	162	145	156	171	165	1513	1484	1767	1683	2517	2006	945	1629	
ICEAP00068	103	116	109	112	100	106	95	105	159	179	168	145	144	145	140	153	869	1260	992	1389	2910	2183	312	1348	
ICEAP554	114	120	115	156	102	129	94	117	166	185	174	221	150	186	169	178	855	975	977	923	2069	1455	411	1047	
ICEAP557	110	121	116	130	100	115	98	112	161	181	170	176	145	162	158	164	1091	1338	1059	1220	2589	1970	674	1382	
ICEAP7035	111	118	115	151	102	127	92	115	166	189	173	164	141	156	203	173	906	1428	1187	1068	2944	1997	599	1389	
ICEAP850	104	118	114	109	125	117	96	110	165	185	171	159	149	157	154	162	1094	866	853	833	1423	1185	661	975	
ICEAP902	106	115	110	134	105	120	91	110	164	183	171	197	149	176	157	170	1280	1194	1082	753	1694	1272	1053	1195	
ICEAP911	108	121	116	119	103	111	100	110	166	186	175	160	150	157	155	163	1174	1054	1031	1138	1864	1544	673	1181	
ICP12012	140	145	154	185	95	137	121	137	173	192	187	269	148	205	223	201	1237	1642	1316	3088	4227	3701	-13	2036	
ICP12023	117	123	114	161	115	139	96	122	171	190	176	271	151	215	203	197	1212	761	797	2105	1923	2049	67	1214	
ICP9135	124	130	124	167	94	130	105	124	169	189	182	223	145	182	206	186	1070	646	939	1309	1377	1297	282	937	
ICPB2039	96	115	102	83	108	97	96	100	149	169	153	146	133	144	139	147	904	619	665	383	947	691	581	687	
ICPB2043	104	119	112	105	91	98	99	104	161	182	174	143	143	142	159	158	1262	1177	1299	1178	1986	1543	803	1281	
ICPB2101	103	118	112	104	117	110	97	108	162	183	165	144	149	153	138	155	832	771	805	980	1761	1358	261	921	
KANCHAN	115	134	129	94	98	95	118	112	155	176	162	142	141	144	140	151	1220	1419	1375	1737	2897	2306	576	1570	
KAT	100	115	113	99	90	93	95	100	152	173	163	144	137	140	140	149	797	1053	840	1151	2468	1857	265	1147	
MARUTI	96	111	105	97	97	96	91	98	157	177	167	146	143	144	140	153	1168	990	1156	936	1613	1246	742	1090	
UG1	111	123	121	127	129	126	99	118	171	195	175	160	143	158	224	179	923	737	1038	721	1373	950	479	847	
UG18	111	124	117	118	100	110	104	111	168	189	181	159	149	153	173	168	1164	968	976	926	1571	1280	733	1072	
UG8	104	115	112	128	113	120	90	110	151	175	178	157	122	128	212	164	1286	720	1067	1264	1145	1185	570	997	
Mean	104	118		112	97		97		160	180		159	142		160		1264	1202		1494	2249		649		
SE±			6.8			7.3		0.6				10.4			8.7		0.9			32.8			377		31.7
CV (%)			7.4			13.6		10.7				8.4			8.4		12.5			33.7			25.3		31

Ka1=Kabete 2009; Ka2=Kabete 2010; Ki1=Kiboko 2009; Ki2=Kiboko2010; LeI=Leldet 2009;μ=individual site mean; Gμ=overall mean

Table 5.6. Regression coefficients for days to flower, days to maturity and grain yield for pigeonpea hybrids

Hybrids	Days to flower	Seed yield	Days to maturity
A2043XUG18	0.70	0.59	0.78
A2043XUG8	0.76	0.75	0.46
A2043XTZ26	0.81	0.36	0.22
A2043XTZ24	0.43	0.64	0.77
A2043XKAT	0.69	0.64	0.22
A2043X12023	0.62	0.43	0.75
A2043X12012	0.63	0.84	0.41
A2043X9135	0.55	0.85	0.67
A2043X7035	0.77	0.58	0.25
A2043X902	0.67	0.55	0.52
A2043X850	0.81	0.59	0.74
A2043X557	0.78	0.72	0.66
A2043X13092	0.77	0.43	0.79
A2043X554	0.62	0.92	0.82
A2043X00068	0.74	0.72	0.76
A2043XASHA	0.69	0.72	0.59
A2039XTZ24	1.22	0.65	0.64
A2039XMARUTI	0.55	0.80	0.58
A2039XKAT	0.53	0.97	0.58
A2039X13092	0.38	0.71	0.46
A2039X12091	0.71	1.31	1.04
A2039X12012	0.63	0.14	0.75
A2039X7035	0.40	1.01	0.77
A2039X2043	0.31	1.08	0.56
A2039X850	0.63	1.39	0.78
A2039X557	0.61	0.54	0.56
A2039X554	0.67	0.90	0.65
A2039XTZ26	0.71	0.72	0.58
A2039X12023	0.63	1.01	0.75

5.3.5 Heterosis for yield

The results showing heterosis values for yield are presented in Table 5.7. The results revealed that mean heterosis for yield varied from -35% (A2039x2043) to 50% (A2043xUG8). Mean heterosis values above 20% were recorded in A2043xUG8 (50%), A2039x7035 (34%), and A2043x850 (25%). Within site analysis recorded heterosis ranging from -32 to 53% at Kabete and -58 to 57% at Kiboko and -68 to 113% at Leldet. The highest positive heterosis (113%) was recorded in A2043xUG8 at Leldet 2009.

Table 5.7. Mean heterosis (%) for pigeonpea yields across and within sites in Kenya

Genotype	Kabete			Kiboko			Leldet	Overall mean
	Season 2009	Season 2010	Mean	Season 2009	Season 2010	Mean	Season 2009	
A2039X12012	11	-48	-19	-49	-66	-58	-2	-31
A2039X12023	3	10	7	-25	-14	-20	-29	-11
A2039X2043	-25	-38	-32	-41	-34	-38	-37	-35
A2039X554	10	24	17	72	33	53	-45	19
A2039X557	8	-13	3	13	-14	-1	-12	-4
A2039X7035	93	-18	38	87	-40	28	48	34
A2039X850	-44	32	-6	11	95	53	-65	6
A2039XKAT	31	8	19	45	-4	20	-24	11
A2039XMARUTI	10	10	10	53	22	38	-12	17
A2043X00068	8	21	14	41	5	23	-19	11
A2043X12012	56	-3	27	-39	-49	-44	72	7
A2043X12023	3	28	16	-33	37	2	8	9
A2043X554	-17	-17	-17	35	8	22	-68	-12
A2043X557	-1	-3	-2	21	-6	8	-14	-1
A2043X7035	1	13	7	14	-1	7	16	9
A2043X850	13	24	19	55	37	46	-5	25
A2043X902	-25	19	-3	3	48	25	-47	0
A2043X911	31	-10	10	104	0	57	-36	18
A2043X9135	-24	1	12	24	40	32	-68	-5
A2043XASHA	-16	-7	-12	-4	6	1	-29	-10
A2043XKAT	14	-9	3	50	-23	14	-21	2
A2043XUG18	11	-2	5	26	-3	12	-3	6
A2043XUG8	50	58	53	11	20	16	113	50



Fig 5.4. Pigeonpea evaluation trial at Kiboko in 2010



Fig 5.5. High pod load on pigeonpea hybrid A2043xTZ24 at Kiboko 2010



Fig 5.6. Different seed colours and sizes of pigeonpea hybrids evaluated at Kiboko, Kabete and Ieldet 2009-2010

5.4 Discussion and conclusions

Pigeonpea is well adapted to the arid and semi-arid tropics where it is mostly grown as a rain-fed crop. Production is therefore dependent on the amount of rainfall during the crop growing season. The significant genotype x environment interaction indicated that variability existed among genotypes and also environments. The experimental sites used had varied available moisture and temperatures that could have had a direct effect on flowering, days to maturity and grain yield. The site with the highest grain yield potential was Kiboko 2010 with an average and maximum yield of 2,249 kg ha⁻¹ and 4,234 kg ha⁻¹ respectively. The high yields could be associated with relatively warm weather conditions, fertile deep soils and no moisture stress as the crop was irrigated. Yields ranging from 500 to 3,500 kg ha⁻¹ are typical for on-station trials in Kenya (Kimani et al., 2003; Onim, 1981). However, visual examination of the GGE scatter plots shows Kabete was the most stable environment for all traits recorded as it was consistently close to the origin relative to other sites.

Days to flowering varied between 77 and 181, with the majority of genotypes flowering after 100 days. The majority of hybrids were early as compared to the parents with maturity varying from 97 to 271 days. Despite pigeonpea possessing a wide range (90-300 days) for maturity, studies by Upadhyaya et al. (2006) found that days to flower was more reliable in estimating maturity duration in pigeonpea as drought, and podborer infestation trigger fresh flower production, which delays the day to maturity. In Kenya pigeonpea maturity groups have been classified as; short duration (90-120 days), medium duration (121-180) and late duration (>180 days) (Mergeai et al., 2001; Silim, 2001). The hybrids in this study could therefore fall in the two broad categories of early (<150 days) and medium duration (151-177 days) groups, while most parents were in the late maturity group (181-271 days). Most of the early maturing genotypes were hybrids and parents of Indian origin, whereas the late maturity types were parents from East Africa. Regression coefficients explain specific response of a hybrid to environmental effects. Hybrids with $b_1=1.0$ have average stability, those with $b_1<1.0$ have above average stability and are insensitive to changes in environmental conditions. However, those with $b_1>1.0$ have below average stability, are specifically adapted to high yielding environments and are sensitive to environmental changes (Finlay and Wilkinson, 1963; Miezán et al., 2004). A2039xTZ24 was the only hybrid with $b_1>1.0$ for days to flower and therefore responsive to environmental changes. The hybrid is adapted to specific sites and should therefore the days to flower will vary for each site. This shows that selection for earliness and evaluation for stability for specific environments for pigeonpea hybrids has great potential in Kenya.

Grain yield of pigeonpea in farmers' fields in Kenya is low, ranging between 200 kg ha⁻¹ to 800 kg ha⁻¹ (Mergeai et al., 2001). In this study, yields were much higher although with variations between sites and seasons. Some hybrids were outstanding, for example at Kabete, the highest yielding hybrid was A2039xTZ24 (2,057 kg ha⁻¹), while at Kiboko, A2043xTZ26 was the best (2,803 kg ha⁻¹). Regression analysis for seed yield showed that A2039x12091 and A2039x850 have below average stability and are specifically adapted to high yielding environments. The others had bi-value significantly below 1.0 implying they had above average stability and are less sensitive to environmental changes. It is therefore important to breed pigeonpea hybrids targeting specific environments. Similar recommendations were given by Saxena (2008), Saxena and Rajni (2001), and Egbe and Vange (2008).

Hybrid vigour or heterosis is important in crop improvement to raise the yield level. In the study, results revealed that mean heterosis for yield varied from -35% (A2039x2043) to 50% (A2043xUG8). Studies by Sreelakshmi et al. (2011) revealed that seed yield showed high heritability, genetic advance and positive correlation with number of primary branches, 50% flowering and number of pods plant⁻¹. The results also suggested that the traits were controlled by additive gene action. The high level of positive heterosis recorded in this study will therefore guide breeders in selection of parents that can be incorporated in a breeding programme. Considerable hybrid vigour over the better parent in pigeonpea has been reported by several workers for grain yield and other characters. Saxena et al. (2010) and Saxena et al (2006) tested experimental hybrids in multilocational trials and reported heterosis ranging between 30% and 80%.

The results of this study have shown the potential for production of pigeonpea hybrids and increased productivity in Kenya is feasible. Most hybrids were in the medium maturity category, were high yielding, uniform plant height (Fig 5.4, Fig 5.5), medium grain size (Fig 5.6) and stable in some environments. However, more research needs to be undertaken to develop even higher yielding hybrids. Hybrids also guarantee uniformity in grain size, which the Indian export market requires. This will be possible if uniform seed is available to farmers by ensuring that large scale production of hybrid seed is done by private seed companies.

References

- Byth, D.E., E.S. Wallis, and K.B. Saxena. 1981. Adaptation and breeding strategies for pigeonpea, pp. 450-465, *In* D. E. Byth and E. S. Wallis (Eds.) ICRISAT/ICAR International Workshop on Pigeonpeas, Vol. 1. ICRISAT, Patancheru, 502 324, Andhra Pradesh, India.
- Chauhan, Y.S., D.H. Wallace, C. Johansen, and L. Singh. 1998. Genotype-by-environment interaction effect on yield and its physiological bases in short-duration pigeonpea. *Field Crops Research* 59:141-150.
- Egbe, M.O., and T. Vange. 2008. Yield and agronomic characteristics of 30 pigeonpea genotypes at Otobi in Southern Guinea Savanna of Nigeria. *Life Science Journal* 5:70-80.
- Finlay, K.W., and G.N. Wilkinson. 1963. The analysis of adaptation in plant breeding programme. *Australian Journal of Agricultural Research* 14:742-753.
- GENSTAT. 2011. GENSTAT 14 (Copyright 2011). Release 14. GENSTAT, Rothamsted Experimental Station. Lawes Agricultural Trust.
- Hayes, J.D., and C.A. Foster. 1976. Heterosis in self-pollinating crops with particular reference to barley, *In* A. Janossy and F. G. H. Lupton (Eds). *Heterosis in plant breeding*. The European Association for Research in Plant Breeding, Budapest, Hungary.
- Holkar, S., Mishra, V.K. and Billore, S.D. 1991. Phenotypic stability of seed yield in pigeonpea (*Cajanus cajan* (L.) Millsp.) under dryland conditions. *Crop Research* 4:247-252.
- Kimani, P.M., G. Mergeai, S.N. Silim, J.P. Baudoin, P.R. Rubaihayo, and M. Jansens. 2003. New regional initiatives in pigeonpea improvement, *In* ICRISAT et al. (Eds.) *Pigeonpea improvement*, Vol. 3. ICRISAT, Nairobi, Kenya.
- Kimani, P.M., A.B. Nyende, and S.N. Silim. 1994. Development of early maturing fusarium wilt resistant pigeonpea cultivars. *African Crop Science Journal* 2:35-41.
- Mallikarjuna, N., and K.B. Saxena. 2005. A new cytoplasmic nuclear male-sterility system derived from cultivated pigeonpea cytoplasm. *Euphytica* 142:143-148.

- Mergeai, G., P. Kimani, A. Mwang'ombe, F. Olubayo, C. Smith, P. Audi, J.P. Baudoin, and A. Roi. 2001. A survey of pigeonpea production systems, utilization and marketing in semi-arid lands of Kenya. *Biotechnology, Agronomy, Society and Environment* 5:145-153.
- Miezan, K., G.A. Milliken, and G.H. Liang. 2004. Using regression coefficient as a stability parameter in plant breeding programmes *Theoretical and Applied Genetics* 54:7-9.
- Onim, J.E.M. 1981. Pigeonpea improvement research in Kenya, pp. 427-436, *In* ICRISAT et al, (Eds.) *Proceedings of the international workshop on pigeonpeas*, Vol. 1. ICRISAT, Patancheru, 502 324 Andhra Pradesh, India.
- Pandey, N., and N.B. Singh. 1998. Stability for seed yield in pigeonpea hybrids. *Legume Research* 21:233-235.
- Primomo, V.S., D.E. Falk, G.R. Ablett, J.W. Tanner, and I. Rajcan. 2006. Genotype x environment interactions, stability, and agronomic performance of soybean with altered fatty acid profile. *Crop Science* 42:37-44.
- Reddy, B.V.S., J.M. Green, and S.S. Bisen. 1978. Genetic male sterility in pigeonpea. *Crop Science* 17:362-364.
- Saxena, K.B. 2000. Pigeonpea, p. 82-112, *In* S. K. Gupta, (Ed.) *Plant breeding theory and techniques*. Agriobios, Jodhpur, India.
- Saxena, K.B. 2008. Genetic improvement of pigeonpea-A review. *Tropical Plant Biology* 1:159-178.
- Saxena, K.B., and R. Rajni. 2001. Pattern analysis for genotype by environment effects for seed weight and grain yield in pigeonpea hybrids. *Indian Journal of Genetics and Plant Breeding* 61:226-231.
- Saxena, K.B., R.V. Kumar, N. Srivastava, and B. Shiyong. 2005. A cytoplasmic-nuclear male-sterility system derived from a cross between *Cajanus cajanifolius* and *Cajanus cajan*. *Euphytica* 145:289-294.
- Saxena, K.B., R. Kumar, D. Shivani. 2010. Development of cytoplasmic-nuclear male sterility, its inheritance, and potential use in hybrid pigeonpea breeding. *Journal of Heredity* 101: 497-503

- Saxena, K.B., R.V. Kumar, K.M. Latha, and V.A. Dalvi. 2006. Commercial pigeonpea hybrids are just a few steps away. *Indian Journal of Pulses Research* 19:7-16.
- Saxena, M.K., U. Saxena, K.B. Saxena, V.S. Khandalkar, R. Sultana. 2011. Profitability and production cost of hybrid pigeonpea seed. *Electronic Journal of Plant Breeding* 2:409-412)
- Sharma, D., and J.M. Green. 1980. Pigeonpea, *In* S. B. S. Tikka, (Ed.) Hybridization of crop plants. Crop Science Society of America, Wisconsin, USA.
- Signor, C.E., S. Dousse, J. Lorgeou, J.B. Denis, R. Bonhomme, P. Carolo, and A. Charcosset. 2001. Interpretation of genotype x environment interactions for early maize hybrids over 12 years. *Crop Science* 41:663-669.
- Silim, S.N. 2001. Strategies and experiences in pigeonpea variety and development for Eastern and Southern Africa, pp. 232 pp, *In* S. N. Silim, et al. (Eds.) Status and potential of pigeonpea in eastern and southern Africa: Proceedings of a regional workshop, 12-15 Sept 2000. ICRISAT, Nairobi, Kenya.
- Singh, M., R.S. Malhotra, S. Ceccarelli, A. Sarker, S. Grando, and W. Erskine. 2003. Spatial variability models to improve dryland field trials. *Experimental Agriculture* 39:1-10.
- Sreelakshmi, C., S.V. Kumar, D. Shivani. 2011. Genetic analysis for yield and its components in hybrid pigeonpea. *Electronic Journal of Plant Breeding* 2: 413-416.
- Sreelakshmi, C., D. Shivani, S.V. Kumar. 2010. Studies on genotype x environment interaction and stability in white seeded pigeonpea (*Cajanus cajan* L.) genotypes. *Legume Research* 33:217-220.
- Upadhyaya, H.D., L.J. Reddy, C.L.L. Gowda, K.N. Reddy, and S. Sube. 2006. Development of a mini core subset for enhanced and diversified utilization of pigeon pea germplasm resources. *Crop Science* 46:2127-2132.

Research overview, implications of the findings and the way forward

The aim of the study was to develop high yielding pigeonpea hybrids that are resistant to fusarium wilt and possess consumer preferred characteristics. The study was divided into three main parts. The first was to examine the various stakeholders and their core functions and identify characteristics of the market preferred pigeonpea varieties to be considered in the hybrid breeding programme. The second part was to evaluate cytoplasmic male sterile (CMS) lines for stability in Kenya. The third part was to evaluate pigeonpea hybrids for general resistance to fusarium wilt and adaptability to different environments and earliness.

Using stakeholder analysis methodology, the roleplayers and their core functions in the pigeonpea value chain were identified, the value chain defined, preferred pigeonpea characteristics identified and constraints affecting pigeonpea listed and ranked. Key stakeholders identified in the pigeonpea value chain were categorized into seven groups; production, trade/processing, research, information dissemination, seed, quality assessment, and policy making. However, most of these stakeholders work independent of each other and this is one of the reasons why pigeonpea productivity and commercialization has possibly remained low.

Important pigeonpea traits preferred by farmers and processors/exporters for both domestic and export markets included white seed, large seed size and medium maturity. The varieties currently grown and consumed in Kenya possess these attributes but farmers acknowledged that they give low yields. The unavailability of quality seed in sufficient quantities of high yielding varieties, and lack of market were cited as the main factors negatively affecting pigeonpea production.

The stakeholder analysis methodology used in this study clearly demonstrated that it is possible to develop a highly client-oriented breeding programme from the on-set. The analysis showed that pigeonpea hybrids can be a viable commercial option in Kenya. The requirements of the key players are incorporated and this can lead to targeted research that will save on resources and ultimately guarantee success. It will also improve the co-ordination of activities of different roleplayers in the chain, ensure high product quality, and ultimately improved livelihoods. Stakeholder analyses have not been used in the past. The study has shown that such an analysis can be used as a participatory tool in plant breeding programmes in order to solicit valuable information from all the roleplayers in the chain.

Six CMS lines with their maintainers were screened for pollen sterility/fertility. The CMS lines ICPA2043 and ICPA2039 were considered highly stable as no significant quantities of viable pollen were observed across the locations and seasons. The two lines recorded the acceptable levels of male sterility (>95%) across all sites and seasons. Both CMS lines were subsequently crossed with improved varieties to develop hybrids that were evaluated in a fusarium wilt infested field and across environments in Kenya. In the pot inoculation studies, ICPA2043 showed high levels of resistance to three fusarium wilt isolates tested.

Laboratory analysis of *F. udum* isolates sampled from infected plants from the wilt-sick field at Kiboko was done using potato dextrose agar (PDA) and carnations leaf agar (CLA). Based on morphological and cultural characteristics, three distinct isolate groups named ISO-A (light pink and cottony mycelia and purple substrate), ISO-B (orange cottony mycelia and cream/whitish substrate, and ISO-C (light pink scanty mycelia and plum-purple substrate) were identified on potato dextrose agar, and were used in the pot inoculation trials. Inoculation of pigeonpea seedlings showed differential reaction of the genotypes. The variation in the reactions of the same genotype to different isolates indicates that physiological races exist. Pigeonpea genotypes were evaluated under Kiboko wilt-sick field, known to inhabit several races of *F. udum*. Resistant genotypes recorded were Maruti, ICPB2043, ICPA2043xICP13092, ICPA2043xICEAP7035, ICPA2043xICEAP850, and ICPA2043xAsha. The stability of the CMS lines and resistance to the three isolates of fusarium wilt opens the way for further studies on the possibilities of commercial production of hybrid seed.

Pigeonpea genotypes were evaluated across environments to test for their stability for yield and earliness. The highest yielding environment was Kiboko with an average and maximum yield of 2,249 kg ha⁻¹ and 4,234 kg ha⁻¹ respectively. Most hybrids were in the medium duration group with days to maturity ranging from 147 to 186. Highest yielding hybrids were A2043xTZ26 and A2039xTZ24 with mean yields 2,803 kg ha⁻¹ and 2,057 kg ha⁻¹ respectively. Mean yields for the best performing parents were 2,036 Kg ha⁻¹ for ICP12012 and 1,629 kg ha⁻¹ for Asha. Mean heterosis for yield varied from -35% (A2039x2043) to 50% (A2043xUG8) with a minimum of -68 % and maximum 113 %. In Kenya, the potential for production and commercialization of hybrid pigeonpea is feasible due to high hybrid vigour, medium maturity genotypes and stable and high yielding parents recorded in the study.

From the results, several lessons can be learnt and used to plan the way forward:

- Input from target clients is important for a successful breeding programme. The involvement of all roleplayers in the pigeonpea value chain facilitates greater ownership of the process and hence can increase adoption of new technologies, such as hybrid seed.
- Pigeonpea production constraints are as many as they are diverse. A multidisciplinary team of scientists and research institutions across borders will enable to address these constraints systematically and these will yield quality outputs that for the benefit of all.
- The research findings show that there is potential for use of CMS technology in Kenya for production of pigeonpea hybrids. Hybrid pigeonpea can have the advantage over open-pollinated varieties in terms of increased grain yield, greater disease resistance and evolution of business partnerships, especially with private seed companies.
- There is a need to develop more hybrids of the available CMS lines with other R-lines in order to further improve the yield.
- On-farm trials will be needed in the main pigeonpea areas to evaluate performance of hybrids in large plots under farmers' field conditions.

However, the backbone of any hybrid breeding technology is to establish an efficient seed production system, and this is the next challenge that needs to be addressed. Availability of genetically pure seeds of improved genotypes is critical for realizing their productivity in different agro-ecological zones. The benefits of new hybrids cannot be fully realized until sufficient quantities of genetically pure and healthy seeds are commercially produced. Because of the out-crossing nature of pigeonpea, a safe isolation distance for production of quality seeds of parental lines and hybrids is essential. This will require large scale seed production of female line (A/B), restorer line (R), and hybrid (AxR) combination. The development of hybrid seed production system under local conditions will be essential prerequisite for the success of hybrid pigeonpea technology in Kenya.

The main challenge is farmers accepting of the new technology. The increase in yield of the hybrids when compared with open-pollinated varieties should be such a degree that it justifies the purchase of the more expensive hybrid seed by the producers. The acceptance of hybrids will be made easier if the grain is uniform and complies with market standards and will therefore fetch a higher price. The study has shown the potential of hybrid pigeonpea in Kenya. The future introduction of the technology could encourage expansion of pigeonpea production in the area and make the pigeonpea sector more internationally competitive.

APPENDICES

Appendix 1. Preparation of Carnation Leaf Agar (CLA)

The procedure was as developed by Leslie and Summerell (2006). Freshly harvested young carnation (*Dianthus caryophyllus* (L.) leaves were cut into 5 x 5 mm pieces and dried in an oven at 45-55°C for 2 h. The leaf pieces were then wrapped in aluminium foil and sterilized for 5 min in a micro-wave oven. Carnation leaf agar was prepared by placing several of the sterile leaf pieces in a petridish and floating them on 2% water agar cooled and used for culturing the isolates.

Appendix 2. Analysis of variance for reaction of pigeonpea genotypes under field conditions at KARI Kiboko 2009/2010

Source of variation	d.f.	s.s.	m.s.	v.r	F pr.
Rep					
Season	1	2781.5	2781.5	2.61	0.353
Genotype	72	186262.6	2587.0	9.91	<.001
Genotype x season	1	177.7	177.7	0.68	0.410
Genotype x season	72	35140.2	488.1	1.87	<.001
Error	217	56675.8	261.2		