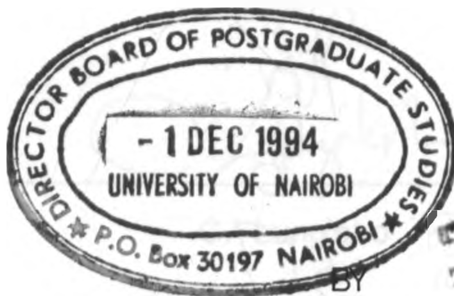
FRUIT YIELD AND QUALITY OF RIDGE GOURD (*Luffa acutangula* (L) Roxb) AS INFLUENCED BY PLANT DENSITY AND VARIETAL DIFFERENCES AS ASCERTAINED BY ISOZYME ANALYSIS



BY

CHARLES LUNG'AHO

A thesis submitted in partial fulfilment for the degree of

MASTER OF SCIENCE IN AGRONOMY

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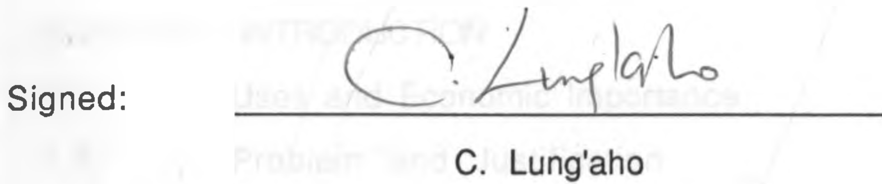
University of Nairobi
Faculty of Agriculture.

1993.

Declaration

I, Charles Lung'aho, hereby declare that the work presented in this thesis is based on research carried out by me between 1991 and 1992, and that it has not been submitted for a degree in this or any other university.



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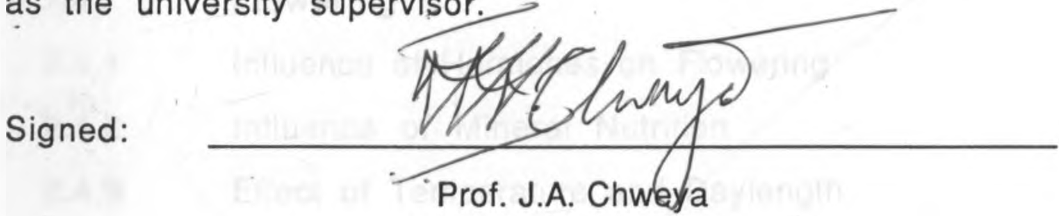

Signed: ______________________

Prof. J.A. Chweya

Date: 20/9/94

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ABSTRACT

Luffa acutangula is a potentially important export crop for Kenya. Little is known of its optimal production practices, genetics or fruit quality. Three studies were conducted at the Institutes for Applied Research of Ben Gurion University of the Negev in Israel between the months of October 1991 and September 1992.

The first study was conducted in a greenhouse during winter and summer seasons with the objective of determining differences in flowering, fruiting and fruit quality characteristics of six cultivars of *Luffa acutangula*.. The experimental design was Randomized complete block with 4 replicates. Results showed that both flowering and fruiting were enhanced during long days experienced in the summer season. Significant differences between cultivars were found in flowering, fruiting and fruit quality characteristics. Yields ranged between 0.338 to 0.917 kg/plant in winter and 2.488 to 2.912 kg/plant in summer. Fruits grown in winter were found to have significant differences in electrical conductivity, total soluble solids and reducing sugars while those grown in summer showed significant differences in pH and reducing sugars.

A field study was conducted during the summer season with the objective of understanding the influence of plant density on the yield and fruit quality of *L.acutangula*.. The experimental design was Randomized complete block. Three planting densities (5,000 , 10,000 and 15,000 plants/ha) and three cultivars ("Kenya", "DDR" and "67871") were studied.

CHAPTER 1

1 INTRODUCTION:

1.1 Uses and Economic Importance:

Luffa acutangula is variously referred to as ridge gourd, ridged loofah, chinese okra, vegetable gourd or turia (Chaundry,1967; Tindall,1987; Benzioni *et al.*,1988). It is a climbing annual (Plate 1) grown primarily for its immature fruits which are eaten raw, pickled or cooked. The immature fruits which may show morphological differences depending on the cultivar (Plate 2) are good sources of vitamin C and iron (Morton,1967; Wills et al.,1984) and are commonly used in soups and curries (Tindall,1987). Mature fruits are bitter and inedible, but are reported to be used for medicinal purposes. The leaves are also edible (Tindall,1987). The seeds are a good source of iron, magnesium, and phosphorous and contain high amounts of essential amino acids such as lysine (Kamel and Blackman,1982). However, seeds of some varieties are bitter due to high levels of bitter steroids like Cucurbitacin B (Watt and Breyer-Brandwijk, 1962).

The pure seed oil is tasteless and contains 68 percent of glycerides of oleic and linoleic acids, thus providing a good substitute for other vegetable oils such as from olive, safflower, and rape seed (Martin, 1979; Girgis and Said, 1968; Kamel and Blackman, 1982).

Plate 1: A Crop of *Luffa acutangula* growing under Field Conditions

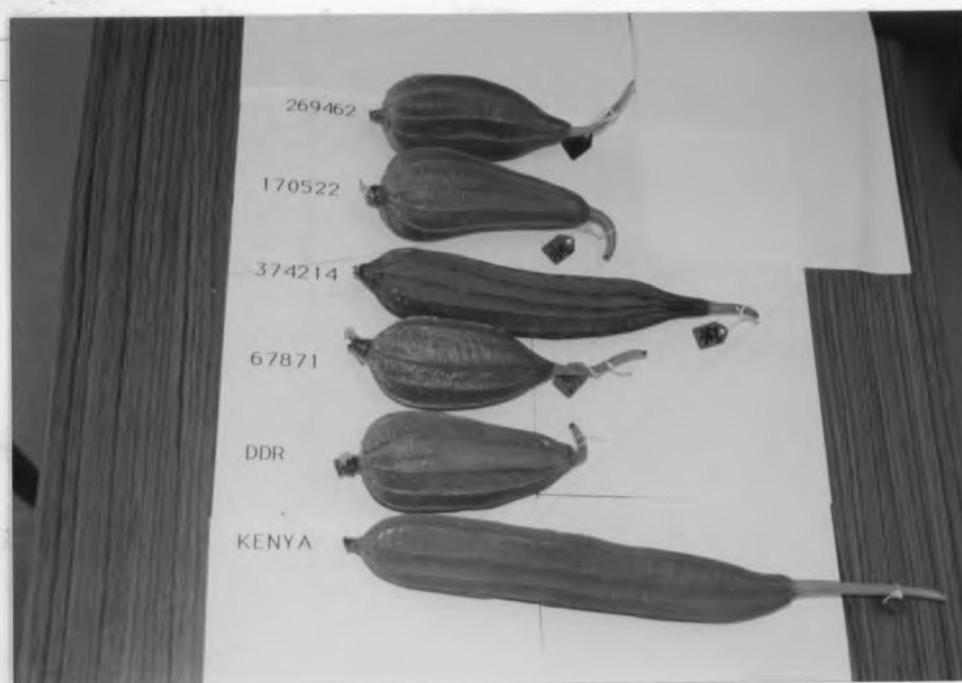

Plate 2: Immature Fruits of six Cultivars of *Luffa acutangula*

It has been categorized as a semi-drying oil (Kamel and Blackman, 1982). The oil cake, due to bitterness and probable toxicity, is not edible, but has a potential of being employed as a fertilizer material due to its high content of proteins and phosphorous (Potterfield, 1955). The toxicity is attributable to saponins which yield sapogenins (Watt and Breyer-Brandwijk, 1962; Varshey and Beg, 1977).

In Kenya, *L.acutangula* is only important as an export crop. Domestic consumption is low and is confined to people of the Asian community (Chweya, personal communication).
Important production areas include; Kiboko, Kibwezi, Makindu, Mtito Andei, Kilifi District, Mwea Tabere, and Mombasa District (Benzioni, et al, 1988). On the world scene, India, Japan, Malasia and Phillipines are among the main cultivation centres.

Kenya exports her produce to United Kingdom, Netherlands and Germany (Joy, 1987). Export figures are not documented but the value is probably worth several million dollars a year (Mendlinger, personal communication).

Demand for the fruit in the export market is high throughout the year (Benzioni et al.,1988), and market prices can be as high as $3.00/Kg (Mendlinger, personal communication).

1.2 Problem and Justification.

Despite its long history and its present extensive cultivation in Kenya, little is known about the optimal production practices, physiology, genetics, or economic potential of *L.acutangula*. Cultural practices are not documented and Kenyan farmers obtain agronomic advice from traders who are engaged in exporting the crop (Benzioni et al, 1988). The traders are not trained agronomists and their advice may not always be correct. Production practices are therefore diverse and yields correspondingly low (Benzioni, et al, 1992). The quality of the exported fruits is also variable. Yet fruit quality is an important factor in determining the prices fetched in the market.

It is against such a background that this study was undertaken.

1.3 Research Objectives.

1. To study the variation in flowering and fruiting characteristics of *L.acutangula.*
2. To evaluate the effect of planting density on the yield of *L.acutangula.*
3. To evaluate the variation in fruit quality of *L.acutangula.*
4. To determine genetic variation between various cultivars of *L.acutangula.*

CHAPTER 2.

2. LITERATURE REVIEW

2.1 Origin, Distribution, and Nomenclature:

The origin of *L.acutangula* is uncertain (Heiser,1988). It is believed to be native to the old world tropics (Jeffrey,1980). South East Asia has been suggested as a probable centre of origin (Heiser and Schilling,1988). Africa is proposed as a second domestication centre (Potterfield, 1955).

L.acutangula has a pantropic distribution (Tindall, 1987) between latitudes 40 °C and 30 °C South, but mainly around South East Asia, Africa, and South America (Jeffery, 1980). Unlike other cucurbits, it is adapted to hot humid tropics (Purseglove,1968; Herklot, 1972).

Jeffery (1980) states that the genus *Luffa* belongs to the sub-tribe *Luffinae* of the tribe *Benincaseae* . Cogniaux and Harms (1924) found 8 species of *Luffa*, namely ; *L.cylindrica* , *L.aegyptica*, *L.graveoleus*, *L.amara*, *L.echinata*, *L.acutangula*, *L.variegata* and *L.operculata*. Of these, only *L.acutangula* and *L.aegyptica* are widely cultivated.

2.2 Climatic Requirements and Cultural Practices.

There is little information in the literature on the climatic requirements and cultivation practices of *L.acutangula*. Reports have indicated that *L.acutangula* is well adapted for growth at elevations less than 500 m above sea level and that it requires soils with high organic matter content for optimal yields (Tindall, 1987). Fruits can be realised in 40-70 days after

sowing with individual plants producing 15-20 fruits (Tindall, 1987).

A survey conducted in Kenya (Benzioni *et al.*, 1988) revealed that cultural practises used in cultivation of *L.acutangula* are varied. They reported that plantings are made in rectangular holes measuring 60cm length x 60cm breadth x60cm depth although holes of size 100cm width x100cm breadth x 60cm depth were also encountered. Plant spacings were found to range between 1.8-5.0 m for the inter-rows and 1.0-4.0 m for the intra rows. The use of inorganic fertilizers was limited.

Establishment of the crop can be by direct sowing or transplanting (Benzioni *et al.*,1988, Tindall, 1987). In Kenya, seedlings are raised in polythene sleeves (of about 10cm in diameter and 15cm deep) and transplanted at 2 months old stage. The seedling mixture used in the sleeves is top soil mixed with manure in a 1:1 ratio.

Most of the farmers in Kenya grow the crop under furrow irrigation. All plants are trained and little attention is paid to pest and disease control (Benzioni *et al.*, 1988).

2.3 Germination.

Optimum conditions for germination of *L.acutangula* seeds are not documented. Okusanya (1978) investigating the effects of light and temperature on germination of *L.aegyptica* reported that at constant temperature, germination was best at 21°C , while alternating temperatures of 21 and 31°C and 15 and 41°C caused higher germination than the most favourable constant germination temperature. Similarly, Salma *et al.* (1985),

observed an 80% germination in *L.acutangula* at alternative and maximum temperatures of 18 and 30°C. In a related study, Huyskens (1991), observed a germination percentage of 60 at a constant temperature of 25°C and 0% germination at temperatures below 13 and above 40°C. On a further study, Huyskens (1991), observed that partial removal of the seed coat on the caruncle end increased both the rate and percentage of germination while vernalization reduced it. These results agree with the findings of Fursa and Gvozdera (1971) who reported that scarification of seeds kept in moist sand at 30-35°C produced good germination in *L.cylindrica*.

It therefore appears that *L.acutangula* seeds have a hard seed coat which tends to inhibit germination unless certain treatments are applied.

2.4 Flowering.

In *Cucurbitaceae*, sex expression of flowers is monoecious (separate staminate and pistillate flowers on the same plant). However, distribution and development of these two sex types differ with the species and sometimes with the cultivar. Hormones, mineral nutrition, temperature, and photoperiod have also been incriminated in regulating these flower types. In *L.acutangula*, andromonoecious as well as gynomonoecious plants are known to occur (Robinson, 1979).

2.4.1 Influence of Hormones on Flowering.

There are contradictory reports on the influence of growth regulating substances on sex expression of *L.acutangula*. Bose

and Nitsch (1970), reported that seed treatment with gibberellic acid induced femaleness while Krishnamoothy and Bhatia (1976), observed that foliar application of gibberellic acid to plants induced maleness. Ghosh and Basu (1984) also reported that endogenous gibberellins seem to favour maleness.

The effect of auxins in promoting femaleness seems to be more consistent. Satyanayarana and Rangaswami (1959) reported that femaleness was induced with the application of auxins to *L.acutangula*. These results are consistent with the findings of Bose and Nitsch(1970), Ghosh *et al.* (1981), and Ghosh and Basu (1984).

Ethrel has been found to be effective in inducing femaleness (Saimbhi, 1978; Ghosh and Basu, 1984). It is postulated that endogenous concentration of auxin may, infact, determine the endogenous concentration of ethylene, and ethylene would then be an intermediate effector molecule that promotes femaleness.

The role of cytokinins in sex regulation of *Luffa* plants is not quite clear as contradictive results have been reported. Takahashi *et al.* (1980), observed that application of a synthetic cytokinin (Benzyladenin) to the shoot apex of *L.cylindrica* from the 2-leaf stage suppressed differentiation of flower buds and reduced both the number of nodes bearing a staminate inflorescence and pistillate flowers on the main stem. In contrast, Bose and Nitsch (1970) in an earlier study had reported that soaking seeds of *L.acutangula* in cytokinins markedly promoted femaleness even under long days which are known to inhibit flowering in cucurbits. It therefore, appears that cytokinins may promote femaleness when applied to the floral

primordia or in the seeds or culture medium before the plant is developed. When applied to the whole plant, they have no effect in promoting femaleness (Takahashi *et al.*, 1980). This is in agreement with the findings of Cleland and Tanaka (1982) and Srivivasan *et al* (1979). It seems therefore that application of cytokinins in the infloresence, or in the seed or in the culture medium is thought to raise the level of cytokinins in the vicinity of the floral primordia resulting in flowering.

The exact mechanism underwhich these growth regulating substances affect sex-expression is unknown. It has however, been postulated that sex-expression in monoecious plants is controlled by an endogenous "auxin-gibberellin" balance in the vicinity of the floral primordia leading to the formation of female or male flowers as sex differentiation takes place in the floral primordia during ontogeny (Atsmon and Galun, 1960; Nitsch *et al.*, 1952). When the level of auxins in the floral primordia is higher than the level of gibberellins, the plant develops female flowers, while high levels of gibberellins relative to auxins causes the plant to develop staminate flowers.

2.4.2 Influence of Mineral Nutrition.

There is a general lack of precise information on the role of mineral nutrition on the flowering behaviour of *L.acutangula.* Omini and Hossan (1987), working with *L.aegyptica* observed that potassium containing nutrient treatments (Potassium, Nitrogen-Potassium, and Phosphorous-Potassium) promoted staminate flowering while phosphorous and nitrogen containing treatments (Nitrogen, Phosphorous, Nitrogen-Phosphorous, and

Nitrogen-Phosphorous-Potassium) promoted femaleness and reduced staminate flowering. These results are consistent with the findings reported for monoeciuos cucurbits (Hall, 1949; Heslop and Harrison, 1972; Dhaparidze,1976).

Omini and Hossan (1987), explain their results by suggesting that an increase in uptake of nitrogen and phosphorous results in the increase in auxin and/or cytokinin contents, thus altering the hormonal balance of the plant. Their postulate seems to be based on the fact that the precursor of auxin is the amino acid tryptophan and that of cytokinin is the purine base, adenine (Wareing and Phillips, 1978). Increased availability of nitrogen and phosphorous in the plant can cause an increase in both tryptophan and adenine as well as other amino acids, proteins, enzymes and nucleotides, and nucleic acids in the apex of the plant (Salisbury and Ross, 1985). This leads to increased activity of the meristem and young leaves (Wareng and Phillips, 1978), leading to an increase in auxin and cytokinin content in these regions. This increase in hormones in turn promotes early development of floral primordia into female flower buds.

2.4.3. Effect of Temperature and Daylength:

Daylength and temperature strongly influence sex expression in cucurbits (Whitaker and Davis,1962; Shfriss and George, 1965; Rudich et al., 1976). However, in *L.acutangula* contradictive results on the female flower habit have been documented. Kaushik and Sharma (1974); Yonemori and Fujieda (1985), and Takahashi and Saito, (1986) observed that short days and low night temperature (<20°C) promoted female flower

production, while Gosh and Basu (1983) reported that long days led to enhanced female flower production. Male flowers were stimulated under long days. Similarly, Huysken (1991), observed that staminate flowers were induced by low temperatures and short daylengths while both staminate and pistillate flowers were suppressed outside the range 17-35°C.

2.5. Effect of Plant Density on Yield:

Few studies have been conducted on the effect of plant density on the yield of *Luffa* species. Consequently, no optimum plant density is documented.

In Kenya, the stand density of *L.acutangula* is variable as both the inter-row and intra-row spacings vary. Benzioni *et al.* (1988), reported that the inter-row spacings range between 1.8-5.0 m while the intra-row spacing ranges between 1.0-4.0 m. These spacings give plant densities of between 500 and 5555 plants/ha. A study conducted on *L.aegyptica* (Ko *et al.*, 1978) demostrated that a spacing of 1.5 x0.9 m can give optimum yields of about 9600 gourds/ha. No mention is made of the yield in kg/ha. Similarly, Kvaratskheliya (1985), reported that a plant spacing of 2x1 m gave a commercial yield of 292.7 kg/ha per year as an average of 5 years. In *L.acutangula,* was observed that a plant density of 20,000 plants/ha gave higher yields than a density of 10,000 plants/ha (Huyskens,1991). However, the fruits obtained were smaller in size.

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2.6 Fruit Quality:

Quality in fruits is that combination of characteristics which makes them desirable to the consumer. Fruits are ordinarily chosen by appearance because other methods for determination of quality are rarely available to the casual purchaser. Unfortunately, the eating quality of many fruits cannot be accurately determined by the visible characteristics like size, shape, colour, and freedom from external defects. To determine such real quality factors like flavour, texture, and freedom from internal defects other measurements must be done. These include; total soluble solids(TSS), titratable acidity(TA), reducing sugars, electrical conductivity(EC), and pH.

Little is known about the factors that affect the fruit quality of *L.acutangula* . Benzioni *et al.*, (1992), found that untrained plants had fruits with higher TSS and reducing sugars than fruits from trained plants.

In musk melons (*Cucumis melo*) which are also members of *Cucurbitaceae* work on fruit quality is well documented. It has been established that the fruit quality of melons is markedly influenced by cultural practises, growing season, and genotype. Zeng *et al.* (1988) reported that basal application of potassium sulphate and top dressing with the same resulted in an increase in fruit sugar content, better colour, and flavour. Similarly, Yadav (1984), observed that both irrigation and nitrogen fertilization influenced the yield and quality of musk melons. In a related study, Satish *et al.* (1988), observed that phosphorous and nitrogen treatments caused an increase in the dry matter(DM) content of melons.

Variation of fruit quality with cultivar has also been documented. Gurdeep *et al.* (1987), reported differences in the TSS, DM, and acidity in various lines of musk melons they studied.

Moschin *et al.* (1987), demonstrated that low night temperature (10°C) resulted in poorer quality and yields of melons than a night temperature of 16°C under greenhouse conditions. These findings are similar to the results of Wacquant (1989), who reported larger fruits and faster fruit development at a night temperature of 19°C when compared to a night temperature of 15°C. Temperatures outside the range 35-40°C were found to decrease the total sugar content. Welles *et al.* (1988) also observed an increase of soluble solids content(SSC) of musk melons with rise in temperature.

2.7 Genetic Variation:

Genetic variation among plants is important as it provides the raw material essential for improving agronomic traits such as disease resistance, drought tolerance, yields and quality (Simmonds, 1986). It also assists in producing cultivars with wider ecological adaptation

There is little information in literature on genetic variation in *L.acutangula*, although germplasm material exists in several gene banks including the National Gene Bank of Kenya.

Biochemical markers offer a more precise method for distinguishing cultivars than morphological, phenological and physiological characteristics (Moore and Collins, 1983). This is partly because reliable identification of cultivars using

classical methods based on morphological and physiological traits has become increasingly difficult due to the large number of lines being released and the convergence of these lines on a few of the most desirable traits (Weeden,1984). Time and resource requirements for grow out tests and their depedance on "normal" enviromental conditions make such procedures impractical for routine screening. Further, morphological differences between species are very difficult to interpret genetically. In addition, the results are often the consequence of different development environments and expressivity may be operational.

Electrophoretic techniques are widely used for analysis of genetic variation of proteins . Horizontal starch gel electrophoresis is a process of forced diffusion in an electric field. Protein molecules are moved in an electric field through a starch medium by applying an electric gradient. The principle being that proteins in solution at a pH above their isoelectric points have a net negative charge and will move towards the anode when placed in an electric field; proteins in solution at a pH below their isoelectric points have a net positive charge and will move towards the cathode; Protein molecules with a similar charge will move at the same rate. Upon applying a gradient, proteins assume different charges, often with a different net sign at different pHs, and their rate of migration through the gradient differs in proportion to their charge and molecular weight. This results in separation of the different proteins into bands which are then resolved by staining or spectrophotometry to provide visible prints called zymograms. The bands are called

isozymes (i.e molecular forms of an enzyme). Usually when genetically similar tissues of the same age are assayed they give identical prints. However, differences will show in the prints if these tissues are genetically unrelated. It is such variation that is of importance in distinguishing different lines and cultivars. Any differences in the bands of the sample imply that they are not identical and will be an indication that they carry different genetic material.

Application of isozymes was outlined by Pierce and Brewerbaker (1973) and included cultivar identification. Isozyme analysis has subsequently been reported for cultivar identification for several clonally propagated species including apples (Weeden and Lamb,1985), pineapple (Dewald *et al.*,1988), and sweet potatoes (Kennedy and Thompson,1991). They have also been used to distinguish among different classes of beans (Bassiri and Adams,1978), barley (Fedak, 1974; Bassiri, 1976), wheat (Kessarda *et al.*, 1973), and maize (Cardy and Kanneberge, 1982).

CHAPTER 3:

3. MATERIALS AND METHODS:

3.1 Site and Location:

The experiments were conducted between the months of October 1991 and September 1992 in Beer Sheva, Israel at the Institutes for Applied Research of Ben Gurion University of the Negev . The institutes are located 31°14'N latitude in the Negev desert at an altitude of 280 metres above sea level. The climate is characterised by hot dry summers lasting from April to October and cool, wet winters in the month of November to March.

3.2 Planting Material and Germination:

The seeds used were obtained from the Regional Plant Introduction Station (U.S.A) and East African Seeds Company (Kenya) (Table 1). A morphological description of the cultivars was not given. They were one year old and had previously been stored in paper bags which had been kept in the dark at room temperature (25°C)

Previous research done at the Institutes had shown that partial removal of the seed coat improved germination (Benzioni *et al.*,1988). For all the studies, therefore, a small incision was made on the seeds at the caruncle end inorder to hasten imbibition of water. The seeds were then put between moist filter papers (Whatman no.4) in petri dishes. The petri dishes were then sealed with parafilm and kept in an incubator

at 31 °C. After the appearance of the radicle, normally after two - three days, the seeds were transfered into seedling trays which were then placed in the greenhouse. The seedling mixture consisted of vermiculite, peat, and perlite in a 1:1:1 ratio. The seedlings were watered once every day until the appearance of the first true leaf afterwhich Hoagland solution was applied twice a week and watering reduced to five times a week. The composition of the Hoagland solution is given in Appendix 1. Transplanting was done when the seedlings attained the two true leaf stage.

Selection of planting material was based on availability of seed.

Table 1: *Luffa acutangula* Cultivars Investigated and their Sources

Cultivar	Country of origin	Source
170552	Turkey	Regional Plant Introduction Station, U.S.A
269462	Pakistan	"
374214	USA	"
67871	Hungary	"
DDR	Germany	"
"KENYA"	Kenya	East African Seeds Company, Kenya.

3.3 Experiment I - Greenhouse Experiment:

This experiment aimed at understanding the variation in the flowering pattern, yield and fruit quality of six cultivars of *L.acutangula* when grown in winter and summer seasons.

The study was conducted during the winter and summer seasons in a greenhouse. The greenhouse was equipped with a cooling system consisting of a centrifugal fan drawing cool air from the outside through a water drenched wall located on the greenhouse end opposite the fan. A heating system consisting of PVC piping in which hot water flowed was also employed during the cooler months of winter.

3.3.1 Experimental Design and Treatments:

The experiments were laid out as a randomized complete block design with four blocks. The treatments consisted of six lines of *L.acutangula,* viz,

L_1="67871" L_4="374214"

L_2="269462" L_5="DDR"

L_3="Kenya" L_6="170552"

3.3.2 Procedure:

The potting mixture consisted of vermiculite, peat, and perlite in a 1:1:1 ratio. The mixture was prepared by shredding the peat by hand, followed by addition of vermiculite. After thorough mixing, the perlite was added and the mixing done again. The uniform mixture was then used to fill the pots.

Transplanting was done into 15-litre pots, after liberally watering the potting mixture. The system of transplanting

used was that of root block in which the seedlings were first watered, then lifted up from the seedling tray together with their seedling medium still attatched to the roots. Holes were then made into the pots by hand. The seedlings were placed in the pots such that the seedlings' root block was level with the potting mixture. This was followed by immediate watering.

3.3.3 Cultural Practices:

A drip irrigation system with low pressure emitters (0.1Mpa) was used for both irrigation and fertigation. The plants were watered with 0.5 litres of water and 0.5 litres of Hoagland solution every other day. These levels were increased upto a maximum of 1 litre of water and 1 litre of Hoagland solution as the plants grew. Once every three weeks the pots were drained of accumulated salts by watering with distilled water for 3 hours. The quantites of water and Hoagland solution given to the plants were automatically regulated by the "Drip thinker" computer.
The electrical conductivity of the water used for irrigation was approximately 1.2 dS/m (700 ppm).

The plants were trained on a nylon netting frame to a height of two metres during the growing season.

Pest and disease control was achieved by prophylatically spraying with an insecticide Endosulphan according to manufacturers recommendation for the control of white flies Benomyl was sprayed against downy and powdery mildews.

3.3.4. Data Collected:

Data collected included date to first male and female flowers, total number of fruits, percent fruit set, total yield and marketable yield. The number of male and female flowers was recorded every day starting at the onset of flowering until the end of the experiment. Total number and yield of fruits was recorded during every harvest which was usually done every 3-4 days. At harvest, marketable and non-marketable fruits were sorted out.

Percent fruit set was calculated using data for number of female flowers produced and the number of fruits that were harvested.

Determinations for fruit quality included: Total soluble solids (TSS), pH, electrical conductivity(dS/m), acidity(meq/gfw), and reducing sugars(mg/gfw).

TSS was determined from a drop of fruit juice using a refractometer (Bousch and Lomb). The other determinations were conducted on a fruit extract of 10g of fresh weight material which was homogenised with 5 ml of double distilled water, centrifuged at 5000xg at 4 ^{o}C for 20 minutes, and then filtered. The pH was measured in the filtrate by a digital meter (El Hamma instruments PSB-737) while EC was measured by a digital conductometer(H-250). Titratable acidity was measured by titrating 3ml of the filtrate with 0.05N NaoH with 1% phenolphthalene as the indicator. Reducing sugars were determined as described by Sumner (1921). A 0.6 ml aliquat of the extract was added to 2.5 ml of the Sumner reagent, and the mixture boiled for 5 minutes. The

mixture was then cooled at room temperature (25 ^{o}C) and 3 ml of double distilled water added. The reducing sugars were then determined spectrophotometrically by measuring the absorbance at 550 nm with glucose as the standard.

3.3.5 Data Analysis:

Analysis of variance was carried out and where significant F-values were obtained at 5% level, Duncan Multiple Range Test (DMRT) was used to separate the means at the 5% level.

3.4. Experiment 2- Field Experiment:

The study was conducted in the summer season between the months of April and September, 1992. The objective was to understand the influence of planting density on the yield, yield components and fruit quality of three *L.acutangula* cultivars.

During the study period, the climate was characterised by hot sunny days. No rainfall was recorded. The average maximum and minimum temperatures were 37 ^{o}C and 14 ^{o}C respectively.

3.4.1 Experimental Design and Treatments:

The experiment was a 3 X 3 factorial laid out as a randomized complete block design with 3 blocks, giving a plot total of 27.

The treatments consisted of 3 cultivars of *L.acutangula* and 3 plant densities viz;

L_1= "Kenya" D_1= 5000 Plants/Ha

L_2= "DDR" D_2= 10000 Plants/Ha

L_3="67871" D_3= 15000 Plants/Ha

This gave 9 treatment combinations:

1. L_1D_1	4. L_2D_1	7. L_3D_1
2. L_1D_2	5. L_2D_2	8. L_3D_2
3. L_1D_3	6. L_2D_3	9. L_3D_3

3.4.2 Procedure:

The experimental plot was previously under *L.acutangula* (1990), *Vernonia ssp.* (1991), and fallow in winter 1992.

In early March, the experimental plot was ploughed and harrowed. The plot was then applied with 1500 kg/ha of cow and chicken manure in a ratio of 3:1, and 700 kg/ha of single superphosphate as pre-fertilizer treatments. These fertilizers were incorporated into the soil at a depth of 40 cm using a rotorvator. Raised beds measuring 2 Metres wide were then prepared. A single drip line with 2 l/hr drippers spaced every 1.0 m, 0.5 m, and 0.33 m depending on the treatment was assembled in each plot. A week before transplanting the field was liberally watered until weeds germinated. The plots were then sprayed with a herbicide, 2-4 D, at manufacturers recommended rates.

Seedlings were started as in experiment 1. Transplanting was done on 20/4/92 after 4 weeks in the seedling trays. In order to minimize transplanting shock, seedling trays were carried to the field and transplanting done early in the morning when the temperatures were relatively low. The system of transplanting was that of "root block" as described in section 3.2.1 above.

3.4.3 Cultural Practices.

Irrigation and fertigation were by a drip irrigation system. Plants were initially watered every 2 days with 0.5 litres of water. Later, this was adjusted upwards as the plants grew. Appendix 2 shows the watering regime and the amount of fertilizer given at each fertigation. During fertigation fertilizer (N:P:K) 20:20:20 was applied to the plants at each irrigation. The plants were grown as trained.

Pest and disese control procedures were as in experiment 1.

3.4.4 Data Collected:

Data recorded during the experimental period included date to first male and female flowers, total number of fruits, percent fruit set, total yield and marketable yield. Determinations for fruit quality were as described in experiment 1.

3.4.5 Data Analysis:

Analysis of variance was carried out and where significant F-values were obtained at 5% level, Least

significant differences (LSD) were used to separate the means at the 5% level.

3.5 Experiment 3: Electrophoretic Analyses

The aim of this study was to genetically characterise six cultivars of *L.acutangula* using isozyme polymorphism.

Fresh leaf samples representing six cultivars of *L.acutangula* (Table 1) were obtained from greenhouse grown plants. Eight plants per cultivar were sampled. The samples consisted of young leaf tissue measuring approximately 1 cm^2 in area and were taken from the plants 8 days from germination.

All samples were collected on the morning of the run in test-tubes and transported to the laboratory in ice.

3.5.1 Sample Preparation:

Homogenisation of samples was achieved by mechanical grinding using a cold mortor and pestle. The grinding took place in six drops of double distilled water. A fresh set of mortor and pestle was used for each sample.

3.5.2 Starch Gel Preparation:

A day before the actual run, starch gels were prepared as described by Gottlieb (1973), with several modifications. 43.75 g of potato starch (Sigma Chemicals Company, Lot number 100 H0492(s-4501)) in 350 ml of gel buffer produced 12.5% starch gels which were found suitable.

Gel preparation was achieved by first mixing the starch in 150 ml of gel buffer in a 100 ml beaker, then pouring out the solution in an erlenmeyer flask. The resulting mixture was frequently swirled. The remaining 200 ml of gel buffer was then brought to boil on a hot plate in a 1-litre volumetric flask. The hot gel buffer was then poured into the cold starch/gel solution shaking vigorously. The mixture was then brought to boil while swirling frequently inorder to avoid the starch setting. Evacuation of gas was also undertaken during this time. The mixture was assumed to have boiled sufficiently when it showed the following characteristics: an increase in the boiling rate, a change in colour to clear, decreased viscosity, and the appearance of relatively large bubbles.

After boiling the hot degassed suspension was immediately but gradually poured into a leveled gel mound measuring 18cm X 15cm X1cm (270cm^3). This was usually done in a smooth motion that filled all the corners of the mould evenly. Occasional bubbles were lifted out using a needle immediately after pouring. The gels were then cooled at room temperature (25 ^oC) until they became opaque (20-30 minutes), after which they were put in the refrigerator for further cooling (1 hour). The gels were then covered with with plastic wrap to prevent dessication of the surface, and left overnight in refrigerator(4 ^oC).

Two gel buffer systems were employed in order to enhance enzyme resolution. Table 2 gives the composition of the buffers.

Table 2: Buffer Systems and their Composition.

Buffer	Electrode buffer		Gel buffer	
Poulik	Boric acid	18.5g/L	Trisma-base	9.2g/L
	NaoH	2.4g/L	Citric acid	1.0g/L
	pH 8.6			pH 8.7
Tris-maleate	Trisma-base	12.1g/L	Dilute the electrode	
	Maleic acid	11.6g/L	buffer with distilled	
	EDTA	2.7g/L	H_20 in a ratio of	
	$MgCl_2$	2.0g/L	1:20 respectively	
	pH 7.4			

3.5.3 Loading the Gel:

Following removal from the refrigerator, the gel was unwrapped and a straight cut which served as the origin made 5 cm from the cathodal end. Paper wicks (Whatman filter paper no. 3) measuring 0.4 X 1cm were then dipped into the raw extract, blotted on tissue paper to remove excess solution, and then inserted 2-3 mm apart into the slice across the gel using a pair of forceps. Loading was such that the wicks touched the base of the gel mould. 15 wicks were loaded per gel. To denote the front of the tris-maleate gel during the run the first wick inserted at the left hand side of the gel mould was dipped into a bromophenol blue dye solution and not the raw extract.

3.5.4 Running the Gel:

The loaded gel was then positioned above and between the cathodal and anodal ends of a tray that had been filled with the electrode buffer (also called tank buffer). Cellulose sponges (one cathodal and one anodal) were used to provide a contact bridge between the electrode buffer and the gel. The sponges were first moistened with the electrode buffer and applied so as to cover about 2cm on each end of the gel. The sponges were placed parallel to the wicks thus ensuring a straight front. Plastic wrap was then placed over the gel and sponges inorder to reduce surface dessication. A glass plate was added on top of the assembly to hold the sponges uniformly against the gel. Some ice in a plastic container was also placed on top of the gel to ensure that the gel was run at non denaturing conditions.

The voltage at which the gels were ran varied depending on the gel buffer. Poulik gels were ran at 200V while the Tris-maleate gels were ran at 150V

3.5.5 Preparation of Stains:

Stains for each enzyme were prepared just before the end of the run. This facilitated immediate gel staining thus avoiding the decline in enzyme activity coupled with their diffusion into the gel. However, cofactors, tetrazolium salts, and coupling enzymes were added just prior to staining. Enzymes and reagents were dispensed with pippetors.

3.5.6 Enzymes Assayed:

Six enzyme systems namely; Glulose-6-phosphate dehydrogenase (G-6-PDH), malate dehydrogenase (MDH), esterase (EST), shikimic acid dehydrogenase (SHDM), phosphoglucoisomerase (PGI), and peroxidase (PRX) were assayed. The composition of their staining recipes is shown in Table 3. Poulik gels were used to assay G-6-PDH, EST, SHDH, PGI and PRX. Tri-maleate gels were used to assay MDH.

3.5.7 Slicing the Gel:

After the gels had run for a distance of about 8-10 cm from the origin , they were removed from the electrophoretic assembly and the ends of the gel trimmed at the gel front on the anodal side and the wicks removed from the cathodal end. The gel was then inverted onto a plexiglass plate and sliced horizontally into three slices of thickness 1.5 mm. The slicer used was made of piano wire, the principle being similar to wire cheese cutters (Harris and Hopkinson, 1976; Werth, 1985). After each clean cut, the top portion was removed so as to get the sliced piece which was then placed cut surface up in a staining bath measuring 21cm x12cm x5cm.

Prior to slicing, a cut was made at the left hand corner of the gel so as to distinguish the location of the origin and the first sample.

Table 3: Staining Solutions for Enzymes Assayed:

Ingredient	Amount
1. Glucose-6-phosphate dehydrogenase (G-6-PDH)	
0.5 M Tris-HCl (pH 7.1)	20 ml
Double dustilled water	80 ml
1 M $MgCl_2$	2 ml
Glucose-6-phosphate	200 mg
NADP	30 mg
NBT	20 mg
PMS	10 mg
2. Malate dehydrogenase (MDH)	
0.2 M Tris-HCl (pH 8.0)	80 ml
Malate substrate	10 ml
NAD	60 mg
NBT	40 mg
3. Esterase (EST)	
0.1 M Phosphate buffer (pH 6.25)	50 ml
Fast blue RR salt	100 mg
Beta-Naphthyl acetate (in 70% acetone water)	100 mg

Table 3 cont'd

Ingredient		Amount
4. Shikimic acid dehydrogenase (SHDH)		
0.1 M Tris-HCl (pH 7.5)		100 ml
Shikimic acid		100 mg
NADP		14 mg
MTT		10 mg
PMS		4 mg
5. Phosphoglucoisomerase (PGI)		
0.2 M Tris-HCl (pH 8.0)		70 ml
Fructose-6-phosphate		40 mg
1 M $MgCl_2$		10 ml
Glucose-6-phosphate dehydrogenase		40 units
NADP		20 mg
NBT		30 mg
PMS		10 mg
6. Peroxidase(PRX)		
Beaker 1:	30% H_2O_2	1 ml
	DI H_2O	60 ml
Beaker 2:	Potassium Iodide	0.4g
	DI H_2O	60 ml
	Acetic acid	4 drops

3.5.8 Staining:

Once a given slice was ready for staining, the final co-factors ($MgCl_2$, NAD, NADP, MTT, NBT, and PMS) were then dispensed quickly into the staining bath for final mixing. Repipetters were used. The staining solution was made such that it fully covered the gel. The staining baths were then incubated in an oven at 37 ^oC to hasten band development.

The staining baths were opaque and covered inorder to prevent evaporation during incubation and help avoid cross contamination of gels by stains with volatile components (e.g acetone for esterase).

After the bands were stained, they were removed from the oven and fixed overnight with 10% acetic acid. Photographing and data collection was then done.

3.5.9 Data Collected:

The distance that the bands had moved from the origin was noted. The thickness and intensities of the bands were also noted. The number of loci for each enzyme was noted. The band closest to the origin was designated as number 1 and so on. Zymograms were then drawn and comparisons made.

CHAPTER 4 .

4. RESULTS.

4.1 Greenhouse Experiments.

4.1.1 General:

Flower buds appeared first on the lower part of the stem and side branches and progressed towards the apex. In rare instances some male flowers appeared on the tendrils of the plant. Flowering began at the second node and continued till the end of the experiment.

Staminate flowers occured in clusters of about 12 buds which opened in succession. Some solitary flowers were also observed. However, upto 6 flower buds from the same inflorescence could open at the same time.

Pistillate flowers occurred singly beginning at about the fourth node of the main stem but not at a fixed location on side branches. Production continued until the conclusion of the experiment.

4.1.2 Days to First Male Flower:

Significant differences in the number of days to first male flower were observed between cultivars both in winter and summer (p=0.05). Table 4 shows that in winter cultivars "67871" and "269462" flowered significantly earlier (30 days) than "DDR" and "170522". Cultivars "374214" and "Kenya" were not significantly different from "67871", "269462", "DDR" and "170552".

Table 4: Differences in the Number of Days to First Male and Female Flowers Between Various Cultivars Luffa acutangula of Grown in the Greenhouse in 1991 and 1992

	WINTER 1991		SUMMER 1992	
CULTIVAR	DAYS TO FIRST MALE FLOWER	DAYS TO FIRST FEMALE FLOWER	DAYS TO FIRST MALE FLOWER	DAYS TO FIRST FEMALE FLOWER
67871	29.75b	33.25b	29.25b	27.00a
269462	30.00b	39.00a	22.25b	23.75a
374214	30.25ab	33.75b	18.50c	21.25a
DDR	33.75a	33.00b	22.25b	23.75a
170552	34.50a	33.50b	24.25b	23.25a
KENYA	33.00ab	43.00a	18.25c	22.25a
C.V. (%)	7.02	8.40	9.17	17.05

Values with same letters within columns imply no-significant at 5% level of probability using Duncan's Multiple Range Test.

flow

In summer, "374214" and "Kenya" flowered significantly earlier than cultivars "170552", "DDR", "269462" and "67871" at 18.50 and 18.25 days after transplanting respectively. Cultivar "67871" was significantly late in its days to first male flower than all the cultivars at 29.25 days after transplanting. In general male flowers were produced starting at 22 days after transplanting.

4.1.3. Days to First Female Flower:

Cultivar differences in the number of days to first female flower in winter were significant but differences in summer were not significant (p=0.05).

In summer, the cultivars produced the first female flower between 21 and 27 days after transplanting. In winter, the cultivars produced the first female flower between 33 and 43 days after transplanting. Table 4 shows that in winter cultivars "Kenya" and "269462" were significantly late in producing their first female flower at 43 and 39 days after transplanting respectively compared to cultivars "170552", 'DDR", "374214" and "67871".

4.1.4. Number of Male Flowers/Plant:

The differences in number of male flowers per plant between cultivars were significant in both seasons (p=0.05). In winter, cultivar "269462" had a significantly higher number of male flowers (71.25 flowers) than cultivars "67871", "374214", "DDR", "170552", and "Kenya" (Table 5). The number of male flowers/plant ranged between 7.25 ("DDR") and 71.25 ("269462").

Table 5: Differences in the Number of Female Flowers, Number of Male Flowers, Total Number of Fruits and Percent Fruit Set Between the Various Cultivars of *Luffa acutangula* Grown in the Greenhouse in Winter, 1991

CULTIVAR	NO. OF MALE FLOWERS	NO. OF FEMALE FLOWERS	RATIO OF MALE TO FEMALE FLOWERS	TOTAL NUMBER OF FRUITS	% FRUIT SET
67871	29.25b	8.38b	5a	4.50a	58.3a
269462	71.25a	13.25a	5a	7.00a	52.8a
374214	16.00b	9.00b	2a	5.50a	62.2a
DDR	7.25b	5.63b	1a	4.3a	78.4a
170552	35.00b	13.3a	3a	6.25a	47.7a
KENYA	35.25b	13.63a	3a	6.5a	50.0a
C.V (%)	53.11	25.16	69.45	23.41	28.57

Values with same letters within columns imply non-significant at 5% level of probability using Duncan's Multiple Range Test

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In summer, cultivars "67871" and "269462" had a significantly higher number of male flowers per plant (322.58 and 335.49 respectively) than cultivars "374214", "DDR", "170552" and "Kenya" (Table 6). The table also shows that cultivars "170552" and "374214" were significantly poorer in male flower production compared to the other four cultivars. They produced 55.59 and 109.42 male flowers per plant respectively.

4.1.5. Number of Female Flowers/Plant:

The differences in the number of female flowers per plant between cultivars were significant in winter but not in summer (p=0.05).

In winter, cultivars "DDR", "67871" and "374214" had significantly lower numbers of female flowers/plant than cultivars "269462", "170552" and "Kenya" (Table 5). In general the number of female flowers/plant ranged between 5.63("DDR") and 13.63("Kenya") in winter while the range was 18.67("DDR") to 25.67 ("374214") female flowers/plant in the summer.

4.1.6. Ratio of Male to Female Flowers:

Significant differences were not found between cultivars for the ratio of male to female flowers in the winter season but in summer the cultivars showed significant differences (p=0.05).

In winter, the ratio ranged between 1:1("DDR") to 5:1("269462" and "67871"). Table 6 shows that in summer

cultivars "67871" and "269462" had significantly higher ratios (15:1 and 14:1 respectively) than cultivars "170552", "374214" and "Kenya" which had ratios of 3:1, 4:1 and 8:1 respectively. Among the cultivars examined, "Kenya" and "DDR" were intermediate in their ratios (8:1 and 11:1 respectively).

4.1.7. Number of Fruits/Plant

Significant differences in the number of fruits/plant were not found between cultivars in both seasons (p=0.05). Table 5 shows that in winter the number of fruits/plant ranged between 4.3 to 7.0 fruits/plant. In summer, the range was 14.09 to 17.42 fruits/plant.

4.1.8. Percent Fruit Set:

Percent fruit set, defined as the percent female flowers that developed into fruits , did not differ between cultivars in winter but in summer, significant differences were observed between cultivars at the 95% level of confidence.

In winter fruit set varied from 47.7% ("170552") to 78.4%("DDR"). Table 6 shows that in summer, "DDR" had a significantly higher fruit set (81.1%) than cultivars "67871", "269462", "374214", "170552" and "Kenya" while cultivar "374214" had a significantly lower fruit set than "170552". Fruit set ranged between 56.9% and 81.1% in summer.

4.1.9 Total Fruit Yield/Plant:

Cultivar differences in fruit yield were significant in winter but not in summer(p=0.05) .

In the winter, yields ranged between 0.338 kg/plant("DDR") and 0.917 Kg/plant ("Kenya"). The seasons' average was 0.626 kg/plant. Table 7 shows that "Kenya" and "170552" had significantly higher fruit yields (0.917 kg/plant and 0.816 kg/plant respectively) than cultivars "67871", "374214", and "DDR", while "DDR" and "67871"had significantly lower fruit yields (0.338 kg/plant and 0.374 kg/plant respectively) than cultivars "269462", "170552" and "Kenya". Cultivars "269462" and "374214" were not found to be significantlly different from each other.

During the summer season fruit yield ranged from 2.488 Kg/plant("DDR") to 2.912 Kg/plant ("67871").

4.1.10 Mean Fruit Weight/Plant:

Significant differences in mean fruit weight/plant were observed between the cultivars in winter but not in summer (p=0.05).

In winter , mean fruit weight ranged between 57.56 g ("DDR") and 153.28 g ("Kenya").

Table 7 shows that "Kenya" had a significantly higher mean fruit weight (153.28 g) than cultivars "170552", "DDR", "374214", "269462" and "67871" while "DDR" and "67871" were found to have significantly lower fruit weights(57.56 g and 83.36 g respectively) than "269462", "170552" and "Kenya".

Table 6: Differences in the Number of Female Flowers, Number of Male Flowers, Total Number of Fruits and Percent Fruit set Between the Various Cultivars of Luffa acutangula Grown in the Greenhouse in Summer, 1992

CULTIVAR	NO. OF MALE FLOWERS	NO. OF FEMALE FLOWERS	RATIO OF MALE TO FEMALE FLOWERS	TOTAL NUMBER OF FRUITS	% FRUIT SET
67871	322.58a	22.58a	15a	17.42a	70.0bc
269462	335.49a	24.17a	14a	15.08a	63.2bc
374214	109.42bc	25.67a	4c	14.09a	56.9c
DDR	213.75b	18.67a	11ab	14.92a	81.1a
170552	55.59c	19.75a	3c	15.42a	80.1b
KENYA	176.83b	24.75a	8bc	15.08a	61.0bc
C.V (%)	34.97	22.78	38.20	20.90	17.25

Values with same letters within columns imply non-significant at 5% level of probability using Duncan's Multiple Range Test

Table 7: Differences in Fruit Yeild, Mean Fruit Weight and Fresh Biomass Between Various Cultivars of *Luffa acutangula* Grown in the Greenhouse in 1991 and 1992

	WINTER 1991			SUMMER 1992		
CULTIVAR	FRUIT YIELD PER PLANT (kg)	MEAN FRUIT WEIGHT (g)	BIOMASS (g)	FRUIT YIELD PER PLANT (g)	MEAN FRUIT WEIGHT (g)	BIOMASS (g)
67871	0.374c	83.36cd	80c	2.912a	165.65a	880a
269462	0.783ab	119.55b	145b	2.837a	187.58a	859a
374214	0.527bc	102.30bc	180ab	2.511a	179.10a	866a
DDR	0.338c	57.56d	94c	2.488a	166.13a	771a
1705552	0.816a	127.22b	218a	2.670a	173.44a	1051a
KENYA	0.917a	153.28a	174ab	2.761a	185.99a	974a
C.V (%)	28.13	19.68	21.37	13.39	6.51	18.59

Values with same letters within columns imply non-signifcant at 5% level of probability using Duncan's Multiple Range Test.

In summer, the fruit weight varied between 165.65g("67871") and 187.58g("269462"). The seasons average was 176.31g.

4.1.11. Above Ground Biomass (g/plant):

Significant differences in above ground fresh biomass were observed between cultivars in winter but not in summer(p=0.05).

Table 7 shows that in winter "170552", "Kenya" and "374214" had significantly higher biomass than "67871" and "DDR". In both seasons cultivar "170552" had the highest biomass production (218 g in winter and 1051 g/plant in summer). "67871"was the lowest producer in winter (80 g) while "DDR" was the lowest producer in summer (771 g).

Average biomass production in winter was 148 g/plant while in summer it was 900 g/plant.

4.1.12. Fruit Quality Determinations.

4.1.12.1 Electrical Conductivity (EC):

In winter significant differences in EC were observed between the cultivars (p=0.05). Table 8 shows that in winter fruits of "Kenya" had a significantly higher EC (2.41 dS/m) than cultivars "170522" and "269462" which had ECs of 2.11 dS/m and 1.94 dS/m respectively. Fruits of cultivars "67871", "374214", and "DDR" were found not to be significantly different from each other in their ECs. Cultivar "269462" was found to have a significantly lower EC (1.94 dS/m) compared to the cultivars "67871", "374214" "DDR" and "Kenya". Fruit EC of cultivar "170552" did not differ significantly from that of cultivar "269462.

In winter, fruit EC ranged between 1.94 dS/m ("269462") and 2.68 dS/m ("DDR"). Table 9 shows that in summer the cultivars did not show significant differences from each other and that fruit EC ranged from 2.69dS/m to 3.09 dS/m.

4.1.12.2. pH:

Fruit pH did not show significant differences among the cultivars in winter, but in summer significant differences were observed (p=0.05). The range of pH values for winter was 6.35 to 6.52 while in summer it was 6.05 to 6.68.

In summer, fruits of "DDR" had a significantly higher pH(6.68) than cultivar "269462"(6.05) (Table 9). pH of cultivars "67871", "374214", "DDR", "170552" and "Kenya" did not differ significantly from each other.

Table 8: Differences in Fruit Quality Attributes Between the Various Cultivars of *Luffa acutangula* Grown in the Greenhouse in Winter, 1991

CULTIVAR	EC (ds/m)	pH	TITRATABLE ACIDITY (meq/gfw)	TSS(%)	REDUCING SUGARS (mg/gfw)
67871	2.44ab	6.52a	0.011a	3.35bc	35.25bc
269462	1.94c	6.42a	0.014a	4.38a	55.38a
374214	2.42ab	6.35a	0.014a	3.71b	27.17c
DDR	2.68ab	6.40a	0.012a	3.96ab	26.10c
170552	2.11bc	6.39a	0.010a	3.46bc	39.25b
KENYA	2.41a	6.39a	0.014a	3.27c	30.13bc
C.V. (%)	11.00	2.15	25.41	10.40	16.86

Values with same letters within columns imply non-significant at 5% level of probability using Duncan's Multiple Range Test.

Table 9: Differences in Fruit Quality Attributes Between the Various Cultivars of *Luffa acutangula* Grown in the Greenhouse in Winter, 1991

CULTIVAR	EC (ds/m)	pH	TITRATABLE ACIDITY (meq/gfw)	TSS(%)	REDUCING SUGARS (mg/gfw)
67871	2.81a	6.54a	0.019a	3.98a	38.52ab
269462	2.90a	6.05b	0.016a	4.30a	43.99a
374214	3.12a	6.43ab	0.017a	3.75a	32.08b
DDR	2.76a	6.68a	0.013a	3.90a	35.74ab
170552	2.69a	6.49ab	0.014a	4.03a	36.43ab
KENYA	3.09a	6.40ab	0.017a	4.03a	32.56b
C.V. (%)	9.34	4.25	77.46	16.10	16.20

Values with same letters within columns imply non-significant at 5% level of probability using Duncan's Multiple Range Test.

4.1.12.3. Titratable Acidity: (TA)

There were no significant differences between the cultivars for titratable acidity of fruits both in winter and summer. The values ranged between 0.010 and 0.014 meq/gfw in winter while in summer the range was 0.013 meq/gfw to 0.019 meq/gfw.

4.1.12.4. Total Soluble Solids(TSS):

Fruits grown in winter showed significant differences in TSS between the cultivars, but those grown in summer did not (p=0.05).

In winter, fruits of cultivars "269462" had a significantly higher TSS (4.38%) than those of cultivars "67871", "374214", "170522" and "Kenya" but they did not differ significantly from those of cultivar "DDR" (Table 8). The table also shows that cultivars "67871", "374214", "170552" and "DDR" were found not to be significantly different from each other. Cultivar "269462" had the highest TSS (4.38%) while "Kenya" had the lowest (3.27%). During the summer, cultivar "269462" still had the highest TSS (4.30%) while cultivar "374214" had the lowest (3.75%).

On average the TSS were higher in summer (4.00%) when compared to winter (3.69%).

4.1.12.5 Reducing Sugars:

Significant differences between cultivars in the reducing sugars of fruits were observed during both seasons (p=0.05). In winter, fruits of cultivar "269462" had a

significantly higher level of reducing sugars (55.38 mg/gfw) than all the other cultivars examined (Table 8). However, fruits of cultivars "67871", "374214", "DDR" and "Kenya" were not significantly different from each other. Cultivar "170552" had a significantly higher level of reducing sugars (39.25 mg/gfw) than cultivar "DDR" and "374214" which had reducing sugars of 26.10 and 27.17 mg/gfw respectively. Levels of reducing sugars in winter ranged between 55.38 and 26.10 mg/gfw.

Table 9 shows that during the summer, fruits of cultivar "269462" had a significantly higher level of reducing sugars (43.99 mg/gfw) than cultivar "374214"(32.08 mg/gfw) and "Kenya" (32.56 mg/gfw). However, fruits of cultivar "67871", "374214", "Kenya", "DDR", and "170552" were not significantly different from each other. During the season, the reducing sugars of the fruits ranged between 32.08 mg/gfw and 43.99mg/gfw.

4.2. Field Experiments

4.2.1. Effect of Plant Densitv on Davs to First Male and Female Flowers of the Various Cultivars.

Plant density did not influence the appearance of either the first male or female flower. The interaction between density and cultivar was also not significant for both days to first male and first female flower (p=0.05). In general, the first male flower opened within 28 to 34 days from transplanting while pistillate flowers appeared between 30 and 33 days after transplanting.

4.2.2 Effect of Plant Densitv on Total Yield of the Various Cultivars:

Total yield was influenced by plant density only. The interaction between density and cultivar was not significant (P=0.05).

Figure 1 shows that increasing plant density resulted in increased total yield. Doubling of plant density from 5,000 to 10,000 plants/ha increased the yields by almost 50 percent. This trend was observed in all the cultivars. Table 10 shows that doubling plant density from 5,000 to 10,000 plants/ha improved the yields(although not significantly) from 12.23 to 21.20 t/ha,while doubling the plant density from to 10,000 to 15,000 plants/ha resulted in a significant increase in yields from 21.20 to 30.27 t/ha.

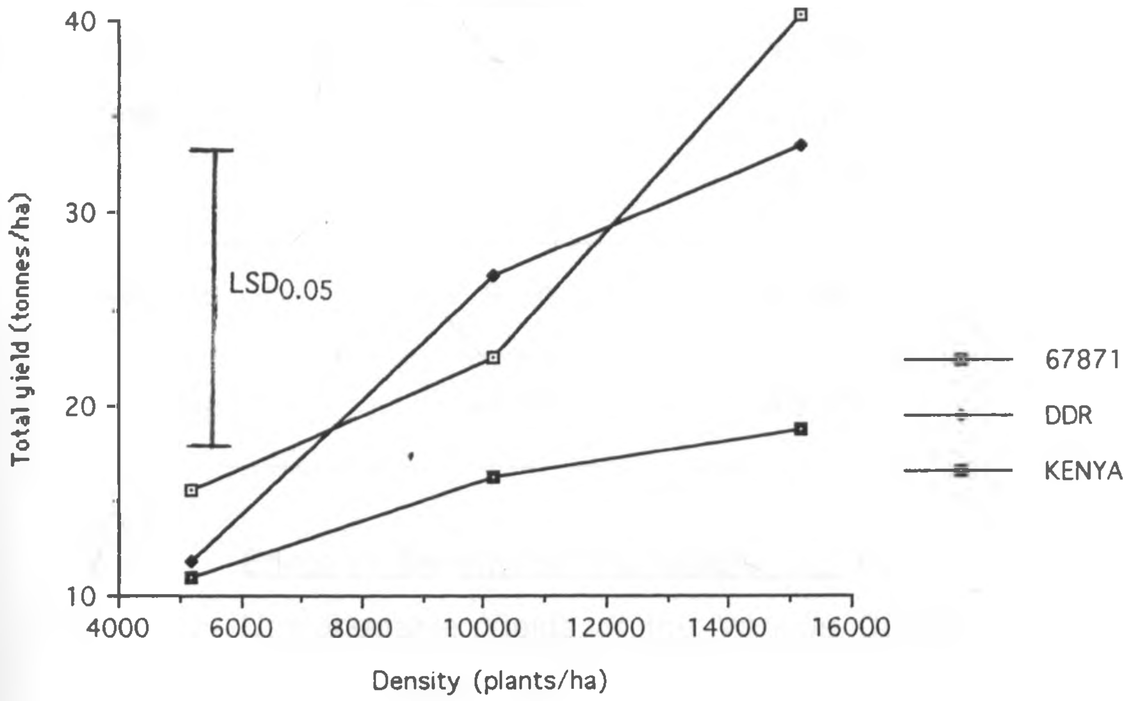

Fig. 1 Effect of Plant Density on Total Yield of *L.acutangula* Grown in Summer 1992

Table 10: Effect of Density on Total yield and Marketable Yield of *Luffa acutangula* grown in Summer 1992:

DENSITY	TOTAL YIELD (t/ha)	MARKETABLE YIELD (T/ha)
5,000	12.23	11.36
10,000	21.20	20.03
15,000	30.27	28.72
$LSD_{0.05}$	9.04	8.74
C.V. (%)	42.60	43.65

4.2.3 Effect of Density on Marketable and Percent Unmarketable Yields of the Various Cultivars:

The differences between cultivars and the interaction between cultivars and density for marketable yield were not significant. However, the effect of density was significant(P=0.05).

Table 10 shows that increasing plant density from 5000 to 10000 plants/ha or from 10000 to 15000 plants/ha did not cause a significant increase in marketable yields. However, tripling plant density from 5000 to 15000 plants/ha significantly increased yields by over 100%. Figure 2 shows that marketable yields increased almost linearly with density.

The effect of plant density and the differences between cultivars for percent unmarketable yield were not significant. In general, percent unmarketable yield ranged from 1.2 to 9.9%.

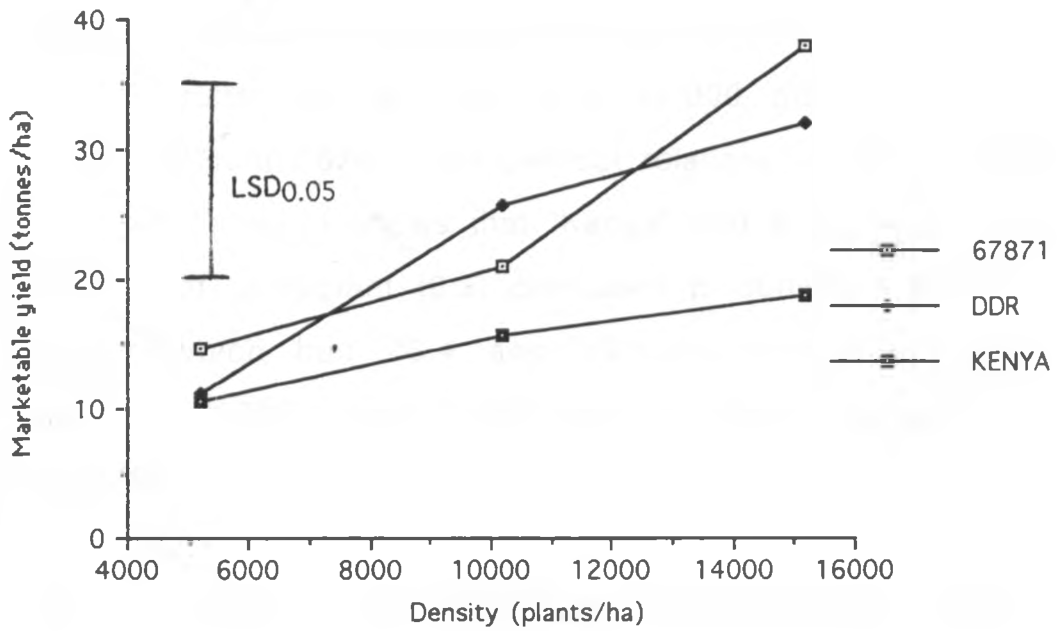

Fig. 2 Effect of Plant Density on Marketable Yield of *L.acutangula* Grown in Summer 1992

4.2.4. Effect of Density on Mean Number of Fruits/Plant of the various Cultivars

Significant differences between cultivars in mean number of fruits per plant were observed. The effect of density and the interaction between density and cultivar were not significant.

Fig 3 shows that for cultivar "Kenya" increasing plant density resulted in decreased number of fruits per plant from 6.3 to 4.5 fruits/plant at 5,000 and 15,000 plants/ha respectively. For "DDR" and "67871" no clearcut relationship was discernible.

Table 11 shows that "Kenya" had a significantly lower number of fruits/plant (6.3) compared to cultivars "67871" and "DDR" which had 16.4 and 15.1 fruits/plant respectively. Cultivars "67871" and "DDR" did not differ significantly from each other.

4.2.5. Effect of Plant Density on Mean Fruit Weight/Plant of the Various Cultivars.

The main effect of density and the interaction between density and cultivar were not significant. However, significant differences between cultivars were observed (p=0.05).

Figure 4 shows that for cultivars "67871"and "DDR", increasing density from 5,000 to 10,000 plants/ha resulted in an increase in fruit weight but after 10,000 plants/ha fruit weight tended to decrease for "DDR" but increased for "67871". In contrast, for "Kenya" increasing density from 5,000 to 10,000 plants/ha resulted in decreased fruit weight. However, the fruit weight tended to increase when density was increased from 10,000 to 15,000 plants/ha.

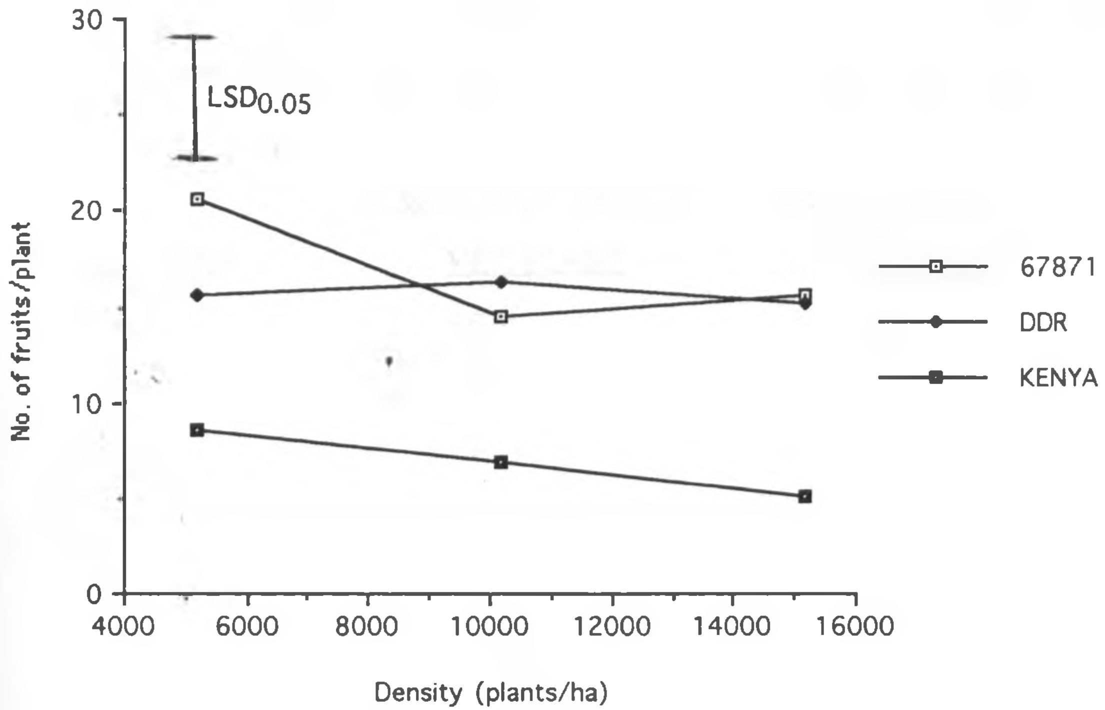

Fig. 3 Effect of Plant Density on Number of Fruits/Plant of *L.acutangula* Grown in Summer 1992

Table 11 shows that "Kenya" had a significantly higher mean fruit weight (267.7g) compared to cultivars "67871" and "DDR" which had fruit weights of 158.5g and 151.4g respectively.

Table 11: Effect of Cultivar on Number of Fruits/Plant and Mean Fruit Weight of *Luffa acutangula* Grown in Summer 1992:

CULTIVAR	NUMBER OF FRUITS PER PLANT	MEAN FRUIT WEIGHT (g)
67871	16.4	158.5
DDR	15.1	151.4
KENYA	6.3	267.7
LSD0.05	3.7	17.6
C.V. (%)	29.43	9.13

4.2.6 Effect of Cultivars and Density on Above Ground Dry Biomass:

Main effects of cultivar and density were significant but their interaction was not (p=0.05). Table 12 shows that increasing density resulted in increased dry biomass for cultivars "Kenya" and "67871". For "DDR" density had little effect on dry biomass. "Kenya" had the highest dry shoot biomass/plant at all densities, which was more than twice the lowest at any density.

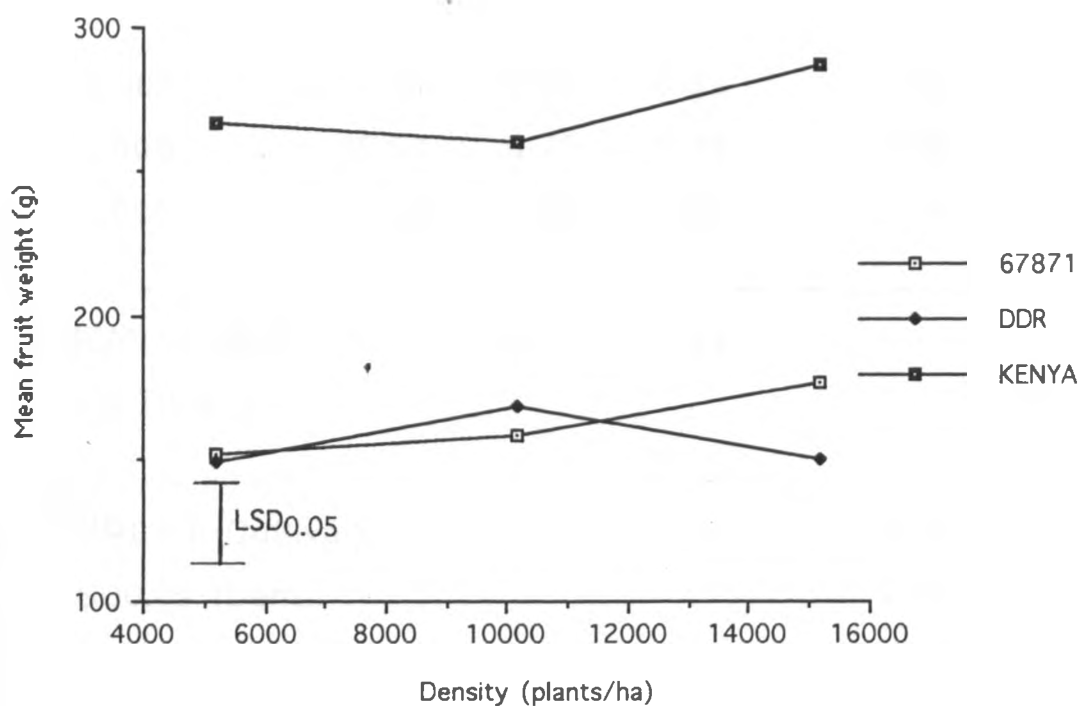

Fig. 4 Effect of Plant Density on Mean Fruit Weight of *L.acutangula* Grown in Summer 1992

Table 12: Effect of Cultivar and Density on Above Ground Dry Biomass of *Luffa acutangula* Grown in Summer, 1992.

	DRY SHOOT BIOMASS (g/plant)			
DENSITY	67871	DDR	KENYA	MEAN VALUES (DENSITY)
5,000	O.41	0.66	0.83	0.63
10,000	0.56	0.70	1.49	0.92
15,000	1.16	0.66	1.89	1.24
MEAN VALUES (CULTIVARS)	0.71	0.67	1.41	
$LSD_{0.05}$ (Cultivar)				0.56
LSD0.05 (Density)				0.56
C.V. (%)				34.7

4.2.7. Fruit Quality Determinations.

4.2.7.1. Percent Total Soluble Solids (TSS):

Percent TSS was significantly influenced by density and cultivar treatments. The interaction between cultivar and density was not significant (p=0.05). Fig 5 shows that increasing plant density resulted in decreased %TSS.

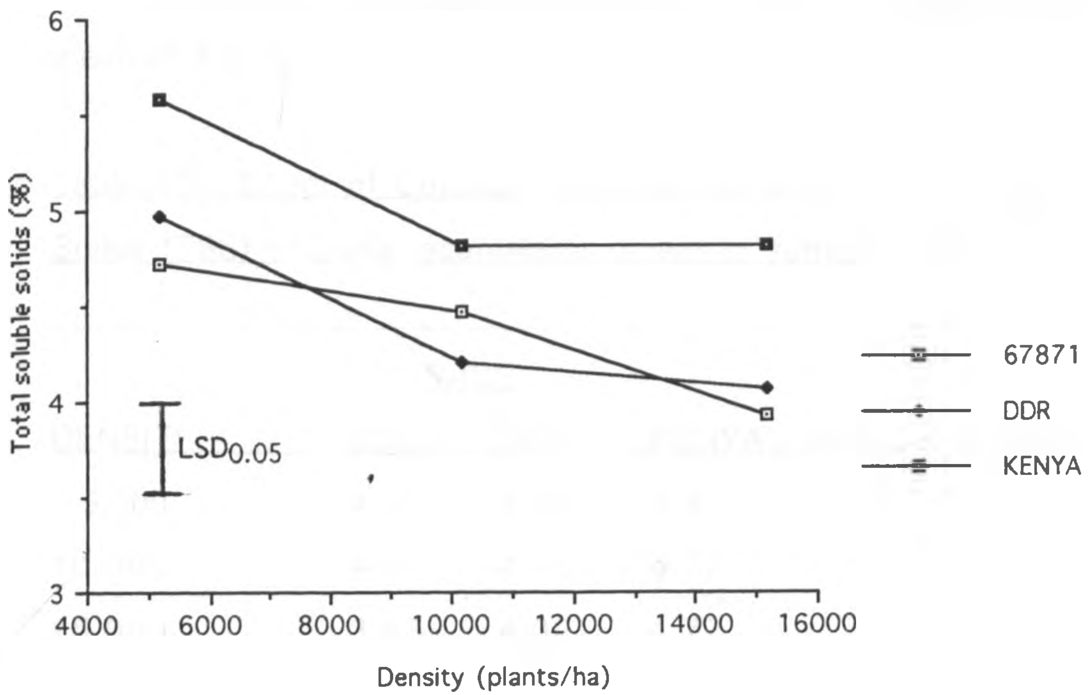

Fig. 5 Effect of Plant Density on Total Soluble Solids of *L.acutangula* Fruits Grown in Summer 1992

"Kenya" had the highest %TSS at all densities and was significantly different from the other cultivars at 5000 and 15000 plants/ha (Table13).

The range for all the cultivars was 3.88% to 5.53%, with a mean of 4.57%.

Table 13: Effect of Cultivar and Density on Percent Total Soluble Solids (TSS) of *Luffa acutangula* grown in summer 1992:

	%TSS			
DENSITY	67871	DDR	KENYA	MEAN VALUES(DENSITY)
5,000	4.67	4.92	5.53	5.04
10,000	4.42	4.15	4.77	4.44
15,000	3.88	4.02	4.77	4.22
MEAN VALUES (CULTIVAR)	4.32	4.36	5.02	
LSD0.05(Mean values)				0.48
LSDO.O5(Cultivar)				0.28
LSD0.05(Density)				0.28
C.V. (%)				19.24

4.2.7.2. Electrical Conductivity (EC)

Fruit EC was not influenced by plant density. The main effect of cultivar was significant but the interaction between density and cultivar was not significant (p=0.05).

Table 14 shows that "Kenya" had a significantly higher EC (3.34 dS/m) compared to cultivars "67871" and "DDR" which had values of 2.88 dS/m and 2.85 dS/m respectively. Cultivars "67871" and "DDR" did not differ significantly from each other.

4.2.7.3. pH :

Fruit pH was not significantly influenced by plant density, but the differences between cultivars were significant. Fig 6 shows that increasing plant density tended to depress fruit pH of cultivars “DDR” and “67871”.

Table 14 shows that "Kenya" had a significantly lower pH (6.4) compared to cultivars "67871" and "DDR" which had pH values of 6.7 and 6.6 respectively. Cultivars "67871" and "DDR" did not differ significantly from each other.

4.2.7.4. Acidity and Reducing Sugars:

Both acidity and reducing sugars were not influenced by the density and cultivar treatments.

Acidity ranged from 0.008 to 0.014 meq/gfw with a mean of 0.011 meq/gfw. Reducing sugars ranged from 36.78 to 47.57 mg/gfw with a mean of 43.09 mg/gfw.

Table 14: Effect of Cultivar on Electrical Conductivity (EC) and pH of *Luffa acutangula* Grown in Summer 1992:

CULTIVAR	EC (dS/m)	pH
67871	2.88	6.7
DDR	2.85	6.6
KENYA	3.34	6.4
$LSD_{0.05}$	0.25	0.1
C.V. (%)	25.68	3.25

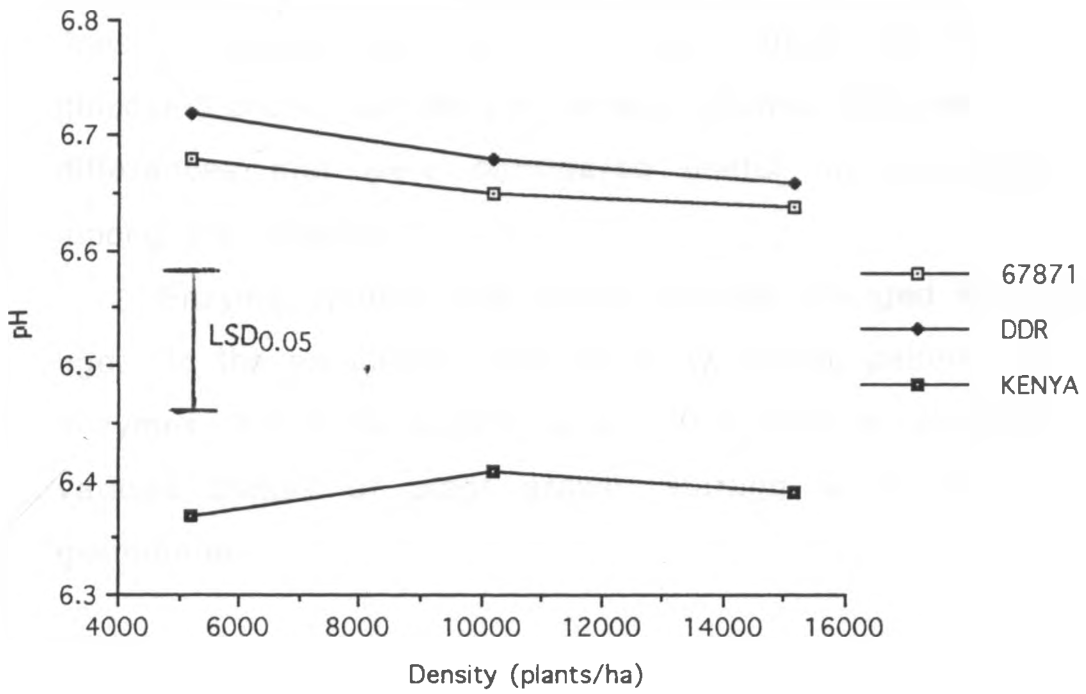

Fig. 6 Effect of Plant Density on pH of *L.acutangula* Fruits Grown in Summer 1992

4.3. Electrophoretic Analyses:

Isozyme patterns observed for various enzymes among the six cultivars of *L.acutangula* examined are illustrated in Fig 7 to 12. The patterns of phosphogluco isomerase, shikimic acid dehydrogenase, and malic acid dehydrogenase did not show any differences with respect to genetic variation among the lines. However, zymograms for peroxidase, esterase, and glucose-6-phosphate dehydrogenase showed somewhat clear differences and were considered useful for distinguishing among the cultivars.

Enzyme systems and protein profiles changed with plant age. In the paragraphs that follow zymogram patterns of the enzymes that were examined are described as observed at various stages of plant growth starting at 8 days after germination.

4.3.1. Esterase:

Esterase stained two major anodal bands which were present in all the cultivars at 8 weeks after transplanting (Fig. 7). Their Rf values were 0.76 for Est1 and 0.86 for Est2. Both bands had a thickness of 0.5 cm and appeared 30 minutes after staining. The colour of the bands was reddish brown.

Both bands exhibited varying intensities depending on the cultivar. Cultivar "374214" had a dark Est2 and a light Est1. For cultivar "269462" both Est1 and Est2 were dark. The cultivar "67871" had a dark Est1 and a light Est2 while cultivar "170552" exhibited a light Est1 and a very dark Est2. Both the cultivars "DDR" and "Kenya" showed very dark Est1 and Est2.

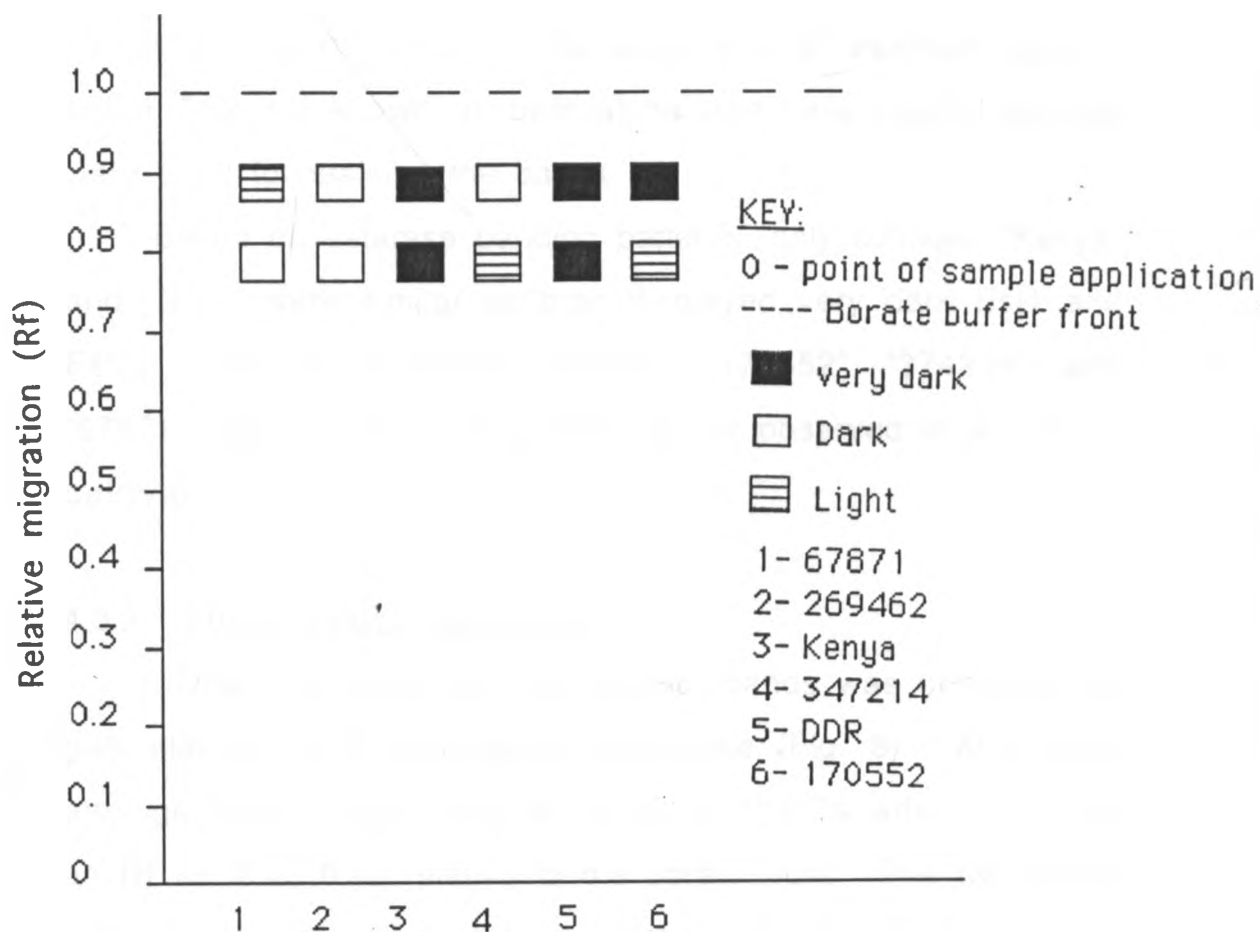

Fig.7 Schematic Diagram of Esterase Isozyme Bands of Leaf Samples of *L.acutangula* Grown in the Greenhouse at 8 Weeks after Transplanting

At earlier stages of plant growth no differences were observed in the esterase phenotypes both in terms of the Rf values and band intensity. The exact kind of esterase seen in figure 7 is not known as both alpha and beta napthyl acetate were used for staining the bands.

Based on esterase banding patterns, only cultivars "Kenya" and "DDR" were similar as both displayed very dark Est1 and Est2. However, cultivars "269462", "170552", "374214", and "67871" had unique banding patterns not observed in any other cultivar.

4.3.2. Phosphogluco isomerase:

One phenotype with two distinct bands was observed for gels stained for Phosphogluco isomerase (Fig. 8). At 8 days after germination PGI1 had an Rf value of 0.24 while PGI2 had an Rf value of 0.46 relative to the borate front. The two bands differed both in thickness and intensity. PGI1 had a thickness of 1.0 cm and was very darkly stained while PGI2 had a thickness of 0.7 cm and was lightly stained.

The PGI phenotype was stable and did not change much with the age of the plant. Usually the bands stained purple and appeared within 30 minutes of staining.

4.3.3. Malate Dehydrogenase:

Figure 9 shows that one phenotype with two distinct bands was observed on gels stained for malate dehydrogenase at 11 weeks after transplanting. MDHI had an Rf value of 0.3 while MDH2 had an Rf of 0.67 relative to the bromophenol blue dye marker.

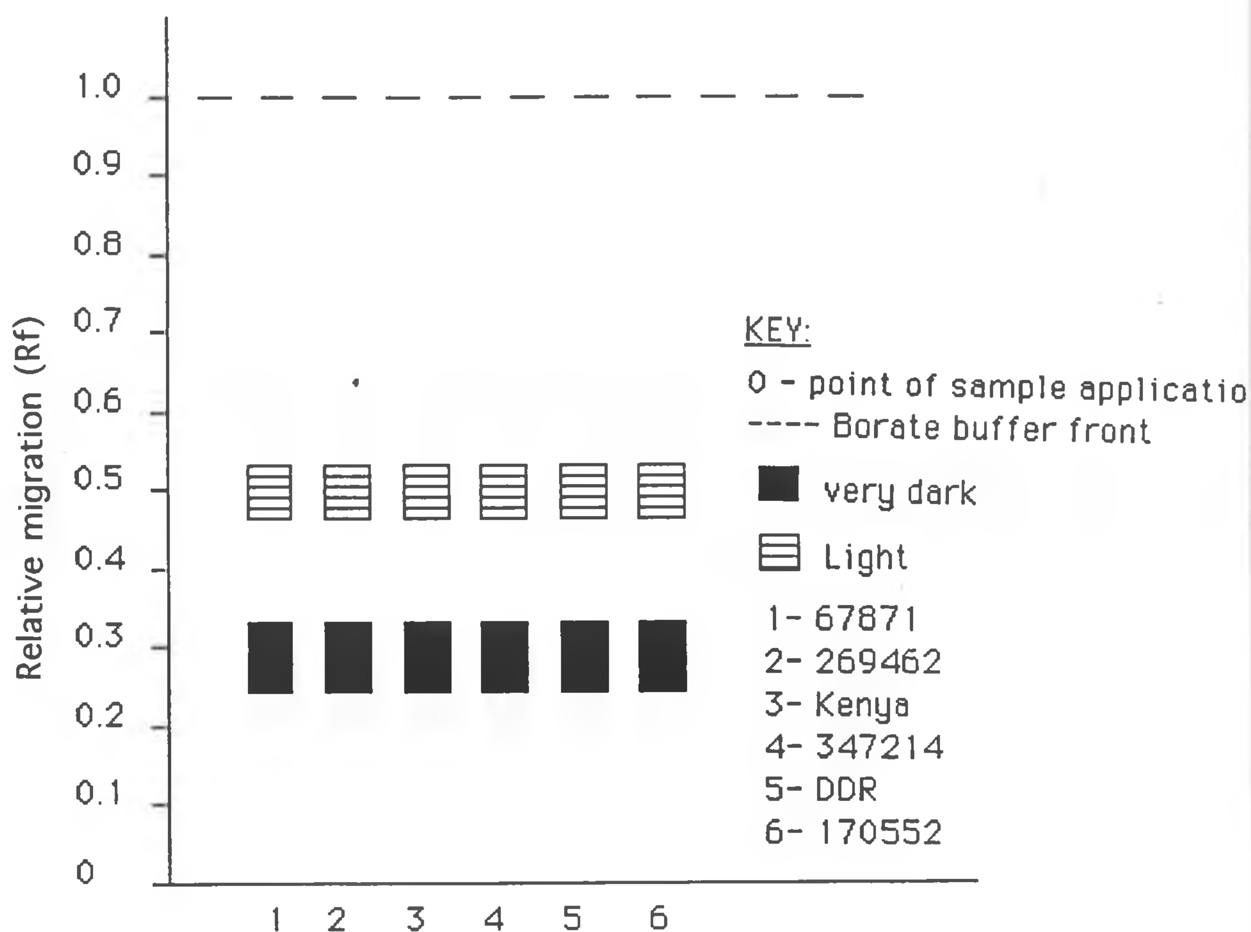

Fig.8. Schematic Diagram of Phosphogluco Isomerase Isozyme Bands of Leaf Samples of *L.acutangula* Grown in the Greenhouse at 8 Days after Transplanting

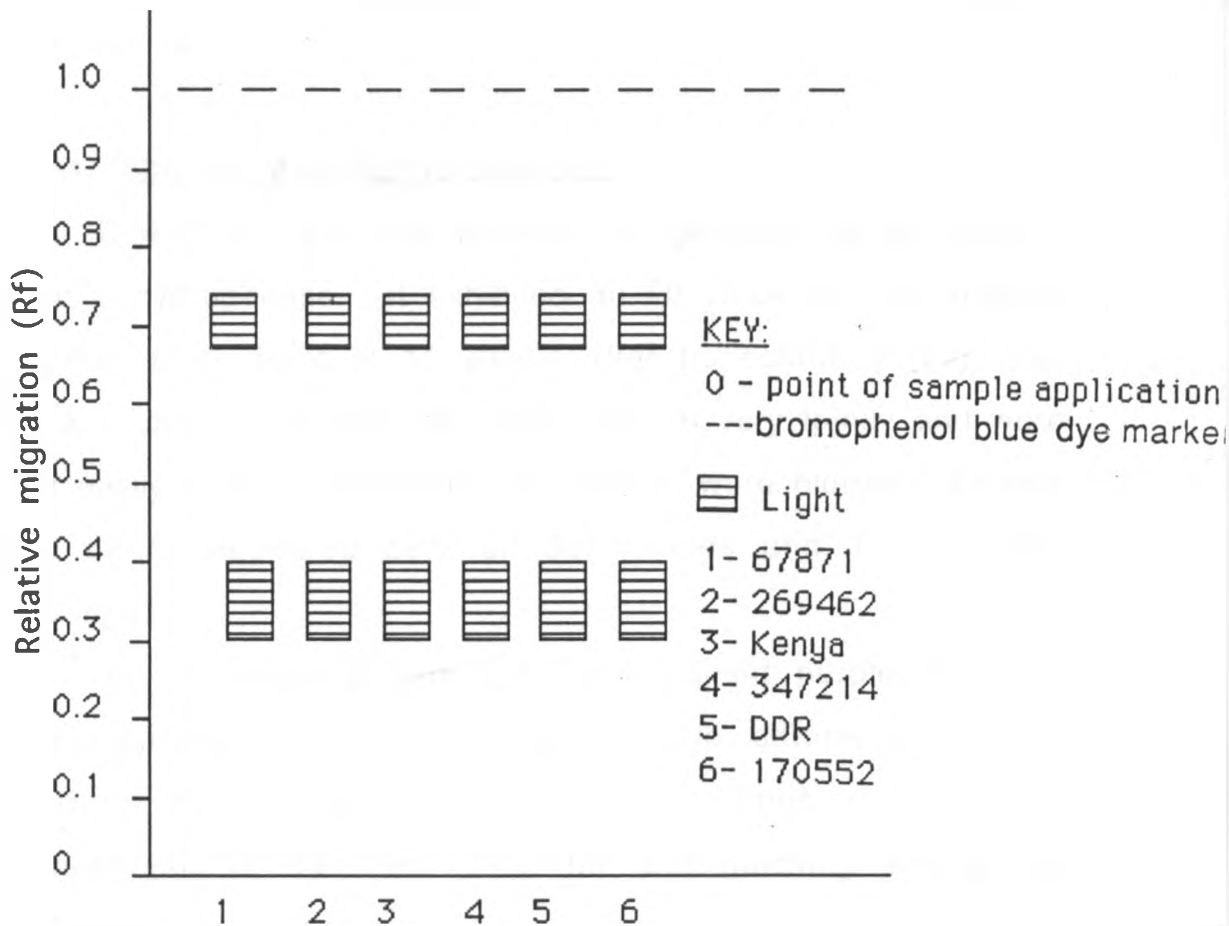

Fig.9. Schematic Diagram of Malate Dehydrogenase Isozyme Bands of Leaf Samples of *L.acutangula* Grown in the Greenhouse at 11 Weeks after Transplanting

MDH1 exhibited a band thickness of 1.0 cm while MDH2 had a band thickness of 0.8 cm. The MDH bands stained purple and were stable through out the plant growth and usually took at least 1 hour after staining for them to appear.

Due to lack of variation in the banding patterns of the six cultivars MDH was not considered useful for identifying among the cultivars.

4.3.4. Shikimic Acid Dehydrogenase:

One major band was observed on gels stained for shikimic acid dehydrogenase. It exhibited an Rf value of 0.88 relative to the borate front at 11 weeks after transplanting (Fig. 10). At a younger plant age the bands stained very faintly and were not easily visible. Sometimes the bands were blurred. Usually the bands appeared between 30 minutes and 1 hour after staining.

At 11 weeks of age the band stained purple and was monomorphic in all the cultivars. No differences in the intensity of the bands was observed. Thus shikimic acid dehydrogenase was not useful for distinguishing among the cultivars.

4.3.5. Peroxidase:

Peroxidase stained one anodal band which was present in all the cultivars (Fig.11). The band had the same intensity in all the cultivars. Some bands were observed but are not shown in figure 11 as they were unclear and therefore considered not useful in terms of distinguishing the cultivars.

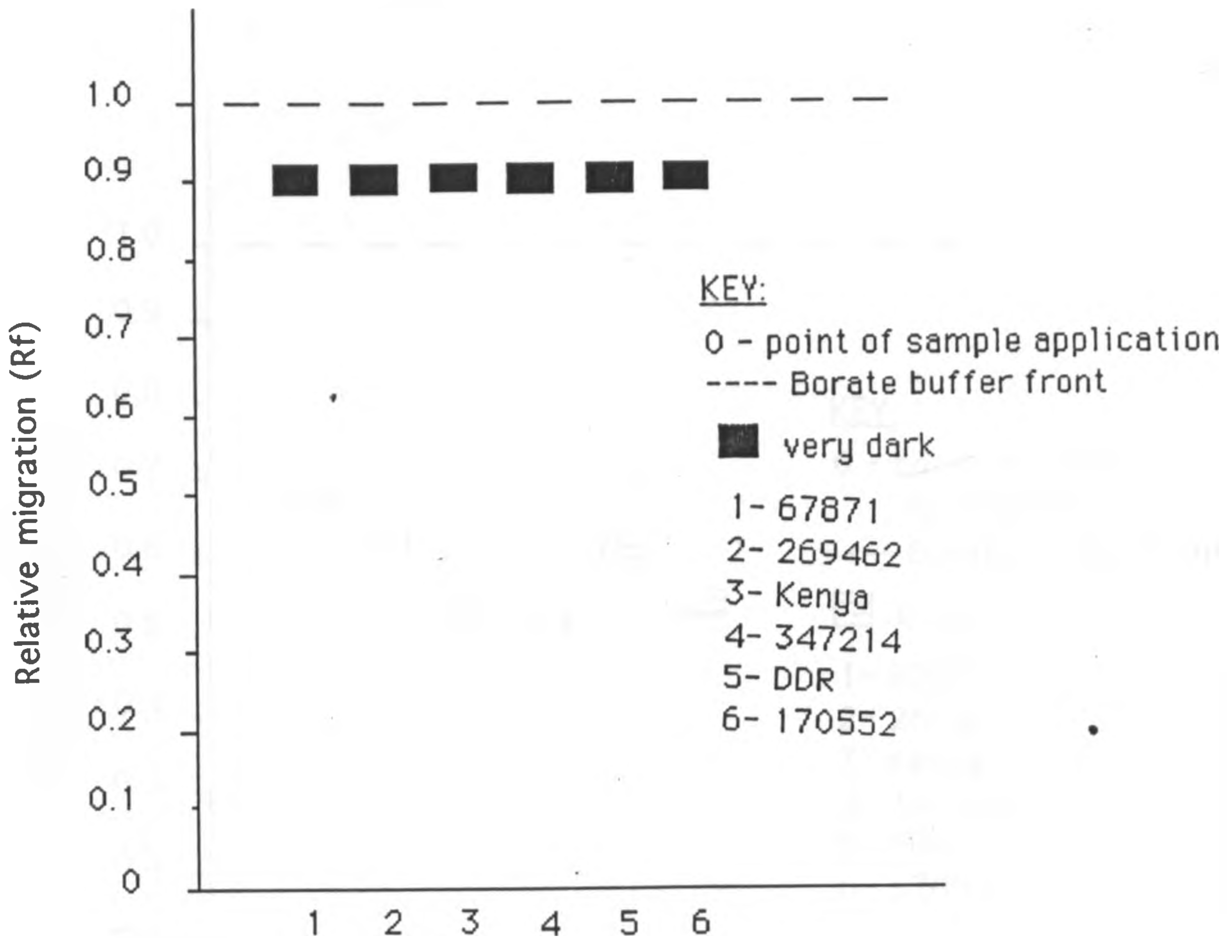

Fig.10. Schematic Diagram of Shikimic Acid Dehydrogenase Isozyme Bands of Leaf Samples of *L.acutangula* Grown in the Greenhouse at 11 Weeks after Transplanting

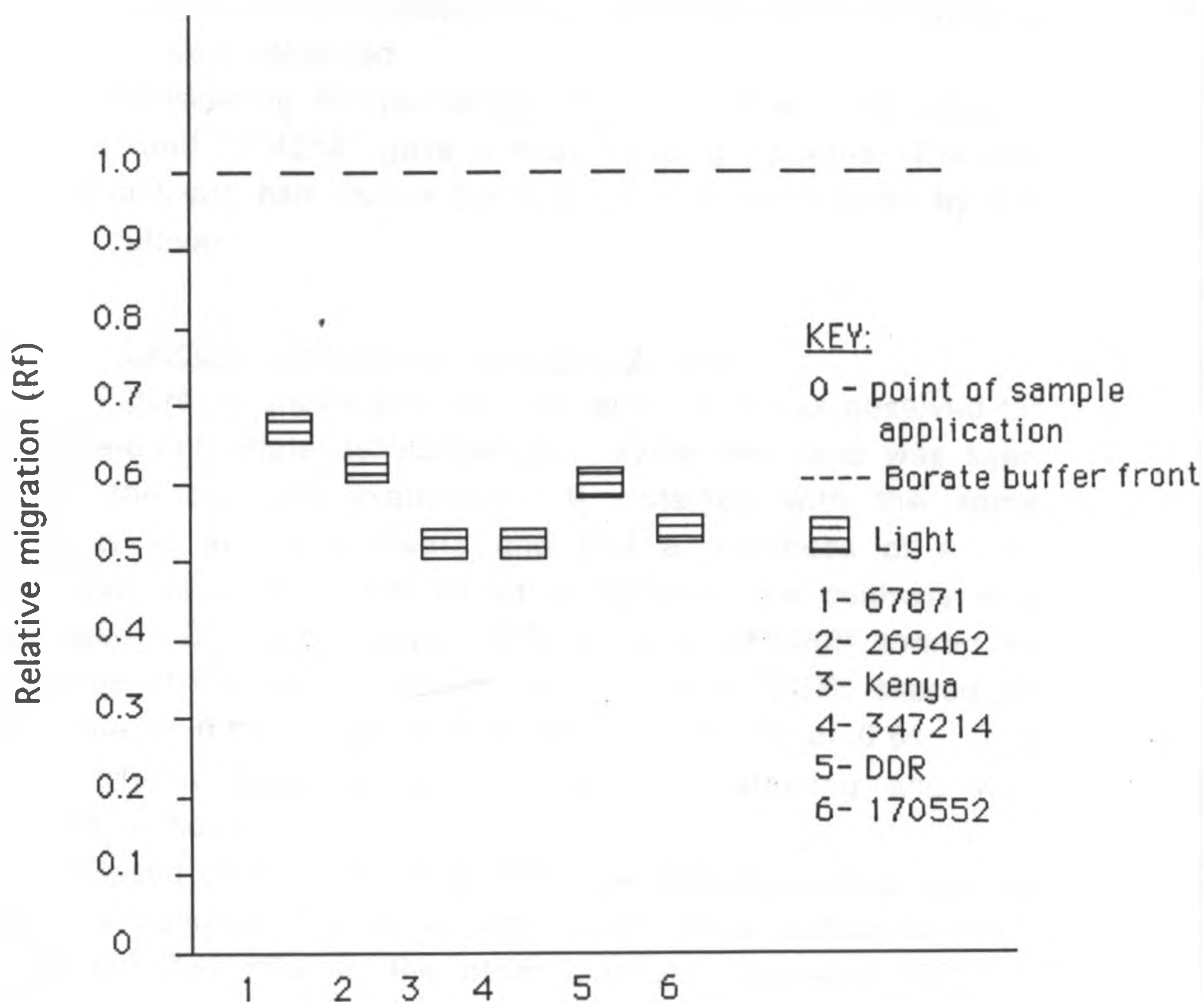

Fig.11. Schematic Diagram of Peroxidase Isozyme Bands of Leaf Samples of *L.acutangula* Grown in the Greenhouse at 11 Weeks after Transplanting

The major band had a thickness of 4 cm in all the cultivars. But the Rf varied depending on the cultivar. Cultivar "67871" had an Rf of 0.65 relative to the borate front while cultivar "269462" had an Rf of 0.60. Cultivars "DDR" and "170552" had Rfs of 0.58 and 0.52 respectively. In contrast cultivars "Kenya" and "374214" had an Rf of 0.5.

Usually the bands appeared within 1-2 minutes after staining. They were blue in colour and were easily observable upto 10 weeks after transplanting. Afterwards no peroxidase isozymes were detected.

Considering the peroxidase isozyme alone ,only cultivars "Kenya" and "374214" gave similar banding patterns. The rest of the cultivars had unique banding patterns not shared by any other cultivar.

4.3.6. Glucose-6-Phosphate Dehydrogenase:

Figure 12 shows that only one phenotype was observed for glucose-6-phosphate dehydrogenase. Only one band was seen in all the cultivars examined. It stained with the same intensity in all the cultivars and had a thickness of 4 cm. However, variation in the Rf value between the cultivars was observed. Cultivars "Kenya", "67871", and "269462" shared an Rf value of 0.6, while cultivars "374214" and "DDR" shared an Rf value of 0.55. Cultivar "170552" had an Rf of 0.61. The band usually appeared within 1 hour of staining and was reddish in colour.

Based on the glucose-6-phosphate dehydrogenase banding patterns cultivars "374214" and "DDR" were similar to each other but dissimilar to the other cultivars. Cultivars "67871", "269462" and "Kenya" were also similar to each other but dissimilar to the other cultivars. Cultivar "170552" differed from all the other cultivars.

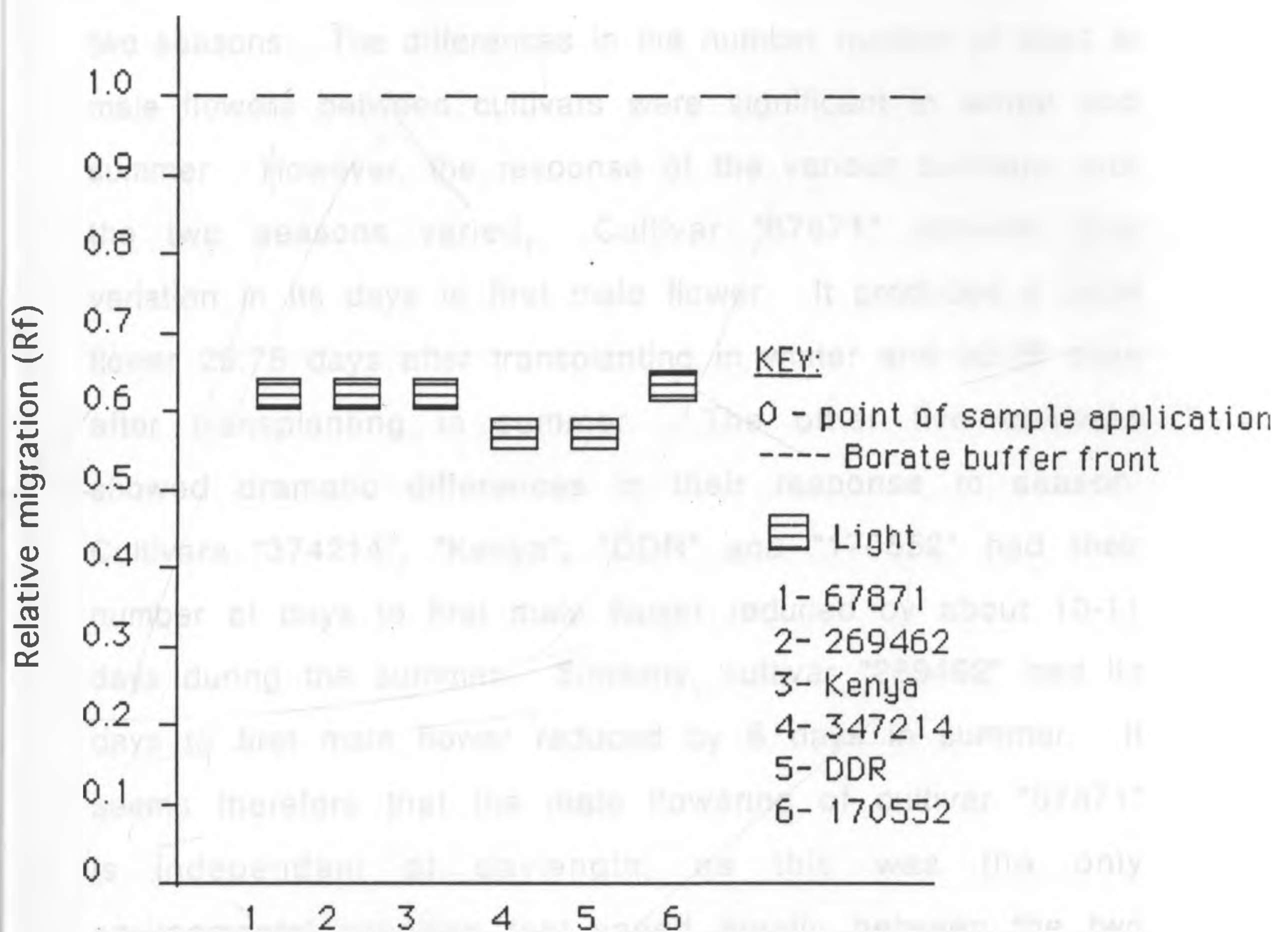

Fig.12. Schematic Diagram of Glucose-6-Phosphate Dehydrogenase Isozyme Bands of Leaf Samples of *L.acutangula* Grown in the Greenhouse at 8 Weeks after Transplanting

CHAPTER 5

DISCUSSION

5.1 Flowering and Fruiting:

The results obtained in the present study demonstrated that there was considerable variation in the flowering behaviour of the cultivars between themselves and over the two seasons. The differences in the number number of days to male flowers between cultivars were significant in winter and summer. However, the response of the various cultivars over the two seasons varied. Cultivar "67871" showed little variation in its days to first male flower. It produced a male flower 29.75 days after transplanting in winter and 29.25 days after transplanting in summer. The other five cultivars showed dramatic differences in their response to season. Cultivars "374214", "Kenya", "DDR" and "170552" had their number of days to first male flower reduced by about 10-11 days during the summer. Similarly, cultivar "269462" had its days to first male flower reduced by 8 days in summer. It seems therefore that the male flowering of cultivar "67871" is independent of daylength, as this was the only environmental condition that varied greatly between the two seasons. Temperature did not vary much (Appendix 3). The cultivar may therefore be termed as day neutral in its male flowering behaviour. The other five cultivars had their days to first male flower reduced in summer although flowering still occured during the winter. These cultivars may therefore be termed as being facultative long day plants. i.e. they flower under short day conditions, but the flowering is markedly enhanced under long day conditions. This result is not

surprising as Salisbury and Ross (1985) reported that different varieties of the same species may behave differently in their response to photoperiod, as this response is controlled by only a single gene.

All the six cultivars had their days to first female flower drastically reduced in summer compared to winter. For instance, "Kenya" produced its first female flower at 43 days after transplanting in winter while it only took 22.5 days during the summer, meaning that the number of days to production was reduced by almost 100%. Cultivars "170552" and "DDR" had their days to first female flower reduced by 10 days while "374214" had its days reduced by 12.5 days. The days of "269462" to first female flower were reduced by 15.25 days. Cultivar "67871" did not show much variation with season as its days to first female flower was reduced by only 6 days during the summer. This suggests that "67871" was relatively day-neutral in its pistillate flower production while the other cultivars seem to be facultative long day plants. In general the response of female flower production to season was less dramatic than that of male flower production.

These results are similar to what is already known. They show that the plants first produced male flowers before pistillate flowers. Benzioni *et al.* (1992) working in Israel also found that long days and high temperatures promoted flowering in *L.acutangula.* Similarly, Kaushik and Sharma (1974) and Rudich (1985) found that long days and high temperature promote the development of both male and female flowers in monoecious cucumber cultivars (*Cucumis sativus L.*) and andromonoecious muskmelons (*Cucumis melo*).

The number of male flowers/plant increased dramatically from winter to summer for all the cultivars except "170552" which showed a moderate increase. Its number of male flowers/plant increased from 35 in winter to only 55.59 in summer. The other cultivars showed a more positive response with change of season from winter to summer. For instance cultivar "67871" had its male flowers/plant increased from 29 in winter to 322 in summer. This positive response is probably attributable only to better vegetative growth in summer as evidenced by the high shoot biomass in summer compared to winter . For the other five cultivars the positive response is probably attributable to shorter days to first male flower coupled with increased vegetative growth during the summer. The higher vegetative growth may have resulted in increased male flower buds which in turn ensured that more male flowers opened. The earlier days to first male flower ensured that the production span was longer in summer thus leading to increased number of male flowers in summer compared to winter.

Similar observations were made by Kaushik and Sharma (1974) and Rudich (1985). They reported that long days and high temperature promote the development of male flowers in monoecious cucumber cultivars(*Cucumis sativus*) and andromonoecious muskmelons(*Cucumis melo*). Prakash (1974), also reported a similar phenomena in the male flowering of *Momordica charantia*.

Although the number of female flowers/plant followed a similar trend as that of number of male flowers/plant the response was not quite as dramatic. For cultivars "67871",

"374214" and "DDR" the number of female flowers/plant was almost tripled with change of season from winter to summer, while for "Kenya" and "170552" it was almost doubled. For "170552" the number of female flowers/plant was only increased by 50% from winter to summer. This flowering behaviour can be explained by shorter days to first female flower (except for line "67871") coupled with increased vegetative growth in summer. Early female flower production in summer may have led to more female flowers per unit time during the summer compared to winter. Better vegetative growth in summer meant that more female flower buds developed leading to increased number of female flowers/plant. For "67871" the increase in female flowers/plant in summer is attributable to only the increase in vegetative growth in summer as the cultivar showed only little difference in the time taken to produce its first female flower over the two seasons. The behaviour of cultivar "170552" cannot be clearly explained since the increase in vegetative growth in summer did not seem to affect its number of female flowers/plant. It is probable, therefore, that the cultivar had its female flowering potential exploited to the full in both seasons. Alternatively, high numbers of female flower buds cauld have been developed in summer but fail to open. This could then result in a lower number of female flowers/plant.

The finding that more female flowers are produced in summer compared to winter is supported by Kaushik and Sharma (1974), Ghosh and Basu (1983), Rudich (1985) and Benzioni *et al.*(1992).

The ratio of male to female flowers generally increased from winter to summer for all the cultivar except "170552". This is probably because the increase in the number of male flowers from winter to summer was far greater than the increase in the number of female flowers from winter to summer. This was particularly true for cultivar "DDR" which had its ratio increased from 1:1 to 11:1 male flowers : female flowers. Cultivars "67871", "269462", "374214" and "Kenya" had their ratios increased by 5 (500%). However, for "170552" the ratio was unchanged and this is directly attributable to the male and female flower flowering behaviour discussed above.

The finding that long days increase the flower ratio in *L.acutangula* is supported by Saito and Ito (1961) and is also known in several other cucurbits (Free 1970, Nitstch *et al.* 1952). A similar behaviour in flower ratio has also been observed in *L.cylindrica* and *Cucumis melo* (Dubey 1983, Mann and Robinson 1950, Martin 1979). In *L.acutangula* several flower ratios have been reported (Ghosh and Basu 1984, Rao and Raj 1975 and Kaushik and Bisaria 1973) because the flowering behaviour is very sensitive to even little changes in the environment surrounding the plant. This includes hormones, temperature and mineral nutrition of the plant. Results of the present are similar to what Benzioni *et al.* (1992) found.

Percent fruit set increased from winter to summer for all the cultivars except "374214" which had its fruit set slightly decreased from 62.2% in winter to 56.9% in summer. In general, fruit sets were lower in winter compared to summer. This is probably due to insufficient pollination during winter when

there were fewer number of male flowers/plant observed compared to summer. Occassional break downs of the heating system in the greenhouse in winter could also have contributed to low temperatures which may have adversely affected the fruit sets. Fruit set of " 170552" was almost doubled from 47.7% to 80.1% with change of season from winter to summer. This was a direct result of improved pollination due to higher numbers of male flowers/plant in summer. Over the two seasons "Kenya" and "374214" showed low fruit sets compared to the rest of the cultivars . The two cultivars were also similar in their fruit shape characteristics. (Probably the pistillate flowers opened much earlier than staminate flowers, so that by the time pollination was done the stigma was no longer receptive). Yet they were prolific male flower producers, which suggests that the pollen for pollination was not the limiting factor. Another probable reason is that these 2 lines posses pollen which is infertile. The results obtained might also be due to the effects of temperature and daylength on photosynthesis and assimilate distribution or due to low stigma receptivity under unfavourable temperature (Katijav 1971). Variation in these factors has been reported to cause a wide range in fruit set (between 5% and 66% in *Cucumis melo* and *Cirullus lanatus* (Maestro and Alvarez, 1988, Mann and Robinson, 1950). As in muskmelons the brief duration of pollen viability, might be a factor in influencing the fruit set of *L.acutangula* , particularly in cultivar "374214" (McCollum *et al.* 1987, Pratt *et al* 1977).

In general fruit production was lower in winter compared to summer due to lower % fruit set in winter. In summer there was a marked improvement in fruit set which

resulted in a favourable increase in fruit number/plant. Little variation was observed in total number of fruits/plant between cultivars over the two seasons. This indicates that % fruit set did not also vary much between cultivars over the two seasons. This is in agreement with Tindall (1987) who reported that *Luffa acutangula* plants produce an average of 15-20 fruits/plant under favourable conditions.

Mean fruit weight improved with change of season from winter to summer for all the cultivars. Cultivars "67871" and "DDR" showed particularly remarkable improvements. This coupled with increased fruit number/plant resulted in improved total yields. The improved mean fruit weight is probably attributable to better vegetative growth in summer as evidenced by high shoot biomass which implies that there was increased photosynthetic area which was capable of supporting heavier fruits due to increased photosynthsates. Similar observations have been reported in *Cucumis Sativus* (Pharr *et al*, 1985 and Schapendok *et al* 1984).

Fruit yield increased dramatically from winter to summer. This is attributable to increased fruit set in summer which caused an increase in fruit number/plant which eventually led to improved yields. "DDR" and "67871" had particularly high fruit yields in summer compared to winter. This is attributable to higher vegetative growth in summer, which was able to support higher fruit number/plant. This hypothesis is supported by Pharr *et al.* (1985) and Schapendok *et al*. (1984) who suggested that the fruiting potential of a pickling cucumber plant may be limited by the plants net photosynthetic capacity. Similar observations were also made

by Widers and Price (1989) who reported a high correlation(r=0.877) between leaf lamina dry weight and fruit growth rate of pickling cucumbers (*Cucumis sativus*). Knavel (1991) also reported that varieties of musk melons which had higher dry biomass tended to yield more than those with lower biomass.

5.2 Fruit Quality.

Total soluble solids ranged from 3.27% to 4.38% in winter while in summer they ranged from 3.75% to 4.30%. Thus on average TSS were slightly higher in summer compared to winter. During winter, cultivar "269462" which had the highest TSS (4.38%) also had the highest level of reducing sugars (55.38 mg/gfw) while cultivars "Kenya", "170552", "DDR" and "374214" which had low TSSs also had low levels of reducing sugars. But the correlation between the two parameters was not significant at the 5% level.

In summer cultivar "269462" which had the highest TSS also had the highest level of reducing sugars (43.99mg/gfw) while cultivar "374214" which had the lowest TSS (3.75%) also had the lowest level of reducing sugars (32.08 mg/gfw). But again the correlation between the two parameters was not significant at the 5% level. This data then suggest that the greater proportion of the TSS was not reducing sugars. The data also indicate that refractive index measurements cannot be used as indicators of levels of reducing sugars and one may need to resort to other measurements. These results differ from those of Benzioni *et al.*(1992) who found total soluble

solids of *L.acutangula* to be significantly correlated with reducing sugars.

Fruit pH remained relatively unchanged over the two seasons except for cultivars "170552", "DDR" and "374214" in which it increased slightly from winter to summer. However, cultivar "269462" had its pH decreasing slightly from winter to summer. In general the pH of the fruits was on the slightly acidic range bordering on neutral. (6.05-6.68). Titratable acidity was quite low and showed a slight increase from winter to summer.

Although pH and titratable acidity are measures of acid content, and thus would be expected to be highly correlated, the results of the present study indicate otherwise. Infact both pH and titratable acidity seem to be either high or low at the same time. This finding agrees with what Anderson (1957) reported but differs from the findings of Stevens (1972) who reported that pH and titratable acidity were negatively correlated in the tomato cultivars he studied.

Both pH and potential acidity contribute to tartness (Harvey 1920). Total acidity and pH should be closely related, but at times the relationship is not good. Anderson (1957) found that pH and acidity are not always inversely related and that in some tomato varieties both values are relatively high. Lower and Thompson (1967) also found poor correlation between pH and acidity in the tomato lines they studied.

EC of the fruits increased from winter to summer. Cultivar "269462" had its EC increased from 1.94 dS/m to 2.90 dS/m. In contrast that of "DDR" only increased from 2.68 dS/m to 2.76 dS/m. For cultivars "170552", "347214" and "67871"

EC was only increased by 0.5 dS/m. These results show that the electrical conductivity of *Luffa acutongula* fruits is cultivar dependant.

5.3 Planting Density

Plant density did not influence either days to first male flower or first female flower. This result is not surprising because by the time female and male flower buds opened, the plants were still young and not much vegetative growth had taken place. Thus stress to the plants due to close spacing did not occur at the onset of flowering

Increasing plant population resulted in increased total and marketable yields. The percent unmarketable fruit was not affected by the treatments. The positive effect of density on yield is attributable directly to the increase in fruit weight. Although the fruit number decreased with density, this had a lesser effect on yield than the increase in fruit weight that was observed.

Contrary to expectations, the percent unmarketable fruit did not differ significantly with plant density even at the wider spacing in which sunscorched fruits would have been greater. This can be partly explained by the conditions under which the experiment was performed. All plants were grown as trained which probably reduced direct radiation which can lead to sunscorched fruits.

Cultivar "Kenya" was more vegetative than the other cultivars as is supported by the higher dry shoot biomass but it was also a low yielder. It therefore seems that this cultivar was a less efficient fruit yielder than the other

cultivars. It invested a lot of energy in luxurious vegetative growth at the expense of fruit yield. It could only support few but heavier fruits. Similar observations were made by Benzioni *et al* (1992). In addition too much vegetative growth could result to inaccessibility of bees to pistillate flowers leading to low fruit sets. Due to the high vegetative mass self shading is likely to arise. The foliar competition for light energy at high density in general and cultivar "Kenya" in particular may also be growth limiting during fruiting as has been observed in squashes and cucumbers (Buwalda *et al*, 1986 and Ramirez, *et al* 1988). However, in pickling cucumbers foliar competition or microenvironmental modification does not seem to alter net photosynthetic efficiency of the leaves (Widders and Price, 1989). The lower fruit to shoot ratio at high density indicates that productive efficiency is adversely affected by neighbouring plants. The present study did not attempt to elucidate the effect of neighbouring plants on the growth and productivity of individual plants thus this effect cannot be discussed, although the nature of this interaction is known to be either competitive or allelopathic (Salisbury and Ross, 1985). Cultivars "DDR" and "67871" had a lower vegetative mass thus exposing most of their leaves to greater photosynthetic activity and subsequently greater potential for supporting female flower production. They also had their pistillate flowers readily accessible to bees. These results are in agreement with those obtained by other workers(Huyskens,1991, Benzioni, *et al* 1988) which indicate that high plant population(20,000 plants/ha) gave yields of upto 35 t/ha.

The present study had marketable yields of upto 28.72 t/ha at 15000 plants/ha.

Density had no effect on fruit quality attributes like pH, titratable acidity, reducing sugars and electrical conductivity. Total soluble solids tended to decrease as density increased . Of the three cultivars examined "Kenya" always had the highest total soluble solids at all densities. Thus the differences observed were mainly due to the different characteristics of the cultivars. The finding that pH and titratable acidity of the fruits were not affected by density is similar to that of Benzioni *et al.* (1992). The increase of % total soluble solids with density also fits to what is already known. It is expected that at high plant density competition sets in, plants shade each other, resulting in decreased photosynthetic activity which leads to less sugars being deposited in the fruits. This results in a lower total soluble solids value in the fruits.

5.4. <u>Electrophoretic Analyses.</u>

The results of this study demonstrate that *L.acutangula* cultivars may be reliably distinguished by means of their isozyme phenotypes when a combination of at least two isozyme systems was employed. However, none of the isozyme systems studied could singly be used to distinguish the cultivars. As already indicated zymograms for Shikimic acid dehydrogenase, Phosphogluco isomerase and malate dehydrogenase did not show any variation among the cultivars, and were therefore considered not useful for distinguishing among the cultivars. Also the peroxidase phenotype was only useful upto 10 weeks after transplanting. Beyond this no

peroxidase activity was observed. This result was not surprising as it has been documented that peroxidase activity shows temporal fluctuations during plant development, although such fluctuations are not genetically defined (O'Sullivan et al.,1972, Sigel *et al.*,1967).

A combination of esterase and peroxidase phenotypes was sufficient to place the six cultivars into six separate groups. Similarly, a combination of esterase and glucose-6-phosphate dehydrogenase or peroxidase and glucose-6-phosphate dehydrogenase was sufficient to distinguish among the cultivars.

The findings of this study are in conformity to the results of Bassiri (1976) who readily identified 12 barley cultivars by means of esterase alone. Fedak (1974) also demonstrated that 55 Canadian barley cultivars could be identified by complementing the alpha-amylase, esterase, and acid phosphatase patterns with kernel and plant morphological characters.

In general, similarity in morphology was not always reflected by a corresponding parallel in allozyme phenotype. The cultivars "67871" and "DDR" which were grouped as being similar in having a short pear-like fruit showed differences in their esterase, peroxidase, and glucose-6-phosphate phenotypes. Similarly, cultivars "269462" and "170522" both of which had a long pear-like fruit displayed the same glucose-6-phosphate phenotype but were dissimilar in their esterase and peroxidase phenotypes. Cultivars "374214" and "Kenya" both of which had a long thin fruit showed differences

in their esterase and glucose-6-phosphate dehydrogenase phenotypes but were similar in their peroxidase phenotypes.

This seems to suggest that fruit shape alone may not be the only character that is of importance in distinguishing the cultivars or the enzyme loci studied are not linked to the genes controlling fruit shape. Alternatively, the cultivars examined may have very little genetic diversity probably due to a common ancestry. Such a hypothesis is supported by the fact that isozyme bands for shikimic acid dehydrogenase, phosphogluco isomerase, and malate dehydrogenase were identical.

The seeds used to grow sample plants were the F_1 generation of the original population. It is possible that outcrossing could have occured resulting in rather uniform populations. A further explanation of the low genetic variability is that often a large number of isozyme bands can occur in different species but most of them may be 'monomorphic' i.e. present in all cultivars of that species (Bassiri *et al.*,1977).

CHAPTER 6

6. CONCLUSION AND SCOPE FOR FURTHER RESEARCH WORK

6.1 CONCLUSION.

I. These results showed that summer grown plants flower earlier, yield more and have fruits of superior quality than winter grown plants. This suggests that it is not economical to produce *L.acutangula* in winter due to extra expenses involved e.g. heating of the greenhouses.

2. Both marketable and total yields increased with plant density, but an optimum plant density was not established as yields continued to increase at all densities examined. This trend was observed for all the three cultivars studied. However, plant density had no effect on fruit quality. These results then suggest that it may be possible to grow *L.acutangula* at higher planting densities than 15000 plants/ha without reducing yields or affecting the quality of the fruits. Farmers in Kenya traditionally grow the cultivar "Kenya" at a wide spacing that varies between 1.8-5.0 m for the inter-rows and 1.0-4.0 m for the intra-rows. Results of the present study indicate that farmers should try alternative cultivars like "DDR" and "67871" as they tended to yield more and did not produce excessively large and heavy fruits compared to "Kenya". Their other advantage is the higher fruit number/plant. The reason why these two cultivars are not grown in Kenya is probably because they have not been introduced to the growing areas.

3. Electrophoretic studies showed that genetic variation existed between the *L.acutangula* cultivars studied based on a combination of esterase and peroxidase phenotypes or esterase and glucose-6-phosphate dehydrogenase phenotypes or peroxidase and glucose-6-phosphate dehydrogenase phenotypes. This suggests strongly that it is possible to biochemically distinguish among *L.acutangula* cultivars using leaf tissue.

6.2. <u>SCOPE FOR FURTHER RESEARCH WORK</u>

In view of so much inconclusive agronomic findings, it is apparent that there is a need for further research work on *L.acutangula*. Work on cultivar x environmental interactions can shed some light into the adaptability of the cultivars in different environments. Work on time of planting should be carried out to determine the appropriate planting time for different cultivars under rainfed conditions. Further work should be carried out to determine the irrigation regimes and watering requirements of different cultivars in different agroecological zones and their effect on yield and fruit quality.

Given that various cultivars of *L.acutangula* have different growing habits, further work should be done to determine suitable intra and inter row spacings for these cultivars with a view of optimising yields.

The need for fertilizer work on *L.acutangula* is underlined by the fact that few Kenyan farmers employ inorganic fertilizers in the production of the crop. This is mainly due to the fact that work on fertilizer requirements of

the crop is not documented. Studies relating the nutrient status of different soils to the levels of the nutrients in different parts of the *L.acutangula* plant should indicate the extent and types of nutrients absorbed by the *L.acutangula* plant. Suffient levels of different nutrients can then be arrived at and this can provide a guide as to the fertilizer types and levels to be applied in different environments. Methods and the appropriate times of fertilizer application should also be studied.

Germination of *L.acutangula* seeds is reported to be poor. In the present study, seeds were first germinated in seedling trays and the crop established by transplants. Studies should therefore be done to determine the optimum conditions that are required for for proper germination of *L.acutangula* seeds. Factors that affect the viability of the seeds should also be looked into.

Studies should also be done to determine the range of fruits weights which give the best quality and therefore the best market value.

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Appendix 1. Composition of Hoagland Solution.

Nutrient	mg/litre of water
$Ca(NO_3)_2.4H_2O$	590
KNO_3	25
$MgSO_4.7H_2O$	246
KH_2PO_4	68
Fe Sequestrene	25
H_3BO_3	1.45
$MnCl_2. 4H_2O$	0.9
$ZnCl_2$	0.06
$CuCl. 2H_2O$	0.024
MoO_3 (85%)(Molybdic acid)	0.02

Appendix 2: Quantities of Water and Fertilizer N.P.K. 20-20-20 Applied to the Field Experiment at Two Day Intervals

WEEK	QUANTITY OF WATER (m^3)	QUANTITY OF FERTILIZER (g)
1.	0.1	100
2.	0.2	100
3.	0.3	150
4.	0.4	150
5.	0.6	200
6.	0.6	200
7.	0.8	300
8.	0.8	300
9.	0.8	300
10.	1.0	400
11.	1.0	400
12.	1.0	400
13.	1.0	400

Appendix 3: Temperature Regimes During the Experiments.

	GREENHOUSE EXPERIMENTS					
	WINTER		SUMMER		FIELD EXPERIMENT	
WEEK	MINIMUM	MAXIMUM	MINIMUM	MAXIMUM	MINIMUM	MAXIMUM
1	16.6	31.6	18.3	34.3	14.4	42.0
2	15.0	32.0	17.O	29.3	13.0	39.0
3.	14.9	31.6	15.7	32.0	12.0	39.0
4.	15.6	27.4	15.0	31.6	13.0	41.4
5.	16.3	30.6	17.2	31.5	14.8	38.2
6.	16.7	30.1	17.1	36.7	14.5	39.3
7.	16.9	31.0	17.8	31.8	13.8	42.0
8.	15.5	27.2	18.1	29.6	12.4	38.2
9.	13.0	28.9	17.6	30.4	14.9	36.0
10.	14.9	32.0	18.3	29.0	13.1	37.2
11.	15.4	28.7	19.0	32.2	15.3	37.1
12.	13.8	31.3	19.3	31.0	16.4	34.7
13.	11.3	29.9	19.2	32.0	18.5	35.5
14.	12.2	3O.2	16.5	32.0	18.6	35.8
15.	14.7	29.3	17.0	31.5	18.4	34.0
16.	15.3	30.0	18.5	31.0	18.2	35.1

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