# EVALUATION OF ELITE BREEDING LINES OF FLUE-CURED TOBACCO FOR FIELD AND MARKET PERFORMANCE

OFFICE LOCARNO PIOUS MULEKANO

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OFFICE LOCARNO PIOUS MULEKANO

A DISSERTATION SUBMITTED TO
THE FACULTY OF BIOLOGICAL AND AGRICULTURAL SCIENCES
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CO-PROMOTER: DR. TOM B. VORSTER

October 12, 1999.

To the late my father

You sustained severe sunburns in those cotton fields in pains-taking effort of setting me on this narrow path to the destination you foresaw!

Kwa malemu bambo wanga

Munazunzika ndi dzuwa lowamba koopsya m'minda ija ya thonje mkuyesetsa kolimba kundilozera kanjira aka ka masomphenya anu!

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# Declaration

The work contained herein is original of the author. A part thereof or its entirety has not been submitted elsewhere for any course. The institutions, authorities, colleagues, friends and relatives acknowledged or quoted may not be held responsible for any clarifications that may be sought.

# EVALUATION OF ELITE BREEDING LINES OF FLUE-CURED TOBACCO FOR FIELD AND MARKET PERFORMANCE

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Magister Institutionis Agrariae
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Sustainable Plant Genetic Resources Management.

## Abstract

Eleven elite breeding lines of flue-cured tobacco, OD1, OD2, ODT1, ODT8, ODT19, ODT82, ODT92, ODT100, OD313, OD490 and OD486B were evaluated for their field and market performance at the Tobacco and Cotton Research Institute in the 1998/99 growing season. The currently accepted cultivar, TL33, was used as a control.

Crop growth duration, photosynthetic competence, plant height at topping, number of leaves per plant, leaf area, and yield were investigated as parameters of field performance. Leaf quality, nicotine and reducing sugar concentrations and monetary returns per hectare were investigated as parameters of market performance.

The correlation analyses of the parameters of field performance showed that plant height at topping and whole-plant leaf area might be the most important

yield components of these elite breeding lines. Non-significant differences existed between any one of the elite breeding lines and TL33, in terms of cured leaf yields, concentrations of nicotine and reducing sugars in the leaves, cured leaf quality and market income. The Non-significant differences could be attributed to either the restricted genetic advance that is due to the common ancestry and the limited genetic base of *Nicotiana tabacum* or the inherent inaccuracy of one trial at a single locality.

A combined analysis of data from the trial at Rustenburg and other similar trials at Groblersdal, Potgietersrus and Vaalwater was conducted so that accurate information could be arrived at for meaningful conclusions.

The combined analysis showed significant differences among the localities and among the entries. ODT92, ODT82, OD2 and OD1 produced significantly higher yields than TL33 across the four localities. However, the four elite breeding lines were not significantly different from each other. ODT82, ODT92 and OD2 gave significantly higher market income per hectare than TL33, but the three elite breeding lines did not differ significantly. The interaction between the localities and the entries were non-significant.

The Additive Main effects and Multiplicative Interaction (AMMI) analysis showed that the first Interaction Principal Component Analysis (IPCA1) was non-significant. However, the AMMI analysis predicted that ODT82 and ODT92 would be the best-adapted genotypes at Groblersdal, Potgietersrus and Rustenburg while ODT92 and OD2 would be the best-adapted genotypes at Vaalwater in productivity and economic viability.

ODT82 and ODT92 were recommended for on-farm trials at Groblersdal, Potgietersrus and Rustenburg. ODT92 and OD2 were recommended for on-farm trials at Vaalwater. The three elite breeding lines would undergo the on-farm trials pending their release as commercial cultivars at their respective localities.

A holistic approach to crop improvement in multiple locality experiments with designs that maximise genetic effects might be a panacea to experimental irregularities and low producer income levels that prohibit investment.

Research programmes may need to have linkages with the concerned industry, have clear research objectives and adhere to the acceptable procedures of recommendation development.

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Illustrations

# List of abbreviations

| | |
|---|---|
| AMMI | Additive Main effects and Multiplicative Interaction |
| ARC | Agricultural Research Council |
| CV | Coefficient of variation |
| DT | Dry bulb thermometer reading |
| DTES | District Trial Evaluation System |
| DTPM | Days to physiological maturity |
| FLES | Fixed Location Evaluation System |
| GM | General mean |
| Ha | Hectare |
| HR | Hours |
| IPCA | Interaction Principal Component Analysis |
| IPCA1 | First Interaction Principal Component Analysis |
| $IPCA1_G$ | Genotype First Interaction Principal Component Analysis |
| $IPCA1_L$ | Locality First Interaction Principal Component Analysis |
| Kg | Kilograms |
| LA | Leaf area |
| LAN | Limestone ammonium nitrate |
| LL | Leaf length |
| LSD | Least significant difference |
| $LW_m$ | Maximum leaf width |
| $M_g$ | Genotypic means |
| MKTV | Magaliesburg Tobacco Growers' Cooperative |
| $M_l$ | Locality means |
| NLP | Number of leaves per plant |
| NS | Not significant |
| PHT | Plant height at topping |
| PTK | Potgietersrus Tobacco Growers' Cooperative |
| R | Rand (South African monetary unit) |
| Rep | Replication |
| RH | Relative humidity |
| SLA | Single leaf area |
| TCRI | Tobacco and Cotton Research Institute |
| TMV | Tobacco mosaic virus |
| WPLA | Whole plant leaf area |
| WT | Wet bulb thermometer reading |
| Y | Genotypic performance |

# CHAPTER 1

## Introduction

Tobacco, *Nicotiana tabacum*, L. of the *Solanaceae* family, originated in South America. It is probably the most widely cultivated non-food crop in the world. Over 33 million people worldwide engage themselves in tobacco production, particularly in the third world countries of Africa. This drug crop is consumed as a smoke, a snuff or a chew for its stimulant alkaloid, nicotine. Upon consumption, the nicotine influences the intellect, stimulates the imagination and improves the endurance of the consumer (Chaplin, 1977; Collins and Legg, 1977; and Keller, 1976). The crop is classified as flue-cured, burley, dark air-cured and oriental. These classes differ in their genetic make-up, production, curing and use. However, all tobaccos have a common ancestral gene pool (Wernsman and Rufty, 1988). Flue-cured and burley tobaccos are the most produced and utilised classes.

The first European person to be introduced to tobacco was Christopher Columbus when he was given tobacco as a gesture of friendliness by the inhabitants of the Americas in 1492. Today, tobacco is grown worldwide from the latitude 45° N to the latitude 40° S under a wide range of climatic and edaphic conditions. The varying conditions under which tobacco is grown result in the localization and specialization in certain types of grades for particular tobacco products (Keller, 1976).

The Portuguese and other sailors brought tobacco to the natives of Southern Africa. When Jan Van Riebeck came to the Cape in 1652, the Hottentots were already using tobacco. Initially, the suitable areas for tobacco production in the inland of South Africa were found in Magaliesburg, Northern Transvaal and the Eastern Lowveld, where it was originally grown on a subsistence basis. The growth of the mining industry and the influx of foreign miners, who brought

sophisticated tobacco consumption methods like cigars and cigarettes, led to commercial production of tobacco. In 1937, a tobacco research farm was established near Rustenburg. In 1953, the tobacco research farm became the Tobacco Research Centre, now known as the Tobacco and Cotton Research Institute (TCRI). Today, the tobacco production areas in South Africa include the Northern Province, the Mpumalanga Lowveld, the Eastern Cape, the Western Cape, the North West Province and certain areas of KwaZulu-Natal (Figure 1.1) (Van Wyk, 1985).

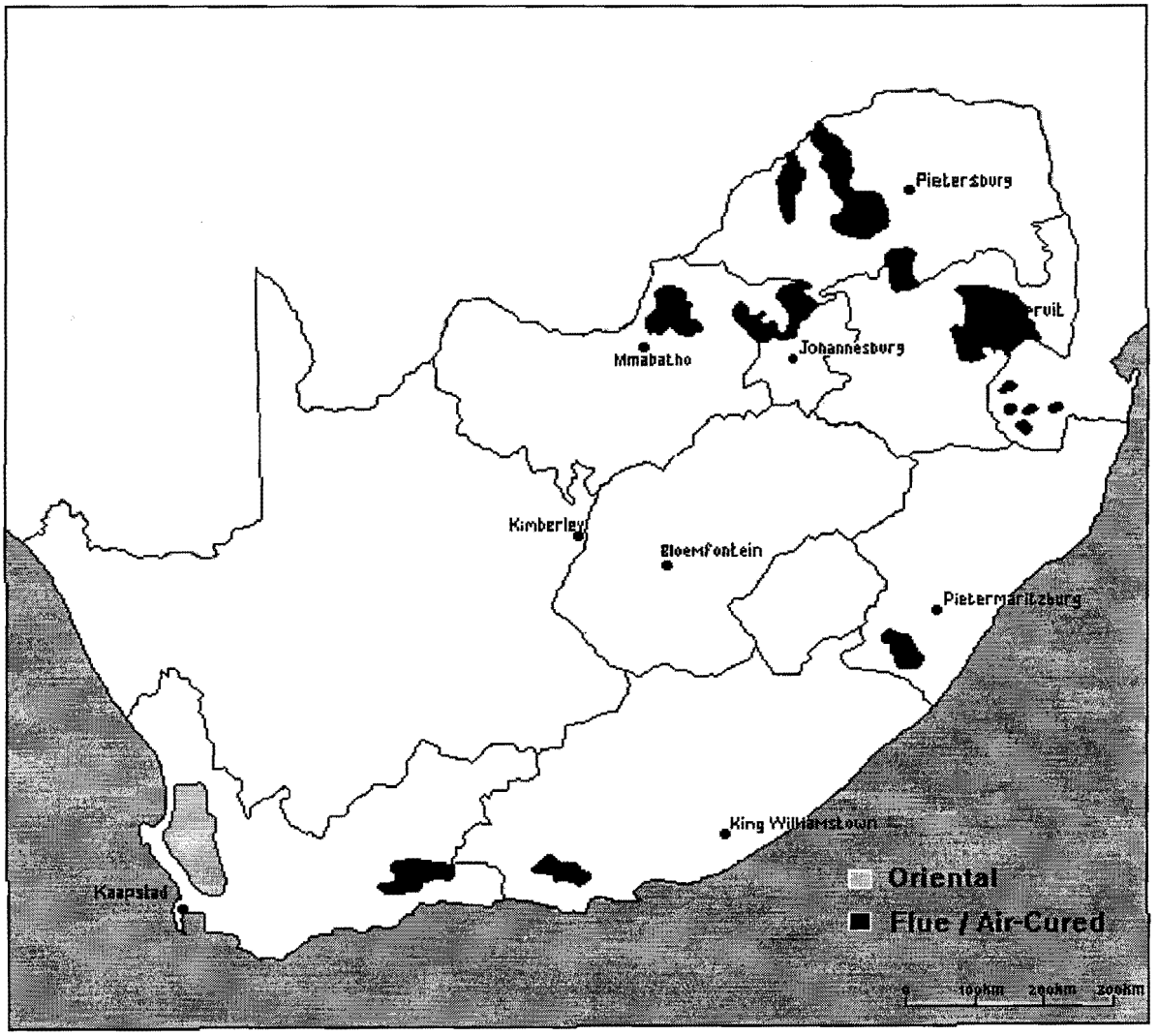



**Figure 1.1:** Tobacco producing areas in South Africa

The labour-intensive nature and profitability of the crop make its production a large-scale job-creating enterprise (Anonymous, 1996). The six provinces invest about R1 billion per year in tobacco farming, generating jobs for over 35000 people, over 63000 wholesalers and about 460000 retailers of tobacco products. Tobacco is easy and not costly to transport because it has a low weight and a low volume/value ratio. Tobacco is not easily perishable, unlike other agricultural products. It is a lucrative crop. In Zimbabwe, tobacco is 22 times as profitable as cotton, 57 times as profitable as maize and 59 times as profitable as soybeans (Anonymous, 1996).

South Africa features on the map of the world economy as the 25$^{th}$ most important tobacco producer (Anonymous 1996). The agricultural sector contributed 4.0% of the total gross domestic product (GDP) in 1998 (Orkin, 1998) (Figure 1.2).

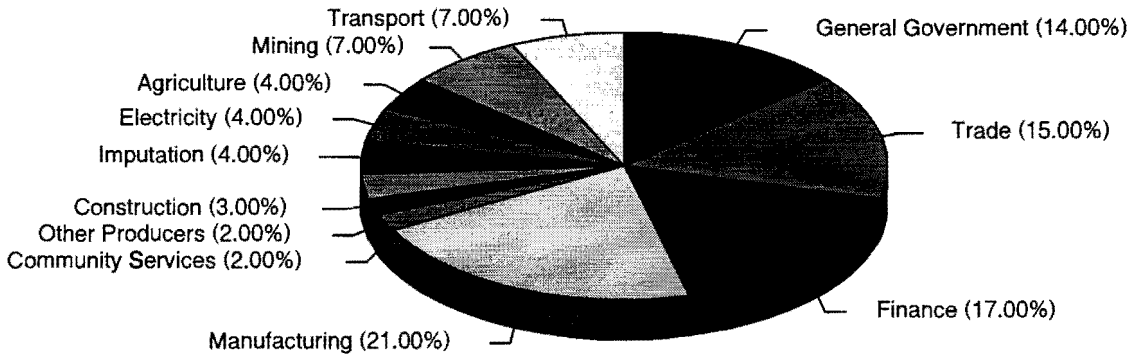



**Figure 1.2:** Contributions of different economic sectors to total GDP at current prices for the first three-quarters of 1998: redrawn from Statistics SA. 1998.

In most parts of the world, tobacco is grown profitably on light soils. In South

Africa, it is mostly grown on heavy black soils (Akehurst, 1968), although today more light soils are also used. Generally, most parts of South Africa receive unreliable rainfall during the tobacco-growing period (Anonymous, 1993). Because of these factors, tobacco produced in South Africa is of a relatively low quality. Attempts to improve on quality by supplementing the inadequate rainfall result in the escalation of the production costs. To compound these problems, the activities of the anti-smoking lobby, who stress the deleterious effects of tobacco smoking on human health, have resulted in a reduction in tobacco consumption (Table 1.1).

**Table 1.1:** The decline in the local consumption of tobacco in South Africa from 1991 to 1992. {Source: Trends in the agricultural sector. (51):9. Department of Agriculture. Republic of South Africa}

| Tobacco products | 1991 | 1992 |
|---|---|---|
| Cigarettes | 33 639 000 | 32 509 000 |
| Pipe tobacco | 7 496 000 | 6 552 000 |
| Snuff | 704 000 | 1 084 000 |
| Roll-tobacco | 49 000 | 23 000 |
| Cigars | 5 000 | - |
| Total | 41 893 000 | 40 168 000 |

The low-quality tobacco led to reduced demand and prices on both the local and export markets. The price of burley tobacco dropped by 35% during the period 1991-1992. Consequently, burley tobacco production was discontinued throughout the country. South Africa experienced a dwindling of both the local and the export tobacco markets in 1992 (Anonymous, 1996).

New technologies, which could improve tobacco yield and quality and reduce production costs, would revive the tobacco industry in South Africa. Tobacco breeding for yield and quality is intended to revive and sustain the industry.

# CHAPTER 2

## Literature review

Plant breeders use plant genetic resources to create new genetic variation. The genetic variation becomes the raw material for crop improvement. Therefore, genetic variation is a prerequisite for sustainable crop production. The explosion of the global population elevates the demand for crop products. Paradoxically, the world is experiencing heavy plant genetic erosion at this very time of high demand for crop products. This trend calls for the management of plant genetic resources to rescue the genetic resources that are continuously being lost.

Categories of biodiversity management systems include *in situ*, *in situ*/on-farm, and *ex situ* conservation. Farmers are already practising *in situ*/on-farm agrobiodiversity conservation at considerable economic sacrifice to the benefit of formal plant breeding systems (Swaminathan, 1997). Therefore, the access to plant genetic resources is a benefit-sharing process between the conservers and the users of agrobiodiversity. Symbiotic linkage between conservers of agrobiodiversity and the commercial industry is probably the promising pathway to the identification, collection, conservation, and sustainable utilization of plant genetic resources. Faith in this symbiotic linkage 'is the assurance of things hoped for, the conviction of unseen realities' (Hebrews 11:1) in the abolition of poverty among the disadvantaged farmers. The evaluation of the elite breeding lines of flue-cured tobacco for field and market performance was for the benefit of the tobacco growers in partial fulfilment of the requirement for the symbiotic linkage.

Increased genetic variability and gains from selection may be results of introgression of diverse germplasm into the present crop genetic base (Thompson and Nelson, 1998). Scientists postulate that *Nicotiana tabacum* L. arose as a single chance hybrid between the progenitors *Nicotiana sylvestris and*

*Nicotiana tomentosiformis*. A review of the commercial tobacco cultivars shows a limited germplasm base. Flue-cured tobacco cultivars show a close genetic relationship. Tobacco plant breeders have reshuffled and recombined a common base of genetic factors. Therefore, it is logical to expect that tobacco cultivars would have similar genetic backgrounds and that the genetic advance would be restricted (Keller, 1976).

Although other factors are of vital importance, yield dominates the objectives of all plant breeding programmes (Stoskopf et al., 1993; Wallace and Yan, 1998; Simmonds, 1987). Intensive investigation is focussed on cultivar structure improvement to achieve this dominant objective (Kostova and Kurteva, 1997). Conventional breeding methods have enabled tobacco researchers to develop a number of high yielding tobacco cultivars (Narayaran et al, 1998). High yields of acceptable quality are to be produced if the high initial capital outlay, farm structure maintenance and crop management costs incurred by the farmer are to be justified (Dippenaar et al., 1991).

The progress in breeding for improved tobacco yield and leaf quality has been quite difficult to assess due to the confounding effects of genetic improvement and improved production technology (Wernsman and Rufty, 1988). Large interactions between the genotype and the environment retard the progress of obtaining gains from selection (Comstock and Moll, 1963). The interactions between the genotype and the environment may constitute a limiting factor in the estimation of the variance components and in the efficiency of the selection programmes (Sprague, 1966). The narrow genetic base, in tobacco, that restricts the progress of genetic gains, compounds the difficulty of assessing the genetic advance. Therefore, it is essential for the breeder to design his testing procedures in such a way as to maximise the genetic effects relative to the environmental and interaction effects (Miller et al., 1958).

Breeders need to make selections in the environments in which the useful

genetic variability is best expressed, the environmental effects on heritable characters not withstanding (Meredith, 1984). Heritability is the ratio of the genetic variance to the total variance, quantitatively expressed as H = Vg/(Vg +Ve) (Allard, 1960; and Breese, 1968), where H is the heritability, Vg is the genetic variance, and Ve is the environmental variance.

Genetic variance is that part of the phenotypic variance, which can be attributed to the genotypic differences among the phenotypes. The variance of the interaction between the genotype and the environment is the part of the phenotypic variance attributable to the failure of the differences between the genotypes to be the same in the different environments (Dudley and Moll, 1969). Therefore, heritability varies with the environmental factors. This emphasises Meredith's idea that the breeders need to make selections in the environments in which the useful genetic variability is best expressed.

A slight negative relationship exists between yield and quality of flue-cured tobacco. Quality decreased when the cured-leaf yield was more than 2000 kg/ha in DH10 and 2500 kg/ha in Drava. The highest value of a DH10 crop was realized in the season when the highest yields were produced, while Drava reached its highest value in the season during which it produced the highest quality (Smalcelj, 1998).

The problem of the negative relationship between yield and quality has spurred in-depth studies of yield and quality components in other crops also. A new high-yielding cultivar of field pea (*Pisum sativum* L.), Crown, consistently yielded better than the standard cultivar, Whero, by 28.2%, because of its short stature and relatively prostrate growth habit (Jermyn and Russell, 1998). A high variance of 51.9% was detected in the yield of a common buckwheat cultivar. The effect of year was significant in characters like plant height and oil and potassium contents (Michalova et al., 1998). High yielding sugar cane cultivars, PS80-847 and PS80-960 exceeded the target yield for the area in comparison with the

control cultivar, M442-51. PS80-960 gave the highest yield and sugar content (11.8%) with straight stems and large stem diameter (Mudefar and Suhardi, 1998). Photosynthesis, chlorophyll fluorescence and symbiotic nitrogen fixation were studied in soybean (*Glycine max* L.) to identify selection criteria for genetically induced cold tolerance. The objective was to enhance the yielding potential of soybean in South Africa (Van Heerden and Kruger, 1998). The drought adaptation mechanism in the tobacco cultivars TL33, CDL28, GS46, and Elsoma, could, in part, be attributed to chlorophyll ***a*** fluorescence (Van Rensburg, 1991). The chlorophyll ***a*** fluorescence values, which are good indicators of photosynthetic efficiency, showed that the more drought-tolerant cultivars, GS46 and Elsoma, had a fast initial decline in photosynthetic efficiency that stabilized as water stress became acute. The less drought-tolerant cultivars, TL33 and CDL28, showed a slow continuous decline.

In Turkish tobacco, the leaf area has the greatest positive direct effect on cured-leaf yield, followed by the number of leaves per plant (Kara and Esendal, 1996). Much as many plant breeding programmes have concentrated on dealing with genetic traits responsible for high yields in turns, a holistic approach is the best means of improving yield (Wallace and Yan, 1998). An operating system of traits is the final determinant of yield levels. It is further argued that from the physiological and genetic viewpoints, biomass accumulation is the major yield component followed by partitioning of photosynthates. Number of days to maturity is another major yield component. Therefore, improved adaptation and number of days required to reach maturity are the most advocated criteria for acceptance of new crop cultivars.

Plant breeders agree that some genotypes do well over a wide range of ecological zones, while others are environment-specific for optimum performance. Some regard the attainment of augmented and stabilized yields as the most important goal in plant breeding (Soliman and Allard, 1991). On the other hand, others employ multiple comparison experimental procedures in

regional yield trials to identify genotypes well suited to particular environmental conditions (Piepho, 1995). Such procedures serve as tools for developing site-specific recommendations for particular cultivars. Suitable genotypes are those that do not differ from or are better than the currently recommended cultivar in the area. Therefore, it is important to evaluate breeding materials under the conditions, which are similar to those in which the materials will eventually be used (Allard and Bradshaw, 1964). It is ideal to breed, for every locality, the genotype that is best adapted to that environment (Hill, 1975).

The objective of this work was to evaluate the elite breeding lines of flue-cured tobacco for their field and market performance in Rustenburg. Evaluation of tobacco breeding lines needs to be done over a period of two seasons at four different locations with four replications per location and year (Wernsman and Rufty, 1998). Additionally, an adequate test for genotypic performance should cover such characters as maturity, plant height, number of leaves per plant, cured leaf yield, grade index and reducing sugar and nicotine concentrations of the leaf (Wernsman and Rufty, 1998).

However, Greeff (1986) argued that both fixed location evaluation systems (FLES) and district trial evaluation systems (DTES) are equally efficient as methods of evaluating the performance of cultivars. The genetic yielding potential is best shown with the FLES. The extra years of testing may not really be necessary and fewer trials than are normally conducted would still provide the same results (Greeff, 1986).

# CHAPTER 3

## The conventional method of tobacco cultivar development

All the classes of tobacco; flue-cured, burley, dark air-cured and oriental differ in their genetic make-up, production, curing techniques and use although they have a common ancestral gene pool (Wernsman and Rufty, 1988).

Tobacco is a prolific, inbred, and seed-propagated allotetraploid (4x) with large perfect flowers that are easy to emasculate. Therefore, the plant can be selfed or cross-pollinated at will in a breeding programme. The pedigree breeding method is employed in almost all the breeding programmes. The back cross breeding method is useful in breeding for disease resistance. The hybrid breeding in flue-cured tobacco is used mainly for rapid results in the combination of characters to expedite the progress in the breeding programme.

The objectives of tobacco breeding programmes include improvement in yield, quality and consequently income per hectare; disease resistance; ease of handling and curing; and chemical constituents while meeting the demands of the grower, the manufacturer and the consumer.

Hybrids could provide rapid results in achieving these objectives because of heterosis. Unfortunately, all flue-cured tobacco inbred lines register low heterosis in their $F_1$ hybrids (Aycock, 1980). The magnitude of heterosis in burley tobacco is higher than that in flue-cured tobacco. Burley tobacco hybrids have higher growth rate, yielding potential and quality than those of their parental inbred lines and can carry multiple disease resistance if the parental inbred lines are prudently selected. Consequently, the monetary returns per hectare, which are realised from a hybrid crop readily offset the additional cost of hybrid seed. Therefore, about 60% of the American, European and Zimbabwean burley

tobacco crop comprises single-cross hybrids (Legg and Collins, 1971; and Vorster, personal communication).

## 3.1 Desirable characters that need improvement in flue-cured tobacco

Cultivars that give high yields of acceptable quality have producer appeal, as the ultimate goal of the producer is to generate high income per hectare. Characteristics of the cured leaf such as the size, wholesome appearance, colour tone, elasticity and feel are important farm-gate quality attributes (Hawks, 1970).

Tobacco growers favour cultivars that suffer the least disease catastrophes. Some of the catastrophic tobacco diseases in South Africa are shown in Table 3.1. Certain genetic factors responsible for black shank, bacterial wilt and tobacco mosaic virus (TMV) resistance have undesirable pleiotropic effects on plant performance (Chaplin and Mann, 1978; Legg et al, 1982). $F_1$ hybrids from a cross of susceptible and resistant inbred lines may resemble the resistant parent and display mid-parent genetic expression or partial dominance in yield and quality with respect to the susceptible parent. Knowledge of plant genetic sources of disease resistance and modes of inheritance of the resistance enable tobacco breeders to develop new genotypes with multiple disease resistance.

**Table 3.1:** Some of the catastrophic tobacco diseases in South Africa and plant genetic resources for resistance (Wernsman and Rufty, 1988).

| Disease | Pathogen | Sources of resistance | Mode of inheritance |
|---|---|---|---|
| Black shank | *Phytophthora parasitica, var. nicotianae* | *Nicotiana tabacum,* Fla 301<br>*Nicotiana longiflora* | Dominant, oligogenic<br>Dominant, monogenic |
| Bacterial wilt | *Pseudomonas solanacearum* | *Nicotiana tabacum,* T1 44 A | Recessive, oligogenic |
| Root knot | *Meloidogyne incognita* | *Nicotiana tabacum,* T1 706 | Dominant, monogenic |
| Mosaic | Tobacco mosaic virus | *Nicotiana glutinosa* | Dominant, monogenic |

Other characteristics that are favourable to growers are ease of handling and curing. Favourable handling characteristics include a negative reaction to factors, which induce early flowering and reduce the number of leaves per plant. Other favourable characteristics are the dormancy of basal axillary buds that reduces the labour required for sucker control, and anchorage that reduces incidences of lodging. Uniform ripening of the leaf blade and leaf elasticity that keeps the leaf on the plant and enables the leaf to sustain little breakage on mechanical impact are other characteristics that contribute to the acceptability of the cultivar. Uniformly ripe tobacco leaf is desirable for the curing process. The temperature and humidity regimes (Schedule 4.3) observed during the leaf colouring phase of curing may have a deleterious impact on the cured leaf yield and quality of flue-cured tobacco leaf that is not uniformly ripe (Hawks, 1978).

As far as the manufacturer is concerned, the acceptability of a cultivar depends on a high cigarette out-turn. Tobacco leaf of high filling power produces firm cigarettes without using such large quantities of leaf as to make it expensive for the manufacturer and hard for the smoker to draw through. Cured tobacco leaf that is not elastic breaks to unusable fine leaf materials during handling and becomes a source of loss to the manufacturer. The large proportions of leaf main veins that are removed during the manufacturing process also contribute to manufacturing losses. Although the fine tobacco leaf materials and the leaf main veins are now being used in cigarette manufacturing as reconstituted tobacco, they are not as favourable as the actual leaf (Hawks, 1970).

The tobacco consumer is concerned about cigarette combustibility, aroma and smoke flavour. The desirability of these factors is affected by the balance among the chemical constituents of the tobacco leaf, particularly nicotine, reducing sugars and total nitrogen. However, nicotine, the stimulant alkaloid, is the basis for smoking (Collins and Hawks, 1993; Wernsman and Rufty, 1988; and Hawks, 1970). Table 3.2 shows the acceptable concentration ranges of the chemical constituents of concern in flue-cured tobacco leaf.

**Table 3.2:** Acceptable ranges of chemical concentrations in flue-cured tobacco leaf (Hawks, 1970)

| Chemical constituent | Nicotine | Sugar | Total Nitrogen | Ether extracts | Bases | Chlorides | Ash |
|---|---|---|---|---|---|---|---|
| Concentration range (%) | 1.5-3.5 | 8-18 | 1.4-2.7 | 6-8 | 0.3-0.5 | <1 | 10-18 |

## 3.2 Problems encountered in flue-cured tobacco breeding

There are two major problems encountered in breeding for yield and quality in flue-cured tobacco. Firstly, the assessment of genetic gains is a painstaking exercise because tobacco has a narrow genetic base, which restricts genetic advance. Secondly, nicotine and total nitrogen concentrations of the leaf are negatively correlated to yield (Wernsman and Rufty, 1988). It is speculated that there will be a market demand for the tobacco leaf with a low nicotine concentration (Papenfus, personal communication). The future market demand for the tobacco leaf with a low nicotine concentration may ease the burden. The unstable recessive alleles that readily mutate to dominant states, which are responsible for the demethylation of nicotine to nornicotine and the transformation of leaf colour to cherry red have been problematic (Wernsman and Rufty, 1988). Breeding programmes may take advantage of this naturally occurring phenomenon to satisfy the speculative market demand for leaf with low nicotine.

Most of the important agronomic characters and chemical constituents of flue-cured tobacco are quantitatively inherited; and they show additive genetic variation (Matzinger and Wernsman 1979). Dominance and epistasis are infrequent. Heterosis among hybrids from homozygous inbred lines is low. $F_1$ hybrids show intermediate parental genetic expression. Therefore, flue-cured

tobacco breeding programmes are geared towards the production of pure lines rather than hybrids.

## 3.3 Crucial issues in tobacco cultivar development

Three major issues are considered in tobacco cultivar development. The breeder needs to know the desired characters, their mode of inheritance and their plant genetic resources. The identities of the desired characters and the most effective breeding plan need to be well known. The long-term repercussions of the breeding plan need to be considered in the light of future crop improvement and the vulnerability of the new cultivars through genetic erosion.

## 3.4 Hybridization

The breeder would start a breeding programme by carefully selecting parental lines of heterogeneous pedigrees. Ideally, the parental lines would collectively display all the desirable characters that the new cultivar needs to have. Parental lines for hybridization are normally restricted to the same tobacco class. The parental lines are selected in such a way that a population with high genetic variation including individuals with high genotypic value can be developed. However, acceptable plant genetic resources for resistance to certain diseases have not been identified in the species, *Nicotiana tabacum*. Interspecific hybridization attains the incorporation of the desired characters into a cultivar of interest. Requirements for interspecific hybridization are the identification of the sources of the resistance and the identification of the qualitative mode of inheritance of the resistance. Dominant alleles are another requirement for easy tracing of the characters in segregating populations. To allow continuity of crop improvement endeavours and avoid possible genetic erosion, the interspecific hybrids should not have reproductive isolation mechanisms from *Nicotiana tabacum* and its progenitors, *Nicotiana sylvestris* and *Nicotiana tomentosiformis.* Recombination of the tobacco genome and the alien genome should be

repeatable. In cases of interspecific incompatibilities, genetic 'bridging' crosses (crosses with related species that can be hybridized) are made. The resulting hybrids need back crossing (Simmonds, 1987; and Stoskopf et al, 1993). Tobacco resistances to black shank, black root rot, blue-mould, tobacco mosaic virus and wild fire are products of the interspecific hybridization (Wernsman and Rufty, 1988).

Artificial hybridization involves choosing and plucking a flower that will undergo anthesis within 24 hours from the terminal panicle of the male parent. The flowers are allowed to open in a protected environment. Flowers that are about to open on the female parent are selected and emasculated using a pair of forceps. Pollen from the flower of the male parent is dusted onto the stigma of the emasculated female parent. A five-centimetre section of a soda drinking straw, sealed on one end, is used to enclose and protect the pollinated stigma and the style. A record of the hybridization is made on a marking tag that is tied around the pedicel of the pollinated flower. About 60 hours after pollination, the corolla dies and falls off together with the protective soda drinking straw. The fruit ripens 21-25 days later. Almost 3-4 weeks after the fruit has ripened, the capsules will be ready for harvesting, drying and threshing. Self-pollination is achieved by covering the unopened flowers with a plastic mesh bag. The genetic variation is manifested in the $F_2$ and the subsequent generations.

Individual plants from the genetically variable population are selfed to partition the genetic variation among families and individual plants within families. Plants with desirable characters are selected (Table 3.3). Selection methods include the pedigree method, bulk selection method and single seed descent. The pedigree method is the most popular method of selection in cultivar development.

Selection and advancement of generations continue until the desirable characters become stable in the selected lines. The stable breeding lines are evaluated for characters of interest in a bulk block (B block), which does not have

an elaborate experimental design. This is done in a glasshouse or in the field. Promising breeding lines are selected and advanced to preliminary replicated trials to evaluate them for their agronomic performance. Randomised designs and control cultivars are used in at least three locations.

The characters to be evaluated include the days that the plants take from transplanting to reach physiological maturity, plant height at topping, and the number of leaves per plant. The leaf yield, leaf quality which is measured as grade index, and the concentration of reducing sugars and nicotine in the leaf are other important characters to be evaluated. Smoking tests may also be conducted. Notes on incidences of premature flowering, ground suckers, uniform ripening and leaf breakage are also important.

Bulking of breeder-seed of the promising breeding lines is done along with the replicated trials. The superior breeding lines are advanced to elite breeding lines trials where the lines are evaluated over a wide range of environments. The elite breeding lines that seem capable of acceptance by the grower, the manufacturer and the consumer are selected for on-farm trials to develop cultivar-site recommendations. The elite breeding lines that pass the on-farm trials would be released for commercial production.

At the time of developing recommendations, multiplication of seed of the candidate cultivars is done by certified seed producers in readiness for distribution to the growers according to the site recommendations.

**Table 3.3:** A typical conventional tobacco cultivar development programme in flue-cured tobacco.

| Year | Breeding activity | Environment |
|---|---|---|
| 1 | Hybridization of the selected parents. | Glasshouse/field |
| 2 | Natural self-pollination of $F_1$ hybrids. | Glasshouse/field |
| 3 | $F_2$ population is subjected to selection pressure for traits of interest and plant type. | Field |
| 4 | $F_3$ population is subjected to selection pressure. Superior plants in the best lines are selected for traits of interest and plant type. | Field |
| 5 | $F_4$ (as for $F_3$) | Field |
| 6 | $F_5$ lines are evaluated for traits of interest in a bulk block (B block). Best lines and plants within lines are selected for further evaluation in the next generation. | Glasshouse/field |
| 7 | $F_6$ lines are put in replicated trials for agronomic evaluation. | Field<br>Three locations. |
| 7 | Bulking of seed of stable lines. | Test sites |
| 8 | Evaluation of $F_7$ elite breeding lines in small test plots. | Multiple test-plots. |
| 9 | On-farm trials and small plot tests of $F_8$ elite breeding lines. | Multiple ecological zones. |
| 10 | Seed multiplication of the new cultivar by certified seed producers. | Multiple ecological zones. |
| 11 | Distribution of certified seed to farmers. | Multiple ecological zones. |

## 3.5 Hybrid-breeding and cytoplasmic male-sterility

Flue-cured tobacco plant breeders use hybrid-breeding technique to combine many desirable characters faster than when the pedigree method is used. The

heterogeneous and the collectively desirable character-rich parental lines are crossed to produce the $F_1$ hybrid seed. The crop from the $F_1$ hybrid seed will be uniform with multiple desirable characteristics.

Some growers have the tendency of using recycled seed. Recycled hybrid-seed will give segregating populations in which undesirable characters may show up to the detriment of the farm output. Cytoplasmic male-sterility makes it impossible for the tobacco growers to recycle the hybrid-seed. The grower buys the $F_1$ hybrid-seed every year to exploit the in-built multiple characteristics. Cytoplasmic male-sterility is a hereditary character that is usually determined by non-chromosomal genetic factors usually located in the chloroplasts or the mitochondria in the cytoplasm. Cytoplasmic characters display maternal inheritance. The most prolific parental line is selected and converted to cytoplasmic male-sterility. Two methods that are commonly used in the development of male-sterile lines are the back cross method and the *in vitro* protoplast fusion. In the back cross method, an existing sterile line is crossed with the cultivar that is to be converted to male-sterility so that the male-sterility gene is incorporated into that cultivar of interest. The male-sterile offspring is then back crossed to the parent of interest to concentrate the genes of interest. In the *in vitro* protoplast fusion method, somatic cells are isolated from both the parental cultivar of interest and the male-sterile parent. The nuclei are extracted from the cells. The nucleus from the cell of the parent of interest is inserted into the cytoplasm of the male-sterile parent. Since the cytoplasmic male-sterility is based on the chloroplasts or the mitochondrion in the cytoplasm, the plant that develops from this 'hybrid' cell is a male-sterile plant. This 'hybrid' cell is cultured *in vitro* to produce plantlets, which are male-sterile. Somatic embryogenesis is then conducted on the male-sterile plantlets to produce male-sterile clones. The male-sterile clones are grown and artificially pollinated with pollen from the male-fertile version of the cultivar to produce desirable quantities of the male-sterile seed for distribution to the growers.

## 3.6 Modern tobacco plant breeding and future prospects

The art and science of plant breeding uses genetic variation as a raw material. The wider the genetic base the higher the number of possible recombinations from which to select the desirable characters. Conventional plant breeding manipulates the available genetic variability within the confines of the crop species at the fixed ploidy level (Simmonds, 1987). Reproductive isolation mechanisms make certain genetic variability unavailable to the breeder for exploitation. This is one of the major limitations of the conventional breeding technique. An array of accessory breeding techniques that employ molecular biology is in use to circumvent the limitations of conventional breeding. These accessory techniques include polyploidy, wide crossing, haploidy, mutagenesis and *in vitro* techniques, including genetic engineering. Conventional plant breeding and the accessory techniques make up the components of modern plant breeding (Stoskopf et el., 1993). Because of modern plant breeding, the South African tobacco industry has the hope for revival. Tobacco growers will be able to exploit different characteristics in new cultivars and in hybrids that will be produced massively via male sterile lines possibly in all the classes of tobacco. Genetically modified tobaccos with various desirable attributes will be made available to the grower. Whether the genetically modified tobacco will be acceptable to the industry will have to be determined.

## 3.7 Release of new tobacco cultivars

Typically, the breeder provides sufficient supporting data in the proposal for the release of the candidate elite breeding lines. A variety release committee, usually composed of tobacco growers, leaf dealers, manufacturers, consumer representatives and tobacco breeders, examines the data of the candidate elite breeding lines in comparison with those of the currently accepted cultivar(s). The committee makes a decision on the release of the candidate elite breeding lines as cultivars for commercial production.

### 3.8 Maintenance of tobacco breeder-seed

Self-pollination of the desired plants maintains cultivar purity. The use of polyethylene mesh bags to cover the inflorescence accomplishes self-pollination. The seed lot from the selfed population is stored as breeder-seed. A storage chamber operating at 10°C and 50% relative humidity will keep tobacco seed viable for at least 10 years. Seed for commercial multiplication of planting material is obtained from the breeder-seed lot. When the viability and the quantity of the stored breeder-seed drop, the seed lot is multiplied to restore the recommended viability and quantity in storage. Rogueing of any plants that do not conform to type, including cherry-red mutants, is obligatory in breeder-seed multiplication plots. This seed multiplication exercise may take place once in five years depending on the demand for the breeder-seed.

### 3.9 Production and marketing of commercial tobacco seed

Commercial seed is produced from breeder-seed and distributed by private seed agencies or plant breeding organisations. The seed of different cultivars can be multiplied in adjacent seed plots as long as each seed plot is surrounded by at least four rows of intact plants of the same cultivar as that of the seed plot crop. If other classes of tobacco are grown in the same locality, a seed plot should be isolated from any other tobacco crop of a different class by a minimum of 400 metres (Wernsman and Rufty, 1988).

# CHAPTER 4

## Materials and methods

The principal objective of tobacco plant breeding programmes is to improve yield per hectare and quality for high economic gains (Stoskopf et. al., 1993; Wallace and Yan, 1998; Simmonds, 1987). Breeding for any desirable characteristic in tobacco can be either a direct or an indirect means of achieving the above objective.

### 4.1 Background work

Based on the above objective, various crosses of carefully selected parental genotypes have been made by the Tobacco and Cotton Research Institute (TCRI) of the Agricultural Research Council (ARC). Advancement of generations and selections for desirable characteristics have been conducted simultaneously in a series of trials at the ARC-TCRI. The promising breeding lines namely OD1, OD2, OD490, ODT92, ODT19, ODT8, ODT82, OD486B, ODT100, ODT1 and OD313 have been advanced to elite breeding lines (Table 4.1).

### 4.2 Current work

In the current work, the elite breeding lines were evaluated for their field and market performance. The days that the plants take from transplanting to physiological maturity, plant photosynthetic competence, plant height at topping, number of leaves per plant, leaf areas and yield were investigated as parameters of crop field performance. Cured leaf colours, nicotine and reducing sugar concentrations of the leaf, grade indices of the cured leaf and the monetary returns per hectare were investigated as parameters of crop market performance.

**Table 4.1:** The elite breeding lines and their pedigrees

| Elite breeding line | Pedigree |
|---|---|
| OD1 | (C411 x TL33) x (SpG28 x TL33) |
| OD2 | 17/17/8/30 x OD212/8 |
| OD490 | SpG80 x 10/43 |
| ODT92 | K326 x TL9hon |
| ODT19 | Island Gold x M1 |
| ODT8 | K326 x 33/94 sub |
| ODT82 | K326 x OD272 |
| OD486B | 17/17/8/30 x OD212/8 |
| ODT100 | K326 x OD212 |
| ODT1 | Island Gold x OD272 |
| OD313 | Virginia 115 x {(C347 x TL33) x (SpG28 x TL33)} |
| TL33 (Control) | SpG28 x A23 |

OD2 and OD486B are different selections from the same parentage.

## 4.3 Tobacco nursery management

Seedling production involved sowing tobacco seeds in a complex germination medium made of vermiculite, peatmoss and perlite in compartmentalised seed-germination trays in a glasshouse. The tray compartments were filled with the germination medium and watered lightly. 0.2g of tobacco seed of a particular genotype was then broadcast onto the compartmentalized seedling tray by hand.

An automated overhead spray-irrigation system was used to water the nursery. This watering system is used at the ARC-TCRI because it demands a lower labour requirement than the use of pipes and watering cans. Soon after sowing, the watering was light and frequent. The watering became heavier but less frequent as the seedlings grew to keep seedling growth rate steady and constant.

After germination, when the seedlings were about five centimetres tall, they were thinned and spaced to one seedling per compartment to reduce competition so that a strong, healthy and uniform stand of 128 seedlings per tray could be achieved.

Nursery fertilizers, fungicides, insecticides and a surfactant were administered by drenching according to Schedule 4.1. The fertilizers included Multifeed™, calcium nitrate ($C_aNO_3$) and limestone ammonium nitrate (LAN). Apron™C and Virikop™ are the fungicides that were used to combat powdery mildew, wildfire and damping-off. Baytan™ was used to control systemic fungal disease-causal agents. Orthene™ and Endosulfan™ are the insecticides that were used to keep insect damage under control. Bladbuff™, a surfactant, was used to enable the chemicals to stick to the leaf surfaces for a lengthy effective action.

**Schedule 4.1:** Fertilizer application, and disease and insect pest control programme used in the seed tray nursery.

| Chemical and quantity | Day | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 7 | 14 | 21 | 28 | 35 | 42 | 49 | 56 | 63 | 70 |
| $C_aNO_3$ 400g | + | + | + | + | + | + | + | + | + | + | + |
| Multifeed™ 50g | | + | + | + | + | + | + | + | + | + | + |
| LAN 208g | | | + | + | | + | + | + | | + | + |
| $KNO_3$ 224g | | | | | + | | | + | | | |
| Apron™C 30g | + | | | + | | | + | | | + | |
| Baytan™ 8ml | | + | | | + | | | + | | | + |
| Virikop™ 100g | | | | | | + | | | + | | |
| Endosulfan™ 40g | | | | + | | | | + | | | |
| Orthene™ 80g | | + | | | | + | | | | + | |
| Bladbuff™ 20ml | + | + | | | + | + | + | + | + | + | + |
| Water 40ml | + | + | + | + | + | + | + | + | + | + | + |

The drenching programme started one week after the thinning and spacing of the seedlings in the seed trays. The chemical mixture was applied at the rate of 15 litres per 50 seedling trays that covered 11.39 square metres of nursery area. Each tray contained 128 seedlings.

Weeding was done by pulling the weeds out as soon as the weeds appeared. A pair of forceps was used to pull out the weeds before the weeds developed large root volumes. Weeds with large roots damage the tobacco seedlings in the process of weeding.

The top leaves of the seedlings were clipped off regularly to maintain uniformity in seedling height within a genotype in a seed tray and to rejuvenate the fast-growing genotypes. A lawn mower fitted with height adjusters and suspended on a bench was used to clip the seedlings. The seedling tray was pushed across the bench but under the running machine to clip off the top leaves of the seedlings. The height of the machine was adjusted depending on the general height of the seedlings in the tray to avoid damaging the apical meristems. Rejuvenating the fast-growing genotypes and maintaining uniformity in seedling heights were important to avoid unnecessary variation in the experimental seedlings.

Three weeks before transplanting, the seedlings were taken from the glass house into the open and deprived of water to harden them off. Hardening off enables the seedlings to survive the shocks of transplanting and the harsh conditions of the field. A well-hardened seedling would not snap easily when bent. Hardened seedlings withstand the handling pressure in the processes of transporting them from the nursery to the field and transplanting.

## 4.4 Land preparation

The trial was conducted on 0.1 hectare of land at the ARC-TCRI. The ARC-TCRI is located at Kroondal near Rustenburg in the North West Province of South

Africa. Rustenburg is located at an altitude of 1157 metres above sea level, latitude of 25° 43' South and a longitude of 27° 18' East.

The land had been grown with *Setaria sphacelata*, a rotational grass locally known as manna grass, during the previous season. Deep ploughing and discing had been done almost six months before transplanting.

Two sets of soil samples were taken from the land in a random manner at three different soil depths: 0-20cm, 20-40cm and 40-60cm before ridging was done. One of the two soil samples was taken as a back-up sample. The soil depth of up to 60cm was chosen because tobacco is a dicotyledonous plant with a tap root system. Therefore, the growth of a tobacco plant is influenced by the soil condition mostly within this soil depth. The soil samples were sent to the soil chemistry laboratory for soil chemical analysis. The results of soil chemistry analysis are presented in table 4.2.

**Table 4.2:** Results of soil chemistry analysis (p.p.m. - parts per million).

| Soil depth | 0-20 cm | 20-40 cm | 40-60 cm | Average |
|---|---|---|---|---|
| pH | 5.91 | 6.15 | 6.26 | 6.11 |
| % sand | 38.00 | 28.00 | 27.00 | 31.00 |
| % silt | 8.00 | 6.00 | 9.00 | 7.67 |
| % clay | 54.00 | 66.00 | 64.00 | 61.33 |
| N p.p.m. | 4.00 | 2.00 | 2.00 | 2.67 |
| P p.p.m. | 5.00 | 1.00 | 0.00 | 2.00 |
| K p.p.m. | 163.00 | 88.00 | 83.00 | 111.33 |
| Ca p.p.m. | 773.00 | 680.00 | 580.00 | 677.67 |
| Mg p.p.m. | 1080.00 | 1400.00 | 1480.00 | 1320.00 |
| Na p.p.m. | 8.00 | 13.00 | 15.00 | 12.00 |
| Cl p.p.m. | 1.00 | 1.00 | 1.00 | 1.00 |
| Zn p.p.m. | 1.44 | 0.60 | 0.56 | 0.87 |

According to Akehurst, 1968, about 70% of the tobacco crop in South Africa is grown on soils with 50-60% clay, 20-35% sand and 5-30% silt.

Flue-cured tobacco crop favours a pH range of 5.5-6.5 (Akehurst, 1968). According to the soil sample analytical results, the pH value was within the favourable range for the growth of flue-cured tobacco crop. Therefore, there was no need for soil pH adjustments before conducting the trial.

Table 4.2 shows that the soil at this trial site is a clay soil. Soil nematode counting is not a routine for the heavy clay soils of ARC-TCRI because nematodes are most prevalent on light, sandy soils (Akehurst, 1968; and Van Biljon, personal communication). In tobacco, the threshold level of nematode infestation per 100 cubic centimetres of soil, when that nematode species occurs alone, is very low. The threshold level is even lower when two or more nematode genera are present. Crops, like tobacco, that are highly susceptible to nematode infestation allow a rapid build-up of nematode populations from the lowest population densities at transplanting (Keetch and Heyns, 1982; and Clayton, 1958). Based on this notion, a precautionary measure was taken by applying 12 kg of Temik™ G per hectare by banding at the time of ridging to control nematodes that might have possibly been in the soil.

Nitrogen, phosphorus and potassium are the most important major nutritional elements for flue-cured tobacco. The residual amounts (Table 4.2) detected in the soil at the trial site were quite negligible considering the total flue-cured tobacco requirements for proper growth and productivity. Table 4.3 shows the average mineral removals from the soil by a flue-cured tobacco crop, which gave an average yield level of 3769.22 kg/ha (Van Dierendonck 1959). This yield level would be acceptable at the ARC-TCRI and other similar ecological zones even now, considering the average yield level of the currently accepted cultivar, TL33 (2986.22 kg/ha) (Table 5.6).

**Table 4.3:** Average mineral removals (kg/ha) from the soil by a flue-cured tobacco crop (Dierendonck, 1959)

| Mineral | N | P | K | Ca | Mg | Crop yield (kg/ha) |
|---|---|---|---|---|---|---|
| Quantity (kg) | 74 | 22 | 133 | 106 | 27 | 3769.22 |

Fertilizer application regime in this trial was aimed at meeting the flue-cured tobacco fertilizer requirements as much as possible. Therefore, Schedule 4.2 was formulated and followed accordingly.

**Table 4.4:** Contribution of applied fertilizers to N, P and K levels (kg/ha) in the soil according to Schedule 4.2.

| Fertilizer | | 2:3:4: (33) | LAN | KNO3 | 1:0:1 (38) | LAN | Total |
|---|---|---|---|---|---|---|---|
| Contribution | N | 48.00 | 42.84 | 12.32 | 17.48 | 21.84 | 142.48 |
| | P | 96.00 | 00.00 | 00.00 | 00.00 | 00.00 | 96.00 |
| | K | 120.00 | 00.00 | 12.32 | 17.48 | 00.00 | 149.80 |

The values of the applied N, P and K were higher than those recorded by Dierendonck. A fertilizer regime that would give relatively higher N, P and K values (Table 4.4) was chosen to allow for nutrient losses. Despite the low rainfall patterns at the ARC-TCRI exemplified by the rainfall during this season (Figure 4.2), the rains come in heavy storms that tend to be erosive, and likely to cause loss of nutrients through run-off and leaching.

Calcium, sulphur and magnesium are rarely deficient in most soils and their importance is masked by the quantities included in the nitrogen, phosphorus and potassium fertilizer compounds. Trace elements are also rarely deficient (Akehurst, 1968). Therefore, calcium, sulphur, magnesium and the trace elements were not considered in the formulation of the fertilizer application regime.

Ridges were made 120cm apart simultaneously with basal dressing fertilizer application (Schedule 4.2) and Temik™ application at 12 kg/ha for a precautionary measure against nematodes. The trial was laid out in a randomised design, replicated three times (Figure 4.1). The randomisation was done by using AGROBASE version of randomisation. Each gross plot consisted of five ridges. Each ridge was five metres long. Planting holes were marked 50cm apart along the ridges.

At the northern and eastern ends of the trial, there were other tobacco trials. On the southern end, the trial bordered on a natural bush. On the western end, there was a rotational crop, sunhemp.

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **3** | **6** | **9** | **12** | **15** | **18** | **21** | **24** | **27** | **30** | **33** | **36** |
| 102 | 106 | 109 | 112 | 208 | 210 | 212 | 202 | 304 | 305 | 307 | 310 |
| **2** | **5** | **8** | **11** | **14** | **17** | **20** | **23** | **26** | **29** | **32** | **35** |
| 111 | 103 | 107 | 110 | 205 | 201 | 206 | 204 | 306 | 303 | 312 | 301 |
| **1** | **4** | **7** | **10** | **13** | **16** | **19** | **22** | **25** | **28** | **31** | **34** |
| 104 | 108 | 105 | 101 | 203 | 209 | 207 | 211 | 309 | 311 | 302 | 308 |

→ North

**Figure 4.1:** Experimental field plan – Randomised design

The numbers **1-36** represent plot numbers. The numbers 101-112, 201-212, 301-312 represent replications and treatments. The first digits represent the replication numbers, while the second and the third digits together represent the treatment numbers (Table 4.5).

**Table 4.5:** The treatment (T) numbers and the corresponding genotypes (G).

| T | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| G | OD 1 | OD 2 | OD 486B | TL 33 | OD 490 | ODT 92 | ODT 1 | ODT 100 | OD 313 | ODT 19 | ODT 8 | ODT 82 |

## 4.5 Transplanting

Tobacco needs to be transplanted in the field when the soil is at field capacity. The soil moisture at transplanting (November 26, 1998) was equivalent to approximately 103.4 mm/30 days=3.4mm of rainfall per day (Figure 4.2). This soil moisture would not meet the initial crop water-requirements.

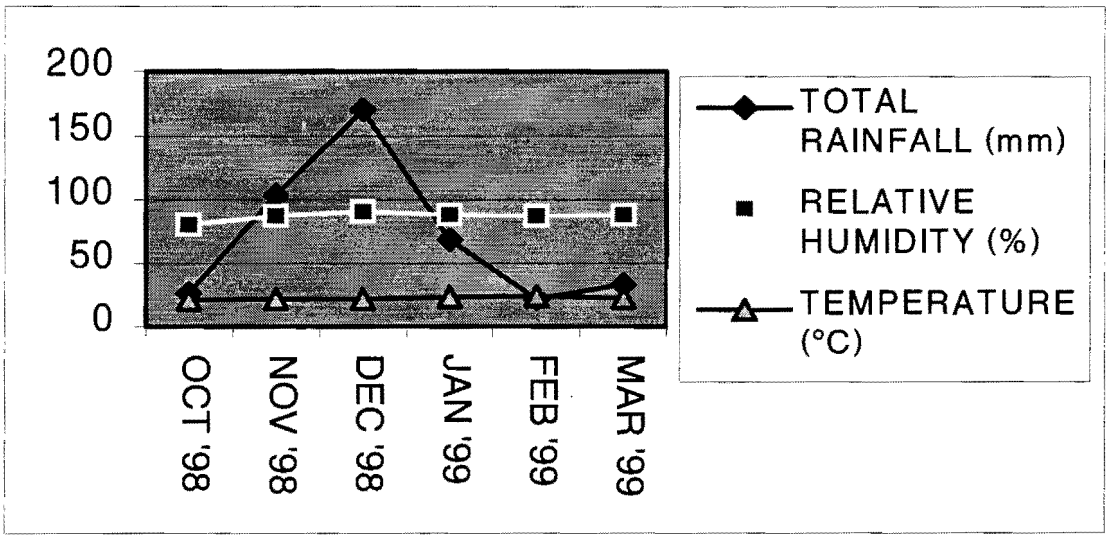



**Figure 4.2:** Weather pattern at the ARC-TCRI during 1998/1999 season.

The y-axis has a common scale of 0-200.

Therefore, arrangements were made for the trial to be water-transplanted. One to two litres of water was poured from a water pipe into each planting hole. Immediately, a planter holding the seedling tray in the left hand used the right hand to pull out from the tray a seedling with an intact mass of nursery growth medium around the roots. The seedling was centrally stuck into the wet soil at the bottom of the planting hole, making sure that the meristem was not submerged in water. As soon as the water was absorbed into the soil at the base of the planting hole, the planting hole was filled with dry soil with the seedling shoot at the centre free of water and mud.

In this manner, ten seedlings were transplanted per ridge. Therefore, with the five ridges per plot, the plot consisted of 50 plants.

Black shank is a disease that is prevalent in the soils of the ARC-TCRI. Therefore, 360g of Ridomil™ WP mixed with 15 litres of water was applied at the rate of 50 millilitres of the mixture per plant after the transplanting operation to control the disease. This application rate required 20.83 kg of Ridomil™ WP per hectare.

## 4.6 Field management

25-38mm of rainfall in a seven to ten-day period, 30-32°C of day temperatures and 18-21°C of night temperatures are necessary conditions for maximum growth of tobacco plants (Hawks, 1978; and Collins and Hawks, 1993). The rainfall regime at the ARC-TCRI (Figure 4.2) was inadequate to meet the crop-water requirement. Therefore, sprinkler irrigation was used, whenever necessary, to supplement the inadequate rainfall. The temperatures were within the required ranges for flue-cured tobacco production (Akehurst, 1968; and Hawks, 1970).
According to the nutritional requirement of flue-cured tobacco (Table 4.3), and the residual nutrient status of the trial site (Table 4.2), Schedule 4.2 for fertilizer application was followed. Split fertilizer application procedure was adopted to minimise excessive nutrient leaching losses that would possibly occur if all the required nutrients were applied in one dose. Akehurst (1968) underlined the necessity of adopting the split application procedure to ensure correct soil nutritional status that influences tobacco cured leaf quality.

**Schedule 4.2:** Fertilizer application regime

| Fertilizer | Application rate (kg/Ha) | Time of application | Type of Application | Mode of application |
|---|---|---|---|---|
| 2:3:4 (33) | 800 | At ridging | Basal dressing | Banding |
| LAN | 153 | 3 weeks AT* | First top dressing | Dollop |
| KNO3 | 88 | 6 weeks AT* | Second top dressing | Dollop |
| 1:0:1 (38) | 92 | 9 weeks AT* | Third top dressing | Dollop |
| LAN | 78 | 12 weeks AT* | Fourth top dressing | Dollop |

AT* = After transplanting

Weeding was done regularly by means of a hoe. When the crop canopy became so closed that using a hoe would lead to leaf damage or leaf drop, any weeds that persisted were pulled out by hand.

When 50% of the plants in any one plot reached the extended bud stage of flowering, the plants were topped. The extended bud along with the flag leaf was clipped off at an angle to prevent stagnation of suckercide on the cut surface. The suckercide, Fair 85™, diluted at 150ml/5 litres of water, was applied at the rate of 8ml per plant (3.88 litres/ha) on the cut surface. The topping angle allowed the suckercide to flow down the stem, 'burning' the axillary buds, until the bottom-most axillary bud. Any surviving suckers were removed by hand.

4.7 Field data collection

Observations of plant and leaf characteristics were recorded from ten randomly selected plants in each net plot. The plant and leaf characteristics included days from transplanting to physiological maturity, chlorophyll ***a*** fluorescence and plant height at topping. Numbers of leaves per plant and leaf lengths and widths were recorded. Leaf areas (LA) were derived from the formula: LA = LL x LWm x factor

(Suggs et al, 1960), where LL = leaf length, LWm = maximum leaf width, and the factor was 0.634. Whole-plant leaf area (WPLA) was calculated using the formula: WPLA = LA x NLP where LA = mean (single) leaf area, and NLP = number of leaves per plant.

4.8 The visual evaluation of the elite breeding lines by the representatives of the tobacco industry.

Representatives of the tobacco growers' cooperatives, extension officers, leaf dealers and manufacturers were invited to evaluate the different elite breeding lines at the ARC-TCRI. Unfortunately, this particular trial was not evaluated because of time constraint. However, in a similar exercise conducted in 1997/1998 growing season, a similar trial with some of its entries also found in this trial, was evaluated. The representatives recorded the scores of the elite breeding lines on evaluation cards (Appendix 3) using the scale of 1-5. One represents a line, which is totally unacceptable. Five represents a perfect line.

4.9 Harvesting of the tobacco leaf

Signs of maturity start to appear in the bottom-most leaves. The signs include yellowing of the leaf; the drooping of the leaf to a right or an obtuse angle with the stem; and twisting and curling of the leaf. A dull, mottled and wrinkled surface of the leaf (Photographs in Appendix 8) and the brittleness of the lamina were yet other easily noticeable signs of the maturity of the leaf.

Harvesting of the leaf was started seven days after the last topping operation. One reaping was done per week. Three to four bottom-most leaves were plucked at every reaping. The harvested leaves were clipped in bulk racks. The racks with the tobacco leaf were suspended on rails mounted on a tractor-trailer ready for transportation to the curing ovens. The harvested tobacco leaf was always covered to protect it against the scorching effect of the hot sun. Harvesting of the

leaf was completed in six reaping operations.

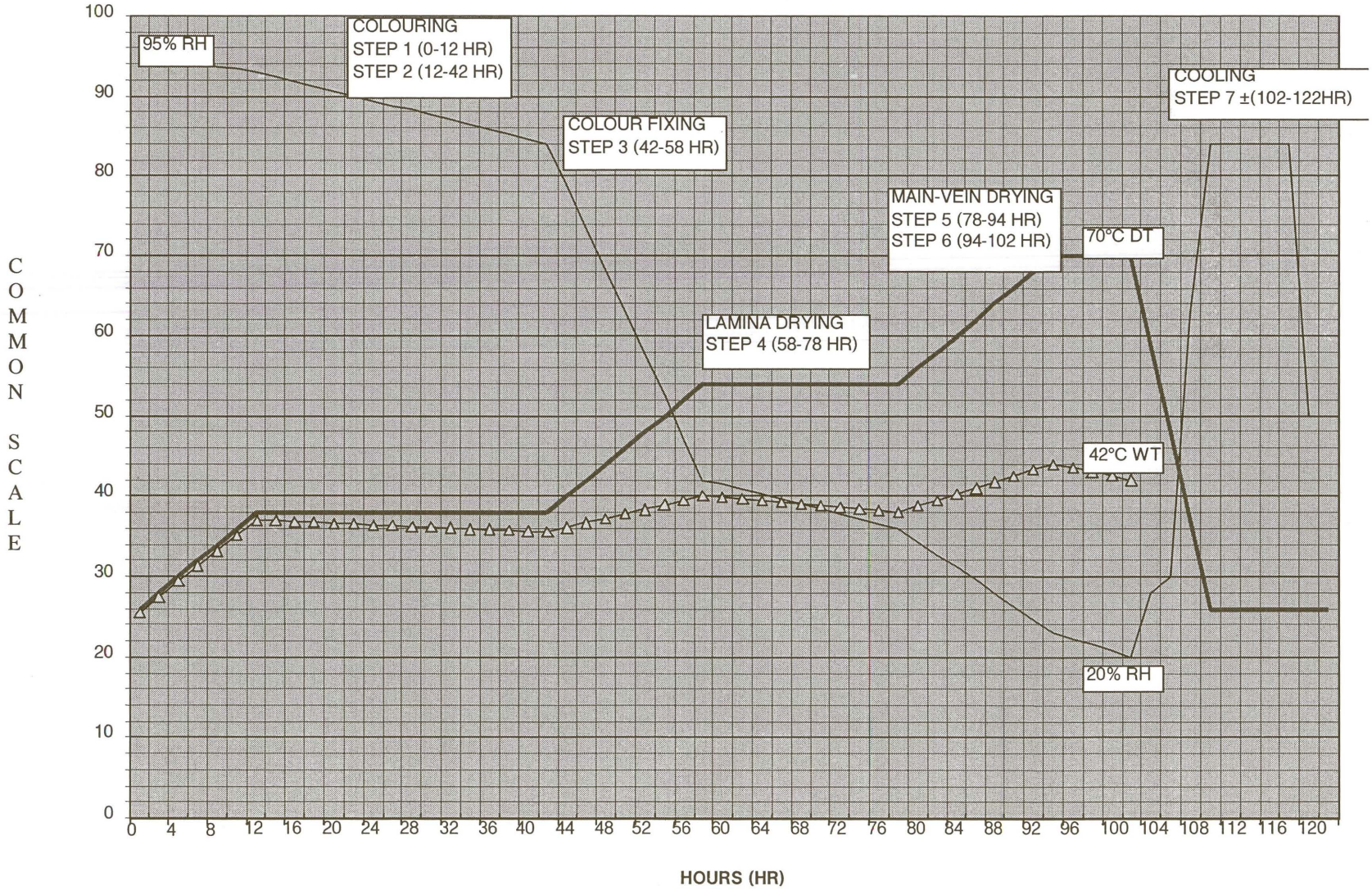
## 4.10 Post-harvest management of the tobacco leaf

The racks with the tobacco leaf were packed in a bulk oven until the oven was full. The bulk oven operates on a hot-water system. The curing of the leaf was accomplished by means of hot air, pre-heated by a water heat exchanger, using coal as fuel. Schedule 4.3 was used as a guide to the tobacco curing procedure.

**Schedule 4.3:** A guide to the curing procedure of flue-cured tobacco

DT Dry bulb thermometer temperature reading (°C) WT Wet bulb thermometer temperature reading (°C)

RH Relative humidity (%)



The cured leaf is normally brittle. However, it is highly hygroscopic. Therefore, the leaf was conditioned by mist sprays in readiness for grading.

The grading was done according to leaf colours, quality degree, curing pattern, body, grain, maturity, damage and the position of the leaf on the stalk in accordance with the description of the grades (Anonymous, 1968).

The previous year's tobacco grade indices (cents/kg) were used to determine the monetary returns per hectare.

Data on leaf yield, leaf quality expressed as grade indices, and monetary returns were determined from whole net plots and expressed in units per hectare wherever necessary.

Cured leaf samples representative of all leaf positions on the stalk were taken and sent to the biochemistry laboratory for determination of nicotine and reducing sugar concentrations in the leaf.

### 4.11 Statistical analyses of data

A computer statistical program written by Vorster (personal communication) was used to perform the analyses of variance for all the sets of data. Vorster's program transforms the raw data into tables of means and then uses the tables of means to perform the analyses. The completed analyses show the performance of the treatments and the corresponding statistics, ready for interpretation (Appendices 1, 2, 4, 5, 6, and 7).

The correlation analyses of the parameters of field performance were performed by means of a calculator. The parameters included the days that the plants took to reach physiological maturity, plant height at topping, number of leaves per plant, whole-plant leaf area and yield. The aim of carrying out the correlation

analyses was to isolate the parameters that constitute the most important yield components of the elite breeding lines being tested.

Statistical comparisons of the field and market performance of the elite breeding lines and the control, TL33, were made at the 0.05 level of probability, unless otherwise stated.

The results from a single trial at Rustenburg could not be accurate enough to serve as the basis for meaningful conclusions because of the inherent inaccuracy of a single trial at a single locality. Therefore, further investigation took into account three other similar trials at Groblersdal in Mpumalanga Province, Potgietersrus in the Northern Province and Vaalwater also in the Northern Province.

The plant breeding department of the ARC-TCRI evaluated the same elite breeding lines at the other three aforementioned localities during the same season, 1998/1999. The same experimental design, number of replications, plot sizes and the control cultivar as those used in the trial at Rustenburg were used at the other three localities.

The trials at the other three sites were carried out according to the protocol (unpublished) recorded in 'LNR Tabak Proefboek: Afdeling Teling' (Vorster, 1998) which was, basically, the same as that followed in conducting the trial at Rustenburg. Therefore, it is not necessary to discuss the details of the procedures of the other three similar trials.

With the kind permission of the plant-breeding department of the ARC-TCRI, a combined analysis, using the AGROBASE, was run on yield, quality and income data from the other three localities and those from the trial at Rustenburg.
ARC-TCRI has up to eleven trial sites for the evaluation of the elite breeding lines the choice of which was dictated by the demand from certain tobacco growers'

co-operatives and the differences in climate among the sites. The data from all these sites could not be used in the combined analysis as some trials had entries, which were not included in the trial at Rustenburg. Furthermore, the trials at some sites suffered hail damage so that it would be an unfair comparison towards such sites if they were to be included in the combined analysis.

From the aforementioned factors, it may be understood that the use of data from Groblersdal, Potgietersrus and Vaalwater was not by design, but rather by the mere fact of the unavailability of data from identical trials in other localities.

The combined analysis showed that ODT82, ODT92, OD2 and OD1 performed significantly better than TL33 across the localities and that they could do equally well at all the localities. This generalised observation aroused the curiosity to examine the genotype-locality interaction variance. The Additive Main effects and Multiplicative Interaction (AMMI) analysis was carried out mainly to investigate the genotype-locality interaction variance.

# CHAPTER 5

## Results and discussion

### 5.1 The field performance of the elite breeding lines in the Rustenburg trial

No significant differences were detected among the genotypes in the number of days that the plants took from transplanting to physiological maturity (DTPM) (Table 5.1). This may imply that a grower would not incur additional field operational costs for substituting any one of the elite breeding lines for TL33. The differences in DTPM among replications were significant possibly due to border effects. The trial was located at the edge of the field, which boundaries on a natural bush with tall trees (Figure 4.1). Therefore, the trial might have suffered the border effect of the natural bush. It was difficult to avoid the border effect because this was the only piece of land that was available for tobacco trials according to the rotation system at the ARC-TCRI. However, efforts were made to orient the trial in such a way that only the first replication was close to the natural bush. The second replication was behind the first replication. The third replication was the furthest away from the natural bush. The first replication might have been the source of the significant differences in the DTPM among the replications.

Chlorophyll ***a*** fluorescence measurements showed non-significant differences among both the genotypes and the replications. According to Dippenaar et al. (1991), the photosynthetic rate of a green tobacco leaf is already at the maximum at a low light intensity. The hypothesis that the performances of the elite breeding lines would deviate from that of TL33 due to differences in leaf photosynthetic efficiencies was rejected at both the 0.05 and 0.01 probability levels (Table 5.1).

Table 5.1 shows differences in plant height at topping that occurred among the genotypes and replications. ODT82 (124.27 cm), OD2 (122.47 cm), ODT92

(122.20 cm), ODT100 (119.67 cm), and OD490 (118.73 cm) gave crop stands of significantly taller plants than TL33 at both 0.05 and 0.01 probability levels. The differences in PHT among replications occurred possibly due to the border effect of the natural bush on the first replication.

The differences in the number leaves per plant (NLP) were highly significant among the genotypes. ODT82 (25), OD486B (24) and ODT100 (24) gave a statistically higher number of leaves per plant than the control, TL33 (22), by 13.6%, 9.1%, and 9.1% respectively. At the 0.01 level of probability, ODT82 produced more leaves than TL33 (Table 5.1). The highly significant differences in the NLP among the treatments suggest that the treatments have different genetic backgrounds although from within the same American gene pool (Table 4.1). No significant differences existed among the replications. Physiologically, the number of leaves that a plant initiates and is able to produce is a genetically determined character (Dippenaar, personal communication). Therefore, it was not surprising to note that non-significant differences in the NLP existed among replications despite the effect of the adjacent natural bush that affected the performance of the plants in the first replication.

Table 5.2 shows lengths, widths and areas of the second-bottom leaves, which represent the lugs (the bottom leaves on the plant). The differences in the second-bottom leaf lengths were highly significant among the genotypes. Replications also showed statistical differences. OD490, OD1, and OD2 produced second-bottom leaves of statistically longer laminae (62.13cm, 59.30cm, and 58.37cm respectively) than those of TL33 (46.40cm). At the 0.01 level of probability, only OD490 showed a highly significant difference from TL33. The differences in the widths of the second-bottom leaves were statistically high among the genotypes, but not significant among the replications. However, none of the elite breeding lines produced leaves, which were statistically wider than those of TL33 (23.07cm). A comparison of the surface areas of the second-bottom leaves showed highly significant differences among the genotypes.

OD490, OD2, OD486B, and OD1 gave second-bottom leaves with larger leaf areas (1556.13cm$^2$, 1406.83cm$^2$, 1250.10cm$^2$ and 1204.60cm$^2$ respectively) than those of the control, TL33 (683.03cm$^2$). At the 0.01 probability level, OD490, OD2 and OD486B still demonstrated the potential to give second-bottom leaves with larger areas than those of TL33.

Table 5.3 illustrates statistical differences that occurred in the lengths of the tenth leaves, which represent the cutters (the lower-middle leaves on the plant) and the main leaves (the upper-middle leaves on the plant) of the genotypes. OD1 produced longer tenth leaves (77.03cm) than the control, TL33 (66.97cm). There were no significant differences between TL33 and each of the other elite breeding lines. Table 5.3 demonstrates that OD2 gave wider tenth-leaf blades (39.97cm) than TL33 (30.93cm). The other elite breeding lines had non-significant differences in the tenth-leaf widths from those of TL33. The tenth-leaf areas were highly variable among the genotypes. OD2 gave significantly larger tenth-leaf areas (1909.47cm$^2$) than those of TL33 (1320.20cm$^2$). No statistical differences were detected between TL33 and each of the other elite breeding lines.

Highly significant differences from TL33 were noted among the elite breeding lines in the lengths of the eighteenth-leaves, which represent the strips (the top-most leaves on the plant) (Table 5.4). ODT92 (73.37cm), ODT19 (72.33cm), ODT100 (71.87cm), and ODT82 (71.67cm) gave longer strips than TL33 (56.30cm). The widths of the eighteenth-leaves also showed highly significant differences. OD1 and OD2 gave greater eighteenth-leaf widths (32.33cm and 32.33cm respectively) than TL33 (23.13cm). Table 5.4 also shows the considerable variation that occurred among the genotypes in the eighteenth-leaf areas. ODT92 produced the eighteenth leaves with larger areas (1476.60cm$^2$) than those of TL33 (825.47cm$^2$).

There were highly significant differences in single-leaf areas among the genotypes and replications. OD490, OD2, and OD1 had significantly larger single-leaf areas (1585.70cm$^2$, 1565.10cm$^2$, and 1429.43cm$^2$ respectively) than those of TL33 (942.90 cm$^2$) (Table 5.5). At the 0.01 level of probability, OD2 and OD490 gave leaves with statistically larger single-leaf areas than those of TL33. Mean whole-plant leaf areas, WPLA, also showed highly significant differences among the genotypes and the replications (Table 5.5). Whole-plant leaf areas which were significantly larger than those of TL33 (20408.95cm$^2$) were obtained with OD1 and OD490 (33263.11cm$^2$ and 32350.00cm$^2$ respectively). All the other elite breeding lines displayed whole-plant leaf areas that were non-significantly different from those of TL33.

A tobacco grower who substitutes either OD1 or OD490 for TL33 would increase the total leaf area per plant by 62.98% or 58.51% respectively (Table 5.5).

Significant differences were detected in the yield levels among the genotypes (Table 5.6). These differences were based on the statistical comparisons between the highest and the lowest performing genotypes. However, none of the elite breeding lines yielded significantly better or worse than the currently ruling cultivar, TL33 (2986.22 kg/ha). The yields were non-significantly different among replications despite the border effect on the first replication, possibly because the greater WPLA in plants of the other replications might have been accompanied by a decrease in weight per unit area (Akehurst, 1968). The effect of the natural bush might have had an impact only on the crop that was closest to the natural bush in the first replication.

Unmarketable throwaway masses described as dip masses arise from heavy disease infection, insect damage and physical damage. Harvesting the leaf when it is immature or overripe may result in large dip masses coming out of the curing oven. Poor curing process and post-cure handling of the leaf may also give rise to large dip masses. The dip masses did not differ among both the genotypes

and the replications. Consequently, the differences in the marketable yield levels followed a trend that was similar to that of the total yield levels (Table 5.6).

The correlation analyses (Table 5.7) showed that plant height at topping (PHT) and whole-plant leaf area (WPLA), PHT and yield, and WPLA and yield had significant correlations at 0.10 probability level. This may imply that PHT and WPLA are the most important yield components in these elite breeding lines.

Table 5.8 illustrates the scores of the elite breeding lines according to the visual evaluation by the representatives of the tobacco industry. ODT82, OD490, OD2, ODT100 and OD1 were generally rated better than TL33.

5.2 The market performance of the elite breeding lines in the Rustenburg trial

The F-values of the leaf quality components (Table 5.9) indicated non-significant differences among the genotypes and replications except for the Z-grade (closed grain) among the replications. This Z grade is usually of a poor quality, thin-bodied leaf with weak colour intensity, which might be punctuated with green tinges. The Z-grade is associated with harvesting of immature leaf. Possibly, some leaves in the first replication might have been harvested prematurely. The immaturity of the leaf might have been masked by the forced aging of the plants due to the border effect of the natural bush that shared boundaries with the first replication. The forced aging was evidenced by the significant differences in days from transplanting to physiological maturity and plant height at topping among the replications (Table 5.1).

There were non-significant differences among the entries and the replications in the concentration of nicotine in the cured leaves. The concentration of reducing sugars showed highly significant differences among the genotypes. However, no statistical differences could be detected between any one of the elite breeding lines and the control, TL33 (Table 5.10). The breeding lines conform to the

acceptable nicotine/reducing sugar concentration ratio of approximately 1:8, which is routinely identified in flue-cured tobacco (Hawks, 1978).

According to Table 5.11, no statistical differences in grade indices (cents/kg) existed among the genotypes. The highly significant differences in grade indices among the replications might have been a direct reflection of the highly significant differences in the Z-grade among the replications (Table 5.9).

There were significant differences in total and market income among the genotypes. The differences were based on the comparison between the highest and the lowest total and market income levels. The comparisons between each elite breeding line and TL33 did not show any significant differences at all.

The non-significant differences observed between the elite breeding lines and the control, TL33, could be explained from the narrow genetic basis as well as from the inaccuracy inherent in one experiment at a single locality. Referring to the pedigrees of the elite breeding lines and the control (Table 4.1), two features can be identified. Firstly, some parents are common in certain pedigrees. Secondly, many pedigrees show that the parents were well-adapted cultivars of American gene pool. Therefore, the non-significant differences could be a consequence of common ancestry and use of well-adapted and closely related cultivars as parents to create new populations from which to make selections. The use of well-adapted parents results in cultivars that are closely related, and the use of closely related parents results in the reduction of genetic diversity and gains from selection (Thompson and Nelson, 1998; Bowman et al, 1984). On the other hand, the differences among the genotypes may be large enough to show up with experimentation that is more accurate than one at a single locality.

## 5.3 Genotype-locality interaction

Table 5.12 shows the mean yield, quality and income levels of the elite breeding lines at Groblersdal, Potgietersrus, Rustenburg and Vaalwater. It might be of interest to note that no significant differences existed in yield, quality and income between any one of the elite breeding lines and TL33 at the four individual sites.

### 5.3.1 Combined analysis of data from the four localities using the AGROBASE program

Table 5.13 shows the results of the combined analysis of the data from the four localities. The differences in the marketable yields, quality and market income levels of the entries due to the localities were highly significant. This strongly suggests that the localities are different.

The differences in yields among the entries across the localities were highly significant. ODT92 (2904.25 kg/ha), ODT82 (2873.44 kg/ha), OD2 (2826.31 kg/ha) and OD1 (2607.03 kg/ha) produced significantly higher yields than TL33 (2142.36 kg/ha) by 36%, 34% and 32% and 22% respectively. At 0.01 probability level, the difference between any one of ODT92, ODT82 and OD2 and TL33 was significant (Table 5.13). ODT92, ODT82, OD2 and OD1 were not themselves significantly different in their yielding potentials.

The grade indices, expressed as cured leaf quality and measured in cents per kilogram of leaf, did not show any statistical differences among the entries across the localities (Table 5.13).

There were highly significant differences in market income per hectare among the entries across the localities. ODT82 (R46256.03/ha), ODT92 (R43575.84) and OD2 (R40850.28/ha) were significantly more financially rewarding than TL33 (R32105.58/ha) by 44%, 36% and 27% respectively (Table 5.13). However,

ODT82, ODT92 and OD2 were not statistically different from each other. AT 0.01 probability level, ODT82 and ODT92 generated more income per hectare than TL33.

The genotype-locality interaction was non-significant for yield, cured leaf quality and income.

The combined analysis using the AGROBASE program (Table 5.13) showed three important things. Firstly, the localities are different. Secondly, the performances of the elite breeding lines did not change significantly with localities. Thirdly, although there were highly significant differences among the entries, the three elite breeding lines; ODT82, ODT92 and OD2 that were economically better than TL33 could do equally well in all the four localities.

The third general observation aroused the curiosity for further investigation of the interaction variance to see if there is any basis for the preference of specific elite breeding lines at certain localities.

### 5.3.2 The AMMI analysis

The results of the AMMI analyses confirmed the significant differences among the localities and among the entries and the non-significant interaction between the localities and the entries in terms of both the yield and income levels.

The partitioning of the interaction variance showed that the first Interaction Principal Component Analysis (IPCA1) was non-significant at 5% probability level for both yield and income levels (Table 5.14 and Table 5.15).

Although IPCA1 was not significant for both yield and income, the AMMI analysis demonstrated its potential application in predicting the genotypic adaptability to particular localities. The biplots (Figure 5.1 and Figure 5.2) provide graphical

summaries of the interactions among the genotypes and the localities. Various agriculturally important interaction patterns may be perceived from the biplots.

The main effects and the IPCA scores tend to be indicative of the agricultural qualities of the localities and the adaptability of the genotypes to the particular localities.

The biplot in conjunction with the AMMI model equation could serve as a useful tool for estimating the performance of the genotypes in the different localities being studied. The AMMI model equation says that the estimated genotypic performance, $Y = M_G + M_L - GM + (IPCA1_G \times IPCA1_L)$. $M_G$ is the genotype mean, $M_L$ is the locality mean, GM is the general mean, which together make the additive component of the AMMI model equation. The genotype IPCA1 ($IPCA1_G$) and the locality IPCA1 ($IPCA1_L$) make the multiplicative component of the AMMI model equation (Gauch and Zobel, 1996; Smith, 1995).

ODT82 (2873 kg/ha) and ODT92 (2904 kg/ha) produced almost equal mean yields (Figure 5.1 and Table 5.13) across localities. However, the AMMI selections (AMMI Table 1) predicted that ODT82 would be the best-adapted genotype at Potgietersrus while ODT92 would be the best-adapted genotype at Vaalwater.

The AMMI biplots of genotype and environment IPCA scores versus mean yields (Figure 5.1) and income (Figure 5.2) explain the reasoning behind the differences in the adaptability of the different genotypes to the different localities.

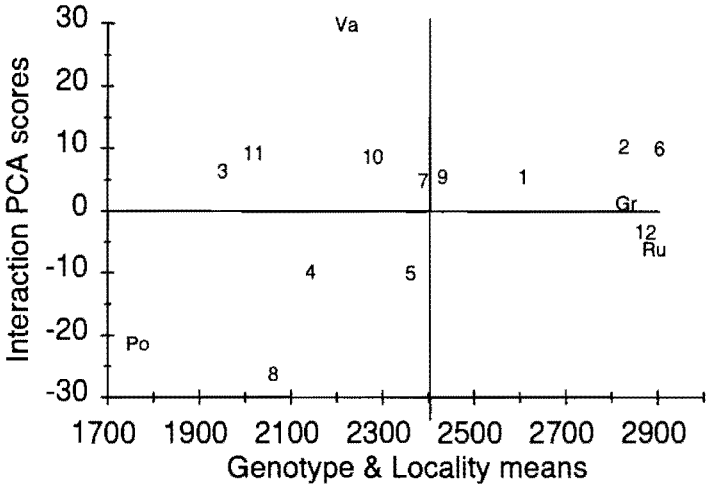



**Figure 5.1:** Plot of IPCA scores versus yield means for genotype and locality

Localities: Gr Groblersdal; Po Potgietersrus; Ru Rustenburg; Va Vaalwater

The treatments in Figure 5.1 have been represented by numbers 1-12 as follows, according to Table 4.5, for clarity.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | OD1 | 2 | OD2 | 3 | OD486B | 4 | TL33 |
| 5 | OD490 | 6 | ODT92 | 7 | ODT1 | 8 | ODT100 |
| 9 | OD313 | 10 | ODT19 | 11 | ODT8 | 12 | ODT82 |

**AMMI Table 1:** Table of the AMMI genotype selections for adaptability to particular localities in terms of yield according to Figure 5.1

| Locality | AMMI selections | | | |
|---|---|---|---|---|
| | Best | Second best | Third best | Fourth best |
| Groblersdal | ODT92 | ODT82 | OD2 | OD1 |
| Potgietersrus | ODT82 | ODT92 | ODT100 | OD2 |
| Rustenburg | ODT82 | ODT92 | OD2 | OD1 |
| Vaalwater | ODT92 | OD2 | ODT82 | OD1 |

Figure 5.1 explains that ODT82 and ODT92 tend to be similar by main effects, but differ by interaction. Potgietersrus and Rustenburg differ by both the main

effects on the x-axis and the IPCA scores on the y-axis, but both had negative IPCA scores. ODT82 also had a negative IPCA score. Therefore, the multiplicative component of the AMMI model for ODT82 at both localities had a positive effect on yield. ODT92 had a positive IPCA score. The multiplicative component of the AMMI model for ODT92 at Potgietersrus and Rustenburg had a negative effect on yield. Hence, the AMMI selection for ODT82 as the best-adapted genotype at Potgietersrus and Rustenburg. Similarly, Groblersdal, Vaalwater and ODT92 had positive IPCA scores. The multiplicative component of the AMMI model for ODT92 at Groblersdal and Vaalwater had positive effects on yields. The multiplicative component of the AMMI model for ODT82 at Groblersdal and Vaalwater had a negative effect on yields. Hence, the AMMI selection for ODT92 as the best-adapted genotype at Groblersdal and Vaalwater.

Although ODT92 had a negative effect on yield at Potgietersrus and Rustenburg, the AMMI selections rated it the second best-adapted genotype at Potgietersrus and Rustenburg due to its high level of the main effect. Similarly, ODT82 was rated the second best-adapted genotype at Groblersdal.

OD2 and ODT92 tended to be similar in interaction on the y-axis, but different in main effects on the x-axis. The AMMI selections rated OD2 second to ODT92 in its adaptability at Vaalwater possibly due to the high level of its main effect and its positive IPCA score. Similar arguments account for the subsequent AMMI selection of genotypes for particular localities.

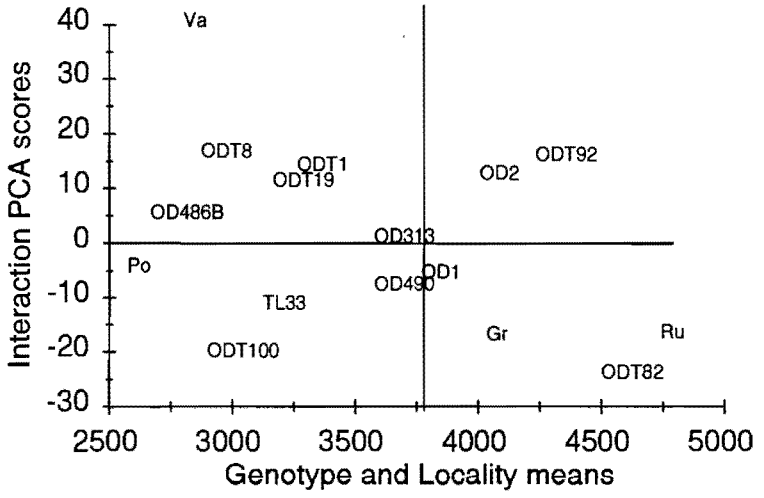



**Figure 5.2:** Plot of IPCA scores versus income means (in tens) for genotype and locality.

Localities: Gr Groblersdal Po Potgietersrus,
Ru Rustenburg Va Vaalwater

**AMMI Table 2:** Table of the AMMI genotype selections for adaptability in particular localities in terms of income according to Figure 5.2.

| Locality | AMMI selections | | | |
|---|---|---|---|---|
| | Best | Second best | Third best | Fourth best |
| Groblersdal | ODT82 | ODT92 | OD1 | OD2 |
| Potgietersrus | ODT82 | ODT92 | OD2 | OD1 |
| Rustenburg | ODT82 | ODT92 | OD1 | OD2 |
| Vaalwater | ODT92 | OD2 | ODT1 | ODT19 |

Figure 5.2 shows that although Groblersdal, Potgietersrus and Rustenburg tend to differ mostly by the main effects on the x-axis, they have negative IPCA scores. ODT82 had a high level of the main effect and a negative IPCA score. The multiplicative component of the AMMI model for ODT82 at Rustenburg, Groblersdal and Potgietersrus had positive effects on income levels. ODT92 had

its main effect just below that of ODT82, but had a positive IPCA score. The multiplicative component of the AMMI model for ODT92 at Groblersdal, Potgietersrus and Rustenburg had negative effects on income levels, whilst at Vaalwater it had a positive effect on income. Therefore, the AMMI selected ODT82 as the best-adapted genotype at Groblersdal, Potgietersrus and Rustenburg; and ODT92 at Vaalwater.

The multiplicative component of the AMMI model for ODT92 at Groblersdal, Potgietersrus and Rustenburg had negative effects on income levels. However, the AMMI selections indicate ODT92 as the second best-adapted genotype to Groblersdal, Potgietersrus and Rustenburg possibly due to the high level of the main effect.

OD2 had a positive IPCA score and tended to be similar to ODT92 in interaction. Therefore, the multiplicative component of the AMMI model for OD2 at Vaalwater had a positive effect on income. Hence, the AMMI selection for OD2 as the second best-adapted genotype to Vaalwater.

Likewise, the level of the main effects and the IPCA scores explain the agricultural qualities of the localities and the adaptability of the other different genotypes to the particular localities.

# CHAPTER 6

## Conclusions and recommendations

The objective of this work was to evaluate the elite breeding lines of flue-cured tobacco for their field and market performance.

The single trial at Rustenburg showed that plant height at topping and whole-plant leaf area could be the most important yield components of the elite breeding lines. The nicotine and reducing sugar concentrations of the elite breeding lines conform to the acceptable levels according to the control, TL33. The smoke flavour and aroma profile of the elite breeding lines may meet the demands of the consumer. The similarity in texture between the elite breeding lines and TL33 might imply that the filling power and the cigarette out-turn of the elite breeding lines may be similar to that of TL33 to the economic benefit of the manufacturer. Non-significant differences existed in yield, quality and income between the elite breeding lines and TL33. These results could not be accurate enough to serve as the basis for meaningful conclusions and recommendations because of the inherent inaccuracy of one trial at a single locality.

More accurate observations of the performance of the elite breeding lines could be obtained when the data from the trial at Rustenburg were combined with the data from other similar trials at Groblersdal, Potgietersrus and Vaalwater in a combined analysis.

The combined analysis demonstrated that ODT92, ODT82, OD2 and OD1 had the potential to produce significantly higher economic yields than TL33 across the localities. ODT82, ODT92 and OD2 would be significantly more economical than TL33. The localities were significantly different. The genotype-locality interaction was non-significant.

Although the IPCA1 was non-significant, the pattern of interaction in the AMMI analysis predicted that ODT82 and ODT92 would be the most economically viable genotypes at Groblersdal, Potgietersrus and Rustenburg. Similarly, ODT92 and OD2 would be the most economical genotypes for Vaalwater. This pattern of interaction seems to be interesting enough to warrant further investigation of the adaptation of the lines over a wider range of environments.

In tobacco, income is dependent on both yield and quality. However, yield dominates the objectives of all plant breeding programs (Stoskopf et al., 1993; and Simmonds, 1987). Therefore, the programmes might not be fully addressing the problems of low producer income to which the low investment in the tobacco industry could be attributed. Dippenaar et al. (1991) suggested that high yields of acceptable quality need to be produced so that a grower may realise high income to meet the production costs and invest more in the business of tobacco production. Therefore, a holistic approach of crop improvement as advocated by Wallace and Yan (1998), where the traits responsible for yield and quality are pursued concurrently, may be worthwhile.

The single trial at Rustenburg alone could not show any differences between the elite breeding lines and TL33. A combined analysis of data from four different localities detected some differences among the entries. These results tended to be in contrast with Greeff's work (1986) on cotton where few trials in a fixed location evaluation system and multiple district trial evaluation system are equally efficient methods of evaluating the performance of cultivars. In tobacco, a satisfactory evaluation of cultivars should involve as many sets of data from as many different localities as possible to eliminate the inaccuracy inherent in one or few trials at a single or few localities.

The ARC-TCRI flue-cured tobacco-breeding programme can be commended for its linkage with the industry. The research-industry linkage enables the researchers to identify the problems of the industry for research action while

keeping the industry well informed about the research developments. Consequently, the proportion of laggards may be small to the benefit of the industry and the national economy.

The ARC-TCRI flue-cured tobacco research programme uses large plot sizes in the evaluation of elite breeding lines. Five ridges with ten plants per ridge allow the collection of many different sets of data from both the destructive and non-destructive sampling procedures without sacrificing the accuracy of results from acceptable sample sizes.

The ARC-TCRI has eleven sites for evaluating elite breeding lines. Such a large number of sites may generate volumes of data that are large enough to collectively detect genotypic differences effectively. However, the non-uniformity of entries in some sites, makes the combined analysis difficult. Data from each site or few sites are analysed separately and conclusions made accordingly. According to the findings of this work, such a programme harbours the inherent inaccuracy of one or few trials at a single or few localities.

This exercise has demonstrated that numerical comparisons of data without restraint by statistical inference procedures may be deceptive. Statistical inferences provide reliable leads to in-depth investigation for refined results that may reveal valuable information necessary for meaningful conclusions and recommendations. Different statistical tools may complement each other in describing genotypic responses to environmental qualities. Eager search for statistical significance may be viewed as statistical pedantry as Greeff (1986) believed, but with proper restraint, it provides a scientific basis for conclusive recommendations.

## Summary

Tobacco production is a profitable and job-creating agricultural enterprise. South Africa is the 25th most important tobacco producer in the world.

The problem of the tobacco industry in South Africa is production of low-quality leaf due to unreliable rainfall and poor soils. The low-quality leaf and the activities of the anti-smoking lobby led to the dwindling of local and export markets.

Breeding for high yields of acceptable quality underpins the revival and sustainability of the tobacco industry in South Africa. The objective of this work was to evaluate the elite breeding lines of flue-cured tobacco for their field and market performance.

Eleven elite breeding lines of flue-cured tobacco; OD1, OD2, ODT1, ODT8, ODT19, ODT82, ODT92, ODT100, OD313, OD490 and OD486B were evaluated at the ARC-TCRI in Rustenburg in 1998/1999. TL33 was used as a control. Crop growth duration, photosynthetic competence, plant height at topping, number of leaves per plant, leaf area and yield were investigated as parameters of field performance. The nicotine and reducing sugar concentrations, cured-leaf quality and monetary returns per hectare were investigated as parameters of market performance.

Correlation analyses revealed that plant height at topping and whole-plant leaf area might be the most important yield components of the elite breeding lines.

There were non-significant differences in the marketable yields, nicotine and reducing sugar concentrations, cured leaf quality and market income between the elite breeding lines and TL33.

The non-significant differences could be attributed to either the restricted genetic

advance that is due to the common ancestry and the limited genetic base of *Nicotiana tabacum* or the inherent inaccuracy of one trial at a single locality.

No meaningful conclusions could be made from these findings from one trial at a single locality. Data from three other similar trials at Groblersdal, Potgietersrus and Vaalwater were combined with those from the trial at Rustenburg. A combined analysis was run so that accurate information regarding the performance of the elite breeding lines could be arrived at for meaningful conclusions.

The combined analysis showed that significant differences existed among the localities and among the entries. ODT92 ODT82, OD2 and OD1 produced significantly higher yields than TL33 across the four localities. However, the four elite breeding lines were not significantly different from each other. ODT82, ODT92 and OD2 gave significantly higher market income per hectare than TL33 across the four localities, but the three elite breeding lines were not significantly different from each other. The differences in the performance of the elite breeding lines due to the interaction between the localities and the entries were non-significant.

The Additive Main effects and Multiplicative Interaction (AMMI) analysis was employed to partition the genotype-locality interaction variance for further investigation. The first Interaction Principal Component Analysis (IPCA1) was non-significant. However, the AMMI analysis predicted that ODT82 and ODT92 would be the best-adapted genotypes to Groblersdal, Potgietersrus and Rustenburg in terms of both yields and financial rewards. ODT92 and OD2 were predicted to be the most productive and economically viable genotypes for Vaalwater.

ODT82 and ODT92 were recommended for on-farm trials at Groblersdal, Potgietersrus and Rustenburg, while ODT92 and OD2 were recommended for

on-farm trials at Vaalwater pending their release as commercial flue-cured tobacco cultivars at those respective localities.

A holistic approach to crop improvement, where the traits responsible for high yields and quality are pursued concurrently, may alleviate the problem of low producer income to which the low investment in the tobacco industry could be attributed.

A number of trials at different localities would be recommended to eliminate inherent inaccuracy of single trials at single localities.

Research programmes may need to be open to the opinions from the industry and be flexible to accommodate demands from the industry without losing sight of the objectives of the programmes. It is a requirement to adhere to the scientific procedures of arriving at conclusions to make sound recommendations.

## Tables of results

**Table 5.1:** Days to physiological maturity, chlorophyll *a* fluorescence, plant height (cm) at topping and number of leaves per plant of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Genotype | Days to maturity | Chlorophyll *a* fluorescence | Plant height at topping | Leaves per plant |
|---|---|---|---|---|
| ODT82 | 57 | 0.76 | 124.27** | 25** |
| OD486B | 58 | 0.79 | 112.23 | 24* |
| ODT100 | 56 | 0.70 | 119.67** | 24* |
| ODT1 | 55 | 0.81 | 111.80 | 23 |
| OD1 | 58 | 0.73 | 111.47 | 23 |
| ODT8 | 56 | 0.79 | 110.47 | 23 |
| OD313 | 56 | 0.77 | 104.93 | 22 |
| ODT92 | 55 | 0.69 | 122.20** | 22 |
| ODT19 | 54 | 0.79 | 115.03 | 22 |
| TL33 | 54 | 0.79 | 108.40 | 22 |
| OD2 | 55 | 0.53 | 122.47** | 20 |
| OD490 | 57 | 0.78 | 118.73** | 20 |
| F-value (Reps) | * | NS | * | NS |
| F-value (Gen.) | NS | NS | ** | ** |
| LSD (5%) | 4.46 | 0.36 | 7.53 | 1.76 |
| LSD (1%) | 5.43 | 0.43 | 9.15 | 2.14 |
| CV (%) | 3.14 | 18.84 | 2.57 | 3.06 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.2:** Lengths, widths and areas of the second-bottom leaves of the elite breeding lines at the ARC-TCRI in 1998/1999 season.

| Genotype | Second-leaf length (cm) | Second-leaf width (cm) | Second-leaf area ($cm^2$) |
|---|---|---|---|
| OD490 | 62.13** | 39.47 | 1556.13** |
| OD2 | 58.67* | 37.77 | 1406.83** |
| OD486B | 56.53 | 23.07 | 1250.10** |
| OD1 | 59.30* | 31.93 | 1204.60* |
| ODT1 | 52.43 | 27.57 | 919.67 |
| ODT19 | 54.53 | 25.67 | 893.63 |
| ODT92 | 57.03 | 22.20 | 802.47 |
| OD313 | 46.57 | 23.53 | 694.17 |
| TL33 | 46.40 | 23.07 | 683.03 |
| ODT82 | 48.00 | 19.53 | 603.30 |
| ODT100 | 47.30 | 20.00 | 602.47 |
| ODT8 | 45.23 | 18.27 | 523.27 |
| F-value (Reps) | * | NS | NS |
| F-value (Gen.) | ** | ** | ** |
| LSD (5 %) | 11.59 | 17.03 | 432.25 |
| LSD (1 %) | 14.09 | 20.70 | 525.55 |
| C.V. (%) | 8.61 | 25.71 | 18.29 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.3:** Lengths, widths and areas of the tenth leaves of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Genotype | Tenth-leaf length (cm) | Tenth-leaf width (cm) | Tenth-leaf area ($cm^2$) |
|---|---|---|---|
| OD2 | 75.27 | 39.97* | 1909.47* |
| OD490 | 75.33 | 38.80 | 1856.97 |
| ODT92 | 76.83 | 37.73 | 1838.00 |
| ODT19 | 73.67 | 36.77 | 1725.40 |
| OD1 | 77.03* | 34.33 | 1676.60 |
| ODT8 | 68.83 | 33.17 | 1448.43 |
| OD486B | 71.73 | 31.57 | 1443.77 |
| ODT1 | 68.70 | 32.63 | 1425.23 |
| TL33 | 66.97 | 30.93 | 1320.20 |
| ODT100 | 67.63 | 30.40 | 1306.03 |
| ODT82 | 70.07 | 26.93 | 1203.87 |
| OD313 | 63.30 | 29.53 | 1187.10 |
| F-value (Reps) | NS | NS | NS |
| F-value (Gen.) | * | ** | ** |
| LSD (5 %) | 10.01 | 8.58 | 567.47 |
| LSD (1 %) | 14.00 | 10.43 | 689.95 |
| C.V. (%) | 6.34 | 10.04 | 14.58 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.4:** Lengths, widths and areas of the eighteenth leaves of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Genotype | Eighteenth-leaf length (cm) | Eighteenth-leaf width (cm) | Eighteenth-leaf area ($cm^2$) |
|---|---|---|---|
| ODT92 | 73.37* | 31.67 | 1476.60* |
| ODT19 | 72.33* | 31.40 | 1449.60 |
| OD1 | 68.23 | 32.33* | 1407.13 |
| OD2 | 66.67 | 32.33* | 1379.00 |
| OD490 | 65.33 | 32.17 | 1343.97 |
| ODT82 | 71.67* | 27.97 | 1281.70 |
| ODT100 | 71.87* | 24.90 | 1137.27 |
| OD486B | 61.13 | 26.47 | 1026.87 |
| ODT8 | 58.90 | 23.60 | 884.27 |
| TL33 | 56.30 | 23.13 | 825.47 |
| ODT1 | 56.13 | 23.00 | 820.43 |
| OD313 | 55.20 | 22.53 | 786.70 |
| F-value (Reps) | NS | NS | NS |
| F-value (Gen.) | ** | ** | ** |
| LSD (5 %) | 15.01 | 9.07 | 631.75 |
| LSD (1 %) | 18.25 | 11.83 | 768.11 |
| C.V. (%) | 9.10 | 13.83 | 21.54 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.5:** Single-leaf areas and whole-plant leaf areas of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Genotype | Single-leaf area ($cm^2$) | Whole-plant leaf area ($cm^2$) |
|---|---|---|
| OD1 | 1429.43* | 33263.11* |
| OD490 | 1585.70** | 32350.00* |
| OD2 | 1565.10** | 32025.53 |
| ODT92 | 1372.37 | 30652.76 |
| ODT19 | 1356.20 | 29841.47 |
| OD486B | 1240.27 | 29296.47 |
| ODT82 | 1029.63 | 25502.56 |
| ODT1 | 1055.20 | 24698.28 |
| ODT100 | 1015.27 | 23894.27 |
| ODT8 | 952.00 | 21625.42 |
| TL33 | 942.90 | 20408.95 |
| OD313 | 889.33 | 20126.96 |
| F-value (Reps) | ** | ** |
| F-value (Gen.) | ** | ** |
| LSD (5 %) | 474.96 | 11868.09 |
| LSD (1%) | 577.48 | 14429.84 |
| C.V. (%) | 15.51 | 17.28 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.6:** Total yield (kg/ha), dip or throwaway mass (percentage of total yield) and marketable yield (kg/ha) of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Genotype | Total yield (kg/ha) | Dip mass (% total yield) | Marketable yield (kg/ha) |
|---|---|---|---|
| ODT82 | 3836.11 | 6.14 | 3602.11 |
| OD1 | 3796.89 | 12.28 | 3326.56 |
| OD2 | 3709.78 | 11.72 | 3283.22 |
| ODT92 | 3384.22 | 8.42 | 3104.11 |
| OD490 | 3288.59 | 7.00 | 3059.33 |
| OD313 | 3168.89 | 10.67 | 2829.89 |
| ODT19 | 3403.33 | 18.14 | 2782.89 |
| OD486B | 3006.33 | 10.84 | 2685.67 |
| ODT1 | 2914.56 | 8.04 | 2669.67 |
| TL33 | 2986.22 | 11.13 | 2666.89 |
| ODT100 | 3072.11 | 15.06 | 2593.11 |
| ODT8 | 2496.00 | 17.67 | 2106.22 |
| F-value (Reps) | NS | NS | NS |
| F-value (Gen.) | * | NS | * |
| LSD (5%) | 1156.96 | 12.40 | 1173.65 |
| LSD (1%) | 1406.69 | 15.08 | 1426.98 |
| C.V. (%) | 13.96 | 42.62 | 15.93 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

Total yield: Total cured leaf mass, dip (throwaway) mass inclusive

Marketable yield: Total cured leaf mass, dip mass exclusive

Dip mass: Unmarketable throwaway mass

**Table 5.7:** The correlation of the parameters of field performance of the elite breeding lines and the control, TL33, at the ARC-TCRI in the 1998/1999 season.

| Parameters | r-values |
|---|---|
| Plant height at topping and number of leaves per plant | -0.028 |
| Plant height at topping and whole plant leaf area | 0.519* |
| Plant height at topping and yield | 0.569* |
| Plant height at topping and days to physiological maturity | 0.023 |
| Number of leaves per plant and whole-plant leaf area | -0.351 |
| Number of leaves per plant and yield | -0.086 |
| Number of leaves per plant and days to physiological maturity | 0.372 |
| Whole-plant leaf area and yield | 0.565* |
| Whole-plant leaf area and days to physiological maturity | 0.301 |
| Yield and days to physiological maturity | 0.265 |

* Significant at 0.10 probability level

**Table 5.8:** The visual evaluation of the elite breeding lines by the representatives of the tobacco industry in the referred 1997/1998 replicated trial at the ARC-TCRI (Scale: 1-5. 1 represents a line that is totally unacceptable. Five represents a perfect line).

| | Representatives of the tobacco industry | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Entry | PTK 1 | PTK 2 | MKTV 1 | MKTV 2 | MKTV 3 | MKTV 4 | MKTV 5 | Other1 | Other2 |
| ODT82 | 3.33** | 2.98 | 4.58 | 4.59** | 3.67 | 5.21** | 3.67 | 4.67* | 4.07 |
| OD496 | 3.00* | 2.62 | 4.54 | 3.67** | 3.99 | 4.61** | 4.67** | 5.00** | 4.96** |
| OD490 | 3.33** | 2.62 | 3.85 | 3.77** | 3.71 | 3.96 | 4.33** | 5.00** | 3.99 |
| OD312 | 3.33** | 2.04 | 4.77* | 2.46 | 3.61 | 4.57** | 3.67 | 5.00** | 4.32 |
| OD2 | 3.00* | 3.01 | 3.96 | 3.56* | 3.97 | 3.57 | 4.00* | 4.67* | 3.39 |
| ODT101 | 2.67 | 2.54 | 4.10 | 3.39* | 4.00 | 3.96 | 4.00* | 4.00 | 3.99 |
| ODT100 | 2.33 | 3.04 | 3.85 | 3.62** | 3.32 | 3.74 | 4.00* | 4.00 | 3.66 |
| OD1 | 2.00 | 2.39 | 3.96 | 3.59* | 2.98 | 3.67 | 3.67 | 4.00 | 4.09 |
| OD489 | 2.33 | 2.27 | 3.31 | 3.69** | 3.38 | 3.49 | 3.67 | 3.67 | 3.28 |
| ODT9 | 2.00 | 2.74 | 3.57 | 2.97 | 2.99 | 3.33 | 3.67 | 3.67 | 2.28 |
| OD313 | 2.33 | 2.74 | 4.15 | 2.97 | 2.67 | 3.39 | 3.00 | 3.67 | 3.07 |
| ODT107 | 2.33 | 1.60 | 3.46 | 2.90 | 3.30 | 3.28 | 3.33 | 4.00 | 2.93 |
| ODT94 | 2.00 | 2.66 | 4.02 | 2.38 | 3.29 | 3.31 | 3.00 | 3.33 | 2.63 |
| ODT6 | 2.33 | 3.13 | 3.25 | 3.07 | 2.34 | 3.31 | 3.00 | 3.33 | 2.76 |
| ODT22 | 2.33 | 2.95 | 3.27 | 2.64 | 2.94 | 2.71 | 3.00 | 3.67 | 3.30 |
| ODT11 | 2.00 | 1.71 | 3.73 | 2.18 | 2.99 | 2.63 | 3.33 | 3.33 | 3.07 |
| ODT16 | 2.67 | 2.70 | 3.23 | 2.39 | 3.35 | 2.90 | 3.00 | 3.67 | 3.29 |
| ODT95 | 2.33 | 2.37 | 2.84 | 2.54 | 2.70 | 2.76 | 3.33 | 3.33 | 2.70 |
| ODT2 | 2.33 | 2.36 | 3.27 | 2.23 | 2.75 | 3.06 | 2.67 | 3.33 | 3.05 |
| ODT12 | 2.33 | 1.56 | 3.12 | 1.97 | 2.06 | 2.65 | 3.00 | 3.67 | 3.24 |
| ODT8 | 2.33 | 2.71 | 2.92 | 2.41 | 2.61 | 2.40 | 3.00 | 3.67 | 2.60 |
| ODT4 | 2.00 | 2.34 | 3.27 | 2.64 | 3.04 | 2.84 | 2.00 | 3.33 | 2.74 |
| TL33 | 1.67 | 2.49 | 3.39 | 2.18 | 2.68 | 2.87 | 2.33 | 3.00 | 2.86 |
| ODT89 | 1.67 | 2.12 | 2.94 | 1.95 | 2.35 | 2.10 | 2.67 | 2.33 | 2.12 |
| ODT90 | 2.00 | 1.64 | 1.98 | 1.23 | 1.32 | 1.02 | 1.67 | 2.67 | 1.30 |
| Average | 2.4 | 2.49 | 3.57 | 2.84 | 3.04 | 3.25 | 3.27 | 3.76 | 3.19 |
| F-value (Reps) | ** | NS | NS | NS | NS | NS | NS | NS | * |
| F-value (Gen.) | ** | NS | ** | ** | NS | ** | ** | ** | ** |
| LSD (5%) | 1.12 | 1.55 | 1.29 | 1.21 | 1.96 | 1.27 | 1.60 | 1.53 | 1.64 |
| LSD (1%) | 1.34 | 1.84 | 1.54 | 1.44 | 2.33 | 1.51 | 1.90 | 1.83 | 1.96 |
| C.V. (%) | 19.5 | 25.8 | 15.06 | 17.70 | 26.81 | 16.26 | 20.37 | 16.98 | 21.44 |

NS Not significant * Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

PTK (Afrikaans) Potgietersrus Tobacco growers' Cooperative

MKTV (Afrikaans) Magaliesburg Tobacco growers' Cooperative

**Table 5.9:** The cured leaf quality components (expressed as percentages) of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Genotype | Quality components | | | | | |
|---|---|---|---|---|---|---|
| | Orange | Light orange | Lemon | Closed grain | Bottom leaf | Broken and unmarketable |
| OD2 | 0.85 | 11.06 | 48.24 | 4.35 | 35.50 | 0.00 |
| OD1 | 0.00 | 6.09 | 67.45 | 1.59 | 24.66 | 0.22 |
| ODT1 | 0.00 | 3.28 | 53.58 | 6.56 | 36.58 | 0.00 |
| OD313 | 0.00 | 3.22 | 62.12 | 2.06 | 32.32 | 0.28 |
| ODT19 | 0.00 | 2.60 | 49.26 | 9.93 | 38.21 | 0.00 |
| ODT100 | 0.00 | 2.25 | 57.10 | 2.70 | 32.23 | 5.72 |
| ODT82 | 0.00 | 1.52 | 71.73 | 1.32 | 24.93 | 0.50 |
| OD486B | 0.00 | 1.10 | 61.95 | 5.69 | 28.60 | 2.66 |
| OD490 | 0.00 | 0.48 | 69.00 | 3.69 | 26.55 | 0.29 |
| ODT8 | 0.00 | 0.00 | 63.69 | 1.45 | 34.56 | 0.30 |
| ODT92 | 0.00 | 0.00 | 59.40 | 2.49 | 38.11 | 0.00 |
| TL33 | 0.00 | 0.00 | 47.97 | 7.10 | 40.70 | 4.23 |
| F-value (Reps) | NS | NS | NS | ** | NS | NS |
| F-value (Gen.) | NS | NS | NS | NS | NS | NS |
| LSD (5 %) | 1.08 | 14.38 | 27.86 | 13.83 | 28.92 | 7.53 |
| LSD (1 %) | 1.32 | 17.49 | 33.88 | 16.81 | 35.16 | 9.16 |
| C.V. (%) | 600.00 | 214.46 | 18.45 | 133.15 | 34.68 | 250.15 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.10:** The cured leaf quality components (nicotine and reducing-sugar concentrations of the leaf expressed as percentages) of the elite breeding lines at the ARC-TCRI in the 1998/1999 season

| Genotype | Nicotine | Reducing sugars |
|---|---|---|
| ODT92 | 2.41 | 20.28 |
| OD486B | 2.52 | 22.29 |
| ODT8 | 2.57 | 17.71 |
| ODT82 | 2.65 | 20.77 |
| OD2 | 2.75 | 22.68 |
| OD1 | 2.81 | 22.44 |
| OD490 | 3.00 | 28.90 |
| OD313 | 3.04 | 27.31 |
| TL33 | 3.27 | 21.76 |
| ODT1 | 3.29 | 19.72 |
| ODT100 | 3.36 | 20.99 |
| ODT19 | 3.37 | 19.55 |
| F-value (Reps) | NS | NS |
| F-value (Gen.) | NS | ** |
| LSD (5 %) | 1.03 | 7.78 |
| LSD (1 %) | 1.26 | 9.45 |
| C.V. (%) | 13.91 | 13.86 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.11:** The grade indices (cents/kg), total income (R/ha) and market income (R/ha) of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Genotype | Grade index (cents/kg) | Total income (R/ha) | Market income (R/ha) |
|---|---|---|---|
| ODT82 | 1756.37 | 67374.21 | 63293.50 |
| OD1 | 1708.53 | 64970.41 | 57028.18 |
| OD490 | 1748.49 | 57546.99 | 53564.39 |
| ODT92 | 1731.54 | 58427.26 | 53529.81 |
| OD2 | 1572.61 | 58664.61 | 52020.53 |
| OD313 | 1698.49 | 53858.05 | 48068.26 |
| ODT100 | 1653.53 | 50479.21 | 42847.01 |
| ODT19 | 1528.67 | 51815.30 | 42549.52 |
| OD486B | 1565.70 | 47133.76 | 42160.68 |
| ODT1 | 1575.23 | 45436.04 | 41880.97 |
| TL33 | 1572.88 | 46877.96 | 41833.98 |
| ODT8 | 1697.39 | 42517.54 | 35856.95 |
| F-value (Reps) | ** | NS | NS |
| F-value (Gen.) | NS | * | * |
| LSD (5 %) | 255.29 | 22858.16 | 23213.99 |
| LSD (1 %) | 310.39 | 27792.15 | 28224.78 |
| C.V. (%) | 6.07 | 16.70 | 19.04 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

Total income = Total yield (dip mass inclusive) x Average grade index

Market income = Total yield (dip mass exclusive) x Average grade index

Average grade index is the average price per kilogram of cured leaf

**Table 5.12:** The mean yield (kg/ha), quality (cents/kg) and income (Rands/ha) of the elite breeding lines at all the four localities in the 1998/1999 season.

| Entry | Sites | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Groblersdal | | | Potgietersrus | | | Rustenburg | | | Vaalwater | | |
| | Yield | Quality | Income | Yield | Quality | Income | Yield | Quality | Income | Yield | Quality | Income |
| ODT82 | 3571.56 | 1666.60 | 59209.26 | 2032.56 | 1585.14 | 32223.60 | 3602.11 | 1756.37 | 63293.50 | 2287.56 | 1330.14 | 30297.77 |
| ODT92 | 3293.67 | 1279.11 | 41883.11 | 2246.33 | 1673.05 | 37443.44 | 3104.11 | 1731.54 | 53529.81 | 2972.89 | 1375.40 | 41447.00 |
| OD2 | 3306.00 | 1416.66 | 46997.42 | 1928.22 | 1284.78 | 24856.15 | 3283.22 | 1572.61 | 52020.53 | 2787.78 | 1395.74 | 39527.02 |
| OD1 | 2998.00 | 1495.91 | 45106.04 | 1701.89 | 1244.22 | 21477.26 | 3326.56 | 1708.53 | 57028.18 | 2401.67 | 1242.83 | 30310.10 |
| OD490 | 2748.44 | 1547.54 | 41465.55 | 1896.11 | 1390.87 | 26362.45 | 3059.33 | 1748.49 | 53564.39 | 1740.44 | 1512.41 | 26613.48 |
| OD313 | 3108.78 | 1472.20 | 46153.71 | 1593.78 | 1432.48 | 23053.09 | 2829.89 | 1698.49 | 48068.26 | 2193.33 | 1433.66 | 30711.00 |
| ODT1 | 3012.56 | 1233.67 | 37264.99 | 1681.11 | 1421.52 | 24014.21 | 2669.67 | 1575.23 | 41880.97 | 2192.00 | 1424.26 | 31599.72 |
| ODT19 | 2657.56 | 1421.24 | 37632.52 | 1448.11 | 1455.59 | 21208.45 | 2782.89 | 1528.67 | 42549.52 | 2227.33 | 1342.49 | 30114.67 |
| TL33 | 2494.00 | 1559.97 | 38460.34 | 1814.56 | 1633.17 | 29511.94 | 2666.89 | 1572.88 | 41833.98 | 1594.00 | 1121.89 | 18616.08 |
| OD486B | 2127.11 | 1476.96 | 30337.11 | 1131.56 | 1520.20 | 17294.70 | 2685.67 | 1565.70 | 42160.68 | 3013.11 | 1235.27 | 36672.81 |
| ODT100 | 2418.11 | 1442.50 | 34624.21 | 2171.89 | 1457.29 | 31640.90 | 2593.11 | 1653.53 | 42847.01 | 1065.67 | 1208.59 | 12694.82 |
| ODT8 | 2243.89 | 1436.33 | 30271.91 | 1551.22 | 1591.77 | 25408.48 | 2106.22 | 1697.39 | 35856.95 | 2173.11 | 1235.50 | 27486.07 |
| F-value (Rep) | * | NS | NS | NS | NS | NS | NS | ** | NS | NS | NS | NS |
| F-value (Entry) | NS | NS | NS | NS | NS | NS | * | NS | * | NS | NS | NS |
| LSD 5% | 1671.58 | 450.53 | 26333.70 | 1285.49 | 403.71 | 20370.38 | 1173.65 | 255.29 | 23213.99 | 2204.15 | 441.21 | 32234.50 |
| LSD 1% | 2032.39 | 547.78 | 32017.89 | 1562.96 | 490.85 | 24767.37 | 1426.98 | 310.39 | 28224.78 | 2679.92 | 536.45 | 39192.39 |
| CV % | 23.18 | 12.17 | 25.36 | 28.58 | 10.75 | 30.52 | 15.93 | 6.07 | 19.04 | 38.98 | 13.11 | 42.66 |

NS Not significant * Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.13:** The mean marketable yield, quality and market income from the AGROBASE combined analysis of variance for the data from the four localities in the 1998/1999 season.

| Entry | Yield (kg/ha) | Quality (cents/kg) | Income (R/ha) |
|---|---|---|---|
| ODT82 | 2873.44** | 1584.56 | 46256.03** |
| ODT92 | 2904.25** | 1514.78 | 43575.84** |
| OD2 | 2826.31** | 1417.45 | 40850.28* |
| OD1 | 2607.03* | 1422.87 | 38480.39 |
| OD490 | 2361.08 | 1549.83 | 37001.47 |
| OD313 | 2431.44 | 1509.21 | 36996.51 |
| ODT1 | 2388.84 | 1413.67 | 33689.97 |
| ODT19 | 2278.97 | 1437.00 | 32876.29 |
| TL33 | 2142.36 | 1471.98 | 32105.58 |
| OD486B | 2239.36 | 1449.53 | 31616.33 |
| ODT100 | 2062.20 | 1440.48 | 30451.74 |
| ODT8 | 2018.61 | 1490.25 | 29755.85 |
| Mean | 2427.82 | 1475.13 | 36138.02 |
| F-value (Locality) | ** | ** | ** |
| F-value (Entry) | ** | NS | ** |
| F-value (Locality x Entry) | NS | NS | NS |
| LSD (5%) | 454.23 | 113.08 | 6989.38 |
| LSD (1%) | 647.13 | 161.10 | 9957.46 |
| CV (%) | 27.59 | 11.30 | 28.52 |

NS Not significant

* Significant differences (0.05 probability level)

** Highly significant differences (0.01 probability level)

**Table 5.14:** The combined analysis of variance for marketable yield data from the four localities in the 1998/1999 season–AMMI model.

| Source | df | SS | MS | F | P>F |
|---|---|---|---|---|---|
| Total | 143 | 89925472 | 628849 | | |
| Treatment | 47 | 56857929 | 1209743 | 3.883 | 0.0000** |
| Locality | 3 | 32617348 | 10872449 | 34.896 | 0.0000** |
| Block | 8 | 5649642 | 706205 | 2.267 | 0.0297* |
| Genotype | 11 | 14973478 | 1361225 | 4.369 | 0.0000** |
| Interaction | 33 | 9267103 | 280821 | 0.901 | 0.6223 |
| IPCA | 13 | 5768534 | 443733 | 1.424 | 0.1644 |
| Residual | 20 | 3498570 | 174928 | 0.561 | 0.9286 |
| Error | 88 | 27417901 | 311567 | | |

Noise is 6.2% of the treatment sum of squares

* Significant differences

** Highly significant differences

**Table 5.15:** The combined analysis of variance for market income data from the four localities in the 1998/1999 season–AMMI model

| Source | Df | SS | MS | F | P>F |
|---|---|---|---|---|---|
| Total | 143 | 275157899 | 1924181 | | |
| Treatment | 47 | 187587082 | 3991215 | 4.504 | 0.0000** |
| Locality | 3 | 113712383 | 37904128 | 42.777 | 0.0000** |
| Block | 8 | 9594317 | 1199290 | 1.353 | 0.2283 |
| Genotype | 11 | 42653108 | 3877555 | 4.376 | 0.0000** |
| Interaction | 33 | 31221591 | 946109 | 1.068 | 0.3932 |
| IPCA | 13 | 14990615 | 1153124 | 1.301 | 0.2275 |
| Residual | 20 | 16230975 | 811549 | 0.916 | 0.5688 |
| Error | 88 | 77976500 | 886097 | | |

Noise is 8.7% of the treatment sum of squares

* Significant differences

** Highly significant differences

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# Appendices

**Appendix 1:** Computer tabulated raw data on the field performance of the elite breeding lines and the control, TL33, at the ARC-TCRI in the 1998/1999 season.

| PLOT | BLOC | REP | ENTR | DTPM | NLP | PHT | FV/FM | 2LL (cm) | 2LW (cm) | 2LA (cm2) | 10LL (cm) | 10LW (cm) | 10LA (cm2) | 18LL (cm) | 18LW (cm) | 18LA (cm2) | SLA (cm2) | WPLA (cm2) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 4 | 55 | 21.50 | 100.50 | 0.79 | 47.60 | 25.10 | 757.50 | 69.80 | 31.90 | 1411.70 | 60.60 | 22.90 | 879.80 | 825.50 | 17748.25 |
| 2 | 1 | 1 | 11 | 55 | 22.10 | 107.90 | 0.77 | 46.10 | 16.60 | 485.20 | 68.80 | 28.30 | 1234.40 | 63.60 | 25.20 | 1016.10 | 884.30 | 19543.03 |
| 3 | 1 | 1 | 2 | 53 | 20.00 | 118.10 | 0.65 | 56.30 | 33.70 | 1188.60 | 77.10 | 39.30 | 1921.00 | 61.60 | 31.80 | 1241.90 | 1379.00 | 27580.00 |
| 4 | 1 | 1 | 8 | 55 | 24.00 | 119.40 | 0.52 | 45.20 | 20.70 | 593.20 | 66.60 | 31.20 | 1317.40 | 69.10 | 22.30 | 976.90 | 1137.30 | 27295.20 |
| 5 | 1 | 1 | 3 | 54 | 24.10 | 114.50 | 0.78 | 53.80 | 34.60 | 1180.20 | 76.50 | 34.60 | 1678.10 | 60.60 | 25.30 | 972.00 | 1026.90 | 24748.29 |
| 6 | 1 | 1 | 6 | 55 | 21.70 | 121.00 | 0.76 | 58.70 | 21.90 | 815.00 | 76.70 | 35.20 | 1711.70 | 71.20 | 26.10 | 1178.20 | 1476.60 | 32042.22 |
| 7 | 1 | 1 | 5 | 55 | 20.40 | 119.40 | 0.72 | 64.00 | 42.90 | 1740.70 | 74.90 | 36.30 | 1723.80 | 74.30 | 36.10 | 1700.50 | 1344.00 | 27417.60 |
| 8 | 1 | 1 | 7 | 54 | 23.20 | 113.70 | 0.81 | 57.80 | 31.80 | 1165.30 | 71.30 | 34.40 | 1555.00 | 61.50 | 24.90 | 970.10 | 820.70 | 19040.24 |
| 9 | 1 | 1 | 9 | 57 | 21.40 | 100.30 | 0.81 | 49.10 | 22.30 | 694.20 | 68.30 | 30.20 | 1307.70 | 52.40 | 26.10 | 867.10 | 786.70 | 16835.38 |
| 10 | 1 | 1 | 1 | 56 | 23.60 | 110.40 | 0.68 | 63.20 | 35.10 | 1406.40 | 77.80 | 31.80 | 1568.50 | 70.00 | 31.90 | 1415.70 | 1407.10 | 33207.56 |
| 11 | 1 | 1 | 10 | 52 | 21.90 | 112.40 | 0.81 | 57.00 | 28.30 | 1022.70 | 78.20 | 39.30 | 1948.40 | 76.60 | 35.50 | 1724.00 | 1449.60 | 31746.24 |
| 12 | 1 | 1 | 12 | 56 | 25.10 | 122.90 | 0.75 | 44.10 | 17.40 | 486.50 | 63.50 | 25.00 | 1006.50 | 74.80 | 30.30 | 1436.90 | 1281.70 | 32170.67 |
| 13 | 1 | 2 | 3 | 60 | 22.00 | 108.80 | 0.78 | 62.70 | 39.50 | 1570.20 | 76.70 | 31.20 | 1517.20 | 60.50 | 24.70 | 947.40 | 1443.80 | 31763.60 |
| 14 | 1 | 2 | 5 | 60 | 19.90 | 117.90 | 0.81 | 59.10 | 38.40 | 1438.80 | 79.80 | 40.10 | 2028.80 | 65.70 | 30.40 | 1266.30 | 1857.00 | 36954.30 |
| 15 | 1 | 2 | 8 | 57 | 23.10 | 120.90 | 0.79 | 53.90 | 21.00 | 717.60 | 71.10 | 32.20 | 1451.50 | 76.90 | 26.50 | 1292.00 | 1306.00 | 30168.60 |
| 16 | 1 | 2 | 9 | 56 | 23.70 | 107.70 | 0.76 | 46.40 | 25.00 | 735.40 | 60.20 | 26.10 | 996.20 | 51.20 | 20.20 | 655.70 | 1187.10 | 28134.27 |
| 17 | 1 | 2 | 1 | 57 | 22.90 | 112.60 | 0.71 | 62.50 | 30.40 | 1204.60 | 78.70 | 36.10 | 1801.20 | 63.30 | 27.50 | 1103.60 | 1676.60 | 38394.14 |
| 18 | 1 | 2 | 10 | 54 | 22.30 | 118.30 | 0.78 | 58.10 | 26.40 | 972.50 | 74.70 | 38.80 | 1837.60 | 77.90 | 30.60 | 1511.30 | 1725.40 | 38476.42 |
| 19 | 1 | 2 | 7 | 57 | 23.40 | 110.60 | 0.81 | 52.10 | 21.50 | 710.20 | 66.10 | 27.40 | 1148.30 | 51.20 | 23.20 | 753.10 | 1425.20 | 33349.68 |
| 20 | 1 | 2 | 6 | 53 | 22.60 | 122.70 | 0.66 | 60.80 | 22.30 | 859.60 | 78.00 | 38.60 | 1908.80 | 71.30 | 34.60 | 1564.10 | 1838.00 | 41538.80 |
| 21 | 1 | 2 | 12 | 60 | 24.40 | 124.80 | 0.80 | 55.60 | 23.20 | 817.80 | 78.70 | 29.40 | 1466.90 | 73.30 | 33.20 | 1542.90 | 1203.90 | 29375.16 |
| 22 | 1 | 2 | 11 | 57 | 22.70 | 113.50 | 0.79 | 43.70 | 19.40 | 537.50 | 68.20 | 32.00 | 1383.60 | 51.70 | 22.50 | 737.50 | 1448.40 | 32878.68 |
| 23 | 1 | 2 | 4 | 53 | 21.50 | 116.30 | 0.81 | 39.80 | 20.60 | 519.80 | 60.00 | 27.90 | 1061.30 | 54.40 | 24.20 | 834.60 | 1320.20 | 28384.30 |
| 24 | 1 | 2 | 2 | 56 | 20.40 | 124.50 | 0.79 | 65.50 | 41.40 | 1719.20 | 72.70 | 36.40 | 1677.70 | 62.20 | 28.70 | 1131.80 | 1909.50 | 38953.80 |
| 25 | 1 | 3 | 9 | 56 | 22.20 | 106.80 | 0.75 | 44.20 | 23.30 | 652.90 | 61.40 | 32.30 | 1257.40 | 62.00 | 21.30 | 837.30 | 694.20 | 15411.24 |
| 26 | 1 | 3 | 6 | 56 | 22.90 | 122.90 | 0.66 | 51.60 | 22.40 | 732.80 | 75.80 | 39.40 | 1893.50 | 77.60 | 34.30 | 1687.50 | 802.50 | 18377.25 |
| 27 | 1 | 3 | 4 | 55 | 22.10 | 108.40 | 0.78 | 51.80 | 23.50 | 771.80 | 71.10 | 33.00 | 1487.60 | 53.90 | 22.30 | 762.00 | 683.00 | 15094.30 |
| 28 | 1 | 3 | 11 | 55 | 23.80 | 110.00 | 0.82 | 45.90 | 18.80 | 547.10 | 69.50 | 39.20 | 1727.30 | 61.40 | 23.10 | 899.20 | 523.30 | 12454.54 |
| 29 | 1 | 3 | 3 | 60 | 25.10 | 113.40 | 0.81 | 53.10 | 29.70 | 999.90 | 62.00 | 28.90 | 1136.00 | 62.30 | 29.40 | 1161.20 | 1250.10 | 31377.51 |
| 30 | 1 | 3 | 5 | 55 | 21.00 | 118.90 | 0.81 | 63.30 | 37.10 | 1488.90 | 71.30 | 40.00 | 1818.30 | 56.00 | 30.00 | 1065.10 | 1556.10 | 32678.10 |
| 31 | 1 | 3 | 2 | 56 | 21.00 | 124.80 | 0.81 | 54.20 | 38.20 | 1312.70 | 76.00 | 44.20 | 2129.70 | 76.20 | 36.50 | 1763.30 | 1406.80 | 29542.80 |
| 32 | 1 | 3 | 12 | 55 | 24.80 | 125.10 | 0.72 | 44.30 | 18.00 | 505.60 | 68.00 | 26.40 | 1138.20 | 66.90 | 20.40 | 865.30 | 603.30 | 14961.84 |
| 33 | 1 | 3 | 7 | 55 | 23.60 | 111.10 | 0.81 | 47.40 | 29.40 | 883.50 | 68.70 | 36.10 | 1572.40 | 55.70 | 20.90 | 738.10 | 919.70 | 21704.92 |
| 34 | 1 | 3 | 8 | 56 | 23.60 | 118.70 | 0.79 | 42.80 | 18.30 | 496.60 | 65.20 | 27.80 | 1149.20 | 69.60 | 25.90 | 1142.90 | 602.50 | 14219.00 |
| 35 | 1 | 3 | 1 | 60 | 23.40 | 111.40 | 0.80 | 52.20 | 30.30 | 1002.80 | 74.60 | 35.10 | 1660.10 | 71.40 | 37.60 | 1702.10 | 1204.60 | 28187.64 |
| 36 | 1 | 3 | 10 | 55 | 21.60 | 114.40 | 0.79 | 48.50 | 22.30 | 685.70 | 68.10 | 32.20 | 1390.20 | 62.50 | 28.10 | 1113.50 | 893.60 | 19301.76 |

DTPM Days to physiological maturity
NLP Number of leaves per plant
PHT Plant height at topping
FV/FM Chlorophyll a fluorescence
2LL Second leaf length
2LW Second leaf width
2LA Second leaf area
10LL Tenth leaf length
10LW Tenth leaf width
10LA Tenth leaf area
18LL Eighteenth leaf length
18LW Eighteenth leaf width
18LA Eighteenth leaf area
SLA Single leaf area
WPLA Whole plant leaf area

**Appendix 2** Analysis of variance of the parameters of field performance of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| ENTRIES | DTPM | NLP | PHT | FV/FM | 2LL (cm) | 2LW (cm) | 2LA (cm2) | 10LL (cm) | 10LW (cm) | 10LA (cm2) | 18LL (cm) | 18LW (cm) | 18LA (cm2) | SLA (cm2) | WPLA (cm2) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OD1 | 57.67 | 23.30 | 111.47 | 0.73 | 59.30 | 31.93 | 1204.60 | 77.03 | 34.33 | 1676.60 | 68.23 | 32.33 | 1407.13 | 1429.43 | 33263.11 |
| OD2 | 55.00 | 20.47 | 122.47 | 0.53 | 58.67 | 37.77 | 1406.83 | 75.27 | 39.97 | 1909.47 | 66.67 | 32.33 | 1379.00 | 1565.10 | 32025.53 |
| OD486B | 58.00 | 23.73 | 112.23 | 0.79 | 56.53 | 23.07 | 1250.10 | 71.73 | 31.57 | 1443.77 | 61.13 | 26.47 | 1026.87 | 1240.27 | 29296.47 |
| TL33 | 54.33 | 21.70 | 108.40 | 0.79 | 46.40 | 23.07 | 683.03 | 66.97 | 30.93 | 1320.20 | 56.30 | 23.13 | 825.47 | 942.90 | 20408.95 |
| OD490 | 56.67 | 20.43 | 118.73 | 0.78 | 62.13 | 39.47 | 1556.13 | 75.33 | 38.80 | 1856.97 | 65.33 | 32.17 | 1343.97 | 1585.70 | 32350.00 |
| ODT92 | 54.67 | 22.40 | 122.20 | 0.69 | 57.03 | 22.20 | 802.47 | 76.83 | 37.73 | 1838.00 | 73.37 | 31.67 | 1476.60 | 1372.37 | 30652.76 |
| ODT1 | 55.33 | 23.40 | 111.80 | 0.81 | 52.43 | 27.57 | 919.67 | 68.70 | 32.63 | 1425.23 | 56.13 | 23.00 | 820.43 | 1055.20 | 24698.28 |
| ODT100 | 56.00 | 23.57 | 119.67 | 0.70 | 47.30 | 20.00 | 602.47 | 67.63 | 30.40 | 1306.03 | 71.87 | 24.90 | 1137.27 | 1015.27 | 23894.27 |
| OD313 | 56.33 | 22.43 | 104.93 | 0.77 | 46.57 | 23.53 | 694.17 | 63.30 | 29.53 | 1187.10 | 55.20 | 22.53 | 786.70 | 889.33 | 20126.96 |
| ODT19 | 53.67 | 21.93 | 115.03 | 0.79 | 54.53 | 25.67 | 893.63 | 73.67 | 36.77 | 1725.40 | 72.33 | 31.40 | 1449.60 | 1356.20 | 29841.47 |
| ODT8 | 55.67 | 22.87 | 110.47 | 0.79 | 45.23 | 18.27 | 523.27 | 68.83 | 33.17 | 1448.43 | 58.90 | 23.60 | 884.27 | 952.00 | 21625.42 |
| ODT82 | 57.00 | 24.77 | 124.27 | 0.76 | 48.00 | 19.53 | 603.30 | 70.07 | 26.93 | 1203.87 | 71.67 | 27.97 | 1281.70 | 1029.63 | 25502.56 |
| **Statistics** | | | | | | | | | | | | | | | |
| F-Value (Reps) | * | nb | * | nb | * | nb | nb | nb | nb | nb | nb | nb | nb | ** | ** |
| F-Value (Gen) | nb | ** | ** | nb | ** | ** | ** | * | ** | ** | ** | ** | ** | ** | ** |
| LSD Gen (0.05) | 4.46 | 1.76 | 7.53 | 0.36 | 11.59 | 17.03 | 432.25 | 11.51 | 8.58 | 567.47 | 15.01 | 9.73 | 631.75 | 474.96 | 11868.09 |
| LSD Gen (0.05) | 5.43 | 2.14 | 9.15 | 0.43 | 14.09 | 20.70 | 525.55 | 14.00 | 10.43 | 689.95 | 18.25 | 11.83 | 768.11 | 577.48 | 14429.84 |
| C.V. | 3.14% | 3.06% | 2.57% | 18.84% | 8.61% | 25.71% | 18.29% | 6.34% | 10.04% | 14.58% | 9.10% | 13.83% | 21.54% | 15.51% | 17.28% |

DTPM Days to physiological maturity
2LW Second leaf width
18LL Eighteenth leaf length
NLP Number of leaves per plant
2LA Second leaf area
18LW Eighteenth leaf width
PHT Plant height at topping
10LL Tenth leaf length
18LA Eighteenth leaf area
FV/FM Chlorophyll a fluorescence
10LW Tenth leaf width
SLA Single leaf area
2LL Second leaf length
10LA Tenth leaf area
WPLA Whole plant leaf area

**Appendix 3:** An evaluation card used by the representatives of the tobacco industry in the field evaluation of the elite breeding lines at the ARC-TCRI.

| FLUE-CURED TOBACCO<br>ELITE BREEDING LINES TRIAL: 1998/1999 | | | | | |
|---|---|---|---|---|---|
| | SCORE | | | | |
| ENTRY CODE | 1 | 2 | 3 | 4 | 5 |
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |
| 5 | | | | | |
| 6 | | | | | |
| 7 | | | | | |
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| 9 | | | | | |
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| 27 | | | | | |
| 28 | | | | | |
| 29 | | | | | |
| 30 | | | | | |
| 31 | | | | | |
| 32 | | | | | |
| 33 | | | | | |
| 34 | | | | | |
| 35 | | | | | |
| 36 | | | | | |

**Appendix 4:** Computer tabulated raw data of yields (kg/ha), quality components (leaf colours) and income (Rands/ha) of the elite breeding lines at the ARC-TCRI in the 1998/1999 season

| PLOT | BLOC | REP | ENTRY | Marketable yield | Market income | Average price | Total yield | Total income | Throwaway mass | Quality components | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | M | O | J | L | Z | X | AF |
| 1 | 1 | 1 | 4 | 2059.67 | 33172.18 | 1610.56 | 2489.67 | 40097.59 | 17.27 | 0.00 | 0.00 | 0.00 | 41.93 | 7.87 | 39.99 | 10.21 |
| 2 | 1 | 1 | 11 | 941.67 | 15761.79 | 1673.82 | 1274.67 | 21335.60 | 26.12 | 0.00 | 0.00 | 0.00 | 60.85 | 0.00 | 39.15 | 0.00 |
| 3 | 1 | 1 | 2 | 2890.67 | 42459.37 | 1468.84 | 3372.00 | 49529.40 | 14.27 | 0.00 | 0.00 | 28.86 | 34.27 | 0.00 | 36.87 | 0.00 |
| 4 | 1 | 1 | 8 | 2192.67 | 37630.70 | 1716.21 | 2487.67 | 42693.51 | 11.86 | 0.00 | 0.00 | 0.00 | 56.90 | 0.00 | 43.10 | 0.00 |
| 5 | 1 | 1 | 3 | 3037.33 | 50047.14 | 1647.73 | 3253.33 | 53606.24 | 6.64 | 0.00 | 0.00 | 0.00 | 68.91 | 1.70 | 25.90 | 3.49 |
| 6 | 1 | 1 | 6 | 2742.67 | 50338.09 | 1835.37 | 3105.33 | 56994.36 | 11.68 | 0.00 | 0.00 | 0.00 | 68.52 | 0.89 | 30.59 | 0.00 |
| 7 | 1 | 1 | 5 | 3310.33 | 59876.34 | 1808.77 | 3461.33 | 62607.59 | 4.36 | 0.00 | 0.00 | 0.00 | 59.62 | 0.00 | 40.38 | 0.00 |
| 8 | 1 | 1 | 7 | 3134.00 | 53897.61 | 1719.77 | 3330.33 | 57274.09 | 5.90 | 0.00 | 0.00 | 0.00 | 57.89 | 0.00 | 42.11 | 0.00 |
| 9 | 1 | 1 | 9 | 2951.33 | 50650.71 | 1716.20 | 3305.67 | 56731.78 | 10.72 | 0.00 | 0.00 | 9.66 | 69.58 | 4.42 | 16.34 | 0.00 |
| 10 | 1 | 1 | 1 | 2987.33 | 49537.68 | 1658.26 | 3357.33 | 55673.23 | 11.02 | 0.00 | 0.00 | 15.88 | 63.88 | 0.00 | 20.24 | 0.00 |
| 11 | 1 | 1 | 10 | 2866.67 | 49540.62 | 1728.16 | 3373.33 | 58296.64 | 15.02 | 0.00 | 0.00 | 0.00 | 48.34 | 0.00 | 51.66 | 0.00 |
| 12 | 1 | 1 | 12 | 3904.33 | 73238.93 | 1875.84 | 4109.33 | 77084.39 | 4.99 | 0.00 | 0.00 | 3.37 | 71.68 | 0.00 | 23.44 | 1.50 |
| 13 | 1 | 2 | 3 | 2465.00 | 40311.53 | 1635.36 | 2831.67 | 46307.84 | 12.95 | 0.00 | 0.00 | 3.31 | 61.92 | 7.38 | 25.40 | 1.99 |
| 14 | 1 | 2 | 5 | 3016.00 | 51630.76 | 1711.90 | 3338.43 | 57150.48 | 9.66 | 0.00 | 0.00 | 1.44 | 66.78 | 7.96 | 23.83 | 0.00 |
| 15 | 1 | 2 | 8 | 2900.00 | 50905.38 | 1755.36 | 3211.33 | 56370.39 | 9.69 | 0.00 | 0.00 | 6.75 | 62.16 | 0.00 | 15.82 | 15.28 |
| 16 | 1 | 2 | 9 | 2751.67 | 48321.51 | 1756.08 | 3152.00 | 55351.70 | 12.70 | 0.00 | 0.00 | 0.00 | 61.30 | 0.00 | 38.70 | 0.00 |
| 17 | 1 | 2 | 1 | 3771.33 | 67596.32 | 1792.37 | 3988.33 | 71485.77 | 5.44 | 0.00 | 0.00 | 0.00 | 61.06 | 0.00 | 38.29 | 0.65 |
| 18 | 1 | 2 | 10 | 2662.33 | 41865.64 | 1572.52 | 3229.67 | 50787.06 | 17.57 | 0.00 | 0.00 | 7.79 | 66.31 | 0.00 | 25.90 | 0.00 |
| 19 | 1 | 2 | 7 | 2117.33 | 36863.23 | 1741.02 | 2218.00 | 38615.86 | 4.54 | 0.00 | 0.00 | 6.66 | 70.72 | 2.74 | 19.88 | 0.00 |
| 20 | 1 | 2 | 6 | 3169.67 | 54543.37 | 1720.79 | 3424.33 | 58925.65 | 7.44 | 0.00 | 0.00 | 0.00 | 54.83 | 1.63 | 43.54 | 0.00 |
| 21 | 1 | 2 | 12 | 3250.67 | 58889.10 | 1811.60 | 3483.67 | 63110.13 | 6.69 | 0.00 | 0.00 | 0.00 | 76.68 | 0.00 | 23.32 | 0.00 |
| 22 | 1 | 2 | 11 | 2605.00 | 45985.33 | 1765.27 | 3124.33 | 55152.98 | 16.62 | 0.00 | 0.00 | 0.00 | 77.49 | 0.00 | 22.51 | 0.00 |
| 23 | 1 | 2 | 4 | 2740.33 | 42557.83 | 1553.02 | 2942.67 | 45700.10 | 6.88 | 0.00 | 0.00 | 0.00 | 51.06 | 9.22 | 37.25 | 2.47 |
| 24 | 1 | 2 | 2 | 3802.67 | 65034.46 | 1710.23 | 4139.67 | 70797.95 | 8.14 | 0.00 | 2.55 | 0.44 | 46.93 | 1.94 | 48.14 | 0.00 |
| 25 | 1 | 3 | 9 | 2786.67 | 45232.54 | 1623.18 | 3049.00 | 49490.68 | 8.60 | 0.00 | 0.00 | 0.00 | 55.48 | 1.77 | 41.93 | 0.83 |
| 26 | 1 | 3 | 6 | 3400.00 | 55707.96 | 1638.47 | 3623.00 | 59361.75 | 6.16 | 0.00 | 0.00 | 0.00 | 54.85 | 4.95 | 40.20 | 0.00 |
| 27 | 1 | 3 | 4 | 3200.67 | 49771.92 | 1555.05 | 3526.33 | 54836.19 | 9.24 | 0.00 | 0.00 | 0.00 | 50.91 | 4.22 | 44.88 | 0.00 |
| 28 | 1 | 3 | 11 | 2772.00 | 45823.74 | 1653.09 | 3089.00 | 51064.05 | 10.26 | 0.00 | 0.00 | 0.00 | 52.73 | 4.34 | 42.03 | 0.90 |
| 29 | 1 | 3 | 3 | 2554.67 | 36123.39 | 1414.02 | 2934.00 | 41487.22 | 12.93 | 0.00 | 0.00 | 0.00 | 55.01 | 7.99 | 34.50 | 2.51 |
| 30 | 1 | 3 | 5 | 2851.67 | 49186.05 | 1724.82 | 3066.00 | 52882.91 | 6.99 | 0.00 | 0.00 | 0.00 | 80.60 | 3.10 | 15.44 | 0.86 |
| 31 | 1 | 3 | 2 | 3156.33 | 48567.76 | 1538.74 | 3617.67 | 55666.48 | 12.75 | 0.00 | 0.00 | 3.88 | 63.52 | 11.11 | 21.49 | 0.00 |
| 32 | 1 | 3 | 12 | 3651.33 | 57752.47 | 1581.68 | 3915.33 | 61928.11 | 6.74 | 0.00 | 0.00 | 1.20 | 66.82 | 3.97 | 28.02 | 0.00 |
| 33 | 1 | 3 | 7 | 2757.67 | 34882.07 | 1264.91 | 3195.33 | 40418.17 | 13.70 | 0.00 | 0.00 | 3.19 | 32.13 | 16.95 | 47.73 | 0.00 |
| 34 | 1 | 3 | 8 | 2686.67 | 40004.95 | 1489.02 | 3517.33 | 52373.73 | 23.62 | 0.00 | 0.00 | 0.00 | 52.25 | 8.11 | 37.77 | 1.87 |
| 35 | 1 | 3 | 1 | 3221.00 | 53950.54 | 1674.96 | 4045.00 | 67752.23 | 20.37 | 0.00 | 0.00 | 2.39 | 77.40 | 4.76 | 15.45 | 0.00 |
| 36 | 1 | 3 | 10 | 2819.67 | 36242.30 | 1285.34 | 3607.00 | 46362.21 | 21.83 | 0.00 | 0.00 | 0.00 | 33.15 | 29.80 | 37.05 | 0.00 |

O : Orange; J : Light orange; L : Lemon; Z : Closed grain or sponge; X : Bottom leaf; AF : Leaf broken to fine mass
Throwaway mass expressed as percentage of the total yield.

**Appendix 5:** Analysis of variance of the yields (kg/ha), quality components (leaf colours), grade indices (average prices in cents/kg) and income (Rands/ha) of the elite breeding lines at the ARC-TCRI in the 1998/1999 season

| PLOT | ENTRY | Marketable yield | Market income | Average price | Total yield | Total income | Throwaway mass | Quality components O | J | L | Z | X | AF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | OD 1 | 3326.56 | 57028.18 | 1708.53 | 3796.89 | 64970.41 | 12.28 | 0.00 | 6.09 | 67.45 | 1.59 | 24.66 | 0.22 |
| 2 | OD 2 | 3283.22 | 52020.53 | 1572.61 | 3709.78 | 58664.61 | 11.72 | 0.85 | 11.06 | 48.24 | 4.35 | 35.50 | 0.00 |
| 3 | OD 486B | 2685.67 | 42160.68 | 1565.70 | 3006.33 | 47133.76 | 10.84 | 0.00 | 1.10 | 61.95 | 5.69 | 28.60 | 2.66 |
| 4 | TL 33 | 2666.89 | 41833.98 | 1572.88 | 2986.22 | 46877.96 | 11.13 | 0.00 | 0.00 | 47.97 | 7.10 | 40.70 | 4.23 |
| 5 | OD 490 | 3059.33 | 53564.39 | 1748.49 | 3288.59 | 57546.99 | 7.00 | 0.00 | 0.48 | 69.00 | 3.69 | 26.55 | 0.29 |
| 6 | ODT 92 | 3104.11 | 53529.81 | 1731.54 | 3384.22 | 58427.26 | 8.42 | 0.00 | 0.00 | 59.40 | 2.49 | 38.11 | 0.00 |
| 7 | ODT 1 | 2669.67 | 41880.97 | 1575.23 | 2914.56 | 45436.04 | 8.04 | 0.00 | 3.28 | 53.58 | 6.56 | 36.58 | 0.00 |
| 8 | ODT 100 | 2593.11 | 42847.01 | 1653.53 | 3072.11 | 50479.21 | 15.06 | 0.00 | 2.25 | 57.10 | 2.70 | 32.23 | 5.72 |
| 9 | OD 313 | 2829.89 | 48068.26 | 1698.49 | 3168.89 | 53858.05 | 10.67 | 0.00 | 3.22 | 62.12 | 2.06 | 32.32 | 0.28 |
| 10 | ODT 19 | 2782.89 | 42549.52 | 1528.67 | 3403.33 | 51815.30 | 18.14 | 0.00 | 2.60 | 49.26 | 9.93 | 38.21 | 0.00 |
| 11 | ODT 8 | 2106.22 | 35856.95 | 1697.39 | 2496.00 | 42517.54 | 17.67 | 0.00 | 0.00 | 63.69 | 1.45 | 34.56 | 0.30 |
| 12 | ODT 82 | 3602.11 | 63293.50 | 1756.37 | 3836.11 | 67374.21 | 6.14 | 0.00 | 1.52 | 71.73 | 1.32 | 24.93 | 0.50 |
| **Statistics** | | | | | | | | | | | | | |
| F-value (Reps) | | NS | NS | ** | NS | NS | NS | NS | NS | NS | ** | NS | NS |
| F-value (Entry) | | * | * | NS | * | * | NS | NS | NS | NS | NS | NS | NS |
| LSD Entry (0.05) | | 1173.65 | 23213.99 | 255.29 | 1156.96 | 22858.16 | 12.40 | 1.08 | 14.38 | 27.86 | 13.83 | 28.92 | 7.53 |
| LSD Entry (0.01) | | 1426.98 | 28224.78 | 310.39 | 1406.69 | 27792.15 | 15.08 | 1.32 | 17.49 | 33.88 | 16.81 | 35.16 | 9.16 |
| C.V. | | 15.93% | 19.04% | 6.07% | 13.96% | 16.70% | 42.62% | 600.00% | 214.46% | 18.45% | 133.15% | 34.68% | 250.15% |

O : Orange; J : Light orange; L : Lemon; Z : Closed grain or sponge; X : Bottom leaf; AF : Leaf broken to fine mass
Throwaway mass expressed as percentage of the total yield.

**Appendix 6:** Computer tabulated raw data of the concentration of nicotine and reducing sugars of the leaves of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Plot | Block | Rep. | Entry | % Nicotine | % Reducing sugars |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 4 | 3.94 | 22.86 |
| 2 | 1 | 1 | 11 | 2.58 | 18.28 |
| 3 | 1 | 1 | 2 | 2.74 | 21.20 |
| 4 | 1 | 1 | 8 | 3.42 | 23.54 |
| 5 | 1 | 1 | 3 | 2.31 | 21.93 |
| 6 | 1 | 1 | 6 | 2.63 | 19.84 |
| 7 | 1 | 1 | 5 | 3.44 | 31.54 |
| 8 | 1 | 1 | 7 | 3.57 | 21.61 |
| 9 | 1 | 1 | 9 | 3.52 | 23.90 |
| 10 | 1 | 1 | 1 | 2.55 | 20.06 |
| 11 | 1 | 1 | 10 | 4.07 | 21.09 |
| 12 | 1 | 1 | 12 | 2.39 | 22.32 |
| 13 | 1 | 2 | 3 | 2.42 | 17.21 |
| 14 | 1 | 2 | 5 | 2.62 | 25.23 |
| 15 | 1 | 2 | 8 | 2.58 | 20.23 |
| 16 | 1 | 2 | 9 | 2.81 | 30.93 |
| 17 | 1 | 2 | 1 | 2.96 | 26.02 |
| 18 | 1 | 2 | 10 | 2.76 | 17.32 |
| 19 | 1 | 2 | 7 | 2.79 | 22.98 |
| 20 | 1 | 2 | 6 | 2.62 | 19.43 |
| 21 | 1 | 2 | 12 | 2.46 | 19.19 |
| 22 | 1 | 2 | 11 | 2.67 | 16.14 |
| 23 | 1 | 2 | 4 | 2.96 | 21.02 |
| 24 | 1 | 2 | 2 | 2.79 | 25.73 |
| 25 | 1 | 3 | 9 | 2.80 | 27.11 |
| 26 | 1 | 3 | 6 | 1.99 | 21.57 |
| 27 | 1 | 3 | 4 | 2.90 | 21.39 |
| 28 | 1 | 3 | 11 | 2.46 | 18.70 |
| 29 | 1 | 3 | 3 | 2.83 | 27.72 |
| 30 | 1 | 3 | 5 | 2.94 | 29.64 |
| 31 | 1 | 3 | 2 | 2.73 | 21.10 |
| 32 | 1 | 3 | 12 | 3.09 | 20.81 |
| 33 | 1 | 3 | 7 | 3.51 | 14.56 |
| 34 | 1 | 3 | 8 | 4.08 | 19.20 |
| 35 | 1 | 3 | 1 | 2.91 | 21.23 |
| 36 | 1 | 3 | 10 | 3.27 | 20.24 |

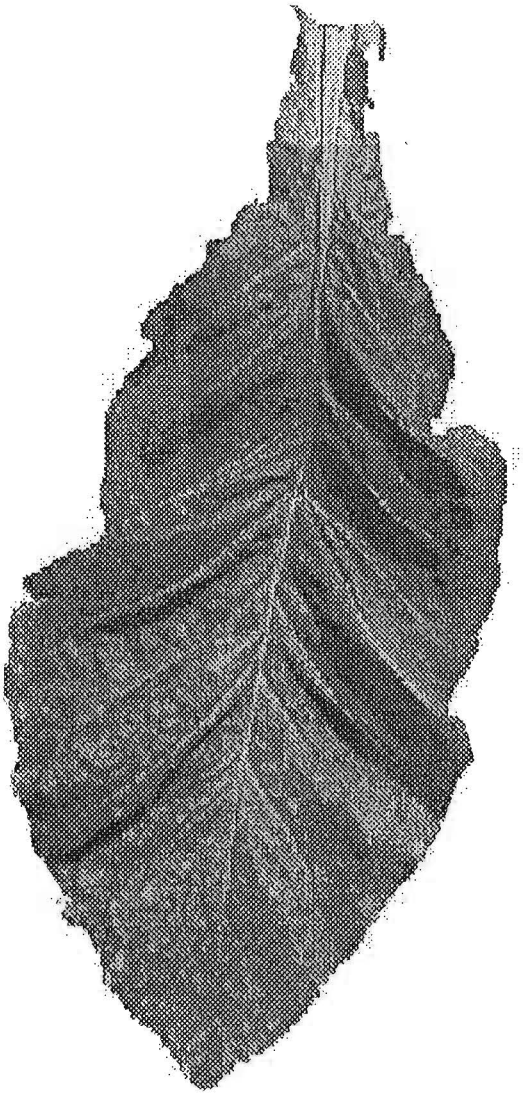
**Appendix 7:** Analysis of variance of the concentration of nicotine and reducing sugars in the leaves of the elite breeding lines at the ARC-TCRI in the 1998/1999 season.

| Entry | % Nicotine | % Reducing sugars |
|---|---|---|
| OD1 | 2.81 | 22.44 |
| OD2 | 2.75 | 22.68 |
| OD486B | 2.52 | 22.29 |
| TL33 | 3.27 | 21.76 |
| OD490 | 3.00 | 28.80 |
| ODT92 | 2.41 | 20.28 |
| ODT1 | 3.29 | 19.72 |
| ODT100 | 3.36 | 20.99 |
| OD313 | 3.04 | 27.31 |
| ODT19 | 3.37 | 19.55 |
| ODT8 | 2.57 | 17.71 |
| ODT82 | 2.65 | 20.77 |
| **Statistics** | | |
| F-value (Reps) | NS | NS |
| F-value (Entry) | NS | ** |
| LSD Entry (0.05) | 1.03 | 7.78 |
| LSD Entry (0.01) | 1.26 | 9.45 |
| C.V. (%) | 13.91 | 13.86 |

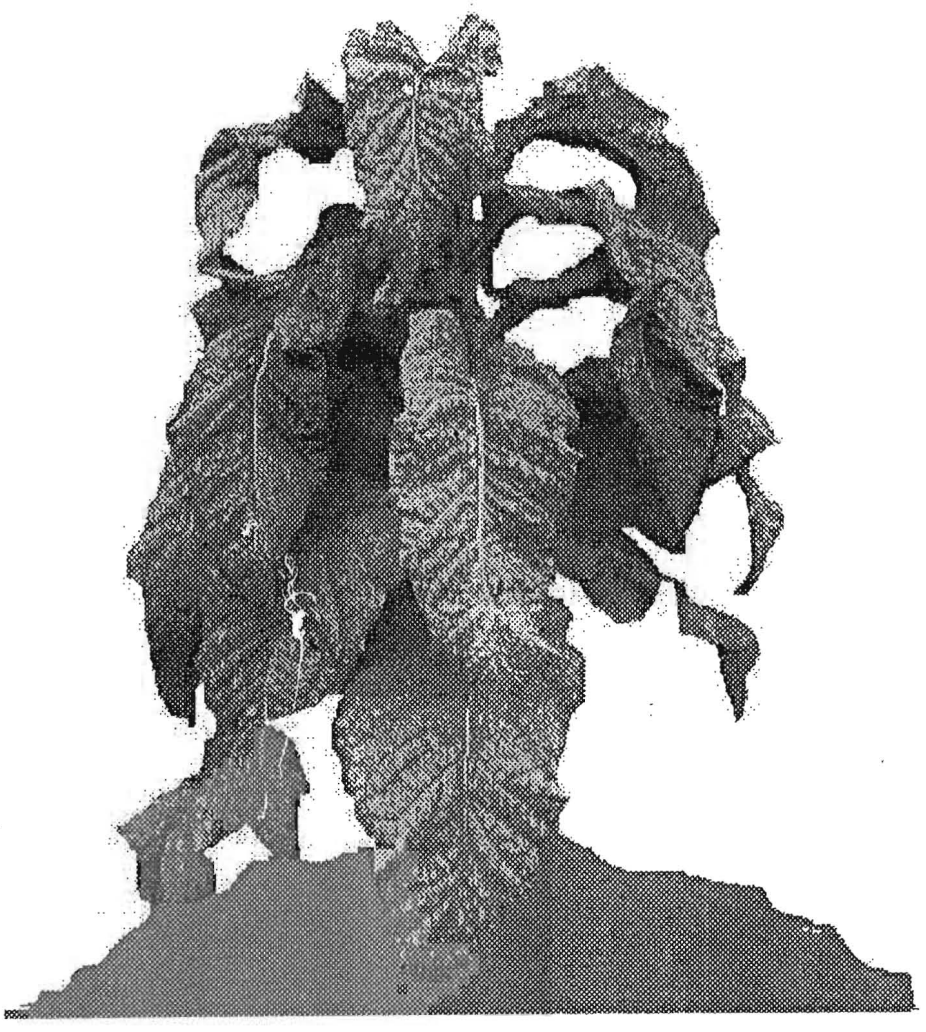
**Appendix 8:** Pictorial characteristic features of the ripe leaves of TL33 and the breeding lines; ODT82, ODT92 and OD2 as observed at the ARC-TCRI in the 1998/1999 season

TL33

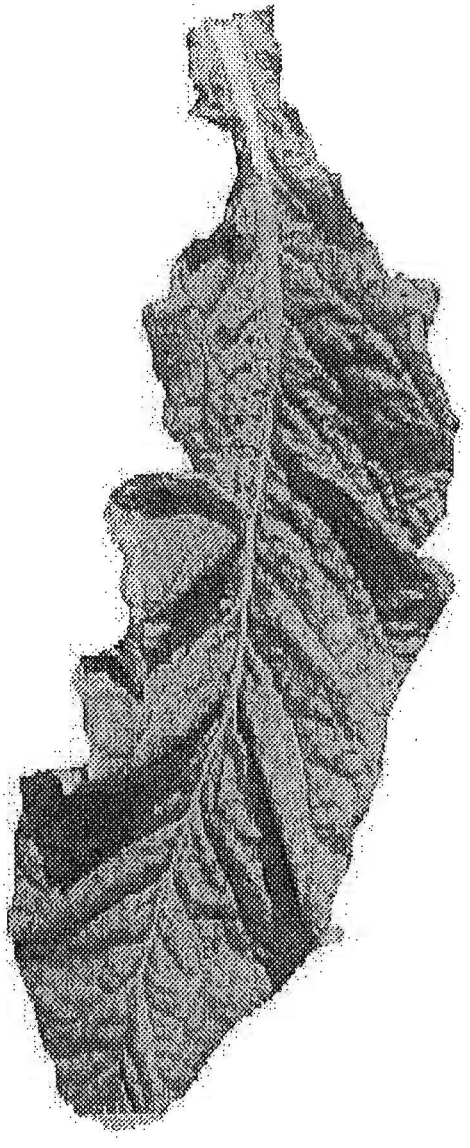
# TL33



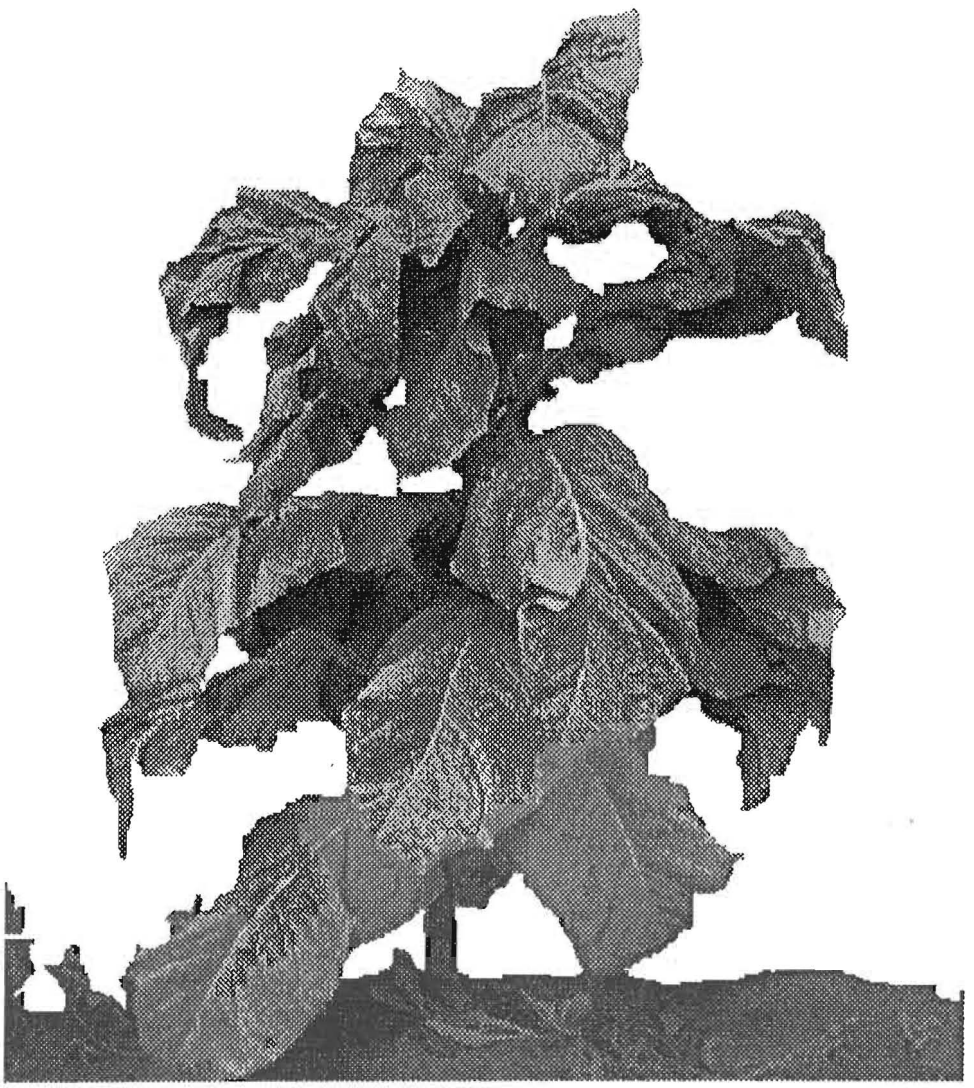
# ODT82



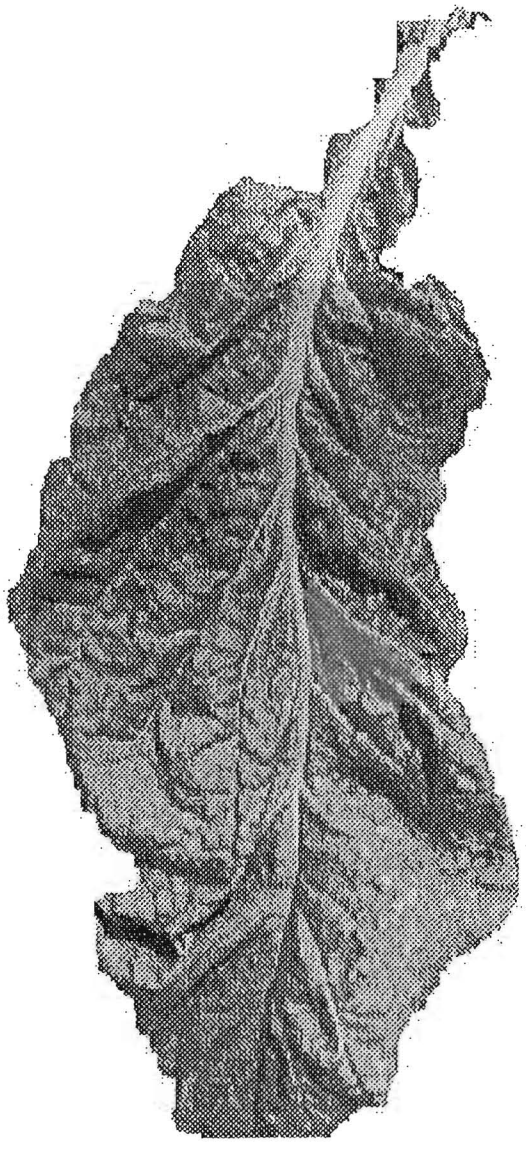
# ODT82



# ODT92



# ODT92



# OD2

# OD2