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 *Nicotiana longiflora*	Dominant, oligogenic 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 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).