AP 11 - Verrier - Tobacco breeding program of Bergerac Tobacco Institute : methods, results and future prospects.

Authors: VERRIER J-L.; ANO G.; BLANCARD D.; TANCOGNE, J.; VIDAL, B.; DELON, R.

The tobacco breeding program of Institut du Tabac (SEITA, ALTADIS) : methods, results and future prospects.

VERRIER, J.-L.*, ANO, G.**, BLANCARD, D.***, TANCOGNE, J.*, VIDAL, B.* DELON, R.*

* *ALTADIS - Institut du Tabac, Bergerac, France.*

** *INRA, Guadeloupe, France.*

*** *INRA, Bordeaux, France.*

Abstract

Tobacco breeding at Tobacco Institute of Bergerac started with TMV resistance, then increased with the blue mold epidemic of years 60, which necessitated creation and release of new, blue mold resistant varieties. In order to diversify the French production, it soon became necessary to create flue-cured and burley varieties, adapted to the French climatic conditions and resistant to the prevailing diseases in France. A comprehensive breeding approach was implemented, not only taking into consideration diseases resistances and early leaf maturity, but also trying to improve leaf quality (as assessed by expert panels) and chemical equilibrium. Joint collaboration with growers cooperatives through ARREAT, then ANITTA, supported a replicated field trial program, which led to releases of new cultivars. In year 1999, 78% of the French flue-cured acreage, and 47% of the burley crop, were planted with F1 hybrids coming from this program. The main features of the breeding strategy underlying these results will be presented. Based on field trial data obtained at Bergerac over a 6 years period, relative contribution of each variety to different quantitative traits, including tar and nicotine from smoking tests, have been estimated. From these data, potential impact of new variety releases on leaf usability of the French production will be discussed.

1. Introduction

Renewal of tobacco varieties in France was first caused by the blue mold epidemic of years 60 and 70. The dark air-cured variety PBD6, originating from the cross Paraguay P48 x Bel 61-10, replaced the traditional dark air-cured Paraguay's (1). Later, decrease of the consumption of "brunes" cigarettes led to introducing burley and flue-cured types. Varieties from Germany (Virginia SCR, also called VD) or from the United States (MN944, K326...) were used. Susceptibility to the prevailing diseases (Black root rot due to the fungus *Thielaviopsis basicola*, also named *Chalara elegans*, vein necrosis due to the virus PVY), and/or late maturity, appeared to be important limitations. The breeding program of Institut du Tabac (SEITA-ALTADIS) aimed at creation of adapted cultivars, with early maturity, good quality potential, and both resistance to *T. basicola* (= *Chalara elegans*) and PVY.

Resistances to powdery mildew (*Erysiphe cichoracearum*), blue mold (*Peronospora tabacina*) and mosaic (TMV) were also considered.

2. New variety development

The indispensable trial network for correctly evaluating putative varieties and releasing the best ones was provided by ARREAT, then ANITTA, associations involving ALTADIS-SEITA, and growers co-operative union (UCAPT).

3. Breeding program

The breeding effort is equally shared between dark air-cured, flue-cured and burley. Breeding methods used for dark air-cured and burley are globally similar. These methods are classical breeding strategies and do not involve genetic modifications (GMO's). Varieties released during the past 5 years are mainly flue-cured and burley, then the following will concentrate on these two types.

4. Burley germplasm base

It mainly involves 8 lines, originating from the United States, Australia, Zimbabwe and Germany. Among these lines, 4 derive from non-burley types (VAM, TB22, Ovens 62, BEL 61-10), and have been used in order to get disease resistances. They likely contributed to increase the overall genetic variability. Among the burley lines, it soon appeared that Kentucky 17 was able to produce, in the absence of PVY, high quality, aromatic burley leaves in the French conditions. The burley program aimed at reaching such quality, or higher, together with disease resistances. Despite their resistances to *T. basicola* and PVY, TN86 and TN90 did not produce in ARREAT / ANITTA trials as high quality as Kentucky 17. Their yield potential, and the important fact that PVY resistance in these lines is associated with secreting glandular trichomes, were important contributions.

Table 1. Burley germplasm base.

Country	Name	Resistance to prevailing diseases in France	Originator
U.S.A.	**Burley 21**		Heggestad, 1966
	Kentucky 17	*Th. basicola* + TMV	Collins et al. 1978
	TN86	*Th. basicola* + PVY	Miller 1987
	TN90	*Th. basicola* + PVY + TMV	Miller 1991
	BEL 61-10	*P. tabacina* + TMV	Clayton *
Australia	**Ovens 62**	*P. tabacina*	Wark
Zimbawbe	**TB22**	*E. cichoracearum*	Smeeton & Ternouth
Germany	**VAM**	PVY	Koelle 1958

(*) supposed originator

5. Flue-cured germplasm base

It mainly involves 15 lines. Among these, Virginia SCR, also called VD, was the only one allowing production of filler flue-cured tobacco in France with some security. This seems to be due to early growth at spring (in the absence of *T. basicola*), relatively early leaf maturity, good yellowing ability, PVY resistance and secreting glandular trichomes. Complex crosses were made, involving VD, the lines 76C16 and 72C18 carrying immunity to *T. basicola* from *N. debneyi* origin, and other lines as contributors for flue-cured quality or disease resistance.

Table 2. Flue-cured germplasm base.

Country	Line	Resistance to main diseases in France	Originator or sponsor
Germany	**Virginia SCR (VD)**	PVY	Carstens & Seehofer 1956
	Perevi	PVY + *P. tabacina*	Reisch 1973
Zimbabwe	**Kutsaga 51E**	E. cichoracearum	1976
	TB22	*E. cichoracearum*	Smeeton & Ternouth
Canada	**76C16, 72C18**	Th. basicola	Anon. 1977
	Islangold, **Delgold**		White & Pandeya 1982 Pandeya & White 1981
Australia	**Ovens 62**	P. tabacina	Wark
U.S.A.	**BEL 61-10**	*P. tabacina* + TMV	Clayton *
	Coker 86	TMV	Coker
	K326, K399, **MN944,** **NC13,** **Speight G-140,** **Speight G-28**		N.K. 1981, 1979 N.K. 1972 NCSU Speight, 1969 Speight

(*) supposed originator

6. Breeding method : pedigree selection

The breeding scheme used is based on the classical pedigree selection, with at least 5 generations of selfing to obtain inbred material. Cytoplasmic male sterility from *N. suaveolens* origin is then used, in order to get F1 male sterile hybrids that are submitted to the ANITTA trial network. Best new inbred lines are backcrossed to *N. suaveolens* cytoplasm in order to extend the array of male sterile lines to be used as females in further combinations.

Haplo-diploidisation methods (anther cultures) have also been tried, in particular in the flue-cured part of the program.

Since the first crosses, more than 2 cycles of pedigree breeding have been achieved. 21 new flue-cured and 19 new burley lines have been converted to male sterility.

7. Breeding method : converters discarded

In every selfed plant from the field nursery, median leaves are harvested, air-cured and

analysed for the ratio nornicotine / total alkaloids. Seed harvest from converter plants (ratio higher than 15 %) is systematically discarded.

8. Breeding method : disease resistance / leaf usability

Disease resistances are tested as early as possible during breeding. Selected, resistant plants are selfed to get further progenies. As soon as they are stable for the main disease resistance genes, these progenies are tested for their leaf quality potential. From each progeny to be studied, 30 plants are topped to produce leaves in a standard way for tobacco production, while sister plants, in another nursery, are selected and selfed.

9. Assessing genotypes : disease resistance tests

Disease resistance tests have been developed with specific regard to their use in a breeding scheme, and to the genes to be revealed. This has been conducted with an important contribution from D. BLANCARD (2) (4). Except for blue mold, these tests may be performed on young plants, which is easier and allows a higher selection pressure. For blue mold, a late season method is used, where plants reach bolting at beginning of September and are not sprayed with anti-oomycete fungicides. Natural contamination occurred each year since 1993 and provided sufficient disease pressure for selection.

Several tests may be successively applied to the same plant population, however, this ends up with a longer vegetative cycle, and greater difficulties to get seed from selected plants.

10. Assessing genotypes : leaf usability

Not only quality, but also traits linked to a "safer" tobacco, are parts of the breeding goal. This is why the term leaf "usability", which comprises both aspects, is used in this presentation. Because of too low number of plants per genotype, and cost of the determinations, important traits linked to usability, for example tar yield from cigarettes, could not be assessed. Attention has been given to correlated traits (reducing sugar content, ashes, total alkaloid content).

Simple and heritable traits are linked to the overall leaf usability. For example, the vein necrosis disease caused by PVY has an important impact on the leaf chemical equilibrium, in particular for burley tobacco, with higher shares of total nitrogen and nitrates in the dry matter. This non-desirable change occurs to a much lower extent in lines carrying the resistance to the PVY conferred by the "va" gene. (3).

Trichome secretion is also roughly evaluated for each line (score 1 to 5 given from hand feeling of medianes and top leaves), in order to discard non secreting lines, which are likely to produce poor flavour and leaf quality. For flue-cured, earliness of leaf maturation has also been taken as an essential trait to be selected.

11. Field experiments to compare progenies

To ensure that environmental variability will not be too high compared to the genetic

differences to reveal, the following is applied:

- Floating bed system for producing seedlings, mechanical planting,
- As homogeneous as possible soil and agronomic practices,
- Standard phytosanitary protection provided to the crop.
- Check variety regularly inserted between progenies, so that every observation made on a progeny may be compared to the nearest check.

Notations are made for traits linked to adaptation or leaf usability (early growth, ground and axillary suckers...).

12. Flue-cured harvesting and curing

Cutters and leaves are primed in each progeny. Each stalk position is harvested when mature, and entered into a yellowing chamber. Days from planting to priming, and yellowing phase duration, are registered with help of bar codes.

13. Burley harvest and curing

20 plants / progeny are stalk harvested and air-cured. After curing, leaves are separated in 4 stalk positions.

14. Industrial quality assessment

Every sample (about 30 to 75 leaves) is described and scored. The sample is treated as a single unit, and is identified by a bar code. Coded description parameters are proposed by a computer system (dialogue boxes), so that not only a global score, but also a more detailed description, are registered. Optional free comments may also be entered.

15. Chemical balance

Following examination, leaves are randomly taken from samples that are to be submitted to NIRS (Near Infrared Reflectance Spectrometry) for evaluation of chemical traits. Whole leaves are ground to a 500 µ powder. In one reading, the NIRS system gives an estimate of total alkaloids, total nitrogen, ashes, and reducing carbohydrates (the latter only for flue-cured).

16. Results : flue-cured

Since 1998 all new filler flue-cured varieties entered in ANITTA trials had both resistance to PVY and *T. basicola*.

Data obtained at the Bergerac location from 1994 to 1999 have been gathered in a database. They all come from replicated trials (4 rep.), and smoking tests have been systematically run to compare varieties (replications gathered). Using a linear model with a constant term, a year effect, a trial effect (when several trials in the same year), and a variety effect, it was possible to estimate the effect of each variety for each trait (least square estimate). This is due to the

fact that many varieties have been repeated from one year to the next. Figures shown here are the sum of the constant term and varieties effects.

4 significant released varieties, which have been tested for at least 2 years, are considered.

Going from the earliest released (VD) to the latest (ITB30804), the following trends are shown:

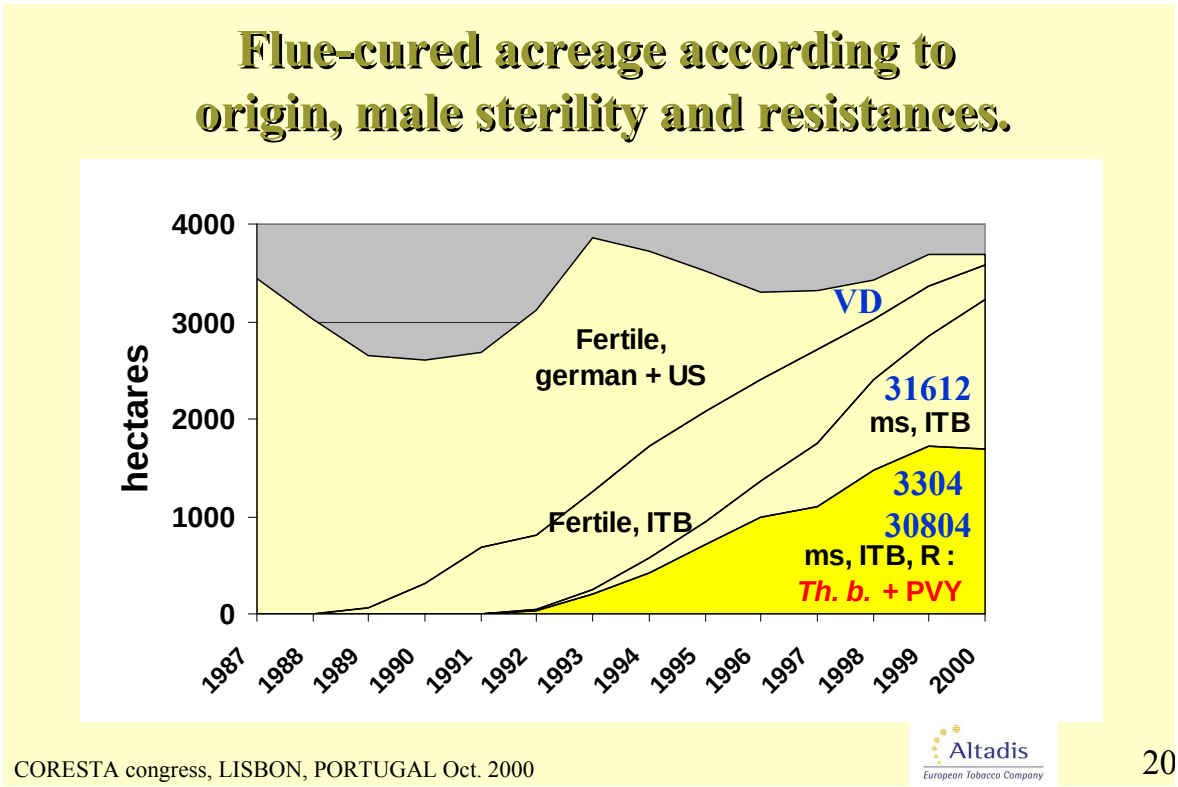
- Sharp increase for industrial leaf quality scores,
- No clear trend for yield,
- Decrease for average harvest date (earlier leaf maturity),
- Sharp decrease for reducing sugars, decrease for tar per cigarette.
- Slight increase for nicotine per cigarette. Values are all clearly below 1 mg/cigarette. These trends are confirmed when considering every tested variety, comprising non-released.

Table 3. Flue-cured results. Data from ANITTA trials 1994 - 1999, Bergerac location.

	Indust. quality score	Leaf total yield	Average harvest date	Red. carbo hydrate	Tar /cig	nicotine /cig	*Th.*
	1-5	T/ha	days	% dm	mg	mg	
VD	2,2	3,7	110	25	19,1	0,43	
ITB 3304	2,8	4,3	116	21	16,3	0,23	
ITB 31612	3,3	3,8	100	17	13,5	0,71	
ITB 30804	4,1	3,7	99	16	14,4	0,57	

17. Flue-cured acreage

Sterile varieties from the breeding program now represent more than 80% of the total acreage, to be compared to zero in 1991. Among these, double resistant sterile varieties account for about 50%, mainly with ITB 3304 and the new ITB 30804. The remaining part of the sterile varieties is mainly ITB 31612. It is supposed that this variety would decrease in the years to come due to lack of PVY resistance, and would be replaced by ITB 30804 which has the same early leaf maturity, and both resistances.



18. Results : burley

All new varieties from the program entered in ANITTA trials since 1996 had both resistances to PVY and *T. basicola*.

Data obtained at the Bergerac location from 1992 to 1999 have been treated in the same way as with flue-cured (see above). 5 significant released varieties tested in these trials for at least 2 years are considered. Going from the earliest released (BB16) to the latest (ITB 502), the following trends are shown:

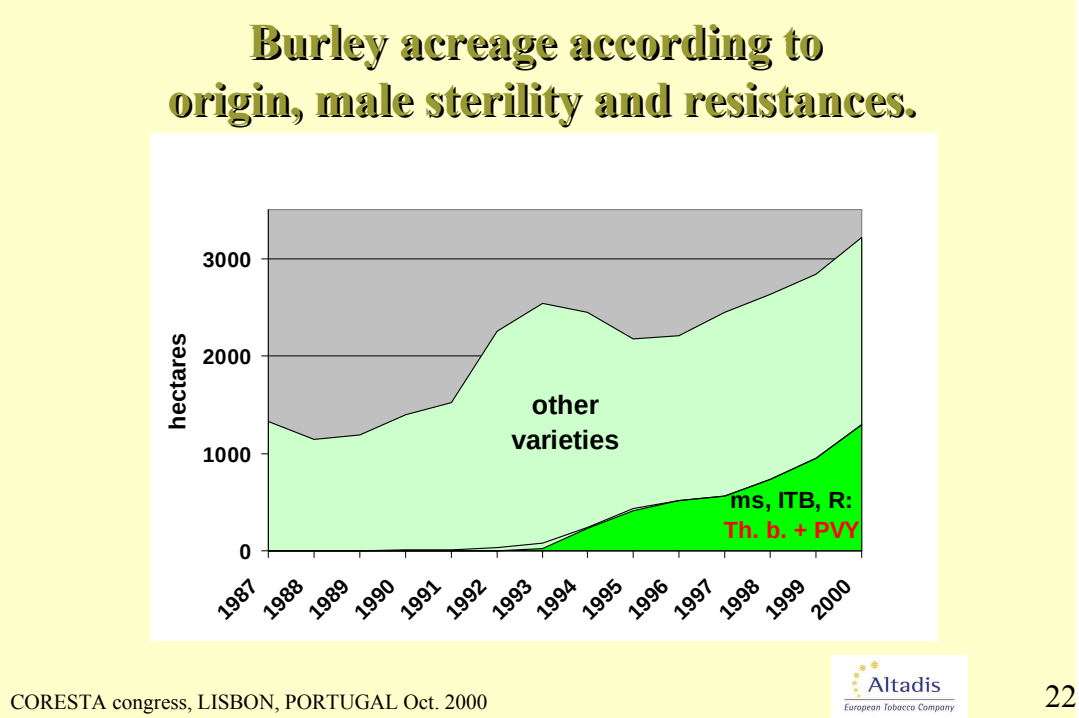
- Industrial leaf quality scores matching Kentucky 17 level with the latest varieties,
- Better yields with the three last varieties,
- Tar per cigarette at around 13 mg, the latest release ITB 502 showing the lowest tar.
- No clear trend for nicotine per cigarette except the latest ITB 502, which seems lower.

Table 4. Burley results. Data from ANITTA trials 1992 - 1999, Bergerac location.

	Indust. quality score	Leaf total yield	Tar /cig	nicotine /cig	*Th. bas.*	PVY	Ye relea
	1-5	T/ha	mg	mg			
BB16	2,4	3,1	15,7	2,6	S	S	19
Kentucky 17	3,8	3,3	13,0	2,6	R	S	19
ITB 2604	3,5	3,6	14,4	2,7	R	R	19
ITB 501	3,9	3,4	13,3	2,8	R	R	19
ITB 502	3,7	3,5	12,4	2,3	R	R	19

19. Burley acreage

Burley acreage has increased more quickly than flue-cured in the past 5 years. This increase is essentially devoted to the newest varieties from the program, mainly ITB501 and ITB 502.



20. Conclusions

It is felt that the following facts have been important for reaching these results:

1. *Understanding of breeding goal - partnership*. Defining a breeding goal requires a forecast of what type and quality of tobacco will be needed, which diseases will be important, how will evolve the farming techniques, in the next 5 to 10 years. Partnership between members of the tobacco industry, understanding of the importance of both disease resistance and leaf quality, allowed taking orientations that proved to be efficient.
2. *Germplasm base adapted to the breeding goals*. Resistances to the main diseases, as well as leaf quality potential, were brought by the germplasm used when starting the program. In flue-cured, consistently earlier leaf maturity than VD could be reached, whereas none of the lines entered when starting were earlier than VD. Heavy selection for leaf maturity in flue-cured has been performed.
3. *Multi-trait breeding.* Every trait of interest has been considered as soon as possible in the breeding process.
4. *System for assessing plant families during breeding.* Constant effort has been involved in increasing heritability of traits being selected. The program continuously benefited from technical improvements (floating beds - mechanical planting - bar-code system for registration of samples, NIRS method...).

In the particular context within which this breeding program takes place (filler flue-cured production in a cooler climate than usual for flue-cured , "va" allele linked to secreting trichomes...) the following conclusions are clear. This does not preclude that the same conclusions should arise in other contexts:

1. No adverse link between disease resistance genes used and leaf usability.
2. Selecting for industrial leaf quality has not been contradictory to reduction of tar potential.
3. Disease resistance and climatic adaptation seem essential to reach leaf usability.

21. Future prospects

Breeding method improvement: N. africana maternal haploids?
The pedigree breeding method used in the program remains a very classical scheme. Despite it's efficiency, it has a cost linked to the necessity of repeating the disease resistance tests up to fixation of resistance genes, and repeating evaluation of new progenies, which may deviate from former ancestors, up to reaching a stable inbred line. Cost structure of a method based on haplo-diploids is different, and total cost might be lower, especially when using PCR tests to assess the presence of disease resistance genes in the haploids to be doubled.

Genetic variability to be introduced: resistance to premature bolting, early growth after planting. Low climatic adaptation is still a limiting factor for the tobacco production in northern Europe.

Genotype assessment during breeding : New tools (NIRS - PCR markers...).
Due to its predictive nature, the NIRS method could be extended for predicting more complex traits than chemical determinations, for example tar potential. Development of PCR markers, at the beginning for disease resistance genes, would be helpful in particular for those tests which are less heritable or require adult plants (PVY, blue mold).

Results already obtained and enhancements of efficiency that may arise with new technical implementations are bases for a reasonable hope.

References

(1) SCHILTZ, P. 1967. Création de Nicotiana tabacum résistant à Peronospora tabacina Adam. Thesis, Bordeaux, p. 1-191.

(2) BLANCARD, D. Maladies du tabac.p. 1-375. INRA Paris 1998, ISBN 2-7380-0751-1.

(3) VERRIER, J.-L., CAILLETEAU, B., DELON, R., TEPFER M. - Impact du PVY nécrotique et CMV sur rendement et qualité de différentes variétés de tabac - CORESTA, Suzhou, Chine, 10- 14 Oct. 1999

(4) DELON, R., VERRIER, J.-L., BLANCARD, D. 1995. Francia : La ricerca di varietà resistenti alle malattie - Il Tabacco Italiano, 1995, 4, 9-17.

(5) DELON, R. -1986 - La stérilité mâle chez le tabac : son utilité pour la production d'hybrides F1. Annales du tabac, n° spécial, sect. 2, p. 39-44.