

**61<sup>st</sup> TSRC      Charlotte (N.C.)**

**Simple and reliable method  
for the quantification of  
proteins in tobacco.**

***BREGÉON Bernard***



# Objective :

**Reevaluate an analytical approach developed in the 70's in order to supply an effective tool in the understanding of the formation mechanisms of the main nitrogenous compounds in the smoke.**

# Structure:

- #1 - Analytical principle.
- #2 - Description of the method.
- #3 - Validation.
- #4 - Tobacco applications.
- #5 - Components specificity of analysis.
- #6 - Relationships between method results and smoke data.
- #7 - Conclusions.

# Analytical principle:

Modified version of the Erich Wegner method published in 1955 in Tabak- Forschung.

**Protein Nitrogen is based on the nitrogen difference of the coagulated extract obtained with and without pepsin-aided digestion.**

**Protein N = Coagulable N – Residual N**

*The analysis allows to estimate the nitrogen in proteins without having other coagulable nitrogenous forms, such as, brown pigments.*

# Protein Nitrogen analysis:

## Way A

### 1- Degradation of residual tobacco enzymes:

0.5 g of tobacco in powder in 25 ml of de-ionized water boiled in an oil bath (130°C). Cooling and acidification: pH: 1.55, HCl N.

### 2 – Protein digestion:

Addition of 20 mg of pepsin in 25 ml water (pH: 1.55) Heating and stirring at 50°C for 6 hours (water bath). Cooling and neutralization (NaOH N)

### 3- Coagulation of Nitrogen compounds:

Addition of 10 ml of trichloroacetic acid (18.75 g / 100 ml) heating in an oil bath (130°C). Cooling and let it rest (1 night).

### 4- Separation of insoluble compounds:

Filtration (glass filter funnel)  
Drying of filtration residue (oven 1H at 110°C)

## Res N

### 5- Nitrogen determinations:

CHN analyzer ( LECO)

## Way B



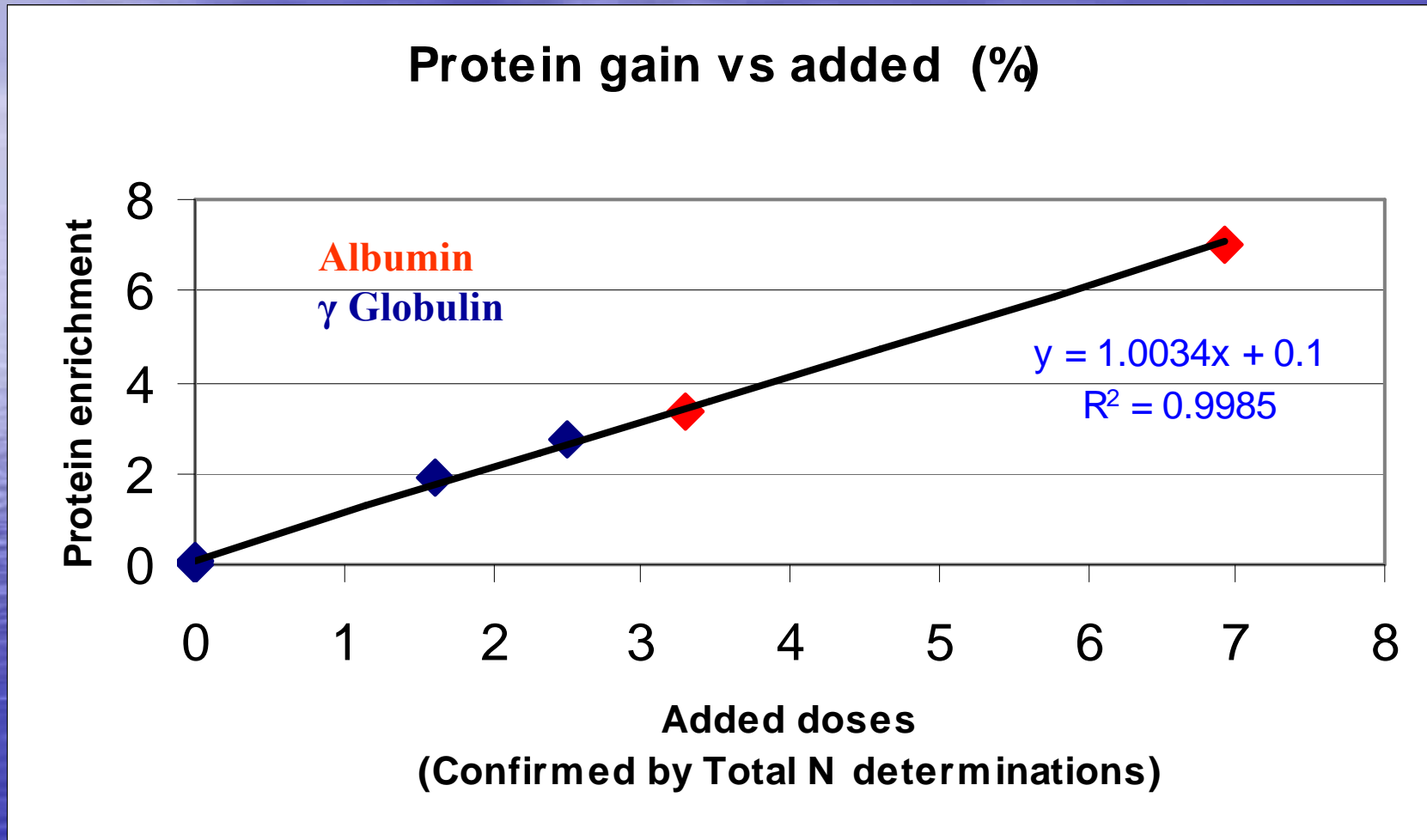
**By-passed step**



## Coag N

# Method validation:

Proteins amount in % = Prot N \* 6.24

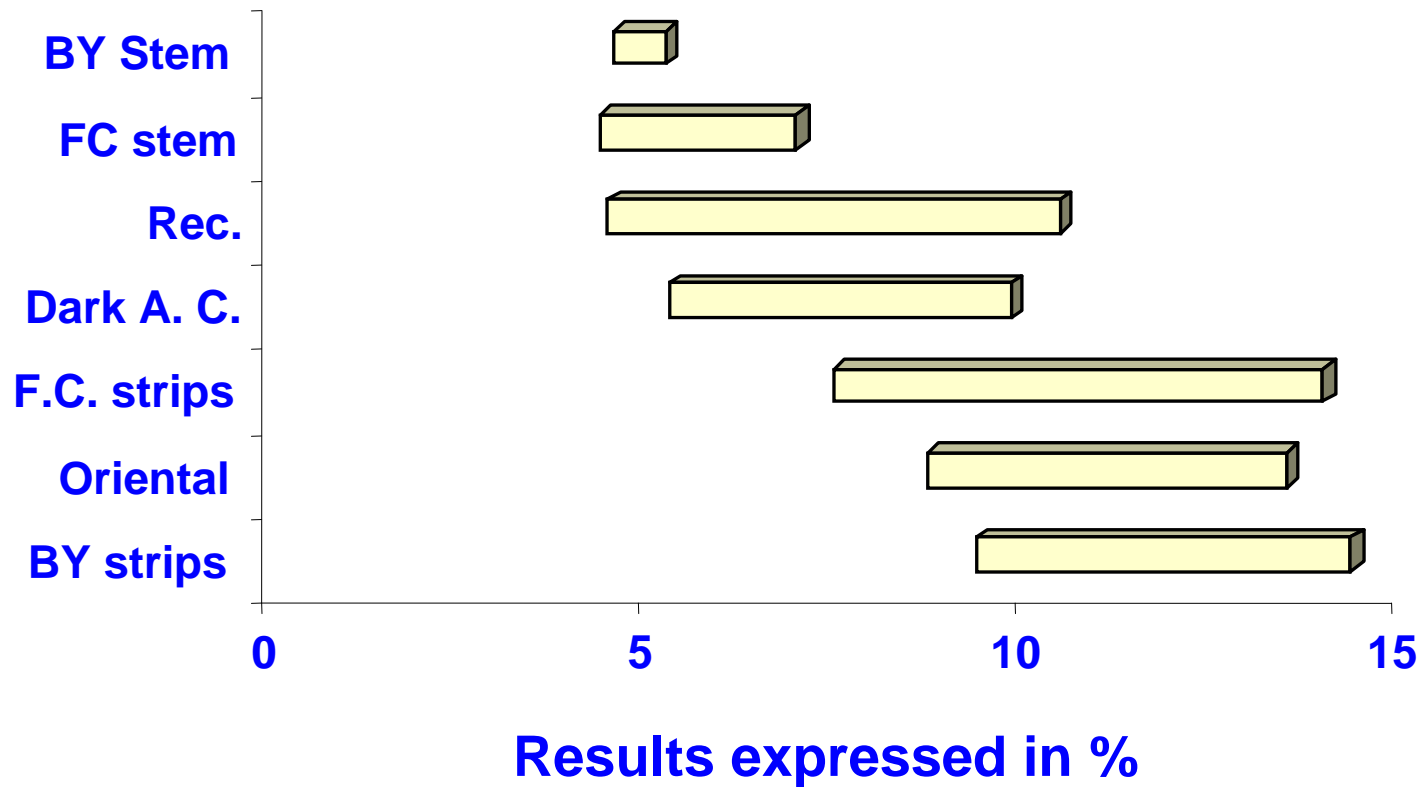


Enrichment protocol: 2 doses of Albumin: 3.5 and 7% and 2 doses of  $\gamma$  Globulin: 1.5 and 2.5% spiked on US blend.

# Tobacco results:

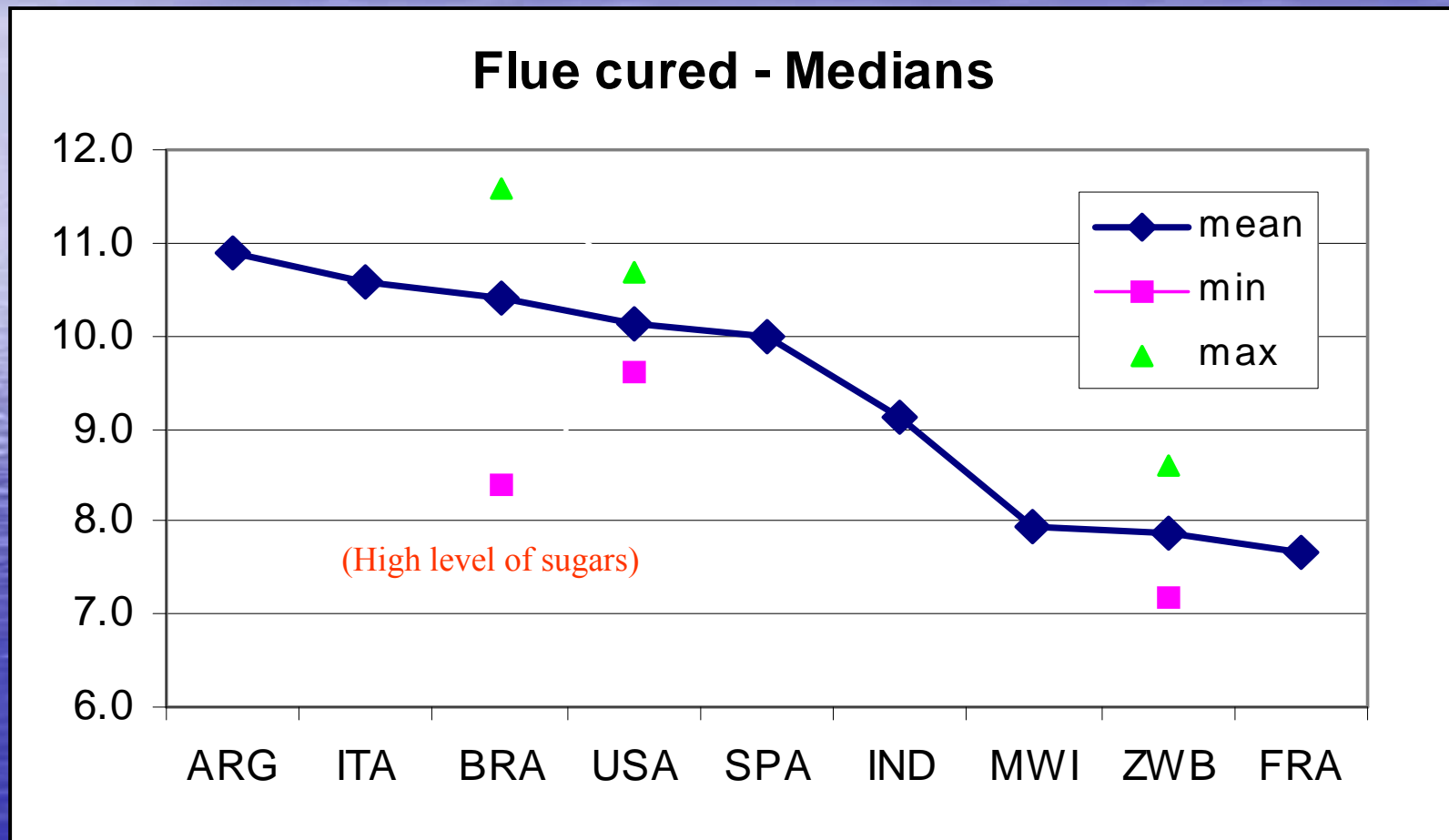
Proteins amount in % = Prot N \* 6.24

## Range of Protein amounts in tobacco



# Crop origin effect / F.C. Medians:

Results expressed in % of Protein

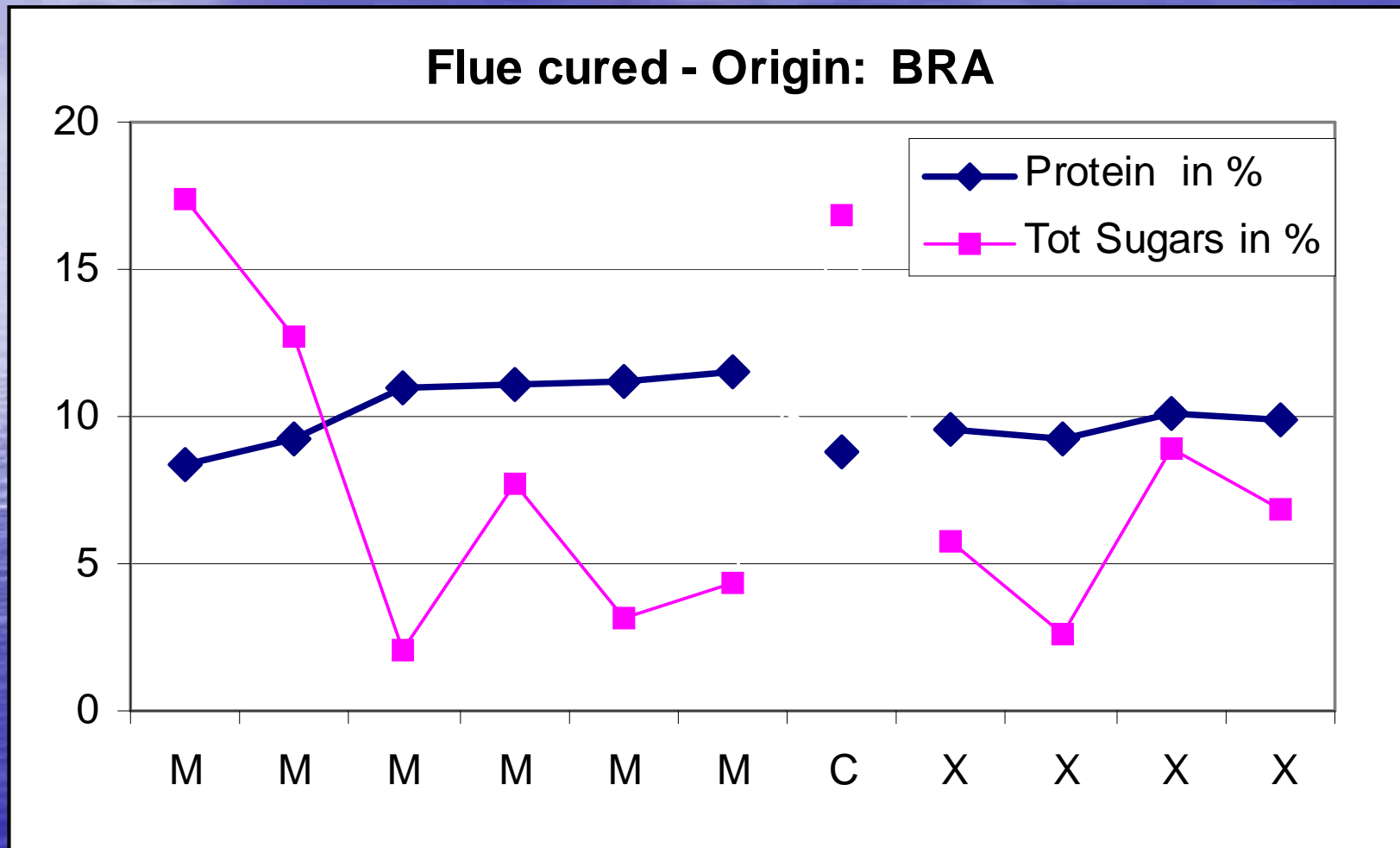


Great incidence of origin on protein amounts.



# Grade incidence / Flue Cured:

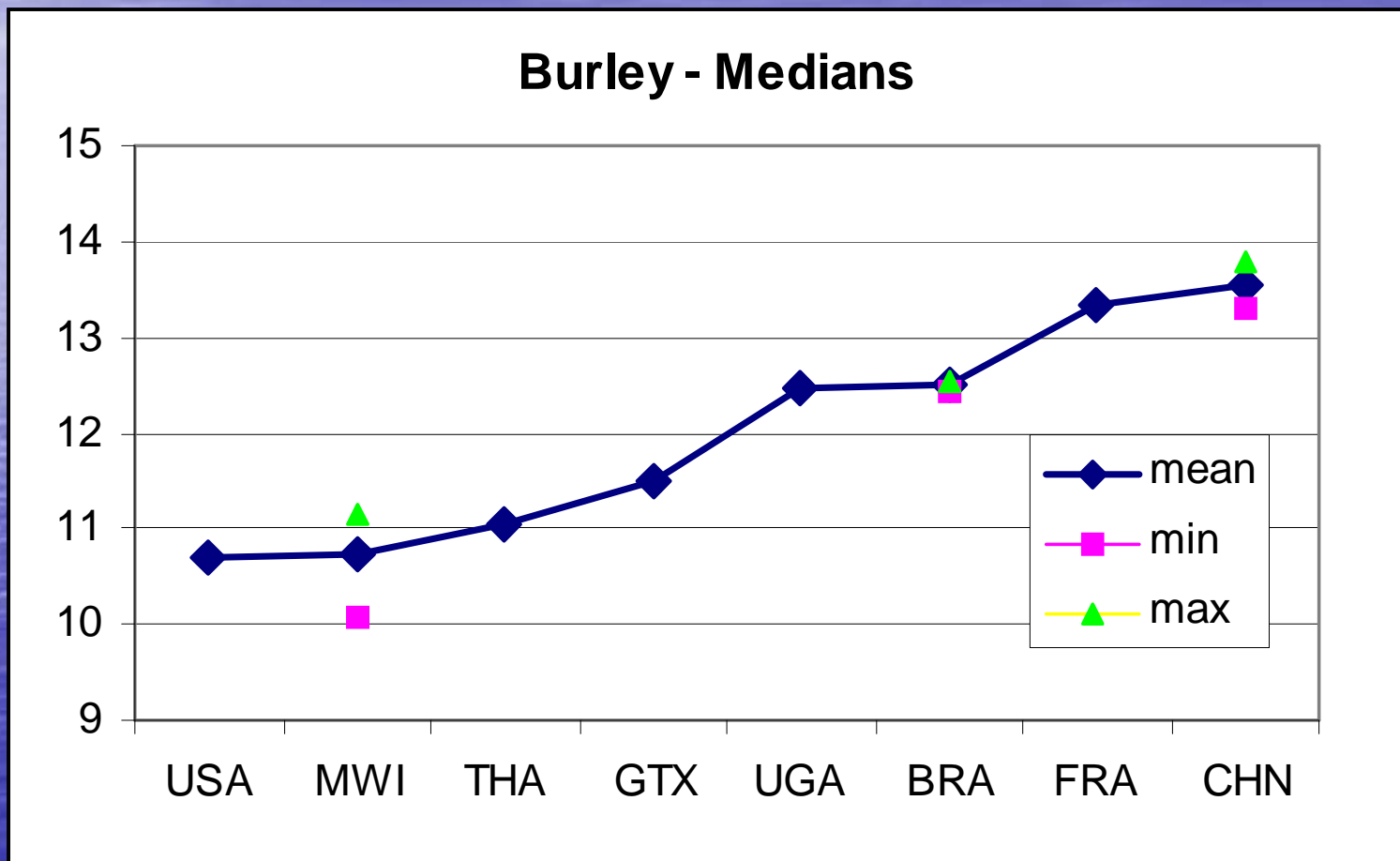
Results expressed in % of Protein



Ripeness more influent than stalk position on protein amounts.

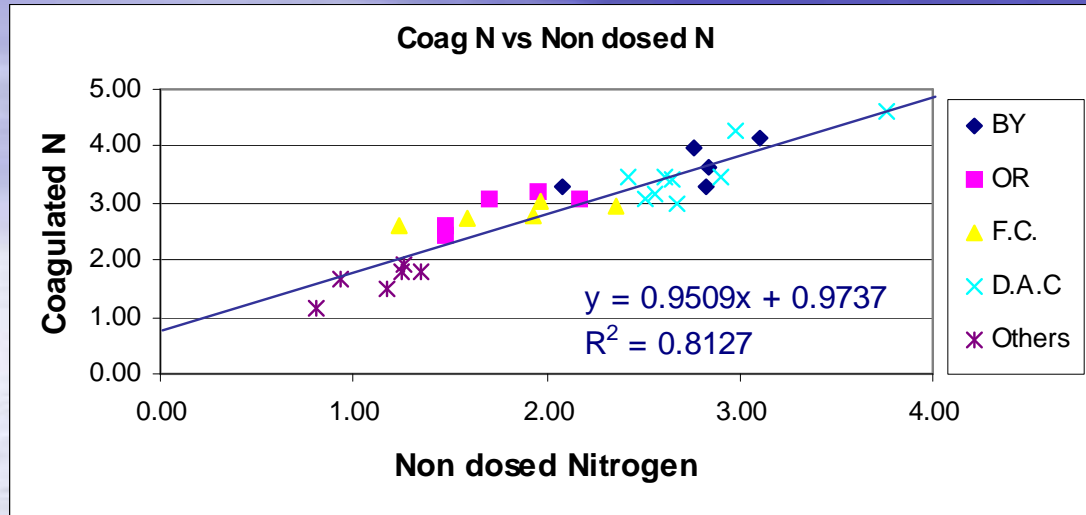
# Crop origin effect / BY medians :

Results expressed in % of Protein



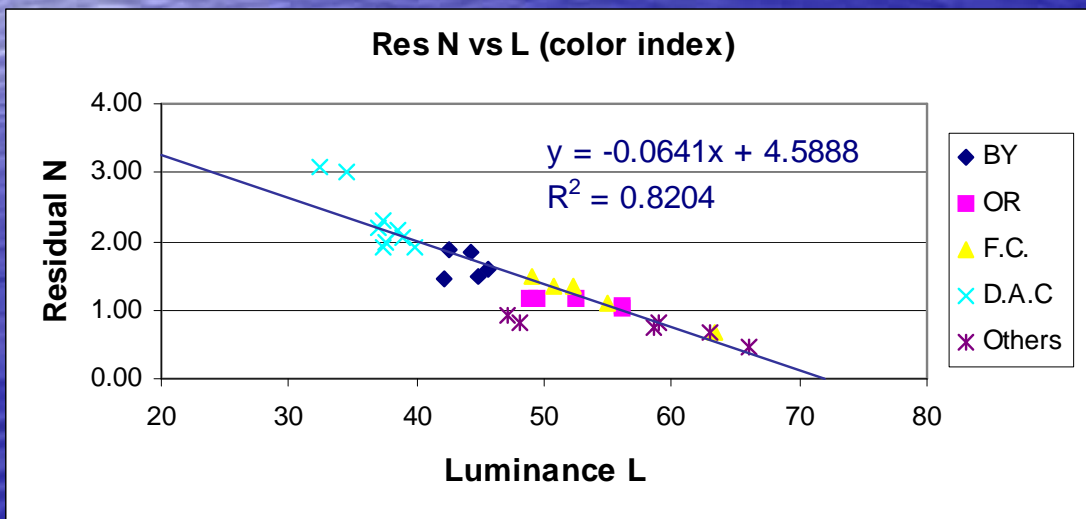
Great incidence of origin on residual protein amounts.

# Components specificity of analysis :



Coagulated N ~  
Non dosed N ~  
*Proteins + Pigments*

**Non dosed N = TN – Sum of dosed N components (all expressed in N %)**

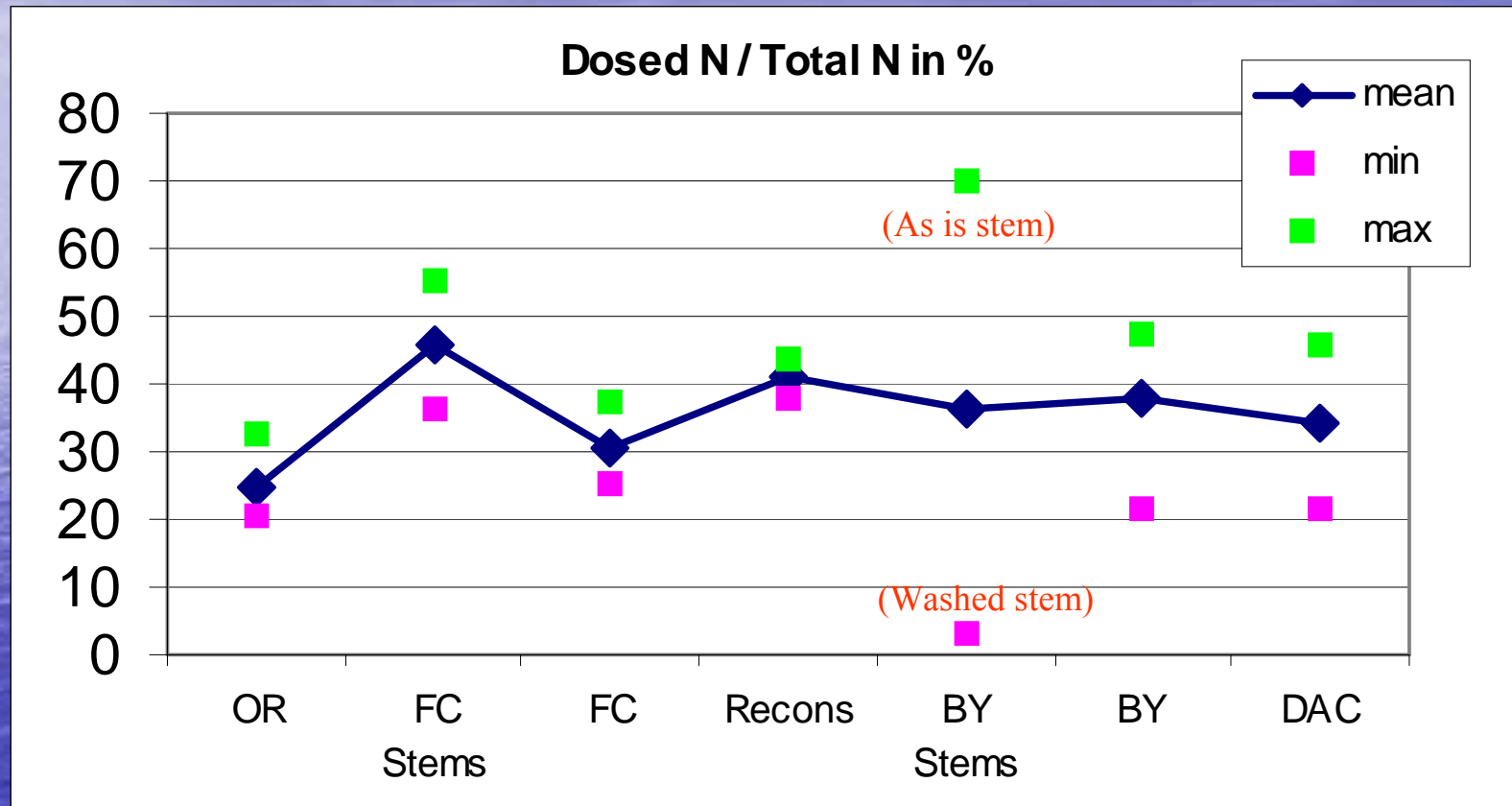


Residual N ~  
Color index L ~  
*Pigments*

**46 representative lots  
including Dark Air Cured**

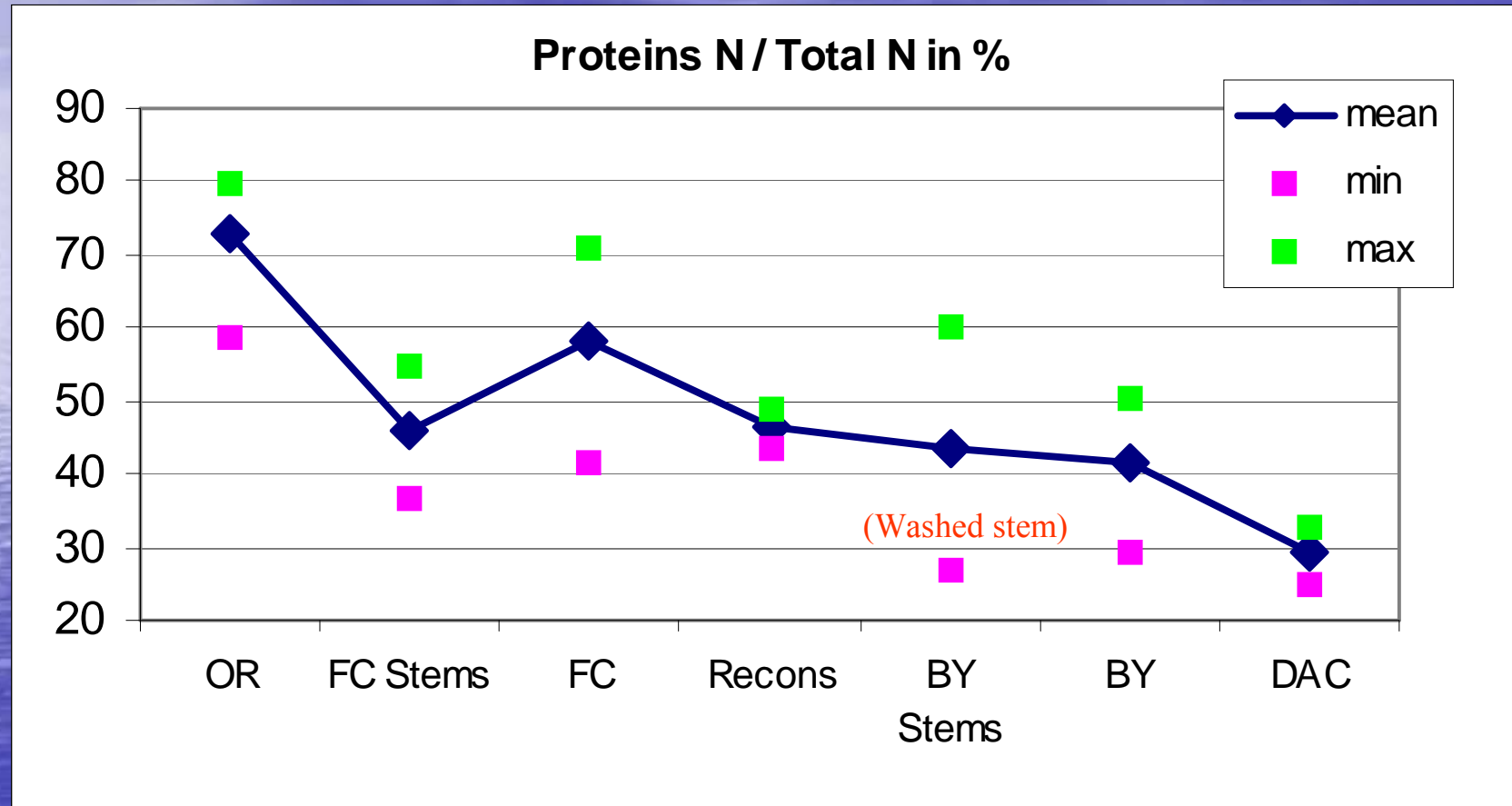
# Nitrogen precursors balance:

$$\text{Total N \%} = \text{Dosed N \%} + \text{Protein N \%} + \text{Pigment N \%}$$



Similar proportion of dosed N compounds in each type of tobacco.  
Global average ~ 36 %.

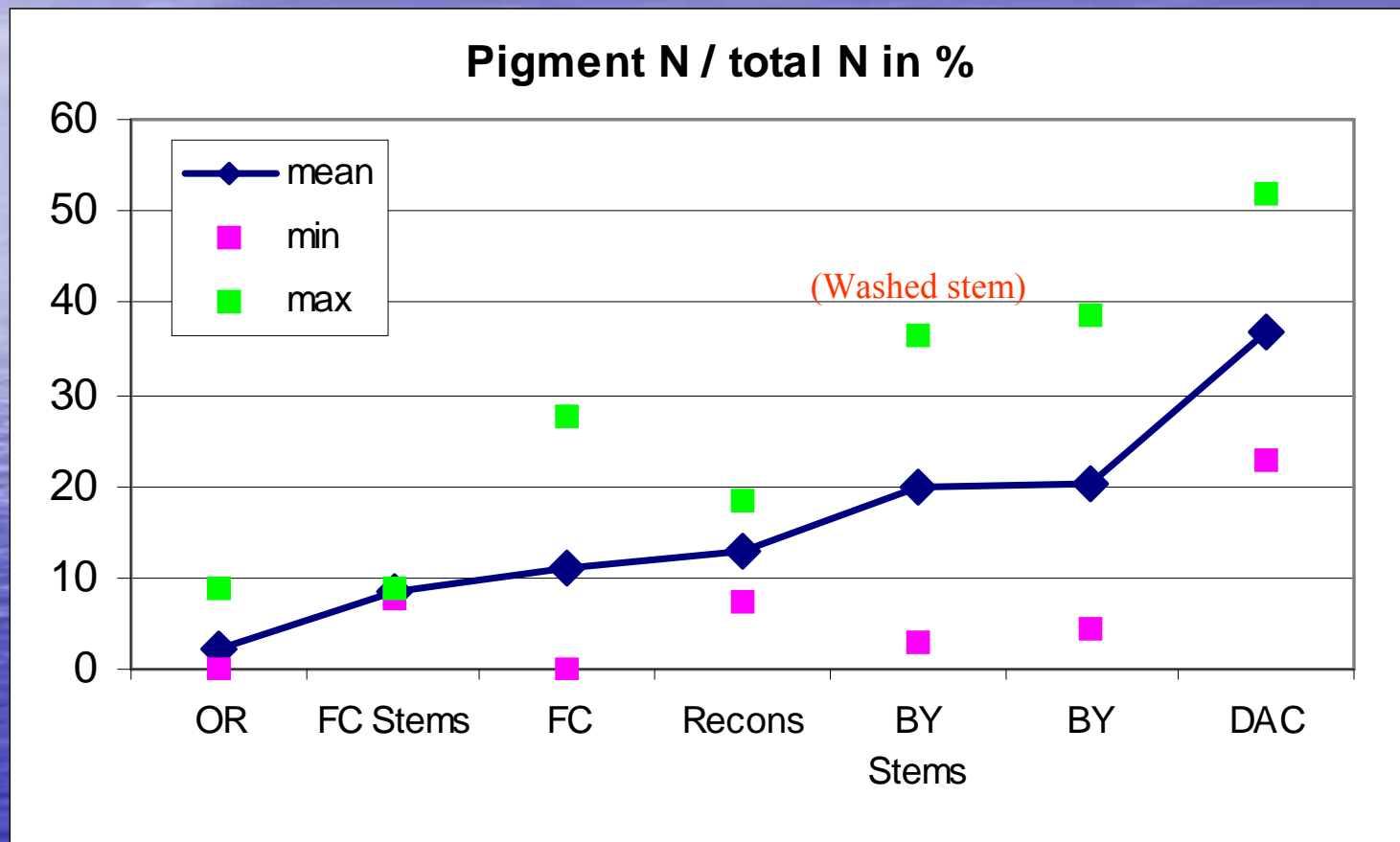
# Protein N contribution:



Great difference in protein relative contribution for each type of tobacco. Important protein richness in sun-cured lots.

# Pigment N contribution:

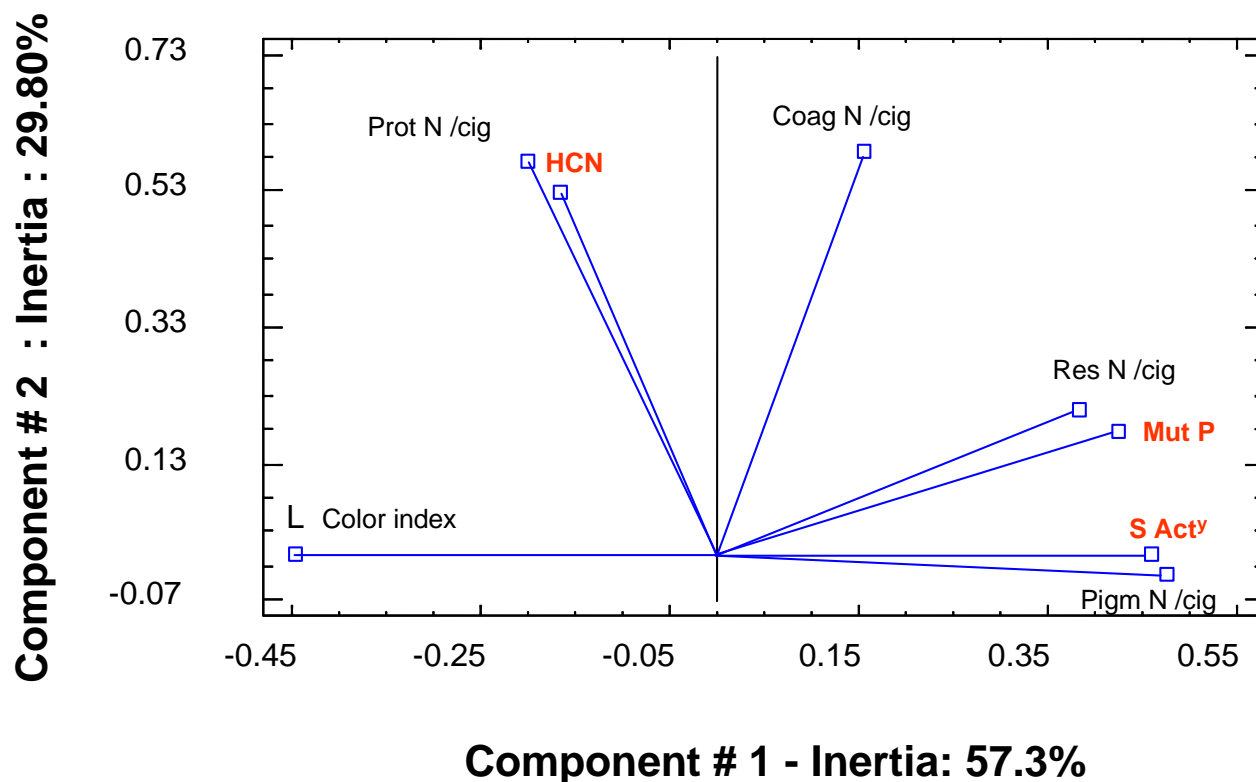
Pigment N = Non dosed N – Protein N.



Great difference in Pigment N relative contribution according to tobacco evolution.

# Relationships between method results and smoke data :

## Correlation circle



**46 representative lots including Dark Air Cured.**

R <sup>2</sup>	Relationship
83	S Acty vs L
76	S Acty vs Pigm N / cig
71	Mut P vs Res N / cig
85	HCN vs Prot N / cig

**Ames test (TA 98 strain + S9):**

**S Acty in Rev /mg of TPM and Mut P in Rev for 1/50 cig.**

**HCN in µg/cig.**

# Conclusions:

## **Efficient method for proteins determination in tobacco.**

- > Approach validated by spiking.
- > Simultaneous quantification of pigment nitrogen.

## **Analytical components highlight importance of pigment nitrogen in air cured tobacco.**

- > No optimal modelling of protein N, despite observed correlations.

## **Tobacco results confirm or reveal:**

- > HCN and proteins relationship.
- > Possible implication of pigment nitrogen on Ames response (TA98 strain + S9).