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STUDIES ON RELATION BETWEEN LEAF AND SMOKE CONSTITUENTS OF FCV TOBACCO

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Significant positive correlations were observed between smoke total particulate matter (TPM) and leaf nicotine, solanesol & petroleum ether extractives (PEE); smoke solanesol and leaf nicotine & solanesol. A positive correlation was obtained between smoke solanesol and leaf PEE. Linear regression equations were developed between TPM, tar & nicotine and different leaf constituents to predict the smoke constituents. In the case of Karnataka Light Soils (KLS) and Northern Light Soils (NLS) samples, the frequencies of chi-square (X^2) values for TPM and tar were in the range of 0.00 - -0.99 were 73.3 & 85.0% and 79.1 & 88.4%, respectively. For nicotine in KLS and NLS samples, X^2 values in the range of 0.00 - 0.49 were 100.0 and 92.86%, respectively. Thus, the selected regression equations can be employed for predicting TPM, tar and nicotine in smoke using the values of leaf nicotine, reducing sugars, chlorides, potassium, PEE and solanesol.

INTRODUCTION

In view of the awareness of the associated health hazards, the challenge for the tobacco scientists is to identify the harmful constituents in tobacco and tobacco smoke and develop methods to reduce them. An obstacle to lower the condensate delivery in tobacco by plant breeding process involves assaying a large number of samples (Chaplin and Spurr Jr., 1982). The particulate matter index (PMI) method tends to alleviate this, but a better method is needed. Further, in the conventional smoke analysis for estimation of TPM, tar and nicotine, at least 3 kg of leaf lamina is required for making cigarettes followed by the standard protocol of conditioning, selection, smoking and estimation of moisture & nicotine by GC. Further, a deviation of ± 20% is allowed in the estimated values of the characteristics. These are the limiting factors involving large number of individual plants to be evaluated in the breeding programme. A multiple regression technique involving selected chemical analysis of cured leaf has been tried. The correlations between smoke constituents and leaf constituents and regression equations using selected leaf constituents for predicting smoke TPM, tar and nicotine were tried.

MATERIALS AND METHODS

A total number of 103 FCV tobacco samples, 60 from Karnataka Light Soils (KLS) and 43 samples from Northern Light Soils (NLS) were collected during 2006-07, 2007-08 and 2008-09 crop seasons for analysis of leaf [nicotine, reducing sugars, chlorides, potassium, solanesol and petroleum ether extractives (PEE)] and smoke [total particulate matter (TPM), tar, nicotine, carbon monoxide and solanesol] constituents (Gangadhar, 2010). The values of smoke constituents and leaf constituents were expressed on mg per gram dry tobacco smoked and mg per gram dry tobacco basis, respectively. Region-wise correlation coefficients were calculated. The region-wise estimated, predicted and the x^2 values of samples are tabulated.

RESULTS AND DISCUSSION

Correlation coefficients

In KLS samples, smoke TPM has significant positive correlation with smoke nicotine, tar and solanesol. Similarly, smoke nicotine has significant positive correlation with smoke tar and

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solanesol. Smoke TPM has high significant positive correlation with leaf nicotine, solanesol and PEE. Further, smoke solanesol has significant positive correlation with leaf nicotine and PEE (Table 1). In the case of leaf constituents, nicotine has significant positive correlation with solanesol (0.674) and PEE (0.441) while PEE has significant positive correlation (0.494) with solanesol.

Significant positive correlation was observed in the case of smoke TPM with nicotine, tar and solanesol in NLS samples and a similar trend exists in respect of nicotine with tar and solanesol; tar with solanesol (Table 2). TPM has significant positive correlation with leaf nicotine, solanesol and PEE. Significant positive correlation was observed between smoke solanesol and leaf nicotine. Smoke TPM, nicotine and tar have significant negative correlations leaf potassium. Among the leaf constituents, nicotine has significant positive correlation with solanesol (0.560) and PEE (0.572), similarly PEE has significant positive correlation (0.491) with solanesol.

Employing cigarettes of chemically defined leaf tobacco, a study found positive correlation between the resin content in the leaf and yield of smoke condensate (0.69**) and a similar trend in relation to the nicotine content of the leaf (Binopoulos *et al.*, 1965). In the study conducted by Tso *et al.* (1982), leaves from 8 stalk positions from 4 burley tobaccos were tested. A total of 116 variables including leaf and smoke characteristics, were determined. Simple correlations and multiple regressions among selected variables were obtained to examine the relationship among these variables.

Tso *et al.* (1982) and Kameswara Rao *et al.* (1985) have reported a negative correlation between TPM and PEE. However, in the present study, a significant positive correlation was observed between TPM and PEE & solanesol and between tar and PEE, in agreement with the findings of Binopoulos *et al.* (1965). Solanesol, as a major component of tobacco lipids accounting for 10-15% of hexane soluble fraction, is implicated as a precursor of smoke PAH. Swain

Table 1: Corrélation coefficients – KLS samples

Smoke constituents	Smoke constituents: Nicotine	Smoke constituents: Tar	Smoke constituents: Solanesol	Leaf constituents: Nicotine	Leaf constituents: PEE	Leaf constituents: Solanesol
TPM	0.619**	0.978**	0.515**	0.610**	0.562**	0.607**
Nicotine		0.579**	0.828**	0.816**	0.379**	0.656**
Tar			0.449**	0.581**	0.543**	0.573*
CO			0.461**	0.276*	0.103	0.337**
Solanesol				0.695**	0.279*	0.692**

Table 2: Corrélation coefficients – NLS samples

Smoke constituents	Smoke constituents: Nicotine	Smoke constituents: Tar	Smoke constituents: Solanesol	Leaf constituents: Nicotine	Leaf constituents: Potassium	Leaf constituents: PEE	Leaf constituents: Solanesol
TPM	0.792**	0.970**	0.439**	0.710**	-0.538**	0.707**	0.475**
Nicotine		0.657**	0.384**	0.910**	-0.455**	0.521**	0.505**
Tar			0.395**	0.586**	-0.542**	0.691**	0.383*
CO			0.543**	-0.163		0.048	0.012
Solanesol				0.315*		0.149	0.326

et al. (1961) reported that the major portion of the PEE was resinous material, the others being paraffins, polyenes esters, solanesol, sterols, tocopherols and fatty acids. Schlotzhauer *et al.* (1976) observed that solanesol contributes to above 30% of smoke PAH and 40% of smoke BaP, emphasizing the desirability of low solanesol levels in tobacco. Watson *et al.* (2004) developed a method to assess cigarette smoke intake by estimating solanesol in cigarette butt. Phani Kiran (2008) reported a significant positive correlation between solanesol in leaf with smoke constituents viz., TPM (0.76), tar (0.73), nicotine (0.86), carbon monoxide (0.77) and solanesol (0.94), which could help in obtaining a regression equation to predict smoke TPM based on leaf solanesol content. These findings corroborate the positive correlation between TPM and leaf PEE & solanesol, found in the present study.

Regression equations

Regression equations were fitted between TPM, tar & nicotine and different leaf constituents in samples from a particular region to predict the smoke constituents.

KLS samples

TPM = 35.8532 + 2.7773 S + 0.2979 P - 0.0478 R - 0.2425 K . **R^2 = 0.478****

TAR = 25.6600 + 0.2939 N + 1.0541 S + 0.1379 P - 0.0945 K. **R^2 =0 .468****

NIC = 0.2481 + 0.1514 N - 0.0028 R + 0.0230 K. **R^2 = 0.704****

NLS samples

TPM = 27.9712 + 0.5973 N + 0.0983 S - 0.4821 K + 0.2096 P **R^2 = 0.682****

TAR = 33.2954 + 0.2055 N - 0.0244 R - 0.4258 K + 0.1720 P **R^2 = 0.608****

NIC = 0.4518 + 0.2054 N - 0.0093 S - 0.0666 K **R^2 = 0.859****

(S: Solanesol; N: Nicotine; K: Potassium; R: Reducing sugars; P: Petroleum Ether Extractives)

Chi-square (χ^2) test

Using the selected regression equations, predicted TPM and tar values of samples from different regions were estimated. The region-wise estimated, predicted and the χ^2 values of samples are tabulated (Table 3). The χ^2 is a non-parametric test. The value χ^2 describes the magnitude of the discrepancy between theoretical and observed values (Snedecor and Cochran, 1967). It is inferred that if the sum of χ^2 values are less than the critical value at 95% level of significance, the null hypothesis of no difference between the observed and expected (predicted) value of TPM and tar exists.

It is observed that the frequencies of smaller χ^2 values for TPM in the range of 0.00-0.99 were high (KLS: 73.3%; NLS: 79.07%). The frequencies of higher χ^2 values were: 1.00-1.99 (10.34 – 13.95%) and 2.00-2.99 (4.65 – 13.79%). Similar trends were observed in the case of values of tar: 0.00-0.99 (KLS: 85.0%; NLS: 88.37%). The frequencies of higher χ^2 values were: 1.00-1.99 (6.98 – 11.67%) and 2.00-2.99 (2.33 – 5.17%).

Yate's correction was applied for nicotine and χ^2 values were calculated and the critical values are given in Table 4.

It is observed from the frequency of distribution of χ^2 values for nicotine that the frequencies of smaller χ^2 values for nicotine in the range of 0.00-0.49 were high (KLS: 100%; NLS: 92.86%). The frequencies of higher χ^2 values were: 0.50-0.99 (0.00 – 7.14%) and 1.00-1.49 (0.00 – 9.09%).

Table 3: Chi-square calculated and critical values for TPM and Tar

Region	Number of samples	TPM		Tar	
		X^2 calculated value	Critical value	X^2 calculated value	Critical value
KLS	60	41.74	79.08	24.49	79.08
NLS	43	25.56	55.76	20.54	55.76

Table 4: Chi-square calculated and critical values for nicotine

Region	Number of samples	Nicotine	
		X^2 calculated value	Critical value
KLS	60 (22 after addition)	2.06	32.67
NLS	43 (28 after addition)	4.20	40.11

The selected regression equations can be employed for predicting TPM, tar and nicotine in smoke using the values of leaf nicotine, reducing sugars, chlorides, potassium, PEE and solanesol. As evidenced by the frequency of distribution of x^2 values, the prediction can be reasonably precise. Thus, the equations can be of value to the plant breeders for screening large population in the breeding of varieties with low TPM/tar.

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