Indian Journal of Agricultural Sciences **83** (10): 1052–7, October 2013/Article

Effect of different levels of nitrogen and leaf position on biochemical quality constituents of FCV tobacco (*Nicotiana tabacum*) grown in northern light soils of Andhra Pradesh

C CHANDRASEKHARARAO[1], K SIVA RAJU[2], H RAVISANKAR[3], M ANURADHA[4] and KASTURI KRISHNA[5]

Central Tobacco Research Institute, Rajahmundry, Andhra Pradesh 533 105

Received: 16 April 2012; Revised accepted: 26 August 2013

ABSTRACT

Field experiment was conducted during 2008-10 at CTRI RS Jeelugumilli on sandy loam soil, to study the effect of nitrogen levels (20, 40, 80, 120, 160 and 200 kg N/ha) and leaf position (P, X, L and T) on bio-chemical quality constituents of FCV tobacco (*Nicotiana tabacum* L.). Chlorophyll a, chlorogenic acid, rutin, petroleum ether extractives (PEE), free amino acids (FAA) and proline contents increased with increase in nitrogen up to 120 kg N/ha and decreased beyond 120 kg N/ha. The FFA content decreased significantly with increase in nitrogen application from 40 to 200 kg N/ha. Chlorophyll b, rutin, FAA and proline contents increased significantly with increase in leaf position from bottom to top (P to T). With increase in nitrogen from 120 to 200 kg/ha, the starch content decreased and lowest content was observed in 200 kg/ha. Tobacco from T position contained nearly 2.9 times higher levels of FAA over the P position. Nitrate nitrogen content increased with increase in nitrogen level up to 180 kg N/ha in all leaf positions, whereas there was a marginal decrease at 200 kg N/ha. Tobacco from L position at 120 kg N/ha showed maximum accumulation of quality constituents. The recommendations of 120 kg N/ha for the variety Kanchan grown in northern light soils of Andhra Pradesh was found to be optimum for the higher yield with balanced quality constituents with higher levels of aroma compounds.

Key words: FCV tobacco, Leaf position, Nitrogen, Quality constituents

Flue-cured tobacco (FCV) (*Nicotiana tabacum* L.) is one of the important quality conscious commercial crops grown in India. Tobacco contributes about ₹ 20,000 crores as excise duty and about ₹ 4000 crores towards foreign exchange. High yielding FCV tobacco variety Kanchan is being grown in Andhra Pradesh and Karnataka. Chemical quality parameters, viz. nicotine, reducing sugars and chlorides and biochemical constituents, viz. starch, petroleum ether extractives, polyphenols, free amino acids and free fatty acids are some of the leaf constituents responsible for aroma and quality of tobacco and these parameters are influenced by quantity and source of manures, position of leaf on stalk, climatic conditions, cultural practices, genotypes and method of curing (Long and Weybrew 1981).

Nitrogen is the key nutrient in tobacco fertilization and tobacco is sensitive to nitrogen nutrition. In tobacco, leaf being the economic product, inadequate or excess nitrogen show adverse effect on growth and chemistry of flue-cured tobacco. From the seedling stage to final harvest, the soil nitrogen regimes affect the process of plant development and chemistry of cured tobacco more than any element (McCants and Woltz 1967). Weybrew *et al.* (1983) reported that, the interplay of nitrogen and carbohydrate metabolism, as influenced by nitrogen nutrient management that predetermined the quality and chemical composition of cured leaf. Inadequate levels of nitrogen causes premature yellowing of leaves, which when cured are generally pale in colour, close grained and thick bodied and their smoke is flat, insipid and imbalance of quality constituents. An increase in nitrogen generally produce higher yields of tobacco but leaf maturity is delayed and cured leaf will be dark brown to black in colour, dry and chaffy, and have a strong and pungent smoke (Court *et al.* 1984). Increasing the rate of nitrogen application increases the amount of ash, nicotine, total protein nitrogen and petroleum ether extracts and decreases in sugars. Nitrate concentration increases in the leaf as the nitrogen fertilization increases (Sims and Atkinson 1975). The concentration of nitrate nitrogen in tobacco leaf influences the health related smoke constituents. Tobacco is multi-level harvesting crop and leaves are harvested from the bottom when ever the leaves are matured. So, the leaves present in the middle (L

[1] Principal Scientist (ccsrao30@rediffmail.com); [2*] Principal Scientist (e mail: rajuks2002@yahoo.co.in); [3] e mail: hravisankar@india.com; [4] e mail: radhama2k2@yahoo.com; [5] Principal Scientist (e mail: krishna_kasturi@yahoo.com), CTRI, Rajahmundry

position) and top (T position) will remain more days on the plant and chemistry of these leaves will be different from lower leaves. Tobacco variety Kanchan is grown in Northern light soils of Andhra Pradesh and Karnataka is an exotic introduction with high yield showing response to higher levels of nitrogen. The tobacco grown in this region is semi-flavorful and maximum quantity is exported. The objective of the present work is to study the effect of different levels of nitrogen and leaf position on biochemical quality constituents in FCV tobacco.

MATERIALS AND METHODS

Field experiment was conducted during 2008-10 at research farm of CTRI Research station, Jeelugumilli, West Godavari district in Andhra Pradesh under semi-arid tropical climate. The soil of experimental site was *Typic Haplustalfs*, sandy loam in surface layers (0-22.2 cm) and sandy clay in deep layers (22.5-45 cm), with pH 6.30 and electrical conductivity 0.20 dS/m. Treatments comprised of 6 nitrogen levels, viz. 20, 40, 80,120,160 and 200 kg/ha (here after mentioned as 20N, 40N, 80N, 120N, 160N and 200N), replicated four times in randomized block design. All package of practices for growing FCV tobacco (cv. Kanchan) in Northern light soils (NLS) were followed and nitrogen was applied in three splits in the ratio of 1:2:1 at 10, 25 and 40 days after planting. Phosphorous was applied as basal dose along with half dose of potassium. Remaining half dose of potassium was applied at 40 days of planting. Cured leaf samples were collected from different primings replication wise and pooled according to the plant position, viz. Priming (P), Lugs and Cutters (X), Leaf (L) and Tips. Midribs were separated from the leaf and lamina was dried in the hot air oven at 60°C for 6 hours, powdered, passed through 40 micron mesh and used for chemical analysis.

The powdered samples were analyzed for biochemical constituents, viz. chlorophyll pigments, carotenoids (Hiscox and Iscrelston 1979), petroleum ether extractives (Andersen *et al*. 1977), free fatty acids (Chu *et al.* 1972), starch (Gaines and Meudt 1968), polyphenols (Siva Raju *et al*. 2005), nitrate nitrogen by using salicylic acid-sulphuric acid reagent (Padmavathy 2008), proline (Bates *et al*. 1973) and free amino acids (Sadasivam and Manikam 1992). The data were statistically analyzed (Panse and Sukhatme 1957).

RESULTS AND DISCUSSION

Chlorophyll pigments

Break down of the chlorophyll pigments was one of the important biochemical transformations during curing to get desired colour of the tobacco. The chlorophyll a content varied from 0.056 to 0.138 mg/g among the various nitrogen treatments and leaf positions (Table 1). Chl a content was significantly higher at 120N compared to the lower levels of nitrogen and was significantly lower than the higher levels of nitrogen (160N and 200N). Chlorophyll b content increased significantly with increased application of nitrogen from 40N to 160N and thereafter there was a significant decrease. Chlorophyll a and b content increased with increase in leaf position from bottom to top (P to T). Court and Hendel (1982) reported that Chlorophyll a and b concentrations in the cured leaf were at about 1% of the amount measured at harvest. The chemical breakdown products of pigments during the curing have been reported to give rise numerous flavor components and improves the colour of the cured leaf (Weeks 1986).

Carotenoids

Carotenoids content was highest (0.616 mg/g) in the L position at 160N whereas it was lowest (0.371 mg/g) in the X position at 20N (Table 2). Carotenoid content was significantly higher at 120N compared to the lower levels of nitrogen and it was decreased significantly at 200N. Carotenoid content was on par at 120 N and 160N application in all leaf positions and higher than 80N. Among the leaf

Table 1 Effect of nitrogen levels on chlorophyll pigments in cured tobacco leaf

Nitrogen (kg/ha)	Chlorophyll a (mg/g)					Chlorophyll b (mg/g)				
	Leaf position					Leaf position				
	P	X	L	T	Mean	P	X	L	T	Mean
N20	0.056	0.084	0.087	0.089	0.078	0.102	0.109	0.132	0.140	0.120
N40	0.078	0.081	0.092	0.094	0.085	0.102	0.105	0.136	0.145	0.121
N80	0.078	0.082	0.096	0.101	0.089	0.131	0.136	0.142	0.171	0.144
N120	0.089	0.094	0.110	0.120	0.103	0.130	0.143	0.200	0.210	0.171
N160	0.123	0.126	0.131	0.136	0.129	0.166	0.177	0.214	0.216	0.193
N200	0.124	0.131	0.138	0.128	0.130	0.136	0.143	0.146	0.163	0.147
Mean	0.09	0.10	0.108	0.111		0.127	0.136	0.162	0.174	
			SEm (±)	*CD (at 5%)*		*SEm (±)*	*CD (at 5%)*			
Nitrogen			0.003	0.009		0.003	0.008			
Position			0.002	0.007		0.002	0.007			
Nitrogen × Position			0.064	NS		0.063	0.017			

Table 2 Effect of nitrogen levels on carotenoids and starch in cured tobacco leaf

Nitrogen (kg/ha)	Carotenoids (mg/g) Leaf position					Starch (mg/g) Leaf position				
	P	X	L	T	Mean	P	X	L	T	Mean
N20	0.445	0.371	0.387	0.424	0.406	7.00	11.6	12.60	14.60	11.45
N40	0.437	0.441	0.499	0.493	0.475	5.80	12.20	13.20	7.80	9.75
N80	0.490	0.509	0.524	0.469	0.498	3.80	12.40	18.60	10.60	11.35
N120	0.545	0.558	0.589	0.568	0.565	4.60	7.20	11.20	8.40	7.90
N160	0.567	0.588	0.616	0.550	0.580	4.00	8.20	12.60	11.40	9.05
N200	0.524	0.524	0.526	0.458	0.534	3.60	4.80	5.80	5.40	4.89
Mean	0.501	0.515	0.523	0.499		4.8	9.436	12.34	9.69	
			SEm (±)	*CD (at 5%)*		*SEm (±)*	*CD (at 5%)*			
Nitrogen			0.011	0.031		0.09	0.25			
Position			0.093	NS		0.073	0.205			
Nitrogen × Position			0.022	0.063		0.181	0.502			

positions, the L position contains maximum content of carotenoids. Carotenoids are precursors to many of the volatile aroma components of tobacco in addition to major colour pigments (red-orange to yellow). The carotenoids have been found in all types of tobacco and photo-oxidative degradation of carotenoids lead to the formation of many compounds which influence the aroma of tobacco (Enzell 1977).

Starch

Starch present in the mature leaves is hydrolyzed to reducing sugars during curing, the level of which has a profound influence upon tobacco quality. The starch content increased significantly with increase in leaf position from P to L in each nitrogen treatment and decreased from L to T position (Table 2). Starch content was maximum (18.36 mg/g) in L position at 80N whereas it was lowest (3.60 mg/g) in P position at 200N. Starch content was significantly higher in L position compared to other positions. With increase in nitrogen from 120N to 200N, the starch content was decreased and lowest content was observed in 200N level of nitrogen. Among all treatments and positions, the starch content varied between 0.3 to 1.8%. Decrease in starch accumulation with increase in nitrogen application in burley and FCV tobacco was reported by Sims and Atkinson (1971). FCV tobacco with starch content below 5% was regarded as good quality character (Long and Weybrew 1981).

Polyphenols

The major phenols in tobacco are chlorogenic acid and rutin which play an important role in quality of tobacco. Chlorogenenic acid and rutin contents increased significantly with increase in nitrogen application up to 120N (Table 3). Chlorogeneic acid content was at par in P and X position whereas it was increased significantly from X to L and T positions. At 120N level of nitrogen, the chlorogenic acid content in T position was 27.86% and 27.57% higher than the P and X positions respectively. Rutin content increased significantly with increase in leaf

Table 3 Effect of nitrogen levels on polyphenols in cured tobacco leaf

Nitrogen (kg/ha)	Chlorogenic acid (mg/g) Leaf position					Rutin (mg/g) Leaf position				
	P	X	L	T	Mean	P	X	L	T	Mean
N20	11.42	15.28	18.13	20.19	16.26	11.25	12.53	13.53	14.81	13.03
N40	15.87	17.01	21.73	20.98	19.12	11.38	12.41	14.52	15.69	13.50
N80	18.79	22.23	23.79	23.86	22.16	12.82	14.26	16.33	21.76	16.31
N120	22.54	22.59	28.71	28.72	25.67	13.25	16.41	18.41	22.39	17.68
N160	18.16	13.18	13.12	17.37	15.45	13.74	14.00	16.24	18.12	15.52
N200	19.00	16.36	9.50	14.54	14.84	13.48	18.79	18.91	11.40	15.64
Mean	17.65	17.77	19.16	21.08		12.70	14.73	16.32	17.37	
			SEm (±)	*CD (at 5%)*		*SEm (±)*	*CD (at 5%)*			
Nitrogen			0.131	0.365		0.11	0.305			
Position			0.107	0.298		0.089	0.249			
Nitrogen x Position			0.263	0.73		0.22	0.61			

Table 4 Effect of nitrogen levels on PEE and FFA in cured tobacco leaf

Nitrogen (kg/ha)	Ether soluble extracts (%)					Free fatty acids (mg/g)				
	Leaf position					Leaf position				
	P	X	L	T	Mean	P	X	L	T	Mean
N20	4.88	5.78	5.84	5.82	5.58	95.90	113.52	199.06	183.50	146.0
N40	5.20	6.10	6.12	6.06	5.86	166.12	198.40	245.46	205.60	203.9
N80	5.04	6.22	7.68	7.24	6.54	156.12	159.74	191.40	163.07	167.6
N120	5.86	6.50	7.38	7.30	6.76	96.47	119.64	159.59	132.57	127.1
N160	6.04	6.44	7.32	7.06	6.71	92.12	112.59	142.36	120.43	116.8
N200	5.34	5.98	7.54	7.16	6.50	86.12	84.12	105.90	93.75	92.4
Mean	5.39	6.17	6.98	6.77		115.51	131.37	172.72	149.79	
			SEm (±)	*CD (at 5%)*		*SEm (±)*	*CD (at 5%)*			
Nitrogen			0.077	0.214		3.35	9.30			
Position			0.063	0.175		2.74	7.59			
Nitrogen × Position				0.154	0.428		6.71	18.61		

position from bottom to top (P to T). At all nitrogen levels, the rutin content increased from P to T position with the exception of 200N level where there was decrease in the rutin content from L to T position. Increase in phenol content with ascending stalk position and increase in nitrogen fertilization has been reported (Sims and Atkinson 1975). Phenolic constituents have long been considered to be important for tobacco leaf quality. Lower levels of polyphenols are preferred in FCV tobacco as more attention has been diverted towards their role as precursors of dihydroxybenzene compounds of tobacco smoke (Snook and Schlolzheuer 1998). Catechol, one of the most potent cocarcinogens found in cigarette smoke condensate is a major pyrolitic product of chlorogenic acid and rutin.

Lipids

The FFA content decreased significantly with increase in nitrogen application from 40N to 200N (Table 4). Among the leaf positions, the FFA content increased significantly with increase in leaf position from P to L and decreased significantly from L to T position. In each leaf position, the FFA content decreased significantly from 40N to 120N. In L position at 120N, the FFA content was 65.4% and 41.4% higher than P and X positions respectively.

PEE content increased significantly with increase in nitrogen application from up to 120N and there after decreased significantly (Table 4). With increase in leaf position from P to L, the PEE content increased significantly and it was at par with T position. The differences among the flue-cured varieties in petroleum ether extractives and fatty acids have been reported (Chu *et al*. 1972). Grunwald *et al*. (1977) reported lower levels of crude lipids in bottom leaves and increase with in the leaves ascending stalk position. Higher levels of PEE are positively correlated with aroma in FCV tobacco (Grunwald *et al*. 1977).

Free amino acids

Higher levels of FAA are preferred in FCV tobacco as they react with sugars at high temperatures to form Amadori compounds responsible for aroma. The transformation of leaf proteins into free amino acids and ammonia during curing contribute significantly to flue-cured tobacco quality (Frankenburg *et al*. 1953). FAA and proline contents increased significantly with increase in nitrogen application from 40N to 120N and thereafter decreased significantly (Table 5). Tobacco in T position contained nearly 2.9 times higher levels of FAA over the P opposition. The FAA content increased significantly from L to T position with increase in nitrogen levels up to 120N and thereafter there was a significant decrease from L to T position. Proline content was significantly higher at 120N compared to other nitrogen levels (Table 5). In all positions, the proline content was increased by more than 10 fold by increase in nitrogen levels from 20N to 120N. The major amino acid in FCV tobacco, proline, seems to be an anomaly in that as much as a 25 fold increase has been observed during the curing schedule of flue-curing of tobacco (Weybrew *et al*. 1966). They observed increase in proline accounted for more than 65% of the increase in free amino acids which can not be explained on the basis of proteolysis.

Nitrate nitrogen

Nitrate nitrogen content varied between 2.02 and 3.96 mg/g among the various levels of nitrogen and leaf positions (Table 5). The maximum content of nitrate nitrogen was in T position with 160N. Nitrate nitrogen content increased significantly with increased level of nitrogen application from 40N to 160N and decreased thereafter. Among the leaf positions, nitrate nitrogen content increased significantly from P to X and L to T positions. Maximum nitrate nitrogen was in T position. Burton and Walton (1989) have reported

Table 5 Effect of nitrogen levels on proline and free amino acids in cured tobacco leaf

Nitrogen (kg/ha	Proline (mg/g) Leaf position					Free amino acids (mg/g) Leaf position					Nitrate nitrogen (mg/g) Leaf position				
	P	X	L	T	Mean	P	X	L	T	Mean	P	X	L	T	Mean
N20	0.26	0.34	0.42	0.83	0.462	0.078	0.98	1.21	2.36	1.16	2.02	2.94	3.00	3.21	2.795
N40	0.44	0.43	0.43	0.21	0.626	0.127	1.19	1.28	2.66	1.31	2.04	2.98	3.16	3.60	2.945
N80	0.60	1.04	2.77	4.95	2.340	0.170	2.88	6.92	11.88	5.46	2.16	3.02	3.26	3.58	3.005
N120	2.92	3.98	4.66	8.94	5.125	6.30	9.96	11.64	14.88	10.69	2.63	3.42	3.64	3.85	3.301
N160	2.91	3.61	5.70	7.30	4.881	5.98	8.54	13.66	10.56	9.68	2.84	3.64	3.92	3.96	3.593
N200	2.46	2.81	4.72	5.20	3.885	5.23	6.88	11.44	9.92	8.36	2.74	2.98	3.08	3.64	3.110
Mean	1.59	2.03	3.11	4.79		2.981	5.07	7.69	8.71		2.40	3.16	3.28	3.64	
			SEm (±)	CD (at 5%)		SEm (±)	CD (at 5%)				SEm (±)	CD (at 5%)			
Nitrogen			0.05	0.14		0.074	0.205				0.678	0.188			
Position			0.04	0.12		0.605	0.167				0.055	0.153			
Nitrogen × Position			0.10	0.28		0.148	0.410				0.135	NS			

a wide variation in nitrate content of cured tobacco depending on genotype, cultural practices and curing method. Variation in nitrate content in different genotypes of FCV tobacco varieties has been reported (Siva Raju *et al*. 2005). Nitrate nitrogen of tobacco has a great influence on the levels of tobacco specific nitrosamines.

Thus in the present study, different levels of nitrogen at different leaf positions influenced the biochemical constituents. Many of the constituents responsible for aroma was maximum in L position (and on par with T position with exceptions) at 120 kg N/ha or on par with higher levels of nitrogen. An increase in nitrogen may produce higher yields of tobacco but leaf maturity is delayed and the recommendation of optimum nitrogen fertilization depends not only on the yields but also on the grade index and quality constituents in the cured leaf. Higher quantity and good quality leaf will be generally from L position and good quality leaf can also be obtained from T position by proper tip management with optimum level of nitrogen fertilization. The recommendations of 120 kg N/ha for the variety Kanchan grown in northern light soils of Andhra Pradesh was found to be optimum for the higher yield with balanced quality constituents and higher levels of aroma compounds.

REFERENCES

Andersen R A, Tso T C and Chaplin J F. 1977. Variation of soluble phenolic compounds in flue-cured tobacco cultivars with stalk position. *Agronomy Journal* **64**: 417–20.

Bates R, Waldern R P and Teare I D. 1973. A rapid determination of free proline for water stress studies. *Plant and Soil* **39**: 205–7.

Burton H R and Walton L. 1989. Changes in chemical composition of burley tobacco during senescence and air-curing. 3. Tobacco specific nitrosamines. *Journal of Agriculture and Food Chemistry* **37**: 426–30.

Chu H, Tso T C and Chaplin J F. 1972. Higher fatty acids of flue-cured tobacco leaves varying in stalk position. *Agronomy Journal* **64**: 280–2.

Court W A and Hendel J G. 1982. Changes in leaf pigments during senescence . *Canadian Journal of Plant Sciences* **64**: 229–32.

Court W A, Elliot J M and Hendel J G. 1984. Influence of applied nitrogen fertilizer on certain lipids, terpens and other characteristics of flue-cured tobacco. *Tobacco Science* **28**: 69–72.

Enzell C R. 1977. Recent progress in the chemistry of sun cured tobaccos. (In:) *Proceeding of American Chemical Society symposium,* New Orleans. pp 37–77.

Frankenburg W G, Gottscho A M, Kissinger S S, Bender D and Ehrlich M. 1953. Analytical studies on harvested tobacco leaves. *Analytical Chemistry* **25**: 1 784–98.

Gaines T P and Meudt W J. 1968. Adaptation of the iodine stain method for determining the starch in flue-cured tobacco. *Tobacco Science* **12**: 130–3.

Grunwald C, Sims J L and Sheen S J. 1977. Effect of nitrogen fertilization and stalk position on chlorophyll, carotenoids and certain lipids of three tobacco genotypes. *Canadian Journal of Plant Sciences* **57**: 525–35.

Hiscox J D and Iscrelston‘s, C P. 1979. A method for extraction of chlorophyll from leaf tissues without maceration. *Canadian Journal of Botany* **57**: 1 332–4.

Long R C and Weybrew J A. 1981. Major chemical changes during senescence and curing. *Recent Advances in Tobacco Science* **7**: 40–7.

McCante C B and Woltz W G. 1967. Growth and mineral nutrition of tobacco. *Advances in Agronomy* **19**: 211–65.

Padmavathi D. 2008., Chemical investigations on tobacco specific nitrosamines (TSNAs) in Indian tobacco., *Ph D Thesis*, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur.

Panse V G and Sukhatme P V. 1957. *Statistical Methods of Agricultural Workers*. ICAR, New Delhi

Sadasivam S and Manikam M. 1992. Biochemical Methods, II edition, pp 143–4. New age International Publishers, New Delhi.

Sims J L and Atkinson O W. 1971. Nitrogen composition of burley tobacco. III. Effect of nitrogen, suckering practice and harvest date on concentration and distribution of nitrogenous constituents. *Tobacco Science* **15**: 67–70.

Siva Raju K, Sarala K and Rao R V S. 2005. Variation in polyphenol content of flue-cured tobacco varieties at different primings. *Tobacco Research* **29**: 138–42.

Snook M E and Schlozheuer W S. 1988 Evaluation of low polyphenol tobacco for pyrolytic production of catechol. (*In*) *Tobacco Chem. Res. Conf. 42*, Abstr. 24, New Orleans, p 28.

Weeks W W. 1986. Carotenoids: A source of flavour and aroma. Biogeneration of aromas. ACS Symposium Series 317. Parliment T H and Croteau R (Ed). American Chemical Society, Washington DC, pp 157–66.

Weybrew W A, wan Ismail W A and Long R C. 1983. The cultural management of flue-cured tobacco quality. *Tobacco Science* **27**: 56–65.

Weybrew J A, Woltz W G and Johnson W H. 1966. Changes in the free amino acids of tobacco during curing. (In) *Proceedings of the 4th International Tobacco Scientific Congress,* Athens, Greece, pp 716–22.