Tobacco Research 41(2) : 55-61, 2015

EFFECT OF NITROGEN AND LEAF POSITION ON QUALITY CONSTITUENTS OF *LANKA* TOBACCO (*NICOTIANA TABACUM*, L.) GROWN IN ANDHRA PRADESH

K. SIVA RAJU, D. DAMODAR REDDY, C. CHANDRASEKHARA RAO AND Y. SUBBAIAH

ICAR-Central Tobacco Research Institute, Rajahmundry, 533 105, Andhra Pradesh, India

(Received on 26st August, 2015 and accepted on 2nd November, 2015)

***Lanka* tobacco, an indigenous air-cured tobacco used for cheroot making, is famous for its characteristic pungent taste and strong aroma and is cultivated on alluvial flood plains of Godavari River in East and West Godavari, and Khammam districts of Andhra Pradesh. A field experiment was conducted during 2010-11 in Rekhapalli, Khammam district, Andhra Pradesh to study the effect of nitrogen levels (Recommended practice; N1=300 kg/ha and farmers practice; N2=1000 kg/ha) and leaf position (bottom, middle and top) on biochemical quality constituents in *Lanka* tobacco. Nicotine, reducing sugars (RS), starch, proline, petroleum ether extractives (PEE) and acid value (positively related to quality) decreased whereas chlorophyll a, chlorogenic acid, rutin and nitrate nitrogen (negatively related to quality) increased with an increase in nitrogen level from 300 to 1000 kg/ha. Nicotine, RS, chlorogenic acid, PEE, starch, nitrate nitrogen and proline contents increased whereas rutin content decreased with increase in leaf position from bottom to top. Nicotine content decreased by 10.9% in the top leaves with increase in nitrogen levels from 300 to 1000 kg/ha. Reducing sugar content varied from 0.46 to 1.41% among the leaf position and nitrogen levels. Reducing sugars and proline contents decreased significantly by 14.39 and 79% respectively, whereas chlorogenic acid content increased by 17.68% with increased levels of nitrogen from 300 to 1000 kg/ha. The chlorogenic acid content in the top leaves was 36.79 and 40% higher than the middle and bottom leaves, respectively. The maximum content of nitrate nitrogen (4.06 mg/g) was in the top position leaves under 1000 kg N/ha. Nitrate nitrogen content increased by 82.53, 48.16 and 129.45% in top, middle and bottom position leaves, respectively, with increase in nitrogen from 300 to 1000 kg/ha. Results revealed that in *Lanka* tobacco accumulation of quality constituents was maximum in the top position followed by middle position at 300 kg N/ha. The Recommendation of 300 kg N/ha for *Lanka* tobacco was found to be optimum for higher yields with balanced quality constituents for its pungent taste and strong aroma compared to farmer's practice (1000 kg N/ha).**

Key words: *Lanka* tobacco, Leaf position, Nitrogen, Quality characters

INTRODUCTION

Lanka tobacco (*Nicotiana tabacum* L.) is an indigenous air-cured tobacco type used for cheroot making, is cultivated on alluvial flood plains (Entisols) of Godavari and Krishna rivers in East and West Godavari districts, Krishna and Khammam districts of Andhra Pradesh. Country cheroot made of *Lanka* tobacco is liked very much by the people as it has a characteristic pungent taste and strong aroma. Chemical quality parameters *viz*., nicotine, reducing sugars (RS) and chlorides and biochemical constituents *viz*., starch, petroleum ether extractives (PEE), 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 of manures, position of leaf on stalk, climatic conditions, cultural practices, genotypes and method of curing (Long and Weybrew, 1981). A change in any of these factors can markedly alter the leaf composition and thus effect the smoke quality. 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 (McCarts and Woltz, 1967). From the time of harvest to that of consumption, the tobacco leaf undergoes several processing steps. Some of these steps involve biochemical and thermal transformation which change the chemical composition and flavour properties. Nitrate concentration increases in the leaf as the nitrogen fertilization increases. The concentration of nitrate nitrogen in tobacco leaf influences the health related smoke constituents.

Tobacco is multilevel harvesting crop and leaves are harvested from the bottom whenever the leaves are matured. Hence, the leaves present in the middle (L position) and top (T position) will remain more days on the plant and chemistry of these leaves will be different from lower leaves. *Lanka* tobacco derives its name from the islands or 'Lankas' of Godavari and Krishna rivers during heavy floods. Even though CTRI recommended 300 kg N/ha, farmers used to apply 1000 kg N/ha in the form of urea expecting good quality and quantity. Urea being applied to the soil surface by broadcasting, considerable loss of nitrogen as ammonia into the atmosphere results in wastage and pollution of environment. The objective of the present work is to study the effect of nitrogen and leaf position on biochemical quality constituents in *Lanka* tobacco.

MATERIALS AND METHODS

A field experiment was conducted during *rabi* season in 2011-12 in Rekhapalli, Khammam district, Andhra Pradesh. Lanka soils are moderately alkaline (pH 7.8 to 8.4) with low CE (10 to 12 m.e./100 g soil). The tobacco variety *Lanka* was grown with recommended fertilizer package of practice by CTRI (300 kg N/ha) and the practice followed by the farmers (1000 kg N/ha). The cured leaf samples were collected replication wise from nitrogen treatments and different leaf positions on the stalk (bottom, middle and top). Midribs were separated from the leaf and lamina was dried in the hot air oven at 60^{o}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 Iscrelstons, 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 nicotine, reducing sugars and chlorides (Harvey *et al.*, 1969). The data were statistically analyzed (Panse and Sukhatme, 1957).

RESULTS AND DISCUSSION

Chlorophyll a (Chl a) content varied from 0.602 to 1.032 mg/g among the leaf positions and nitrogen treatments (Table 1). Chl a content increased significantly (42.37%) with increase in nitrogen levels from 300 to 1000 kg/ha and with increase in leaf position from bottom to top. Chl a content in top leaves was 7.6 and 20.6% higher than the middle and bottom leaves respectively. Chl a content increased with increase in leaf position from bottom to top in each nitrogen level and was maximum in the top leaves. There was non-significant variation in Chl b content in either leaf positions or nitrogen levels (Table 1). Carotenoid content varied from 0.676 to 0.767 mg/g among the leaf positions and nitrogen levels (Table 1). There was non-significant increase in carotenoid content with increase in nitrogen from 300 to 1000 kg/ha. Top position leaves contained significantly higher levels of carotenoids over the bottom leaves. Break down of the chlorophyll pigments was one of the important biochemical transformations during curing to get desired colour of the tobacco. Chandrasekhararao *et al.* (2013) reported an increase in Chlorophyll a, Chl b and total chlorophyll content with increase in leaf position from bottom to top on stalk and a decrease with increase in nitrogen levels. Court *et al.* (1982) reported that Chlorophyll a and b concentrations in the cured leaf were about 1% of the amount measured at harvest. Carotenoids are precursors to many of the volatile aroma components of tobacco in addition to being the major colour pigments (red-orange to yellow). The chemical breakdown products of pigments during the curing have been reported to give rise to numerous flavor components (Shi *et al.*, 2012).

The starch content varied between 2.14 and 8.12 mg/g among the leaf positions and nitrogen levels (Table 1). Starch content increased

Table 1: Effect of nitrogen levels and leaf position on pigments and starch in *Lanka* tobacco

Leaf position	Chlorophyll a (mg/g)			Chlorophyll b (mg/g)			Carotenoids (mg/g)			Starch (mg/g)		
	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean
Top	0.765	1.032	0.899	0.23	0.24	0.24	0.766	0.767	0.767	8.12	5.32	6.72
Middle	0.678	0.992	0.835	0.21	0.21	0.21	0.755	0.750	0.753	5.68	4.62	5.15
Bottom	0.602	0.888	0.745	0.20	0.22	0.21	0.676	0.753	0.715	4.83	2.14	3.48
Mean	0.682	0.971		0.213	0.23		0.732	0.757		6.21	4.03	
	SEm±	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm(±)**	**CD (P=0.05)**	
Nitrogen	**0.009**	**0.027**		**0.012**	**0.038**		**0 .06**	**0.17**		**0.36**	**1.08**	
Leaf position	**0.034**	**0.108**		**0.016**	**0.065**		**0.01**	**0.04**		**0.55**	**1.25**	
Nitrogen x Leaf position	**0.048**	**0.134**		**0.015**	**0.049**		**0.04**	**0.13**		**0.08**	**0.26**	

significantly with increase in the leaf position from bottom to top at each nitrogen level. The starch content decreased by 34.48, 18.66 and 55.69% in top, middle and bottom position leaves, respectively with increase in nitrogen level from 300 to 1000 kg/ha. At 300 kg N/ha, the top leaves contained 30.04 and 40.51% of starch compared to middle and bottom leaves, respectively. Decrease in starch accumulation with increase in nitrogen application in FCV tobacco was reported by Chandrasekhara Rao *et al.* (2014). The starch content increased significantly with increase in leaf position from bottom to middle on the stalk and decreased from middle to top position. It was also reported that with increase in nitrogen from 120 to 200 kg/ha, the starch content decreased and the lowest content was observed in 200 kg N/ha (Chandrasekhara Rao *et al.*, 2013). In FCV tobacco with starch content below 5% is regarded as a good quality character.

Nicotine content varied from 2.89 to 5.13 % among the leaf positions and nitrogen levels (Table 2). Nicotine content decreased with increase in nitrogen levels from 300 to 1000 kg/ha and at each leaf position. Nicotine content increased significantly with increase in leaf position from bottom to top. Top position leaves contained 21 and 36.6% higher levels of nicotine compared to middle and bottom leaves, respectively. The nicotine content decreased by 10.9% in the top leaves with increase in nitrogen levels from 300 to 1000 kg/ha. The interaction between nitrogen levels and leaf position was significant. Reducing sugars (RS) content varied from 0.46 to 1.41% among the leaf positions and nitrogen levels (Table 2). RS content increased significantly with increase in the leaf position from bottom to top. Top leaves contained 1.78 and 2.07 times higher levels of RS over middle and bottom positions respectively. Except in the middle position, RS content decreased with increased levels of N. Chloride content varied from 0.23 to 0.47% among the leaf positions and nitrogen levels. Chloride content decreased with increase in nitrogen levels (Table 2). Top leaves showed maximum chlorides compared to the lower position leaves. In FCV tobacco, generally, carbohydrates fraction was more (reducing sugars; 8.6 – 27.0%) when compared to the air-cured burley tobacco, which contains lower levels of free sugar. Nicotine, sugars, sugar/nicotine and chlorides in the leaf are important chemical quality parameters in FCV tobacco.

The major polyphenols in tobacco are chlorogenic acid and rutin which play an important role in the quality of tobacco. Chlorogenic acid content increased by 17.68% with increased levels of nitrogen from 300 to 1000 kg/ha (Table 3). Top position leaves contained significantly higher chlorogenic acid content when compared to bottom and middle leaves. Chlorogenic acid content in the top leaves was 36.79 and 40% higher than the middle and bottom leaves, respectively. Rutin content increased significantly (14.76%) with increase in nitrogen application from 300 to 1000 kg/ha (Table 3). There were no specific trends in the rutin content among the leaf positions and rutin content was significantly higher in the top leaves compared to the middle and bottom leaves at 1000 kg N/ha.

Top position leaves showed significantly higher levels of phenols compared to middle and bottom position leaves. Phenolic constituents in the tobacco are affected by genotypes, increase in fertilizers, curing method and leaf position on the stalk (Siva Raju *et al.*, 2003; Chandrasekhara Rao *et al.*, 2013). 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

Table 2: Effect of nitrogen and leaf position on nicotine, RS and chlorides in *Lanka* tobacco

Leaf position	Nicotine (%)			Reducing sugars (%)			Chlorides (%)		
	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean
Top	5.13	4.57	5.13	1.41	0.81	1.11	0.47	0.32	0.39
Middle	4.25	3.82	4.03	0.46	0.78	0.62	0.31	0.23	0.27
Bottom	3.62	2.89	3.25	0.57	0.50	0.54	0.45	0.27	0.36
Mean	4.52	3.76		0.813	0.70		0.41	0.27	
	SEm(±)	**CD (P=0.05)**		**SEm(±)**	**(P=0.05)**		**SEm(±)**	**CD (P=0.05)**	
Nitrogen	**0.04**	**0.12**		**0.05**	**0.16**		**0.06**	**0.18**	
Leaf position	**0.11**	**0.36**		**0.02**	**0.06**		**0.15**	**0.48**	
Nitrogen x Leaf position	**0.16**	**0.51**		**0.03**	**0.09**		**0.02**	**0.07**	

Table 3: Effect of nitrogen levels and leaf position on phenols and nitrate nitrogen in *Lanka* tobacco

Leaf position	Chlorogenic acid (mg/g)			Rutin (mg/g)			Nitrate nitrogen (mg/g)		
	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean	300 kg N/ha	1000 kg N/ha	Mean
Top	3.02	5.08	4.05	7.60	8.92	8.26	2.52	4.60	3.56
Middle	2.72	2.40	2.56	6.94	8.26	7.60	2.45	3.63	3.04
Bottom	2.56	2.30	2.43	7.59	8.23	7.91	1.46	3.35	2.40
Mean	2.77	3.26		7.38	8.47		2.14	3.86	
	SEm±	**CD (P=0.05)**		**SEm(±)**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**	
Nitrogen	**0.03**	**0.14**		**0.107**	**0.35**		**0.05**	**0.18**	
Leaf position	**0.08**	**0.29**		**0.117**	**0.36**		**0.19**	**0.62**	
Nitrogen x Leaf position	**0.1**	**0.36**		**0.17**	**0.49**		**0.03**	**0.09**	

(Snook and Schlolzheuer, 1988). Catechol, one of the most potent co-carcinogens found in cigarette smoke condensate is a major pyrolitic product of chlorogenic acid.

Nitrate nitrogen content increased significantly with increase in nitrogen levels and leaf positions from bottom to top on the stalk (Table 3). The maximum content of nitrate nitrogen (4.60 mg/g) was in the top position leaves at 1000 kg N/ha. Nitrate nitrogen content increased by 82.53, 48.16 and 129.45% respectively in top, middle and bottom position leaves with increase in nitrogen, levels from 300 to 1000 kg/ha. Wide variation in nitrate content of cured tobacco as influenced by genotype, cultural practices and curing method was reported (Siva Raju *et al.*, 2005). Nitrate nitrogen in tobacco has a great influence on the levels of tobacco specific nitrosamines. Chandrasekhara Rao *et al.* (2013) reported that 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 in FCV tobacco. Raja Rao and Suryanarayana (1988) reported accumulation of higher levels of nitrate nitrogen in air-cured burley tobacco genotypes and it was considered to be a genetic factor.

Petroleum ether extractives (PEE) content varied from 6.02 to 9.43% among the leaf positions and nitrogen levels (Table 4). With increased application of nitrogen from 300 to 1000 kg/ha, the PEE content decreased by 10.4%. Top and middle position leaves contained significantly higher levels of PEE over bottom leaves. At each leaf position, the PEE content decreased non-significantly with increase in nitrogen levels. Acid value increased with increase in leaf position from bottom to top and decreased with increase in nitrogen levels from 300 to 1000 kg/ha (Table 4). Chandrasekhara Rao *et al.* (2013) reported increase in PEE content with increased application of nitrogen from 40 to 120 kg/ha and decreased with further increase in nitrogen levels. Siva Raju and Sarala (2013) reported lower levels of crude lipids in bottom leaves which increased with increase in leaf position on the stalk (bottom to top) and higher levels of PEE are positively correlated with aroma in FCV tobacco.

Proline content varied from 0.99 to 2.88 mg/g among the leaf positions and nitrogen levels (Table 4). Proline content decreased significantly (79%) with increase in nitogen levels from 300 to 1000 kg/ha whereas, an increase with increase in leaf position from bottom to top was observed. Top position leaves contained significantly higher levels of proline compared to middle and bottom leaves and the middle position leaves contained significantly higher levels over the lower leaves. In FCV tobacco an increase in proline content

Table 4: Effect of nitrogen and leaf position on PEE, acid value and proline in *Lanka* tobacco

Leaf position	**PEE (%)**			**Acid value (%)**			**Proline (mg/g)**		
	300 kg N/ha	**1000 kg N/ha**	**Mean**	**300 kg N/ha**	**1000 kg N/ha**	**Mean**	**300 kg N/ha**	**1000 kg N/ha**	**Mean**
Top	9.43	8.36	8.89	0.235	0.167	0.201	2.88	1.19	2.04
Middle	8.48	7.51	7.99	0.151	0.162	0.157	1.78	1.14	1.46
Bottom	6.25	6.02	6.13	0.133	0.151	0.142	1.25	0.99	1.12
Mean	8.05	7.29		0.173	0.16		1.97	1.10	
	SEm±	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**	
Nitrogen	**0.005**	**0.015**		**0.02**	**0.06**		**0.12**	**0.37**	
Leaf position	**0.35**	**1.04**		**0.001**	**0.004**		**0.21**	**0.68**	
Nitrogen x Leaf position	**0.49**	**1.36**		**0.002**	**0.005**		**0.03**	**0.09**	

with increase in nitrogen application from 40 to 120 kg/ha and with increase in leaf position on the stalk from bottom to top was reported (Chandrasekhara Rao *et al.*,2013). Transformation of leaf proteins into free amino acids and ammonia during curing contribute significantly to tobacco quality. Higher levels of free amino acids are preferred in FCV tobacco as they react with free sugars at high temperatures to form Amadori compounds which are responsible for flavour.

Thus in the present study, leaf position and nitrogen levels showed variation in the chemical and biochemical quality constituents in *Lanka* tobacco. In general, the total quality constituents were more in 300 kg N/ha treatment when compared to 1000 kg N/ha. Middle and top position leaves showed nearly similar contents of quality constituents compared to bottom position leaves. Chemical constituents which are positively related to quality (nicotine, RS, starch, proline, and PEE) decreased whereas constituents negatively related to quality (chlorophyll a, chlorogenic acid, rutin and nitrate nitrogen) increased with an increase in nitrogen level from 300 to 1000 kg/ha. All the quality constituents increased with increase in leaf position from bottom to top except rutin. The recommendation of 300 kg N/ha for *Lanka* tobacco was found to be optimum for higher yields with maximum quality constituents for its pungent taste and strong aroma compared to farmer's practice (1000 kg N/ha).

REFERENCES

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

Bates, R., R.P. Waldern and I.D. Teare. 1973. A rapid determination of free proline for water stress studies. **Plant and Soil** 39: 205-07.

Chandrasekhara Rao, C., K. Siva Raju, H. Ravisankar, M. Anuradha and S. Kasturikrishna. 2013. Effect of different levels of nitrogen and leaf position on biochemical quality constituents of FCV tobacco grown in northern light soils of Andhra Pradesh. **Indian J Agric. Sci**. 83: 1052-7.

Chandrasekhara Rao, C., K. Siva Raju M. Anuradha and H. Ravisankar. 2014. Effect of nitrogen levels and leaf position on carbohydrate and nitrogen metabolism in FCV tobacco (*Nicotiana tabacum*). **Indian J. Plant Physiol.** 19: 244-9.

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

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

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

Harvey, W.R., H.M. Stahr and W.C. Smith. 1969. The method of extraction for simultaneous analysis of chlorides, nicotine and reducing sugars in tobacco using auto analyzer. **Tob. Sci.** 13: 13-5.

Hiscox, J.D. and C.P. Iscrelstons. 1979. A method for extraction of chlorophyll from leaf tissues without maceration. **Canadian J. Bot**. 57: 1332-4.

Long, R.C. and J.A. Weybrew. 1981. Major chemical changes during senescence and curing. **Recent Adv. Tob. Sci**. 7: 40-7.

McCarts, C.B. and W.G. Woltz. 1967. Growth and mineral nutrition of tobacco. **Adv. Agron**. 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 P.V. Sukhatme. 1957. *Statistical Methods of Agricultural Workers*. ICAR, New Delhi.

Raja Rao. D.Ch. and Y.V. Suryanarayana, 1988.

Non flue-cured tobacco types in India. Central Tobacco Research Institute, Rajahmundry. p. 42.

Shi H.Z., S.L. GU, W.D. Duan, J.A. Wang, H. Qua, Q.H. Liu, F. SU and G.S. Liu. 2012. Degradation of plastid pigment and its relationship with volatile catabolite content in cured leaves of different genotypes of flue-cured tobacco. **China Agricul. Sci.** 45: 3346-56.

Siva Raju, K., K. Sarala and R.V.S. Rao. 2003. Variation in polyphenol content of flue-cured tobacco varieties at different primings. **Tob. Res**. 29: 138-42.

Siva Raju, K., K. Giridhar and C. Chadrasekhara Rao. 2005. Effect of fertilization and topping levels on biochemical constituents of FCV tobacco grown in Karnataka light soils. **Tob. Res**. 31: 79-85.

Siva Raju, K. and K. Sarala. 2013. Variation in ether soluble extractives of flue-cured tobacco in different primings. **Tob. Res**. 39: 73-5.

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