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NEUTRAL VOLATILE AROMA CONSTITUENTS OF *LANKA* TOBACCO (*NICOTIANA TABACUM* L.) IN RELATION TO NITROGEN SUPPLY AND LEAF POSITION

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***Lanka* tobacco, an indigenous air-cured cheroot tobacco grown predominantly on alluvial flood plains of river Godavari in Andhra Pradesh, is known for its characteristic pungent taste and strong aroma. 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 neutral volatile aroma constituents in *lanka* tobacco. The neutral volatile compounds detected by GC-MS included degradation products of carotenoids, thunberganoids, terpenoids and neophytadiene. As shown by their relative content in neutral volatile fractions, neophytadiene (36%), thunbergol (2.35%), megastigmatrienone isomers (6.86%), 3-hydroxysolavetivone (8.47%), solavetivone (1.44%), solanone (1.41%) and Z-Abienol (1.37%) were some of the major compounds that significantly contribute to smoke flavour. The neophytadiene content was higher in N1 treatment (11.8%) over N2 treatment. The four isomers of megastigmatrienone were present in all the leaf positions in both the nitrogen treatments and megastigmatrienone isomer-2 was in maximum content. Megastigmatrienone isomers content increased with change in leaf position from bottom to top with exceptions. The total phenylalanine related compounds were more in N1 over the N2 treatment. Maximum content of neutral volatiles were present in the middle (98.53%) and top position (98.88%) leaves compared to bottom leaves (70.41%) in N1 treatment. Thus the leaf position showed significant effect on neutral volatile compounds, whereas the variation between two nitrogen levels in aroma compounds was significant only in bottom position leaf . Results revealed that tobacco from both middle and top positions showed maximum accumulation of neutral volatile aroma constituents at 300 kg N/ha.The recommendation of 300 kg N/ha for *lanka* tobacco was found to be optimum for accumulation of maximum neutral volatile aroma constituents for its pungent taste and strong aroma compared to farmer's practice (1000 kg N/ha).**

Key words: *Lanka* tobacco, leaf position, Nitrogen, NVAC

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is an important commercial crop of India with an area of about 4 lakhs ha and a production of about 700 million kg. Different types of tobaccos grown commercially are distinguished largely by region of production, distinct morphological features of the variety, chemical and physical characteristics and intended use in product manufacturing. *Lanka* tobacco (*Nicotiana tabacum* L.), 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. *Lanka* tobacco derives its name from the islands or 'Lankas' of Godavari and Krishna rivers during heavy floods.

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 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. The physical and chemical properties of leaf tobacco are influenced by the genetics, cultural practices, soil type and nutrients, weather conditions, stalk position, harvesting and curing procedures (Katahata *et al.*,

2007; Wu and Weeks, 1992; Deckmyn and Impens, 1997; Liu and Xian, 1993; Nielson, 1992). A change in any of these factors can markedly alter the leaf chemical composition and thus affect smoke quality. From the time of harvest to that of consumption, the leaf of tobacco plant undergoes several processing steps. Some of these steps involve biochemical and thermal transformations which changes the chemical composition and flavour properties. Murthy *et al.* (1962) reported that Lanka soils (islands) formed by silting of rivers greatly influence the characteristic smoking quality of tobacco. 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, which leading to waste of important nitrogen fertilizer and pollution of the environment.

Quality constituents *viz.*, reducing sugars, nicotine, total nitrogen, starch, phenols, free fatty acids, total petroleum extractives *etc.* are some of the constituents which play an important role in the quality of tobacco and they may not reflect the entire aroma constituents. Tobacco aroma consists of a large number of minor components. Most of these compounds are thought to be generated by the oxidative degradation of terpenoids such as carotenoids, thunberganoids, labdanoids and cyclic polyisoprenoids during the curing and ageing process. Many studies have focused on analysis of aroma components in tobacco. Xiefeng Ye (2011) reported 31 aroma compounds in different types of FCV tobacco varieties grown in China. The objective of the present work is to study the effect of different levels of nitrogen and leaf position on aroma composition of *lanka* tobacco.

MATERIALS AND METHODS

A field experiment was conducted during *rabi* season in 2010-11 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) and the farmers are applying urea as nitrogen source on the soil surface by broadcasting method. The tobacco variety *lanka* was grown with recommended fertilizer package of practice by CTRI (300 kg N/ha) and the practices followed by the farmers (1000 kg N/ha). The cured leaf samples were collected from nitrogen treatments and different leaf position on the stalk (bottom, middle and top). Three representative samples from each treatment were taken mixed and two samples per each treatment were analyzed and mean of the two values was given.

Midribs were separated from the leaf and lamina was dried in the hot air oven at 60°C for 6 h, powdered, passed through 40 micron mesh and used for analysis of neutral volatile aroma compounds. For extraction of neutral volatiles, 10 g of tobacco leaf powder, 250 ml of phosphate buffer (0.2 M, pH 6.8) and 5 g of anhydrous sodium sulphate were taken in 1 l round bottom flask and steam distilled. Distillate (250 ml) was collected into a 500 ml volumetric flask containing 250 ml dichloromethane (DCM). This mixture was taken into a separating funnel, mixed for 10 min and the DCM layer was separated. To the DCM, 50 ml of 5 M tartaric acid solution was added, mixed for 10 min and the DCM layer was collected by passing through anhydrous sodium sulphate and DCM was concentrated to 5 ml in Buchi flash evaporator and 1µl of the sample was injected for GC-MS analysis. The analysis was carried out using a ZB-5 capillary column (length: 30 m, diameter: 0.25 mm, film thickness: 0.25 µm) fixed in GC-MS (SHIMADZU QP 2001 Plus) with injector temperature of 250°C and oven temperature programmed from 60 to 210°C. The samples were injected maintaining a split ratio of 1:15. In MS, Electron Ionization (EI) mode was used with ion source and interface temperatures maintained at 200 and 250°C, respectively. Total ion Chromatograms (TIC) of the samples was recorded between 50 to 500 m/z at a scan speed of 2500. As authentic standards of the compounds are not available for quantification, the area normalization method was adopted and the proportion of a particular compound in the total neutral volatile fraction was calculated.

RESULTS AND DISCUSSION

The compounds identified were the degradation products of carotenoids and thunberganoids, neophytadiene and terpenoids. The degradation products of carotenoids and thunberganoids had a characteristic aroma and were thought to be key flavour components in neutral volatiles. Total neutral volatile aroma compounds (NVAC) in all leaf positions (bottom, middle and top) and N treatments were calculated

for comparison. Total NVAC increased from bottom to middle position and not much variation was observed from middle to top positions leaves on the stalk in both N treatments. The NVAC increased by 28.76% with change in leaf position from bottom to middle in N1 treatment whereas it was increased by 2.9% in N2 treatment.

In both the nitrogen treatments, bottom position leaves contained more number of NVAC compared to middle and top position leaves (Tables 1 and 3). In the leaf samples from bottom position, 30 and 24 compounds were identified in N1 and N2 treatments, respectively. Caryophylllene oxide, nootkatone, nootkatane, pseudosolasodine acetate, nerolidol, trans-caryophyllene and duvatriendiol present in bottom position leaves of N1 treatment were absent in N2 treatment and 5 compounds present in N2 were absent in N1 treatment. Middle position leaves of N2 treatment contained solavetivone, clovene, isolongifolene, iso-velleral, viridifolorol and solanocapsin and they were absent in N1 treatment whereas benzaldehyde, beta damascone, 3-oxo-alpha-ionol and nootkatone present in N1 were absent in N2 treatments. In top position leaves, 22 and 21 NVACs were identified in N1 and N2 treatments, respectively. Neryl acetone, benzaldehyde, benzyl alcohol and solavetivone present in top position leaves of N1 treatment were absent in N2 treatment, whereas

Table 1: Neutral volatile profile of *lanka* tobacco- Bottom position leaf - 300 kg N/ha

RT (min)	Compound	Area	Area (%)
3.89	Furfural	10117	0.08
6.34	Benzaldehyde	19262	0.16
8.01	Benzyl alcohol	69084	0.57
8.27	Phenylacetaldehyde	357867	2.93
9.99	Phenylethyl alcohol	152825	1.25
14.46	Indole	108248	0.89
16.40	Solanone	104734	0.86
18.16	Beta Damascone	11895	0.10
20.93	Beta Ionone	97128	0.79
23.50	Megastigmatrienone	134407	1.10
24.01	Megastigmatrienone	907207	7.42
24.44	Diethyl phthalate	18797	0.15
25.01	Megastigmatrienone 4	170291	1.39
25.35	Megastigmatrienone	922476	7.55
25.82	3-Oxo-Alpha-Ionol	97203	0.80
28.07	Caryophyllene oxide	111801	0.91
30.97	Nootkatone	95477	0.78
31.00	(+) Nootkatane	17116	0.14
31.02	Solavetivone	31064	0.25
31.63	Rishitin	267653	2.19
32.12	Neophytadiene	3593049	29.39
33.70	Pseduosolasodine diacetate	9167	0.07
34.65	Nerolidol	113647	0.93
34.71	Trans – Caryophyllene	22500	0.18
35.87	Dibutyl phthalate	201576	1.65
36.86	Retinol, acetate (CAS) Vitamin A acetate	795272	6.50
38.62	Thunbergol	172605	1.41
39.68	Duvatriendiol	2428698	19.87
40.41	Phytol	1136677	9.30
42.01	(12 Z) – Abienol	47945	0.39
		12225788	100.00

nerolidol and abienol present in top leaves of N2 were absent in N1 treatment (Tables 2 and 4).

Carotenoid related compounds (CRC)

The carotenoids degradation compounds *viz.*, beta damascene, beta ionone, four isomers of megastigmatrienone and 3-oxo-alpha-ionone which were related to aroma, were identified in all leaf positions and in both nitrogen levels (Table 5). Damacenones and damascones were known as aroma compounds. The total carotenoid related compounds (CRC) increased with change in leaf position from bottom to top in both the nitrogen treatments. There was not much variation in CRC between middle (28.9%) and top leaf position (31.73%) ether in N1 or N2 treatments. With increase in N application from 300 to 1000 kg N/ ha, the CRC increased by 34.56% in bottom position leaves of N1 treatment whereas there was no variation between middle and top position leaves in both the nitrogen treatments. The proportion of total megastigmatrienones was also increased with change in leaf position from bottom to top but maximum content was observed in top position leaves (28.26 and 30.52% in N1 and N2, respectively) and they were marginally higher than middle position leaves in the respective nitrogen treatments (Table 5).

Megastimgatrienone 2 was maximum among the four isomers. Presence of inones and four isomers of megastimgatrienone were reported in 13 FCV tobacco varieties grown in China (Xiefeng *et al.,* 2011) and burley tobacco (Takane Fujimori, 1978). Tobacco varieties with higher levels of degradation compounds of carotenoids showed a positive correlation with tobacco flavour (Wei *et al.*, 2005). The total content of degradation products of carotenoids in FCV tobacco increased from 1.5 g N/pot to 3.5 g N/pot and decreased with increase in N to 5.5 g N/pot (Fei Yun *et al.*, 2013). Megastigmatrienone is a degradation product of lutein, and has been regarded as the salient feature of good quality tobacco (Wei *et al.*, 2004).

Table 2: Neutral volatile profile of *lanka* tobacco- Top position leaf - 300 kg N/ha

RT (min)	Compound	Area	Area (%)
6.33	Benzaldehyde	6416	0.08
8.01	Benzyl alcohol	37459	0.49
8.27	Phenylacetaldehyde	228345	2.96
9.99	Phenylethyl alcohol	133405	1.73
14.46	Indole	86812	1.13
16.40	Solanone	82349	1.07
18.15	Beta Damascone	19428	0.25
19.56	Neryl acetone	6627	0.09
20.94	Beta Ionone	27914	0.36
23.48	Megastigmatrienone	170624	2.21
24.01	Megastigmatrienone	1091893	14.16
25.01	Megastigmatrienone 4	166833	2.16
25.34	Megastigmatrienone	921031	11.94
25.82	3-Oxo-Alpha-Ionol	49801	0.65
30.97	Solavetivone	56523	0.73
31.63	Rishitin	106268	1.38
32.12	Neophytadiene	3060574	39.68
33.74	3-Hydroxysolavetivone	462064	5.99
34.68	Farnesyl acetate 3	41980	0.54
35.87	Dibutyl phthalate	139790	1.81
38.63	Longifolenaldehyde	38624	0.50
40.41	Phytol	779038	10.10
		7713798	100.00

Table 3: Neutral volatile profile of *lanka* tobacco- Bottom position - 1000 kg N/ha

RT (min)	Compound	Area	Area (%)
3.89	Furfural	8464	0.06
6.33	Benzaldehyde	23106	0.16
8.01	Benzyl alcohol	72886	0.52
8.27	Phenylacetaldehyde	507573	3.61
9.99	Phenylethyl alcohol	183132	1.30
10.74	Ketoisophorone	16247	0.12
14.45	Indole	125373	0.89
16.40	Solanone	219565	1.56
18.15	Beta Damascone	30739	0.22
19.54	Neryl Acetone	31412	0.22
20.93	Beta Ionone	71800	0.51
23.48	Megastigmatrienone	269659	1.92
24.01	Megastigmatrienone	1613915	11.47
25.01	Megastigmatrienone 4	245554	1.75
25.34	Megastigmatrienone	1291461	9.18
25.82	3-Oxo-Alpha-Ionol	102385	0.73
26.98	Ethyl Benzaldehyde	16858	0.12
30.97	Solavetivone	178935	1.27
31.63	Rishitin	380418	2.70
32.12	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol	4482960	31.87
33.74	3-Hydroxysolavetivone	1191433	8.47
35.41	Iso-Velleral	422644	3.00
35.85	Dibutyl phthalate	200229	1.42
37.25	Beta-N-Methyl Ionone	54814	0.39
38.58	Thunbergol	222353	1.58
40.18	13(16)14-Labdien-8-Ol	34616	0.25
40.41	Phytol	1836687	13.06
42.01	(12 Z) – Abienol	231757	1.65
		14066975	100.00

Thunberganoid related compounds

The compounds formed by the degradation of thunberganoids have the organoleptic properties. Solanone, thunbergol, pseudo-solanedione were present in all leaf positions and in both the nitrogen treatments (Table 5). Solanone content was higher in all leaf positions in N2 treatments when compared to N1. Thunbergol level was maximum (2.35%) in middle leaf position in N2 treatment.

Terpenoids

This group includes mono, di and sesquiterpinoids and their products. Solavetivone, rishitin and 3- hydroxy solavetivone were the sesquiterpenoids present in all leaf positions and in both the nitrogen treatments. The proportion of solavetivone increased with increase in leaf position from bottom to top and nitrogen level from N1 to N2 (Table 5). Rishitin was present in all the samples but no trends were observed with respect to leaf position but the proportion was higher in N2 over the N1 treatment. 3-hydroxy solavetivone was not detected in bottom position leaf in N1 treatment but it was maximum in middle leaf position in both the nitrogen treatments. The labdanoid Z- abienol was identified in all samples except in top position leaves of N1 treatment. Abienol content decreased with change in leaf position from bottom to top in N2 treatment.

Phenylalanine related compounds

Phenylalanine metabolism is an important process affecting the tobacco flavour. The

Table 4: Neutral volatile profile of *lanka* tobacco- Top position leaf- 1000 kg N/ha

RT (min)	Compound	Area	Area (%)
8.26	Phenylacetaldehyde	77522	0.83
9.99	Phenylethyl alcohol	97463	1.05
12.63	2-Phenoxyethanol	5942	0.06
14.45	Indole	78284	0.84
16.40	Solanone	122990	1.32
17.02	Nerolidol	27740	0.30
18.15	Beta Damascone	9611	0.10
20.93	Beta Ionone	40864	0.44
23.48	Megastigmatrienone	272433	2.93
24.01	Megastigmatrienone	1775921	19.13
25.01	Megastigmatrienone 4	254554	2.74
25.34	Megastigmatrienone	1459550	15.72
25.82	3-Oxo-Alpha-Ionol	82037	0.88
31.63	Rishitin	108307	1.17
32.12	Neophytadiene	2963435	31.92
32.53	D-Nerolidol	152950	1.65
33.74	3-Hydroxysolavetivone	429907	4.63
35.85	Dibutyl phthalate	126164	1.36
38.60	Thunbergol	217935	2.35
40.41	Phytol	882881	9.51
41.97	(12 Z) – Abienol	98208	1.06
		9284698	100.00

metabolites of phenylalanine are aroma components which could enhance the flower-scent like floral aroma in flue-cured tobacco (Liu and Shi, 2010). The total phenylalanine related compounds (benzaldehyde, phenylacetaldehyde, benzyl alcohol, phenylethyl alcohol) were more in N1 over the N2 treatment. Total phenylalanine compounds decreased with increase in leaf position from bottom to top in N2 treatment whereas they were maximum in N1 treatment (Table 7). Benzaldehyde was present in all the leaf position in N1 treatment whereas it was not detected in middle and top position leaves in N2 treatment. Phenylacetaldehyde was present in all the leaf positions and in both the N treatments and its proportion was maximum in bottom leaf positions in N2 treatment. Benzyl alcohol was present in all the leaf positions with higher values except, top position in N2 treatment in bottom position leaves. Phenylethyl alcohol content decreased with change in leaf position from bottom to top in N1 treatment whereas it was decreased from bottom to middle position leaves in N2 treatment. The results showed that 300 kg N/ha was optimum for the formation of phenylalanine related aroma compounds.

Neophytadiene

Neophytadiene was maximum among all the compounds identified and its proportion varied from 29.39 to 39.68% and 31.87 to 31.92% among the leaf positions in N1 and N2 treatments, respectively (Table 5). Neophytadiene increased by 25.84% from bottom to middle position leaf in N1 treatment whereas it was decreased by 0.32% from middle to top position. In N2 treatment, there was very little variation in neophytadiene content among the leaf position (31.87 to 32.19%). Neophytadiene was present in large quantities and it was suggested that they were derived from phytol but the experimental evidence was not reported (Takane Fujimori *et al.*, 1978) whereas, Fei Yun *et al.* (2013) reported that neophytadiene is the degradation products of pigments. Neophytadiene was the abundant component of NVAC in FCV and burley tobacco and it enhances the flavour and aroma of FCV tobacco (Fei yun *et*

al., 2013) and its degradation and conversions may lead to the formation of aroma compounds (Zhou *et al.*, 2008).

Furfural, one of the Maillard reaction products was identified only in bottom position leaves in both the nitrogen treatments and it was higher in N1 treatment (Table 1 and 3). The contents of degradation products of phenylalanine were higher than those of aroma components generated from the Maillard reaction between amino acids and sugar.

Thus in the present study, leaf position and nitrogen levels showed variation in the NVAC in *lanka* tobacco. In general, the total NVAC were more in N1 treatment when compared to N2 treatment. Middle and top position leaves showed nearly similar contents of NVAC compared to bottom position leaves. Shi *et al.* (1998) reported that the aroma components transformed from degradation product of phenylalanine, carotenoid and Siebel triethenoid increased with the increased nitrogen level in FCV tobacco but the nitrogen levels applied to *lanka* tobacco were entirely different from FCV tobacco in quantity and source of nitrogen. Results revealed that tobacco from both middle and top position showed maximum accumulation of neutral volatile aroma constituents at 300 kg N/ha. The recommendation of 300 kg N/ha for *Lanka* tobacco was found to be optimum for higher yields with maximum neutral volatile aroma constituents for its pungent taste and strong aroma compared to farmer's practice (1000 kg N/ha).

REFERENCES

Deckmyn, G. and I. Impens. 1997. Combined effects of enhanced UV- B radiation and nitrogen deficiency on the growth, composition and photosynthesis of rye (*Secale cereale*). **Plant Ecol.** 128: 235-40.

Table 5: List of common NVAC in diffferentleaf position and N levels in *lanka* tobacco

RT (min)	Compound	Neutral volatile aroma fraction (%)							
		300 kg N/ha			Mean	1000 kg N/ha			Mean
		B	M	T		B	M	T	
6.34	Benzaldehyde	0.16	0.14	0.08	0.13	0.16	ND	ND	0.16
8.01	Benzyl Alcohol	0.57	0.42	0.49	0.49	0.52	0.44	ND	0.48
8.27	Phenylacetaldehyde	2.93	3.00	2.96	2.96	3.61	1.57	0.83	2.00
9.99	Phenylethyl Alcohol	1.25	1.31	1.73	1.43	1.30	0.90	1.05	1.08
14.46	Indole	0.89	ND	1.13	1.01	0.89	0.74	0.84	0.82
16.40	Solanone	0.86	1.25	1.07	1.06	1.56	1.35	1.32	1.41
18.16	Beta Damascone	0.10	0.35	0.25	0.23	0.22	ND	0.10	0.16
20.93	Beta Ionone	0.79	0.65	0.36	0.60	0.51	1.07	0.44	0.67
23.50	Megastigmatrienone	1.10	1.88	2.21	1.73	1.92	1.23	2.93	2.03
24.01	Megastigmatrienone	7.42	12.07	14.16	11.22	11.47	7.83	19.13	12.81
25.01	Megastigmatrienone 4	1.39	1.74	2.16	1.76	1.75	1.29	2.74	1.93
25.35	Megastigmatrienone	7.55	11.47	11.94	10.32	9.18	7.20	15.72	10.70
25.82	3-Oxo-Alpha-Ionol	0.80	0.74	0.65	0.73	0.73	ND	0.88	0.81
31.02	Solavetivone	0.25	ND	0.73	0.49	1.27	3.15	ND	2.21
31.63	Rishitin	2.19	0.60	1.38	1.39	2.7	7.69	1.17	3.85
32.12	Neophytadiene	29.39	39.81	39.68	36.29	31.87	32.19	31.92	31.99
33.74	3-Hydroxysolavetivone	ND	6.54	5.99	6.27	8.47	11.57	4.63	8.22
35.87	Dibutyl Phthalate	1.65	2.15	1.81	1.87	1.42	2.41	1.36	1.73
38.62	Thunbergol	1.41	2.19	ND	1.80	1.58	1.53	2.35	1.82
40.41	Phytol	9.30	11.75	10.1	10.38	13.06	8.30	9.51	10.29
42.01	(12 Z) - Abienol	0.39	0.47	ND	0.43	1.65	1.39	1.06	1.37

B: Bottom; M: Middle; T: Top;

Fei Yun, Guoshun Liu, Hongzhi Shi and Xiaowen Yang. 2013. Interactive effects of light intensity and nitrogen supply on the neutral volatile aroma components and organic acids of flue-cured tobacco. **J. Food Agric. Environ**. 11: 1187-94.

Katahata, S.I., M. Naramoto, Y. Kakubari and Y. Mukai. 2007. Seasonal changes in photosynthesis and nitrogen allocation in leaves of different ages in evergreen understory shrub *Daphniphyllum humile*. **Trees** 21: 619-29.

Lin, J., R.L. Rouseff, S. Barros and M. Naim. 2002. Aroma composition changes in early season grapefruit juice produced from thermal concentration. **J. Food Agric. Chem.** 50: 813-9.

Liu, B.Z. and K.F. Xian. 1993. Study on the neutral flavour constituents of different stalk positions, maturity and colour of Yunnan flue-cured tobacco. **Acta Tob. Sin**. 1: 46-53.

Liu, J. and D.C. Shi. 2010. Photosynthesis, chlorophyll fluorescence, inorganic ion and organic acid accumulations of sunflower in responses to salt and salt-alkaline mixed stress. **Photosynthetica** 48: 127-34.

Mc Cants, C.B. and W.G. Woltz. 1967. Growth and mineral nutrition of tobacco. **Adv. Agron**. 19: 211-65.

Murthy, G.S., D.M. Gopinath and V.V. Ramana Rao. 1962. Lanka tobacco: Its cultivation and curing. **Indian Tob**. 12:183-91.

Nielson, M.T. 1992. Inheritance of a diterpene constituent in tobacco trichome exudates. **Crop Sci**. 32: 1148-50.

Shi, H.Z., J.F. Han, Y.T. Wang and Q.H. Liu. 1998. Relationship between contents of essential oil components and flavor quality of tobacco leaf at different levels and sources of nitrogen. **China Tob. Sci**. 2:1-5 (in Chinese with English abstract).

Takane Fujimori, Reiko Kasuga, Hajime Kaneko and Masao Noguchi. 1978. Neutral volatile components of burley tobacco. **Beitrage Zur Tabakforsch. Int.** 9: 317-21.

Wei, F.J., G.S. Liu, Y.F. Yang, F. Wang, Y.J. Li and Q.Y. Guo. 2005. Relations between carotenoids and aromatic components from the carotenoids in flue-cured tobacco (*Nicotiana tabacum* L.) leaves at different mature periods. **Scientia Agric. Sinica** 38:1882-9 (in Chinese with English abstract).

Wei, S., I.M. Marton, D. Dekel, E. Shalitin, Lewinsohn, B.A. Bravdo and O. Shoseyov. 2004. Manipulating volatile emission in tobacco leaves by expressing *Aspergillus niger* â-glucosidase in different sub-cellular compartment. **Plant Biotech. J.** 2: 341-50.

Weybrew, W.A., W.A. Wan Ismail and R.C. Long. 1983. The cultural management of flue-cured tobacco quality. **Tob. Sci**. 27: 56-65.

Wu, Z. and W.W. Weeks. 1992. Contribution of neutral volatile to flavor intensity of tobacco during smoking. **Agric. Food Chem**. 40: 1917- 21.

Xiefeng, Y.E., Goushun Liu, Hongen Liu and Shiqing Li. 2011. On model of aroma quality evaluation for FCV tobacco based on principal component analysis. **J. Food Agric. Environ.** 9: 501-4.

Zhou, K., Q.M. Zhou and X.L. Hu. 2008. Advance in aroma substances in flue-cured tobacco. **China Tob. Sci**. 29: 58-61(in Chinese with English abstract).