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EFFECT OF GENOTYPE AND LEAF POSITION ON PIGMENTS AND THEIR DEGRADATION DERIVATIVES IN BURLEY TOBACCO

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Leaf pigments and their derivatives play an important role in the colour and aroma of tobacco. Chemical and biochemical quality constituents of tobacco (*Nicotiana tabacum* L.) are influenced by leaf position on stalk, cultural practices, genotypes and curing methods. Leaf curing is an important and essential post-harvest process in tobacco production which is likely to have a bearing on leaf quality, particularly in terms of pigments. The present study, therefore, assessed the effects of genotype and leaf position on pigments and their derivatives in air cured burley tobacco. The pigments identified in cured burley tobacco leaf included chlorophylls, carotenoids, pheophytin, chlorophyllide, protoporphyrin and magnesium protoporphyrin. Total chlorophyll (Tchl) content varied from 50.9 to 130 µg/g among the leaf positions and varieties. Tchl content was significantly higher in the variety Banket A1 compared to YB4 and middle position leaves (3rd pick) showed significantly higher levels of Tchl compared to the lower and top position leaves. Carotenoid content varied from 131 to 199 µg/g among the leaf positions and genotypes. The variety YB4 showed higher levels of carotenoids but lower levels of more polar carotenoids (MPC) and less polar carotenoids (LPC) than variety Banket A1. The carotenoid content increased with increase in leaf position with exception at 2nd pick and top position leaves showed maximum carotenoids. The variety Banket A1 showed significantly higher levels of Pheophytin a (Phe a) compared to YB4. Pheophytin b content was 2.3 times higher in the variety Banket A1 compared to YB4. Variety Banket A1 showed higher levels of MgPP and protoporphyrin compared to variety YB4. Chlide a content between the two varieties ranged from 0.06 to 1.73 mg/g whereas chlide b content varied from 1.06 to 2.80 between the varieties and among the picks. The variety YB4 showed significantly higher content of Chlide b compared to Banket A1. Pheophorbide compounds were not detected in both the varieties. The end product of the chlorophyll degradation may be chlide in tobacco and the degradation of chlorophyll pigments after curing was greater in YB4 compared to variety Banket A1. Thus, the advanced breeding line YB4 showed lower levels of chlorophyll pigments indicating the higher breakdown which may be leading to the formation of higher levels of aroma compounds.

Key words: Breakdown compounds, Burley tobacco, Genotypes, Leaf position, Pigments

INTRODUCTION

Chemical and biochemical quality parameters responsible for aroma and quality of tobacco are influenced by manures, leaf position on stalk, climatic conditions, cultural practices, genotypes and method of curing (Long and Weybrew, 1981). The chemical composition of the final product is strongly influenced, not only by genetic factors and growth conditions, but also by post harvest processing, eg. sun, air, smoke, flue, pit curing etc. The objective of curing the tobacco is to maintain and enhance the potential quality embodied in the harvested leaf and also to provide an environment conducive to the transformation of the leaf into a high quality cured tobacco product. This is achieved through control of the chemical and biochemical conversions and removal of moisture during curing. Burley tobacco, leaves are harvested by priming 3-4 leaves at a time when the leaves are matured and are shade cured. These processes lead to the creation of typical tobacco aroma and the most important part in the generation and transformation of various biochemical compounds. Air-curing is different from flue-curing as there is no control intemperature and moisture. Hence, formation of degradation products in tobacco during air-curing is entirely different from flue-curing. The rate of decline in all the leaf pigments, but especially

chlorophyll, which are observed during maturation of tobacco in the field are greatly accelerated during flue-curing (Long and Weybrew,1981). The breakdown products of pigments during the curing have been reported to give rise to numerous flavor components (Weeks, 1986). Burley, a light air-cured, cigarette tobacco type used for blending purpose in cigarette manufacture and is grown in Andhra Pradesh, Telangana, Karnataka and Orissa states in India. In the present paper, effect of leaf position and genotypes on the chlorophyll pigments and their degradation derivatives in burley tobacco reported.

MATERIALS AND METHODS

Cured leaf samples of variety the Banket A1 and the advanced breeding line YB4 were collected during 2012 season from experimental plots at Vinukonda. Leaf samples from different leaf position on the stalk (bottom, middle and top) were collected separately and the mid-ribs were removed and dried in the hot air oven at 60°C for 3 to 6 h, powdered and passed through 40 micron mesh sieve. Leaf powder (250 mg) was extracted with 5 ml of 80% acetone, centrifuged at 1,500 g for 5 min. The supernatant was separated and the absorbance was measured at 663.6, 646.6 and 440.5 nm, the major absorption peaks of chlorophyll a and b and carotenoids (Car), respectively. Content of the pigments was calculated using the equations of Porra *et al.* (1989) and Holm (1954), respectively and the values obtained were reported as µg/g dry weight.

Chl a =12.25 A663.6-2.55 A646.6 (µg/ml)
Chl b= 20.31 A646.6-4.91 A663.6 (µg/ml)
Chl a+b = 17.76 A646.6 +7.34 A663.6 (µg/ml)
Car = 4.69 A440.5 – 0.267Chl a+b (µg/ml)

Supernatant obtained was mixed with equal volume of hexane, vortexed and kept undisturbed until the interface appeared. The upper phase contains less polar compounds dissolved in hexane and the lower phase contains more polar compounds dissolved in acetone. The upper and lower phases were separated. The lower fraction was used to measure the absorbance at 575, 590 and 628 nm. The equation of Kahn *et al.* (1976) was used to estimate the content of protoporphyrin IX (PPIX) and magnesium-protoporphyrin IX (MgPP).

PPIX = 196.25 A575 - 46.6 A590 - 58.68 A628 (nmole)
MgPP = 61.81 A590 – 23.77 A575 - 3.55 A628 (nmole)

Chlorophyllide (Chlide a and b) content was estimated by the method of McFeeters *et al.* (1971) in acetone fraction at 667, 650 and 440.5 nm and more polar carotenoids (MP Car) were measured by the equation of Holm (1954) and Porra *et al.* (1989).

Chlide a = A667/74.9 (mM)
Chlide b = A650/47.2 (mM)
MP Car = 4.69 A440.5 – 0.267 Chl a+b (µg/ml)

The upper hexane fraction was dried with nitrogen and the pellet was dissolved in 80% acetone. The chlorophyll molecules were destroyed with 50 µl of 12.5% HCl. The absorbance was measured at 665.4, 653.4 and 470 nm which are the major absorption peaks of pheophytin a, b and less polar carotenoids (LP Car), respectively. The content of pheophytin a, b and LP Car were calculated according to the equation of Lichtenthaler (1987)

Phe a = 22.42 A665.4 – 6.81 A653.4 (µg/ml)
Phe b = 40.17 A653.4 – 18.58 A665.4 (µg/ml)
LP Car = (1000 A470 – 4.28 A665.4 – 4.78 A653.4)/ 164 (µg/ml)

RESULTS AND DISCUSSION

The total chlorophyll (TChl) content varied from 43.57 to 130 µg/g among the leaf positions and varieties (Table 1). The total chlorophyll content significantly increased with increase in leaf position (3rd pick) and decreased with further increase in leaf position. The variety Banket A1 showed maximum Chl compared to the variety YB4. The variety Banket A1 showed maximum total chl (130 µg/g) in 3rd pick compared to other picks in both varieties. Chlorophyll a (Chl a) varied from 20.13 to 50.13 µg/g among the picks and varieties (Table 1). The 3rd pick showed maximum Chl a content in both the varieties. Chl a content increased significantly with increase in leaf position from 1st to 3rd pick and decreased with increase in leaf position on the stalk. Banket A1 showed significantly higher contents of Chl a compared to YB4. The Chl a content was at a par

Table 1: Effect of genotype and leaf position chlorophyll pigments in burley tobacco (µg/g)

Pick	Chl a		Mean	Chl b		Mean	Total chl		
	YB4	Banket A1		YB4	Banket A1		YB4	Banket A1	
1	22.84	20.82	21.83	29.73	22.52	26.12	56.85	43.57	50.21
2	27.79	34.42	31.11	31.81	44.03	37.92	51.25	81.45	66.35
3	29.93	50.13	40.03	44.26	58.80	51.53	51.58	130.00	91.16
4	27.43	30.80	29.11	31.81	44.98	38.39	50.93	77.20	64.06
6	20.13	23.46	21.80	31.60	28.80	30.20	68.86	50.53	59.70
Mean	25.62	31.93		33.84	39.82		55.89	76.69	
	SEm±	CD (P=0.05)		SEm±	CD (P=0.05)		SEm±	CD (P=0.05)	
Varieties	**1.04**	**3.10**		**1.06**	**3.15**		**1.6**	**4.8**	
Pick	**1.65**	**4.90**		**1.60**	**4.90**		**2.5**	**7.6**	
Varieties x pick	**2.30**	**6.94**		**2.30**	**7.04**		**3.65**	**10.8**	

among 2, 3 and 4th picks of YB4 whereas there was a significant variation among the leaf position of Banket A1. The chl b content significantly increased with increase in leaf position from 1st to 3rd pick and thereafter it decreased with increase in leaf position (Table 1). In both the varieties the chl b content was increased upto 3rd pick and decreased with further increase in leaf position. The variety Banket A1 showed 17.67% higher chl b content compared to YB4.

Chlide a content between the two varieties ranged from 0.53 to 1.73 µg/g whereas chlide b content varied from 1.06 to 2.80 µg/g between the varieties and among the picks (Table 2). Both Clide a and Chlide b contents increased up to 3rd pick and decreased on further increase in leaf position on the stalk. The variety YB4 showed significantly higher content of Chlide b compared to Banket A1 (Table 2). PPIX content varied from 16.98 to 33.46 µg/g and there was no variation between the varieties and among the picks 1, 2 and 4 (Table 2).

Pheophytin a (Phe a) content increased significantly with increase in leaf position from bottom to middle position (1st to 3rd pick) and decreased with further increase in leaf position (Table 3). The middle position leaf contained significantly higher content of Phe a compared to other leaf positions. The variety Banket A1 showed significantly higher content of Phe a compared to YB4. The middle position leaves of Banket A1 contained significantly higher level of (34.5 µg/g) Phe a compared to other leaf positions in both the varieties (Table 3). The pheophytin b (Phe b) content also increased significantly with increase in leaf position from bottom to middle and decreased with further increase in leaf positions (Table 3). Middle position leaves contained significantly higher content of Phe b. Banket A1 showed 2.3 times higher Phe b compared to YB4. The middle position leaves of Banket A1 showed maximum and significantly higher content of Phe b over the other leaf positions in both the varieties.

MgPP content varied from 2.99 to 6.99 µg/g between the varieties and among leaf positions (Table 3). The variety Banket A1 showed significantly higher levels of MgPP compared to variety YB4. MgPP content increased significantly with increase in leaf position i.e. from Ist to 3rd pick and thereafter it decreased significantly with increase in leaf position.The variety YB4 showed significantly higher levels of MgPP copared to Banket A1 in each pick except 6th pick.

The carotenoid content varied from 131 to 199 µg/g among the leaf positions and varieties (Table 4). Carotenoid content increased with increase in leaf position with exception at 2nd pick and top leaf (6th pick) showed maximum content of carotenoids. The variety YB4 showed non-significantly higher levels of carotenoids over the variety Banket A1. The more polar carotenoids (MPC) content decreased with increase in leaf

Table 2: Effect of genotype and leaf position on chlide and protoporphirin in burley tobacco (µg/g)

Pick	Chlide a		Mean	Chlide b		Mean	PPIX		Mean
	YB4	Banket A1		YB4	Banket A1		YB4	Banket A1	
1	0.86	0.86	0.86	1.60	1.60	1.60	24.04	23.30	23.67
2	1.20	1.00	1.10	2.13	1.46	1.80	24.00	22.65	23.47
3	1.73	1.26	1.50	2.80	1.70	2.25	28.95	33.46	30.88
4	0.80	0.86	0.83	1.53	1.56	1.55	25.30	26.95	26.13
6	0.60	0.53	0.56	1.13	1.06	1.10	16.98	20.31	18.64
Mean	1.04	0.90		1.84	1.48		23.72	25.39	
	SEm±	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**	
Varieties	**0.07**	**NS**		**0.1**	**0.3**		**0.59**	**NS**	
Pick	**0.11**	**0.34**		**0.16**	**0.49**		**0.9**	**2.7**	
Varieties x pick	**0.16**	**NS**		**0.23**	**NS**		**1.32**	**NS**	

Table 3: Effect of genotype and leaf position on MgPP and pheophytin in burley tobacco (µg/g)

Pick	MgPP		Mean	Phe a		Mean	Phe b		Mean
	YB4	Banket A1		YB4	Banket A1		YB4	Banket A1	
1	4.47	3.21	3.84	7.81	22.1	14.98	8.39	17.32	12.85
2	5.32	3.64	4.48	9.29	23.3	16.31	10.52	19.73	15.13
3	6.99	5.44	6.21	10.4	34.5	22.53	11.06	24.70	17.88
4	4.70	4.65	4.68	8.20	18.9	13.56	7.73	17.10	12.42
6	2.99	3.23	3.10	5.20	20.6	12.93	3.06	14.93	9.00
Mean	4.89	4.03		8.19	23.9		8.15	18.76	
	SEm±	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**		**SEm±**	**CD (P=0.05)**	
Varieties	**0.15**	**0.46**		**0.5**	**1.6**		**0.37**	**1.11**	
Pick	**0.07**	**0.24**		**0.8**	**2.6**		**0.59**	**1.77**	
Varieties x pick	**0.57**	**1.7**		**1.2**	**3.6**		**0.84**	**2.50**	

position from bottom to top (Table 4). The bottom position leaf (1st pick) showed significantly higher levels of MPC compared to upper position leaves. The variety Banket A1 showed significantly higher levels of MPC compared to YB4. The interaction effect between leaf positions and varieties was non-significant.

Less polar carotenoids (LPC) content decreased with increase in leaf position from bottom to top (1st to 6th pick) (Table 4). LPC content was at a par among the first 3 picks and was significantly higher than the other two picks. The variety Banket A1 showed 93.3% higher levels of LPC compared to the variety YB4. In both the varieties, the bottom position leaves showed maximum and significantly higher content of LPC with exception of 3rd pick of Banket A1. The content of MPC was higher than that of LPC.

Chlorophyll pigments offer an all-oriented network of interception, reflection and protection for plant body, allowing plant to use sunlight and cut off its photodamage. The biosynthesis of plant pigments are very complex processes and more than fifteen biochemical reactions are involved in the biosynthesis of chlorophyll originated from precursor glutamate and eight steps in the degradation pathway from chlorophyll to pheophorbide (Reinbothe and Reinbothe, 1996).

Table 4: Effect of genotype and leaf position on carotenoid in burley tobacco (µg/g)

Pick	Carotenoids		Mean	Less polar carotenoids (LPC)		Mean	More polar carotenoids (MPC)		Mean
	YB4	Banket A1		YB4	Banket A1		YB4	Banket A1	
1	162	155	158	32.4	74.3	53.3	137.6	160.0	148.8
2	140	131	135	30.4	53.2	41.8	124.0	154.3	139.1
3	197	176	186	26.8	60.0	43.4	117.0	144.6	130.8
4	199	190	195	18.9	37.2	28.0	125.6	130.6	128.1
6	199	199	199	18.4	21.0	19.7	99.6	118.6	109.1
Mean	179	170		25.4	49.1		120.8	141.6	
	Em±	CD (P=0.05)		SEm±	CD (P=0.05)		SEm±	CD (P=0.05)	
Varieties	**2.5**	**7.5**		**0.59**	**NS**		**3.0**	**8.9**	
Pick	**4.0**	**11.9**		**0.9**	**2.7**		**4.7**	**14.0**	
Varieties x pick	**5.6**	**NS**		**1.32**	**NS**		**6.7**	**NS**	

In tobacco, leaf pigments play an important role in the quality and colour of tobacco as the chlorophyll pigments were degraded maximum leading to the formation of aroma compounds. The changes of chloroplast pigment and contents of degradation products were studied in leaves at different maturities in flue-cured tobacco (Zhao *et al.*, 2009) whereas Zhao *et al.* (2010) reported the effect of irrigation and fertilizers on degradation of chromoplast pigments in FCV tobacco. In the present study, pigments identified included chlorophylls, carotenoids, pheophytin, chlorophyllide, protoporphyrin and magnesium protoporphyrin. The variety YB4 showed significantly lower levels of chlorophyll pigment and higher levels of carotenoids compared to the variety Banket A1. It may be due to the genetic character of variety YB4. The tobacco varieties with higher contents of carotenoids and their degradation compounds showed a positive correlation with tobacco flavour (Wei *et al.*, 2005).

A number of factors are involved during the curing process including genetic traits which makes one of the important aspects in production of flavored tobacco. Therefore, genotypes have their own variables which influence the final composition of the cured leaf. The major pigments in green tobacco are chlorophyll a, chlorophyll b, lutein, â-carotein, neoxanthine and violaxanthin. Degradation of chlorophylls during yellowing stage of flue-curing follows a first-order reaction but during air-curing there was no control of the temperature and humidity which makes a difference between the flue-cured and air cured tobacco quality. Court and Hendel (1982) reported that chlorophyll a and b concentrations were about 1% of the amount measured at harvest in flue-cured tobacco. Siva Raju and Krishnamurthy (2011) and Siva Raju *et al.* (2012) reported that the pigment contents were higher in smoke and pit-cured chewing tobacco compared to air-cured and flue-cured tobacco.

The degradation products of carotenoids include a number of aroma compounds in tobacco. Total amount of pigment degraded and aroma compounds in the leaf are affected by the type of curing (Shi *et al.*, 2012). Different genotypes had different pigment degradation products and had significantly positive correlations with the contents of volatile degraded aroma compounds in cured leaves, while there were no negative correlations with the contents of pigment residue in cured leaves (Ma *et al.*, 2006). The results revealed that the end product of the chlorophyll degradation may be chlide in tobacco and the degradation of chlorophyll pigments was greater in the variety YB4 compared to Banket A1 as indicated by the lower levels of pigments. Thus, the advanced breeding line YB 4 showed lower levels of chlorophyll pigments indicating higher

breakdown which may be leading to the formation of higher levels of aroma compounds.

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