Polyphenol Accumulation in Chlorophyll Mutants of Tobacco under Two Cultural Practices*

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INTRODUCTION

Leaf quality and labor costs are two major concerns for tobacco production in the United States. One possible solution to the latter problem may be the use of breeding programs that select genotypes for uniform ripening of the leaves from all stalk positions. This reduces the number of primings and facilitates mechanized harvesting. The use of chlorophyll-deficient mutants to achieve these objectives has been suggested (3, 4).

Polyphenols in tobacco contribute directly to the color of the leaf and possibly to cigarette smoking flavor and aroma (7). These chemical and physical characteristics serve as criteria of leaf quality. A comparison of dark and Burley tobaccos showed a positive correlation between polyphenol and chlorophyll quantities in leaf lamina (10). Burley, as a chlorophyll-deficient mutant, differs from dark tobaccos by two recessive factors, yb_1 and yb_2 (5), and is low in polyphenol content (12). Polyphenol content in tobacco leaf is also influenced by nitrogen fertilization (13, 14). Since culture of flue-cured tobacco involves application of less nitrogen fertilizer than culture of Burley tobacco, the degree of interaction between cultural practice and chlorophyll-deficient genotype should determine leaf quality.

The objective of the present investigation was to study the accumulation of polyphenols in a number of chlorophyll-deficient mutants during the growing season and after curing under cultural practices for flue-cured and

Burley tobacco. The activities of polyphenoloxidase (PPO) and peroxidase (PRO) responsible for polyphenol oxidation in the leaves were also examined.

MATERIALS AND METHODS

Field experiments were conducted at the Oxford Tobacco Research Station, Oxford, North Carolina, and at the Agricultural Experiment Farm of the University of Kentucky, Lexington, Kentucky, in 1975. The Oxford experiment used flue-cured tobacco, *Nicotiana tabacum* L., cultivars NC95 and SC58 and the two isogenic lines NC95 — pale yellow (Py) and SC58 — yellow green (yg). It was carried out with four replications of 10 plants per entry under conventional cultural practices for flue-cured tobacco, which included application of 78 kg of nitrogen per ha (70 lb. per acre). Line NC95-Py was selected from the BC₅F₃ generation of crosses between NC95 and T.I. 1372, and the dominant factor Py is characterized by rapid leaf yellowing at maturity (3). The seed of SC58-yg, obtained from Dr. T. J. Mann, North Carolina State University, carries the yg gene of Consolation (9) after a series of backcrosses to SC58. In contrast to the Py gene, the yg gene is a recessive one that limits chlorophyll accumulation in the early stage of leaf growth.

Beginning 4 weeks after transplanting, eight leaf discs (13 mm in diameter) from each of three leaves at middle stalk positions of 10 plants were sampled at 2-week intervals. When the normal cultivars were judged to be ripe for harvest, leaf-disc samples were taken from leaves at the top, middle and bottom stalk positions. Leaves from the same stalk positions were sampled after flue-curing.

* Paper No. (78-11-207) of the Journal Series of Kentucky Agricultural Experiment Station and No. (5944) of the North Carolina Agricultural Research Service. The research was supported in part by Plant Science Research Division, ARS-USDA, Contract No. 12-14-7001-573 and in part by the University of Kentucky Tobacco and Health Research Institute Project No. 24110.

Received: 26th April 1979 — accepted: 16th July 1979.

The experiment at Lexington, Kentucky, used the same four flue-cured cultivars plus "Burley 21" as an additional entry of chlorophyll-deficient genotype (yb₁ yb₁ yb₂ yb₂) and was conducted with four replications of 25–30 plants per entry. Cultural practices followed the conventional Burley methods, which included application of 224 kg of nitrogen per ha (200 lb. per acre). Leaf discs were taken from leaves at middle-stalk positions 4 weeks after transplanting and every 2 weeks thereafter throughout the growing season as in the Oxford experiment. Mature leaves from middle-stalk positions were primed for simulated air-curing in a growth chamber according to the conditions previously described (11). Leaf samples of both experiments were bulked on a replicated row basis, freeze-dried, pulverized to pass through a 40 mesh screen, and stored in a desiccator over CaSO₄ in a freezer until they were extracted for chemical analysis. A 0.1 g sample of leaf powder was extracted with 50 ml of 80 % acetone for spectrophotometric measurement of chlorophyll content by the method of Arnon (1). Total chlorophyll is the sum of chlorophyll a and chlorophyll b. Carotenoids were quantified by the procedures de-

scribed in A.O.A.C. (8). Polyphenol quantification employed Soxhlet extraction of 0.5 g of leaf powder with 80 % isopropanol, followed by concentration of isopropanol extract *in vacuo* and two-dimensional paper chromatography. Chlorogenic acid (3-caffeoylquinic acid) and isomers were spectrophotometrically measured at 328 nm, whereas rutin (quercetin-3-rutinoside) was determined in 0.1 M AlCl₃ at 416 nm. Coumarins, scopolin and scopoletin, were detectable only in mature and cured leaves; their concentrations were spectrophotometrically measured at 345 nm. Total polyphenol concentration is the sum of the chlorogenic acid, rutin, scopolin and scopoletin. Assays of PPO and PRO in leaf extract were performed by spectrophotometric methods, with 3,4-dihydroxyphenylalanine and p-phenylenediamine-H₂O₂ as the respective substrates. Procedures of polyphenol quantification and oxidase preparation and assay have been detailed in a previous report (11). Data from the two experiments were treated separately by analysis of variance as a split-plot experiment in which cultivars and chlorophyll-deficient mutants are regarded as whole plots and sampling dates as subplots.

Table 1. Changes in chlorophyll and polyphenol concentration during the growing season and after curing in the midstalk leaves of chlorophyll genotypes of tobacco cultured according to practices for flue-cured tobacco at Oxford, North Carolina.

Chlorophyll genotype	Sampling date*	Concentration (mg/g dry weight)			
		Total chlorophylls	Chlorogenic acid	Rutin	Total polyphenols**
NC 95	6/27	5.98	20.20	4.80	25.00
	7/11	8.87	21.06	5.56	26.69
	7/25	3.59	30.66	7.46	39.03
	8/12	1.55	37.40	9.46	47.82
	after flue-curing	0.48	33.70	1.50	41.75
NC 95-Py	6/27	6.54	21.38	4.94	26.32
	7/11	8.15	27.43	5.69	33.27
	7/25	2.99	24.40	5.61	30.63
	8/12	0.90	38.50	8.70	48.55
	after flue-curing	0.31	24.60	5.37	32.15
SC 58	6/27	6.25	26.18	5.57	31.75
	7/11	7.71	24.00	5.38	29.46
	7/25	4.01	32.48	6.13	39.33
	8/12	1.98	41.87	6.85	49.62
	after flue-curing	0.39	38.54	6.32	47.00
SC 58-yg	6/27	2.38	28.54	5.96	34.50
	7/11	5.84	27.06	5.20	32.41
	7/25	2.82	27.64	5.89	34.37
	8/12	1.91	38.18	6.61	46.03
	after flue-curing	0.59	32.15	4.20	37.97
Least-significant difference among genotypes for the same sampling date	p = 0.05	1.06	7.56	1.15	10.65
	p = 0.01	1.54	11.00	1.68	15.50

* Plants were topped and primed on 7/25 and 8/12, respectively.
 ** Includes chlorogenic acid, rutin, scopolin and scopoletin.

RESULTS AND DISCUSSION

Irrespective of chlorophyll genotypes, the leaves from the middle-stalk positions of plants cultured under conditions for flue-cured tobacco had the highest concentration of chlorophylls 6 weeks after transplanting, and the concentration declined thereafter (Table 1). Cultivars NC95 and SC58 showed a similar pattern of chlorophyll accumulation during the growing season. Leaf yellowing occurred at maturity. It was especially evident in upper leaves, which had chlorophyll concentrations of 0.69 and 2.51 mg/g dry weight in NC95-Py and NC95, respectively (data not shown). Differences between these two genotypes were less in the lower leaves. Thus, the Py gene enhances the uniformity of yellowing or ripening of the leaves at all stalk positions. This enhancement may be a desirable characteristic for mechanized harvesting or for reducing the number of primings.

The difference in chlorophyll concentration between SC58 and SC58-yg was significant from transplantation to the time of topping but was less apparent at priming. Within a SC58-yg plant, upper leaves were lighter green than those from lower stalk positions. This gradation is in keeping with the original description of the yg mutant (9). The similarity in chlorophyll concentration in mature leaves of SC58 and SC58-yg may be attributed either to a more rapid loss of chlorophyll in the normal genotype or to a slow rate of chlorophyll degradation in the mutant. Chlorophylls disappeared rapidly during flue-curing, and the residual amounts in the cured leaf of the four tobacco genotypes were comparable.

Table 1 also presents the polyphenol concentrations in the samples. Total polyphenols increased slowly during early growth and reached a maximum at the time of harvest. Differences among the cultivars and chlorophyll-deficient mutants at any given sampling date were not significant. Mature leaves of NC95 and NC95-Py from middle-stalk positions contained significantly more rutin than those of SC58 and SC58-yg. Within the pairs of

isogenic lines, these phenolic compounds accumulated in the same pattern.

Three priming dates (Table 2) represent the leaves harvested from bottom, middle and top stalk positions when ripe. Since interactions between priming date and chlorophyll genotype were not statistically significant, the concentrations of plastid pigments and polyphenols in the plants of the Oxford experiment are given in Table 2 as averages for the four entries on each priming date. Plastid pigment and chlorogenic acid concentrations were highest in the first-primed green leaves and declined with subsequent primings. This relationship differs from the positive correlation between leaf stalk position and the content of chlorophylls and chlorogenic acid reported earlier (14). Priming as leaves ripen minimizes the difference in physiological age of the leaves and also restricts to a degree the translocation of metabolites between leaves of different stalk positions. The late-primed leaves from upper stalk positions are usually senescent with decreased soluble polyphenols. In contrast to the pattern for chlorogenic acid, the concentration of rutin and scopolin increased with lateness of priming date. Flue-cured leaves all contained sizable concentrations of scopolin and scopoletin indicating that polyphenolic compounds in tobacco leaf are not synthesized and degraded in the same manner at different leaf ages.

Except for Burley 21, the chlorophyll genotypes cultured under conditions for Burley tobacco reached a maximum level of chlorophylls at the time of topping, but total polyphenol concentration did not change significantly from one sampling date to the next (Table 3). Air-curing decreased the quantity of chlorophylls and polyphenols. Cultivar SC58 and its isogenic yg line tended to contain more polyphenols than the NC95 genotypes during the growing season. On the average, the flue-cured cultivars and isogenic chlorophyll-deficient mutants were 50 % higher than Burley 21 in total polyphenol concentration in green and air-cured leaves.

Table 2. Effect of priming date and flue-curing on the concentration of plastid pigments and polyphenols in green and flue-cured leaves of tobacco cultured according to practices for flue-cured tobacco at Oxford, North Carolina.

Kind of leaf	Priming date	Plastid pigments		Polyphenols				
		Total chlorophylls	Carotenoids	Chorogenic acid	Rutin	Scopolin	Scopoletin	Total
mg/g dry weight								
Green	7/31	1.65	0.25	50.88	7.15	0.49	0	58.52
	8/12	1.58	0.16	38.98	7.90	1.11	0	47.99
	8/28	1.17	0.16	36.24	9.00	1.62	0	46.86
Flue-cured	7/31	0.53	0.07	29.50	4.83	1.28	0.62	36.23
	8/12	0.44	0.05	32.25	5.53	1.34	0.61	39.71
	8/28	0.50	0.08	30.89	8.62	1.38	0.89	41.77
Least-sig- nificant difference	p = 0.05	0.53	0.05	7.77	1.15	0.28	0.23	10.70
	p = 0.01	0.77	0.07	11.31	1.68	0.40	n.s. *	15.57

* n.s. = not significant.

Table 3. Changes in chlorophyll and polyphenol concentration during the growing season and after curing in the midstalk leaves of chlorophyll genotypes of tobacco cultured according to practices for Burley tobacco at Lexington, Kentucky.

Chlorophyll genotype	Sampling date*	Concentration (mg/g dry weight)			
		Total chlorophylls	Chlorogenic acid	Rutin	Total polyphenols**
NC 95	7/17	4.52	21.63	6.87	28.61
	7/31	5.66	22.48	6.32	29.17
	8/14	6.75	20.42	7.84	29.55
	8/28	4.42	18.94	5.32	25.50
	9/11	2.49	24.54	5.61	31.89
	after air-curing	1.28	16.81	3.62	22.87
NC 95-Py	7/17	3.92	18.91	6.89	25.93
	7/31	5.63	18.76	5.83	25.04
	8/14	5.05	21.81	5.99	28.81
	8/28	2.29	16.60	5.42	22.97
	9/11	1.20	18.69	5.04	25.02
	after air-curing	0.39	12.45	2.68	17.30
SC 58	7/17	4.61	27.78	6.73	34.63
	7/31	5.15	26.50	6.65	33.54
	8/14	5.95	25.37	6.84	33.44
	8/28	3.62	22.08	5.42	28.61
	9/11	1.84	28.70	6.08	36.18
	after air-curing	0.85	15.88	4.25	21.93
SC 58-yg	7/17	1.93	26.49	7.30	33.93
	7/31	2.74	25.58	5.76	31.79
	8/14	3.62	27.51	6.98	35.28
	8/28	3.11	22.18	5.32	28.40
	9/11	2.08	28.95	5.04	34.94
	after air-curing	0.62	15.42	4.02	21.30
Burley 21	7/13	3.48	13.55	4.76	18.39
	7/31	4.82	14.88	4.27	19.37
	8/14	3.86	17.10	4.94	22.48
	8/28	2.62	11.43	3.33	15.21
	9/11	1.72	13.36	3.33	17.20
	after air-curing	0.28	6.68	3.37	10.82
Least-significant difference among genotypes for the same sampling date	p = 0.05	0.49	6.58	1.13	7.29
	p = 0.01	0.74	9.97	1.71	11.05

* Plants were topped and harvested on 8/14 and 9/11, respectively.

** Quantity includes chlorogenic acid, rutin, scopolin and scopoletin.

The average concentration of plastid pigments and polyphenols over all sampling dates under the two cultural conditions are summarized in Table 4. The yg mutant accumulated significantly less chlorophyll than the recurrent parent SC58 under both cultural conditions. Chlorophyll a/b ratio in the immature leaf of SC58-yg grown under both cultural conditions reached as high as 7.8 (data not shown), but declined progressively with time to a level comparable to the other flue-cured cultivars. The difference in chlorophyll concentration between NC95-Py and NC95 was significant under practices for Burley tobacco but not under practices for flue-cured tobacco. The non-significance of the difference under the latter conditions is attributable to a low nitro-

gen application rate that caused NC95 leaves to turn yellow rapidly at maturity. The higher rate of nitrogen fertilizer used in the Burley region prolonged leaf greening, so the difference in chlorophyll concentration between these genotypes was more prominent. Under either cultural condition, the chlorophyll a/b ratios of NC95 and NC95-Py were around 3.5 throughout the growing season. It has been suggested that the mode of action for the Py gene is an early breakdown of chloroplasts as leaves approach maturity (6). This breakdown would cause leaf yellowing but would not alter the chlorophyll a/b ratio.

Chlorophyll content in Burley 21 was nearly as low as that in SC58-yg, whereas its chlorophyll a/b ratio re-

Table 4. Comparison of plastid pigment and polyphenol concentrations in tobacco chlorophyll genotypes under two cultural practices, averaged from samples taken during the growing season and after curing.*

Cultural method**	Chlorophyll genotype	Plastid pigments				Polyphenols				
		Chloro- phyll a	Chloro- phyll b	Total chloro- phylls	Caro- tenoids	Chloro- genic acid	Rutin	Scopolin	Scopo- letin	Total
mg/g dry weight										
Practices for flue- cured tobacco	NC 95	2.18a	0.67a	2.85a	0.31a	34.71a	7.95a	0.94a	0.13a	43.73a
	NC 95-Py	1.83ab	0.54a	2.37ab	0.28ab	29.17a	6.42a	1.00a	0.34a	36.93a
	SC 58	2.06a	0.64a	2.70a	0.31a	35.12a	6.64a	0.84a	0.19a	42.79a
	SC 58-yg	1.31b	0.37b	1.68b	0.22b	32.77a	5.69b	0.81a	0.28a	39.55a
Practices for Burley tobacco	NC 95	3.32a	0.90a	4.22a	0.50a	21.25ab	6.18a	0.98a	0.09a	28.50a
	NC 95-Py	2.58b	0.68b	3.26b	0.40b	18.69bc	5.55a	0.82a	0.08a	25.14b
	SC 58	3.00a	0.81a	3.81a	0.47a	25.95a	6.29a	0.85a	0.07a	33.16a
	SC 58-yg	1.95c	0.43c	2.38c	0.32c	25.37a	5.83a	0.69a	0.07a	31.96a
	Burley 21	2.21c	0.58bc	2.79c	0.38b	12.83c	4.00b	0.35b	0.06a	17.24c

* Within each column and cultural method, means followed by a letter in common are not significantly different at the 1% level of probability, according to Duncan's multiple range test.

** The practices for flue-cured tobacco were used at Oxford, N.C., and those for Burley tobacco at Lexington, Ky.

sembled those of flue-cured cultivars. These results indicate metabolic differences between genotypes yg₁ yg₂ and yb₁ yb₂ for chlorophyll deficiency. Low carotenoid content was common for all chlorophyll-deficient mutants, among which the yg had the lowest. Carotenoids in chloroplasts are regarded as photoprotective agents that protect chlorophyll against photosensitization (2). The association of low carotenoids with low chlorophyll content in these mutants, especially the yg₁ yg₂ genotype, might be expected.

With the exception of low rutin concentration in SC58-yg, all chlorophyll genotypes grown under conditions for flue-cured tobacco had similar levels of individual and total polyphenols. Under practices for Burley tobacco, chlorogenic acid accumulated more in NC95, SC58 and SC58-yg, less in NC95-Py, and the least in Burley 21. The latter was also significantly lower than the others in rutin and scopolin content. The four flue-cured entries were 37% higher in total polyphenols when grown under the conditions for flue-cured tobacco as compared with the conditions for Burley tobacco. This represents increases of 44 and 12% in chlorogenic acid and rutin, respectively. The increase in polyphenols is probably due to the low nitrogen fertilizer rate with the practices for flue-cured tobacco, since polyphenol accumulation has been demonstrated in tobacco under low nitrogen rates (13, 14).

It has been suggested that a positive correlation between chlorophyll and polyphenol content in tobacco leaves may be attributed to the conversion of L-phenylalanine to phenylpyruvate by δ -aminolevulinic acid transaminase for chlorophyll biosynthesis. In this case, phenylpyruvate is a by-product and can be readily converted to cinnamic acid subsequently to polyphenols (10). The present results with Burley 21, NC95 and SC58 confirm such a positive correlation. However, the high polyphenol con-

centration in the chlorophyll-deficient mutants NC95-Py and SC58-yg requires a different explanation. As mentioned above, chlorophyll deficiency in NC95-Py may be a result of rapid chloroplast breakdown in mature leaves. This phenomenon does not interfere with chlorophyll biosynthesis during leaf growth; hence, a depletion of the phenolic precursor phenylpyruvate is not expected. The comparable levels of polyphenols in SC58 and SC58-yg throughout the growing season may also indicate that these genotypes do not differ from each other in rate of chlorophyll biosynthesis. The low concentration of carotenoids in the upper leaves of SC58-yg suggests some type of light-dependent chlorophyll degradation. This is further substantiated by leaf greening toward the lower stalk positions. On the basis of the time of chlorophyll gene expression and polyphenol accumulation, one may conclude that the yb₁ and yb₂ genes in Burley 21 limit chlorophyll biosynthesis and the Py and yg genes accelerate chlorophyll degradation. Experiments are in progress to verify this conclusion by comparison of the rates of chlorophyll biosynthesis and degradation in these chlorophyll-deficient mutants.

Both cultural practices and curing methods affected the activities of PPO and PRO in tobacco leaves (Table 5). Under cultural conditions for flue-cured tobacco, PPO and PRO activities peaked before or at the time of flower bud initiation (7/11) and decreased significantly by the time of topping (7/25). The activity of PPO increased slightly in the primed leaves (8/12) of all entries except SC58. The difference in the activities of both oxidases between NC95 and NC95-Py was non-significant at most sampling dates, whereas SC58-yg distinguished itself from SC58 by maintaining a high PRO level late in the growing season. The significance of high PRO activity in conjunction with the yg genotype remains to be determined. Flue-curing caused drastically

Table 5. Comparison of polyphenoloxidase (PPO) and peroxidase (PRO) activity (A) during the growing season and after curing in the midstalk leaf of chlorophyll genotypes of tobacco under cultural practices for flue-cured and Burley tobacco.

Chlorophyll genotype	Practices for flue-cured tobacco*			Practices for Burley tobacco**		
	Sampling date	PPO	PRO	Sampling date	PPO	PRO
ΔA per 100 mg dry weight per min						
NC 95	6/27	1.08	110.00	7/17	1.42	91.00
	7/11	1.45	167.00	7/31	0.66	219.14
	7/25	0.46	78.00	8/14	1.26	259.00
	8/12	0.64	77.25	8/28	1.77	189.00
	after flue-curing	0.10	0.38	9/11	1.58	287.00
				after air-curing	2.05	281.85
NC 95-Py	6/27	0.90	136.00	7/17	0.83	131.99
	7/11	1.13	160.00	7/31	0.73	267.74
	7/25	0.46	78.00	8/14	0.68	184.24
	8/12	0.68	87.25	8/28	0.85	214.48
	after flue-curing	0.10	0.52	9/11	1.33	406.00
				after air-curing	1.83	288.54
SC 58	6/27	1.52	156.00	7/17	0.93	153.71
	7/11	0.83	219.00	7/31	0.74	260.84
	7/25	0.39	59.00	8/14	1.09	189.14
	8/12	0.37	61.75	8/28	1.35	204.00
	after flue-curing	0.05	0.43	9/11	1.55	238.00
				after air-curing	1.12	183.23
SC 58-yg	6/27	1.31	144.50	7/17	0.98	155.00
	7/11	1.07	153.00	7/31	0.53	200.60
	7/25	0.28	150.10	8/14	1.09	189.00
	8/12	0.53	136.50	8/28	1.29	246.00
	after flue-curing	0.05	0.30	9/11	1.40	259.00
				after air-curing	0.98	107.10
Burley 21				7/17	0.40	67.00
				7/31	0.55	144.24
				8/14	2.05	170.59
				8/28	1.23	141.00
				9/11	2.43	119.00
				after air-curing	0.83	71.82
Least-significant difference among genotypes for the same date	p = 0.05	0.16	22.35		0.42	55.18
	p = 0.01	0.23	32.51		0.63	83.59

* At Oxford, N.C.

** At Lexington, Ky.

reduced activities of both oxidases as would be expected because of the high temperature (71 °C; 160 °F) during curing.

Oxidase activities were higher in ripe green leaves of plants grown in the Burley tobacco region than those of plants in the flue-cured tobacco region. This difference probably reflects again the high nitrogen rate applied in Burley tobacco culture. Among the chlorophyll-deficient genotypes, the mature leaf of Burley 21 was the lowest in PRO but the highest in PPO activity. In air-cured leaves, NC95, NC95-Py and SC58 retained higher PPO and PRO activities than Burley 21 and SC58-yg. The latter two chlorophyll mutants had comparable levels of these oxidases in cured tissues. During the growing sea-

son the oxidase activities were similar in SC58 and SC58-yg; variation between NC95 and NC95-Py was far greater. Leaf senescence caused by the expression of Py gene may explain this variation. The high level of both oxidases in the air-cured leaf of flue-cured cultivars may permit continuation of polyphenol oxidation during storage.

The relationship between concentrations of chlorophyll and polyphenol under the two cultural practices can also be examined by the coefficients of partial correlation between the leaf components under investigation (Table 6). The concentrations of chlorophyll and polyphenol were positively correlated under practices for Burley tobacco but negatively correlated under practices

Table 6. Coefficients of partial correlation between pairs of chemical constituents in the leaf of tobacco chlorophyll genotypes at all sampling dates. ⁺

Chemical constituent	Coefficient of partial correlation				
	Total Chlorophylls	Chlorogenic acid	Rutin	Total polyphenols	Polyphenol-oxidase
Chlorogenic acid	-0.39** -0.32**				
Rutin	-0.28* 0.60**	0.50** 0.70**			
Total polyphenols	-0.30** 0.35**	0.83** 0.99**	0.57** 0.77**		
Polyphenoloxidase	0.64** -0.29**	-0.11 -0.11	0.02 -0.25**	-0.09 -0.11	
Peroxidase	0.71** -0.08	-0.08 0.01	-0.06 -0.15	0.01 -0.01	0.83** 0.24**

⁺ The upper and lower correlation coefficients of each pair represent the values with cultural practices for flue-cured and Burley tobacco, respectively.
* and **: significant at the 5 % and 1 % levels of probability, respectively.

for flue-cured tobacco. Chlorophyll content in the flue-cured region was positively correlated with the activities of both oxidases, which were also positively correlated with each other. Of interest is the lack of correlation between polyphenols and oxidases. The greater the concentration of PPO and PRO in tobacco leaves, the faster might be leaf browning owing to a rapid rate of polyphenol oxidation. In addition, high oxidase activities may result in a low content of soluble polyphenols in the leaf. Uniform ripening of tobacco leaves, yellowing, or both, with a low concentration of soluble polyphenols, are desirable for homogenized leaf curing, which is a new process for tobacco production with the potential for reducing labor costs and improving leaf quality in terms of lowering cigarette-smoking hazards (15).

Although the present results cannot be delineated from the effects of soil, climate and other environmental factors in each experimental location, the results at least demonstrated differences in phenotypic expression of chlorophyll-deficiency genes as they interacted with cultural methods. Aside from the usefulness of these genes for production of flue-cured tobacco as reported in the literature (4), the Py and yg genes may also improve leaf quality and yield per acre in the production of Burley tobacco. The addition of Py gene into the Burley genotype should enhance uniformity of leaf maturation within a plant. An accelerated maturation of upper leaves permits early harvesting; as a result, the loss of bottom leaves is reduced. However, the recombination of Py gene with the Burley genotype presents a difficulty in identification. For the recombinants of yg and Burley chlorophyll genes, the yg gene is expressed as a distinct yellow-green color at the seedling stage. The interaction of these chlorophyll genes in a Burley plant would accelerate yellowing of the upper leaves as does the Py gene. Breeding programs to incorporate these chlorophyll-deficiency genes into Burley cultivars are in progress. In view of a decrease in starch and sugar and an

increase in α -amino nitrogen in NC95-Py as compared with the recurrent parent NC95 under practices for flue-cured tobacco (3), the influence of Py and yg genes on leaf chemical composition in Burley tobacco should be evaluated.

SUMMARY

Quantitative variation in plastid pigments and polyphenols during leaf growth and after curing have been studied with several chlorophyll-deficient genotypes of tobacco. Under culture for flue-cured tobacco, the chlorophyll-deficient pale-yellow (NC95-Py) and yellow-green (SC58-yg) lines did not differ in polyphenol accumulation from the corresponding normal green recurrent parents NC95 and SC58. A negative correlation was evident between concentrations of polyphenol and chlorophyll. Decrease of the latter during leaf growth was accompanied with a decline of PPO and PRO activities. When the chlorophyll-deficient genotypes were grown under conditions for Burley tobacco, concentrations of chlorophyll and polyphenol maintained a steady level and were positively correlated, while the oxidases tended to increase. Burley 21 contained the lowest amount of polyphenols among the chlorophyll mutants, although the low chlorophyll content of its mature leaves was comparable to that of SC58-yg. Use of cultural practices for flue-cured tobacco caused an accumulation of polyphenols in green leaves. There was a greater loss of soluble polyphenols during air-curing than during flue-curing. Results indicate that the interaction of cultural practices with chlorophyll-deficient genes affects the metabolism of chlorophylls and polyphenols during leaf growth and maturation. Introduction of these genes into tobacco cultivars may provide a means of enhancing uniformity of leaf maturity and modifying certain leaf chemical constituents.

ZUSAMMENFASSUNG

An mehreren chlorophyllarmen Tabakgenotypen wurde die quantitative Veränderung des Pigmentgehaltes der Plastiden und des Polyphenolgehaltes im Verlauf des Wachstums und nach der Trocknung des Blattgutes untersucht. Bei Anwendung der für „flue-cured“-Tabak gebräuchlichen Anbaumethoden unterschieden sich die chlorophyllarmen weißgelben (NC95-py) und gelbgrünen (SC58-yg) Zuchtstämme in der Polyphenolbildung nicht von den entsprechenden grünen Ausgangsstämmen NC95 und SC58. Zwischen der Polyphenol- und der Chlorophyllkonzentration zeigte sich eine negative Korrelation. Die Abnahme des Chlorophyllgehaltes der Blätter in der Wachstumsperiode ging mit einem Rückgang der Aktivität der Polyphenoloxidase (PPO) und der Peroxidase (PRO) einher. Wenn die chlorophyllarmen Genotypen unter für Burley-Tabake üblichen Anbaumethoden gezogen wurden, blieben die Chlorophyll- und die Polyphenolkonzentrationen bei positiver Korrelation zueinander konstant, während die Oxidaseaktivität tendenziell zunahm. Unter den Chlorophyll-Mutanten enthielt Tabak der Sorte Burley 21 die geringste Menge an Polyphenolen, obwohl der niedrige Chlorophyllgehalt seiner reifen Blätter dem des SC58-yg-Zuchtstammes vergleichbar war. Die für „flue-cured“-Tabak üblichen Anbaumethoden führten zu einer vermehrten Bildung von Polyphenolen im grünen Blatt. Bei Lufttrocknung trat ein größerer Verlust an löslichen Polyphenolen ein als bei Heizröhrentrocknung. Die Ergebnisse der Untersuchung zeigen eine Beeinflussung des Chlorophyll- und Polyphenolstoffwechsels während des Wachstums und der Reifung der Blätter durch die gegenseitige Wechselwirkung zwischen landwirtschaftlichen Anbauverfahren und genetischer Determinierung zu geringem Chlorophyllgehalt. Durch die Einbeziehung dieser genetischen Determinierung in die Züchtung von Tabakstämmen kann eine größere Einheitlichkeit des Reifungsprozesses aller Blätter einer Pflanze und eine Veränderung bestimmter chemischer Blattinhaltsstoffe erreicht werden.

RÉSUMÉ

On a étudié la variation quantitative des pigments plastides et des polyphénols pendant la croissance des feuilles de tabac et après le «curing» sur différents génotypes présentant une déficience en chlorophylle. Lorsqu'elles sont cultivées comme le tabac «flue-cured», les variétés jaune pâle (NC95-py) et jaune vert (SC58-yg) déficientes en chlorophylle présentent la même accumulation de polyphénols que les variétés vertes apparentées NC95 et SC58. On a établi une corrélation négative entre les concentrations de polyphénols et de chlorophylle. La diminution de cette dernière lors de la croissance des feuilles est accompagnée d'un ralentissement de l'activité de la polyphénol-oxidase (PPO) et de la peroxydase (PRO). Lorsque les génotypes déficients en chlorophylle sont cultivés de la même façon que le tabac Burley, les concentrations de

chlorophylle et de polyphénols se maintiennent à un niveau régulier et mettent en évidence une corrélation positive, tandis que les oxydases ont tendance à augmenter. Le Burley 21 contenait le moins de polyphénols parmi les mutants «chlorophylle», bien que la faible teneur en chlorophylle de ses feuilles à leur maturité fût comparable à celle de la variété SC58-yg. La pratique de la culture «flue-cured» a provoqué une accumulation de polyphénols dans les feuilles vertes. La perte en polyphénols solubles a été plus importante pendant le séchage à l'air que pendant le «flue-curing». Les résultats montrent que l'interaction des pratiques culturales avec des gènes de déficience en chlorophylle influence le métabolisme des chlorophylles et des polyphénols pendant la croissance et la maturation des feuilles. L'introduction de ces gènes dans des cultivars de tabac peut constituer un moyen d'augmenter l'uniformité de la maturation des feuilles et de modifier certains composants chimiques de la feuille.

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