DIFFERENCES IN AMINO ACID CONTENTS BETWEEN FLUE-CURED TOBACCO AND BURLEY TOBACCO BOTH CURED WITH FLUE-CURING AND AIR-CURING METHOD

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Importance of Amino Acids

- Nitrogen metabolism.
- Alkaloid biosynthesis.
- Precursors for aroma components.
  - **Precursors for non-enzymatic browning reaction, combining with sugars to form sugar-amino acid compounds followed by Maillard reaction to produce many aroma components such as pyran, pyrazine, pyrrole, pyridine**
  - **Substrate for pyrolysis to produce some aroma compound.**

    phenylalanine → Benzyl alcohol,
    Phenyl acetaldehyde,
    Benzyldehyde,

Importance of Amino Acids

- Tasty agents

  sweet type: proline, glycine, alanine

  fresh type: aspartic acid, glutamic acid

  aromatic type: phenylalanine, tyrosine

The constitution, proportion and contents of individual amino acid may have significant impact on the flavor quality and style of tobacco.

Previous Study

- In different tobacco types, total and individual amino acid varies greatly

  --Dr. Bush found a higher content of aspartic acid in burley than in flue-cured tobacco

  --Davenport et al.(2007) reported that there are big differences in the contents of amino acids between burley and flue-cured tobacco, proline is enriched in flue-cured tobacco, while aspartic acid and glutamic acid are rich in burley tobacco

Previous Study

- The investigation was also conducted in our laboratory to compare the contents of amino acids among burley, flue-cured, Oriental, sun-cured tobaccos and found significant differences in total and individual contents of AA.

  The result showed that the highest content in flue-cured tobacco and oriental tobacco is Pro and Glu and accounted for 31.01%-46.75% of total amino acids; while in burley tobacco, maryland tobacco and sun-cured tobacco, Asp and Glu were the dominant amino acids and accounted for 34.43%-38.69% of total.

QUESTION

- What is the cause of the difference in amino acid contents between flue-cured tobacco and burley tobacco?

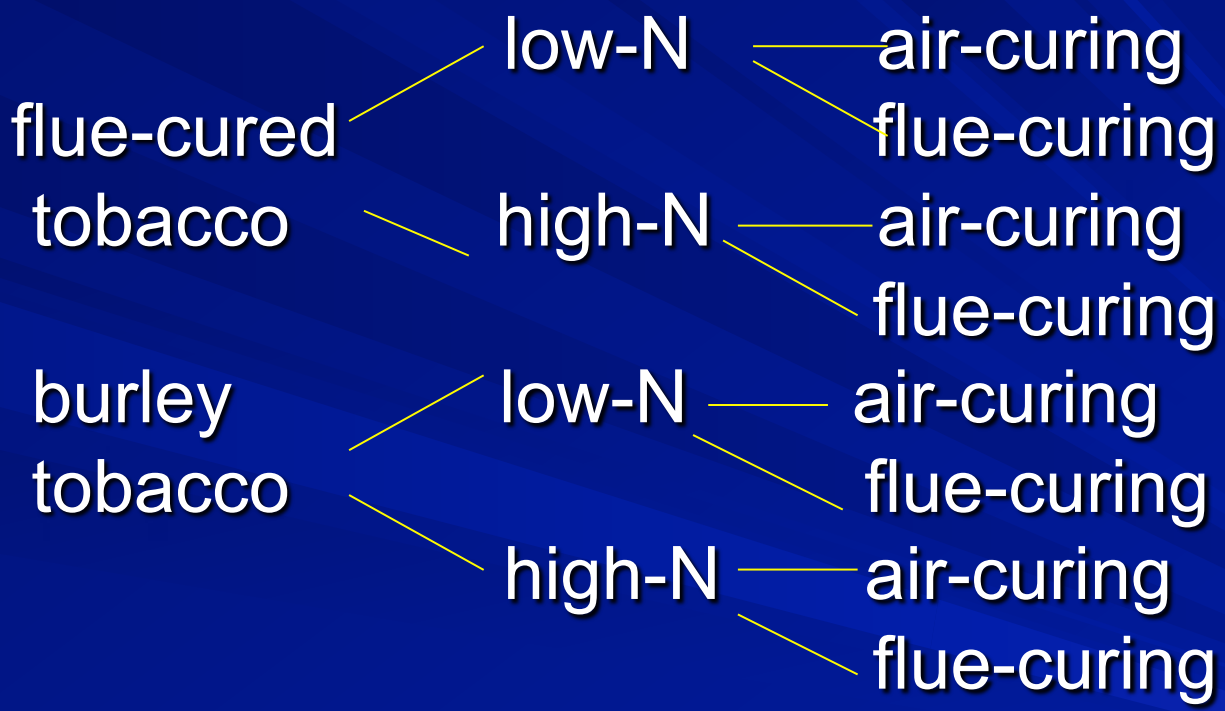
  Possible factors:

  -- genetic background

  -- curing method

  -- nitrogen applying amount

Experimental Design



Low-N: 90kg/ha; High-N: 200kg/ha

Material and method

- Cultivar:

  Flue-cured tobacco: Yunyan87

  Burley: TN86

- Method:

  Amino acids contents were determined by S-433D (SYKAM, Germany) amino acids analyzer

Results

- Effects of curing methods on the content of amino acid in the flue-cured tobacco and burley tobacco

Upper leaves; Low-N

Amino acids	Burley tobacco		b/a	Flue-cured tobacco		b*/a*	a/a*	b/b*
	Air-curing a	Flue-curing b		Air-curing a*	Flue-curing b*			
aspartic acid	3.55	3.47	0.98	0.67	0.70	1.04	5.30	4.96
glutamic acid	1.60	1.57	0.98	1.03	0.93	0.90	1.55	1.69
serine	0.26	0.32	1.23	0.19	0.18	0.95	1.37	1.78
arginine	0.33	0.44	1.33	0.19	0.22	1.16	1.74	2.00
glycine	0.37	0.43	1.16	0.24	0.25	1.04	1.54	1.72
threonine	0.32	0.47	1.47	0.20	0.20	1.00	1.60	2.35
proline	0.96	2.42	2.52	0.92	2.09	2.27	1.04	1.16
alanine	0.36	0.43	1.19	0.24	0.23	0.96	1.50	1.87
valine	0.08	0.11	1.38	0.00	0.05	--	--	2.20
methionine	0.22	0.42	1.91	0.18	0.43	2.39	1.22	0.98
isoleucine	0.04	0.07	1.75	0.00	0.05	--	--	1.40
leucine	0.20	0.27	1.35	0.12	0.11	0.92	1.67	2.45
phenylalanine	0.50	0.68	1.36	0.27	0.26	0.96	1.85	2.62
histidine	0.39	0.44	1.13	0.15	0.19	1.27	2.60	2.32
lysine	0.22	0.27	1.23	0.12	0.10	0.83	1.83	2.70
tyrosine	0.43	0.64	1.49	0.38	0.31	0.82	1.13	2.06
total	9.83	12.45	1.27	4.90	6.30	1.29	2.00	1.98

Middle leaves

Amino acids	Burley tobacco		b/a	Flue-cured tobacco		b*/a*	a/a*	b/b*
	Air-curing a	Flue-curing b		Air-curing a*	Flue-curing b*			
aspartic acid	3.46	3.26	0.94	0.47	0.53	1.13	7.36	6.15
glutamic acid	1.75	1.74	0.99	0.79	0.78	0.99	2.22	2.23
serine	0.24	0.21	0.88	0.16	0.18	1.13	1.50	1.17
arginine	0.25	0.30	1.20	0.14	0.19	1.36	1.79	1.58
glycine	0.33	0.30	0.91	0.18	0.24	1.33	1.83	1.25
threonine	0.29	0.27	0.93	0.15	0.18	1.20	1.93	1.50
proline	0.59	1.25	2.12	0.62	1.40	2.26	0.95	0.89
alanine	0.27	0.27	1.00	0.19	0.25	1.32	1.42	1.08
valine	0.05	0.09	1.80	0.00	0.05	--	--	1.80
methionine	0.25	0.48	1.92	0.16	0.41	2.56	1.59	1.17
isoleucine	0.03	0.05	1.67	0.00	0.05	--	--	1.00
leucine	0.16	0.20	1.25	0.07	0.11	1.57	2.29	1.82
phenylalanine	0.39	0.42	1.08	0.23	0.32	1.39	1.70	1.31
histidine	0.35	0.33	0.94	0.12	0.15	1.25	2.92	2.20
lysine	0.17	0.15	0.88	0.10	0.15	1.50	1.70	1.00
tyrosine	0.39	0.49	1.26	0.28	0.20	0.71	1.39	2.45
total	8.97	9.81	1.09	3.66	5.19	1.42	2.45	1.89

Upper leaves; Low-N

Amino acids	Burley tobacco		b/a	Flue-cured tobacco		b*/a*	a/a*	b/b*
	Air-curing a	Flue-curing b		Air-curing a*	Flue-curing b*			
aspartic acid	3.55	3.47	0.98	0.67	0.70	1.04	5.30	4.96
glutamic acid	1.60	1.57	0.98	1.03	0.93	0.90	1.55	1.69
serine	0.26	0.32	1.23	0.19	0.18	0.95	1.37	1.78
arginine	0.33	0.44	1.33	0.19	0.22	1.16	1.74	2.00
glycine	0.37	0.43	1.16	0.24	0.25	1.04	1.54	1.72
threonine	0.32	0.47	1.47	0.20	0.20	1.00	1.60	2.35
proline	0.96	2.42	2.52	0.92	2.09	2.27	1.04	1.16
alanine	0.36	0.43	1.19	0.24	0.23	0.96	1.50	1.87
valine	0.08	0.11	1.38	0.00	0.05	--	--	2.20
methionine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2.39	1.22	0.98
isoleucine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	--	--	1.40
leucine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.67	2.45
phenylalanine	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.85	2.62
histidine	[illegible]	[illegible]	1.13	0.15	0.19	1.27	2.60	2.32
lysine	0.22	0.27	1.23	0.12	0.10	0.83	1.83	2.70
tyrosine	0.43	0.64	1.49	0.38	0.31	0.82	1.13	2.06
total	9.83	12.45	1.27	4.90	6.30	1.29	2.00	1.98

At the same nitrogen level, the contents of aspartic acid and glutamic acid were nearly unchanged when burley tobacco were cured by the two curing methods; this was also true for flue-cured variety, there was no significant difference between air curing and flue-curing.

Upper leaves

Amino acids	Burley tobacco		b/a	Flue-cured tobacco		b*/a*	a/a*	b/b*
	Air-curing a	Flue-curing b		Air-curing a*	Flue-curing b*			
aspartic acid	3.55	3.47	0.98	0.67	0.70	1.04	5.30	4.96
glutamic acid	1.60	1.57	0.98	1.03	0.93	0.90	1.55	1.69
serine	0.26	0.32	1.23	0.19	0.18	0.95	1.37	1.78
arginine	0.33	0.44	1.33	0.19	0.22	1.16	1.74	2.00
glycine	0.37	0.43	1.16	0.24	0.25	1.04	1.54	1.72
threonine	0.32	0.47	1.47	0.20	0.20	1.00	1.60	2.35
proline	0.96	2.42	2.52	0.92	2.09	2.27	1.04	1.16
alanine	0.36	0.43	1.19	0.24	0.23	0.96	1.50	1.87
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	--	--	2.20
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	2.39	1.22	0.98
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	--	--	1.40
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.92	1.67	2.45
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.96	1.85	2.62
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.27	2.60	2.32
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.83	1.83	2.70
tyrosine	0.43	0.64	1.49	0.38	0.31	0.82	1.13	2.06
total	9.83	12.45	1.27	4.90	6.30	1.29	2.00	1.98

For proline, it was a quite different story, when burley tobacco were cured by different curing methods the contents of proline and methionine in flue-curing group were greatly enhanced compared with air-curing method. The proline content was increased 152% in flue-cured burley tobacco. For flue-cured tobacco variety the content of proline was also increased in flue-curing method, with the increasing rate of 127%.

Upper leaves

Amino acids	Burley tobacco		b/a	Flue-cured tobacco		b*/a*	a/a*	b/b*
	Air-curing a	Flue-curing b		Air-curing a*	Flue-curing b*			
aspartic acid	3.55	3.47	0.98	0.67	0.70	1.04	5.30	4.96
glutamic acid	1.60	1.57	0.98	1.03	0.93	0.90	1.55	1.69
serine	0.26	0.32	1.23	0.19	0.18	0.95	1.37	1.78
arginine	0.33	0.44	1.33	0.19	0.22	1.16	1.74	2.00
glycine	0.37	0.43	1.16	0.24	0.25	1.04	1.54	1.72
threonine	0.32	0.47	1.47	0.20	0.20	1.00	1.60	2.35
proline	0.96	2.42	2.52	0.92	2.09	2.27	1.04	1.16
alanine	0.36	0.43	1.19	0.24	0.23	0.96	1.50	1.87
valine								
methionine								
isoleucine								
leucine								
phenylalanine								
histidine	0.39	0.44	1.13	0.15	0.19	1.27	2.60	2.32
lysine	0.22	0.27	1.23	0.12	0.10	0.83	1.83	2.70
tyrosine	0.43	0.64	1.49	0.38	0.31	0.82	1.13	2.06
total	9.83	12.45	1.27	4.90	6.30	1.29	2.00	1.98

- When the comparison was made between two tobacco types under same curing method , we can see that the aspartic acid and glutamic acid contents in burley tobacco were significantly higher than that in flue-cured tobacco variety, it was true both for air-curing and flue-curing methods, indicating that the genetic background plays an important role in leading to the difference in aspartic acid and glutamic acid content.

Upper leaves

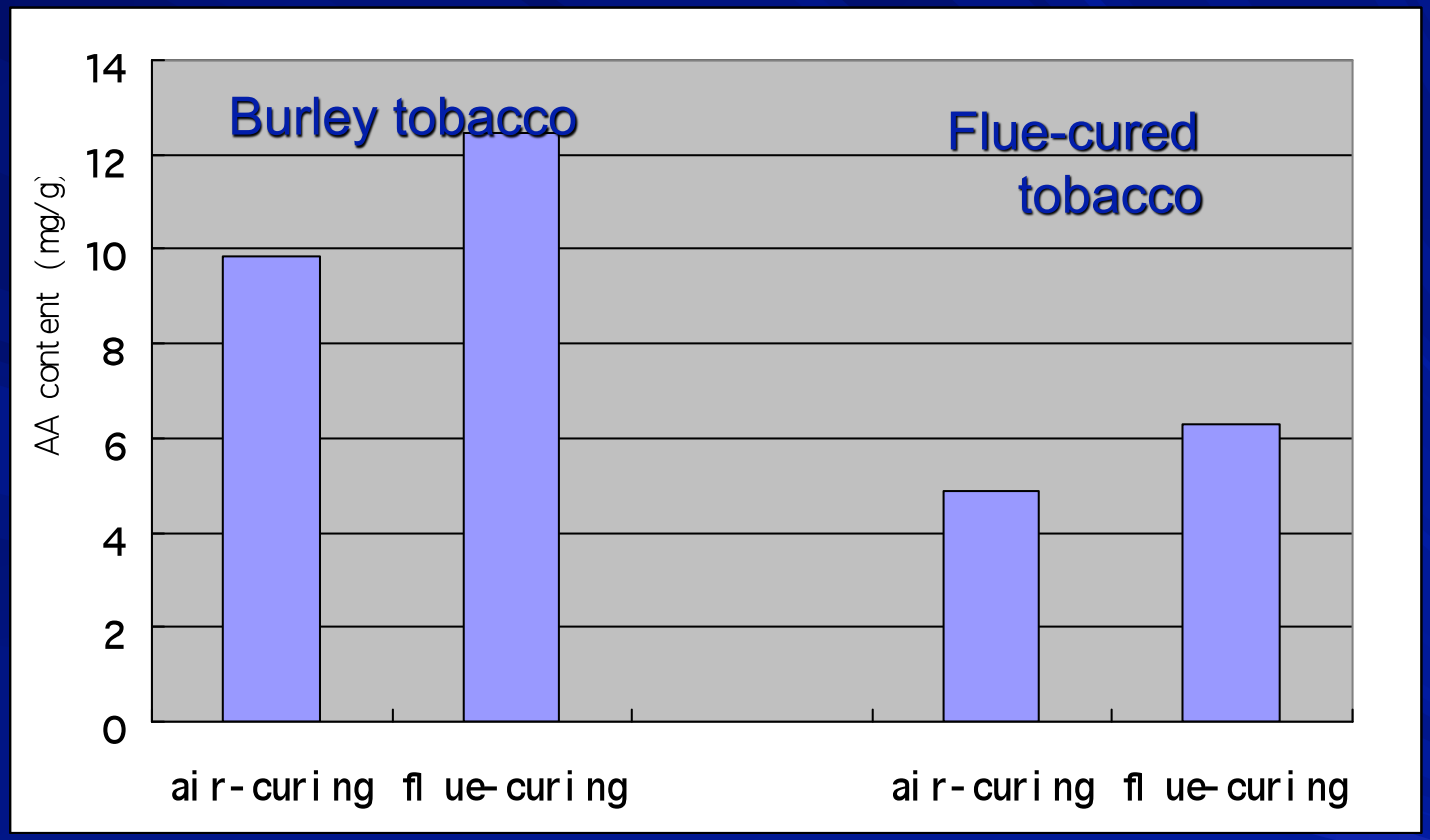
Amino acids	Burley tobacco		b/a	Flue-cured tobacco		b*/a*	a/a*	b/b*
	Air-curing a	Flue-curing b		Air-curing a*	Flue-curing b*			
aspartic acid	3.55	3.47	0.98	0.67	0.70	1.04	5.30	4.96
glutamic acid	1.60	1.57	0.98	1.03	0.93	0.90	1.55	1.69
serine	0.26	0.32	1.23	0.19	0.18	0.95	1.37	1.78
arginine	0.33	0.44	1.33	0.19	0.22	1.16	1.74	2.00
glycine	0.37	0.43	1.16	0.24	0.25	1.04	1.54	1.72
threonine	0.32	0.47	1.47	0.20	0.20	1.00	1.60	2.35
proline	0.96	2.42	2.52	0.92	2.09	2.27	1.04	1.16
alanine	0.36	0.43	1.19	0.24	0.23	0.96	1.50	1.87
valine	0.08	0.11	1.38	0.00	0.05	--	--	2.20
methionine	0.22	0.42	1.91	0.18	0.43	2.39	1.22	0.98
isoleucine	0.04	0.07	1.75	0.00	0.05	--	--	1.40
leucine	0.20	0.27	1.35	0.12	0.11	0.92	1.67	2.45
phenylalanine	0.50	0.68	1.36	0.27	0.26	0.96	1.85	2.62
histidine	0.39	0.44	1.13	0.15	0.19	1.27	2.60	2.32
lysine	0.22	0.27	1.23	0.12	0.10	0.83	1.83	2.70
tyrosine	0.43	0.64	1.49	0.38	0.31	0.82	1.13	2.06
total	9.83	12.45	1.27	4.90	6.30	1.29	2.00	1.98

Upper leaves

Amino acids	Burley tobacco		b/a	Flue-cured tobacco		b*/a*	a/a*	b/b*
	Air-curing a	Flue-curing b		Air-curing a*	Flue-curing b*			
aspartic acid	3.55	3.47	0.98	0.67	0.70	1.04	5.30	4.96
glutamic acid	1.60	1.57	0.98	1.03	0.93	0.90	1.55	1.69
serine	0.26	0.32	1.23	0.19	0.18	0.95	1.37	1.78
arginine	0.33	0.44	1.33	0.19	0.22	1.16	1.74	2.00
glycine	0.37	0.43	1.16	0.24	0.25	1.04	1.54	1.72
threonine	0.32	0.47	1.47	0.20	0.20	1.00	1.60	2.35
proline	0.96	2.42	2.52	0.92	2.09	2.27	1.04	1.16
alanine	0.36	0.43	1.19	0.24	0.23	0.96	1.50	1.87
valine	0.08	0.11	1.38	0.00	0.05	--	--	2.20
methionine	0.22	0.42	1.91	0.18	0.43	2.39	1.22	0.98
isoleucine	0.04	0.07	1.75	0.00	0.05	--	--	1.40
leucine	0.27	0.44	1.63	0.27	0.40	0.92	1.67	2.45
phenylalanine	0.31	0.47	1.52	0.32	0.49	0.96	1.85	2.62
histidine	0.39	0.42	1.13	0.15	0.19	1.27	2.60	2.32
lysine	0.22	0.27	1.23	0.12	0.10	0.83	1.83	2.70
tyrosine	0.43	0.64	1.49	0.38	0.31	0.82	1.13	2.06
total	9.83	12.45	1.27	4.90	6.30	1.29	2.00	1.98

- When comparing the proline content between the two tobacco types under the same curing method, we can see that the difference in proline content was not significant between burley tobacco and flue-curing tobacco, indicating that the proline content was not greatly related to genotypes.

Total amino acid content



Total amino acid contents were generally higher in burley tobacco than in flue-cured tobacco type, while for the same tobacco type, flue-curing was conducive to increasing total amino acid contents.

- Differences in amino acids between air-cured burley tobacco grown at high nitrogen level and that grown at low N level

Air-cured burley tobacco

Amino acids	Upper leaves			Middle leaves		
	Low N	High N	Increment（%）	Low N	High N	Increment（%）
aspartic acid	3.55	4.17	17.5	3.46	3.73	7.8
glutamic acid	1.60	2.29	43.1	1.75	1.85	5.7
serine	0.26	0.26	0	0.24	0.38	58.3
arginine	0.33	0.38	15.6	0.25	0.33	32.0
glycine	0.37	0.38	2.7	0.33	0.39	18.2
threonine	0.32	0.37	15.6	0.29	0.34	17.2
proline	0.96	1.45	51.0	0.59	0.92	55.9
alanine	0.36	0.36	0	0.27	0.42	55.6
valine	0.08	0.09	12.5	0.09	0.11	22.2
methionine	0.22	0.32	45.5	0.25	0.35	40.0
isoleucine	0.04	0.04	0	0.03	0.00	-
leucine	0.20	0.24	20.0	0.16	0.20	25.0
phenylalanine	0.50	0.48	-4.0	0.39	0.54	38.5
histidine	0.39	0.45	15.4	0.35	0.37	5.7
lysine	0.22	0.20	-9.1	0.17	0.27	58.8
tyrosine	0.43	0.41	-4.7	0.39	0.52	33.3
Total	9.83	11.89	20.1	9.01	10.72	19.0

Air-cured flue-cured tobacco

Amino acids	Upper leaves			Middle leaves		
	Low N	High N	Increment (%)	Low N	High N	Increment (%)
aspartic acid	0.67	0.96	43.3	0.47	0.74	57.5
glutamic acid	1.03	1.58	53.4	0.79	1.29	63.3
serine	0.19	0.26	36.8	0.16	0.21	31.3
arginine	0.19	0.25	31.6	0.14	0.22	57.1
glycine	0.24	0.33	37.5	0.18	0.30	66.7
threonine	0.20	0.29	45.0	0.15	0.22	46.7
proline	0.92	1.54	67.4	0.62	1.48	138.7
alanine	0.24	0.36	50.0	0.19	0.31	63.2
valine	0.00	0.00	--	0.00	0.06	--
methionine	0.18	0.21	16.7	0.16	0.18	12.5
isoleucine	0.00	0.00	--	0.00	0.03	--
leucine	0.12	0.14	16.7	0.10	0.14	40.0
phenylalanine	0.27	0.41	51.9	0.23	0.37	60.9
histidine	0.15	0.21	40.0	0.12	0.20	66.7
lysine	0.12	0.20	66.7	0.10	0.18	80.0
tyrosine	0.38	0.37	-2.6	0.28	0.33	17.9
total	4.90	7.11	45.1	3.69	6.26	69.9

amino acids content in the leaves grown under high nitrogen nutrition were higher than that in the leaves grown under low nitrogen nutrition

Summary

- Different kinds of amino acids were affected by different factors. Under the same N fertilization level, contents of proline was mainly affected by the curing methods while not by the tobacco genotype. This may be due to the lost of water, salt stress happened during the flue-cured process and thus reaction induced the accumulation of large quantity of proline which is an important stress-responsive amino acid in plant .
- Tobacco genotype was the primary factor could affect the contents of aspartic acid and glutamic acid at same nitrogen level and aspartic acid was the most significant different amino acid between the burley tobacco and flue-cured tobacco.

Conclusion

- Nitrogen fertilization level also affect amino acid content in the cured leaves. When cured by air-curing method, the contents of most amino acids in cured leaves were higher under high nitrogen condition than that under low nitrogen condition. The amino acids mostly affected by nitrogen level were glutamic acid, proline, alanine and lysine. Also the upper leaves contained more amino acid compared with the middle position leaves.

Conclusion

Answer to the previously raised question:

- The higher content of aspartic acid and glutamic acid in burley tobacco was mainly due to genetic difference and partly due to higher N fertilization.
- The higher content of proline in flue-cured tobacco is almost exclusively due to flue-curing, and if not for the low N was applied, the proline content in flue-cured tobacco would even much higher.

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Thank you!

谢谢各位!