The Influence of Mineral Nutrients on Growth and Alkaloid Levels in *Lycopersicum esculentum*

See-Ryun CHUNG
Yeungnam University

Mineral Nutrient溶液이 Tomato의 成長과 Tomatine Alkaloid 生成量에 미치는 影響

鄭 時 鍊
嶺南大學校

石英 pot에 tomato를 심어 20種類로 design된 mineral nutrient 溶液을 9週間 供給하여, 栽培中의 成長効果를 觀察하였든바 NS3 group에서 最大効果를 나타내었고, 한편 收穫한 各試驗區의 tomatine alkaloid의 含量을 測定한 結果는 KMg 18 group에서 最大含量을 나타내는 것을 알았다.

Introduction

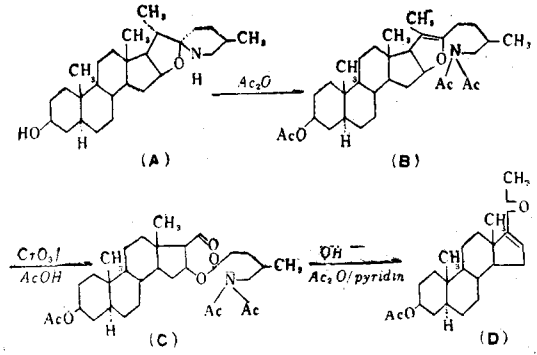
Tomatine, $C_{50}H_{83}O_{21}N$, a steroidal alkaloid having antibiotic, fungicidal, and insecticidal activity, was first isolated by Fontaine and co-workers (1948) from tomato plant and found that by acid hydrolysis it liberates a tetrasaccharide moiety composed of one mole each of D-xylose and D-galactose, two D-glucose units as the β-lycotetraose form, and an aglycone portion tomatidine, $C_{27}H_{45}N$ (Roberta, 1950; Prelog 1953, 1960; Kuhn 1957, Schreiber 1968).

Sato et al (1951) and Kuhn et al (1952) reported, respectively, that tomatidine degraded to a pregnane derivative, 3β-acetoxy-5α-pregn-16-en-20-one(D) and the enantiomeric 5-methyl-2-piperidones (Schrreiber 1968) as side products. On the other hand, Sato (1964) reported microbiological hydroxylation of tomatidne.

Treatment of tomatidine (A) with acetic anhydride yielded an unsaturated N, N, O-triacetylfurost-20-ene derivative (B), and oxidation of B with chromic acid anhydride leading to 16μ-acyloxy-20-oxypregnanes (C) and subsequent hydrolysis and reacetylation resulted in the formation of D (D has nearly the same structure as the human pregnancy hormone, progesterone, secreted by the corpus luteum).

These results led to an attempt to improve the procedures and to increase the yields, especially with regard to the tomatidine and solasodine degradation, since the solanum spirosolane alkaloids appeared to be quite convenient and a most promising source as an intermediate starting material for the industrial synthesis of hormonal steroids (Schreiber 1968).

In this study, the author tried to survey the optimum concentration between many interactions as well as the maximum yield treated with various mineral nutrient solutions and thus effected on growth and aklaloid biosynthesis by means of the method of systematic variations and the binary systems which were established by professor Homès and Homès-Van Schoor of the University of Brussels (1961, Vol I & 1966, Vol II, and 1969).



D=3β-acetoxy-5α-pregn-16-en-20-one,
progesterone=3β-oxy, 5(4)-en.

Materials and Methods

1. Mineral nutrient solutions

Mineral nutrient solutions were prepared as described previously by the author (Chung 1971) and the concentration of total ions, pH, and electric conductivities in each treatment are expressed in Table 1 and 2.

2. Green-house experiments

Experimental plants, *Lycopersicum esculentum*, sown on March 26, 1970 and cultivated for three weeks

Table 1. Concentration of total ions in 1000 meq. (3000 meq. in 10 liter of distilled water) in each treatment, A/C=1.083.

Treatments	NO_3^-	SO_4^{--}	PO_4^{---}	K^+	Ca^{++}	Mg^{++}
NS 1	0	415.9	104.0	200.2	200.2	79.7
NS 2	104.0	311.9	104.0	200.2	200.2	79.7
NS 3	208.0	208.0	104.0	200.2	200.2	79.7
NS 4	311.9	104.0	104.0	200.2	200.2	79.7
NS 5	415.9	0	104.0	200.2	200.2	79.7
NP 6	52.0	104.0	363.9	200.2	200.2	79.7
NP 7	104.0	104.0	311.9	200.2	200.2	79.7
NP 8	208.0	104.0	208.0	200.2	200.2	79.7
NP 9	311.9	104.0	104.0	200.2	200.2	79.7
NP 10	363.9	104.0	52.0	200.2	200.2	79.7
KCa 11	359.8	80.1	80.1	0	384.1	96.0
KCa 12	359.8	80.1	80.1	96.0	288.0	96.0
KCa 13	359.8	80.1	80.1	192.0	192.0	96.0
KCa 14	359.8	80.1	80.1	288.0	96.0	96.0
KCa 15	359.8	80.1	80.1	384.1	0	96.0
KMg 16	359.8	80.1	80.1	0	96.0	384.1
KMg 17	359.8	80.1	80.1	96.0	96.0	288.0
KMg 18	359.8	80.1	80.1	192.0	96.0	192.0
KMg 19	359.8	80.1	80.1	288.0	96.0	96.0
KMg 20	359.8	80.1	80.1	384.1	96.0	0
Control	0	0	0	0	0	0

(mean height 3 cm) in a sand media were planted in plastic pots 180 ml of capacity filled with pure Norwegian quartz with one plant per pot. These plants were grown until they died or until May 28 and harvested while in flowering. There was no control of the length of day and night, but the temperature and humidity were controlled in the green house at approximately between 27°C to 33°C each day, 18°C to 24°C each night and 65 %, respectively.

During the cultivating periods, they were supplied with double deionized water and various mineral nutrient solutions prepared as shown previously. The dose given each plant was 120 meq. of the major solutions and 50 ml of additional minor element solutions (Oligo solution, Chung 1971) was supplied.

Table 2. Details of pH values and electric conductivities of each binary interaction, Unit of e.c.; milli MHO, measured by conductivity meter, type CDM 2d, Copenhagen

Treatments		pH.	e.c.	Treatments		pH.	e.c.
NS	1	5.85	9.00	KCa	11	5.10	11.25
NS	2	5.40	10.00	KCa	12	5.25	11.75
NS	3	5.50	11.25	KCa	13	5.45	12.75
NS	4	5.40	12.00	KCa	14	5.90	13.75
NS	5	5.30	13.25	KCa	15	7.00	14.75
NP	6	9.65	6.25	KMg	16	5.75	11.50
NP	7	7.75	7.25	KMg	17	6.00	12.25
NP	8	6.15	9.50	KMg	18	6.05	13.00
NP	9	5.40	12.00	KMg	19	5.90	13.75
NP	10	5.25	12.75	KMg	20	5.70	14.50

3. Observations and Measurements

For the cultivating periods in the green-house, several growth potentials were measured such as the height of plant, the appearance of flowers and the number of flowers as well as the general phenomena of the plants; for example, changing color, death of the plant in a specific treatment, dryness of leaves in a certain nutrient condition, the falling of leaves, etc.

At the end of cultivation, when harvested, the plants were defoliated into six segments: that is, old leaves, young leaves, growing leaves, flower parts, stem, and root system and the fresh and dry weight of each of the six parts was measured.

4. Chemical experiments

Plant material: *Lycopersicum esculentum*, harvested and divided into six segments, were dried immediately at 70°C in a drying chamber and then at 105°C until a constant weight was obtained (B.P.C. 1968) Thus the resulting materials were used for chemical analysis.

Isolation and assay of alkaloid: The total alkaloid was determined by a direct method in a microisolation procedure, 20 g. of moderately coarse powdered material was extracted with 150 ml of 80 % methanol for 48 hours and condensed in a vacuum distillator (Glassapparatefabrik Flawil Type YRYr 65/45, Switzerland) at 60°C. The solvent free extract was boiled with 70 ml of distilled water, and this clarified solution was precipitated with a sufficient volume of 10 % ammonia water. The crude alkaloid thus ontained was washed, dried, weighed, and reprecipitated from 6 % acetic acid solution in a dilute methanol solvent and then recrystallized from dilute methyl alcohol. In each of these procedures, the test for complete extraction of alkaloids was undertaken.

Identification: Attempts to identify exactly the alkaloid from the extracted solutions or precipitated materials using various alkaloid precipitate or color reagent were used from time to time (Chung 1971).

Quantitative estimation: Determination of the alkaloid levels were carried out by the method described by Gracza (1962).

Results and Discussion

1. Growth

The growth in all interactions when plotted formed rather uniform curves. In each case one treatment showed the highest difference. There was not any marked differences in growth rate in contrasy with various solutions of mineral nutrients until the fifth week, but after this period large differences appeared (Fig. 1-1, 1-2, 1-3, 1-4).

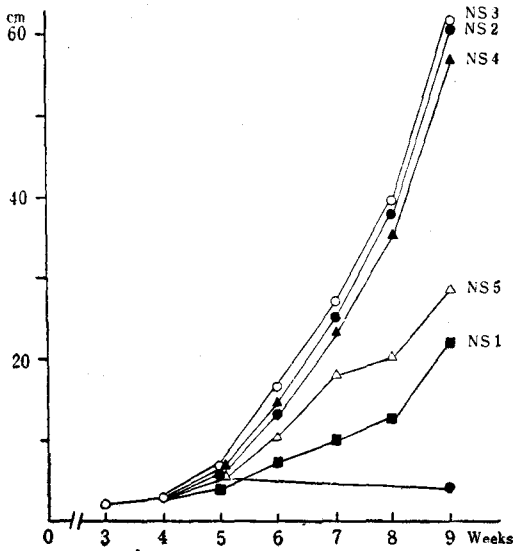


Fig. 1-1. A comparison of the growth rate by height per plant in NO_3^- and SO_4^{--} interactions.

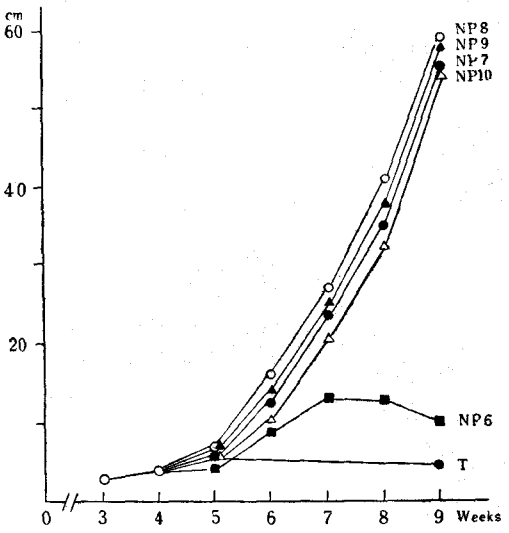


Fig. 1-2. A comparison of the growth rate by height per plant in NO_3^- and PO_4^{---} interactions.

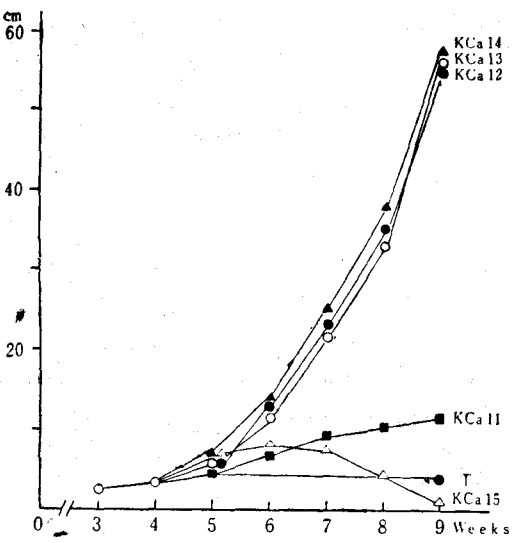


Fig. 1-3. A comparison of the growth rate by height per plant in K^+ and Ca^{++} interactions.

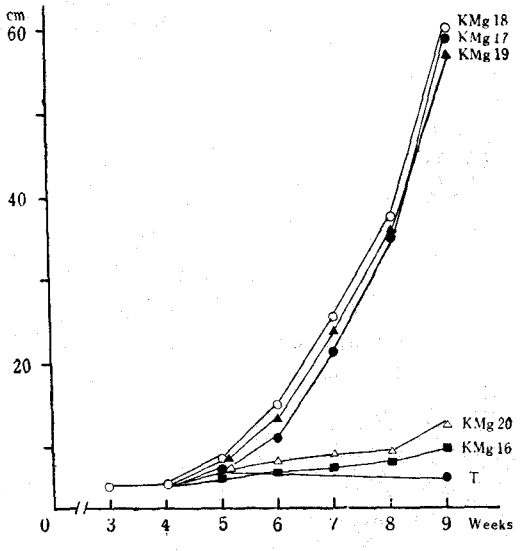


Fig. 1-4. A comparison of the growth rate by height per plant in K^+ and Mg^{++} interactions.

The appearence of the flower and the mean number of the flowers were observed from the first appearence date to harvest at three day intervals. These results are summarized in Table 3. There are, however, no flowers in treatments NS 1, NP 6, KCa 11, KCa 15, KMg 16, KMg 20 and Control group.

Table 3. The number of days from sowing to flowering, the percentage of flowering plants, and the mean mumber of flowers per flowering plant in green house experiments DF: Dates of flowering, PF: percentage of flowering plant, HF: mean number of flowers, T: control group, Mean data from 10 repetitions

Treatments	DF 51		DF 54		DF 57		DF 60		DF 63	
	PF	HF	PF	HF	PF	HF	PF	HF	PF	HF
NS 1	—	—	—	—	—	—	—	—	—	—
NS 2	—	—	10	1.0	80	2.0	100	5.5	100	10.4
NS 3	—	—	60	2.0	70	3.1	100	6.4	100	10.6
NS 4	—	—	30	2.0	70	2.3	100	6.4	100	10.4
NS 5	—	—	—	—	10	2.0	30	2.3	30	2.3
NP 6	—	—	—	—	—	—	—	—	—	—
NP 7	—	—	—	—	60	1.8	80	5.0	80	7.3
NP 8	10	1.0	40	2.8	90	3.2	100	6.5	100	10.2
NP 9	—	—	30	2.0	70	2.3	100	6.4	100	10.4
NP 10	—	—	30	2.0	40	1.0	90	4.4	90	5.8
KCa 11	—	—	—	—	—	—	—	—	—	—
KCa 12	—	—	10	2.0	70	2.4	90	5.5	90	9.8
KCa 13	—	—	—	—	40	1.8	100	5.8	100	10.4
KCa 14	—	—	40	1.5	70	2.3	90	5.1	90	7.2
KCa 15	—	—	—	—	—	—	—	—	—	—
KMg 16	—	—	—	—	—	—	—	—	—	—
KMg 17	—	—	—	—	30	1.3	100	3.7	100	5.4
KMg 18	—	—	50	1.4	90	2.0	100	6.0	100	10.8
KMg 19	—	—	40	1.5	70	2.3	90	5.1	90	7.2
KMg 20	—	—	—	—	—	—	—	—	—	—
T	—	—	—	—	—	—	—	—	—	—

From the fifth week, there was also an interesting sign of nutrient deficiency or unbalanced excess of nutrients in several treatments; for example, NS 1 group plants were very slow in their growth and the leaves either changed to a pale yellow or fell, NS 2 groups were generally in a good condition but some young leaves dried on the edge, NP 6 groups were very similar to those of the NS 1 groups but the sprouts dried out entirely and 40 % of the plants died during the cultivating, KCa 11 group plants grew vrey slowly and the leaves curled backward and fell while still green. Frenching symptom was appeared from fifth week in KCa 15 treatment and 100 % of this group plant died from the seventh to ninth week. Frenching symptom appeared in this study was very similar to that of reported by Karracker et al. (1934) on tomatoes and petunias (for about Frenching see pages 26-27 of Chung 1971). KMg 16 group plants behaved very similarly to the KCa 11 group, KMg 20 groups grew only their stems and 20 % of them had died. But in the control group (Treatment T) which supplied only double deionized water, the devolution phenomena appeared from the fifth week, nevertheless, no plants died.

The gradient of growth potential of the plants segments expressed as weight after nine weeks in culture can be noted in the root system as well as in aereal parts: old leaves, young leaves, growing leaves, flower parts, and the stem. The fresh and dry weight is summarized in Table 4.

2. Alkaloid

Many research workers, hitherto, have cited yields of the alkaloid product from the dried plant material, which varied from 0.5 % to 5.0 %, and they have cited that the highest yield was obtained from

Table 4. Total fresh and dry weight of the aerial parts and the root system
I. H. =Index of hydration

Treatments	Total aerial parts			Root system		
	Fw	Dw	I. H.	Fw	Dw	I. H.
NS 1	6.00	0.71	88.17	5.46	0.52	90.48
NS 2	79.77	9.79	87.73	28.48	3.39	88.09
NS 3	109.26	12.36	88.69	40.18	4.61	88.53
NS 4	112.28	11.99	89.32	37.98	4.31	88.65
NS 5	34.44	4.28	87.54	8.90	0.99	88.88
NP 6	6.40	0.69	89.22	1.25	0.13	89.60
NP 7	64.00	6.73	89.48	17.72	1.81	89.79
NP 8	108.88	12.10	88.88	39.66	4.62	88.35
NP 9	112.28	11.99	89.32	37.98	4.31	88.65
NP 10	93.66	9.54	89.81	33.10	3.74	88.70
KCa 11	1.30	0.15	88.46	1.02	0.09	91.18
KCa 12	106.52	11.13	89.55	33.50	3.90	88.36
KCa 13	105.92	10.27	90.30	32.96	3.27	90.08
KCa 14	106.30	10.13	90.47	37.34	3.66	90.20
KCa 15	—	—	—	—	—	—
KMg 16	1.10	03.12	89.09	0.96	0.10	89.58
KMg 17	103.68	10.65	89.73	29.76	3.14	89.45
KMg 18	117.82	12.32	89.54	40.30	4.36	89.18
KMg 19	106.30	10.13	90.47	37.34	3.66	90.20
KMg 20	6.10	0.75	87.70	2.46	0.20	91.87
T	0.34	0.03	91.18	0.44	0.05	88.64

Table 5. Total alkaloid in per cent for dried leaves
OL: old leaves, YL: young leaves
GL: growing leaves, m: mean values

Treatments	OL	YL	GL	m
NS 1	—	—	—	—
NS 2	0.54	0.69	1.81	1.01
NS 3	0.65	0.68	4.12	1.82
NS 4	0.69	0.76	0.71	0.72
NS 5	—	—	—	—
NP 6	—	—	—	—
NP 7	0.55	0.73	0.75	0.68
NP 8	0.89	0.76	3.27	1.64
NP 9	0.69	0.76	0.71	0.72
NP 10	0.57	0.40	0.22	0.40
KCa 11	—	—	—	—
KCa 12	0.84	0.36	0.66	0.62
KCa 13	0.78	0.37	1.00	0.72
KCa 14	0.73	0.55	3.20	1.49
KCa 15	—	—	—	—
KMg 17	1.74	0.70	3.72	2.05
KMg 18	1.23	0.67	4.32	2.07
KMg 19	0.73	0.55	3.20	1.49
KMg 20	—	—	—	—
T	—	—	—	—

Lycopersicum esculentum. In these plants which contained tomatine, the amounts of alkaloid present decreased significantly during September and October (Brink 1951, Kuhn 1952, and Prelog 1953).

This seasonal effect on growth and alkaloid synthesis in *Lycopersicum esculentum* are further studied and will report hereafter.

In this study, the author found that the amount of the total alkaloids for dried leaves varied from 0 to 2.07 %.

A comparinson of the total alkaloid synthesizing activity of each treatment between old leaves, young leaves, and growing leaves are shown in Table 5. There seemed to be a slight increasing tendency toward growing parts of dhe plant. Although, the level of synthesizing activity varied somewhat from treatment to treatment. Clearly, the plants in the interactions NS 3 and KMg 18 were most active in both growth and alkaloid synthesis.

It is very remarkable that nutrients that are rich in nitrogen (60 to 69.2 %) usually produce marked increase of weight when applied to *Lycopersicum esculentum* (Table 4). Nevertheless, the effect of nitrogenous nutrients on the alkaloid synthesizing activity is still ambiguous. But, certainly, in a good cultural condition, or well balanced nutrient conditions promoted better yield of the alkaloids. Fig. 2 shows alkaloid amounts in averages in four binary interactions.

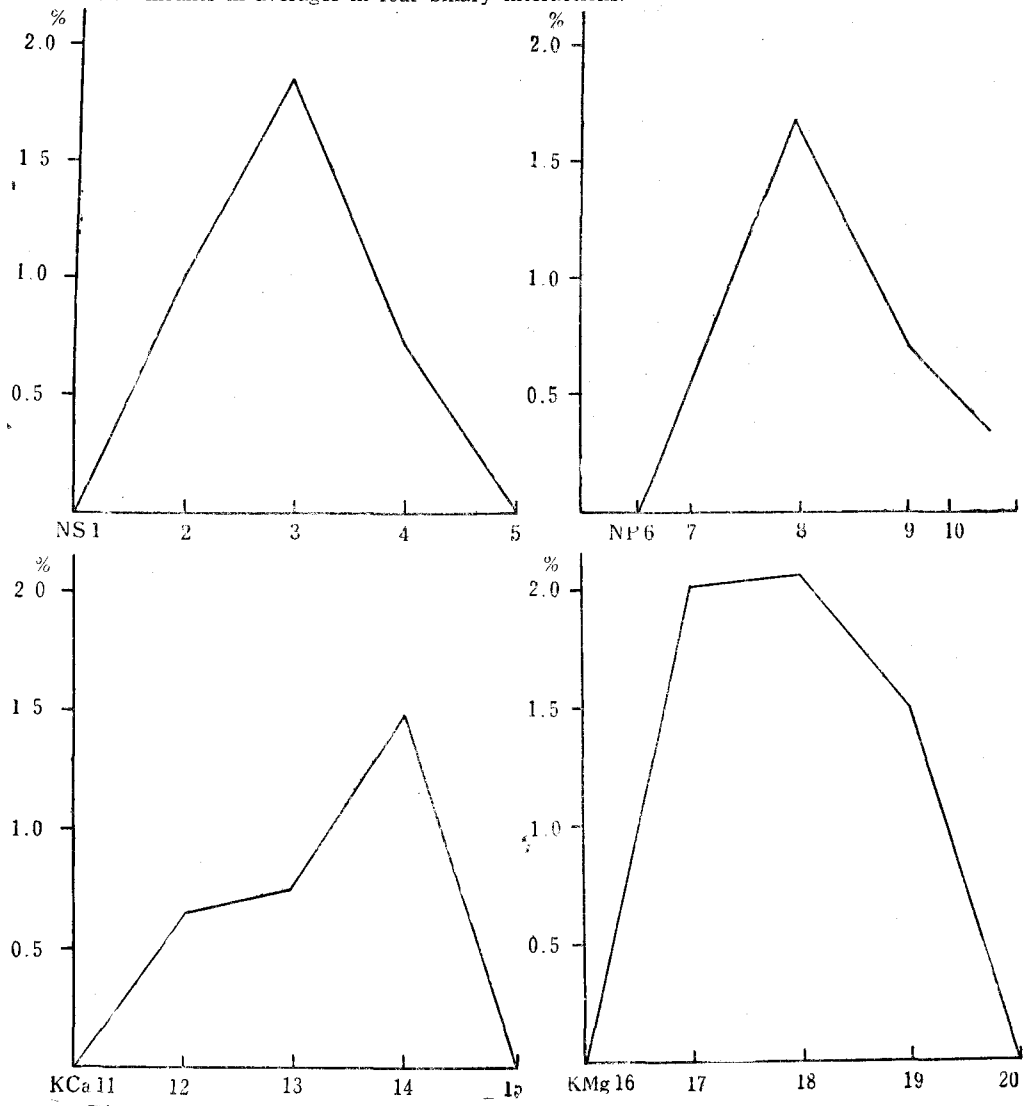


Fig. 2. Comparison fo the alkaloid levels in four different binary interactions.

Summary

Twenty kinds of minerel nutrient solution were prepared and supplied to the tomatoes planted in Norwegian quartz pots.

These plants were cultured for nine weeks and several physiogical phenomena were observed duirng the growing period, and after harvest, the alkaloid contents were determined.

The highest growth potential was in NS 3 group while the highest alkaloid content wasein KMg 18 group.

References

Brink, N.G. et al. 1951. Isolation of tomatidine from the roots of the Rutgers tomato plant. J. Am. Chem. Soc. **73**, 4018.

British Pharmaceutical Codex. 1968.

Chung, S.R. 1943. The influence of various mineral nutrient solutions on growth and alkaloid Synthesis in Solanaceae. 1971. Ph.D. theses, University of Brussels, 1-163.

Fontaion, T.D. et al. 1948. Arch. Biochem. **18**, 467.

Gracza, L. und Szasz, K. 1962. Bestimmung des Steriodalkaloid-Gehaltes einiger Solanaceen-Drogen mit Hilfe einer neuen, Kolorimetrischen Methode. Archiv. der Pharmazie. **299**, 859-860.

Homès, M.V. 1961. "Systematic" methods in the determination of nutrient requirements of plants. Ann Phys. Vgt., Univ. of Brussels, **6**, 99-136.

Homès, M.V. L'alimentation minrale equilibre des vgtaux. Universa, Belgique. vol Ⅰ, 1-298, 1961. *et vol* Ⅱ, 1-425, 1966.

Homès, M.V. 1969. et Van Schoor, G. La nutrition minrale des vgtaux Masson & cie, Paris, 1-162.

Karracker, P.E. et al. 1934. Eull Ky. Agric. Exp. Stn. **349**, 63-109.

Kuhn, R. et al. 1952. Abbau von Tomatidin zum Tigogeninlaction. Chemische Perichte, **85**, 416-424.

Kuhn R. et al. 1957. Die Konstitution der Lycotetraose. Chemische Berichte, **90**, 203-218.

Prelog, V. et al. 1953. The chemistry of Solanum and Veratrum Alkaloids. The Alkaloids, Ⅲ. Manske, 247-312.

Prelog, V. et al. 1960. Steroid Alkaloids, The Alkaloids, Ⅶ. Manske, 343-361.

Roberta, M. et al. 1950. Arch Biochem. **27**, 461.

Sato, Y. et al. 1951. Tomatidine, a steroid secondary amine. J. Am. Chem. Soc. **73**, 878-880.

Sato, Y. et al. 1964. The microbial hydroxylation of Tomatidine. J. Grg. Chem. **29**, 198-201.

Schreiber, K. 1968. Steroid Aakillds, The Alkaloids, X. Manske, 2-192.