

Chemical Constituents of Flue-cured Tobacco (*N. tabacum*) as Affected by Application of Hormones

by

R. V. MISRA, HARI SHANKER and A. N. PATHAK*

Although the effect of hormones on elongation of plants is well recognized (Brian *et al*, 1954, Gray, 1957; Marth *et al* 1956; Yabuta *et al* 1941) little information is available concerning its effect on the chemical composition of plants (Stove and Yamaki, 1957). It has been shown by many investigators (Schmuck, 1924; Kovalenko, 1935; Sastry, 1953) that within certain limits quality in the flue-cured type is directly proportional to sugars and inversely to nitrogenous constituents. Taking sugar and nitrogen figures, it appears that the Indian leaves generally contain less total sugars and more nitrogen than what is desirable.

Stuart (1938) and Mitchell and Stuart (1938—'39) while using IAA found an accumulation of carbohydrates in bean cuttings. Similarly Wost (1949), Greulach and Singh (1949), and Mc-Illarth (1950) using 2, 4-D, NAA and Maleic-hydrazide respectively noted an increase in the carbohydrate content of hormone treated plants. Dastur and Bhatt (1956) found a greater accumulation of cane-sugar and starch in the leaves of the hormone treated cotton plant. They did not find any effect of hormone on the nitrogen content of stems and leaves, but the reproductive parts showed consistently higher nitrogen content than control. Yabuta *et al* (1943) and Parups (1959) reported a decreased nicotine content in hormone treated tobacco plants.

Keeping the above facts in view, it was considered desirable to investigate fully the effect of some hormones on the chemical constituents and subsequently on the quality of tobacco.

Material and Methods: Tobacco plants (*N. tabacum* var. *Harrison special*) were grown under green house conditions in pots containing 20 kg. soil. Each pot contained single plant. The soil was taken from Students' Instructional Farm, Government Agricultural College, Kanpur. Pots were fertilized at 15 lb. N, 50 lb. P₂O₅ and 40 lb. potash per acre, supplied as Calcium-ammonium nitrate, Super phosphate and Potassium sulphate respectively. The nurseries were sown on 14th September, 1966 and the harvesting was done on 1st February, 1967. Standard planting, cultivation, harvesting and curing practices were followed. Topping was not done. The experiment included the following treatments:

* Dept. of Agrl. Chemistry and Soil Science, Govt. Agricultural College, Kanpur.

Received on 20-6-1967.

Hormones used	Rates of application (mg. per plant)	Time of application (days after planting)
1. Gibberellic Acid (GA)	... Control, 0.5, 1.0, 1.5, 2.0	25, 40, 50
2. Napthalene acetic Acid (NAA)	... -do-	-do-
3. Indole - Acetic Acid (IAA)	... -do-	-do-
4. Indole - Butyric Acid (IBA)	... -do-	-do-

The plants were sprayed with aqueous solutions of hormones. Control plants were sprayed with distilled water. The treatments were replicated four times and pots randomized within each replicate. The pots were rotated periodically, to reduce the effects of localized environmental conditions in the green house.

Composite samples of the leaves were prepared from all the four replications. The leaves were analysed for Starch (Colorimetric method, using iodine), Sucrose and Reducing sugars (Iodimetry) by the standard methods as described by Ward and Johnston (1962). Total nitrogen was determined by the Kjeldahl method for tobacco samples containing nitrates as described by A. O. A. C. (1960). Samples were analysed for nicotine by the method of Cundiff and Markunas (1955).

Results and Discussion: The results of the experiment are being presented in the graphical form.

I. CARBOHYDRATES: Analysis of cured tobacco leaves, sprayed with four chemical hormones *viz.*, GA, NAA, IBA and IAA for carbohydrates revealed that these hormones produced some effect on the carbohydrate content. It can be observed that application of hormones has resulted in increased carbohydrate content of the leaves. The tobacco leaves have been analysed for following carbohydrates:

- (a) Starch
- (b) Reducing sugars
- (c) Sucrose

(a) *Starch:* Hormone application has resulted in increased starch content of leaves. This may be probably due to the fact that the leaves of hormone treated plants had a higher rate of photosynthesis per unit

area than the control plants. The results are in agreement with the findings of Dastur and Bhatt (1956). Keeping in view their effectiveness in bringing about an increase in starch content, the various hormones can be placed in the following order :



Increasing rates of application have further contributed to the increase in starch content of leaves. A general increase is noted in the starch content of leaves with increasing rates of application. As regards the time of application, medium and late applications were most effective in increasing the starch content of tobacco leaves. Maximum increase of 54.3 per cent for starch was observed with the application of 1.5 mg. GA per plant 50 days after planting. Further increase in the rate of GA application *viz.*, 2.0 mg. per plant, at the same date brought negligible decrease in the starch content of leaves.

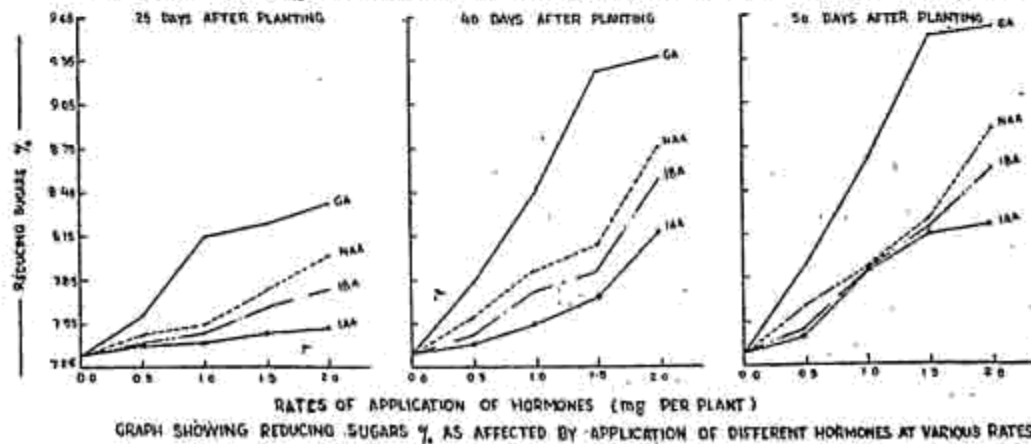
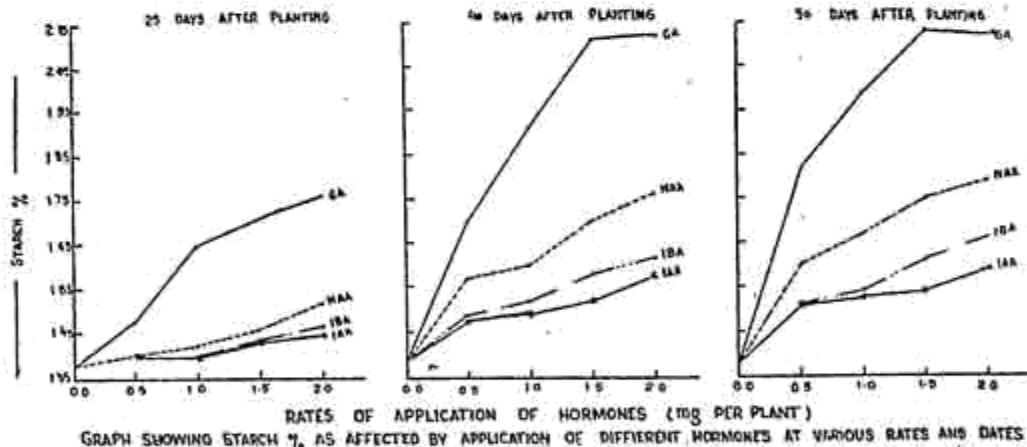
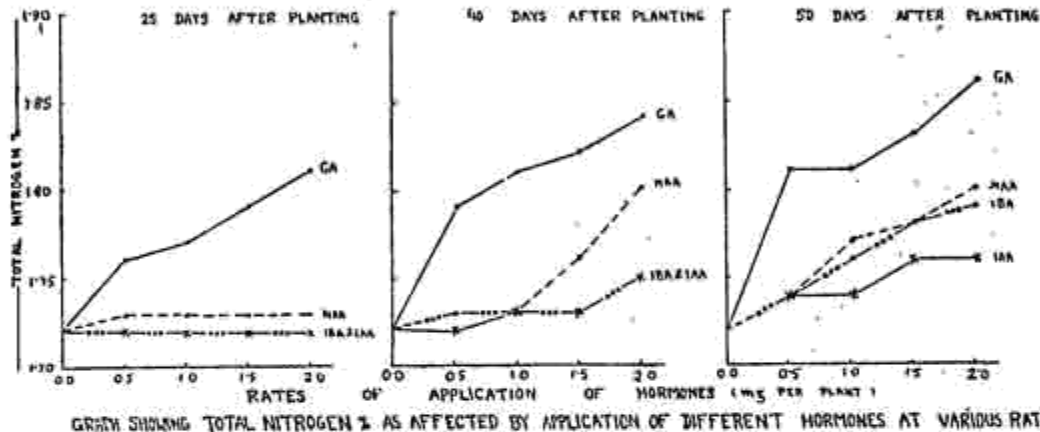
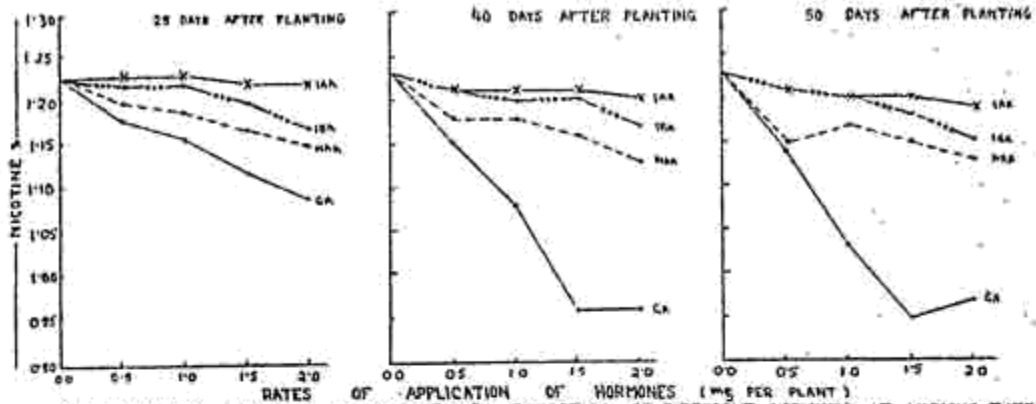
(b) *Reducing sugars* : The effect of application of various hormones has a pronounced effect upon the sugar content of tobacco leaves. Reducing sugar content of tobacco leaves increases with the application of hormones. Of the hormones used, GA exercises the most significant effect in increasing the reducing sugar content of leaves. Other hormones are less effective in increasing the reducing sugar level of the leaves. The order of the hormones as regards their effectiveness in increasing the reducing content is as follows :



Further increase is noted in the reducing sugar level of the leaves with increased rates of application. Late and medium time of applications have proved most effective in increasing the reducing sugar content of the leaves. Maximum increase of 30.3 per cent for reducing sugars was observed with the application of 2.0 mg. GA per plant, 50 days after planting.

(c) *Sucrose* : As is evident from the graph, the application of hormones has helped tobacco leaves to accumulate more sucrose. The highest level of sucrose has been found in tobacco leaves treated with GA. Other hormones, *viz.*, NAA, IBA and IAA were less effective in increasing the sucrose content than GA. The hormones in order of their effectiveness are as under :





Significant increase in the sucrose content of the leaves has been noted with higher rates of hormone application. Medium and late application of hormones are more effective in bringing about an increase in sucrose content than the early application. Medium and late application are more or less similar in their effect. The application of 2.0 mg. GA per plant, 50 days after planting has given the maximum increase of 27.3 per cent for sucrose. The results are supported by the findings of Dastur and Bhatt (1956).

II. NICOTINE: The graph reveals that nicotine content decreases with the application of hormones. The most effective hormone in reducing the nicotine content is GA. The other hormones are less effective and their order of effectiveness is $NAA > IBA > IAA$. The effect of rate and time of application is also visible. The effectiveness of hormones increases with the increase in rate and late date of application. 1.5 mg. GA per plant, applied 50 days after planting has been most effective in reducing the nicotine content, bringing a reduction of 22.7 per cent. Higher rate of GA application *viz.*, 2.0 mg. per plant, at the same date has resulted in 21.1 per cent reduction, which is slightly less than the reduction obtained by application of 1.5 mg. GA per plant. The results are in corroboration with the findings of Yabuta *et al* (1943) and Parups (1959).

III. TOTAL NITROGEN: Like the carbohydrates (Starch, Reducing Sugars and Sucrose) total nitrogen content of tobacco leaves also increases with hormone application. But the magnitude of increase is considerably less in their comparison. A maximum increase of 7.5 per cent is noted due to application of 2.0 mg. GA, 50 days after planting. The order of effectiveness of hormones is same as for carbohydrates *i. e.* $GA > NAA > IBA > IAA$. The rates and dates of hormone application affect the total nitrogen content of the leaves and increase occurs with higher rates and late date of application.

Since the carbohydrates (Starch, Reducing Sugar and Sucrose) and nitrogen content of tobacco are influenced by hormone application and, that the quality of flue-cured type is directly proportional to sugars and inversely to nitrogenous constituents, it is evident that the application of hormones also affects the quality of tobacco. It is observed from the experiment that an increase occurs in carbohydrate and nitrogen content of hormone treated plants but the magnitude of increase in nitrogen content is considerably less than the carbohydrates. This suggests that application of hormones improves quality in flue-cured types.

Indian flue-cured tobacco mostly has its nicotine within range of 1 to 1.5 per cent (Anonymous 1956). According to Garner (1951), while a distinctly high nicotine content (about 3 per cent) is usually objectionable,

it is also true that a very low content of nicotine in a tobacco produce—perhaps less than 1.5 per cent—will be unsatisfactory, despite the popular belief to the contrary. A number of measures for increasing the nicotine content of flue-cured type have been discussed by Gopalachari and Gopinath (1965). It is observed from the experiment that the nicotine content decreases with the application of hormones. Thus, it can be concluded that in case the nicotine content of Indian flue-cured type is controlled effectively, the hormone treatment (particularly GA) of tobacco can be used as an effective treatment for improving its quality.

Summary: Effect of some hormones *viz.*, Gibberellic Acid, Naphthalene Acetic Acid, Indole-Butyric Acid and Indole Acetic Acid, on the chemical constituents of tobacco was studied. It was observed that application of hormones increased the starch, reducing sugars and sucrose content of the cured leaves. Also, there was an increase in the nitrogen content of leaves though to a considerably lesser degree than that of carbohydrates. A decrease was noted in the nicotine content with the application of hormones. Gibberellic Acid was the hormone which brought about the greatest changes in the contents of chemical constituents. The most effective rate and time of application was 2.0 mg. per plant after 50 days of planting.

REFERENCES

- | | |
|--|---|
| Anon | 1956 <i>Ann. Rep.</i> 1955—1956. Central Tobacco Research Institute, Rajahmundry. |
| A. O. A. C. | 1960 <i>Official methods of Analysis</i> , 9th Ed. |
| Blick, R. T. J. | 1953 Physical and chemical characteristics of typical American and Nelson tobacco leaf samples. <i>New Zealand J. Sci. & Tech.</i> , 25 : 53-62. |
| Brian, P. W., G. W. Elson,
H. G. Hemming and
Margaret Radley | 1954 The plant growth promoting properties of Gibberellic Acid, a metabolic product of the fungus. <i>Gibberella fujikuroi</i> . <i>J. Sci. Food Agr.</i> , 5 : 602-12. |
| Cundiff, R. H. and
P. C. Markunas | 1965 Determination of nicotine, nornicotine and total alkaloids in tobacco. <i>Anal. Chem.</i> , 27 : 1650-3. |
| Darkis, F. R., L. F. Dixon,
F. A. Wolf and P. M. Gross | 1936 Correlations between chemical composition and stalk position of tobacco produced under varying weather conditions. <i>Ind. Eng. Chem.</i> , 28 : 1214. |
| Dastur, R. H. and Jayendra Bhatt | 1956 Effect of chemical hormones on the carbohydrate and nitrogen contents of cotton plant. <i>Indian J. Agr. Sci.</i> 26 : 39-79. |
| Garner, W. W. | 1951 <i>The production of tobacco</i> . Blackiston Co., Philadelphia, 715-8. |
| Gopalachari, N. C. and
D. M. Gopinath | 1965 Review of the factors influencing nicotine in tobacco. 15 : 145-58. |

- Gray, R. A. 1957 Alternation of leaf size and shape and other changes caused by Gibberellins in plants. *Amer. J. Bot.*, **44** : 674-82.
- Greulach, V. A. and Sucha Singh 1949 Effects of Naphthalene Acetic Acid and Naphthalene Acetamide on the development of cotton plant. *Amer. J. Bot.*, **36** : 646-51.
- Kovalenko, E. I. 1935 State Inst. Tobacco and Makhorka. *Ind. Krasnodar (U. S. S. R.) Bull*; **125** : 147.
- Marth, P. C., W. V. Audia and J. W. Mitchell. 1956 Effects of Gibberellic Acid on growth and development of plants of various genera and species. *Bot. Gaz.*, **118** : 106-11.
- Mc-Illarth, W. G. 1950 Response of the cotton plant to Maleic hydrazida. *Amer. J. Bot.*, **37** : 816-9.
- Mitchell, J. W. and Stuart. N. W. 1938-39 Growth in metabolism of Bean cuttings subsequent to Rooting with Indole Acetic Acid. *Bot. Gaz.*, **100** : 627-50.
- Parups, E. V. 1959 Influence of Gibberellic acid on the nicotine content of Cigar tobacco, *Canad. J. Plant Sci.*, **38** : 48-55.
- Sastry, A. S. 1951 The starch content of the green leaf as an index of quality in flue-cured tobacco. *Indian Tobacco.*, **1** : 249.
- 1924 Chemical changes during flue curing and their bearing on the quality of leaf of cigarette tobacco. *Proc. Indian Acad. Sci.*, **38** (B) : 1-25.
- Schmuck, A. 1924 State Inst, Tobacco Investigations. *Krasnodar (U. S. S. R.) Bull.* **24**.
- Stove, B. B. and Toshio Yamaki 1957 The history and physiological action of the Gibberellins. *Ann. Rev. Plant Physiol.*, **8** : 181-216.
- Stuart, M. W. 1938 Nitrogen and carbohydrate metabolism of kidney Bean cuttings as affected by treatment with Indole Acetic Acid. *Bot. Gaz.*, **100** : 198-212.
- Ward, G. M. 1942 Physiological studies with the tobacco plant. *Can. Dept. Agric. Tech. Bull.*, **37** : 41.
- Ward, G. M. and F. B. Johnston 1962 *Chemical methods of plant analysis. Research Branch. Canada Dept. Agric.*
- Wost, D. J. 1949 Response of the Buck wheat to treatment with 2, 4-D. *Amer. J. Bot.*, **36** : 673-5.
- Yabuta, T., Y. Sumiki, K. Aso and Hayashi 1941 Biochemical studies of bakanae fungus. XIII. The effects of Gibberellin on special components and special tissues of plants. 4-Action of Gibberellin on tobacco seedlings. (Section 1). *J. Agr. Chem. Soc. Japan.*, **17** : 1001-4.
- Yabuta, T., Y. Sumiki and T. Takahashi 1943 Biochemical studies on bakanaefungus. XVI. The effects of Gibberellin on special components and special tissues of plants. 5. Action of Gibberellin on tobacco seedlings (Section 2). *J. Agr. Chem. Soc. Japan.*, **19** : 396.