MATERIALS AND METHODS
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The experimental plot is a portion of the unirrigated hill slope, of Farm No.1 of the Department of Agricultural Botany, Gauhati University, Assam.

The soil of the farm is laterite and brown, with pH ranging between 6 to 7.3

Preparation of the soil: Before the suckers are planted the land was well prepared. The hard crust of the soil in the field was subjected to two ploughings. The weeds and other organic matter were turned under soil following thorough harrowing and hoeing of the land.

Plant Materials used: The suckers of the "Queen" variety were used in the experiment which were obtained from Kabikuchi Govt. agricultural Research Station Assam.

Plantation: Double row system of plantation was adopted. There were 350 plants in the field. Suckers were planted during rainy season i.e. in the month of June 1969. The suckers used were more or less uniform in size and of same age, planted in the pits already kept ready for use. Half basket field rotted cowdung manure was added in each pit. Before planting few lower leaves were removed from the suckers for rooting easily.
Irrigation: Just after planting foliage of the suckers were watered at evening. In the early stage of the plant irrigation was done every evening for 15 days. After this period watering was done once in a week for two months and then interval between two irrigation was extended for 15 days till the commencement of rain.

Interculture: Weeding was done from time to time with Khurpi and soil near by plant was kept loose for aeration. Twice in experimental period spading was done.

During this operation much care was taken in order to save the plant from injury and for this no spading was done when plants had luxurious growth.

Preparation of Auxin Solution: 50 mg. i.e. 0.05 gms crystals of auxin was first weighed on a clean watch glass, whose weight was already noted and then transferred to 100 ml volumetric flask for boiling in a few c.c. of water. Calculated amount of N/10 KOH was added drop by drop till the last crystal of the chemical was dissolved imparting a clear solution. The stock solution was kept in refrigerator for overnight. In the next day pH of this aqueous solution was brought to
neutral point (pH 7) by addition of N/10 HCl solution after which the volume of auxin solution was brought to the mark. On the day of treatment the desired concentration for use was made by dilution. (The concentration of the stock solution was 500 ppm).

In the case of calcium carbide treatment the solid calcium carbide was placed directly upon the growing stem apices of the plants. This added carbide by reaching with moisture or rain water liberated Acetylene gas which in turn promoted early flowering.

The spraying of the aqueous auxin solution was made with a pipette in 20 ml per plant into the heart of the plant (stem apex and leaf-bases).

**Field Records**: Flowering percentage following auxin treatment, the date of flowering, the time interval from the date of auxin treatment to flower formation, all these were recorded treatment-wise.

**In Laboratory**: Chemical Analysis and the method employed:

The induced metabolic changes in the chemical composition of Pineapple leaves after the application of auxin were estimated by using suitable chemical methods.
(1) Estimation of sugars:

Different sugars were extracted by crushing 10 gms of fresh pineapple leaves in a mortar and boiled for few minutes with distilled water. The extract was then filtered through cotton and made upto a volume of 250 ml. 50 ml of this sugar solution was purified by adding equal volume of 5\% Ba (OH)\(_2\) and 5\% Zn SO\(_4\) solution and filtered. The filtrate was made upto a volume of 250 cc. This purified sugar solution was then used in the estimation of total and reducing sugars.

Estimation :- Sugar estimation was made by the iodometric Technique of Somogyi (1945).

Somogyi's Reagent was prepared as follows:

28 gms of anhydrous Na\(_2\)HPO\(_4\) and 40 gms of Rochelle salt (Sodium Potassium tartarate) was dissolved in 700 ml. of distilled water to which 100 ml of N.NaOH was added. 80 ml of 10\% Copper sulphate solution was added while stirring. Finally 180 gms of Anhydrous sodium sulphate was added and when dissolved the solution was diluted to 1 litre and allowed to stand for a day or two during which the impurities separated out. The clear top portion of the solution was decanted and the remainder was filtered through a filter paper. 20 ml of N.KIO\(_3\), was added per litre of this reagent.
Analytical procedure:

(i) Reducing sugar: 5 ml. of purified sugar extract was heated with 5 ml of Somogyi's reagent in a pyrex test tube. After cooling in a ice-bath, 1 c.c. of 2.5% KI solution was added by running it from a pipette down the wall of the container without disturbing the sugar solution. Then 1.5 ml of \(2\text{NH}_2\text{SO}_4\) was added rapidly with thorough shaking so that the entire content of the beaker could be acidified at once. Quick titration was made against \(0.005\ N \text{Na}_2\text{S}_2\text{O}_3\) solution (diluted freshly from \(0.01\ N \text{Na}_2\text{S}_2\text{O}_3\) stock solution) till the solution become faintly straw colored. Then a drop of starch indicator was added to the solution and finally titration was completed by making the solution colourless with further addition of Thiosulphate solution. Together another set of tubes were maintained similarly in which 5 ml of distilled water was added instead of sugar solution (Blank).

The amount of sugar in 5 ml. of solution (extract) was determined as follows:

(A) \(0.005\ N \text{Na}_2\text{S}_2\text{O}_3\) ml - required to titrate sample with sugar.

(B) \(0.005\ N \text{Na}_2\text{S}_2\text{O}_3\) ml - required to titrate sample without sugar.

Amount of sugar in 5 ml. of Sugar solution

\[= (B-A) \times 0.135 \text{ mg. of glucose.}\]

\((1\ \text{ml. of } 0.005\ N \text{Na}_2\text{S}_2\text{O}_3 \text{ corresponds to } 0.135\ \text{mg of glucose.})\)
(ii) Total sugar : To estimate the total sugar 20 ml of purified sugar extract was boiled in water bath for 15 minutes after addition of .3 ml of conc. HCl. After cooling the solution was neutralized with solid Na₂CO₃. It was then diluted to 100 ml and determination was done in the same way as in the case of reducing sugar.

1. Reducing sugar $\% = \frac{(B-A) \times 0.135 \times 250 \times 250 \times 100}{5 \times 50 \times 10 \times 1000} \times \frac{20 \times 50 \times 10 \times 1000}{5 \times 50 \times 10 \times 1000} \times (B-A) \times 0.34\%$

2. Total sugar $\% = \frac{(B-A) \times 0.135 \times 100 \times 250 \times 250 \times 100}{5 \times 20 \times 50 \times 10 \times 1000} \times \frac{5 \times 50 \times 10 \times 1000}{5 \times 50 \times 10 \times 1000} \times (B-A) \times 1.7\%$

3. Non Reducing sugar $\% = (\text{Total sugar} - \text{Reducing sugar})$

Estimation of Total Titratable Acid :-

10 grams of Pineapple leaves were weighed out and grained in a mortar and boiled for a few minutes with distilled water. The extract was then filtered through the Cotton Wool and the ppt. was washed with hot water several times. The extract was titrated.
against 0.1 Normal NaOH (N/10) using phenolphthalein as indicator.

In this titration extract was taken in a beaker. The extract was allowed to run until it was decolourised.

1 ml of N/10 NaOH = 6.4 mg of Acid.

Multiplication of this factor by the titratable value gave the amount of acid content per gram of the fresh leaves.

Estimation of Nitrogen :-

(1) Preparation of reagent :-

(1) Catalyst: - 1 gm of CuSO₄, 8 gms of potassium sulphate, and 1 gm of Salamium dioxide grind each separately and mixed thoroughly.

(2) N/38 HC1: 318 ml of Conc HC1 diluted to 10 litres of water.

(3) Conway’s indicator: - 6 ml of methyl red (0.16% in 95% alcohol), 12 ml of bromocrysol green (0.04% in water), 6 ml of 95% alcohol.

(iv) Salicylic - Sulphuric acid mixture: Dissolve 5 gms of Salicylic acid (Pure) in 100 ml of conc H₂SO₄ acid.

(v) 2% Boric Acid: Dissolve 20 gms of boric acid in water and made the volume 1 litre.
Analytical Procedure:

Method which was adopted for the estimation of Nitrogen content in the leaves consist of three steps:

Digestion: 100 mgs of dried (Pineapples leaves) powdered material was taken in a small Kjeldahl flask. To it, 1 c.c. of Salicylic Sulphuric acid mixture was added, and allowed to stand for 20 minutes. Then 0.3 gms of Na-Thiosulphate was added and heated gently until fumes appear. After cooling 0.6 gms of catalyst and 0.7 c.c. of H₂SO₄ acid was added and heated until the digest was apple green in colour. At the same time a blank experiment (without material) was also conducted.

Distillation: The distillation was carried in Micro-Kjeldahl steam distillation apparatus. To proceed with the distillation the apparatus was steamed out for about 10 minutes with water boiling vigorously in the steam generating flask. The digest was introduced into the apparatus through the side tube and the digestion tube was washed out twice with about 1 c.c. of distilled water each time. The ground glass stop was replaced and an excess quantity of 40% NaOH was added to the funnel. The digest was allowed to distill in 5 c.c. of boric acid contained
in a 50 c.c. conical flask. After first few drops of distill the top of the condenser was raised above the surface of the acid. Distillation was continued until about 25 c.c. collected.

**Titration:** Two drops of conway's indicator were added to the distillate and was titrated against N/28 HCl until a faint pink colour was obtained. The volume of HCl required was noted and the quantity of N$_2$ present was calculated and expressed in terms of percentage.

**Calculations:** The amount of nitrogen present in 25ml of the distillate determined as follows:

(A) The volume of $\frac{N}{28}$ HCl (ml) required to titrate 25 ml of distillate with material (N$_2$)

(B) The volume of $\frac{N}{28}$ HCl (ml) required to titrate 25 ml of distillate without material.

Therefore, amount of nitrogen present in 25 ml of the distillate is $(A-B) \times 0.5$ mg of nitrogen.

(1 ml of $\frac{N}{28}$ HCl corresponds to 0.5 mg Nitrogen.)

Now 25 ml of the distillate is originated from 100 mgs of dried powder.

\[ \therefore \text{The nitrogen percentage is } (A-B) \times 0.5 \text{ mg}. \]

The data of flowering p.c., and interval of time were statistically analysed.