Phytohormones and Nitrogen Deficiency in Paddy

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Introduction: Phytohormones are growth-regulating chemicals found in minute quantities in plants. They are capable of influencing specific physiological processes at parts whither they had been transferred. Hormones influence plants in four important ways, (1) causing physiological and morphological differences in the plant parts which bear roots, leaves, flowers, and fruits, (2) determining the form of a plant (3) producing tropisms just as phototropism, geotropism etc., and (4) controlling growth. All stages of growth and especially the cell enlargement phase are greatly influenced by hormones.

Nowadays synthetic hormones are being put to a variety of uses to help the farmer and the fruit grower, as in parthenocarpy or seedless fruit-setting, prolonging or breaking of dormancy in tuber plants as and when required, prevention of lodging, prevention of pre-harvest fruit drop, rooting of cuttings and eradication of weeds. Overcoming certain nutrient deficiencies in soil was also found possible by means of synthetic growth-hormones.

Review of literature: Avery, Burkholder and Creighton (1936) showed that a deficiency of nitrogen causes reduced hormone content in plants like Helianthus and Nicotiana, but the deficiencies of Ca. Mg. S. and P. did not show such effects.

Folke Skoog (1940) showed no relationship between the deficiencies of Ca. and Mg. with hormone concentration, in tomatoes.

Eaton (1940) showed that boron deficiency affected hormones in cotton and that indole-acetic acid could be used to overcome the deficiency to some extent.

Avery et al (1937 — 45) and Riker (1939) showed similar results and observed that zinc deficiency reduced plant hormones.

The present work was planned to find out the relationship between nitrogen, hormones and growth in paddy. The work was carried out at the Plant Physiology Laboratory, in the College of Agricultural Research, Benares Hindu University.

Materials and Methods: Forty-eight cement pots were taken and waxed inside, the drainholes being closed with cement mortar. Sand washed with hydrochloric acid and water previously, was used to fill them up. Eight pots were used to grow Sahdia variety of paddy, applying tap water alone. When 6 inches high, the plants were transplanted (five in each) into each of the remaining 42 pots. Six pots were kept as control and were supplied with a normal nutrient solution based on Turner's formula (1922) modified by Beckenbach, Wadleigh and Shive (1936) and further modified by the author. Here the stock solution B was replaced by Hoagland and Snyder's (1933) stock solution B which possesses all the necessary minor elements (Refer Table I). The remaining 36 pots were supplied with N-deficient solution based on the same formula (refer Table II).

On 5-10-1949, a month after starting the experiment, the 36 pots supplied with N-deficient solution were divided into 6 groups. One group was given 4 times the usual amount of nitrogen according to Turner's formula. The second was given normal nutrient solution. The third was supplied with one-tenth the normal nitrogen. The fourth, fifth and sixth groups were supplied with alpha-naphthalene-acetic acid in concentrations of 1/25,000, 1/50,000 and 1/100,000 respectively (refer to table II).

The hormone content present in paddy at the shoot tips was observed at fortnightly intervals. The hormones were first extracted and the concentrations of hormones then determined by the "Pea test" and expressed in terms of degrees. The methods adopted for extraction and determination of hormones from paddy shoots were as follow:

Extraction of Hormones: This was done according to the simplified method of hormone extraction suggested by Overbeek (1938). Ether purified by shaking with ferrous sulphate, Ca O and H₂ O to remove traces of peroxide and distilled later was used. The shoot tip of paddy was cut and immediately placed in a chilled beaker and covered over by ice to inactivate enzymes from destroying auxins. After sufficient chilling nearly 1 gm. of shoot tip was weighed quickly and placed in a beaker containing 40 cc. of purified ether and allowed to stand overnight in a refrigerator. Next day this ether was evaporated till 1 or 1.5 cc. of residue was left. This was diluted to 20 cc. and used for the "Pea Test".

Assaying the hormones: F. W. Went's method (1934) of pea test was employed and the French sugar variety of peas was used. The requisite number of pea seeds were soaked in water for 6 hours and were then planted in moist sand in a dark chamber provided with a red light. The plants would be ready within 7—9 days, when they grow 10—12 cm. high, developing two nodes each bearing a scale leaf and one at the top bearing a leaf. Selection was made of such plants whose internodes between the terminal leaf and terminal bud were less than 5 cm. in length. Such plants were uprooted, washed in water and then the tops were cut 5 mm. below the terminal bud. Later, the stem was split centrally lengthwise with a sharp safety razor blade for 3 cm. length. Another cut was given a few mm. below the split stem portion and then washed in flowing tap water for 1 hour. Thus split sections of pea were obtained. At least six of them were transferred to each petri dish containing about 20 cc. of the extracted hormone solution. The acidity of this solution under test should never exceed pH 4.

Shadowgraphs were taken of these sections placed in each petri dish, separately, after 12—15 hours or overnight. The pea sections were quickly dried in the folds of a filter paper and placed on Velox or bromide paper in a dark room and exposed to 60. c. p. electric bulb for 2 seconds at four feet distance.

The angles of curvature were then measured with a transparent plastic protracter placing it on a shadowgraph. The angle to be measured is that subtended by the tangents drawn to the extreme curved tip and at that particular point on the stem where the direction of curvature changes. Thus two angles on either side of a split section were obtained and averaged.

Results: The hormone content fell markedly in N-deficient plants and reached a zero value on 4—10—1949 (refer table III) nearly a month after starting the experiment, whereas the control showed a steady increase.

When N-deficient plants were supplied with nitrogen, the hormone content showed an increase which even exceeded the control value in the case of 4N. Similarly, application of synthetic hormone increased the phytohormone, the highest concentration of it reaching a level higher than even the control value (refer table IV).

The yield and vegetative vigour were better in control than in the treated plants. High doses of N delayed grainsetting and increased vegetative growth. (refer tables V and VI)

Discussion: The results show that there is a definite relation-ship between nitrogen, auxins and the growth of the plant. Nitrogen deficiency is followed by auxin reduction which is accompanied later by decrease in growth. Application of nitrogen as well as alphanaphthalene acetic acid increased the phytohormone content. At this time application of nitrogen in higher quantities delayed grainsetting due to undue increase of vegetative growth. But the synthetic hormone increased yield, though vegetative growth was not affected by it beyond a certain extent. This can be explained by Kraus and Kraybill's hypothesis (1913) of C/N ratio, where nitrogen is increased greatly and carbohydrates are not proportionately increased. Hence a major portion of the carbohydrates are synthesised into amino-acids, leaving very little for grain-setting resulting in rank vegetative growth. Increase in phytohormone by application of nitrogen may be due to increased protoplasm and chlorophyll pigment which increase auxin synthesis, and the decrease of auxin in N-deficient plants may be due to abnormal oxidation capacity in N-deficient plants (Folke Skoog, 1940).

The increase of hormones by the synthetic substance is due to the setting free of the plant hormone from its storage form, being itself a free acid. The chemical structure of this substance is also found similar to a phytohormone.

Summary:

- 1. Nitrogen content, auxin production and growth are correlated.
- 2. N-deficiency decreases auxin production due to inactivation or destruction of plant hormones caused by increased activity of oxidising enzymes.
- 3. Application of alpha-naphthalene acetic acid one month after transplanting to the N-deficient plants increases plant hormones to the level of control. Yield is increased, whereas vegetative growth is not increased beyond a certain extent.
- 4. Nitrogen in higher doses increases vegetative growth without any favourable effect on yield when applied one month after transplanting to the N-deficient plants. Phytohormones are increased by the application of nitrogen to a level nearabout that of the control.

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TABLE I.
Turner, nutrient solutio
Stock solution-A.

Reagents	Gme./litre for each.	Volume molar concentration	No. of c.c. per litre.
KN03	50.5	0.5	2
Ca (NO3) H2O	118.1	0.5	2
Mg (NO3)2 6H2O	128-1	0.5	2
KH2 PO4	68-1	0.5	2
Ca (H2 PO4) 2	126.1	0.5	- 2
Mg (H2 PO4)2	109 2	0.2	2
K2 804	87.1	0.5	-2
CaSO4-2H2O	17.2	0.1	10
Mg SO4 7H2O	123.3	0.2	2
Distilled water	### J#1		974

Stock solution-B.

Reagents	Gms./18 litres solution	
LiC1	0.5	I cc. of stock '8' is added to
CuSO4H2O 5H2O	1.0	litre of stock solution 'A' and 1 co
Zn 804	1.0	of freshly prepared 0.5% Ferri
H3 BO3	11.0	Tartrate solution is added to this
A12 (SO4) 3	1.0	just before application to plants.
SnCl 2. 2H2O	04	The second of th
MnC12, 4H 2O	7.0	
NiSO4. 6H2O	1.0	
Co (NO3) 2. 6H 2O	1.0	· d
TiO2	1.0	i.
KI	1.0	
KBr	0.5	•
Distilled water	18 litres.	•1

TABLE II

Composition of Nutrient solutions-cc. of Stock solution required to make 1 litre of culture solution.

Balts	Volume molar concentration of	of stock		N.	Series	
	stock solution.	solution.	4 N.	1 N.	0.1N	0.0 N
KN03	0.2	50.2	8.0 cc.	20	0 2	0.0
Ca (NO3)2	05	118-1	80	2.0	0.2	0.0
Na (NO3)	0.2	128-2	8.0	2.0	0.2	0.0
Mg2 (PO4) KH2 PO4	0.5	68'1	2.0	2.0	2.0	2.0
Ca (H2PO4)2	0.5	126.1	2.0	2.0	2.0	20
Mg (H2P04) 2	0.5	109.2	2.0	2.0	2.0	2.0
K2 SO4	0.5	87-1	2.0	2.0	2.0	2.0
CaSO4	0.5	17.2	10.0	10.0	-10.0	10.0
MgSO4. 7H2O	0.5	123.3	20	2.0	2.0.	2.0

TABLE III - Pea test for hormones. Mean angle of curvature.

Date.	Control.	N. deficient.
4-9-49	29.92	29·8°
940	58.0℃	10.10
—10—149°.::	73.75	0.00

TABLE IV-Pea test for hormones

Treatments	Mean angle of 19—10—49	curvature on 4-10-49
Control	-91·4°	116·3°
4 N -	63.3	139.0
1 N	38.8°	144.0
0·1 N	6:5°	61.3
1/25,000 naphthalene acetic acid	63.8	140.3
1/50,000 ,,	39.50	110.3
1,1100,000	7.10	65.0
1/100,000	* · · · · · · · · · · · · · · · · · · ·	#743/F 1

TABLE V. Fresh weight, dry weight and ash weight per plant of 20-11-49.

Treatments	Fresh wt.	Dry wt.	% dry wt.	Ash in	% ash on dry basis.
7.7.4	in gme.	in gms		ģms.	5,
Control	1528-0	509.6	33.0	76.44	15.0 .
4 N	1232.5	320.4	26.1	- 36:90	12.6
1 N	1006-4	381.6	26.3	41'02	10.7
0 N	890.0	169*1	19.0	16.11	9.5
1/25,000 Naph.	1052-0	236-9	25.2	31.79	13.6
1/50'000 ,	945.0	239.3	25.0	20.68	13 0
1/109,000	763 0	105.4	16.1	10:10	9.9

TABL VI.

Yield per pot of 5 plants expressed in gms
24.0
15-5
20.4
17.6
25.6
22:0
17·D

BIBLIOGRAPHY.

- 1. Appleman-Sci. 48, 1918. 2. Armstrog, H.E. and E. J. Armstrong-Ann. Bot. 25, 1911. Avery G. S.-Bull. Torrey Bot. Club. 62, 1935. Avery Jr. G.S. and P.R. Burkholder-Bull. Torrey Bot. Club. 63-1936. do. and H.B. Creighton, Ann. J. Bot. 24, 1937 (51-58) 5. Ann. J. Bot. 24, 1937 (226-232) 6. do. 7. do. Ann. J. Bot. 24, 1937 (553-557) do. 8. Proc. Nat. Acad. Sci 22, 1936. 9. Avery and Pottorf-Ann. J. Bot. 1, 1945. Avery et al-Ann. J. Bot. 28, 1941. 11. Bauguoss L. C. Ann. J. Bot. 22, 1935. 12. Bayliss. W. M. & E. Starling-Proc. Roy. Soc. 73, 1904. 13. Beckenbach J. R. Wadleigh C. H. & Shive, J.W. Soil Sci. 41, 1936. Borgor and Avery-Ann. J. Bot. 21, 1944. Bonner, J. - J. Gen. Physiol. 17-1933. 16. Proc. Nat. Acad. Sci. 19-1933. 17. Protoplasma 21-1934. 18. Proc. Nat. Acad-Sci. 29-1933. Bonner & K. V. Thinnann-J. Gen. Physiol. 16-1938. 20. Boysen Jensen P-Growth hormones in Plants-1936-Translated by G. S. Avery Jr. & P.R. Burkholder. 21. -Ann. Rev. Biochem. 7-1938. 22. Bunzel H.H.-J. Agr. Res. 2, 1914. 23. J. Biol. Chem. 28-1917. 24. Cailhajan M.H. Publ. Acad. Sci. USSR. 1937. Clark M. A. - Plant Physiol. 1-1926. 26. Cholodny N G.-Herbage Rev. 3 - 1935. Cooper W. C .- Plant Physiol. 10-1935. 28. Curtis O. F-Cornell - Univ. Agr. Expt. Sta. Mem 14-1918. 29. Dolk H.E. and Thimann K.V.-Agr. Expt. Sta. Mem. 14-1918. 30. Eaton F. M. Bot. Gaz - 101 - 1940. 31. Garden F. E. and Marth. P.C. - Bot. Gaz. 99-1937. 32. do. Sci. 90-1933. 33. Gericke W.F. - Bot. Gaz. 78 - 1924. 34. Goodwin R. H - Ann. J. Bot. 24-1937
- Graham R.J.D. and L.B. Stewart Proc. XI Int. Hort. Cong. 1930, 1931.
 Greogory, F.G.-& Purvis O.N-Nature 138-1936.
- 37. Gustafson, F.G. Proc. Nat. Acad. Sci. 22-1936.
- 38. do. Am. J. Bot. 25-1936.
- 39. Haagensmidt A.J. & F.W. Went-Proc. Kan. Akad. Wetendsch. 35-1935.
- 40. Haagensmidt A.J. Am. J. Bot. 29-1942.
- 41. Havas, L. J. Nature 157-1946.
- 42. Heath O.V.S. New Phytol. 7-1949.

```
43. Hitchcock, A. E. W. Crocker & P.W. Zimmermann-Contr-Boyce Thomsom
                                                                 Inst. 6-1935
 44. Hitchcock, A. E. & P. W. Zimmermann.
                                                        do.
 45. Howlett F.E. Ghio Agr. Expt. Sta. Bull 1943.
 46. Hurd Karrer-J. Agr. Res. 29-1930.
 47. Hurd A.M - J. Agr. Ros, 25 - 1923.
 48. Kogl-F. Rep. Bri. Assoc. 1933.

    Kraus E. J. & Kraybill - Ore Sta Bull. 1948-1918.

 50. Leoman A. C., Pamphl. Soc Agr. Biol. Soc. 6, 1933.
 51. Leomen L. H. & V. G. Lilly - Am. J. Bot. 21, 1937.
 52. Lochwing W.F. & Bauguess L.C - Sci. 84-1936.
 53. Marmer D.R - Am. J. Bot. 1538-1937.
 54. Mc. Call A.G. J. Am. Soc. Agr. 15-1925.
 55. Michenor H. D., Sci. 82-1935.
 56. Miller E. C. Plant Physiology, McGraw Hill Book Co., 1938.
 57. Overbeek J. Van - Proc. Nat. Acad. Sci. 21-1935.
 58.
                                             21-1938.
                        Am. J. Bot. 26-1941.
 59.
 60.
                        Nature 155-1945.

    Purvis O.N-Ann. Bot. Lond. N.S. 8-1944.

62. Purvis O. N. & Gregory F.G-Ann. Bot. 1. 1937.
 63. Ramiah K - 'Rice in India' 1937.
 64. Sen R. - Curr-Sci. 9-1940.
 65. Skoog F-J. Gon. Physiol. 20-1937.
... 66. Ann. J. Bot. 40-1940.
67. Snow R.- New Phytol 21-1932.
68. Southwick L - Proc. Am. Soc. Hort Sci. 42-1943.
69. Thimmana K. V. & J. Bonner - Proc. Nat. Acad. Sci. 18-1932.
70.
                               ...
                                     Proc. Roy. Soc, 113, 1933.

    Thimmann K.V - J. Gen. Physiol. 19-1938.

                      Ann. Rev. Biochem. 4, 1935.
72.
            55
 73.
                       Ann. J. Bot. 29, 1942.
74. Thomas Swarbrick - New Biol. 5-1948.
  75. Went F.A.F.C. - Nature - 132-1933.

    76. Went F.W. - Proc. Kow. Akad. Wetensch. 30-1926.

                       38
77. Went F.W.
                   Bot. Rev. 1 - 1935.
  78.
        **
              Proc 25th Ann. Celebration Univ. Soc. Calif. 1936.
 79.
  80. Went. F. W. & K. V. Thimmann - Phytohormones - Macmillan & Co.,
81. Went F. W. - Ann. Rev. Biochem. 8, 1939.
                   Bot. Gaz. 99-1939.
  82.
                    Bull Torry - Bot. Club. 66-1939.
83.
        9.9
                    Bot. Rev. 11-1945.
  84.
        100
                   Plant Physiol, 22-1047.

    Whyte R.O. Crop Production & Environment - 1946.

  87. Zimmermann - Contr. Boyce, Thompson, Inst. 19-1942.
```