Pakistan J. Bot. 2 (1): 37-43

BIOCHEMICAL STUDIES ON DEVELOPING AND RIPENING BANANA

M.A.A. KHAN and M. ANWARUL HAQUE

Department of Botany, University of Dacca, East Pakistan.

Abstract

The contents of auxin, N and phosphates were determined at various stages of development of Sagar Kala variety of banana fruit. In the first 30 days auxin could not be detected in the fruit but N and inorganic phosphate contents decreased. Auxin was, however, found present in uniform quantities in bananas from 45 to 90 days old when the level of N and inorganic phosphate remained almost unchanged. In the third stage i.e. ripening stage from 90 to 105 days there was over 3-fold increase of both auxin and N. A reduction in the inorganic phosphate content took place during this period with a corresponding increase in the level of organic phosphate—an observation highly suggestive to the formation of energy rich organic phosphate compounds associated with the climacteric rise of respiration during ripening.

Introduction

It is a common observation that the end of life of a fruit is preceded by a period of great activity although the growth is over and the ripening is much influenced by the parent plant and the earlier stages of development of the fruit from its initiation through the maturation. Since 1950 considerable research work has been done on the various aspects of fruits and a considerable information is available about the physical and chemical changes that occur in the ripening fruit. The failure of dinitrophenol-treated tomatoes to ripen normally indicated that energy was required for ripening process (Marks *et al.* 1957). The increase in the amount of protein was observed by many workers in fruits during the climacteric rise of respiration (Rowan *et al.* 1958, Hulme *et al.* 1963). Crane (1948) reported that the application of auxin in fig fruits led to earlier ripening.

The present biochemical study on the estimation in the contents of auxin, nitrogen, organic and inorganic phosphate in Sagar Kala, a local commercial variety of banana at its various stages of development is aimed at knowing more about the ripening process without trying to answer at this stage the final question as to what it is that sets in motion the changes in fruits during ripening.

Materials and Methods

The age of the fruit, a parthenocarpic banana which is locally known as Sagar Kala (*Musa sapientum* Linn.) was counted from the day of the opening of bracts.

Alternate slices from the bananas were taken for the extraction of different substances. For auxin about 100 gms of tissues for each sample were extracted in 1200 ml of diethyl ether freed from peroxides (Werner 1933). The extract was allowed to evaporate to dryness under fan at the room temperature. The residue was dissolved with 3 ml of ethanol and the volume was made to 15 ml with demineralized water. Quantitative measurements of the effect of auxin on straight growth of coleoptiles of maize (*Zea mays* var. Min. 806) seeds were made according to the method described by Machlis and Torrey (1959).

Indole-3-acetic acid (IAA) was estimated chemically also in the extract following the technique of Gordon and Webber (1951). The banana extract used for the bioassay was yellowish in colour. So the extract before chemical assay, was cleared by washing with benzene (benzene is miscible with ethanol but when plenty of water is present, the alcohol-waterpart separates from the benzene). Benzene washing was tested with pure IAA and there was no difference in spectrophotometric reading before and after washing.

Alternate transverse slices of bananas were dried in an oven at 70°C. The dried slices were powdered and used for the determination of nitrogen following the colorimetric nesslerization method of Thompson and Morrison (1951).

Phosphates were estimated following the method of Fiske and Row (1925). Phosphates were extracted in trichloroacetic acid solution. The inorganic phosphate was measured from the extract without digestion and the total phosphate was measured after sulphuric acid digestion. The organic phosphate was obtained after subtracting the inorganic phosphate from the total phosphate.

Seven samples of bananas at 15, 30, 45, 60, 75, 90 and 105 days were collected from the plant before extraction. Yellowing started after 90 days. At 105 days bananas were fully yellow when they were cut off from the plant and stored and the last sample was extracted after 7 days i.e. from overripe bananas of 112 days old.

Experimental Results

Experiment for auxin:

The bioassay (3.3 gm of fresh wt of tissue/assay) failed to trace auxin in 15 and 30 days old bananas (Fig. 1). The level of auxin was almost same in 45, 60, 75 and 90 days old banana but increased several folds in the ripe (yellow) bananas of 105 days old and then declined slightly in 112 days old bananas.

Similar to bioassay the chemical assay (6.6 gm of fresh wt of tissue/assay) also failed to detect any IAA in 15 and 30 days old bananas (Fig. 2). The IAA content varied from 157 μg in 45 days old bananas to 236μg in 90 days old bananas. It increased to 820 μg in the yellowing bananas.

Experiment for nitrogen:

The nitrogen content in 15 days old bananas was 890 mg per 100 gm dry weight (Fig. 3). It gradually decreased to 360 mg in 45 days old bananas and remained almost unchanged in bananas from 45 to 90 days old. The nitrogen content increased to 1446 mg in 105 days old bananas.

Experiment for phosphates:

The inorganic phosphate content in 15 days old bananas was 75 mg per 100 gm fresh weight (Fig. 4). It decreased to 55 mg in 30 days old bananas and remained more or less same in bananas upto 90 days old. It further decreased to 35 mg in 105 days old bananas and then increased slightly in 112 days old bananas.

The organic phosphate content varied from 20 to 30 mg in the bananas between 15 and 90 days old. It increased to 59 mg in 105 days old bananas and then fell slightly in 112 days old bananas (Fig. 4).

The total phosphate content was 96 mg in the 15 days old bananas and then it decreased gradually to 70 mg in the 45 days old bananas. It then slowly increased and reached 90 mg in 105 days old bananas (Fig. 4).

Discussion

Changes in the auxin content:

Auxin was not detected in 15 and 30 days old bananas in our experiment both by the bioassay and chemical assay (Figs. 1,2). Biale (1954) also observed that the measurable quantities of IAA did not appear in some cases before fruit growth was well under way and Khalifah (1966) obtained only 18 μg of IAA in 300 gms of tissue of young bananas of 6.5 cm long.

The auxin level which remained unchanged in 45 to 90 days old bananas, increased about four times in 105 days old bananas (Figs. 1,2). There are many reports that the fruit maturity and ripening are marked by a very low auxin content (Luckwill 1953, Wright 1956). But in the ripening bananas a sharp increase of auxin took place, the significance of which was not known. However,

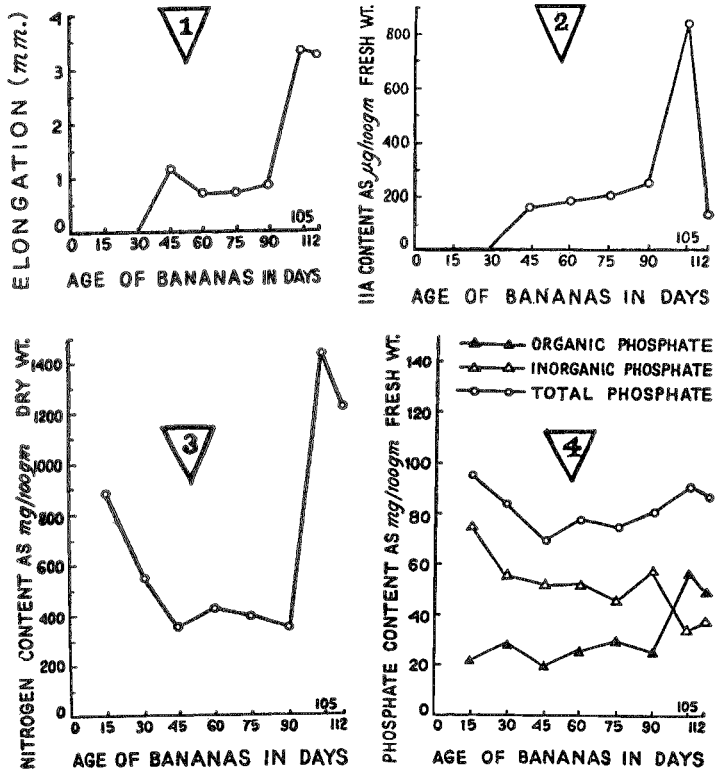

Fig. 1. Changes in the elongation of coleoptile sections in the test solution containing 0.5 ml banana extract (3.3 gm fresh wt).

Fig. 2. Changes in the content of IAA in bananas.

Fig. 3. Changes in the content nitrogen in bananas.

Fig. 4. Changes in the contents of phosphates in bananas.

auxins are known to accelerate starch hydrolysis in some tissues (Mitchell *et al.* 1940a, Mitchell and Whitehead 1940b, Gall 1948), to stimulate respiration (Commoner and Thimann 1941) and enzyme activities (Newcomb 1951, Teubner *et al.* 1952). Biale (1954) reported that IAA could be made by the oxidation of tryptophan known to be present in the pollen. One possibility of the increase of IAA in the banana during ripening might be due to the oxidation of tryptophan in the climacteric period. The fruit auxins are known mainly to be localized in the seeds but in the parthenocarpic banana it should be present in other parts of the fruit.

Changes in the nitrogen content:

The nitrogen level decreased from the young banana to the mature banana (Fig. 3). According to Biale (1964) the proteins are the chief constituents of the young undifferentiated cells and with cell enlargement vacuoles appear and the protein content on a weight or volume basis is diluted.

The nitrogen content increased remarkably in the ripening (yellowing) banana (Fig. 3). Hulme *et al.* (1963) and Rowan *et al.* (1958) reported that the protein content increased in fruits during the climacteric rise of respiration. According to Hulme *et al.* (l.c.) the increase in the protein content during the climacteric rise is due to the actual synthesis of enzymes (carboxylase and malic enzymes). Hulme (1954) found a close parallel between the drift in the rate of respiration and in the protein nitrogen/nonprotein nitrogen ratio in apples. The increase of nitrogen observed in the ripening bananas could be explained on the same explanation as postulated by Hulme (l.c.).

Changes in the phosphate content :

The level of total phosphate was almost same in both the 15 days old young banana and 105 days old ripening banana. It was lower in the mature banana (Fig. 4). The inorganic phosphate content decreased from 75 mg to 55 mg in banana from 15 to 30 days old when the loss of 13 mg of inorganic phosphate was not accounted for by the rise of organic phosphate and then it remained unchanged upto 90 days. The most significant change occurred in 105 days old bananas when inorganic phosphate decreased and organic phosphate increased concurrently (Fig. 4). Here the gain of 12 mg of organic phosphate was not accounted for by the loss of inorganic phosphate. It is suggested that this excess phosphate was absorbed by the fruit from the other parts of plants.

The rise of organic phosphate in the ripening banana observed here is considered to have resulted from the formation of the high energy phosphate

compounds. Several workers have reported in various fruits that the oxidative phosphorylation continues throughout the ripening process (Millerd *et al.* 1953, Marks *et al.* 1957, Biale 1960).

Acknowledgments

We thank Dr. M.M. Rahman, Department of Biochemistry, University of Dacca for help during the preparation of this paper. We also thank the authorities of the Agricultural Experimental Station, Texas and the Department of Agriculture, Minnesota, U.S.A. and the Agricultural Research Institute, Dacca, East Pakistan for supplying maize seeds.

References

Biale, J.B. 1964. Growth, maturation and senescence in fruits. Science **146**: 80.

———1960. Enc. Plant Physiol. (W. Ruhland, ed.) Springer-Verlog, Berlin. **12**: 536.

———1954. The ripening of fruits. Scientific American **190**(5): 40.

Commoner, B. and K.V. Thimann. 1941. On the relation between growth and respiration in the *Avena* coleoptile. J. Gen. Physiol. **24**: 279.

Crane, J.C. 1948. Fruit growth of four fig varieties as measured by diameter and fresh growth. Proc. Amer. Soc. Hort. Sci. **52**: 287.

Fiske, E.H. and Y. Subba Row. 1925. The colorimetric determination of phosphorous. J. Biol. Chem. **66**: 375.

Gall, H.J.F. 1948. Effects of 2, 4-D on starch digestion and reducing activity in bean tissue culture. Bot. Gaz. **110**: 319.

Gordon, S.A. and R.P. Webber. 1951. Colorimetric estimation of indoleacetic acid. Plant Physiol. **36**: 192.

Hulme, A.C. 1954. Studies of nitrogen metabolism of apple fruits. The climacteric rise in respiration in relation to changes in the equilibrium between protein synthesis and breakdown. J. Exptl. Bot. **5**: 159.

———, J.D. Jones and L.S.C. Wooltorton. 1963. The respiration climacteric in apple fruits. Proc. Roy. Soc. (London), B. **158**: 514.

Khalifah, R.A. 1966. Indole-3-acetic acid from the developing banana fruit. Nature **212** (5069): 1471.

Luckwill, L.C. 1953. Studies of fruit development in relation to plant hormones. II. The effect of naphthalene acetic acid in fruit set and fruit development in apples. Hort. Soc. Sci. **28**(1): 25.

Machlis, L. and J.G. Torrey. 1959. Plants in action. p. 20, W.H. Freeman and Co., San Francisco, U.S.A.

Marks, J.D., R. Bernloho and J.E. Varner. 1957. Esterification of phosphate in ripening fruit. Plant Physiol. **32**: 259.

Millerd, A., J. Bonner and J.B. Biale. 1953. The climacteric rise in fruit respiration as controlled by phosphorylative coupling. Plant Physiol. **28**: 521.

Mitchell, J.W., E.J. Kraus and M.R. Whitehead. 1940a. Starch hydrolysis in bean leaves following spraying with alpha naphthalene acetic acid emulsion. Bot. Gaz. **102**: 97.

——— and M.R. Whitehead. 1940b. Starch hydrolysis in bean leaves as affected by application of growth regulating substances. Bot. Gaz. **102**: 293.

Newcomb, E.H. 1951. Effect of auxin on ascorbic oxidase activity in tobacco pith cell. Proc. Soc. Exptl. Biol. Med. **76**(3): 504.

Rowan, K.S., H.K. Pratt and R.N. Robertson. 1958. Relationship of high energy phosphate, protein synthesis and the climacteric in ripening avocado and tomato. Aust. J. Biol. Sci. **11**: 329.

Teubner, F.G., E. Marre and A.E. Murneck. 1952. A.I.B.S. meeting. Plant Physiology Section, Cornell Univ., Ithaca, N.Y.

Thompson, J.F. and G.R. Morrison. 1951. Determination of organic nitrogen. Anal. Chem. **23**(ii): 1153.

Werner, E.A. 1933. Simple and rapid procedure for the purification of ether and of acetone. Analyst. **58**: 334.

Wright, S.T.C. 1956. Studies of fruit development in relation to plant hormones. J. Hort. Sci. **31**: 196.