Nucleic Acid Metabolism in *Eriophyes*-Induced *Zizyphus* Gall & Normal Stem Calli in Culture

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Nucleic acid metabolism as influenced by auxin and its precursors was investigated using callus tissues from *Zizyphus jujuba* Lamk. stem galls induced by *Eriophyes cernuus* Massee. and normal stem established and maintained on modified Murashige and Skoog's (MS) medium. Increase in concentrations of α-naphthalene acetic acid (NAA) to the extent of 10 mg/litre in the medium increased the RNA content of normal tissue while no appreciable change was recorded in the gall tissue. Substitution of NAA with L-tryptophan (5 mg/litre) in the medium induced increased synthesis of RNA in the gall tissue alone. Both the tissues grew after replacing NAA and L-tryptophan with increased concentration of zinc sulphate (15 mg/litre). DNA content of the gall tissue was significantly higher to that of the normal tissue. Maximum amount of DNA was obtained after 20 days growth. Subsequently it decreased to a minimum on the last harvest taken after 50 days growth. The DNA content of the normal tissue remained steady throughout its growth. Addition of increased concentrations of NAA (10.0 mg/litre), L-tryptophan (5.0 mg/litre) and zinc sulphate (15.0 mg/litre) separately in the medium did not change appreciably the general pattern of DNA contents of the normal and gall tissues. Important relationship of the nucleic acids with the nature of the diseased and normal growth of the tissues was established.

IMPORTANCE of nucleic acid content in the growth of normal and diseased higher plants is established[1-4]. Plant tissue culture technique appears important in elucidating the role of nucleic acids in cellular growth[5]. Young cultures of excised tobacco tissues are marked with active synthesis of RNA and DNA[6]. Direct relationship between growth rate and RNA content of *Phylloxera* gall and normal grape stem single cell clones in cultures has been established[4]. Abnormal growth hormone metabolism in gall tissue has been observed[7] and, therefore, the present study has been undertaken to evaluate the effect of some of the growth factors like NAA, L-tryptophan and zinc sulphate on the nucleic acid (RNA and DNA) contents of the normal and gall tissues during various periods of tissue growth.

The experimental material consisted of the callus tissue from *Zizyphus jujuba* Lamk. stem gall induced by *Eriophyes cernuus* Massee., and normal stem, established and maintained on modified Murashige and Skoog[8] (MS) medium for 3 weeks, which was the zero day for the transplants and contained the RNA and DNA values obtained after 3 weeks growth.

NAA (1 and 10 mg/litre), L-tryptophan (5 mg/litre) and zinc sulphate (15 mg/litre) were incorporated separately in the auxin free but 0.04 mg/litre kinetin containing MS medium, before autoclaving as per requirement of the treatment. *p*H of the medium was adjusted to 5.8. The cultures grown in 100 ml Erlenmeyer flasks were incubated in fluorescent lighted (1000 lux) air conditioned room at 26 ± 2°C and 60% relative humidity. Tissue harvests were made after every 10 days up to 50 days of tissue growth for nucleic acid estimation.

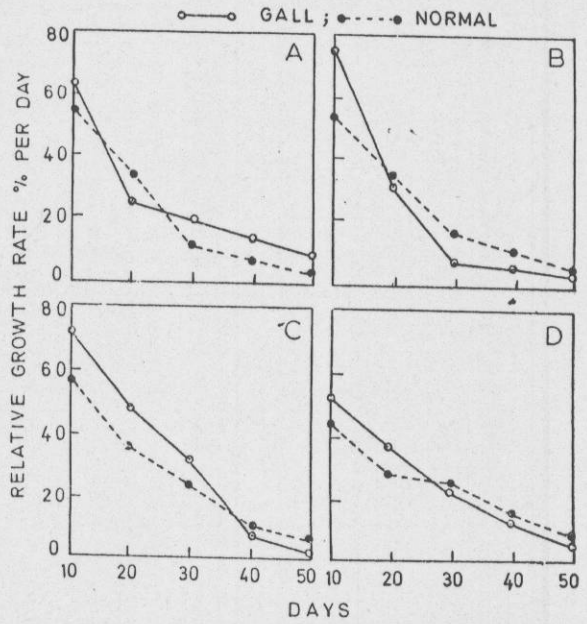

Fig. 1 — Relative growth rate per cent per day of normal and gall tissues at different growth periods and with different growth factors in MS medium [Growth factors (mg/litre): (A) NAA, 1; (B) NAA, 10; (C) L-tryptophan in the medium without NAA, 5; and (D) zinc sulphate in the medium without NAA and L-tryptophan, 15]

Extraction of nucleic acids was made following Ogur and Rosen methods as modified by Arya *et al*[9]. RNA estimation was done by orcinol test for ribose sugars[10]. DNA was estimated by diphenylamine colour reaction for deoxypentose sugars as reported by Burton[11] and Kupila *et al*[12]. Optical density of the colour developed was determined against a blank treated in the same manner.

Per cent relative growth rate per day of normal and gall tissues is given in Fig. 1 (A-D). The amounts (mg/g wet weight tissue) of RNA and DNA yielded by normal and gall tissues during different periods (10 to 50 days) of growth with different growth factors are given in Fig. 2 (A-D). Nucleic acid estimations earlier than 10 days could not be made for lack of availability of enough tissues during this period.

RNA content of the tissues in the present study is directly influenced with external application of auxin in the medium. Results show that RNA content of the normal tissue increased with the increase in the concentration of NAA (10 mg/litre) in the medium (Fig. 2B) while no appreciable change was recorded in the gall tissue. This difference in normal and gall tissues indicates that probably increased concentrations of NAA are concerned with increased synthesis of RNA in the normal tissue. Negative response

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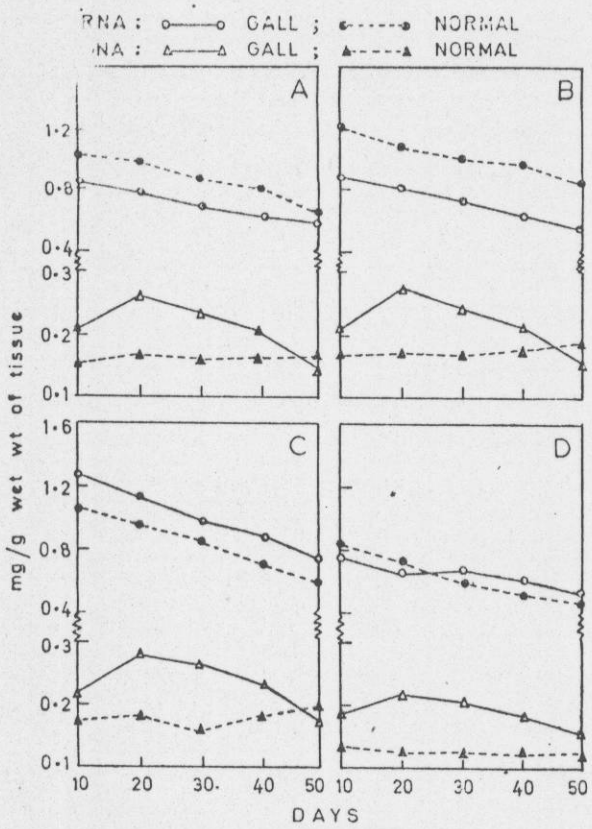

Fig. 2 — RNA and DNA contents (mg/g wet weight of the tissues) of normal and gall tissues at different growth periods and with different growth factors supplemented in MS medium [Growth factors (mg/litre) : (A) NAA, 1; (B) NAA, 10; (C) L-tryptophan in the medium without NAA, 5; and (D) zinc sulphate in the medium without NAA and L-tryptophan, 15]

of gall tissues with increased concentration of NAA needs explanation. However, increased concentration of NAA in the medium did not reduce the RNA content of the gall tissue. Interesting results were obtained with L-tryptophan (5 mg/litre) in the medium. This compound induced increased synthesis of RNA in the gall tissue alone (Fig. 2C) suggesting its positive role in the RNA metabolism of the gall tissue.

With zinc sulphate (15 mg/litre) in the medium RNA contents of normal and gall tissues were much lower (Fig. 2D) as compared with NAA or L-tryptophan in the medium. This probably indicates that zinc sulphate has relatively limited role in RNA synthesis. DNA content of the gall tissue was initially higher than that of normal tissue but the yield of DNA in the gall tissue declined in contrast with that of normal tissue in which it remained steady. This was a significant difference in the DNA pattern of the two tissues. Additions of different concentrations of NAA (1 and 10 mg/litre), L-tryptophan (5 mg/litre) and zinc sulphate (15 mg/litre) separately in the medium did not change appreciably the general pattern of DNA contents of the normal and gall tissue. The behaviour of the normal tissue was in agreement with the general concept that the chromosomal nucleic acid was constant from cell to cell. Proper explanation for decline in the yield of DNA of the gall tissue can be advanced at this stage on the basis of continuous increase in the ratio of larger sized and relatively inactive oval cells to smaller spherical cells (unpublished). Certain important changes in the nuclear material including changes in the chromosome number and cytoplasm may be responsible for decline in the yield of DNA[13]. The probability of increase in the deoxyribounclease (DNA-ase) activity in the gall tissues can not be ruled out.

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