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# ISOLATION AND ESTABLISHMENT OF TISSUE CULTURE FROM NEMATODE INDUCED ROOT GALLS AND NORMAL ROOTS OF TOMATO

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Tissue cultures from nematode induced root galls and normal tissues of tomato (*Lycopersicon esculentum Mill)* were isolated and established by testing various physico-chemical requirements. Out of the various media used, the gall and normal tissues were isolated on Wisconsin 'D' medium and finally established on revised MS- medium. Both the tissues grew well in dark at 26 ± 2°C and pH 5.8. It was also observed that, at various intervals of growth, a definite correlation exists between wet (fresh) weight and dry weight of these tissues..

**Keywords:** Gall tissues; *Lycopersion esculentum* ; Medium; Nematode; Physico-chemical requirements.

## Introduction:

Plant tissue culture can be regarded as an important tool in the study of fundamental aspects of normal and abnormal growth[1,2]. The growth of the tissues that are cultured *in vitro* depend on a large number of physical and chemical factors[3,4]. Determination of such factors for healthy growth of callus tissues is a necessary prelude for studies which involve complex biological process.

Abnormal growths induced by virus, bacteria and insects have been studied *in vitro* but the nematode induced galls received less attention. There is no report on the culture of nematode induced root gall tissues of tomato. Therefore, in the present study attempts were made to isolate and establish callus tissues from nematode induced root galls and normal roots of tomato (*Lycopersicon esculentum* Mill). The basic physico-chemical requirements such as growth medium, temperature, pH, light and growth criteria were determined to obtain uniform growth of both the tissues.

## Materials and Methods

Callus tissues from nematode (*Meloidogyne incognita*) induced root galls and normal roots of tomato (*L. esculentum)* were isolated using root galls (ca 5-10x2.5mm) and healthy seeds, respectively. The root galls and seeds were surface sterilized with 0.1% (w/v) mercuric chloride solution and after thorough washing in sterile glass distilled water, these were then aseptically transferred to 40 ml of solidified Wisconsin 'D' medium[5]. The callus formation in root gall explants and root (hypocotyl) portion of the seedling was observed as white protuberances after incubation of 15-25 days. The gall and normal callus, so produced, were allowed to grow along with the mother explants for 2-3 subcultures on Wisconsin 'D' medium. The callus tissues were then excised and placed on fresh Wisconsin 'D' - medium where it produced undifferentiated mass of cell.

In order to determine the suitable medium for continuous good growth the gall and normal tissues were grown on various semisynthetic and synthetic media, viz., Tobacco (T) medium[5], Wisconsin 'C' medium[5], Wisconsin 'D' medium[5], Yeast extract (YE) medium[5], Tobacco high salt (THS) medium[6], Revised tobacco (RT) medium[7] and Murashige and Skoog's (MS)

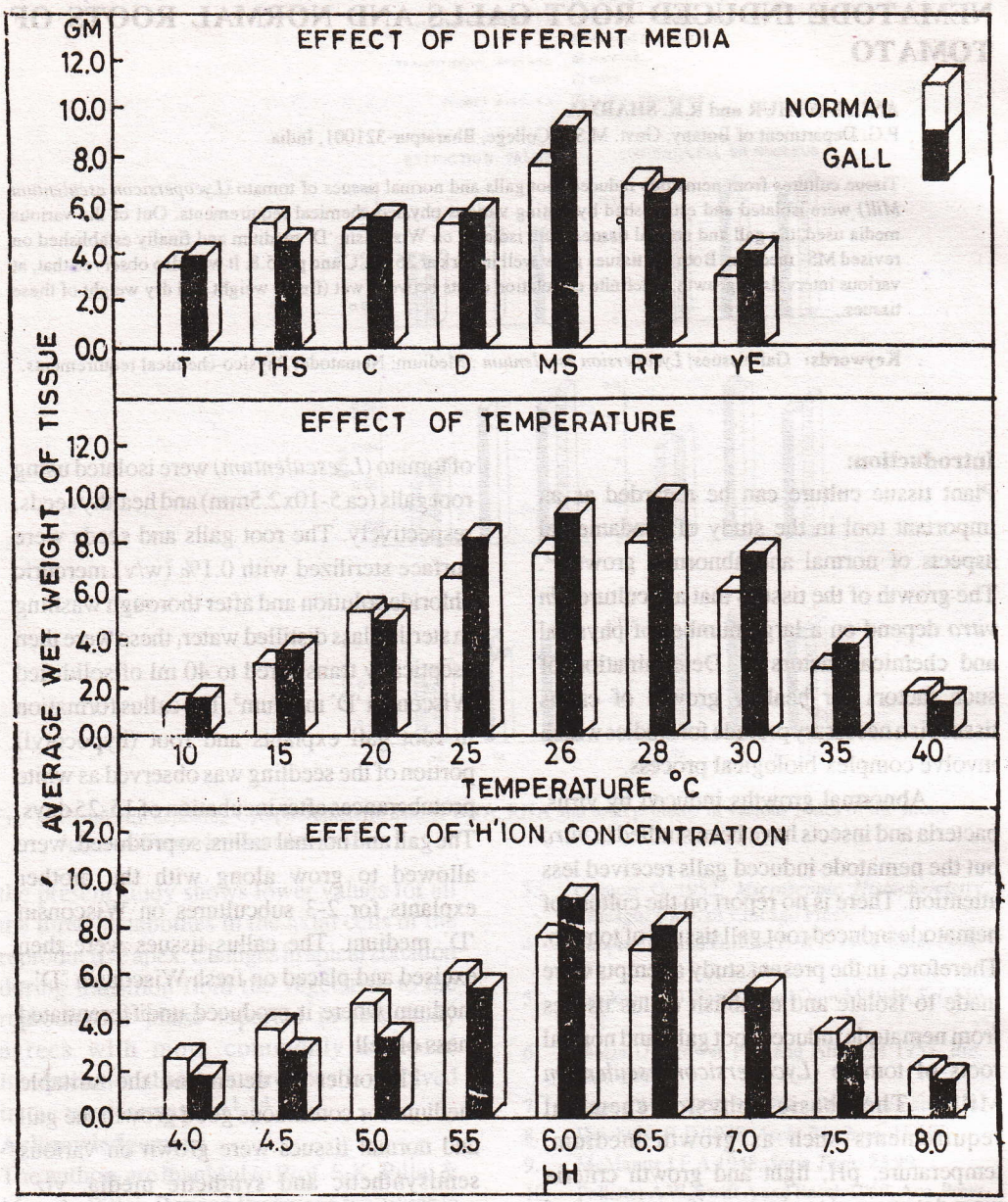


Fig. 1 : Comparative growth of gall and normal tissues of *Lycopersicon esculentum* on different nutrient media, temperatures and pH.

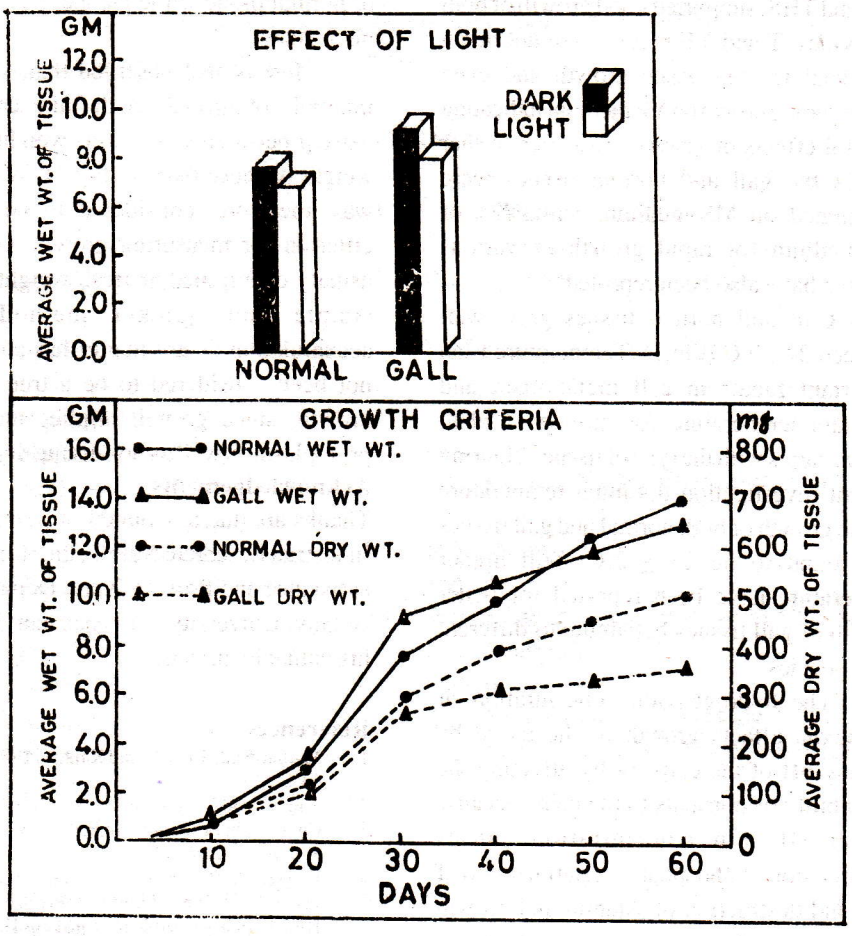


Fig. 2. : Comparative growth of gall and normal tissues of *Lycopersicon esculentum* in light and dark conditions; and wet/dry weight at different periods of growth.

medium[8]. Modified MS-medium[1] was finally used for maintenance and other experimental studies of gall and normal tissues.

Effect of temperatures (10-40°C) and ph (4.0-8.0) was observed on the growth of both the tissues. These tissues were harvested at different intervals for determining wet (fresh) weight and then dried at 60°C for recording the dry weight. Effect of light on tissues growth was also studied by subjecting them in light (4000 lux 16 hrs/ day).

**Results and Discussion**

Growth of gall and normal tissues varied with the compositions of different media (Fig. 1). The best growth of gall (9.2 g/flask) and

normal tissue (7.6 g/flask) was recorded on MS-medium, whereas other media viz, RT, D, C and THS, supported good growth of both the tissues. T and YE media were not much beneficial for the tissue growth and even tissues turned brown on YE medium indicating harmful effects of yeast extract. For further studies the gall and normal tissues were maintained on MS-medium. Suitability of MS-medium for rapid growth of various cultures have also been reported [3,4,9].

Gall and normal tissues grew well between 24-30 C (Fig.1). Temperature is an important factor in cell metabolism and optimum temperature for growth of plant tissues varies with the type of tissue [10]. During present investigation optimum temperature for the growth of both normal and gall tissues was found to be 26 ± 2 C. Still higher temperature have been reported for better growth of gall tissues belonging to different plant species[11].

The hydrogen ion concentration of the media affects growth of the tissue by altering pH of the cells, or by affecting the availability of nutrients to the cells, because higher 'H' ion concentration causes precipitation of phosphates, gelatinization of agar and destruction of vitamin and growth regulators [12]. The gall and normal tissues showed good growth on the media from pH 5.5 - 6.5 with a maximum at pH 5.8-6.0 (Fig.1). Interestingly it was observed that growth of gall tissue was less as compared to normal tissue over pH ranges from 4.0-5.5 and 7.5-8.0. The pH 4.0 - 4.5 rendered the medium semi solid which resulted in the poor growth of sunken tissues.

Growth of both the tissues was observed to be better in the dark as compared to that in light conditions (Fig.2). Under the influence of light a change in colour from creamish to brown-red was observed in case of normal tissue while the gall tissue remain unchanged.

It was also observed that, at various intervals of growth, a definite correlation existed between wet (fresh) weight and dry weight of these tissues (Fig.2). Wet weight was, therefore, considered to be a better criterion for measuring growth. Growth is usually determined as fresh weight, being a simple and quicker method[13]. The accumulation of dry matter has some times not been considered to be a true index of growth, since growth implies increase in protoplasm as well as non-living dry matter[14].

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