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In-vitro propagation of *Solanum lycopersicum L.*

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Abstract
Solanum lycopersicum L. is a major vegetable crop which has a tremendous popularity and grown in all parts of the country. Tomato seeds were used as the explant in the In-vitro propagation. The explants were further transferred into Murashige and Skoog media (M.S media). The explants germinated within a week and further callus formation was observed after 10 days.The early stage of shoot initiation was observed after 2 weeks in prior to the callus formation. The results are summarised, discussed and presented in this paper.

Keywords: *Solanum lycopersicum* L., *In vitro* propagation, seed culture, Tomato

Introduction
In the present research paper, *In vitro* propagation of *Solanum lycopersicum* L. was performed. Tomatoes are good source of vitamin C and other antioxidants. Tomato seed is a convenient research object because of its relatively simple structure (Henk *et al*., 1998) Cultures are conducted in aseptic environment with the assurance of production of disease-free plants and without of risk of reinfection (Karim and Kayum, 2007) [3]. The most important factor for the yield of tomato seeds is successive healthy plants. Germination of the seed is a critical stage, because the rest of the plant life is directly depended upon the rate of its germination. (Qadir and Shahzadi, 1969) [8]. Compared to the traditional nursery bed, seedlings that are raised using portray germinate early and are vigorous (Duraisamy V M, 2017) [2]. This technique has the potential to be applied in unfavourable environmental conditions and enables the production in low cost, high quality, virus free tomato plants. In the modern world, tomatoes are one of the vital horticultural cash crops.

Material and Methods
The present research work was conducted at Tissue culture laboratory, Department of Botany, St. Joseph's college, Bangalore. The plant material used was Tomato (*Solanum lycopersicum* L.). It belongs to the family Solanaceae. The seeds were purchased from Seedscare Agro India. The seeds were surface sterilized with 10% teepol solution and it was been washed for around 15mintues. Later, it was been washed thoroughly under running tap water until the foam formation disappears. Then the seeds were transferred into a container which is filled with auto claved distilled water. The seeds were been transferred from auto clave distilled water to a bottle containing 0.1% mercuric chloride and further washed for 15minutes. Subsequently, the seeds were accompanied by 3 times rinsed with double distilled water. Transfer the seeds to hydrogen peroxide solution with the wash of less than 1 minutes and further autoclaved distilled water.

Fig 1: 1. Tomato Seeds; 2. Tomato flower; 3. Tomato plant

Nutrient Medium

Table 1: Growth hormones

1.	BAP	2mg/L
2.	IAA/2,4 D	2mg/L

The different combinations of four types of modified MS media were prepared and tried i.e., MS1, MS2, MS3, MS4 with various combination of growth regulators. Out of these, the best result was observed in MS1 and the combination of MS1 medium along with hormones as mentioned in table 1.

Result

Table 2: Concentration of growth regulator

Media	Auxin/ Conc mg/L	Cytokinin/Conc Mg/L	After10days	After 24 days	After 40 days
MS 1	2 mg/L	2mg/L	Seeds germinated	Callus formation seen	Shoot arises
MS 2	3 mg/L	3mg/L	No Growth	No Growth	No Growth
MS 3	4 mg/L	Mg/L	No Growth	No Growth	No Growth
MS 4	5 mg/L	5 mg/L	No Growth	No Growth	No Growth

As mentioned in table 2, a total of 4 medium with various concentration of growth regulators was used in this research. The best result was obtained from MS 1 out of all the other medium. In MS 1 after 10 days initiation of seed germination was observed, followed by massive induction of callus was seen after 24 days. After 40 days of culture, shoot formation was been observed. The callus observed was in cream colour. In the other medium there was no growth observed. Explants can be inoculated on the culture media in polar (straight up, with the physiological base in the medium) or poplar (upside down, physiological base out of the medium) orientation. (Poonam Bhatia *et al*., 2004) [7]. The polar orientation regenerates shoot than the non-polar orientation. In tomato adventitious shoot regeneration can be achieved either directly or indirectly through an intermediate callus phase (Geetha *et al*., 1998) [11]. The time period required for the successful competition of tissue culturing depends upon the type of explant used.

Discussion

In this study, the tomato seeds were cultured using modified MS 1 media. Both IAA and BAP growth regulators were used in order to induce shoot development in the seed cultivation. Different surface sterilization chemicals such mercuric chloride, hydrogen peroxide, teepol were used for sterilization of tomato seeds and found that 0.1% mercuric chloride for 15minutes was more effective resulting in high germination rate with no contamination. The dose of cytokinin is known to be critical in multiple shoot induction. (Abdellatef and Khalafallah, 2007) [18]. An efficient sterilization procedure needs to be done in order to establish an efficient *in vitro* regeneration protocol in. (Duzyaman *et al*., 1994) [12] found that the degree of shoot regeneration was in the order of leaves ≥ cotyledons≥ hypocotyls, and all cultivars responded similarly. (Plastira and Perdikaris, 1997) [14] reported differential regeneration frequency of various explants in the order of hypocotyl> cotyledon> leaf. Tomato callus responds differently to varying nutrient concentrations. (Cano *et al*., 1990) [15] used two modified MS media (viz. NK and NB). Four major cytokinins viz. zeatin, 2-iP, BA, and kinetin, can be used either separately or in combination with auxins (Table 1) for organogenesis in tomato (Poonam Bhatia *et al*., 2004) [7]. High concentration levels of cytokinin with lower levels of auxin have always improved callus induction and hence response of tomato in absence of auxin supplemented with additional cytokinin was evaluated (Vageeshbabu and Boopal, 2016) [4] but in this study cytokinin and auxin was used in equal proportion. Although seed germination is an internally regulated process influenced by genotype external factors such as light, temperature, moisture and the presence of certain chemical compounds (phytohormones or organic acids) also strongly influenced this process (Finkelstein, 2004; Kucera *et al*., 2005) [16, 17]. In this experiment, germination percentage increased when the seeds were soaked in water for 24 hours. This protocol produces disease free healthy plants in large scale within a short period of time.

Fig 2: Seed Germination

Fig 3: Callus formation with initial stage of shoot

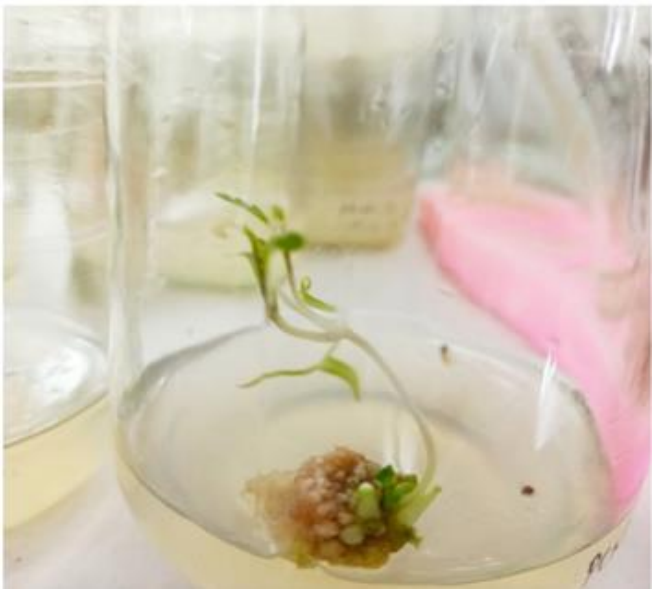

Fig 4: Shoot Development in 24 days

Fig 5: Shoot development in 40 days

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