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Review Article

FACTOR AFFECTING TISSUE CULTURE EXPERIMENTS OF WOODY PLANT: A REVIEW

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Abstract: ***Tissue culture is primarily used for plant propagation and is often referred to as micropropagation. Another way to define the plant tissue culture is the culture of plant cells or plant tissues in a synthetic culture medium under controlled aseptic conditions. The controlled conditions give the culture a suitable micro environment for the successful growth. The tissue culture techniques are successfully used to overcome barriers for plants which are difficult to propagate. Many factors can affect the in vitro establishment as well as micropropagation of different woody plants. Type of explants, physiological status of explant, genotype and age of donor plant, media, plant growth regulators, photoperiod, antioxidants etc. This paper reviews all these aspects related to tissue culture experiment of woody plants.***

Key words: Tissue culture, woody plants.

INTRODUCTION

Plant tissue culture forms the backbone of plant biotechnology, which is comprised of micropropagation, induction of somaclones, somatic hybridization, cryopreservation and regeneration of transgenic plants. Plant cell and tissue culture has already contributed significantly to crop improvement and has great potential for the future [1]. Research efforts in plant cell and tissue culture have increased dramatically worldwide in recent years including efforts made in developing nations. Plant cell and tissue culture is defined as the capability to regenerate and propagate plants from single cell, tissue and organ under sterile and controlled environmental conditions [2,3]. The controlled conditions provide the culture an environment conducive for their growth and multiplication. These conditions include proper supply of nutrients, pH of medium, adequate temperature and proper gaseous and liquid environment. Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis [4]. Endangered, threatened and rare species have successfully been grown and conserved by micropropagation because of high coefficient of multiplication and small demands on number of initial plants and space. In India, tissue culture research began nearly five decades ago with the first report on production of test tube fertilization [5].

The tissue culture techniques are successfully used to overcome barriers for plants which are difficult to propagate. Many factors can affect the *in vitro*

establishment as well as micropropagation of different woody plants. Type of explants, physiological status of explant, genotype and age of donor plant, media, plant growth regulators, photoperiod, antioxidants *etc.* This paper reviews all these aspects related to tissue culture experiment of woody plants.

Factor affecting *in vitro* culture of woody plants:

Effect of plant growth regulators: Four broad classes of growth regulators, namely auxins, cytokinins gibberellins and abscisic acid are important in plant tissue culture. The callus induction and organogenesis of tissues become feasible only on the addition of one or more of these classes of hormones to a medium. The type and extent of organogenesis in plant cell cultures depend on the ratio of auxin to cytokinin [6].The ratio of growth regulators required for root and shoot induction is not the same universally. Generally both (auxin and cytokinin) are added to the culture media to promote morphogenesis.

The idea that morphogenesis could be manipulated by chemical treatment, was truly refined after the discovery of Kinetin and it was hypothesized by Skoog and Miller [6] that it is the auxin:cytokinin ratio which controls the development in cultural tissues. The hypothesis has been tested with large number of cell and tissue culture and many deviations have been observed. It is now well established that plethora of other factors modify or negate the response of auxin:cytokinin ratio.

Effect of cytokinins: Cell division is initiated when cytokinins are added to a culture medium. It induces shoot formation as well as axillary shoot proliferation and inhibits root formation. When a low auxin to cytokinin ratio added or provided to medium, adventitious and axillary shoot proliferation occurs. The most striking influence of bud breaks and shoot multiplication has been found with cytokinins [7]. BAP and Kn are the most common used cytokinins for micropropagation. In the current investigation both cytokinins, when incorporated singly in the basal medium, induced shoot bud at all levels in nodal segment and shoot apex explants. Maximum shoot bud induction was observed when nodal segment and shoot apex explants were inoculated on basal medium containing 2.0 and 2.5 mg/l BAP, respectively. These results were in accordance with results obtained by Kumari [8]. with respect to plant growth regulator (BAP). She observed maximum rate of shoot multiplication at 5.0 mg/l BAP in *Bauhinia variegate.* However in the present study maximum shoot proliferation was observed at 2.0 and 2.5 mg/l BAP. This variation might be due to genera differences. Role of BAP for shoot induction was also observed by Ali et al. [9] in *Psidium guajava*, Widiyanto et al. [10] in *Tecton grandis*, Golozan and Shekatendeh [11] in pomegranate, Kumar and Singh [12] in *Prosopis cineraria*, Borthakur et al. [13] in *Albizza chinesis*, Choudhari et al. [14] in *Syzygium cuminii* and Bensaad and Milad [15] in *Punica granatum.* These results were in close agreement with present study. However range of BAP supplementation was 0.5 – 10.0 mg/l might be due to different genera and type of explant used.

Effect of auxins: In nature different hormones of auxin group play a key role in elongation of stem, internodes, tropism, apical dominance, cell division, abscission and rooting. Auxins differ in their physiological activity and to the extent that they are bond to cells, metabolized or move through tissues. Exposure to high levels of auxins particularly 2, 4-D results in suppressed morphogenic activity and promote rapid proliferation of cells. In culture medium, auxin is usually added for callus induction and cell growth, somatic embryogenesis, root, shoot initiation and stimulation of growth from shoot apices and shoot tip cultures. At a low concentration auxins promote root formation while at a high concentration it promotes callus induction.

Auxins are mostly used for rooting and callus induction. Rooting response of shoot was reported to be controlled by growth regulators in the medium [16], basal salt composition [17-19]. For most of the species auxin is required to induce root. NAA and IBA are most commonly used for root induction [16]. By the use of the IBA in many plants such as *Lycopersicon esculentum* [20], *Hydichium roxburgii* [21], *Carnation* [22] *in vitro* rooting was obtained.

Interactive effect of cytokinins and auxins: The ratio of auxins and cytokinins is important with respect to morphogenesis in the culture system. For embryogenesis, callus initiation and root initiation the requisite ratio of auxins to cytokinin is high, while the reverse leads to axillary and shoot proliferation.

A systematic approach to organogenesis *in vitro* started when Skoog and Coworker [6,23-25],

demonstrated that in tobacco differentiation of shoot/root can be induced more or less by manipulation of the balance of IAA and adenine/Kinetin in the culture medium. On the basis of their observations, Skoog and Miller [6] rejected the concept of specific organ forming substances proposed by Went [26] and regarded organ formation to be determined by quantitative interaction i.e. ratios rather than absolute concentration of substances participating in growth and development. A high level of auxin to cytokinin is root promoting, whereas the opposite condition, favors' shoot bud induction. This so called Skoog-Miller model holds good for many species.

Effect of explant: Explants are small pieces of plant parts or tissues that are aseptically cut and used to initiate a culture in a nutrient medium. Plant parts like shoots, leaves, stems, flowers, roots *etc*. can be used as explant in plant tissue culture. The source of explants has been considered a critical variable for *in vitro* culture in pomegranate. All explants are not equal in terms of regenerability. It is likely that different selective pressures would be exerted against different explants. This could result in different frequencies and spectrums of regeneration among plants from different explants.

Type of explant is one of the important factors in optimizing the tissue culture protocol. Type of explants like leaf, petiole, cotyledonary leaf, hypocotyle, epicotyle, embryo, internode and root explant significantly effect on tissue culture process of plants [27-33]. This may be due to the different level of endogenous plant hormones present in the plants parts.

Effect of media: Growth and morphogenesis of plant tissues *in vitro* are largely governed by the composition of the culture media. Although the basic requirements of cultured plant tissues are similar to those of whole plants, in practice, nutritional components promoting optimal growth of a tissue under laboratory conditions may vary with respect to the particular species. Media compositions are therefore formulated considering specific requirements of a particular culture system. The composition of a medium is formulated considering the specific requirements of a given culture system. The media used may be solid or liquid in nature. The selection of solid/liquid medium is dependent on the better response of a culture. Different types of media are used in laboratory for specific purpose i.e. White's medium for root culture, MS medium for organogenesis and regeneration, B_5 medium for cell suspension and callus culture, N_6 medium for cereal anther culture, Nitsch's for anther culture *etc*.

The composition of growth medium is an important factor affecting growth and morphogenesis of plant tissues. Plant tissue culture medium consists of macronutrients, micronutrients, vitamins, amino acids or other nitrogen supplements, carbon sources, organic supplements, solidifying agents and growth regulators. Murashige and Skoog [34] is the most commonly used medium in plant tissue culture. The B_5 [35], N_6 [36] and Nitsch and Nitsch [37] have been widely used for many plant species. Moreover, for culture of woody species, the DKW [38] and the WPM medium [39] are used. The growth medium is selected for the purpose of tissue culture and for the plant species [40].

Effect of antioxidants: Under *in vitro* culture condition there is problem of browning of media in many plant species (woody plants) due to leaching of some phenolic substances and/or secondary metabolites from cut surfaces of explants. These compounds oxidize later and turn the media brown. After oxidation these component become toxic to the explants and results in retardation of growth and eventually leads to complete failure to survivability of the explants. Medium browning is a major problem in pomegranate due to the exudation of high amount of phenols, especially in mature explants (nodal segment and shoot apex). In perennial fruit crops like pomegranate, establishment of explants requires special procedures to escape the problem that associated with exudation of phenolic compounds from cut surface. Different attempts has been made to eliminate browning problem in woody plant species like pre-socking of explants in antioxidants (activated charcoal, PVP, ascorbic acid, citric acid *etc*.) solution, incorporation of oxidants into medium, incubation of culture in to dark period and frequent subculturing of explants. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals. Free radicals can cause damage or cell death. Antioxidants remove free radical intermediates and inhibit other oxidation reaction.

Under *in vitro* condition accumulation of inhibitory substances and/or secondary metabolites in the growth medium is a major problem more frequently

associated with micropropagation and root formation in woody perennial [41]. These substance leaches from the cut surface of the explant and oxidizes later results in turning of media brown. Browning of tissue process is caused by the oxidation of tannin and polyphenols and the formation of guinones which are highly reactive and toxic to the tissue. Phenolic compounds contain at least one hydroxyl group on the benzene ring. Several oxidases such as monophenolase (Tryosinase), polyphenol (Catecholoxidase) oxidized the hydroxyy group resulting in the formation of guinone and water [42], [43]. Plant tissue contains these substances in separate pools or compartments. During tissue wounding or senescence these pools are integrated and oxidation process is initiated [44]. After oxidation these component become toxic to the explants and results in retardation of growth and eventually lead to complete failure to survivability of the explants. Medium browning is a major problem in pomegranate due to the exudation of high amount of phenols, essentially in nodal segment and shoot apex explants [45].

Effect of photoperiod: Photoperiodism is the physiological reaction of organisms to the length of day or night. Photoperiodism can also be defined as the developmental responses of plants to the relative lengths of the light and dark periods. Hence, it should be emphasized that photoperiodic effects relate directly to the timing of both the light and dark periods.

The ability to co-ordinate certain developmental processes to particular times of the year when environmental conditions are likely to be more favorable confer distinct advantages. Timing reproduction to spring time so that vulnerable young offspring have the maximum possible time to develop before experiencing the harsh conditions of winter, for example, would result in a greater survival rate of the offspring. There is thus a selective advantage for plants and animals that have acquired mechanisms enabling them to sense seasonal differences through the detection and response to changes in photoperiod. The photoperiod is the amount of light and darkness in a daily cycle of 24 h. Photoperiod controls many developmental responses in animals, plants and even fungi. The response to photoperiod has evolved because day length is a reliable indicator of the time of year, enabling developmental events to be scheduled to coincide with particular environmental conditions [46].

Several aspects such as wavelength (quality), intensity (quantity) and duration of light are important factors affecting plant growth [47]. Although light is an important factor in micropropagation, reports on the effect of artificial light intensities on plant growth, particularly of orchids, are rather scarce. This is chiefly because the higher light intensity necessary for some plants to mature are difficult to achieve and because of the space required by some plants at this stage. For relatively short time periods, plant performance probably reflects the photosynthetic process.

Generally, plant growth and development are affected by both internal factors including genotype and plant hormones and external factors such as light, duration, temperature and moisture supply. This result may be due to the interaction between light intensity and internal factors which directly affect plant growth. The suitable light intensity and duration will give the best result of product [48].

Photoperiodism is the physiological reaction of organisms to the length of day or night. Photoperiodism can also be defined as the developmental responses of plants to the relative lengths of the light and dark periods. Hence, it should be emphasized that photoperiodic effects relate directly to the timing of both the light and dark periods.

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