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Effect of media and media components on shoot proliferation and root development in micropropagation of pomegranate (*Punica granatum* L.)

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Abstract
In this study the effect of media, media components and growth regulators in different concentrations were tested for shoot and root development in micropropagation of pomegranate. The explants were collected from healthy plants which were authenticated in the morning, third node from the shoot tip. For shoot development The MS media was supplemented with different concentrations of alpha-naphthalene acetic acid (NAA), indole-3-butyric-acid (IBA), 6-benzyl aminopurine (BAP) and kinetin and their combinations. For rot development MS media was supplemented with different concentrations of alpha-naphthalene acetic acid (NAA), indole-3-butyric-acid (IBA) was tested. It is observed that MS media when supplemented with ADS 15mg/l + NAA 2.0 mg/l + BAP 2.0 mg/l had given highest maximum shoot proliferation, number of shoots, number of leaves along with highest number of nodes and intermodal length and survival per cent, followed by MS media supplemented with ADS 15mg/l + NAA 1.0 mg/l + BAP 2.0 mg/l. MS media when supplemented with IBA 0.6 mg/l had given maximum number of roots per shoot, highest number of shoots producing roots and more number of new leaves followed by MS media supplemented with 0.6 mg/l NAA.

Keywords: Media, media components, Sterilization, micropropagation, pomegranate, shoot proliferation, root growth

Introduction
Pomegranate (*Punicagranatum* L.) Var. Bhagwa belonging to the family Punicaceae is grown in tropical and subtropical countries. The name Pomegranate is derived from two Latin words Pomum meaning apple and granatus meaning full of seeds. Pomegranate is commercially cultivated for fresh consumption of fruits. It is highly nutritive and is rich in proteins, fats, fiber, carbohydrates, minerals like Fe, Ca and antioxidant components like phenols, pigments and tannins. Apart from its high demand for fresh fruits and juice, the processed products like pomegranate wine, tea and candy are also gaining more importance in the world trade. The tree is also valued for its pharmaceutical properties because it cures diseases like dyspepsia and leprosy. The rind of the fruit and the bark of pomegranate tree are also used as a traditional remedy against diarrhea, dysentery and intestinal parasites. Fruit rind, bark of the stem and the roots are widely used in tannin production (Pal *et al*., 2014) [17]. Pomegranate has the ability to withstand harsh and adverse climatic conditions. The versatile adaptability, hardy nature, low maintenance cost, production of steady but high yields, better keeping quality, fine table and therapeutic values have lead to a steady increase in the area and production of pomegranate in India. To get true to type planting material, pomegranate is commercially propagated by stem cuttings (hard wood cutting) or by air layering. However, it has several limitations like low success rate, very slow procedure and new plants require at least one year for establishment and only limited number of plants can be produced through these methods. This results in non-availability of plantlets throughout the year.
In developed countries micro propagation is being commercially employed for mass production of disease free planting material for many fruit crops. Micro propagation offers the possibility of rapid clonal production of plantlets on commercial scale where, the demand is high and supply is low or for establishment of planting material for special uses in a compressed time frame Plant growth regulators are not nutrients, but they influence growth and development of plants.

They are generally produced naturally in plants; hence they are added selectively to culture media. Since phyto-hormones are the key compounds in tissue culture studies; hence a good deal of effort is required to determine their optimum levels (Skoog and Miller 1957) [21]. The cytokinin 6-benzylamino-purine (BAP) is the most commonly used growth regulator for shoot regeneration in a variety of plants. In some cases, mixed cytokinins have been used in combination for shoot regeneration systems. In pomegranate to a combination of cytokinin(s) and auxin has been employed to for culture initiation and shoot proliferation/multiplication; and addition of auxin has been found essential for obtaining good rooting. Different factors favouring root initiation have been tried. Auxin IBA was found the most effective of all auxin types. In contrast, IAA though being natural was least effective as it got degraded due to light. In pomegranate, Drazeta (1997) [4] found that MS medium supplemented with 0.1 mg/l IBA was most effective for rooting.

The selection or development of the culture medium is vital for success in tissue culture. In general, medium contains inorganic compounds like salts, and organic compounds like plant growth regulators, vitamins, carbohydrates, hexitols and a gelling agent. In addition, the medium can also include amino acids, antibiotics or natural complexes.

Various mineral salt formulations have been used for *in-vitro* cultures. However, full strength minerals salts are not always optimum and different formulations may work better at different stages. Modifications with respect to different constituents like phytohormones, sucrose, agar concentrations and other additives like PVP, AC, coconut milk, *etc*., are usually done in order to ensure a better *In-vitro* response. In the past, several workers have studied different factors affecting *in vitro* plant regeneration of pomegranate and other crops. It is very difficult to produce plants *in- vitro* in case of woody plants. Attempt is made to study the effect of media and media components for better shoot proliferation and root development.

Materials and Methods

Basal Media: Yang and Ludders (1993) [26] tried different media, *viz*., MS, Gamburg's B5 and WPM for *in vitro* propagation of pistachio (*Pistacia vera* L.) and observed that, MS media resulted in significantly better shoot formation than other media. As compared to MS media less shoot proliferation was observed in mangosteen cultures on WPM.

Sucrose

The majority of media used for *in vitro* shoot regeneration and rooting invariably contains 2-3% (w/v) sucrose as a source of energy and osmotic agent. Root induction is inhibited when sucrose is omitted from the media. Adventitious root formation is a high energy requiring process and inhibition of rooting by sucrose omission can be explained by starvations of heterotrophic shoots. Rahman *et al*., (1992) [28] reported increased rooting with 40 g/l of sucrose as compared to 20 g/l and 60 g/l of sucrose.

Agar

The solidifying agent used in tissue culture is agar, which is the costliest ingredient and often contains impurities which affect the growth of the cultured plant tissue and organ. So many researchers use more purified forms of agar like plytagar, bacto-agar or meragar. Physical and chemical analysis revealed large differences in response of cultures to types of agar used.

The optimum concentration of agar is 0.8 to 1% (w/v).

In *Malus*, Singh (1982) reported promotive effect of agar at low concentrations on tissue growth. This may be due to increased uptake and availability of nutrients through leaves or tissues submerged in the media.

Decrease in rooting performance with increasing agar concentration and 15 g/l of agar inhibits completely. The optimum concentration for root induction was 6 g/l in rose (Rahman *et al*., 1992) [28].

Plant Growth Regulators

Growth regulators (Phytohormones), are not nutrients but they influence growth and development. Generally growth regulators are produced naturally in plants. But in cultures they are added selectively to culture media as sufficient quantities of growth regulators are not manufactured in cultures. As the phytohormones play a key role in tissue culture studies, efforts are required to determine optimum levels. The formation of adventitious shoots or roots was first determined by Skoog and Miller (1957) [21] through discovery of the regulation of organ formation i.e., shoots and roots by changing the ratio of cytokinin / auxin, when the ratio of cytokinin / auxin is high, it favours the formation of shoot but root formation is inhibited. The reverse favours the root formation, whereas the intermediate ratio induces unorganized growth of callus tissue. Regulation of plant growth and development including plant regulation from isolated cell and tissue is under the control of hormones. Among various hormones, auxins as IAA, IBA, NAA and 2, 4-D, cytokinins as 6- Benzyl amino purine (BAP), Kinetin, Zeatin, 2-iso pentenyl amino purine (2 ip), TDZ, gibberellins and ethylene are important.

Generally among cytokinins, benzyladenine is the most commonly used at a concentration up to 25 µm for shoot regulation in a variety of explants. Sometimes, mixed cytokinins are used in combination for shoot regeneration system of various fruit trees, *viz*., Bael, jamun (Yadav *et al*., 1990) [25], Ber, aonla and kiwifruit. For root development, auxins such as 1AA, 1BA and NAA have been used individually or in combination.

Patil *et al*., (2011) [18] reported that healthy and well-formed plants from nodal explants were developed in pomegranate var. Bhagwa when nodal segments were cultured on two different media at full strength MS medium and WPM. The media was prepared as a basal medium supplemented with 0.2 to 2.0 mg/l BAP, 0.1 to 1.0 mg/l NAA, 0.5 to 2.5 mg/l silver nitrate ($AgNO_3$) and 10 to 50 mg/l adenine sulphate for establishment. The nodal explants grown on MS medium containing 1.8 mg/l BAP, 0.9 mg/l NAA, 1.0 mg/l silver nitrate and 30 mg/l adenine sulphate shown highest proliferation rate (10 to 15 shoots/explant) in establishment stage. The number of leaves per explant was also maximum (15 to 20 leaves/explant) on proliferation medium containing 0.4 mg/l BAP and 0.3 mg/l NAA. The plantlets grown on MS medium were found to have better survival compared to WPM medium. Rooting response was same in media with 0.5 mg/l NAA and 0.5 mg/l IBA, whereas roots were thicker in the medium containing 0.5 mg/l IBA.

The media supplemented with different concentrations (2.3, 4.7, 9.2 and 18.4 µM) of Kinetin (Kn) along with 0.54 µM NAA in proliferation stage. WPM proved to be more efficient when compared to MS medium. Highest number of nodes, shoot length and leaf number were recorded at 9.2 µM concentration of Kn and half strength WPM supplemented with 5.4 µM NAA was effective for rooting of shoots.

Other Additives

Many other additions to nutrient media serve various purposes. Antioxidants are added if there is excessive browning of the explant and they retard oxidation of the explant. Some of the antioxidants are citric acid, ascorbic acid, pyragallol and phloroglucinol. Sometimes when there is excessive tissue discolouration of the medium and explant, two absorbents, polyvinylpyrolidone and activated charcoal (0.1-0.3%) are used. Addition of adenine sulphate improved shoot multiplication in *Nepenthes khasaina* and *Acacia catechu*.

Amin and Jaiswal, (1988) reported that, PVP has been included in media to avoid explant browning. PVP absorbs phenols through hydrogen bonding by thus preventing oxidation of phenols.

Activated charcoal (AC) is also added to media of plant tissue culture because of its beneficial effects on many aspects of *in-vitro* regeneration. Activated charcoal have been attainted to various factors such as, darkening the media removal of inhibitory compounds adsorption of growth regulations; adsorption of agar impurities; adsorption of phenols produced by the tissues and gradually releases the adsorbed products.

The positive effect of activated charcoal on the rootability of the micro-shoots in the rooting expression media can be attributed to the reduction of light at the base of the shoots, thus providing an environment conductive to the accumulation of photo-insensitive auxin or cofactors.

Liwu *et al*., (2003) reported half-strength MS medium with 0.5 mg/l NAA + 1.0 mg/l activated charcoal + 20 g/l sucrose was optimum for the induction of adventitious roots. Addition of 0.1 per cent activated charcoal to the primary medium was efficient for the absorption of phenolic substances, prevention of explant browning and acceleration of rooting.

Cultural Environment

The factors of culture environment influenced the *in vitro* growth and differentiation of plant tissue cultures. The cultural environment, which is the result of interaction between plant material, cultural media, type of culture vessel and external environment of the culture room, has tremendous influence on a tissue culture system. This may include the physical form of the media, pH, humidity, gaseous atmosphere, light and temperature.

Physical form of Media

The agar solidified media is most successful but, the use of liquid media was increasingly evaluated for the tissue culture purpose (Keathley, 1983) [8]. The advantages of liquid media for enhancing shoot multiplication, growth, somatic embryogenesis or rooting have been reported for several species. Through the mechanism involved in this improved performance is not known, may be the absence of gelling agent increasing the availability of water and dissolved substances to the explant could be the reason.

pH of the Media

The pH of the plant tissue culture media is generally adjusted to P^H 5.5 to 6. Below 5.5, the agar will not gel properly and above 6.0, the gel may be too firm (Murashige, 1973). Media pH generally drops by 0.6 to 1.3 units after autoclaving. Cultures of some plant tissues cause a pH drop over time that is attributed to the production of organic acids or nitrogen utilization. The media pH was influenced by inorganic salts, carbohydrate sources, gelling agent, activated charcoal and medium storage method. Media P^H can be adjusted by adding 1.0 or 0.1N Hcl or NaOH while stirring the medium. Always P^H adjustment should be done before adding the agar.

The leaf callus of kiwifruit grew better at P^H 7.0 and 7.5, but shoot regeneration and survival were strongly reduced at P^H higher than 5.7. Shoot production increased by reducing the pH to 5.0 in cherry. In some species low pH induced root development. In rose the best pH for rooting was 5.5 and at 7.5 pH root development was completely inhibited (Rahman *et al*., 1992) [28]. Low pH along with dark treatment stimulates rooting. In *Prostanthera straitiflora* and *Coreade cumbeus* low pH (4.0) and dark period of two weeks found optimum for root induction (Williams *et al*., 1985).

Light

The quality and intensity of light affects, the shot growth and morphogenesis in addition to having a role in photosynthesis (Hughes, 1981). Plants cultured *in-vitro* and supplied with carbohydrates as they are heterotrophic. Ziv, (1991) reported that, light regulates the size of leaves and stem as well as morphogenic pathway and therefore, is implicated in pigment formation and vitrification. In some species, the effect of photoperiod may or may not be pronounced. There is no distinct difference in root and plantlet formation between the light 18 hour photoperiod and dark grown cultures of *Prosopis tamaruga* (Jordon, 1987). In blackcurrant higher light levels during proliferation stage of culture increased and time of root emergence. Wang (1992) reported that irradiation has a strong effect on shoot multiplication in pear with an increase in photosynthetic photon flux (PPF) from 10 to 30 µmole m^{-2} sec^{-1}, shoot number and length, shoot fresh and dry weights were increased.

The influence of total darkness versus a 16/8 photoperiod ad of auxins added to the culture medium on the *in vitro* root formation capacity of *Acacia mangium* microshoots of juvenile and mature origin. Rooting of the mature clone was significantly increased by exposing the microshoots to auxins (4 and 6 µM IAA or IBA) in darkness, while the promoting effect of darkness combine with 4 µM IAA was more time restricted for the juvenile origin microshoots. Maintaining the microshoots off both origins on auxins supplemented medium in darkness resulted in a greater number of adventitious roots formed than the standard 16/8 lighting conditions. On the other hand, light stimulated root elongation.

Tyagi *et al.* (2006) investigated effects of various concentrations of maleic hydrazide (MH) (2.0, 4.0, 6.0 and 8.0 mg/l) and three light treatments (16-h, 24-h, 0-h) on *in vitro* rhizome formation and conservation of ginger (*Zingiber officinale* Rosc. cv. Rio de janerio). *In vitro* rhizome formation occurred in all the above treatments. A significant effect of light treatment was observed on survival of culture but not on rhizome formation. More than 50 per cent culture survived upto 14 months on control of medium less than 16 hours and 24 hours light conditions as compare to 20 per cent culture on same medium incubated under dark. A total of 33 genotypes of cultivated and wild species of *Zingiber* were subsequently tested for conservation through *in vitro* rhizome formation on control medium under 16 hours light condition.

Singh and Patel (2014) tested various level of light intensity in pomegranate by keeping the constant temperatures 26 $\pm$ 2° C and 16/8 hour light/dark period in the incubation room. Maximum number of shoots per explant and highest length of shots was recorded under 3000 lux intensity.

Temperature

The temperature influences the growth and development of

explants *in-vitro* just as in the case of *in vivo*. Temperature controls the relative humidity in culture vessel, as it is having an indirect effect on vitrification. Better root and plantlet development in *Prosopis tamaruga* at temperature of 25^{0}C and 30 °C than at 20 °C in MS media containing NAA (0-10 mg/l). When compared to 20^{0}C and 25^{0}C the culture incubation at 30 °C had higher degree of browning and leaf shedding.

The temperature of root zone is important to enhance root growth during acclimatization. For increasing the humidity around the cuttings and for good root activity the media should be warmer than the air. Wang (1992) observed that rooting percentage and the number of roots increased with increased temperature during darkness between 5 and 25^{0}C in pear. A further increase in dark temperature up to 30^{0}C reduced rooting percentage and root number.

Humidity

The growth, development and photosynthesis of plantlets *in-vitro*, are influenced by humidity in the vessel and osmotic potential of media which influences the water relations of plantlets *in vitro*. In plum, the reduction of relative humidity in *in vitro* culture during proliferation resulted in a progressive reduction in weight of shoot cluster and in number of shoots produced. When the relative humidity was reduced during rooting of the shoots the rooting process was delayed.

Results and Discussion

The results revealed that presence of NAA and BAP in combination resulted in good proliferation, (Fig-1) whereas, poor proliferation was recorded when they were used alone. Organogenesis depends upon the auxin / cytokinin ratio and not on their absolute concentrations (Murashiage & Skoog, 1962) [10]. Synergistic effects of BAP and NAA will lead to cell division, formation of nodular meristemoids and finally adventitious shoot bud regeneration. The results were in accordance with the findings of Murkute *et al.* (2004) [13], who reported more shoots per culture (1.9) with 1.0 B.A.P. + 0.5 NAA g/l, in pomegranate. Zimmerman and Swartz, (1994) suggested that NAA and BAP combinations were rewarding in many fruit species. Naik *et al*. (1999) [14] reported cytokinin is effective for shoot regeneration when used in combination with an auxin.

The number of shoots developed per explant was highest in MS medium that used highest concentration of NAA and BAP are known to induce highest adventitious shoots (Murkute *et al.*, 2002) [12]. Naik and Chand (2003) [16] and Kanwar *et al.* (2010) [19] reported 57 per cent and 63 per cent shoot regeneration frequency in explants of pomegranate, whereas there was no shoot proliferation observed in MS medium without any growth regulator. Similar results reported by Naik *et al*. (1999 and 2000) [14] in nodal explant in pomegranate. The addition of ADS (60 mg/l) along with other growth regulators was most effective in inducing shoot multiplication (Table-1) (Fig-2).

The highest shoot length was observed when MS medium supplemented with BAP and NAA and no shoot growth in case of MS medium without any growth regulators. Similar results were obtained by Murkute *et al.*, 2004 [13], as well as Gangamma and Arlikatta (2005) in Jack fruit and Patil *et al.* (2011) [18] in pomegranate (Table-2) (Fig-3). *in vitro*

It can be observed from trend concerning the maximum number of leaves on proliferation in the medium containing 0.4 mg/l BAP and 0.3 mg/l NAA (Fig-3) (Patil *et al*., 2011) [18], while they reported that there was no leaf formation when MS media was not supplemented with growth regulators. Patil *et al.* (2011) [18] reported highest number of leaves was obtained when MS medium supplemented with BAP 2.5 mg/l. It is observed and concluded that MS media when supplemented with ADS 15mg/l + NAA 2.0 mg/l + BAP 2.0 mg/l had given highest maximum shoot proliferation, number of shoots, number of leaves along with highest number of nodes and intermodal length and survival per cent, followed by MS media supplemented with ADS 15mg/l + NAA 1.0 mg/l + BAP 2.0 mg/l.

The minimum number of days (15.33) were recorded in full strength MS medium supplemented with NAA 0.6 mg/l followed by MS medium supplemented with IBA 0.6mg/l (17.66), the possible reason for early root initiation by NAA and IBA might be due to their involvement in ethylene biosynthesis (Arteca, 1990). These results were in conformity with the findings of Naik *et al.* (1999) [14] and Murkute *et al*., (2004) [13].

Addition of auxins to the medium induced rooting in the regenerated shoots. Similar results were reported by Naik *et al.* (1999) [14] that IBA was best rooting auxin. Same results were also reported by Chaugule *et al.* (2007) [3] in pomegranate, Yadav and singh (2011) [25] in wood apple and Ashrafuzzaman *et al.* (2012) [2] in jack fruit. No root development was observed when MS medium was not supplemented with any auxins (Table-3) (Fig-4).

It was observed that per cent of shoots giving roots was highest in MS medium + IBA 0.6 mg/l (T_7) (96.66 per cent) followed by MS medium + IBA 0.6 mg/l (T_7) and MS medium + NAA 0.6 mg/l (T_4) (both 86.66 per cent). The results proved that IBA was found to be best auxin for root development. Similar results were reported by Murkute *et al.* (2004) [13] in pomegranate var. Ganesh as rooting in regenerated shoots from cotyledon derived callus was highest when half strength MS medium supplemented with IBA (Table-4) (Fig-5).

The root length was maximum in case of MS media supplemented with NAA 0.6 mg/l as NAA has been best auxin for root development, similar results was reported by Kantharajan *et al*., 1998; Naik and Chand, 2003 [16]. Root development was not recorded in MS medium without any growth regulators.

Table 1: Number of shoots developed per explant (at 20th day, 40th day and 60th day)

S. No.	Treatments	Number of days			Mean
		20 days	40 days	60 days	
1.	T_1- MS medium + ADS 15 mg/l + NAA 0.0 mg/l + BAP 0.0 mg/l	0.00 (0.70)	0.03 (0.72)	0.10 (0.77)	0.04 (0.73)
2.	T_2- MS medium + ADS 15 mg/l + NAA 0.0 mg/l + BAP 1.0 mg/l	0.03 (0.72)	0.16 (0.81)	0.23 (0.85)	0.14 (0.79)
3.	T_3- MS medium + ADS 15 mg/l + NAA 0.0 mg/l + BAP 2.0 mg/l	0.06 (0.75)	0.30 (0.89)	0.50 (0.99)	0.28 (0.87)
4.	T_4- MS medium + ADS 15 mg/l + NAA 1.0 mg/l + BAP 0.0 mg/l	0.06 (0.75)	0.10 (0.77)	0.16 (0.81)	0.10 (0.77)
5.	T_5- MS medium + ADS 15 mg/l + NAA 1.0 mg/l + BAP 1.0 mg/l	0.36 (0.92)	0.73 (1.10)	0.83 (1.15)	0.64 (1.05)
6.	T_6- MS medium + ADS 15 mg/l + NAA 1.0 mg/l + BAP 2.0 mg/l	0.50 (0.99)	0.86 (1.16)	1.16 (1.28)	0.84 (1.14)
7.	T_7- MS medium + ADS 15 mg/l + NAA 2.0 mg/l + BAP 0.0 mg/l	0.40 (0.94)	0.63 (1.06)	0.66 (1.07)	0.56 (1.02)

8.	T_8-MS medium + ADS 15 mg/l + NAA 2.0 mg/l + BAP 1.0 mg/l	0.40 (0.94)	0.76 (1.12)	0.93 (1.19)	0.69 (1.08)
9.	T_9- MS medium + ADS 15 mg/l + NAA 2.0 mg/l + BAP 2.0 mg/l	0.50 (0.99)	1.16 (1.29)	1.33 (1.35)	0.99 (1.21)
	Mean	0.25 (0.85)	0.52 (0.99)	0.65 (1.05)	
	S. Em. ±	0.04	0.03	0.03	
	CD (P = 0.05)	0.12	0.08	0.1	

Table 2: Length of shoots in cm (at 30th day and 60th day)

S. No.	Treatments	Number of days		Mean
		30 days	60 days	
1.	T_1- MS medium + ADS 15 mg/l + NAA 0.0 mg/l + BAP 0.0 mg/l	0.00	0.00	0.00
2.	T_2- MS medium + ADS 15 mg/l + NAA 0.0 mg/l + BAP 1.0 mg/l	0.08	0.15	0.11
3.	T_3- MS medium + ADS 15 mg/l + NAA 0.0 mg/l + BAP 2.0 mg/l	0.15	0.26	0.20
4.	T_4- MS medium + ADS 15 mg/l + NAA 1.0 mg/l + BAP 0.0 mg/l	0.11	0.27	0.19
5.	T_5- MS medium + ADS 15 mg/l + NAA 1.0 mg/l + BAP 1.0 mg/l	0.78	1.4	1.09
6.	T_6- MS medium + ADS 15 mg/l + NAA 1.0 mg/l + BAP 2.0 mg/l	0.72	1.26	0.99
7.	T_7- MS medium + ADS 15 mg/l + NAA 2.0 mg/l + BAP 0.0 mg/l	0.65	1.18	0.91
8.	T_8-MS medium + ADS 15 mg/l + NAA 2.0 mg/l + BAP 1.0 mg/ll	0.78	1.8	1.29
9.	T_9- MS medium + ADS 15 mg/l + NAA 2.0 mg/l + BAP 2.0 mg/l	1.1	1.88	1.49
	Mean	0.49	0.91	
	S. Em. ±	0.15	0.27	
	CD (P = 0.05)	0.45	0.8	

Table 3: Number of roots developed per shoot

S. No.	Treatments	Days		Mean
		20 days	30 days	
1.	T_1- MS medium	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
2.	T_2- MS medium + NAA 0.2 mg/l	3.33 (6.14)	6.66 (12.28)	4.99 (9.21)
3.	T_3- MS medium + NAA 0.4 mg/l	6.66 (12.28)	13.33 (21.14)	9.99 (16.71)
4.	T_4- MS medium + NAA 0.6 mg/l	16.66 (23.85)	23.33 (28.78)	19.99 (26.31)
5.	T_5- MS medium + IBA 0.2 mg/l	0.00 (0.00)	3.33 (6.14)	1.66 (3.07)
6.	T_6- MS medium + IBA 0.4 mg/l	10.00 (15.00)	20.00 (26.56)	15.00 (20.78)
7.	T_7- MS medium + IBA 0.6 mg/l	23.33 (28.78)	26.66 (30.99)	24.99 (29.88)
	Mean	8.57 (12.29)	13.33 (17.98)	
	S. Em. ±	4.62	3.64	
	CD (P = 0.05)	14.02	11.04	

Table 4: Per cent of shoots giving roots

S. No.	Treatments	Days		Mean
		20 days	30 days	
1.	T_1- MS medium	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
2.	T_2- MS medium + NAA 0.2 mg/l	3.33 (6.14)	43.33 (35.85)	23.33 (20.99)
3.	T_3- MS medium + NAA 0.4 mg/l	36.66 (31.92)	73.33 (59.21)	54.99 (45.56)
4.	T_4- MS medium + NAA 0.6 mg/l	86.66 (68.85)	83.33 (66.14)	84.99 (67.49)
5.	T_5- MS medium + IBA 0.2 mg/l	0.00 (0.00)	13.33 (13.07)	6.66 (6.53)
6.	T_6- MS medium + IBA 0.4 mg/l	46.66 (38.06)	86.66 (68.85)	66.66 (53.45)
7.	T_7- MS medium + IBA 0.6 mg/l	86.66 (72.78)	96.66 (83.85)	91.66 (78.31)
	Mean	37.13 (31.10)	56.66 (46.71)	
	S. Em. ±	10.49	8.99	
	CD (P = 0.05)	31.82	27.27	

Table 5: Length of roots in cm

S. No.	Treatments	Days		Mean
		20 days	30 days	
1.	T_1- MS medium	0.00	0.00	0.00
2.	T_2- MS medium + NAA 0.2 mg/l	0.60	1.00	0.80
3.	T_3- MS medium + NAA 0.4 mg/l	0.50	1.30	0.90
4.	T_4- MS medium + NAA 0.6 mg/l	2.46	2.86	2.66
5.	T_5- MS medium + IBA 0.2 mg/l	0.00	0.23	0.12
6.	T_6- MS medium + IBA 0.4 mg/l	2.13	2.56	2.35
7.	T_7- MS medium + IBA 0.6 mg/l	1.90	2.70	2.30
	Mean	1.08	1.52	
	S. Em. ±	0.31	0.33	
	CD (P = 0.05)	0.95	0.99	

Conclusions

The present investigation revealed that It is observed that MS media when supplemented with ADS 15mg/l + NAA 2.0 mg/l + BAP 2.0 mg/l had given highest maximum shoot

proliferation, number of shoots, number of leaves along with highest number of nodes and intermodal length and survival per cent, followed by MS media supplemented with ADS 15mg/l + NAA 1.0 mg/l + BAP 2.0 mg/l.

MS media when supplemented with IBA 0.6 mg/l had given maximum number of roots per shoot, highest number of shoots producing roots and more number of new leaves followed by MS media supplemented with 0.6 mg/l NAA.

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