International Journal Of

Recent Scientific Research

ISSN: 0976-3031

Volume: 7(11) November -2015

STANDARDISING AXENIC POMEGRANATE EXPLANT PROCESSING FOR MICROPROPAGATION

Manasa Satheesh and Sridharan T.B

THE OFFICIAL PUBLICATION OF
INTERNATIONAL JOURNAL OF RECENT SCIENTIFIC RESEARCH (IJRSR)
http://www.recentscientific.com/ recentscientific@gmail.com

ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 6, Issue, 11, pp. 7544-7549, November, 2015

International Journal of Recent Scientific Research

RESEARCH ARTICLE

STANDARDISING AXENIC POMEGRANATE EXPLANT PROCESSING FOR MICROPROPAGATION

Manasa Satheesh and Sridharan T.B

School of Biosciences and Technology, VIT University, Vellore

ARTICLE INFO

Article History:
Received 15th August, 2015
Received in revised form 21st September, 2015
Accepted 06th October, 2015
Published online 28st November, 2015

Key words:
Bhagwa, MS, WPM, explants, regeneration, ANOVA

ABSTRACT

Bhagwa variety pomegranate (*Punicagranatum*) is a very common commercial crop and favorite fruit crop. In spite of this, production of pomegranate is very low in India as farmers are facing serious threat of diseases attacking pomegranate. Farmers are not confident to step forward and overcome this issue as diseases are capable of affecting the whole farm in less time. Alternative option to overcome the issue and increase the productivity is Micropropagation. It helps in mass production in less time; produces disease free, pest free plant. In this study, the best axenic explant of the pomegranate was standardized by using one way ANOVA, Student-Newman-Keuls Multiple Comparisons Tests by comparison with other explants. Best survival percentage of 82% was found in Young nodal explants using 10% Sodium hypochlorite treatment for about 3 minutes followed by 78% using 0.1% Mercuric chloride treatment for 10 min. Shoot regeneration was observed in MS medium in all the explants rather in McCown'sWPM medium. Highest regeneration response was 82% in Apical shoot tips followed by 63% in young nodal explants and 21% in mature nodal explants.

Copyright © Manasa Satheesh and Sridharan T.B., 2015, This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

The pomegranate is one of the first five cultivated foods in the world. Pomegranate (*Punicagranatum*) is native to the Middle East and South Asia. Bhagwa variety has high acceptance in European market (Babak Valizadeh Kaji *et al*., 2013). Bhagwa tree grows to about five and eight meters tall. It is cultivated at a commercial scale in vast regions across Indian sub-continent, Iran, Caucuses, and Mediterranean regions for its fruits. It has been categorized in 2 families within the *Lythraceae* family, of genus *Punica* and its scientific name is *Punicagranatum* and dwarf pomegranates (*Punicagranatum* L. *'Nana'*) belong to the Punicaceae family (Pushprajsingh and R M. Patel, 2014).The varieties of pomegranate in demand are Mridula, Bhagwa, Ruby, Arakta and Ganesh grown commercially. The superiority to the yield will be shown by Jalore Seedless, G 137 and Ganesh (Soumendra K. Naik *et al*., 2000).

Micropropagation Over Conventional Vegetation

Bacterial blight of pomegranate affects leaves, twigs, and fruits. Infected fruit and twigs are potential sources of primary inoculums (Surinder Kumar, Jitender Kumar Kanwar, 2006). Disease buildup is rapid from July to September. Severity increases during June and July and reaches a maximum in September and October and then declines. Hence, In vitro propagation of pomegranate has been reported through axillary shoot proliferation from nodal segments and also using several parts of the plant which eliminates the disease cause and increases the early maturity of the crop (Raj Deepika and Kamlesh Kan, 2010).

Nutritional Benefit

The fruit is a rich source of minerals, vitamins, antioxidant polyphenols, and tannins. Apart from its demand for fresh fruits and juice, the processed products like wine and candy are also gaining importance in world trade (R. Lokesh *et al*., 2014). One cup of fruit (174 grams) containsiber: 7 grams, Protein: 3 grams, Vitamin C: 30% of the RDA, Vitamin K: 36%, Folate: 16%, Potassium: 12%. (Jaya Singh *et al*., 2014). It fights against Breast Cancer, Prostate Cancer, Lowers Cholesterol, Maternal consumption of Pomegranate also cures Brain damage, regular intake of pomegranate juice decreases the risk of heart stroke, gives immunity (F. Soukhak *et al*., 2011). Each part of the plant has several health benefits. The rind of the pomegranate when ground into powder and diluted with oil cures anal itching (R. Kumar *et al*., 2009). (Jaime A. Teixeira da Silva *et al*., 2013).

Corresponding author:* **Manasa Satheesh
School of Biosciences and Technology, VIT University, Vellore

MATERIALS AD METHODS

Sample collection

The plant of interest "Bhagwa" Pomegranate plants – 10 numbers were procured from Indian Institute of Horticultural Research (IIHR), Hesaraghatta, Bangalore, Karnataka. The plants are labeled and maintained at Genewin Biotech, Hosur, a DBT certified Tissue culture Laboratory – Green house premises. Mother plant pedigree is developed for the future use of conserving its Germplasm. Potted plants procured are maintained in Green house till the end of research. All glasswares and working materials being used were washed thoroughly, dried and autoclaved at 15 psi for 1 hour.

Explant sterilization

The explants preferred for the micropropagation technique were Nodes and Auxillary shoot tips. New emerging shoot stalks of 100 numbers consisting of 6-7 nodes with healthy auxillary shoot tip were excised from the established Mother plant pedigree. The collected shoot stalks were defoliated using secateurs and divided into 3 samples based on the texture and color (C. Sandeep *et al.,* 2013).

1. Soft and tender shoots found on the upper portion of the shoot stalk – Auxillary shoot tips
2. White moderately thick region found on the middle portion of the shoot stalk – Young nodes (YN)
3. Dark very hard region found on the lower portion of the shoot stalk – Mature nodes (MN)

From the defoliated stalks, the nodes were trimmed to 2 – 3 cm length and the auxillary shoot tips of 0.5 – 1.5 cm in length were excisedusing secateurs. The explants were processed in the production laboratory into 2 phases:

Phase 1: Non-clean room practice

The Nodes and Auxillary shoot tips were washed thoroughly in running tap water for 5 min in order to free the dust particles from the explants followed by antiseptic solution treatment using Savlon (1%) for 5 minutes which reduces the possibility of contamination and continued with sterile Demineralized water (DM) wash twice. The explants further treated with antibacterial agent Sterptocycline (0.15%) and Carbendazim (0.1%) for 15-20 minutes.

Phase 2: Clean room practice

Phase 2 surface sterilization involved the detergent wash using Polysorbate 20 for 20 min. DM water wash was done thoroughly till the foam disappeared. Sterilization ended up with the use of surface sterilanttrials. The explants were decanted using DM water where complete removal of surface sterilant was ensured.

Trial 1: It covered the trials of Sodium hypochlorite (NaoCl) which contains chlorine as active gradient with 5.5%. 10%, 20% and 30% of the prepared NaoCl solutions exposed for 3, 5, 7 minutes on the explants.

Trial 2: It covered of the trials of Mercury(II)chloride ($HgCl_2$)at various concentrations 0.1% and 0.2% exposed for 5, 10, 15 minutes on the explants.

***Trial 3*:** It covered the trials using 70% and 90% ethanol exposed for 3, 5, 7 minutes on the explants.

The efficiency of the surface sterilant was compared with the explants.

Preliminary study media (PSM)

According to Vijay M. Patil *et al*., 2011, Jaya Singh *et al*., 2014, the surface sterilized explants are inoculated in PSM which consists of MS + 6BAP – 1.8 mg/l

Media preparation

The universally accepted medium namely Toshio Murashige and Folke Skoog (MS) medium was used for the research work with slight modifications. Also, since the selected species is Bhagwapomegranate which is a woody plant, Mc Cown's Woody Plant Medium (WPM) was also chosen for the comparison with MS media for the research.

Table1 Composition of MS media

INGREDIENTS	g/l (MS)	g/l (WPM)
Ammonium nitrate	1.65	0.4
Potassium nitrate	1.90	-
Potassium dihydrogenortho phosphate	0.17	0.1807
Magnesium sulphatehepta hydrate	0.37	0.17
Calcium chloride dehydrate	0.44	0.0725
Manganese (II) sulfate monohydrate	0.022	22.3 mg
Zinc sulfate monohydrate	0.009	8.6 mg
Boric acid	0.007	6.2 mg
Copper sulfatepentahydrate	0.003	0.25 mg
Ferrous sulfate	0.278	-
Na_2EDTA	0.372	-
Ascorbic acid	0.05	0.05
Citric acid	0.025	0.025
Stock solutions*	1 ml	1 ml
Calcium nitrate	-	0.386
Ferric EDTA	-	37 mg
Inositol	0.1	8.6 mg

* **Stock solutions:** Nicotinic acid – 500 mg/l; Thiamine Hydrochloride – 1000 mg/l; Pyridoxine Hydrochloride – 500 mg/l; Cobaltous chloride – 25 mg/l; Glycine – 2000 mg/l; Potassium Iodide – 830 mg/l; Sodium molybdate – 250 mg/l

The final pH of the media was set to 5.8 using either NaOH or HCl. The prepared media was poured into bottles of about 50 ml and capped with lids and placed in trays and autoclaved at 121 °C, 15 psi for 20 minutes. The autoclaved media was checked for the contamination and used for the research work after a couple of weeks.

Table 2 Effect of growth regulator combinations of in MS and WPM media under study

Media	Combinations of Growth Regulators	
	Auxin	Cytokinnin
MS	NAA – 0.01%	6BAP – 2.22 µM
	0.05%	4.44 µM
	0.1%	6.66 µM
	0.5%	8.88 µM
WPM	NAA – 0.01%	6BAP – 2.22 µM
	0.05%	4.44 µM
	0.1%	6.66 µM
	0.5%	8.88 µM

The growth regulators combinations mentioned above were accurately weighed and added to the media.

Tools for dissection

The Surface sterilization and Initiation was carried under Laminar Air flow conditions provided with personal hygiene of wiping the hands with 70% alcohol very frequently and also the surface area. The tools used for the dissection are Foreceps and blade which is supported by a holder. The tools are kept sterile by using the Glass bead Steripot sterilizer of temperature 245±5 °C which helps in sterilizing the tools every 15 min.

RESULTS AND DISCUSSION

Table 3 Effect of exposure to 10% NaOCl in PSM

Concentration Of NaOCl (%)	Exposed Time (min)	M%FC±SD	M%BC±SD	M%NR±SD	M%SE±SD
		Apical shoot tips			
10	3	15±8.4^{b}	18±10.6^{a}	6±2.1^{h}	61±41^{b}
10	5	5±0^{e}	4±0.7^{e}	52±33.2^{d}	39±24^{e}
10	7	3±2.8^{g}	1±4.2^{g}	77±49.4^{a}	19±8.4^{g}
		Nodes			
		Young Nodes (YN)			
10	3	8±3.5^{d}	7±2.8^{c}	3±0^{i}	82±55.8^{a}
10	5	7±1.4^{d}	6±0.7^{d}	28±16.2^{f}	59±38.1^{b}
10	7	4±2.1^{f}	3±2.8^{f}	65±41^{c}	28±14.8^{f}
		Matured Nodes (MN)			
10	3	18±10.6^{a}	16±9.1^{b}	14±7.7^{g}	52±34.6^{c}
10	5	9±2.8^{c}	6±0.7^{d}	41±25.4^{e}	44±27.5^{d}
10	7	5±1.4^{e}	6±0.7^{d}	70±44.5^{b}	19±8.4^{g}

Key: SD – Standard deviation; M%FC – Mean % of Fungal contaminants; M%BC - Mean % of Bacterial contaminants; M%DE - Mean % of Death Explants; M%NR - Mean % of Not Responded; The means followed by the same letters along the columns are not significantly different from each other ; Student-Newman-Keuls Multiple Comparisons tests

When the explants were exposed to 10% sodium hypochlorite for 3, 5, 7 min, the highest survival rate was recorded in YN of 82% at 3 min followed by Apical shoot tips of about 61% at 3 min and MN of about 52% at 3 min. 3min was the comfortable time for the explants to survive independent of the efficiency. At above 3 min, it was found that the rate of contamination gradually decreased with increase in the rate of non responding explants.

Table 4 Effect of exposure to 20% NaOCl in PSM

Concentration Of NaOCl (%)	Exposed Time (min)	M%FC±SD	M%BC±SD	M%NR±SD	M%SE±SD
		Apical shoot tips			
20	3	24±14.8^{c}	29±18.3^{a}	18±10.6^{f}	29±18.3^{b}
20	5	17±8.4^{e}	19±9.8^{c}	52±33.2^{d}	12±4.9^{e}
20	7	7±0^{g}	9±1.4^{f}	75±48^{b}	9±1.4^{f}
		Nodes			
		Young Nodes (YN)			
20	3	26±16.2^{b}	25±15.5^{b}	15±8.4^{g}	34±21.9^{a}
20	5	17±8.4^{e}	18±9.1^{d}	49±31.1^{e}	16±7.7^{d}
20	7	6±0.7^{g}	11±2.8^{e}	72±45.9^{c}	11±2.8^{e}
		Matured Nodes (MN)			
20	3	32±20.5^{a}	28±17.6^{a}	19±11.3^{f}	21±12.7^{c}
20	5	18±9.1^{d}	17±8.4^{d}	54±34.6^{d}	11±4.2^{e}
20	7	8±0.7^{f}	4±2.1^{g}	82±53^{a}	6±0.7^{g}

Key: M%FC – Mean % of Fungal contaminants; M%BC - Mean % of Bacterial contaminants; M%SE - Mean % of Survival Explants; M%NR - Mean % of Not Responded; SD – Standard deviation; The means followed by the same letters along the columns are not significantly different from each other ; Student-Newman-Keuls Multiple Comparisons tests

When the explants were exposed to 20% sodium hypochlorite for 3, 5, 7 min, the highest survival rate was recorded in YN of 34% at 3 min followed by Apical shoot tips of about 29% at 3 min and MN of about 21% at 3 min. 3min was the comfortable time for the explants to survive independent of the efficiency. At above 3 min, it was found that the rate of contamination gradually decreased with increase in the rate of non responding explants.

Table 5 Effect of exposure to 30% NaOCl in PSM

Concentration of NaOCl (%)	Exposed Time(min)	M%FC±SD	M%BC±SD	M%NR±SD	M%SE±SD
		Apical shoot tips			
30	3	32±20.5^{a}	28±17.6^{a}	23±14.1^{h}	17±9.8^{b}
30	5	28±16.2^{b}	25±14.1^{b}	37±22.6^{f}	10±3.5^{d}
30	7	9±1.4^{f}	7±0^{e}	78±50.2^{b}	6±0.7^{f}
		Nodes			
		Young Nodes (YN)			
30	3	28±17.6^{b}	28±17.6^{a}	21±12.7^{i}	23±14.1^{a}
30	5	22±12^{c}	20±10.6^{c}	41±25.4^{e}	17±8.4^{b}
30	7	11±2.8^{e}	5±1.4^{f}	73±46.6^{c}	11±2.8^{d}
		Matured Nodes (MN)			
30	3	32±20.5^{a}	26±16.2^{b}	28±17.6^{g}	14±7.7^{c}
30	5	19±9.8^{d}	17±8.4^{d}	56±36^{d}	8±2.1^{e}
30	7	6±0.7^{g}	3±2.8^{g}	87±56.5^{a}	4±2.1^{g}

Key: M%FC – Mean % of Fungal contaminants; M%BC - Mean % of Bacterial contaminants; M%SE - Mean % of Survival Explants; M%NR - Mean % of Not Responded;SD – Standard deviation; The means followed by the same letters along the columns are not significantly different from each other ; Student-Newman-Keuls Multiple Comparisons Tests

When the explants were exposed to 10% sodium hypochlorite for 3, 5, 7 min, the highest survival rate was recorded in YN of 23% at 3 min followed by Apical shoot tips of about 17% at 3

Table 6 Effect of exposure to 0.1% Mercuric chloride in PSM

Concentration of Mercuric Chloride (%)	Exposed Time (min)	M%FC	M%BC	M%NR	M%SE
		Apical shoot tips			
0.1	5	27±15.5^{a}	28±16.2^{a}	26±14.8^{f}	19±9.8^{f}
0.1	10	15±3.5^{c}	17±4.9^{d}	29±13.4^{e}	39±20.5^{c}
0.1	15	4±7.7^{f}	3±8.4^{g}	81±46.6^{a}	12±2.1^{h}
		Nodes			
		Young Nodes (YN)			
0.1	5	26±14.8^{a}	22±12^{b}	10±3.5^{h}	42±26.1^{b}
0.1	10	5±3.5^{f}	5±3.5^{f}	12±1.4^{g}	78±48^{a}
0.1	15	2±9.1^{g}	4±7.7^{g}	68±37.4^{b}	26±7.7^{e}
		Matured Nodes (MN)			
0.1	5	23±12.7^{b}	21±11.3^{c}	38±23.3^{d}	18±9.1^{g}
0.1	10	13±2.1^{d}	12±1.4^{e}	41±21.9^{c}	34±16.9^{d}
0.1	15	7±5.6^{e}	5±7^{f}	68±37.4^{b}	20±3.5^{f}

Key: M%FC – Mean % of Fungal contaminants; M%BC - Mean % of Bacterial contaminants; M%NR - Mean % of Not Responded; M%SE - Mean % of Survival Explants; SD – Standard deviation; The means followed by the same letters along the columns are not significantly different from each other ; Student-Newman-Keuls Multiple Comparisons Tests

Table 7 Effect of exposure to 0.2% Mercuric chloride in PSM

Concentration Of Mercuric Chloride (%)	Exposed Time (min)	M%FC	M%BC	M%NR	M%SE
		Apical shoot tips			
0.2	5	26±14.8^{a}	22±12^{a}	15±7^{h}	37±22.6^{c}
0.2	10	23±9.1^{b}	18±5.6^{b}	42±22.6^{f}	17±4.9^{f}
0.2	15	10±3.5^{e}	8±4.9^{e}	71±39.5^{b}	11±2.8^{g}
		Nodes			
Young Nodes (YN)					
0.2	5	10±3.5^{e}	10±3.5^{d}	14±25.4^{h}	66±43.1^{a}
0.2	10	7±2.1^{f}	4±4.2^{g}	44±24^{e}	45±24.7^{b}
0.2	15	6±6.3^{f}	6±6.3^{f}	61±32.5^{c}	27±8.4^{e}
Matured Nodes (MN)					
0.2	5	18±9.1^{c}	16±7.7^{c}	34±20.5^{g}	32±19^{d}
0.2	10	12±1.4^{d}	10±0^{d}	58±33.9^{d}	16±4.2^{f}
0.2	15	7±5.6^{f}	5±7^{g}	78±44.5^{a}	10±3.5^{g}

Key: M%FC – Mean % of Fungal contaminants; M%BC - Mean % of Bacterial contaminants; M%NR - Mean % of Not Responded; M%SE - Mean % of Survival Explants; SD – Standard deviation; The means followed by the same letters along the columns are not significantly different from each other ; Student-Newman-Keuls Multiple Comparisons Tests

min and MN of about 14% at 3 min. 3min was the comfortable time for the explants to survive independent of the efficiency. At above 3 min, it was found that the rate of contamination gradually decreased with increase in the rate of non responding explants.

When the explants were exposed to 0.1% Mercuric chloride for 5, 10, 15 min, the highest survival rate was recorded in YN of 78% at 10 min followed by Apical shoot tips of about 39% at 10 min and MN of about 34% at 10 min. 10 min was the comfortable time for the explants to survive independent of the efficiency. At above 10 min, it was found that the rate of contamination gradually decreased with increase in the rate of non responding explants.

When the explants were exposed to 0.2% Mercuric chloride for 5, 10, 15 min, the highest survival rate was recorded in YN of 66% at 10 min followed by Apical shoot tips of about 37% at 10 min and MN of about 32% at 10 min. 10 min was the comfortable time for the explants to survive independent of the efficiency. At above 10 min, it was found that the rate of contamination gradually decreased with increase in the rate of non responding explants.

Table 8 Effect of exposure to 70% Mercuric chloride in PSM

Concentration Of Ethanol (%)	Exposed Time (min)	M%FC	M%BC	M%NR	M%SE
Apical shoot tips					
70	3	26±16.2^{b}	23±14.1^{b}	38±24.7^{e}	13±7^{c}
70	5	18±9.1^{d}	20±10.6^{d}	45±28.2^{d}	17±8.4^{b}
70	7	4±2.1^{g}	6±0.7^{g}	78±50.2^{b}	12±3.5^{c}
Nodes					
Young Nodes (YN)					
70	3	25±15.5^{b}	23±14.1^{b}	33±21.2^{f}	19±11.3^{a}
70	5	20±10.6^{c}	21±11.3^{c}	40±24.7^{e}	19±9.8^{a}
70	7	9±1.4^{f}	6±0.7^{g}	69±43.8^{c}	16±6.3^{b}
Matured Nodes (MN)					
70	3	28±17.6^{a}	24±14.8^{a}	39±25.4^{e}	9±4.2^{d}
70	5	13±5.6^{e}	11±4.2^{e}	69±45.2^{c}	7±1.4^{e}
70	7	7±0^{f}	8±0.7^{f}	81±52.3^{a}	4±2.1^{f}

Key: M%FC – Mean % of Fungal contaminants; M%BC - Mean % of Bacterial contaminants; M%NR - Mean % of Not responded; M%SE - Mean % of Survival Explants; SD – Standard deviation; The means followed by the same letters along the columns are not significantly different from each other ; Student-Newman-Keuls Multiple Comparisons tests

Table 9 Effect of exposure to 90% Mercuric chloride in PSM

Concentration Of Ethanol (%)	Exposed Time (min)	M%FC	M%BC	M%NR	M%SE
Apical shoot tips					
90	3	26±16.2^{a}	24±14.8^{a}	39±25.4^{f}	11±5.6^{b}
90	5	17±8.4^{c}	19±9.8^{c}	56±36^{d}	8±2.1^{c}
90	7	5±1.4^{f}	6±07^{e}	82±53^{b}	7±0^{d}
Nodes					
Young Nodes (YN)					
90	3	25±15.5^{a}	23±14.1^{b}	35±22.6^{g}	17±9.8^{a}
90	5	20±10.6^{b}	18±9.1^{c}	51±32.5^{e}	11±4.2^{b}
90	7	7±0^{e}	4±2.1f	79±50.9^{b}	10±2.1^{b}
Matured Nodes (MN)					
90	3	26±16.2^{a}	25±15.5^{a}	41±26.8^{f}	8±3.5^{c}
90	5	13±5.6^{d}	16±7.7^{d}	65±42.4^{c}	6±0.7^{d}
90	7	5±1.4^{f}	4±2.1^{f}	87±56.5^{a}	4±2.1^{e}

Key: M%FC – Mean % of Fungal contaminants; M%BC - Mean % of Bacterial contaminants; M%NR - Mean % of Not responded; M%SE - Mean % of Survival Explants; SD – Standard deviation; The means followed by the same letters along the columns are not significantly different from each other ; Student-Newman-Keuls Multiple Comparisons tests

When the explants were exposed to 90% Ethanol for 3, 5, 7 When the explants were exposed to 70% Ethanol for 3, 5, 7 min, the highest survival rate was recorded in YN of 19% at 3 and 5 min followed by Apical shoot tips of about 17% at 5 min and MN of about 9% at 3 min. Efficiency was comparatively lower when compared to sodium hypochlorite and mercuric chloride. min, the highest survival rate was recorded in YN of 17% at 3 min followed by Apical shoot tips of about 11% at 3 min and MN of about 8% at 3 min. Efficiency was comparatively lower when compared to sodium hypochlorite and mercuric chloride.

Table 10 Effect of shoot regeneration from nodes and apical buds using various concentrations of NAA and 6BAP in MS media

Explants	Growth Regulators NAA(%)+6BAP(µm)	Shoot Regeneration Response %	Shoot Number	Quality
Apical Shoot Tips	0.01 + 2.22	34±22.4^{g}	0.54	+
	0.01 + 4.44	35±23.1^{g}	0.73	+
	0.01 + 6.66	35±23.1^{g}	0.61	+
	0.01 + 8.88	36±23.8^{g}	0.6	+
	0.05 + 2.22	44±27.9^{f}	0.65	+
	0.05 + 4.44	48±30.8^{e}	0.67	+
	0.05 + 6.66	47±30^{e}	0.92	+
	0.05 + 8.88	47±30^{e}	0.91	+
	0.1 + 2.22	52±32^{d}	0.92	+
	0.1 + 4.44	67±42.6^{c}	1.13	++
	0.1 + 6.66	82±53.2^{a}	3.23	+++
	0.1 + 8.88	79±51.1^{a}	2.67	++
	0.5 + 2.22	54±31.9^{d}	1.45	++
	0.5 + 4.44	65±39.6^{c}	1.22	++
	0.5 + 6.66	80±50.2^{a}	2.87	++
	0.5 + 8.88	76±48.8^{b}	2.54	++

	Growthregulators NAA(%)+6bap(µm)	Response (%)		Shoot Number		Quality	
		YN	MN	YN	MN	YN	MN
Nodes	0.01 + 2.22	38±25.3^{g}	9±4.7^{e}	2.10	1.12	+	+
	0.01 + 4.44	37±24.5^{g}	8±4^{e}	2.10	1.47	+	+
	0.01 + 6.66	39±26^{g}	10±5.5^{d}	2.21	1.61	+	+
	0.01 + 8.88	39±26^{g}	11±6.2^{d}	2.24	1.65	+	+
	0.05 + 2.22	46±29.3^{f}	11±4.6^{d}	2.36	1.65	+	+
	0.05 + 4.44	45±28.6^{f}	12±5.3^{d}	2.31	1.71	+	+
	0.05 + 6.66	48±30.8^{e}	12±5.3^{d}	2.47	1.74	+	+
	0.05 + 8.88	50±32.2^{d}	13±6^{d}	2.87	1.98	++	+
	0.1 + 2.22	54±33.4^{c}	15±5.8^{c}	2.94	2.07	++	+
	0.1 + 4.44	56±34.8^{b}	16±6.6^{b}	2.95	2.14	++	+
	0.1 + 6.66	63±39.8^{a}	21±10.1^{a}	3.29	2.54	+++	++
	0.1 + 8.88	61±38.4^{a}	20±9.4^{a}	2.99	2.45	+++	++
	0.5 + 2.22	52±30.4^{d}	14±3.6^{c}	2.91	2.09	++	+
	0.5 + 4.44	54±31.9^{c}	14±3.6^{c}	2.95	2.09	++	+
	0.5 + 6.66	60±36.1^{a}	19±7.1^{a}	2.97	2.41	++	++
	0.5 + 8.88	57±34^{b}	17±5.7^{b}	2.94	2.31	++	++

Key: SD – Standard deviation; means followed by the same letters along the columns are not significantly different from each other; Student-Newman-Keuls Multiple Comparisons tests; + - very small; ++ - shoot emerged at least 1 cm; +++ - more than one healthy shoot

Results indicated that single node explants responded better than shoot tips based on number of growing shoots, number of leaf per explant and shoot length (Bensaad, Z.M. and Milad, K.M., 2015). The work carried out during January 2014 to may 2014.the shoot initiation was found to be better on BAP (0.5 to 2mg/L) highest average growth response. And the regeneration of the shoot was better on 0.9mg/L NAA (Jaya Singh *et al*., 2014).In this study, shoot regeneration was observed in MS media with higher rate of 82% in apical shoots followed 63% in YN and 21% in MN; highest shoot height was 3.29 cm in YN, 3.23 in apical shoots. Very healthy shoots was observed in Young nodes though the shoot regeneration response was not high. Apical shoot regeneration response was high but the quality was low.

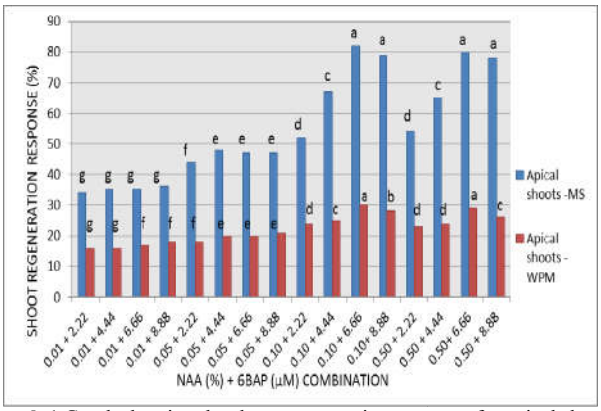
Shoot proliferation was induced in nodal segments on MS media containing various combinations of 6-BAP (0-2 mg/l) and NAA (0-1 mg/l). Maximum number of shoots was induced with 6BAP 1.5 mg/l and NAA 0.5 mg/l and shoot length was 7.7 cm (Usharani T.R *et al*., 2014).The highest number of shoot per explants was observed on MS medium containing 1.8 mg/L

Table 11 Effect of shoot regeneration from nodes and apical buds using various concentrations of NAA and 6BAP in WPM media

Explant	Growth Regulators NAA(%)+6BAP(µM)	Response %	Shoot Number	Quality
Apical Shoot tips	0.01 + 2.22	16±9.7^{g}	0.57	+
	0.01 + 4.44	16±9.7^{g}	0.64	+
	0.01 + 6.66	17±10.4^{f}	0.71	+
	0.01 + 8.88	18±11.1^{f}	0.74	+
	0.05 + 2.22	18±9.5^{f}	0.98	+
	0.05 + 4.44	20±11^{e}	1.27	++
	0.05 + 6.66	20±11^{e}	1.24	++
	0.05 + 8.88	21±11.7^{e}	1.4	++
	0.1 + 2.22	24±12.2^{d}	1.4	++
	0.1 + 4.44	25±12.9^{c}	1.43	++
	0.1 + 6.66	30±16.5^{a}	1.56	++
	0.1 + 8.88	28±15^{b}	1.55	++
	0.5 + 2.22	23±9.9^{d}	1.41	+
	0.5 + 4.44	24±10.6^{d}	1.46	+
	0.5 + 6.66	29±14.2^{a}	1.51	++
	0.5 + 8.88	26±12.1^{c}	1.47	+

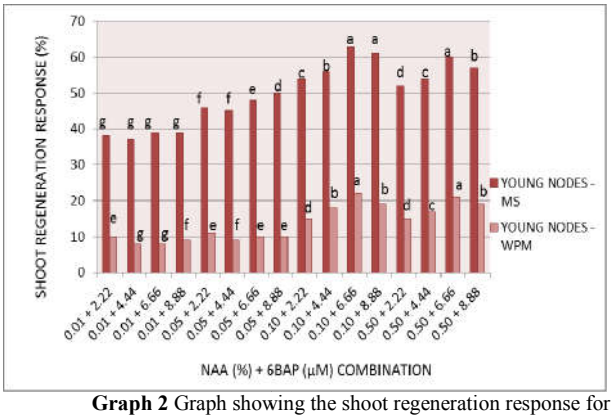
	Growthregulators	Response (%)		Shoot Number		Quality	
	NAA(%)+6BAP(µM)	YN	MN	YN	MN	YN	MN
Nodes	0.01 + 2.22	10±5.5^{e}	1±0.8^{e}	1.19	0.41	+	+
	0.01 + 4.44	8±4^{g}	3±0.^{d}	1.21	0.49	+	+
	0.01 + 6.66	8±4^{g}	3±0.5^{d}	1.21	0.44	+	+
	0.01 + 8.88	9±4.7^{f}	2±0.1^{d}	1.09	0.54	+	+
	0.05 + 2.22	11±4.6^{e}	2±1.7^{d}	1.07	0.53	+	+
	0.05 + 4.44	9±3.2^{f}	1±2.4^{e}	1.12	0.51	+	+
	0.05 + 6.66	10±3.9^{e}	1±2.4^{e}	1.51	0.76	+	+
	0.05 + 8.88	10±3.9^{e}	2±1.7^{d}	1.45	0.74	+	+
	0.1 + 2.22	15±5.8^{d}	4±1.8^{c}	1.54	0.81	+	+
	0.1 + 4.44	18±8^{b}	5±1.1^{c}	1.81	0.86	+	+
	0.1 + 6.66	22±10.8^{a}	8±0.9^{a}	1.84	0.94	++	++
	0.1 + 8.88	19±8.7^{b}	7±0.2^{a}	1.8	0.94	+	+
	0.5 + 2.22	15±4.3^{d}	4±3.4^{c}	1.74	0.85	+	+
	0.5 + 4.44	17±5.7^{c}	6±2^{b}	1.64	0.87	+	+
	0.5 + 6.66	21±8.5^{a}	7±1.3^{a}	1.81	0.93	++	++
	0.5 + 8.88	19±7.1^{b}	6±2^{b}	1.79	0.87	+	+

Key: SD – Standard deviation; means followed by the same letters along the columns are not significantly different from each other; Student-Newman-Keuls Multiple Comparisons Tests; + - very small; ++ - shoot emerged atleast 1 cm; +++ - more than one healthy shoot

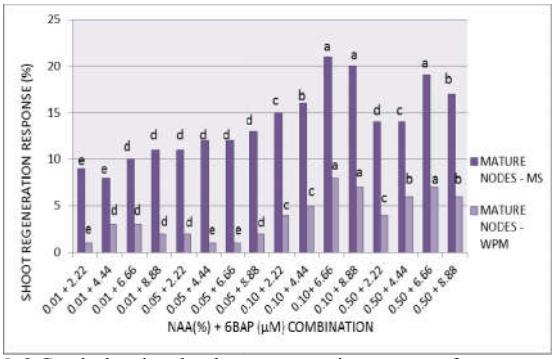


Graph 1 Graph showing the shoot regeneration response for apical shoots MS and WPM

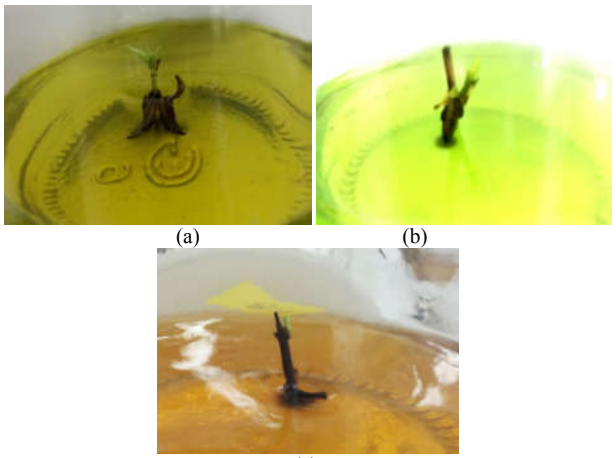
BAP, 0.9 mg/L NAA In both WPM and MS medium when different levels of alone BAP and NAA were tried, WPM medium showed poor proliferation response compared to MS medium (Vijay M. Patil *et al*., 2011). In this study, shoot regeneration was observed in WPM media with higher rate of 30% in apical shoots followed 22% in YN and 8% in MN; highest shoot height was 1.84 cm in YN, 1.56 cm in apical shoots. Very healthy shoots was observed in Young nodes though the shoot regeneration response was not high. Apical shoot regeneration response was high but the quality was



Graph 2 Graph showing the shoot regeneration response for young nodes MS and WPM

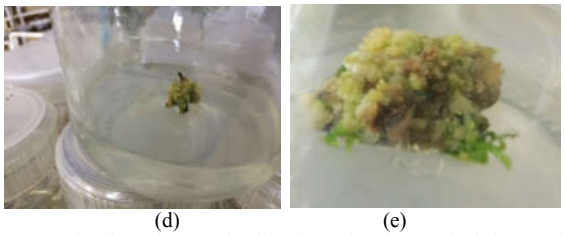


Graph 3 Graph showing the shoot regeneration response for mature nodes MS and WPM



(a) (b) (c)

Figure 1 (a) Shoot arised form apical bud (b) Shoot arised from young nodes (c) Shoot arised from mature nodes



(d) (e)

Figure 2: (d) Slight shoot and callus formation from apical shoot at low concentration of NAA and 6BAP (e) Callus formation from mature nodes at high concentrations of 6BAP and NAA

low. WPM media did not favor the shoot regeneration for all the selected explants unlike MS which showed higher results. Though pomegranate is a woody plant, Woody plant medium did not help in the regeneration. MS media was found to be best for the regeneration of shoots for the selected explants.

Acknowledgement

My sincere thanks to my guide Dr. T. B. Sridharan, VIT University, Vellore who guided me technically in carrying out my Research work successfully.

CONCLUSION

From the study, axenic explant standardization was done and the best healthy explant tolerant to contamination was found as Young nodes. Though the shoot regeneration response was higher in Apical shoot tips of about 82% and Young nodal explants responded to 78% only, the healthy shoots and highest shoot number was observed in Young nodal explants. Moreover, quality of the shoots was very weak, pale green in color as apical shoot tip are soft in nature. The shoots appeared dark green and found to be strong in Young nodes. Mature nodes are good in quality but the contamination rate was higher with lower regeneration response.

References

1. Alireza Bonyanpour and Morteza Khosh-Khui, (2013). Callus Induction and Plant Regeneration in *Punicagranatum* L. '*Nana'* from Leaf Explants. J. Central European Agri, 14(3), 75-83.
2. Babak Valizadeh Kaji, Ahmad Ershadi, and Masoud Tohidfar, (2013). In vitro propagation of two Iranian commercial pomegranates (*Punicagranatum* L.) cvs. 'Malas Saveh' and 'Yusef Khani'. Physiol Mol Biol Plants, 19(4), 597–603.
3. Jaime A. Teixeira da Silva, Tikam Singh Rana, Diganta Narzary, Nidhi Verma, Deodas Tarachan Meshram, Shirish A. Ranade, (2013).Pomegranate biology and biotechnology: A review. Scientia Horticul., 160, 85 – 107.
4. Jaya Singh, Saurabh Gupta, Paras Khoshe, (2014).*In vitro* Regeneration of Pomegranate (*Punicagranatum* L.) from Nodal Explant. Int. J. Adv Pharm, Biol. Chem., 3(3).
5. R. Kumar, M.R. Shamarao Jahagirdar, S.T. Yenjerappa and H.B. Patil, (2009). Epidemiology and management of bacterial blight of pomegranate caused by *Xanthomona saxonopodis* pv. *Punicae*. Acta Horticulture. (ISHS), 818, 291-296.
6. R. Lokesh, Erayya, K.M. Kumaranag, N. Chandrashekar and A.N.A. Khan, (2014). *In vivo* Efficacy of some Antibiotics against Bacterial Blight of Pomegranate caused by *Xanthomona saxonopodis*pv. *Punicae.* Int. Res. J. Biol. Sci, 3(1), 31 – 35.
7. Vijay M. Patil, G. A. Dhande, Dipak M. Thigale and J. C. Rajput, (2011). Micropropagation of pomegranate (*Punicagranatum* L.)' Bhagava' cultivar from nodal explant. Afri J. Biotechnol., 10(79), 18130-18136.
8. Soumendra K. Naik, Sitakanta Pattnaik, Pradeep K. Chand, (2000). High frequency axillary shoot proliferation and plant regeneration from cotyledonary nodes of pomegranate (Punicagranatum L.). Scientia Horticul, 85, 261-270.
9. Pushpraj singh and R M. Patel, (2014). Factors influencing in vitro growth and shoot multiplication of pomegranate. The Bioscan, 9 (3): 1031-1035.
10. Raj Deepika and Kamlesh Kanwar, (2010). *In vitro* regeneration of *Punicagranatum* L. plants from different juvenile explants. J. Fru. Orna. Plant Res., 18 (1), 5-22
11. C. Sandeep and C.K. Suresh, (2013). Sterilization Protocol and Control of Polyphenol Exudation in Micropropagation of Pomegranate Cv. Bhagwa. Biosci. Biotechnol. Res. Asia, 10(1), 305-310.
12. Usharani.T.R, Meenakshi.S, Vijayalakshmi, Sunisha. C, Mohandas Sukhada, (2014). Invitro shoot regeneration from nodal explants of Pomegranate (Punicagranatum L.) cv, Bhagwa. Adv. Appl. Res., 6 (1), 66-71.
13. Bensaad, Z.M. and Milad, K.M., (2015). Influence of type of explant, salt composition of basal medium and cytokinins on micropropagation of pomegranate (*Punicagranatum*' khadouri'). Acta Hortic. 1089, 91-96.
14. F. Soukhak, A. Khalighi, S. A. Ghaemmaghami, (2011). Study of Direct Adventitious Shoot Regeneration in Pomegranate (Punicagranatum cv. MalasSaveh) through Cotyledonary Explants. Int. J. Agri. Sci. Res., 2 (3).
15. Surinder Kumar, Jitender Kumar Kanwar, (2006). Regeneration ability of petiole, leaf and petal explants in gerbera cut flower cultures in vitro. Folia Horticul., 18(2), 57 – 64.

How to cite this article:
Manasa Satheesh and Sridharan T.B., Standardising Axenic Pomegranate Explant Processing For Micropropagation. *International Journal of Recent Scientific Research Vol. 6, Issue, 11, pp. 7544-7549, November, 2015*

****

ISSN 0976-3031