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COMPARATIVE STUDY OF DIFFERENT GROWTH REGULATORS FOR EFFICIENT PLANT REGENERATION IN GRAPES

Sadia Tehrim*, M. Yasin Mirza** and G. Mustafa Sajid*

ABSTRACT:- The grape (*Vitis vinifera* L.) is the most valuable and widely cultivated horticultural crop worldwide. In the present study, different hormonal combinations in regeneration media were studied to determine the best combination for in vitro morphogenesis of grapevine accessions viz., 4494(1) Kowar Sufaid, 4472(12) Harker Agoon and 4492(1) Kowar Naray. When levels of benzylaminopurine (BAP) were increased in the basal MS media for culturing the grape plantlets, there was a corresponding increase in the number of shoots and root mass. Additionally, the shoot proliferation response to increasing level of BAP was found genotype dependent. Naphthaleneacetic acid (NAA) also showed a positive effect on in vitro growth responses (shoot proliferation and root induction) when combined with BAP. The media containing NAA, BAP and kinetin accelerated the growth in terms of shoot mass in two accessions, 4494(1) Kowar Sufaid and 4472(12) Harker Agoon and again the culture response was genotype dependent. There was a difference in the growth responses of the three accessions studied. The best rooting response was obtained on 75% MS media containing 0.7 mgl^{-1}, NAA.

Key Words: Vitis vinifera; Induction Media; Regeneration; Growth Hormones; Micropropagation; Shoot Proliferation; Root Induction; Pakistan.

INTRODUCTION

The grape (*Vitis vinifera* L.) is one of the earliest domesticated and widely grown fruit crop in world which can be eaten raw and also used for making juice, wine, jam, jelly and raisins. According to the historical records, the cultivation of grapevine, *Vitis vinifera* subsp. v*inifera* (which was evolved from its wild relative, *Vitis vinifera* subsp. *sylvestris*) was started 6,000-8,000 years ago in the Near East (McGovern, 2003). In the beginning, grapes have been propagated through both sexual (seed) and asexual means where seed was the most common form of propagation because of its trouble-free transport. *V. vinifera* exhibits tremendous level of genetic diversity which is due to its long cultivation history and by intentionally and unintentionally made crosses (Riaz et al., 2013).

In Pakistan, grape fruit crop is grown over 13,000 ha with the production of 49000t per year (Anonymous, 2011). Additionally, in Pakistan the major contribution for grape production both in area and production is from Balochistan province. The grape varieties are grown in highland

*Institute of Agri-Biotechnology and Genetic Resources, National Agricultural Research Centre, Islamabad, Pakistan.
** Oilseed Research Programme, Crop Sciences Institute, National Agricultural Research Centre, Islamabad, Pakistan.
Corresponding author: tehrim.sadia@gmail.com

areas of the province. In the country, almost all grape varieties (scion or rootstock) are conventionally propagated through stem cutting, layering and grafting. However, these conventional techniques are looking no more efficient due to some major problems including seedling heterozygosity, long space and time requirement, seed dormancy and limited yield. Moreover, many fungal and bacterial diseases also affect the crop production (Jaskani et al., 2008). In Pakistan, some of the grape varieties are facing risk of extinction due to genetic drift, bad agronomic practices and deficiency of conservation strategies. High mortality and low rooting response make this crop very difficult to grow vegetatively (Sajid et al., 2003). In this regard, good quality and increased production in grapes can be obtained through tissue culture practices (such as micropropagation) to provide true to type, rapid multiplication and disease free plants (Stern, 1943; Lewandowski, 1991 and Sajid et al., 2003).

Plant tissue culture is a well established technique in plant science to grow and study the cells, tissues and organs on an artificial medium, static or liquid, under controlled environment. Recently plant tissue culture has gained significant importance in various fields like clonal propagation, conservation of germplasm, phytopathology, haploid and triploid production, embryo rescue, in vitro pollination and fertilization, somatic hybridization and cybridization, somaclonal and gametoclonal variant selection, genetic manipulation and industrial production of plant metabolites (McCown, 2000). In vitro micropropagation is a practice in the plant tissue culture to reproduce asexually a large number of identical plant genotypes. In the world, almost hundreds of millions of plants including 50,000 plant varieties are micro propagated annually (Vasil, 1994). For the propagation of woody plant species, the micropropagation method is very practical and its efficiency relies on shoot multiplication rate and their rooting percentage. In grapes, rapid and efficient micropropagation practices need to combine both shoot multiplication and their rooting (Beauchesne, 1982; Jaskani et al., 2008). For grape in vitro cultivation, various micropropagation protocols using different growth hormones and explants were reported by many researchers (Jiang and Jin, 1995; Compton and Gray, 1995; Meyerson et al., 1995; Doroshenko, 1996; Gok et al., 1997; Sajid et al., 2008; Jaskani et al., 2008). Axillary buds are considered as most successful explant source for angiospermic plants like grape (Vasil and Thorpe, 1994).

The purpose of this study is to employ the combinations of different growth hormones for grape micropropagation and to find out the best possible conditions for successful in vitro shoot proliferation and root induction.

MATERIALS AND METHOD

Plant Materials and Explants Source

In vitro cultures of grape (*V. vinifera*) germplasm were established in the gene bank of Plant Genetic Resources Programme of the Institute of Agricultural Biotechnology and Genetic Resources (IABGR), National Agricultural Research Centre (NARC),

Islamabad. These cultures were derived from the buds taken from the Grape Clonal Repository located at the Centre. The nodal segments of 0.5 to 1.0cm were excised aseptically from in vitro established cultures and transferred on to defined media containing specific growth hormone regimes for in vitro morphogenesis and root induction. Six in vitro established grape accession viz., 4492(1) Kowar Naray, 4472(12) Harker Agoon, 4494(1) Kowar Sufaid, 4472(1) Gang Agoon Sufaid, 4489(1) Kowar Jangli and 4472(18) Singh Lok Agoon were used in the study.

Media and Culture Incubation Conditions

All the six grape germplasm accessions were grown on 75% MS medium supplemented with 3% sucrose, vitamins and varying levels of hormonal regimes (Murashige and Skoog, 1962). The pH of media was adjusted at 5.8 and then sterilized by autoclaving at 121°C for 17 min. For proliferation, the glass jars were used because cultures initiated in larger flasks grow and develop better than those in small flasks. The larger flasks offer enough space for dilution of toxic gases like ethylene and carbon dioxide evolved during incubation and more amounts of nutrient media are available to the explants per unit area (Pierik, 1987). After transferring the cultures on specified media, they were incubated in the growth room maintained at 25 $\pm$ 2°C and illuminated with 1000-2000 lux of light under 16hL/8hD periods.

Sub Culturing

Plant cultures were multiplied for the culture maintenance and proliferation to generate adequate cultures for further experiments. Single isolated shoots were excised aseptically from the cultures of five in vitro established accessions namely 4492 (1) Kowar Naray, 4472 (12) Harker Agoon, 4489 (1) Kowar Jangli, 4472 (18) Singh Lok Agoon and 4494 (1) Kowar Sufaid. These shoots cultured on the 75% media containing MS salts, sucrose (30gl^{-1}), myo-inositol (0.1 gl^{-1}), vitamins, BAP (0.5 mgl^{-1}) and agar (8 gl^{-1}).

Hormonal Combinations used for Grape Growth Rate and Proliferation

In this study, different combinations of benzylaminopurine (BAP) (0.05, 0.20, 0.25, 0.50, 0.75, 1.0, 2.0 mgl^{-1}); naphthaleneacetic acid (NAA) (0.0, 0.1, 0.2, 0.3 mgl^{-1}); indolebutyric acid (IBA) (0.2 mgl^{-1}) and kinetin (0.5, 1.0, 2.0 mgl^{-1}) were employed for three grape accessions [4492 (1) Kowar Naray, 4494 (1) Kowar Sufaid and 4472 (12) Harker Agoon] in combination with MS medium to find their effect on grape growth rate and proliferation.

Root Induction

Twelve combinations of IAA, NAA and IBA were also tested to study their effect on root induction. Media was enriched either with 5 different levels of IAA (0, 0.1, 0.3, 0.7, 1.0 mgl^{-1}), 5 different levels of NAA (0, 0.1, 0.3, 0.7, 1.0 mgl^{-1}) or 5 different levels of IBA (0, 0.2, 0.5, 1.0, 2.0 mgl^{-1}) for one grape accession 4472(1) Gang Agoon Sufaid tested in this study.

Plant Growth Parameters

After 4-6 weeks data on viability (%), mortality (%), contamination (%),

shoot number explants^{-1}, shoot length (cm), number of nodes plant^{-1}, shoot mass (g) and root mass (g) were used to analyze the growth rate, proliferation and root induction.

Growth responses were compared between accessions and hormonal regimes in all the experiments. The experiment was designed as completely randomized design with four replications in each experiment. The data collected were subjected to statistical analysis (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

Culture Multiplication

Two cycles of sub-cultures were carried out for shoot proliferation. In first cycle of subculture, culture multiplication was conducted on MS media supplemented with BAP (0.5 mgl^{-1}) to generate adequate plant cultures for further experimentation. The sprouting from nodal segments was started after 4-5 days of culture initiation. In first cycle, the highest shoot number (2.28) and shoot length (2.9 cm) was achieved in 4494(1). Whereas, maximum number of nodes were recorded in 4472(12) and highest shoot mass (1.51 g) was harvested in 4492(1). Maximum root mass (4.52 g) was recorded in 4494(1) accession. In second subculture, maximum shoot length (2.30 cm) was gained in 4489(1). While maximum shoot number (2.66), number of nodes (3.87) and shoot mass (5.27 g) were observed in 4492(1). Therefore on the basis of exceptional growth responses, three grape accessions viz., 4492 (1) Kowar Naray, 4494 (1) Kowar Sufaid and 4472 (12) Harker Agoon were selected for use in further experiments.

Effect of BAP on Growth Rate and Proliferation

When BAP was increased from 0.05 to 2.0 mgl^{-1}, maximum shoot number (4.66), number of nodes plant^{-1} (6.71), shoot mass (3.51g) and root mass (1.04 g) were obtained in 4494 (1) on MS media. However, maximum shoot length (4.97 cm) was achieved with 0.05 mgl^{-1} concentration of BAP. In accession 4472 (12), maximum shoot number and shoot mass was achieved with 2.0 mgl^{-1} of BAP, maximum shoot length and root mass was achieved with 0.05 mgl^{-1} of BAP and maximum number of nodes were obtained with 0.75 mgl^{-1} of BAP. Accession 4492 (1) showed highest shoot length (8.0 cm), number of nodes plant^{-1} (11.0) and root mass (0.95 g) on MS media containing 0.05 mgl^{-1} of BAP (Table 1).

Combined Effect of NAA and BAP on Growth Rate and Proliferation

Combined effect of NAA and BAP was studied putting the two growth regulators in the same media.

A constant level of NAA (0.1 mgl^{-1}) and different concentrations of BAP (0.05, 0.20, 0.75, 2.0 mgl^{-1}) were employed in MS medium to determine their combined effect on grape growth rate. In the accession 4492 (1), 33.3% mortality was observed in the medium containing 0.1 and 0.2 mgl^{-1} of NAA and BAP, respectively while the accession 4472 (12) showed 100% viability response with all NAA + BAP combinations. In 4492 (1) and 4472 (12), no contamination was observed except one combination in 4492 (1). Maximum shoot number of 3.66 was achieved in 4472 (12) on medium containing 0.1 mgl^{-1} of NAA and 2.0 mgl^{-1} of BAP (Figure, 1a). The media

Table 1. In vitro growth responses of grape accessions affected by different concentrations of BAP in MS media

Accession	Treatment (mgl^{-1})	Mortality (%)	Viability (%)	Contamination (%)	Shoot number $explant^{-1}$	Shoot length (cm)	No. of nodes $plant^{-1}$	Shoot mass (g)	Root mass (g)
4494(1) Kowar Sufaid	0.05	0	75	25	1.33	4.97	4.5	0.55	0.34
	0.20	0	50	50	1.5	4.40	5.0	0.28	0
	0.75	0	100	0	4.0	3.16	6.06	2.41	0.24
	2.00	0	75	25	4.66	3.81	6.71	3.51	1.04
	Mean	0	75 [b]	25 [a]	2.86 [a]	4.09 [b]	5.58 [b]	1.68 [b]	0.41 [a]
	Range	0	50-100	0-50	1.5-4.66	3.16-4.97	4.5-6.7	0.28-3.51	0-1.04
4472(12) Harker Agoon	0.05	0	100	0	1.5	8.50	5.67	1.07	0.61
	0.20	0	66.6	33.3	1.0	2.45	4.5	0.42	0
	0.75	0	100	0	4.0	2.24	6.17	2.03	0
	2.00	0	100	0	4.33	2.72	4.85	3.60	0
	Mean	0	91.6 [a]	8.3 [b]	2.70 [a]	3.97 [b]	5.29 [b]	1.78 [b]	0.15 [b]
	Range	0	66.6-100	0-33.3	1.0-4.33	2.24-8.50	4.5-6.17	0.42-3.60	0-0.61
4492(1) Kowar Naray	0.05	0	33.3	33.3	1.0	8.0	11.0	1.90	0.95
	0.20	0	100	0	1.66	4.5	4.8	1.25	0.24
	0.75	0	66.6	33.3	2.0	3.23	6.25	1.49	0.21
	2.00	0	33.3	66.6	3.0	3.13	6.33	9.78	0.53
	Mean	0	58.3 [b]	30.3 [a]	1.92 [b]	4.72 [a]	7.09 [a]	3.61 [a]	0.48 [a]
	Range	0	33.3-100	0-66.6	1.0-3.0	3.13-8.0	4.8-11.0	1.25-9.78	0.21-0.95

Means followed by same letters do not differ significantly at 5% level

Table 2. In vitro growth responses of grape accessions affected by different concentrations of BAP and constant level (0.1 mgl^{-1}) of NAA in MS media

Accession	Treatment (mgl^{-1})	Mortality (%)	Viability (%)	Contamination (%)	Shoot number $explant^{-1}$	Shoot length (cm)	No. of nodes $plant^{-1}$	Shoot mass (g)	Root mass (g)
4494(1) Kowar Sufaid	0.1:0.05	0	75	25	1.0	10.30	8.0	1.22	1.07
	0.1:0.20	0	75	25	1.66	2.68	4.60	0.69	0
	0.1:0.75	25	50	25	3.0	2.92	4.83	0.72	0
	0.1:2.00	0	100	0	3.5	3.19	7.07	1.67	0.01
	Mean	6.25^{b}	41.25^{b}	18.75^{a}	2.29^{a}	4.77^{a}	6.12^{a}	1.07^{b}	0.27^{b}
	Range	0-25	50-100	0-25	1.0-3.5	2.68-10.3	4.60-8.0	0.69-1.67	0-1.07
4472(12) Harker Agoon	0.1:0.05	0	100	0	1.0	2.16	3.33	0.89	0.19
	0.1:0.20	0	100	0	1.33	2.30	3.25	2.13	0.09
	0.1:0.75	0	100	0	3.0	2.90	5.50	2.04	1.10
	0.1:2.00	0	100	0	3.66	2.89	5.91	3.35	0
	Mean	0	100^{a}	0	2.24^{a}	2.56^{b}	4.49^{b}	2.10^{a}	0.35^{a}
	Range	0	100	0	1.0-3.66	2.16-2.90	3.25-5.91	0.89-3.35	0-1.10
4492(1) Kowar Naray	0.1:0.05	0	33.3	66.6	1.0	1.50	1.0	0.61	0
	0.1:0.20	33.3	66.6	0	1.5	2.43	2.33	0.83	0.01
	0.1:0.75	0	100	0	1.33	3.98	6.0	1.32	0.95
	0.1:2.00	0	100	0	3.0	3.43	5.0	4.06	0.61
	Mean	8.32^{a}	74.97^{a}	16.65^{a}	1.72^{b}	2.83^{b}	3.56^{c}	1.71^{a}	0.39^{a}
	Range	0-33.3	33.3-100	0-66.6	1.0-3.0	1.5-3.98	1.0-6.0	0.61-4.06	0-0.95

Means followed by same letters do not differ significantly at 5% level

Table 3. In vitro growth responses of grape accessions affected by different concentrations of BAP and constant level (0.3 mgl^{-1}) of NAA in MS media

Accession	Treatment (mgl^{-1})	Mortality (%)	Viability (%)	Contamination (%)	Shoot number $explant^{-1}$	Shoot length (cm)	No. of nodes $plant^{-1}$	Shoot mass (g)	Root mass (g)
4494(1) Kowar Sufaid	0.3:0.05	25	75	0	1.0	8.50	10.00	1.63	3.04
	0.3:0.20	0	75	25	2.0	7.25	5.50	1.54	3.92
	0.3:0.75	0	50	50	3.0	4.33	4.00	3.99	13.06
	0.3:2.00	0	100	0	4.25	2.61	5.12	5.35	0.00
	Mean	6.25[a]	63.75[b]	18.75[b]	1.81[b]	5.67[a]	6.15[a]	3.12[a]	5.00[a]
	Range	0-25	50-100	0-50	1.0-4.25	2.61-8.5	4.0-10.0	1.54-5.35	0-13.06
4472(12) Harker Agoon	0.3:0.05	0	33.3	66.6	1.0	1.20	1.00	0.26	0.00
	0.3:0.20	0	50	50	1.5	7.03	6.33	3.35	0.57
	0.3:0.75	0	100	0	3.0	2.28	5.16	1.74	0.00
	0.3:2.00	0	100	0	2.5	3.06	4.20	0.69	0.00
	Mean	0	70.82[a]	29.15[a]	2.0[b]	3.39[b]	4.17[b]	1.51[b]	0.14[b]
	Range	0	33.3-100	0-66.6	1.0-3.0	1.2-7.03	1.0-6.33	0.26-3.35	0-0.57
4492(1) Kowar Naray	0.3:0.05	0	33.3	66.6	1.0	8.50	8.00	1.98	1.00
	0.3:0.20	0	66.6	33.3	2.5	2.80	4.00	1.36	0.22
	0.3:0.75	0	100	0	3.33	4.14	5.60	3.63	3.00
	0.3:2.00	0	100	0	5.33	3.17	4.18	9.01	1.01
	Mean	0	74.97[a]	24.97[a]	3.04[a]	4.65[a]	5.44[a]	3.99[a]	1.06[b]
	Range	0	33.3-100	0-66.6	1.0-5.33	2.8-8.5	4.0-8.0	1.36-9.01	0.22-3.00

Means followed by same letters do not differ significantly at 5% level

Table 4. Effect of different hormonal regimes of NAA (0 or 0.3mgl^{-1}) and BAP (0.25, 0.50, 0.75, 1.0mgl^{-1}) in MS media on in vitro growth responses of grape accessions

Accession	Treatment (mgl^{-1})	Mortality (%)	Viability (%)	Contamination (%)	Shoot number explant^{-1}	Shoot length (cm)	No. of nodes plant^{-1}	Shoot mass (g)	Root mass (g)
4494(1) Kowar Sufaid	0.0:0.25	0	66.6	0	3.00	2.38	3.66	0.99	0.04
	0.0:0.50	0	100	0	2.83	2.54	4.47	4.13	0.15
	0.0:0.75	0	100	0	2.83	2.44	4.70	4.37	0.06
	0.0:1.00	0	100	0	4.66	2.60	3.14	5.17	0.00
	0.3:0.25	0	100	0	1.50	2.11	2.44	1.19	0.52
	0.3:0.50	0	100	0	2.83	2.23	2.88	5.39	0.71
	0.3:0.75	0	83.3	16.6	3.00	2.36	3.60	3.21	0.85
	0.3:1.00	0	83.3	16.6	3.00	2.44	4.46	3.52	0.03
	Mean	0	91.65^{b}	4.15^{b}	2.95^{b}	2.38^{a}	3.66^{b}	3.49^{b}	0.29^{b}
	Range	0	66.6-100	0-16.6	1.5-4.66	2.11-4.70	2.44-4.70	0.99-5.39	0-0.85
4472(12) Harker Agoon	0.0:0.25	0	100	0	3.33	2.04	3.20	2.48	0.30
	0.0:0.50	0	100	0	2.00	2.33	3.66	2.84	0.00
	0.0:0.75	0	100	0	2.33	1.86	3.86	0.94	0.00
	0.0:1.00	0	100	0	3.00	2.78	3.55	2.73	0.00
	0.3:0.25	0	100	0	2.33	2.01	2.86	0.69	0.00
	0.3:0.50	0	66.6	33.3	2.00	2.05	3.00	1.35	0.00
	0.3:0.75	0	66.6	33.3	2.50	1.78	3.00	0.89	0.00
	0.3:1.00	0	100	0	3.00	2.05	3.33	1.54	0.00
	Mean	0	91.65^{b}	8.32^{a}	2.56^{b}	2.11^{a}	3.52^{b}	3.30^{b}	0.03^{c}
	Range	0	66.6-100	0-33.3	2-3.33	1.78-2.78	2.86-3.86	0.69-2.84	0-0.30
4492(1) Kowar Naray	0.0:0.25	0	100	0	2.66	2.98	4.50	1.84	0.00
	0.0:0.50	0	100	0	6.00	2.72	4.05	12.60	2.35
	0.0:0.75	0	100	0	6.33	2.68	3.00	7.31	1.03
	0.0:1.00	0	100	0	6.00	2.51	3.61	4.33	0.28
	0.3:0.25	0	100	0	1.00	2.56	3.66	1.72	1.89
	0.3:0.50	0	100	0	3.00	2.24	3.55	2.84	1.26
	0.3:0.75	0	100	0	3.00	2.44	4.55	4.28	3.09
	0.3:1.00	0	66.6	33.3	3.00	2.17	5.33	2.51	0.00
	Mean	0	95.82^{a}	4.16^{b}	3.87^{a}	2.53^{a}	4.03^{a}	4.67^{a}	1.23^{a}
	Range	0	66.6-100	0-33.3	1.0-6.33	2.17-2.98	3.0-5.33	1.72-12.6	0-3.09

Means followed by same letters do not differ significantly at 5% level

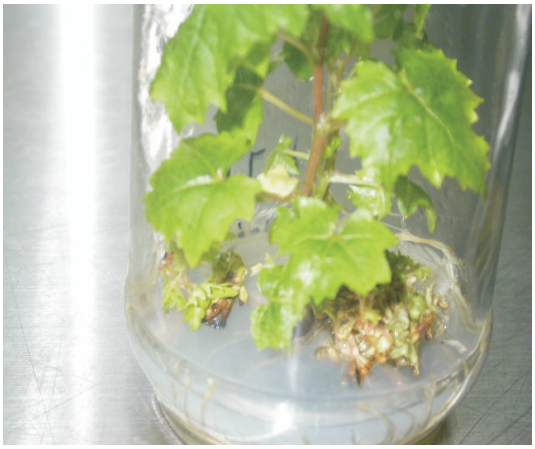

Figure 1(a). Well rooted and fully grown in vitro plant of grape germplasm accession, 4472 (12)

containing 0.1 mgl^{-1} of NAA and 0.05 mgl^{-1} of BAP in accession 4494 (1) was the best combination to obtain highest shoot length and number of nodes per plant with mean values of 10.3 cm and 8.0, respectively. However, maximum shoot mass with mean value of 4.06 g was obtained in accession 4492(1) on the media containing 2.0 mgl^{-1} of BAP and 0.1 mgl^{-1} of NAA (Table 2).

A second constant level, of NAA (0.3mgl^{-1}) and different concentrations of BAP (0.05, 0.20, 0.75, 2.0 mgl^{-1}) were studied. A 100% viability, highest shoot number/explant (5.33) and shoot mass (9.01g) was obtained in 4492(1) with the medium containing NAA (0.3mgl^{-1}) and BAP (2.0mgl^{-1}). In all the accessions, no contamination was observed when grown on media containing NAA 0.3 mgl^{-1} and BAP 2.0 mgl^{-1}. Highest root mass (13.06g) was observed in accession 4494 (1) on the medium containing NAA (0.3mgl^{-1}) and BAP (0.75mgl^{-1}). Maximum shoot length (8.5 cm) was obtained in accessions 4492(1) and 4494(1) on media containing NAA (0.3 mgl^{-1}) and BAP (0.05 mgl^{-1}) (Table 3).

In another study BAP level was increased from 0.25, 0.50, 0.75 and 1.0 mgl^{-1} either without NAA or with 0.3 mgl^{-1} of NAA in the MS media. It was observed that BAP affected the growth of cultures depending upon whether or not NAA was present (Table 4). In the presence of NAA (0.3 mgl^{-1}), with (0.25 mgl^{-1}) BAP in the media resulted in the decline of shoot number in grape accession 4492 (1). Similarly, 100% viability and no contamination were observed in this genotype. In 4492 (1), maximum shoot length (2.98 cm) was obtained when 0.25 mgl^{-1} BAP was used with no NAA. Among all accessions studied maximum shoot mass (12.6 g) was noted in 4492 (1) on media containing BAP 0.50 mgl^{-1} without NAA. Maximum root mass (3.09g) was obtained in 4492 (1) on media containing 0.75 mgl^{-1} BAP with 0.3 mgl^{-1} NAA.

In present study, when a single hormone BAP was increased from 0.05 to 2.0 mgl^{-1}, there was a progressive increase in culture response. A similar finding was observed in another study where a very low level of benzyladenine (0.05 mgl^{-1}) had a stimulating effect on shoot growth and development, and the viability of regenerants (Doroshenko, 1996). Jaskani et al. (2008) reported that better shooting (80%) was observed on MS medium with 0.05 mgl^{-1} BAP whereas Chen et al. (2000) have found that BAP in the medium at a concentration of 1mgl^{-1} favors shoot differentiation and improves the quality of plants. The accumulative effect of two growth regulators on the morphogenesis of grape accessions in

present study was found excellent in accession 4494(1) to obtain highest shoot length and number of nodes per plant using 0.1mgl^{-1} and 0.05mgl^{-1} of NAA and BAP, respectively. Tapia and Read (1998) also observed that variation in growth responses of grapes accessions was highly genotype dependent and BAP levels at (0.5 or 1.0 mgl^{-1}) combined with 0.01 mgl^{-1} of NAA proved to be the best medium for shoot proliferation.

Effect of BAP, NAA, IBA and Kinetin on Growth Rate and Shoot Proliferation

Different combinations of four hormones containing IBA (0.2 mgl^{-1}), BAP (1.0 mgl^{-1}), NAA (0.2 mgl^{-1}) and kinetin (0.5, 1.0 and 2.0 mgl^{-1}) were tested to find out the response of three accessions. Maximum shoot number per plant (5.0) and shoot mass (10.2 g) were obtained with the accession 4494(1) on media containing the NAA 0.2, BAP, 1.0 and K 1.0 mgl^{-1}, and the media containing 0.2 mg of IBA and 2.0 mgl^{-1} of Kinetin was found suitable for obtaining maximum shoot length (6.33 cm) and 100% viability in 4492(1). Maximum nodes (7.5) and shoot length (4.98 cm) with 100% viability was obtained on the MS media containing 0.2 mgl^{-1} of IBA and 1.0 mgl^{-1} of kinetin in 4494(1). Maximum root mass (4.8 g) was obtained with the MS media containing 0.2, 1.0 and 1.0 mgl^{-1} of IBA, BAP and kinetin, respectively in 4494 (1) (Table 5).

In present study, when NAA was used in combination with BAP and kinetin, it stimulated the growth of cultures and maximum shoot mass of 10.2 g and 4.6 g was obtained from 4494 (1) and 4472 (12), respectively. In another study, the presence of IAA or NAA in the media promoted shoot length even in the presence of BAP and NAA was more effective in promoting shoot length of accession viz., Sundar Khani (Sajid et al., 2006). The cultures in present studies did not show such kind of responses. This may be credited to genotypic differences. In other study of grape establishment, half strength MS media was more suitable for meristem establishment as reported by Choi et al. (1992). However, they recommended the full strength MS media supplemented with BAP and IAA for maximizing the shoot proliferation. They also used kinetin to study the effect on shoot multiplication and no notable effect was found. In sugarcane cultures. Saira et al. (2005) reported that BAP affects the shoot mass as its concentration is increased along with the auxin in the media. Responses among banana cultivars are influenced by hormone type, concentration and cultivar as recommended by Hamide and Pekmezei (2004) and Shirani et al. (2009). Moreover, Buah et al. (2010) observed that media supplemented with 4.5 mgl^{-1} of BAP induced the highest number of shoots after eight weeks of culture in two banana cultivars and BAP had the highest shoot induction response in both cultivars, followed by kinetin and 2ip. Furthermore the degree of efficiency of shooting was dependent on the type of hormone and the plant cultivar.

Effect of IAA, NAA and IBA on Root Induction

Different concentrations of IAA, NAA and IBA incorporated in 75% solid MS media were compared to study root induction efficiency. It was

Table 5. In vitro growth responses of grape accessions affected by different concentrations of IBA, BAP, Kinetin and NAA in MS media

Accession	Treatment (mgl^{-1})*	Mortality (%)	Viability (%)	Contamination (%)	Shoot number $explant^{-1}$	Shoot length (cm)	No. of nodes $plant^{-1}$	Shoot mass (g)	Root mass (g)
4494(1) Kowar Sufaid	0.2:0.5	0	75	25	1.0	3.50	5.66	0.79	0.29
	0.2:1.0	0	100	0	1.0	4.98	7.50	0.74	0.85
	0.2:2.0	0	75	25	1.33	3.78	2.30	0.73	2.42
	0.2:1.0:1.0	0	100	0	3.5	4.08	4.79	3.28	4.80
	0.2:1.0:1.0	25	25	50	5.0	4.20	6.20	10.20	2.51
	Mean	5^{b}	75^{b}	20^{a}	2.36^{a}	4.11^{a}	5.28^{b}	6.80^{a}	2.17^{a}
	Range	0-25	25-100	0-50	1.0-5.0	3.5-4.98	2.30-6.20	0.73-10.2	0.29-4.80
4472(12) Harker Agoon	0.2:0.5	0	100	0	1.33	2.80	3.50	0.18	0.03
	0.2:1.0	0	100	0	1.33	2.87	4.00	1.56	0.09
	0.2:2.0	25	50	25	1.50	2.70	2.00	0.46	0.11
	0.2:1.0:1.0	33.30	66.6	0	4.50	2.38	3.33	0.99	0.00
	0.2:1.0:1.0	0	66.6	33.3	3.50	2.99	6.43	4.60	0.04
	Mean	11.66^{a}	76.64^{b}	11.66^{b}	2.43^{a}	2.74^{b}	3.84^{b}	1.55^{b}	0.05^{b}
	Range	0-33.3	50-100	0-33.3	1.33-4.50	2.70-2.99	2.0-6.43	0.18-4.60	0.0-0.11
4492(1) Kowar Naray	0.2:0.5	0	66.6	33.3	1.0	3.50	3.50	1.42	0.57
	0.2:1.0	0	100	0	1.0	5.66	5.00	0.36	0.50
	0.2:2.0	0	100	0	1.0	6.33	5.66	2.47	1.60
	0.2:1.0:1.0	0	66.6	33.3	4.0	4.68	6.50	4.34	0.76
	0.2:1.0:1.0	0	100	0	2.33	3.71	4.71	3.46	1.38
	Mean	0	86.64^{a}	13.32^{b}	1.86^{b}	4.77^{a}	5.00^{a}	2.41^{b}	0.96^{b}
	Range	0	66.6-100	0-33.3	1.0-4.0	3.50-6.33	3.50-6.50	0.36-4.34	0.50-1.60

** 0.2:0.5 (IBA:K), 0.2:1.0 (IBA:K), 0.2:2.0 (IBA:K), 0.2:1.0:1.0 (IBA:B:K), 0.2:1.0:1.0 (NAA:B:K). Means followed by same letters do not differ significantly at 5% level*

Table 6. In vitro root induction in grape accession 4472(1) grown on MS media containing different levels of IAA, NAA and IBA

Treatment (mgl^{-1})	Mortality (%)	Viability (%)	Contamination (%)	Shoot number $explant^{-1}$	Shoot length (cm)	No. of nodes $plant^{-1}$	Shoot mass (g)	Root mass (g)
MS Control	0	80	20	1.25	5.70	4.60	0.27	0.06
IAA								
0.1	0	100	0	1.0	7.80	5.00	0.31	0.13
0.3	0	60	40	1.33	6.63	4.25	0.41	0.34
0.7	0	40	60	1.5	5.30	2.66	0.78	0.14
1.0	0	80	20	1.0	9.87	6.25	0.66	0.32
Mean	0	72[b]	28[a]	1.21[a]	7.06[a]	4.55[a]	0.48[b]	0.19[b]
Range	0	40-100	0-60	1.0-1.5	5.30-9.87	2.66-6.25	0.27-0.78	0.06-0.34
NAA								
0.1	0	80	20	1.0	6.55	4.00	1.16	0.67
0.3	0	60	40	1.0	4.83	3.66	0.41	0.82
0.7	0	80	20	1.0	3.95	3.25	0.52	1.19
1.0	0	60	20	1.0	1.13	1.00	0.17	0.45
Mean	4[a]	72[b]	24[b]	1.0[b]	4.43[b]	3.30[b]	0.50[b]	0.63[b]
Range	0-20	60-80	20-40	1.0	1.13-6.55	1.0-4.0	0.17-1.16	0.45-1.19
IBA								
0.2	0	80	20	1.0	8.65	5.50	0.43	0.32
0.5	0	80	20	1.0	9.13	5.75	0.93	0.49
1.0	0	80	20	1.0	4.93	2.75	0.43	0.44
2.0	0	80	20	1.0	9.00	6.25	1.27	1.07
Mean	0	80[a]	20[b]	1.0[b]	7.48[a]	4.97[a]	0.66[a]	1.47[b]
Range	0	80	20	1.0	4.93-9.13	2.75-6.25	0.27-1.27	0.32-1.07

Means followed by same letters do not differ significantly at 5% level

observed that there was an increase from 0.06 to 0.34g in root mass by increasing the concentrations of IAA. The maximum root mass (0.34g) was obtained with the 0.3 mgl^{-1}. With NAA (0, 0.1, 0.3, 0.7, 1.0 mgl^{-1}) best rooting was obtained at the concentration of 0.7 mgl^{-1} of NAA. Root mass was increased from 0.06 to 1.19g by increasing the concentrations of NAA. The highest rooting response (1.07 g) was obtained at the highest level of IBA (2.0 mgl^{-1}) (Table 6 and Figure 1b).

There was a strong linear relationship between rooting response and IBA levels. As the concentration of IBA was increased, the rooting response improved accordingly. Jiang and Jin (1995) also reported that IAA was a suitable additive for the rooting of plantlets with the optimum concentrations being 0.5-1.0 mgl^{-1}. Choi et al. (1992) documented that the best media for rooting contained 0.5 and 1.0 mgl^{-1} NAA. Similarly, Lewandowski (1991) obtained 95% rooting of micro-cuttings on MS having a combination of IBA and NAA. Compton and Gray (1995) have observed that the root production was 76-83% when transferred to medium with 1mg NAA or IBA, respectively. On the contrary, Gok et al. (1997) have reported root induction on hormone free media or the media containing IBA. The findings of Jaskani et al. (2008) also indicated that media having 10 µM IBA proved the best for root formation in grapes micro shoots.

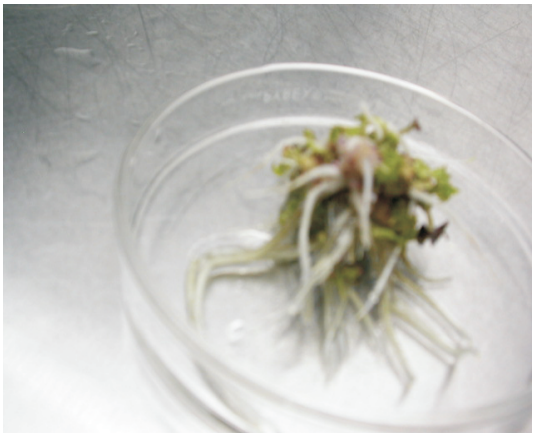

Figure 1(b). Roots mass harvested from grape accession grown in vitro on MS media containing NAA (0.7mgl^{-1})

This is a comprehensive study as it covers the influence of different hormonal combinations along with the type of media which contribute well in the micropropagation of grape fruit crop. The media optimized in the present study is applicable on a commercial scale production of true to type grape plants.

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