Asian Journal of Chemistry; *Vol. 25, No. 1 (2013), 287-291*

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13001

Effect of Different Cytokinins and Auxins on Microopropagation and Callogenesis in Amaryllus (*Amaryllis vittata*)

AAMIR ALI*, RABIA SHAHEEN NIAZI, SAIRA YASMIN, ABDUL MAJID and NAIMA HUMA NAVEED

Department of Biological Sciences, University of Sargodha, Sargodha, Pakistan

*Corresponding author: E-mail: aamirali73@hotmail.com

(*Received*: 2 November 2011; *Accepted*: 20 July 2012) AJC-11864

In vitro propagation of *Amaryllis vittata* was carried out to explore the regenerative and callogenic potential of different explants (both meristem and twin scales were used as explants) and to study the influence of different concentrations and combinations of various phytoharmones along with varying environmental conditions like temperature and light on *in vitro* shoot initiation, formation, multiplication and rooting. Shoot formation response (92 %) from meristem was obtained on Murashige and Skoog's medium (MS) containing 2.5 mg/L of kinetin after 11 days of inoculation. When twin scales were used as explants 92 % shoot initiation response was obtained on MS medium supplemented with 2 mg/L 6-benzylamino purine (BAP) + 0.25 mg/L α-naphthalene acetic acid (NAA). For multiplication of *in vitro* developed shoots, the best response was obtained on MS medium containing 2 mg/L BAP + 0.25 mg/L NAA with 4.4 shoots per culture vial having an average shoot length of 4.18 after 10 days of inoculation. For rooting, best response was obtained on ½MS medium supplemented with 1.0 mg/L NAA + 0.5 mg/L indole butyric acid (IBA) where 4.4 shoots were obtained having an average shoot length of 3.7 cm in about 10 days of explants inoculation. A temperature of 26 ºC and photoperiod of 16 h and 8 h dark in every 24 h cycle proved best for *in vitro* culturing of amaryllis.

Key Words: Cytokinins, Auxins, Micropropagation, Amaryllus.

INTRODUCTION

World-wide the Amaryllis has greatest economic value as ornamental, flavouring and phycotropic plant[1,2]. It is most suitable for planting in border, shrubbery and in pots[3]. Amaryllis plants are also a source of number of alkaloids of considerable medicinal value, pseudolycorine has antiviral activity and Narciclasine has antimitotic activity[4]. Simlarly, lycorine isolated from many Amaryllis plants has been studied for its inhibitory activities against human immunodeficiency virus (HIV-1), severe acute respiratory syndrome-associated corona virus (SARS-CoV), poliovirus, coxsackie virus, measles virus and herpes simplex virus type 1 (HSV-1)[5,6]. Lycorine in some plant species have also been reported to inhibit *Plasmodium falciparum* growth[7].

Amaryllis is also attacked by several viral and fungal diseases and pests, which cause extensive damage to the plants and bulbs. The common means of Amaryllis propagation are from seeds and by vegetative methods. Seed being very small in size have low rate of germination and seed propagation also results in variation. In vegetative method of propagation, plant once infected by pathogen can transfer it generation after generation. These conventional methods of propagation are not only time consuming but also they do not ensure healthy, true to type and disease-free plants.

Plant tissue culture is providing new means for the conservation and rapid propagation of valuable, rare, endangered and disease free plants[8] that are true to type and disease-free[9]. The present research work was planned to analyze the effect of various phytoharmones on *in vitro* propagation of Amaryllis using different type of explants under variable environmental conditions (temperature and light).

EXPERIMENTAL

For *in vitro* propagation, bulbs were used as explants which were obtained from plant nursery situated on university road, Sargodha. The undesired leaves, root segments and dry upper bulb scales were removed with the help of sterilized scalapel. As meristematic portion and inner scales of Amaryllis has covering of rolled scales, therefore, these explants are naturally sterilized. Hence, it is not necessary to adopt an elaborate protocol for disinfection. However, to prevent any contamination from outer covering of scales, surface sterilization was carried out. For this purpose explants were first washed thoroughly with running tap water for 5 min. Then they were dried with sterilized paper to remove all traces of tap water. This was followed by treating explant with 1 % sodium hypochlorite solution for 15 min. Then explants were washed thoroughly 3 times with autoclaved double distilled water. The sterilized explants were then inoculated by proper

TABLE-1
EFFECT OF DIFFERENT HORMONES ON *in vitro* Shoot INITIATION AND FORMATION *Amaryllis vittata*

Media (MS)	Concentration (mg/L)	Days of shoot formation	Shoot length (cm)	Rate of shoot formation (%)	Days of shoot formation	Shoot length (cm)	Rate of shoot formation (%)
		Apical meristem			Twin scales		
Basal		20.8 ± 1.16^{k}	1.52 ± 0.38^{ij}	24	30.2 ± 1.32^{l}	0.88 ± 0.52^{i}	28
BAP	1.0	16 ± 0.894^{j}	1.8 ± 0.32^{ghi}	28	21.8 ± 0.97^{kl}	0.94 ± 0.34^{i}	44
BAP	1.5	15.2 ± 0.7^{hi}	2.22 ± 0.34^{fgh}	32	18.2 ± 1.32^{ijkl}	$2.6 \pm 0.0.24^{cde}$	52
BAP	2.0	14.2 ± 0.74^{gh}	2.08 ± 0.3^{fgh}	44	18.4 ± 0.8^{ijkl}	3.42 ± 0.27^{ab}	56
BAP	2.5	13 ± 1.09^{ef}	2.8 ± 0.40^{de}	56	16 ± 0.894^{fgh}	$3.66 \pm 0.0.3^{a}$	76
BAP	3.0	$12 \pm 0.0.89^{cdef}$	3.02 ± 0.33^{cde}	64	13.4 ± 1.01^{b}	3.9 ± 0.26^{a}	88
BAP	3.5	10.8 ± 0.74^{b}	3.92 ± 0.48^{ab}	68	14.4 ± 0.48^{cd}	2.9 ± 0.41^{cd}	80
BAP	4.0	8 ± 0.632^{a}	3.92 ± 0.24^{ab}	84	15.2 ± 0.77^{def}	$1.8 \pm 0.0.27^{h}$	72
BAP	4.5	11 ± 0.632^{c}	3.56 ± 0.36^{abc}	60	17.8 ± 0.74^{hij}	1.94 ± 0.28^{gh}	68
Kinetin	1.0	15.8 ± 0.74^{j}	0.96 ± 0.19^{j}	16	17 ± 0.63^{hi}	0.9 ± 0.38^{i}	20
Kinetin	1.5	14.6 ± 0.8^{hi}	1.46 ± 0.24^{ij}	24	16.2 ± 0.74^{fgh}	1.26 ± 0.19^{i}	52
Kinetin	2.0	13.4 ± 0.48^{fg}	2.18 ± 0.27^{fgh}	72	14.8 ± 0.97^{cde}	2.44 ± 0.25^{def}	68
Kinetin	2.5	11 ± 0.63^{c}	3.38 ± 0.33^{bcd}	82	13.6 ± 0.8^{b}	3.08 ± 0.34^{bc}	84
Kinetin	3.0	12.8 ± 0.97^{def}	2.42 ± 0.44^{efg}	64	14.4 ± 0.48^{cd}	2.78 ± 0.20^{cde}	72
BAP + Kinetin	0.5 + 0.5	16.2 ± 0.74^{j}	2.64 ± 0.4^{ef}	56	17.8 ± 0.74^{hij}	1.9 ± 0.24^{gh}	56
BAP + Kinetin	1.0 + 0.5	$15.4 \pm 0.48i^{j}$	2.84 ± 0.6^{de}	60	15.4 ± 0.4^{efg}	2.36 ± 0.3^{efg}	64
BAP + Kinetin	1.5 + 0.5	12.6 ± 0.64^{cdef}	3.84 ± 0.3^{ab}	88	14 ± 0.89^{c}	3.1 ± 0.17^{bc}	84
BAP + Kinetin	2.0 + 0.5	14.8 ± 0.74^{hi}	3.6 ± 0.34^{ab}	64	16.6 ± 0.8^{ghi}	2.9 ± 0.25^{cd}	72
BAP + NAA	1.0 + 1.0	19.2 ± 0.74^{j}	1.1 ± 0.40^{j}	60	20.8 ± 1.6^{jkl}	2.4 ± 0.42^{def}	36
BAP + NAA	2.0 + 0.25	11 ± 0.632^{c}	4.02 ± 0.29^{a}	84	13.2 ± 0.7^{a}	3.46 ± 0.28^{ab}	92
BAP + NAA	2.0 + 0. 5	15.4 ± 1.01^{j}	2.08 ± 0.3^{fghi}	36	15 ± 0.632^{def}	2.02 ± 0.3^{fgh}	76
BAP + NAA	2.0 + 1.0	11.6 ± 0.28^{cdef}	1.7 ± 0.70^{hi}	56	19 ± 0.63^{jklm}	1.7 ± 0.36^{h}	88
LSD (0.05)		1.12	0.54		1.24	0.44	

Means followed by different letters in the same column differ significantly at p = 0.05 according to Duncan's new multiple range tests.
BAP = 6-Benzylamino purine; NAA = α-Naphthaleneacetic acid.

dissecting and sizing the meristem (1.0-5.0 mm) and scales (0.5-1.0 cm) on Murashige and Skoog's medium (MS)[10] supplemented with different concentrations of 6-benzylamino purine (BAP), kinetin and α-naphthaleneacetic acid (NAA) either alone or in combination with each other. Varying concentrations and combinations of BAP, Kinetin and NAA were also tested for multiplications of well developed shoots. For *in vitro* rooting MS medium containing different concentrations of NAA and indolebutyric acid (IBA) were used either alone or in combination with each other. Explants were inoculated in both solid and liquid medium and pH of the medium was adjusted in the range of 5.7-5.8 with 1.0 N solution of NaOH or HCl. 3 % sucrose was used in all the culture media and solidification of medium was carried out with 0.6 % agar. The medium was autoclaved at 121 ºC and 15 Ibs/Inch^2 pressure for 15 min. The data was recorded for number of days for shoot initiation from scales and shoot formation from meristem, number of shoot per culture vial, days for *in vitro* shoot multiplication, days for root induction and days for callus induction.

The experimental design was completely randomized with five replicate cultures for each hormonal treatment and each experiment was repeated thrice. Duncan's new multiple range tests for significance between mean were applied for analysis of variance (ANOVA)[11].

RESULTS AND DISCUSSION

For *in vitro* shoot initiation and formation from twin scales and meristem, MS medium was supplemented with different concentrations of BAP and Kinetin either alone or in combination with each other or with NAA. Two type of explants were used in present study *i.e.* meristem and twin scales. Data presented in Table-1 depicts that when shoot apical meristem was inoculated on MS basal medium, an average of 1.52 shoots were formed after 20.8 days of inoculation. However when BAP was added in MS medium, shoot formation response was enhanced and at 4.0 mg/L of BAP, 84 % shoot formation response was obtained after 8 days of meristem inoculation having an average shoot length of 3.92 cm. By further increase in the concentration of BAP, not only the rate of shoot formation was decreased but time taken for shoot formation was also increased. In case of twin scale explants best results were obtained on MS medium containing 3.0 mg/L of BAP *i.e.* 88 % shoot formation after 13.4 days of explants inoculation having an average shoot length of 3.9 cm. This difference in requirement of growth hormones may be due to the difference in endogenous growth hormones in both type of explants. Fidalgo *et al.*,[12] also reported the use of twin scales as appropriate starting material for micropropagation. Similarly, Aslam *et al.*,[13] reported the scales of bulbous plants to be the most suitable explants for micropropagation.

Different concentrations of Kinetin ranging from 1-3 mg/L were also used in MS medium but no good results as compared to BAP were obtained (Table-1). MS medium was also supplemented with different concentration of BAP with Kinetin or NAA. Among these combinations 2.0 mg/L BAP + 0.25 mg/L NAA provided best results for twin scale explants which were 92 % shoot initiation after 13.2 days of inoculation having an average shoot length of 3.46 cm but for meristematic explants results were similar as were obtained by using 4.0 mg/L BAP *i.e.* 84 % shoot formation response. Zhang *et al.*,[14] reported up to 90 % shoot induction response when MS medium was

TABLE-2
EFFECT OF DIFFERENT HORMONES ON *in vitro* SHOOT MULTIPLICATION

Media (MS)	Concentration (mg/L)	Days of shoot multiplication	No of shoots per culture vial	Average shoot length (cm)
Basal		28.6 ± 1.49^{i}	1 ± 0.632^{g}	1.48 ± 0.35^{fg}
BAP	1.0	20.2 ± 0.74^{i}	1.2 ± 0.4^{g}	1.9 ± 0.236^{f}
BAP	1.5	16.4 ± 0.489^{gh}	2.2 ± 0.4^{ef}	2.6 ± 0.17^{de}
BAP	**2.0**	**12.4 ± 0.806^{de}**	**4 ± 0.632^{abc}**	**3.9 ± 0.37^{ab}**
BAP	2.5	14.6 ± 0.489^{f}	3.2 ± 0.748^{cd}	3.1 ± 0.22^{cd}
Kinetin	1.0	16 ± 0.89^{gh}	2.2 ± 0.74^{ef}	1.06 ± 0.27^{g}
Kinetin	1.5	14.8 ± 0.74^{g}	2.8 ± 0.4d^{e}	2.4 ± 0.36^{e}
Kinetin	**2.0**	**11.2 ± 0.97^{c}**	**4 ± 0.88^{abc}**	**3.9 ± 0.17^{ab}**
Kinetin	2.5	12.8 ± 0.48^{e}	3.2 ± 0.48^{cd}	3.88 ± 0.25^{ab}
BAP + Kinetin	0.5 + 0.5	14.6 ± 0.64^{f}	4.4 ± 0.48^{ab}	2.52 ± 0.44^{e}
BAP + Kinetin	1.0 + 0.5	13.8 ± 0.74^{f}	2.6 ± 0.8^{de}	3.1 ± 0.46^{cd}
BAP + Kinetin	**1.5 + 0.5**	**10.8 ± 0.97^{b}**	**4.8 ± 0.4^{a}**	**3.58 ± 0.27^{bc}**
BAP + Kinetin	2.0 + 0.5	12.2 ± 0.4^{cd}	1.4 ± 0.48^{fg}	3.14 ± 0.67^{c}
BAP + NAA	1.0 + 1.0	18.2 ± 0.74^{hi}	1.4 ± .529^{fg}	1.46 ± 0.28^{fg}
BAP + NAA	1.0 + 2.0	17.6 ± 1.01^{gh}	2.2 ± 0.7^{ef}	2.58 ± 0.30^{f}
BAP + NAA	**2.0 + 0.25**	**12.2 ± 0.4^{cd}**	**3.8 ± 0.4^{bc}**	**3.83 ± 0.31^{ab}**
BAP + NAA	2.0 + 0.5	14 ± 0.894^{f}	2.4 ± .48de	3.44 ± 0.28^{bc}
Kinetin + NAA	1.0 + 1.0	17.4 ± 0.48^{gh}	2.4 ± 0.48^{de}	1.92 ± 0.32^{f}
Kinetin + NAA	2.0 + 0.5	12.6 ± 0.8^{f}	3.2 ± 0.4^{cd}	3.08 ± 0.29^{cd}
Kinetin + NAA	**2.0 + 0.25**	**10.2 ± 0.74^{a}**	**4.4 ± 0.88^{ab}**	**4.18 ± 0.21^{a}**
Kinetin + NAA	2.0 + 1.0	14.2 ± 0.748^{f}	2.2 ± 0.74^{ef}	3.24 ± 0.18^{c}
LSD (0.05)		**1.12**	**0.83**	**0.46**

Means followed by different letters in the same column differ significantly at p = 0.05 according to Duncan's new Multiple range tests.
BAP = 6-Benzylamino purine; NAA = α-Naphthaleneacetic acid.

supplemented with 2.0 mg/L BAP + 1.0 mg/l NAA, but in present investigation better results with more number of shoots *i.e.* 3.7 shoots per culture vial were obtained at lower concentration of NAA *i.e.* 0.25 mg/L. It was further observed in present study that 3.0 mg/L BAP proved best for *in vitro* shoot formation and initiation from both type of explants used *i.e.* meristem and twin scales (Table-1).

Yi-min and Su-Juan[15] reported that high concentration of sucrose is important for shoot initiation and formation. According to them maximum shoot initiation response was obtained by supplementing ½MS medium with 2.0 mg/L BAP + 1.0 mg/L NAA and using 45 g/L of sugar. Similarly Azadi and Khosh[16] also used MS medium containing 0.1mg/L BAP + 0.1 mg/L NAA with a higher concentration of sucrose *i.e.* 6 % to obtain the best shoot initiation response, however in present study 3 % sucrose was found to be sufficient enough to get good shoot formation response.

Once shoot formation has been achieved successfully they were further shifted on fresh MS medium supplemented with different growth regulators for *in vitro* shoot multiplication. For this purpose MS medium was supplemented with different concentrations and combinations of different cytokinins (BAP and Kinetin) either alone or in combination with an auxin (NAA). Statistics of Table-2 gives an account that uniform shoot multiplication response was obtained at 2.0 mg/L of BAP as well as at 2 mg/L of kinetin. Combination of BAP with NAA also provided good shoot multiplication response but number of shoots was less as compared to other combinations however their shoot length was found to be more. It can be adjudged from the present finding that for efficient shoot multiplication response it is not only the concentration that matters most but also the type of cytokinin used. According to Yew *et al.*,[17] relatively high concentrations of cytokinins should be present to observe high multiplication rates but in present study low concentration of BAP as well as Kinetin produced the optimum results. Our findings are also strengthened by the findings of Kohut *et al.*,[18] who also used low concentration of BAP *i.e.* 1.0 mg/L for optimum shoot formation response.

In present study cytokinin-cytokinin and cytokinin-auxin combination was also used for *in vitro* shoot multiplication. It was observed that 2.0 mg/L Kinetin + 0.25 mg/L NAA proved to be the best combination for shoot multiplication with 4.0 shoots per culture vial after 10.2 days of inoculation having an average shoot length of 4.18 cm (Table-2). Although, in present study a high amount of Kinetin (2.0 mg/L) was used in combination with a low concentration of NAA (0.25 mg/L) for shoot multiplication whereas according to Binh *et al.*,[19] a lower concentration of Kinetin (0.5 mg/L) along with same amount of NAA as used in present study, produce shoots in some species and genus of *Amaryllidaceae*. While Bansude *et al.*,[20] reported that when Kinetin (1-2 mg/L) was used in combination with NAA (0.5 mg/L), it resulted in the induction of somatic embryogenesis in Agave, another member of family *Amaryllidaceae*. So, it can be inferred that different species and genus of family *Amaryllidaceae* have different response to the combination of Kinetin and NAA.

The present study also aimed to find out the best media composition for *in vitro* rooting. Both half and full strength MS media were supplemented with varying concentrations of different auxins *i.e.* IBA and NAA either alone or in combination with each other. In present study, although roots were obtained in full strength MS basal medium but number of roots per plant were less and time taken for rooting was also more. Best results were obtained in ½ MS medium supplemented with 1.0 mg/L of NAA and 0.5 mg/L of IBA with 4.4 roots per plant after 10.6 days of inoculation (Table-3). Zhang *et al.*,[14]

TABLE-3
EFFECT OF DIFFERENT AUXINS ON *in vitro* ROOTING OF *Amaryllis vittata*

Media (MS)	Concentration (mg/L)	Day of root formation	No of average roots	Average root length (cm)
½MS		16 ± 0.632^{m}	1.4 ± 0.8^{ghij}	0.68 ± 0.32^{l}
Basal		18.6 ± 0.8^{m}	1.8 ± 0.4^{fghi}	0.74 ± 0.29^{l}
½MS + NAA	0.5	12.8 ± 0.74^{cdefg}	3.4 ± 0.48^{abcd}	1.02 ± 0.33^{kl}
½MS + NAA	1.0	10.6 ± 0.8^{a}	3.2 ± 0.74^{bcde}	2.44 ± 0.56^{cdefg}
½MS + NAA	1.5	13.6 ± 0.48^{efghi}	2.6 ± 0.48^{cdef}	3.68 ± 0.25^{a}
½MS + NAA	2.0	15.8 ± 0.74^{m}	2.4 ± 0.64^{defg}	3.5 ± 0.12^{a}
MS + NAA	0.5	16.6 ± 0.48^{m}	1.8 ±0.97^{fghi}	2.48 ± 0.32^{cdef}
MS + NAA	1.0	14.8 ± 0.97^{jkl}	2.4 ± 0.48^{defg}	2.54 ± 0.31^{cde}
MS + NAA	1.5	14.6 ± 1.01^{ijkl}	2.8 ± 0.74^{bcdef}	1.94 ± 0.30^{fghij}
MS + NAA	2.0	13.8 ± 0.74^{fghi}	2.8 ± 0.97^{bcdef}	1.78 ± 0.24^{hij}
½MS + IBA	0.5	13.4. ± 0.8^{defghi}	2.2. ± 0.74^{efg}	1.92 ± 0.44^{ghij}
½MS + IBA	1.0	12.8 ± 0.74^{cdefg}	3.4 ± 0.8^{abcd}	2.68 ± 0.34^{cd}
½MS + IBA	1.5	10.8. ± 0.97^{bc}	3.8 ± 0.4^{ab}	3.3 ± 0.46^{ab}
½MS + IBA	2.0	11 ± 0.632^{bcd}	2.8 ± 0.979^{bcdef}	2.92 ± 0.28^{bc}
MS + IBA	0.5	14.4. ± 0.48^{hijkl}	0.8 ± 0.74^{ij}	0.54 ± 0.26^{l}
MS + IBA	1.0	14.8 ± 0.74^{jkl}	1.4 ± 0.48^{ghij}	1.08 ± 0.58^{kl}
MS + IBA	1.5	13.8 ± 0.97^{ghijk}	2.4 ± 0.8^{defg}	1.48 ± 0.24^{jk}
MS + IBA	2.0	15.6 ± 0.8^{m}	1.8 ± 0.74^{fghi}	1.7 ± 0.307^{hij}
½MS + NAA + IBA	0.5 + 0.5	12.6 ± 0.48^{bcdef}	2.2 ± 0.4^{efg}	2.62 ± 0.27^{cd}
½MS + NAA + IBA	1.0 + 0.5	10.6 ± 0.8^{a}	4.4 ± 0.48^{a}	3.7 ± 0.47^{a}
½MS + NAA + IBA	1.5 + 0.5	11.2 ± 0.97^{bcde}	3.6 ± 0.8^{abc}	2.3 ± 0.28^{defgh}
½MS + NAA + IBA	2.0 + 0.5	13 ± 0.89^{cdefgh}	2 ± 0.632^{fgh}	2.06 ± 0.34^{efgh}
MS + NAA + IBA	0.5 + 0.5	14.2 ± 0.74^{hijkl}	1 ± 0.894^{hij}	1.6 ± 0.29^{ij}
MS + NAA + IBA	1.0 + 0.5	14 ± 0.89^{hijkl}	2.4 ± 0.48^{defg}	0.9 ± 0.37^{l}
MS + NAA + IBA	1.5 + 0.5	15.4 ± 0.48^{l}	0.6 ± 0.48^{j}	1.64 ± 0.28^{ij}
MS + NAA + IBA	2.0 + 0.5	15.2 ± 0.74^{kl}	1.8 ± 0.74^{fghi}	0.86 ± 0.32^{l}
LSD (0.05)		1.08	0.98	0.48

Means followed by different letters in the same column differ significantly at p = 0.05 according to Duncan's new multiple range tests.
MS = Murashige and Skoog's medium; NAA = α-Naphthaleneacetic acid; IBA = Indolebutyric acid.

TABLE-4
EFFECT OF DIFFERENT HORMONES FOR CALLUS INDUCTION FROM DIFFERENT EXPLANTS

Media	Concentration (mg/L)	No. of test tubes inoculated	No of test tubes showing callus induction		Days for callus induction		Rate of callus induction	
			L	S	L	S	L	S
MS Basal		5	0 ± 0^{f}	0 ± 0^{e}	0 ± 0^{e}	0 ± 0^{d}	0	0
MS + 2,4-D	1.0	5	1.8 ± 0.74^{de}	1 ± 0.63^{d}	18.4 ± 0.8^{d}	16 ± 1.41^{c}	36	20
	2.0	5	3.4 ± 0.48^{b}	2 ± 0.89^{bcd}	15 ± 1.41^{d}	14.6 ± 1.62^{b}	68	40
	3.0	5	2.6 ± 0.48^{bcd}	4.2 ± 0.97^{a}	13.8 ± 0.74^{c}	11 ± 0.63^{a}	52	84
	4.0	5	4.6 ± 0.48^{a}	3 ± 0.63^{b}	12.8 ± 0.97^{b}	13.2 ± 0.748^{a}	92	60
MS + BAP + NAA	1.0 + 0.2	5	2 ± 0.89^{bcd}	2.6 ± 0.48^{bc}	16.6 ± 0.8^{d}	16 ± 0.89^{c}	40	52
	1.0 + 0.5	5	2.4 ± 0. 8^{cd}	1.8 ± 0.74^{cd}	13.2 ± 0.74^{c}	13.8 ± 0.74^{ab}	48	36
	2.0 + 0.25	5	1 ± 0.63^{e}	3 ± 0.63^{b}	14.6 ± 0.48^{cd}	10.8 ± 0,74^{a}	20	60
	2.0 + 0.5	5	3.2 ± 0.74^{bc}	2.8 ± 0.74^{bc}	12.6 ± 1.01^{a}	15.4 ± 1.35^{b}	64	56
LSD (0.05)			0.91	0.99	1.23	1.46		

Means followed by different letters in the same column differ significantly at p = 0.05 according to Duncan's new multiple range tests.
L = leaf; S = Twin scales; MS = Murashige and Skoog's medium; 2,4-D = 2,4-Dichlorophenoxyacetic acid; BAP = 6-Benzylamino-purine; NAA = α-Naphthaleneacetic acid.

reported *in vitro* rooting in *Amaryllis spp*. without any growth regulators and without the use of specialized rooting medium.

Callogenesis: In tissue culture studies the formation of callus is a prerequisite for organogenesis. The experimental result regarding callus induction and growth showed that the callus formation varies with type of explants and also the media composition. This is due to the fact that plant genes are not only involved in plant growth and development they also govern the inheritance of callus growth[21]. Among all the treatments the highest callus induction from leaf explants was obtained in 4.0 mg/L of 2, 4-D[22] but twin scales showed best response in 3.0 mg/l of 2, 4-D. Naik *et al.*[22], used 2.0 mg/L of 2, 4-D in bulbous plant *Ornithogalum virens* to obtain callus induction up to 75 % in greater span of time *i.e.* 2-3 weeks. When BAP was used in combination with NAA, a wide variation occurred in days to callus initiation and percentage of callus formation from different explants. The best response in combination of BAP and NAA was shown by leaf explants in medium containing 2.0 mg/L of BAP and 0.5 mg/L of NAA with 64 % response after 12.6 days of inoculation (Table-4).

The present study also adjudged the optimum temperature and light conditions for *in vitro* propagation of Amaryllus. The best shoot formation response was obtained at 26 ± 1 ºC with 16 h light and 8 h dark period in every 24 h cycle (Table 5a

TABLE-5a
EFFECT OF DIFFERENT TEMPERATURE CONDITIONS ON *in vitro* SHOOT FORMATION

Temperature (ºC)	23 ± 1	24±1	25±1	26±1	27±1	28±1	29±1	30±1
Meristem	++	+++	+++	++++	+++	+++	++	+
Twin scales	++	+++	+++	++++	+++	+++	++	+

+ = Poor; ++ = Fair; +++ = Good; ++++ = Excellent

TABLE-5b
EFFECT OF DARK AND LIGHT REGIMES ON *in vitro* SHOOT FORMATION

Photoperiod (light/dark) (h)	No of test tubes inoculated	No of test tubes showing shoot formation	Day of shoot formation	Rate of shoot formation (%)
16/8	10	8.9 ± 0.89[a]	7.6 ± 0.8[a]	89
8/16	10	5.0 ± 0.49[b]	11.8 ± 0.74[b]	50
LSD (0.05)		1.86	0.96	

Means followed by different letters in the same column differ significantly at p = 0.05 according to Duncan's new multiple range test

and b). Peijun[23] described that the best temperature of the culture was 25 ± 1 ºC and it was provided with 16 h illumination intensity everyday at about 3000 lux illumination intensity. After 30 days of culture the rate of initiation reached to 86.4 %. These results are in corrboration with our findings. Best response for *in vitro* growth was obtained at 16 h light and 8 h dark period after 7.6 days of explants inoculation (Table 5a and b).

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