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Annals of Biological Research, 2013, 4 (2):69-71
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

In vitro rooting of amaryllis (*Hippeastrum johnsonii*), a bulbous plant, via NAA and 2-iP

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ABSTRACT

This study showed the in vitro rooting of a bulbous plant (Amaryllis) via singular effect of 2-iP and NAA. For rooting of bulblets, different concentrations of 2-iP (0, 14, 16 and 18 mg l^{-1}) and NAA (0, 14, 16 and 18 mg l^{-1}) were used. A well developed root system was achieved in the MS medium containing 16 mg l^{-1} 2-iP and 4 mg l^{-1} NAA. Rooting is a crucial step for successful micropropagation. The highest root length (2.71 cm), longest root (3.53 cm) and root number (2.77) were obtained in the MS medium supplemented with 4 mg l^{-1} NAA. The plantlets containing roots were acclimatized (90% survival percentage) by transferring them to plastic pots containing cocopeat, compost and sand.

Keywords: Bulblets, Plantlets, Ornamental plants, Plant growth regulators.

INTRODUCTION

Amaryllis (*Hippeastrum johnsonii*) is an ornamental bulbous flowering plant belongs to the family Amaryllidaceae. It has large and showy flowers with many bright colours [12]. Amaryllis is native to Central and South America, and is easily grown in the tropical and subtropical regions [11]. Propagation can be accomplished by using seed, offset bulblets and twin scaling [14]. Conventional propagation of *Hippeastrum johnsonii* by bulb offsets is slow, seasonal and variable [13]. In addition, normally a plant produces two to three bulblets in a year of growth [1]. For rooting of *Hippeastrum hybridum* plantlets, Siddique et al. [12] used different concentrations of NAA, singly. Study of Siddique et al. [12] on rooting of *Hippeastrum hybridum* plantlets showed that the well developed root system was achieved in the MS media supplemented by 0.2 mg l^{-1} NAA. Study of Kaviani et al. [7] on *in vitro* propagation of *Matthiola incana* (Brassicaceae), an ornamental plant, demonstrated that the addition of NAA and NAA along with KIN in culture media was effective for increasing the number of root and root length. Some studies showed the positive effect of NAA on rooting [5, 8]. Rooting is a crucial step to the success of micropropagation. Without effective root system plant acclimatization will be difficult and the rate of plant propagation may be severely affected. The aim of this study was to induce rooting of Amaryllis (*Hippeastrum johnsonii*) using different concentrations of 2-iP and NAA.

MATERIALS AND METHODS

Bulbs of Amaryllis (*Hippeastrum johnsonii*) were collected from a greenhouse in Abas Abad city, located in northern part of Iran. Bulbs were washed under running tap water with a few drops of liquid soap for 20 min, and

then they were immersed in fungicide solution of Carboxytyrame (2 g l^{-1}) for 2 min. Bulbs were thoroughly rinsed with sterile distilled water for three times, followed by soaking in sodium hypochlorite solution at 10% for 20 min along with some drops of Tween-20. Bulbs were thoroughly rinsed with sterile distilled water for 15 min. Then, bulbs were transferred in the aseptic condition under a laminar air flow cabinet and immersed into ethanol 70% for 10 sec. followed by soaking in 1% mercuric chloride solution for 12 min, then transferred to 20% sodium hypochlorite solution for 10 min. Finally, bulbs were washed 3-4 times by double distilled water and then separated into so-called twin scales, consisting of a basal plate and two to four scales. The twin scales of size 15 mm was used as explants. In this study, MS [9] medium was used. The medium pH was adjusted to 5.7 before autoclaving at 121°C, 1.2 kg cm^{-2} for 20 min. The explants were cultured on MS basal medium supplemented with different levels of 1-naphthaleneacetic acid (NAA) (0, 1, 2 and 4 mg l^{-1}) and 2-isopentenyl-aminopurine (2-iP) (0, 14, 16 and 18 mg l^{-1}). Three explants were inoculated in each glass dishes. Cultures were incubated in a growth chamber at 25±2°C, 70-80% relatively humidity. Samples were subcultured each 14 days. Data (root length, longest root and root number) were recorded after 6 wk from the first inoculation. The plantlets containing roots were acclimatized (90% survival percentage) by transferring them to plastic pots containing cocopeat, compost and sand with equal volumes and then they were kept in a greenhouse under 75% shade and 80% relatively humidity. The statistical analysis was completely randomized block design (R.C.B.D). The recorded data were statistically analyzed using SPSS software, and the means were compared using the Least Significance Difference Test (LSD) at 5% level.

RESULTS AND DISCUSSION

Based on Table 1 (analysis of variance), significant ($p \leq 0.01$) differences were found among various concentrations of 2-iP and NAA in increasing root number. Among different concentrations of NAA, 4.0 mg l^{-1} represented the best response to root number (2.77). The treatment without NAA took minimum root number (1.25). Among different concentrations of 2-iP, maximum root number (2.33) was calculated in explants grown on medium containing 16 mg l^{-1} (Table 2). Average number of roots was minimum (1.25) in absence of NAA. Data in Table 2 shows that in most cases, the number of root was least in the base of bulblets grown in media without NAA. The highest root length (2.71 cm) and longest root (3.53) were measured with treatment 4 mg l^{-1} NAA (Table 2). 2-iP at 16 mg l^{-1} induced the maximum length (2.03 cm) with the longest roots (2.60 cm). Lowest and smallest root length (1.75 cm) was measured with control plantlets (Table 2). Data in Table 2 shows that in most cases, the length of root was shortest in the base of bulblets grown in media without NAA.

Current study showed that NAA in combination with 2-iP is effective on increasing the number and length of roots. There are some findings in agreement with our study [12]. Siddique et al. [12] showed that NAA at 0.2 mg l^{-1} come out with the best (99.61%) response to rooting in *Hippeastrum hybridum*. Higher concentrations of NAA did not showed good result in case of response to rooting. The treatment NAA at 0.2 mg l^{-1} took minimum days (6.21) for root induction. In case of length of root, highest root length (8.75 cm) was measured with treatment 0.2 mg l^{-1} NAA. These researchers also found that NAA at 0.2 mg l^{-1} yielded the maximum number with the longest roots (9.33 cm). Study of Kaviani et al. [7] on *in vitro* propagation of *Matthiola incana* (Brassicaceae), an ornamental plant, demonstrated that the addition of NAA and KIN in culture media was effective for increasing the number of root and root length. Some studies showed the positive effect of NAA on rooting (5, 8]. In accordance with our findings, some studies showed the positive effect of cytokinins on rooting [2]. Contrary to our findings, root formation was inhibited in the medium culture of *Lilium longiflorum* Georgia containing BA [3]. In accordance with our results, the lowest rooting of *Bambusa arundinacea* was observed in medium without cytokinins [10]. Studies of Isutsa [4] on micropropagation of *Passiflora edulis* varieties showed that the shoots did not initiate roots on all IBA-augmented media but they initiated roots only on NAA-augmented medium. In a study on *in vitro* micropropagation of orchid [6], NAA stimulated root growth. In conclusion, suitable concentrations of 2-iP and NAA induced the maximum root number and root length.

Table 1. Analysis of variance of the effect of 2-iP and NAA on rooting of *Hippeastrum johnsonii*

Source of variation	df	Longest root length (cm)	Root length (cm)	Root number
2-iP	3	3.0632^{**}	0.5711^{ns}	0.0277^{**}
NAA	3	43.7495^{**}	19.0963^{**}	3.1365^{**}
Error	-	0.7077	0.4315	0.7587
CV (%)	-	38.08	35.45	44.32

**: significant at 1%, ^{ns}: not significant

Table 2. Mean comparison of the effect of 2-iP and NAA on rooting of *Hippeastrum johnsonii*

Treatments (mg l^{-1})	Longest root length (cm)	Root length (cm)	Root number
0 2-iP	1.97[b]	1.75[a]	1.61[c]
14 2-iP	2.25[ab]	1.83[a]	1.94[b]
16 2-iP	2.60[a]	2.03[a]	2.33[a]
18 2-iP	2.08[b]	1.78[a]	1.97[b]
0 NAA	1.07[d]	1.07[d]	1.25[d]
1 NAA	1.57[c]	1.46[c]	1.58[c]
2 NAA	2.64[b]	2.14[b]	2.25[b]
4 NAA	3.53[a]	2.71[a]	2.77[a]

Means followed by the same letters in each column are not significantly different by LSD test in 5% level

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