In vitro propagation of *Matthiola incana* (Brassicaceae)-an ornamental plant

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Abstract

Tissue culture techniques are applied for micropropagation and production of pathogen-free plants. Successful *in vitro* propagation requires an understanding of specific requirements and precise manipulation of various factors. Direct plant production from cultured explants is important to minimize somaclonal variation in regenerated plants. In this study, an efficient protocol for micropropagation of *Matthiola incana* using shoot tips is presented. Seeds from mother plants were germinated on MS medium without growth regulators. Shoot tips from *in vitro* germinated seedlings were subcultured on solid MS medium supplemented with kinetin (KIN)(0, 0.5, 1 and 2 mg/L) and naphthalene acetic acid (NAA)(0, 0.5, 1 and 2 mg/L). Four-week-old *in vitro* plants, obtained from micro-cuttings, showed successful shooting and rooting. MS medium supplemented with 2 mg/L KIN without NAA resulted in the best shoot length (1.166 cm) and largest number of node (4.64). When the shoot tips were inoculated in the medium containing 2 mg/L NAA without KIN and medium containing the combination of 1 mg/L NAA + 2 mg/L KIN, the best result was observed for root number (1.85) and root length (5.2 cm). Moreover, fresh weight, dry weight and chlorophyll content of plants were calculated.

Keywords: Auxin, Brassicaceae, cytokinin, *Matthiola incana*, root and shoot induction.
Abbreviations: KIN_kinetin; MS_Murashige and Skoog; NAA_naphthalene acetic acid.

Introduction

Matthiola incana (Brassicaceae) is an important ornamental plant. Micropropagation has been proven to be an extremely useful technique for clonally propagation of many species, especially ornamental plants. It is well known that several factors can affect *in vitro* micropropagation (George and Debergh, 2008). Most important of these parameters are the plant growth regulators content in the culture media (Gomes and Canhoto, 2003). Plant growth regulators act like signals to stimulate, inhibit or regulate growth in the developmental programs of plants (Mercier et al., 1997). Cytokinins are usually used on the micropropagation media to stimulate axillary shoot proliferation (Chawla, 2009; El-Agamy, 2009). However, the ideal concentrations differ from species to species and need to be established accurately to obtain the effective rates of multiplication. Rooting is a crucial step to the success of micropropagation. Auxins enhance the germination, root induction and seedling growth of many species (Gautam et al., 1983; Isutsa, 2004; Kalimuthu et al., 2007; Jain and Ochatt, 2010; Hashemabadi and Kaviani, 2010; Eeckaut et al., 2010; Casas et al., 2010; Barakat and El-Samak, 2011).

The number of papers dealing with the *in vitro* cloning of *Matthiola incana* is scarce. Multiple shoot buds were differentiated from cotyledon explants of *Matthiola incana*, cultured on medium containing BAP and NAA (Gautam et al., 1983; Hamidoghli et al., 2011). Plantlets were regenerated from protoplast culture of *Matthiola incana* in medium supplemented with BAP, 2,4-D and NAA (Hosoki and Ando, 1989). Different organs of *Matthiola incana* exhibit differential morphogenic potential. Probably, the change in response depends on the exogenous and endogenous plant growth regulators (Gautam et al., 1983). Nowadays, studies generally analyze the effect that a plant growth regulator exercises on the explants after a short period of time, and not its influence on later development (Feito et al., 1994; Moncaleán et al., 2003). Thus, the effects of different concentrations of KIN and NAA on regeneration of shoot and root in *Matthiola incana* was studied in this paper.

Results

We studied the effect of different concentrations of KIN and NAA on micropropagation of *Matthiola incana*, an ornamental plant. Studied characteristics were shoot length, node number, root number, root length, fresh weight, dry weight and chlorophyll content. The results are summarized in Tables 1, 2 and 3. Our data revealed that there are differences in the effect of the different concentrations of KIN, NAA and interaction between these two growth regulators on the characters. Shoot tips were excised and transferred on MS medium containing KIN (0-2 mg/L) and NAA (0-2 mg/L). Subsequently, within the next 3-4 weeks, differences were observed. The medium supplemented with 2 mg/L KIN without NAA resulted in the best shoot length (1.166 cm) and largest number of node (4.64)(Table 1 and Figs. 1 and 2). Data analysis showed that the effect of KIN, NAA and KIN × NAA were significant on the length of shoot and the number of node (p≤0.01)(Table 4). Our results indicated an overall significant positive correlation (r=0.855,

Table 1. Effect of different concentrations of KIN on some traits of *Matthiola incana*.

Traits / Treatments	Shoot length	Node No.	Root No.	Root length	Fresh weight	Dry weight	Chlorophyll content
KIN 0	0.836[a]	2.53[b]	0.85[a]	1.30[ab]	0.9275[a]	0.09335[a]	33.706[a]
KIN 0.5	0.657[b]	2.28[b]	0.42[a]	0.94[b]	0.5105[b]	0.05325[c]	25.804[b]
KIN 1	0.737[ab]	2.49[b]	0.75[a]	1.162[ab]	0.9505[a]	0.06965[b]	34.212[a]
KIN 2	0.8595[a]	2.97[a]	0.81[a]	1.91[a]	0.678[ab]	0.0681[b]	30.658[ab]

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test
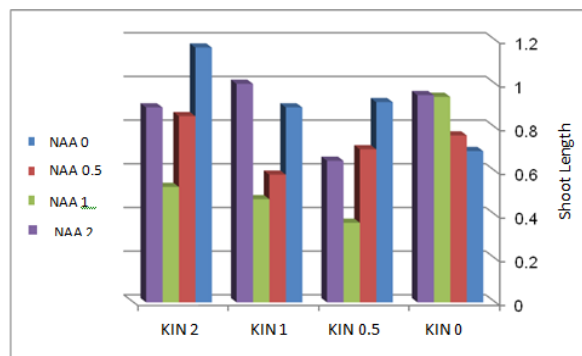

Fig 1. Effect of different concentrations of KIN and NAA on shoot length of *Matthiola incana*.

Table 2. Effect of different concentrations of NAA on some traits of *Matthiola incana*.

Traits / Treatments	Shoot length	Node No.	Root No.	Root length	Fresh weight	Dry weight	Chlorophyll content
NAA 0	0.9165[a]	3.39[a]	0.51[b]	0.8[b]	0.6235[ab]	0.0645[b]	27.883[b]
NAA 0.5	0.725[b]	2.12[c]	0.54[b]	0.892[b]	1.017[a]	0.0764[a]	31.791[ab]
NAA 1	0.576[c]	1.87[c]	0.76[ab]	1.64[a]	0.584[b]	0.0596[b]	27.146[b]
NAA 2	0.872[a]	2.89[b]	1.02[a]	1.98[a]	0.842[ab]	0.08385[a]	37.56[a]

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test
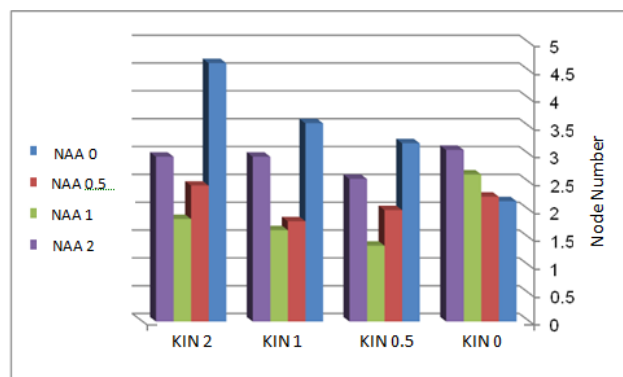

Fig 2. Effect of different concentrations of KIN and NAA on node number of *Matthiola incana*.

p=0.0001) between shoot length and node number, as well as between shoot length and dry weight (r=0.641, p=0.01) and there was no positive correlation between shoot length and root number, root length, fresh weight and chlorophyll content (Table 5). A significant positive correlation (r=0.383, p<0.05) of node number with dry weight was also observed. When the shoot tips were inoculated in the medium containing 2 mg/L NAA without KIN and medium containing the combination of 1 mg/L NAA + 2 mg/L KIN,

the best result was observed for root number (1.85) and root length (5.2 cm), respectively (Tables 2 and 3, and Figs. 3 and 4). This result was comparatively better than root number (0.36) and root length (0.41 cm) of control. Analysis of variance showed that the effect of KIN was not significant on the root number, while the effect of NAA and KIN × NAA on the root number and root length were significant (p≤0.05 and p≤0.01, respectively)(Table 4). Our results indicated a significant positive correlation (r=0.831, p=0.0001) between

root number and root length, as well as between root number and chlorophyll content (r=0.477, p=0.01)(Table 5). A significant positive correlation of root number with fresh weight (r=0.361, p=0.05) and dry weight (r=0.277, p= 0.05) was also observed. There was no positive correlation between root length and dry weight (Table 5). The highest fresh weight (1.69 g) and dry weight (0.102 g) were found when we used 1 mg/L KIN + 0.5 mg/L NAA and 1 mg/L NAA without KIN, respectively (Tables 3 and 2, and Figs. 5 and 6). The most chlorophyll content (46.83) was determined in medium supplemented with 1 mg/L NAA + 2 mg/L KIN (Table 3, Fig. 7). This result was comparatively better than the growth of control. Data analysis showed that the effect of KIN, NAA and KIN × NAA were no significant on the fresh weight but on the dry weight was significant (p≤0.01) (Table 4). The effect of KIN on the chlorophyll content was significant at the probably level of 5%, but the effect of NAA and KIN × NAA on that were significant at the probably level of 1% (Table 4). Current study demonstrated significant positive correlation between root length and chlorophyll content (r=0.477, p=0.01) also between root length and fresh weight (r=0.241, p=0.05), and there was no positive correlation between root length and dry weight (Table 5). There was a positive correlation between fresh weight with dry weight (r=0.370, p=0.05) and chlorophyll content (r=0.317, p=0.05), as well as between dry weight and chlorophyll content (r=0.225, p= 0.05).

Discussion

Our results indicated that there are differences in the effect of the different concentrations of KIN for shoot length and node number. Cytokinins are usually used on the micropropagation media to stimulate axillary shoot proliferation (Nitsch et al., 1967; Almeida et al., 2005; Debnath and McRae, 2001; Van Staden et al., 2008; Chawla, 2009; Jain and Ochatt, 2010). Similar to our findings, many researchers showed that cytokinin (KIN) induced multiple shoot formation and shoot length (Sajina et al., 1997b; Mathai et al., 1997; Luo et al., 2009; Gomes et al., 2010). Gomes et al. (2010) found that KIN was more effective in promoting shoot growth of *Arbutus unedo* L. than other cytokinins.

In current study, the highest rates of shoot production were obtained when shoot tips were cultured on the medium supplemented with 2 mg/L KIN without NAA. In accordance with our finding, Gomes et al. (2010) showed that NAA was unable to improve the multiplication rate. Best results were achieved on media without NAA. Some species may require a low concentration of auxin in combination with high levels of cytokinins to increase shoot proliferation (Van Staden et al., 2008). Contrary to our results, studies of Fuller and Fuller (1995) on the micropropagation of *Brassica* spp. showed that the most shoot percentage (88.3%) obtained in medium containing 2 mg/L IBA + 4 mg/L KIN. Studies of Tatari Vernosefadrani et al. (2009) on micropropagation of *Gerbera jamesonii* using different growth regulators showed that the most proliferation and plantlets length obtained in medium containing 2 mg/L KIN. Contrary to our results, studies on *Bambusa arundinacea* showed that the highest multiplication was shown in medium without KIN (Nayak et al., 2010). However, Rout et al. (1990) observed that the rate of growth in *Rosa* spp. is very poor in a hormone-free medium. Our findings demonstrated that the addition of NAA and NAA + 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 (Gautam et al., 1983; Xilin, 1992;

Hammaudeh et al., 1998; Lee-Epinosa et al., 2008; Jain and Ochatt, 2010). 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 (Gonçalves et al., 1998). Current study showed the positive effect of KIN on root induction and root length. The largest number of root obtained in media containing 2 mg/L NAA and 1 mg/L NAA + 2 mg/L KIN, respectively. Also, the most root length was obtained in medium supplemented with 1 mg/L NAA + 2 mg/L KIN. Some studies showed the positive effect of cytokinins on rooting (Gomes et al., 2010).

Contrary to our findings, root formation was inhibited in the medium culture of *Lilium longiflorum* Georgia containing BA (Han et al., 2004). Also, Fuller and Fuller (1995) demonstrated that the most percentage of explants regeneration with root percent (65.0%) in *Brassica* spp. obtained in culture medium supplemented with 2 mg/L IBA without KIN. In accordance with our results, the lowest rooting of *Bambusa arundinacea* was observed in medium without KIN (Nayak et al., 2010). Studies of Gautam et al. (1983) on micropropagation of *Matthiola incana* by cotyledon explants revealed that a combination of auxin-cytokinin is antagonistic to the individual response of both and produced only a callus mass. Studies of Gomes et al. (2010) on *Arbutus unedo* L. showed that shoots produced on higher cytokinin-containing medium are more amenable to root induction than shoots obtained with the lowest concentrations of BA. A review of the literature clearly points out to a negative effect of cytokinins on shoot rooting (Van Staden et al., 2008), although a positive role has been occasionally referred (Nemeth, 1979; Bennett et al., 1994). Our study showed positive role of KIN on rooting. Study on *Zinnia elegans thumbelina* revealed that the most length of root obtained on MS medium supplemented with 2 µM KIN (Mahmoudzadeh et al., 2009). The studies of Gautam et al. (1983) on *in vitro* regeneration of plantlets from somatic explants of *Matthiola incana* showed only a few shoots developed on explants reared on MS medium supplemented with 0.1 mg/L KIN. Also, NAA (1 and 4 mg/L) induced profuse rooting in explants. Studies of Isutsa (2004) 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 (Kalimuthu et al., 2007) NAA stimulated root growth. Hartmann et al. (1997) have recommended brief exposure to auxins for root induction and not for prolonged growth. Our studies demonstrated the positive effect of NAA on both root induction and root length. The present investigation revealed that the medium supplemented with certain concentrations of KIN and NAA influenced on shoot multiplication and root initiation of *Matthiola incana*.

Materials & methods

Seeds of *Matthiola incana* obtained from Mohaghegh-e-Ardabili University, Iran. The seeds were washed thoroughly under running tap water and a few drops of hand washing for 10 min. After three times rinses with distilled water, seeds were disinfected with a 20% NaOCl aqueous solution and Tween-20 for 10 min then rinsed three times in sterile distilled water (10 min each).

Table 3. Effect of different concentrations of KIN and NAA on some traits of *Matthiola incana*.

Treatments	Shoot length	Node No.	Root No.	Root length	Fresh weight	Dry weight	Chlorophyll content
NAA 0 × KIN 0	0.692^{cdefg}	2.16^{efg}	0.36^{cd}	0.41^{d}	0.74^{b}	0.0772^{c}	34.16^{abcd}
NAA 0.5 × KIN 0	0.764^{bcdef}	2.24^{defg}	0.21^{d}	0.53^{d}	0.95^{ab}	0.094^{b}	35.65^{abc}
NAA 1 × KIN 0	0.94^{abc}	2.64^{cde}	1.24^{abc}	1.36^{cd}	1.01^{ab}	0.1024^{a}	19.34^{ef}
NAA 2 × KIN 0	0.948^{abc}	3.08^{bc}	1.85^{a}	3.24^{b}	1.01^{ab}	0.0998^{ab}	45.68^{a}
NAA 0 × KIN 0.5	0.916^{abcd}	3.2^{bc}	0.56^{cd}	0.72^{cd}	0.52^{b}	0.0548^{h}	26.22^{cde}
NAA 0.5 × KIN 0.5	0.7^{cdefg}	2^{efgh}	0.36^{cd}	1.04^{cd}	0.73^{b}	0.074^{cd}	28.03^{cde}
NAA 1 × KIN 0.5	0.364^{h}	1.36^{h}	0.4^{d}	0.5^{d}	0.19^{b}	0.0216^{i}	10.08^{f}
NAA 2 × KIN 0.5	0.648^{defg}	2.56^{cdef}	0.76^{bcd}	2^{bc}	0.61^{b}	0.0626^{fg}	38.88^{abc}
NAA 0 × KIN 1	0.892^{abcd}	3.56^{b}	0.68^{cd}	1.08^{cd}	0.57^{b}	0.0572^{gh}	29.86^{cde}
NAA 0.5 × KIN 1	0.584^{efgh}	1.8^{fgh}	1.6^{ab}	2.288^{bc}	1.69^{a}	0.0658^{ef}	43.46^{ab}
NAA 1 × KIN 1	0.472^{gh}	1.64^{gh}	0.4^{d}	1.2^{d}	0.58^{b}	0.0584^{gh}	32.33^{bcde}
NAA 2 × KIN 1	1^{ab}	2.96^{bcd}	0.72^{bcd}	1.28^{cd}	0.96^{ab}	0.0972^{ab}	31.19^{bcde}
NAA 0 × KIN 2	1.166^{a}	4.64^{a}	0.44^{cd}	0.8^{cd}	0.67^{b}	0.0688^{def}	21.28^{def}
NAA 0.5 × KIN 2	0.852^{bcde}	2.44^{cdef}	0.2^{d}	0.54^{d}	0.7^{b}	0.0718^{cde}	20.02^{ef}
NAA 1 × KIN 2	0.528^{fgh}	1.84^{fgh}	1.8^{a}	5.2^{a}	0.55^{b}	0.056^{gh}	46.83^{a}
NAA 2 × KIN 2	0.892^{abcd}	2.96^{bcd}	0.8^{bcd}	1.4^{cd}	0.79^{b}	0.0758^{cd}	34.49^{abc}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

Table 4. Analysis of variance (ANOVA) for the effect of different concentrations of KIN and NAA on some traits of *Matthiola incana*.

Chlorophyll content	Dry weight	Fresh weight	Root length	Root No.	Node No.	Shoot length	df	Source of variations
298.12*	0.00549**	0.88763^{ns}	3.450**	0.7685^{ns}	1.68**	0.174**	3	KIN
454.59**	0.00244**	0.81470^{ns}	6.608**	1.116*	9.781**	0.476**	3	NAA
610.38**	0.00095**	0.32161^{ns}	12.106**	2.468**	1.904**	0.172**	9	KIN × NAA
100.54304	0.0003473	0.3845981	1.33592	0.402	0.298	0.0378225	64	Error
32.2471	26.21485	20.8948	7.03464	9.6	21.26	25.17949		c.v. (%)

**: Significant at $\alpha = 1\%$, *: Significant at $\alpha = 5\%$, ns=Non sense

Table 5. Simple correlation of the effect of KIN and NAA on some traits of *Matthiola incana*.

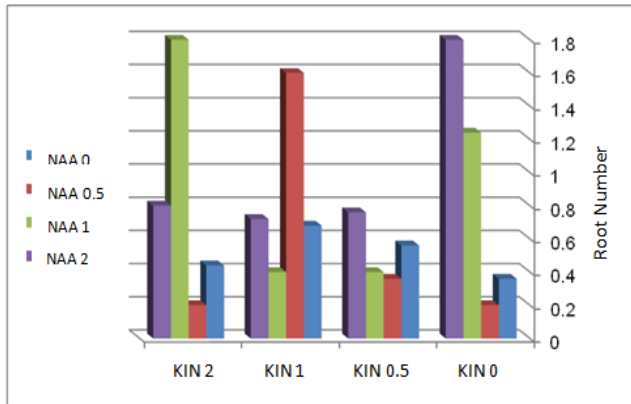| Traits	Shoot length	Node No.	Root No.	Root length	Fresh weight	Dry weight	Chlorophyll content
Shoot length	1.00						
Node No.	0.855**	1.00					
Root No.	0.134	0.115	1.00				
Root length	0.029	0.035	0.871**	1.00			
Fresh weight	0.194	0.079	0.361*	0.241*	1.00		
Dry weight	0.641**	0.383**	0.277*	0.157	0.370*	1.00	
Chlorophyll content	0.039	-0.003	0.433**	0.477**	0.317*	0.225*	1.000

Fig 3. Effect of different concentrations of KIN and NAA on root number of *Matthiola incana*.
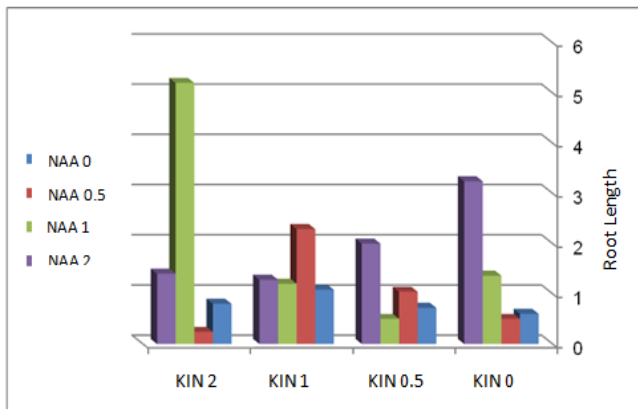

Fig 4. Effect of different concentrations of KIN and NAA on root length of *Matthiola incana*.
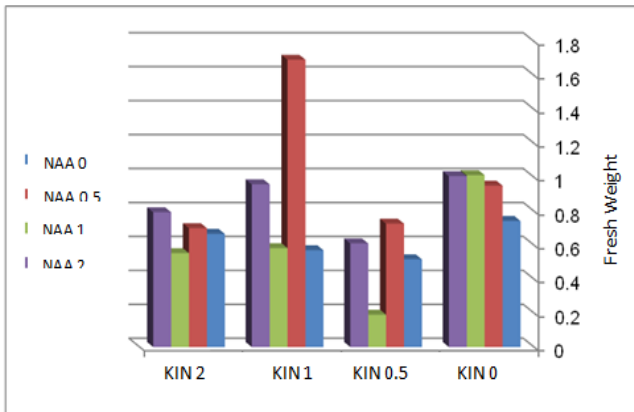

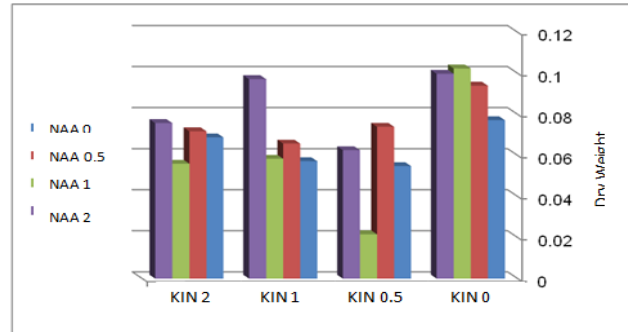**Fig 5.** Effect of different concentrations of KIN and NAA on fresh weight of *Matthiola incana*.

Fig 6. Effect of different concentrations of KIN and NAA on dry weight of *Matthiola incana*.
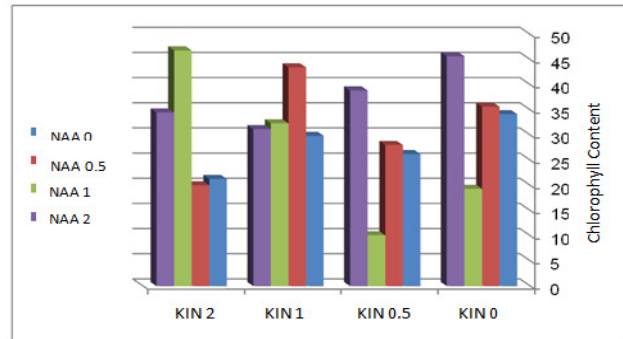

Fig 7. Effect of different concentrations of KIN and NAA on chlorophyll content of *Matthiola incana*.

At the end, seeds were sterilized for 2 min in 70% ethanol followed by three times rinses with sterile distilled water (15 min each). Seeds had gelatinous state, thus they were put on the filter paper for drying and gel removing. Five seeds were cultivated in culture flasks on MS (Murashige and Skoog, 1962) basal medium without growth regulators. Shoot tips were isolated from 4-week-old plants and after removing the extra leaves, they were cultivated on MS medium supplemented with 0.5, 1 and 2 mg/L of KIN and 0.5, 1 and 2 mg/L of NAA (16 treatments). The media were adjusted to pH 5.7-5.8 and solidified with 7 g/L Agar-agar. The media were pH adjusted before autoclaving at 121°C, 1 atm. for 30 min. The cultures were incubated in growth chamber whose environmental conditions were adjusted to 25±2°C and 75-80% relative humidity, under a photosynthetic photon density flux 50 µmol/m^2/s with a photoperiod of 14 h per day. Characters including shoot length, node number, root number, root length, fresh weight, dry weight and chlorophyll content were calculated after 30 days. The experimental design was RCBD. Each experiment was carried out in five replicates and each replicate includes five specimens (totally; 25 specimens for each treatment). Data were subjected to ANOVA (analysis of variance) and significant differences between treatments means were determined by LSD test.

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