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Direct shoot regeneration, callus induction and plant regeneration from callus tissue in Mose Rose (*Portulaca grandiflora* L.)

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Abstract

This experiment was performed to optimize the medium for tissue culture of Mose Rose. Different tissue culture approaches such as shoot regeneration from nodal segments, callus formation from leaf explants and plant regeneration from callus were investigated in this study. All the explants were cultured on MS basal medium supplemented with 30 g/l sucrose, 8 g/l agar and different plant growth regulators. Nodal segments of Mose Rose were cultured on media containing different levels of BAP or kinetin (0, 5 and 10 µM) to direct shoot regeneration study. Leaf explants were cultured in different combination of BAP (at three levels: 2.5, 5 and 10 µM) and NAA (at two levels: 5 and 10 µM) to examine callus formation. After the callus formation the formed calli were cultured on different combinations of BAP and NAA for shoot regeneration. BAP at three levels (5 and 10 and 15 µM) and NAA at two levels (5 and 10 µM) in all possible combinations (totally 6 hormonal treatments) were used for shoot regeneration from callus. The results showed that the treatment containing 10 µM BAP was found to be the best one for shoot regeneration from nodal segments. The treatment with 10 µM NAA in combination with 10 or 5 µM BAP were found to be suitable treatments for callus production from leaf explants, as well. Moreover, 15 µM BAP alone or in combination with 5 µM NAA were found to be the best treatments for shoot regeneration from callus.

Key words: shoot regeneration; NAA; Mose Rose; micro propagation; callus; BAP

Abbreviations: BA- 6-benzylaminopurine; NAA-naphthaleneacetic acid; MS- Murashige and Skoog; 2, 4-D-Dichlorophenoxy acetic acid

Introduction

Mose Rose (*Portulaca grandiflora* L.) is an ornamental plant widely growing in temperate climates which grows up to 30 cm tall. The leaves are thick and fleshy, up to 2.5 cm long, arranged alternately or in small clusters (Huxley, 1992). Mose Rose is a widely used ornamental plant for landscaping and in-door decorations (Ogale et al., 2000). Besides, Petals, steams and other parts of this plant contain a lot of beta-alanin instead of anthocyanin which is used as natural colorant in food industries, pharmaceuticals and cosmetics. It also has shown antifungal and antimicrobial activities. (Bhuiyan and adachi, 2002). Optimization of tissue culture medium can be useful for further studies for this plant, such as *in vitro* polyploidization in order to improve their ornamental qualities (production of polyploidy plant with enlarged leaves or flowers), or to investigate the in vitro production of its effective materials such as beta-alanin or other compounds. Callus formation can be useful for several aims such as establishment of cell suspension culture (Kumar and kanwar 2007; Vasil et al, 1990), protoplast culture (Dunbar and Stephens, 1991), induction of embryogenic callus (Kulkarni et al, 2002) and gene transformation (Frame, et al. 2000). Moreover shoot regeneration from callus tissue is required for regeneration of modified or genetically transformed plants. There are only some reports about tissue culture of Mose Rose. For instance, Rossi-Hassani and Zryad (1995) used 2,4-D (1 to 5 µM) for callus production from stem segments, and also they used combination of 2 µM kinetin and 1 µM 2,4-D for direct root formation from leaf or petal explants in this plant. Bhuiyan and Adachi (2002) used different levels of thidiazuron, BAP and kinetin in tissue culture of this plant, as well, but they used only hypocotyl explants in their study. It seems that tissue culture studies are

Table 1. Comparing the different levels of BAP and kinetin on shoot regeneration from stem nodal segments of Mose Rose (data was recorded six weeks after culture).

Hormone	Concentration (µM)	Regeneration (%)
BAP	0	0[c]
	5	16[b]
	10	48[a]
Kinetin	0	0[c]
	5	12[b]
	10	32[a]

Different letters within the columns indicate significant differences (Duncan multi range test, P‹= 0.05)

largely deficit for Mose Rose, therefore we decided to investigate the callus induction from leaf explants and plant regeneration from callus in some treatments containing plant growth regulators. Moreover, it is believed that adventitious shoot regeneration from callus may causes somaclonal variation (Kumar and kanwar, 2007), while direct shoot regeneration from leaf or stem explants may eliminate such undesirable variations (Kaul et al., 1990). Therefore we decided to study direct shoot regeneration from nodal segments, as well.

Materials and methods

Endemic form of mature Mose Rose plants (*Portulaca grandiflora*) were gathered from the campus of Sari agricultural university (in Mazandaran province in the north of Iran) and washed under tap water for 30 minutes to eliminate mud waste, and subsequently immersed in ethanol 70% for 45 seconds. Then under sterile condition plant materials were transferred to 1% sodium hypochlorite solution in a sealed bottle and gently agitated. After 15 minutes disinfectant solution was decanted and plant materials were washed with sterile distilled water three times. The plant materials were placed on sterile filter paper and then cut as explants to be cultured. Three different experiments were performed in this study mentioned below including direct shoot regeneration from nodal segments, callus induction from leaf explants, and finally shoot regeneration from the leaf-derived calli.

Direct shoot regeneration from nodal segments

Nodal segments were cultured on test tubes containing 10 ml MS (Murashige and Skoog., 1962) basal medium supplemented with BAP or kinetin each in three levels (0, 5 and 10 µM). 50 nodal segments were considered for each treatment. Moreover, some nodal segments were cultured on hormone-free MS basal medium as a control. The test tube incubated at 25 ±2 º C in photo period of 16h light / 8h dark. Shoot regeneration percentage was recorded in the third week after culture. The regenerated shoots were transferred to conical containers to grow more.

Callus induction

For callus induction, the leaf explants with the length of about 1.5 cm were wounded with scalpel and cultured in Petri dishes containing 20 ml MS basal medium supplemented with 30 g/l sucrose, 8 g/l agar and plant growth regulators. NAA at two levels (5 and 10 µM) and BAP at three levels (2.5, 5 and 10 µM) were used as auxin and cytokinin sources, respectively. All possible combinations among these levels were considered as treatments so 6 treatments were made. Five replications were considered for each treatment. Eight leaf explants were cultured in each Petri dish. Petri dishes were sealed with para film and incubated at 25 ±2 º C in dark and sub cultured every three weeks.

Shoot regeneration from callus

After callus formation from leaf explants the formed calli were transferred into conical containers containing 100 ml MS basal medium supplemented with 8g/l agar, 30 g/l sucrose and plant growth regulators. BAP at three levels (5, 10 and 15 µM) and NAA at two levels (0 and 5 µM) were used as cytokinin and auxin sources respectively. All possible combinations among these levels were considered as hormonal treatments (on the whole 6 hormonal treatments). Five segments of calli (with the dimensions of about 1cm^3) were placed in each conical container. Moreover ten calli with the same dimensions were cultured in two conical containers (five calli per container) containing 100 ml MS basal medium without any hormones as control. The conical containers were sealed with sterile aluminum foil and incubated at 25±2 ºC in photoperiod of 16 h light/8 h dark. The explants were subcultured every three weeks. The number of regenerated shoots per callus was recorded in the sixth week after callus culture.

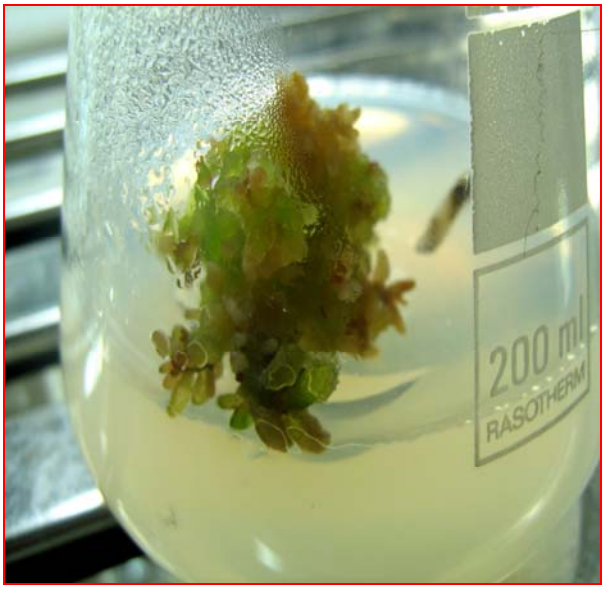

Fig1. Multiple shoot regeneration from a nodal segment of Mose Rose in the medium containing 10 µM BAP in the fifth week after culture

Table 2. Comparison of different hormonal treatments on shoot regeneration from callus in Mose Rose (data was recorded six weeks after transferring to regeneration media).

Treatment	Number of shoots per callus	Treatment	Number of shoots per callus
15 µM BAP+ 5 µM NAA	9±3 long shoots+ numerous dense-short shoots	10 µM BAP alone	4±2 long shoots
15 µM BAP alone	7±2 long shoots+ numerous dense-short shoots	5 µM BAP+ 5 µM NAA	Callus proliferation without any shoot regeneration
10 µM BAP+ 5 µM NAA	4±2 long shoots+ numerous short shoots	5 µM BAP alone	Callus proliferation without any shoot regeneration

Results and discussion

Shoot regeneration from nodal segments

About a week after culture, some stem segments in the treatment containing 10 µM BAP initiated to form shoots. The shoots emerged from nodal part of explants in the form of small leaf clusters. In the next weeks these shoots proliferated rapidly and formed dense cluster of leaves (Fig 1). Shoot regeneration percentage was determined in the sixth week after culture (table1). Shoot regeneration from nodal segments in the media containing 10 µM kinetin occurred lately. Also shoot regeneration percentage in this treatment (32%) was less than treatment containing 10 µM BAP (48%). Shoot regeneration percentages in 5 µM BAP and 5 µM kinetin were 16% and 12% respectively. Bhuiyan and Adachi (1995) reported that in equivalent concentrations BAP is better than kinetin for shoot regeneration from hypocotyl explants of *Portulaca* species, which it corresponds with our results of nodal segments culture of *Portulaca grandiflora*. We found that low level (5 µM) of these hormones (BAP and kinetin) is not suitable for shoot regeneration from nodal segments of *Portulaca grandiflora*. Bhuiyan and Adachi (1995) obtained the similar results with hypocotyl explants, as well. Similarly to hypocotyl explants, no shoot regeneration was observed in the control treatment and all explants became brown and died in the third week. It seems that BAP in 10 µM and higher levels is suitable for shoot regeneration in many plants. For example, Das and Pal (2005) used 3 mg/l BAP (equivalent to 13.3 µM) for shoot regeneration from lateral buds of *Bambusa balcooa*. Alexandrova et al (1996) used 13 µM BAP for shoot regeneration from nodal segments of *Panicum virgatum*, as well.

Callus formation from leaf explants

In most of the treatments callus initiation from leaf explants occurred about two weeks after culture, but the best callus formation and growth rate was observed in the medium containing 10 µM NAA in combination with 10 or 5 µM BAP. The formed calli in these treatments were more friable than those in the others (Fig 2a and 2b). Generally with neglect of BAP concentration, callus formation in the media containing 10 µM NAA was much better than those containing 5 µM NAA. Since callus formation and proliferation in the medium containing 10 µM NAA and 2.5 µM BAP was better than those containing 5 µM NAA in combination with 2.5, 5 or10 µM BAP. Also, callus formation was not suitable when BAP used in higher level than NAA. It seems that high levels of NAA are necessary for callus formation and using BAP can accelerates this process. We found that the media cont aining 10 µM NAA in combination with 10 or 5 µM-BAP are suitable for callus induction from leaf explants of Mose Rose. Safdari and Kezemitabar (2009) achieved the best callus production in the media containing 10 µM IBA (instead of NAA) in combination with 10 or 5 µM BAP. They reported that the media containing IBA alone is not able to callus formation from leaf explants of wild purslane (*Portulaca oleracea*). Similar to our results for Mose Rose, they found that callus formation from leaf explants is not suitable when BAP is used in higher concentration than auxin.

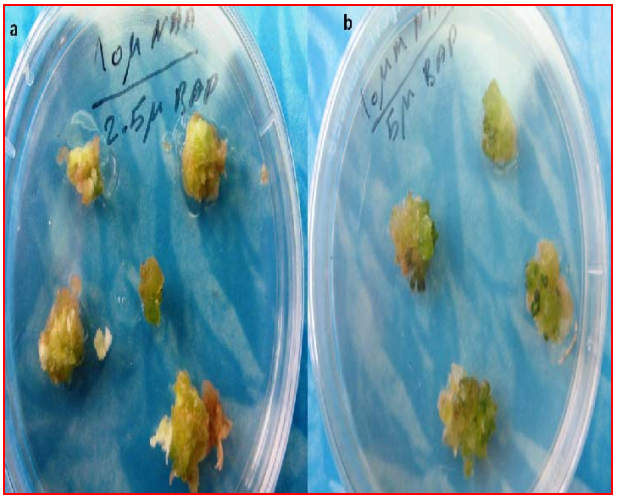

Fig 2. The formed calli in medium containing 10 µM NAA plus 5 µM BAP was more friable than that containing 10 µM NAA plus 2.5 µM BAP.

Shoot regeneration from callus

After transferring the formed calli to shoot regeneration media, purple or green spots appeared on their surface in some treatments (Fig3-a) which subsequently converted to shoot primordia and then to shoots with

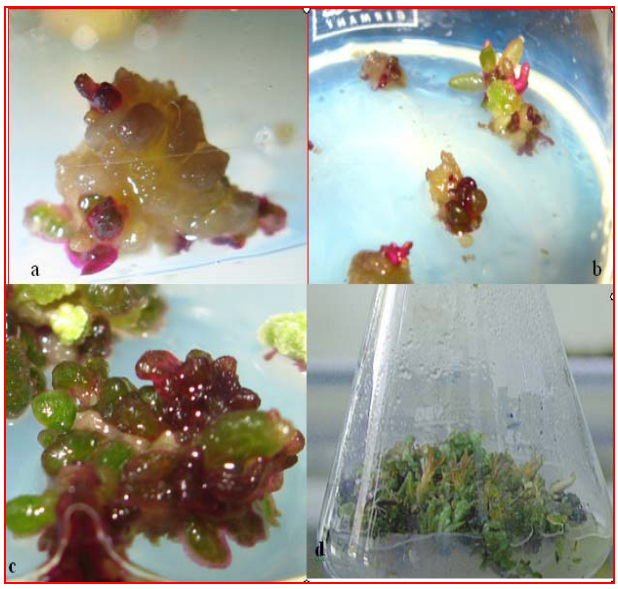

Fig 3. Purple spots on callus surface regenerate shoots (a). Regenerated shoots from callus in the medium containing 15 µM BAP (b). All of callus surface converted to shoots in the medium containing 15 µM BAP plus 5 µM NAA (c). Densely shoot regeneration from callus in the medium containing 10 µM BAP plus 5 µM NAA in the sixth week after (d).

Fig 4. Shoot regeneration from callus of Mose Rose in the medium containing 15 µM BAP plus 5 µM NAA (a), and in medium containing 15 µM BAP alone (b). Regenerated shoots with 10±3 leaves are seen in both pictures.

several leaves (Fig 3-b). In the next weeks, shoot formation was observed (Fig 3-c). Shoot regeneration in the medium containing 10 µM BAP plus 5 µM NAA was better than the medium containing 10 µM BAP alone. A sample of regenerated shoots in the sixth week of callus culture in 10 µM BAP plus 5 µM NAA has been shown in Fig 3-d. Among all the treatments, the treatment containing 15 µM BAP alone or in combination plus 5 µM NAA were found to be the best ones for shoot regeneration from callus. Regenerated shoots in these treatments were longer than those in the other treatments and had about 10± 3 leaves (Fig 4-a, 4-b). Callus proliferation had predominate response on the media containing 5 µM BAP alone or in combination with 5 µM NAA. Formed calli in these treatments were green and friable but were not able to regenerate shoots. The results of shoot regeneration from callus in different treatments have been shown in table 2. Bhuiyan and Adachi (2002) achieved suitable shoot formation in *Portulaca grandiflora* by transferring the hypocotyl-derived callus to MS medium without plant growth regulator, whereas we found that the presence of cytokinin is necessary for shoot regeneration from leaf-derived calli, since the calli which were placed in MS medium without plant growth regulator failed in regenerating shoots. Moreover, we found that in high concentrations (10 or 15 µM) of cytokinins, addition of NAA promotes shoot regeneration from callus, which it corresponds with the results of Rossi- Hassani and Zryad (1995). It seems that high concentrations of BAP are necessary to shoot regeneration in Mose Rose. Also It can be deduced that it in higher levels of BAP (10 or 15 µM) Shoot regeneration is promoted by adding of NAA, while in lower concentration of BAP (5 µM) it helps the callus proliferation. Some researchers have reported that an appropriate combination of NAA and BAP stimulated shoot formation (Tokuhara and Mii, 1993; Tisserat and Jones, 1999; Roy and Banerjee, 2003; Janarthanam and Seshadri (2008). Our findings confirm their results.

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