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Full Length Research Paper

Plant tissue culture study on two different races of purslane (*Portulaca oleracea* L.)

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This study was performed on two races of purslane; agronomic purslane and wild one. All the explants were cultured on MS basal medium supplement with 30 g/l sucrose, 8 g/l agar and different plant growth regulator. Petioles, shoot tips and leaves of wild purslane and also leaves of agronomic purslane were cultured in different concentrations and combinations of IBA and BAP (Both in the same four levels, 0, 1, 5 and 10 µM; collectively 16 treatments). On the other hand, nodal segments and shoot tips of agronomic purslane were cultured in different concentrations (4.44, 8.88 and 13.32 µM) of BAP or kinetin. The regenerated were rooted in the media containing three levels of IBA or IBA (0.0, 2.5 and 5 µM). Results showed that the treatments containing10 µM IBA in combination with 10 or 5 µM BAP are suitable for callus induction from leaves of wild purslane. Direct shoot regeneration from shoot tips or petiole explants of wild purslane was observed only in 10 µM IBA alone. Also, BAP at level 8.88 µM was found to be the best treatment to shoot regeneration from nodal segments of agronomic purslane and IBA at level 2.5 µM was found to be the best treatment for rooting of regenerated shoots in both races of purslane.

Key words: *Portulaca oleracea*, microproagation, callus induction, BAP, NAA, IBA.

INTRODUCTION

Purslane (*Portulaca oleracea L.*) is a pharmaceutical plant that grows in warm and moist regions of north hemispher. There are two types of purslane: one with sparse growth with branches on the earth that grows as a weed plant (wild purslane) and the other one has straight growth known as agronomic purslane (Zargari, 1981). Purslane has wide range of pharmaceutical importance. For example it is used as anti bacterial and antivirus matters in China (Okwuasaba et al., 1986). Also it has wound healing and muscle relaxant activities in mouse muscles (Okwuasaba et al., 1986; Okwuasaba et al., 1987a, b; Okwuasaba et al., 1987; Rashed. et al., 2003). So far extensive studies have been performed about purslane, such as determination of it effective materials (Liu et al., 2000), but information about tissue culture of this plant is largely deficit. Therefore, we decided to determinate the best hormonal treatment for callus induction from leaf explants, determination of the best explants to shoot regeneration and subsequently determination of the best hormonal treatments for root induction from regenerated shoots.

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Abbreviations: **BAP,** 6-Benzylaminopurine; **NAA,** naphthalene acetic acid; **IBA,** indole-3 butyric acid.

MATERIALS AND METHODS

Preparation and sterilization of plant materials and culture media

Mature plants of wild purslane were collected from their natural habitat in surroundings of Sari, Mazandaran province in Iran. Also, the seeds of agronomic purslane were obtained form Gachsaran, Kohgiluye- buyerahmad province and cultured in greenhouse to prepare the needed explants. In order to sterilize, plant materials were washed with many drops of dish washing liquid under tap water for about 30 min and then immersed in 70% ethanol for 45 s. After this stage, plant materials were transferred to 1% sodium hypo chlorite solution in a sealed bottle under sterile condition, gently agitated for 20 min and then rinsed three times with sterile distilled water. Plant materials were transferred on sterile filter

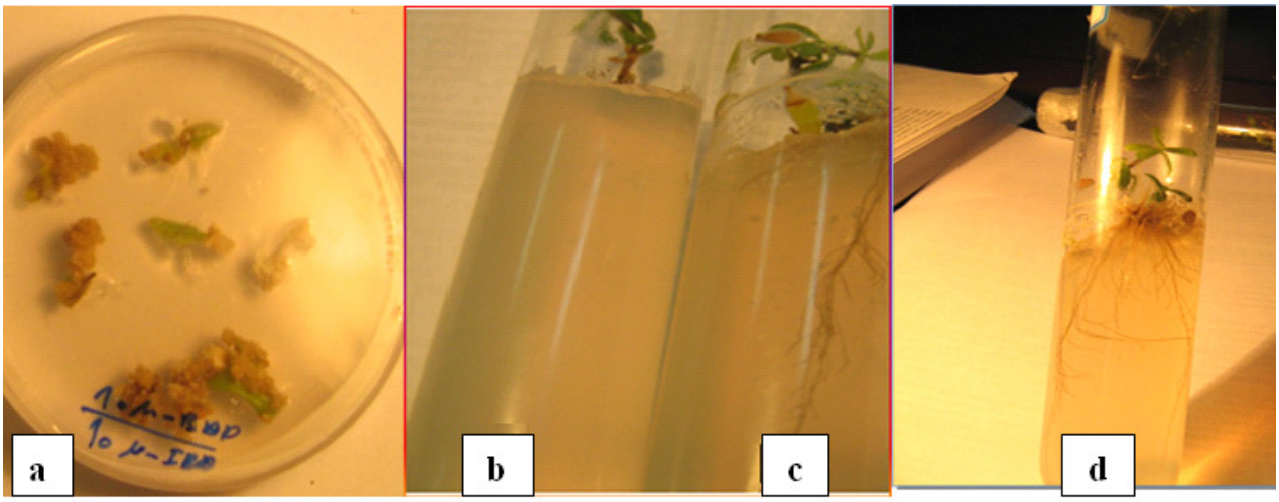

Figure 1. Calli formation from leaves of wild purslane in 10 µM IBA/10µM BAP (**a**), shoot regeneration from petiole explant of wild purslane in 10 µM IBA alone (**b**), root formation in 2.5 µM IBA (**c**), and a whole plantlet regeneration from shoot tip explant (**d**).

papers and cut as explants to culture. MS basal media supplemented with 30 g/l sucrose, 8 g/l agar and different plant growth regulator was prepared in 500 ml conical bottles. pH was adjusted to 5.8 and then were autoclaved for 20 min at 121°C.

Tissue culture of wild purslane

The leaves (with the size of about 1 × 1.5 cm), shoot tips (with the dimensions about 1 × 1 ×1.5 cm) and petioles (with the length of about 1 cm) of wild purslane were separately cultured in Petri dishes containing 25 ml MS basal medium supplemented with 30 g/l sucrose, 8 g/l agar and plant growth regulators. IBA and BAP each in four levels (0, 1, 5 and 10 µM) were used as auxin and cytokinin sources, respectively. All possible combination among these levels considered were used as treatments so 16 hormonal treatments was made. Six explants were placed in each Petri dish. These experiments were performed by factorial arrangement based on complete randomized design with five replications. Petri dishes were incubated at 25 ± 1ºC in dark. Fresh weights of formed calli from leaf explants were estimated (by transferring them on a pre weighted sterile aluminum foil and weighting again) and compared in the third week after culture. More over, IBA and NAA each in three levels (0.0, 2.5 and 5 µM) was separately used to rooting the petiole- derived shoots.

Tissue culture of agronomic purslane

Leaf explants with the size of approximately 1 × 1.5 cm were excised and cultured in different concentration and combination of IBA and BAP like as leaf culture of wild purslane and incubated in 25 ± 1ºC at dark. But nodal segments (with the length of about 1.5 cm) and shoot tips (with the dimensions about 1 cm × 1 × 1.5 cm) of agronomic purslane were cultured based on complete randomized design with five replication in vessel tubes containing 10 ml MS basal medium supplemented with 30 g/l sucrose, 8 g/l agar and BAP or Kinetin, each in four levels (0.0, 4.44, 8.88 and 13.32 µM). The vessels incubated at 25 ± 1ºC in photoperiod of 16 h light/8 h dark. After shoot regeneration from nodal segments they were transferred to free hormone MS medium to more growth. All the obtained data were analyzed with software SAS based on Duncan test at level 1%. After ten days the developed shoots were transferred to the media containing different levels (0.0, 2.5 and 5 µM) of IBA or NAA to compare the root formation in these treatments.

RESULTS

Results of tissue culture of wild purslane

Callus initiation from leaf explants of wild purslane was observed in some treatments about a week after culture. Generally, in most of the treatments, root formation was predominant response in culture of leaf explants. The treatments containing 10 µM IBA in combination with 5 or 10 µM BAP and also that containing 5 µM IBA in combination with 5 µM BAP were found to be the best ones for callus initiation from leaf explants of wild purslane. Callus initiation in these treatments was earlier than others. Callus proliferation rate in these treatments was more than others as well (Figure 1a). No callus formation was observed in the treatments containing BAP or IBA alone or in the free hormone treatment (control). The results of leaf culture are summarily listed in the Table 1. About three weeks after culturing the petiole explants of wild type, the light- tender shoots emerged at the base of explants in the treatment containing 10 µM IBA alone (Figure 1b). Generally, shoot regeneration performance from petioles was low (approximately 20%). The regenerated shoots were transferred to MS basal medium with out any hormones in photoperiod of 16 h light/8 h darkness to more growth. After ten days, green-long shoots were transferred to root induction medium. The formed roots in 2.5 µM IBA were delicate, long and dense (Figure 1c). The formed roots in 2.5 µM NAA were tick and relatively shorter than those formed in 2.5 µM IBA. Root formation was not suitable in 5 µM of these

Table 1. Leaf culture of wild purslane.

Treatment	Days taken to callus initiation	Medium callus fresh weight (gr/per Petri dish) (21 days after culture)	Response
10 µM BAP+10 µM IBA	7 - 9	1.69	Very suitable callus formation
10 µM BAP+ 5 µM IBA	8 -11	0.65	Low callus formation with many roots
10 µM BAP+1 µM IBA	9 - 11	0.35	Low callus formation with some roots
10 µM BAP alone	No callus formation	0.00	No response
5 µM BAP +10 µM IBA	7 - 9	1.69	Very suitable callus formation
5 µM BAP+ 5 µM IBA	7 - 9	1.55	Very suitable callus formation
5 µM BAP+1µM IBA	9 - 11	0.33	Low callus formation with some roots
5 µM BAP alone	No callus formation	0.00	No response
1 µM BAP+10 µM IBA	7 - 9	0.89	Callus formation with many roots
1 µM BAP+ 5 µM IBA	8 - 10	0.22	Low callus formation with many roots
1 µM BAP+1 µM IBA	8 - 10	0.21	Very low callus formation with some roots
1 µM BAP alone	No callus formation	0.00	No response
10 µM IBA alone	No callus formation	0.00	Many roots with out callus formation
5 µM IBA alone	No callus formation	0.00	Many roots with out callus formation
1 µM IBA alone	No callus formation	0.00	Some roots with out callus formation
MS free hormone	No callus formation	0.00	No response

hormones. Also, the formed roots in 5 µM IBA were delicate, but were lower in density. Generally, 5 µM NAA was found not suitable treatment to root induction in compare with the other treatments; formed roots were tick and with very low density. When apical Meristems (shoot tips) were cultured in different concentrations and combinations between IBA and BAP, different responses were observed. Root formation was a prevalent response in most of the treatments. No root formation was observed in the treatments containing BAP alone. Shoot induction following root formation occured only in treatment containing 10 µM IBA alone. Shoot regeneration performance in this treatment was approximately 50%. Root system of these plantlets was both long and dense (Figure 1d). The shoot tips in 10 µM IBA/10 µM BAP, 10 µM IBA/ 5 µM BAP and 5 µM IBA/5 µM BAP showed suitable callus formation, but those cultured in 10 µM BAP / 5 µM IBA showed low callus formation with few roots.

Tissue culture results of agronomic purslane

As against that of wild purslane, leaf explants of agronomic purslane did not have any response to different concentrations and combinations of IBA and BAP. These leaves became brown and died about three weeks after culture (Figure 2). Some shoot tips in 8.88 µM or 13.32 µM BAP or in 13.32 µM kinetin produced 1 - 3 shoots per explant, but in other treatments no response was observed. Shoot regeneration percentages for these treatments were 15, 20 and 17%, respectively. Despite the shoot tips, nodal segments of agronomic purslane showed suitable response to BAP or kinetin. Results of variance analysis revealed that shoot formation from nodal segments in agronomic purslane is significantly impacted by BAP. Also there were significant differences among the used levels of BAP. The maximum shoot regeneration was observed in 8.88 µM BAP (78%). Moreover, shoot regeneration percentages for 0.0, 4.44 and 13.32 µM BAP were 39, 52 and 67%, respectively. Results of variance analysis for kinetin showed that it also has significant effects on shoot regeneration from nodal segments of agronomic purslane (at level 1%). The maximum shoot regeneration for kinetin was observed at level 13.32 µM (71%). Shoot regeneration percentages in 0.0, 4.44 and 8.88 µM kinetin were 40, 49 and 57%, respectively (Table 2). Among all the treatments used for shoot inducing from nodal segments, that containing 8.88 µM BAP was found to be the best (78%). Shoots regeneration in 13.32 µM BAP was nearly equal to 13.32 µM kinetin (67 and 71%, respectively). A sample of regenerated shoots has been shown in Figure 3a. There were no significant differences between shoot regeneration percentages in 4.44 and 8.88 µM BAP or between 0.0 and 4.44 µM BAP at level 1%, but it was significant between the levels of 0.0 and 8.88 µM of this hormone. Besides shoot formation, some of nodal segments in 4.44 µM BAP or kinetin formed the green calli at the base of explants and then expanded throughout the explants. In comparison with 4.44 µM BAP, callus formation was less in 4.44 µM kinetin. The results of nod culture are summarily listed in Table 2.

About a week after transferring the regenerated shoots to root induction media, most of them initiated to root formation at their bases, but root regeneration and

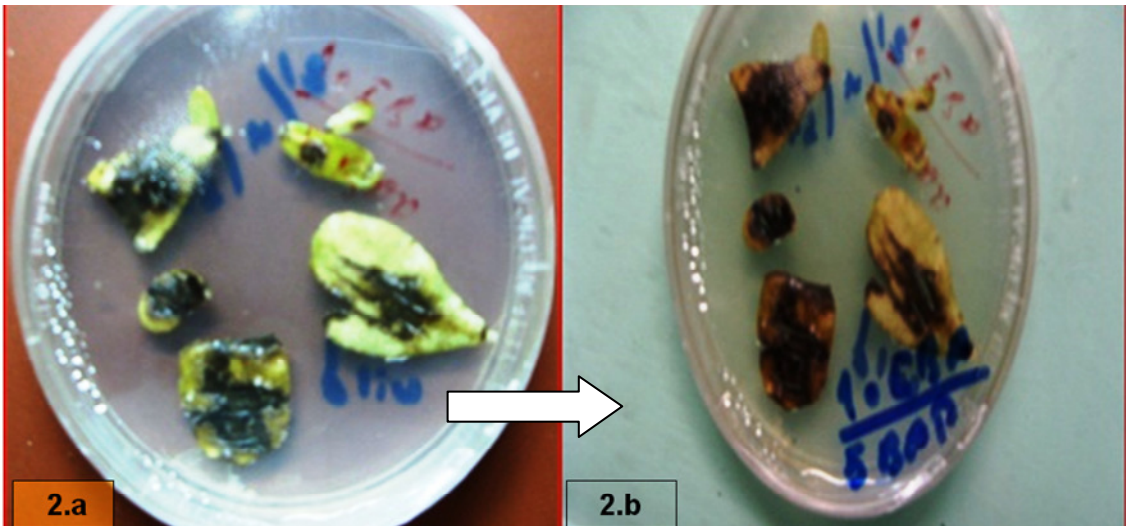

Figure 2. Leaf explants of agronomic purslane failed in forming callus in different combination of IBA and BAP. **2.a** shows the cultured leaves in 10 µM IBA / 5 µM BAP a week after culture and **2.b** shows the same leaves three weeks after culture.

Table 2. Comparing the different levels of BAP and kinetin on shoot regeneration from nodal segments of agronomic purslane (data was recorded in the third week after culture).

treatment		Days taken to shoot initiation	Number of auxiliary shoots	Length of auxiliary shoot (cm)	Shoot regeneration percentage
Kinetin	4.44 µM	9 - 14	2 - 3	2.5 - 4	49%
	8.88 µM	8 - 12	2 - 3	3.5 - 8	57%
	13.32 µM	7 - 10	2 - 5	5 – 8.5	71%
control	-	≥ 9	1 - 3		About 40%
BAP	4.44 µM	9 - 13	2 - 3	3.5 – 5.5	52%
	8.88 µM	5 - 8	4 - 6	6 - 9.5	78%
	13.32 µM	7 - 9	3 - 6	4.5 – 8.5	67%

development was not identical in all the treatments. The maximum root regeneration percentage occurred in 2.5 µM IBA and then in 2.5 µM NAA (90 and 80% respectively). Moreover, root regeneration percentages for 5 µM IBA and 5 µM NAA were 68 and 48%, respectively. A sample of roots formed in 2.5 µM IBA has been shown in Figure 3b. Effects of different levels of these hormones on root induction from regenerated shoots have been compared in diagram 1. Besides the difference in root regeneration percentage, generally the roots formed in the media containing IBA were more delicate, dense and longer than those formed in media containing NAA. The comparative data of root regeneration has been provided in Table 3 for both races of purslane in different levels of IBA and BAP.

DISCUSSION

Callus production is a primary stage in many tissue culture processes, as establishment of cell suspension cultures (Kumar and kanwar, 2007; Rodriguez-Garay and Rubluo, 1992; Gurel et al., 2002; Ngara et al., 2008), indirect somatic embryos (Barna and Wakhlu, 1993; Kulkarni et al., 2002; Rahman et al., 2006) and many other applications. Various hormonal treatments and explant sources have been used to this aim in different plants. Some researchers have used only auxins (Ramgareeb et al., 2001; Rossi-Hassani and Zaryad), whereas some have used the combinations of auxins and cytokinins together (Aswath and Choudharv, 2002; Jayanthi and mandal, 2001; Sharry and Saliva, 2006). We used different combinations of BAP and IBA for callus induction in purslane. In some plants such as *Solanum trilobatum* it has been reported that adding BAP to the medium containing coconut milk significantly improves callus formation (Alagumanian et al., 2004). It was found that the presence of BAP not only was necessary to callus induction from leaf explants of wild purslane but improves callus proliferation as well, so that the treatments without

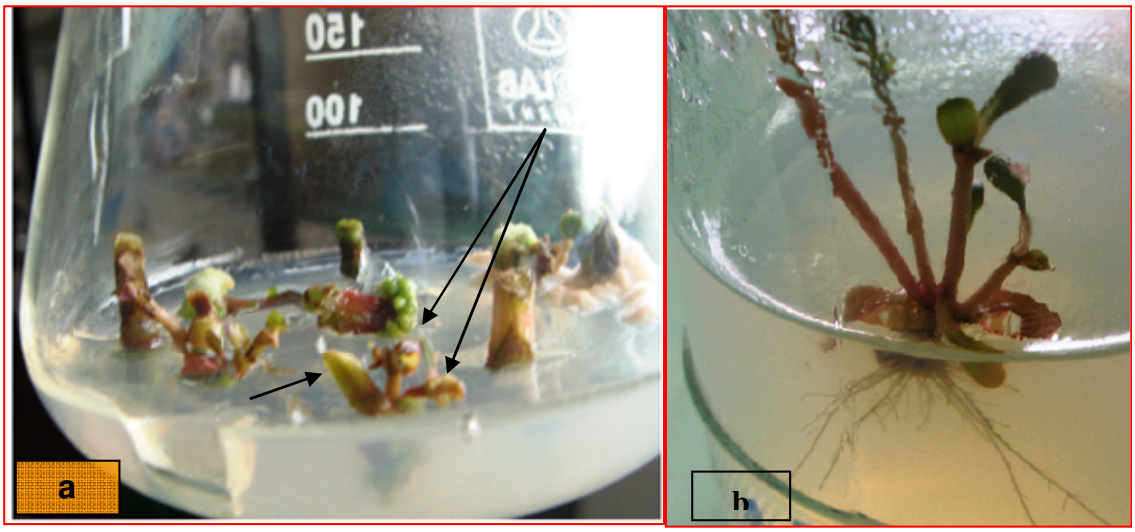

Figure 3. The short arrow in the left panel indicates a nodal segment in treatment containing 8.88 µM BAP, and the long arrows indicate the newly regenerated shoots from it (**a**). Root formation in 2.5 µM IBA (**b**); four long roots and many hair roots are seen easily.

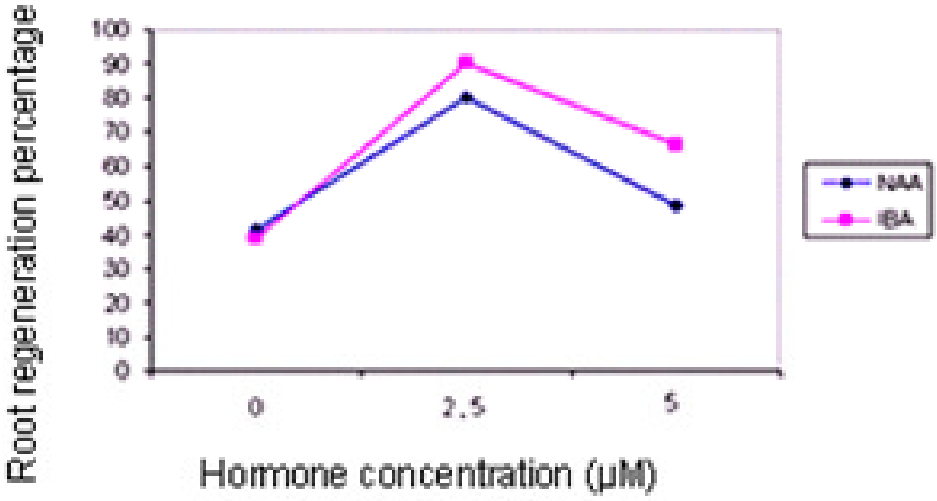

Diagram 1. Comparing the different levels of IBA and NAA on root regeneration from regenerated shoots of agronomic purslane (data was recorded after 30 days).

BAP failed in callus formation. Suitable combination of IBA and BAP can also improve callus formation in purslane. Callus formation in leaf culture of this plant was not suitable when BAP used in higher level than IBA (for example 5 µM IBA in combination with 10 µM BAP). Generally, it can be deduced that in combina-tions among the higher levels (5 and 10 µM) of these hormones callus formation is suitable when IBA is used in equal or higher level than BAP (Table 1).

Direct root formation from leaf explants of wild purslane was better when IBA used alone, in combination with low level of BAP (1 µM) or in the treatments in which BAP was in higher level than IBA (Table 1). Direct shoot regeneration can be used for regenerating poly ploid plants from explants exposed to ploy ploidy inducer agents such as colchicin (Escandon et al., 2005) or amiprofos-methyl (APM) (Jakse et al., 2003). At medium concentration (8.88 µM), BAP was found to be better than kinetin in shoot regeneration from nodal segments of agronomic purslane, but at higher level (13.32 µM) they were nearly similar (Table 2). Bohidar et al. (2008) used different con-centrations of kinetin and BAP (0.0, 1.0, 2.0 and 3 mg/l) (nearly equivalent to 0.0, 4.44, 8.88 and 13.32 µM used in our study) to shoot induction from nodal explants of *Ruta graveolens*. They achieved the maximum shoot regeneration (96.6%) in 1 mg/l BAP (equivalent to 4.44

Table 3. Comparison of root regeneration between agronomic and wild purslane using different levels of IBA and NAA.

	Treatment	Days taken to root initiation	Root length (cm) 10 days after initiation	Number of roots (in the forth week after culture)
Wild purslane	Free hormone	≥ 17	2.5 – 3.5	1 - 3 roots with too few hair roots
	2.5 µM IBA	5 - 8	7 - 10	3 - 6 with numerous root hairs
	5 µM IBA	7 - 9	6.5 - 9	2 - 3 with some hair roots
	2.5 µM NAA	5 - 8	4.5 - 7	2 - 3 with a few hair roots
	5 µM NAA	7 - 10	3.5 -5	1 - 3 with few hair roots
Agronomic purslane	Free hormone	≥ 21	2 – 3.5	1 - 2 roots with too few hair roots
	2.5 µM IBA	6 - 8	7 -9	3 - 6 with many hair roots
	5 µM IBA	7 - 10	5 – 7.5	1 - 3 roots with many hair roots
	2.5 µM NAA	6 - 8	6 – 8.5	2 - 3 with some hair roots
	5 µM NAA	8 - 10	3 - 4.5	1 - 3 roots with too few hair roots

µM) whereas the maximum shoot regeneration percentage (78%) in purslane belonged to 8.88 µM BAP and it was low in 4.44 µM of this hormone (39%). We used only cytokinins for shoot formation from nodal segments, whereas some researchers have achieved suitable shoot regeneration in combinations of auxin and cytokinin (Barwal et al., 1996; Kosir et al., 2004). It was also found that both IBA and NAA at medium concentration (2.5 µM) were better than the higher concentration (5 µM) for root regeneration from regenerated shoots. Nevertheless, in equal concentrations of these hormones IBA was more effective than NAA (Diagram 1). Bohidar et al. (2008) used different levels of IBA or NAA to root induction from regenerated shoots of *R. graveolens*. They found that IBA is generally better than NAA for root induction and development in this plant. Also, they reported that the cultured shoots on media with out auxins failed in root formation. Similarly we found that IBA is generally better than NAA for root induction in purslane, but unlike the *R. graveolens*, root formation in this plant occurred in the media without any auxins (about 40%). Indeed, application of auxins encouraged root induction in this plant. In purslane, IBA especially at low concentrations (1 mg/l, equivalent to 5 µM and lower) proved to be a suitable auxin to root inducer as in many other plants, for example in bamboo (Das and Pal, 2005), *Mucuna prriens* (Faisal et al., 2006), and *Solanum trilobatum* (Alagumanian et al., 2004).

The results of this experiment revealed that there are tangible differences between these two types of purslane in response to tissue culture approaches, such that different explants or hormonal treatments were suitable to different aims for each type. For example, in the case of wild purslane, callus formation from leaf explants was very effective in most combinations of IBA and BAP, whereas leaf explants of agronomic purslane did not have any responses to all the combination of these hormones. However, nodal segments in 8.88 µM BAP are good explants for shoot regeneration in agronomic purslane. Both petioles and shoot tips in 10 µM IBA are also suitable explants for micropropagation of wild purslane. In addition, 2.5 µM IBA is a suitable hormone concentration for root induction from regenerated shoots in both wild and agronomic purslane.

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