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Original Article

Physiological Responses of *Melissa* the Effects of Growth Regulators on Induction of Callus Tissue and Regeneration of Harmala (*Peganum harmala L.*)

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ABSTRACT

There has been limited information on the in-vitro propagation of Harmal. In this research, leaf, hypocotyl and embryo samples of this plant were examined for callus formation and regeneration. The present experiment was conducted as factorial based on a completely randomized design with three replications in two separate experiments with different hormonal treatments. In the first experiment, MS medium contained growth with different concentrations of Benzylaminopourine ((BAP) 0, 0.5, 1 and 2 mg/L) in combination with Naphthalene acetic acid (NAA) at concentrations (0, 0.25 and 0.5 mg/L. In the second experiment, BAP with concentrations (0, 0.5, 1 and 2 mg/L) in combination with 2,4-Dichlorophenoxyacetic acid (2, 4-D) with concentrations (0, 25 and 0.5 mg / L). The results of the first experiment showed that the highest callus and regeneration for the Harmal a plant was the combination of BAP hormone (0.5 mg/L) with NAA (0.5 mg/L). In this research, leaf, hypocotyl and embryo samples of this plant were examined for callus formation and regeneration. The results of the second experiment, on the other hand, showed that callus of explants was carried out in most of the BAP and 2,4-D hormonal treatments, and the highest regeneration was observed in the treatment of 1 mg/L BAP with 0.5 mg/L 2,4-D. High BAP concentration in the presence of NAA decreased callus formation and appeared to be better for NAA callus enhancement. The high concentration of BAP in the presence of NAA reduced callus formation and it can be said that NAA was better for increasing callus production. It was found that in the presence of 2,4-D and BAP, leaf explants were more efficient for regeneration and callus formation compared to hypocotyl and embryo explants. In the medium supplemented with 0.5 mg/L 2,4-D and 1 mg/L BA for the leaf and hypocotyl explants, Maximum callus induction and shoot regeneration was obtained.

INTRODUCTION

The Harmala, scientifically named *Peganum harmala* is an herbaceous perennial herb of the Zygophyllaceae family. Harmala is spread in North Africa, the Mediterranean, Pakistan, and Iran [1]. This plant grows as a perennial shrub (30-100 cm height) with short creeping roots, white flowers and round seed capsules carrying more than 50 black seeds [2]. In the importance of the medicinal herb, it is normally enough that the dried seeds of the herb contain 35% protein, 17% oil and alkaloids Harmaline, Harmine, Harmalol, Harman, Peganine (Wazinein), Isopeganine, Dipeganine, DeoxyVazine Nun. In 17 percentage oil and alkaloid the proportion of alkaloids Harmaline, Harmine, Harmalmol, and Peganine (Wazi Sin) are about 3.8 to 5.8 percentage [3].

The tendency to use medicinal plants to produce medicine is increasing in most countries and our country is no exception to this rule. Improper and indiscriminate exploitation of genetic resources, in addition to lowering the amount of traditional production, has caused the reduction of these resources. Most medicinal plants cannot be produced using conventional methods, and other methods such as asexual propagation have been

invented for plant propagation [4]. The global need for aromatic and medicinal plants is currently high and growing [5]. Most medicinal plants are not easily reproduced using conventional methods and the asexual reproduction method has been invented, which is a useful method of producing such plants and is, nowadays, widely used for these purposes [6]. The compounds in Harmala have different medicinal effects. Among the properties of Harmala, we can mention increasing milk secretion, improving rheumatism, helping to treat tumoral and cancer cells. Also, reported that this plant has anti-tumor, antioxidant, antimicrobial, antispam, anti-HIV, antifungal effects as well as immune system stimulatory and blood sugar decreasing properties [1].

In some explants [7], auxin and in others, naphthalene acetic acid [8] alone cause the regeneration of new shoots, but in most of the researches, naphthalene acetic auxin in combination with Benzylaminopourine [9, 10 and 11] or thidiazoron [12 and 13] was able to induce stem regeneration well.

For successful application of the tissue culture techniques in plant breeding, the potential of each plant for callus induction and plant regeneration must be determined [14]. Understanding the mechanism of plant regeneration in vitro is useful for genetic manipulation, physiological studies and alkaloid's production. Chemical, mineral, growth hormones and regulators are also important factors in plant differentiation and growth [15]. Tissue culture and regeneration of medicinal plants is usually used for mass propagation purposes, conservation and production of active compounds for herbal and medicine manufacturing [16]. In a study on embryonic callus formation, different concentrations of 2,4- dichlorophenoxyacetic acid (2,4-D) and naphthalene acetic acid (NAA) were used separately and showed that the highest amount of callus was due to high concentrations of auxin and cytokinin (1-2 mg/L) on *Bunium persicum* plants [17].

In the research on Milk Thistle (*Silybum marianum L. Gaertn.*) results revealed that the highest percentage of callus induction and silymarin accumulation (14.4%) were observed with 0.5 and 1 mg/L 2,4-D and Kin in root explant after one month [18]. In a study on *Dracocephalum kotschyi*, two growth regulators of NAA and 6-benzylaminopurine (BAP) were found to be more effective in both callus production and regeneration [19]. The combined effect of these two compounds on the induction of undifferentiated callus of Dracocephalum kotschyi and the production of root webs has also been demonstrated [20]. In another study on grape (*Vitis vinifera* L.), it was found that all three leaf specimens, branch, and middle meristem had the highest callogenesis in the simultaneous combination of BAP and NAA growth regulators in MS and LS medium [21]. In a respective study on tulip (*Fritillaria imperialis* L.) in the same mainstream, the highest callus weight was obtained from bud specimens in media containing 0.5 mg/L NAA [22].

The culture medium containing 1 mg/L kinetin in combination with 1 mg/L NAA produced more direct and indirect onions than other media and averaged 5.5 onions sample thumbnail per Fritillariaim perialis L. [23].

Fatima *et al*., [24] in an experiment on finger plant (*Digitalis purpurea*) showed that leaves were the best sample thumbnail for callus in relation to hypocotyl, stem, and root and were also an effective source of regeneration. With quite the same results;Kumari and Chandra [7], showed that the leaves highest levels of secondary metabolites. The current study, by presenting a suitable method for inducing callus and regeneration of harmala, could be used for future studies regarding the use of genetic engineering to increase the production of effective material in this plant as well as the use in suspension culture for effective material production.

Accordingly, this study aimed to determine the most suitable explants and determine the best combination of hormone concentration in the culture of explants to achieve the highest number of callus formation and regeneration.

MATERIALS AND METHODS

The seeds of harmala were prepared from Khorasan Razavi Agricultural Research and Natural Resource Center. The experiment was carried out in 2016 at the tissue culture laboratory of Department of Agriculture, Payame Noor University of Mashhad. Seeds were disinfected with 30% alcohol for 1 minute and sodium hypochloride with 2% concentration for 5 minutes. Seeds were washed with sterile distilled water three times between disinfection steps. Finally, they were drained with

sterile filter paper. Then, 40 disinfected seeds were cultured in MS medium [25] that was contained 30 g sucrose and 8 g agar at pH 5.7 to 5.8 in each seed per bottle. The bottles were kept in a growth chamber at 25 °C for 16 h light and 8 h darkness. After germination stage, germinated seeds were kept in the growth chamber for 1.5 months until, the stem reaches about two centimeters and at this time, sterile seedlings were prepared for preparation of leaf and hypocotyl explants and finally for culture in the target medium.

In this experiment to determine the most suitable explants and determine the best combination of hormone concentration in the culture of explant to achieve the highest number of callus formation and regeneration, we conducted as a factorial experiment. In this research, leaf, hypocotyl and embryo samples of this plant were examined for callus formation and regeneration.

A factorial experiment based on completely randomized design with three replications in two separate experiments with different hormonal treatments we conducted.

MS medium containing growth regulators was tested in two experiment; The first experiment with different concentrations of Benzylaminopourine (BAP) (Control, 0.5, 1 and 2 mg/L) in combination with Naphthalene acetic acid (NAA)at concentrations Control, 0.25 and 0.5 mg/L. The second experiment of BAP with concentrations control, 0.5, 1 and 2 mg/L in combination with 2,4-D ichlorophenoxyacetic acid (2, 4-D)with concentrations control, 0.25 and 0.5 mg/L and interaction of NAA with BAP was used.

Different samples of leaf, hypocotyl and cut embryos were used for culture as well. For this purpose, the space of each petri dish was divided into three equal portions, with each sample separately divided into three samples and each petri dish was also considered as a replicate.

In the first experiment, the effect of different concentrations of BAP and NAA and in the second experiment the effect of different concentrations of 2,4-D and BAP on callus formation and shoot regeneration of three explants were studied.

Method of Preparation of Explants for Tissue Culture

Leaf Explants

Initially, the required tools and the laminar hood environment were sterilized and disinfected (all appliances were autoclaved at 121 °C for 20 minutes and used after cooling). Then with a scalpel, seedlings containing leaves of appropriate size were selected and separated, so that the tail of the leaf shall not move with the leaf. The sides of the leaf were scratched with a scalpel, then it was transported to the prepared culture medium with different hormones. The explants were cultured in a culture medium that was exactly in contact with it.

Hypocotyl Explants

The seedlings that had previously been cut off leaves, their hypocotyls were also cut and separated by scalpel, so that the primary bud was not transported with the hypocotyl. It was then transported to the prepared culture medium, and it contained different hormones. The explants were cultured in a culture medium that was exactly in contact with it.

Embryo-Explants

Seeds of Harmala were first sterilized in 30% alcohol for 1 minute and 2% sodium hypochloride for 5 minutes and were washed 3 times with distilled water. After washing, the seeds were cultured in Petri dishes containing hormone-free MS medium and kept in the growth chamber for 48 h. During this time the seeds swelled, and the seed shell softened. Then, with Scalpel No. 10, cut the seeds under completely sterile conditions under the laminar hood and with the pressure on the middle of the seed, the embryo, then, came out easily. The embryo was finally slowly extracted with forceps and placed on a hormone culture medium.

After preparing all the explants and transferring them to the culture medium, the petri dish door sealed with parafilm and transferred to a growth chamber at 25 ± 2 °C for 16 h light and 8 h darkness. By daily observing the slightest contamination in the cultured samples, they were removed from the growth chamber. After four weeks, calluses and regeneration numbers were counted, and the data were then analyzed on statistics basis.

Statistical Analysis

The factorial experiment was conducted in a completely randomized design with three replications. SAS software (9.1) and Excel software were used to draw charts. LSD with 0.01% finally, the probability level was used to compare the means.

RESULTS

One and two weeks after culture of explants on MS medium containing different hormone treatments, the callus initiation and somatic embryogenesis occurred, respectively (Fig. 3-5). Somatic embryos were maturated and regenerated in the same medium after the consumption of hormone content and decreasing the level of auxin in the medium.

First Experiment

In the first experiment, the results showed that the effects of BAP, NAA, and explants (leaf, hypocotyl and cut embryos) and interaction effects of NAA on BAP on callus formation and regeneration were significant at 0.01 level (Table 1).

Shoot regeneration and callus induction were not observed on MS basal medium without hormones. Interaction of BAP and NAA on callus formation of all explants showed that the highest mean number of callus formation at 0.01 levels was related to 0.5 mg/L NAA and 0.5 mg/L BAP treatment. The mean comparison of the data of BAP and NAA interaction effects on regeneration showed that the highest regeneration was related to 0.5 mg/L NAA and 0.5 mg/L BAP treatment (Table 2). Interaction effects of BAP (1 mg/L) and explants in callus formation showed that the highest number of callus formation was observed in leaf explants with 2.41 calluses but in 0.5 mg/L BAP treatment with hypocotyl treatment showed no significant difference (Table 3).

Investigation of interactions of BAP and explants in regeneration showed that the highest interaction of BAP and explants of leaf, hypocotyl, and embryo at concentration of 0.5 mg/L and the least of this effect on leaf, hypocotyl, and embryo explants at concentration one mg/L^1 were observed (Table 3 and Fig. 1). By examining the interaction between NAA and explants in callus formation, we observed that the highest leaf callus formation was in 0.25 mg/L, and hypocotyl was 0.5 mg/L NAA and the lowest in control treatment. Also, the highest interaction of NAA with explants was observed at a concentration of 0.5 mg/L and the lowest in control treatment (Table 3 and Fig. 2). Examination of the interaction between NAA and explants in regeneration showed that the highest amount of regeneration was observed in leaf, hypocotyl and embryo at a concentration of 0.5 mg/L and the least in control (Table 3 and Fig. 3).

Table 1 Analysis of variance of BAP, NAA and microbial sample for callus induction and shoot regeneration.

S.O.V		Mean squares	
	df	Regeneration	Callus
BAP	3	2.47 **	1.2 4 **
Micro sample	2	2.12 **	5.77 **
NAA	2	7.67 **	16.58 **
BAP*Micro sample	6	0.07 ^{ns}	0.28 ^{ns}
BAP*NAA	6	4.58 **	0.49 ^{ns}
NAA* Micro sample	4	0.09 ^{ns}	0.36 ^{ns}
BAP*NAA*Micro sample	12	0.15 ^{ns}	0.20 ^{ns}
Error	72	0.21	0.20

*, ** and ns are significant at the level of 0.05 and 0.01 and non-significant, respectively.

Table 2 Interaction effects of BAP and NAA on the mean number of calluses and shoot regeneration in leaf, hypocotyl and embryo explants.

NAA	BAP	Number of regenerations	Number of calluses
0	0	0?	0?
	0.5	0.24 g	0.9 j
	1	0.24 g	1.25 i
	2	1.33 bc	2.6 d
0.25	0	1.55 b	2.01 e
	0.5	1.38 b	2.75 c
	1	0.7 e	2.73 c
	2	0.41 f	1.32 g
0.5	0	1d	2.93 b
	0.5	2.55 a	3 a
	1	1.14 cd	2.93 b
	2	0.8 e	1.73 f

Lsd	-	0.2	0.06

Means of column followed with the same letter are not significantly different (P≤0.01).

Table 3 The effects of BAP and NAA on the mean number of callus formation and shoot regeneration in leaf, hypocotyl and embryo explants.

		Leaf		Hypocotyl		Embryo		
	Concentration	callus	regeneration	callus	regeneration	callus	regeneration	LSD
NAA	0	1.44 a	0.58 e	1.25 b	1.1 bc	0.78 d	0.25 f	0.08
	0.5	2.75 a	1.13 d	2.41 b	1.1 d	1.57 c	0.72 e	0.13
	1	2.61 a	1.63 c	2.5 a	1.4 c	2.05	1 d	0.11
BAP	0	2 a	1 d	1.85 b	1 d	1.23 c	0.53 e	0.09
	0.5	2.37 a	1.52 c	2.37 a	1.45 c	2 b	1.1 d	0.1
	1	2.41 a	0.9	2.17 b	0.75 e	1.32 c	0.32 f	0.09
	2	2.37 a	1.1 d	1.85 b	0.75 e	1.42 c	0.64 f	0.11

Means of column followed with the same letter are not significantly different (p≤0.01).

Table 4 Interaction effects of NAA, BAP and type of explants on the mean number of callus formation and regeneration.

		Number regeneration			Number callus		
NAA	BAP	Embryo	Hypocotyl	Leaf	Embryo	Hypocotyl	Leaf
0	0	0	0	0	0	0	0
	0.5	0	0.37 e	0.37 f	0.65 f	1 e	1 e
	1	0	0.37 e	0.37 f	0.65 f	1.37 bc	1.7 bc
	2	1 c	1 d	1.68 b	2 c	2.75 a	3 a
0.25	0	1 c	1 d	1.68 b	2 c	3 a	3 a
	0.5	1.37 b	1.37 c	1.32 c	2.32b	3 a	3 a
	1	0.38 e	0.38 f	1 d	1.32 e	2.8 a	3 a
	2	0.38 e	0.37 f	0.68 e	0.7f g	1.3 c	2 b
0.5	0	0.68 d	1.67 b	1.37c	1.66 d	2.7 a	3 a
	0.5	2 a	2 a	3 a	3 a	3 a	3 a
	1	0.68 d	1.67 b	1.37c	2 c	2.7 a	2.7 a
	2	0.68 d	1.67 b	1d	1.67 d	1.67 b	2 b
	Lsd	0.30	0.29	0.27	0.30	0.35	0.32

Means of column followed with the same letter are not significantly different (p≤0.01).

Table 5 Analysis variance of BAP, 2,4-D and explants for Callus formation.

SOV	df	Mean squares	
		Regeneration	Callus
BAP	3	0.44 ^{ns}	1.95 **
Micro sample	2	2.56 **	1.92 **
NAA	2	4.7 **	19.17 **
BAP*Micro sample	6	0.15 ^{ns}	0.54 **
BAP*NAA	6	2.7 **	9.49 **
NAA* Micro sample	4	0.06 ^{ns}	0.078 ^{ns}
BAP*NAA*Micro sample	12	0.13 ^{ns}	0.12^{12}
Error	72	0.21	0.15

*, ** and ns are significant at the level of 0.05 and 0.01 and non-significant, respectively.

Table 6 Interaction effects of 2,4-D and BAP on the mean number of callus formation in the explants.

2,4-D			0			0.25			0.5				
BAP	0	0.5	1	2	0	0.5	1	2	0	0.5	1	2	Lsd
Callus number	0	1 d	1.45 c	2.67 b	2.83 a	3 a	2.67 b	1.57 b	2.83 a	3 a	3 a	1 d	0.2

Means of column followed with the same letter are not significantly different (p≤0.01).

Table 7 Interaction effects of 2,4-D and BAP and type of explants on the average number of callus formation and regeneration of explants.

2 4 D	BAP	Number of regenerations			Number of calluses		
		Embryo	Hypocotyl	Leaf	Embryo	Hypocotyl	Leaf
0	0	0	0	0	0	0	0
	0.5	0	0.3 d	0.3 c	1 d	1 ef	1 d
	1	0	0.3 d	0.3 c	1 d	1.7 d	1.7 c
	2	0.7 c	0.7 c	0.25	2.33 c	2.7 ab	3 a
0.25	0	0.65 c	1.1 b	1.77 b	2.7 ab	3 a	3 a
	0.5	0.3 d	0.65 def	1 c	3 a	3 a	3 a
	1	0.3 d	0.65 def	0.65 d	2.33 c	2.7 ab	3 a
	2	0	0.65 def	0.5	0.7 c	2 cd	2 b
0.5	0	1 b	1 b	1 c	2.7 ab	3 a	3 a
	0.5	0.65 c	1.77 a	1.77 b	3 a	3 a	3 a
	1	1.77 a	2 a	2.33 a	3 a	3 a	3 a
	2	0	0.3 d	0.65 d	0.7 c	1.3 e	2 b
	Lsd	0.23	0.28	0.32	0.33	0.33	0.33

Means of column followed with the same letter are not significantly different (P≤0.01).

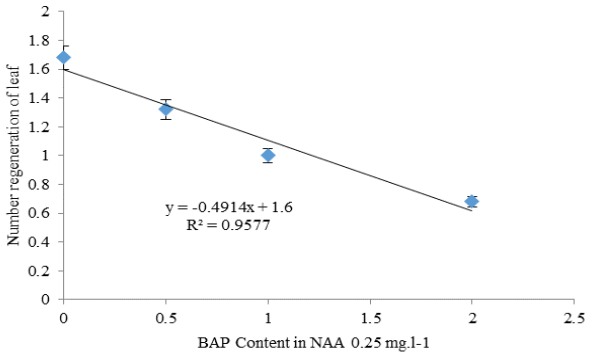

Fig. 1 The effects of NAA 0.25 mg/L and BAP content on regeneration of leaf.

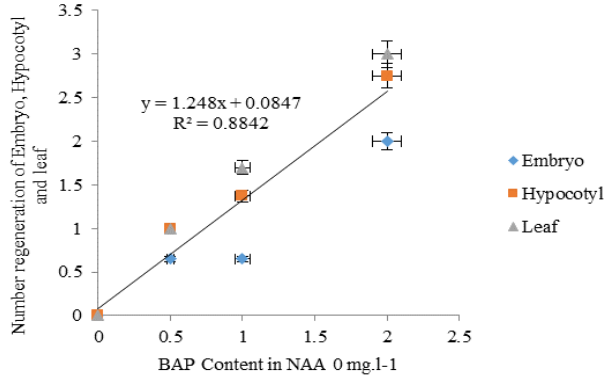

Fig. 2 The effect of BAP content on callus formation of Embryo, Hypocotyl and leaf.

According to Figs 1 and 2, it was found that with increasing BAP levels together NAA (0.25 mg/L), the number of regenerations of leaf explants decreased. However, in the case of net BAP consumption, the number of calluses in different samples (Embryo, Hypocotyl and Leaf) increased.

Interaction effects of NAA, BAP and explants on callus formation showed that the highest amount of callus formation between hormones and a micro embryo was observed at concentrations of 1 mg/L NAA and 0.5 mg/L BAP (Table 4).

By studying the interactions of hormones NAA, BAP, and explants in regeneration, it was found that the maximum number of interactions between hormones and explants of hypocotyl and embryo were in concentrations of 1 mg/L NAA and 0.5 mg/L BAP (Table 4).

Second Experiment

In the second experiment, the effects of BAP, 2,4-D, and explants on callus production and direct regeneration in the medicinal plant of *P. harmala L.* were investigated. The analysis variance showed that the effect of BAP on callus formation and 2,4-D and explants on callus formation and regeneration were significant at 0.01 level. Interaction of explants in BAP on callus formation and interaction of 2,4-D hormones in BAP on callus formation and regeneration were significant at 0.01 level.

Fig. 3 Calligraphy and regeneration of leaf explants. Interaction between BAP and Leaf Sample at 0.5 mg/L.

Fig. 4 Calligraphy and microenvironment regeneration in hypocotyl of 0.5 mg/L NAA.

Fig. 5 Calligraphy and regeneration of fetal explanted related to the interaction between NAA and microbial samples at concentrations of 0.5 mg/L.

No significant differences were observed in the other interactions (Table 5). The level of callus induction and shoot regeneration enhanced by increasing the concentration of 2,4-D to 0.5 mg/L. The highest frequency of callus induction and shoot regeneration was achieved at 1 mg/L BAP, however, callus induction and shoot regeneration decreased at higher concentration and had suppressing effect.

By examining the interactions of 2,4-D, BAP and explants in regeneration, it was found that the highest amount of regeneration was achieved between hormones and a explant of leaf, hypocotyl, and embryo in treatment with 1 mg/L BAP and 0.5 mg/L 2,4-D per liter.

DISCUSSION

Plant growth regulators are important are regarded as an important factor affecting cell growth, differentiation and embryogenesis in tissue culture [26].

In vitro extension methods, there are required components of plant genetic resource management and are becoming increasingly important for the protection of low and endangered plant types [27]. In addition, there are different studies which display the role of 2,4-D on the excitation of embryonic callus [24 and 28].

In this study it was found that between somatic embryogenesis and division in pH gradient were affected by stress or 2,4-D. pH of cytoplasm and vacuole increases during embryogenesis there is a high correlation [24]. Increasing the concentration of 2,4-D up to 10 mg/L in *Medicago sativa L.* caused the number of embryos was increased [28].

Investigation of interactions of BAP and explants in regeneration showed that the highest interaction of BAP and explants of leaf, hypocotyl, and embryo at concentration of 0.5 mg/L and the least of this effect on leaf, hypocotyl, and embryo explants at concentration one mg/L was observed as well. Cytokinins are among the best growth regulators for regeneration in the culture medium, and among them, BAP has been identified as the most important compound [29].

By studying the interactions of hormones NAA, BAP, and explants in regeneration, it was found that the maximum number of interactions between hormones and explants of hypocotyl and embryo was in concentrations of 1 mg/L NAA and 0.5 mg/L BAP. In tissue culture and cell culture studies, growth and morphogenesis were characterized by the type and concentration of growth regulators and their interactions as well [30].

Mean comparison of interaction effects of BAP and 2,4-D on callus formation showed that the highest mean number of callus formation were observed in treatments of 0.5 mg/L BAP × 0.25 mg/L 2,4-D, 0.5 mg/L BAP × -0.5 mg/L 1 mg/L BAP × 0.5 mg/L D-2,4 and the lowest was related to control treatment. BAP caused improper bud production but with the addition of auxin hormone, the number of buds increased significantly [31]. In study on *Fittonia verschaffeltii* regeneration the highest number of shoots (5.9 per explant) and shoot length (7.95 cm) were observed for the node explant at MS medium supplemented with 2 mg/L BAP + 1 mg/L IBA and MS medium containing 1mg/L BAP + 1 mg/L IBA, respectively [32].

In a study on *Passi floraedulis*, it was found that after 8 weeks at the concentrations of 1 mg/L BAP and 2 mg/L 2,4-D the highest callus formation was produced [33]. On the whole, it can be said that

optimizing the concentration of growth regulators in the medium, and applying different mediums with a specific concentration of sucrose, can tangibly improve regeneration. Studies have shown that 2,4-D has higher auxin activity than other auxins and induce callus production and somatic embryogenesis [34 and 35].

In the research on Milk Thistle (*Silybum marianum L. Gaertn*.) results revealed that by increasing 2,4-D concentration along with Kin, has been increased significantly the callus induction in root explants. The highest percent of callus induction were observed in root explants grown on MS medium supplemented with 1, 1.5 and 2, 1.5 mg/L 2,4-D and Kin, respectively [18].

Each plant in vitro needs different plant growth regulators with certain concentrations. Because of the importance of optimizing the conditions within the culture environmental conditions for micro propagation studies, a suitable protocol for induction and regeneration of callus could be studied in future based on the use of genetic engineering. Increase production of the effective substance in medicinal plants as well as use in suspension culture for production of effective substance production can also be under further studies. According to experiments on tissue culture of the medicinal plant harmala that were performed in two completely separate experiments, the highest rate of induction of callus formation and Regeneration of specimens for tissue culture of the plant in the first experiment, was observed Hormonal concentrations of 0.5 mg/L BAP and 0.5 mg/L NAA. High BAP concentration in the presence of NAA decreased callus formation as well and appeared NAA to be better for callus formation.

CONCLUSION

The highest induction of Callus induction and regeneration in the samples in the second experiment was observed in 1 mg/L BAP and 0.5 mg/L 2, 4-D treatment. Increasing BAP concentration in the presence of 2, 4-D tangibly decreased callus formation and lack of BAP caused good regeneration. The hormone 2, 4-D seemed to be necessary to increase regeneration. Generally, 2, 4-D and BAP hormones can be used for callus formation and NAA and BAP hormones for regeneration as well.

Harmala is a valuable herb and medicinal plant. Studying and identifying the regeneration mechanism of this plant has industrial and medical importance. The use of 2, 4-D and BAP growth regulators were the best procedure for callus formation and the leaf explants had the best callus production among all explants, and it was found that the leaf explants was more efficient for regeneration and callus formation than the hypocotyl and embryo samples. In this experiment, explant of embryo axis was performed for the first time and different concentrations of BA to induce regeneration of callus and shoot in medicinal plant *P. harmala L*. In the present experiment, a method has been developed in which regeneration of this medicinal plant by hormones can be a step towards the enrichment of genetic engineering.

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