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Full Length Research Paper

Determination of callus induction of the artichoke and optimization of callus producing culture

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In the present research, the effects of different culture media and hormone concentrations on the callus production of different organs of the artichoke were investigated. The obtained seedlings were transferred to half-strength MS medium. Only non-contaminated seedlings were used for preparation of root, leaf and petiole explants. The SH, MS and B5 media containing 0.8% agar, 3% sucrose and 0, 0.5, 0.75 and 1 (mg/l) 2,4-D were used for callus induction. The samples were kept in 25±2°C and at dark conditions. No callus formation was observed in hormone free medium. The experiment was performed based on a completely randomized plan and in 3 replications. According to the results, it was found that the values of callus formation and callus dry mass (dry weight) were statistically different (α<0.05) among various media. So, the highest callus formation was observed in leaf explants which was planted in B5 medium containing 1 (mg/l) 2,4-D. Callus dry mass in the petiole explant in the B5 medium with supplemented 0.5 (mg/l) concentration of hormone was significantly higher than that of the other media.

Key words: Artichoke, explant, 2,4-D, culture media, callus induction.

INTRODUCTION

Artichoke is one of the oldest medicinal plants and is a perennial plant of the Asteraceae family. The food value of artichoke is related to the phenolic compounds, inulin and salt minerals. Since ancient times, extracts of *Cynara scolymus* L. 'artichoke' have been used for treating different diseases because of their hepatoprotective quality (Adzet et al., 1987).

2,4-D is a plant hormone from auxin family and a chlorophenoxy herbicide. According to EPA (2005), 2,4-D kills plants by increasing three characteristics of the plant: the plasticity of the cell walls, the amount of proteins being made in the plant, and the amount of ethylene being produced by the plant. The effect of these changes is to cause cells to divide and the plant to grow uncontrollably. The end result is that the tissues of the plant are damaged and death occurs (Cox, 2006).

Plant growth regulators and culture media are one of the most important factors affecting cell growth, differentiation and metabolite formation (Liang et al., 1991). The appropriate concentration of the medium is one of the critical determinants in controlling callus growth and metabolite production. To produce cell dry mass as well as secondary metabolites from medicinal plants, it is important to establish the optimal culture conditions (chemical and physical environments) for the plant species used. The individual levels of auxin and cytokinin in the media used influenced the growth and regulation of cell metabolism. In addition, oxidative stress also plays important role in the production of secondary metabolites in plants (Dixon and Paiva, 1995). The aim of this study is to determine the callus induction of the artichoke and optimize a callus producing culture.

MATERIALS AND METHODS

Surface sterilization

First, the viability of seeds of *C. scolymus* L. was ensured

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by germination percentage test. After that, seeds were soaked in 200 (mg/l) GA3 for 24 h. Then seeds were washed by water. Treated seeds were surface sterilized with 70% metanol for 10 seconds, then they were washed with sterilized water. Next the seeds were placed in 20% sodium hypochlorite for 20 min and rinsed with autoclaved double distilled water 3 times in laminar flow hood (Michalak, 2006). Some seeds were cultured and the rest were stored in sterile glass bottle.

In vitro seed germination and callus production

For germination, surface sterilized seeds were placed in the Petri dishes containing filter paper, and were incubated in culture room at 23±2°C with 16 h photoperiod. After peeling off sprout coats, the obtained sprouts were placed individually in (10×15 cm) culture tubes containing 2 cm of solidified (0.8% agar) half-strength MS medium culture (1/2 MS). The culture tubes were transferred to the culture room at 23±2°C for four weeks.

The disease-free seedlings were used as the initial material for preparation of root, leaf and petiole explants (1×1.5 cm). Prepared explants were cultured in culture tubes (10×15 cm) containing 30 ml of each of the B5 (Schenk and Hildebrandt, 1972), SH (Gamborg et al., 1968) and MS (Murashige and Skoog, 1962) culture media containing 8 (g/l) agar, 30 (g/l) sucrose and different 2,4-D concentrations. All of the calluses were transferred to a dark culture room with temperature of 23±2°C.

In order to determe callus dry weight, calluses were placed separately in oven at 70°C, until their dry weight became constant.

Statistical analysis

Multi-factorial experiment was conducted in a completely randomized plan with three replications for each 24 treatments. The results were subjected to variance analysis in SPSS 17.0.; Microsoft Excel was used for tables' preparation.

RESULTS AND DISCUSSION

Interaction effect of medium, hormone and explant on fresh and dry weights

Statistical analysis showed that the highest amount of fresh weight was in B5 media with hormone concentration of 0.75 (mg/l) in leaf explant. But in level ($\alpha<0.05$), there was no significant difference between these treatments: SH medium with hormone concentration of 0.75 (mg/l) in petiole explant, MS medium with hormone concentration of 0.75 and 1 (mg/l) in leaf explant, and MS medium with hormone concentration of 0.5 (mg/l) in root explant (Table 1).

The frequency of callus induction and type of callus varied depending on explant used as well as type and level of auxin used (Prakash and Gurumuithi, 2010).

In the findings of Fatima et al. (2009) on Woolly foxglove (*Digitalis lanata* L.) plant and Legha et al. (2012) on marigold plant (*Calendula officinalis* L.), it was resulted that increase in 2, 4-D hormone was related to increase in dry weight. But, in the present study, dry weight was obtained from 1 (g) fresh callus and then data were compared together. According to Table 1, B5 medium with hormone concentration of 0.5 (mg/l) in petiole explant had the highest amount of dry weight. But in level ($\alpha<0.05$), there was no significant difference between these treatments: SH medium with hormone concentration of 0.5 (mg/l) in petiole explant, SH and B5 media with hormone concentration of 0.75 (mg/l) in leaf explant, MS medium with hormone concentration of 1 (mg/l) in leaf explant (Table 1).

At lower levels of 2, 4-D, the callus was minor, loosed and white in color, while at higher concentration, the callus was major and hard (Prakash and Gurumuithi, 2010). But in this study, it was observed that in root explants, the callus was soft, formless and white in color, but in leaf and petiole explants, the callus was compact and brown in color (Figure 1). So the smooth form of the callus is as a result of the less density of cells, followed by the lowest amount of dry weight. This result coincided with harvectes of Mannan et al. (2012).

In determining the effect of explant type and different plant growth regulators on callus induction, Savita et al. (2010) discovered that callusing has not increased with the increase of hormone concentrations in all of the explants, rather a certain hormone concentration for each explants caused more callusing. In this way, between the concentrations of 1, 2, 4 and 6 (mg/l) 2,4-D, maximum callus induction (98.66%) was observed in leaf segments on MS medium supplemented with 2, 4-D (4 mg/l). For nodal segments, maximum callus induction (96%) was observed in 2, 4-D (1 mg/l). In root segments, it was 48.66% on MS medium supplemented with 2, 4-D (2 mg/l) (Savita et al., 2010).

Effect of 2, 4-D in the measure of fresh and dry weights

In this study, data obtained indicate that there is no difference between concentrations 0.75 and 1 (mg/l) of 2,4-D, in the amount of produced callus ($\alpha<0.05$). This shows that lower concentration (0.5 mg/l) of hormone was not suitable for cellular production (Table 1). These results thus explain the fact that the important role of auxin (2, 4-D) is related to division, elongation and distinguishing of cells into cellular, texture or explant levels (Ranjan et al., 2003). But in the case of dry weight, there was a different status. Statistical analysis showed that there was no significant difference ($\alpha<0.05$) between none of the treatments. This shows that different

Table 1. Reciprocal effect of media, hormone and explant in amount of fresh and dry weight (per of 1(g) fresh weight).

culture Media	Hormone	Explant	Fresh weight (g)	Dry weight (g)
		L*	6.306^{ef}	0.094^{de}
	0.5	P	3.248^{kl}	0.119^{ab}
		R	5.140^{g-i}	0.066^{g-j}
		L	5.590^{f-i}	0.119^{ab}
SH	0.75	P	8.444^{ab}	0.102^{cd}
		R	5.765^{f-h}	0.070^{g-i}
		L	6.312^{ef}	0.104^{b-d}
	1	P	5.137^{g-i}	0.092^{d-f}
		R	3.413^{kl}	0.047^{k}
		L	7.072^{de}	0.101^{cd}
	0.5	P	3.486^{kl}	0.133^{a}
		R	5.102^{hi}	0.067^{g-j}
		L	4.844^{ij}	0.117^{a-c}
B5	0.75	P	3.019^{l}	0.094^{de}
		R	3.152^{l}	0.057^{i-k}
		L	8.796^{a}	0.108^{b-d}
	1	P	5.673^{f-i}	0.081^{e-g}
		R	5.996f^{g}	0.062^{h-k}
		L	5.797^{f-h}	0.072^{g-i}
	0.5	P	4.816^{ij}	0.072^{g-i}
		R	8.619^{ab}	0.051^{jk}
		L	8.272^{a-c}	0.077^{f-h}
MS	0.75	P	7.893^{b-d}	0.0046^{k}
		R	7.451^{cd}	0.0056^{i-k}
		L	8.345^{ab}	0.069^{g-i}
	1	P	4.115^{jk}	0.105^{b-d}
		R	6.059^{f}	0.069^{g-i}
p-value			0.000	0.001
LSD (5%)			0.893	0.0168

* L (leaf), P (petiole), R (root).

* Each value in the table is the average of three replicates. Values sharing the same letter in each column are not significantly different from each other by protected LSD analysis (α<0.05).

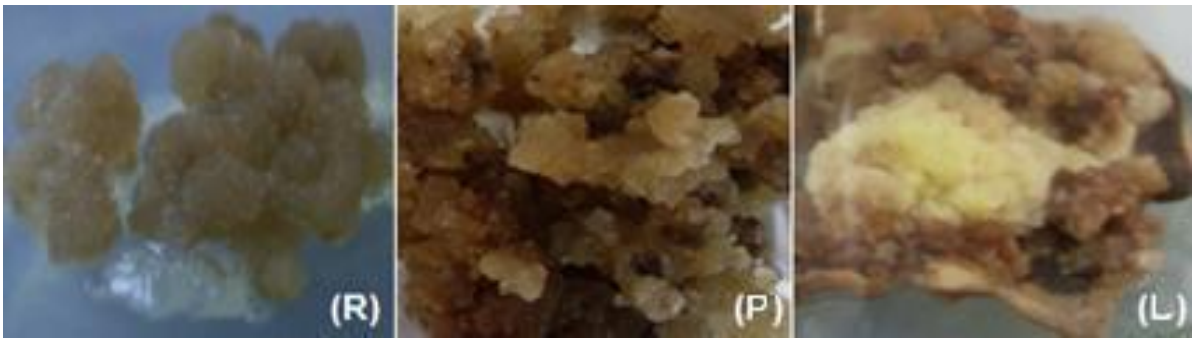

Figure 1. Callusing of different explants (R: Root, P: Petiole, L: Leaf).

Table 2. Effect of 2, 4-D in measure of fresh and dry weights (per 1 (g) of fresh weight).

Hormone	Fresh weight (g)	Dry weight (g)
0.5	5.509[b]	0.086[a]
0.75	6.048[a]	0.083[a]
1	5.983[a]	0.083[a]
p-value	0.000	0.383
LSD (5%)	0.00044	n.s

* Each value in the table is the average of three replicates. Values sharing the same letter in each column are not significantly different from each other by protected LSD analysis (α<0.05).

Table 3. Effect of media in measure of fresh and dry weights (per 1 (g) of fresh weight).

Culture media	Fresh weight (g)	Dry weight (g)
SH	5.484[b]	0.091[a]
B5	5.237[c]	0.092[a]
MS	6.818[a]	0.069[b]
p-value	0.007	0.000
LSD (5%)	0.298	0.0056

* Each value in the table is the average of three replicates. Values sharing the same letter in each column are not significantly different from each other by protected LSD analysis (α<0.05).

Table 4. Effect of explant in measure of fresh and dry weights (per 1 (g) of fresh weight).

Explant	Fresh weight (g)	Dry weight (g)
Leaf	6.815[a]	0.096[a]
Petiole	5.092[c]	0.094[a]
Root	5.633[b]	0.061[b]
p-value	0.000	0.000
LSD (5%)	0.298	0.0056

* Each value in the table is the average of three replicates. Values sharing the same letter in each column are not significantly different from each other by protected LSD analysis (α<0.05).

concentrations of the hormone have no effect on the callus structure. So it is clear that the difference in the amount of fresh weight of callus is related to their water content (Table 2).

Influence of media in the measure of fresh and dry weights

There was a significant difference between measure of fresh and dry weights of calluses among media. MS medium had produced the highest amount of fresh weight. But about the amount of dry weight, the results showed that MS medium had produced very lower quantity in comparison to SH and B5 media (Table 3). The media structure was the reason for the different production rates between fresh and dry weights.

This result shows that MS medium produced fresh calluses with more water but the calluses of B5 and SH media were hard and contained more cell mass. One of the most important factors governing the callus is the composition of the culture medium. However, the basic nutrient requirements of the cultured plant cells are very similar to those of the whole plants (Zouzou et al., 2008).

Several media formulations are commonly used for the majority of all cell and tissue culture work. With evaluation of media formulations, this result was obtained that different concentrations and types of nutrients offer different products such that the MS medium contains a high concentration of the nutrients, while B5 medium contains the lowest concentration of the nutrients. So this diversity is bound to different products of callus.

Influence of explant in the measure of fresh and dry weight

By comparing all of the treatments, it was identified that leaf explant was very prosperous in callusing. Also the highest amount of dry weight was in leaf and petiole explants (Table 4).

This result shows that explant type and its anatomical structure has a significant role in callus production. Such variations can be indicative of the physiological condition of the explant, which is determined by genetic factors (Nagarathna et al., 1991).

With regards to Table 4 and comparison of dates, it can be concluded that water content in callus of root explant was more than that of other explants. Different callusing abilities in various explants were reported in many plants (Ishii et al., 1998; Zouine et al., 2004).

Conclusion

The results of this study showed that substantial callusing can also be synthesized in cultures containing only one type of hormone (2, 4-D), but none of the treatments could produce callus at the zero concentration of hormone; so they were ignored for continuous test. In other words, the hormone is the callusing motivator. Also, the conditions for production of callus of artichoke (*C. scolymus* L.) were optimized.

Callusing was dependent on medium formulation, growth regulator concentrations and explant type. In this study, it was concluded that the leaf explant would be the best explant for production of fresh and dry callus if the reciprocal effect of the influencing factors is ignored. Also MS (in the only effect of media) and B5 media produced more callus than SH medium.

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