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Evaluation of the Effect of Cytokinine and Auxin Plant Growth Regulator on Callus Induction and Plant Regeneration on Medicinal plant *Convolvulus arvensis* L.

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ABSTRACT: The *Convolvulus arvensis* L plant is a type of Convolvulaceae. Many secondary metabolites such as Saponins, flavonoids and caffeic acid, alkaloids and lipids are found in this plant. Pharmacologic studies suggest that the pure extract of *Convolvulus arvensis* L causes inhibition of growth of cancer cells, inhibition of growth of blood veins and increasing the performance of immune system in our bodies. Proliferation of this plant through tissue culturing technique and genetic manipulation can facilitate the increase of secondary production in the plant. According to medicinal values this plant has, some experiments was carried out in order to determine the best condition of formation of callus from micro samples of leaves and roots and Internodes in various levels of 4-D, 2 zero, 0.2 and 0.4 micro molar and zero Kinetin, 0.2 and 0.8 micro molar alone or in combination with each other by a factorial method in format of a completely random design with 3 repetition and 3 instances in each repetition.

Based on results, we can state that the combination of 2,4-D and kinetin can yield the best callus induction especially in micro samples of the root. On the other hand the best callus induction was obtained in the presence of IBA and Kinetin as well.

Key words: *Convolvulus arvensis* L, Cytokinine, Auxin, callus induction, regeneration

INTRODUCTION

Convolvulus arvensis L is one of 250 species of Convlovulaceae (Korendkaly, 2012). *Convolvulus arvensis* L is a few years aging plant, its stem is long, climbing and it wraps around its surrounding trees and in case there was no such surrounding trees to wrap around, it grows lied on the ground (Mir Heydar, 1375). Its leaves are arc and egg like triangle shaped with sharp points. Its flower is ordered and five green leaflets and five pink or white interconnected Petals are found in each flower and inside it there are five flags which are in the form of decussate with respect to corolla pieces. Its Pistil is interconnected two pointed, its fruit is in the form of a capsule and its seed contains a little albumen (Khosravi Farsani, 1389).

Pharmacologic studies carried out suggest that this plant has a diverse effect on different parts of the body and can be used for producing various medicines. The pure extract of *Convolvulus arvensis* L causes inhibition of growth of tumor cells, inhibition of growth of blood veins and increasing the performance of body's defense system (Korendkaly, 2012). Also if used moderately it can induce relaxation and has anti bacterial anti fungal properties and causes relief in intestinal and uterine pain. The resins existing in its root is highly diuretic, laxative and purgative (Tilotson, 2001). Injecting the alkaloid extract of *Convolvulus arvensis* L to mice and cat results in an increase in blood pressure and increase in blood circulation in Coronary vessels (Manj *et al*, 2002). The use of dried *Convolvulus arvensis* L and its effect on the performance of Secretion of bile from the liver was tested on white mice. Results of this test demonstrated that usage of dried plant can be useful for elimination of acute hepatitis and liver dysfunctions. The use of *Convolvulus arvensis* L with a dose of 0.4-2.8 mg/ml causes elimination of duodenal smooth muscle spasm in rabbits (Manadhar, 2002).

Medicinal plants are considered as one of the most important medicinal resources due to their secondary metabolites and they have been used since thousands of years ago.

The world's health organization estimated that more than 80 percent of people use medicinal plants in a traditional manner or a modern manner; moreover many of chemical medicines are produced by modeling after plant materials (Tripati, 2003).

Culture of medicinal plants doesn't seem economic because of low concentration of secondary metabolites in the plant, limited natural resources, increased destruction of forests, meadows, gardens, destruction of diverse herbal and animal species, issues concerned with domestication and agricultural culturing of this plants, the low speed of producing secondary metabolites and long time required for producing, and it seems necessary for rapid and mass production of the secondary metabolites and medicinal materials to optimally use herbal tissue culturing methods (Habibi Khaniani *et al*, 1384). The biologic science offers an opportunity through cell, tissue or organ culturing in vitro to obtain the desired combination. Increasingly application of cell and herbal organ culturing resulted in producing a large scale of plant metabolites based on this method (Ramach and ravishankar, 2002).

Torrey (1958) carried out in vitro studies on formation of root and Seedling from roots separated from *Convolvulus arvensis* L. among all growth factors investigated by adding to food environment, merely kinetin results in producing organs from root pieces of *Convolvulus arvensis* L. It is produced in the presence of 0.1 mg/l of kinetin and in presence of numerous primordial in the chopped off end of the root but in darkness, stimulation of kinetin was less. According to Torrey, apparently kinetin induces the beginning of germination in the cultured root pieces of *Convolvulus arvensis* L and increases this effect. Elizabeth D. Earle and John G. Torrey also during some experiments determined that presence of Auxin (2,4-d) is necessary for inducing growth of callus but there is no necessity for presence of kinetin. No wide research has been carried out yet about culturing tissues of *Convolvulus arvensis* L, however it seems we can increase the amplification factor by tissue culturing far more than what occurs in nature. (Maleki Nasab and Kazemi Tabar, 1394). In this study, we investigated the possibility of producing callus and regeneration of the *Convolvulus arvensis* L plant in vitro from different parts of the plant and in various concentrations.

MATERIALS AND METHODS

Callus Inducing experiments were carried out in form of a completely random design with three repetitions and three samples were carried out in each repetition in order to study and compare the callus induction in micro samples of leaves, root and internodes of the *Convolvulus arvensis* L plant under different levels of 2,4-D (zero, 0.2, 0.4 mg/l) and kinetin (zero, 0.2, 0.8 mg/l). The second experiment was carried out following the first one such that calluses with the best hormone combinations which caused formation of the most calluses were used for evaluation. An experiment on the basis of a completely random form with three repetitions was carried out for indirect regeneration (by callus).

Various combinations of growth regulators were as follows: kinetin in level one and 0.5 mg/l, 0.8 mg/l of kinetin together with 0.2 mg/l of 2,4-D, 0.5 mg/l of IBA, 0.5 mg of IBA together with one mg/l of BAP, 0.2 mg/l of IBA together with 0.4 mg/l of kinetin, 0.5 mg of IBA together with one mg/l of kinetin and 0.5 mg/l of IBA together with 1.5 mg/l of kinetin were used as control from a hormone free culturing environment.

The herbal materials used in this study were collected from fields of agriculture Sciences University and natural resources of Sari. To disinfect the micro sample of *Convolvulus arvensis* L, first different parts of the plant were washed with water and then were placed in a distilled water solution and a few droplets of dishwashing liquid for 20 minutes and then were rinsed with distilled water. And then in the culturing room and under a hood for the rest of disinfection, samples were placed for 2-3 minutes in benomyl solution and were shaken. After that we shake them in 5 %hypochlorite sodium solution with a few droplets of phenytoin for 2-3 minutes and then disinfect them with 70 percent alcohol for 20 seconds and finally wash them three times with sterile distilled water.

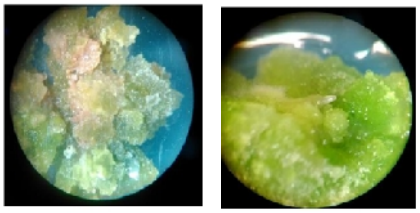
Culturing environment and dishes from autoclave devise along with culturing environment were used for disinfection. The rest of required tools such as Clamps, scalpel and forceps normally were disinfected by being placed in 96 percent ethanol and heating and cooling after the autoclave process while working and while using. Before the job began, the surface of the device was disinfected using 70 percent alcohol and then for disinfection of its surface and internal space of device a UV lamp was used to ensure disinfection of internal space of the device. It should be noted that all the required tools when working were already disinfected by alcohol and moved to inside of the device. Also hand were completely cleaned and disinfected with alcohol before the job began so that no pollution infiltrates inside of the device. Fluorescent lamps and ventilation devices were lit until the completion of the work.

A basic MS culturing environment was used (Murashik vaskoog, 1962). In each dish of culturing, three micro samples were placed in the culturing environment and were kept in the growth room with a 25±2 centigrade temperature and in darkness. After about 20 days, rather huge culluses were observed. Culturing environments were changed in order to produce root, stem and leaves and samples were placed in the light.

CONCLUSION AND DISCUSSION

The reaction of micro samples was investigated in vitro in culturing situation after 20 days passed. In the basic culturing environment without application of herbal hormones was used as control, no callus was observed during investigation. In the culturing environment using micro samples of the root, the most calluses were for 0.2 concentration of 2,4-D and 0.8 of kinetin. Of course in 0.2 concentration of 2,4-D and 0.2 kinetin, those calluses were obtained which were less in comparison with the 0.2 concentration of 2,4-D and 0.8 of kinetin. Higher and less concentrations didn't result in callus induction in micro sample of the root. Therefore it can be said that the best concentration of callus induction using micro sample of the root are 0.2 2,4-D and 0.8 of kinetin.

Result of callus inductions using micro samples of internodes demonstrated that in all level of hormones used, callus inductions weren't successful and only in 0.2 concentration of 2,4-D and 0.2 of kinetin happened with a low efficiency. In vitro culturing depends on different factors and different levels of internal hormones, concentration of external growth regulators and also contrary effect of these factors are effective of the response of micro sample to callus induction (Torres, 1989). Therefore, using other micro samples of internodes and different concentration of hormones might be interesting for callus induction.



(a) (b)

Fig. 1. a) producing callus b) calluses during germinating.

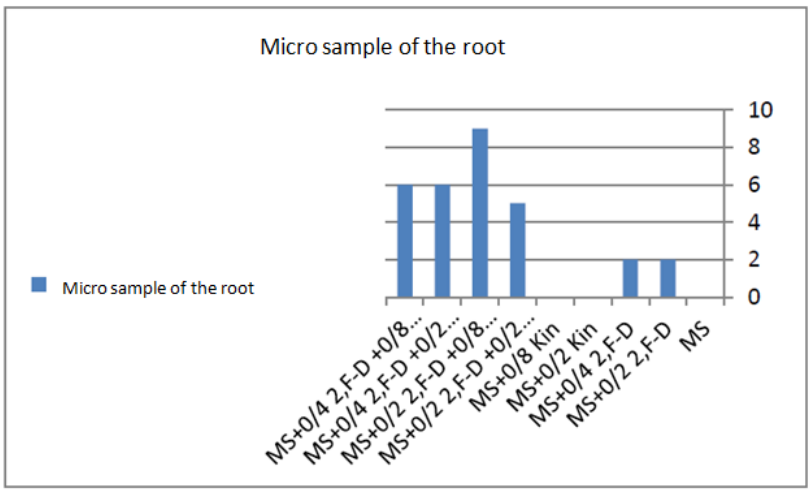


Fig. 2. Calluses produced from micro sample of the root.

The highest number of calluses in micro sample of leave belonged to 0.2 concentration of 2,4-D and 0.8 of kinetin. In 0.2 concentrations of 2,4D and 0.4 of kinetin and also in 0.4 concentration of 2,4-D and 0.2 of kinetin we had the calluses which were less in number than 0.2 concentration of 2,4-D and 0.8 of kinetin. Maleki Nasab and Kazemi Tabar (1394) showed that the lowest used concentration of 2,4-D had the most influence in callus induction. In the regeneration experiment, the most value of regeneration was obtained from a 0.5 mg/l concentration of IBA and a 1.5 mg/l of kinetin. Regeneration also took place in 0.5 mg/l concentration of IBA and one mg/l of kinetin but no callus regeneration was observed in any other concentration.

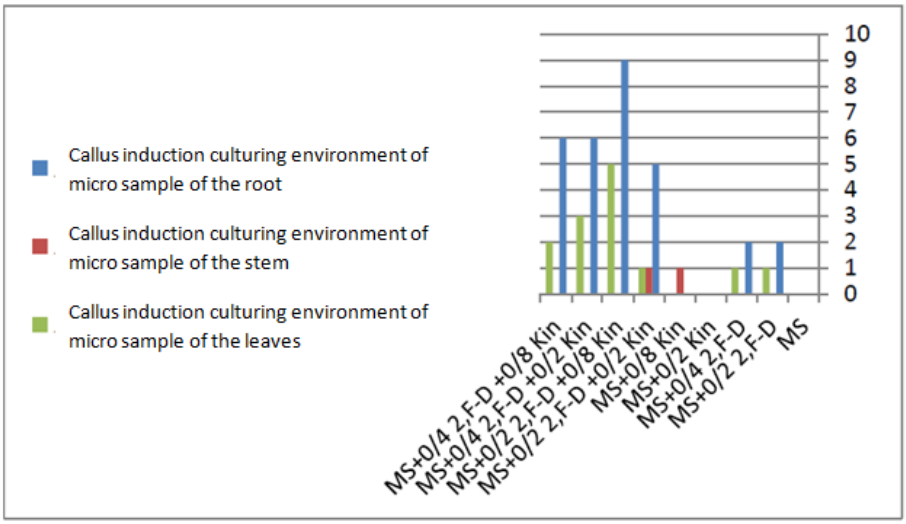


Fig. 3. Comparison of number of produced calluses in different concentrations.

Fig. 4. Regeneration in different concentrations.

Fig 5. Regeneration from callus.

Valizadeh *et al* (2007) showed that using the micro sample of hypoctile of Caravay in culturing environment of MS or one mg/l of 2,4-D, regeneration of the stem occurs after callus induction and without having to do culturing. In addition, more callus inductions were observed in 0.1 mg/l concentration of 2,4-D and 2 mg/l of kinetin (Bagheri *et al*, 1392).

In general, according to lack of studies in the field of callus induction and regeneration of *Convolvulus arvensis* L plant it is clear that achieving effective and repeatable methods for regeneration and production of the plants devoid of diseases and minimum genetic diversity in a short time can be effective as a requirement of genetic engineering in this plant and also in studies such as protecting genetic sources, investigating the types of tensions or shortening the modification duration of this plant.

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