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Contribution of Polymers to the Vegetative Propagation of *Hypericum perforatum* L.

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In present study, the contribution of two hydrophilic polymers to the vegetative propagation of *Hypericum perforatum* L.. has been investigated. Poly(acrylamide-g-ethylene diamine tetraacetic acid) polyelectrolyte hydrogels (AAm EDTA JEL4) and poly(N-vinyl-2-pyrrolidone/itaconic acid) (VPITA JEL4) polymers were used with seven different growth mediums. The seedlings were propagated with meristem tissue culture in Murashige and Skoog medium. The two polymers increased seedling survive and plant growth after transplantation. Polymers contributed to the vegetative propagation of Saint John's wort especially due to growth medium and increased survive of transplants. Growth mediums also effected the survive and growth rate of seedlings and contribution of polymers. When it thought that the secondary metabolites are found abundantly at early growth stages of young plants, this is an important contribution. The polymers contributed particularly in aridity.

Key Words: Polymer, Hypericum, Vegetative propagation, Tissue culture.

INTRODUCTION

Saint John's wort (*Hypericum perforatum* L.) is a shrubby, perennial flowering plant belonging to the family Hypericaceae. It is widely distributed in all of the world and widely used for its medicinal qualities. Historically, this plant has been used for its sedative, antiinflammatory, antibactericide, antiviral and antidepressant qualities. This plant has nearly a 200 year history of use in traditional folk medicine for the treatment of various ailments[1,2] and it is used for shocks, hysteria, gastritis, hemorrhoids and wounds. St. John's wort has been shown to help; ward off infections,

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stress and chronic pain, increase energy levels, inhibit the replication of viruses and several physiological processes. *Hypericum perforatum* L. 0.3-1.2 m herb is gaining increasing popularity as a medicinal plant[3,4] and there is great interest in its medicinal properties.

St. John's wort is best known for the treatment of mild to moderate depression[5] and clinical studies have supported the effectiveness of its extract[6,7]. Extract of the crude drug are widely used in the treatment of mild and moderate depression by increasing the concentration of neurotransmitters in the central nervous system[8]. *Hypericum perforatum* L. bioactive constituents are not completely understood. Possible candidates include hypericin, pseudohypericin and releated bisanthraquinones.

These secondary metabolites are part of specific plant defense systems[9] and these phototoxic anthraquinons exhibiting antimicrobial, antiviral and antiherbivore properties *in vitro*[10,11]. Furthermore its antitumor[12,13], anticancer[14,15] and antidepression[16] properties. The clinical studies showed that it is also anti HIV agent[15-17].

St. John's Wort is an economically important plant due to its secondary metabolite contents. For instance, sales of monopreparations and multi-ingredient preparations of this plant in the USA in 1997 reached some 200 million US dollars[18]. The situation in European countries is similar[19] and 570 million US dollars worldwide annually[20].

The use of hypericin and pseudohypericin in medicinal therapy has attracted considerable interest to investigate *Hypericum perforatum* L.. It is because one of the plants used in drug production industry intensively and its propagation is very important. Germination is a critical stage in the life cycle of weeds and crop plants and often controls population dynamics with major practical implications[21]. But, germination rate and generative propagation of St.John's wort is very difficult due to seed dormancy[22]. Its seeds exhibit both exogenous and endogenous dormancy[23]. The seeds need different exogenously treatment for breaking endogenous dormancy[23,24]. Therefore vegetative methods are more useful for propagation and this plant prefers to be propagated vegetatively[25]. Tissue culture and cell suspension culture are widely used for vegetative propagation. But both low seedling survive and difficulties in transplantations make this plant production difficult, especially under water stress or in aridity. For this reasons alternative methods or growth mediums must be used to increase the seedling survive.

In the early 1980's water-absorbing polymers or hydrogels were introduced for agricultural use[26]. These hydrophilic polymers vary in effectiveness depending on the transplanting situation they are used in and properties of growth medium. When they used correctly and in ideal situations they increase seedling survive, plant growth and water-holding capacity of soil[27-31].

The main aim of this study is to determine the possibilities of survive of seedlings at early stages of development due to excess of quinoid compounds in young plants and to determine the contribution of polymer or different media factors. Because, the seedlings are susceptible to environmental conditions and dried in short time after transplantation and the surviving time is very important for its contents that used as drug material.

EXPERIMENTAL

Plant material one initial explants, stem segments (10 mm in height), was obtained from young shoots, 20-25 cm in height of *Hypericum perforatum* L. plants grown wildly in Mayis University campus area. Stem segments was surface sterilized in a 20 % (v/v) commercial sodium hypochlorite solution containing 0.05 % (v/v) Tween20 for 20 min, followed by rinsing in sterile distilled water. The experiments were carried out at the beginning of the summer in 2002 in Samsun province.

Callus initiation: The explants were cultured in media supplemented with the same culture constituent in magenta boxes (Fig. 1).

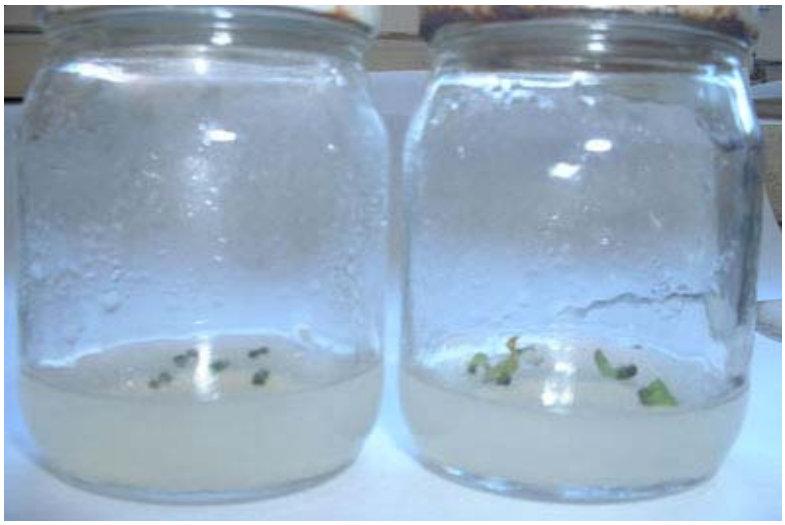

Fig. 1. Initiation of meristem culture

The callus initiation medium contained Murashige and Skoog[32] (MS) salts and vitamins. The pH was adjusted to 5.8 before autoclaving at 121°C for 20 min at 1.2 atm pressure, sucrose concentration (30 g L^{-1}), plant growth regulators (kinetin and 2,4-dichloro phenoxy acetic acid) and its concentrations (0.5, 1 and 1.5 mg L^{-1}) were tested. All chemicals were purchased

from Sigma. Cultures were kept in darkness at 26 ± 2°C for 8 weeks without sub-culturing. Six explants were cultured per 25 mL magenta box.

Shoot induction and multiplication: For shoot induction, calli were transferred to shoot induction medium containing MS salts and vitamins, 1 mg L^{-1} of BA and 30 g L^{-1} of sucrose, pH 5.8 and kept under the same conditions employed in callus initiation for 6 weeks[33] (Fig. 2). Transferred to the photoperiod (8/16 light/dark,1200 lux. After 5-7 weeks, the number of shoots per treatment was recorded.

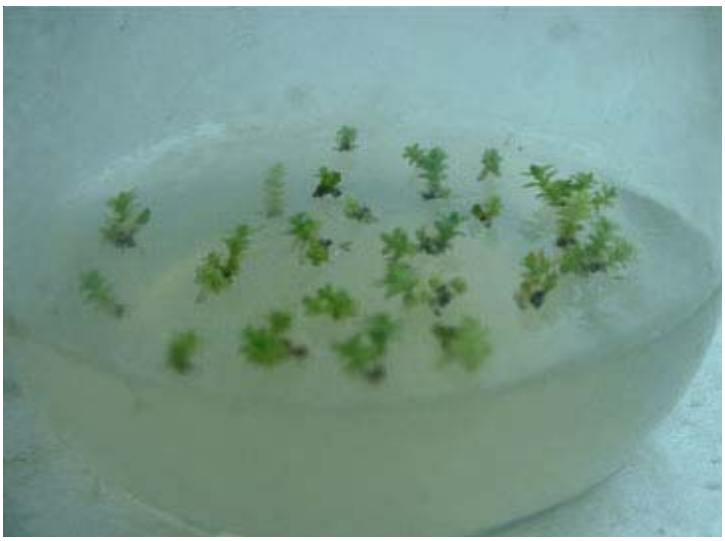

Fig. 2. Rooted seedlings in cultured meristem tissues

Rooting: To induce roots, elongated shoots were excised and transferred into MS medium supplemented with 30 g L^{-1} of sucrose and 1 mg L^{-1} of IAA, pH 5.8. Four shoots were placed in a magenta box (30 mL media) per treatment. Data were recorded after 4 weeks of culture. After removal from magenta boxes, the rooted plantlets were washed with tap water to remove rooting medium debris. The seedlings were transplanted into boxes containing a different mixtured medium with or without polymers (Table-1). After 10 d, the acclimated plants were transferred to a greenhouse, maintained under partial shade and irrigated daily or weekly. The percentage of survival was recorded during 3 weeks intervals.

Polymers; Polymer-1: Poly(acrylamide-g-ethylene diamine tetraacetic acid) polyelectrolyte hydrogels (AAm EDTA JEL4)[34] and **Polymer-2:** Poly (N-vinyl-2-pyrrolidone/itaconic acid) (VPITA JEL4)[35] were synthesized

TABLE-1
GROWTH MEDIUMS AFTER TRANSPLANTATION

Control	Polymer-1(Aam EDTAJEL4)	Polymer-2 (VPITA JEL4)
Sand	Sand+Polymer-1	Sand+Polymer-2
Soil	Soil+Polymer-1	Soil+Polymer-2
Perlite	Perlite+Polymer-1	Perlite+Polymer-2
Sand+Soil	Sand+Soil+Polymer-1	Sand+Soil+Polymer-2
Sand+Perlite	Sand+Perlite+Polymer-1	Sand+Perlite+Polymer-2
Soil+Perlite	Soil+Perlite+Polymer-1	Soil+Perlite+Polymer-
Sand+Soil+Perlite	Sand+Soil+Perlite+Polymer-1	Sand+Soil+Perlite+Polymer-2

by reported method. These polymers were placed in pots so as to allow maximum contact with the roots of seedlings. The pots were watered profusely after transplantation to allow the hydrogels to absorb water.

The results were analyzed statistically and the contribution of polymers to vegetative propagation of St. John's wort.

RESULTS AND DISCUSSION

Seedlings of *Hypericum perforatum* L. are oversensitive to environmental conditions and seedlings survived 2-3 d in six control groups and maximum 5 d only in mixed medium. The seedling transplanted to polymer used medium survived more than control (Table-2). The survival of seedlings changed due to polymers and mediums properties. In sand and perlite, polymer-1 increased survival time of transplants more than polymer-2 (Table-2). In all mixed mediums polymer-2 increased survival times more than polymer-1 (Table-2). Polymer-1 and polymer-2 both affected survival time and increased considerable time than control. Polymer-1 was more effective especially in sand and in perlite. It increased liveliness time 4.5 fold in sand and 6 folds in perlite more than control. If it think over the drug materials isolated from this plants are found especially at early stage of development[36]. Taking this into account, it must be considered an important contribution for its vegetative propagation. In spite of Böttcher *et al.*[37] reported different environmental or crop conditions effect the rate of hypericin in St. John's Wort. Ksouth *et al.*[36] suggested that the presence of hypericin and adhyperforin was first proved in the *Hypericum perforatum* seedlings at early stage of development.

The maximum survival time was in perlite with polymer-1 as 18 d (Table-2). After pots transferred to natural conditions, transplants were dried (Fig. 3). The usual goal of adding hydrogels to the growth mediums is to add water holding capacity (especially in sandy soils) and when they used in ideal situations will have at least 95 % of their stored water available for

TABLE-2
SURVIVE AND GROWTH OF SEEDLING IN DIFFERENT MEDIUMS

Different growth medium with or without polymers	Survival of plants (d)	Plant height (cm)
Sand	**2a**	**3.1a**
Sand + Polymer-1	9b	4.8b
Sand + Polymer-2	4a	9.8c
Soil	**4a**	**9.2c**
Soil + Polymer-1	5c	5.9d
Soil + Polymer-2	6c	4.1b
Perlite	**3a**	**8.0c**
Perlite + Polymer-1	18d	9.5c
Perlite + Polymer-2	10b	5.5d
Sand + Soil (1:1)	**2a**	**5.2d**
Sand + Soil + Polymer-1	6c	5.9d
Sand + Soil + Polymer-2	7c	7.1cd
Sand + Perlite (1:1)	**2a**	**8.4c**
Sand + Perlite + Polymer-1	12e	7.5cd
Sand + Perlite + Polymer-2	13e	7.4cd
Soil + Perlite (1:1)	**2a**	**7.6cd**
Soil + Perlite + Polymer-1	12e	7.3cd
Soil + Perlite + Polymer-2	13e	8.5c
Sand + Soil + Perlite (1:1:1)	**5c**	**6.5d**
Sand + Soil + Perlite + Polymer-1	9b	6.8cd
Sand + Soil +Perlite + Polymer-2	10b	11.4e

*Values in vertical columns followed by a different letter are significantly different at the 0.05 level.

Fig. 3. Transplants at natural conditions after 3 weeks

plant absorption and improve the physical properties of soil[38,39]. Most effectiveness of polymers has been seen in sand, in perlite or their mixture (Table-2) that both mediums are granular and there are large spaces between particles. In our opinion the polymers penetrate these areas and increased retention of water in mediums that becomes available for plants and thus the seedlings could be survived. Such effect contributes to young plants after transplantation.

Growth rates and differences between the polymers or controls are shown in Table-2. The present results showed that seedling survival was affected by polymers in all growth media after transplantation and can use in vegetative propagation process. But, there is no apparent correlation was found between polymer using and plant growth rates. However, the results indicate significant differences between growth media. For example, most growth rate has been observed in soil control group. Only polymer-2 increased the growth of plants in mixed medium (sand+soil+perlite+polymer-2) significantly (Table-2).

It is supposed that hydrophilic polymers can contribute to propagation of St. John's Wort especially used with other elicitors. For example, some elicitors such as lactic acid[40] and Jasmonic acid[41] can promote growth of transplants. And some synthetic hydrogels containing indolbutyric acid (IBA) as rooting auxin can be used for plant production[42].

Frantz *et al.*[43] suggested that water-holding polymers can be used in plant production. They effect the plants, organs, flowering, fresh and dry masses differently. The transplants can be supplied by this elements for its development for a more extended period.

As a result, it can be concluded that the hydrophilic polymer can be used for propagation of *Hypericum perforatum* L. and contributed to survival of transplants. Its seedlings are oversensitive to environmental conditions especially to aridity during transplantation, polymers can reduce this problem and increased survival time of transplants.

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