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Effects of Polymers and Growth Mediums on in vitro Plantlets of Winter Squash (*Cucurbita maxima* Duch. ex Lam.) and Pumpkin (*Cucurbita moschata* Duch. ex Poir.) in Acclimatization

Ertan Sait Kurtar[1*], Ahmet Balkaya[2] and Nilgün Özbek Okumuş[3]

[1]*Bafra Higher School of Vocational Education, Ondokuz Mayis University, Samsun, Turkey*
[2]*Horticulture Department of Agriculture Faculty, Ondokuz Mayis University, Samsun, Turkey*
[3]*Radiaiton Oncology Department, Faculty of Medical Science, Ondokuz Mayis University, Samsun, Turkey*

Abstract

The aim of this study was to determine the effects of hydrophilic polymers (hydrogels) and growth mediums on survive and growth of in vitro plantlets of winter squash and pumpkin during acclimatization. AAm EDTA JEL4, VPITA JEL4 and PCrA 0.18JEL3 were used with eight different growth mediums. The plantlets were propagated by in vitro embryo culture in solid E20A medium via irradiated pollen technique. Plantlets were transplanted to: (1) soil (2) soil with polymers (3) sand (4) sand with polymers (5) perlite (6) perlite with polymers (7) peat moss (8) peat moss with polymers. Growth mediums with polymers increased rate of survival and growth of in vitro plantlets of winter squash and pumpkin during acclimatization. Transplanted plantlets were survived in growth mediums with polymers range from 1.2 to 3.3 times more than controls.

Key Words: Hydrophilic polymer, in vitro acclimatization, seedling survival and growth, winter squash, pumpkin.

INTRODUCTION

Hydrophilic polymers (hydrogels) work by absorbing and storing water and nutrients in a gel form and these are used specially in horticulture. 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 [1].

Hydrogels have at least 95% of their stored water available for plant absorption, eliminate the moisture stress, improve the physical properties of soil and can reduce nutrients losses from soils

[2]. Hydrogels can absorb hundreds or thousand of times their weight in water and increase retention of large quantities of water in soil that becomes available for plants [1]. Thus, seedling survives and plant growth rate could be increased [3].

Embryo culture is one of the unique technique of tissue culture is used for principal experiments in biology (embryogenesis), plant propagation from un-germinated seeds (dormancy and seed sterility), embryo rescue (haploidy and hybridization) and forceful analyses of seeds vigour. In this technique, seeds that contain different maturing stages embryos were extracted from fruits and these embryos were cultured in aseptic and special conditions (*in vitro*) with different mediums [4]. High relative humidity and temperature, low CO_2 and low light intensity, high sugar and ethylene concentration during *in vitro* culture result in the formation of seedlings of abnormal morphology, anatomy (little development of cuticle wax layer, malfunction of stomata, less stomata density, vitrification, small leaf area and less development of palisade tissue) and physiology (low transpiration rate, low net photosynthetic rate and high respiration rate) [5,6].

After transfer from the *in vitro* to the *ex vitro* conditions, these seedlings might be easily impaired by sudden changes in environmental conditions. They have little cuticle development and they can not control the water status because of low hydraulic conductivity of roots and root-stem connections [7]. Also many seedlings die during this period, when they are transplanted in greenhouse or a field conditions, and so acclimatization period is necessary to increase the survival percentage of seedlings and correct the abnormalities [6]. During acclimatization, the environment is changed gradually with time, starting with the near *in vitro* environment and finishing with the near greenhouse or field environment [8,9].

Although, tissues culture techniques are used for plant propagation widely today, both low seedlings survival rate and difficulties in transplantations make this plant production difficult, especially under inappropriate and undesirable conditions (water stress or in aridity) [5,10]. For this reasons alternative methods or growth mediums should be used to increase the young seedling survival during acclimatization.

The main aim of this study is to determine the possibilities of contributions of hydrogels and different growth media factors on survival and growth at early stages of *in vitro* plantlets of winter squash (*Cucurbita maxima* Duchesne ex Lamonier) and pumpkin (*Cucurbita moschata* Duch. ex Poir.) during acclimatization stress.

MATERIALS AND METHODS

Materials

Six winter squash (57SI06, 57SI21, 55BA02, 55BA03, 55ÇA06 and G14) and five pumpkin genotypes (55BA01, 14YE01, 14YE02, 14BO01 and G9) were used as plant materials. Genotypes were selected from the Black Sea region of Turkey (genetic material used in the project was funded by TUBITAK-TOVAG -104O144), except “G14” and “G9”genotype which were provided by the Turkish Seed Gene Bank, Menemen, Izmir.

Poly (acrylamide-g-ethylene diamine tetraacetic acid) polyelectrolyte hydrogels (AAm EDTA JEL4) [11] **(P1),** Poly (N-vinyl-2-pyrrolidone-itaconic acid) (VPITA JEL4) [12] **(P2)** and Poly (N-vinyl-2-pyrrolidone-crotonic acid) (PCrA 0.18JEL3) [11] **(P3)** were used as hydrogels. P1, P2 and P3 can retain at least 16, 24 and 153 times its weight of water, respectively, and slowly release it, approximately 6 d (Figure 1). These polymers were placed in cups so as to allow

maximum contact with the roots of plantlets. The cups were watered profusely after transplantation to allow the polymers to absorb water.

Pollen irradiation and pollination

Female flowers were isolated with white cloth bags (15 x 10 cm) and male flowers were collected around noon on the day before anthesis. Anthers without filaments were irradiated in small cardboard boxes (5 x 7 x 2 cm) by Cobalt 60 gamma-rays at doses of 50, 100, 200 and 300 Gray (1 Gray = 100 Rad). On the morning (between 7.00-9.00a.m.) of the following day (0^{th} day), female flowers were pollinated with irradiated pollen and then isolated again.

Harvest, extraction and *in vitro* embryo culture

Harvested fruits were washed in tap-water and kept in 2% sodium hypochlorite solution for 30 minutes to eliminate micro-organisms. Prior excision, the laminar air-flow cabinet was disinfected with UV (Ultraviolet) light for 15 minutes following 70% ethanol application. The fruit surface was then flame sterilised using ethanol and seeds were extracted in disinfected cabinet. Embryos were excised from seeds and cultured in magenta boxes or culture glasses which contained approximately 25 ml solid E20A medium [13]. Magenta boxes and culture glasses were incubated in a growth chamber [16 hour photoperiod (6000 lux) at 28 $\pm 1^{o}$C] (Figure 2a). After 5 to 15 days, mini-plantlets (having root and shoot) were transplanted individually to other boxes or tubes.

Multiplication, transplantation and acclimatization

After 27 to 41 days, well-generated plantlets (Figure 2b) were multiplied via micro-cutting to increase plantlets number (Figure 2c). For this purpose, plantlets were divided into cuttings have a leaf and a node. After the plantlets were obtained by the multiplication, plantlets underwent an acclimatization process while under in vitro conditions. Firstly, the covers of magenta boxes and culture glasses were gradually opened over a period of 8-10 days. Plantlets were removed initially and cleaned in tap-water to eliminate excess agar on the roots. The roots were then rinsed three times with distilled water and soaked for 10 minutes in 0.2% solution of fungicide (Maxim XL035FS) to prevent contamination at the beginning of the acclimatization process. The plantlets were transplanted into white plastic cups (150 cm^3) which were filled with sterilised different growth medium with or without polymers (Table). Each cup was closed with a transparent cup and the plants were acclimatized in the growth cabinet [16 hour photoperiod (6000 lux) at 28 $\pm 1^{o}$C] at high relative humidity (approximately 95%) (Figure 2d). The transparent cups were gradually opened and removed after 6 days. The humidity with in the growth cabinet was reduced periodically at intervals of 5% over a period of 2 days, until humidity reached the greenhouse or field conditions (60-65%) (Figure 2e).

Experimental design

8 applications (soil for control, soil with polymers, sand for control, sand with polymers, perlite for control, perlite and polymers, peat moss for control and peat moss with polymers) were used at 3 replications and each replication contains 8 plantlets. The survival rate (%) and length (cm) of plantlets were recorded at the end of the acclimatization. Plantlets survival is defined as the rate of living plantlets. Plantlets length was measured from the surface of medium to the apex.

RESULTS AND DISCUSSION

During acclimatization, rate of survive plantlets in mediums with polymers were higher than controls (Table), except peat moss + P3 medium. The number of survival plantlets changed with polymers and growth mediums properties. In soil P2, in sand P3, in perlite P1 and P2, and in peat moss P1 were increased number of survival plantlets more than the other polymers for winter squash and pumpkin. Polymers were increased number of survival plantlets range from 1.2 (perlite + P3) to 3.3 (sand + P3) times more than controls. While P3 was more effective in sand and it increased survival plantlets at 3.3 times (41.2%) more than control (12.5%), it has negative effect in peat moss and it reduced rate of survival plantlets. 16.7% plantlets survived in soil; while it was 37.5% in P3 medium.

Average growth rates (plantlets length) and differences between the polymers and controls are shown in Table. While the significantly differences were found between growth medium and polymer, plant growth rates were not affected correlatively with using polymer in all growth medium. The highest plantlets length of 8.5 cm was obtained in peat moss + P1 medium. Length of plantlets grown in peat moss and perlite were the higher than soil and sand, and the most growth rate derived from peat moss in control group because of water holding and conservation capacity. In soil all polymers, in soil, perlite and peat moss P1 and P2 gave the higher rates than control. P3 has the highest water holding capacity on the contrary had negative effects on both number of survive plantlets and growth in peat moss. It is assumed that the highest water holding capacity caused shortage of oxygen and increase fungus infection in growth medium, then slow progress in plant growth and finally wilting.

Main aim of polymers adding is raise water holding and retention capacity of growth mediums (especially in sandy soils), thus plantlets were regulated water-root connection rapidly and continued their liveliness in unfavourable conditions. It is clear that polymers were the most effective in sand (Table 1) that medium is granular and there are large spaces between particles. The polymers penetrate these areas and increased retention of water in mediums that becomes available for plants and thus the plantlets could be survived [14]. Hydrogels have been used to increase plantlets and transplants survival especially in the arid conditions [15-17].

Some polymers have some plant growth promoter and plant defence regulator such as lactic acid [18], Jasmonic acid [19], indolbutyric acid [20] and gibberelic acid [21]. For example, hormonal analyses of oligo-chitosan (polyacrylamide hydrogel) indicated the presence of hormones; auxin, cytokinin and gibberelic acid. These compounds are known to increase the root plant and decrease in the drop off of flowers. Also, some of these polymers can preserve their own shape and can be used for many times. In spite of polymers have various advantageous effects; some studies have shown little or no advantage with hydrogel addition [22-25]. The variations in polymers effects are also dependent on crops species, type of polymers and growth mediums [14,18,26,27].

Table 1: Survival rate and length of plantlets in different mediums

	Survival rate (%)		Length (cm)	
Medium	**WS**	**P**	**WS**	**P**
Soil	16.7 f	12.5 f	6.7 c	6.9 c
Soil + P1	33.3 c	29.2 cd	7.1 c	7.0 c
Soil + P2	37.5 bc	37.5 bc	7.3 b	7.6 b
Soil + P3	29.2 c	25.0 d	7.5 b	7.2 b
Mean	**29.2 b**	**27.1 b**	**7.2 b**	**7.2 b**
Sand	12.5 f	12.5 f	6.2 d	6.3 d
Sand + P1	29.2 c	33.3 c	6.8 c	7.0 c
Sand + P2	33.3 c	33.3 c	7.1 c	7.1 c
Sand + P3	37.5 bc	41.2 b	7.3 b	7.5 b
Mean	**28.1 b**	**31.1 b**	**6.9 c**	**7.0 c**
Perlite	29.2 c	25.0 d	7.3 b	7.4 b
Perlite + P1	41.2 b	45.8 b	7.6 b	7.8 a
Perlite + P2	45.8 b	45.8 b	7.4 b	7.5 b
Perlite + P3	33.3 c	29.2 c	7.1 c	7.3 b
Mean	**36.3 a**	**36.5 a**	**7.4 b**	**7.5 b**
Peat moss	29.2 c	33.3 c	7.8 a	8.0 a
Peat moss + P1	54.2 a	50.0 a	8.4 a	8.5 a
Peat moss + P2	41.2 b	45.8 b	8.1 a	8.2 a
Peat moss + P3	20.8 e	25.0 d	7.1 c	7.4 b
Mean	**36.4 a**	**38.5 a**	**7.9 a**	**8.0 a**
Overall	**32.7**	**33.3**	**7.39**	**7.50**

**Values in vertical columns followed by a different letter are significantly different at the 0.05 level.; WS: Winter squash; P: Pumpkin; P1: Polymer-1 (AAm EDTA JEL4); P2: Polymer-2 (VPITA JEL4); P3: Polymer-3 (PCrA 0.18JEL3)*

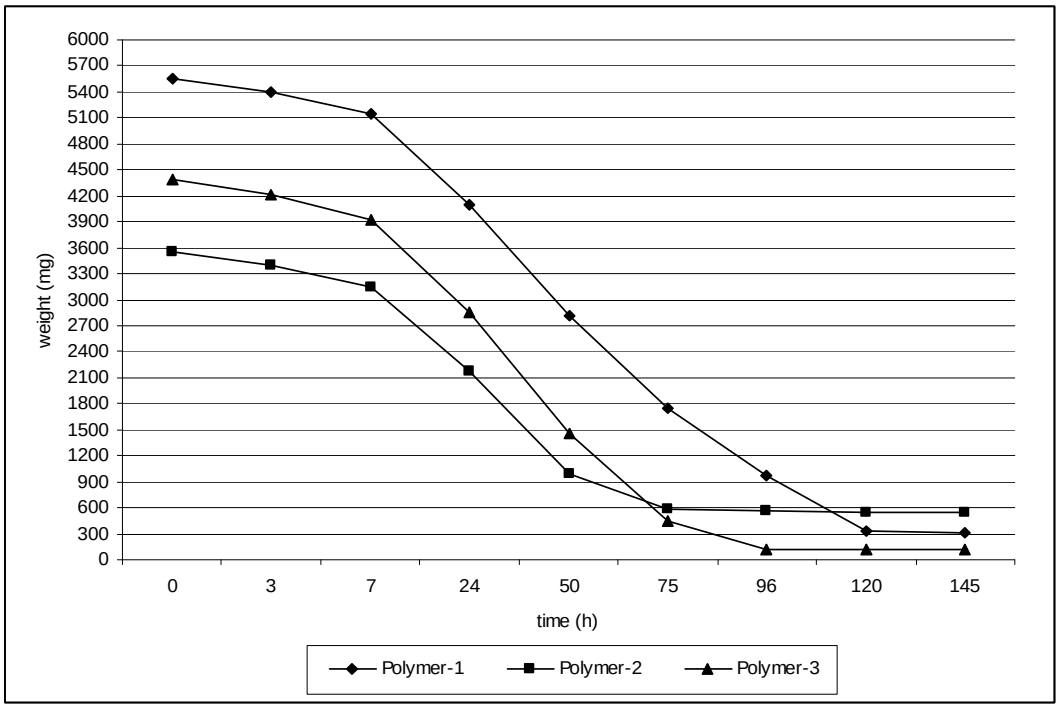

Figure 1. Water release rates of polymers

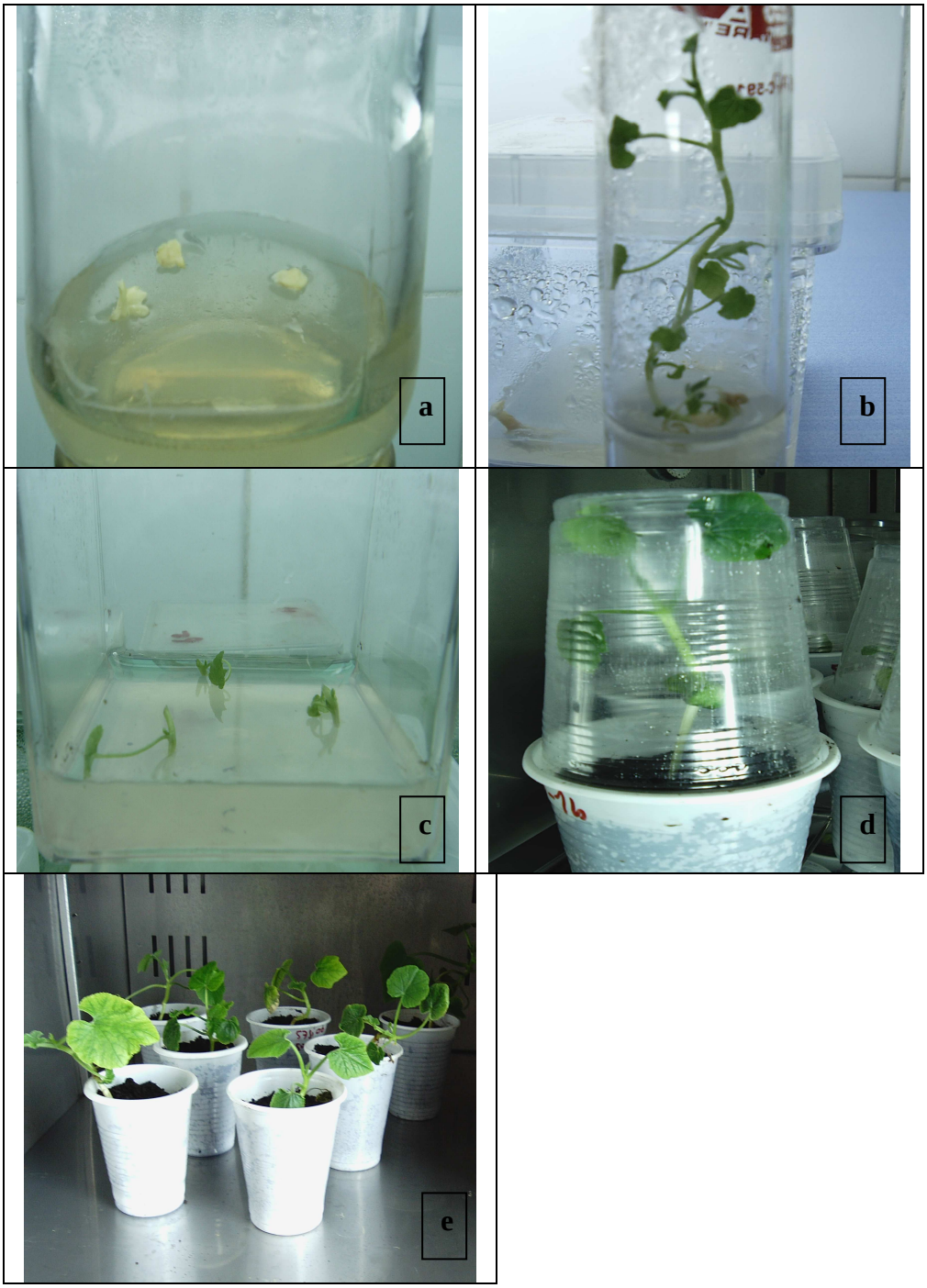

Figure 2. Embryos in culture glass (a), a well generated plantlet (b), multiplication via micro-cutting (c), acclimatization of plantlets in the growth cabinet (d, e)

CONCLUSION

Addition of soil conditioners such as these polymers (hydrogels), have good water-retention capacity, can be reduced sensitivity and increased adaptation ability of plantlets, thereby enabling supports to be made in early survival and subsequent growth under unfavourable environmental conditions during acclimatization stress. The results of the present work indicated that hydrophilic polymers can be used for acclimatization of in vitro plantlets of winter squash and pumpkin forcefully and enhanced to survival and growth of plantlets.

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