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Effect of Plant Growth Regulators on Osmotically Stressed Callus Cultures of Some *Capsicum annuum* Var. *grossum* L. Cultivars

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Abstract: In this research, *in vitro* studies were realized on the *Capsicum annuum* L. varieties which is very important crop for Turkey. *Capsicum annuum* L. seedlings were grown from seeds which surfaced sterilized with 70% ethanol for 4 min 10% NaOCl (household bleach) solution for 6 min. The seeds were sown on the surface of hormone free MS (Murashige-Skoog) medium, pH adjusted to 5.8, the medium was solidified with 0.8% of bactoagar and 3% of sucrose used for carbon and energy source. After that, the cultures of the seeds germinated under 16 h photoperiod at 25°C. Cotyledone and hypocotyl explants from 3 weeks old seedlings were excised approximately 1 cm length segments. These explants were cultured on MS basal medium for callus formation. For determination to find what is the effects of drought stress or lowering osmotic potential conditions in the medium on the callus formation and response of the biomass induction. Polyethyleneglycol (PEG 3350) in 10% concentration were added to the MS medium and then biomass measurement were realized in 3 weeks interval from the callus. Two cultivars which belonging to the variete *grossum* (bell peppers) showed different responses against to the drought stress conditions. In the normal MS medium, biomass formation was very fast but when PEG used for changing the osmotic potential of the MS medium the biomass formation decreased because of the drought stress conditions, after that MS medium was supplemented with auxines (NAA) and cytokinins (KIN) alone and together with PEG 3350 for recovering the biomass formation. As a result, auxines (NAA) and cytokinins (KIN) showed different recovering levels in two cultivars when they used alone or together. These growth regulators can use for to manage the drought stress conditions in the field studies before sowing or germination and this can be help to increase the plenty and productivity of some varieties for *Capsicum annuum* against drought stress.

Key words: *Capsicum annuum*, tissue culture, Polyethyleneglycol, NAA, KIN

INTRODUCTION

All of the species which belonging to the *Solanaceae* family are very important and economic species for Turkey and all around the world. For example; *Nicotiana tabacum* (Tobacco), *Lycopersicon esculentum* (Tomato), *Solanum melongena* (Eggplant), *Solanum tuberosum* (Potato). All of the *Capsicum annuum* L. (pepper) varieties have economic importance for Turkey and some varieties of *C.annuum* has problem with water stress conditions in the some parts of Turkey. There is very close relations between plant growth regulators and effects of drought stress on plant. Endogenous hormone levels in plant tissues are effected from environmental stresses. Plant growth regulators has very different effects on the plants and because of that these substances using in a very large amount for field and *in vitro* studies. Some experiments showed us that NAA (Naphtalen Acetic Acid) were stimulated water absorbtion and cell wall plasticity. In *in vitro* drought

stress experiments polyetylenglycol, NaCl, mannitol and sucrose which decrease medium's water potential were used oftenly. This substances change the osmotic potential of the medium. In this research we tried to find some useful approaches for solution of this problem (drought stress) in *in vitro* conditions and for this reason we used plant tissue culture technics and plant growth regulators in different concentrations and combinations. Because of the drought stress agricultural fields have very serious value loss. Most of the places have problems because of the water salinity in worldwide. In this kind agricultural fields crop growing becomes problem. Because of exceeding drought stress the soils inconvenient to cultivation occupy in Turkey. Most of the researchers have used poliethyleneglycol because of it's high molecular weight. PEG molecules are large in size and this feature of the molecules inhibits the enter to the plasmalemma and symplast with this way metabolic products doesn't effect directly^[1]. *C. annuum* L. is drought sensitive species. Some of the researches on

in vitro studies for *C. annuum* varieties plant growth regulators have been using for increase the tolerance against drought sensitivity^[2]. Concentration of polyethyleneglycol from 15 to 30% were increased the drought tolerance of cell line but this tolerance has been lost in subcultured cell line which adapted to polyethyleneglycol medium which doesn't include osmotic conditions^[3]. The effect of polyethyleneglycol on the development of cell colony, osmotic potential and ion change in red pepper were realized. One of the cell colony have been developed in concentration of 20 and 25% PEG 8000. The other cell colony have been highly developed biomass in 5 and 10% PEG 8000^[4].

MATERIALS AND METHODS

In this research two cultivars (Kandil Dolma, Yağlık 28) belonging to *Capsicum annuum* L. Var. *grossum* were used. Certificated seeds of two cultivars were obtained from Aegean Agricultural Research Institute. Certificated seeds of two cultivars surface sterilized with 70% ethanol for 4 min 10% NaOCl (household bleach) solution for 6 min and then distilled in distilled water for 30 min. The seeds were sown on the surface of hormone free sterilized MS^[5] nutrient media containing 3% sucrose, 0.8% agar and ph adjusted to 5.8 before autoclaving. All process have realized under aseptic conditions in the laminar airflow cabinet. After that the seeds germinated under 16 h photoperiod at 25±2°C in growth chamber. Cotyledone and hypocotyl explants from 3 weeks old seedlings were excised approximately 1 cm. length segments. These explants were cultured on MS basal medium for callus formation. Between 0.8 -2 g callus were placed on flasks for observing biomass changing. Callus cultures were subcultured every three weeks onto the freshly prepared MS medium. Biomass changing were recorded at the end of each three weeks culture period till the fourth subculture. Biomass media consisted of MS medium alone and supplemented with auxins like NAA, cytokinin like KIN with concentrations 10 ppm L⁻¹ were used. Drought stress conditions were realized adding 10% polyethyleneglycol (PEG-3350) to the MS medium (Table 1).

RESULTS AND DISCUSSION

Callogenesis in Kandil Dolma and Yağlık 28 cultivars have developed from the both explants types in MS medium. Effect of different medium ingredients on biomass changing in two cultivars were obtained. Callus cultures were subcultured every three weeks onto the freshly prepared MS medium. In a other research

Table 1: *In vitro* medium types for biomass obtaining from callus

Medium	Group name
MS+9a10 PEG-3350	Drought stress
MS+9a10 PEG-3350+10 ppm NAA	PGR effect
MS+9a10 PEG-3350+10 ppm NAA +10 ppm KIN	PGR effect

Table 2: *In vitro* biomass changing of *C. annuum* Var. *grossum* Cv. Kandil Dolma. Data were given as the increase in biomass over 3-week passage in gram

Medium ingredients	Subcultures				
	0	1st	2nd	3rd	4th
MS	6.8	9.1	13.7	19.7	25.3
MS+9a10 PEG-3350	4.1	4.4	5.1	6.8	8.3
MS+9a10 PEG-3350 +10 ppm NAA	7.2	7.8	11.2	15.9	19.6
MS+9a10 PEG-3350 +10 ppm NAA+10 ppm KIN	6.8	5.3	7.3	9.8	11.6

Table 3: *In vitro* biomass changing of *C. annuum* Var. *grossum* Cv. Yağlık 28. Data were given as the increase in biomass over 3-week passage in gram

Medium ingredients	Subcultures				
	0	1st	2nd	3rd	4th
MS	3.4	4.5	9.3	12.8	16.2
MS+9a10 PEG-3350	4.1	4.4	7.0	9.7	11.4
MS+9a10 PEG-3350 +10 ppm NAA	7.7	6.7	8.7	11.0	12.9
MS+9a10 PEG-3350 +10 ppm NAA+10 ppm KIN	4.3	3.1	3.7	4.3	4.60

subculturing period has used in every four weeks^[6]. Callus formation rate have stabilized in 17-19th days. Same callogenesis medium without changing for six weeks have been effected on callus appearance like changing colour and structural corruptions. Drought tolerance in tomatoes cell line have been increased with using PEG concentration between 15 and 30%^[3]. PEG treatment in *C. annuum* L. cell colony has been changed ion balance^[4]. According to some of the researchers osmotic potential increasing which medium supported with 5-10% polyethyleneglycol were stimulated nicotiana callus growing, protein and chlorophyl content. When polyethyleneglycol concentration increased to 30% inhibition of callus growing and the other parameters were realized^[7]. During all subcultural period biomass increasing have been inhibited when MS medium supported with 10%PEG in the Kandil Dolma and Yağlık 28 cultivars. *In vitro* biomass changing of *C. annuum* Var. *grossum* cultivar Kandil Dolma (Table 2) and cultivar Yağlık 28 (Table 3) datas were given as the increase in biomass over 3-week passage in gram. Biomass in the beginning for Kandil Dolma cultivar between 4.1-7.2 g and for Yağlık 28 cultivar 3.4-7.7 g in 5 flasks.

In vitro biomass changing percentage of *C. annuum* Var. *grossum* cultivar Kandil Dolma (Table 4) and cultivar Yağlık 28 (Table 5). Total *in vitro* biomass increasing percentage of *C. annuum* var. *grossum* cultivar Kandil Dolma (Table 6) and cultivar Yağlık 28 (Table 7). Results of this study indicate that biomass changing in two cultivars have shown differences in *in vitro* conditions because of differences their endogenous hormone levels.

Table 4: *In vitro* biomass changing percentage of *C. annuum* Var. *grossum* Cv. Kandil Dolma

Medium ingredients	Percentage of biomass increasing \pm SE*			
	1st	2nd	3rd	4th
MS	51.1 \pm 7.2	51.3 \pm 7.7	45.1 \pm 5.7	29.1 \pm 3.1
MS+%l0 PEG-3350	19.7 \pm 2.7	41.2 \pm 6.4	29.2 \pm 3.3	26.8 \pm 2.5
MS+%l0 PEG-3350+10 ppm NAA	44.9 \pm 6.9	74.5 \pm 9.4	44.4 \pm 6.4	23.9 \pm 3.4
MS+%l0 PEG3350+10 ppm NAA+10 ppm KIN	39.8 \pm 4.6	41.6 \pm 6.8	35.7 \pm 5.3	16.0 \pm 2.3

* Value indicates the number of callus \pm SE of three independent determinations, involving 15 replicates

Table 5: *In vitro* biomass changing percentage of *C. annuum* Var. *grossum* Cv. Yağlık 28

Medium ingredients	Percentage of biomass increasing \pm SE*			
	1st	2nd	3rd	4th
MS	58.6 \pm 7.3	157.0 \pm 13.5	37.5 \pm 5.9	29.1 \pm 4.1
MS+%l0 PEG-3350	33.3 \pm 6.8	81.1 \pm 6.9	34.5 \pm 4.2	26.8 \pm 3.5
MS+%l0 PEG-3350 +10 ppm NAA	12.1 \pm 2.2	42.7 \pm 6.2	59.3 \pm 6.2	23.9 \pm 3.4
MS+%l0 PEG-3350 +10 ppm NAA+10 ppm KIN	28.4 \pm 4.7	27.8 \pm 5.6	13.1 \pm 2.4	6.7 \pm 4.9

*Value indicates the number of callus \pm SE of three independent determinations, involving 15 replicates

Table 6: Total *in vitro* biomass increasing percentage of *C. annuum* Var. *grossum* Cv. Kandil Dolma at the end of 4th subculture

Medium ingredients	Total biomass increasing (%) \pm SE*
MS	305.8 \pm 36.3
MS+%l0 PEG-3350	124.8 \pm 20.4
MS+%l0 PEG-3350 +10 ppm NAA	190.2 \pm 33.9
MS+%l0 PEG-3350 +10 ppm NAA+10 ppm KIN	113.5 \pm 9.32

*Value indicates the number of callus \pm SE of three independent determinations, involving 15 replicates

Table 7: Total *in vitro* biomass increasing percentage of *C. annuum* Var. *grossum* Cv. Yağlık 28 at the end of 4th subculture

Medium ingredients	Total biomass increasing (%) \pm SE*
MS	325.3 \pm 29.3
MS+%l0 PEG-3350	184.6 \pm 19.5
MS+%l0 PEG-3350 +10 ppm NAA	62.6 \pm 7.1
MS+%l0 PEG-3350 +10 ppm NAA+10 ppm KIN	88.3 \pm 9.3

*Value indicates the number of callus \pm SE of three independent determinations, involving 15 replicates

MS medium which supported with auxin and cytokinin together have negative effect on biomass increasing comparing with auxin alone in Kandil Dolma cultivar. This is show that cytokinin which added to the MS medium have sinerjistic effect with PEG for inhibition of biomass formation in Kandil Dolma cultivar. But in Yağlık 28 cultivar when MS medium supported with auxin and cytokinin together biomass increasing were realized. In this case sinerjistic effect of PEG and NAA were inhibited with adding KIN to the MS medium. Endogenous hormone levels in both cultivars are effected from drought stress conditions differently and independently. Biomass decreasing have observed in drought stressed tissues. Thus, it has been thought that singly or together external applications of NAA and KIN would optimize physical metabolic conditions favorable for drought stress. While the growth regulator NAA stimulated biomass increasing in Kandil dolma cultivar, growth regulator KIN stimulated biomass increasing in Yağlık 28 cultivar. At the end of our

research we declared that drought stress can be recovered in agricultural fields with pretreatment of seeds or seedlings before planting.

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