ACTA SCIENTIARUM POLONORUM

Acta Sci. Pol. Hortorum Cultus, 19(5) 2020, 115–127

https://czasopisma.up.lublin.pl/index.php/asphc ISSN 1644-0692 e-ISSN 2545-1405 DOI: 10.24326/asphc.2020.5.12

ORIGINAL PAPER

Accepted: 7.11.2019

DO JASMONIC ACID AND ACTIVATED CHARCOAL INCREASE THE *in vitro* DEVELOPMENT OF ORANGE CARROT (*Daucus carota* L. subsp. *sativus* Hoffm.) AND PURPLE CARROT (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.)?

Tugce Ozsan, Gulsun Elif Vural, Ahmet Naci Onus

Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya, Turkey

ABSTRACT

Present study aimed to reveal the effects of jasmonic acid and activated charcoal on *in vitro* carrot plantlet regeneration by using Murashige and Skoog (MS) medium supplemented with BAP, NAA, activated charcoal and jasmonic acid at various concentrations. To serve the purpose, *in vitro* carrot seed germination, shoots, cotyledons, and first leaves formation of orange and purple carrot plantlets were investigated. During the experiments, root size, weight, and size of petiole diameter, hyperhydricity and callus formation rate were recorded. Experimental results revealed that combination of jasmonic acid and activated charcoal in medium had a positive effect especially on the first stage of developmental processes such as seed swelling and germination, cotyledon and first leaf formation as well as having positive effects on above-ground internode elongation, petiole and plantlet height.

Key words: carrot, early plant growth, jasmonates, plant tissue culture

INTRODUCTION

There are several species that produce and accumulate vitamins, carotenoids and anthocyanins in high quantities in storage roots. Carrots are one of these species and also among the first 10 plants in the world with high economic value [Luby et al. 2014, Wang et al. 2017]. Carrot is one of the ideal model plants used in genetic and molecular genetic studies [Yazawa et al. 2004, Grebenstein et al. 2013, Jourdan et al. 2015, Wang et al. 2015]. Having a very important place in terms of human nutrition, carrots are a good source of additional nutrients with high fiber content such as good vitamins A, B and K, magnesium, antioxidants, pectin [Nowacka and Wedzik 2016]. In addition, since it contains high amount of bioactive components (flavonoids, α- and β-carotene), the immune system and the eyesight are positively affected.

Carrot plant botanically is classified into two groups: carotene containing group (orange carrots – *Daucus carota* ssp. *sativus*) and anthocyanin containing group (purple carrots – *Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) [Dereli 2010].

Activated charcoal is used in many fields of *in vitro* studies such as micropropagation, protoplast culture, somatic embryogenesis, androgenesis, gynogenesis, callus formation, root formation, shoot formation, proliferation and prolongation [Thomas 2008]. It is reported that when dark conditions by using activated charcoal in culture plates during several stages such as *in vitro* seed germination, rooting and tuber formation were provided, the development of vegetative organs were promoted [Thomas 2008]. By providing *in vitro* dark conditions, petiole and tuber formation and

onus@akdeniz.edu.tr

development were positively affected [Thomas 2008]. It should be noted that carrot germination takes quite long time (20–25 days, depending on environmental factors) if the seeds are directly sown into soil [Hanci and Cebeci 2012].

Jasmonates are the main plant hormones that regulate the development and defense processes in plants. They play a role in defending against disease and pests, regulation of many physiological and developmental processes such as root development, tuber formation, fruit ripening, aging and pollen development [Lorenzo and Solano 2005]. Jasmonates contribute in synthesis of various enzymes (chitinase enzyme and proteinase inhibitors) and proteins. Storage proteins present in onions, tubers, and seeds are important for the survival of plants. These proteins, known as vegetative storage proteins, are controlled by certain genes. In recent years, it has been stated that jasmonates play an important role in plant development. The development based on this idea is the discovery that the genes encoding vegetative storage proteins are regulated by jasmonates [Creelman and Mullet 1997]. It is reported that jasmonate levels are increased when plants are under stress. However, the effects of jasmonates (jasmonic acid, methyl jasmonate, and tuberonic acid) on the biochemical functions of plants are yet to be clarified in details [Baktır 2015].

The endogen methyl jasmonate contributes to initiate and regulate the growth and development processes of plants and the level of methyl jasmonate increase when any stress condition is encountered. As a result of cell division and growth, plants' root and shoot growth are suppressed. Externally applied jasmonic acid has similar effects. However, this effect varies depending on the application dose, plant species, plant age, physiological condition of the plant, and since the jasmonic acid signals work together with other plant growth regulators and the effect may be antagonistic or synergistic. Therefore, present study is conducted to reveal the effects of various concentrations of jasmonic acid and activated charcoal on *in vitro* carrot plants growth and development.

MATERIALS AND METHODS

Two different carrot types, standard Nantes type orange (Naval F_1) and purple carrot (Purple Sun F_1) cultivars, were investigated. The seeds were surface sterilized for 15 minutes in a 15% sodium hypochlorite solution and then left in 70% alcohol for 15 seconds. Afterwards, seeds were rinsed 3 times with sterile distilled water. After sterilization, 15 seeds were cultured in plates, kept 3 days in dark conditions, and then transferred to the growth chamber with 8 hours photoperiod and 3000 lux illumination at 24 ±2°C.

In the study, 9 different nutrient media combinations of jasmonic acid and activated charcoal were used as shown in Table 1. MS [Murashige and Skoog 1962] (Duchefa Biochemie) supplemented with 30 g L^{-1} sucrose (Duchefa Biochemie), 0.5 mg L^{-1} naphthalene acetic acid (NAA, Duchefa Biochemie) and 2.5 mg L^{-1} benzyl aminopurine (BAP, Duchefa Biochemie) were used. Plant growth regulators were constant in all media combinations tested. The differences between the media combinations were created by adding activated charcoal (2 g L^{-1} and 0 g L^{-1}) [Kenar 2013] and jasmonic acid (0.5, 1, 2 and 5 mg L^{-1}) [Ahmadi et al. 2014] – Table 1.

All observations were started with seed sowing and continued until all measurements were completed. The petiole length was measured from the point where it combines with root to the highest point at which the plantlet grows, the root length was measured from the lowest point of the plantlet to the point where the root combines with the petiole. The petiole diameter measurement was conducted with a digital caliper, while the petiole length and root length were measured with millimeter paper and ruler.

Three pots with 15 seeds were employed for each medium and the study was conducted with three replications. The data were subjected to a two-way ANOVA with interaction using the JMP 8.1 packet program. The significant differences between treatments were determined with the LSD test.

RESULTS AND DISCUSSIONS

In the present study, developments on orange carrot and purple carrot seeds after culturing were daily observed and recorded (Tab. 2). Results revealed that seeds of both carrot types started to swell within 2 days in all media combinations. Seeds were germinated through 3 days except for seeds that were cultivated in media 5, 6 and 7. On day 4 in the orange

Table 1. The media combinations used in the study

Media codes	Medium 1 (OC_1–PC_1)	Medium 2 (OC_2–PC_2)	Medium 3 (OC_3–PC_3)	Medium 4 (OC_4–PC_4)	Medium 5 (OC_5–PC_5)	Medium 6 (OC_6–PC_6)	Medium 7 (OC_7–PC_7)	Medium 8 (OC_8–PC_8)	Medium 9 (OC_9–PC_9)
Activated charcoal (g L^{-1})	2	2	2	2	–	–	–	–	–
Jasmonic acid (mg L^{-1})	0.5	1	2	5	0.5	1	2	5	–

* Media combination codes: OC – orange carrot, PC – purple carrot

Table 2. Daily change parameters of orange carrot and purple carrot seeds in different media combinations

Parameters/media	Orange carrot (OC)									Purple carrot (PC)								
	OC_1	OC_2	OC_3	OC_4	OC_5	OC_6	OC_7	OC_8	OC_9	PC_1	PC_2	PC_3	PC_4	PC_5	PC_6	PC_7	PC_8	PC_9
Seed swelling/ day	1	1	2	1	2	2	2	1	1	1	1	2	1	2	2	2	1	1
Beginning of germination/ day	3	3	3	3	6	6	4	3	3	3	3	3	3	6	6	7	3	3
Root formation/ day	4	4	4	4	7	7	5	4	4	4	4	4	4	7	7	8	4	4
Hypocotyl formation/ day	5	5	4	4	8	8	6	5	5	5	5	4	4	8	8	8	5	5
Cotyledon formation/ day	6	6	6	6	9	9	8	8	6	6	6	6	6	9	10	9	10	6
Epicotyl formation/ day	12	8	8	8	15	17	16	20	13	9	9	12	8	14	15	15	19	11
Leaf formation/ day	14	10	10	10	20	21	21	–	17	11	11	14	10	21	18	22	–	14
Color change/ day	21	22	20	21	–	–	–	–	–	15	15	15	15	21	–	–	–	–
Foliar yellowing/ day	17	45	21	–	–	–	–	–	–	–	–	21	–	28	–	–	–	–
Abnormal plant formation/ day	–	–	–	–	23	25	24	21	–	–	–	–	–	22	20	23	20	–

carrot, the first hypocotyl formation was observed in media 3 and 4 after swelling. While on day 6, cotyledons formed in media 1, 2, 3, 4, and 9, epicotyl formation started on day 8, in media number 2, 3 and 4 for orange carrot; in medium 4 for the purple carrot.

At the end of the 3rd week of the study, the thickening in the root zone was recorded. After the 2nd–3rd week, the color formation in the storage roots started to be seen and in all 4 media combinations, containing activated charcoal, color changes peculiar to orange carrot and purple carrot were observed. Since there were no adventitious roots formation in media number 6, 7, 8 and 9 in orange carrot and purple carrot, leaves could not develop but instead abnormal plantlets, callus, and hyperhydricity took place.

In general, the cotyledons formed in the activated charcoal-free media combinations were found to be thicker and the plantlets with thick cotyledons were short in a comparison to plantlets formed in media combinations with activated charcoal. Results also clearly showed that, petiole of the plantlets developed in media combinations with activated charcoal was thin, the branching rate was high, the root development was quite good and the tuber taproot and lateral hairy roots were very intense (Tab. 2, Figs 1 and 2). Similar results were also reported by Koda and Kikuta [2001] as they reported that JA added to media positively affected the tuber formation in potato.

In terms of seed germination and cotyledon formation, the responses of orange carrot and purple carrot seeds were statistically insignificant, while the media combinations showed statistically significant differences in their interaction with orange and purple carrots (Tab. 3). When the effects of media combinations on the germination rates were examined, the best germination rate was recorded in medium number 4 (MS + 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA + 5 mg L^{-1} jasmonic acid + 2 g L^{-1} activated charcoal; 98.9%). Regarding cotyledon formation rates, the highest formations were determined in media number 2 and 4 (MS + 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA + 1 mg L^{-1} jasmonic acid + 2 g L^{-1} activated charcoal; MS + 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA + 5 mg L^{-1} jasmonic acid + 2 g L^{-1} activated charcoal, respectively; 95.6%). Media combinations with activated charcoal and control medium gave the best results in terms of seed germination and cotyledon formation. Based on the experimental results, it is possible to say that jasmonic acid and activated charcoal have no positive effect on seed germination. In previous studies it is reported that jasmonic acid and methyl jasmonate prevent germination of seeds that are not in dormancy state but encourage germination of seeds in dormancy state [Creelman and Mullet 1997]. Similar responses were obtained in many studies in which effects of jasmonic acid on seed germination and seedling growth were investigated [Wilen et al. 1990, Kepczynski et al. 1999]. Moreover, it is clear from the studies that germination and development slow down by increasing jasmonic acid concentration [Koda and Kikuta 1991, Parthier 1991, Tsai et al. 1997] and it is not possible to reverse these negative reactions [Çavusoglu and Kabar 2006, Çavusoglu et al. 2007]. In present study, it is thought that the reason of low germination rate in media combinations with various concentrations of jasmonic acid with no activated charcoal is due to negative effects of jasmonic acids. But adding activated charcoal into the nutrition media prevents negative effects of jasmonic acid. Activated charcoal is known to contribute to growth-promoting conditions in plants. Its contribution is seen in the processes from embryo formation to plant regeneration by adsorbing certain growth-promoting vitamins, cytokinins, auxins [Rathinapriya et al. 2019]. Due to the use of the storage substances present in the seed germination stage, it is thought that low amount of activated charcoal present in the plant regeneration process following germination contributes to the plant regeneration process by tolerating the decreasing substances.

In terms of hyperhydricity rate and callus formation, there were no statistically significant differences between the carrot types and the carrot types x media interactions while there were differences among the media combinations (Tab. 3). The highest rate of hyperhydricity was recorded in plantlets grown in medium number 5 (MS + 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA + 0.5 mg L^{-1} jasmonic acid) – Figs 3e and 4e). It is possible to assume that the reason for the high rate of hyperhydricity in the nutrient medium 5 can be attributed to adverse effects of jasmonic acid, even at the low concentration and absence of activated charcoal. On the other hand, while activated charcoal significantly inhibited callus formation, the absence of acti-

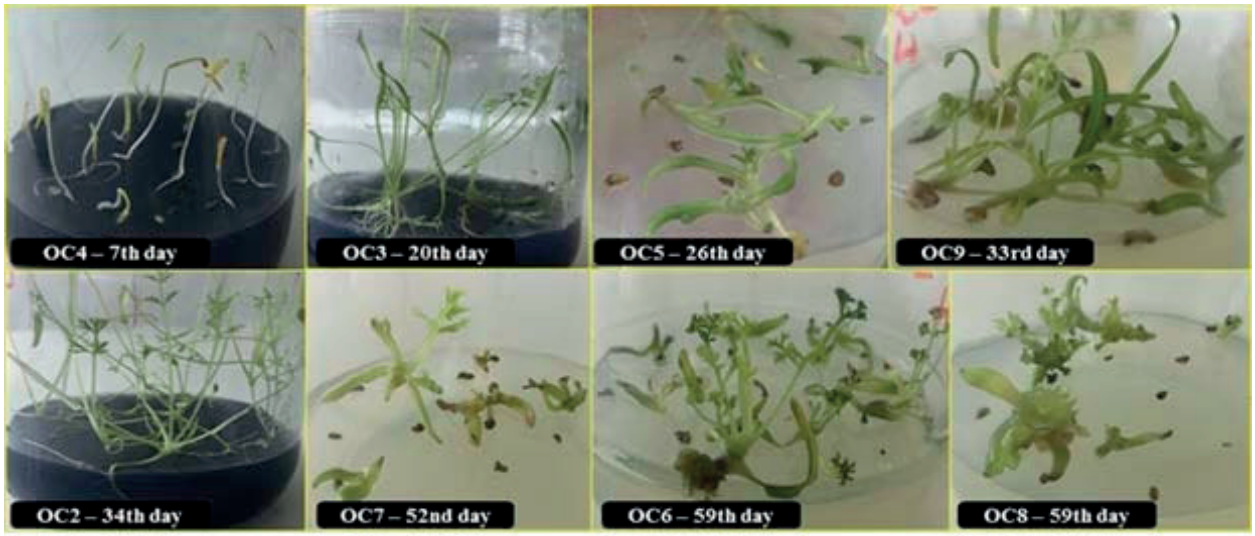

Fig. 1. Orange carrot (OC) plantlet development following the seeding in different media combinations (Tab. 1 – media combinations)

Fig. 2. Purple carrot (PC) plantlet development following the seeding in different media combinations (Tab. 1 – media combinations)

vated charcoal in other media combinations triggered the callus formation (Figs 3e–3i and 4f, 4g, 4i).

When the leaf formation rate was examined, there were statistically significant differences (Tab. 4) between two carrot types as the purple carrot formed high percentage of leaf. In terms of relationship between media combinations and leaf formation, the highest leaf formation was detected in medium number 4 (MS + 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA + 5 mg L^{-1} jasmonic acid + 2 g L^{-1} activated charcoal), while the medium number 8 (MS + 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} NAA + 5 mg L^{-1} jasmonic acid + 30 g L^{-1} sucrose) which in

Table 3. General results of seed germination (%), cotyledon formation (%), hyperhydricity (%), callus formation (%)

Parameters		Orange carrot				Purple carrot			
		seed germination (%)	cotyledon formation (%)	hyper--hydricity (%)	callus formation (%)	seed germination (%)	cotyledon formation (%)	hyper--hydricity (%)	callus formation (%)
Media codes	M1	86.7$^{a–d}$	84.4$^{a–d}$	2.2	0	93.3^{abc}	93.3^{ab}	6.7	0
	M2	97.8^{ab}	95.6^{ab}	8.9	2.2	95.6^{ab}	95.6^{ab}	6.7	0
	M3	93.3^{abc}	93.3^{ab}	0	2.2	88.9$^{a–d}$	88.9^{abc}	6.7	0
	M4	100^{a}	97.8^{a}	2.2	0	97.8^{ab}	93.3^{ab}	6.7	0
	M5	86.7$^{a–d}$	77.8$^{a–d}$	62.2	33.3	68.9^{ef}	66.7^{de}	42.2	24.4
	M6	88.9$^{a–d}$	77.8$^{a–d}$	26.7	33.3	55.6^{fg}	55.6^{ef}	46.6	28.9
	M7	82.2$^{b–e}$	71.1^{cde}	31.1	13.3	75.6^{de}	75.6$^{b–e}$	46.6	17.8
	M8	51.1^{g}	42.2^{f}	24.4	17.8	77.8^{cde}	77.8$^{a–d}$	44.4	20.0
	M9	93.3^{abc}	88.9^{abc}	11.1	37.8	95.6^{ab}	95.6^{ab}	4.4	66.7

Media	M1	M2	M3	M4	M5	M6	M7	M8	M9
Average seed germination (%)	89.9^{ab}	96.7^{a}	91.1^{a}	98.9^{a}	77.8^{c}	72.2^{cd}	78.9^{bc}	64.4^{d}	94.4^{a}
	LSD types = N.S.	LSD media*** = 11.9	LSD types × media** = 16.9			orange carrot: 86.7	purple carrot: 83.2		
Average cotyledon formation (%)	88.9^{a}	95.6^{a}	91.1^{a}	95.6^{a}	72.2^{b}	66.7^{b}	73.3^{b}	59.9^{b}	92.2^{a}
	LSD types = N.S.	LSD media*** = 14.5	LSD types × media* = 5.8			orange carrot: 80.9	purple carrot: 82.5		
Average hyper-hydricity (%)	4.4^{c}	7.8^{c}	3.3^{c}	4.4^{c}	52.2^{a}	36.7^{b}	38.9^{ab}	34.4^{b}	7.8^{c}
	LSD types = N.S.	LSD media*** = 15.5	LSD types × media = N.S.			orange carrot: 18.8	purple carrot: 23.5		
Average callus formation (%)	0^{e}	1.1^{e}	1.1^{e}	0^{e}	28.9^{bc}	31.1^{b}	15.6^{d}	18.9^{cd}	52.2^{a}
	LSD types = N.S.	LSD media*** = 10. 8	LSD types × media = N.S.			orange carrot: 15.6	purple carrot: 17.5		

Different letters in the same column and rows indicate a statistically significant difference at *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, respectively. N.S. – not significant

Table 4. General results of leaf formation (%), shoot formation/ plantlet, leaf formation/ plantlet

Parameters		Orange carrot			Purple carrot		
		leaf formation (%)	shoot formation/ plantlet	leaf formation/ plantlet	leaf formation (%)	shoot formation/ plantlet	leaf formation/ plantlet
Media codes	M1	77.8$^{a–d}$	5.3	5.5	91.1^{ab}	4.9	5.2
	M2	77.8$^{a–d}$	5.5	5.8	88.9^{ab}	4.9	4.9
	M3	93.3^{a}	6.1	6.4	86.7^{abc}	4.8	4.8
	M4	93.3^{a}	6.5	6.6	93.3^{a}	4.5	4.7
	M5	51.1^{e}	1.1	1.1	62.2^{de}	1.1	1.1
	M6	77.8$^{a–d}$	6.8	6.8	55.6^{e}	7.2	7.3
	M7	55.6^{e}	2.5	3.5	71.1^{b-e}	1.6	2.3
	M8	24.4^{f}	0	0	66.7^{cde}	0	0
	M9	77.8$^{a–d}$	3.9	4.3	93.3^{a}	6.2	6.5

Media	M1	M2	M3	M4	M5	M6	M7	M8	M9
average leaf formation (%)	84.4^{a}	83.3^{a}	89.9^{a}	93.3^{a}	56.7^{bc}	66.7^{b}	63.3^{b}	45.6^{c}	85.6^{a}
	LSD types* = 7.0	LSD media*** = 14.9	LSD types × media* = 21.1			orange carrot: 69.9^{b}	purple carrot: 78.8^{a}		
average shoot formation/ plantlet	5.1^{b}	5.2^{b}	5.5^{ab}	5.5^{ab}	1.1^{cd}	6.9^{a}	2.0^{c}	0^{d}	5.1^{b}
	LSD types = N.S.	LSD media*** = 11.9	LSD types × media = N.S.			orange carrot: 4.2	purple carrot: 3.9		
average leaf formation/ plantlet	5.3^{a}	5.3^{a}	5.6^{a}	5.6^{a}	1.1^{c}	7.1^{a}	2.9^{b}	0^{c}	5.4^{a}
	LSD types = N.S	LSD media***= 1.8	LSD types × media = N.S.			orange carrot: 4.5	purple carrot: 4.1		

Different letters in the same column and rows indicate a statistically significant difference at *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, respectively. N.S. – not significant

Table 5. General results of petiole diameter (mm), petiole length (cm), root length (cm)

Parameters		Orange carrot			Purple carrot		
		petiole diameter (mm)	petiole length (cm)	root length (cm)	petiole diameter (mm)	petiole length (cm)	root length (cm)
Media codes	M1	2.48	13.5	11.3	2.15	10.1	13.5
	M2	2.54	10.0	9.4	2.52	10.9	13.2
	M3	2.46	10.8	11.3	2.54	11.7	16.1
	M4	2.69	10.9	10.7	1.82	10.6	11.6
	M5	0.65	0.8	0.2	0.59	0.8	0.2
	M6	3.21	2.5	0.6	3.15	2.3	0.6
	M7	1.08	0.9	0.3	0.41	0.6	0.2
	M8	0	0	0	0	0	0
	M9	2.48	3.7	1.3	3.66	5.1	1.8

Media	M1	M2	M3	M4	M5	M6	M7	M8	M9
average petiole diameter (mm)	2.31^{bc}	2.53^{abc}	2.50^{abc}	2.26^{c}	0.62^{d}	3.18^{a}	0.75^{d}	0^{d}	3.07^{ab}
	LSD types = N.S. LSD media*** = 0.77 LSD types × media = N.S.					orange carrot: 1.95	purple carrot: 1.87		
average petiole length (cm)	11.8^{a}	10.5^{a}	11.2^{a}	10.8^{a}	0.8^{d}	2.4^{c}	0.7^{d}	0^{d}	4.4^{b}
	LSD types = N.S. LSD media*** = 1.54 LSD types × media = N.S.					orange carrot: 5.90	purple carrot: 5.78		
average root length (cm)	12.4^{ab}	11.3^{ab}	13.7^{a}	11.2^{b}	0.2^{c}	0.6^{c}	0.2^{c}	0^{c}	1.6^{c}
	LSD types* = 1.16 LSD media*** = 2.45 LSD types × media = N.S.					orange carrot: 5.0^{b}	purple carrot: 6.4^{a}		

Different letters in the same column and rows indicate a statistically significant difference at *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, respectively. N.S. – not significant

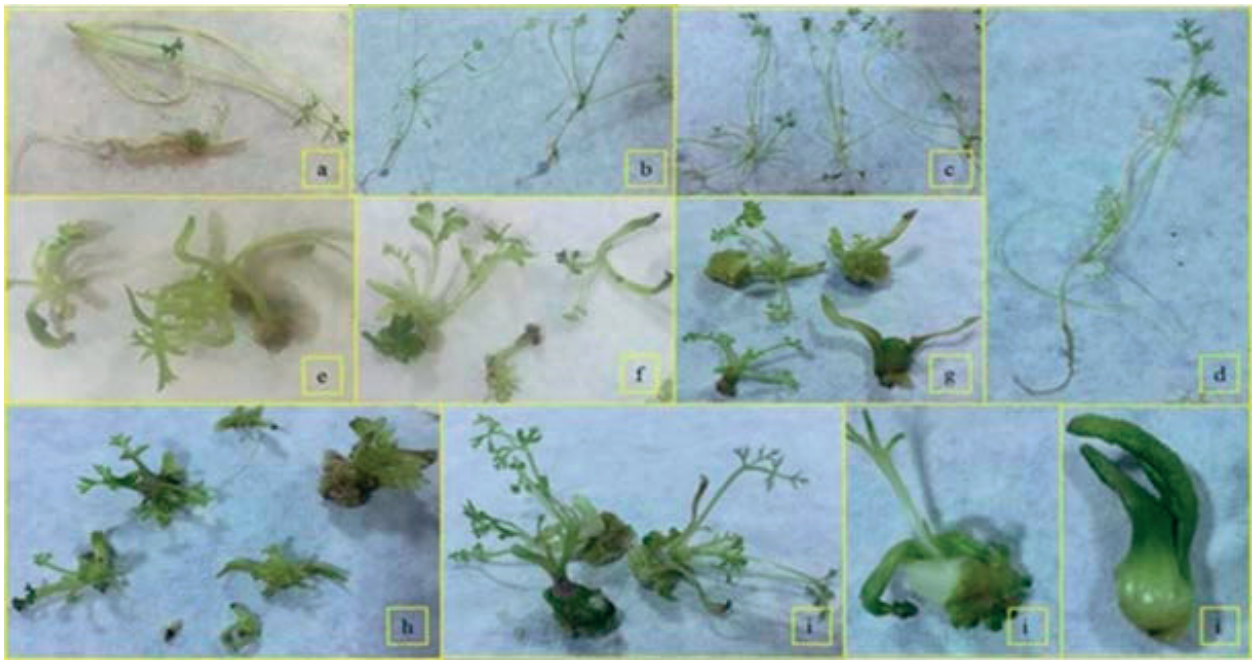

Fig. 3. Day 60th, observations made before measurements in different media combinations in orange carrot; **a.** Medium 1, **b.** Medium 2, **c.** Medium 3, **d.** Medium 4, **e.** Medium 5, **f.** Medium 6, **g.** Medium 7, **h.** Medium 8, **i.** Medium 9

Fig. 4. Day 60th, observations made before measurements in different media combinations in purple carrot; **a.** Medium 1, **b.** Medium 2, **c.** Medium 3, **d.** Medium 4, **e.** Medium 5, **f.** Medium 6, **g.** Medium 7, **h.** Medium 8, **i.** Medium 9

Table 6. General results of petiole weight (g), root weight (g), total plantlet weight (g)

Parameters		Orange carrot			Purple carrot		
		petiole weight (g)	root weight (g)	total plantlet weight (g)	petiole weight (g)	root weight (g)	total plantlet weight (g)
Media codes	M1	0.22	0.08	0.29	0.09	0.07	0.17
	M2	0.17	0.05	0.21	0.10	0.09	0.20
	M3	0.16	0.08	0.23	0.14	0.16	0.30
	M4	0.20	0.12	0.33	0.11	0.10	0.21
	M5	0.03	0.007	0.03	0.02	0.005	0.03
	M6	0.14	0.07	0.21	0.13	0.08	0.21
	M7	0.03	0.02	0.11	0.02	0.02	0.03
	M8	0	0	0	0	0	0
	M9	0.11	0.30	0.41	0.13	0.28	0.41

Media	M1	M2	M3	M4	M5	M6	M7	M8	M9
average petiole weight (g)	0.16^{a}	0.14^{a}	0.15^{a}	0.16^{a}	0.02^{b}	0.14^{a}	0.02^{b}	0^{b}	0.12^{a}
	LSD types* = 0.030 LSD media*** = 0.064 LSD types × media = N.S.					orange carrot: 0.12^{a}		purple carrot: 0.08^{b}	
average root weight (g)	0.08^{bc}	0.07^{bcd}	0.12^{b}	0.11^{b}	0.006^{de}	0.07^{bc}	0.02^{cde}	0^{e}	0.29^{a}
	LSD types = N.S. LSD media*** = 0.067 LSD types × media = N.S.					orange carrot: 0.080		purple carrot: 0.090	
average total plantlet weight (g)	0.23^{b}	0.21^{b}	0.27^{b}	0.27^{b}	0.03^{c}	0.21^{b}	0.07^{c}	0^{c}	0.41^{a}
	LSD types = N.S. LSD media*** = 0.11 LSD types × media = N.S.					orange carrot: 0.21		purple carrot: 0.17	

Different letters in the same column and rows indicate a statistically significant difference at *** $P \leq 0.001$; ** $P \leq 0.01$; * $P \leq 0.05$, respectively. N.S. – not significant

the presence of the same concentration of jasmonic acid had the lowest percentage of leaf formation. Although medium number 4 and number 8 had same concentration of jasmonic acid (5 mg L^{-1}) the major difference was the presence of activated charcoal in media number 4 and that is why it is assumed that activated charcoal prevented the negative effect of jasmonic acid in medium number 4.

When the average shoot formation, leaf formation and petiole diameter were examined, differences only in media combinations were recorded, while there were no significant differences in carrot types and the carrot types x media interactions (Tabs 4, 5 and Figs 3f, 4f). The obtained results from medium 6 were promising for shoot development. While activated charcoal is not used in this nutrient medium, it is believed that the cytokinin (BAP) used in combination with 1 mg L^{-1} jasmonic acid is a positive response.

When the petiole length was examined, statistical differences only in media combinations were observed, but no significant differences in carrot types and the carrot types × media interactions were recorded (Tab. 5 and Figs 3a, 4a). As is seen from Table 5 medium containing the lowest jasmonic acid concentration was found successful in terms of petiole length measurements. In previous studies it was reported that presence of jasmonic acid reduced the shoot length [Gaspar et al. 1996]. Findings of present study are therefore in agreement with previous studies.

The statistical significant differences were determined among media combinations and carrot types in terms of plantlet root length (Tab. 5). As reported in previous studies, in addition to its effect on seed germination, JA also prevented root development [Berger et al. 1996]. As can be seen from the Table 5 in terms of root development, purple carrot had better results than the orange carrot. The shortest root length was recorded in media combinations with no activated charcoal (Figs 3 and 4), supporting previous studies [Ravnikar and Gogala 1990, Gaspar et al. 1996]. In previous studies, it has been also reported that JA inhibits the elongation of the root formation in potato node cultures [Ravnikar et al. 1992] and garlic root formation [Ravnikar et al. 1993]. On the other hand Sarkar et al. [2006] reported that MeJA had a stimulating effect on potato root development.

In terms of petiole weight, root weight and total plantlet weight; statistically significant differences were recorded between carrot types and media combinations in terms of petiole weight (Tab. 6). When the average plantlet root weight and average total plantlet weight were evaluated, differences only among media combinations were recorded, but no significant differences in carrot types and carrot types x media interactions were found (Tab. 6). Similar results were also reported in previous studies. For example, according to Sarkar et al. [2006], when fresh weight of potato tubers were measured, the stimulating effect of JA was seen only in one variety, and there were not any differences among the media combinations. However, the use of high concentrations of JA caused a decrease on tuber growth and triggered morphological changes in tuber shapes [Sarkar et al. 2006]. *In vitro* bulb formations in garlic [Ravnikar et al. 1993], tuber formation in potato [Koda et al. 1991, Pelacho and Mingo-Castel 1991, Sarkar et al. 2006] have been reported to be promoted through the use of various concentrations of jasmonates. Therefore, based on the results of these studies, it can be said that jasmonates promote the formation of storage organs in tuberous plants [Teixeira da Silva 2012].

CONCLUSION

Jasmonates are known the main plant hormones regulating the development and defense processes in plants. As shown in present study, well-developed *in vitro* plantlets were obtained when jasmonic acid and activated charcoal were used together. Callus formation, abnormal vegetative growth, and hyperhydricity were only observed in media combinations containing jasmonic acid with no activated charcoal.

It is also found that the use of jasmonic acid together with activated charcoal is desirable at the first stages of plant developments such as seed swelling and germination, cotyledon and first leaf formation. Jasmonic acid's growth and development inhibitory properties were overcome by adding activated charcoal into the medium. For example presence of activated charcoal in medium dramatically reduced to seed germination time from 20 days to 3 days. Root formation rate and the root volume were found to be better in media combinations containing activated charcoal. It is thought

that trying various concentrations of jasmonic acid and activated charcoal in media combinations is a necessity for *in vitro* and *in vivo* orange and purple carrot development studies.

REFERENCES

Ahmadi, B., Shariatpanahi, M.E., da Silva, J.A.T. (2014). Efficient induction of microspore embryogenesis using abscisic acid, jasmonic acid and salicylic acid in *Brassica napus* L. Plant Cell Tiss. Organ Cult., 116, 343–351. DOI: 10.1007/s11240-013-0408-x

Baktır, İ. (2015). Jasmonateler (Yasemin Asitleri). Hormonlar, Bitki Gelişim Düzenleyicileri, Özellikleri ve Tarımda Kullanımları [Jasmonates (Jasmine Acids). Hormones, Plant Growth Regulators, Properties and Uses in Agriculture]. İstanbul, Türkiye: Hasad Yayıncılık, sf. 90–92 [in Turkish].

Berger, S., Bell, E., Mullet, J.E. (1996). Two methyl jasmonate-insensitive mutants show altered expression of AtVsp in response to methyl jasmonate and wounding. Plant Physiol., 111, 525–531. DOI: 10.1104/pp.111.2.525

Creelman, R.A., Mullet, J.E. (1997). Biosynthesis and action jasmonates in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol., 48, 355–381.

Çavusoglu, K., Kabar, K. (2006). Does Jasmonic Acid Prevent The Germination of Barley Seeds? SDÜ Fen Edebiyat Fakültesi Fen Dergisi (E-Dergi), 1(12–2), 35–41.

Çavusoglu, K., Kabar, K., Kılıç, S. (2007). Effects of Some Plant Growth Regulators on Jasmonic Acid Induced Inhibition of Seed Germination and seedling Growth of Barley. SDÜ Fen Edebiyat Fakültesi. Fen Dergisi (E-Dergi), 2(1), 53–59.

Dereli, U. (2010). Siyah Havuç Suyu Konsantresi Üretimi ve Depolanması Sürecinde Fenolik Maddelerdeki Değişimler ve Bu Değişimlerin Antioksidan Aktivite ile İlişkisi. Yüksek Lisans Tezi. Ankara Üniversitesi, Fen Bilimleri Enstitüsü.

Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, D.M., Thorpe, T.A. (1996). Plant Hormones and Plant Growth Regulators In Plant Tissue Culture. *In vitro* Cell. Dev. Biol.-Plant, 32, 272–289.

Grebenstein, C., Kos, S.P., de Jong, T.J., Tamis, W.L.M., de Snoo, G.R. (2013). Morphological markers for the detection of introgression from cultivated into wild carrot (*Daucus carota* L.) reveal dominant domestication traits. Plant Biol., 15, 531–540.

Hanci, F., Cebeci, E. (2012). Siyah Havuç (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) Yetiştiriciliği. Tarım Türk Dergisi, 7, 36–40 [in Turkish].

Jourdan, M., Gagné, S., Dubois-Laurent, C., Maghraoui, M., Huet, S., Suel, A., Hamama, L., Briard, M., Peltier, D., Geoffriau, E. (2015). Carotenoid content and root color of cultivated carrot: a candidate-gene association study using an original broad unstructured population. PLoS One, 10:e0116674, 1–19. DOI: 10.1371/journal.pone.0116674

Kenar, S. (2013). Patateste (*Solanum tuberosum* L.) *in vitro* Mikrotuberizasyon Üzerine Jasmonik Asit – Giberellik Asit Etkileşiminin Araştırılması. Yüksek Lisans Tezi, Hacettepe Üniversitesi, Ankara, Turkey [in Turkish].

Kepczynski, J., Bialecka, B., Kepczynska, E. (1999). Ethylene biosynthesis in Amaranthus caudatus seeds in response to methyl jasmonate. J. Plant Growth Regul., 28, 59–65. DOI: 10.1023/A:1006201625637

Koda, Y., Kikuta, Y., Takazi, H., Tsujino, Y., Sakamura, S., Yoshihara, T. (1991). Potato tuber-inducing activities of jasmonic acid and related compounds. Phytochemistry, 30(5), 1435–1438. DOI: 10.1016/0031-9422(91)84180-Z

Koda, Y., Kikuta, Y. (1991). Possible involvement of jasmonic acid in tuberization in yam plants. Plant Cell Physiol., 32, 629–633.

Koda, Y., Kikuta, Y. (2001). Effects of jasmonates on *in vitro* tuberization in several potato cultivars that differ greatly in maturity. Plant Prod. Sci., 4(1), 66–70. DOI: 10.1626/pps.4.66

Lorenzo, O., Solano, R. (2005). Molecular players regulating the jasmonate signalling network. Curr. Opin. Plant Biol., 8, 532–540. DOI: 10.1016/j.pbi.2005.07.003.

Luby, C.H., Maeda, H.A., Goldman, I.L. (2014). Genetic and phenological variation of tocochromanol (vitamin E) content in wild (*Daucus carota* L. var. *carota*) and domesticated carrot (*D. carota* L. var. *sativa*). Hortic. Res., 1(15), 1–6. DOI: 10.1038/hortres.2014

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15, 473–97.

Nowacka, M., Wedzik, M. (2016). Effect of ultrasound treatment onmicrostructure, colour and carotenoid content in fresh and dried carrot tissue. Appl. Acoust., 103, 163–171. DOI: 10.1016/j.apacoust.2015.06.011

Pelacho, A.M., Mingo-Castel, A.M. (1991). Jasmonic acid induces tuberization of potato stolons cultured *in vitro*. Plant Physiol., 97, 1253–1255. DOI: 10.1104/pp.97.3.1253

Parthier, B. (1991). Jasmonates, new regulators of plant growth and development: Many facts and few hypotheses of their action. Bot. Acta, 104, 446–454. DOI: 10.1111/j.1438-8677.1991.tb00257.x

Rathinapriya, P., Satish, L., Rameshkumar, R., Pandian, S., Rency, A.S., Ramesh, M. (2019). Role of activated charcoal and amino acids in developing an efficient regener-

ation system for foxtail millet (*Setaria italica* (L.) Beauv.) using leaf base segments. Physiol. Mol. Biol. Plants, 25(2), 533–548. DOI: 10.1007/s12298-018-0619-z

Ravnikar, M., Gogala, N. (1990). Regulation of potato meristem development by jasmonic acid *in vitro*. J. Plant Growth Regul., 9, 233–236. DOI: 10.1007/BF02041968

Ravnikar, M., Vilhar, B., Gogala, N. (1992). Stimulatory effects of jasmonic acid on potato node and protoplast culture. J. Plant Growth Regul., 11(1), 29–33. DOI: 10.1007/BF00193840

Ravnikar, M., Žel, J., Plaper, I., Špacapan, A. (1993). Jasmonic acid stimulates shoot and bulb formation of garlic *in vitro*. J. Plant Growth Reg., 12(2), 73–77. DOI: 10.1007/BF00193236

Sarkar, D., Pandey, S.K., Sharma, S. (2006). Cytokinins antagonize the jasmonates action on the regulation of potato (*Solanum tuberosum*) tuber formation *in vitro*. Plant Cell, Tissue Organ Cult., 87, 285–295. DOI: 10.1007/s11240-006-9166-3

Teixeira da Silva, J.A. (2012). Jasmonic Acid, but not Salicylic Acid, Improves PLB Formation of Hybrid Cymbidium. Plant Tissue Cult. Biotech., 22(2), 187–192. DOI: 10.3329/ptcb.v22i2.14209

Thomas, D. (2008). The role of activated charcoal in plant tissue culture. Biotechnol. Adv., 26, 618–631. DOI: 10.1016/j.biotechadv.2008.08.003

Tsai, S.J., Juengel, J.L., Wiltbank, M.C. (1997). Hormonal regulation of monocyte chemoattractant protein-1 messenger ribonucleic acid expression in corpora lutea. Endocrinology, 138, 4517–4520. DOI: 10.1210/endo.138.10.5577

Wang, G.L., Que, F., Xu, Z.S., Wang, F., Xiong, A.S. (2017). Exogenous gibberellin enhances secondary xylem development and lignification in carrot taproot. Protoplasma, 254, 839–848. DOI: 10.1007/s00709-016-0995-6

Yazawa, K., Takahata, K., Kamada, H. (2004). Isolation of the gene encoding carrot leafy cotyledon1 and expression analysis during somatic and zygotic embryogenesis. Plant Physiol. Biochem., 42(3), 215–223. DOI: 10.1016/j.plaphy.2003.12.003

Wilen, R.W., Mandel, R.M., Pharis, R.P., Holbrook, L.A., Moloney, M.M. (1990). Effects of abscisic acid and high osmoticum on storage protein gene expression in microspore embryos of *Brassica napus*. Plant Physiol., 94, 875–881. DOI: 10.1104/pp.94.3.875