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Effect of different activated charcoal and casein hydrolysate concentrations on shoot regeneration of *in vitro* tomato

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ABSTRACT

This study was conducted to compare the capacity regeneration of two genotypes of tomato (Agayad 7 and Jawahar) using particular explants (hypocotyl and cotyledon) under different concentrations of activated charcoal (0, 1, 5, 10 mg/l) and casein hydrolysate (0, 50, 100, 200, 500, 1000 mg/l). Our results show that cotyledon and hypocotyl of 'Jawahar' genotype significantly affected plantlets regeneration than 'Agayad 7'. Furthermore, the addition of 5mg/l activated charcoal to the nutritional media helped to increase shoot regeneration for both genotypes, mainly with cotyledon plantlets. Plantlets regeneration increased significantly by mixing 5 mg/l of activated charcoal with 100mg/l casein hydrolysate in 'Agayad 7' and with 100mg/l casein hydrolysate in 'Jawahar'. Notably, the best plantlet fresh weight (10.20g) was produced by mixing 1mg/l of activated charcoal with 200 mg/l of casein hydrolysate using hypocotyl of 'Jawahar' genotype. In addition**,** culturing 'Jawahar' and 'Agayad 7' explants on MS medium contains 1mg/l of activated charcoal resulted better shoot height compare to other concentrations. Meanwhile, 500mg/l casein hydrolysate presented better shoot height (11.67 and 12.02 cm) with cotyledon and hypocotyl of 'Jawahar' genotype, respectively. In respect to the effect of treatments on the percentage of regenerated shoots from cotyledon, 1 mg/l of activated charcoal revealed better significant increase (93.21 and 83.34) with 'Jawahar and Agayad 7', respectively. While, mixing 10 mg/l of activated charcoal with 200mg/l casein hydrolysate increased percentage of hypocotyl-regenerated shoots (98.15%). In conclusion, this study explore that addition of activated charcoal to fresh media is beneficial for development of shoots while casein hydrolysate did not significantly affect organogenesis for hypocotyl explant. The efficiency of the organogenesis depended on the interaction among genotype, activated charcoal and casein hydrolysate concentrations.

Key words: *Solanum lycopersicum, hypocotyl, cotyledon, tissue culture*

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically important vegetable crops grown worldwide that recognized as a highly nutritious food rich in vitamins, minerals, fibers, fatty acids and cholesterol free (Mathieu *et al.,* 2009; Aldrich *et al.,* 2010 and Anthon *et al.,* 2011). Plant tissue culture technique reported as importance techniques for quick multiplication and breeding work since the 1930s. Many workers use tissue culture for many different purposes like callus induction, anther culture, protoplast culture and somatic embryogenesis (Bhatia *et al.,* 2004). Environmental stresses, insects and diseases significantly reducing tomato productivity both in open field and greenhouse. Therefore, there is an urgent need for biotechnological methodology to sustain tomato production (Liza *et al.,* 2013).

In vitro regeneration of cultivated tomato has been a subject of research because of its commercial value and flexibility for more enhancements via genetic manipulation (Evans, 1989 and Raziuddin *et al.,* 2004). Murashige and Skoog (MS) is the most tissue culture generation used medium with a high content of nitrate, ammonium and potassium. Sometimes it requires supplementation of additional substances in the medium like activated charcoal and casein hydrolysate (Vinod *et al.,* 2009). The presence of activated charcoal in the nutritional medium balances plants' growth regulators, other materials inside the nutritional medium; it also helps to stimulate tissue cultivated, differentiated and modified (Abdulwahed 2013).

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However, application of additives is adapted to the objectives of the experimental studies and the particular plant species taken. Accordingly, several studies on plant regeneration from a wide range of tissues and organs of cultivated tomato germplasm have been conducted (Zapata *et al.,* 1981 and Mohamed *et al.,* 2010). However, the influence of explant on growth and development of organs is varied and depends on several factors like age, size and type of the used explant (Bansal and Gokhale 2012). Gubis *et al.,* (2005) evaluated the effect of different plant growth regulators, plant growth of three tomato cultivars from hypocotyl and cotyledon explants. The author reported that hypocotyl was the most responsive explant, with 92% regeneration and 0.18 - 0.38 shoot production per explant. In connection, Amber *et al.,* (2010) found maximum regeneration and better significant increase in number of shoot primordial of tomato cv. Avinash was obtained with hypocotyl explant than leaf discs derived calli. In addition, shoot apex, nodal and root segments were successfully used for callus induction and regeneration (Jatoi *et al.,* 2001).

Genotypes including species and cultivars play a determinant role in the *in vitro* induction of shoots. For instance, the regeneration capacity of the Brazilian industrial tomato cultivars IPA-5 and IPA-6 were studied using four compositions of culture media. The highest frequency of shoot bud formation was 100% with 'IPA-5' and 65% with 'IPA-6' (Fari *et al.,* 2000). Also, thirteen tomato cultivars and regeneration capacity of six types of explants were compared (Gubis *et al.,* 2003). The total number of shoot primordial produced in all types of explants was higher in 'Hana' and 'Premium' cultivars and lower in 'UC 82' and 'Money marker' cultivars. The present study aims to evaluate the potential of improving shoot regeneration response including the quality of shoots regenerated from tomato hypocotyl and cotyledons from two genotypes using different concentrations of activated charcoal and casein hydrolysate.

Materials and methods

This study was carried out at the Tissue Culture Laboratory of Vegetable Crops Department, Faculty of Agriculture Cairo University, Giza, Egypt, during the period of 2009 and 2010.

Explant material

Two tomato genotypes 'Agayad 7' and 'Jawahar' (Suez Canal for Commercial Development, Giza Egypt) were used in this study. Seeds were surface sterilized by 70% ethanol for one minute then immersed in 20% NaOCl for 15 minutes, thus rinsed three times with sterile water. Five seeds were placed in jars filled with 80 ml of solid MS (Murashige and Skoog, 1962) free hormone medium and incubated in growth chambers at 25^0C, with 16/8-h light /dark photoperiod.

Culture media

Hypocotyl and cotyledon explants were isolated from two weeks old seedlings and cultured in modified MS medium contains MS salts, 3 % (w/v) sucrose, B5 vitamins (Gamborg *et al.,* 1968), 1% (w/v) agar and 3mg/l zeatin. Different concentrations of activated charcoal (0,1,5,10 mg/l) and casein hydrolysate (50,100,200,500,1000 mg/l) were supplemented to MS medium; while pH was adjusted at 5.8 using 1 M NaOH or 0.25 M HCl. Jars were placed in a controlled environment room using completely randomized design, with three replicates per treatment. Every replicate contained 3 jars with three explants. Shoot regeneration and callus formation were observed. Data were collected on number of shoots, fresh weight of shoots, height of shoots and percentage of regeneration.

Statistical Analysis

Data were subjected to analysis of variance, a randomize complete design with three factor was used (factorial system) for analysis all data with three replications for each parameter. The treatment means were compared by least significant difference (L.S.D.) test as given by Snedecor and Cochran (1976) by used Mstat-c program**.**

Acclimatization

Acclimatization was achieved by transferring shoots of 3 mm in length to $^1/_2$ MS medium supplemented with 0.5mg/l NAA. After two weeks, plantlets with well-developed root system were transferred to sterilize vermiculite in plastic cups and irrigated with ¼ MS solution. After acclimatization for three weeks, the plants were fit to grow under green house conditions.

Results and Discussion

Effect of genotype, activated charcoal and casein hydrolysate on cotyledon explant

1. Number of regenerated shoots

Data in Table (1) show that number of regenerated shoots of 'Jawahar' genotype (14.12) was significantly higher than 'Agayad 7' genotype (12.27). Activated charcoal at 1 and 5 mg/l seemed to act as a promoter of organogenesis with 'Jawahar' and 'Agayad 7'. The magnitude of the response to the addition of casein hydrolysate was significantly varied between the two genotypes. According to the effect of interactions, combination of 5 mg/l activated charcoal and 100 mg/l casein hydrolysate resulted the best number of regenerated shoots (14.39) compare to other combinations.

Table 1: Effect of genotype, different concentrations of activated charcoal (AC) and casein hydrolysate (CH) on number of shoots regenerated from cotyledon.

Genotype	CH (mg/l)	AC (mg/l)				Mean
		0	1	5	10	
Agayad 7	0	12	12.3	11.5	11.83	11.91
	50	11.67	11.77	13.03	10.01	11.62
	100	12.77	12.6	14.17	12.17	12.93
	200	12.83	12	12.87	12.17	12.47
	500	13.13	11.5	12.67	11.77	12.27
	1000	12.63	11.97	13.33	11.83	12.44
Mean		**12.51**	**12.02**	**12.93**	**11.63**	**12.27**
Jawahar	0	13.87	16	15.33	14.43	14.91
	50	12.67	14.37	14.77	12.33	13.53
	100	13.9	14	14.6	13.33	13.96
	200	13.67	14.43	13.73	14	13.96
	500	14.00	14.67	13.77	15.53	14.49
	1000	14.67	13.57	13.00	14.33	13.89
Mean		**13.79**	**14.51**	**14.2**	**13.99**	**14.12**
	0	12.94	14.15	13.42	13.13	13.41
	50	12.17	13.07	13.9	11.17	12.58
	100	13.34	13.3	14.39	12.75	13.45
	200	13.25	13.22	13.3	13.09	13.22
	500	13.57	13.09	13.22	13.65	13.38
	1000	13.65	12.77	13.17	13.08	13.17
Mean		**13.15**	**13.27**	**13.57**	**12.81**	

LSD value at 0.05: Genotype (G)= sig.; CH concentration (B)= 0.68; AC concentration (C)= 0.55; G×B= 0.96; G×C = 0.78; B×C= 1.36; G×B×C= 1.92.

2. Fresh weight of regenerated shoots

Date presented in Table (2) reveal that the differences for fresh weight of plantlets were non-significant due to genotype and different activated charcoal concentrations. Shoots fresh weight was increased significantly by the addition of casein hydrolysate. The highest fresh weight of 'Agayad's 7' plantlets (8.27g) was obtained on MS media mixed with 1000 mg/l of casein hydrolysate. Wheanwhile, application of casein hydrolysate at 500 and 1000 mg/l to MS media resulted the highest fresh weight of 'Jawahar' (8.83g and 8.60g, respectively). The interaction between genotypes, activated charcoal and casein hydrolysate showed the highest fresh weight of plantlets (10.30g) with 1mg/l activated charcoal and 500 mg/l of casein hydrolysate in 'Jawahar' genotype.

Table 2: Effect of genotype, different concentrations of activated charcoal (AC) and casein hydrolysate (CH) on the fresh weight (g) of regenerated shoots from cotyledon.

Genotype	CH (mg/l)	AC (mg/l)				Mean
		0	1	5	10	
Agayad 7	**0**	6.87	5.20	6.30	7.23	6.40
	50	5.72	7.10	6.02	5.70	6.14
	100	5.10	6.80	8.17	6.43	6.63
	200	6.57	7.00	8.20	8.57	7.59
	500	5.90	5.23	6.80	7.23	6.29
	1000	7.97	7.57	8.10	9.43	8.27
Mean		**6.36**	**6.48**	**7.27**	**7.43**	**6.88**
Jawahar	**0**	9.17	8.20	6.63	8.90	8.23
	50	9.67	7.90	8.02	7.37	8.24
	100	7.40	8.80	9.17	6.27	7.91
	200	8.40	9.00	8.90	7.50	8.45
	500	9.23	10.30	7.73	8.07	8.83
	1000	8.63	8.57	9.87	7.33	8.60
Mean		**8.75**	**8.80**	**8.39**	**7.57**	**8.38**
	0	8.02	6.70	6.47	8.07	7.32
	50	7.70	7.50	7.02	6.54	7.19
	100	6.25	7.80	8.67	6.35	7.27
	200	7.49	8.00	8.55	8.04	8.02
	500	7.57	7.77	7.27	7.65	7.57
	1000	8.30	8.07	8.99	8.38	8.44
Mean		**7.56**	**7.64**	**7.83**	**7.51**	

LSD value at 0.05: Genotype (G)= ns.; CH concentration (B)= 0.88; AC concentration (C) = ns.; G×B= 2.35; G×C= 2.35; B×C= 1.76; G×B×C= 2.49.

3. Height of the regenerated shoots

As shown in Table (3), non-significant differences between the two genotypes or activated charcoal on shoot height were found. Concerning casein hydrolysate, concentration of 500 mg/l resulted the best shoot height (11.79 cm) than other treatments. For the interaction effect, results showed non-significant differences either between activated charcoal or casein hydrolysate and the genotypes. On the other hand, the longest shoot (17.50 cm) resulted from 1mg/l activated charcoal. The results of three ways interaction showed that the longest (19.00 cm) shoots were produced by culturing the explant of 'Jawahar' on MS medium contain 100 mg/l of casein hydrolysate and without

additives of activated charcoal. However, 'Agayad 7' produced the longest shoots (16.67 cm) when MS media supplemented with 1mg/l activated charcoal alone.

Table 3: Effect of genotype, different concentrations of activated charcoal (AC) and casein hydrolysate (CH) on the height (cm) of regenerated shoots from cotyledon.

Genotype	CH (mg/l)	AC (mg/l)				Mean
		0	1	5	10	
Agayad 7	0	11.00	16.67	8.67	12.67	12.25
	50	11.67	8.17	10.00	7.00	9.21
	100	10.33	11.33	8.00	9.33	9.75
	200	8.33	9.00	8.33	11.67	9.33
	500	7.67	11.67	14.33	14.00	11.92
	1000	8.67	10.00	12.67	14.33	11.42
Mean		**9.61**	**11.14**	**10.33**	**11.50**	**10.65**
Jawahar	0	5.33	18.33	7.67	13.00	11.08
	50	12.67	8.67	10.67	11.33	10.83
	100	19.00	11.00	7.67	8.33	11.50
	200	8.67	8.00	9.33	11.00	9.25
	500	8.67	9.33	14.33	14.33	11.67
	1000	9.33	10.00	13.00	13.00	11.33
Mean		**10.61**	**10.89**	**10.45**	**11.83**	**10.94**
	0	8.17	17.50	8.17	12.84	11.67
	50	12.17	8.42	10.34	9.17	10.03
	100	14.67	11.17	7.84	8.83	10.63
	200	8.50	8.50	8.83	11.34	9.29
	500	8.17	10.50	14.33	14.17	11.79
	1000	9.00	10.00	12.84	13.67	11.38
Mean		**10.11**	**11.02**	**10.39**	**11.67**	

LSD value at 0.05: Genotype (G)= ns.; CH concentration (B)= 2.29; AC concentration (C)= ns.; G×B= ns.; G×C= ns. ; B×C= 4.95; G×B×C= 6.49.

4. Percentage of regenerated shoots

Table (4) shows non-significant differences in organogenic responses between genotypes. Similarly, application of different activated charcoal and casein hydrolysate did not stimulate shoot response. Analysis of variance showed significant differences between activated charcoal and genotypes for percentage of regenerated shoots. The highest percentage of regenerated shoots was reached by mixing MS media with 1mg activated charcoal at /l for 'Jawahar' (93.21%). On contrast, the magnitude of the response to the addition of casein hydrolysate was varied with both genotypes. However, casein hydrolysate at 500 mg/l recorded the best results with 'Jawahar' (91.68%) and 'Agayad 7' (88.89%) compare to other concentrations. Interaction between genotype, activated charcoal and casein hydrolysate did not show significant variations.

Effect of genotype, activated charcoal and casein hydrolysate on hypocotyl explant

1. Number of regenerated shoots

The genotype 'Agayad 7' has produced a higher number of shoots per explant (13.76) if compared to 'Jawahar' (12.60), as shown in Table (5). The differences in number of shoots per explant were significant due to the interaction effect between genotypes and activated charcoal. The highest number of regenerated shoots (14.29) were obtained when 'Jawahar' cultured with 10 mg/l activated charcoal or 50 mg/l casein hydrolysate (14.53). However, 500 mg/l casein hydrolysate significantly increased shoots production and plant regeneration of 'Agayad 7' (14.07) or 'Jawahar' (14.13) than other concentrations. In addition, the highest number of shoots per explant (15.33) were found with 'Jawahar' explants cultured on MS medium contains 50mg/l casein hydrolysate combined with 10mg/l activated charcoal. No significant differences in the response of the genotypes for the number of shoots per explant.

Table 4: Effect of genotype, different concentrations of activated charcoal (AC) and casein hydrolysate (CH) on the percentage of regenerated shoots from cotyledon.

Genotype	CH (mg/l)	AC (mg/l)				Mean
		0	1	5	10	
Agayad 7	**0**	88.90	100.00	77.80	72.23	84.73
	50	77.80	83.33	72.23	77.80	77.79
	100	72.23	77.80	83.33	72.23	76.40
	200	88.90	83.33	66.67	66.70	76.40
	500	83.33	83.33	88.90	100.00	88.89
	1000	72.23	72.23	66.67	88.90	75.01
Mean		**80.57**	**83.34**	**75.93**	**79.64**	**79.87**
Jawahar	**0**	66.67	88.90	100.00	100.00	88.89
	50	88.90	100.00	83.33	88.90	90.28
	100	83.33	100.00	88.90	66.67	84.73
	200	66.67	77.77	88.90	88.90	80.56
	500	77.80	100.00	88.90	100.00	91.68
	1000	77.77	92.60	88.90	75.00	83.57
Mean		**76.86**	**93.21**	**89.82**	**86.58**	**86.62**
	0	77.79	94.45	88.90	86.12	86.82
	50	83.35	91.67	77.78	83.35	84.04
	100	77.78	88.90	86.12	69.45	80.56
	200	77.79	80.55	77.79	77.80	78.48
	500	80.57	91.67	88.90	100.00	90.29
	1000	75.00	82.42	77.79	81.95	79.29
Mean		**78.71**	**88.28**	**82.88**	**83.11**	

LSD value at 0.05: Genotype (G)= ns.; CH concentration (B)= ns.; AC concentrations (C)= ns.; G×B= ns.; G×C= 14.91; B×C= 25.83; G×B×C= ns.

Table 5: Effect of genotype, different concentrations of activated charcoal (AC) and casein hydrolysate (CH) on number of regenerated shoots from hypocotyl.

Genotype	CH (mg/l)	AC (mg/l)				Mean
		0	1	5	10	
Agayad 7	0	12.00	12.47	12.43	12.17	12.27
	50	12.17	12.50	13.90	10.00	12.14
	100	12.53	12.07	13.17	12.83	12.65
	200	13.43	12.20	13.17	12.63	12.86
	500	14.13	12.43	12.80	12.53	12.97
	1000	14.10	12.00	12.50	12.30	12.73
Mean		**13.06**	**12.28**	**13.00**	**12.08**	**12. 60**
Jawahar	0	13.33	13.33	14.00	14.00	13.67
	50	14.93	14.87	13.00	15.33	14.53
	100	14.00	14.03	13.33	13.73	13.77
	200	13.90	13.23	12.67	14.57	13.59
	500	14.00	14.00	12.00	14.13	13.53
	1000	13.43	13.63	12.83	13.97	13.47
Mean		**13.93**	**13.85**	**12.97**	**14.29**	**13.76**
	0	12.67	12.90	13.22	13.09	12.97
	50	13.55	13.69	13.45	12.67	13.34
	100	10.27	13.05	13.25	13.28	12.46
	200	13.67	12.72	13.92	13.60	13.48
	500	14.07	13.22	12.40	13.33	13.26
	1000	13.77	12.82	12.67	13.14	13.10
Mean		**13.00**	**13.07**	**13.15**	**13.19**	

LSD value at 0.05: Genotype (G)= sig.; CH concentration (B)= ns.; AC concentration (C)= ns.; G×B= 1.06; G×C= 0.87; B×C= 1.49; G×B×C= 2.12.

2. Fresh weight of regenerated shoots

No significant differences in the response of the genotypes due to the fresh weight of plantlets were found (Table 6). Also, different casein hydrolysate concentrations had non-significant effect on the fresh weigth of shoots. Activated charcoal improved shoots fresh weight at 5 mg/l (8.68g) followed by reduction in shoot fresh weight at 10 mg/l (7.94g). Increasing concentration of casein hydrolysate in 'Agayad 7' up to 200 mg/l led to significant increase in fresh weight (9.26g) compared to tnon-treated plants (8.98g). Meanwhile, 5 mg/l activated charcoal significantly enhanced 'Jawahar' shoots fresh weigth compare to other concentrations. In addition, the highest fresh shoot weigth (10.20g) was obtained with 'Agayad 7' grown on MS medium contains 1mg/l activated charcoal mixied with 200 mg/l of casein hydrolysate, or 500 mg/l with 'Jawahar' (9.43g).

3. Height of the regenerated shoots

Date in Table (7) show non-significant differences between gynotypes in term of plantlets height. Activated charcoal had non- significant effect on the shoot height while casein hydrolysate affected shoots height significantly compare to control. The genotype 'Agayad 7' reveal significant increase (10.50 cm) with 5 mg/l activated charcoal compare to other concentrations. The longest shoot was produced in 'Jawahar' (12.08 cm) and 'Agayad 7' (11.75 cm) grown on MS media mixed with

casein hydrolysate at 500 mg//l. Concerning the three ways interaction, combination between 500 mg/l casein hydrolysate, and 5 or 10 mg/l activated charcoal produced the longest shoots (13.67 cm).

4. Percentage of regenerated shoots

Significant differences between 'Agayad 7' and 'Jawahar' in term of percentage of regenerated shoots were observed in Table (8). We recorded higher percentage of regenerated shoots of 'Jawahar' (90.86%) compared to 'Agayad 7' (7.21%). Meanwhile, activated charcoal had significant decrease in percentage of regenerated shoots compared to control. Casein hydrolysate had non-significant effect on the shoot percentage. Concerning the interaction between the genotype and activated charcoal concentration, data indicates significant reduction 'Agayad 7' with increasing activated charcoal concentrations. On contrast, 10 mg/l activated charcoal resulted the best shoot percentage of 'Jawahar' (96.30%) than other concentrations. Casein hydrolysate at 50 m/l showed higher shoot percentage (84.03%) with 'Agayad 7'. Combination between 200 mg/l casein hydrolysate and 10 mg/l activated charcoal showed the best percentage of regenerated shoots (98.15%).

Table 6: Effect of genotype, different concentrations of activated charcoal (AC) and casein hydrolysate (CH) on the fresh weigth (g) of regenerated shoots from hypocotyl.

Genotype	CH (mg/l)	AC (mg/l)				Mean
		0	1	5	10	
Agayad 7	**0**	9.93	8.97	9.57	7.43	8.98
	50	6.03	9.23	7.27	9.50	8.01
	100	7.77	9.70	7.70	8.20	8.34
	200	8.83	10.20	8.03	9.97	9.26
	500	7.00	8.23	9.73	6.03	7.75
	1000	8.33	7.43	8.87	6.57	7.80
Mean		**7.98**	**8.96**	**8.53**	**7.95**	**8.36**
Jawahar	**0**	7.00	8.73	9.37	6.70	7.95
	50	8.17	7.23	8.73	9.10	8.31
	100	8.00	9.00	9.20	6.67	8.22
	200	6.80	8.20	7.00	9.63	7.91
	500	8.03	9.43	8.73	9.27	8.87
	1000	8.20	7.80	9.63	6.20	7.96
Mean		**7.70**	**8.40**	**8.78**	**7.93**	**8.20**
	0	8.47	8.85	9.47	7.07	8.47
	50	7.10	8.23	8.00	9.30	8.16
	100	7.89	9.35	8.45	7.44	8.28
	200	7.82	9.20	7.52	9.80	8.59
	500	7.52	8.83	9.23	7.65	8.31
	1000	8.27	7.62	9.25	6.39	7.88
Mean		**7.85**	**8.65**	**8.68**	**7.94**	

LSD value at 0.05: Genotype (G)= ns.; CH concentration (B)= ns.; AC concentration (C)= 0.55; G×B=0.95; G×C= 0.77; B×C= 1.34; G×B×C= 1.89.

Table 7: Effect of genotype, different concentrations of activated charcoal (AC) and casein hydrolysate (CH) on the height (cm) of regenerated shoots from hypocotyl.

Genotype	CH (mg/l)	AC (mg/l)				Mean
		0	1	5	10	
Agayad 7	**0**	10.33	12.00	10.33	8.00	10.17
	50	7.33	7.33	9.00	13.00	9.17
	100	9.00	9.33	7.67	8.33	8.58
	200	10.00	9.67	12.33	10.00	10.50
	500	10.67	9.00	13.67	13.67	11.75
	1000	11.17	9.67	10.00	7.33	9.54
Mean		**9.75**	**9.50**	**10.50**	**10.06**	**9.95**
Jawahar	**0**	6.00	12.00	10.67	11.00	9.92
	50	7.67	8.00	9.00	9.67	8.58
	100	13.00	11.67	8.00	8.00	10.17
	200	9.00	10.33	12.33	9.67	10.33
	500	10.67	10.33	13.67	13.67	12.08
	1000	11.67	12.00	11.00	13.33	12.00
Mean		**9.67**	**10.72**	**10.78**	**10.89**	**10.51**
	0	8.17	12.00	10.50	9.50	10.04
	50	7.50	7.67	9.00	11.34	8.88
	100	11.00	10.50	7.84	8.17	9.38
	200	9.50	10.00	12.33	9.84	10.42
	500	10.67	9.67	13.67	13.67	11.92
	1000	11.42	10.84	10.50	10.33	10.77
Mean		**9.71**	**10.11**	**10.64**	**10.47**	

LSD value at 0.05: Genotype (G)= ns.; CH concentration (B)= 1.45; AC concentration (C)= ns.; G×B= 2.05; G×C= ns.; B×C= 2.89; G×B×C= 4.10.

Our work shows that cotyledon and hypocotyl of 'Jawahar' genotype significantly affected plantlets regeneration than 'Agayad 7', while 1 and 5 mg/l activated charcoal increased shoot regeneration of cotyledon plantlets than hypocotyl. On contrast, plantlets regeneration presented differ response with casein hydrolysate. In this respect, it is important to mention that application of activated charcoal to tissue culture media adsorbs toxic and phenolic tissue exudates in culture, which prevents inhibition of growth, browning and promotes embryogenesis (Van Winkle and Pullman, 1998 and Tomas, 2008). Moreover, casein hydrolysate is a source of calcium, phosphate, several microelements, vitamins and mixture of amino acids (Narayanswamy, 2007). In connection, Bhatia and Ashwath (2008) studied effects of activated charcoal (0, 1, 5 and 10 mg/l), ascorbic acid (30, 60, 120, 240 and 480 µM) and casein hydrolysate (0, 50, 100, 200, 500 and 1000 mg L^{-1}) for morphogenesis of *Lycopersicon esculentum* cv. Red Coat cotyledons. The media contained higher concentrations of activated charcoal and ascorbic acid found to produce longer shoots.

Iapichino (2003) found that single node cuttings in the tuberization medium supplemented with 8g/l activated charcoal and 80g/l sucrose gave a tuberization percentage of 94% and the highest weight of microtuber potato cv. Arinda microtuber. Meanwhile, Yan *et al.* (2006) reported that higher rooting and survival ratio of *Cypripedium falvum* were obtained when growing medium was supplemented with 0.6 -1 g/l activated charcoal. In connection, Ozkum and Tprdamaz (2007) reveal that application 2.5g/l of activated charcoal to the culture medium on pepper (*Capsicum annuum*) presented the highest numbers of embryos (12.5 embryos/100 anthers) with MS medium containing 4 mg NAA/l+1 mg/l BA. Benamar *et al.,* 2012 found that adding 2g/l of activated charcoal to culture

media improved *in vitro* embryos germination and growth of *Pistacia vera* seedlings. The improvement percentage of growth was 44.4% for aerial parts and 67.4% for roots. In the current study a reduction in shoot response was observed at high concentrations of activated charcoal or casein hydrolysate either with cotyledon or hypocotyl explants. These reductions might be due to the adsorption of growth inhibitory substances along with the plant growth regulators by activated charcoal. Casein hydrolysate did not significantly affect organogenesis for used explant with some exceptions. This might be attributed to the low concentrations of casein hydrolysate used in this experiment. In this respect, Malabadi and Staden van (2006) have been successfully used casein hydrolysate in tissue culture of *Pinus patula* and concluded that addition of casein hydrolysate significantly reduced callus response. One contrast, addition of casein hydrolysate (100mg/l) to growing culture increased the shoot number of *Elaeocarpus sphaericus* (Sakalani *et al.,* 2015). While, 500mg/l casein hydrolysate promoted shoot multiplication rate significantly, eliminated the basal callus formation problem and necrosis faced during the later stage of *Quercus serrata* Thunb shoot proliferation (Pandeya and Tamta 2014).

Table 8: Effect of genotype, different concentrations of activated charcoal (AC) and casein hydrolysate (CH) on the percentage of regenerated shoots from hypocotyl.

Genotype	CH (mg/l)	AC (mg/l)				Mean
		0	1	5	10	
Agayad 7	**0**	88.90	66.67	61.10	72.23	72.23
	50	88.90	75.00	83.33	88.90	84.03
	100	88.90	72.23	72.23	72.23	76.40
	200	72.23	88.90	72.23	96.30	82.42
	500	83.33	83.33	66.67	66.67	75.00
	1000	83.33	72.23	77.77	83.33	79.17
Mean		**84.27**	**76.39**	**72.22**	**79.94**	**78.21**
Jawahar	**0**	100.00	100.00	88.90	100.00	97.23
	50	88.90	100.00	72.23	100.00	90.28
	100	100.00	88.90	77.77	100.00	91.67
	200	100.00	83.33	75.00	100.00	89.58
	500	83.33	88.90	88.90	77.77	84.73
	1000	83.33	100.00	83.33	100.00	91.67
Mean		**92.59**	**93.52**	**81.02**	**96.30**	**90.86**
	0	94.45	83.34	75.00	86.12	84.73
	50	88.90	87.50	77.78	94.45	87.16
	100	94.45	80.57	75.00	86.12	84.04
	200	86.12	86.12	73.62	98.15	86.00
	500	83.33	86.12	77.79	72.22	79.87
	1000	83.33	86.12	80.55	91.67	85.42
Mean		**88.43**	**84.96**	**76.62**	**88.12**	

LSD value at 0.05: Genotype (G)= sig.; CH concentration (B)= ns.; AC concentration (C)= 10.72; GxB= 18.56; G×C= 15.16; B×C=ns.; G×B×C= 37.13.

Conclusion

This study explore that addition of activated charcoal to fresh media is beneficial for development of shoots while casein hydrolysate did not significantly affect organogenesis for hypocotyl explant. However, the efficiency of the organogenesis depended on the interaction among genotype, activated charcoal and casein hydrolysate concentrations.

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