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Callus culture, somatic embryogenesis and direct bulblet formation *of Allium rotundum L*

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Key words: *Allium*, direct bulblet formation, somatic embryogenesis, callus.

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Abstract

Wild leek *(Allium rotandum L.)* is a perennial plant of *Allium* genuse and in the family of *Alliaceae* known to be a rare medicinal plant. The current study used Ilam and Kordestan genotypes, MS basic medium with the different auxin growth regulators (NAA, 2, 4-D) and cytokinin (BAP, 2ip, Kin) Meanwhile, the attributes of direct bulblet formation, callus induction, and somatic embryogenesis were inspected. The results of inspecting the analyzed attributes showed that Kordestan genotype started to induction callus in a shorter time in comparison with that of Ilam's, furthermore, it has more callus in an environment containing 1 milligram per liter 2,4-D and 0.5 milligram per liter Kin. In this test, the attribute of direct bulblet formation was also scrutinized. The results of experiment indicate that the most number and percentage of direct bulblet formation were found in Kordestan genotype in the culture medium C (0.24 milligrm per 2, 4-D liter, and 0.5 milligram per 2ip liter).Embryogenesis was not observed in both culture mediums C (0.24 milligram per liter and 0.5 milligram in BAP liter) and B (6 milligrams in 2,4-d liter and 3 milligrams in BAP liter). The highest percentage of somatic embryogenesis was in the use of 2,4-D with twice more cytokinin.

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Introduction

Under the present cultivation of medicinal plants as an important branch of the farmers is presented for the extraction and production of raw materials that are used in making Medications available, occurs. *Allium* is wild medicinal plant is commonly known as wild leek (Mehrabi *et al*, 2012). And comprises 450 species widely distributed in the northern hemisphere (lonzotti, 2006). This wild medicinal plant is commonly known as wild leek. The genus is mainly restricted to regions that are seasonally dry, with center of divercity in south west/ central asia especially Zagros Mountains in IRAN (Mehrabi *et al*, 2012). These especies are characterized by a specific flavor and are used for cooking (teda, 1988). *Allium* has traditional dietary and medicinal application as anti-infection agent (lawson 1991- Lawson 1998) in vitro evidence of the antimicrobial activity of many bacterial (Cavallito and Billy, 1944. Rees *et al*, 1993. Ross *et al*, 2001; Onyeagba *et al*, 2004; Alves De Moura *et al*, 2005; Shalabyte *et al*, 2006), fungi (Adetumbi *et al*, 1986) and viruses (Weberb*et al*.1992) this plant is available. *Allicin* has the wide range of biological and pharmacological activities, such as anticoagulation, antihypertensive, antimicrobial, antibiotic, antiparasitic, antimycotic, antiviral, antitumoral, anti-oxidant, anti-aging, antiplatelet, detoxifies heavy metals and immune enhancer (Amagase, 2006; Iciek *et al*., 2009; Jacob, 2006; Munchberg *et al*, 2007). The propagation rate of garlic in the field is approximately five to ten percent per year; therefore it takes many years to produce sufficient number of seed bulbs for practical cultivation of new variety (Nagakubo T, Nagasawa A, Ohkawa H, 1993). The propagation of garlic is by division of the individual cloves of its bulbs. Because garlic almost never produces fertile seeds, it must be propagated vegetatively. A variety of studies have reported *in vitro* somatic embryogenesis, plant regeneration and micropropagation of *A. sativum* from the culture of several explants for multiple propagations (Bockish *et al*, 1997; Novak, 1990). A protocol for somatic embryogenesis requires the induction callus, and embryogenic regeneration from callus has been demonstrated only for a limited number of genotypes (Phillips and Luteyn 1983). The purpose of this study, effect of type and concentration of the regulator growth and achieving favorable medium for callus induction, Direct induction of Bulblet somatic embryogenesis is a wild leek plant explants. Plant regeneration through somatic embryogenesis is a rare phenomenon, it has several advantages over organogenesis and appears to be most promising for future large scale, rapid plant propagation; though it is a rare phenomenon (Ignacimuthu S (1995). Ignacimuthu S (1996).

Material and methods

The bulblets (*Allium rotundum L*) collected from native habitate of Illam and Kordestan used in this research. The bulblets initially rinsed by tap water 5 minutes and in order to surface sterilization used 70% alcohol and 1% hypochlorite sodium (with shaking) for 40 seconds and 15 minutes, respectively. Finally materials plants rinsed three times with sterile distilled water each 5 minutes. In this study for callus induction, evaluation direct bulblet formation and somatic embryogenesis, explants prepared from disinfected plants and cultured in sterilized petri dish with base culture medium of MS (Murashige & Skoog, 1962), including 3% sucrose, 0.8% Agar, and different growth regulators. Hormonal compounds was used in this experiment included: 6mg/L NAA, and 3 mg/L BAP (A medium), 6 mg/L 2,4-D, 3 mg/L BAP (B medium), 0.24 mg/ L 2,4-D and 0.5 mg/L 2ip (C medium) and 1mg/L 2,4-D and 0.5 mg/L Kin (D medium). Finally Petri dishes contain explants kept in dark condition for two weeks and then transferred to condition for callus induction with photoperiod of 8/16 (Night/ Day) at 25±1 c^{o}.

Statistical analysis

The results were achieved using analysis of variance and mean comparison of experimental treatments by SAS software (Ver.9.1) and Exel.

Result and discussion

Direct Bulblet formation

The response of genotypes in four media include different components of growth regulation, were

different. In all culture medium except B (3mg/L BAP and 6 mg/L 2, 4,-D), direct Bulblet formation of both genotypes occurred (Table 1-Fig 3) which is probably caused use high level of 2,4,-D (Table 1). In other experiment by (mehrabi *et al*, 2012) the percent of direct bulblet formation was equal 0% when used 2,4-D. The result of this study show that the percent of direct bulblet production of Illam genotype in A, C and D media was same and different have not seen, whereas the highest percent of bulblet formation have seen in C medium (Table 1-Fig 1). (Ishioka *et al*, 1993) showed that the highest percent of bulblet formation (7.4%) obtained from leaf explants of *A. longiflorum* with 10 mg/L BAP and 10 mg/L NAA. (Azadi *et al*, 2007), reported 5.41% bulblet formation from bulblet explants of *A. ledebourii* in medium containing 0.1 mg/L BA and 0.1 mg/L NAA. In the other hand (Niimi, 1985) showed that the addition BA and NAA to medium culture not affected bulblet formation in *L. rubellum*. The result obtained (Mehrabi *et al*, 2012) showed that NAA and BAP influence on bulblet formation in *A. scorodoprasum rotundum L.* Furthermore the results of this study show that the duration of bulblet formation in Illam genotype in a medium was lower. In the other word this genotype in a medium culture has shown earlier direct bulblet production, but both genotypes in C media culture later other treatment components bulblet production occured (Table 1- Fig 2).

Table 1. Effects of growth regulator combinations on bulblet formation in different genotype of *A.rotundom*.

		Traits evaluated	
Genotype	Medium	Bulblet formation	Percent bulblet
	A	$47.00^{ab} \pm 1.15$	$33.33^{b} \pm 0.00$
Kurdestan	B	———	———
	C	$56.50^{a} \pm 4.90$	$66.66^{a} \pm 23.75$
	D	$38.50^{bc} \pm 3.75$	$33.33^{b} \pm 0.00$
	A	$21.00^{d} \pm 9.23$	$33.33^{b} \pm 0.00$
Ilam	B	———	———
	C	$54.00^{ab} \pm 0.66$	$33.33^{b} \pm 0.00$
	D	$24.00^{cd} \pm 1.13$	$33.33^{b} \pm 0.00$

At least one common letter in each column means no significant difference at 5% level of is (Duncan's multiple range tests).

Table 2. Effects of growth regulator combinations on callus induction in different genotype of *A.rotundom*.

		Traits evaluated		
Genotype	Medium	Time to callus induction	Callus volume	Callus percent
	A	$32.6^{a} \pm 1.38$	$17.20^{abc} \pm 1.62$	$53.32^{ab} \pm 7.69$
Kurdestan	B	$38.50^{ab} \pm 0.95$	$21.50^{ab} \pm 1.49$	$50.00^{ab} \pm 5.55$
	C	$39.50^{ab} \pm 0.86$	$9.25^{c} \pm 0.94$	$41.66^{b} \pm 4.81$
	D	$13.75^{e} \pm 0.43$	$27.25^{a} \pm 2.60$	$58.33^{ab} \pm 4.80$
	A	$35.75^{bc} \pm 1.08$	$18.50^{abc} \pm 1.40$	$41.66^{b} \pm 4.81$
Ilam	B	$32.33^{cd} \pm 0.96$	$15.66^{bc} \pm 2.26$	$44.44^{b} \pm 6.41$
	C	$29.25^{d} \pm 1.13$	$17.75^{abc} \pm 1.32$	$41.66^{b} \pm 4.81$
	D	$17.75^{e} \pm 0.82$	$25.75^{ab} \pm 3.18$	$83.33^{a} \pm 9.62$

Callus production

with respect the results of this study deduced which callus formation in genotype of Kordestan earlier Illam has been happened (Table 2- Fig 4). The highest percent of callus induction had seen in Illam genotype in D medium (Table 2- Fig 5). Also consider that the highest callus volume is in D medium and Krdestan genotype (Table 2- Fig 6). The result of (Mehrabi *et al*, 2012) show that highest percent of callus in medium culture of MS as well as growth regulator (3 mg/L 6-BAP and 1 mg/L 2,4-D) has been occur. (Kamstaity *et al*, 2004) indicated that BAP have highest effect on callus. In according this finding, the increase of BAP concentration from 0.9 to 4.4 has

highest effect on callus formation. With respect of this reports, observed that ouxins: 2, 4-D, NAA and Cytokinin: BAP affect callus of *Alliacea* family. (Gamborg *et al*, 1968) medium containing various combinations of BAP, 2,4-D, NAA and 2ip for callus induction. In other experiment by (Mehrabi *et al*, 2012), the higest callus induction percent of 80% was observed with a combination of 4mg/l NAA+ 6mg/l BAP. Over all for characters callus production percent, duration to callus induction and callus volume, D medium culture is (1 mg/L 2,4-D and 0.5 mg/L Kin) the best medium of point view callus induction and using this culture medium contain this concentration of growth regulations could produce many callus in short time.

Table3. Genotypes reaction to different media.

		Traits evaluated		
Genotype	Medium	Callus induction	Bulblet formation	Somatic embryogenesis
	A	+	+	+
	B	+	-	-
Kurdestan	C	+	+	-
	D	+	+	+
	A	+	+	+
Ilam	B	+	-	-
	C	+	+	-
	D	+	+	+

Not all traits evaluated + View traits evaluated.

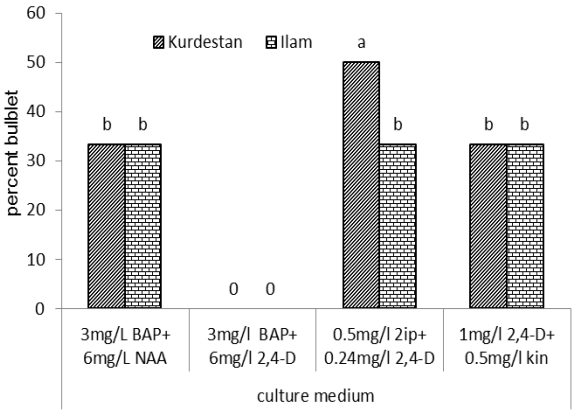

Fig. 1. Effect of different components of growth regulators on induction percent bulblet of different genotypes.

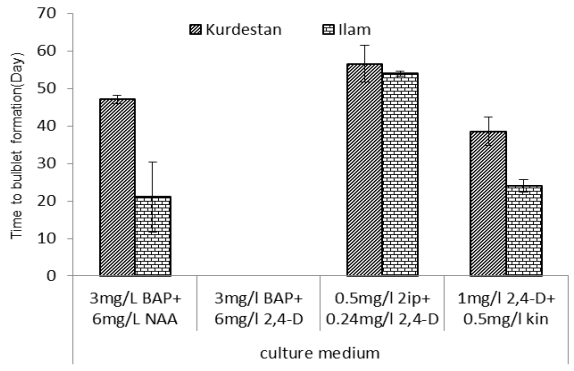

Fig. 2. Effect of different components of growth regulators on time to induction direct bulblet of different genotypes.

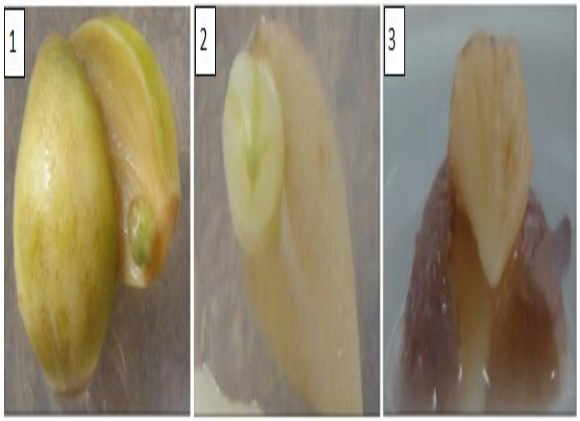

Fig. 3. Effect of culture media with different type and concentrations of growth regulators on bulblet direct induction.

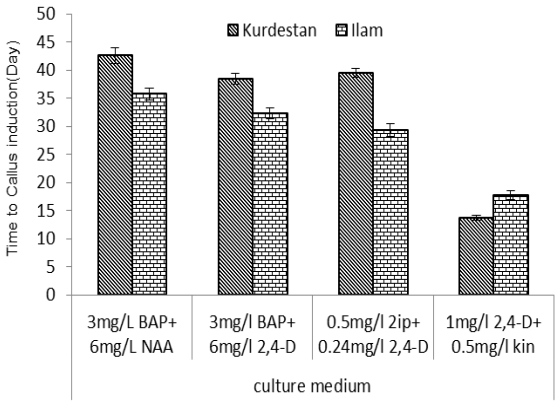

Fig. 4. Effect of different media with different

concentrations of growth regulators on time to induction callus of different genotypes.

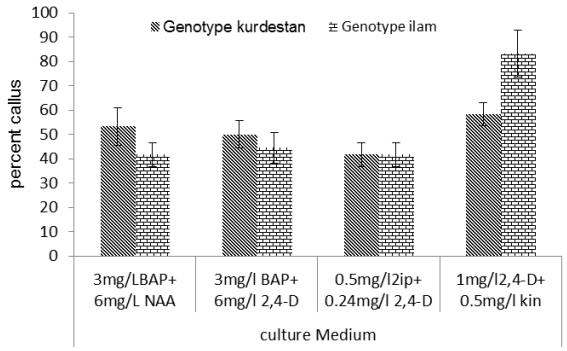

Fig. 5. Effect of different media with different concentrations of growth regulators on percent callus induction of different genotypes.

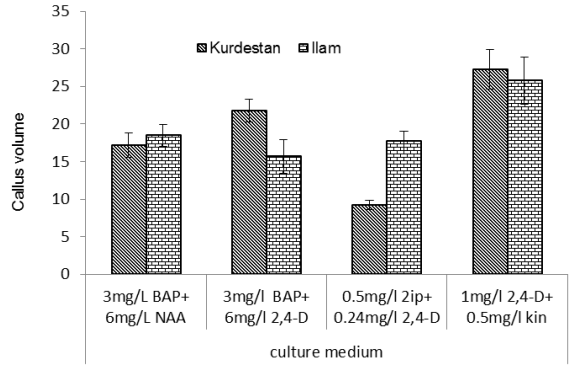

Fig. 6. Effect of different media with different concentrations of growth regulators on callus volume of different genotypes.

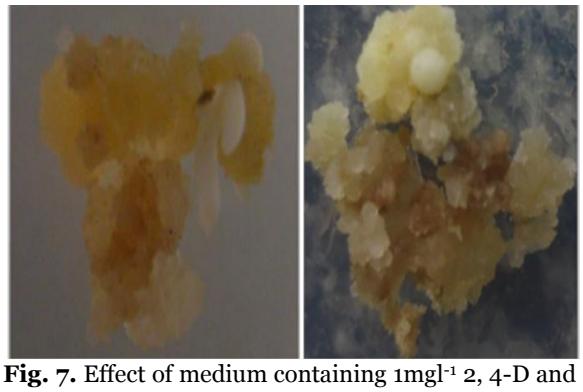

Fig. 7. Effect of medium containing 1mgl^{-1} 2, 4-D and 0.5mgl^{-1} Kin.

Somatic embryogenesis

the result of study showed that in B and C medium have not been seen somatic embryogenesis. In A and D medium that ratio auxin/ccitokinin was two-fold, somatic embryogenesis observed and highest percent was in D medium (1mg/L 2,4-D and 0.5 mg/L Kin) that probably caused use 2,4-D (Table 3- Fig 7). (S.J. Sata *et al*, 2000), reportet somatic embryogenesis formation from basal part of garlic in medium containing 2,4-D(0.5-1 mg/l) and kin(0.5-1 mg/l) both were present in the basal medium. The frequency of somatic embryogenesis was highest (60%) i.e. 45–48 tubes out of 75 induced 20 or more somatic embryos per explants with 2,4-D and kinetin at 1.0 mg/L and 0.5 mg/L,respectively, the results of our testing has been inconsistent.

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