



In vitro propagation of Tunisian local garlic (*Allium sativum* L.) from shoot-tip culture

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ABSTRACT

Purpose: Tunisian garlic is widely threatened by the attack of several viruses genus. For this purpose, a reliable protocol was established for rapid *in vitro* propagation of local garlic (*Allium sativum* L.) for large-scale production of virus-free plants and high quality bulblets. **Research method:** Well disinfected shoot-tips of 1 mm were used as explants and cultivated on MS basal solid media enriched with various growth regulators: 6-Benzylaminopurine, α -Naphthalene-acetic acid, Kinetin, Indole-3-butyric acid and 2-isopentenyladenine for assessment of shoot formation, shoot proliferation and bulb formation. **Findings:** Among the different phytohormone concentrations and combinations, MS basal medium without any growth regulators (M₀) was found optimal for shoot-tip initiation (96% explants development) and plantlets elongation (56.26 mm). For shoot proliferation, the M₁ culture medium containing 1 mg L⁻¹ BAP and 0.25 mg L⁻¹ NAA was the best, giving a multiplication rate of 1.7 plantlets/explant. Shoots on M₀ culture medium formed bulblets earlier. Multiple bulblets per explants were obtained on medium M₂₂ containing 2 mg L⁻¹ Kin and 0.1 mg L⁻¹ NAA. Separated bulblets were transferred individually on bulbification media. Non-dividable bulblet was developed in various sizes. **Research limitations:** Bulblet acclimatization step needs to be well studied for high quality cloves production. **Originality/value:** This efficient optimized *in vitro* protocol will be successfully applied for large multiplication of virus-free garlic cultivars.

INTRODUCTION

Garlic (*Allium sativum* L.) is an ancient crop that was originally domesticated in Central Asia (Vieira et al., 2015). It is one of the most important and widely cultivated *Allium* crops worldwide, which is utilized for food and medicinal purposes (Hammami & El May, 2012; Khoshtinat et al., 2017). Garlic is mainly propagated by vegetative methods and its improvement through breeding programs is limited due to difficulties in flower induction (Haider et al., 2015). As garlic bulbs cannot be stored for more than 6-8 months, maintenance is usually done by planting them in the field every year. However, infection with different pathogens and pests makes the maintenance of the field a costly affair (Mishra et al., 2014). Prolonged conservation in the field leads to decreased yields and sometimes, to their total destruction by the accumulation of viruses in the bulbs (Asha Devi et al., 2007). Systemic viral infection of garlic plants causes significant losses in crop production around the world (McDonald et al., 2004; Perotto et al., 2010). Furthermore, vegetative propagation facilitates the transmission of several virus species throughout the plant life cycles (Parrano et al., 2012). Due to the difficulties in flower induction in this species, breeding programs have been limited to clonal selection and production of virus-free materials via meristem culture (Mahajan, 2016). In vitro techniques have been used in the conservation of endangered plants in recent years (Sarasan et al., 2006). Shoot-tip culture has been used for decades for virus removal in vegetatively propagated plants (Ghaemizadeh et al., 2014) and from garlic vitroplants (Pramesh & Baranwal, 2015).

Virus-free clones produced through meristem culture showed higher yield with improved quality (Taskin et al., 2013). Therefore, the tissue culture techniques have got the high potential for the improvement of garlic in respect of yield and quality (Mukhopadhyay et al., 2005). Also, it can be employed for its propagation and genetic improvement research (Haider et al., 2015). The use of meristems or shoot-tips as explant for micropropagation of multiple bulblet formation is more suitable than another source of explants (Roksana et al., 2002).

In Tunisia, farmers are using garlic cloves from a previous crop as seeds in a household small-scale production system (Jabbes et al., 2011), potentially leading to a high virus infection rate in the crops; which lead to an extensive reduction in productivity and quality. Shoot-tip culture is a perfect and benefit tool for producing virus-free garlic. However, no research work related to in vitro regeneration of Tunisian garlic was reported. Recently, programs to produce certified virus-free materials of popular cultivars are being carried out in Tunisia in collaboration with the National Gene Bank. Therefore, the main objective of this study was to establish and to optimize a reliable protocol for in vitro regeneration of virus-free Tunisian garlic from shoot-tip culture for large scale production and yield increase of good quality bulbs. With this protocol, steps of shoot-tip initiation, shoot multiplication, and bulblet formation were studied.

MATERIALS AND METHODS

The study was carried out at the Biotechnology Laboratory of the Regional Research Centre on Horticulture and Organic Agriculture in Chott-Mariem, Sousse. Tunisian local garlic, provided by the National Gene Bank, was used as plant material. In fact, local garlic bulbs were stored for two weeks at 4 °C to stimulate the sprouting process, thereafter they were separated into single cloves (Fig. 1) and then washed with running tap water for 10 min. The outer, dry, papery bulb scales of the cloves were removed. Healthy cloves were surface-sterilized by placing them in 70% ethanol for 5 min, then immediately disinfected by 10%

sodium hypochlorite solution (NaOCl), containing two drops of Tween 20 per 100 ml, for 30 min with frequent agitation. The cloves were rinsed three times, for 5 min, with sterile distilled water.

Shoot-tips, about 1 mm in size, were excised from the aseptic cloves under a laminar air-flow hood, with the help of a microscope. The explants, consisted of the shoot meristem and one or two leaf primordia, were rapidly cultured on the appropriate initiation medium.

Shoot regeneration and development

Shoot-tip explants were cultured on MS basal medium (Murashige and Skoog, 1962) with 13 different combinations of plant growth regulators, including BAP, NAA, IBA and Kin (Table 1). 30 g L⁻¹ sucrose and 7 g L⁻¹ Agar (Bio Basic Inc.) were added to these media. The explant development on the media was measured by the percentage of shoot-tip development, shoot length and callus formation.

Shoot proliferation

The regenerated plantlets from shoot-tips were transferred to a proliferation medium. In this phase, the effects of BAP and 2ip were examined. The NAA concentration was fixed at 0.25 mg L⁻¹ while the concentrations of BAP and 2ip were 1 and 2 mg L⁻¹. The number of shoots per explant was counted. The media used for the multiplication step were: M₀: MS medium without growth regulator

M₁: MS + 1 mg L⁻¹ BAP + 0.25 mg L⁻¹ NAA

M₃: MS + 2 mg L⁻¹ BAP + 0.25 mg L⁻¹ NAA

M₁₅: MS + 1 mg L⁻¹ 2ip + 0.25 mg L⁻¹ NAA

M₁₆: MS + 2 mg L⁻¹ 2ip + 0.25 mg L⁻¹ NAA

Bulb formation

Vigorous emerged plantlets from proliferated shoots were used to induce *in vitro* bulb formation. The diameter of formed bulblets was measured two, four and six weeks after plantlets planting on the bulbification media using a caliper. Media used for bulbification step were:

M₀: MS medium without growth regulator

M₂₀: MS + 0.5 mg L⁻¹ BAP + 0.1 mg L⁻¹ NAA

M₂₂: MS + 2 mg L⁻¹ kin + 0.1 mg L⁻¹ NAA



Fig. 1. Local garlic (*Allium sativum*) bulbs and cloves, the source of explants

Table 1. Different media used for initiation of garlic meristem

Medium code	BAP (mg L ⁻¹)	NAA (mg L ⁻¹)	Kin (mg L ⁻¹)	IBA (mg L ⁻¹)
M0	0	0	0	0
M1	1	0.25	0	0
M2	1.5	0.25	0	0
M3	2	0.25	0	0
M4	0	0.25	1	0
M5	0	0.25	2	0
M6	0	0.25	3	0
M7	1.5	0	0	0
M8	1.5	0.1	0	0
M9	1.5	0.5	0	0
M10	1.5	0	0	0.1
M11	1.5	0	0	0.25
M12	1.5	0	0	0.5

MS: Murashige Skoog medium, BAP: 6-Benzylaminopurine, NAA: α -Naphthalene acetic acid, kin: Kinetin, IBA: Indole-3-butyric acid.

Media preparation and culture condition

The pH of all media was adjusted to 5.8 prior to autoclaving for 20 min at 121 °C and 1.1 kg cm⁻². Cultures were maintained in a growth chamber at 25±1 °C under 16/8 hours photoperiod with a light intensity of 50 μ mol m⁻² s⁻¹ provided by cool white fluorescent lamps.

Data Analysis

All experiments were carried out in a completely randomized design with three replications. The analysis of variance (ANOVA) and the mean comparisons ($P \leq 0.01$), Tukey's range test, was carried out using SPSS (SPSS Inc., ver. 20.0).

RESULTS AND DISCUSSION

Shoot-tip development and plantlet regeneration

Shoot buds started to develop on the MS medium within two weeks from shoot-tip culture (Fig. 2). Results indicated that high rates of good quality shoots were easily regenerated from shoot-tips of garlic. Regeneration occurred on all the tested media, indicating that a high frequency of shoot regeneration can be obtained from shoot-tip culture of Tunisian garlic. The effects of growth regulators were monitored by scoring the percentage of regenerated shoots and observing the morphological differences among the regenerants.

Tukey's multiple range tests showed that media effects on shoot-tip evolution were significantly different in all evaluated parameters and the percentage of shoot regeneration varied from 53 to 100% (Table 2). The highest regeneration rates were recorded on media M8 (100%), M9 (96%), M0 (96%) and M6 (94%). Whereas, the lowest regeneration rate was obtained with medium M5 containing 2 mg L⁻¹ kin and 0.25 mg L⁻¹ NAA. Generally, among all media tested, the growth regulator-free medium, M0, was distinctly the better. All combinations of growth regulator were inferior (or same) to the growth regulator-free medium M0, which offered the highest shoot regeneration rate of 96% and the highest shoot length (5.6 cm in 4 weeks). Similar results were also observed by Haque et al. (2003) for the use of MS free-hormone medium for garlic regeneration. In contrast, Luciani et al. (2006) and Mehta et al. (2013) reported the non-efficacy of the free-hormones medium in high garlic shoot regeneration from shoot-tips. In fact, they indicated that shoot-tips have great potential to induce multiple shoots when cultured on MS media containing very low concentrations of NAA, IBA, IAA and 2,4-D and higher concentrations of BAP and Kin; shoot initiation started on all treatments but BAP and Kin showed the best responses, after 3 weeks.



Fig. 2. Garlic (*Allium sativum*) shoot-tip initiation on medium M1, (Bar = 2 mm)

Table 2. Effect of various establishment media on the shoot-tip development of Tunisian local garlic

Medium	Callus rate (%)	Regeneration rate (%)	Shoot length (mm)
M0	4.0 ± 4.00 † ^e	96.0 ± 4.30 ^{ab}	56.26 ± 4.50 ^a
M1	50 ± 13.00 ^{abc}	56.0 ± 12.80 ^{bc}	24.77 ± 5.66 ^{bcd}
M2	36.0 ± 15.20 ^{bcd}	64.0 ± 15.20 ^{abc}	12.73 ± 2.80 ^{cd}
M3	80.0 ± 10.70 ^a	60.0 ± 13.10 ^{abc}	11.9 ± 2.06 ^{cd}
M4	23.0 ± 12.20 ^{bcd}	85.0 ± 10.40 ^{abc}	30.04 ± 5.48 ^{bc}
M5	47.0 ± 12.50 ^{abcd}	53.0 ± 12.50 ^e	15.48 ± 5.84 ^{bcd}
M6	6.0 ± 5.90 ^e	94.0 ± 6.00 ^{ab}	21.16 ± 4.11 ^{bcd}
M7	10.0 ± 5.60 ^{cde}	90.0 ± 6.00 ^{abc}	32.14 ± 4.22 ^b
M8	86.0 ± 9.71 ^a	100 ± 0.0 ^a	10.77 ± 1.58 ^d
M9	54.0 ± 100 ^{ab}	96.0 ± 4.20 ^{ab}	16.3 ± 2.19 ^{bcd}
M10	9.0 ± 6.30 ^{de}	82.0 ± 8.40 ^{abc}	14.57 ± 1.66 ^{bcd}
M11	0.0 ± 0.00 ^e	87.0 ± 7.20 ^{abc}	14.61 ± 1.46 ^{bcd}
M12	4.0 ± 4.00 ^e	87.0 ± 7.00 ^{abc}	12.25 ± 1.42 ^{cd}
	**	**	**

** Highly significant differences (Tukey, $P \leq 0.01$); †Means with a different letter in a column are statistically different.

On the other side, the callus rates were significantly high on M3 (80%) and M8 (86%) and low on M0 and M12 (4%). No callus formation in all explants was observed with M11. Also, no shoots were regenerated from the calli on the various media tested.

Shoot cultures and plantlets were obtained directly from excised shoot apices, with no necrosis and without any callus formation, which is very beneficial for regeneration of shoots and genetic stability of vitroplantlets (Gimenez et al., 2016). These results were similar to that found by Haque et al. (2003) and Bekheet (2006) who reported regeneration of garlic shoots with no callus and necrosis. Given that a large callus formation can induce somaclonal variations in the regenerants. Thus, Haque et al. (2003) reported that a method of regeneration involving no callus formation phase is preferred for the production of clones of true-to-type. In the same context, Asha Devi et al. (2007) indicated the advantage of using 2ip is mainly that the induced shoots were healthy and with no callus at the base of explants. On the other hand, M0 is selected to be the best medium for the initiation phase (96% regeneration rate and only 4% callus formation), better than M8 (100% regeneration rate, 86% callus formation) and M9 (96% regeneration rate, 54% callus formation). As it was reported by Ikeuchi et al. (2013) and Hassan et al. (2014), this result is evident since no exogenous hormones were added to the medium to induce abundant callus formation.

Otherwise, it was noticed that initiation media containing 1.5 mg L⁻¹ BAP improves shoot-tip development and leads to high shoot regeneration rates (Table 2), and the best results were obtained on media with NAA. In fact, the inclusion of NAA at a concentration of

0.1 mg L⁻¹ (M8) or 0.5 mg L⁻¹ (M9) increased shoot regeneration percentages and gave comparable results to those recorded on growth regulator-free medium. Whereas, lower plantlet regeneration rates and shoot length (12.25 to 14.61 mm) were observed by the addition of IBA (media M10, M11, and M12) (Table 2). The combination of 1.5 mg L⁻¹ BAP and 0.5 mg L⁻¹ NAA showed 96% of local garlic regeneration in two weeks but small shoot elongation of 1.63 cm in 4 weeks. However, Roksana et al. (2002) proved similar shoot initiation of garlic with the same combination, in liquid media, in six days and up to 7.2 cm length after 21 days only.

Media composition has a significant effect on the shootlets length. The highest plantlets (56.26 mm) were obtained on hormone-free M0 medium. Also, long plantlets (32.14 mm) were induced on medium M7, without auxins. The shortest vitroplantlets were regenerated on M8 medium with only 0.1 mg L⁻¹ NAA.

Although there were highly significant differences between media effects on shoot-tip evolution and regeneration, there was no clear and stable relationship between the increase in growth regulators and the regeneration rates of Tunisian garlic. This seems contrary to the results of Haque et al. (2003) which found a gradual decrease in the regeneration frequency with increasing growth regulator concentrations. In the same way, Gull et al. (2014) and Izquierdo-Oviedo et al. (2016) indicated that kinetin was found as an ideal phytohormone for shoot formation in garlic. It was also noted that by increasing the concentration of kinetin to 1 mg L⁻¹ or 1.5 mg L⁻¹ in MS media, a significant increase in shoot length was observed, but further increase in the concentrations showed a decline in the shoot length.

In this study, the M6 medium containing 3 mg L⁻¹ Kin and 0.25 mg L⁻¹ NAA showed a high regeneration rate of 94% after two weeks. A similar regeneration rate was also achieved by Gull et al. (2014) on MS medium supplemented with 1.5 mg L⁻¹ Kin. They also indicated that BAP was a potent phytohormone for shoot induction in garlic but the combination of BAP and Kin was not as much suitable. The efficiency of Kin in the culture medium, for garlic explant development, was mentioned by Mehta et al. (2013) and Manjunathgowda et al. (2017) who found that MS medium containing 1 mg L⁻¹ Kin was the best for in vitro regeneration of *Allium sativum*.

Shoot proliferation

To multiply garlic vitroplantlets (Fig. 3), five media with different compositions of hormones were tested for the rapid proliferation of the regenerated shoots from the previous stage (Table 3). The cultures were incubated on the media under the previously mentioned conditions for four weeks. During this period, significant differences were observed for shoot number registered on each medium (Table 3).

After four weeks of culture on the proliferation media, the medium M1 enriched with 1 mg L⁻¹ BAP and 0.25 mg L⁻¹ NAA gave the best results with a multiplication rate of 1.7 plantlets/explants (Table 3). In a similar purpose, this hormonal composition was applied by Bekheet (2006) for garlic proliferation and it leads to the best multiplication rates, the highest shoot length, and fresh mass. In the same context, Mehta et al. (2013) proved that incorporation of BAP or Kin into MS medium supported multiplication of shoots in culture. Shyab (2017) also reported that high plant proliferation rate, from garlic calli, was achieved via medium supplemented with BAP, Kin, and 2.4-D. In our conditions, the best shoot multiplication rate (1.7) of local garlic was relatively low. This may be related to the genotype of the cultured cultivar. Same behavior of proliferated garlic explant was reported by Asha Devi et al. (2007) who indicated that genotype plays a very important role in the response to garlic culture under in vitro conditions. Roksana et al. (2002) also noticed that the response of garlic shoot-root proliferation was varying with genotype.

In the same context, MS free growth regulator medium (M0) seems efficient for shoot proliferation since it offered a high shoot number of 1.59 plantlets/explant and it was found slightly better than the other tested media (M3, M15, and M16), containing BAP and 2ip (Table 3). Similar observations were recorded by [Ayabe and Sumi \(1998\)](#) who found multiple tissue-cultured shoots consistently differentiated from a single clove during one month of culture on a phytohormone free Linsmaier and Skoog medium using the stem disc as explant. In contrast, [Roksana et al. \(2002\)](#) noticed that no shoot proliferation was observed in medium without any growth regulators. They proved that the use of phytohormone is essential for shoot multiplication and proliferation. Also, [Gad El Hak et al. \(2011\)](#) found the lowest number of plantlets/explant when explants were grown on the complete MS hormone-free medium.

In the other side, the addition of a high concentration of BAP (2 mg L⁻¹) or the use of 2ip seems significantly with no effects on shoot multiplication increase (Table 3) since almost no plant proliferation was recorded (1.05 to 1.07 plantlets/explant). During all the multiplication period, no necrosis and no callus were noticed on the shoots.

M15 and M16 media containing 1 and 2 mg L⁻¹ 2ip respectively, with 0.25 mg L⁻¹ NAA did not offer a high shoot proliferation level (1.07%) since [Roksana et al. \(2002\)](#) found a highest mean of shoot proliferation of 9.8% at 21 days interval on medium with only 0.5 mg L⁻¹ 2ip and 0.25 mg L⁻¹ NAA. This confirms the hypothesis of the use of low concentrations of 2ip and NAA for high percentages of shoot proliferation ([Zheng et al., 2003](#)). The ability of 2ip to give rise to multiple shoots in garlic has been cited by [Koch and Salomon \(1994\)](#). Similarly, [Asha Devi et al. \(2007\)](#) found that 2ip was very effective in inducing shoot multiplication in the wide range of studied genotypes.

Bulblet formation

For bulblet formation and evolution, M0 (hormone-free), M20 and M22 media supplemented with BAP, 2ip and NAA composition were tested. During the first week of plant transfer on bulbification media, shoots on free-growth regulator medium had swelled at their bases indicating bulblet formation (Fig. 4-a). During the same period, shoots on the bulbification M20 and M22 media, supplemented with NAA and BAP or Kin, did not show any swelling. Therefore, shoots cultured on free-growth regulator medium were formed bulblets earlier. After 2 weeks of shoot culture on these media, it was evident that there was bulblet development in all media. Similar observations were made by [Asha Devi et al. \(2007\)](#) and [Metwally et al. \(2014\)](#) who also indicated that free-hormones media induced early bulb induction and reduced, consequently, plantlet multiplication rates.



Fig. 3. Multiplication of garlic (*Allium sativum*) vitroplants on the proliferation medium

Table 3. Effect of media composition on shoot multiplication of Tunisian local garlic

Medium	Medium composition	Shoot number
M0	MS free-growth regulator medium	1.59 ±0.19* † ^{bc}
M1	MS + 1 mg L ⁻¹ BAP + 0.25 mg L ⁻¹ NAA	1.7±0.3 ^a
M3	MS + 2 mg L ⁻¹ BAP + 0.25 mg L ⁻¹ NAA	1.05±0.05 ^{*c}
M15	MS + 1 mg L ⁻¹ 2ip + 0.25 mg L ⁻¹ NAA	1.07±0.07 ^{*c}
M16	MS + 2 mg L ⁻¹ 2ip + 0.25 mg L ⁻¹ NAA	1.07±0.07 ^{*c}

*: Significant differences (Tukey, P≤ 0.05); †Means with a different letter within a column are statistically different.

In this study, all tested bulbification media were enriched with 3% sucrose. This amount of carbon source seems relatively low to induce large vitrobulbs, if compared with what was used in other bulbification protocols. In this context, Haque et al. (2003) found that 12% sucrose was the best for bulblet weight only and hormone-free MS medium was the best for bulblet diameter and length. Dixit et al. (2013) reported a proper maturation of bulblets occurred on Gamborg’s medium containing 6% sucrose.

Despite a non-significant effect of media composition on bulbification development, variations on bulblet size and growth were registered (Table 4). The largest bulblets (7.1 cm) were obtained after six weeks of evolution with an M22 medium, containing 2 mg L⁻¹ Kinetin. On this medium, multiple bulblets related to plantlets cluster were obtained (Fig. 4-b). The smallest bulblets were regenerated on M20 medium, with only 0.5 mg L⁻¹ BAP. On M0 medium, we observed better bulb development and we obtained relatively large bulbs (6.9 cm).

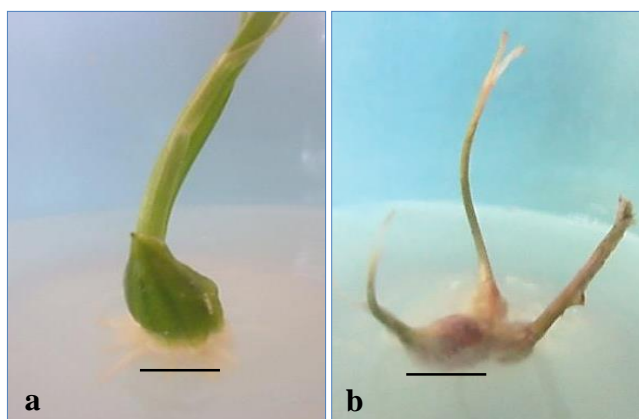


Fig. 4. Garlic plantlet derived bulblet in its base on medium M0 (a), multiple vitrobulbs of garlic on medium M22 (b), (Bar = 5 mm)

Table 4. Evolution of Tunisian garlic vitrobulbs on bulbification media during 2, 4 and 6 weeks

Medium	Bulb diameter (cm)		
	2 weeks	4 weeks	6 weeks
M0	5.9 ± 0.46 ^{ns}	6.43 ± 0.44 ^{ns}	6.9 ± 0.51 ^{ns}
M20	4.88 ± 0.18 ^{ns}	5.67 ± 0.42 ^{ns}	5.93 ± 0.23 ^{ns}
M22	4.76 ± 0.61 ^{ns}	6.16 ± 0.46 ^{ns}	7.1 ± 0.51 ^{ns}

M0: MS growth regulator-free medium; M20: MS + 0.5 mg L⁻¹ BAP + 0.1 mg L⁻¹ NAA; M22: MS + 2 mg L⁻¹ kin + 0.1 mg L⁻¹ NAA; ns: not significant differences.

Three large bulblets per explant were obtained on M22 medium (Figure 4-b), with Kin and NAA; while Roksana et al. (2002) reported the induction of multiple bulblet formation on both liquid and semi-solid MS medium by the effect of 2ip and NAA. The formation of multiple bulblets from single explants is the most desirable criteria of the in vitro technique. In fact, the proliferated developed bulblets were separated as individual cloves and cultured on MS medium with BAP, Kin, and NAA to assure further bulblet formation. The same results were obtained by Roksana et al. (2002), on the bulbification medium, after dividing multiple bulblets into individual cloves. Finally, small, non-dividable bulblets were obtained on the 3 bulbification media after the first two weeks of their culture. Similar bulb behavior was recorded by Gull et al. (2014) on MS medium who found small non-dividable bulblets of 1 cm, after one month. Metwally et al. (2012) also supported our results as they noticed that the bulbs were small and non-divided in the first regeneration. The number of cloves per bulb was 2.4, 45.8 and 54 at the second, third and fourth vegetative generations, respectively. Generally, the highest bulb development rates were achieved during the last three or four vegetative generations (Robledo-Paz & Tovar-Soto, 2012). So, garlic plantlets derived from tissue culture takes four vegetative generations (four years) to reach the commercial size. These developed plantlets are considered as a new source for breeding and improvement of garlic crop.

CONCLUSION

An optimized reliable protocol for rapid regeneration of garlic plants from shoot-tip culture was established. With this protocol, essentially the steps of shoot regeneration, plantlet proliferation, and bulblet formation were studied. Thus, the present optimized *in vitro* protocol will be successfully applied for large multiplication and for important *in vitro* management programs of local garlic for gene banks. Also, it will be efficient for viral sanitation program of local garlic cultivars, which seems to be an important agricultural input to improve garlic yield.

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ازدیاد درون شیشه‌ای سیر محلی تونس (Allium sativum L.) با استفاده از کشت نوک ساقه

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چکیده:

سیر تونس به طور گسترده‌ای توسط حمله چندین نوع ویروس تهدید می‌شود. از اینرو یک پروتکل قابل اعتماد برای ازدیاد سریع درون آزمایشگاهی سیر محلی (*Allium sativum L.*) به منظور تولید مقادیر زیادی از گیاهان بدون ویروس و گلبرگ‌های با کیفیت بالا ایجاد شد. ریزنمونه‌های ۱ میلی‌متر به خوبی مورد استفاده قرار گرفتند و در محیط جامد MS محیطی غنی شده با تنظیم کننده‌های رشد مختلف شامل ۶- بنزیل امینوبورین، α - نفتالین استیک اسید، کینتین، اسید ایندول ۳- بوتیریک و ۲- ایزوپنتیل آدنین کشت شدند و ارزیابی تشکیل ریشه، پرآوری شاخه و تشکیل پیازچه انجام شد. در میان غلظت‌های مختلف فیتوهورمون مصرفی و ترکیبات آنها، محیط کشت بر اساس MS بدون هیچ تنظیم کننده رشد (M0) برای آغازش نوک ساقه (۹۶٪ رشد ریزنمونه) و طویل شدن گیاهچه‌ها (۵۶/۲۶ میلی‌متر) بهینه بود. برای پرآوری ساقه، محیط کشت M1 حاوی ۱ میلی‌گرم در لیتر BAP و ۲۵/۰ میلی‌گرم در لیتر NAA بهترین بود، که حاصل آن ۱/۷ گیاهچه در نمونه بود. شاخه‌ها بر روی محیط کشت M0 زودتر پیازچه تشکیل دادند. تکثیر پیازچه‌ها در گیاهان درون محیط کشت M22 شامل ۲ میلی‌گرم کاینیتین و ۱/۰ میلی‌گرم اسید نفتالین استیک بدست آمد. پیازچه‌های جدا شده، به صورت مجزا بر روی محیط کشت استقرار منتقل شدند. پیازچه‌های تقسیم شده در اندازه‌های مختلفی رشد کردند. این پروتکل در شرایط درون شیشه‌ای آزمایشگاهی بهینه‌سازی شد و به طور موفقیت آمیزی برای ازدیاد انبوه ارقام عاری از ویروس سیر بکار برده خواهد شد.

کلمات کلیدی: *Allium sativum*، اکسین، پیازچه، سیتوکینین، ریز ازدیادی