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The Effect of KIN and 2,4-D on *In vitro* Propagation of Garlic (*Allium sativum* L.*)*

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Authors' contributions

This work was carried out in collaboration between all authors. Author FM designed the study and performed the statistical analysis. Authors HH and MEH wrote the protocol and managed the analyses of the study. Author FK wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The research work was conducted to observe *in vitro* propagation of garlic in the Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Bangladesh from the period of July 2015 to June, 2016, to determine the effect of Kinetin (KIN) and 2, 4-Dichlorophenoxy acetic acid (2, 4-D) in Murashige and Skoog (MS) medium where disease free, healthy and sterilized basal part of clove (2-3 cm) was used as explants. The highest shoot regeneration percentage (100%) was obtained from the treatment of 3.0 mg/L of KIN alone and also from all combination of 2.5, 3.0 mg/L KIN with 1.0, 1.5, 2.0, 2.5 mg/L 2, 4-D. All treatments of KIN and 2, 4-D either alone or in combination, showed one number of shoot after 3 weeks of inoculation. The highest shoot length (34.50 cm) was observed in 3.0 mg/L KIN treatment whereas in combined treatments, 3.0 mg/L KIN+ 2.0 mg/L 2, 4-D showed highest 33.73 cm shoot length after 3 weeks from inoculation. Besides, maximum root induction percentage (100%) was observed by 2.5 mg/L KIN + (1.0, 1.5) mg/L 2, 4-D. The highest number of roots (10.67) were developed by 3.0 mg/L KIN+ 1.5 mg/L 2, 4-D. After transferring the plantlets in the field condition, 86% survival plants were recorded.

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Finally, the *in vitro* regeneration protocol of garlic described herein can potentially be used as a tool in molecular breeding programs for improvement of different cultivars and genotypes of garlic.

Keywords: In vitro; propagation; garlic; KIN; 2,4-D.

1. INTRODUCTION

Garlic (*Allium sativum* L.) belonging to the family Alliaceae is an important and widely cultivated culinary crop. It is considered to be originated in central Asia, especially Mediterranean region [1]. Nowadays China, Bangladesh, India, South Korea, Spain, Egypt, Thailand and Turkey are the leading producers of this crop. The garlic bulbs are normally divided into numerous fleshy sections called clove, is the most commonly used part of the plant for consumption (raw or cooked).

Bangladesh was ranked 4th in world garlic production in 2014. Totally 3,45,725 tones of garlic was produced in 57049 hectare of land and total yield per hectare (ha) was 60601 hg/ha in 2015. Among the other plant spices, garlic ranked second in terms of production in the same years and it covered about 15 percent of the total plant production area [2].The per capita garlic consumption was set 3.34 kg for the fiscal year in 2014-2015. It has been estimated to rise up to 3.61 kg for the year 2016-17. The total domestic demand of garlic was 0.527 million tones in 2015. In 2020-21 it is assumed to be raised in 0.721 million tons [3].

Garlic is an important spices crop, which is rich in carbohydrates, protein and phosphorus used against various conventional ayurvedic treatments. Garlic supplementation is known to boost the function of the immune system and it has a significant impact on reducing blood pressure in people with high blood pressure. Garlic contains antioxidants that may help prevent Alzheimer's disease and dementia. It has biological applications as like antibiotic, anticancer, antithrombotic and in lipid lowering cardiovascular disorders [4,5]. It is used for diabetes [6] and sickle cell anemia [7] patients.

Garlic has a low efficient of multiplication due to its sexual sterility, potential for transmission of viral diseases. Garlic is mainly propagated through vegetative means and the improvement of garlic through breeding programs is limited due to difficulties of inducing flowering [8,9]. Many of the elite garlic cultivars are susceptible to diseases caused by viruses, nematodes and fungi and suffer from insect pests [10]. The low propagation rate and the continuous accumulation of deleterious viruses produced in the field have promoted the development of in vitro propagation of garlic [11]. Therefore *in vitro* propagation would be one of the key technologies for sustainable supply of this important plant source. The regeneration of plants from tissue culture is imperative and essential technique of biotechnological research and sometimes genetic manipulation of plants are achieved through this technique. Somatic embryogenesis and organogenesis have long been studied in garlic [12]. The most important factors affecting plant regeneration are explant type, the physiological condition of the explant, genotype and the growth regulator combination used in the culture medium. Regarding callus differentiation and plant development determination by growth regulators, several reports have shown the effects of the synthetic auxins, picloram (4-amino-3,5,6-trichloropicolinic acid) and 2,4-D (2,4-dichlorophenoxyacetic acid), on different garlic cultivars [13,14].Genetic improvement programs and genetic research will largely benefit from efficient protocols for garlic plant transformation. Herein, we focused to explore the variability of *in vitro* responses among different varieties of garlic. So, the present study was undertaken with following objectives:

1. To establish an efficient *in vitro* regeneration protocol for garlic.
2. To identify the best concentration of hormone for *in vitro* regeneration.

2. MATERIALS AND METHODS

The present study was carried out in the Biotechnology Laboratory of the Department of Biotechnology, Sher-e-Bangla Agricultural University, Sher-e Bangla Nagar, Dhaka-1207, Bangladesh during July, 2015 to June, 2016 using healthy, disease free *Allium sativum* L. as experimental materials which were collected from Agargaon nursery, Dhaka and the basal part of clove was used as explants.

Murashige ans Skoog (MS) medium [15] contained different phytohormones such as KIN (1.0, 1.5, 2.0, 2.5, 3.0, 3.5 55 mg/L) for shoot induction and 2,4-D (1.0, 1.5, 2.0, 2.5 mg/L) with KIN for shoot and root formation. The pH was adjusted to 5.8 before placing in microwave oven which was used for melting agar (semi solidifying agent). Stock solutions of hormones were prepared ahead of media preparation and stored at 4°C temperature.

Garlic cloves were separated from compound bulb and pealed manually. The cloves were washed firstly under running tap water and then with sterilized distilled water for several times and air dried. For sterilization, they were treated with 70% ethanol for 1-2 minutes and then rinsed with autoclave distilled water for 3-4 times. After that, the explants were cut into suitable size (1 to 2 cm) and immersed in 0.5% HgCl2 solution with 3 to 4 drops of Tween-20 for 1 minute with constant hand agitation. Finally, the basal part of cloves were washed 3-4 times with sterilize distilled water. The isolated and surface sterilized basal parts of clove were inoculated carefully to each of the culture tube containing 50 mL of MS medium supplemented with different concentrations of hormones as per treatment through maintaining aseptic condition inside the laminar air flow cabinet. The cultures were incubated at the 25±1°C temperature and under 16 hour photoperiod conditions with light intensity of 3000 lux for proper growth and development of culture. The observations on development pattern of shoots were made throughout the entire culture period. Data recording was started after 3 weeks from *in vitro* culture.

For the maintenance of proliferating shoots, the entire samples of *in vitro* shoot were cut into small pieces so that each piece would contain about one shoot and subcultured into a similar fresh medium. The subculturing was done at the interval of 20-25 days.

In vitro proliferated micro shoots were separated and each of the micro shoot was placed on culture medium, which was supplemented with particular concentration of hormone for shoot differentiation. Newly formed shoots with adequate length were excised individually from the culture vial and transferred to rooting media.

Regenerated plantlets were transplanted to pots (10×15cm) containing sandy soil and cow dung in 1:1 ratio. Occasional spray of water was applied to prevent sudden desiccations and maintain high humidity (70%) around the plantlets. Initially the plantlets were hardened in growth chamber. Then after 2 weeks, exposed to lower humidity and higher light intensity in shade house. Finally, after 21 days plantlets were transferred to natural environment.

Some observations were performed to evaluate the effect of different treatments on shoot and root proliferation after 1-3 weeks after inoculation (WAI). The observations were made on number of shoots, length of shoot and number of roots. The experiment was conducted in Completely Randomized Design (CRD) with three replications in culture room. Data were statistically analyzed by analysis of variance (ANOVA) technique and differences among treatment means were compared by using Duncan's multiple range test (DMRT) at 5% probability level using MSTAT-C program.

3. RESULTS AND DISCUSSION

Two separate experiments were conducted for the rapid micro propagation of the garlic. The objective of the present study was to develop a regeneration protocol of garlic.

3.1 The Effect of KIN on Shoot Proliferation in Garlic

This experiment was conducted under laboratory condition to evaluate the effect of different plant growth regulators on shoot proliferation in garlic. In the current investigation the relative ratio of auxin to cytokinin has been used. Significant variation was found on percent of explants showing shoot induction, number of shoots per explants and length of shoot, imposing doses of KIN. Maximum (100%) shoot induction was in 3 mg/L KIN, whereas the lowest (50%) induction was found at control condition (Table 1). The findings of present study are not supported by Khan et al. [16] where they found 10 mg/L BAP with highest shoot regeneration (56.80%) while working with proliferation from callus. This result corroborated with the findings of Kudou et al. [17] who reported that BAP was the most effective stimulator for shoot formation.

Different KIN concentrations did not have influence on the variation of number of shoots per explants. 1 shoot per explants was found at 0 to 3.5 mg /L concentration of KIN (Table 1). The records were taken on weekly basis up to 3 weeks after initiation. Mahajan et al. [18] found 4.34 numbers of shoots per explant with 1 mg/L

BAP while using cloves of garlic as explants. He observed that average number of shoots per explant was less when the MS medium was supplemented with BAP and Kinetin both as compared to medium supplemented with either BAP or Kinetin alone. The variation may be due to in vitro cultures of garlic are greatly influenced by the genotype. Different genotypes of garlic also showed variations in time required for plant regeneration [8].

The maximum average length of shoot 33.43 cm was noticed from the 3.0 mg/L KIN which was statistically similar with 2.5 mg/L KIN (32.50 cm) and statistically different from rest of treatments; whereas the minimum (18.0 cm) in case of lack of hormone (Table 1 & Fig. 1). Mahajan et al. [18] observed that BAP 1.0 mg/L gave average 6.9 cm of shoots from cloves of garlic after 28 days of initiation.

3.2 The Combine Effect of KIN + 2,4-D on Shoot and Root Regeneration

Individual doses of KIN showing most potential performance was used as the basis of setting of different hormonal combination along with 2,4-D. Significant variations of different concentrations of KIN and 2, 4,-D showed on regeneration potentiality, number of shoots per explants, shoot length, number of root in Garlic (Tables 2-3 & Plate 1). With 2.5 mg/L KIN + 1.0 mg/L 2,4-D; 2.5 mg/L KIN + 1.5 mg/L 2,4-D; 2.5 mg/L KIN + 2.0 mg/L 2,4-D; 2.5 mg/L KIN + 2.5 mg/L 2,4-D ; 3.0 mg/L KIN + 1.0 mg/L 2,4-D; 3.0 mg/L KIN + 1.5 mg/L 2,4-D; 3.0 mg/L KIN + 2.0 mg/L, 2,4-D; 3.0 mg/L KIN + 2.5 mg/L 2,4-D. 100% shoot initiation was found. The lowest regeneration potentiality was seen in control treatment (60%). Yanmaz et al. [12] showed that lower auxin and cytokinin concentrations were effective for shoot induction. In medium, 0.1 mg/L BA and 0.1 mg/L. IAA were sufficient doses for Tunceli garlic.

After 3rd week of inoculation no significant variation was not observed on different concentration of KIN+2,4-D on the number of shoots per explant. One shoot per explant was observed in every treatment. Mahajan et al. [18] showed that maximum numbers of shoots were obtained in MS medium supplemented with 1.0 mg/L of Kinetin (5.0 shoots per explant). The results of our observation are consistent with the findings of Yanmaz et al. [12].

The highest average length of shoot 33.7 cm was noticed with 3.0 KIN+ 1.5mg/L 2,4-D, which is statistically similar with 2.0 mg/L KIN + 2.0 mg/L 2,4-D;2.0 mg/L KIN + 2.5 mg/L 2,4-D; 3 mg/L KIN + 1mg/L 2,4-D mg/L; 3 KIN + 2.0 mg/L 2,4-D ; 3 mg/L KIN + 2.5 mg/L 2,4-D whereas the minimum 11.3 cm average shoot length was found in control. According to Mahajan, [18] the average shoot length was maximum in the medium supplemented both with 0.5 mg/L Kinetin and 0.5 mg/L BAP (9.8 cm) while the MS medium supplemented with 1.0 mg/L Kinetin and 1.0 mg/L BAP gave lowest average shoot length of 2.3 cm.

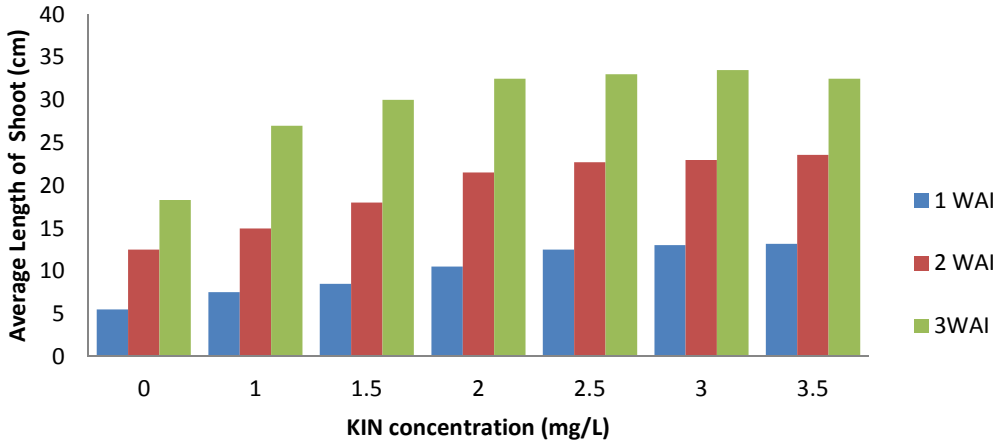


Fig. 1. Effect of KIN on length of shoot in garlic

Table 1. Effect of KIN on the shoot proliferation in garlic

KIN (mg/L)	Number of explants inoculated	Shoot initiation potentiality (%)	Number shoots per explants			Length of shoot (cm)		
			After 1 week	After 2 week	After 3 week	After 1 week	After 2 week	After 3 week
Control	25	50	1	1	1	5.50 e	12.50 f	18.00 e
1.0	25	88	1	1	1	7.50 d	15.00 e	27.00 d
1.5	25	90	1	1	1	8.50 c	18.00 d	28.33 c
2.0	25	95	1	1	1	10.50 b	21.50 c	30.33 b
2.5	25	98	1	1	1	12.50 a	22.70 b	31.33 b
3.0	25	100	1	1	1	13.00 a	23.00 ab	33.43 a
LSD(0.05)						0.83	0.64	1.15
CV (%)						4.64	3.85	4.25

Values in the column are the means of thee replicates. Mean values, in a column with the same letters are not statistically different from each other at 5% level by DMRT.

A **B** **C**

Plate 1. Effect of 3.0 mg /L KIN+ 1.5 mg /L 2,4-D on shoot regeneration in garlic-(A) after 2 days of initiation; (B), after a week of initiation; (C) after 3 weeks of initiation

Table 2. Effect of KIN and 2,4-D on shoot regeneration

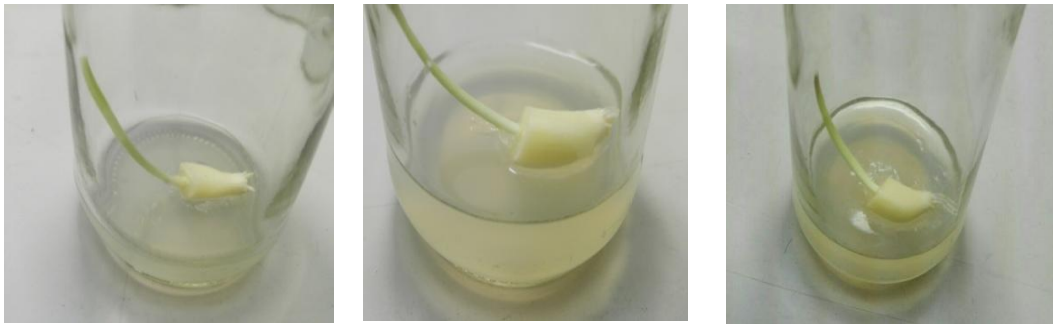
Treatments	Concentrations (mg/L)	Number of explants inoculated	Percent of explants showing shoot induction	Number of shoot			Shoot length		
				After 1 week	After 2 weeks	After 3 Weeks	After 1 week	After 2 weeks	After 3 weeks
Control	0.0	20	60	1	1	1	4.067 i	9.1k	11.33g
	1.0+1.0	20	87	1	1	1	11.3 h	19.7j	25.53ef
	1.0+1.5	20	88	1	1	1	12.5 g	22.7i	24.4f
	1.0+2.0	20	93	1	1	1	13.5 f	22.97 hi	25.33e f
	1.0+2.5	20	95	1	1	1	14.3 e	23.70 ghi	24.87 f
	1.5+1.0	20	95	1	1	1	13.6 f	24.50 efgh	25.57 ef
	1.5+1.5	20	97	1	1	1	15.3 d	24.00 fghi	25.33 ef
	1.5+2.0	20	98	1	1	1	16.5 c	24.73 efg	26.07 ef
Kin+2,4-D	1.5+2.5	20	89	1	1	1	16.6 c	25.6 bcde	26.67de
	2.0+1.0	20	87	1	1	1	18.7 a	25.4 cdef	28.90 bc
	2.0+1.5	20	92	1	1	1	17.3 b	26.3 abcd	28.07 cd
	2.0+2.0	20	94	1	1	1	15.3 d	26.7 abc	29.83 b
	2.0+2.5	20	97	1	1	1	15.6 d	26.6 abc	29.8 b
	2.5+1.0	20	100	1	1	1	15.3 d	27.8 a	29.87 b
	2.5+1.5	20	100	1	1	1	15.6 d	27.03 ab	30.4 b
	2.5+2.0	20	100	1	1	1	15.7 d	27.33 a	32.57 a
	2.5+2.5	20	100	1	1	1	13.07 f	26.7 abc	32.73 a
	3.0+1.0	20	100	1	1	1	13.5 f	24.8 defg	33.47 a
	3.0+1.5	20	100	1	1	1	15.47 d	26.33 abcd	33.7 a
	3.0+2.0	20	100	1	1	1	13.53 f	24.6 efg	33.57 a
	3.0+2.5	20	100	1	1	1	12.47 g	23.77 ghi	32.73 a
	LSD(0.05)						0.5373	1.398	1.47
	CV(%)						2.29	3.49	3.17

Values in the column are the means of three replicates. Mean values, in a column with the same letters are not statistically different from each other at 5% level by DMRT.

Table 3. Effect of KIN and 2,4-D on root regeneration

Treatments	Concentrations (mg/L)	Number of explant inoculated	Percent of explants showing root induction	Number of roots per explants					
				After 1 week		After 2 weeks		After 3 weeks	
	Control	20.00	87	0.00		2.667 h		5 g	
	1.0+1.0	20.00	88	5.00	def	6.00	Defg	8.00	def
	1.0+1.5	20.00	93	5.33	cdef	7.33	Abcd	8.67	bcde
	1.0+2.0	20.00	95	5.33	cdef	5.33	Fg	8.00	def
	1.0+2.5	20.00	95	5.33	cdef	4.67	G	6.67	f
	1.5+1.0	20.00	97	4.33	efg	6.67	bcdef	8.00	def
	1.5+1.5	20.00	98	5.33	cdef	6.33	Cdef	8.67	bcde
	1.5+2.0	20.00	89	5.00	def	6.33	Cdef	8.33	cde
	1.5+2.5	20.00	87	3.33	g	5.33	Fg	7.67	def
KIN+2,4-D	2.0+1.0	20.00	92	6.00	abcd	8.67	A	9.67	abc
	2.0+1.5	20.00	94	4.33	efg	7.33	Abcd	8.67	bcde
	2.0+2.0	20.00	97	5.67	bcde	6.67	bcdef	8.00	def
	2.0+2.5	20.00	100	6.67	abc	5.67	Efg	7.67	def
	2.5+1.0	20.00	100	5.00	def	8.00	Ab	9.67	abc
	2.5+1.5	20.00	98	4.00	fg	7.00	Bcde	8.33	cde
	2.5+2.0	20.00	99	6.33	abcd	8.00	Ab	8.00	def
	2.5+2.5	20.00	95	6.33	abcd	7.00	Bcde	7.33	ef
	3.0+1.0	20.00	97	5.67	bcde	7.00	Bcde	10.00	ab
	3.0+1.5	20.00	99	7.00	ab	8.00	Ab	10.67	a
	3.0+2.0	20.00	98	7.33	a	7.67	Abc	9.00	bcd
	3.0+2.5	20.00	87	6.33	abcd	7.67	Abc	9.00	bcd
		LSD (0.05)		1.37		1.39		1.26	
		CV (%)		15.88		12.65		9.17	

Values in the column are the means of three replicates. Mean values, in a column with the same letters are not statistically different from each other at 5% level by DMRT.



A B C

Plate 2. Effect of 3.0 mg/L of KIN and 1.5 mg/L of 2,4-D on roots regeneration in garlic: Rooting after (A) 2 days, (B) 7 days and (C) 2 weeks

With 2.5 mg/L KIN + 1.0 mg/L 2,4-D; 2.5 mg/L KIN + 1.5 mg/L 2,4-D the highest percentage (100%) of root induction was recorded. The control treatment showed lowest percentage (87%) of root induction (Table 2 & Plate 2). Metwally et al. [9] indicated that *in vitro* rooting of garlic is easily achieved on MS medium without PGR. However Tapia [19] reported that garlic roots formed well on MS medium supplemented with KIN and IAA. Kapoor et al. [20] found evidence that MS medium without any plant growth regulators is best for root induction in garlic. This finding showed similarity with that of Shuto et al. [21] who reported that garlic callus initiates roots on hormone-free MS medium. The highest number of roots per explants (10.67) was found in 3.0 mg/L KIN +1.5 2,4-D at 3 WAI (Table 3), which was statistically different with other treatments. The lowest number of roots per explants was obtained in controlled treatment. Mahajan et al. [18] found effective rooting of plantlets on MS media supplemented with 0.1 mg/L NAA. He suggested that lower level of NAA (0.1 mg/L) resulted in elongated but fewer roots in *in vitro* regeneration. Metwally et al [9] observed highest number of roots was observed on MS media containing 0.1 mg/L NAA, after 5-7 days of culturing. In addition, root length was also affected by NAA in the medium. A lower level of NAA (0.1 mg/L) resulted in elongated but few roots.

3.3 Acclimatization of Plantlets

The plantlets were taken for acclimatization after 21 days of culture. In culture room 100% plantlets survived (Table 4). After that the plantlets were shifted to shade house where humidity was 70% RH and sunlight was indirect. Transparent plastic sheet were used to cover the top of the pots in the shade house. The plantlets grew for 7 days at room temperature with periodic watering (2 days interval), about 80% of the plantlets survived. Afterwards the plantlets were planted to the soil in different pots of bigger size following potting and depotting. The plantlets were watered daily basis. Soil was mulched on its upper layer occasionally whenever necessary. In such atmosphere 86% plants were survived (Table 4 & Plate 3). Regenerated plants were found morphologically similar to the mother plant. According to Mahajan, [18], the rooted *in vitro* plants transferred to small plastic cups resulted in 90% survival rate after three weeks of transplantation. Khan et al. [16] found about 75% of survival in field condition after regenerating *in vitro*.

Plate 3. Acclimatization of regenerated plantlets of garlic

Table 4. Survival rate of *in vitro* regenerated plants of garlic

Acclimatization	No. of plants transplanted	Duration of observation	No. of plants survived	Survival rate (%)
In growth chamber	20	7days	15	100
In shade house	20	7 days	12	80
In pot culture condition	20	7 days	13	86

4. CONCLUSION

The results from the experiment implies that combination of KIN and 2,4-D cause slightly better responses regarding parameters observed than use of KIN alone. The experiments showed high regeneration potentiality with satisfactory shooting and rooting with very short duration of time. The findings can be beneficial for regeneration of diseases free plantlets for conventional breeding and development of more precise *in vitro* regeneration protocol as well. An *in vitro* regeneration protocol has been developed in Garlic. Better performance for shoot and root formation in garlic was observed in the combined effect of KIN and 2,4-D doses than individual effect of KIN. The protocol thus optimized for the plant regeneration from local garlic varieties provides a reliable propagation technique of garlic and will be of great use in genetic engineering or in molecular breeding program for improvement of garlic genotypes.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Thompson HC, Kelly WC. In: Vegetable crops. McGraw-Hill Book Co., Inc. New York. 1957;368-370.
2. BBS (Bangladesh Bureau of Statistics). Yearbook of Agricultural Statistics of Bangladesh, Statistics Division, Ministry of Planning, Government of the People's Republic of Bangladesh, Dhaka, Bangladesh; 2015.
3. The Financial Express, 2015. Available:http://thefinancialexpress-bd.com (Accessed on 20/05/2017 (online)
4. Banerjee SK, Maulik SK. Effect of garlic on cardiovascular disorders: A review. The J. Nutri. 2002;1(1):4.
5. Khan N, Alam MS, Nath UK. *In vitro* regeneration of garlic through callus culture. J. Biol. Sci. 2004;4(2):189–91.
6. Collins HA. Garlic and cardiovascular disease. In: Functional foods, diet, cardiovascular disease and diabetes. Arnoldi A,Dodds F, (Eds.). Woodhead Publishings, U.K. 2004;240-295.
7. Takasu J, Uykimpang R, Sunga MA, Amagase H, Niihara Y. Aged garlic extract therapy for sickle cell anemia patients. BMC Blood Disorders. 2002;2:3.
8. Barandiaran X, Martin N, Rodriguezconde MF, Di pietro A, Martin J. An efficient method for callus culture and shoot regeneration of garlic (*Allium sativum* L.). Hort. Sci. 1999a;34:348-349.
9. Metwally EI, Denary ME, Dewir YH, Naidoo Y. *In vitro* propagation of Garlic through adventitious shoot regeneration. African J. Biotech. 2014;13(38):3892-3900.
10. Verbeek M, Van Dijk, Van P, Well EMA. Efficiency of eradication of four Viruses of Garlic (*Allium sativum L.)* by meristem-tip culture. *Eur.J. Pathol*. 1995;101:231-239.
11. Kellar ERJ, Senula A. Micropropagation and chryopreservation of garlic (*Allium sativum* L.). In: Lambardi M, et al., editors. Protocols for micropropagation of selected economically-important horticultural plants, methods in molecular biology, Springer, NY, USA. 2013;994:353-368.
12. Yanmaz R, Yazar E, Kantoglu KY, Alper A. *In vitro* plant regeneration and bulblet formation of Tunceli garlic (*Allium tuncelinum*) (Kollman) by shoot and root culture. J. Food, Agri. & Env. 2010;(3&4): 572-576.
13. Zheng SJ, Heinken B, Krens FA, Kik C. The development of an efficient cultivar-independent plant regeneration system from callus derived from both apical and non-apical root segments of garlic (*Allium sativum L.).* In vitro Cell. Dev. Biol. Plant. 2003;30(3):288-292.

14. Luciani GF, Mary AK, Pellegrini C, Curvetto NR. Plant-Cell. Tissue Org. Cult. 2006;87(2):139-143.
15. Murashige T, Skoog F. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum. 1962;15:473-497.
16. Khan N, Alam MS, Nath UK. *In vitro* regeneration of garlic through callus culture. Bangladesh J. Biol. Sci. 2014; 4(2):189-191.
17. Kudou R, Fujime Y, Amimoto K. Effects of plant growth regulators and sampling positions on organ formation of garlic. Technical Bulletin of Faculty of Agriculture, Kagawa University. 1995;47(10):15-2.
18. Mahajan R, Sharma K, Bandryal S, Jamwal P, Billowria P. *In vitro* propagation and cryopreservation of snow mountain garlic endemic to Himalayan region. Inter. J. Adv. Biotech and Res. 2013;4(3):372-379.
19. Tapia MI. Callus induction, organogenesis and *in vitro* plant regeneration of garlic (*Allium sativum L.*) A*gro. Ciencia.* 1996; 12(20):149-154.
20. Kapoor R, Nasim SA, Mahmooduzzafar Mujib A. Establishment of efficient method for callus culture and shoot regeneration of local Indian garlic. J. of Ecobiotech. 2011; 3(12):14-17.
21. Shuto H, Abe T, Sasahara T. *In vitro* propagation from apex-derivedcalli in Chinese chive (*Allium tuberosum* Rottler) and (*Allium sativum* L.). Japan J. Breed. 1993;43:349-354.

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