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Effect of growth regulators and genotypes on *in vitro* regeneration of garlic (*Allium sativum* L.)

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Abstract

The present research was conducted to assess the interaction of growth regulators and genotypes on *in vitro* regeneration from root tips explants of local and exotic cultivars of garlic. Root tips were cultured on MS medium supplemented with different combinations of NAA and BAP. Among those combinations 1.0 mg l⁻¹ NAA and 4.0 mg l⁻¹ BAP was found to be the best hormonal supplement for the highest percentage of shoot regeneration (67.33%) in Chinese genotype. Chinese genotype had higher number (5.50) of longer shoots than the local genotypes. Rooting performance was also better in Chinese genotype.

Keywords: *In vitro* regeneration, Garlic, Growth regulators, Genotypes

Introduction

Garlic (*Allium sativum* L.) is an aromatic herbaceous annual spice and one of the most important bulb crops belonging to the family Alliaceae. It is the second most widely used *Allium* after onion (Bose and Som, 1990) with the characteristic pungent smell. Garlic has been used as a spice and condiment in many countries. It is popular all over the world as an important spice for different dishes. It has also been used in traditional medicine for more than 4000 years (Srivasta *et al.*, 1995). It has been known as a folk medicine since ancient times. Fresh garlic and preparations from garlic extract possess antimicrobial and cancer preventing activity. Aqueous extract of garlic cloves significantly reduce cholesterol level (Augusti, 1977). It is also used as a popular remedy for various diseases. According to the Unani and Ayurvedic, garlic is successfully used as a medicine for infection of stomach and intestine, desentry, typhoid, cholera and diseases of lungs (Chopra *et al.*, 1985). In recent times, garlic oil and garlic powder are prepared from it for adding flavour to the curries (Pruthi, 1976).

Garlic is sexually sterile and propagated vegetatively. The vegetative propagation has aided in virus transmission and resulted in poor rate of multiplication. Production of virus free plant has been possible by meristem culture. *In vitro* propagation is considered as efficient way of rapid multiplication of disease free high quality garlic plants. Many protocols have been suggested using shoot tips (Abo El-Nil, 1977; Nagasawa and Finer, 1988; Novak, 1983), basal plate (Koch *et al.*, 1995) and root tips (Mayer and Simon, 1998; Ali and Metwally, 1992; Shuto *et al.*, 1993; Haque *et al.*, 1997, 1998). However, very little attention has been given in Bangladesh. Based on the above information, the present study was undertaken to compare regeneration potential of a local and exotic cultivars of garlic using root tips as explants.

Materials and Methods

The present research was conducted in the Laboratory of Department of Biotechnology, Bangladesh Agricultural University, Mymensingh during the period from January to May 2007. Two different genotypes of garlic (Local & Chinese) were used in the present investigation to study their direct regeneration potential from root tips.

Healthy bulbs of two garlic genotypes were selected. Cloves were separated from the bulbs. The outer scale leaves were removed. The diseased cloves were discarded. Healthy cloves were surface-sterilized by placing them in 70% ethanol for 30 sec and 0.1% sodium hypochlorite solution containing 2 drops of Tween 20 per 100 ml for 20 minutes with frequent agitations. They were washed three times in sterile distilled water and aseptically cultured on water-agar medium for sprouting. Roots were collected from vigorously growing plantlets. MS medium (Murashige and Skoog, 1962) was used as basal medium for callus induction and plantlet regeneration. The root tips including meristems were used as explants. Young root tips measuring 2 to 3 mm in length were separated with the help of a sterile scalpel and forceps and placed onto the sterile culture media supplemented with BAP (2.0 & 4.0 mg l⁻¹) and NAA (0.5 & 1.0 mg l⁻¹) for regeneration. Five root tips were inoculated in each vessel and plugged with cork as well as non absorbent cotton. Following inoculation, all the cultures were maintained in a 16 h photoperiod, having light intensity of 2000-3000 lux from white fluorescent light at temperature of 25±1°C. Subcultures were carried out at regular intervals. All instruments, glassware and culture media were sterilized by autoclaving.

Data were collected on number of days required for shoot regeneration, length of shoot, number of roots plantlet⁻¹, days required for root initiation and length of roots. The experiments were arranged in Completely Randomized Design (CRD) with 4 replications. Data were statistically analyzed and the difference between the pair of means was evaluated at 5% level of significance by Duncan's Multiple Range Test (Gomez and Gomez, 1984).

Results and Discussion

Investigation on *in vitro* regeneration of these genotypes were accomplished by using root tips as explants, cultured on MS medium supplemented with different concentrations and combinations of NAA and BAP.

NAA at 0.5 mg l⁻¹ and BAP at 2.0 mg l⁻¹ was effective enough in initiating shoots in both varieties (Table 1). Among the genotypes, exotic genotype was better than local genotype for shoot induction from root tips. This result agree well with that of Ali (2006), who reported highest regeneration in Chinese cultivar of garlic on medium supplemented with 0.5 mg l⁻¹ NAA with 2.0 mg l⁻¹ BAP. Using the concentration of 1.0 mg l⁻¹ NAA and 4.0 mg l⁻¹ BAP, the best performance for shoot induction from root tips was achieved in this study (Fig. 1 A&B). In this concentration Chinese cultivar performed highest regeneration (67.70%) compared to local cultivar (60%). Similar result was reported by Haque *et al.* (2000), where higher regeneration was found in Japanese and Chinese cultivar compared to other genotypes. Barandiaran *et al.* (1999) also reported genotypic variation in the regeneration while working with 20 garlic genotypes.

Table 1. Combined effects of NAA, BAP and genotype on regeneration from root tips

Variety	Hormone concentration (mg l ⁻¹)		Regeneration (%)	Days for shoot regeneration
	NAA	BAP		
Local	0.5	2.0	27.33 b	38.17 a
	1.0	4.0	60.00 a	24.67 b
Chinese	0.5	2.0	24.33 b	37.33 a
	1.0	4.0	67.70 a	22.17 b
LSD _(0.05)			6.25	0.87

In the present experiment the genotypic response to different concentrations and combinations of NAA and BAP was observed. Chinese garlic genotype produced highest number of shoots (5.5) per explant on media containing 1 mg l^{-1} NAA + 4 mg l^{-1} BAP within short duration (29.83 days) (Table 2). Shoot length (5.83 cm) also higher in Chinese genotype in same concentration. But the lowest number of shoots (2.83) and lowest shoot length (4.17 cm) was observed in both local and Chinese genotype with 0.5 mg l^{-1} NAA and 2.0 mg l^{-1} BAP combination. Haque *et al.* (1999, 2000) reported similar kinds of direct regeneration of multiple shoots using NAA and BAP in different combination.

Table 2. Effects of different combinations of NAA, BAP on the genotype for shoot initiation

Variety	Hormone concentrations (mg l^{-1})		No. of shoots/ explant	Shoot length (cm)
	NAA	BAP		
Local	0.5	2.0	2.83 c	4.17 b
	1.0	4.0	4.67 b	4.50 b
Chinese	0.5	2.0	2.83 c	4.17 b
	1.0	4.0	5.50 a	5.83 a
LSD (0.05)			0.751	0.852

The genotypic response to different concentrations and combinations of NAA and BAP was observed. Chinese garlic genotype produced highest number of roots (5.83) per shoot on media containing 1.0 mg l^{-1} NAA and 4.0 mg l^{-1} BAP within short duration (29.50 days), whereas the lowest was obtained in local genotype with 0.5 mg l^{-1} NAA and 2.0 mg l^{-1} BAP combination (Table 3). Reports are available on garlic shoot develop roots on hormone free medium (Haque *et al.*, 1997; Barringer *et al.*, 1996). Root length (1.53 cm) also higher in Chinese genotype in the same concentration. The lowest root length (0.83 cm) was observed in Chinese genotype with 0.5 mg l^{-1} NAA and 2.0 mg l^{-1} BAP combinations.

Table 3. Effect of different combinations of NAA, BAP on the genotype for root initiation from shoot

Variety	Hormone concentrations (mg l^{-1})		No. of roots	Root length (cm)	Days to root initiation
	NAA	BAP			
Local	0.5	2.0	3.33 b	1.18 b	44.00 a
	1.0	4.0	5.00 ab	1.08 b	31.17 c
Chinese	0.5	2.0	4.00 ab	0.93 c	41.50 b
	1.0	4.0	5.67 a	1.35 a	29.50 d
LSD(0.05)			1.84	0.12	1.02

After sufficient development of shoot and root systems, the small plantlets were taken out from the culture vessels with proper care without damaging the roots. The agar around the roots was washed off by tap water to prevent microbial infection. The plantlets were then transplanted in small pots containing 50% cowdung and 50% soil. When the plantlet grew to a height of 5-6 cm, they were transferred to plastic pots. The growth condition of plantlets was satisfactory and the survivability of plant was 75%.

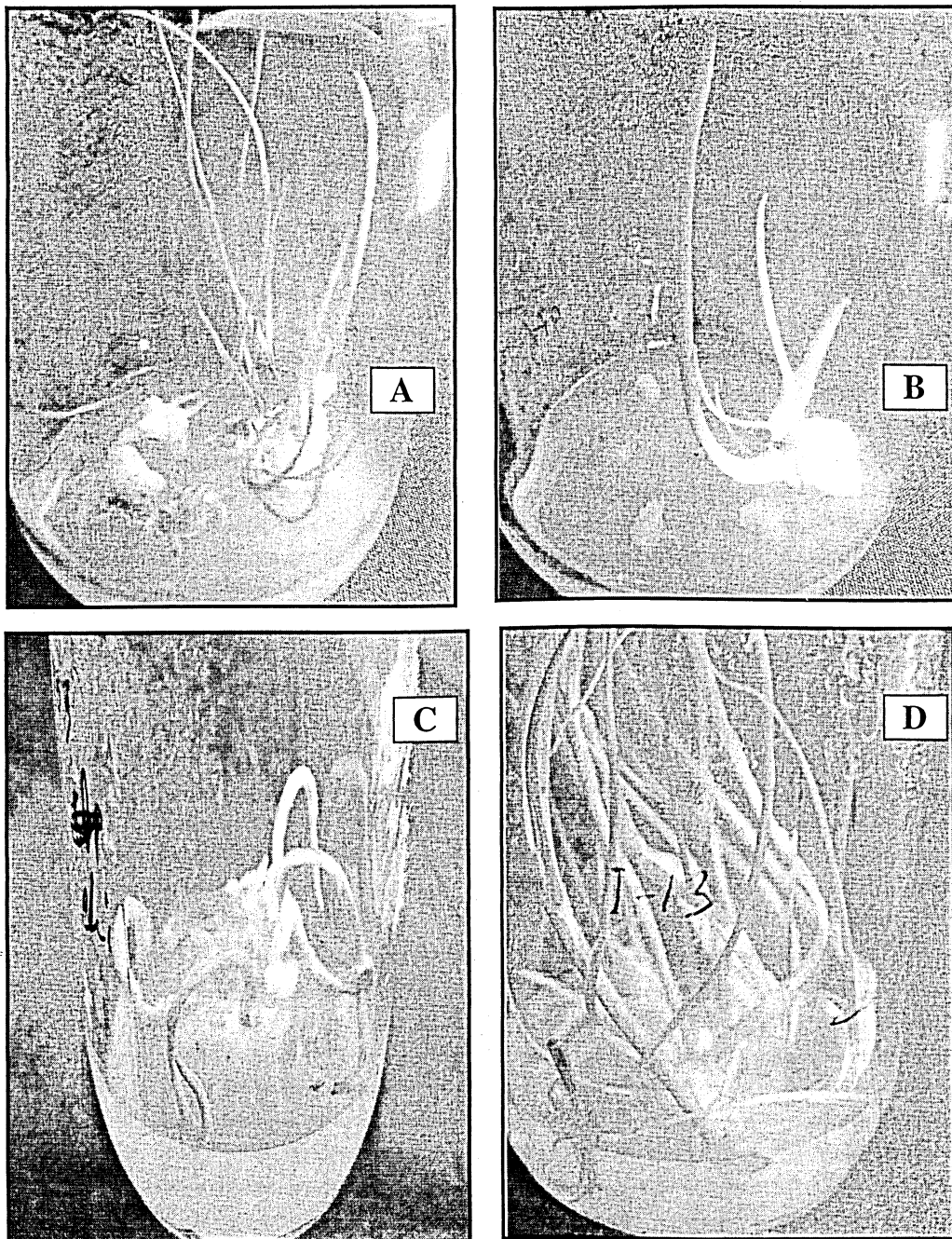


Fig 1. Shoot regenerated from root tips of local and exotic cultivars of garlic

- A. Chinese variety with 1.0 mg l^{-1} NAA and $4.0 \text{ BAP mg l}^{-1}$ at 30 DAI
- B. Local variety with 1.0 mg l^{-1} NAA and $4.0 \text{ BAP mg l}^{-1}$ at 30 DAI
- C. Local variety with 1.0 mg l^{-1} NAA and $4.0 \text{ BAP mg l}^{-1}$ at 45 DAI
- D. Chinese variety with 1.0 mg l^{-1} NAA and $4.0 \text{ BAP mg l}^{-1}$ at 45 DAI

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