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Full Length Research Paper

Bulb and vegetative characteristics of garlic (*Allium sativum L.*) from *in vitro* culture through acclimatization and field production

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This study reports the field performance of tissue-cultured garlic plants (cultivar Balady) upon their acclimatization for four successive generations. Bulb weight reached 0.7, 3.4, 62.1 and 87.9 g at the first, second, third and fourth vegetative generations, respectively. The bulb was small and non-divided in the first vegetative generation. The number of cloves per bulb was 2.4, 45.8 and 54.0 at the second, third and fourth vegetative generations, respectively. Bulb weight and cloves number in the original cultivar were 76.4 g and 55.2 cloves per bulb, respectively. The bulb development rate was high for three vegetative generations. However, it was the closest between the third and the fourth vegetative generation in which garlic plants reached a comparable size of the original cultivar. This study indicates that garlic plantlets derived through 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.

Key words: Acclimatization, bulbs, cultivation, garlic, tissue culture.

INTRODUCTION

Garlic (*Allium sativum* L., Liliaceae) is an important and widely cultivated crop, which is known for its culinary and medicinal use. Garlic has been cultivated vegetatively because of its sexual sterility (Etoh, 1985). Vegetative propagation of garlic is achieved through division of the ground bulbs and/or aerial bulbs; therefore, the multiplication rate is fairly low. Also, due to difficulties of inducing flowering, improvement of this crop through breeding programs is limited (Barandiaran et al., 1999). Many of the elite garlic cultivars are susceptible to diseases caused by viruses, nematodes and fungi and suffer from insect pests (Davies, 1994; Verbeek et al., 1995). The low propagation rate and the continuous accumulation of deleterious viruses produced in the field have promoted the development of several protocols for in vitro propagation, describing high multiplication rate and the production of virus-free plants (Nagakubo et al., 1993; Seabrook, 1994; Koch et al., 1995; Mohamed-Yassen et al., 1995; Haque et al., 1997, 2003; Ayabe and Sumi, 1998; Myers and Simon, 1998, 1999; Robledo-Paz et al., 2000; Kim et al., 2003; Luciani et al., 2006). However, transplantation of tissue-cultured garlic plants to field continues to be a bottleneck in commercialization of in vitro propagation of garlic. To the best of our knowledge, there is no information on the field performance of the tissue-cultured garlic plants. Therefore, the objective of this study was to evaluate the bulb and vegetative characteristics of tissue-cultured garlic plants during four successive generations in Balady cultivar from test tube

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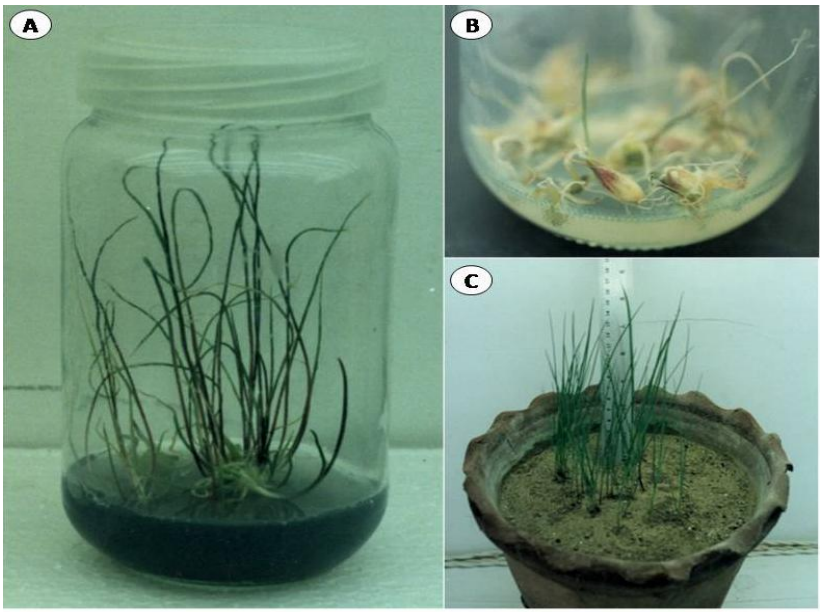

Figure 1. Acclimatization of garlic plantlets *A. sativum* Balady cultivar. (A) *In vitro* rooting; (B) Bulb formation *in vitro*; (C) Acclimatized plantlets.

through commercial size.

MATERIALS AND METHODS

Callus formation and plantlets regeneration

Root segments (0.8 to 1.2 cm) of garlic (*A. sativum* cv. Balady) containing the apical meristems were cultured on Murashige-Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 1.1 mg/L **2**,**4**-**Dichlorophenoxyacetic acid** (2,4-D) + 2.0 mg/L Kinetin (Kin) + 1.7 mg/L indole-3-acetic acid (IAA) for 8 weeks. Then, all calli were transferred to a differentiation medium which consists of MS medium supplemented with 2.0 mg/L 6-benzyladenine (BA) + 1.0 mg/L 1- naphthalene acetic acid (NAA) for 4 weeks and were sub-cultured twice. The calli were incubated at 25 ± 1°C with a 16 h photoperiod at 35 µmol/m^2/s photosynthetic photon flux (PPF) provided by cool white fluorescent tubes.

Root formation and plant acclimatization

Proliferated shoots obtained through organogenic calli were individually separated and inoculated to cylindrical culture jars (375 ml capacity) containing 35 ml MS medium without plant growth regulators (PGRs). The medium was supplemented with 3% (*w/v*) sucrose and was solidified with 0.8% (*w/v*) agar. The pH of the medium was adjusted to 5.8 before autoclaving (at 121°C and 1.2 kg cm^{-2} pressure for 15 min). All the culture jars were maintained for 4 weeks at 25 ± 1°C with 16 h photoperiod at 35 µmol/m^2/s PPF provided by cool white fluorescent tubes. For pre-acclimatization of garlic plantlets, the leaves were trimmed and the plantlets were sub-cultured for 4 weeks. The jars were subjected to a light intensity of 35 µmol/m^2/s PPF for 2 weeks followed by 50 µmol/m^2/s PPF for 2 weeks. The plantlets were carefully cleaned from the medium and were washed with tap water. They were then transplanted into sterilized clay pots (20 cm diameter) containing a mixture of peat moss:silt:sand (1:1:1, *v/v/v*). The leaves were trimmed into half size and the pots were incubated in a growth chamber. The environment in the growth chamber was adjusted to 25 ± 1°C air temperatures, 50 µmol/m^2/s PPF with a 16 h photoperiod provided by cool white fluorescent tubes and 40 to 50% relative humidity. The pots were covered with a clear polyethylene bags for the first week, and the polyethylene was gradually removed and the plantlets were grown for 3 weeks. The acclimatized plants were then transferred to a greenhouse for 6 weeks before their planting in an open field.

Statistical analysis

Bulb and vegetative characteristics in terms of plant height, pseudo-stem length, number of leaves, plant fresh weight, bulb weight and diameter and number of cloves were recorded. Data were subjected to Duncan's multiple range test using **Statistical Analysis System** (SAS) program (Version 6.12, SAS Institute Inc., Cary, USA).

RESULTS AND DISCUSSION

The regenerated garlic plantlets formed a well-developed root system within 7 to 8 days upon their culture on MS medium without PGRs (Figure 1A). Each plantlet had 2 to 5 roots with 2 to 3 cm in length. Previous reports indicatedthat *in vitro* rooting of garlic is easily achieved on MS medium without PGRs (Lu et al., 1982; Metwally and Zanata, 1996). However, Tapia (1987) reported that garlic roots formed well on MS medium supplemented with Kin and IAA. Exposure of garlic plantlets to 50 µmol/m^2/s PPF for 2 weeks proved useful for its acclimatization. It is well known that increasing light intensity reduces leaf length, length and width of cell and stomata index, while it increases leaf thickness (Rahim and Fordham, 1991). At the end of the acclimatization

Table 1. Growth and bulb development of tissue cultured garlic (Balady cultivar) plants during four successive generations.

Generation	Plant height (cm)	Pseudo stem length (cm)	Number of leaves/plant	Plant fresh weight (g)	Bulb weight (g)	Bulb diameter (cm)	Number of cloves/bulb
First	20.0^{dZ}	6.0^{d}	4.0^{d}	0.8^{e}	0.7^{e}	0.5^{c}	1.0^{c}
Second	55.0^{c}	11.0^{c}	6.0^{c}	41.0^{d}	3.4^{d}	1.9^{b}	2.4^{c}
Third	90.8^{b}	32.7^{b}	9.8^{b}	98.0^{c}	62.1^{c}	5.7^{a}	45.8^{b}
Fourth	101.0^{a}	35.2^{b}	12.8^{a}	122.8^{a}	87.9^{a}	6.3^{a}	54.0^{a}
Original cultivar	104.0^{a}	41.3^{a}	13.0^{a}	114.2^{b}	76.4^{b}	5.8^{a}	55.2^{a}

^{Z}Same letters within each column show no significant differences by Duncan's multiple range test at 5% level.

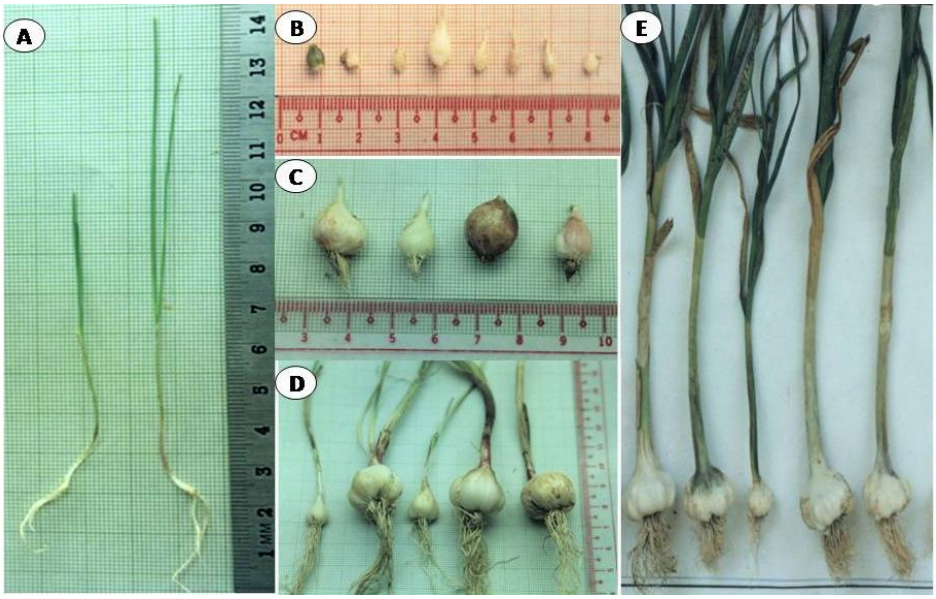

Figure 2. Development of garlic bulbs during four successive generations in Balady cultivar. (A) Rooted garlic plantlets; (B) Small bulbs derived during root formation in jars; (C) Non-divided bulbs derived from the first vegetative generation in pots; (D) Bulbs derived from the second vegetative generation in the field; (E) Bulbs derived from the third vegetative generation in the field.

stage, each plantlet gave a small and a non-divided bulb ranging from 0.2 to 1.2 cm in diameter (Figure 1B and C). The obtained plantlets differed in the shoot size; green color darkness, number of leaves and skin color of bulblet (white, light purple and purple). Such observations were also reported by Metwally and Zanata (1996).

The vegetative and bulb characteristics of garlic plants during four successive generations are presented in Table 1 and Figure 2. Plant height was developed from 20 cm in the first vegetative generation to 55.0 cm in the second one, then it reached 90.8 cm in the third. Afterward, it became 101.0 cm in the fourth vegetative generation. Pseudo-stem length was 6.0, 11, 32.7 and 35.2 cm for the first, second, third and fourth vegetative generation, respectively. Likewise, the number of leaves per plant was developed from 4.0 leaves per plant in the first vegetative generation to 12.8 leaves per plant in the fourth vegetative generation. The development of plant fresh weight which was recorded was 0.8, 41.0, 98.0 and 122.8 g for the first, second, third and fourth vegetative generation, respectively. The average bulb weight of garlic plants derived from tissue culture was also developed from 0.5 g in the first vegetative generation to 3.4 g in the second then, became 62.1 g in the third generation, while in the fourth generation, the average bulb weight reached 87.9 g. For the number of cloves per bulb, the results showed that garlic plants derived from tissue culture produced small and non-divided bulbs. The plants produced 2.4 cloves per bulb in the second season. Afterwards, number of cloves per bulb developed to 45.8 in the third vegetative generation and 54.0 in the fourth one. Also, bulb diameter of garlic plants was 0.4, 1.9, 5.1 and 6.3 cm for the first, second, third and fourth vegetative generation, respectively.

Generally, the rate of bulb and vegetative development was wider between the first and second vegetative generations and between the second and third ones. However, the development rate was the closest between the third and fourth generation in which garlic plants reached the commercial size. Previous studies on *in vitro* cultured lily bulblets revealed that the transition from juvenile to vegetative adult is characteriz-ed by increased mitotic activity in the apical meristem, followed by stem elongation (Langens-Gerrits et al., 2003). This step was related to weight of the bulblets (Niimi, 1995; Langens-Gerrits et al., 2003) and their size (Matsuo and Arisumi, 1978). It seems that a number of growing seasons are necessary for tissue cultured bulblets to reach the adult and commercial size. For example, tissue cultured bulbs of Narcissus showed that the size of flowering bulbs is reached after their third or fourth growing season (Hanks, 1993). The number of these growing cycles is dependent on the environmental conditions and is also cultivar-dependent (Hanks, 1993). Squires and Langton (1990) reported that a high proportion of Narcissus cultivar, Tete-a-Tete bulbs, reached the flowering size after two growing seasons. For garlic, it has been reported that the tissue cultured Japanese cultivar, Fukuchi-howaito, which ordinary has only 5 to 6 cloves, reached a comparable size and weight to those obtained by the usual clove cultivation after only one growing season (Ayabe and Sumi, 1998). However, the authors did not provide any information on the weight and/or size of bulbs. In this study, tissue cultured garlic plantlets cultivar, Balady, which has 54 to 55 cloves, reached the commercial size after four vegetative generations. These garlic plants derived through tissue culture are considered a new source for garlic breeding.

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