Micropropagation of Garlic (*Allium sativum*) by Bulblet Chipping In Vitro©

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INTRODUCTION

Garlic (*Allium sativum*) is well known as one of the noticeable foods which have many medicinal effects for human health from ancient times. In Mie Prefecture, a local cultivar 'Iki-wase' traditionally has been cultivated. Recently, it has been shown that this cultivar has highly effective functions as an antioxidant. Because of this, we decided to develop a rapid and effective propagation method for 'Iki-wase'. Several tissue culture methods have been reported. However, those methods have some problems, such as the occurrence of somatic variation (Luciani et al., 2006), the need for long-term cultivation (Nagakubo et al., 1993), and the necessity of mastering skillful techniques (Ayabe and Sumi, 1998). In this report, we tried to apply the bulblet chipping method used for nerine (Ezura, 1993) and amaryllis (Yanagawa, 1988) to multiply garlic bulbs rapidly without somatic variations.

MATERIALS AND METHODS

Bulblets obtained through callus culture from stem-disc explant of 'Fukuchi-howaito' were used for the experiments on the medium supplements and the procedure for bulblet chipping. The medium which contained Murashige and Skoog salts and organic compounds (Murashige and Skoog ,1962) supplemented with 0.1 $mg \cdot L^{-1}$ benzyladenine (BA), 50 $g \cdot L^{-1}$ sucrose, and 8 $g \cdot L^{-1}$ agar (pH 5.8) was used as the basal medium. Ten milliliters of medium was poured into Ø20 × 120-mm-glass test tubes, sealed with double-layered aluminum foil, and sterilized by autoclaving (120 °C, 15 min). All cultures were maintained at 24±2 °C, 16-h light (40 $\mu mol \cdot m^{-2} \cdot s^{-1}$PPF under cool white fluorescent lamps) per 8-h dark cycle. Examined factors were as follows; BA concentration (0, 0.1, 0.5, 1.0 $mg \cdot L^{-1}$), sucrose concentration (50, 80 $g \cdot L^{-1}$), improvement of culture vessel ventilation (closure with or without an air permeable microfilter), and cutting size of bulblets (longitudinally divided into 1 (no cut), 2, 4, or 8 segments (Fig. 1).

Next, the effect of liquid rotary culture on the enlargement of bulblets was examined. Various size bulblets of 'Fukuchi-howaito' were inoculated into 100-ml Eelenmeyer flasks that contained 40 ml of the basal medium without agar. The flasks were cultured at 23±2 °C, continuous light (56 $\mu mol \cdot m^{-2} \cdot s^{-1}$PPF under cool white fluorescent lamps) for 2 months.

Finally, effects of low temperature treatment to mother bulbils for chipping on the bulblet formation and its enlargement were examined. Bulbils of 'Iki-wase' with or without low temperature treatment (5 °C, 40 days) were cut into quartered segments, and they were cultured on the basal medium.

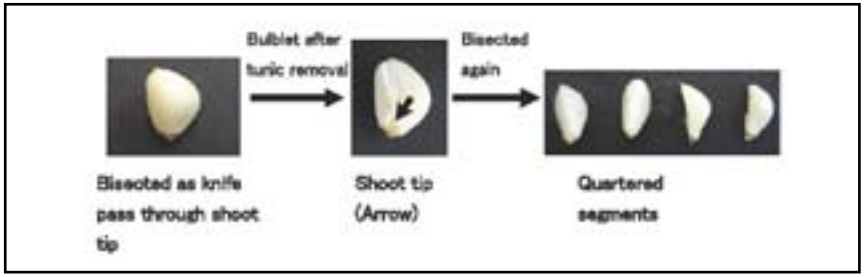

Figure 1. Chart of the cutting method of bulblets.

RESULTS AND DISCUSSION

Concentration of BA, sucrose, and ventilation rate elevation of culture vessel showed little effect on the bulblet formation and its enlargement. Chipping of bulblets was obviously effective on increasing shoot and bulblet number per mother bulblet because of apical dominance breaking (Table 1). When those bulblets were subcultured on basal (agar) medium, leaves and roots of bulblets grew vigorously but these were not adequate for acclimatization. For the purpose of promotion of bulblet enlargement, liquid-rotary culture was tested. Small bulblets (3 mm in diameter) grew rapidly during liquid rotary culture without vigorous growth of leaf and root organs, and showed bulb enlargement within 2 months (Table 2).

The cultivar, 'Iki-wase', is known as a type for warm climate, that is, its chilling requirement is not high. However, low temperature treatment of bulblets before chipping was very effective on bulb formation and enlargement (Table 3). The procedure proposed in this report (Fig. 2) has several merits compared with past reports as follows: low probability of occurrence of somatic variation (bulblets formed directly not through callus culture) on the quarter segment after apical dominance breaking), and relatively short time for the technique—this culture system consists of only three steps: (1) bulblet chipping (2 months), (2) bulb enlargement by liquid rotary culture (2 months), and (3) chilling treatment before chipping (40 days).

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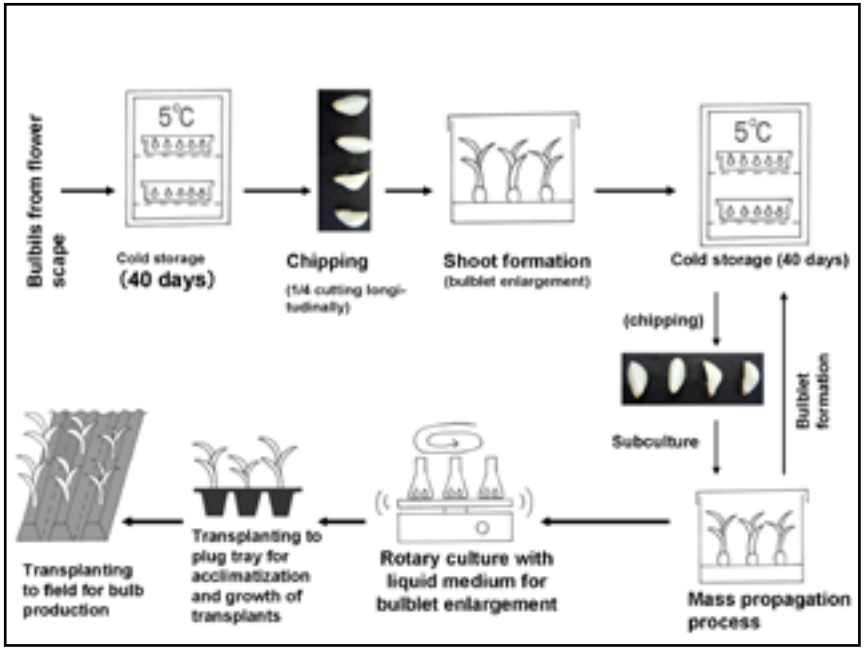

Figure 2. Scheme of the proposed micropropagation system for garlic 'Iki-wase' (a local cultivar in Mie Prefecture) through bulblet chipping.

Table 1. Effects of bulb chipping (explant) size n the shoot formation and bulblet enlargement.

Explant Size	Total FW (mg)	Root FW (mg)	Plant height (mm)	Shoot diameter (mm)	Bulblet diameter (mm)		Shoot number	
					Maximum	Minimum	Explant	A bulb
1/1	807±100	32±0	205±31	3.2±0.2	7.4±0.4	6.9±0.3	1.0±0.0	1.0
1/2	223±3.0	69±20	69±21	1.7±0.2	5.0±0.4	4.1±0.3	1.2±0.2	2.4
1/4	229±40	74±20	47±17	1.4±0.3	6.3±0.4	4.2±0.2	1.3±0.1	5.2
1/8	77±10	18±10	17±4	0.7±0.3	3.8±0.4	2.8±0.3	1.3±0.2	10.4

Table 2. Bulb enlargement through rotation of liquid culture (culture period 2 months).

Initial bulblet size		Total FW (mg)	Root FW (mg)	Plant height (mm)	Shoot diameter (mm)	Bulblet diameter (mm)	
Diameter (mm)	FW (mg)					Maximum	Minimum
3	58	460±90	9.0±0.0	53.5±6.7	3.5±0.3	6.0±0.3	5.6±0.3
5	138	738±50	5.8±0.0	65.9±3.7	4.2±0.3	6.7±0.2	6.2±0.2
7	182	1112±70	8.0±0.0	56.4±7.7	4.5±0.7	9.8±0.6	9.1±0.5
9	362	1117±390	7.0±0.0	38.4±8.8	3.7±0.9	11.7±1.1	10.7±1.0

Table 3. Effect of low temperature (5 °C) treatment before bulblet chipping on bulb formation and its enlargement (n = 72).

Low temp. (5 °C) treatment period (days)	Contamination (%)	Bulb formation		Shoot (unenlarged)		Root formation (%)	No response (%)
		(%)	No./explant	(%)	No./explan		
0 (control)	30.6	25.5	1.1±0.1	36.2	1.3±0.1	12.8	25.5
40	18.4	64.5	1.6±0.2	11.3	1.1±0.1	0.0	24.2