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Preliminary evaluation of transgenic tomato plants expressing *Escherichia coli* heat-labile toxin B subunit grown under *in vivo* condition

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ABSTRACT

In this study, three LTB (Escherichia coli heat-labile toxin B subunit) transgenic tomato lines (#1-3) were grown in the pot to characterize their growth and development, and evaluate fruit and LTB subunit productivity. Generally, the growth and development of transgenic tomato lines are not significantly different with non-transgenic tomato cultivar, "311" (commercial cultivar as reference). All of them took 120 days to final harvest, and number of fruit is near equivalent. *However, fruit quality characteristics are relatively different and line #3 has highest values of dry matter (6.02%), reducing sugar (2.51%) and degree Brix (6.40%). The vitamin C and acidity of all transgenic lines and the control are the same content. LTB subunit only expressed in two lines #1 and #3 with contents of 1.04% and 1.19% of total soluble protein, respectively.*

Keywords: Fruit, growth and development, LTB, transgenic tomato.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the major vegetable plants and was be grown around the world. The tomato fruit is rich in lycopene, a carotenoid in the same family as β-carotene, which may have beneficial health effects. The LTB (*Escherichia coli* heat-labile enterotoxin B subunit) of enterotoxigenic *E. coli* (ETEC) is known to be a potent mucosal adjuvant [1, 2]. It is considered a subunit vaccine candidate to be used against ETEC-induced diarrhea [3]. Tomato plant usually used for transformation of antigen genes to develop edible plant-based vaccines since its fruit is fresh edible. There were several reports on the introduction of the CTB (cholera toxin B subunit) gene into tomato plants, and these transgenic plants expressed the CTB subunit in leaves and fruits, which could specifically bind to GM1-ganglioside receptor, a special receptor for CTB and LTB subunits [4, 5, 6].

The purpose of this study is to characterize the growth and development of LTB-transgenic tomato lines under *in vivo* condition, and evaluate their fruit and LTB subunit productivity.

MATERIALS AND METHODS

Plant materials

Three transgenic tomato (*Lycopersicon esculentum* L. cv. 311) lines #1-3 developed with the construct of pMYO51 vector through *Agrobacterium tumefaciens*-mediated transformation for LTB expression [7, 8].

Physiological and biochemical analysis

Characteristics of the growth and development of plant were determined following Grodzinxki and Grodzinxki [9]. Total carotenoid content in the tomato fruits is determined spectrophotometrically at 470 nm. Acidity of tomato juice is measured by acid neutralization reaction [10]. Reducing sugar and vitamin C content are determined by dinitrosalicylic acid and iodine titration methods, respectively [11]. Degree Brix is measured using an ATAGO N1 refractometer (Japan). ELISA analysis for LTB subunit was performed similarly as a previous report on CTB expression in the tomato fruit [6]. Total soluble protein (TSP) concentration was determined by the method of Bradford using bovine serum albumin as a standard. The sample was read at wave length of 595 nm against the blank [12].

Statistical analysis

All experiments were conducted with a minimum of nine replicates and all experiments were repeated three times. The data were analyzed as means ± standard error followed by comparisons of the mean by Duncan's test ($p<0.05$) using the SAS program.

RESULTS AND DISCUSSION

Growth and development

The stages of growth and development of LTB-transgenic tomato lines were showed in table 1. Generally, there were no significant differences between transgenic plants and the control. The first harvest time was in a range of 65-69 days, and all of them took 120 days to final harvest. Figure 1 illustrates flowering and fruiting LTB-transgenic plants.

Data in table 2 showed that the height, the number of flowers and fruits of LTB-transgenic plants are similar with the control. However, other characteristics such as leaf area and number of inflorescences are slight difference. For two important characteristics of the growth and development to be harvest time and number of fruits, our results showed that there were no significant difference between LTB-transgenic plants and the control.

Table 1. Stages of growth and development of LTB-transgenic tomato plants

Lines	Stages of growth and development (days)				
	Branching	Flowering	Fruiting	First harvest	Final harvest
1	13	36	42	67	120
2	10	34	41	67	120
3	12	31	38	65	120
Control	14	37	43	69	120

Control: non-transgenic tomato plant

Table 2. Characteristics of growth and development of LTB-transgenic tomato plants

Lines	Plant height (cm)	Leaf area (cm^2)	No of inflorescences	No of flowers	No of fruits
1	59.00^{ab}	43.87^{a}	26.33^{b}	87.00^{a}	19.67^{a}
2	63.00^{ab}	32.93^{b}	24.00^{b}	83.33^{a}	17.00^{a}
3	67.67^{a}	44.99^{a}	31.67^{a}	78.67^{a}	15.33^{ab}
Control	60.33^{ab}	41.71^{a}	27.33^{b}	91.00^{a}	17.00^{a}

Different letters in a column indicate significantly different means (Duncan's test, p<0.05).

Figure 1. LTB-transgenic tomato plants

Fruit characteristics

Except two lines #1 and #3 showed that the fruits have highest average weight (92.53 g and 80.11 g, respectively). Generally, other characteristics of tomato fruit form are not different between LTB-transgenic lines and the control (Table 3 and Figure 2).

Data on tomato fruit quality in table 4 indicated that transgenic lines are the same vitamin C content and acidity as the control. Line #3 has dry matter, reducing sugar, degree Brix and LTB subunit reached highest values of 6.02%, 2.51%, 6.40% and 1.19% of TSP, respectively. LTB subunit expressed at high levels in fruits of two lines #1 and #3, but it was not found in fruit of line #2. However, in an early report, LTB subunit strongly expressed in *in vitro* leaves of line #2 [8].

Table 3. Fruit form of LTB-transgenic tomato plants

Lines	Fruit weight (g)	Fruit diameter (cm)	Fruit shape	Fruit colour
1	92.53^{a}	6.50^{a}	slightly-flattened circle	Light red
2	71.88^{c}	5.80^{b}	slightly-flattened circle	Light red
3	80.11^{b}	6.70^{a}	slightly-flattened circle	Light red
Control	66.59^{c}	6.10^{ab}	slightly-flattened circle	Light red

Table 4. Fruit quality of LTB-transgenic tomato plants

Lines	Dry matter (%)	Reducing sugars (%)	Vitamin C (mg/100g)	Acidity (%)	Brix (%)	Carotenoid (mg/100 g)	LTB (% TSP)
1	5.52^{b}	1.35^{d}	44.30^{a}	0.73^{a}	4.83^{c}	2.60^{c}	1.04^{b}
2	5.07^{c}	2.27^{b}	40.07^{a}	0.77^{a}	4.20^{d}	4.30^{a}	-
3	6.02^{a}	2.51^{a}	45.83^{a}	0.83^{a}	6.40^{a}	3.30^{b}	1.19^{a}
Control	5.41^{b}	2.19^{c}	43.13^{a}	0.79^{a}	6.00^{b}	3.60^{b}	-

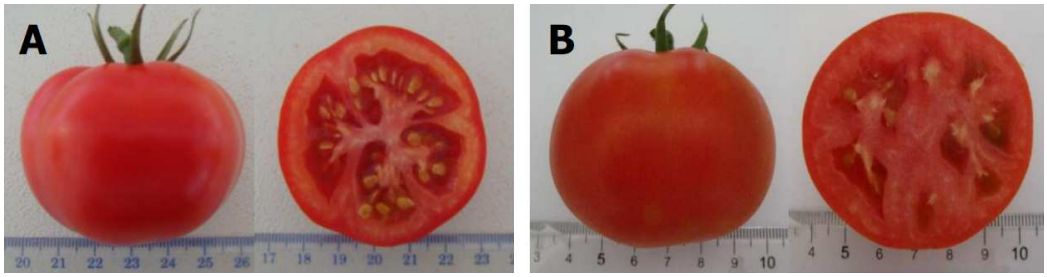

Figure 2. Fruits of non-transgenic tomato plant (A) and LTB-transgenic tomato plant (B)

CONCLUSION

Results of preliminary evaluation of three LTB-transgenic tomato lines grown under *in vivo* condition showed that their growth and development are similar with the control (cultivar 311). However, some characteristics of fruit form and quality are relatively different, and only two lines #1 and #3 produce LTB subunit in fruit.

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