Tomatine is a steroidal glycoalkaloid in tomato plants (Lycopersicon esculentum) and other Lycopersicon and Solanum species. Tomatine is used as an indicator to evaluate the safety of transgenic tomatoes by FDA in U.S.A. We have developed a facile and rapid method for absorptiometric measurement of the tomatine content. This method was used to measure the tomatine content of fruits of a transgenic tomato cultivar (Lycopersicon esculentum) which contained an antisense polygalacturonase gene (anti-PG). We found that the tomatine content in the fruits of transgenic and non-transgenic tomatoes was very similar. The data were also compared with those of other tomato cultivars, “KAGOME 77” (L. esculentum) and “KAGOME 88” (L. esculentum).

Key words: tomatine; transgenic tomato; absorptiometric measurement

Tomatine, which has anti-fungal activity, is a steroidal glycoalkaloid present in tomato plants (Lycopersicon esculentum) and other Lycopersicon and Solanum species. Tomatine operates as an inhibitor of cholinesterase and has cytotoxic activities. Tomatine is utilized as a precursor in the synthesis of steroidal pharmaceuticals and as a ligand for affinity chromatography. The role of tomatine in vivo is not well understood, but it is presumed to present resistance to microorganisms and insects.

Recent advances in genetic engineering technology have made it possible to endow plants with useful characteristics. The tomato plant has been transformed with genes that slow fruit softening and present greater resistance to microorganisms.

Guidance for assessing the safety of foods or food components from transgenic plants indicates that the major constituents of the recombinant should be substantially equivalent to those of the host. There should thus be no variation in the amount of the major constituents in a transgenic tomato as compared with the host tomato control and natural range. It is therefore necessary to measure the tomatine content in mature fruits of transgenic tomato to evaluate the substantial equivalence.

The common methods of HPLC, GC, MS, etc. that are used for measuring tomatine content cannot be applied to assess many samples simultaneously. We have developed a facile and rapid method for measuring the tomatine content by an absorptiometer. Although the lower limit of tomatine determination with our method is 0.1 mg/100 g, we conclude that this level of tomatine concentration is adequate for evaluating the food safety of tomato fruits. In this paper, we describe the determination of tomatine content in transgenic tomato fruits in comparison with the host by using our developed method.

Transgenic tomato plants (Lycopersicon esculentum) which contained an antisense polygalacturonase gene (anti-PG) and the non-transgenic type (L. esculentum) were cultivated in an isolated field at the Research Institute of Kagome Co. After harvesting the fruits at two stages of ripeness (mature stage and turning stage), the tomatine content was measured. As the non-transgenic controls, “KAGOME 77” (L. esculentum) and “KAGOME 88” (L. esculentum) cultivars were cultivated on the farm at the Research Institute of Kagome Co.

Tomato (L. esculentum) variety KGM 963 was transformed with the antisense PG gene. The level of PG activity of transgenic tomato fruits was measured by the spectrophotometric method reported by Gross, the PG activity of transgenic tomato fruits at the mature stage being found to be 0.6% of the non-transgenic tomato level.

Tomato fruit samples were homogenized for 3 minutes in an Excel auto-homogenizer (Nippon Seiki), frozen and then lyophilized for 2 days. The freeze-dried material was reduced to a fine powder and stored at -20°C until needed for measurement.

The tomatine content in tomato fruits was measured by the absorptiometric method previously developed by us.

Tomato powder (5 g) from whole fruit (mature stage or turning stage) was placed in a 50-ml centrifuge tube and then extracted at 50°C for 20 minutes with 30 ml of 50% methanol/2% acetic acid. Three extracts of each sample were combined and extracted with hexane until the hexane phase was colorless. The aqueous phase was concentrated at 75°C by rotary evaporator (Tokyo Rika) and after adding 30 ml of 1.5% hydrochloric acid and 10 ml of chloroform to the resulting residue, it was hydrolyzed for two hours at 80°C on a water bath. The samples were cooled and the chloroform phase was recovered. The chloroform phase was washed with a saturated sodium bicarbonate solution until the wash became colorless. The chloroform phase was then rinsed with water and dried with anhydrous sodium sulfate. The extract was evaporated at 40°C, the resulting residue being dissolved with dichloromethane and made up to 10 ml.

Out of this solution, 1 ml was further diluted to 10 ml
Tomatine Content in Transgenic Tomatoes

30 ml of 2% AcOH/50% MeOH
Extract at 50°C
Wash with hexane
Hydrolyse with 1.5N HCl (80°C for 2hr)
Extract with CHCl3
Wash with sat. NaHCO3 and H2O
Evaporate
Make up to 10ml with CHCl3
5ml of 0.2mM BTB (Na salt)
Shake and separate

Lower layer
1ml of 0.01M NaOH in MeOH
Measure at 620nm

Fig. Absorptiometric Measurement of Tomatine.
AcOH, acetic acid; MeOH, methanol; BTB, bromothymol blue BTB (Na salt) was prepared as described by A. O. A. C. 14 with dichloromethane and to this, 5 ml of a 0.2 mM aqueous bromothymol blue solution (sodium salt) was added, the mixture being shaken for 5 minutes in a separatory funnel. The lower layer (dichloromethane layer) was collected in a test tube and shaken after 1 ml of 0.01 M sodium hydroxide in methanol had been added to it. The blue color that developed was immediately measured at 620 nm (Figure).

The calibration curve for calculating the tomatine content was prepared with a tomatidine standard that had been obtained from tomatine. To prepare the tomatidine, 100 ml of water and 50 ml of a 10% sulfuric acid solution were added to 735 mg of tomatine (Funakoshi), this then being hydrolyzed for two hours at 100°C. The reaction mixture was extracted with dichloromethane and successively washed with saturated sodium carbonate and water. The dichloromethane phase was dried with anhydrous sodium sulfate and evaporated at 45°C. The resulting residue was chromatographed in a column of silica gel with 100:1 dichloromethane-methanol to afford 266 mg of tomatidine. The structure of tomatidine was investigated by 13C-NMR spectroscopy (Varian), the calibration curve prepared in the range of 0-3 mg/100 g of tomatidine being linear in this range. Tomatine in the tomato sample was quantified from the response factors obtained from the tomatidine standards.

Table I shows the tomatine content in the fruits (n=6) of transgenic and non-transgenic tomatoes measured by the absorptiometric method. It was found that the tomatine content in the transgenic tomato fruits with anti-PG was very similar to that in the non-transgenic cultivars at both the stages that were evaluated. These data indicate that there is little effect of transgenic modification on the tomatine content of tomato fruits regardless of their ripening state. In addition, there was no difference in the tomatine content of tomato fruits at the mature stage and that of the "KAGOME 77" and "KAGOME 88" cultivars (Table II), the level being within the normal range for tomatine content in tomato fruits.

Table I. Tomatine Content in Transgenic and Non-transgenic Tomato Fruits

<table>
<thead>
<tr>
<th>Tomato</th>
<th>Tomatine contenta (mg/100 g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Turning stage (S.D.)</td>
</tr>
<tr>
<td>Transgenic cultivar</td>
<td>1.7 (0.4)</td>
</tr>
<tr>
<td>Non-transgenic cultivar</td>
<td>1.4 (0.3)</td>
</tr>
</tbody>
</table>

a Transgenic tomato had the antisense polygalacturonase gene introduced.

Table II. Tomatine Content in "KAGOME77" and "KAGOME88" Tomato Fruits

<table>
<thead>
<tr>
<th>Tomato cultivar</th>
<th>Tomatine contenta (mg/100 g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Turning stage (S.D.)</td>
</tr>
<tr>
<td>KAGOME 77</td>
<td>3.4 (0.5)</td>
</tr>
<tr>
<td>KAGOME 88</td>
<td>2.7 (0.4)</td>
</tr>
</tbody>
</table>

a Average (n=6)

One of the safety indicators for transgenic tomatoes is the tomatine level in tomato fruits, and this was evaluated by the simple and rapid method of absorptiometric measurement.

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References