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Evaluation of agronomic and fruit quality traits of miraculin transgenic tomato

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Abstract. *Carsono N, Desiana N, Nurrizqi FM, Elfakhriano IF, Anas, Sari S, Kusumiyati, Ohsawa R, Shimono A, Ezura H. 2022. Evaluation of agronomic and fruit quality traits of miraculin transgenic tomato. Biodiversitas 23: 2004-2009.* Tomato cv. Moneymaker has been inserted with miraculin gene which can modify sour taste becomes sweet in the human tongue and, thus, it has potential as a sweetener. Evaluation of agronomic and fruit quality traits for this special transgenic tomato is a major concern that should be performed as substantial equivalence. The study's objective was to compare agronomic and fruit quality traits of transgenic miraculin tomato with those of its wild-type. The experiment employed a randomized block design, one genotype factor with two levels (transgenic vs. non-transgenic tomato cv. Moneymaker), and each was four replicates. Data were subjected to the Student t-test. The study revealed no significant differences between the transgenic tomato plants cv. Moneymaker and the wild type for important agronomic traits such as plant height, stem diameter, leaf area index, fruit weight, fruit weight per cluster, fruit weight per plant, fruit diameter, number of fruits per cluster, number of fruits per plant, and harvest index of miraculin. Some fruit quality traits such as fruit shelf-life, fruit hardness, total soluble solids, and content of glucose, sucrose, and fructose were equivalent to those of its counterpart, suggesting that miraculin transgene did not affect the expression of the observed traits. The miraculin transgenic tomato traits were more likely identical to those of wild-type except for the existence of the miraculin transgene. This will open the possibility for this special transgenic tomato to undergo further environmental safety assessment in the field test facility as a part of the biosafety assessment of genetically modified organism (GMO).

Keywords: cv. Moneymaker, miraculin, substantial equivalence, tomato, transgenic crop

INTRODUCTION

Miraculin is a natural low-calorie sweetener (Wong and Kern 2011) that can be used as an alternative sweetener for people who have diabetes, obesity, hyperglycemia, and caries (Wang et al. 2010). Miraculin was able to turn a sour taste on the tongue into a sweet taste (Misaka 2013). It is difficult to find miraculin products in the market, which could be due to the difficulty in cultivating it outside its origin of location and the very limited amount of production. Sugaya et al. (2008) and Sun et al. (2007) have successfully produced genetically modified (GM) or transgenic tomato cv. Moneymaker expressing miraculin under the control of 35S CaMV (Cauliflower mosaic virus) promoter. A transgenic tomato plant is considered the most suitable plant for mass production of miraculin since miraculin produced in this transgenic tomato plant is in relatively larger quantity as compared to that of the transgenic strawberries (Sugaya et al. 2008), and the gene silencing does not occur on the next generation as it did in transgenic lettuce (Sun et al. 2006). Besides, the miraculin expressed in tomato fruit is stable because of the acidic pH in the fruit of tomato (Agius et al. 2018). The information mentioned above convinces us to use tomato as a suitable plant for the mass production of miraculin. However, the utilization of transgenic crops should follow the regulation of biosafety assessment of transgenic crops in countries where this special tomato is grown.

Organization of Economic Cooperation of Development (OECD) (1993) explained that assessing the risk of the biosafety of transgenic crops should use the concept of substantial equivalence, where genetically organisms (GMO) are substantially equivalent to the product of non-GMO origin except the nature of the trait engineered (ISAA 2016; Matsaunyane and Dubery 2018). Risk assessment considerations for transgenic crops on the environment followed the Cartagena Protocol, the European Union, and countries that grow transgenic crops such as Argentina, Australia, Canada, China, and the USA. Some parameters were observed weediness, invasiveness, and the impact of transgenic crops on the soil environment (Turrini et al. 2015; Singh and Dubey 2017), agricultural cultivation systems, and non-target organisms (Naranjo 2014).

Transgenic plants, created by using recombinant DNA technology, have been developed by many laboratories worldwide for various purposes, from searching DNA sequence/gene function to creating transgenic plants for genetic improvement of valuable traits. Biosafety assessment of transgenic plants has been a major concern of our society. Concerns related to environmental safety, such as the possibility of risks that are detrimental to biodiversity, such as possible risks that are harmful to and threaten human health, the possibility of adverse effects as a result of the production of transgenic plants, should be avoided, among others, by performing a substantial equivalence assessment of transgenic plants before they released to the public.

Biosafety assessment is one of the requirements to be met before the public release of genetically modified crops into the society and environment. Biosafety assessment in genetically modified tomatoes includes equivalence test through agronomic traits, invasiveness (environmental safety assessment), and allergenicity and toxicity evaluations (food safety), which have a very important role in the utilization and release of a transgenic plant, both for human health and the environment. All these tests were conducted to determine the potential risk of GM crops to the environment and food safety (Bawa and Anilakumar 2013). Transgenic miraculin tomato has been introduced in Indonesia. However, there is no environmental safety assessment report for this tomato, particularly grown in Indonesia. Therefore, an evaluation of agronomic and fruit quality traits as part of environmental safety assessment through substantial equivalence is essential. This study compared agronomic and fruit quality traits of transgenic miraculin tomato plants cv. Moneymaker with its counterpart. This is the first report concerning environmental safety assessment in terms of substantial equivalence through evaluating agronomic and fruit quality traits of transgenic miraculin tomato versus non-transgenic wild type grown in a biosafety containment of limited facility in Indonesia.

MATERIALS AND METHODS

Plant materials

The seeds of miraculin transgenic and non-transgenic tomatoes, developed by the University of Tsukuba, were used in this experiment. Forty seeds of transgenic and 40 seeds of non-transgenic tomato were used. The experiment was carried out in the Biosafety Containment of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor. The experiment was arranged in a randomized block design. A mono-factor treatment, i.e., genotype was applied, consisted of two levels: transgenic tomato and non-transgenic wild-type tomato, each consisting of four replicates. The tomato seeds were treated in the oven at a temperature of 70 ^{0}C for 2 x 24 hours to eliminate the viruses. The tomato plants were grown in the containment greenhouse facility. The growth media consisted of soil, husk charcoal, and cow manure with a 2 :1:1 ratio. The spacing between transgenic and non-transgenic tomatoes was 70 cm x 50 cm. Watering was done every morning and evening. Installation of stakes was done at the age of 2 weeks after planting (wap) with a length of 1 m-1.5 m. Basal fertilizer was provided by adding 15:15:15 NPK compound fertilizer at a dose of 17.5 g per plant, applied twice at planting and the generative phase of the tomato plants. The hand-picking method was applied to control pests and diseases, and weeding was done manually.

Evaluation of agronomic traits

Agronomic traits evaluated were growth variables (plant height, stem diameter, and leaf area index), yield and yield component variables (fruit weight, fruit weight per cluster, fruit weight per plant, fruit diameter, number of fruits per cluster, number of fruits per plant, and harvest index) and yield quality variables (shelf life, fruit hardness, total soluble solids (Brix meter), glucose, sucrose, and fructose contents). The shelf life of tomatoes was observed after harvest. Measurements were made by taking five samples of transgenic tomatoes and five samples of non-transgenic tomatoes in each replication; the selected tomatoes had the same level of ripeness (red fruit). Fruit samples were stored in plastic perforated plastic and then stored in a closed room at room temperature. The observation was conducted within five days of tomato fruit experiencing senescence (until the fruit is not suitable for the market). The measurement of shelf life was carried out on the first harvest. Data of transgenic and non-transgenic tomatoes were compared using a student t-test performed using SPSS ver. 22.

RESULTS AND DISCUSSION

Growth variables

The growth variables (plant height, stem diameter, leaf area index) of the transgenic and non-transgenic plants were compared. The results showed that the growth variables of transgenic miraculin tomato plants were not significantly different from those of the wild-type tomato, as revealed by the student's t-test. This included plant height (Figure 1), stem diameter (Figure 2), leaf area index (Table 1). Meanwhile, the flower's appearance of the transgenic and non-transgenic plants is presented in Figure 3. The results clearly show that the transgenic and non-transgenic plants had similar agronomic traits.

Yield variables

Number of fruits per cluster and the number of fruits per plant of the miraculin transgenic tomato were similar to those of non-transgenic with the number of fruits per cluster was, respectively, 2.90 and 2.70, while the number of fruits per plant was 8.75 and 8.15, respectively (Table 2). There were no significant differences between transgenic and non-transgenic tomatoes in number of fruits per cluster, number of fruits per plant, fruit weight per plant, fruit weight per cluster, fruit weight per plant (Table 2), fruit diameter, and harvest index (Table 3).

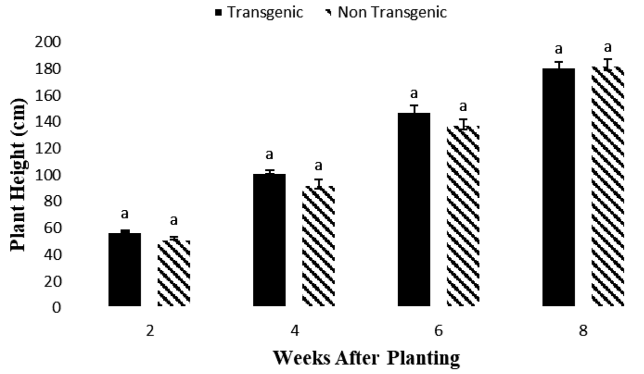

Figure 1. Plant height of transgenic miraculin tomatoes and non-transgenic wild type. Note: Values with the same letter in the same column are not significantly different according to the student's t-test

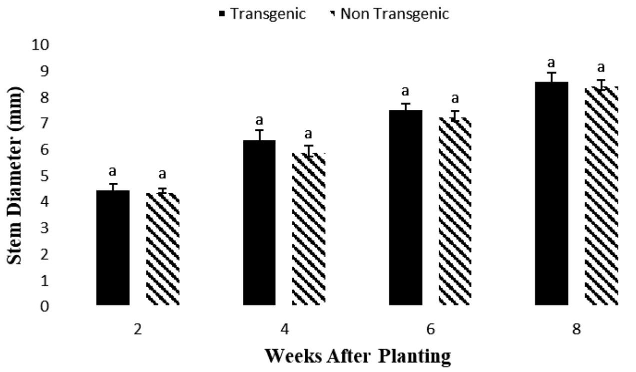

Figure 2. Stem diameter of transgenic miraculin tomato plants and non-transgenic counterpart. Note: Values with the same letter in the same column are not significantly different according to the student's t-test

A B

Figure 3. Flowers. A. Transgenic miraculin tomato. B. Non-transgenic tomato

Table 1. Leaf area index, fruit diameter, shelf-life, fruit hardness, and harvest index of miraculin transgenic and non-transgenic miraculin tomato plants

Tomato type	Leaf area index (LAI)	Fruit diameter (cm)	Shelf-life (day)	Fruit hardness (mm/g/s)	Harvest index
Transgenic	0.02 ± 0.005 a	3.65 ± 0.28 a	15.05 ± 6.53 a	4.38 ± 0.83 a	0.32 ± 1.20 a
Non-Transgenic	0.02 ± 0.005 a	3.84 ± 0.34 a	15.05 ± 6.53 a	4.19 ± 0.68 a	0.26 ± 0.36 a

Remarks: Values with the same letter in the same column are not significantly different according to the student's t-test

Table 2. Number of fruits and fruit weight of miraculin transgenic tomato and non-transgenic plants

Tomato type	Number of fruits		Fruit weight (g)		
	Per cluster	Per plant	Per fruit	Per cluster	Per plant
Transgenic	2.90±0.86 a	8.75±5.82 a	32.33±8.77 a	96.99±34.71 a	269.68±167.48 a
Non-transgenic	2.70±0.70 a	8.15±5.66 a	30.74±8.17 a	92.22±32.96 a	233.65±149.99 a

Remarks: Values with the same letter in the same column are not significantly different according to the student's t-test

Table 3. Total soluble solid and glucose, sucrose, fructose contents of miraculin transgenic tomato and non-transgenic

Tomato type	Total soluble solids (^{0}Brix)	Glucose (%)	Sucrose (%)	Fructose (%)
Transgenic	5.53 ± 0.67 a	4.60 ± 0.27 a	0.92 ± 0.20 a	4.60 ± 0.27 a
Non-Transgenic	5.99 ± 1.06 a	4.56 ± 0.23 a	0.65 ± 0.09 a	4.56 ± 0.23 a

Remarks: Values with the same letter in the same column are not significantly different according to the student's t-test

Fruit quality variables

Transgenic miraculin tomato plants' fruit hardness, glucose, sucrose, and fructose contents were slightly higher than non-transgenic miraculin tomato plants (Table 1 & 3). However, the total soluble solids of transgenic miraculin tomato plants were slightly lower than non-transgenic miraculin tomato plants (Table 5). Furthermore, the shelf life of both had the same duration of 15 days. There were no significant differences in shelf life and fruit hardiness (Table 4), total soluble solid and glucose, sucrose, fructose contents of miraculin transgenic tomato with those of non-transgenic (Table 5) tomato cv. Moneymaker. These results showed that the transgenic and non-transgenic plants had no different appearance on quality traits.

Discussion

In our study, miraculin transgenic tomato plants previously described by Ezura and Hiwasa-Tanase at the University of Tsukuba (2016) have been subjected to environmental safety assessment in terms of substantial equivalence by comparing some agronomic and fruit quality traits of transgenic tomatoes with the non-transgenic wild type grown in the containment greenhouse facility. In the production of miraculin transgenic tomato, the callus induction method was used as a tissue culture technique. Callus itself is an important source of in vitro culture in plant regeneration because each plant cell can form new individuals. Therefore, transgenic plant expressing the miraculin gene has been selected by phenotypic and genetic analyses. However, some transgenic plants developed from callus cells have been reported to have phenotypic and genotypic alteration due to genetic and epigenetic (Miguel and Marum 2011; Rajeevkumar et al. 2015), somaclonal variations (Coronel et al. 2018), retrotransposon activation (Benoit et al. 2019) and transgene insertion effect (Schnell et al. 2015; Dong and Ronald 2021).

The study results revealed no significant differences between transgenic miraculin and non-transgenic tomato on plant height from 2 to 8 weeks after planting (wap; Figure 1). All plant heights of tomato plants were statistically similar. Therefore, it seems likely that no somaclonal variations occurred in the transgenic tomato's plant height. Developmental changes in stem diameter in miraculin transgenic and non-transgenic tomato plants from 2 to 8 wap were identical. The mean diameter of transgenic and non-transgenic at the eight wap was 8.67 mm and 8.48 mm, respectively (Figure 2). A sturdy stem diameter will support plant growth because it functions as a medium for transporting nutrients from leaves to roots or vice versa through vessels in the stems (Zou et al. 2017). The mean leaf area index was the same, i.e., 0.02 (Table 1). Plants with a larger leaf area at the beginning of growth will experience faster growth because they can produce a higher photosynthetic rate than plants with a low leaf area (Jun and Shin 2020).

Plant height (Figure 1), stem diameter (Figure 2), and leaf area index (Table 1) of miraculin transgenic tomato were not significantly different from those of non-transgenic tomato plants. This means that the insertion of the miraculin gene into tomato plants did not affect the tomato plant growth variables, as shown by the growth variables of the miraculin transgenic tomato being substantially equivalent to those of its counterpart. Schauzu (2000) and Murthy et al. (2015) explained that if a novel food or novel food component is found substantially equivalent to an existing food or food component, it can be treated similarly with respect to safety. This is in line with the miraculin transgenic tomato found in this study.

There were no significant differences between miraculin transgenic tomato and its counterpart in yield variables. In addition, there were no significant differences between agronomic performance and quality fruit of transgenic miraculin tomato with those of non-transgenic miraculin tomato cv. Moneymaker suggested that adding the miraculin gene into tomato plants did not affect the tomato fruit yield (Table 2). The fruit diameter was also not significantly different between transgenic and non-transgenic tomatoes, with a diameter of 3.83 cm and 3.65 cm, respectively (Table 3). In addition, the harvest index of transgenic tomatoes was similar to that of non-transgenic tomatoes (Table 3). However, increasing the number of fruits in tomatoes can suppress fruit development which causes the fruit size to be smaller is because the amount of photosynthate supplied by the leaves is not proportional to the number of fruits (Nangare et al. 2016). Ochar (2019) explained that fruit size tends to be smaller if the number of fruits per plant increases. As with fruit weight, increasing the number of fruits per plant will reduce the fruit diameter of the tomatoes.

There were no significant differences between miraculin transgenic tomato and its counterpart on yield quality variables such as shelf life, fruit hardness, total soluble solids, and glucose, fructose, sucrose contents. This indicates that the transformation did not disrupt these traits. Furthermore, total soluble solids and sugar content were not significantly different, and it shows that transgenic tomatoes that have been inserted with miraculin gene only manipulating a sour taste in the tongue into a sweet taste, it does not change the content of total soluble solids and sugar content (glucose, sucrose, and fructose). The results align with Hirai et al. (2011), which state that miraculin protein is not giving sweetness but a protein that converts a sour taste into a sweet taste on the tongue. Therefore, it means that the yield quality of transgenic miraculin tomato was substantially equivalent to its counterpart, and thus, it can be treated in the same manner concerning safety.

The insertion of miraculin gene into cv. Moneymaker tomatoes did not affect the shelf life of tomatoes with the same average shelf life of 15.05 days at 26ºC. Knowledge of shelf life is very important to determine how long a commodity can be stored with fruit quality that consumers accept. The hardness of the fruit will also affect the mechanical damage and shelf life of the fruit. Fruit hardness is influenced by the flexibility of the fruit skin, the thickness of the fruit juice (juice thickness), and the inner structure of the fruit (comparison between the thickness of the pulp and the fruit cavity) (Ambarwati et al. 2012; Uba et al. 2020). According to Ambarwati et al. (2012), fruit hardness occurs due to the pressure of the cell contents on the fruit cell walls. The amount of cell content pressure depends on the concentration of solutes in the cell vacuole. The higher the concentration, the greater the pressure of the cell contents against the cell wall, resulting in a high level of fruit hardness. The fruit hardness of transgenic tomatoes was 4.38 mm/g/s, and non-transgenic tomatoes were 4.19 mm/g/s (Table 4). Xie et al. (2013) found that transgenic tomato co-expressing MADS-box gene *Solanum lycopersicum FYFL*, just began to soften after thirty-two days of harvest, highly comparable with tomato wild-type, which showed soft, dehydrated, and moldy fruit characters. Therefore, it is assumed that transgenic tomato has harder fruits than its wild type. Currently, there is no published report mentioning the fruit hardness of miraculin tomato.

It is known that the total dissolved solids content and sugar content (glucose, sucrose, and fructose) have a strong positive correlation with the sweetness of the fruit (Schwartz et al. 2009; Beckles et al. 2012). Additionally, Schwartz et al. (2009) also stated that the main component of the type of sugar contained in total dissolved solids consists of a combination of fructose and glucose. Our study results showed the total dissolved solids of transgenic miraculin tomatoes in cv. Moneymaker were 5.53 Brix (Table 5), did not much different from the research results by Hirai et al. (2011), which showed that the total of dissolved solids in the fruit of Miraculin transgenic tomatoes in cv. Moneymaker was 5.30 Brix. There were no significant differences between transgenic miraculin and non-transgenic tomato on total soluble solids and glucose, sucrose, fructose contents (Table 5). However, these results are different from that of Gupta et al. (2019), that showed significant differences between transgenic miraculin and non-transgenic tomato on total soluble solids, and also Rashid et al. (2022), who found that sucrose content was significantly higher in the transgenic seedlings than in the non-transgenic, but that there were no obvious changes in fructose and glucose content. However, when the transgenic plant mature fruits were compared to the non-transgenic, the fructose and glucose contents significantly increased, but no changes in sucrose content were observed. The differences in sugar content might be caused by FvTST1 overexpression, which induced glucose, sucrose, fructose contents. Gupta et al. (2019) and Rashid et al. (2022) utilized *A. tumefaciens* to generate transgenic tomato plants, which tend to be random, and the genes may be disrupted, not perfectly expressed overexpressed, however, if there are differences in agronomic characters between the transgenic miraculin tomato and non-transgenic miraculin tomato cv. Moneymaker, then this difference is thought to be due to somaclonal variations, insertional effect, retrotransposon activation, epigenetic, and chromosomal mutations that might occur during the development of transgenic tomato. Somaclonal variation is genetic variation produced through tissue culture (Krishna et al. 2016). Plants produced through tissue culture can produce somaclonal variations resulting in changes in appearance variations in regenerated plants (Sato et al. 2011). The trigger for somaclonal variation can occur due to the oxidative stress experienced by the plant during culture (wounds, exposure to sterilization material during sterilization), method of work (incomplete tissue like protoplasts as an extreme example), tissue culture, and media (imbalance of media components such as high concentrations of growth regulators, sugar from nutritional media as a substitute for photosynthesis in leaves) and tissue culture environment (lighting conditions, disruption of the relationship between high humidity and transpiration) (Smulders and de Klerk 2011; Krishna et al. 2016).

This study reports the results of the evaluation of plant growth, fruit yield, and yield quality traits between the transgenic and non-transgenic (wild type) tomato plants. Genetic transformation of the miraculin gene to tomato cv. Moneymakers do not interfere with the genes that express these traits. It can be concluded that there were no significant differences between transgenic miraculin tomato and non-transgenic tomato miraculin cv. Moneymaker. Transgenic miraculin tomato likely has substantial equivalence with those of non-transgenic wild type. Further biosafety assessment conducted in the limited field test facility and food safety should be performed to further evaluate the environment and food safety assessment as a requirement for the public release of transgenic miraculin tomato. It is expected that a comprehensive biosafety assessment should be done for the next experiment.

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