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Research article

The comparative analysis of agronomic, compositional, and physiological traits of miraculin transgenic tomato in the confined field trial

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Abstract: The miraculin transgenic tomato is a genetically modified (GM) crop that can be used as an alternative for low calories food and a natural non-sugar sweetener. Before the release and distribution, transgenic crop needs to go through an environmental risk assessment (ERA) as a backbone to achieve biosafety. Comparative analysis is a general principle of ERA to identify differences between transgenic crop and its non-transgenic counterpart which may indicate substantial equivalence and unintended effects. This experiment was aimed to compare the agronomic, compositional, and physiological characteristics of miraculin transgenic tomato cv. Moneymaker with non-transgenic tomato. The data obtained were plant height, stem diameter, relative growth rate, chlorophyll content, stomatal conductance, days to 50% flowering, days to fruit maturity, a number of flowers per cluster, a number of fruits per cluster, a number of fruits per plant, fruit weight, fruit diameter, harvest index, total dissolved solids, fructose, glucose, and sucrose contents, and total carotenoids, lycopene, and β-carotene contents. This study found that there were no significant differences between miraculin transgenic and non-transgenic tomato in all variables observed. It suggests that miraculin transgenic tomato is equivalent to its counterpart and unintended effects are not detected as.

Keywords: environmental risk assessment; limits of concern; substantial equivalence; tomato; unintended effects

1. Introduction

The miraculin transgenic tomato is a genetically modified (GM) crop that expresses the miraculin gene. This gene was isolated from miracle fruit (*Synsepalum dulcificum*) and transferred into the genome of the tomato plants cv. Moneymaker mediated by *Agrobacterium tumifaciens* vector [1,2]. Miraculin is a glycoprotein compound that is able to turn sour into sweet taste by binding to taste receptors on the tongue [3,4]. Miraculin transgenic tomato can be used as an alternative food that is low in calories and natural non-sugar sweeteners, especially for diabetics [5].

The release, distribution, and utilization of transgenic crops are determined by regulatory permits since transgenic crops would have effects on the environment, human health and animal health [6]. In Indonesia, Government Regulation No. 21 of 2005 lays out that environmental risk assessment (ERA) is required to prevent the occurrence of adverse risks to biodiversity which may due to the use of transgenic crops. This potential risk is associated with unintended effects, the alteration of plant agronomic traits [7] such as dwarfism, delayed flowering, and decreased productivity [8–10]. Composition and physiological characteristics can also change which is caused by the alteration in synthesis of certain proteins as a result of transgene insertion [11,12]. The potential risk can be assessed by comparing the agronomic, compositional, and physiological characteristics of transgenic plants with its conventional counterparts. These characteristics have to be equivalent except for the modified traits [13].

Minister of Environment of the Republic of Indonesia has issued Regulation Number 25 of 2012 regarding ERA which states that ERA is a stepwise process beginning with testing in laboratory, biosafety containment, to confined field trials (CFT). This regulation is in line with the European Food Safety Authority (EFSA) principle. Field testing of transgenic crops is needed to evaluate the expression of target genes and phenotypic characteristics of plants in actual conditions [13].

Previously, Carsono et al. [14] have evaluated the agronomic characteristics of miraculin transgenic tomato cv. Moneymaker and its origin tomato in biosafety containment which show substantial equivalence between transgenic and non-transgenic tomato plants. Until now there is no report regarding on biosafety assessment of miraculin transgenic tomato on agronomic, compositional and physiological traits that conducted in the confined field trial. As one of the important steps in environmental risk assessment, the further evaluation of transgenic crops in CFT is required to be conducted. The objective of this research was to compare the agronomic, compositional, and physiological traits between miraculin transgenic tomato and non-transgenic tomato in CFT. This study will provide substantial equivalence information and possible unintended effects for further utilization and production of miraculin transgenic tomato.

2. Materials and methods

This experiment was conducted in the CFT at Ciparanje experimental station, Jatinangor, West Java Province, Indonesia, during August-December 2020. The CFT was 780 m above sea level and received 156.63 mm of rainfall monthly, with a daily average temperature of 12.3 ℃ minimum and 32 ℃ maximum and mean relative humidity of 84%. The soil type was inceptisols with neutral

pH (6.8), medium C-organic content (2.14%), and medium total N, K_2O, and P_2O_2 (0.21%, 31.47 mg/100g^{-1},13.97 ppm P). The isolation distance was more than 20 m, in accordance with the implementation regulation on the safety assessment of GMOs in Indonesia. The experiment was arranged in a randomized block design (RBD) with two treatments: miraculin transgenic and non-transgenic tomato cv. Moneymaker. Each treatment was replicated 16 times. Experimental plot was 1.2 m long and 6 m wide with a spacing of 60 cm x 60 cm. An experimental unit consisted of 20 plants with a total of 640 plants.

Before sowing, the tomato seeds were soaked in warm water and 70WP propineb fungicide solution for 12 hours and 30 minutes. The seeds were sown in portray with the mixture of soil and cow manure in a 2:1 ratio. After 6 weeks, the seedlings were transferred into the field. Fertilization using NPK 16:16:16 was applied at transplanting, 30, and 60 days after planting (DAP). Plants were watered once in two days. Manual weeding was done 3 times during the planting season. The stakes were installed when the tomato plants were 21 DAP or 3 weeks after planting (WAP). Tomato was harvested 3 times at the breaker stage phase with intervals of 5 days.

The agronomic traits observed were plant height, stem diameter, relative growth rate (RGR), days to 50% flowering, days to fruit maturity, a number of flowers per cluster, a number of fruits per cluster, a number of fruits per plant, fruit weight per item, fruit diameter, and harvest index. The compositional analysis consists of total dissolved solids (TDS), fructose, glucose, and sucrose contents, and total carotenoids, lycopene, and β-carotene contents. TDS was determined using refractometer (Atago Model 41325). Sugar contents was analyzed by High Performance Liquid Chromatograph (HPLC) method and total carotenoids, lycopene, and β-carotene contents were analyzed using spectrophotometric method. The physiological traits consist of leaf chlorophyll content and stomatal conductance. Chlorophyll content was measured with chlorophyll meter (CCM-200 Plus). Leaf stomatal conductance was measured using leaf porometer (Decagon device, Inc.). The data were analyzed using the independent samples t-test and Limit of Concern (LoC) [6] as presented below.

$$0.5 \leq \frac{\text{Transgenic Plants}}{Non\ Transgenic\ Plants} \leq 1.5 \quad (1)$$

Value > 1.5 = Not equivalent (there is an unintended effect);
Value 0.5–1.5 = Equivalent (there is no unintended effect);
Value < 0.5 = Not equivalent (there is an unintended effect).

3. Results and discussion

EFSA suggests using comparative assessment as a starting point for GMOs' whole risk assessment process. The characteristics of the GM plant are compared with those of its conventional counterpart cultivated under similar conditions. The comparative approach's underlying assumption is that traditionally cultivated non-GM plants have a history of being safe for humans, animals, and the environment [13]. This study was a step to ensure that the miraculin transgenic tomato is as safe as its counterpart. Through risk communication, this kind of information was important to generate consumer acceptance of transgenic food [15].

The results demonstrated that there were no significant differences between miraculin transgenic tomato and non-transgenic tomato in height, stem diameter, and relative growth rate variables (Figure 1). No statistical differences were found in a number of flowers and a number of fruits per cluster (Table 1),

days to 50% flowering, and days to maturity. The traits of a number of fruits per plant, fruit weight, fruit diameter, and harvest index did not show 'any significant differences (Table 2). The days to 50% flowering of miraculin transgenic and non-transgenic tomato revealed similar results in which the two crops flowered on average 28 DAP. There were also similarities in days to maturity between the two crops at 70 DAP. The mean value of fruit set for both crops was 70.97% and 68.51%, respectively indicating that 29.04%–31.49% of the flowers failed to become fruit. The reduced fruit set could be affected by high temperatures and humidity that cause lower pollen viability and release [16,17].

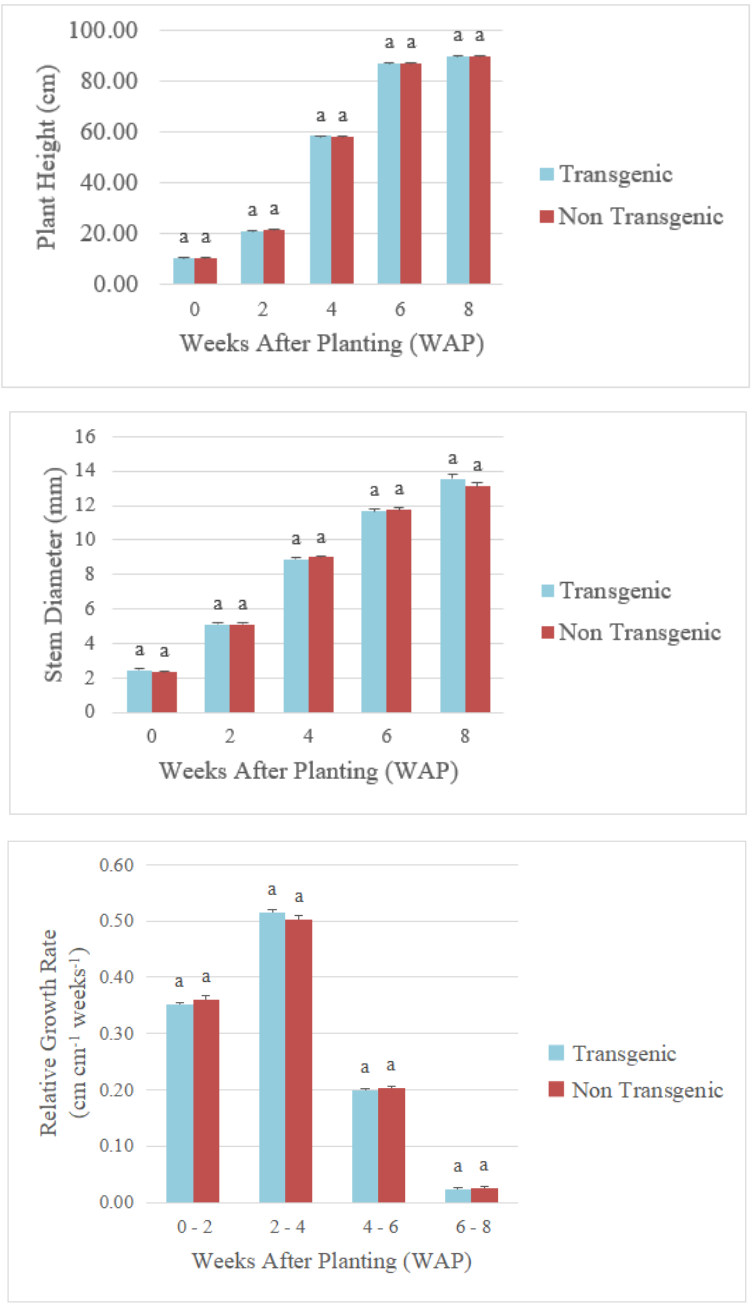

Figure 1. Plant height, stem diameter, and relative growth rates. Values with the same letter in the same column are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80).

Table 1. Number of flower and fruit per cluster of transgenic miraculin tomato plants and non-transgenic miraculin tomato plants cv. moneymaker.

Plants	Number of flowers per cluster	Number of fruits per cluster
Transgenic	4.65 ± 0.11 a	3.30 ± 0.08 a
Non-Transgenic	4.51 ± 0.10 a	3.09 ± 0.08 a

Values with the same letter in the same column are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80).

Table 2. Total fruit**,** fruit weight, fruit diameter, and harvest index of transgenic miraculin tomato plants and non-transgenic miraculin tomato plants cultivar moneymaker.

Plants	Number of fruits per plant(fruit)	Fruit weight (g)	Fruit diameter(cm)	Harvest index
Transgenic	14.32 ± 0.26 a	31.79 ± 0.45 a	30.99 ± 0.29 a	0.30 ± 0.01 a
Non-Transgenic	13.90 ± 0.27 a	32.94 ± 0.40 a	31.81 ± 0.34 a	0.27 ± 0.01 a

Values with the same letter in the same column are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80).

The statistical test showed that there were no significant differences in total dissolved solids, sugar contents, total carotenoids, lycopene content, and β-Carotene between the miraculin transgenic and non-transgenic tomato (Table 3). Chlorophyll content and stomatal conductance results were also not statistically different (Figure 2). This may be because of transgene does not affect biosynthesis pathway of the compound. The research conducted by Kusano et al. (2011) showed that the detected metabolites of miraculin transgenic tomato has 86% of chemical diversity listed in *Solanum lycopersicum* (LycoCyc) database, which indicates the equivalence of transgenic lines with its control [18]. Based on the limit of concern, the agronomic, compositional, and physiological traits of miraculin transgenic tomato and its counterparts were equivalent (Table 4). This showed by the equivalence value that is below the maximum threshold (<1.5) and above the minimum threshold (> 0.5). LoC is acceptability threshold, either quantitatively or qualitatively, for adverse effects on the environment [19]. For field studies, EFSA suggests an effect size of 50% as a possible LoC value [6,20].

Table 3. Total dissolved solid, sugar content, total carotenoids, lycopene content, and β-carotene of transgenic miraculin tomato plants and non-transgenic miraculin tomato.

Plant	Total dissolved solid (^{0}Brix)	Sugar content			Carotenoids (µg/g)	Lycopene (µg/g)	β-Carotene (µg/g)
		Glucose (%)	Fructose (%)	Sucrose (%)			
Transgenic	5.29 ± 0.20a	3.50 ± 0.22 a	2.60 ± 0.13 a	0.03 ± 0.002 a	14.13 ± 0.97 a	24.33 ± 1.72 a	13.95 ± 0.79 a
Non-Transgenic	5.14 ± 0.34a	3.60 ± 0.21 a	2.65 ± 0.11 a	0.03 ± 0.003 a	15.98 ± 1.07 a	29.16 ± 2.05 a	13.69 ± 0.63 a

Values with the same letter in the same column are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80).

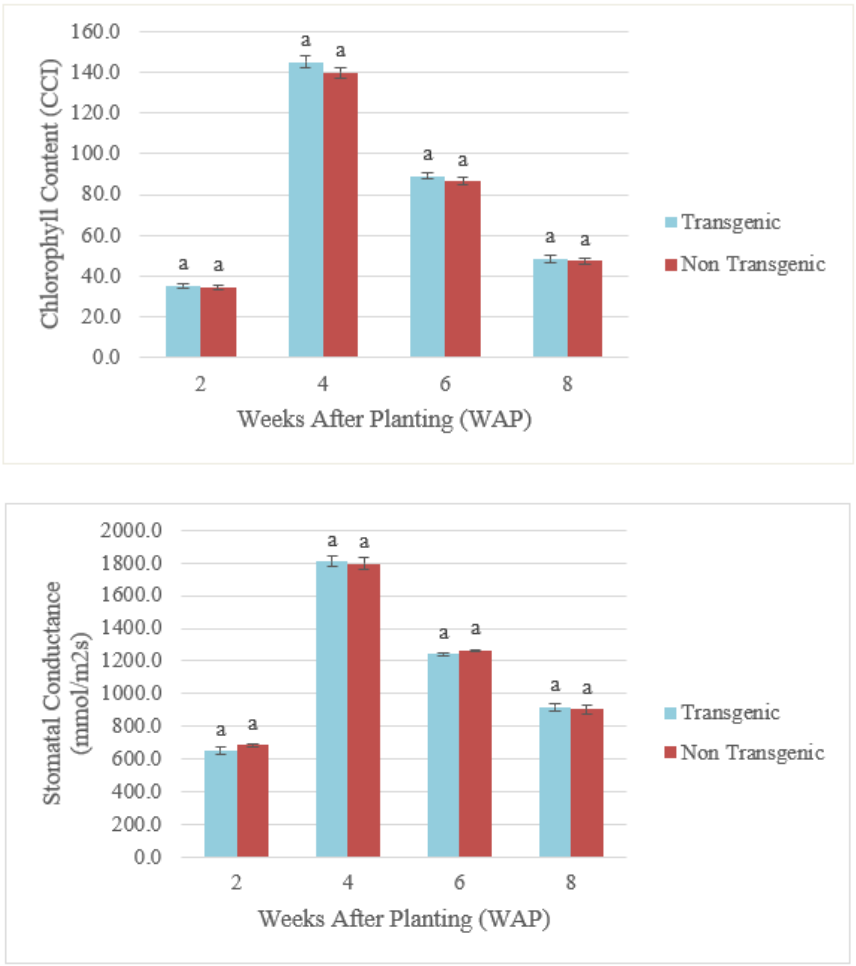

Figure 2. Chlorophyll content and stomatal conductance. Values with the same letter are not significantly different according to student's t-test. Data show mean values with standard error of the means (n = 80).

This study is consistent with the previous research conducted by Carsono et al. [14]. The result shows that the miraculin transgenic tomato was equivalent to its non-transgenic counterpart. This indicates that the occurrence of unintended effects in miraculin transgenic tomato was not detected. The possible reason is because the miraculin gene is genetically stable. Genomic southern blot analysis of transgene confirms stable inheritance of single copy miraculin gene through multiple generations. The insertion of this gene into the tomato plant genome does not cause genome rearrangement which can result in phenotypic alterations [2]. In addition, the transgene might not affect cellular function of many traits or transcription factor, other regulatory proteins or molecules affecting multiple pathways. [7].

The result is also in line with other studies. Comparative field observations of miraculin transgenic tomato and its conventional counterpart have performed in Japan from 2018–2019. Traits evaluated in these field trials included plant morphology and growth characteristics. The statistical analysis over all sites revealed no statistically significant differences [21]. In the case of other environmental risk assessment procedures, such as weediness and invasiveness potential, the miraculin transgenic tomato was also equal to its counterpart. There is no evidence that the introduced miraculin gene by the genetic modification results in increased invasiveness and allelopathic compounds of tomato [22].

Table 4. Limit of concern traits.

No.	Characteristics	Equivalence value	Percentage difference	Note
1.	Plant height (0 WAP)	0.99	1%	Eq
2.	Plant height (2 WAP)	0.98	2%	Eq
3.	Plant height (4 WAP)	1.00	0%	Eq
4.	Plant height (6 WAP)	1.00	0%	Eq
5.	Plant height (8 WAP)	1.03	3%	Eq
6.	Stem diameter (0 WAP)	1.04	4%	Eq
7.	Stem diameter (2 WAP)	1.00	0%	Eq
8.	Stem diameter (4 WAP)	0.99	1%	Eq
9.	Stem diameter (6 WAP)	0.99	1%	Eq
10.	Stem diameter (8 WAP)	1.03	3%	Eq
11.	RGR (2-0 WAP)	1.00	0%	Eq
12.	RGR (4-2 WAP)	1.04	4%	Eq
13.	RGR (6-4 WAP)	1.00	0%	Eq
14.	RGR (8-6 WAP)	0.67	33%	Eq
15.	Days to 50% Flowering	1.00	0%	Eq
16.	Days to Maturity	1.00	0%	Eq
17.	Number of flowers per cluster	1.03	3%	Eq
18.	Number of fruits per cluster	1.07	7%	Eq
19.	Number of fruits per plant	1.03	3%	Eq
20.	Fruit weight	0.97	3%	Eq
21.	Fruit diameter	0.97	3%	Eq
22.	Harvest index	1.10	10%	Eq
23.	Total dissolved solid	1.03	3%	Eq
24.	Fructose content	1.06	6%	Eq
25.	Glucose content	0.98	2%	Eq
26.	Sucrose content	1.00	0%	Eq
27.	Total carotenoids	0.88	12%	Eq
28.	Lycopene content	0.83	17%	Eq
29.	β-carotene content	1.02	2%	Eq
30.	Chlorophyll content (2 WAP)	1.02	2%	Eq
31.	Chlorophyll content (4 WAP)	1.04	4%	Eq
32.	Chlorophyll content (6 WAP)	1.04	4%	Eq
33.	Chlorophyll content (8 WAP)	1.02	2%	Eq
34.	Stomatal conductance (2 WAP)	0.95	5%	Eq
35.	Stomatal conductance (4 WAP)	1.00	0%	Eq
36.	Stomatal conductance (6 WAP)	0.98	2%	Eq
37.	Stomatal conductance (8 WAP)	1.00	0%	Eq

Remarks: WAP = weeks after planting; RGR = relative growth rate; eq = equivalence.

The difference in characteristics between transgenic plants compared to conventional plants may occur due to in vitro culture of target tissue, such as callus, and possibly due to the insertion of transgenes. This process can cause alternation in plant genomic DNA including genetic variations

(mutations), epigenetic variations, and the influence of regeneration techniques that result in somaclonal variations and gene expression due to insertional of the transgene [7,23]. This change is also due to the genetic and environmental interactions [24,25]. Environmental factors such as temperature, humidity, and rainfall affect the phenotype expression in transgenic plants [26]. In this research, there were changes in these characteristics of miraculin transgenic and non-transgenic tomato such as low average number of fruits per plant (14.30 and 13.90) and fruit diameter (30.99 and 31.81 mm). Tomato cv. Moneymaker has the average number of fruits per plant and fruit diameter which are 31.9 and 51.2 mm [27]. These characteristics still meet the principle of equivalence because the changes occur in uniform.

During the experiment, the maximum day temperature is above the optimum temperature (21–29.5 ℃) [28] reached 32 ℃. High temperature can decrease pollination efficiency including pollen viability and tomato fruit production. High temperatures can reduce the rate of DNA synthesis and inhibit the chromosome condensation process which in turn affects the failure of tetrad development during the meiosis stage [29].

In this research, the maximum humidity reached 91%. The suitable humidity level for growing tomato plants is approximately 50–70%. Tomato plants are sensitive to high humidity especially during the generative phase. This condition has impact on decreasing fruit quantity and quality. High humidity can reduce the rate of transpiration which results in the loss of plant cell turgor [28].

The high rainfall during the experiment was also a limiting factor for the growth and development of tomato. High rainfall can decrease the number of fruits due to an increase in the percentage of shed flowers by around 50% [30]. The high humid conditions due to high rainfall are suitable for the development of bacterial wilt disease. *Ralstonia solanacearum* bacteria thrive in soil during the rainy season [31]. These bacteria invade plants through the xylem vessels in the roots and produce exopolysaccharides (EPSs) which can inhibit water transportation from the roots to all plant tissues. This causes the photosynthesis process to be interrupted and the plant withers [32]. This disruption causes the unoptimal size, weight, and a number of fruits [33] and reduces the fruit dry weight and stover up to 26.9–38.2% [34].

4. Conclusions

In this study, the comparative analysis of miraculin transgenic tomato cv. Moneymaker and its origin showed no significant difference in all agronomic, compositional, and physiological traits. This indicates that the miraculin transgenic and non-transgenic tomato were equivalent. Any unintended effects were not detected. Further research is required to assess the miraculin transgenic tomato in multi-locations of field trials.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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