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EVALUATION ON INVASIVENESS AND AGRONOMIC TRAITS OF TRANSGENIC TOMATO WITH MIRACULIN GENE

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ABSTRACT

Tomato cv. Moneymaker has been engineered with a miraculin gene that can be used as an alternative sweetener since it can modify sour taste becomes sweet taste in our tongue when we are eating such tomato. Environmental safety assessment test is important step in assessing the safety of transgenic tomato before it is released to the environment, therefore it is in accordance with sustainability point of views. Evaluation was performed for invasiveness (potentially to be invasive) and agronomic traits of transgenic tomato compared to its counterpart. The evaluation of invasiveness was carried out in biosafety containment of ICABIOGRAD, Bogor by growing a mixture of transgenic and non-transgenic with 0: 100 and 100: 0 (mono-culture); 25: 75; 50: 50; and 75: 25 (poly-culture). Wet and dry biomasses of three weeks old tomato plants were then measured. Aggressiveness of transgenic miraculin tomato was equal with its counterpart as shown in its aggressiveness value -0.17 and 0 (for wet weight) and - 0.32 and 0 (for dry weight) in mixture and mono-culture, respectively. In terms of agronomic traits evaluation, it was found that no significant differences on plant height, stem diameter, fruit weight, fruit diameter and leaf area index of transgenic miraculin tomato and its non-transgenic one, suggesting transgenic miraculin tomato cv. Moneymaker is substantially equivalent to non- transgenic except for the presence of miraculin transgene.

Keywords: Agronomic traits, Environment safey assessment, Invasiveness, Miraculin, Tomato

INTRODUCTION

Transgenic tomato with miraculin gene that isolated from miracle fruits ‹*Richadella dulcifica),* a shrub native to West Africa, has been developed (Sun et a1. 2007). Miraculin was able to turn a sour taste on the tongue into a sweet taste (Kurihara and Beidler, 1968). For example, before enjoying a lemon that tastes very acidic eat the fruits which have glycoprotein miraculin, then the tongue will taste sweet. Therefore, it is also known as miraculin protein modifier sour taste to taste sweet. This will provide an alternative sweetener especially for diabetic patients and other consumers who like to search for a healthy food.

Previous studies have successfully generated tomato plant that can express miraculin under the control of 35S promoter (Sugaya et al., 2008; Sun et al., 2006, 2007). These studies have paved the way for mass producing miraculin. Different with the miraculin expressed in *Escherichia coli* (Matsuyama et al., 2009) and *Aspergillus oryzae* (Ito et al., 2007), there was no reduction in activity observed on the sweet taste miraculin expressed in plants (Hirai et al., 2010b). Genetically Modified (GM) tomato plant is considered as the most suitable plant for mass production of miraculin. Miraculin produced in relatively large quantities in transgenic tomatoes compared with transgenic strawberries (Sugaya et at., 2008), and gene silencing did not occur on the next generation as it did on transgenic lettuce (Sun et al., 2006). In addition, the miraculin expressed in tomato fruit is stable because the pH of the acid contained in the fruit of tomato (Theerasilp and Kurihara, 1989; Gancendo and Luh, 2006). In addition, tomato can also be easily cultivated in Indonesia and harvested with high productivity. The potential of transgenic plants for strengthening our food- and feed security and even energy security is very challenging for scientists all over the world. Transgenic crops have been developed to improve quality life of human by providing high valuable crops and other important agronomic traits such as resistant to biotic (pests and diseases) and a biotic stresses (drought/freezing tolerance) and others benefit traits. With advance of transgenic technology/DNA recombinant technology, genes which are previously not accessible by plant breeders, now become available for genetic improvement or breeding activities. Transgenic crop, as a product of transgenic experiment, will be an alternative choice for current and next challenges especially for dealing with unpredictable environment such as global climate changes. For that reason, some universities and public and private institutes in Japan as well as in Indonesia have actively created many transgenic crops. However only some of them have been commercialized since they have to undertake a biosafety assessment which includes assessing the impact of transgenic plant to environment. An essential step in assessing the potential impact of transgenic miraculin tomato plant to environment and human health that related to its cultivation and practices is environmental and food safety assessment. Environmental safety assessment for transgenic plants or living modified organisms (LMO) includes substantial equivalence through agronomic traits, and weediness in terms of invasiveness trait. The aim of this study was therefore to obtain the invasiveness and agronomic traits of transgenic tomato plants cv. Moneymaker compared with those of non-transgenic counterpart.

MATERIALS AND METHOD

The experiments were conducted at biosafety containment, ICABIOGRAD, Bogor in September 2013 to May 2014. Testing for invasiveness was performed according to method developed for canola

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(Agriculture and Agri-Food Canada, Food Production and Inspection Branch Plant Product Division, 1996). The invasiveness experiment was arranged in a randomized block design with five treatments (two treatments monoculture and three treatments mixture) and repeated 4 times. Varying composition of transgenic and non-transgenic tomatoes was applied i.e.: 0: 100 and 100: 0 (mono-culture); 25: 75; 50: 50; and 75: 25 (poly-culture). A number of 80 seeds were grown in plastic tray with total area 1962.5 cm^2. A wet and dry biomass of three weeks old after planting was then measured. For drying, tomato plants were stored in a drier at 55 ^oC for six days. Calculation of yield relative:

$$r_a = X_{ab}/X_{aa} \quad (1)$$
$$r_b = X_{ba}/X_{bb} \quad (2)$$

X_{ab} = biomass of transgenic (mixture)
X_{ba} = biomass of non-transgenic (mixture)
X_{aa} = biomass of transgenic (monoculture)
X_{bb} = biomass of non-transgenic (monoculture)

Relative yield total (RYT) $= r_a + r_b$ (3)
Aggressiveness (A) $= (r_a - r_b) / RYT$ (4)
A = 0 = equal competitiveness,
A = (+) = high competitiveness,
A = (-) = low competitiveness,

For agronomic traits evaluation, experiment was arranged in randomized block design with two treatments (transgenic and non-transgenic) and repeated four times. A number of 40 plants of transgenic tomato cv. Moneymaker and 40 plants of non- transgenic tomatoes cv. Moneymaker were grown in biosafety containment. Variables observed were plant height (cm), stem diameter (mm), and leaf area index.

Data for invasiveness was analysis based on calculation of invasiveness in canola plants by Plant Biosafety Office Canadian Food Inspection agency (2000). Meanwhile, agronomic trait data were analyzed by student's t-test to compare between two populations (transgenic versus non- transgenic).

RESULTS AND DISCUSSION

Result of invasiveness evaluation can be seen in Table 1 and 2. Based on Table 1 to Table 2, transgenic tomato cv. Moneymaker showed aggressiveness value -0.17 at mixture and 0 at mono-culture, respectively. This suggests transgenic tomato has low competitiveness and equal competitiveness as non- transgenic one. Wet weight of tomato plants showed biomass values -0.10 (Table 1). It means that it has a negative aggressiveness or has a low ability to compete (invasive) to other plants.

In dry weight, biomass is -0.19, aggressiveness at mixture was -0.32 and 0 (zero) at mono-culture (Table 2). Although the values of tomato biomass and its aggressiveness is positive, these results clearly indicated that transgenic tomato did not have any potential to be invasive, because its value still closed to negative and zero, so it is considered that transgenic tomato plants have equal competitiveness as non transgenic tomato plants.

The values were obtained from tomato plants in the Biosafety Containment, both biomass and aggressiveness at wet and dry weight showed all values were below or close to zero, indicating that the planting of transgenic tomato plants do not have the potential to become invasive or cultivation of transgenic tomato plants is safe even if they grown in the mixture.

Figure 1 showed a comparison on plant height between transgenic miraculin tomato and non-transgenic counterpart cv. Moneymaker. It can be seen that there was no large differences between plant height of transgenic miraculin tomato and plant height of non-transgenic tomato miraculin cv. Moneymaker.

Figure 2 showed a comparison for stem diameter between transgenic miraculin tomato with non-transgenic miraculin cv. Moneymaker. It was likely that stem diameter of transgenic miraculin tomato was equal with non-transgenic one.

Table 1. Total of Yield Relative and Aggressivenes at Mixture and Mono-culture of Wet Weight of Tomato Plants

Seed Composition	Shoot Wet Weight of Non-Transgenic	Shoot Wet Weight of Transgenic	Ra	Rb	RYT	A
100NT : 0TR (1)	254.5	0.00				
100NT : 0TR (2)	320.0	0.00				
100NT : 0TR (3)	304.0	0.00	1	0	1	0
100NT : 0TR (4)	370.0	0.00				
Average	312.13	0.00				
75NT : 25TR(1)	285.2	151.0				
75NT : 25TR(2)	202.0	31.0				
75NT : 25TR(3)	36.5	75.0	0.61	0.33	0.94	-0.33
75NT : 25TR(4)	244.0	87.5				
Rata-rata	191.93	86.13				
50NT : 50TR(1)	48.0	113.5				
50NT : 50TR(2)	158.0	161.0				
50NT : 50TR(3)	128.5	225.5	0.43	0.60	1.03	-0.17
50NT : 50TR(4)	202.0	138.5				
Average	134.125	159.63				
25NT : 75TR(1)	76.5	212.5				
25NT : 75TR(2)	141.0	184.0				
25NT : 75TR(3)	97.5	274.5	0.32	0.92	1.24	-0.01
25NT : 75TR(4)	78.5	307.0				
Average	98.38	244.50				
0NT : 100TR(1)	0.00	158.5				
0NT : 100TR(2)	0.00	248.0				
0NT : 100TR(3)	0.00	297.0	0	1	1	0
0NT : 100TR(4)	0.00	356.0				
Average	0.00	264.88				
Biomass		-0.10				
Aggressiveness	Mixture	-0.17				
	Mono-culture	0				

Note: Ra = relative results of non-transgenic tomato;
Rb = relative results of transgenic tomato;
RYT = relative yield total; A = value of aggressiveness.
Biomass = average of all aggressiveness values;
Aggressiveness at polyculture = average value of the aggressiveness of seed composition at poly-culture,
Aggressiveness at monoculture = average value of the aggressiveness of seed composition at mono-culture.

A comparison for leaf area index between transgenic miraculin tomato and its counterpart was presented on Figure 3. It was clear that no differences between leaf area index of transgenic miraculin tomato and its original.

Figure 4 showed a comparison for average fruit weight between transgenic miraculin tomatos with non-transgenic miraculin cv.

Moneymaker. It was likely that average of fruit weight of transgenic miraculin tomato was equal with non- transgenic one.

Figure 5 showed a comparison for average fruit diameters between transgenic miraculin tomato with non-transgenic miraculin cv. Moneymaker. It was likely that average of fruit weight of transgenic miraculin tomato was equal with non- transgenic one.

Results on invasiveness and weediness evaluation of this transgenic miraculin tomato were in accordance with transgenic miraculin strawberry (Sugaya *et al.,* 2008) and transgenic rice cv. Fatmawati (Carsono *et al.,* 2013). These results clearly indicated that transgenic tomato did not have any potential to be invasive, because of their values of biomass and aggressiveness are closed to negative and zero, so it is considered that transgenic tomato plants have equal competitiveness as non transgenic tomato plants.

Table 2. Total of Yield Relative and Aggressivenes at Mixture and Mono-culture of Dry Weight of Tomato Plants

Seed Composition	Shoot Wet Weight of Non-Transgenic	Shoot Wet Weight of Transgenic	Ra	Rb	RYT	A
100NT : 0TR (1)	11.3	0.0				
100NT : 0TR (2)	15.4	0.0				
100NT : 0TR (3)	14.0	0.0	1	0	1	0
100NT : 0TR (4)	21.2	0.0				
Average	15.5	0.0				
75NT : 25TR(1)	7.8	12.5				
75NT : 25TR(2)	2.2	8.4				
75NT : 25TR(3)	5.2	1.9	0.42	0.51	0.93	-0.76
75NT : 25TR(4)	10.8	5.6				
Average	6.5	7.1				
50NT : 50TR(1)	9.3	6.3				
50NT : 50TR(2)	9.3	10.3				
50NT : 50TR(3)	7.1	16.8	0.63	0.78	1.40	-0.11
50NT : 50TR(4)	13.0	9.9				
Average	9.675	10.83				
25NT : 75TR(1)	2.9	11.8				
25NT : 75TR(2)	9.0	11.5				
25NT : 75TR(3)	5.4	16.5	0.36	1.13	1.49	-0.10
25NT : 75TR(4)	5.2	23.0				
Average	5.6	15.7				
0NT : 100TR(1)	0.0	7.7				
0NT : 100TR(2)	0.0	10.3				
0NT : 100TR(3)	0.0	17.5	0	1	1	0
0NT : 100TR(4)	0.0	20.2				
Average	0.00	13.93				
Biomass		-0.19				
Aggressiveness	Mixture	-0.32				
	Monoculture	0				

Note: Ra = relative results of non-transgenic tomato;
Rb = relative results of transgenic tomato;
RYT = relative yield total; A = value of aggressiveness.
Biomass = average of all aggressiveness values;
Aggressiveness at polyculture = average value of the aggressiveness of seed composition at poly-culture,
Aggressiveness at inonoculture = average value of the aggressiveness of seed composition at mono-culture.

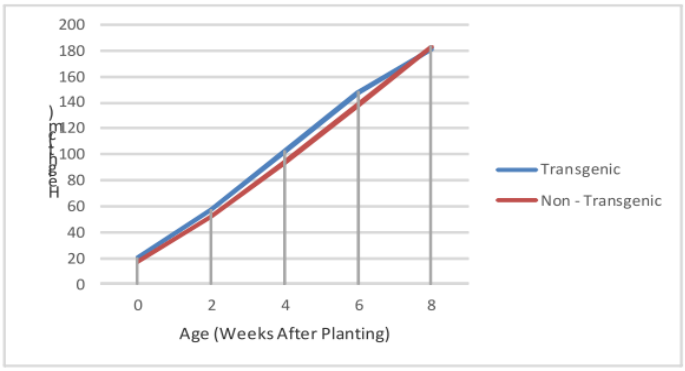

Figure 1. Comparison on Plant Height of Transgenic Miraculin Tomato Plants cv. Moneymaker and Non-Transgenic Counterpart

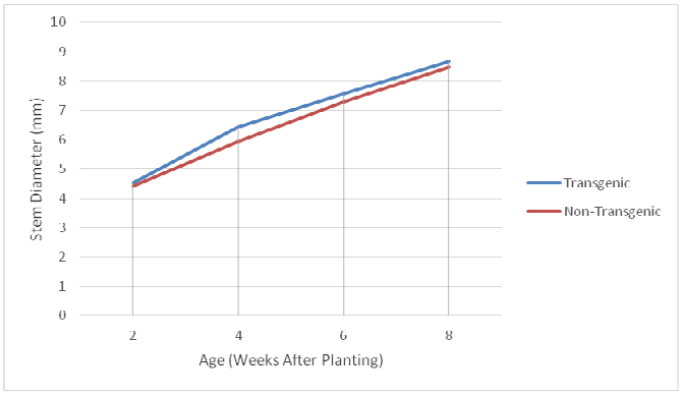

Figure 2. Comparison on Stem Diameter of Transgenic Miraculin Tomato Plants cv. Moneymaker and Non-Transgenic Counterpart

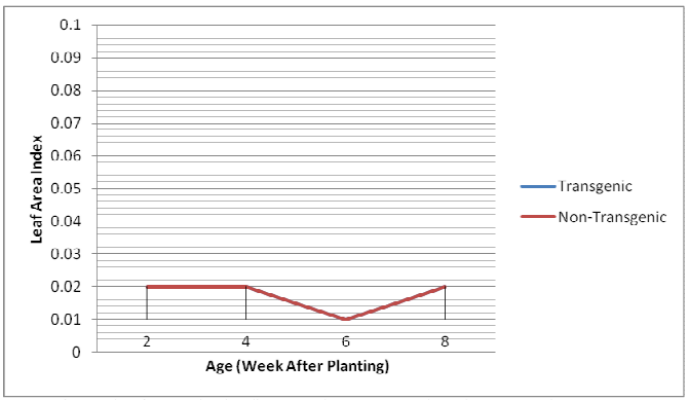

Figure 3. Comparison on Leaf Area Index of Transgenic Miraculin Tomato Plants cv. Moneymaker and Non-Transgenic Counterpart

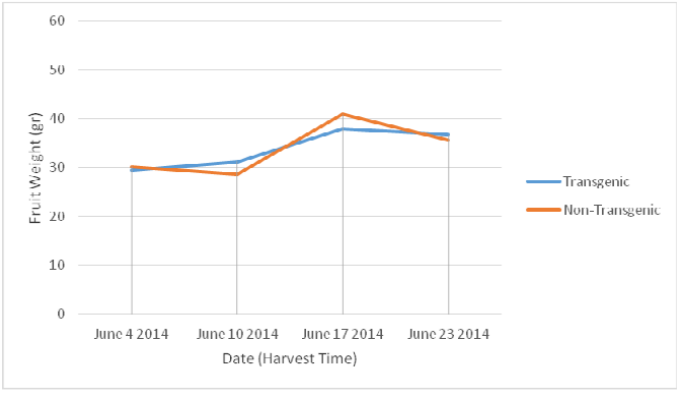

Figure 4. Comparison on Average Fruit Weight of Transgenic Miraculin Tomato Plants cv. Moneymaker and Non-Transgenic Counterpart

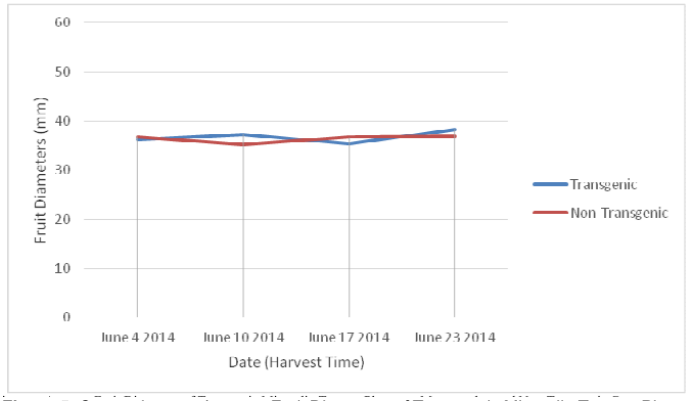

Figure 5. Comparison on Average Fruit Diameters of Transgenic Miraculin Tomato Plants cv. Moneymaker and Non-Transgenic Counterpart

CONCLUSION

Invasiveness testing showed that both biomass and aggressiveness for wet weight and dry weight in transgenic miraculin tomato with non-transgenic miraculin tomato cultivar Moneymaker were negative and close to zero. There was no significant differences on plant height, stem diameter, leaf area index, average of fruit weight and average of fruit diameter of transgenic miraculin tomatoes with those of non-transgenic original one, indicating transgenic miraculin tomato cv. Moneymaker is substantially equivalent to non-transgenic except for the presence of miraculin transgene.

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