

# Biosafety Assessment of Phytase Transgenic Potato in Isolated Field Trials

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## Motivation

Potato (*Solanum tuberosum*) is the most important noncereal food crop in the world. The transgenic potato plants, strain 2-1, were obtained by phytase-gene transformation into their original plants, Kenrebec. Here, we carried out a biosafety assessment of the transgenic potato plants harboring phytase gene in isolated field.

## Material and Methods

- Table 1 shows all items for biosafety assessment. Some of these items that are not considered to have an important environmental effect on potato, however, were excluded in this report.
- In 2004-2005 fall seasons, we first evaluated the morphological and growth characteristics, as well as the effect to ecosystem by performing biosafety assessment of the phytase transgenic potato plants in isolated field at ARI (Figure 1).
- The biosafety assessment of reproductive characteristics on transgenic potato is going to be conducted in the coming plan year.



Figure 1. The biosafety assessment experiment of the phytase transgenic potato in the isolated field at ARI.

## Results and Discussion

### 1. Morphological and growth characteristics

The results showed that the plant growth and yield characteristics of phytase transgenic potato plants were slightly lower than those of non-transgenic potato plants, but their differences were almost not significant in all growth periods (Table 2 and 3). Emergence rate was measured at 2 and 4 weeks after planting. Phytase transgenic potato showed a significantly lower emergence rate than non-transgenic potato at 4 weeks after planting (Table 4).

### 2. Environmental effect

#### (1) Influence on soil microflora

The populations of soil microorganisms were not significantly different between transgenic and non-transgenic potato planting area (data not shown here). Thus, planting phytase transgenic potato had a limited impact on soil microorganisms.

#### (2) Influence on pests and insects

We surveyed visiting insects at the transgenic and non-transgenic potato fields, such as *Myzus persicae*, *Liriomyza bryoniae*, *Spodoptera litura*, *Pieris rapae crucivora*, *Spodoptera exigua*, *Bemisia argentifolii*, and *Thrips palmi*. The population count of Aphid (*Myzus persicae*) was the highest among visiting insects at potato field. There was no difference in Aphid communities between transgenic and non-transgenic potato (Figure 2).

Both transgenic and non-transgenic plants were sensitive to disease incidence of early blight from *Alternaria solani*, although transgenic plants showed a little higher infection rate (87.23%) than non-transgenic plants did (75.21%). Thus, the impact of phytase transgenic potato on pests and insects communities was limited.

Table 1. Items for biosafety assessment on transgenic potato

Evaluation items	Full-containment greenhouse	Semi-containment greenhouse	Isolated greenhouse	Isolated nethouse	Isolated field
1. Confirmation of existence and expression of introduced genes: (1) Existence of the selection maker gene (2) Existence of the phytase gene (3) Expression of the phytase gene (4) Resistance to disease	■				○
2. Morphological and growth characteristics: (1) Morphological characteristics					●
3. Reproductive characteristics: (1) Pollen morphology (2) Pollen fertility (3) Pollen dispersal by wind or insects (4) Longevity of pollen (5) Seed fertility (6) Seed germination (7) Cross compatibility with allied species (8) Pollen scattering range (9) Perenniality	○	○			○
4. Production of allelochemical-like substances: (1) Phenolic acids produced in leaves and stems (2) Phenolic acids released from roots (3) Production of volatile compounds (4) Influence of soil to succeeding crop	○				○
5. Effect to ecosystem: (1) Influence on soil microflora (2) Survey of visiting entomofauna					●
6. Residual Agrobacterium as vector:	○				●

■, finished previously; ●, performed in 2004-2005; ○, being performed in the coming plan year; ○, will be performed in the future plan.

Table 2. The growth and yield characteristics of transgenic and non-transgenic potato plants in different growth periods during the fall season in 2004

Weeks after planting	Cultivar <sup>2</sup>	Main stem length (cm)	Lateral stem no./plant	Upper dry matter		Tuber weight (g/plant)	Tuber number / plant			
				leaves	stems		Total	Small (<50g)	Medium (50-100g)	Large (>100g)
7	WT	31.3±1.2	2.0±0.2	6.8±0.9	3.0±0.3	60.4±10.2	3.3±0.2	3.1±0.1	0.3±0.1	0.0±0.0
	2-1	30.9±0.9	2.0±0.2	7.5±0.7	3.4±0.4	64.6±11.3	3.4±0.4	3.3±0.4	0.1±0.1	0.0±0.0
9	WT	38.5±1.3	1.6±0.2	12.3±0.9	3.6±0.3	215.2±23.9	4.5±0.5	2.6±0.3	1.2±0.3	0.7±0.2
	2-1	38.1±1.0	1.8±0.2	11.8±1.6	3.2±0.4	193.5±19.2	4.3±0.8	2.4±0.7	1.4±0.2	0.4±0.1
14	WT	44.6±1.1	2.2±0.2	8.5±0.5	5.8±0.4	351.8±13.4	4.4±0.2	1.9±0.2	1.1±0.1	1.4±0.1
	2-1	42.4±1.3	2.0±0.2	8.4±0.7	4.8±0.3	339.4±25.8	4.4±0.3	2.0±0.2	1.0±0.1	1.3±0.1

Data indicate mean and its standard error of 6 replicates with each 3, 3, and 20 samples at 7, 9 and 14 weeks after planting, respectively. <sup>2</sup>WT, wild type (non-transgenic) potato (Kenrebec); 2-1, transgenic potato (strain 2-1). For each growth period, no significant difference between cultivars was detected by LSD-test at 5% level.

Table 3. The growth and yield characteristics of transgenic and non-transgenic potato plants in different growth periods during the fall season in 2005

Weeks after planting	Cultivar <sup>2</sup>	Main stem length (cm)	Lateral stem no./plant	Upper dry matter		Tuber weight (g/plant)	Tuber number / plant			
				leaves	stems		Total	Small (<50g)	Medium (50-100g)	Large (>100g)
5	WT	34.0±1.2	2.3±0.2	5.4±0.4	1.4±0.1	12.3±2.6	5.1±0.5	5.1±0.5	0.0±0.0	0.0±0.0
	2-1	32.3±0.9	1.9±0.4	5.0±0.6	1.2±0.3	8.6±4.0	5.0±0.6	5.0±0.6	0.0±0.0	0.0±0.0
8	WT	37.2±1.6	1.5±0.3	6.7±0.7	1.5±0.2	100.8±13.1	3.6±0.2	2.8±0.3	0.8±0.2	0.0±0.0
	2-1	35.4±1.2	1.7±0.2	6.3±0.2	1.2±0.1	99.7±6.6	3.7±0.4	2.9±0.5	0.8±0.1	0.0±0.0
15	WT	38.1±0.9	2.6±0.2	15.4±0.8	6.9±0.5	388.0±26.6	5.0±0.4 a	2.1±0.3	1.4±0.2 a	1.5±0.1
	2-1	36.1±0.8	2.3±0.1	14.0±0.9	4.8±0.7	336.3±24.4	4.1±0.5 b	1.6±0.4	1.1±0.2 b	1.4±0.1

Data indicate mean and its standard error of 6 replicates with each 5, 4, and 20 samples at 5, 8 and 15 weeks after planting, respectively. <sup>2</sup>WT, wild type (non-transgenic) potato (Kenrebec); 2-1, transgenic potato (strain 2-1). For each growth period, no significant difference between cultivars was detected by LSD-test at 5% level, excluding tuber number at harvest; a,b indicate significant difference between cultivars at 5% level.

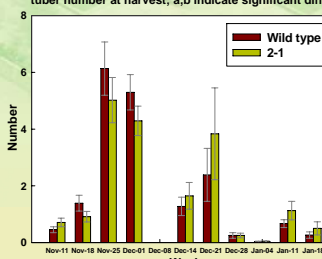


Figure 3. The population count of Aphid (*Myzus persicae*) at the transgenic and non-transgenic potato field in 2005.

Table 4. The emergence rate of transgenic and non-transgenic potato in 2005 fall season

Cultivar <sup>2</sup>	emergence rate (%)	
	2 WAP <sup>3</sup>	4 WAP
WT	58.3±8.9 a	95.5±2.0 a
2-1	56.2±8.3 a	90.8±1.9 b

<sup>1</sup>Data indicate mean and its standard error of 6 replicates. <sup>2</sup>WT, wild type (non-transgenic) potato (Kenrebec); 2-1, transgenic potato (strain 2-1). <sup>3</sup>WAP, weeks after planting. Within columns, means followed by the different letter are significantly different by LSD-test at 5% level.

