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REPRODUCTIVE FITNESS OF OUTCROSSED HYBRIDS BETWEEN TRANSGENIC BROCCOLI (*BRASSICA OLERACEA*) CARRYING THE IPT TRANSGENE AND CONVENTIONAL VARIETIES OF KALE, BROCCOLI AND CAULIFLOWER

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Abstract

Pollens are potential carriers for genetically modified crops to transfer genetic materials horizontally to other plants. For phanerogams, pollen viability and cross-compatibility are critical factors for successful outcross hybridization. To evaluate this possibility, this project investigated pollen viability and pod setting rate by comparing broccoli (*Brassica oleracea* L. var. *italica* Planck) and broccoli transformed with the isopentenyl transferase (*ipt*) gene. Both served as pollen donors and four other varieties as pollen receptors to determine outcross rates. For pollen viability, F_1 progeny was higher ($p \leq 0.05$) for the cross of transgenic *ipt* broccoli with Li Syue significantly by FDA (fluorescein diacetate) assay. Higher successful hybrids were observed for transgenic *ipt* broccoli with Fu Yue, Li Syue and Green King. As pollen properties, number and grain diameter were significantly larger ($p \leq 0.05$) in hybrid combinations of transgenic *ipt* broccoli with Li Syue and Green King significantly ($p \leq 0.05$). The pod setting rates were higher while transgenic *ipt* broccoli served as donor plant. These results analyzing pollen properties between transgenic crops with possible outcross candidates would serve as one of those critical strategies for evaluating environmental biosafety issues for transgenic crops.

Introduction

Transgene escape from genetically modified (GM) corps to Non-GM corps is a big issue for environmental and regulatory concerns (Shinwari *et al.,* 2004). The horizontal gene transfer occurs between transgenic crops and relatives mostly through pollen dispersal (Conner, 2003). Once the GM crops hybridize with weedy relatives and confer biotic/abiotic resistance to them. This may enhance the fitness and survival of weeds, and therefore cause tremendous impact to ecological system (Ellstrand, 2001; Philip *et al.,* 2002).

In vegetable production, *Brassica oleracea* is the species of self-incompatible crop which riches in vitamins, dietary fiber and anti-cancer compounds, such as glucoraphanin and 3', 3'-diindolylmethane and has therefore become an important vegetable crop worldwide (Chakrabarty *et al.,* 2002; Jeffery *et al.,* 2003). Broccoli (*Brassica oleracea* L. var. *italica* Planck), kale (*Brassica oleracea* L. var. *alboglabra*) and cauliflower (*Brassica oleracea* L. var. *botrytis*) are very popular in Taiwan and grown in autumn to provide as vegetable foods, while seed production companies grow kale year around, and broccoli and cauliflower in autumn to support seed production industrial. Our previous research for possible outcross among these popular cultivars focused on overlapped flowering periods, and hybridization rates. Overlapping blooming periods for possible outcross ranged from 3 to 28 days, and outcross rates were observed as 86-100%, 1.1- 42.5% and 0-9% by artificial, pollinator (bee), and natural pollination, respectively (Ting *et al.,* 2010). Further investigations on seed properties altered due to transgenic effects are required to achieve better biosafety evaluation even florets of these crops, such as broccoli and cauliflower, were harvested way before seeds were fully developed.

Outcrosses among Brassica species were reported that three ancestral diploid Brassica species, *B. rapa*, *B. nigra* and *B. olerecea* are capable of hybridizing in all combination to produce *B. juncea*, *B. napus* and *B. carinata* (U, 1935). Even most interspecific hybridization in Brassica family produced immature seeds because of failure of endosperm development. Nevertheless, incompatible interspecific hybridization could still fertilize normally and produced a few seeds (Nishiyama *et al.,* 1991). All these results suggested that transgene introgression could happen between either inter- or intra-specific relatives (Warwick *et al.,* 2003; Sutherland *et al.,* 2006).

Moreover, with the developing genetic engineering, there are many transgenic modified Brassica crops have been created, such as *B. napus* with increasing fatty acid content (Kuntson *et al.,* 1992; Voelker *et al.,* 1992), herbicide resistance (Miki *et al.,* 1990), heavy mental tolerance (Misra *et al.,* 1989), and fungal disease resistance (Bangash *et al.,* 2013; Broglie *et al.,* 1991), while *B. oleracea* with pest resistance (Jin *et al.,* 2000; Chakrabarty *et al.,* 2002), herbicide resistance (Waterer *et al.,* 2001), disease resistance (Kuvshinov *et al.,* 2001), and delayed flower yellowing (Chen *et al.,* 2001). The other concern is the potential impact of introgression between GM and Non-GM plant populations through inter- or intra-specific hybridization may result in increasing weediness (Lu & Snow, 2005; Ahmad *et al.,* 2013), expansion into new habitats (Rieseberg *et al.,* 2007), and assimilation of wild species (Wolf *et al.,*

2001). Therefore, these genetic modified plants would have certain impacts to the environment.

In order to investigate possible environmental impacts by possible outcrosses between genetic broccoli and other popular varieties, broccoli transformed with *ipt* (gene coded for isopentenyltransferase) was employed to serve as transgenic crop (designated as *ipt* broccoli) in this experiment. The ipt transgenic broccoli was previously transformed with *ipt* (isopentenyl transferase) gene driven by a SAG-13 (senescence-associated-gene) promoter to delay senescence. Upon the call of senescence, this *ipt* was expressed and resulted in elevated endogenous cytokinin levels in broccoli that exhibited delayed floral yellowing (Chen *et al.,* 2001).

The potential of elevated cytokinin levels would alter field traits based on its physiological effects in plant growth and development. Other than previous reports on outcross effects on fitness, such as first backcross generation from interspecific hybridizing *B. napus* with the weedy relative *B. rapa* showed high fertility (Mikkelsen *et al.,* 1996), and fitness changes in *B. napus* and hybrids are promoted by herbicide resistant gene drift, the cytokinin effects would alter the pollen properties itself and seed development. All these information would contribute to part of the evaluation for biosafety. It will be interesting to reveal potential effects of transgenic *ipt* broccoli on environmental adaption, and consequently altered the fitness of hybrids by investigating outcrosses with kale, broccoli and cauliflower.

Materials and Methods

Transgenic *ipt* T_0 broccoli plants were obtained by *Agrobacterium tumefaciens*-mediated transformation of the Green King cultivars with pSG766A. This plasmid harbors a chimerical construct over expressing *ipt* (isopentenyl transferase) gene driven by a senescence-associated gene promoter (SAG-13). The kanamycin resistance *npt*II gene with a 35S promoter was used as a selectable marker (Chen *et al.,* 2001; Chan *et al.,* 2009). The *npt*II served a functional antibiotic marker gene used for selection during tissue culture. All elements inside the T-DNA region can be detected by performing polymerase chain reaction (PCR). The T_5 inbred broccoli lines used in this project were created by self-pollination within transgenic lines. The broccoli transformed with the *ipt* gene was designated as transgenic *ipt* broccoli. The non-transgenic broccoli served as control and four closely related varieties, the Chinese kales (*B. oleracea* var. *alboglabra*) 'Fu Yue' (white flower) and 'Bai Ge Lin' (yellow flower), cauliflower (*B. oleracea* var. *botrytis*), 'Li Syue' and broccoli (*B. oleracea* var. *italica*) 'Green King' were used for evaluating the pollen viability and pod setting rate.

Greenhouse experiments: Broccoli seeds from the transgenic broccoli and non-transgenic parental line were pretreated on filter papers for three days, followed by exposure to moist conditions in the laboratory before being planted. Subsequently, the seeds were planted in a pot (4.5 cm × 4.5 cm × 5 cm). The 3-wk-old greenhouse seedlings with four to five leaves were transplanted into a tray (26 cm × 29 cm). Prior to transplantation, N, P_2O_5 and K_2O fertilizers were applied to the test plot at 270, 150, and 200 kg ha^{-1}, respectively. A total of four applications were administered every 20 days. The cultivation of all broccoli were performed in chamber rooms with artificial light control for one month (day / night temperatures were set to 20°C / 10°C, and subsequently they were maintained in an isolated greenhouse until bolting. Transgenic and non-transgenic broccolis were used as pollen donors (male), and four closely related varieties were served as pollen receptors (female). The artificial pollinated flowers were all emasculated before anthesis. The seeds were harvested and planted out for the evaluation of pollen viability of the F_1 progenies.

Analysis for the *ipt* gene of F_1 progenies: Half of the collected seeds were sampled when the number of F_1 hybrid seeds was less than 1000. In the case of collected seeds number was over 1000, one third of seeds were sampled (the maximum sampling error is ± 1.9% under 95% confidence level). All sampled seeds were grown and subjected to further analysis. Total genomic DNA was extracted using GeneMark kit. The PCR reaction was performed using the PCR Master Mix II (5X) kit (GeneMark) to detect the presence of the *npt*II and *ipt* genes. Forward 5'-GAGGCTATTCGGCTATGACTG-3' and reverse 5'-ATCGGGAGCGGCGATACCGTA-3' primers were used to amplify a 700-bp fragment of the *npt*II gene (Chen *et al.,* 2001). The thermal cycles were run with (C1000, Biored) and set as follows: initial denaturation at 94°C for 5 min and followed by 25 cycles of 94°C for 1 min, 58°C for 1 min, and a final elongation step at 72°C for 2 min. For the detection of the *ipt* insert, forward 5'-ACCCATGGACCTGCATCTA-3' and reverse 5'-GGAGCTCAGGGCTGGCGTAACC-3' (Li *et al.,* 1992) were used to amplify a 750-bp fragment. The thermal cycles were set as follows: initial denaturation at 94°C for 2 min, followed by 25 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, and a final elongation cycle at 72°C for 7 min.

Pollen numbers and diameter for donor plants and F_1 hybrids: The pollen grains from donor, receptor and F_1 hybrids were collected separated from mature flowers by tapping pollen from flowers into 1.5 ml tubes. For analyzing pollen number per flower, 1 ml of sterile water was added into each tube and shaken in a mixer for 10 min to suspend pollen in the water. For data collection, 4 plants were sampled and 4 flowers from each plant (n=16). Remove the lens into the eyepiece micrometer. The stage micrometer was engraved on 1 mm of 100 small grids and each grid width of 0.01 mm (10 μm). When one end of the moving stage micrometer scale to overlap into line with the eyepiece micrometer, the eyepiece micrometer small grid size calculated as follows: 10 μm × (number of stage micrometer grids / number of eyepiece micrometer grids). The 4 plants and 10 pollen grains of per plant on average were measured for pollen diameter (Snow *et al.,* 2010).

Hybrids of viability for donor and F_1: Pollen esterase activity and cell membrane integrity were assessed by FDA (fluorescein diacetate) staining. During blooming period, matured pollens were collected. A total of 5 mg of FDA was dissolved in 1 ml of acetone and added to a 16% sucrose solution, and 20 µl was applied to the anther pollen. The FDA-treated pollen was observed under a fluorescence microscope (Heslop-Harrison & Heslop-Harrison, 1970). For examining the pollen viability, 4 plants and 4 flowers per plant were tested. The pollen was observed under an inverted microscope.

Pod setting and seed numbers per pod for donor and F_1 hybrids: Transgenic *ipt* broccoli and non-transgenic broccoli (designated as CK, served as control) were used as pollen donors and four closely related varieties, the Chinese kales (*B. oleracea* var. *alboglabra*) 'Fu Yue' (white flower) and 'Bai Ge Lin' (yellow flower), cauliflower (*B. oleracea* var. *botrytis*), 'Li Syue' and broccoli (*B. oleracea* var. *italica*) 'Green King' were served as pollen receptors followed by artificial pollination, and the pods were subsequently harvested to evaluate the potential outcross. Four plants and 15 flowers per plant were used for both donors and receptors. One receptor plant was then pollinated by one donor plant.

$$\text{Pod setting (\%)} = \frac{\text{Number of pods produced}}{\text{Number of hand pollinated flowers}} \times 100$$

Data analyses: Data were analyzed by analysis of variance (ANOVA) and the least significant difference (LSD) test using the SAS 9.1 software (Anon., 2004).

Results

Pollen traits, viability, pod setting and seed number per pod between transgenic *ipt* and non-transgenic broccoli: As the trait of pollens, current results showed that transgenic broccoli exhibited more pollens per flower than non-transgenic broccoli significantly ($p<0.05$), however the pollen grain diameters were not significantly different (Table 1). The pollen viability of transgenic broccoli was higher than non-transgenic broccoli ($p<0.05$), while the pod setting rate and seed number exhibited no difference compared with non-transgenic broccoli (Table 2).

Table 1. Comparison of the pollen numbers and diameter between transgenic *ipt* broccoli and non-transgenic broccoli.

Donor	Pollen no./flower	Pollen grain diameter (µm)
Transgenic line	916.0 ± 5.7 a[a]	38.5 ± 1.3 a
Non-transgenic line	639.2 ± 16.6 b	38.0 ± 1.1 a

[a] Mean ± standard error (n=4), n means number of plant and 4 flowers of per plant on average were measured for pollen numbers; the pollen diameter were measured of 10 pollen grains of per plant. The means given within a column for each line followed by the same letters are not significantly different at the 0.05 probability level based on the LSD test

Pollen traits of F_1 progenies: For F_1 hybrids, pollen number of between transgenic and non-transgenic broccoli crossed with Bai Ge Lin and Li Syue were no significant different (Table 3). However, pollen number of F_1 progenies showed significant difference ($p<0.05$) when Green King and Fu Yue served as pollen recipients. As the property of pollens, the diameters of pollen grain were ranged from 30.5 to 40.8 µm, and no significant difference was observed among F_1 progenies descended from transgenic and non-transgenic broccoli crossed with Fu Yue and Bai Ge Lin. Only two occasions showed significant larger diameters ($p<0.05$) when the F_1 progenies from transgenic broccoli crossed with Li Syue and Green King.

Table 2. Comparison of the pollen viability, pod setting rate and seed numbers per pod between transgenic *ipt* and non-transgenic broccoli.

Donor	FDA (%)	Pod setting rate (%)	Seed no./Pod
Transgenic line	75.4 ± 3.4 a[a]	57.9 ± 3.2 a	1.8 ± 0.1 a
Non-transgenic line	62.1 ± 2.4 b	54.3 ± 7.5 a	1.5 ± 0.2 a

[a] Mean ± standard error (n=4), n means number of plant and 4 flowers of per plant on average were measured for pollen viability; the pollen diameter were measured of 15 flowers per plant. A receptor was pollinated by one donor. The means given within a column for each line followed by the same letters are not significantly different at the 0.05 probability level based on the LSD test

Table 3. Comparison of the pollen numbers and diameter among F_1 progenies from transgenic *ipt* broccoli and non-transgenic broccoli crossed with four closely related varieties.

Pollen recipient	Pollen donor	F_1 progeny	
		Pollen no./flower	Pollen grain diameter (µm)
B. oleracea var. *alboglabra*– Fu Yue	Transgenic line	3931.2 ± 6.9 a[a]	36.8 ± 0.7 a
	CK	1062.0 ± 8.7 b	35.4 ± 0.5 a
B. oleracea var. *alboglabra*– Bai Ge Lin	Transgenic line	3878.0 ± 13.5 a	37.3 ± 0.5 a
	CK	3948.0 ± 14.9 a	38.0 ± 1.4 a
B. oleracea var. *botrytis*– Li Syue	Transgenic line	1581.2 ± 13.2 a	40.8 ± 1.1 a
	CK	1626.0 ± 15.3 a	30.5 ± 0.3 b
B. oleracea var. *italica*– Green King	Transgenic line	1503.2 ± 15.2 b	37.0 ± 1.1 a
	CK	1662.8 ± 14.3 a	32.3 ± 1.3 b

[a] Mean ± standard error (n=4), n means number of plant and 4 flowers of per plant on average were measured for pollen numbers; the pollen diameter were measured of 10 pollen grains of per plant. The means given within a column for each pollen recipient followed by the same letters are not significantly different at the 0.05 probability level based on the LSD test

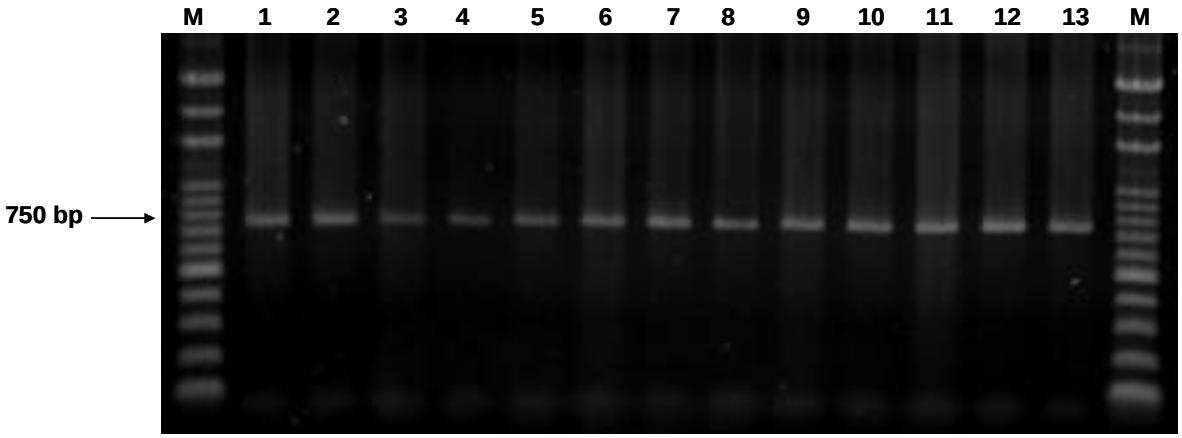

Fig. 1. PCR result for the identification of the *ipt* sequence in the F_1 progenies from transgenic *ipt* broccoli crossed with closely related varieties. Line 1-12: Progenies of closely related varieties x transgenic broccoli; Line 13: Positive control.

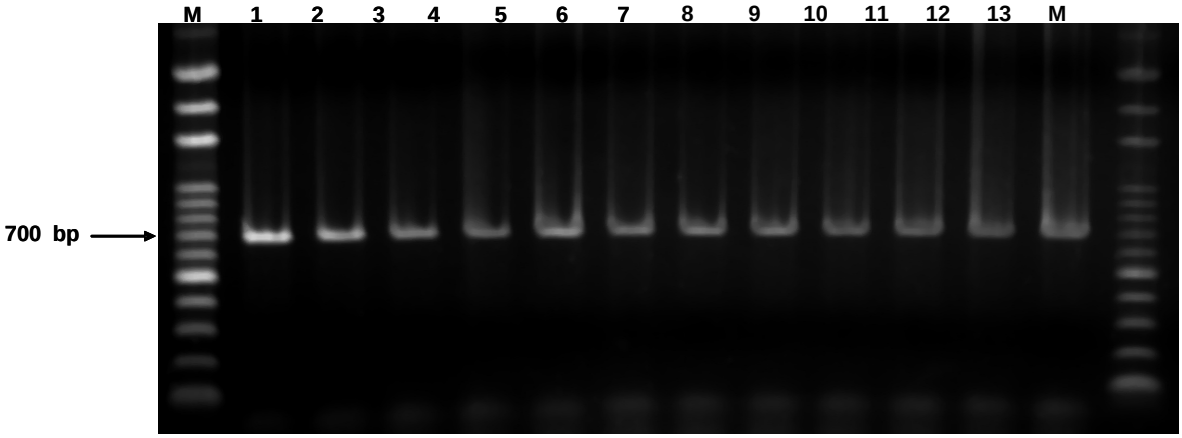

Fig. 2. PCR result for the identification of the *nptII* sequence in the F_1 progenies from transgenic *ipt* broccoli crossed with closely related varieties. Line 1-11: Progenies of closely related varieties x transgenic broccoli; Line 12: Positive control. (transgenic line).

Pollen viability, pod setting and seed numbers per pod of F_1 progenies: To confirm the presence of transgenic *ipt* sequence in transgenic broccoli, the results of PCR screening for *ipt* (750 bp) of F_1 progenies were showed in Fig. 1, and *npt*II (700 bp) in Fig. 2. All pollen viabilities of F_1 progenies were apparently higher ($p<0.05$) when donor parent was transgenic broccoli based on the results of FDA tests (Table 4). For both analyzed pod setting or seed number , except for Green King, pollen viability of F_1 progenies descended from Fu Yue, Bai Ge Lin and Li Syue crossed with transgenic broccoli were all higher compared with those of non-transgenic broccoli ($p<0.05$).

Discussion

Popular brassica cultivars including broccoli, cauliflower, kale and cabbage, were massively sown in autumn and bloomed in the following spring to meet the seed requirement in Taiwan and many other countries. Moreover, in these few years, there have been developing many GM broccolis and valuable varieties all over the world.

For the possible issue on biosafety evaluation for broccolis, some biological factors influencing successful pollination include the ability of the donor plant to produce viable pollens and the duration time for the pollen viability. These two major factors would contribute to the probability of gene flow. If the competitive ability of the pollen grain is poor, its capacity to compete with fresher pollen produced in the vicinity of the receptor plant will be poor. Pollen viability can vary greatly between species but is also dependent on environmental variables, such as temperature and humidity. Pollen viability has been evaluated using a variety of staining techniques (e.g., tetrazolium salts to detect dehydrogenase activity, aniline blue to detect callose in pollen walls and pollen tubes, iodine to determine starch content, and fluorescein diacetate to determine esterase activity and plasma membrane integrity), in vitro and in vivo germination tests, and by analyzing final seed set (Adhikari & Campbell, 1998; Dafni & Firmage, 2000). The analysis method depends on the species and crop specific characteristic (Dafni & Firmage, 2000). A directed method to assess the pollen viability is important for studying the fitness of transgenic crops.

Table 4. Comparison of the pollen viability, pod setting rate and seed numbers per pod among the F_1 progenies from transgenic *ipt* broccoli and non-transgenic broccoli (CK) crossed with four closely related varieties.

Pollen recipient	Pollen donor	F_1 progeny		
		FDA (%)	Pod setting rate (%)	Seed no./Pod
B. oleracea var. *alboglabra*– Fu Yue	Transgenic line	69.4 ± 2.7 a[a]	96.3 ± 1.5 a	17.7 ± 1.7 a
	CK	43.6 ± 3.0 b	74.2 ± 6.3 b	1.4 ± 0.1 b
B. oleracea var. *alboglabra*– Bai Ge Lin	Transgenic line	60.4 ± 2.8 a	90.0 ± 5.6 a	15.7 ± 1.7 a
	CK	33.7 ± 2.3 b	70.6 ± 1.2 b	1.5 ± 0.1 b
B. oleracea var. *botrytis*– Li Syue	Transgenic line	83.6 ± 1.9 a	98.4 ± 1.1 a	15.1 ± 1.1 a
	CK	37.5 ± 2.8 b	69.8 ± 4.3 b	1.5 ± 0.1 b
B. oleracea var. *italica*– Green King	Transgenic line	80.1 ± 1.8 a	30.3 ± 4.6 a	4.1 ± 1.0 a
	CK	34.4 ± 2.4 b	34.9 ± 6.1 a	3.2 ± 0.6 a

[a] Mean ± standard error (n=4), n means number of plant and 4 flowers of per plant on average were measured for pollen viability; the pollen diameter were measured of 15 flowers per plant. A receptor was pollinated by one donor. The means given within a column for each pollen recipient followed by the same letters are not significantly different at the 0.05 probability level based on the LSD test

In this current study and previous report, strategies for biosafety evaluation were attempted to analyze adaptive strategies in *Brassica* by counting the average pollen grain number per flower and the diameter (Yoshihito *et al.,* 2008). Basically, the chance of pollen-mediated gene transfer is closely related to the number and diameter of the pollen from the donor. In our results, the pollen number in transgenic *ipt* broccoli was significantly higher than that of the non-transgenic broccoli ($p<0.05$, Table 2). However, the pollen number of the transgenic F_1 progenies varied with different pollen recipient parents that were cultivar dependent ($p<0.05$, Table 3). The pollen diameter of transgenic *ipt* broccoli showed no significant difference compared with those of the non-transgenic broccolis ($p<0.05$, Table 1), however, the pollen diameters of F_1 progenies from different pollen recipients exhibited similar or higher width ($p<0.05$, Table 3). The results of pollen grain diameter analysis revealed that pollen morphology might be altered during outcrossed with varied species or relatives, and the larger the pollen, the greater the possibility for survival. Some possibilities for altered nature of pollen may due to the inserting of foreign DNA sequence or by crossing from parents with different genetic backgrounds (Wei *et al.,* 2005).

For analysis of pollen activity, staining methods were employed for the advantages of quick and easy handling. These methods have been very useful for quick determination of pollen activity even the results of these techniques might be influenced by the nature of tested pollen samples, and also affected by incubation condition (Ali Khan & Perveen, 2014). Base on the results shown in Table 2, the pollen viability of the transgenic broccoli was higher than that of the non-transgenic line ($p<0.05$) as determined by FDA staining. When comparing the F_1 progenies descended from transgenic and non-transgenic broccolis, the results showed that F_1 progenies from transgenic broccoli as parents all exhibited higher pollen viability ($p<0.05$, Table 4). This staining method is feasible for determining the pollen activity to assess biosafety evaluation for outcrossing potential of transgenic *ipt* broccoli.

Another concern regarding the transformed *ipt* gene would contribute to all these altered reproductive properties. There was no direct evidence can be draw from current project; nevertheless cytokinin biosynthesis was promoted or endogenous cytokinin accumulations were observed whenever *ipt* was expressed in flowers (Chang *et al.,* 2003). Other differences of field performances were also observed, such as plant growth, development, stress tolerance and senescence. Higher yield in the transgenic lines compared with non-transgenic lines (Chan *et al.,* 2009; Liu *et al.,* 2011). Accumulation of stress responsive protein was observed at harvest in transgenic *ipt* broccoli (Liu *et al.,* 2011). Taken altogether, the results from pollen viability evaluation showed that the donor parent transgenic broccoli, Chinese kales (Fu Yue and Bai Ge Lin) and cauliflower of F_1 progenies may have better survival ability due to their enhanced viability, pod setting rate and seed number (Table 4). All these results suggested that these outcrossed F_1 progenies would have better chance to survive based on increased fitness to environment. These results would be helpful for the information required for biosafety evaluation.

The pod setting of transgenic *ipt* broccoli was significantly higher than those of non-transgenic lines ($p<0.05$, Table 2), and similar result for transgenic F_1 hybrid compared with non-transgenic F_1 hybrid ($p<0.05$, Table 4) except for F_1 hybrid from transgenic line crossed with Green King. Seed number analyses were not significant different for both transgenic and non-transgenic parents, while transgenic F_1 hybrid was significantly higher ($p<0.05$, Table 4) than non-transgenic F_1. However, F_1 hybrid from transgenic line crossed with Green King showed no difference ($p<0.05$, Table 4). There were variations among cultivars we tested. Hybrids from broccoli x broccoli showed lower fitness (except for pollen viability) compared with kale x broccoli or cauliflower x broccoli. This results would due to the hybrids obtained from broccoli x broccoli that were intra species (*Brassica oleracea* L. var. *italica* Planck) hybridization sharing common genetic background, while hybrids from kale x broccoli or cauliflower x broccoli

were hybridization between two different species leading to heterosis (outbreeding enhancement). Previous studies showed that sometimes hybrid fitness is higher than both parents (Hauser *et al.,* 2003), while different results in other situation (Arriola & Ellstrand, 1997).

The possibility of successful outcrossing is species, or cultivars dependent. Previous report shown the cross between same genus (*Brassica napus* x *Brassica juncea*) exhibiting hybrid vigor (Huangfu *et al.,* 2011), while Brassica species fail to produce seeds when outcrossed with other relatively species within same genus in our experiment (data not shown). There are several approaches for interspecific hybrids with marker analysis revealed substantial homology between the A-, B- and C-genomes for Brassica (Fayyaz *et al.,* 2014; Prkash & Chopra, 1990; McGrath & Quiros, 1991). In the case of high degree of homology between the A-genomes of the two species and that an exchange of genes, located on the A-genomes, can occur relatively unimpeded by recombination (Huangfu *et al.,* 2011). Crossing within the Brassica complex has shown that sometimes hybrid fitness is higher than both parents (Hauser *et al.,* 2003). This variation in fitness likely reflects the magnitude of genetic differences between their parents, the frequency of genes with antagonistic effects. Hybridization between different crops and their wild-type species occurs at different rates, and even crossed with the same crop but under different conditions, the ratio is different (Mikkelsen *et al.,* 1996; Chèvre *et al.,* 1997, 2000). Moreover, unpredictable transformation results of altered traits due to random insertions of DNA sequence might collaborate into any functional elements such as promoter, enhancer and endogenous genes and thereby changed their traits. Similar situation was observed in the results, the activity of the pollen resulting from the hybridization of the transgenic and non-transgenic broccoli with its related species were consistent with parents, while the numbers of pod setting and seed showed some variations.

In summary, this report focused on pollen biology using transgenic *ipt* broccoli as a study model. Significantly better pollen viability, pod setting rate and seed number were obtained whenever the donor parent is transgenic broccoli, Chinese kale (Fu Yue and Bai Ge Lin) and cauliflowers of F_1 progenies than those of non-transgenic under our experiment condition.

Conclusions

These results analyzing pollen properties between transgenic crops with possible outcross candidates would serve as one of those critical strategies for evaluating environmental biosafety issues for transgenic crops. Moreover, in order to mimic real situation in nature environment, data were collected from overlapped blooming periods and other hybridization percentage between transgenic *ipt* broccoli and its close relatives by artificial, pollinator (bees) and natural pollination of broccoli in Taiwan was published (Ting *et al.,* 2010). Transgenic *ipt* inbred lines also shown better growth, yield and shelf-life compared with non-transgenic inbred line and parental variety (Chan *et al.,* 2009). Taking altogether, these research results not only provided information on the fitness of transgenic broccoli but also revealed possible impacts of transgenic broccoli to our environment.

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