



### บรรณานุกรม

- สำนักส่งเสริม และจัดการสินค้าเกษตร กรมวิชาการเกษตร. 2546. การพัฒนาองค์กร และเกษตร  
ดีที่เหมาะสม (GAP), น. 11-55. ใน การอบรมสัมมนาโครงการพัฒนาสับปะรด ครั้งที่ 3.  
มหาวิทยาลัยเกษตรศาสตร์, กรุงเทพฯ.
- Bhowmik, G. and Bhagabat, A. 1975. Self incompatibility studies in pineapple (*Ananas  
comosus* L.). Indian J. Agric Sci. 19:259-265.
- Coppens d'Eeckenbrugge, G., M.F. Duval and Van Miegroet, F. 1993. Fertility and self-  
incompatibility in the genus *Ananas*. Acta Hort. 334:45-51.
- Davey, M.R., Sriporaya, S., Anthony, P. And J.B Power. 2004. Microprojectile-mediated  
transformation of pineapple. In I.S.Curtis (ed.), Transgenic Crops of the World-  
Essential Protocols, 187-197. Kluwer Publishers, The Netherlands.
- FAO. 2003. Pineapple Production. Food and Agriculture Organization Yearbook  
Production. 52: 161-162.
- FAO. 2007. FAOSTATISTIC DIVISION. <http://www.faostat.fao.org>.
- FAO. 2009. FAOSTATISTIC DIVISION. <http://www.faostat.fao.org>.
- Sriporaya, S., J.B. Power, M.R. Davey and K.C. Lowe. 1999. Towards the genetic  
manipulation of pineapple (*Ananas comosus*): somatic embryogenesis. Journal  
of Experimental Botany, 50: 19. (Suppl).
- Sriporaya, S., Marchant, R., J.B. Power and Davey M.R. 2001. Herbicide tolerant  
transgenic pineapple (*Ananas comosus*) produced by microprojectile  
bombardment. Annals of Botany, 88: 597-603.
- Sriporaya, S. (2001). Genetic Manipulation of Pineapple (*Ananas comosus*). Ph.D.  
Thesis, University of Nottingham, UK.
- Sriporaya, S, S.Keawsompong, P.Insupa, J.B.Power M.R.Davey and P.Srinives. 2006.  
Transgene stability and expression of genetically modified pineapple (*Ananas  
comosus*) under experimental field conditions. Plant Breeding. 125 (4):411-  
413.

Sripaoraya, S. 2009: Pineapple hybridization and selection in Thailand. ISHS Acta Hort. **822**, 57-62.

Sripaoraya, S., Marchant, R., Power, J.B. and Davey, M.R. (2003). Plant regeneration by somatic embryogenesis and organogenesis in commercial pineapple (*Ananas comosus* L). *In Vitro Cell and Developmental Biology-Plant* ,39: 450-454.

## Output ที่ได้จากโครงการ

1. ได้สืบประวัติพันธุ์ลูกผสมชั่วที่ 1 ที่มีศักยภาพเป็นพันธุ์ลูกผสมที่ดีซึ่งเป็นพันธุ์ที่มีลักษณะดี ดังนี้
  - 1.1 ด้านทานสารกำจัดวัชพืชในกลุ่ม Glufosinate ammonium เช่นที่มีชื่อการค้า Bialaphos, Basta X<sup>®</sup> (ในประเทศไทยใช้ชื่อการค้าว่า Basta X<sup>®</sup>)
  - 1.2 มีลักษณะทางเขตรกรรมดีเช่นมีความต้านทานโรค ขอบใบเรียบไม่มีหนามหรือมีเฉพาะปลายใบเหมือนพันธุ์ปัตตาเวีย
  - 1.3 มีคุณภาพทั้งความหวาน เนื้อมีกลิ่นหอม กรอบ เนื้อผลเป็นสีทอง เหมือนพันธุ์ภูเก็ต มีตามลย่อยต้นเหมือนพันธุ์ปัตตาเวีย
  
2. ได้องค์ความรู้เรื่องการถ่ายทอด Transgene คือยีนต้านทานสารกำจัดวัชพืชไบลาฟอส (*bar gene*) ในสับปะรด ซึ่งเป็นงานวิจัยชิ้นแรกที่ทำและกำลังจะออกตีพิมพ์เผยแพร่ โดยมีการถ่ายยีน *bar* จากสับปะรดตัดแปรพันธุกรรมไปสู่สับปะรดพันธุ์การค้า และการถ่ายทอดยีนดังกล่าว เป็นไปตามกฎเมนเดลในสับปะรดลูกผสมชั่วที่ 1 องค์ความรู้ที่ได้นี้กำลังลงตีพิมพ์เผยแพร่

## ภาคผนวก

### 1. Manuscript 1

Manuscript for Journal **เรื่อง** Inheritance of the Bialaphos Resistance (*Bar*) Gene from Genetically Modified Pineapple (*Ananas comosus* L.) to Commercial Cultivars ซึ่งได้รับการตอบรับการลงตีพิมพ์หลังมีการแก้ไข (will be acceptable after moderate revision) และได้ส่งฉบับแก้ไขกลับไปแล้ว ดังเอกสารแนบ

### 2. Manuscript 2

Manuscript for Proceeding of the VII<sup>th</sup> International Pineapple Symposium **เพื่อจะ** ลงพิมพ์ใน Acta Horticulturae **เรื่อง** F<sub>1</sub> Hybrid Pineapple Resistant to Bialaphos Herbicide

### ตารางภาคผนวก

**ตารางภาคผนวกที่ 1** ค่าไค-สแควร์ของลักษณะต้านทานสารกำจัดวัชพืชไบล่าฟอสของสับปะรดลูกผสมชั่วที่ 1 ที่แสดงความต้านทานสารกำจัดวัชพืชไบล่าฟอสและสับปะรดลูกผสมชั่วที่ 1 ที่แสดงความไม่ต้านทานสารกำจัดวัชพืชไบล่าฟอส

Character	Observed	Expected	O-E	(O-E) <sup>2</sup> / E
Herbicide resistance	507	465	42	1.89
Herbicide sensitive	423	465	-42	1.89
Total	930	930	0	3.78 <sup>NS</sup>

df = 2-1 , p < 0.05

**ตารางภาคผนวกที่ 2** เปอร์เซ็นต์การตายของใบสับปะรดหลังฉีดพ่นสารกำจัดวัชพืช Basta X<sup>®</sup> ที่อัตราความเข้มข้น 400 มล./ไร่ ที่ 3, 7 และ 14 วัน ณ คณะเกษตรศาสตร์ ต.ทุ่งใหญ่ อ.ทุ่งใหญ่ จ.นครศรีธรรมราช ปี พ.ศ.2553

พันธุ์	เปอร์เซ็นต์การตายของใบสับปะรดหลัง ฉีดพ่นสารกำจัดวัชพืช Basta-x <sup>®</sup> (วัน)			ผลรวม พันธุ์	ค่าเฉลี่ย
	3	7	14		
พันธุ์ภูเก็ต	30	70	100	200	66.67
พันธุ์ปัตตาเวีย	30	70	100	200	66.67
สับปะรดตัดแปรพันธุ์กรรม	0	0	0	0	0
สับปะรดลูกผสมF <sub>1</sub> (PV x TP)	0	0	0	0	0
สับปะรดลูกผสมF <sub>1</sub> (TP x PV)	0	0	0	0	0
สับปะรดลูกผสมF <sub>1</sub> (PV x PK)	30	70	100	200	66.67
สับปะรดลูกผสมF <sub>1</sub> (PK x PV)	30	70	100	200	66.57
ผลรวมเปอร์เซ็นต์การตาย ของใบ	120	280	400	800	28.57
ค่าเฉลี่ย	17.14	40	57.14		

1 **Inheritance of the Bialaphos Resistance (*Bar*) Gene from Genetically Modified Pineapple**  
2 **(*Ananas comosus* L.) to Commercial Cultivars**

3  
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14  
15 **Abstract**

16 Inheritance of the Bialaphos resistance (*bar*) gene was investigated by sexual  
17 hybridization of the transgenic pineapple cv. 'Phuket' (TP) with the commercial pineapple cvs.  
18 'Pattavia' (PV) and 'Phuket' (PK). Direct crosses, reciprocal crosses, and selfings were made. A  
19 total of 2,437 F<sub>1</sub> seeds, resulted in 1,459 plants were obtained from direct and reciprocal crosses  
20 of TP with PV and PK with PV. Of these, 1172 F<sub>1</sub> seeds and 886 F<sub>1</sub> plants were obtained from  
21 crossing between TP and PV. All selfed parental plants, and both direct and reciprocal crosses  
22 between TP and PK, either failed to set seed or set seeds with no kernels. β-glucuronidase (GUS)  
23 activity as conferred by the *gus* gene, was used in the initial screening for gene transfer into F<sub>1</sub>

24 plants at 2 cm in height. Two hundred and fifty-three from 886 plants obtained from sexual  
25 crosses involving PV and TP parents were checked for GUS activity and 116 were GUS positive  
26 while 137 were GUS negative, giving a ratio of 1:1 by a Chi-square test. When grown in pots,  
27 the GUS positive versus GUS negative F<sub>1</sub> plants showed a corresponding 1 : 1 ratio of resistance  
28 to sensitivity when sprayed with the herbicide Basta™. The hemizygous transgenic plants *bar*/-  
29 transmitted the *bar* gene to the normal -/- (coin herewith as nullizygous) plants in the Mendelian  
30 manner. Thus the *bar* transgene can be fully utilized in the future conventional pineapple  
31 breeding programs.

32

33 **Key words:** transgenic pineapple, inheritance, hybridization, transgene, *bar* gene

34

## Introduction

35

36 Pineapple (*Ananas comosus* L.) is one of the most popular and important fruits in the  
37 world. In 2007, world production of pineapple was 18.87 million tons with an average yield of  
38 19.749 t ha<sup>-1</sup> from a total harvested area of 955,646 ha. Thailand was a major producer and  
39 exporter, generating 2.32 million tons from 96,081 ha, with an average yield of 24.144 t ha<sup>-1</sup>  
40 (FAO, 2009). Thailand is the largest exporter of pineapple products, sending around 600,000  
41 metric tons to the world market, representing about 43% of the world's processed pineapple.

42 Two common pineapple cultivars are grown in Thailand. The most commercially  
43 important cv. for both canning and fresh fruit is 'Pattavia' which is classified into the Cayenne  
44 group. The second cv., 'Phuket', is classified in the Queen group and grown extensively in  
45 southern Thailand for fresh fruit market. Other cvs. that are cultivated in a smaller scale include  
46 'Nanglae' or 'Nampeng' (Cayenne group), 'Intarachit Daeng' and 'Intarachit Khao' (Spanish  
47 group). The cv. Pattavia usually has spineless leaves and flat eyes, in contrast to Phuket which  
48 has spiny leaves and deep eyes. Pineapple has low genetic variation, particularly within the  
49 Cayenne group, partly due to self-incompatibility. Sanewski and Giles (1997) reported a failure  
50 in selective breeding to control blackheart in 'Smooth Cayenne' due to sterility factors.  
51 Consequently, advance in varietal improvement has been very slow. Until recently, the use of  
52 conventional sexual hybridization in combination with biotechnological approaches have helped  
53 accelerating progress in genetic improvement of this fruit crop.

54 Most pineapple breeders make crosses between pineapple groups to generate viable F<sub>1</sub>  
55 hybrids to increase heterosis (hybrid vigor). However, traditional pineapple breeding consumes  
56 time and yet crosses can be made from only between different varieties or groups. The offspring  
57 share characteristics of both parents, and genes are transferred randomly from the parents to the

58 new variety. Current biotechnology, such as tissue culture combined with genetic engineering,  
59 can be exploited to increase genetic variation within pineapple germplasm. In addition, the  
60 biotechnological techniques enable pineapple breeders to target trait loci and thus increase the  
61 efficiency of pineapple breeding for some traditional intractable agronomic problems such as  
62 herbicide and drought resistance, control flowering and ripening trait (Botella et al., 2000), and  
63 to control blackheart disease (Ko et al., 2006). These techniques can directly introduce the  
64 foreign or novel DNA into potentially regenerative cells. Transgenic pineapple plants have been  
65 generated carrying agronomically useful transgenes. An example is the development of plants  
66 genetically modified for Bialaphos herbicide resistance (Sripaoraya et al., 2001), and  
67 subsequently assessed for stability and expression of herbicide resistance under field condition  
68 (Sripaoraya et al., 2006).

69 The present experiment was undertaken as an extension of the previous investigations  
70 (Sripaoraya et al., 2001; Sripaoraya et al., 2006), in order to assess the feasibility of transferring,  
71 by sexual hybridization, agronomically useful genes from transformed plants into commercial,  
72 non-transgenic pineapples. This approach should help introgressing genetic variation into  
73 commercial cultivars. The donor plants were those transformed previously by Biolistics-  
74 mediated gene delivery of the construct pHAC25, carrying the  $\beta$ -glucuronidase (*gus*) and  
75 Bialaphos resistant (*bar*) genes, into leaf bases of the cv. Phuket (Sripaoraya et al., 2001). The  
76 *bar* gene encodes phosphinothricin acetyltransferase (PAT) which converts the active  
77 component, phosphinothricin (PPT), to a non-herbicidal acetylated form by transferring the  
78 acetyl group from acetyl CoA to the free amino group of PPT. This investigation gives the first  
79 report on inheritance of a transgene pineapple into its progeny.

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81

82

## Materials and Methods

83 *Plant materials and sexual hybridization*

84 Transgenic plants of the cv. Phuket (TP) that exhibited stable expression of the *bar* gene under  
85 experimental field conditions (Sripaoraya et al., 2006) were micropropagated from leaf bases  
86 using the culture technique as described earlier by Sripaoraya et al. (2003). Micropropagated TP  
87 plants were transferred to pots and used for sexual hybridization with commercial pineapple  
88 cultivars. Suckers of non-transformed plants of the commercial cvs. Pattavia (PV) and Phuket  
89 (PK), lacking *bar* and *gus* genes (both with the expected genotypes  $-/-$ ,  $-/-$  coin herewith as  
90 double nullizygous), were grown for sexual hybridization using the method of Sripaoraya (2009).  
91 Direct and reciprocal crosses were made between the parental plants (i.e. TP×PV, PV×TP;  
92 TP×PK, PK×TP, PV×PK and PK×PV). The parents were also selfed (i.e. pollinated between  
93 plants of the same cv. TP×TP, PV×PV, PK×PK).

94

95 *Seed germination and seedling establishment*

96 Ripening fruits were harvested, the seeds were collected and germinated according to Sripaoraya  
97 (2009). The  $F_1$  seeds obtained were sown in trays of sand for germination and left grow for 2  
98 months. Seedlings, each 2 cm in height were transferred to plastic bags containing a mixture of  
99 equal parts of coconut sheet (coir) and clay soil.

100

101 *Confirmation of transgene expression in the  $F_1$  progeny*

102 The *gus* and *bar* genes were linked on the same transformation construct (pHAC25) used to  
103 transform plants of the cv. Phuket (Sripaoraya et al., 2001). The histochemical GUS was assayed

104 according to Jefferson et al. (1987) to analyze for transgenes stability and the GUS activity in  
105 order to initially select for F<sub>1</sub> seedlings carrying the transgene. F<sub>1</sub> seedlings, each 12 cm in  
106 height, were sprayed with the commercial herbicide Basta™ (Bayer, Germany) containing  
107 glufosinate ammonium as the active ingredient, at the commercially recommended dose (5 lha<sup>-1</sup>)  
108 in order to confirm the herbicide resistance of the F<sub>1</sub> plants. Chi-square analysis was applied to  
109 determine the segregation ratio of *gus* expression and *bar* gene inherited from TP to commercial  
110 pineapple cultivars. Since TP and PV parents are expected to carry the genotype of *bar*<sup>-/-</sup> and  
111 <sup>-/-</sup>, respectively, the segregation of F<sub>1</sub> progeny is expected to fit a 1: 1 ratio of hemizygous *bar*<sup>-/-</sup>  
112 to nullizygous <sup>-/-</sup>.

113

## 114 **Results and Discussion**

### 115 *Production of F<sub>1</sub> seeds and seedlings*

116 A total of 2,437 F<sub>1</sub> seeds were obtained from sexual hybridization (Table 1). Viable F<sub>1</sub> hybrid  
117 seeds were obtained only from sexual crossing (Fig. 1a-c) of TP with PV and the reciprocal cross  
118 PV with TP, and between the non-transformed parents PV with PK and the reciprocal cross, PK  
119 with PV. Selfing of the transgenic plants (TP×TP) did not generate viable seeds. Likewise,  
120 selfing of non-transformed plants (PV×PV and PK×PK) failed to produce viable seeds. Any  
121 seeds produced through selfing were lacking in kernels and not viable. This confirmed that self-  
122 incompatibility in pineapple occurs in the non-transformed pineapples (Bhowmik and Bhagabat,  
123 1975; Py et al., 1987; Coppens d'Eeckenbrugge et al., 1993; Leal and Coppens d'Eeckenbrugge,  
124 1996; Sripaoraya, 2009). Our work is the first to report on self-incompatibility in transformed  
125 pineapple. The same self-incompatibility was observed from the cross between TP and its non-  
126 transgenic progenitor PK (Table 1). Of the seeds that were produced, 1,459 seedlings were

127 obtained from direct and reciprocal crosses of TP×PV and PV×PK (Table 1, Fig. 1d,e). One  
128 thousand one hundred and seventy-two F<sub>1</sub> seeds and 886 F<sub>1</sub> plants were obtained from both  
129 crosses between TP and PV parents. Eight hundred and ninety-nine F<sub>1</sub> seeds and 728 F<sub>1</sub> plants  
130 were obtained from PV×TP. Since TP was grown in pots, flowers and seeds set from the  
131 reciprocal cross TP×PV were not good, giving 273 F<sub>1</sub> seeds and 158 F<sub>1</sub> plants. Crossing between  
132 normal PV×PK and PK×PV gave a normal seed set of 594 and 653 seeds, and 235 and 338  
133 seedlings, respectively.

134

#### 135 *Expression of the gus and bar genes*

136 GUS histochemical staining of leaves showed that, of 158 seedlings obtained from the TP×PV,  
137 72 were GUS positive revealing stable expression of transgene, while 86 of them failed to  
138 express the *gus* gene (Table 1). The difference in number of seedlings showing *gus* expression  
139 was not significant by Chi-square analysis. Similarly, of 95 seedlings obtained from the  
140 reciprocal cross, PV×TP, 44 were positive for GUS activity, while 51 were GUS negative. Ten  
141 sampled F<sub>1</sub> seedlings, each from the normal parents PV×PK and PK×PV were all GUS negative  
142 with the expected nullizygous -/- in both *gus* and *bar* loci.

143

144 Expression of the *bar* gene was confirmed by spraying the F<sub>1</sub> seedlings with the commercial  
145 herbicide Basta™. All GUS positive seedlings were also herbicide tolerant, retained their  
146 chlorophyll and remained healthy following application of the herbicide. In contrast, the GUS  
147 negative plants were not tolerant to the application of Basta™ and died (Fig. 1f). This confirmed  
148 that *bar* gene was inherited into the F<sub>1</sub> plants through sexual hybridization.

149

150 *Inheritance of Bialaphos resistance in F<sub>1</sub> plants*

151 Chi-square analysis showed that the GUS positive and GUS negative plants exhibited a 1:1 ratio  
152 following Mendel's Law of Inheritance. The same expected ratio was also found for the *bar* gene  
153 controlling Bialaphos resistance. All GUS positive plants are herbicide tolerant indicated that *gus*  
154 and *bar* genes are transmitted together as linked genes. Such information is essential for  
155 assessing the inheritance of transgenes from genetically modified pineapple plants to non-  
156 transgenic plants. This is the first paper that reports inheritance of transgene in pineapple. Since  
157 pineapple takes at least five years from crossing between pineapple varieties to obtain F<sub>1</sub> fruit.  
158 Pineapple propagation by suckers required 13-15 months for Queen group such as Phuket  
159 cultivar or 18-24 months for Cayenne group such as Pattavia cultivar. For crossed seed  
160 propagation, at least 3.5 years per crop is used for pineapple production. So, there is no paper  
161 reported about inheritance of transgene in pineapple thus far. However, in annual crops such as  
162 maize, soybean and rice, inheritance of transgenes were reported up to 5 - 6 generations and the  
163 transgenes were found inherited following Mendel's Law. For example, *mcry3A* transgene in  
164 maize transferred from *Bacillus thuringiensis* var. *tenebrionis* (B.t.t) was inheritable and stable  
165 during the study for five generations. In generation five (T<sub>5</sub>), individual plants were assayed for  
166 the presence of corn rootworm resistance for *mcry3A* gene. The expected Mendelian inheritance  
167 ratio of positive and negative plants for hemizygous triat in T<sub>5</sub> population is 3:1 (Syngenta,  
168 2009). The surviving F<sub>1</sub> seedlings were grown and used for selfing and backcrossing to all  
169 parents in order to further evaluate inheritance of the *bar* gene. F<sub>1</sub> plants exhibiting herbicide  
170 resistance showed segregation in plant morphology and agronomic characteristics such as leaf  
171 spiny, number of suckers and slips and virus wilt resistance. Some progenies showed spiny  
172 leaves as Phuket cultivar while some had smooth leaves with spiny tip as Pattavia. There are

173 slips in some progenies whereas both parents, Phuket and Pattavia, have no slip. Some progenies  
174 produced more aerial suckers than Pattavia but less than Phuket. All F<sub>1</sub> progenies showed  
175 resistance to virus wilt and heart rot diseases. These F<sub>1</sub> progenies will be selected based on their  
176 agronomic characters, including fruit yield and quality. The potential F<sub>1</sub> plants will be multiplied  
177 by tissue culture and evaluated for superior F<sub>1</sub> hybrids in the future.

### 178 **Conclusions**

179 The Bialaphos resistance (*bar*) gene from transgenic pineapple is inherited as a single gene into  
180 commercial, non-transformed pineapple cultivars. F<sub>1</sub> plants showed a 1: 1 ratio of herbicide  
181 resistant to susceptible plants following the Mendelian inheritance.

182

### 183 **Acknowledgement**

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185

### 186 **References**

- 187 Bhowmik, G. and A. Bhagabat. 1975. Self incompatibility studies in pineapple (*Ananas*  
188 *comosus* L.). Indian J. Agric Sci. 19: 259-265.
- 189 Botella, J.R., A.S. Cavaiiario and C.I. Cazzonelli. 2000. Towards the production of transgenic  
190 pineapple to control flowering and ripening. In: S. Subhadrabandhu and P. Chairidchai,  
191 (eds) Proc 3rd Int Pineapple Symp. Acta Hort. 529:115–122.
- 192 Coppens d'Eeckenbrugge, G., M.F. Duval and F. Van Miegroet. 1993. Fertility and self-  
193 incompatibility in the genus *Ananas*. Acta Hort. 334: 45-51.
- 194 FAO. 2009. FAOSTATISTIC DIVISION. <http://www.faostat.fao.org>.
- 195 Jefferson, R.A., T.A. Kavanagh and M.W. Bevan. 1987. GUS fusions:  $\beta$ -glucuronidase

- 196 as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* 6:3901–3907.
- 197 Ko, H.L., P.R. Campbell, M.P. Jobin-D'ecor, K.L. Eccleston, M.W. Graham and M.K. Smith.  
198 2006. The introduction of transgenes to control blackheart in pineapple (*Ananas*  
199 *comosus* L.) cv. Smooth Cayenne by microprojectile bombardment. *Euphytica* 150: 387–  
200 395.
- 201 Leal, F. and G. Coppens d'Eeckenbrugge. 1996. Pineapple, pp. 515-557. In: J. Janick and J.N.  
202 Moore, eds., *Fruit Breeding, Vol. I: Tree and Tropical Fruits*. John Wiley and Sons Inc.,  
203 New York, USA.
- 204 Py, C., J.J. Lacaevilhe and C. Teisson. 1987. *Pineapple, Cultivation and Uses*. Maisonneuve and  
205 Larose, Paris.
- 206 Sanewski, G.M. and J. Giles. 1997. Blackheart resistance in three clones of pineapple  
207 (*Ananas comosus* (L.) Merr.) in subtropical Queensland. *Aust J Exp Agric.* 37: 459–  
208 461. Sripaoraya, S., R. Marchant, J.B. Power and M.R. Davey. 2001. Herbicide-tolerant  
209 transgenic pineapple (*Ananas comosus*) produced by microprojectile bombardment. *Ann.*  
210 *Bot.* 88: 597-603.
- 211 Sripaoraya, S., R. Marchant, J.B. Power and M.R. Davey. 2003. Plant regeneration by somatic  
212 embryogenesis and organogenesis in commercial pineapple (*Ananas comosus* L.). In  
213 *Vitro Cell Dev. Biol.-Plant* 39: 450-454.
- 214 Sripaoraya, S., S. Keawsompong, P. Insupa, J.B. Power, M.R. Davey and P. Srinives. 2006.  
215 Transgene stability and expression of genetically modified pineapple (*Ananas comosus*)  
216 under experimental field conditions. *Plant Breed.* 125: 411-413.
- 217 Sripaoraya, S. 2009. Pineapple hybridization and selection in Thailand. *Acta Hort.* 822: 57-62.
- 218 Syngenta. 2009. MIR604 Maize, Syngenta Agrisure™ RW Maize for Corn Rootworm Control.

219

Syngenta Brochur 2009. Syngenta International AG, Switzerland. [www.syngenta.com](http://www.syngenta.com).

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221 **Table 1** Number of F<sub>1</sub> seeds and seedlings generated, GUS activity and Chi-square analysis of  
 222 the number of F<sub>1</sub> plants from sexually crossing transgenic pineapple of the cv. Phuket (TP) with  
 223 non-transformed plants of the cvs. Pattavia (PV) and Phuket (PK). The results of crossing  
 224 between non-transformed cvs. and selfing are also presented.  
 225

Crosses	No. of seeds	No. of seedlings	Seedlings assessed for GUS activity	Number of F <sub>1</sub> seedlings		$\chi^2$ (1:1)	Apparent genotype of F <sub>1</sub> seedlings <sup>c</sup>	
				GUS <sup>+</sup> <sup>a</sup> BastaR <sup>b</sup>	GUS <sup>-</sup> <sup>a</sup> BastaS <sup>b</sup>		<i>gus</i> /- <i>bar</i> /-	<i>gus</i> /- <i>bar</i> /-
TP×PV	273	158	158	72	86	1.24 <sup>NS</sup>	<i>gus</i> /- <i>bar</i> /-	<i>gus</i> /- <i>bar</i> /-
PV×TP	899	728	95	44	51	0.52 <sup>NS</sup>	<i>gus</i> /- <i>bar</i> /-	<i>gus</i> /- <i>bar</i> /-
PV×PK	594	235	10	-	10	-	- /- - /-	- /- - /-
PK×PV	653	338	10	-	10	-	- /- - /-	- /- - /-
PK×TP	14	0	-	-	-	-		
TP×PK	0	-	-	-	-	-		
TP×TP	0	-	-	-	-	-		
PV×PV	2	0	-	-	-	-		
PK×PK	2	0	-	-	-	-		
∑(PV×TP)	1,172	886	253	116	137	1.74 <sup>NS</sup>		
Total	2473	1,459	273	116	157	-		

226

227 <sup>a</sup> GUS<sup>+</sup>: GUS positive, GUS<sup>-</sup>: GUS negative.

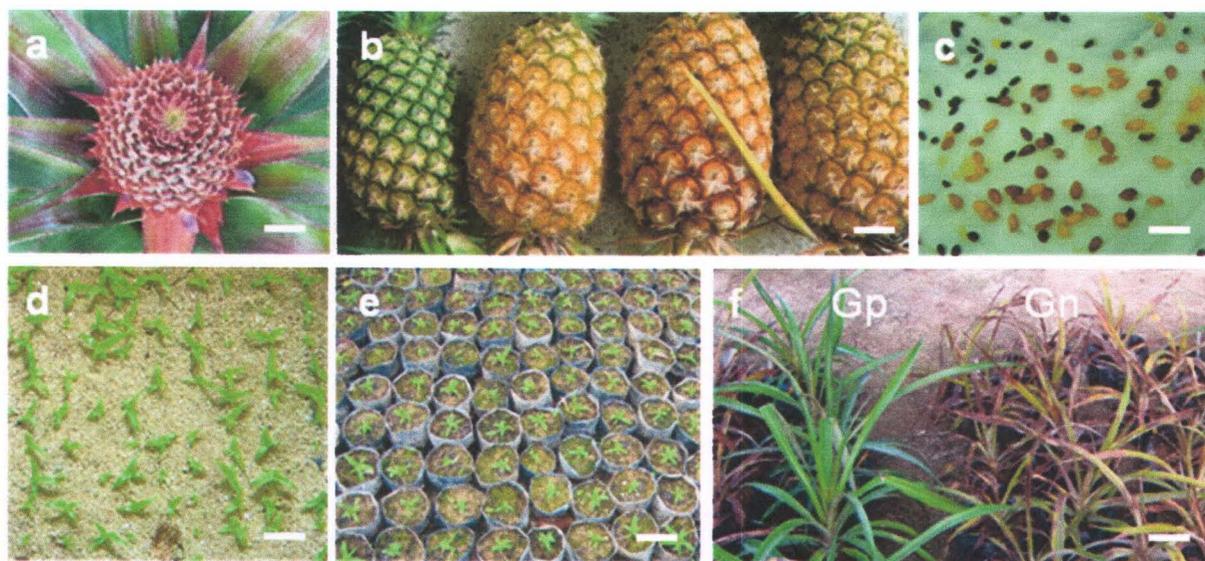
228 <sup>b</sup> BastaR: Bialaphos-resistant, BastaS: Bialaphos-sensitive.

229 <sup>c</sup> *gus* : *gus* gene, *bar* : bialaphos resistant gene.

230 NS: not significant.

231 **Fig. 1** Hybridization of transgenic with non-transformed pineapple plants, and germination of F<sub>1</sub>  
232 seeds. (a) Inflorescence of a transformed plant of the cv. “Phuket” (TP; Bar = 1.4 cm), (b) Fruits  
233 obtained from PV×TP (Bar = 2.7 cm), (c) F<sub>1</sub> seeds from direct (TP×PV) and reciprocal crosses of  
234 PV×TP (Bar = 6 mm), (d) Seedlings of PV×TP 1 month after seed germination (Bar = 2 cm), (e)  
235 Seedlings 1 month after potting in a coir/soil compost in plastic bags and transfer to the  
236 glasshouse (Bar = 5.7 cm). (f) F<sub>1</sub> seedlings sprayed with the herbicide Basta™. All GUS-  
237 positive (Gp) plants were tolerant and retained their chlorophyll (two weeks after application of  
238 the herbicide). GUS-negative plants (Gn) were not tolerant to Basta™, became necrotic and died  
239 (Bar = 3.8 cm).

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## **REFeree EVALUATION SHEET**

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**Manuscript : TJ-AGR-1209-39**

**Date 21 August 2010**

**Title : Sexual transmission of the bialaphos resistance (Bar) gene from genetically modified pineapple (*Ananas comosus* L.) to commercial cultivars**

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### **Evaluation :**

Please circle the option of your choice.

- This manuscript
1. is acceptable in its present form\*
  2. will be acceptable after minor revision\*
  - 3. will be acceptable after moderate revision\***
  4. could be reconsidered after major revision\*
  5. is not acceptable for publication\*

\* Please specify below

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### **Comments (Please continue on a separate sheet if more space is needed):**

#### Comment from Reviewer 1

The manuscript concerns with Mendelian traits inheritance and testing of hybrids between transgenic pineapple and wild type Phuket and Pattavia cultivars. Authors perform direct crosses and reciprocal crosses using transgenic pineapples resistance to bialaphos and assay in the progenies on the trait transfers. The essence to perform hybridization between transgenic plants developed from one commercial cultivar with wild type commercial cultivars available thus far was a must in normal biotech based breeding approach.

In this submitted manuscript, although authors did perform genetic test to bar and gus genes, they provided no detail for other associated characteristics, especially those related with plant morphology and fruit quality (and its analysis), appeared among available progenies. The stabilities of those characteristic in the progenies need also be focused. Those were among criteria important in pine apple breeding. There were several points that authors could make insight discussions for instance the phenomena of incompatibility in some cultivars (instead of just only ref on the mere incidence), the background of GUS activities in non transgenics, plant overall traits before and after crosses.

In the title, the term sexual transmission has a strong meaning of disease transmission as normal used in medical genetics over the meaning of trait inheritance designated here. It should be better to consider an alternative term used instead of the term sexual transmission.

In introduction part, authors need to make minor modification to address how important of the biotech based breeding of pineapple before entering part of Sriporaya's works. More detail on transgenic should be provided.

In the materials and methods, authors did not provide the method reference for both GUS and bar assay except the resistance evaluations.

Results and discussions need more data on plant characteristics. Only trait analysis (Chi square) was not enough to be evaluated for publication.

With the above comments, it is recommended that manuscript should be revised especially on the additional data on the associate phenotypes necessary for general evaluation of pineapples in their breeding program. More discussions were also required

#### Comment from Reviewer 2

1. Does The subject falls within the general scope of the journal?  
Yes.
2. Is this a new original contribution? (For review articles this need not necessarily apply).  
Yes.
3. Are interpretations and conclusion sound, justified by the data and consistent with the objectives?  
Yes.
4. Does the title clearly reflect its contents?  
Yes.
5. Is the abstract sufficiently informative, especially when read in isolation?  
Yes.
6. If applicable, are appropriate keywords given?  
Yes.
7. Is the statement of objectives of the article adequate and appropriate in view of the subject matter?  
Yes.
8. Is the description of materials and methods sufficiently informative to allow replication of the experiment?  
Yes.
9. Are the results clearly presented?  
Yes.
10. Is the organization of the article satisfactory (e.g. no discussion in Results)?  
Yes.
11. Is the article structured in agreement with the Guide for Authors?  
Yes.
12. Does the content justify the length?  
Yes.
13. Are the figures and tables all necessary, complete (e.g. titles) and clearly presented?

Yes.

14. Are the reference adequate and in agreement with the Guide for Authors?

No. The discussion part should be expanded. The author should discuss more and compare this work with other researchers. If there is no report on pineapple, other plants should be compared as well.

15. Is the English correct and understandable to a multidisciplinary and multinational readership?

Yes.



## F<sub>1</sub> Hybrid Pineapple Resistant to Bialaphos Herbicide

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Thailand

**Keywords:** *Ananas comosus* var. *comosus*, *bar* gene, crossing, glufosinate, weed,  
biodegradation

### Abstract

Weeds are the main factor for pineapple production. Pineapple plantation growers spend at least a half of production cost controlling them. The other important factor for production is pineapple cultivar. However, all current commercial pineapple cultivars are sensitive or susceptible to biodegradation herbicide. This research project aims to establish F<sub>1</sub> hybrid pineapple variety resistant to herbicide by conventional breeding. Direct and reciprocal crosses between genetically modified pineapple (TP) carrying bialaphos resistant (*bar*) gene and two commercial pineapple cultivars [cvs. 'Pattavia'(PV) and 'Phuket'(PK)] were made in 2005 at Faculty of Agriculture, Rajamangala University of Technology Srivijaya, Nakhonsithammarat, Thailand. 1,172 F<sub>1</sub> seeds and 886 F<sub>1</sub> plantlets were obtained from the first crossing of both crosses of PV and TP. However, no seeds could be obtained from neither direct nor reciprocal crosses of PK and TP. The 886 F<sub>1</sub> plants were studied and evaluated for agronomic characteristics, fruit quality and bialaphos herbicide resistance. Of 886 F<sub>1</sub> plants, 410 F<sub>1</sub> plants showed resistant to Basta-X herbicide. Furthermore, there are eighteen potential F<sub>1</sub> plants showing other good agronomic characters and good fruit quality. Three promising hybrids, (PV x TP)-51; (PV x TP)-46 and (PV x TP)-34, showed resistant to biodegradation bialaphos herbicide, a high percentage of brix (18-22.2° brix), a fragrant smell, crispy flesh and a smooth leaf with only the tip being spiny. In particular, (PV x TP)-34 hybrids presented pale pink bract and fruit and it gave 22.2° brix, cylindrical fruit shape, dark yellow flesh, as well as pleasant flavor. These potential hybrids will further be propagated and evaluated to confirm the traits and consequently release to farmers.

### INTRODUCTION

Pineapple (*Ananas comosus* L.) is one of the most popular and important fruits in the world. World production in 2007 was 18.8 million tons with an average yield of 19.7 t ha<sup>-1</sup> from a total harvested area of 955,646 ha. Thailand was a major pineapple producer and exporter, generating 2.3 million tons from 96,081 ha, with an average yield of 24.144 t ha<sup>-1</sup> (FAO, 2009). For pineapple production, the main cost is generally from weed control and

fertilizer application. Weeds are the main factor for pineapple plantation and growers spend at least a half of cost controlling them. Farmers use both herbicide and mechanical methods to control weeds. However, all current commercial varieties are sensitive and susceptible to biodegradation herbicide including Thai pineapple cultivars such as “Pattavia” and “Phuket” which are classified into the Cayenne group and in the Queen group, respectively. Pineapple has small genetic variation, particularly within the Cayenne group. Advance in varietal improvement has been very slow. Until recently, the use of both conventional sexual hybridization and biotechnological approaches has helped to accelerate progress in the improvement of this fruit crop. Several research groups are developing basic and applied studies to create new varieties with better agronomic performance. This research project aims to establish F<sub>1</sub> hybrid pineapple variety resistant to biodegradation herbicide and having good yield and good qualities.

Since, there is no resistant biodegradation herbicide gene in any natural pineapple resources. However, pineapple breeders inserted transgenes to pineapple cultivar and obtained transgenic pineapple carrying herbicide resistant transgene such as Sripaoraya et al. (2001) and Espinosa et al. (2002). The donor plants were those transformed previously by Biolistics-mediated gene delivery of the construct pHAC25, carrying the  $\beta$ -glucuronidase (*gus*) and Bialaphos resistant (*bar*) genes, into leaf bases of the cv. “Phuket” (Sripaoraya et al., 2001). The *bar* gene encodes phosphinothricin acetyltransferase (PAT) which converts the active component, phosphinothricin (PPT), to a non-herbicidal acetylated form by transferring the acetyl group from acetyl CoA to the free amino group of PPT. This *bar* gene is stable after evaluation in the field for the first generation of vegetative propagated transgenic pineapple (Sripaoraya et al., 2006). Moreover, biochemical side effects of herbicide FINALE on *bar* gene-containing transgenic pineapple plantlets was studied (Yabor et al., 2008).

The present experiments were undertaken as an extension of the previous investigations, in order to assess the feasibility of transferring, by sexual hybridization, agronomically useful genes from transformed plants into commercial, non-transgenic pineapple cvs., with the long-term aim of using this approach to introgress genetic variation into commercial cvs., consequently select resistant to biodegradation herbicide F<sub>1</sub> hybrid. These investigations are the first on the inheritance of a transgene into the progeny in pineapple.

## **MATERIALS and METHODS**

### **Plant Materials and Sexual Hybridization**

Transgenic pineapple plants of the cv. “Phuket” (TP) that exhibited stability and expression of the *bar* gene under experimental field conditions (Sripaoraya et al., 2006) were micropropagated from leaf bases using culture techniques as described earlier (Sripaoraya et al., 2003). Micropropagated TP plants were transferred to pots and used for sexual hybridization with commercial pineapple cvs. Suckers of non-transformed plants of the commercial cvs. “Pattavia” (PV) and “Phuket” (PK), lacking *bar* and *gus* genes (both with expected genotypes of -/-, -/-), were grown for sexual hybridization using the method of Sripaoraya (2009). Direct and reciprocal crosses were made between the parental plants (*i.e.*



TP x PV, PV x TP; TP x PK, PK x TP, PV x PK and PK x PV). The parents were also selfed (i.e. TP x TP, PV x PV, PK x PK).

### **Seed Germination and Establishment of Seedlings**

Ripening fruits were harvested, the seeds were collected and germinated according to Sripaoraya (2009). The F<sub>1</sub> seeds obtained were sown in trays of sand for germination and left grow for 2 months. Seedlings, each 2 cm in height were transferred to plastic bags containing a mixture of equal parts of coconut sheet (coir) and clay soil.

### **Confirmation of Transgene Expression in the F<sub>1</sub> Progeny**

Since the *gus* and *bar* genes were linked on the same transformation construct (pAHC25) used to transform plants of the cv. “Phuket” (Sripaoraya et al., 2001), GUS activity was used initially to select F<sub>1</sub> seedlings carrying transgenes. F<sub>1</sub> seedlings, each 12 cm in height, were sprayed with the commercial herbicide Basta™ (Bayer, Germany) containing glufosinate ammonium as the active ingredient, at the commercially recommended dose (5 lha<sup>-1</sup>) in order to confirm the herbicide resistance of the F<sub>1</sub> plants. Chi-square analysis was applied to determine the segregation ratio of *gus* expression and *bar* gene inheritance from TP to commercial pineapple cultivars. Since TP and PV parents are expected to carry the genotype of *bar*/- and -/-, respectively, the segregation of F<sub>1</sub> progeny was expected to fit a ratio 1:1 of *bar*/- to -/-.

### **Selection F<sub>1</sub> hybrid pineapples resistant to Bialaphos herbicide**

F<sub>1</sub> hybrid pineapple plants which tolerant to Basta-X herbicide were selected for good agronomic performance.

## **RESULTS and DISCUSSION**

### **F<sub>1</sub> Seeds and Seedling Production**

A total of 2,437 F<sub>1</sub> seeds were obtained from sexual hybridization (Table 1). Viable F<sub>1</sub> hybrid seeds were obtained only from sexual crossing (Fig. 1a) of TP with PV and the reciprocal cross PV with TP, and between the non-transformed parents PV with PK and the reciprocal cross, PK with PV. Selfing of the transgenic cv. did not generate viable seeds. Likewise, selfing of non-transformed cvs. failed to produce viable seeds; any seeds that were produced (PV x PV; PK x PK) lacked kernels and were not viable. This confirmed that self-incompatibility in pineapple occurs in the non-transformed pineapple plants (Bhowmik and Bhagabat, 1975; Py et al., 1987; Coppens d’Eeckenbrugge et al., 1993; Leal and Coppens d’Eeckenbrugge, 1996; Sripaoraya, 2009) and is not the result of transformation. These are also the first experiments to report on self-incompatibility in transformed pineapple. Of the seeds that were produced, 1,459 seedlings were obtained from direct and reciprocal crosses of TP x PV and PV x PK (Table 1, Fig. 1b-c). One thousand one hundred and seventy two F<sub>1</sub> seeds and 886 F<sub>1</sub> plants were obtained from both crosses between TP and PV parents. Eight hundred and ninety nine F<sub>1</sub> seeds and 728 F<sub>1</sub> plants were obtained from PV x TP compared to 273 F<sub>1</sub> seeds and 158 F<sub>1</sub> plants from TP x PV. Since the female parent influences the number of flowers in the inflorescence, PV had more flowers than the TP female.

### **Expression of the *gus* and *bar* Genes**

GUS histochemical staining of leaves showed that of 158 seedlings obtained from the TP x PV, 72 were GUS positive revealing transgene stability and expression. However, 86 of them failed to express the *gus* gene (Table 1). The difference in the number of seedlings showing *gus* expression was not significant by Chi-square analysis. Similarly, of 95 seedlings obtained from the reciprocal cross, PV x TP, 44 were positive for GUS activity, but 51 were negative.

Expression of the *bar* gene was confirmed by spraying seedlings with the commercial herbicide Basta™. All GUS positive seedlings were also herbicide tolerant, retained their chlorophyll and remained healthy following application of the herbicide (Fig.1d). In contrast, the GUS negative plants were not tolerant to the application of Basta™ and died (Fig. 1e). This confirmed that *bar* gene was inherited in the F<sub>1</sub> plants obtained from sexual hybridization.

### **Inheritance of Bialaphos resistance in F<sub>1</sub> plants**

Chi-square analysis showed that the GUS positive and GUS negative plants exhibited a 1:1 ratio following Mendel's Law of inheritance for a pair of genes controlling Bialaphos resistance. Such information is essential for assessing the inheritance of transgenes from genetically modified pineapple plants to non-transgenic plants. The surviving F<sub>1</sub> seedlings were grown to flowering and used for selfing and backcrossing to all parents in order to further evaluate inheritance of the *bar* gene. F<sub>1</sub> plants exhibiting herbicide resistance were selected based on their agronomic characters, including yield and fruit quality.

### **Bialaphos herbicide resistant F<sub>1</sub> hybrid selection**

The 886 F<sub>1</sub> plants were studied and evaluated for agronomic characteristics, fruit quality and bialaphos herbicide resistance. Of 886 F<sub>1</sub> plants, 410 F<sub>1</sub> plants showed resistant to Basta™ herbicide and gave variation for fruit shape, size, color and firm flesh (Fig.2a). There are eighteen potential F<sub>1</sub> plants showing other good agronomic characters and good fruit quality (Table 2, Fig.2b). Three promising hybrids, (PV x TP)-51; (PV x TP)-46 and (PV x TP)-34, showed resistant to biodegradation bialaphos herbicide, a high percentage of brix (18-22.2° brix), a fragrant smell, crispy flesh and a smooth leaf with only the tip being spiny. In particular, (PV x TP)-34 hybrids presented pale pink bract and fruit and it gave 22.2° brix, cylindrical fruit shape, dark yellow flesh, as well as pleasant flavor.

### **CONCLUSIONS**

The Bialaphos resistance (*bar*) gene from transgenic pineapple was inherited as a single gene into commercial, non-transformed cvs. of pineapple. F<sub>1</sub> plants showed a 1:1 ratio of herbicide resistant to susceptible plants. Inheritance and stability of the *bar* gene is being assessed in the F<sub>2</sub> and subsequent seed generations. Three promising hybrids, (PV x TP)-51; (PV x TP)-46 and (PV x TP)-34, showed resistant to biodegradation bialaphos herbicide, a high percentage of brix (18-22.2° brix), a fragrant smell, crispy flesh and a smooth leaf with only the tip being spiny. In particular, (PV x TP)-34 hybrids presented pale pink bract and fruit and it gave 22.2° brix, cylindrical fruit shape, dark yellow flesh, as well as pleasant

flavor. These potential hybrids will further be evaluated to confirm the traits and consequently release to farmers.

#### ACKNOWLEDGEMENTS

This research was supported by the Thailand Research Fund (TRF).

#### Literature Cited

- Bhowmik, G. and Bhagabat, A. 1975. Self incompatibility studies in pineapple (*Ananas comosus* L.). Indian J. Agric Sci. 19:259-265.
- Coppens d'Eeckenbrugge, G., M.F. Duval and Van Miegroet, F. 1993. Fertility and self-incompatibility in the genus *Ananas*. Acta Hort. 334:45-51.
- Espinosa, P., J.C. Lorenzo, A. Iglesias, L. Yabor, E. Menendez, Y. Borroto, L. Hernandez and Arencibia, A. 2002. Production of pineapple transgenic plants assisted by temporary immersion bioreactor. Plant Cell Rep. 21:136-140.
- Food and Agricultural Organization. 2007. www.faostat.fao.org.
- Food and Agricultural Organization. 2009. www.faostat.fao.org.
- Leal, F. and Coppens d'Eeckenbrugge, G. 1996. Pineapple. In: Janick, J. and Moore, J.N. (eds), Fruit Breeding, Vol. I: Tree and Tropical Fruits. John Wiley and Sons Inc., New York, USA. pp. 515-557.
- Py, C., J.J. Lacaevilhe and Teisson, C. 1987. The Pineapple Cultivation and Uses. Maisonneuve and Larose, Paris. 568 pp.
- Sripaoraya, S., R. Marchant, J.B. Power and Davey, M.R. 2001. Herbicide-tolerant transgenic pineapple (*Ananas comosus*) produced by microprojectile bombardment. Ann. Bot. 88:597-603.
- Sripaoraya, S., R. Marchant, J.B. Power and Davey, M.R. 2003. Plant regeneration by somatic embryogenesis and organogenesis in commercial pineapple (*Ananas comosus* L.). In Vitro Cell Dev. Biol.-Plant 39:450-454.
- Sripaoraya, S., S. Keawsompong, P. Insupa, J.B. Power, M.R. Davey and Srinives, P. 2006. Transgene stability and expression of genetically modified pineapple (*Ananas comosus*) under experimental field conditions. Plant Breed. 125:411-413.
- Sripaoraya, S. 2009: Pineapple hybridization and selection in Thailand. Acta Hort. 822:57-62.
- Yabor L., C. Aragon, M. Hernandez, A.D. Arencibia and Lorenzo, J.C. 2008. Biochemical side effects of the herbicide FINALE on bar gene-containing transgenic pineapple plantlets. Euphytica. 164:515-520.

## Tables

Table 1. Number of F<sub>1</sub> seeds and seedlings generated, GUS activity and Chi-square analysis of the number of F<sub>1</sub> plants from crossing sexually transgenic plants of the cv. Phuket (TP) with non-transformed plants of the cvs. Pattavia (PV) and Phuket (PK). The results of crossing between non-transformed cv. and selfing are also presented.

Crosses	No. of seeds	No. of seedlings	Seedlings assessed for GUS activity	Number of F <sub>1</sub> seedlings		$\chi^2$ (1:1)	Apparent genotype of F <sub>1</sub> seedlings <sup>c</sup>
				GUS <sup>+</sup> <sup>a</sup> BastaR <sup>b</sup>	GUS <sup>-</sup> <sup>a</sup> BastaS <sup>b</sup>		
TP x PV	273	158	158	72	86	1.24 <sup>NS</sup>	<i>gus</i> /- <i>bar</i> /-
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PV x PK	594	235	10	-	10	-	- /- - /-
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TP x PK	0	-	-	-	-	-	
TP x TP	0	-	-	-	-	-	
PV x PV	2	0	-	-	-	-	
PK x PK	2	0	-	-	-	-	
∑(PVxTP parents)	1,172	886	253	116	137	1.74 <sup>NS</sup>	
Total	2,473	1,459	273	116	157	-	

<sup>a</sup> GUS<sup>+</sup>: GUS positive, GUS<sup>-</sup>: GUS negative.

<sup>b</sup> BastaR: Bialaphos-resistant, BastaS: Bialaphos-sensitive.

<sup>c</sup> *gus*: *gus* gene, *bar*: bialaphos resistant gene.

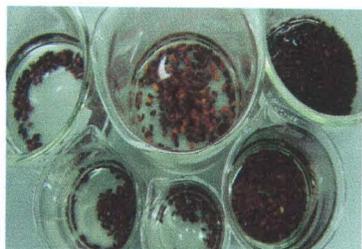
NS: not significant.

Table 2. Brix and agronomic characteristics of Basta™ resistant F<sub>1</sub> hybrids pineapples PV x TP for 18 potential F<sub>1</sub> hybrids.

Hybrid No.	°Brix	wt. (g)		Shape	Fruit size (cm)		Core dia. (cm)	Cup+ depth (cm)	Leaf spiny
		crown	fruit		width	length			
7	17.15	120	1450	cylindrical	12.0	17.0	2.8	1.5	spiny
8	17.5	100	1580	cylindrical	12.5	19.0	3.0	1.1	tip only
13	17.25	130	1420	cylindrical	12.0	18.0	4.5	1.5	spiny
15	17.15	130	720	round	10.5	11.5	2.5	0.7	tip only
17	16.9	130	720	cylindrical	8.9	14.2	2.0	0.7	tip only
23	18.5	130	730	cylindrical	9.2	13.8	2.0	1.0	spiny
24	17.55	70	840	cylindrical	10.0	13.0	2.0	1.0	tip only
25	16.2	80	850	cylindrical	10.0	14.0	2.0	0.9	spiny
<b>34</b>	<b>22.2</b>	<b>140</b>	<b>740</b>	<b>round</b>	<b>8.5</b>	<b>8.0</b>	<b>2.0</b>	<b>0.7</b>	<b>tip only</b>
37	17.4	130	1060	cylindrical	11.5	13.5	2.2	1.1	tip only
39	17.45	280	1160	cylindrical	12.4	18.0	2.4	1.2	tip only
45	18.7	120	850	round	10.5	11.5	2.3	0.7	spiny
<b>46</b>	<b>18.0</b>	<b>430</b>	<b>1900</b>	<b>conical</b>	<b>14.0</b>	<b>18.5</b>	<b>4.0</b>	<b>1.0</b>	<b>tip only</b>
<b>51</b>	<b>18.65</b>	<b>160</b>	<b>920</b>	<b>cylindrical</b>	<b>10.5</b>	<b>12.8</b>	<b>2.3</b>	<b>0.8</b>	<b>tip only</b>
308	17.4	140	700	round	11.5	8.0	4.0	0.9	tip only
312	17.65	130	1340	cylindrical	11.0	18.0	1.5	1.0	spiny
320	17.25	140	680	round	11.5	9.0	3.5	1.1	irregularly
322	17.6	250	990	round	13.0	11.0	5.0	0.6	spiny

## Figures

Fig. 1 Seeds and seedlings from hybridization between transgenic and non-transformed pineapple plants. (a) F<sub>1</sub> seeds from direct and reciprocal crosses of PV x TP (b) germinating F<sub>1</sub> seeds from PV x TP (c) seedlings in plastic bag in greenhouse (d) F<sub>1</sub> plants before spraying Basta™ and (e) plants showed brown and died after 2 weeks herbicide spraying



a



b



c



d



e



**Fig. 2** F<sub>1</sub> hybrid variation of fruit size, color, shape, crown color: a) F<sub>1</sub> hybrid fruits from crossing between PV x TP and b) potential F<sub>1</sub> hybrids from crossing between PV x TP



**a**



**b**

