J. HPT Tropika. ISSN 1411-7525
Vol. 10, No. 2: 91 – 99, September 2010

PYRAMIDING IMPORTANT DISEASE-RESISTANT CHARACTERS BY HYBRIDIZATION OF TRANSGENIC AND NON-TRANSGENIC PEANUTS (*ARACHIS HYPOGAEA* L.)

Dwi Hapsoro[1], Hajrial Aswidinnoor[2], Rusmilah Suseno[3], Jumanto[4] & Sudarsono[2]

ABSTRACT

Pyramiding Important Disease-Resistant Characters by Hybridization of Transgenic and Non-Transgenic Peanuts (*Arachis hypogaea *L.). We have produced transgenic peanut lines carrying a coat protein gene of peanut stripe virus (PStV) and showing resistance to the virus. However, their susceptibility to leafspot disease caused by *Cercospora sp*. and their lower productivity compared to their genetic background cultivar make them commercially less undesireable. The objective of this research was to test whether crossing the transgenic peanut plants with a non-transgenic peanut line WS, which was resistant to leafspot disease and high-yielding, could produce progenies in F2 generation that were resistant to both PStV and leafspot disease as well as of higher yield campared to their transgenic progenitor. If this test was proven, pyramiding novel transgenic and non-transgenic characters in peanut plants by hybridization would probably be a routine procedure in the future. Crosses were made between transgenic peanut plants that were resistant to PStV and non-transgenic peanut line WS. F2 population was evaluated for resistance to PStV and leafspot disease. Number of filled pods, filled pod dry weight per plant, and dry weight of each pod were measured. Result of the experiment showed that some of the plants in F2 population exhibited resistant both to PStV and leafspot disease and produced higher number of filled pods, filled pod dry weight per plant, and dry weight of each pod compared to those produced by their transgenic parent plants.

Key words: peanut, transgenic, resistance, PStV, leafspot, *Cercospora* sp.

INTRODUCTION

We have produced transgenic peanut lines carrying a coat protein gene of peanut stripe virus (PStV) and showing resistance to the virus (Hapsoro *et al*., 2005; Hapsoro *et al*., 2007a, 2007b; Hapsoro *et al*., 2008). The transgene has been proven to be stabile up to seven generations of selfing (Hapsoro *et al*., 2007b). The transgenic peanut lines has been shown to carry the PStV cp transgene and inherited according the Mendel law (Hapsoro *et al*., 2008). Therefore, these transgenic pure lines could be used as parents in a breeding program of pyramiding character of resistance to PStV and other novel characters in peanut plants.

As previously reported, transgenic peanut lines cv. Gajah that are resistant to PStV obtained through genetic engineering showed lower productivity than their non-transgenic counteparts. It has also been shown that the transgenic lines were susceptible to leafspot disease caused by *Cercospora*. This is understandable bacause in fact peanut cv. Gajah has been shown to be susceptible to leafspot disease (Hidajat *et al*., 2000).

To increase its productivity and resistance to leafspot disease, the transgenic peanuts need to be crossed with non-transgenic peanuts that are highly productive and shows resistance to leafspot disease. It has been reported that peanut line WS exhibited resistance to leafspot disease caused by *Cercospora* (Kusumo, 1996). WS is GPNC-WS4, registrated by the North Carolina Agricultural Research Service as peanut germplasm showing resistance to leafspot disease (Stalker & Beute, 1993). Compared to cv. Gajah this line showed higher productivity (Suryami, 2000). Therefore, this line can be used to produce transgenic peanuts resistant to PStV, leafspot disease and of high productivity by crossing them with transgenic peanuts cv. Gajah.

[1] Department of Agronomy, Faculty of Agriculture, The University of Lampung,
Jl. Sumantri Brojonegooro No.1 Bandar Lampung 35145. E-mail: hapsorodwi@yahoo.com
[2] Plant Molecular Biology Laboratory, Faculty of Agriculture, Bogor Agricultural University (IPB Bogor),
Jl. Meranti, Kampus IPB Darmaga, Bogor 16680.
[3] Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University (IPB Bogor),
Jl. Meranti, Kampus IPB Darmaga, Bogor 16680.
[4] BB Biogen, Bogor, Jl.Tentara Pelajar, Bogor.

A breeding program to combine novel characters owned by two self-pollinated cultivars begins with the hybridization of the two that lead to F1 and F2 population. This research aimed to test whether hybridization between transgenic peanut carrying PStV *cp* gene (resistant to PStV, susceptible to leafspot disease caused by *Cercospora* and low productivity) and peanut line WS (susceptible to PStV, resistant to leafspot disease caused by *Cercospora* and high productivity) result in some F2 plants resistant to PStV, resistant to leafspot disease, and of high productivity.

MATERIALS AND METHODS

Plant Materials. Crosses were made between transgenic peanut cv. Gajah containing PStV *cp* gene as male parents and non-transgenic peanut line WS as female parents. Cultivar Gajah, resulted from selection of progenies of crosses of Schwarz-21 and Spanish 18-38 lines, was susceptible to leafspot disease caused by *Cercospora* (Badan Penelitian dan Pengembangan Pertanian, 2010). The transgenic peanut cv. Gajah was resistant to PStV, susceptible to leafspot disease caused by *Cercospora* and of low productivity. WS is GPNC-WS4, a germplasm registrated by the North Carolina Agricultural Research Service and showed moderate resistance to leafspot disease caused by *C. personatum* (Stalker & Beute, 1993). Compared to cv. Gajah, WS showed higher productivity (Suryami, 2000). This line was resulted from an interspecific cross between an *A. hypogaea* parent (PI 261942 or PI 261943) and *A cardenasii* Krap.et Greg. nom.nud. (GKP 10017, PI 262141).

F_1 plants were grown to maturity to produce F_2 seeds. The F_2 plants were grown and evaluated for their agronomic characters and their response to PStV inoculation and leafspot disease. All plants were grown in a mixture of soil and sand (2:1) contained in polybags (45 x 50 cm) and maintained in an insect-proof plastic house. Watering to field capacity was done everyday. Plants were fertilized at 8 weeks after planting with NPK fertilizer (15-15-15) 2 g per plant. Pest and disease control was conducted using Confidor, Kelthane, and Dithane 45.

Transgenic peanut parents were T_4 plants resulted from four generations of selfing of one T_0 plant (one independent primary transformant). One regenerant derived from one transformation event was clonally propagated in vitro through axillary branching. T_0 plants were grown to maturity in plastichouse. $T_{0:1}$ seeds were sown to get T_1 plants that were grown to produce $T_{1:2}$ seeds. $T_{1:2}$ seeds were sown to get T_2 plants that were grown to produce $T_{2:3}$ seeds. Such selfings were conducted until four generations and in so doing T_4 plants were obtained.

Nomenclature of plants employed in this experiment is as follows. T_0 plants were designated as G. T_1, T_2, T_3, and T_4 plants were designated as G (n), G (n.o), G (n.o.p), and G (n.o.p.q), respectively, where n, o, p, and q are cardinal numbers, respectively. As an illustration, G (1) is a T1 plant number 1, G (2) is a T_1 plant number 2, G (3) is a T_1 plant number 3, and so on. G (1.1) is a T2 plant number 1 derived from G (1). G (1.2) is a T_2 plant number 2 derived from G (1). G (2.1) is a T_2 plant number 1 derived from G (2). Arbitrarily, G (9.2.5.2) is a T_4 plant number 2 derived from a T_3 plant G (9.2.5). WS/G (9.2.5.2).2 is an F1 plant number 2 derived from a cross between line WS and G (9.2.5.2). T_4 Plants used as parents in this experiment were those designated as G (8.10.8.1), G (8.10.8.2), G (8.10.8.4), G (8.10.8.5), G (9.2.5.2), and G (9.2.5.5).

Response to PStV. Parent plants, F1 plants, and F2 plants were mechanically inoculated with PStV at least three times, i.e. at 2, 4 and 6 weeks after planting. Further inoculation every two weeks were done on plants that did not show disease symptom to ensure that the plants were resistant. PStV inoculation was also conducted on non-transgenic peanuts cv. Gajah as a control. Inoculum of PStV was maintained and propagated in peanut plants cv. Kelinci which had been inoculated with PStV isolate Bogor that caused severe blotch-stripe symptom in peanut plants cv. Landak (Akin *et al.*, 1999; Avivi, 2000; Yasin, 2001). Inoculation was carried out as described by Culver & Sherwood (1987). The fully-open-youngest leaves were spread with carborundum powder (600 mess) and rubbed with cutton bud previously dipped in inoculum solution. The inoculum was prepared by grinding PStV-infected leaves (0.5 cm in diameter) in 200 μl of phosphate buffer solution pH 7. Effectiveness of the inoculation was evaluated using an indicator plant, i.e *Chenopodium amaranticolor*.

Response to *Cercospora*. Response to *Cercospora* was evaluated without the plants being inoculated as reported by Utomo & Akin (2004) because infestation by leafspot disease occurred naturally. The plant response was grouped into resistant and susceptible. Susceptible plants showed spot symptoms on leaves. The spots were getting bigger then followed by yellowing of leaves (chlorosis) and finally the leaves fell down. Resistant plants showed spots on their leaves and the spot become necrotic and did not expand and the leaves did not show chlorotic symptoms.

Agronomic data. At harvest time, plant height, number of filled pod, and pod dry weight were observed. Pod dry weight measurement was conducted by drying the pods in plastic house under sun light for one week and then weighed.

RESULTS AND DISCUSSION

Number of pod, weight of total pods, and weight of each pod produced by several lines of T_5 and T_6 generations of transgenic peanuts cv.Gajah and the non-transgenic peanuts cv.Gajah as a control were presented in Table 1. Number of pods, weight of total pods, and weight of each pod produced by transgenic peanut plants cv. Gajah was lower than that produced by the non-transgenic one.

In the population of transgenic parents, the mean values of number of pod, total pod dry weight, and dry weight of each pod were 11 pods, 12 g, and 0.9 g, respectevely, while those of non-transgenic parents were 14 pods, 20 g, and 1.4 g respectively (Figure 1, 2, and 3). A cross of those two parents would expectedly result in progenies having mean values of number of pod, total pod dry weight, and dry weight of each pod higher than those of the transgenic parent plants. In fact, a phenomenon called transgresive segregation did occure in the F2 population. Transgresive segregation refers to occurrence of one or more individuals in a population whose performance for a character falls outside the range of the parents of the population (Fehr, 1993). Figure 1, 2, and 3 show that in F2 population there were plants that produced pods heavier and in a larger number than those produced by the transgenic parent plants. For example, there were plants in the F2 population that had number of pod per plants of 29-30 pods, total dry weight of pods per plant of 22-26 g, and dry weight of each pod of 1.6-2.3 g, which were higher than those found in transgenic parent population. Proportion of F2 population producing higher number of pod per plant than the mean number of pod per plant of the transgenic parent population and non-transgenic parent population was 40% and 18%, respectively. The figures were 50% and 1% for total dry weight of pods per plant and 61% and 54% for dry weight of each pod. Figure 4 shows pods of male parent (P2), female parent (P1) and F1 progeny and Figure 5 shows pods produced by plants in F2 population.

The transgenic parent was resistant to PStV (score 0) and susceptible to leafspot disease caused by *Cercospora*, while the non-transgenic parent was susceptible to PStV (score 4) and resistant to *Cercospora* leafspot. Crosses between those two parents led to F1 plants that were less susceptible to

Table 1. Number of pod, weight of total pods, and dry weight of each pod produced by several lines of T5 and T6 generations of transgenic peanuts cv. Gajah and the non-transgenic peanuts cv. Gajah

Lines	Number of pod	Total pod dry wight	Dry weight of each pod
G (8.4.3.1.1)	14.4	14.7	1.1
G (8.10.8.6.1)	8.1	6.2	0.9
G (8.11.6.1.2)	13.4	8.1	0.6
G (8.17.1.1.3)	8.7	7.5	0.9
G (8.17.1.1.5)	12.6	12.2	0.9
G (9.2.5.1.1)	10.9	12.0	1.1
G (9.2.5.1.2)	12.1	11.0	0.9
G (9.4.16.1.3)	11.5	12.2	1.0
G (17.1.8.1.3)	8.9	9.2	1.1
G (8.10.8.4.1.1)	8.5	8.3	1.0
cv. Gajah (non-transgenic)	15.9	20.3	1.3

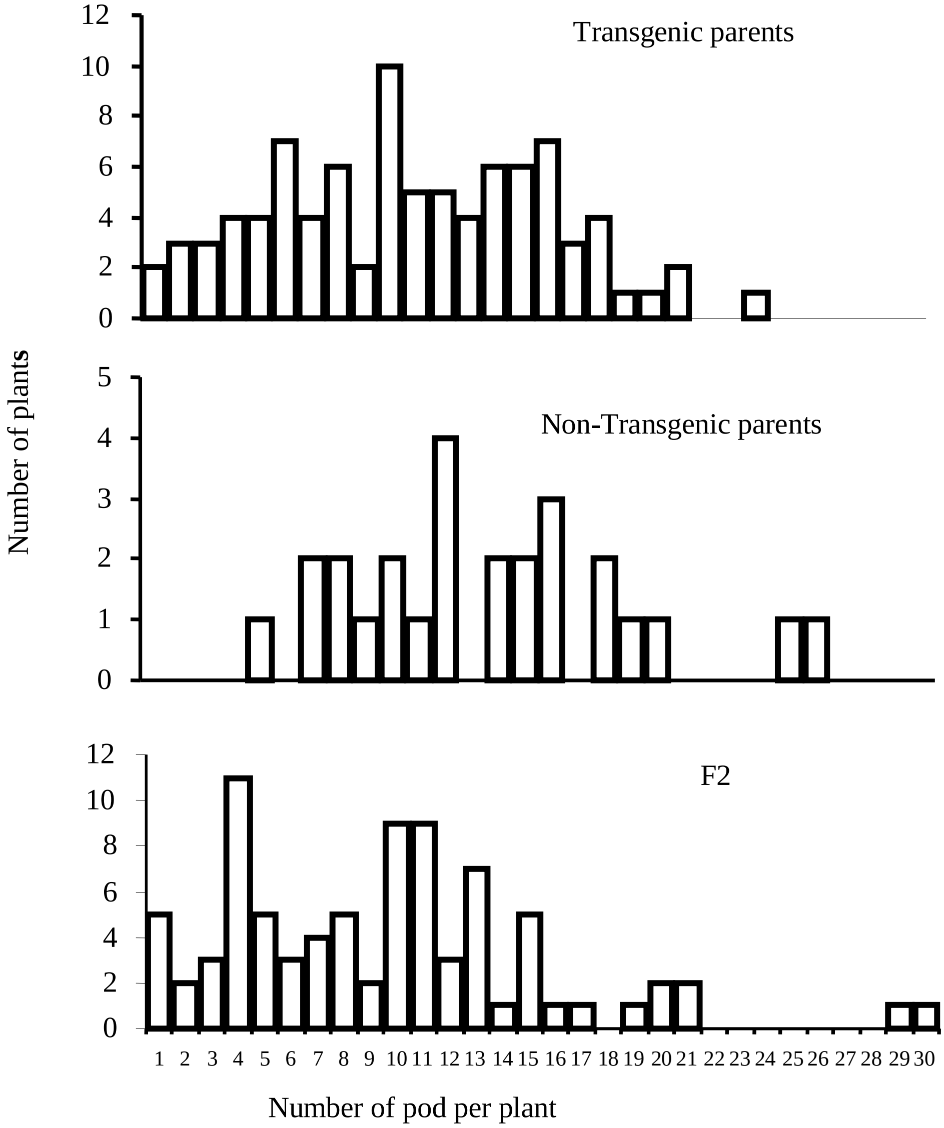

Figure 1. Frequency distribution of number of pod per plant in population of PStV-resistant transgenic peanut plants containing PStV cp gene, of non-transgenic peanut plants WS, and of the F2 generation derived from a cross between the transgenic peanut plants as male parents and the non-transgenic peanut plants as female parents

PStV and resistant to leafspot disease (Figure 6). Among F2 population there were plants showing resistant and susceptible to leafspot disease and exhibiting degree of response to PStV from score 0 (resistant) to score 4 (very susceptible) (Table 2 and Figure 7).

Tranagenic plants containing PStV cp gene used in this experiment showed resistance to PStV, which was stable up to seven generations of selfing and inherited in mendelian manner (Hapsoro et al., 2008). The drawback was that the production was lower than that of peanut plants cv. Gajah from which the transgenic plants derived (Table 1). The low production was indicated by pod dry weight per plant, number of pod, and dry weight of each pod. Other weakness was that the transgenic plants were susceptible to *Cercospora*, a phenomenon that was understandable since the plants cv. Gajah as its genetic background was susceptible to the disease.

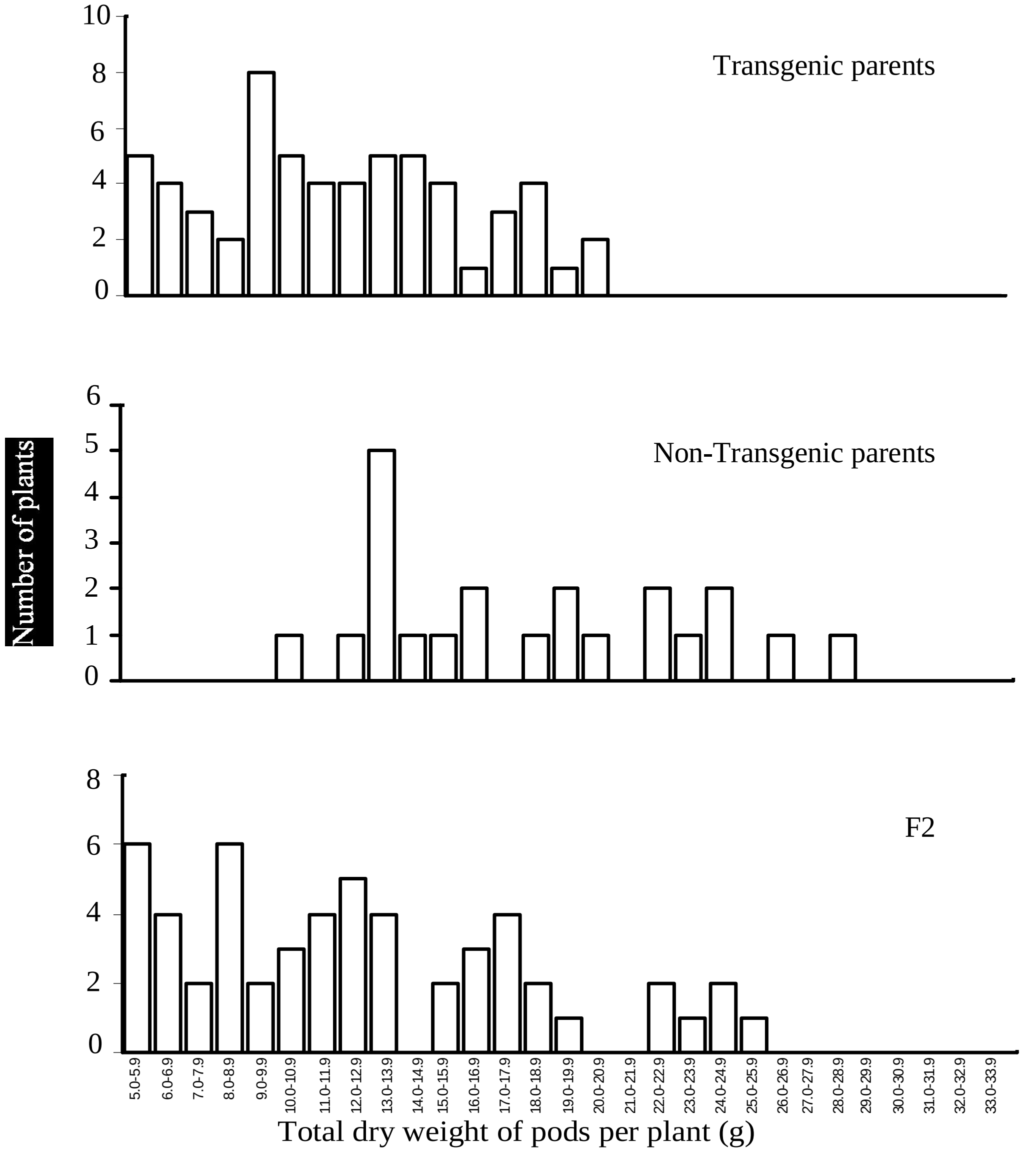

Figure 2. Frequency distribution of total dry weight of pods per plant in population of PStV-resistant transgenic peanut plants containing PStV cp gene, of non-transgenic peanut plants WS, and of the F2 generation derived from a cross between the transgenic peanut plants as male parents and the non-transgenic peanut plants as female parents

To improve the low productivity of the transgenic plants, they were crossed with non-transgenic plants cv. WS that had higher productivity. The F2 population derived from the cross contained some plants that showed higher productivity than its transgenic progenitor. By use of selfing for several generations, these plants would potentially produce high productivity lines. To increase resistance of the transgenic plants to leafspot disease caused by *Cercospora*, they were crossed with non-transgenic peanut plants cv. WS that were resistant to leafspot disease. The cross resulted in F2 plants some of which showed resistance not only to leafspot disease but also to peanut stripe disease. Selfing those F2 plants for several generations would expectedly lead to pure lines exhibiting resistance to both leafspot and stripe diasease. This experiment demonstrated that transgenic character can be treated as a "natural" character in a breeding program, i.e. it can be combined with non-transgenic character using sexual approach. Accordingly, it should also be possible to sexually combine two or more transgenic characters.

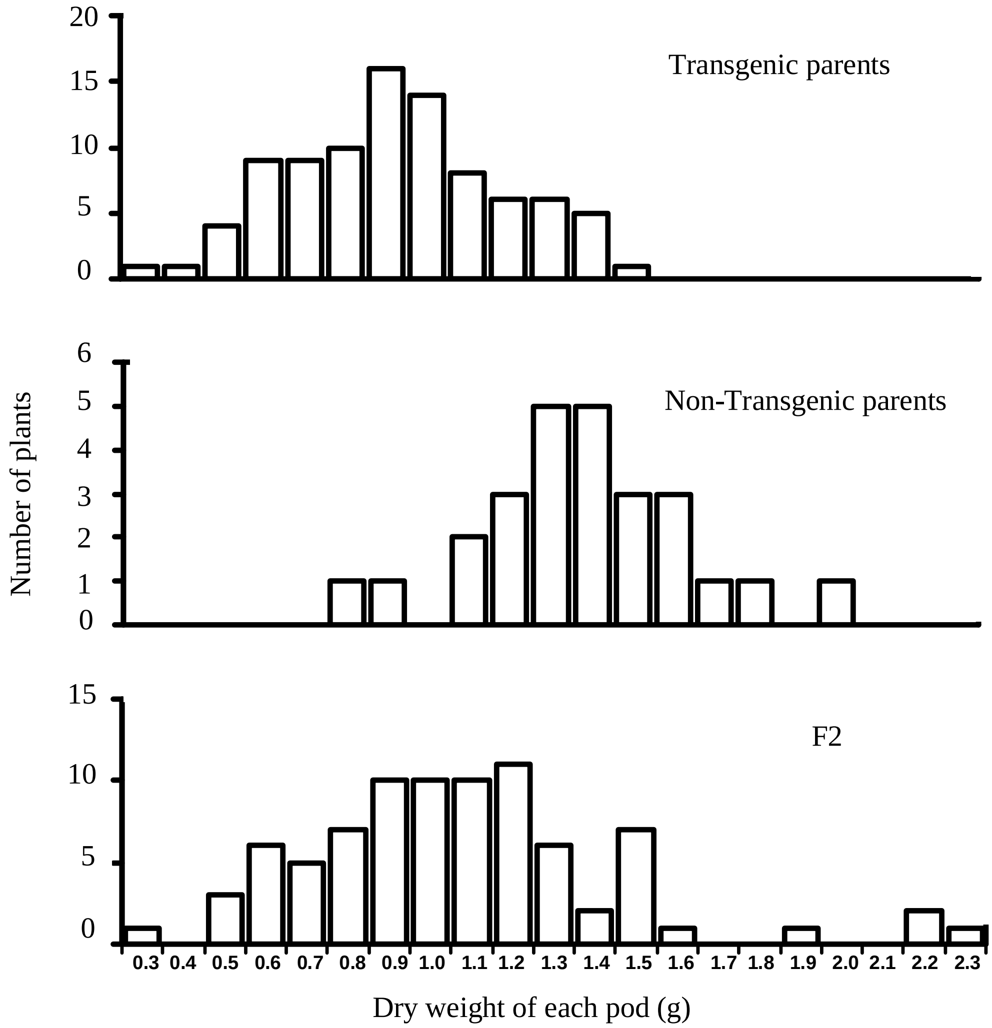

Figure 3. Frequency distribution of dry weight of each pod in population of PStV-resistant transgenic peanut plants containing PStV cp gene, of non-transgenic peanut plants WS, and of the F2 generation derived from a cross between the transgenic peanut plants as male parents and the non-transgenic peanut plants as female parents

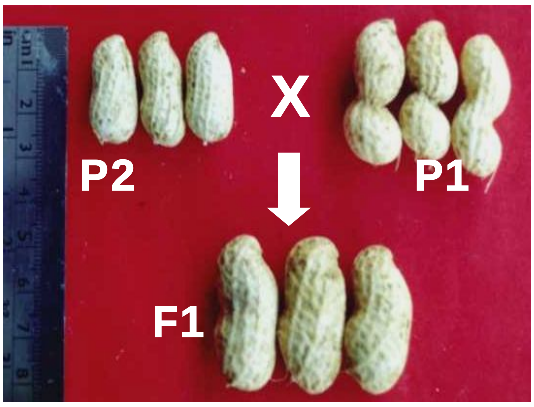

Figure 4. Pods produced by transgenic peanut plants (P2) as male parents and non-transgenic peanut plants cv. WS as female parents (P1) and pods of F1 plants

Table 2. Number of F2 progenies showing response to PStV and *Cercospora*. The F2 population was derived from a cross between transgenic peanut parents that were resistant to PStV and susceptible to leafspot and non-transgenic peanut parents that were susceptible to PStV and resistant to leafspot disease. The transgenic parents contained PStV cp gene

Response to *Cercospora*[a]	Resistant					Susceptible				
Response to PStV (Score)[b]	0	1	2	3	4	0	1	2	3	4
F1 plant number										
WS/G (8.10.8.1).5	1	0	0	0	0	0	1	1	0	0
WS/G (8.10.8.2).1	7	2	1	2	4	1	1	0	2	1
WS/G (8.10.8.2).2	5	0	2	3	3	8	0	0	4	3
WS/G (8.10.8.2).7	3	0	0	1	1	1	0	0	0	2
WS/G (8.10.8.4).1	3	0	1	0	1	0	0	1	0	0
WS/G (8.10.8.5).1	1	0	0	2	3	0	0	0	0	0
WS/G (8.10.8.5).4	0	0	0	1	1	0	0	0	0	0
WS/G (9.2.5.2).2	0	0	0	0	0	0	0	0	0	0
WS/G (9.2.5.5).1	5	0	0	3	1	7	1	0	5	9

Figure 5. Pods produced by individual plants in F2 population derived from a cross between transgenic peanut plants as male parents and non-transgenic peanut plants cv. WS as female parents

Figure 6. A cross between non-transgenic peanut plants cv. WS (P1) , which were resistant to leafspot disease caused by *Cercospora* and transgenic peanut plants (P2), which were susceptible to leafspot disease caused by *Cercospora*, resulted in peanut plants (F1) that were resistant to leafspot disease

Figure 7. F2 peanut plants susceptible (1) and resistant (2 and 3) to *Cercospora* F2 plants were resulted from crosses between non-transgenic plants cv. WS resistant to *Cercospora* and susceptible to PStV and transgenic plants susceptible to *Cercospora* and resistant to PStV

CONCLUSION

Resistance to PStV, which was a transgenic character in peanut, could be combined with resistance to leafspot disease, a non-transgenic character, through hybridization. This demonstrated that transgenic character can be treated just as non-transgenic character in a breeding program employing hybridization.

LITERATURES CITED

Akin HM, Suseno R & Sudarsono. 1999. Variasi biologi dan molekuler strain-strain PStV (*peanut stripe potyvirus*) yang diisolasi dari berbagai pertanaman kacang tanah di Indonesia. *Buletin Hama dan Penyakit Tumbuhan* 11: 56–61.

Avivi S. 2000. Berbagai Tipe Konstruksi Gen *cp* PStV yang Dapat Memproteksi Tanaman *Nicotiana benthamiana* Transgenik terhadap Infeksi PStV pada Kacang Tanah. *Disertasi*. Program Pascasarjana Institut Pertanian Bogor.

Badan Penelitian dan Pengembangan Pertanian. 2010. http://www.puslittan.bogor.net/index.php?bawaan=varietas/gerbang&proses=daftarstok& komoditas=05026&varietas=1. Accessed on 3 January 2010.

Culver JN & Sherwood JL. 1987. Resistance to Peanut Stripe Virus in Arachis germ plasm. *Plant Dis.* 71: 1080–1082.

Fehr WR. 1993. *Principles of Cultivar Development: Theory and Technique*. Volume 1. Macmillian Publishing Company, USA.

Hapsoro D, Aswidinnoor H, Jumanto, Suseno R & Sudarsono. 2007b. Transgene identity and number of integration sites and their correlation with resistance to PStV in transgenic peanuts carrying Peanut Stripe Virus (PStV) coat protein gene. *J.Hama dan Penyakit Tumbuhan Tropika* 7(1): 39–47.

Hapsoro D, Aswidinnoor H, Jumanto, Suseno R & Sudarsono. 2008. Inheritance of resistance to PStV in transgenic peanuts containing cp PStV gene. *J. Hama dan Penyakit Tumbuhan Tropika* 8(1): 31–38.

Hapsoro D, Aswidinnoor H, Jumanto, Suseno R & Sudarsono. 2007a. Resistance to Peanut Stripe Virus (PStV) in Transgenic Peanuts (*Arachis hypogaea* L.) Carrying PStV *cp* Gene Was Stabile up to Seven Generations of Selfing. *Biota* 12 (2): 83–91.

Hapsoro D, Aswidinnoor H, Jumanto, Suseno R & Sudarsono. 2005. Transformasi tanaman kacang tanah (*Arachis hypogaea* L.) dengan gen cp PStV dengan bantuan Agrobacterium. *J. Agrotropika* 10(2): 85–93.

Hidajat JR, Kartaatmadja S & Rais SA. 2000. *Teknologi produksi benih kacang tanah. Pusat Penelitian dan Pengembangan Tanaman Pangan*. Badan Penelitian dan Pengembangan Pertanian.

Kusumo YWE. 1996. Analisis genotipik ketahanan kacang tanah (*Arachis hypogaea* L.) terhadap penyakit bercak daun hitam disebabkan oleh *Phaeoisariopsis personata* (Berk. & Curt.) v. Arx. *Disertasi*. Institut Pertanian Bogor.

Stalker HT & MK Beute. 1993. Registration of four interspecific germplasm lines resistant to *Cercospora arachidicola*. *Crop Sci*. 33: 1117.

Suryami Y. 2000. Pengaruh Mulsa Plastik Perak Hitam terhadap Penyebaran Peanut Stripe Virus (PStV) dan Produksi Kacang Tanah (*Arachis hypogaea* L.) Galur-galur Introgresi. *Skripsi*. Jurusan Budidaya Pertanian Fakultas Pertanian Institut Pertanian Bogor.

Utomo SD & Akin HM. 2004. Ketahanan tiga spesies Arachis terhadap bercak daun akhir *Cercosporidium personatum* Berk.Et Curt. pada pemberian dan tanpa pemberian Mankozeb. *J. Hama dan Penyakit Tumbuhan Tropika* 4(2): 75–82.

Yasin N. 2001. Evaluasi ketahanan tanaman transgenik model *Nicotiana benthamiana* terhadap *peanut stripe virus*. *Tesis*. Program Pascasarjana Institut Pertanian Bogor.