ORIGINAL ARTICLE

Heritability, phenotypic and genotypic correlations of *Peanut bud necrosis virus* (PBNV) reaction parameters in peanut

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Abstract

Tonsomros, Y., Jogloy, S., Wongkaew, S., Akkaseang, C., Kesmala, T. and Patanothai, A.
Heritability, phenotypic and genotypic correlation of *Peanut bud necrosis virus* (PBNV) reaction parameters in peanut
Songklanakarin J. Sci. Technol., 2006, 28(3) : 469-477

Peanut bud necrosis disease (PBND) caused by *Peanut bud necrosis virus* (PBNV) is an important disease of peanut (*Arachis hypogaea* L.) in Thailand especially during the dry season. Host plant resistance is one of the effective methods to control the disease. The objectives of this study were to estimate broad sense heritability and to evaluate phenotypic and genotypic correlation between PBND score and PBND incidence in the F_4 generation of 10 crosses of peanut. A randomized complete block design with 4 replications was used for testing the mentioned F_3 families in F_4 generation at two locations in Kalasin province in the Northeast of Thailand. Characters under study were PBND score and PBND incidence (percent infected plants) evaluated at 30, 40, 50, 60, 70, and 90 days after sowing (DAS). The 50 and 60 day data are reported herein. There were significant differences among crosses for PBND score and PBND incidence. Means for PBND score and PBND incidence of resistant x susceptible group were intermediate between resistant x resistant group and

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Received, 15 July 2005 Accepted, 9 November 2005

susceptible x susceptible one. ICGV 86388 x IC 34 and IC 10 x KK 4 had lower PBND score and PBND incidence than the other crosses.

Heritability estimates for PBND score and PBND incidence evaluated at 50 and 60 DAS were moderate to high, ranging from 0.27 to 0.90, revealing that families that had low PBND score and PBND incidence could be readily identified in the F_4 generation. Phenotypic and genotypic correlations between PBND score and PBND incidence were closely associated, indicating that single parameter evaluation is sufficient. PBND incidence is more suitable than PBND score because of its simplicity.

Key words : PBND score, PBND incidence, heritability estimate

บทคัดย่อ

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อัตราพันธุกรรม สหสัมพันธ์ที่ปรากฏ และสหสัมพันธ์ทางพันธุกรรมของปฏิกิริยาต่อโรคยอดไหม้ในถั่วลิสงที่เกิดจากไวรัส

ว. สงขลานครินทร์ วทท. 2549 28(3) : 469-477

โรคยอดไหม้ที่เกิดจากไวรัสเป็นโรคที่มีความสำคัญต่อการผลิตถั่วลิสงในประเทศไทยโดยเฉพาะอย่างยิ่งในฤดูแล้ง การใช้พันธุ์ต้านทานเป็นวิธีหนึ่งที่มีประสิทธิภาพในการควบคุมโรคยอดไหม้ วัตถุประสงค์ของการศึกษานี้เพื่อประเมินอัตราพันธุกรรมแบบกว้าง และสัมประสิทธิ์สหสัมพันธ์ที่ปรากฏและสหสัมพันธ์ทางพันธุกรรมระหว่างคะแนนความรุนแรงของโรค และเปอร์เซ็นต์ต้นที่เป็นโรค โดยประเมินในลูกผสมชั่วที่ 4 ของถั่วลิสง 10 คู่ผสม ใช้แผนการทดลองแบบสุ่มสมบูรณ์ภายในซ้ำ มี 4 ซ้ำ 2 สถานที่ทดลองในจังหวัดกาฬสินธุ์ ลักษณะที่ศึกษาคือ คะแนนความรุนแรงของโรคยอดไหม้ และเปอร์เซ็นต์ต้นที่เป็นโรคยอดไหม้ โดยประเมินเมื่อถั่วลิสงมีอายุ 30, 40, 50, 60, 70 และ 80 วัน หลังปลูก และได้รายงานผลเฉพาะจากการประเมินเมื่อ 50 และ 60 วัน หลังปลูก

จากผลการทดลองพบว่า มีความแตกต่างกันในระหว่างคู่ผสมอย่างมีนัยสำคัญ ในลักษณะคะแนนความรุนแรงของโรค และเปอร์เซ็นต์ต้นที่เป็นโรค โดยมีค่าเฉลี่ยของคู่ผสมจากการผสม ต้านทาน x อ่อนแอ อยู่ระหว่างค่าเฉลี่ยของคู่ผสมจากการผสม อ่อนแอ x อ่อนแอ และต้านทาน x ต้านทาน คู่ผสม ICGV 86388 x IC 34 และ IC 10 x KK 4 มีคะแนนความรุนแรงของโรคและเปอร์เซ็นต์ต้นที่เป็นโรคต่ำกว่าคู่ผสมอื่น

ค่าอัตราพันธุกรรมในลักษณะคะแนนความรุนแรงของโรค และเปอร์เซ็นต์ต้นที่เป็นโรค ในการประเมินเมื่อ 50 และ 60 วัน หลังปลูก มีค่าปานกลางถึงสูง มีพิสัยตั้งแต่ 0.27 ถึง 0.90 ซึ่งแสดงให้เห็นว่าสามารถที่จะระบุสายพันธุ์ที่มีคะแนนความรุนแรงของโรคและเปอร์เซ็นต์ต้นที่เป็นโรคต่ำในลูกผสมชั่วที่ 4 ได้ ค่าสหสัมพันธ์ระหว่างลักษณะคะแนนความรุนแรงของโรค และเปอร์เซ็นต์ต้นที่เป็นโรค มีความสัมพันธ์กันสูง ทั้งในลักษณะที่ปรากฏและลักษณะทางพันธุกรรม แสดงให้เห็นว่าสามารถประเมินเพียงลักษณะใดลักษณะหนึ่งก็เพียงพอ ลักษณะเปอร์เซ็นต์ต้นที่เป็นโรคน่าจะเป็นลักษณะที่ควรจะประเมินมากกว่าคะแนนความรุนแรงของโรค เพราะประเมินได้ง่ายและสะดวกกว่า

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Peanut bud necrosis disease (PBND) caused by *Peanut bud necrosis virus* (PBNV) is the most economically important disease among virus diseases of peanut in Thailand. It is transmitted by thrips (*Thrip palmi* Karny.). Since the first report in Thailand in 1984, the disease incidence has increased at an alarming rate especially during the dry season (Wongkaew, 1990; Wongkaew and Chuapong, 1996). It is a newly emerging viral disease of peanut that can cause severe losses in

many peanut-producing countries in South Asia and Southeast Asia, including India, Pakistan, Nepal, Myanmar, Thailand, Vietnam and Sri Lanka (Buiel, 1996). In India, yield losses of 89 million US dollars per annum have been estimated (Reddy *et al*., 1995).

PBNV causes a wide range of symptoms in peanut, including mosaic, ringspot, chlorosis, rosette, tip blight and bud blight. Wongkaew (1993) and Reddy *et al*. (1995) classified the visual symptoms into two stages: primary and secondary. The primary symptoms start with chlorotic spot on an infected leaf at the middle of the stem. After that, the vein becomes chlorotic and newly emerging leaves turn mosaic. These leaves can be misshaped, deformed or crooked. Leaf size is much smaller than usual and mostly rosette due to short internodes. Leaves at the top of the plants always droop, and some may show necrosis that can spread throughout the whole leaf. At primary stage, other symptoms such as mosaic, ringspot, oak leaf pattern or chlorotic ringspot may occur. If infection occurs at early growth stage, the infected plants often die and yield no pod

The typical symptoms are mild ringspot, or necrosis at the shoot tip after which yellowing and wilting occur resulting in a drying out of the whole plant. These symptoms can be easily differentiated from other virus disease. The secondary symptoms are stunting, axillary shoot proliferation and malformation of leaflets. If infection occurs in mature plant, the necrosis and other symptoms may be limited to the infected leaves or infected branches. The infected plants may survive and may be able to produce some pods. However, pods are small in number and size, and seed quality is also affected (Wongkaew, 1993).

World germplasm collections have been screened to identify resistant lines. Resistant accessions were found among cultivated peanuts (Dwiwedi *et al*., 1995) and wild *Arachis* species (Dwivedi *et al*., 1995; Reddy *et al*., 2000). These peanut germplasm lines have been used as sources of PBNV resistance in peanut breeding for PBNV resistance in India and Thailand.

A few studies have reported genetic control of PBNV resistance. Buiel (1996) reported that additive gene effects controlled quantitative traits of PBNV resistance. Pensuk *et al*. (2002) reported additive gene actions predominate over dominance and epistatic gene actions. Kesmala (2003) also reported that additive genetic component was important for peanut bud necrosis disease (PBND) incidence (percent infection) and PBND score (severity of disease symptoms). In addition, broad sense heritability estimate for PBND score was low and correlation between PBND score and PBND incidence was high.

As part of the on-going peanut breeding program at Khon Kaen University, this study was conducted with the objectives of evaluating broad sense heritability and estimating phenotypic and genotypic correlation between PBND score and PBND incidence in the materials to be used in peanut breeding for PBND resistance.

Materials and Methods

F_3 seeds of 10 peanut crosses were kindly provided by Dr. Viboon Pensuk (Pensuk *et al*., 2002). These crosses were divided into three groups resistant x resistant, resistant x susceptible and susceptible x susceptible groups. Resistant x resistant group consisted of ICGV 86388 x IC 10 and ICGV 86388 x IC 34. Resistant x susceptible group comprised ICGV 86388 x KK 4, ICGV 86388 x KK 60-1, IC 10 x KK 4, IC 10 x KK 60-1, IC 34 x KK 4 and IC 34 x KK 60-1. Susceptible x susceptible group included JL 24 x KK60-1 and KK 4 x KK 60-1. Descriptions of parental lines and cross regeneration were reported by Pensuk (2002).

Thirty F_3 families of each cross derived from the F_2 plants were randomly chosen and multiplied by selfing to produce ample F_4 seeds at Khon Kaen University's agronomy farm during rainy season in 2001. The 300 families of 10 crosses with 30 families each, including two resistant check cultivars (IC 10 and IC 34) and a susceptible check cultivar (Tainan 9) were evaluated under natural infestation of *Peanut bud necrosis virus* (PBNV) at two hot spots (sites) in Kalasin province in the

Northeast of Thailand during January to May 2002. Experimental site 1 was in Muang district and experimental site 2 was in Rongkhum district. The two sites were about 30 km apart. A randomized complete block design with 4 replications was used in both locations. Each entry was planted in a single row plot with 3 m long on a raised bed, which could accommodate 2 rows. Spacing was 50 cm between rows and 30 cm between plants within the row.

A fungicide (captan; 3a,4,7,7a-tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione) and ethephon ((2-Chloroethyl) phosphonic acid) 0.02% were used for seed treatment to control crown rot disease and break possible seed dormancy because some parents are Virginia type peanut that has seed dormancy. Seeds were over-planted and then seedlings were thinned to obtain one plant per hill. Pre-emergence herbicide (alachlor; acetramide, 2-chloro-N-(2, 6-diethylphenyl)-N-(methoxymethyl)) was sprayed soon after planting at the rate of 500 cc rai^{-1} (0.16 ha). Hand weeding was done at 20 days after planting. Inorganic fertilizers formula 12-24-12 of N-P_2O_5-K_2O at the rate of 25 kg rai^{-1} and gypsum ($CaSO_4$) at the rate of 50 kg rai^{-1} were applied at 30 days after planting. No herbicides or pesticides were used during the crop cycle, and irrigation was applied as needed.

Data collection

Peanut bud necrosis disease (PBND) resistance parameters were recorded as PBND score and PBND incidence (percent infected plants) at 30, 40, 50, 60, 70 and 90 day after sowing (DAS). PBND score was rated by visual observation on individual plants in the plots. Disease score ratings were 1 to 5, where 1= healthy plant, 2= spots on some leaves, 3 = systemic symptoms without stunting, 4 = systemic symptoms with stunting and 5 = severe necrosis or die (Pensuk, 2002), and then the average score was calculated for each plot. PBND incidence was calculated based on the total number of plants in each plot.

At each evaluation date, bamboo sticks were placed near main stems of diseased plants to observe symptom development. The color of bamboo sticks corresponded to each evaluation date. Because there was high outbreak of peanut leaf yellow spot disease in both locations and its symptoms could confound PBND score rating 2, plants with rating 2 that did not show the symptom progress at successive evaluations were rescored as healthy plant.

Data analysis

Analysis of variance and mean comparison

Data were tested for violation of assumptions underlying the analysis of variance. PBND score data were log-transformed and PBND incidence data were arcsine-transformed. Separate analysis of variance was performed for each location according to a randomized complete block design. Entries were considered as fixed effects, while replications and location were random. Error mean squares were tested for variance heterogeneity using Bartlett's method and combined analysis of variance was performed. Duncan's multiple range test was used to compare means (Gomez and Gomez, 1984).

Fifty and 60 day data were reported because of low C.V. values and significant F-values. Mean comparisons of separate analyses and combined analysis for PBND score and PBND incidence followed similar patterns. Therefore, only combined results of 50 and 60 day data are reported herein.

Broad-sense heritability

Estimates of broad-sense heritability for 10 crosses were calculated by partitioning variance components of family mean squares to pooled environmental variance (σ^2_E) and genotypic variance (σ^2_G), and then broad-sense heritability estimate (h^2) was calculated as follows (Srinives, 1982):

Phenotypic and genotypic correlations

Phenotypic and genotypic correlations between PBND score and PBND incidence were calculated as described by Srinives (1982). Mean squares for PBND score (X) and PBND incidence (Y), mean squares of cross products (MCP),

Table 1. Analysis of variance in combined experiments of a randomized complete block design.

S.O.V.	df	MS	EMS
Locations (l)	l-1		
Reps within l	l(r-1)		
Families (f)	f - 1	MF	$\sigma^2 + r\sigma^2_{FL} + rl\sigma^2_F$
F x Locations	(f-1)(l-1)	MFL	$\sigma^2 + r\sigma^2_{FL}$
Pooled error	l(r-1)(f-1)	ME	σ^2

$h^2 = \sigma^2_G/\sigma^2_P$
$\sigma^2_G = \sigma^2_F$
$\sigma^2_P = \sigma^2_F + \sigma^2_{FL}/l + \sigma^2/rl$

Where : h^2 = broad sense heritability
σ^2_G = genotypic variation
σ^2_P = phenotypic variation
r = no. of replications
l = no. of locations
f = no. of families

Table 2. Analysis of variance of cross and cross product

S.O.V.	df	MS Character		MCP	EMS	EMCP
		X	Y			
Locations (l)	l-1					
Reps within l	l(r-1)					
Families (f)	f - 1	M_3'	M_3	$M_3'M_3$	$\sigma^2_E + r\sigma^2_{Fl} + rl\,\sigma^2_F$	$\sigma_{E'E} + r\sigma_{(Fl')\,(Fl)} + rl\sigma_{F'F}$
F x Locations	(f-1)(l-1)	M_2'	M_2	$M_2'M_2$	$\sigma^2_E + \sigma^2_{Fl}$	$\sigma_{E'E} + r\sigma_{(Fl')\,(Fl)}$
Error	l(r-1)(f-1)	M_1'	M_1	$M_1'M_1$	σ^2_E	$\sigma_{E'E}$

Phenotypic correlation $(r_p) = (M_3'M_3) / [(M_3')(M_3)]^{1/2}$
Genotypic correlation $(r_G) = (M_3'M_3 - M_2'M_2) / [(M_3' - M_2')(M_3 - M_2)]^{1/2}$

expected mean squares (EMS) and expected mean of cross products (EMCP) are outlined in Table 2.

Results and Discussion

Mean comparison

Combined analysis of variance across two locations showed that cross x location interactions for PBND score and PBND incidence were not significant (data not reported), indicating that few good test sites are sufficient for the effective test of breeding materials for PBNV resistance. Buiel (1996) also found small genotype x environment interaction for PBND incidence in India. However, the two locations were approximately 30 km apart and environmental conditions were considered very similar. Cross x location interactions might occur if the two locations were more widely separate.

Mean comparisons for PBND score and PBND incidence showed that standard resistant check cultivars (IC 10 and IC 34) and susceptible check cultivar (Tainan 9) were significantly different (Table 3). For PBND incidence, means of most cross populations were not significantly different from susceptible check cultivar, except for the cross ICGV 86388 x IC 10 at 50 and 60 DAS and the cross ICGV 86388 x IC 34 at 50 DAS. Most crosses had higher PBND incidence than the resistant check cultivars (IC 10 and IC 34), except for the cross ICGV 86388 x IC 34 at 50 and

Table 3. Means of peanut bud necrosis disease (PBND) incidence and PBND score in 10 crosses of peanut, two resistant check cultivars and a susceptible check cultivar evaluated at 50 and 60 DAS at two locations in Kalasin.

Genotype	PBND incidence		PBND score[1/]	
	50 DAS	60 DAS	50 DAS	60 DAS
Resistant x resistant				
ICGV 86388 x IC 34	3.46cd	3.71cde	1.090de	1.115cd
ICGV 86388 x IC 10	1.57cd	1.66de	1.053de	1.058cd
Resistant x susceptible				
ICGV 86388 x KK 4	5.47abc	6.40abc	1.151bcd	1.204bc
ICGV 86388 x KK 60-1	3.97bcd	4.56cd	1.099de	1.148cd
IC 10 x KK 4	4.11bcd	4.41cd	1.096de	1.128cd
IC 10 x KK 60-1	3.70bcd	4.04cd	1.108de	1.133cd
IC 34 x KK 4	4.52abc	4.92bcd	1.131cde	1.157cd
IC 34 x KK 60-1	6.16abc	6.77abc	1.153bcd	1.200bc
Susceptible x susceptible				
JL 24 x KK 60-1	10.44a	11.98a	1.289ab	1.380a
KK 4 x KK 60-1	9.42ab	10.94ab	1.266bc	1.363ab
Resistant check cultivar				
IC 10	0.00e	0.00f	1.000e	1.000d
IC 34	2.50de	2.50ef	1.038de	1.050cd
Susceptible check cultivar				
Tainan 9	12.64ab	12.64abc	1.456a	1.556a
F-ratio	12	13	13**	16**
C.V. (%)	37.22	36.52	11.06	11.79

Note: Means in the same column followed by the same letter(s) are not significantly different at 0.05 probability level by DMRT
DAS = days after sowing
[1/] 5 ratings; 1 = healthy plant, and 5 = severe necrosis or die

60 DAS, which was intermediate between IC 10 and IC 34. For PBND score, means of most crosses were significantly lower than that of the susceptible check cultivar and close to the resistant check cultivars, except for the cross JL 24 x KK 60-1 at 50 and 60 DAS and the cross KK 4 x KK 60-1 at 60 DAS.

The low PBND incidence in this experiment (12.64% for susceptible check cultivar) might cause small difference among tested peanut genotypes especially among the resistant x susceptible group and susceptible check cultivar. The families of crosses ICGV 86388 x IC 10 and ICGV 86388 x IC 34 had the lowest PBND score and PBND incidence. They should be retained for further selection if heritability estimates are high.

Heritability

Broad-sense heritability estimates based on family means at 50 and 60 DAS ranged from 0.29 to 0.91 for PBND incidence and 0.27 to 0.90 for PBND score. On average, the families from susceptible x susceptible crosses had lower heritability estimates than resistant x susceptible ones. Among resistant x resistant group, the families from cross ICGV 86388 x IC 34 had lower heritability estimates than the families from cross ICGV 86388 x IC 10 in which the heritability estimates were high for PBND incidence and PBND score (Table 4).

Table 4. Heritability estimates (based on family means) for peanut bud necrosis disease (PBND) incidence and PBND score in the F_4 generation (F_3 family) of 10 crosses of peanut evaluated at 50 and 60 DAS at two locations in Kalasin.

Cross	PBND incidence		PBND score[1/]	
	50 DAS	60 DAS	50 DAS	60 DAS
Resistant x resistant				
ICGV 86388 x IC 34	0.91	0.91	0.89	0.87
ICGV 86388 x IC 10	0.46	0.51	0.27	0.64
Resistant x susceptible				
ICGV 86388 x KK 4	0.90	0.91	0.84	0.90
ICGV 86388 x KK 60-1	0.76	0.76	0.45	0.79
IC 10 x KK 4	0.66	0.63	0.77	0.71
IC 10 x KK 60-1	0.77	0.77	0.79	0.79
IC 34 x KK 4	0.72	0.80	0.70	0.79
IC 34 x KK 60-1	0.77	0.78	0.84	0.83
Susceptible x susceptible				
JL 24 x KK 60-1	0.29	0.48	0.50	0.53
KK 4 x KK 60-1	0.57	0.50	0.50	0.40

Note: DAS = days after sowing
[1/] 5 ratings; 1 = healthy plant, and 5 = severe necrosis or die

The high heritability values in this study were in contrast to the results of Kesmala (2003) and Poldate (2002). They reported low heritability estimates for PBND score based on individual plants in F_2 generation. The contrasting results might be due to the difference in evaluation methods. In this study, the heritability estimates were evaluated based on variation among families within the crosses. Pensuk (2002) reported that non-additive gene effects were important in F_1 and F_2 generations, but smaller than additive gene effects. In contrast, Buiel (1996) found that additive gene effects involved in the inheritance of PBNV resistance, but dominance and epistatic gene effects were not important. The heritability estimates in this study were evaluated in the F_4 generation (F_3 families), when non-additive gene effects, if any, were reduced after three successive generations of selfing. The broad-sense heritability values were, therefore, assumed to be close to narrow-sense heritability estimates, and selection for superior lines should be possible.

The most promising cross appears to be ICGV 86388 x IC 10. With the lowest PBND score and PBND incidence and the high heritability estimates, it should be possible to select superior lines (among families) with relatively small efforts in this cross. Because susceptible parents have good agronomic background, mild selection should also be practised in the crosses involving resistant x susceptible parents. Bi-directional selection for high and low PBND incidence should be carried out to compare the progress from selection in later generations.

Phonotypic and genotypic correlations

Correlation coefficients between PBND score and PBND incidence at 50 and 60 DAS ranged from 0.88 to 0.99 and 0.92 to 1.00 for phenotypic correlation and genotypic correlation, respectively (Table 5). Kesmala (2003) also reported the similar relationship. The high and complete correlation indicated that genetic systems for these traits were closely associated, and thus single evaluation for the trait that is easier to evaluate is sufficient. PBNV incidence is easier to

Table 5. Phenotypic and genotypic correlation between peanut bud necrosis disease (PBND) score and PBND incidence in the F_4 generation (F_3 family) of 10 crosses of peanut evaluated at 50 and 60 DAS at two locations in Kalasin.

Cross	50 DAS		60 DAS	
	r_P	r_G	r_P	r_G
Resistant x resistant				
ICGV 86388 x IC 34	0.88	0.85	0.98	1.00
ICGV 86388 x IC 10	0.97	0.99	0.99	1.00
Resistant x susceptible				
ICGV 86388 x KK 4	0.98	1.00	0.99	1.00
ICGV 86388 x KK 60-1	0.92	1.00	0.98	1.00
IC 10 x KK 4	0.92	1.00	0.93	1.00
IC 10 x KK 60-1	0.90	0.92	0.97	0.99
IC 34 x KK 4	0.94	1.00	0.97	1.00
IC 34 x KK 60-1	0.95	1.00	0.97	1.00
Susceptible x susceptible				
JL 24 x KK 60-1	0.90	1.00	0.95	1.00
KK 4 x KK 60-1	0.94	0.94	0.93	0.90

Note: DAS= days after sowing
r_P = phenotypic correlation, r_G = genotypic correlation

determine than PBND score and should be used as a selection criterion for PBNV resistance.

Conclusion

High heritability estimates and high correlation between PBND score and PBND incidence indicated the possibility to select against these traits simultaneously by selecting among segregating progenies derived from crosses. The cross ICGV 86388 x IC 10 is more promising because its progenies have low PBND score and PBND incidence and high heritability estimates. PBND incidence should be used as a selection criterion for PBNV resistance because of its simplicity in operation.

Acknowledgements

This work was funded by the Senior Research Scholar Project of Professor Dr. Aran Patanothai under the Thailand Research Fund and also supported by Peanut Improvement Project, Department of Agronomy and Plant Breeding Research Center for Sustainable Agriculture, Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002 Thailand.

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