

New Sources of Resistance to Fusarium Wilt, Sterility Mosaic Disease and Phytophthora Blight in Vegetable Pigeonpea Germplasm

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Abstract

Vegetable pigeonpea germplasm collection composed of 224 genotypes representing the wide geographical diversity worldwide was evaluated to identify the sources of resistance to Fusarium wilt, sterility mosaic disease and Phytophthora blight. Screening was done in the wilt and sterility mosaic disease sick plot at International Crops Research Institute for the Semi-Arid Tropics, Patancheru. Twelve genotypes were found resistant to Fusarium wilt (< 10% disease incidence), which originated from five countries. Sterility mosaic disease resistance was found in 30 genotypes that originated from six countries. Thirty four genotypes showed resistant reaction to Phytophthora blight under natural epiphytotic conditions. Combined resistance to wilt and sterility mosaic disease was found in four genotypes (ICPs 7991, 12059, 13257 and 14291). However, only one genotype (ICP 13229) was found to have combined resistance to sterility mosaic disease and Phytophthora blight. The geographically diverse genotypes that are resistant to these diseases should be useful to vegetable pigeonpea disease resistance breeding program.

Keywords: Host plant resistance, *Cajanus cajan*, *Fusarium udum*, *Phytophthora drechsleri* f. sp. *cajani* and Sterility mosaic disease

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is the most versatile grain legume cultivated in more than 25 tropical and sub-tropical countries, which plays an important role in food security, balanced diet and alleviation of poverty because of its diverse usage as a food, fodder and fuel. It is consumed in various forms (*dal*, *sambhar* and immature seeds as fresh vegetable) in different countries. However, being a rich source of protein (~20% crude protein), the use of vegetable pigeonpea as fresh or canned green peas is preferred in parts of India, Africa, Central America and the Caribbeans. The green pigeonpea seeds are considered superior to *dal* in general nutrition (Saxena *et al.* 2010). At present, protein availability among rural masses in the developing world is less than one-third of its normal requirements and with continuously growing population and stagnation of productivity, the pulse program is facing a greater challenge to meet the demand of unprivileged mass.

Globally, pigeonpea is cultivated in about 4.5 million ha, adding 3.48 million tones of grain to the global food basket (FAOSTAT 2009). India is the largest pigeonpea producer in the world with contribution of 75-80 per cent. Although,

India leads the world both in area and production of pigeonpea, its productivity is lower than the world average, which may be attributed to the various biotic and abiotic stresses. Among the biotic stresses, Fusarium wilt and sterility mosaic diseases are the major constraints to pigeonpea production worldwide. Recently, Phytophthora blight is emerging as a potential threat to pigeonpea production especially in regions prone to temporary flooding due to excessive rains (Pande *et al.* 2011).

Fusarium wilt (FW), caused by *Fusarium udum* Butler, is the most devastating disease of pigeonpea reported from all the crop growing regions (Jain and Reddy 1995). In India alone, the loss due to this disease has been estimated at US \$ 71 million (Kannaiyan *et al.* 1984, Reddy *et al.* 1993). The pathogen survives in the soil and on residual plant debris causing yield losses from 30-100% (Kannaiyan *et al.* 1981, Reddy *et al.* 1993). Sterility mosaic disease (SMD), often referred to as "Green Plague", is important disease of pigeonpea in India and can cause yield losses up to 100% (Reddy and Nene 1981, Singh *et al.* 1999, Jones *et al.* 2004). The disease is caused by pigeonpea sterility mosaic virus (PPSMV), which is transmitted by eriophyid mite (*Aceria*

cajani) (Kumar *et al.* 2003). Yield losses due to SMD in India alone, were estimated at 205,000 tons of grains valued at US \$ 76 million (Kanniyan *et al.* 1984). The disease causes mosaic symptoms on leaves, reduction in size and finally complete or partial cessation of reproductive structures. Phytophthora blight (PB) caused by necrotrophic fungus *Phytophthora drechsleri* Tucker f. sp. *cajani*, Kannaiyan *et al.* is reoccurring as an economically important disease of pigeonpea especially when excessive rains fall within a short span of time (Sharma *et al.* 2006, Pande *et al.* 2011). Characteristic symptoms of the disease are water-soaked lesions on the leaves, slightly sunken lesions on stems and petioles, girdling of the stem and finally drying of foliage. Widespread incidence of PB was recorded on improved and local varieties irrespective of cropping system and soil type in Deccan Plateau of India (Sharma *et al.* 2006, Pande *et al.* 2006).

Vegetable germplasm collection maintained in the gene bank at ICRISAT represents vast genetic variation that can be utilized in crop improvement (Upadhyay *et al.* 2010). Developing resistant cultivars is the most effective method to minimize yield losses due to FW, SMD and PB diseases of pigeonpea. Therefore, the present study was undertaken to evaluate vegetable pigeonpea germplasm to identify resistance to FW, SMD and PB that could be utilized specifically in vegetable pigeonpea disease resistance breeding program.

Materials and methods

Seed source

Seed of the 224 vegetable pigeonpea genotypes was obtained from the Genetic Resource Division, ICRISAT, Patancheru, India. Seeds of all the genotypes used as susceptible checks for different diseases were obtained from the Department of Legumes Pathology, ICRISAT, Patancheru, India.

Evaluation for Fusarium wilt resistance

The 224 vegetable pigeonpea genotypes were evaluated in the pigeonpea wilt-sick plot (5×10^6 cfu of *Fusarium udum* / g soil) under artificial epiphytotic conditions at ICRISAT, Patancheru during 2009-10 crop season. Each genotype was planted in two rows of 4m length with a spacing of 60cm between rows and 15cm between plants. The trial was laid out in randomized complete block design with two replications. Susceptible (ICP 2376) and resistant (ICP 8863) checks for wilt was included after every 10 test rows for comparison. Susceptible check also served as an indicator/infecter rows. Plants were scored for wilt incidence at seedling, flowering and pod formation stages by counting the healthy plants (no wilt symptoms) and diseased plants.

Resistance to FW found in wilt-sick plot was confirmed in greenhouse following root dip inoculation technique (Nene *et al.* 1981). Resistant genotypes were raised in polythene bags filled with sterilised river sand in a greenhouse maintained at $25 \pm 3^\circ\text{C}$ for eight days. Wilt susceptible check (ICP 2376) was used for comparison. Inoculum was prepared from a single conidial culture of *F. udum* isolated from wilt-infected plants collected from ICRISAT wilt sick plot. For mass inoculum preparation, a 7-mm disc of actively growing *F. udum* culture was put into a 250ml conical flask containing 100ml of sterilized potato dextrose broth and incubated for seven days in incubator shaker at $25 \pm 1^\circ\text{C}$ and 125 rpm. The culture was then homogenized in sterilized distilled water and adjusted to 6×10^5 conidia ml^{-1} using a haemocytometer for use as an inoculum. Eight-day-old seedlings of each test as well as susceptible genotypes grown in sterilized river sand were uprooted, cleaned with tap water and root inoculated by dipping in inoculum suspension for 1-2 minutes to enable conidia to adhere to the roots. Inoculated seedlings were transplanted in pre-irrigated sterile vertisol and sand (3:1) in pots and incubated in a greenhouse at $25 \pm 3^\circ\text{C}$. Thirty seedlings of each line were tested in three replications (10 seedlings/pot) in a completely randomized design (CRD). Disease incidence was recorded for 60 days after inoculation.

Evaluation for sterility mosaic disease resistance

All the 224 vegetable pigeonpea genotypes were evaluated for SMD in Pigeonpea sterility mosaic disease sick plot during the 2009-10 crop season at ICRISAT, Patancheru. The experimental design was similar as explained above. A SMD susceptible check (ICP 8863) and resistant check (ICP 2376) was included after every 10 test rows. At two-leaf stage, each and every plant of the test entries was inoculated with SMD infested leaves using the leaf staple technique (Nene *et al.* 1981). The SMD infected leaflet (maintained on the susceptible cultivar ICP 8863 in isolated pigeonpea sterility mosaic disease nursery at ICRISAT) was taken and folded on the primary leaf in such a way that its lower surface comes in contact with a primary leaf of the test seedling and then staple with a small paper stapler for successful SMD infection. Susceptible check ICP 8863 was planted in the sick plot one month in advance of the regular planting time to serve as an infecter row in order to have a good source of virus inoculum. The genotypes found resistant to SMD under field conditions were confirmed in the greenhouse following leaf staple technique. Genotypes were screened in the pots in three replications in a CRD and in each replication five plants were maintained. Procedure for inoculation was similar as explained above.

Evaluation for PB resistance

The 224 vegetable pigeonpea genotypes planted in wilt and sterility mosaic disease sick plot were also evaluated for PB under natural epiphytotic conditions at ICRISAT, Patancheru during 2009-10 crop season. Observations on plant mortality due to PB were recorded periodically in two replications planted in RCBD. Percent disease incidence was calculated for each genotype.

Statistical analysis

Data on disease incidence (FW, SMD and PB) was collected from each replication in the randomized experimental block for the field experiment. Based on the disease reaction, the genotypes were grouped as resistant (0-10% of plants mortality); moderately resistant (10.1-20% plant mortality); susceptible (20.1-40% plant mortality) and highly susceptible (>40%). Data collected on disease incidence for each disease were subjected to arc sine transformation to make error variances homogenous. Best linear unbiased predictors (BLUPs) for each genotype were estimated. Analysis of Variance (ANOVA) was carried out to determine the effect of genotypes and their interaction considering replication as fixed and genotype as random using proc mixed procedure of SAS software version 9.2 for Windows (SAS Institute Inc. 2008. SAS/STAT® 9.2 User’s Guide, Cary, NC: SAS Institute Inc).

Results and discussion

Fusarium wilt resistance

The FW incidence in 224 vegetable pigeonpea germplasm lines ranged from 0-100% (Fig. 1). The susceptible check exhibited 100% wilt incidence indicating that screening was reliable. The ‘F’ value calculated from ANOVA was significant at 1% level of significance suggesting that genotypes showed considerable variation for the FW reactions. Description of the statistics of FW incidence is given in Table 1. Based on the mean disease incidence of both the replications, 12 genotypes were found resistant, 16 moderately resistant (10.1-20% incidence), 56 susceptible (20.1-40% incidence) and 139 highly susceptible (40.1-100% incidence) to FW. Among the 12 resistant genotypes, five genotypes ICP’s 7903, 12031, 12059, 12771 and 12775 were found asymptomatic (0% plant mortality) and in seven genotypes (ICP’s 7991, 12841, 13257, 13258, 13618, 14291 and 15137) incidence ranged 2.9-9.5% (Table 2). All the genotypes found resistant in the field also showed resistant reaction in the greenhouse. These resistant genotypes originated from India, Tanzania, Philippines, Kenya and Zaire.

Sterility mosaic disease resistance

The ANOVA exhibited significant ($P < 0.0001$) variation among the 224 vegetable pigeonpea genotypes for SMD

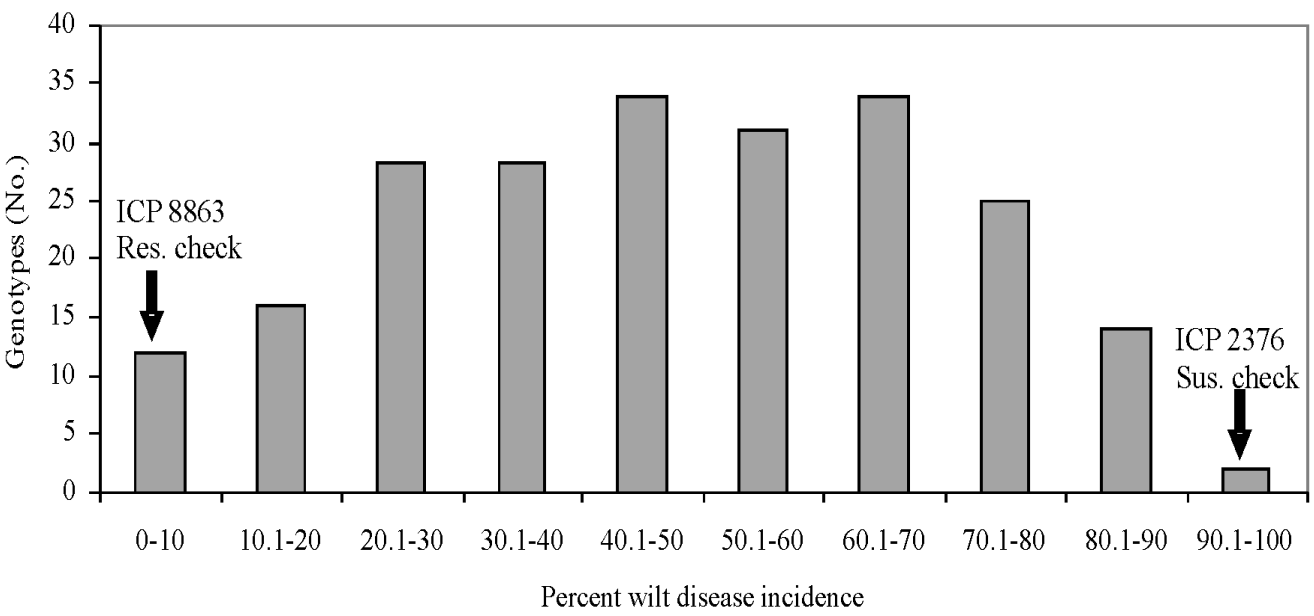


Figure 1. Frequency distributions of per cent disease incidence for Fusarium wilt in 224 vegetable pigeonpea germplasm collection evaluated during 2009-10 crop season. The mean disease incidence for susceptible (ICP 2376) and resistant (ICP 8863) genotypes are indicated by arrows.

(Table 1). Most of the genotypes (68%) showed susceptible to highly susceptible reaction to SMD. Susceptible check ICP 8863 showed 100% SMD incidence. However incidence was 3.8% in the resistant check ICP 2376. Frequency distribution of per cent disease incidence of SMD is given in Fig. 2. Based on the mean of disease incidence in both the replications, 30 genotypes were found resistant (<10% incidence) and 24 moderately resistant (10.1-20% incidence) to SMD. Six genotypes (ICP's 7867, 9151, 12059, 13183, 13593, and 14291) were free from SMD (0% incidence, Table 2). All the 30 resistant genotypes showed resistant reaction in greenhouse. Among the resistant genotypes, nine originated each from India, Tanzania and Kenya and one each from Ethiopia, Trinidad & Tobago and Dominican Republic indicating wide geographical diversity of resistant lines.

Phytophthora blight resistance

Characteristic PB symptoms were observed in the vegetable pigeonpea genotypes under natural epiphytotic conditions. In the 224 genotypes, PB incidence varied from 0 to 100% (Fig. 3). Based on the mean disease incidence of the two replications, 34 genotypes were found resistant and 47 moderately resistant. The ANOVA showed significant ($P < 0.001$) variation among the 224 genotypes evaluated for the PB incidence under natural conditions (Table 1). Six genotypes (ICPs 7916, 12055, 12114, 12161, 13126, 15511) were found asymptomatic with no incidence of PB. The PB resistant genotypes originated from 12 countries (India, Colombia, Kenya, Tanzania, Mozambique, Malawi,

Table 1. Analysis of variance for Fusarium wilt, Sterility mosaic disease and Phytophthora blight incidence in the vegetable pigeonpea germplasm

Covariance parameter	Estimate of variance	Probability (F)
Fusarium wilt		
Genotype	0.035	<.0001*
Replication	-	0.97
Residual	0.093	<.0001*
Sterility mosaic disease		
Genotype	0.037	<0.0001*
Replication	-	0.91
Residual	0.024	<.0001*
Phytophthora blight		
Genotype	0.054	<0.0001*
Replication	-	0.0001*
Residual	0.148	<.0001*

* Statistically significant.

Grenada, Barbados, Venezuela, Zaire, Nigeria and Uganda) representing diverse geographical locations. The PB resistant genotypes identified in the field under natural epiphytotic conditions need confirmation under the controlled environmental conditions.

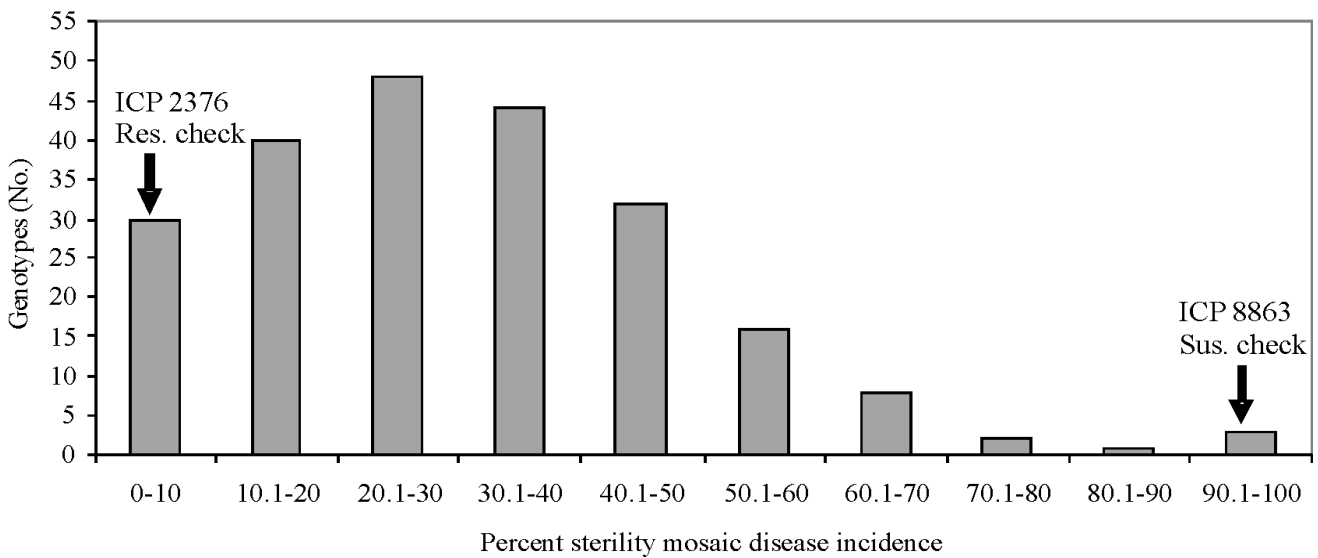


Figure 2. Frequency distributions of per cent disease incidence for sterility mosaic disease in 224 vegetable pigeonpea germplasm collection evaluated during 2009-10 crop season. The mean disease incidence for susceptible (ICP 8863) and resistant (ICP 2376) genotypes are indicated by arrows.

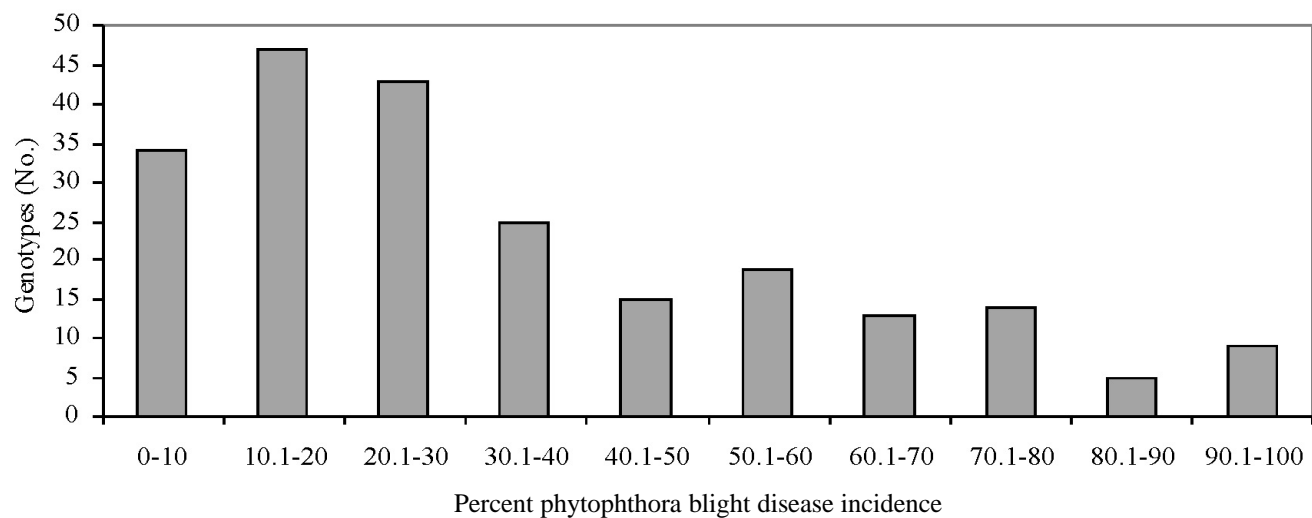


Figure 3. Frequency distributions of per cent disease incidence for *Phytophthora* blight in 224 vegetable pigeonpea germplasm collection evaluated during 2009-10 crop season.

Multiple disease resistance

Among the 224 vegetable pigeonpea germplasm, no genotype was found resistant to more than two diseases. Four genotypes [ICPs 7991 (India), 12059 (Tanzania), 13257 (Kenya) and 14291 (Kenya)] had a combined resistance to both FW and SMD. Only one genotype [ICP 13229 (Kenya)] had combined resistance to SMD and PB. Combined resistance to FW and PB was not found in any of the vegetable pigeonpea germplasm collection (Table 2).

The present study indicated a high level of resistance to FW, SMD and PB diseases in the 224 vegetable pigeonpea germplasm lines evaluated in the wilt and sterility mosaic disease sick plot at ICRISAT, Patancheru during 2009-10 season. Sources of resistance to FW and SMD has been reported in germplasm and breeding lines of pigeonpea by various workers (Nene *et al.*, 1989, Khare *et al.* 1994, Reddy *et al.* 1998, Gwata *et al.* 2006), but to the best of our knowledge, no sources of resistance to these diseases are reported particularly in vegetable pigeonpea. The PB is emerging as a potential threat to pigeonpea production due to change in the frequency and distribution of rainfall and the resistant genotypes reported to this disease in earlier studies (Kannaiyan *et al.* 1981) have shown susceptible reaction (Sharma *et al.* 2006, Pande *et al.* 2006, 2011). In this study, few germplasm lines with resistant reactions to PB have been identified under natural conditions, which need further confirmation in controlled environment.

The use of pigeonpea as fresh vegetable (immature shelled seeds) is preferred in some parts of India particularly in Karnataka and Gujarat. The tribal areas of various states

also prefer vegetable pigeonpea as it is a rich source of protein (Saxena *et al.* 2010). Further, the number of seeds per pod is considered an important yield component in vegetable pigeonpea and is preferred by consumers (Saxena *et al.* 2010). Genotype ICP 138628, which was found resistant to PB in the present study had 10 seeds per pod (Sastry *et al.* 2006) and can be a potential source for meeting such demands. Vegetable pigeonpea germplasm collection evaluated in the present study originated from several countries representing wide geographical diversity (Saxena *et al.* 2010, Upadhayay *et al.* 2010). Therefore, identification of vegetable pigeonpea genotypes with resistance against single disease and/or select combination of two diseases (FW and SMD, and SMD and PB) would permit use of diverse sources for future breeding efforts and ensure a better chance of success in improving the disease resistance of vegetable pigeonpea.

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Table 2. Details of the genotypes showing resistance to individual disease indentified from the vegetable pigeonpea germplasm

Target disease	Disease reaction ^a	
	Asymtomatic	Resistant
Fusarium wilt	ICP 7903, 12031, 12059 , 12771, 12775	ICP 7991 , 12841, 13257 ,13258, 13618, 14291 , 15137
Sterility mosaic disease	ICP 7867, 9151, 12059 , 13183, 13593, 14291	ICP 7893, 7897, 7899, 7991 , 8535, 8692, 9157, 12009, 12019, 12090, 12139, 12747, 12806, 12808, 12813, 12819, 13107, 13229 , 13257 , 13277, 13625, 13774, 13965, 15176
Phytophthora blight	ICP 7916, 12055, 12114, 12161, 13126, 15511	ICP 6523, 7265, 7719, 7889, 7925, 8914, 9189, 10002, 12029, 12034, 12068, 12142, 12799, 12842, 13103, 13162, 13229 , 13241, 13271, 13438, 13599,13613, 13828, 13852, 14104, 15142, 15452, 15530

^aReaction of vegetable pigeonpea genotypes to individual disease is based on the mean of two replications; asymtomatic = 0% plant mortality, resistant = 1 to 10% plant mortality
*Genotypes with asymptomatic/resistant reaction to more than one disease are highlighted in the table

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