SSR Markers Linked to Mite (*Polyphagotarsonemus latus* Banks) Resistance in Jute (*Corchorus olitorius* L.)

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Abstract: The mite is considered as one of the major limiting factors of jute production in Bangladesh. So it is essential to select genotypes with the desired resistance allele combination using DNA markers. Eighty-eight SSR primers were used to screen the mite resistant and sensitive parents. Among them only twenty-one primer pairs showed polymorphism. A genetic linkage map was constructed using these polymorphic primers in one hundred and fifty F_2 populations, derived from a cross between the parents. The SSR markers J-170 and HK-89 have been mapped at 34.1 cM and 35.4 cM, respectively, on either side of the phenotypic marker. These two markers (J-170 and HK-89) along with HK-64 showed 100% selection efficiency in combination.

Keywords: jute; linkage map; mite; SSR

Jute (*Corchorus* species, 2*n* = 14) is an important bast fibre crop (Kundu 1956). It has a special significance in the Indian subcontinent due to the dependence of the livelihood of millions of small and marginal farming families on its production and utilization (Pathak 1991). The genus *Corchorus* comprises about 40 species (Purseglove 1968), among them only two species are widely cultivated. They are *Corchorus capsularis* L. and *C. olitorius* L. They have originated from Indo-Burma and. Africa, respectively (Kundu 1951). These two species are self-pollinating and contain very limited genetic variability with respect to (i) adaptability to different agronomic environments, (ii) fibre quality, (iii) fibre yield, and (iv) susceptibility to diseases and pests (La Farge *et al.* 1997). But unfortunately, these two species do not cross successfully with each other, possibly due to the presence of a strong sexual incompatibility between them (Patel & Datta 1960; Swaminathan *et al.* 1961).

Jute faces both biotic and abiotic stresses. Among them the yellow mite (*Polyphagotarsonemus latus*) is one of the important destructive pests of jute (Pradhan & Saha 1997). Both nymphs and adults suck the sap from younger leaves and shoots. The *C. olitoris* species of jute suffers more from the yellow mite infestation than *C. capsularis* (Das 1989). As a result, both the quality and quantity of the fibre are affected and it is estimated that the loss of jute production due to pest damage alone may be 38% (Keka *et al.* 2008).

Not much is known about plant defence responses to small arthropods that pierce single plant cells and feed on intracellular fluids, such as broad mites (Grinberg *et al.* 2005). One of the most significant advances to occur in the last decade for the development of improved crops is the use of molecular markers to identify and track genes of interest (Tanksley & McCouch 1997; Tanksley *et al.* 1989). Many disease resistance genes linked to SSR markers have already been

identified in different plants, such as southern corn rust (Liu *et al.* 2003; Chen *et al.* 2004), rice brown planthopper (Yang *et al.* 2002). Mite resistance is typically monogenic (Herron & Rophail 1993; Goka 1998), although some cases of polygenic resistance have been reported (Mizutani *et al.* 1988; Keena & Granett 1990; Clark *et al.* 1995). Polygenic resistance is not associated with a typical mechanism of resistance, but only refers to the number of genes involved in resistance (Lindhout 2002).

The genetic analysis in jute was studied in the past using only morpho-physiological traits, such as plant height, harvest index, cambial activity and fibre strength (Palit *et al.* 1996). Such traits are limited in number and are often influenced by the environment, thus making them unsuitable for correct assessment of this analysis. This limitation can largely be overcome by the use of molecular tools, which are unlimited in number and are not influenced by the environment. Therefore, in recent years, considerable emphasis has been placed on the development and use of molecular markers in all major crops (Liu *et al.* 1996; McCouch *et al.* 1997; Röder *et al.* 1998; Sharopova *et al.* 2002; Hirata *et al.* 2006). PCR based markers have been successfully developed such as RFLPs, RAPDs and SSRs (Bradshaw *et al.* 1994; Cheung *et al.* 1997; Jung *et al.* 1999). These markers have been successfully exploited in many crops including jute (Hossain *et al.* 2002, 2003; Qi *et al.* 2003; Basu *et al.* 2004; Roy *et al.* 2006; Akter *et al.* 2008) for genetic diversity analysis as well as genotyping. Among these markers, simple sequence repeats (SSR) have become the markers of choice due to several desirable attributes including their abundance, multiallelic and codominant nature, high level of reproducibility, cross-species transferability etc. (Gupta *et al.* 1996; Gupta & Varshney 2000). Therefore, SSR markers are useful for a variety of applications in plant genetics, genetic linkage mapping, plant breeding and marker aided selection. A genetic map facilitates marker-aided selection thereby speeding up the breeding process (Gong *et al.* 2008). It is crucial for the structural and functional genomic studies of *Corchorus*.

Many linkage maps in plants were constructed using F_2 or back cross or recombinant inbred line (RIL) populations (Burr *et al.* 1988; Gardiner *et al.* 1993; Röder *et al.* 1998; Davis *et al.* 1999; Temnykh *et al.* 2000). But it is better to use an F_2 population for molecular mapping and about 50 F_2 plants are sufficient for a fairly detailed map (Chawla 2002). The aim of this research was to evaluate SSR markers for their link to the mite resistance trait for marker-aided selection and development of a molecular linkage map of jute through the use of a large segregating F_2 population.

MATERIALS AND METHODS

Plant materials

For mapping, previous crossing material was used (Keka *et al.* 2008), consisting of 150 F_2 plants. It was derived from a cross between *Corchorus olitorius* (var. O-7/95), which was reported to be most tolerant to the mite attack, and *Corchorus olitorius* (var. O-72), most sensitive (Zaman *et al.* 2008).

Phenotypic data

A Mite Tolerability Index (MTI), based on the intensity of mite infestation was developed by the Entomology Department of Bangladesh Jute Research Institute. They scored all the F_2 plants between 0 (no mite infestation) to 5 (top shoot dead) for their resistance to the natural mite attack (Table 1).

DNA extraction

Total genomic DNA was isolated from leaves of one-month-old plants, according to Echevarría-Machado *et al.* (2005). The quality and concentration of DNA were checked on agarose gel along with λ DNA and quantities were checked with a Nanodrop ND-1000 Spectrophotometer (Thermo Scientific, USA). The DNA preparation was diluted to50 ng/µl for use as template for PCR amplification.

SSR markers

Eighty-eight SSR primers were used to perform the linkage analysis of 150 F_2 plants. Among them forty-five primers were taken from Mir *et al.* (2008) and the rest were directly designed from the jute genomic library. The microsatellite enriched

Table 1. Rating scale (0–5) of jute after yellow mite infestation

Rating scale	Symptoms of infestation on leaves	Figures
0	no change of colour or curling of leaves (no mite infestation)	
1	slightly brown colour at the base	
2	coppery brown + curling	
3	1–3 leaves dropped	
4	all leaves dropped but top shoot alive	
5	top shoot dead	

library was constructed commercially from Vizon Sci. Inc., BC Research Complex 3650, Westbrook Mall, Vancouver, British Columbia, Canada. Clones containing SSRs were identified and sequenced and then SSR primers were designed from the sequences. Detailed information on the polymorphic SSR primers used in this study is given in Table 2.

Polymerase Chain Reaction (PCR)

SSR analysis was carried out according to Keka *et al.* (2008). Each 25 µl of the reaction mixture contained 50 ng of jute genomic DNA, 10µM of each primer pair, 10 × PCR buffer, 50mM $MgCl_2$, 2mM dNTPs, 0.2 unit Taq DNA polymerase (Invitrogen, USA). Amplification profiles consisted

Table 2. Detailed information on polymorphic SSR primers used in this study

SL No	Primer name		Primer Sequence (5'-3')	Motif	Tm (°C)
1	HK-51	F	ATTTAAGATGCCAGCCATTCCA	(GT)17	56
		R	TGGCTTTAAAGTTATGCATTGATC		
2	HK-54	F	GAATTCAAACACACGCACAC	(TG)12	58
		R	TGGCCAAGTCACCATTGGCA		
3	HK-56	F	GCCAAAATTGTGGGAAGCAC	(GTT)8	58
		R	TGGTGTCGATTCGTTTCTAC		
4	HK-59	F	CTTTTCGAGCTTGATCAGTTACCA	(CTAT)18	60
		R	GACTTTACTTGTACCCATCTCCA		
5	HK-60	F	CCATACTTGCGTTCTGAGGTGCA	(GATA)17GA > 82	60
		R	AATCCTTCCCATACTGGAGATGA		
6	HK-63	F	AATCAGAGTCAGACAGAAGGGA	(CT)14	59
		R	GTCTTACCCATCATCTCAGACA		
7	HK-64	F	GATTGAATGGTTCTGGGTTTCA	(AG)21	58
		R	CAATGTAAGCGCATTCATCAATAG		
8	HK-66	F	AATGTGCTTGTGCGTGGTAGGT	(AG)12	60
		R	TACATTGAGCTGTGTCGATGTC		
9	HK-69	F	CCTTCCCATA CTGGAGATGAGA	(AG)23	60
		R	TACTTGCGTTCTGAGGTGCA		
10	HK-74	F	TTCATTCTGTGT GCAAGAGAAC	(CT)32	57
		R	TACACTCTGAAGTAGCTTCATAC		
11	HK-79	F	CAGTTGAAGTAGTGCAATAGTTAC	(AG)16	58
		R	CCAGCAATTTCAGGCTTTAAC		
12	HK-89	F	GCCAATGAAATCTATGTTTTCGA	(GA)32	55
		R	CTATTTACTCCTATTTACTTGGA		
13	J-3	F	TTTTATCTCTTTCGGCATCATC	(TAT)6	56
		R	ACGCACATTGCACGTGACTC		
14	J-139	F	CCTGTTCAAGAATCACCCCAAAG	(GA)6	60
		R	GCCCGGCTTTAGCCCTTTC		
15	J-170	F	GGTTTGCTTTCCCCGTGGTC	(CAA)4	61
		R	TCAAGCCAGACCCAGACGAAG		
16	J-174	F	CCATGATGCAAAGAATGGATGC	(CAA)7	60
		R	TTGTCCTCCAGCCTCTTGTTCC		
17	J-179	F	GACGCCATTTTGACCCAAGC	(CTT)5	60
		R	GGGATCATCGAAGGGTGAAGC		
18	J-183	F	GGGCAAAACTGTCTATCCACACC	(TTA)4,26bp, (TTTC)4	59
		R	AAAAAGGGCCTAGCCCATAACG		
19	J-184	F	TGCCAATAAAATGGCCAACC	(GAA)5	59
		R	TTGTGGCCTTTTGAGGAGGTG		
20	J-189	F	TTGGGACTAAATTGAGCAAATCG	(CTT)4	58
		R	TTGGCAAGAGCTTGGTGAGG		
21	J-190	F	ATGCCTATCACCGGGCACAC	(AAG)4	60
		R	TGGACTCTGCCTCTTTTCTTCTGG		

Tm – melting temperature of the primers

of 5 min of denaturation at 95°C, 35 cycles of 30 s denaturation at 95°C, 1 min annealing at 52°C–64°C and 1 min extension at 72°C, followed by a final 10 min extension at 72°C (GeneAmp PCR System 9700, Applied Biosystems).

Visualization of PCR products

For obtaining the better resolution of polymorphic bands, amplified PCR products were subjected to polyacrylamide gel electrophoresis. According to Streiff *et al.* (1998) the PCR products were pretreated and run in an 8% polyacrylamide gel using a sequencing gel apparatus (Thermo Scientific, USA). The gels were run in 1X TBE buffer adjusted to pH 8.3 at 150 V for 2.5–3 h. Silver staining of the gels was performed according to Streiff *et al.* (1998).

Scoring and data analysis

The amplified PCR products were scored for further analysis. During scoring, only the intense and clearly resolved amplification products that were reproducible in multiple runs were considered for linkage analysis. Polymorphisms were scored for the presence and absence of bands on polyacrylamide gels. Using 1 Kb + ladder the product sizes were found to vary from 100 to 300 bp.

The PCR amplification products of F_2 plant DNA were scored as A, B, H and null (–) by comparing with PCR amplification products of parental DNA (O-72 and O-7/95). When the bands resembled the mite tolerant parental (O-7/95) DNA, they were designed as A (homozygous). Similarly, when the bands resembled the mite sensitive parental (O-72) DNA, they were designed as B (homozygous). When the bands showed similarity with both the parental (O-7/95 and O-72) DNA, they were designed as H (heterozygous). When no bands were obtained after PCR amplification with specific SSR primer from plant DNA, they were scored null (–).

Linkage map construction

The linkage map of jute SSR markers was constructed using the software MAPMAKER version 3.0 (stat soft 1994) considering 21 SSR markers and one phenotypic marker (M-Phe) with 150 F_2 population. The raw data format contained the polymorphic bands which were scored as A, B, H and null (–). Recombination frequencies were converted to map distances in centimorgans by the Kosambi function (Kosambi 1944).

RESULTS

Polymorphism of SSRs

A total of 88 SSR primers gave amplification products. These were run in a sequencing gel to detect the polymorphism between the parents. Among them only 21 SSR primes showed polymorphism between the parents. This gives a very low percentage of polymorphism between the mite sensitive and resistant parents.

Genotype determination and analysis of data

One hundred and fifty F_2 plant DNA were amplified by the 21 polymorphic SSR primers. The genotypes were determined by comparing their bands with the bands of parental DNA. During scoring, only the intense and clearly resolved bands that were reproducible in multiple trials were considered for linkage analysis (Figure 1).

Results of the segregation test in F_2 generation are given in Table 3. Chi-square test reveals that the markers have effectively segregated from F_1 to F_2 population, which is indicated by the low *P*-value, and it can be inferred at 1% significance level that two different bands for each primer are due to the presence of two different alleles and not to two different polymorphic states of a marker present in the same DNA molecule in the F_1 population.

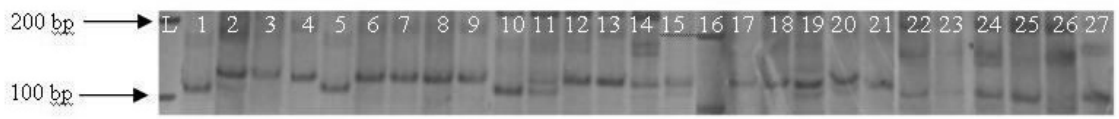

Figure 1. Amplification product after polyacrylamide gel electrophoresis; column 1: mite resistant variety (O-7/95), column 2: mite sensitive variety (O-72) and columns 3-27: F_2 population; L = 1 Kb+ ladder

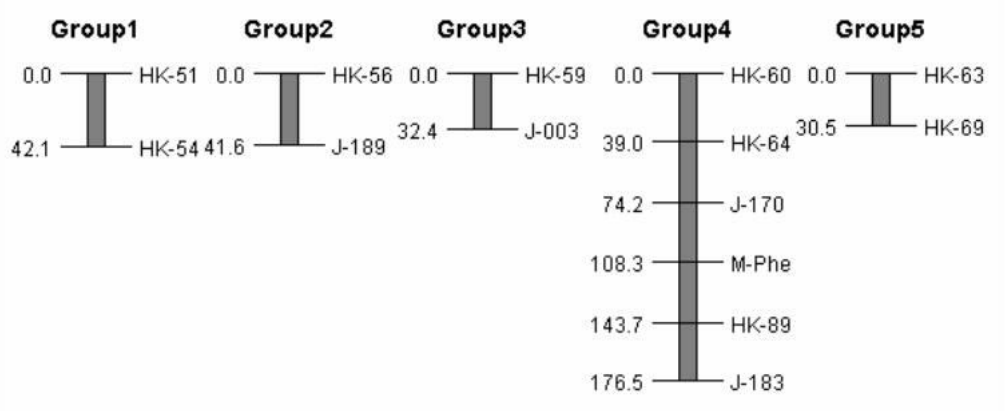

Figure 2. Linkage map showing the distance between markers (in cM)

Construction of linkage map

A linkage map of jute genome was constructed using twenty-one SSR markers and one phenotypic marker (designated by M-phe) with an F_2 population consisting of 150 plants (Figure 2). Linkage analysis was carried out by the nearest neighbouring locus method and a recombination frequency of 0.35. In this method a group is constructed by sequentially combining a locus which shows

Table 3. Results of the segregation test in F_2 generation

Marker	N	Ratio	χ^2	*P*-value
HK-51	129	29:55:45	8.76	0.0125*
HK-54	128	37:53:38	6.47	0.0394*
HK-56	116	37:33:46	25.45	0.0000***
HK-59	129	47:48:34	12.45	0.0020**
HK-60	131	38:44:49	16.35	0.0003***
HK-63	135	40:44:51	17.84	0.0001***
HK-64	136	43:57:36	5.19	0.0748
HK-66	129	45:28:56	40.08	0.0000***
HK-69	123	43:40:40	17.31	0.0002 ***
HK-74	127	25:64:38	5.79	0.0554
HK-79	108	24:48:36	14.64	0.0007 ***
HK-89	110	43:31:36	26.68	0.0000***
J-003	125	39:49:37	9.08	0.0107*
J-139b	135	49:12:74	91.97	0.0000***
J-170	123	41:47:35	10.95	0.0042**
J-174	113	28:41:44	18.95	0.0001***
J-179	125	34:24:64	58.21	0.0000***
J-183	128	47:43:38	15.56	0.0004***
J-184	126	31:44:51	18.80	0.0001***
J-189	123	33:39:51	22.68	0.0000***
J-190	112	46:34:32	25.15	0.0000***
M-Phe[1]	150	41:0:109	221.65	0.0000***

***, **, * and no asterisk designate the significance level which differed at $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P > 0.05$, respectively; [1]M-Phe means phenotypic marker; χ^2 – chi-squared distribution

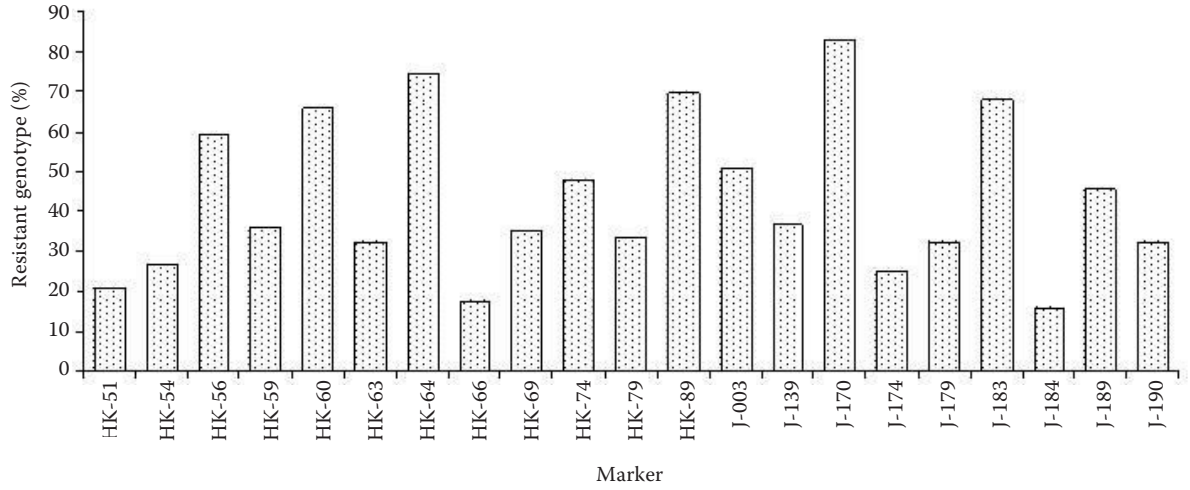

Figure 3. Genetic variation in F_2 population based on tolerance level for twenty-one primers

the smallest recombination value between them. Five linkage groups were observed at a maximum distance of 50 cM. Among the five linkage groups, four groups (LG-1, LG-2, LG-3 and LG-5) are found to contain two markers each and the distances between the markers are 42.1 cM, 41.6 cM, 32.4 cM and 30.5 cM, respectively. The remaining group (LG-4) consists of six markers HK-60, HK-64, J-170, M-Phe, HK-89 and J-183 and the distances among the markers are 39.0 cM, 35.2 cM, 34.1 cM, 35.4 cM and 32.8 cM. The linkage between J-170 and M-Phe remains unbroken even at a more severe recombination frequency of 0.30 at 34.15 cM.

Genetic variation in mite resistant F_2 plants

Genetic variations in F_2 plants against the mite are presented in Figure 3. It reveals that none of the mite tolerant plants possesses 100% A (tolerance) genotype. Thus the mite resistance trait of jute could be a quantitative polygenic trait.

Table 4 reveals that the selection efficiencies of J-170, HK-64 and HK-89 markers are 82.92%, 76.74% and 69.77%, respectively and 100% in combination. Although selection based on single marker is sufficient for breeding purposes, their simultaneous use in combination gives a selection accuracy of 100%.

DISCUSSION

Only the polymorphic primers were used to determine the genotype of the segregating F_2 population in this study. The percentage of polymorphic SSRs reported earlier for mite sensitive and resistant jute was 23.25% (Keka *et al.* 2008). We also observed a similar polymorphism between two parents (23.86%). A close polymorphism rate (17%) of RAPD markers was previously observed between the cold sensitive and resistant jute (Hossain *et al.* 2003).

We present here a primary linkage map of jute based on SSR markers and demonstrate their link-

Table 4. Single and combined effects of markers to identify mite resistant jute genotypes

SSR marker	Number of resistant genotypes (A)*	Number of resistant genotypes (A) on a field level	% of resistant genotypes (A)
J-170	41	34	82.92
HK-64	43	33	76.74
HK-89	43	30	69.77
Combination of three markers	22	22	100

*out of 150 genotypes

age with mite resistance. We found five linkage groups in this study. A preliminary linkage map of jute was constructed in our laboratory by Keka *et al.* (2008). They found two linkage groups using only 10 SSR markers with 35 F_2 populations. But the unique chromosome number of *Corchorus* species is seven ($2n = 14$), so seven linkage groups were expected to represent the whole genome (Zickler *et al.* 1984). Our effort was very limited for linkage mapping and the total number of loci was also very low. The development of microsatellites of jute is a tedious task due to unavailability of the whole genome sequence. In this study only 21 polymorphic SSR markers were used to construct the linkage map. Compared to the previously published ones dense linkage maps in different crops have been constructed using more markers, for example three dense linkage maps of sunflower were constructed using 1089 SSR markers by Yu *et al.* (2003) and a SSR based genetic linkage map of *Cucurbita pepo* L. was constructed by Gong *et al.* (2008) using 532 SSR markers. By increasing the number of markers, a marker dense saturated linkage map of the jute genome can be constructed. It will be helpful in future to identify the locations of genes responsible for the mite resistance trait in jute by positional cloning.

Most of the pest and disease resistant traits are controlled by many genes (Schiff *et al.* 2001; Lindhout *et al.* 2002). They could be genetically complex quantitative traits, involving many genes and environmental effects. In our study, forty-one F_2 plants out of one hundred and fifty were found to be mite tolerant (from field data). Phenotypically they were 100% tolerant but none of the markers showed 100% tolerance genotypically (Table 4). It was observed that all the markers showed at least 20% resistance. Possibly it is so because the mite resistance is a polygenic trait and the presence of only one resistant trait cannot prevent these plants from the mite attack. This was also pointed out by Keka *et al.* (2008).

Among the markers J-170, HK-64 and HK-89 show tolerance in more than 70% of the plants individually but in combination it was 100%. This suggests that they are closely linked to the mite resistant genes. Similar findings were also observed by Wang *et al.* (2005). They reported that the selection efficiencies of SSR2-004 and RM1358 were 96.8% and 92.7%, respectively, and 99.8% in combination. Shalini *et al.* (2007) identified some molecular markers that are associated with mite resistance in coconut (*Cocos nucifera* L.). After combined stepwise multiple regression analysis of data, they showed that a combination of 5 markers can explain 100% association with the mite resistance trait in coconut.

The map presented here provides a good starting point for the production of a saturated linkage map of the jute genome based on SSR markers. This linkage analysis will in future help to isolate the mite resistant genes in jute. The rice genome sequencing has benefited from the development of genetic maps. The first genetic map for rice was published in 1988 (McCouch *et al.* 1988) and it was followed by much denser maps (Causse *et al.* 1994; Harushima *et al.* 1998). Therefore tasks such as the whole genome sequencing of jute will also require the development of such maps with known locations on the chromosomes.

Acknowledgements. This research was financially supported by the US Department of Agriculture (USDA). We are grateful to Bangladesh Jute Research Institute for supplying seed for this study.

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Received for publication October 7, 2009
Accepted after corrections May 21, 2010

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