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Cytogenetic identification of wheat–*Psathyrostachys huashanica* amphiploid × triticale progenies for English grain aphid resistance

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ABSTRACT: English grain aphid (EGA, *Sitobion avenae* Fabricius) is an important pest in wheat (*Triticum aestivum* L.). To develop EGA-resistant varieties, introducing the desirable genes from related species is regarded as an efficient avenue. In this study, the F_1, F_2 and F_3 plants derived from the cross of EGA-susceptible wheat–*Psathyrostachys huashanica* Keng ex Kuo amphiploid (PHW-SA, AABBDDNsNs) and EGA-resistant triticale (Zhongsi 828, AABBRR) were analyzed for EGA resistance. Consequently, PHW-SA was moderately susceptible while Zhongsi 828 and their F_1 hybrids were immune, suggesting that the resistance is dominant. All the F_2 plants showed high resistance or immunity over two years, indicating that EGA resistance genes are more likely carried by the rye (*Secale cereale* L.) chromosomes rather than the genomes A or B of Zhongsi 828. In the F_3 generation, 25 of 239 lines became susceptible. Giemsa C-banding patterns revealed that these F_3 lines had 38–40 chromosomes, including complete rye genome except 5R (and 2R in five lines). Genomic *in situ* hybridization analysis confirmed this result. During meiosis, all the chromosomes formed bivalents. Six bivalents in 20 lines and five bivalents in five lines were characterized from rye. In contrast, their F_2 parental lines had 42 chromosomes (21 bivalents), containing 1R–7R of rye. No *P. huashanica* chromosomes were detected. Therefore, we propose that the rye chromosome 5R may be related to EGA resistance.
Keywords: chromosome constitutions, genetic resources, wide cross

Introduction

Aphid (Hemiptera: Aphididae) is one of the major pests of cereal crops worldwide, especially in temperate regions. They cause a significant loss of yield by consuming the photoassimilates in plant sap and by functioning as the vectors transmitting over 250 plant viruses such as barley yellow dwarf virus (BYDV) (Nault, 1997). In wheat (*Triticum aestivum* L.), the English grain aphid (EGA) is the most important pest, known as an ear feeder in summer. It distributes extensively in Northern and Western China almost every year, and can give rise to a remarkable reduction in wheat yield (up to 70 %) as well as flour quality under heavy infestation (Shi et al., 2009).

As a critical part of the integrated pest management (IPM), the development of aphid-resistant wheat varieties will be a sustainable way in a long term. Many aphid resistance genes have been found and located in wheat. For example, genes *Dn1*, *Dn2*, *Dn5*, *Dn6* and *DnX* located on chromosome 7DS, *Dn4* on 1DS, *Dn8* on 7DL and *Dn9* on 1DL express resistance to the Russian wheat aphid (Liu et al., 2005; Tyrka and Chelkowski, 2004). Genes *Gb3* and *Gbz* on chromosome 7DL function against the greenbug (Weng and Lazar, 2002; Zhu et al., 2004), whilst a single dominant gene *RA-1* on chromosome 6AL is identified against EGA (Liu et al., 2012).

Attention has been paid to broadening genetic variation of crop plants over the past decades. For wheat, there are many related species present in Triticeae. They have the potential to contribute to the development of wheat cultivars with aphid resistance. The resistance to aphid races has been found in some species of *Agropyron* (Tremblay et al., 1989), *Avena* (Weibull, 1986), *Elymus* (Tremblay et al., 1989), *Hordeum* (Weibull, 1987), *Secale* (Anderson et al., 2003), *Triticum* (Lage et al., 2004) and several accessions of triticale (Webster, 1990).

In the present study, a cross was conducted between the EGA-susceptible female parent, wheat–*Psathyrostachys huashanica* Keng ex Kuo amphiploid 'PHW-SA' (AABBDDNsNs) and the EGA-resistant male parent, hexaploid triticale 'Zhongsi 828' (AABBRR). The offspring family (F_1 to F_3 generations) was analyzed for EGA resistance. Interestingly, 25 F_3 lines became high susceptible as compared to their F_2 parental lines and sibs carrying resistance to EGA. The genomic constitutions of these lines were then characterized cytogenetically.

Materials and Methods

Plant materials

A cross between the EGA-susceptible wheat–*P. huashanica* amphiploid 'PHW-SA' ($2n = 8x = 56$, AABBDDNsNs) and the EGA-resistant triticale cultivar 'Zhongsi 828' ($2n = 6x = 42$, AABBRR) was obtained in 2008 (Kang et al., 2011). The F_1 plants were selfed for three generations. As a result, 26 F_2 lines and 239 F_3 lines were produced in 2010 and 2011, respectively. The individuals of F_1 and F_2 generations were cultivated in 2011 as well. A rye cultivar 'Qinling' ($2n = 14$, RR) and *P. huashanica* ($2n = 14$, NsNs) were used as the probes

for genomic *in situ* hybridization. Common wheat 'Chinese Spring' ('CS', 2*n* = 42, AABBDD) was used as the blocker. These plant materials went through the cropping seasons under natural field conditions without any fertilizer or pesticide.

EGA resistance evaluation

The resistance of F_1, F_2 and F_3 plants to EGA was evaluated in two cropping seasons. Only F_2 plants were investigated in 2010, and all the F_1, F_2 and F_3 plants observed in 2011. Evaluation of EGA resistance was carried out under the natural infection conditions as this aphid appears regularly every year. For each line, three spikes of the main shoots were cut at the milky stage and the number of the aphids was counted. The ratios of the average aphid number per spike of each line and the average aphid number per spike of all the lines were calculated, termed as aphid indexes. A 0 (immune) – 6 (highly susceptible) scale was employed to denote the infection severity according to the Painter's method (1951). That is, 0 = immunity, aphid index being 0; 1 = high resistance, aphid indexes ranging from 0.01 to 0.30; 2 = moderate resistance, aphid indexes ranging from 0.31 to 0.60; 3 = low resistance, aphid indexes ranging from 0.61 to 0.90; 4 = low susceptibility, aphid indexes ranging from 0.91 to 1.20; 5 = moderate susceptibility, aphid indexes ranging from 1.21 to 1.50; 6 = high susceptibility, aphid indexes being more than 1.50.

Giemsa C-banding

The Giemsa C-banding technique was adopted to identify individual alien chromosomes. The seeds were germinated at 25 °C, and their roots that were 1 to 2 cm in length were collected. The roots were then fixed in the fresh Carnoy's fixative I (3 volumes of 100 % ethanol + 1 volume of glacial acetic acid) solution for 24 h, after pretreatment in ice-cold water for about 20 h, followed by hydrolyzation in 0.2 mol L^{-1} HCl for 3–4 h. The meristem cells were squashed in 45 % acetic acid and the cover glasses were removed through freezing in liquid nitrogen. Giemsa C-banding was performed using the methods described by Gill et al. (1991) as revised by Wang et al. (2011). The characterization of *S. cereale* and *P. huashanica* chromosomes was made by matching the standard patterns demonstrated by Gill and Kimber (1974), and Zhang et al. (2009), respectively.

Meiotic analysis

Meiotic analysis of pollen mother cells (PMCs) was conducted to understand the chromosome pairing. The growing spikes were removed when the flag leaf sheathes reached around 5 cm length, followed by immediate fixation in Carnoy's fixative II (six volumes of 100 % ethanol + three volumes of chloroform + one volume of glacial acetic acid) solution for 24 h. One of three anthers in a floret during meiosis was squashed in a drop of modified carbol fuchsin. Chromosome pairing was analyzed based on 50 PMCs.

Genomic *in situ* hybridization (GISH)

The genomic constitutions of somatic cells and PMCs were further identified by GISH. The procedures of chromosome preparation were described earlier. For meiotic analysis, the remaining two anthers were used here. The slides were air-dried, post-fixed in 4 % paraformaldehyde for 10 min, denatured in 70 % deionized formamide at 80 °C for 2 min, and then dehydrated in ethanol series (-20 °C, 5 min each in 70 %, 95 % and 100 %).

The genomic DNA of plant materials was isolated by the hexadecyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). The total genomic DNA of rye (RR) and *P. huashanica* (NsNs) were labeled, as the probes with digoxigenin-11-dUTP, by nick translation following the manufacturer protocol (Roche, Mannheim, Germany). The total genomic DNA of CS was autoclaved at 115 °C for 10 min as the blocker (200–500 bp). A sample of 30 µL hybridization solution, which included 50 % formamide, sodium chloride – sodium citrate (SSC) 2X buffer, 10 % dextran sulfate, 0.17 mg mL^{-1} herring sperm DNA (200–500 bp), 50 ng probe DNA and 3,000 ng CS DNA, was used for each slide. GISH was performed by the method described by Chen et al. (1996) and modified by Wang et al. (2011). After being counterstained by propidium iodide (PI, 1.5 µg mL^{-1}) (Vector, California, USA), the hybridization signals on chromosomes were visualized with a fluorescence microscope equipped with green and blue filters. Images were recorded with a CCD (charge-coupled device) camera.

Results

EGA resistance evaluation

The progenies of the F_2 generation in 2010 and F_1, F_2 and F_3 generations in 2011, together with the female parent PHW-SA and male parent Zhongsi 828, were screened for the reaction types to EGA (Table 1). PHW-SA was moderately susceptible whereas Zhongsi 828, F_1 and F_2 plants were immune or highly resistant. Out of 239 F_3 lines, 25 from five F_2 parental lines were highly susceptible to EGA (Figure 1). The other F_3 lines, however, exhibited high resistance or immunity.

Mitotic analysis

The mitotic metaphase cells in root tips of the F_3 lines with high susceptibility to EGA, their F_2 parental plants and sibs were analyzed for the chromosome num-

Table 1 – English grain aphid (EGA) resistance evaluation in the progeny of PHW-SA × Zhongsi 828.

Lines		Aphid indexes	Resistance	Resistance scales
PHW-SA		1.29	Moderate susceptibility	5
Zhongsi 828		0.00	Immunity	0
F_1		0.00	Immunity	0
F_2		0.00–0.28	Immunity–High resistance	0–1
F_3	25 lines	1.57–1.86	High susceptibility	6
	Others	0.00–0.30	Immunity–High resistance	0–1

ber and compositions in order to determine the genetic difference among them (Table 2). The chromosome number of F_2 parental lines was consistently 42. In 25 F_3 lines, 20 had 40 chromosomes and the rest contained only 38 chromosomes.

Giemsa C-banding and GISH were used to characterize the chromosome compositions of these lines. C-banding patterns indicated that five F_2 parental lines included complete R genome of rye (Figure 2A). Among 25 F_3 lines, 20 had each pair of rye genome except 5R (Figure 2B) and five lacked both 5R and 2R. Eight F_3 sibs from the same F_2 parents were selected randomly, and most of them had a complete rye genome (1R–7R) and the remaining ones had 1R or 2R lost. There were no *P. huashanica* chromosomes found in all the examined combinations.

GISH confirmed the results of Giemsa C-banding (Figure 2C–D). Fourteen chromosomes of rye were denoted by yellow-green fluorescence in five F_2 parental lines (Figure 2C). Twelve rye chromosomes were present in 20 F_3 lines (Figure 2D), and ten were detected in five lines. No hybridization signals were found when the DNA of *P. huashanica* was used as the probe.

Figure 1 – The F_3 lines with susceptibility to English grain aphid (EGA). **A** The F_2 parental lines showing resistance to the aphid. **B** The target F_3 lines showing high susceptibility to the aphid.

Meiotic analysis

PMCs in these lines were further observed to understand their chromosome behavior during meiosis (Figure 3). Five F_2 parental lines formed 21 bivalents in the most of PMCs at meiotic metaphase I (MI) (Figure 3A). Twenty and 19 bivalents were observed in 20 (Figure 3B) and five target F_3 lines. At anaphase I, one of F_2 parental lines yielded a pair of lagging chromosomes, and a number of micronuclei were produced at telophase II. The other F_2 parents and F_3 plants progressed normally.

The rye chromosomes during meiosis were visualized by GISH. Seven bivalents were consistently seen in the F_2 parents at MI, clustering with the wheat chromosomes at the equatorial plate (Figure 3C). Six and five bivalents from rye were identified in 20 and five target F_3 lines, suggesting that these chromosomes were homologous (Figure 3D). As the cells reached the anaphase I, these bivalents were able to separate regularly. Overall, it can be summarized that 20 of 25 F_3 lines showing high susceptibility to EGA were nullisomic for 5R and the remaining lines lacked both 2R and 5R, compared with their F_2 parental lines that contained the complete rye genome.

Discussion

The F_1 hybrids of PHW-SA ($2n = 8x = 56$, AABBDDNsNs) and Zhongsi 828 ($2n = 6x = 42$, AABBRR) have 49 chromosomes, comprising AABBDNsR (Kang et al., 2011). After selfing, three genomes (D, Ns and R) might undergo elimination since they could not pair and separate normally in meiosis without their homologous chromosomes. In fact, there were 42 chromosomes in five F_2 parental lines. No chromosomes of *P. huashanica* were found but the complete rye genome (1R–7R) was observed in these lines. Additionally these chromosomes formed 21 bivalents at MI. This suggests that D and Ns genomes had been lost while R genome had been doubled during propagating. Fourteen out of 21 bivalents, theoretically, belong to the A and B genomes of wheat and the remaining ones are the chromosomes of rye. In other words, the F_2 parental lines may be considered as new triticale ($2n = 6x = 42$, AAB-

Table 2 – Chromosome constitutions of the English grain aphid (EGA)-susceptible F_3 lines and their F_2 parental lines.

Lines		Resistance scales	2*n*	Genome constitutions			
				A+B	D	R	Ns
PHW-SA		5	56	28	14	0	14
Zhongsi 828		0	42	28	0	14	0
F_1		0	49	28	7	7	7
F_2 parental lines		0–1	42	28	0	14	0
Target F_3 lines	20 lines	6	40	28	0	12 (5R absent)	0
	5 lines	6	38	28	0	10 (2R and 5R absent)	0

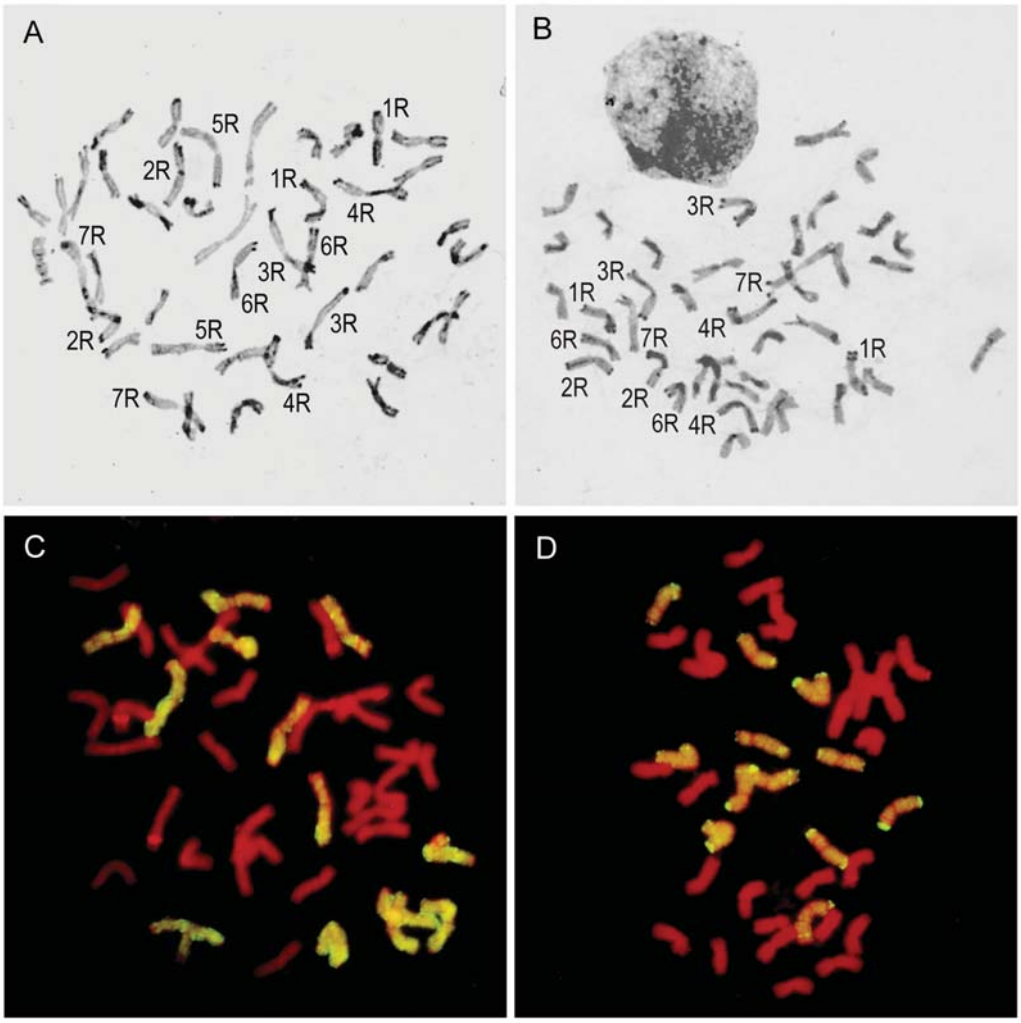

Figure 2 – Giemsa C-banding (**A–B**) and genomic *in situ* hybridization (GISH) (**C–D**) patterns on the mitotic metaphase chromosomes. For GISH analysis, the genomic DNA from rye was used as probe (yellow-green signals) and the genomic DNA from Chinese Spring (CS) used as blocker. **A** C-banding pattern of the F_2 parental lines, showing complete genome of rye (1R–7R). **B** C-banding pattern of the English grain aphid (EGA)-susceptible F_3 lines, showing complete genome of rye with the lack of 5R. **C** GISH pattern of the F_2 parental lines, showing 14 rye chromosomes. **D** GISH pattern of the EGA-susceptible F_3 lines, showing 12 rye chromosomes.

BRR). The absence of wheat D genome could be beneficial because this can reduce the confounding effects of genes on the genome. For example, several major genes conferring aphid resistance have been located on the D genome (Liu et al., 2005; Weng and Lazar, 2002; Zhu et al., 2004). The other aphid resistance genes could thus be detected easily without the effect of these major ones.

Screening for resistance to EGA revealed that the female parent PHW-SA was susceptible whereas the male parent Zhongsi 828 was immune. Their F_1 hybrids showed immunity. One can deduce the existence of certain dominant resistance gene(s) in Zhongsi 828 and successful transmission of those to the hybrids. In the F_2 segregating population all the plants remained at high resistance or immunity to the aphids over two years. This indicates that these genes are less likely on the genome A or B of Zhongsi 828 as there should have been susceptible genotypes in F_2 plants after recombination. Further, only 25 lines in the F_3 generation became highly susceptible to EGA. In contrast to their F_2 parental lines and sibs, they consistently lacked chromosome 5R of rye. It may therefore be speculated that chromosome 5R of rye functions against EGA.

To date about seven aphid resistance genes have been identified in rye. Two greenbug resistance genes, *Gb2* and *Gb6*, and one Russian wheat aphid resistance gene *Dn7* are located on 1RS, and four greenbug resistance genes *Dnx* (*Dnr1*, *Dnr2*, *Dnr3*, and *Dnr4*) located on 1RL, 3RS, 4R and 7R, respectively (Fritz et al., 1999;

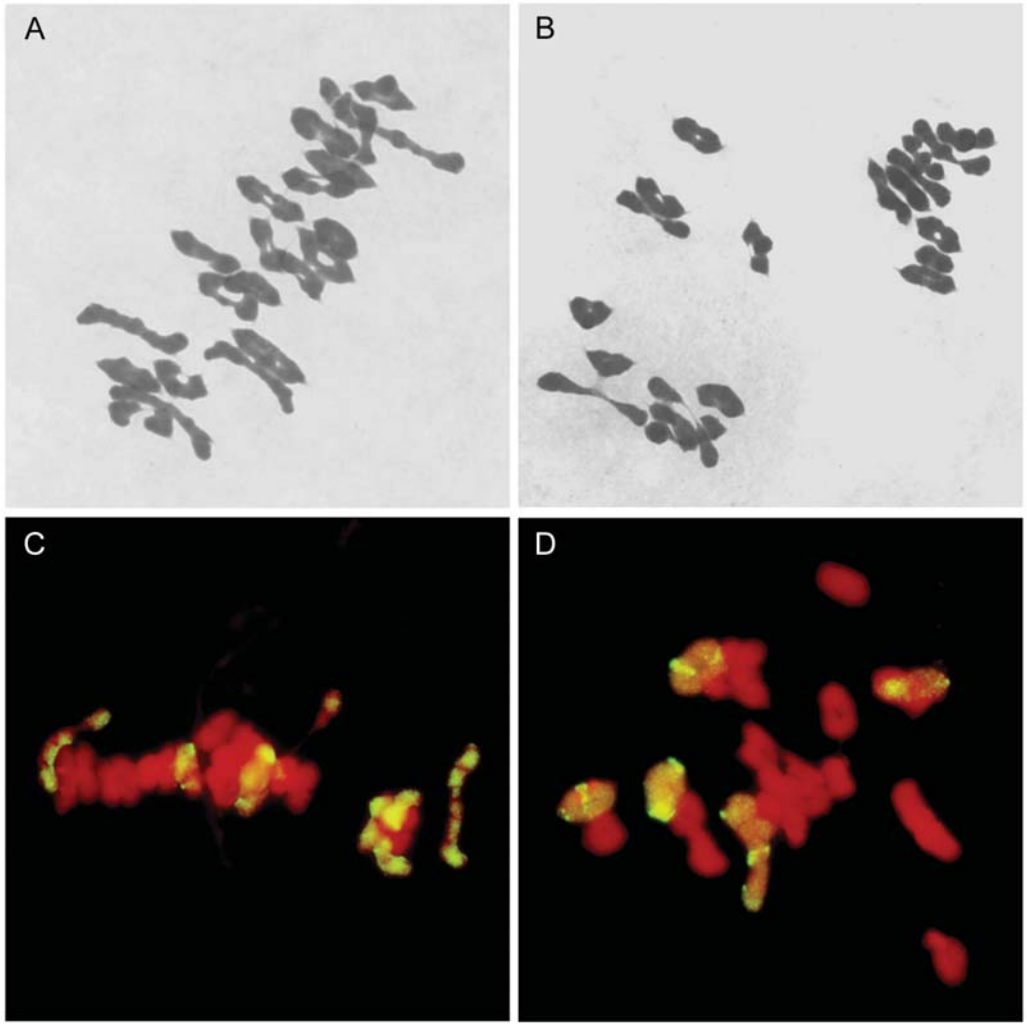

Figure 3 – Chromosome pairing (**A–B**) and genomic *in situ* hybridization (GISH) patterns (**C–D**) at meiotic metaphase I. **A** Chromosome pairing of the F_2 parental lines, $2n = 42 = 4$ II (*rod*) + 17 II (*ring*). **B** Chromosome pairing of the English grain aphid (EGA)-susceptible F_3 lines, $2n = 40 = 5$ II (*rod*) + 15 II (*ring*). **C** GISH pattern of the F_2 parental lines, showing seven rye bivalents. **D** GISH pattern of the EGA-susceptible F_3 lines, showing six rye bivalents.

Lapitan et al., 2007; Marais et al., 1994; Nkongolo et al., 1996; Tyrka and Chelkowski, 2004). Numerous translocations, additions and synthetic triticale have been developed for transfer of these genes to wheat (Hesler, 2005; Marais et al., 1994; Nkongolo et al., 2009, 2011), and a number of molecular maps are available (Anderson et al., 2003; Fritz et al., 1999; Lapitan et al., 2007; Tyrka and Chelkowski, 2004). However, few studies have focused on EGA in spite of its tremendous damage in wheat worldwide. Only one dominant gene responsible for EGA resistance was reported on chromosome 6AL of wheat (Liu et al., 2012). In the present study, it is elucidated that chromosome 5R of rye may carry major gene(s) for EGA resistance. These gene(s) should be different from those reported on 1R, 3R, 4R and 7R. The mechanism of these genes from rye against aphids remains unknown but it is possibly associated with high concentrations of hydroxamic acids (mainly DIBOA) in rye plants (Niemeyer et al., 1992).

Rye has been used widely as a resource for broadening the genetic base of wheat. Over 39 resistance genes against diseases and pests have been found in rye (Tyrka and Chelkowski, 2004). Here we report the existence of the putative genes conferring EGA resistance on chromosome 5R of rye. This offers an opportunity to transfer them to wheat.

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