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Segregation of glutenins in wheat×maize-derived doubled haploid wheat populations

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Abstract. The segregation of both high and low molecular weight glutenin subunits across 7 F_1 wheat (*Triticum aestivum* L.)×maize (*Zea mays* L.) derived doubled haploid populations was examined. The F_1 wheats used in each population were produced from parents of interest to Australian wheat breeding programs. The parents varied by up to 5 glutenin subunit loci. Examination of subunits individually within each population using a chi-square analysis revealed that all but 2 of the 26 pairs of alleles analysed fitted the expected 1 : 1 segregation ratio. Glutenin profiles were examined for each cross individually and all but one (Sonalika/Hartog) fitted the expected Mendelian segregation pattern. The analysis of allele distribution of the 6 glutenin loci across all 7 crosses showed all falling well within expected segregation ratios. Closer examination of parental lines and populations revealed irregularities which conflict with original assumptions and provide a valid explanation for the few segregation distortions observed. It is concluded that wheat×maize-derived doubled haploid populations represent a unbiased assortment of parental gametes on both arms of Group 1 chromosomes.

Additional keywords: Triticum aestivum, *Zea mays*, glutenin subunits, segregation distortion.

Introduction

Doubled haploid populations have become a valuable resource for conventional breeding programs. Their suitability for this purpose depends on them being equivalent to conventionally derived inbred lines in agronomic performance and genetic variability. In addition, the application of doubled haploid technology is an effective means of rapidly generating populations for purposes such as linkage mapping (Moharramipour *et al*. 1997). The evaluation of mapping populations is generally based on the assumption that the progeny of particular crosses represent an unbiased sample of parental genes. Software analysis packages such as MAPMAKER (Lander *et al*. 1987) assume the absence of gamete selection and segregation distortions. Thus, it is important to confirm that doubled haploid populations do not exhibit distorted segregation ratios for those regions of the genome being mapped.

Several haploid production systems are currently available for wheat (*Triticum aestivum* L.). Of these haploid production systems, intergeneric crossing to maize (*Zea mays* L.) (Laurie and Bennett 1986) is currently the most efficient form of haploid production when utilising a diverse range of wheat germplasm (Inagaki and Tahir 1990; Kisana *et al*. 1993). It is generally held that doubled haploid populations produced by wide crossing and subsequent chromosome elimination are more closely aligned to Mendelian gene frequencies than pollen-derived populations (Bjørnstad *et al*. 1993; Tinkler *et al*. 1993; Wang *et al*. 1995). However, few detailed analyses have been made. In particular, there is a need for a more detailed examination of wheat populations derived from the widely used wheat×maize system.

Glutenin storage proteins provide a useful set of markers for genetic studies in wheat, since their properties, chromosome location, and segregation have been widely documented. High molecular weight glutenin subunits are encoded by the Glu-1 loci located on the long arms of chromosomes 1A, 1B, and 1D (Lawrence

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Table 1. Glutenin profiles of the parents used in this study

High molecular weight glutenin subunits nomenclature according to Payne and Lawrence (1983) with low molecular weight glutenin subunits named according to Gupta and Shepherd (1990)

Parent	Origin	Glu-1A	Glu-1B	Glu-1D	Glu-3A	Glu-3B	Glu-3D
Hartog	Australia	a	i	d	b	h	b
Sonalika	India	b	c	a	c	h	a
Klasic	USA	a	i	d	d	h	b
CD87	Australia	b	b	a	b	b	a
QT2200–20	Australia	b	b	a	c	b	b
Neepawa	Canada	b	c	d	e	h	c
Gamenya	Australia	b	i	a	b	b	b
Batavia	Australia	a	b	a	c	b	c
W21MMT70	Australia	b	i	a	b	h	a
Mendos	Australia	b	i	a	b	b	b

and Shepherd 1980; Lawrence 1986). Low molecular weight glutenin subunits are encoded by the Glu-3 loci located on the short arms of chromosomes 1A, 1B, and 1D (Jackson *et al*. 1983). With the availability of fast, economical separation techniques such as 1-dimensional polyacrylamide gel electrophoresis, glutenins provide convenient markers for the investigation of segregation ratios.

This investigation forms part of a much larger study seeking to identify both molecular and protein markers linked to wheat quality parameters of interest for genetic improvement in the northern region of Australia's wheatbelt. Crosses between selected parents have been made and populations of doubled haploid lines developed from these crosses. We investigated the assortment of parental alleles in 7 of these populations, by examining the segregation of both the high and low molecular weight glutenin subunits, with the aim of establishing the suitability of the lines for mapping and related studies. These 7 populations in total comprise 721 individual lines, with each cross segregating for 1–5 glutenin subunits.

Materials and methods

Plant material

Ten wheat lines were selected as parents because of differing grain quality attributes (Table 1). Selected crosses among these parents were made, and the resulting F_1s were then crossed to maize to produce haploid plants using the methods developed by Kammholz *et al*. (1996). Resulting haploid plants were treated at early tillering in an aerated solution comprising 0·05% colchicine, 3% dimethylsulfoxide, and 1 drop/100 mL of Tween 20 for 6 h to facilitate chromosome doubling. A total of 721 doubled haploid lines were developed and increased in the field to provide seed for this and subsequent investigations.

Glutenin extraction and electrophoresis

Five seeds of each doubled haploid line were ground and stored in 1·5-mL Eppendorf tubes. The ground seed (16–24 mg) was subsampled and used for glutenin extraction according to the method of Gupta and MacRitchie (1991). High and low molecular weight glutenin subunits were separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using 5–15% discontinuous gradient gels, in a BioRad Protean IIxi (20 cm by 20 cm by 1 mm) system. Gels were stained with Coomassie Brilliant Blue-R250. Protein bands were described using the nomenclature of Payne and Lawrence (1983) and Gupta and Shepherd (1990).

Data analysis

Gamete segregation was examined using a chi-square analysis. Loci were initially examined individually within a population. Secondly, segregation of glutenin profiles for individual crosses were analysed. This assumes for a given population there are $(2)^n$ possible glutenin profiles, where n equals the number of alleles segregating for a given population. The relative frequency of individual subunits at each of the 6 loci across all 7 populations was then analysed.

Results

Glutenin subunit electrophoretic patterns for each parent used in the present study are illustrated in Fig. 1. These parental profiles are summarised in Table 1. The segregation of high and low molecular weight glutenin subunits within a selection of the CD87/Hartog doubled haploid population is presented in Fig. 2. The 5 segregating glutenin subunits can readily be seen.

Analysis of glutenin subunits individually within a cross revealed that only the Glu-3A locus in the Gamenya/Batavia and Glu-1D locus in the Sonalika/Hartog cross did not fit the expected 1 : 1 segregation ratio. These were significantly different at $P = 0{\cdot}01$ and $P = 0{\cdot}05$, respectively (Table 2). All other subunit distributions fitted the expected 1 : 1 segregation ratio for a single gene within each population.

The highly significant deviation from a random segregation of the Glu-3A locus in the Gamenya/Batavia cross prompted a closer investigation of the Gamenya seed stock used to produce the F_1. When single seed extractions were examined electrophoretically they were

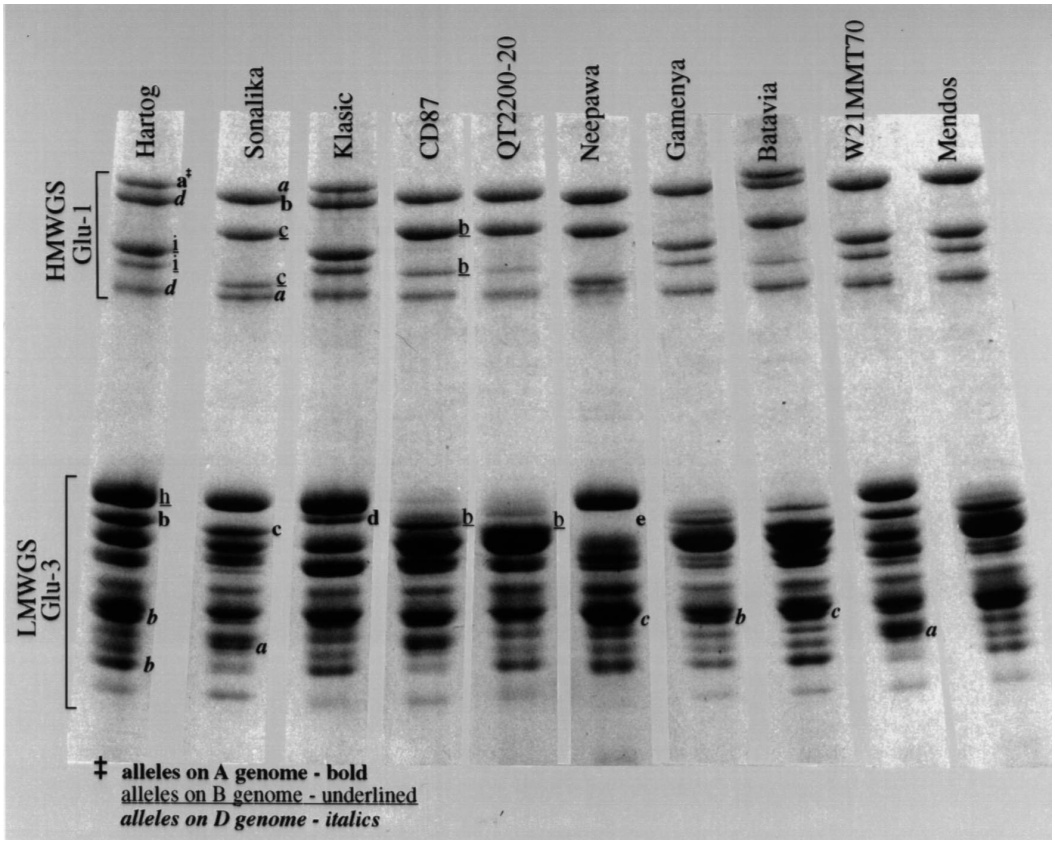

Fig. 1. SDS-PAGE highlighting the high molecular weight glutenin subunits (HMWGS) and low molecular weight glutenin subunits (LMWGS) profiles of the parents used in this study.

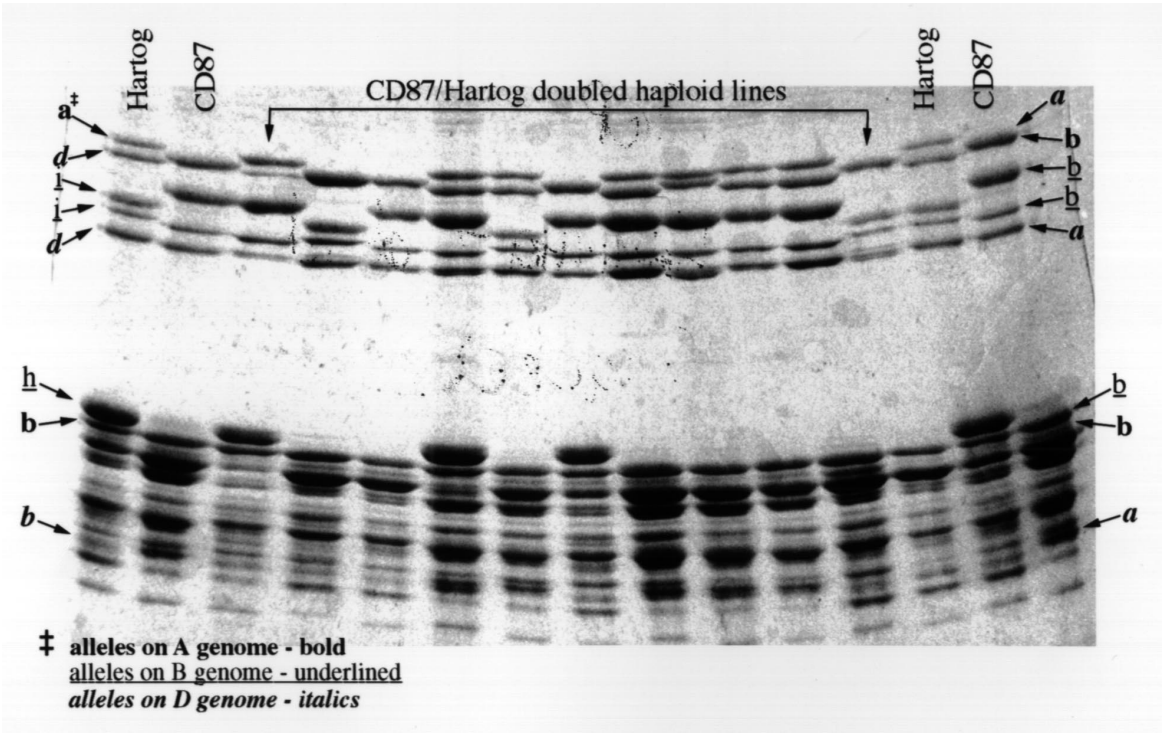

Fig. 2. Segregation of 3 high and 2 low molecular weight glutenin subunits within a random subset of the CD87/Hartog population.

Table 2. Frequency of glutenin subunits within doubled haploid populations

Cross and no. of doubled haploid	Segregating alleles	Frequency of alleles	χ^2 Individual	χ^2 Population
Klasic/Hartog (154)	Glu-3A	71d : 83b	0·94	0·94
W21MMT70/Mendos (97)	Glu-3B	50h : 47b	0·09	1·85
	Glu-3D	45a : 52b	0·51	
Gamenya/Batavia (104)	Glu-1A	47b : 57a	0·96	21·49
	Glu-1B	53i : 51b	0·04	
	Glu-3A	32b : 72c	15·38***	
	Glu-3D	52b : 52c	0·00	
Neepawa/Hartog (101)	Glu-1A	53b : 48a	0·25	11·0
	Glu-1B	54c : 47i	0·49	
	Glu-3A	49e : 52b	0·09	
	Glu-3D	57c : 44b	1·67	
Sonalika/Hartog	Glu-1A	53b : 41a	1·53	57·74**
	Glu-1B	47c : 47i	0·00	
	Glu-1D	58a : 36d	5·15*	
	Glu-3A	49c : 45b	0·17	
	Glu-3D	45a : 49b	0·17	
CD87/Hartog (97)	Glu-1A	48b : 49a	0·01	33·97
	Glu-1B	51b : 46i	0·26	
	Glu-1D	47a : 50d	0·09	
	Glu-3B	49b : 48h	0·01	
	Glu-3D	47a : 50b	0·09	
QT2200-20/Hartog (74)	Glu-1A	38b : 36a	0·05	34·11
	Glu-1B	41b : 33i	0·86	
	Glu-1D	35a : 39d	0·22	
	Glu-3A	33c : 41b	0·86	
	Glu-3B	36b : 38h	0·05	

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

found to be mixed at the Glu-3A locus (Fig. 3). Of the 15 seeds examined, 2 carried the Glu-3Ac allele rather than the Glu-3Ab allele.

Analysis of the segregation of glutenin profiles across each population indicated that only doubled haploids derived from the Sonalika/Hartog F_1 did not fit the expected segregation ratio. Of the 32 (i.e. 2^5) glutenin profiles possible within this cross, 3 were not represented. There was a strong bias toward the female parent profile, which was represented 11 times within the population of 94. When the 2 parental glutenin profiles were removed from the analysis the revised χ^2 value (36·32) was not significant.

Results of the χ^2 analysis of individual glutenin loci across all 7 crosses indicated there were no significant segregation distortions (Table 3). Deviations present in both the Sonalika/Hartog and Gamenya/Batavia crosses were not in evidence.

Discussion

The major goals of the present study were to establish whether gamete selection was present in wheat×maize-derived doubled haploids and to compare these results with those obtained with other doubled haploid technologies in other studies. When the distribution of alleles of individual high and low molecular weight glutenin subunit loci was assessed across 7 populations containing 721 progeny (Table 3) no segregation distortions were evident. However, when the distribution of possible combinations of alleles within single populations is considered, the population derived from the Sonalika/Hartog cross was not randomly distributed at $P = 0{\cdot}001$, with a χ^2 value of 57·74 (Table 2). The strong bias observed toward the Sonalika glutenin profile suggests some selfing of the female parent during F_1 production. When parental glutenin profiles are excluded from the analysis, the revised χ^2 value (36·32) indicates random segregation (95% probability). To confirm the likelihood of maternal selfing during the initial cross, the over-representation of the Sonalika profile is currently being monitored for other groups of markers in this population.

Within the Sonalika/Hartog cross, the frequency of alleles at the Glu-1D locus was biased at $P = 0{\cdot}05$. This locus did, however, segregate randomly for the a and d alleles in 2 other populations in this study, both of which featured Hartog as the male parent (Table 2). If as suspected, maternal selfing did occur during F_1 production of this population, then apparent distortions of the kind observed at the Glu-1D locus

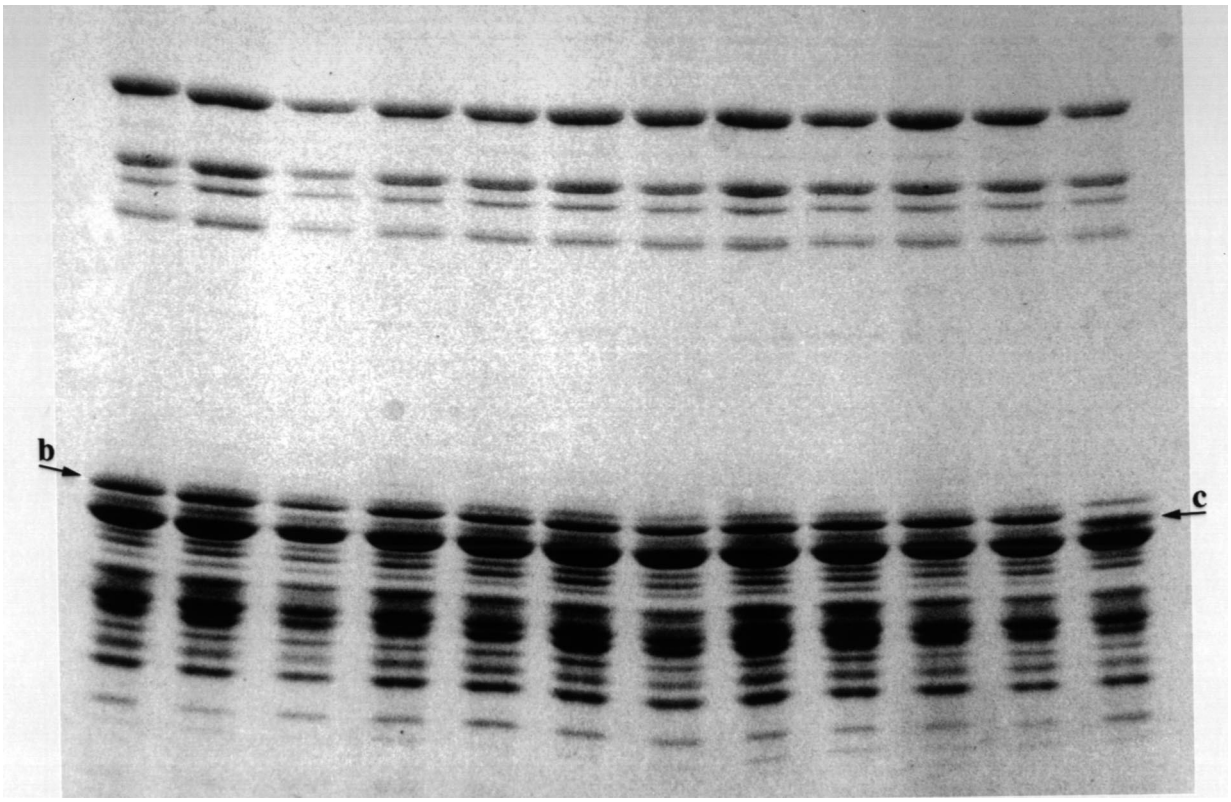

Fig. 3. High and low molecular weight glutenin subunit profiles of single seeds of the Gamenya parent seed stock.

are likely. In any case, the probability of at least 1 Type 1 error in 26 χ^2 analyses of segregating allele pairs is 0·74 at $P = 0{\cdot}05$. We believe the balance of evidence argues against the observed segregation distortion being due to gamete selection during doubled haploid production from the F_1 cross.

One other population, derived from the Gamenya/Batavia cross, contained a highly significant ($P < 0{\cdot}01$) segregation distortion at the Glu-3A locus. A Type 1 error in 26 analyses at this level of significance has a probability of 0·026 and thus is not a legitimate explanation for this result. Four other populations in this study segregated randomly at this locus, with 2 of these populations also segregating for the b and c alleles. One possible explanation is that closely linked genes in the Gamenya/Batavia cross are causing gamete selection during doubled haploid production. Alternatively, we investigated the possibility that one of the parents was segregating at this low molecular weight glutenin subunit locus, since at the time this cultivar was originally developed (1960), low molecular weight glutenin subunit profiles were difficult to determine and were not a criterion for selection. A follow-up screening of individual seeds from the original sample indicated that the Gamenya seed used as the female parent was heterogeneous for Glu3Ab/c (Fig. 3), with all other loci consistent. The Glu-3A locus has been recorded previously as heterozygous in Gamenya (Metakovsky *et al.* 1990). This heterogeneity readily explains the distortion towards the c allele during doubled haploid development. This finding emphasises the importance of screening parent seed carefully when entering into such studies and also highlights the potential effects of residual heterogeneity in certain inbred varieties.

Table 3. Chi-square analysis of individual glutenin subunits across crosses

Loci	Subunits	Frequency		χ^2
		Observed	Expected	
Glu-1A	a	231	235	0·14
	b	239	235	
Glu-1B	b	143	137·5	0·69
	c	101	97·5	
	i	226	235	
Glu-1D	a	140	132·5	0·85
	d	125	132·5	
Glu-3A	b	253	263·5	3·31
	c	154	136	
	d	71	77	
	e	49	50·5	
Glu-3B	b	132	134	0·06
	h	136	134	
Glu-3D	a	137	144	0·75
	b	247	246·5	
	c	109	102·5	

There have been few studies of the distribution of parental gametes in maternal doubled haploids in wheat. Suenaga and Nakajima (1993) examined the segregation of 8 markers in a population of 203 wheat×maize-derived lines. One pair of unspecified alleles studied was located at the Glu-1D locus. Only one of the studied markers, glume pubescence, demonstrated a distorted segregation ratio. This distortion was attributed to a close linkage between glume pubescence and tillering ability which also differed between the parents. Tillering ability is thought to be an important variable in the yield of doubled haploid spikes from haploid plants treated with colchicine (Thiebaut *et al.* 1979; Hassawi and Liang 1991). Colchicine treatment is a necessary component of all wide cross mediated, doubled haploid generation protocols because spontaneous doubling rarely occurs.

In a study by Wang *et al.* (1995), wheat×*Hordeum bulbosum*-derived doubled haploid lines were found to be more representative of the expected parental gamete frequencies than microspore-derived lines. This more representative nature of wide cross derived, doubled haploid lines over those derived through anther culture has also been suggested in studies using *Hordeum vulgare* (Bjørnstad *et al.* 1993; Tinkler *et al.* 1993). Segregation distortions are not uncommon in doubled haploid populations derived via anther culture and microspore-based methods (Graner *et al.* 1991; Guiderdoni 1991; Henry *et al.* 1993; Murigneux *et al.* 1993; Foisset *et al.* 1996). By comparison, the only segregation distortions observed in our study could be attributed to initial undetected heterogeneity in a parent line or to technical errors in establishing one of the crosses. Our findings support the conclusion that wheat doubled haploids derived via the wheat×maize system are suitable for detailed genetic investigations requiring the assumption of random assortment of parental gametes.

For the high and low molecular weight glutenin subunit loci on the long and short arms of chromosome 1, respectively, we have demonstrated a random distribution of alleles across 7 different genetic backgrounds. This indicates that the extensive studies we are presently undertaking in search of other markers for wheat quality attributes can assume the absence of gamete selection at Glu-1 and Glu-3 loci and closely linked regions on chromosome 1. We are currently surveying marker loci on other chromosome groups to confirm both the random segregation of gametes throughout the wheat genome during wheat×maize doubled haploid generation and the suitability of these populations for detailed genetic analyses using available software. When this information is combined with quality data currently being generated from field trials of these populations, possible associations between these markers and end-use quality can be determined for the northern wheat-growing region of Australia.

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