Role of Recombination in the Evolution of Host Specialization Within *Bean yellow mosaic virus*

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ABSTRACT

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Seven complete genomes and 64 coat protein gene sequences belonging to *Bean yellow mosaic virus* (BYMV) isolates from different continents were examined for evidence of genetic recombination using six different recombination-detection programs. In the seven complete genomes and a single complete genome of the related virus *Clover yellow vein virus* (CIYVV), evidence for eight recombination patterns was found by four or more programs, giving firm evidence of their presence, and five additional recombination patterns were detected by three or fewer programs, giving tentative evidence of their occurrence. When the nucleo-

tide sequences of 64 BYMV and one CIYVV coat protein genes were analyzed, three firm recombination patterns were detected in 21 isolates (32%). With another six isolates (9%), tentative evidence was found for three further recombination patterns. Of the 19 firm or tentative recombination patterns detected within and between strain groups of BYMV, and with CIYVV, 12 involved a generalist group of isolates as a parent but none of the other BYMV groups acted as parents more than six times. These findings suggest that recombination played an important role in the evolution of BYMV strain groups that specialize in infecting particular groups of domesticated plants.

Additional keywords: diversity, emergence, host range, origins, *Potyviridae*.

Studies on the molecular evolutionary history of plant viruses provide important information on host ranges and virus emergence, which is helpful when designing strategies for control. Such studies require an understanding of variation caused by factors like recombination, reassortment, new mutations, synergism, genetic drift, founder effects, genetic bottlenecks, selection, and adaptation (4,10,14,29,30). Exchange of genomic segments that occurs during recombination and reassortment has often been associated with host switches, host range expansion, and the appearance of new viruses and virus strains (8,14,16,24,50).

Both intraspecies recombination and interspecies recombination occur commonly and constitute a major evolutionary driver within the potyvirus group, which is the largest and economically most important group of plant viruses (1,13,38). For a new recombinant potyvirus to be generated and prosper, progenitors must co-infect a host and their recombinant progeny must demonstrate adaptations that confer superior fitness to that of parental strains. The ability of potyviruses to infect hosts is controlled by multiple factors such as vector compatibility, vector/host interactions, and synthesis of viral suppressors of gene silencing, but it is thought that, in most cases, host range limitations result from mechanisms that restrict virus movement rather than the inhibition of virus replication within cells (15). Usually, the coat protein (CP) is essential for long-distance movement of potyviruses beyond the inoculated leaves, as well as the recruitment of host-encoded susceptibility factors: its vari-

able-length N-terminal is involved in movement and accumulation within infected plants, and its trypsin resistant “core” region determines cell-to-cell movement (2,9,20,34,51). A number of other potyviral proteins, including the genome-linked protein (VPg), helper component protease (HC-pro) (6), the third protein (P3), and six kilodalton 2 peptide (6K2) are also determinants of host susceptibility, probably through specific interactions with host-encoded proteins (6,19,22,31,39).

Bean yellow mosaic virus (BYMV) (genus *Potyvirus*, family *Potyviridae*) occurs worldwide, infecting domesticated and wild species within several monocotyledonous and dicotyledonous families naturally, often causing economic crop losses. It has a single-stranded plus-sense genomic RNA of about 9.7 kb. The genome has terminal untranslated regions flanked by a single open reading frame. The single large polyprotein is processed into at least 10 proteins (33,46). Wylie et al. (52) analyzed the phylogeny of CP gene sequences of 64 isolates in relation to original natural isolation hosts and geographical distribution and proposed the subdivision of BYMV into seven distinct strain groups. The group with the greatest intra-group genetic diversity also had a generalist host strategy, naturally infecting both monocotyledons and dicotyledons, and wild and domesticated species, and so was called the general group. The six other groups all had specialist host strategies, as the isolates within them were originally obtained from narrow ranges of plant species (1-4/group) all of which were domesticated. Five of these were named after their natural hosts, the monocot, lupin, broad bean, canna, and pea groups. The remaining monotypic group was called W after isolate W originally from white lupin (*Lupinus albus*). Wylie et al. (52) proposed that the general group may represent the original ancestral group from which the specialist groups evolved in different types of domesticated plants in their various centers of origin after the advent of agriculture (23).

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Of 23 BYMV isolates analyzed in a study by Chare and Holmes (4), Sawyer's run test and incongruent phylogenies predicted that recombination in the 5' region of the CP gene involving different broad bean group-derived isolates produced isolate S. This paper reports an analysis of the CP sequences of 64 BYMV isolates, and of the complete genomes of seven BYMV isolates and one of CIYVV to determine whether recombination played a significant role in the evolution of host specialization within BYMV.

MATERIALS AND METHODS

Virus isolates. The CP sequences of 24 BYMV isolates from southwest Australia were reported previously (26,48,51,52). The 40 other BYMV CP sequences, seven complete BYMV genomes and one complete CIYVV genome, were from the GenBank database. Table 1 gives details of each BYMV isolate, including the strain group it belongs to (52), the host it was first obtained from, where it was originally collected, and its GenBank accession number.

Recombination analyses. The 64 BYMV CP sequences and the seven complete BYMV genome sequences were aligned against the corresponding sequence of CIYVV isolate No. 30, because this is the virus most closely related to BYMV. Sequence alignments were computed using ClustalW (44) with default parameters within MEGA 4 (42) using the Maximum Composite Likelihood model for nucleotides. Alignments were checked by eye and adjusted where necessary. The aligned sequences were checked for incongruent relationships resulting from recombination using the programs implemented in Recombination Detection Program (RDP v3.15), which were GENECONV, Bootscan, maximum chi-square, Chimaera, SiScan, and 3Seq (25). Recombination detecting programs that compare sequences only identify approximate positions of recombination sites. Alignments were scanned with default settings for each program using a Bonferroni corrected *P* value cutoff of 0.05. These analyses also determined which nonrecombinant sequences had regions that were closest to regions of recombinant sequences and hence indicated probable ancestral lineages. For simplicity, these are termed parental or ancestral isolates, although, in reality, they were just the most closely related sequences to the set being analyzed. Firm recombination patterns were those detected by four or more different programs, whilst tentative recombination patterns were those detected by three or fewer programs (29).

RESULTS

Recombination analyses. Complete genome. Evidence for presence of eight firm and five tentative recombination patterns was detected in the seven complete BYMV or single complete CIYVV genomes (Table 2; Fig. 1). CP-derived general group isolate, MB4, had firm recombination patterns within its HC-pro, P3, and N1b genes (patterns A, B, and C, respectively). The parental isolates were from unknown and broad bean lineages. Isolate GDD, also in the general group, had firm independent recombination patterns (E and F) within its P3/CI and N1b genes, both apparently derived from recombination between general and monocot group isolates. There was firm evidence of overlapping recombination patterns between closely related isolates, IbG and M11, both members of the monocot group: these recombination patterns (G and H) had general and broad bean, and general and unknown group origins, respectively. Patterns A and G, in the general and monocot groups, respectively, differed in length but shared the same predicted origins in general and unknown groups and had closely similar breakpoints (nts 1839 and 1851, respectively), suggesting they may represent the same recombination event. In the genome of the pea group isolate (CS) there was a tentative

recombination pattern within its CI gene (D), probably derived from CIYVV. Analysis of the full genome of isolate S revealed a single tentative recombination pattern (I) in its CP gene, apparently derived from an unknown parent. There was no evidence of recombination within isolate W, except for one small (62 nucleotides) tentative recombination site (J), apparently derived from CIYVV, in its N1b gene. A firm recombination pattern (M) in the 3' untranslated region (UTR) of CIYVV involving a parental isolate from the pea group, provided further evidence of interspecies recombination history between CIYVV and this group. There were also two tentative patterns near its 3' terminus (K and L), both apparently involving parental isolates within the monocot group and an unknown group.

Coat protein gene. When the nucleotide sequences of 64 CP genes from different BYMV isolates were analyzed for presence of recombination, in 21 isolates (32%) four or more programs detected three recombination patterns (patterns 1, 5, and 6), giving firm evidence of recombination. With six other isolates (9%), three or fewer programs detected three other recombination patterns (patterns 2, 3, and 4), providing tentative evidence that additional recombination was present (Table 3, Fig. 2). Firm evidence of an intra-group recombination pattern between isolates within the general group was detected in the CPs of isolates from wild native and domesticated plants (pattern 1). The proposed parental isolates were also from wild and domesticated plants. Firm recombination pattern 5 represents an intragroup recombination event within the broad bean group while pattern 6 represents intergroup recombination between parental lineages in the general and broad bean groups. Pattern 5 was present only in isolate S and not in other members of the broad bean group, which had a general group background. Pattern 5 was similar, but not identical, to pattern 6 as it had closely similar breakpoints, although the recombinant fragment was larger. Pattern 6 was present in all 18 isolates within the monocot and lupin groups, which strongly suggested that this recombination event was present in a shared ancestor common to these two groups. Among tentative recombination events, isolates within the general and monocot groups apparently recombined to produce isolates within the canna group (pattern 2). Pattern 3 apparently involved recombination between a lupin group isolate and a CIYVV isolate, resulting in the CP found in pea group isolates. For pattern 4, the recombination proposed was intragroup, occurring between general group members resembling isolates derived from the domesticated host species *G. hybrida* and *P. vulgaris*. Recombination breakpoints for patterns 1 to 4 were within the first 100 nucleotides of the variable N terminal region of the CP gene, while the breakpoints of patterns 5 and 6 were close to the core region of the CP. No evidence of recombination was found within the CP of isolate W, the only member of monotypic strain group W.

DISCUSSION

This study involving seven complete genomes and sixty-four CP genes of BYMV provides evidence that recombination is likely to have played an important role in the evolution of specialist strain groups of BYMV adapted to infect limited ranges of domesticated plants (Fig. 3). We used several recombination detection programs to identify regions where recombination had taken place. Evidence for 19 recombination patterns was found to a greater or lesser extent in the complete genomes or CPs of isolates representing all seven strain groups distinguished by natural hosts proposed by Wylie et al. (52). In complete genomes, recombination sites supported by firm evidence were in the HC-Pro, P3, and CI genes in the 5' region of the genome, and in the 3' region within the N1b and CP genes and 3'UTR. Most notably, the data supports the deduction that the general and broad bean groups may be ancestral to two host-specialist groups, the lupin and monocot groups, and to isolate S within the broad bean group. In

addition, there is tentative evidence that recombination between general and monocot group isolates was a factor in formation of the host-specialist canna group. Firm or tentative evidence of inter-species recombination between CIYVV and an unknown

BYMV isolate to form the pea group was found in the CI and CP genes of pea group isolates.

The general group seems likely to have evolved in communities of wild plants containing diverse species. Because of its broad

TABLE 1. *Bean yellow mosaic virus* strain groups and isolates analyzed

Strain group	Isolate ^a	Geographical origin ^b	Original host species	Host family	Accession no.	Original isolate reference
Broad bean	S*	Naracoorte, S. Australia	<i>Vicia faba</i>	Fabaceae	U47033	32
	90-2	Japan	<i>V. faba</i>	Fabaceae	D89545	37
	V124	Kagoshima, Japan	<i>V. faba</i>	Fabaceae	AB041970	47
	FBMj	Manjimup, W. Australia	<i>V. faba</i>	Fabaceae	EU082112	5
	FBD1	Dongara, W. Australia	<i>V. faba</i>	Fabaceae	EU082117	5
	FBD2	Dongara, W. Australia	<i>V. faba</i>	Fabaceae	EU082128	5
	FBD3	Dongara, W. Australia	<i>V. faba</i>	Fabaceae	EU082113	5
	FBI-1	Irwin, W. Australia	<i>V. faba</i>	Fabaceae	EU082116	5
	FBI-2	Irwin, W. Australia	<i>V. faba</i>	Fabaceae	EU082114	5
	FBI-3	Irwin, W. Australia	<i>V. faba</i>	Fabaceae	EU082115	5
Canna	Cgz	Guangzhou, China	<i>Canna</i> sp.	Cannaceae	EF592168	Unpublished
	Chz	Hangzhou, China	<i>Canna</i> sp.	Cannaceae	DQ060521	Unpublished
	Csz	Shenzhen, China	<i>Canna</i> sp.	Cannaceae	EF592169	Unpublished
General	BadgB-1	Badgingarra, W. Australia	<i>Kennedia prostrata</i> ^c	Fabaceae	DQ901433	48
	Diurus	ACT, Australia	<i>Diuris maculata</i> ^c	Orchidaceae	AF185962	12
	KP	The Lakes, W. Australia	<i>K. prostrata</i> ^c	Fabaceae	DQ901432	27
	LCbk-NN	Cranbrook, W. Australia	<i>Lupinus angustifolius</i>	Fabaceae	AF192783	5
	LDw-NN	Dowerin, W. Australia	<i>L. angustifolius</i>	Fabaceae	EU082118	5
	LKoj1-NN	Kojonup, W. Australia	<i>L. angustifolius</i>	Fabaceae	AF19282	5
	LNgn-NN	Narrogin, W. Australia	<i>L. angustifolius</i>	Fabaceae	EU082121	5
	LutKP-1	Kirup, W. Australia	<i>L. luteus</i>	Fabaceae	AF192781	5
	LutKP-2	Kirup, W. Australia	<i>L. luteus</i>	Fabaceae	EU082122	5
	MI	South Perth, W. Australia	<i>Melilotus indica</i>	Fabaceae	X81124	21
	MI-NAT	South Perth, W. Australia	<i>M. indica</i>	Fabaceae	AF434661	51
	MUB-1	Murdoch, W. Australia	<i>K. prostrata</i> ^c	Fabaceae	DQ901431	48
	Pterostylis	Australia	<i>Pterostylis curta</i> ^c	Orchidaceae	AF185960	12
	PvB-1	Mt Pleasant, W. Australia	<i>Phaseolus vulgaris</i>	Fabaceae	EU082123	52
	WHPB-1	Wireless Hill, W. Australia	<i>K. prostrata</i> ^c	Fabaceae	DQ901435	48
	WHPB-2	Wireless Hill, W. Australia	<i>K. prostrata</i> ^c	Fabaceae	DQ901434	48
	BH-8	Hokkaido, Japan	<i>P. vulgaris</i>	Fabaceae	AB041972	47
	Danish	Denmark	<i>Gladiolus</i> sp.	Iridaceae	X53684	3
	E441	Palampur, India	<i>Gladiolus</i> sp.	Iridaceae	AJ844916	Unpublished
	E-92C	Kagoshima, Japan	<i>G. hybrida</i>	Iridaceae	AB029439	47
	GDD*	Utah, USA	<i>Gladiolus</i> sp.	Iridaceae	AY192568	18
	MB4*	Japan	<i>Gladiolus</i> sp.	Iridaceae	D83749	11
	Palampur-1	Palampur, India	<i>Gladiolus</i> sp.	Iridaceae	AM398198	Unpublished
	S-22C	Kagoshima, Japan	<i>G. hybrida</i>	Iridaceae	AB029436	47
	Sb-12C	Kagoshima, Japan	<i>V. faba</i>	Fabaceae	AB029440	47
	Sb-50C	Kagoshima, Japan	<i>V. faba</i>	Fabaceae	AB029437	47
	Solan	Solan, India	<i>Gladiolus</i> sp.	Iridaceae	EF611822	Unpublished
Lupin	LP-1	Hatay, Turkey or Homs, Syria	<i>L. pilosus</i>	Fabaceae	EU082119	21
	LP-2	As above	<i>L. pilosus</i>	Fabaceae	EU082120	48
	Pullman	Pullman, USA	<i>L. luteus</i>	Fabaceae	EU144223	Unpublished
	RLut-1	St Petersburg, Russia	<i>L. luteus</i>	Fabaceae	EU082124	52
	RLut-2	St Petersburg, Russia	<i>L. luteus</i>	Fabaceae	EU082125	52
	RLut-3	St Petersburg, Russia	<i>L. luteus</i>	Fabaceae	EU082126	52
	RLut-4	St Petersburg, Russia	<i>L. luteus</i>	Fabaceae	EU082127	52
Monocot	35-1	Saitama, Japan	<i>Gentiana</i> sp.	Gentianaceae	AB097090	45
	B-33	Saitama, Japan	<i>Gentiana</i> sp.	Gentianaceae	AB097089	45
	E-24N	Kagoshima, Japan	<i>G. hybrida</i>	Iridaceae	AB029438	47
	Hangzhou	Hangzhou, China	Not stated		AJ311371	Unpublished
	IbG*	Kyoto, Japan	<i>Gladiolus</i> sp.	Iridaceae	AB079887	28
	M11*	Kyoto, Japan	<i>Gladiolus</i> sp.	Iridaceae	AB079886	28
	Masdevallia	Germany	<i>Masdevallia</i> sp.	Orchidaceae	AF185961	Unpublished
	No. 4	Saitama, Japan	<i>Gentiana</i> sp.	Gentianaceae	AB079782	45
	S-22N	Kagoshima, Japan	<i>G. hybrida</i>	Iridaceae	AB029435	47
	VM-0	India	<i>Vanilla fragrans</i>	Orchidaceae	AY845011	Unpublished
	VM-23	India	<i>V. fragrans</i>	Orchidaceae	AY845012	Unpublished
Pea	I	Vic, Australia	<i>Pisum sativum</i>	Fabaceae	S71232	43
	CS	Japan	<i>P. sativum</i>	Fabaceae	D00604	41
	P242	Wakayama, Japan	<i>P. sativum</i>	Fabaceae	AB041971	Unpublished
	Suzuki	Japan	Not stated		AB032023	Unpublished
	Verbena	USA	<i>Verbena</i> × <i>hybrida</i>	Verbenaceae	AY520092	17
W	W*	Idaho, USA	<i>L. albus</i>	Fabaceae	DQ641248	— ^d
CIYVV	No.30*	Hokkaido, Japan	<i>V. faba</i>	Fabaceae	AB011819	40

^a Asterisk indicates complete genome sequence.

^b W. Australia, Western Australia; S. Australia, South Australia; Vic, Victoria, Australia; ACT, Australian Capital Territory.

^c Indigenous, undomesticated host species. In the text, native species are included in the “wild” category.

^d Isolate W is from Hampton et al. (52).

TABLE 2. Recombination sites in seven complete genomes of *Bean yellow mosaic virus* (BYMV) and one complete genome of *Clover yellow vein virus* (CIYVV) detected by six recombination-detecting programs

Site no. ^a	Recombinant isolate(s) (strain group) ^b	Recombination site (programs detected by) ^c	Gene region	Parental isolates ^d	Parental isolate groups	<i>P</i> value ^e
A	MB4 (general)	1839-2984 ^f (RGBMCS3S)	HC-pro-P3	Unknown × GDD	Unknown × general	8.3 × 10 ⁻⁴²
B		3072-3271 (RGMC3S)	P3	S × GDD	Broad bean × general	4.4 × 10 ⁻¹¹
C		8349-8509 (RMC3S)	NIb	S × GDD	Broad bean × general	3.2 × 10 ⁻⁸
D	CS (pea)	3893-4129 (MC)	CI	CIYVV × unknown	CIYVV × unknown	5.3 × 10 ⁻³
E	GDD (general)	2935-4195 (RGMS3S)	P3-CI	IbG × MB4	Monocot × general	1.1 × 10 ⁻¹²
F		7689-8529 (RGMC3S)	NIb	M11 × MB4	Monocot × general	5.9 × 10 ⁻¹⁶
G	IbG, M11 (monocot)	1851-2099 (RGMC3S)	HC-pro	S × GDD	Broad bean × general	2.2 × 10 ⁻²⁵
H		1109-8555 (RGBMCS3S)	HC-pro-NIb	Unknown × GDD	Unknown × general	4.6 × 10 ⁻¹²³
I	S (broad bean)	9363-9546 (GB)	CP	Unknown × FBMj	Unknown × broad bean	1.1 × 10 ⁻³
J	W	8334-8396 (R)	NIb	CIYVV × unknown	CIYVV × unknown	4.4 × 10 ⁻³
K	CIYVV	6680-6810 (RMC)	NIa-pro	IbG × unknown	Monocot × unknown	8.0 × 10 ⁻³
L		9352-9539 (GMC)	3'UTR	IbG × unknown	Monocot × unknown	3.1 × 10 ⁻⁴
M		8726-9581 (RGBMCS)	CP-3'UTR	CS × unknown	Pea × unknown	5.4 × 10 ⁻¹⁶

^a Firm recombination patterns (in bold type) were those detected by four or more different programs whilst tentative recombination patterns (in plain text) were those detected by three or fewer programs.
^b CP-determined grouping of isolate shown in parentheses (reference 52).
^c R, RDP; G, GENECONV; B, Bootscan; M, Maxchi; C, Chimaera; S, SiScan; 3, 3Seq.
^d Source of recombinant fragment (minor parent) is listed first, followed by major parent.
^e The reported *P* value is for the program in RDP3.15 (in bold type) and is the greatest *P* value among the isolates calculated for the region in question.
^f Numbers indicate nucleotide positions.

Fig. 1. Recombination patterns between seven *Bean yellow mosaic virus* (BYMV) and one *Clover yellow vein virus* (CIYVV) complete genomes. The location of each potyvirus gene is shown (top). Thirteen recombination patterns were found (A to M). The boxes represent the genomes named, with the proposed group names in parentheses, with the sites and sizes of recombination patterns within them as assessed by the six recombination-detecting programs used. “Firm” and “tentative” recombination patterns are shown in underlined and plain text, respectively.

natural host range (including wild and domesticated plants, and both monocotyledons and dicotyledons) and greater genetic diversity, Wylie et al. (52) proposed that it represented an ancestral strain group from which at least six specialist groups emerged. The occurrence of complex recombination patterns, intra-group (two patterns), and inter-group involving its progeny groups (six patterns), also contributed to its genetic diversity. The general group is predicted to be ancestral in 12 of the 19 firm and tentative recombination patterns involving the genomes and CPs. In contrast, the individual specialist groups were ancestral in no more than six such recombination patterns. Thus, the greater number of patterns in which the general group is implicated is

consistent with it being ancestral to the specialist groups. Recombination analysis of complete genomes representing some of the different groups, and CPs representing each of them, provided clues about the sequence of recombination events that lead to the creation of host-specialist groups. Presence of a firm recombination site common to the CPs of all members of both the monocot and lupin groups (pattern 6), and probably isolate S in the broad bean group (pattern 5), strongly suggests that the monocot and lupin groups share a common ancestor derived from the general and broad bean groups. However, the possibility remains that the reverse scenario might actually have occurred, i.e., that one or more of the specialist groups are ancestral to the

TABLE 3. Recombination sites in coat protein genes of *Bean yellow mosaic virus* (BYMV) isolates detected by six recombination-detecting programs

Site no. ^a	Recombinant isolate(s)	Recombination site (program detected by) ^b	Recombinant strain group	Parental isolates	Parental isolate groups ^c	<i>P</i> value ^d
1	WHPB-2, PvB-1	9-549 ^e (MCS3S)	General	KP × E-92C	General × general	7.9 × 10 ⁻⁷
2	Cgz, Csz	14-463 (S)	Canna	MI × Masdevallia	General × monocot	5.6 × 10 ⁻⁹
3	P242, Verbena, I	42-417 (S)	Pea	CIYVV ^f × RLut-2	CIYVV × lupin	2.2 × 10 ⁻⁴
4	GDD	95-804 (S3S)	General	S-22C × BH-8	General × general	4.3 × 10 ⁻⁹
5	S	459-742 (RGBMS)	Broad bean	FBMj × GDD	Broad bean × general	7.6 × 10 ⁻⁵
6	35-1, B-33, Hangzhou, No.4, Masdevallia, E-24N, S-22N, VM-0, VM-23, IbG, M11, LP-1, LP-2, RLut-1, RLut-2, RLut-3, RLut-4, Pullman	517-716 (RGBMS)	Monocot, lupin	FBMj × GDD	Broad bean × general	7.6 × 10 ⁻⁵

^a Firm recombination patterns (in bold text) were those detected by four or more different programs whilst tentative recombination patterns (in plain text) were those detected by three or fewer programs.
^b R, RDP; G, GENECONV; B, Bootscan; M, Maxchi; C, Chimaera; S, SiScan; 3, 3Seq.
^c Source of recombinant fragment (minor parent) is listed first, followed by major parent.
^d The reported *P* value is for the program in RDP3.15 (in bold type) and constitutes the greatest *P* value among the isolates calculated for the region in question.
^e Numbers indicate nucleotide positions.
^f CIYVV = *Clover yellow vein virus*.

Fig. 2. Recombination patterns within the coat protein genes of 64 different *Bean yellow mosaic virus* (BYMV) and one *Clover yellow vein virus* (CIYVV) isolates as assessed by the six recombination-detecting programs used. Six recombination patterns are shown, numbered 1 to 6. The boxes represent the recombination patterns found. The BYMV group(s) to which each belongs is indicated in parentheses. The sites, sizes, group, and isolate names (in parentheses) are illustrated within the boxes. Underlined and plain text show “firm” and “tentative” recombination patterns, respectively.

Pattern 2, i.e., recombination between isolates within the general and monocot groups, can be interpreted as giving rise to formation of the canna group, although the evidence for this is tentative. Similarly, tentative recombination patterns detected within the pea group (patterns 3 and D) and isolate W (pattern J) support recombination between CIYVV and the lupin group and unknown BYMV isolates. The scenario we propose is that the general group was ancestral to the broad bean group, and that these two groups became ancestral to the monocot and lupin groups (Fig. 3). The monocot group recombined with the general group to form the canna group. Two pea group isolates and

An extensive study of recombination with 92 complete genomes of another potyvirus, *Turnip mosaic virus* (TuMV), revealed significant hotspots of recombination in the first protein (P1) gene and in the cytoplasmic inclusion 6K2/VPg (CI/6K2/VPg) gene region, although sites were found in all other genes except for the 6K1 peptide (29). It was not known whether any of these recombinations affect the phenotype of TuMV. In BYMV, no patterns were found in the P1 gene and only one pattern (H) occurred within the CI/6K2/VPg region. Instead, of the 13 patterns found by complete genome analysis, five were in the HC-pro/P3/CI region, four in the NIb, one in the nuclear inclusion a protease (NIa-pro) and the remainder in the CP/3'UTR. The apparent differences in recombination patterns between TuMV and BYMV may reflect the fewer complete BYMV genomes analyzed. HC-pro, P3, and CP genes are all involved in host range determination in other potyviruses through their roles in mediating virus movement within hosts (6,35,39), but it remains to be seen whether two other genes in which recombination was also found (NIa-pro and NIb) also influence BYMV host range.

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Fig. 3. Proposed scenario in which recombination within and between different *Bean yellow mosaic virus* (BYMV) groups gave rise to further BYMV groups. Proposed direction of transfer of firm and tentative recombination events shown by arrows with solid and dotted lines, respectively. Patterns A to M were from complete genome analyses and patterns 1 to 6 from coat protein gene analyses.

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