ISOLATION OF NEW 1DS/1RS WHEAT_RYE RECOMBINANTS

(ISOLASI REKOMBINAN BARU GANDUM-RYE 1DS/1RS)

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ABSTRACT

The wheat-rye translocation 1DL.1RS has been used to introduce stem rust disease resistance associated with the 1RS chromosome, however, cultivars carrying this translocation exhibit deleterious end-use quality. The aim of the experiment was to screen and isolate additional wheat-rye recombinants, in order to eliminate or reduce these deleterious effects of the rye chromosome 1RS when transferred to wheat. Besides their potential use in wheat improvement, a series of new wheat-rye recombinants could find application in mapping activities with other genes.

Using the presence of the SEC-1 marker as an indication of the 1RS chromosome segment, several suspected recombinants were isolated. The frequency of recovery of suspected recombinants of phenotypes "TRI-D1$^+$GLI-D1$^-$SEC-1$^+$" and "TRI-D1$^-$GLI-D1$^+$SEC-1$^+$" ranged from 0.6 to 6 % in families 1 to 3. However, family 4 showed a much higher than expected frequency of these suspected recombinant chromosomes (24.4%). A model was proposed to explain the nature of Family 4 based on homozygosity for the 1DL.1RS translocation and the presence of two recombinants chromosomes involving the short arm of group 1 chromosomes.

Key words: wheat-rye recombinants, 1DL.1RS translocation lines

ABSTRAK

Translokasi gandum-rye 1DL.1RS digunakan untuk mengintroduksi ketahanan terhadap penyakit karat batang yang terletak pada kromosom 1RS, tetapi kultivar pembawa tranlokasi ini menunjukkan kualitas produk akhir yang jelek. Penelitian ini bertujuan untuk mengidentifikasi dan mengisolasi rekombinan gandum-rye baru, untuk mengurangi efek kurang menguntungkan dari kromosom 1RS ini jika ditransfer ke tanaman gandum. Disamping pentingnya dalam perbaikan kualitas gandum, serangkaian rekombinan akan bermanfaat bagi kegiatan pemetaan gen-gen lain.

Dengan menggunakan marker protein SEC-1 sebagai indikator keberadaan segmen kromosom 1RS, beberapa rekombinan telah diisolasi. Frekuensi rekombinan "TRI-D1$^+$GLI-D1$^-$SEC-1$^+$" dan "TRI-D1$^-$GLI-D1$^+$SEC-1$^+$" berkisar 0.6 – 6% pada famili 1-3, tetapi famili 4 menunjukkan frekuensi jauh lebih tinggi (24.4%). Suatu model dirancang untuk menjelaskan keadaan ini berdasarkan homosigositas translokasi 1DL.1RS dan keberadaan 2 rekombinan kromosom yang melibatkan lengan pendek kromosom grup 1 pada gandum.

Kata kunci: rekombinan gandum rye, galur translokasi 1DL.1RS

INTRODUCTION

The wheat-rye translocation 1DL.1RS has been used to introduce stem rust resistance associated with the 1RS chromosome of "Imperial" rye into wheat (Shepherd, 1973). However, despite the beneficial effects of stem rust resistance associated with this translocation, cultivars carrying this translocation exhibit deleterious end-use quality characteristics such as poor dough mixing tolerance, decreased dough resistance to stretching and dough stickiness. This results in inferior bread-making quality making the dough from these wheat lines unacceptable for commercial exploitation. This quality defect has severely limited the utilisation of this source of stem rust resistance in Australian wheat breeding programs.

It is hypothesised that the quality defect could be eliminated or reduced by further reducing the size of the rye chromosome segment thereby either removing deleterious factors on the rye chromosome (eg. secalins) or re-introducing quality-enhancing genes on the related wheat chromosome arm (1DS). Therefore an active search for additional wheat-rye recombinants was undertaken to test this possibility.

The aim of the experiment was to screen and isolate additional wheat-rye recombinants. Besides their potential use in wheat improvement, a series of new wheat-rye recombinants could find

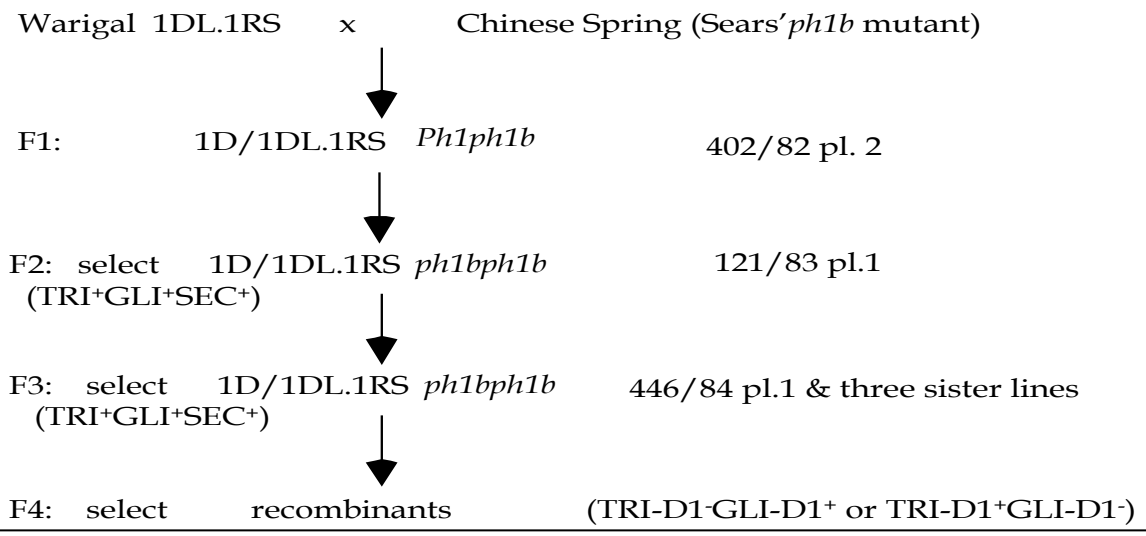

Figure 1. Scheme for production of recombinants between 1DS and 1RS chromosome arms
Gambar 1. Skema pembentukan rekombinan antara kromosom 1DS dan 1RS

application in mapping activities with other genes. Such new recombinants carrying rye chromosome segments of differing sizes could be used for the localisation of other genetic markers (e.g. RFLP, PCR-based) and further characterize the gene order on the 1DS and 1RS chromosome arms (Rogowsky *et al*., 1991). Ideally, a new recombinant with a molecular marker very closely linked to the stem rust resistance gene *SrR* could provide the basis for eventual cloning of that resistance gene using 'chromosome walking' techniques.

MATERIAL AND METHODS

Plant material and crossing procedure

The pedigree of the material tested is shown in Figure 1. The starting material was a 1DL.1RS translocation line isolated in Chinese Spring background (Shepherd, 1973) and subsequently backcrossed to cultivar Halberd and then to cultivar Warigal [(1DL.1RS CS x Halberd^3) x Warigal ^{3}]. Following the strategy of Koebner and Shepherd (1986) this line was crossed to a stock of Sears' *ph1b* mutant in a Chinese Spring background (Sears, 1977) to induce homoeologous recombination. Due to the few number of seeds produced in F_3 generation (progenies of 121/83 plant 1), F_3 plants deduced to be heterozygous fo
the 1DL.1RS translocation and homozygous for *ph1b* were allowed to self-fertilise and the F_4 progeny were screened to isolate recombinants.

Marker loci used in the isolation of recombinant lines

The genetic markers used in the detection and isolation of the new recombinants in the current experiment were the same as used previously by Koebner and Shepherd (1986) to select the primary recombinants. These were the seed storage protein markers TRI-D1 and GLI-D1 (Singh and Shepherd, 1985), which are controlled by genes on the short arm of chromosome 1D of wheat. The TRI-D1 phenotype is controlled by the triticin gene *Tri-D1* which is located proximally on the chromosome 1DS arm.

The γ-gliadin storage protein gene *Gli-D1* is located distally on chromosome arm 1DS. Chromosome arm 1RS of rye is identified by the presence of the storage protein secalin SEC-1 (Shepherd and Jennings, 1971), controlled by *Sec-1*, and the gene for stem rust resistance (*SrR*) shown by Singh *et al.* (1990) to be linked with *Sec-1* on the distal segment of rye 1RS.

The endosperm storage protein markers controlled by genes located on chromosomes 1DS and 1RS can all be visualised simultaneously by single dimension SDS-PAGE (Singh and Shepherd, 1985). The band TRI-1 is controlled by *Tri-D1* alone, while a second band TRI-2 is a hybrid molecule formed from the gene products of *Tri-D1* and *Tri-A1*, a homoeolocus of *Tri-D1* which is located on chromosome arm 1AS (Singh and Shepherd, 1985). Therefore the absence of the *Tri-D1* gene results in the loss of both bands TRI-1 and TRI-2. The storage protein controlled by *Gli - D1* appears as a single band with greater mobility than the TRI-D1 band, while

Sec-1 of rye codes for a group of bands with higher mobility than GLI-D1. These patterns are shown in Figure 2.

RESULTS AND DISCUSSION

Criterion for detection of wheat-rye recombinants

Recombination between the rye chromosome arm 1RS and homoeologous wheat chromosome 1DS would be expected to occur rarely and at random positions along the chromosomes. Occasionally, homoeologous recombination would be expected to occur between the loci *Tri-D1* and *Gli-D1*, which could be detected on protein gels by the breakage of linkage (dissociation) between the markers TRI-D1 and GLI-D1.

Isolation of recombinants showing dissociation of protein markers

A total of 732 F4 progeny seeds from four F3 plants having putative genotype 1D/1DL.1RS *ph1bph1b* were screened with SDS-PAGE to detect additional recombinants. The different phenotypic patterns obtained for the progeny of these populations and the frequency of their occurrence are given in Table 1.

Using the presence of the SEC-1 marker as an indication of the 1RS chromosome segment, several suspected recombinants were isolated. The frequency of recovery of suspected recombinants of phenotypes "TRI-D1$^+$GLI-D1$^-$ SEC-1$^+$" and "TRI-D1$^-$GLI-D1$^+$SEC-1$^+$" ranged from 0.6 to 6 % in families 1 to 3. However, family 4 showed a much higher than expected frequency of these suspected recombinant chromosomes (24.4%).

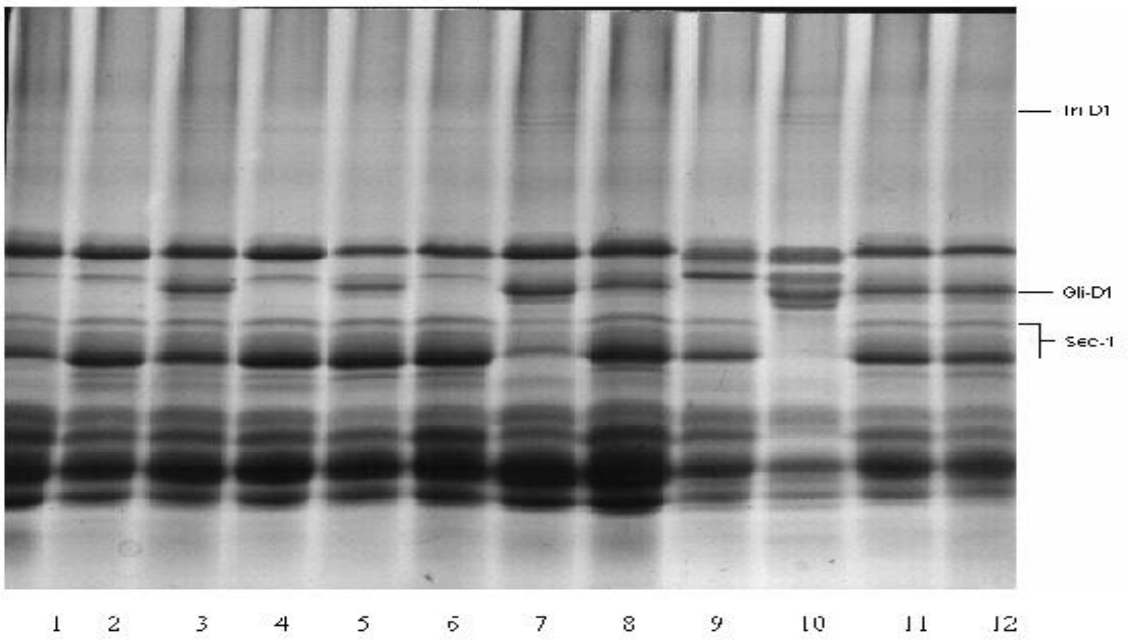

Figure 2. Storage protein phenotype patterns obtained by SDS-PAGE of unreduced protein extracts of F_4 progenies 1D/1DL.1RS *ph1bph1b* and control seeds. Controls: 9) 1DL.1RS translocation (Tri-D1$^-$Gli-D1$^-$Sec-1$^+$) and 10) Normal' Chinese Spring' wheat (Tri-D1$^+$Gli-D1$^+$Sec-1$^-$), progenies screened: 1, 2, 4, 6 (Tri-D1$^-$Gli-D1$^-$Sec-1$^+$), 3, 5, 7, 8, 11, 12 (Tri-D1$^+$Gli-D1$^+$Sec-1$^+$).

Gambar 2. Pola fenotipe protein dari SDS-PAGE progeny F4 1D/1DL.1RS ph1bph1b dan kontrol. Kontrol : 9) translokasi 1DL.1RS (Tri-D1$^-$Gli-D1$^-$Sec-1$^+$) dan 10)gandum 'Chinese Spring' normal (Tri-D1$^+$Gli-D1$^+$Sec-1$^-$); Progeny : 1, 2, 4, 6 (Tri-D1$^-$Gli-D1$^-$Sec-1$^+$); 3, 5, 7, 8, 11, 12 (Tri-D1$^+$Gli-D1$^+$Sec-1$^+$).

Table 1. Endosperm protein phenotypes and their frequency in four *ph1bph1b* 1D/1DL.1RS F_4 families of translocation lines in a Warigal background. Suspected recombinant phenotypes are progenies with dissociation between TRI-D1 and GLI-D1

Tabel 1. Fenotipe protein dan frekuensinya (jumlah biji) pada 4 famili F_4 1D/1DL.1RS ph1bph1b galur translokasi Warigal background. Fenotipe rekombinan adalah progeny yang menunjukkan disosiasi antara TRI-D1 dan GLI-D1

Endosperm Protein Phenotype			Observed Frequency (number of seeds) in Family No.			
TRI-D1	GLI-D1	SEC-1	1	2	3	4*
+	+	-	28	52	50	0
-	-	+	42	30	46	48
+	+	+	56	84	100	122
+	-	+	3	1	5	29
-	+	+	5	0	5	26
+	-	-	0	0	0	0
-	+	-	0	0	0	0
Total screened			134	167	206	225

+ : protein present - : protein absent

* This family came from 446/84 plant 1

In families 1 to 3, most of the progeny showed parental phenotype combinations "TRI-D1$^+$ GLI-D1$^+$ SEC-1$^-$", "TRI-D1$^-$ GLI-D1$^-$ SEC-1$^+$" and "TRI-D1$^+$ GLI-D1$^+$ SEC-1$^+$", representing the transmission of either a normal 1D chromosome or a translocation 1DL.1RS from heterozygous parents. These parental phenotypes could reflect either the presence of unchanged parental chromosomes or the presence of 1 parental chromosome and a recombinant chromosome masked by the presence of the parental-type chromosome. Family 4 was unusual in that it did not show any SEC-1$^-$ progeny suggesting that the parent plant must have been homozygous for *Sec-1* (Figure 3).

Recombinant phenotypes 'TRI-D1$^+$GLI-D1$^-$ SEC-1$^-$'and 'TRI-D1$^-$GLI-D1$^+$SEC-1$^-$' which would reflect the presence of the products of wheat-wheat recombination were not detected. However, these types of wheat-wheat recombinants could still have been produced in these families but their occurrence could be masked by the presence of an unaltered parental chromosome.

Confirmation of suspected recombinants from families 1, 2 and 3

A total of 19 seeds with recombinant phenotypes were detected in Families 1, 2 and 3 (excluding Family 4) (Table 1). In order to confirm their recombinant status, the embryo portions of each grain were planted but only 11 of the 19 embryos germinated. The resulting plants were crossed to Gabo ditelo 1DL and the endosperm protein phenotype from 12 hybrid seeds from each cross was determined in gels to detect their segregation for TRI-D1, GLI-D1 and SEC-1 markers.

In six of the putative recombinants, the wheat chromosome 1D markers segregated independently of the rye chromosome 1R markers in these hybrid seeds and hence were considered not to be wheat-rye recombinants. It is assumed that they arose from recombination between homoeologous wheat chromosomes, and therefore represent wheat-wheat recombinants. Progeny from four of the five remaining plants, showed complete linkage between 1D and 1R markers, indicating likely wheat-rye recombinants. The phenotype of the progeny from the fifth plant indicated that it did not have a recombinant phenotype and had been misclassified in the original screening.

These four additional suspected wheat-rye recombinants were not characterised further, because it was thought at that time that Family 4 might be a more valuable source of crossover products considering the high frequency of putative recombinants produced. A large variety of crossover products could be useful for understanding the basis of quality problem of translocation lines.

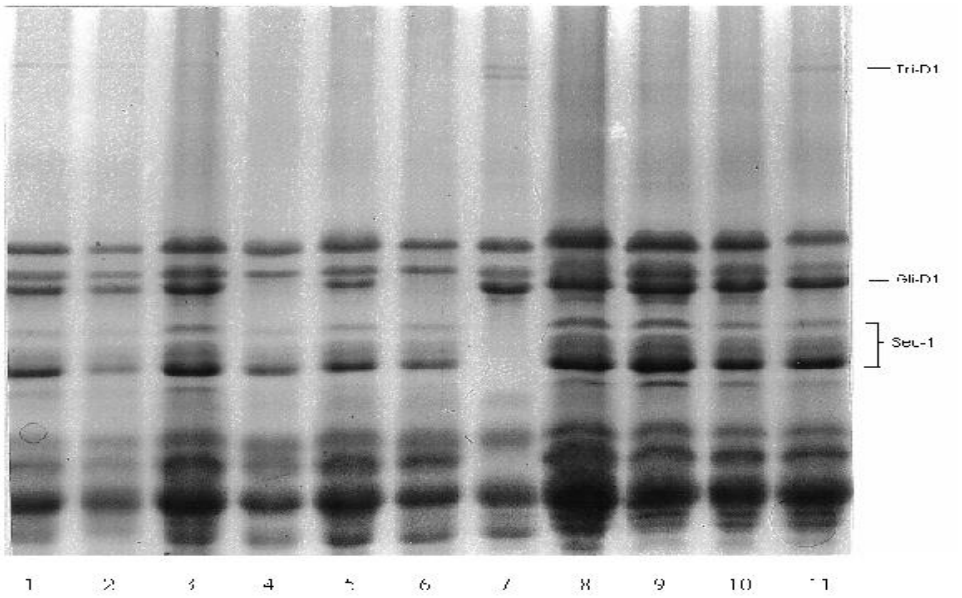

Figure 3. Storage protein phenotype patterns obtained by SDS-PAGE of unreduced protein extracts of Family 4 progenies and control seeds. Controls: (lane 7) Normal' Chinese Spring' wheat (Tri-D1$^+$Gli-D1$^+$Sec-1$^-$) and (6) 1DL.1RS translocation (Tri-D1$^-$Gli-D1$^-$Sec-1$^+$) and suspected recombinants in lanes 5, 8, 9 (Tri-D1$^-$Gli-D1$^+$Sec-1$^+$) and in lane 4 (Tri-D1$^+$Gli-D1$^-$Sec-1$^+$)

Gambar3.Pola fenotipe protein dari SDS-PAGE progeny Famili 4 dan kontrol. Kontrol (7) Normal' Chinese Spring' (Tri-D1$^+$Gli-D1$^+$Sec-1$^-$) dan (6) translokasi 1DL.1RS (Tri-D1$^-$Gli-D1$^-$Sec-1$^+$) dan diduga rekombinan pada 5, 8, 9 (Tri-D1$^-$Gli-D1$^+$Sec-1$^+$) dan pada 4 (Tri-D1$^+$Gli-D1$^-$Sec-1$^+$)

Further investigation of Family 4

Family 4 gave a very high percentage of progeny plants with dissociation phenotypes (suspected recombinants). Among 225 progeny seeds examined, 55 showed dissociation phenotypes (24.4%) with 29 plants being "TRI-D1$^+$GLI-D1$^-$SEC-1$^+$" and 26 plants being "TRI-D1$^-$GLI-D1$^+$SEC-1$^+$" (Table 1). It was of some interest to find whether the high frequency of dissociation phenotypes was due to recombination or some other cause.

Origin of dissociation phenotypes in Family 4

There were two unexpected features observed in the progenies of Family 4. Firstly there was a very high rate of dissociation phenotypes detected and secondly all F_4 progeny were SEC-1$^+$. The first check was to find whether any of these 55 plants with dissociation phenotypes carried wheat-rye recombinant chromosomes and this is normally determined by crossing them to Gabo ditelo 1DL and observing whether the TRI-D1$^-$ or GLI-D1$^-$ markers were co-segregated with SEC-1$^+$ in the progeny. However, given the likelihood that the parent plant was homozygous for *Sec-1*, there would be no segregation for *Sec-1* in this cross and a further generation of selfing would be required with the F_1 plants which were now heterozygous for *Sec-1*. Only 30 out of the 55 embryos showing dissociation phenotypes germinated and the resulting plants (named DP-1 to DP-30) were crossed with Gabo ditelo1DL. From 5 to 16 F_1 progeny seeds from each of the 30 families were screened in gels to record their segregation for protein marker genes.

The 13 plants with TRI-D1$^-$GLI-D1$^+$SEC-1$^+$ dissociation phenotype (Table 2), gave F_1 progeny which segregated for "TRI-D1$^-$GLI-D1$^+$SEC-1$^+$" and "TRI-D1$^-$GLI-D1$^-$SEC-1$^+$" (11 families) and 2 families (DP-1 and DP-8) showed no segregation (all progeny had phenotype TRI-D1$^-$GLI-D1$^+$SEC-1$^+$). However, family DP 6 also produced 2 F_1 progenies with the unexpected phenotypes TRI-D1$^-$GLI-D1$^-$SEC-1$^-$ and TRI-D1$^-$GLI-D1$^+$SEC-1$^-$. Note that all 135 F_1 progenies except these two had the SEC-1$^+$ phenotype indicating again that the Family 4 parent-plant (Table 1) was most likely homozygous for *Sec-1*.

Table 2. Endosperm protein phenotype of F_1 progeny and their frequency from crosses between plants having phenotype $TRI^-GLI^+SEC^+$ and Gabo ditelo 1DL (TRI^- GLI^- SEC^-)

Tabel 2. Fenotipe protein progeny F_1 dan frekuensinya (jumlah biji) dari persilangan antara tanaman dengan fenotipe$TRI^-GLI^+SEC^+$ dan Gabo ditelo 1DL (TRI^- GLI^- SEC^-)

Dissociation phenotype	Endosperm Protein Phenotype of F_1 progeny					
(-++) plant no	-++	--+	+-+	+++	-+-	---
DP 1	10	0	0	0	0	0
DP 2	6	4	0	0	0	0
DP 3	4	8	0	0	0	0
DP 4	3	7	0	0	0	0
DP 5	5	5	0	0	0	0
DP 6	4	6	0	0	1	1
DP 7	4	6	0	0	0	0
DP 8	13	0	0	0	0	0
DP 9	3	5	0	0	0	0
DP 10	3	7	0	0	0	0
DP 11	3	7	0	0	0	0
DP 12	5	5	0	0	0	0
DP 13	5	7	0	0	0	0

These results are consistent with the selected dissociation progeny plants being homozygous for *Sec-1* and heterozygous for *Gli-D1* except for DP-1 and DP-8 which were probably homozygous for *Gli-D1* as well as *Sec-1*. The unusual phenotypes in the progeny of DP-6 could be due to rare aneuploidy (---) or to segregation for *Sec-1* (-+-).

The 17 plants with dissociation phenotype $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^+$ (Table 3) gave F_1 progeny which segregated for the protein phenotypes "$TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^+$" and "$TRI\text{-}D1^-GLI\text{-}D1^-SEC\text{-}1^+$" (15 families) and two families (DP-14 and DP-29) which showed no segregation (all plants $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^+$, except 1 showed $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^-$ in DP-29). However, there were also a few phenotypes $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^-$ and $TRI\text{-}D1^-GLI\text{-}D1^-SEC\text{-}1^-$ in 7 of the families. These segregation patterns suggest that plant DP-14 is homozygous for *Sec-1* and *Tri-D1* (no segregation of either loci) and plants DP-16, DP-17, DP-19, DP-24, DP-26 and DP-27 are homozygous for *Sec-1* and heterozygous for *Tri-D1*.

The presence of $SEC\text{-}1^-$ and $GLI\text{-}D1^-$ phenotypes in progeny of DP-15, DP-18, DP-25, DP-28 and DP-30 suggest that these particular plants are heterozygous for both *Sec-1* and *Tri-D1* and DP-29 homozygous for *Tri-D1* and heterozygous for *Sec-1*. Note presence of $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^-$ individuals in progeny of these 6 DP plants (all heterozygous for *Sec-1*) indicates that these plants at least do not contain wheat-rye recombinant chromosomes.

Table 3. Endosperm protein phenotype of F_1 progenies and their frequency from crosses between plants having phenotype $TRI^+GLI^-SEC^+$ and Gabo ditelo 1DL

Tabel 3. Fenotipe protein progeny F1 dan frekuensinya (jumlah biji) dari persilangan antara tanaman dengan fenotipe $TRI^+GLI^-SEC^+$ dan Gabo ditelo 1DL

Dissociation phenotype (+-+) plant no	Endosperm Protein Phenotype of F_1 progeny					
	-++	--+	+-+	+--	++-	---
DP 14	0	0	11	0	0	0
DP 15	0	3	6	1	0	0
DP 16	0	7	3	0	0	0
DP 17	0	4	1	0	0	0
DP 18	0	1	1	4	0	0
DP 19	0	7	4	0	0	1
DP 20	0	2	6	0	0	0
DP 21	0	6	6	0	0	0
DP 22	0	5	5	0	0	0
DP 23	0	11	5	0	0	0
DP 24	0	3	7	0	0	0
DP 25	0	4	2	3	0	1
DP 26	0	5	5	0	0	0
DP 27	0	6	3	0	0	1
DP 28	0	3	6	1	0	0
DP 29	0	0	11	1	0	0
DP 30	0	4	5	1	0	0

Further progeny test of dissociation phenotypes from Family 4

In order to investigate the nature of Family 4 further, F_1 seeds showing dissociation phenotypes (now heterozygous for *Sec-1*) were analysed, to find whether the dissociation phenotype co-segregated with $SEC\text{-}1^+$ and was therefore linked to it by a recombination event. Two F_1 progeny seeds with dissociation phenotypes ($TRI\text{-}D1^-GLI\text{-}D1^+SEC\text{-}1^+$ and $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^+$) from each cross between the individual original dissociation phenotypes and Gabo ditelo-1DL (from Table 2 and 3) were planted and allowed to self fertilise. However, only 23 seeds could be further tested, due to germination problems. There were F_2 progeny from 7 F_1 plants of the type $TRI\text{-}D1^-GLI\text{-}D1^+SEC\text{-}1^+$ and 16 F_1 plants with the phenotype $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^+$.

With the $TRI\text{-}D1^-GLI\text{-}D1^+SEC\text{-}1^+$ dissociation types, all progenies showed some plants with the marker pattern $TRI\text{-}D1^-GLI\text{-}D1^+SEC\text{-}1^-$ indicating that *Gli-D1* did not co-segregate with *Sec-1* in these plants. With the $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^+$ dissociation types, 15 of the 16 BCF_2 progenies gave some plants with marker pattern $TRI\text{-}D1^+GLI\text{-}D1^-SEC\text{-}1^-$ or $TRI\text{-}D1^-GLI\text{-}D1^-SEC\text{-}1^+$ indicating that *Tri-D1* did not co-segregate with *Sec-1* in these plants. Therefore, the dissociation phenotypes analysed involved wheat-wheat recombination and not wheat-rye recombination. F_2 of family DP-26 showed no segregation indicating either wheat-rye recombinant or chance failure to segregate in a small F_2 family (Table 4).

It seems clear from these results that the parent plant of family 4 (446/84 plant 1 in Figure 1) must have contained wheat-wheat recombinants, in which 1DS chromosome arm had recombined with its homoeologous 1AS or 1BS chromosome arms in an earlier generation. The high frequency of dissociation observed would then result from segregation of these translocated chromosomes in the selfed progeny.

It is possible that the line of Chinese Spring wheat carrying Sears' *ph1b* mutant, used in these crosses, might have carried one or more wheat-wheat recombinant chromosomes as remnants from previous meiotic cycles of homoeologous recombination. Also in this project, the plants were screened in F4 generation and had undergone two cycles of meiosis in *ph1b ph1b* condition increasing the chances of accumulating wheat-wheat translocations.

Table 4. Endosperm protein phenotypes (TRI-D1/GLI-D1/SEC-1) of F_2BC_1 progenies and their frequency obtained from selfing of selected F_1BC_1 seeds

Tabel 4. Fenotipe protein (TRI-D1/GLI-D1/SEC-1) progeny F_2BC_1 dan frekuensinya (jumlah biji) dari selfing biji-biji F_1BC_1

F_1 backcross	Phenotype	Endosperm Protein Phenotype (F_2BC seeds)					
		+-+	--+	-++	+--	-+-	---
F_1 DP4 x ditelo 1DL	-++	0	1	8	0	1	1
F_1 DP5 x ditelo 1DL	-++	0	0	6	0	2	0
F_1 DP6 x ditelo 1DL	-++	0	2	0	0	7	1
F_1 DP8 x ditelo 1DL	-++	0	3	6	0	2	1
F_1 DP8 x ditelo 1DL	-++	0	0	9	0	3	0
F_1 DP9 x ditelo 1DL	-++	0	0	3	0	4	4
F_1 DP10 x ditelo 1DL	-++	0	1	6	0	2	1
F_1 DP14 x ditelo 1DL	+-+	5	1	0	4	0	2
F_1 DP14 x ditelo 1DL	+-+	7	2	0	3	0	0
F_1 DP15 x ditelo 1DL	+-+	7	5	0	0	0	0
F_1 DP17 x ditelo 1DL	+-+	5	2	0	3	0	2
F_1 DP19 x ditelo 1DL	+-+	7	2	2	3	0	0
F_1 DP19 x ditelo 1DL	+-+	1	2	0	1	0	2
F_1 DP20 x ditelo 1DL	+-+	6	4	0	2	0	0
F_1 DP20 x ditelo 1DL	+-+	5	1	0	0	0	0
F_1 DP21 x ditelo 1DL	+-+	7	2	0	1	0	2
F_1 DP21 x ditelo 1DL	+-+	6	4	0	2	0	0
F_1 DP22 x ditelo 1DL	+-+	7	2	0	2	0	1
F_1 DP22 x ditelo 1DL	+-+	9	1	0	2	0	0
F_1 DP26 x ditelo 1DL	+-+	10	0	0	0	0	1
F_1 DP27 x ditelo 1DL	+-+	4	3	0	1	0	0
F_1 DP28 x ditelo 1DL	+-+	10	0	0	1	0	0
F_1 DP29 x ditelo 1DL	+-+	8	1	0	4	0	0

A model to explain this phenomenon

Table 4 clearly shows the isolation of large numbers of TR1-D1$^+$GLI-D1$^-$ and TR1-D1$^-$GLI-D1$^+$ plants in family 4, all with SEC-1 present. As mentioned previously, this family did not segregate for secalin (Table 1). A model was proposed to explain the nature of family 4 (Figure 4) based on homozygosity for the 1DL.1RS translocation and the presence of two recombinants chromosomes involving the short arm of group 1 chromosomes. Segregation of these two recombinant chromosomes would give the high percentage of dissociation phenotypes in F_2.

In this model, chromosome arm 1DL is present in 3 doses allowing the formation of trivalents at meiosis. Hence, it is difficult to predict expected ratios accurately. The presence of trivalents would provide an explanation of why several of the progeny plants were heterozygous for *Sec-1*, even though the parent plant appeared to be homozygous. It should be noted that the 1DS recombinant chromosome shown in this mode could instead bear a terminal deletion of normal 1D and still be Tri-D1$^+$Gli-D1.

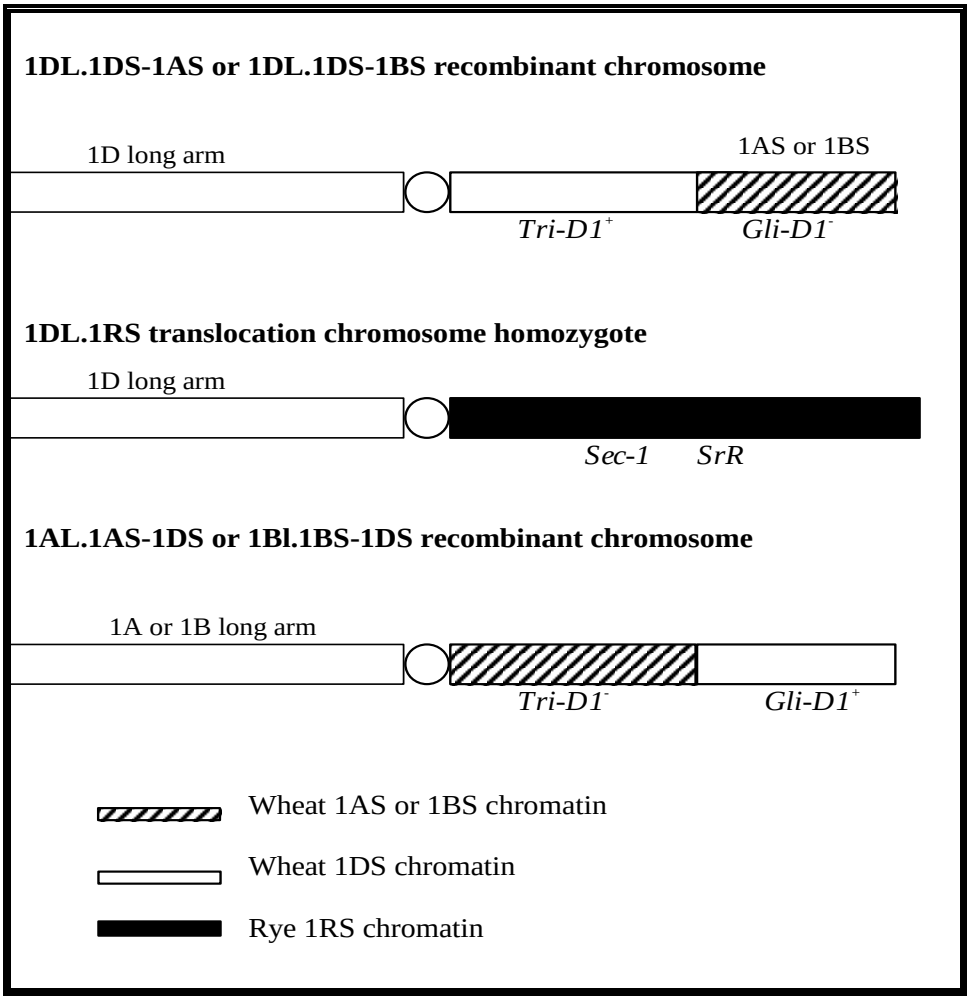

Figure 4**.** Proposed model of chromosome structure of parent plant of family 4 (446/84 plant 1) showing homozygosity for 1DL.1RS and two prior wheat-wheat chromosomal interchanges

Gambar 4. Model rancangan struktur kromosom tetua famili 4 (446/84 tanaman 1) menunjukkan homosigositas 1DL.1RS dan 2 pertukaran antar kromosom gandum.

CONCLUSION

Caution should be used in allowing two generations of selfing in the presence of *ph1bph1b* before selecting for recombinants between alien chromosomes and wheat. Also, it is inefficient to select for wheat-rye recombinants by using dissociation of wheat markers as selection criteria. A better procedure would be to select for dissociation of markers on the alien chromosome itself. In the present case, however, there were only two known markers on the 1RS chromosome, namely, *Sec-1* and *SrR* and they are known to be closely linked limiting their use as selective markers.

REFERENCES

Anugrahwati, DR., 2006. Isolation, characterisation and quality testing of 1DS/1RS wheat-rye recombinants. PhD thesis, School of Agriculture and Wine, University of Adelaide, Australia.

Koebner RMD and Shepherd KW, 1986. Controlled introgression to wheat of genes from rye chromosome arm 1RS by induction of allosyndesis. I. Isolation of recombinants. *Theor Appl Genet*. 73: 197-208

Rogowsky PM, Guidet FLY, Langridge P, Shepherd KW and Koebner RMD, 1991. Isolation and characterisation of wheat-rye recombinants involving chromosome arm 1DS of wheat. *Theor Appl Genet.* 82: 537-544

Sears ER, 1977. An induced mutation with homoelogous pairing in wheat. *Can J Genet Cytol.* 19: 585-593

Shepherd KW and Jennings AC, 1971. Genetic control of rye endosperm proteins. *Experentia* 27: 98-99

Shepherd KW, 1973. Homoeology of wheat and alien chromosomes controlling endosperm protein phenotypes. In: Sears ER and Sears LMS (Eds). Proc 4th Int Wheat Genet Symp. Columbia, MO. pp. 745-760

Shepherd KW, Anugrahwati DR, Langridge P and Francki M, 1994. Isolation and characterization of wheat-rye recombinants and their quality characteristics, A Review. In: Paull J, Dundas IS, Shepherd KW, and Hollamby GJ (Eds). Proc 7th Assembly Wheat Breeding Society of Australia (Addendum). Wheat Breeding Society of Australia, Adelaide Australia.

Singh NK and Shepherd KW, 1985. The structure and genetic control of a new class of disulphide-linked proteins in wheat endosperm. *Theor Appl Genet*. 71: 79-92

Singh NK; Shepherd KW and McIntosh RA, 1990. Linkage mapping of genes for resistance to leaf, stem and stripe rusts and omega-secalins on the short arm of rye chromosome 1R. *Theor Appl Genet.* 80: 609-616