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Effects of phlb Mutant and Double–ditelocentric Lines (T.aestivum L.cv. Chinese Spring) on Transfer of Alien Genetic Materials to Wheat Chromosomes

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Abstract

In the PMCs of the hybrid progenies of phlb mutant x Agrotriticum and DDT lines x Spring Rye, the multivalent and the heteromorphic bivalent predominated cells were relatively frequent (ranged between 30 to 40%) at Metaphase, chromosomal bridges were also present at Anaphase Ⅰ, especially Anaphase Ⅱ, some possible reasons are discussed. The similarity and difference of meiotic behavior between phlb and DDT lines as one of parents in distant hybrid are also compared. It can be concluded that phlb mutant involves in facultative heterochromatinization and DDT lines are mainly effects of constitutive heterochromation, all of them take on the facilitated breakage, fusion, and removal of heterochromatin chracteristics, they all are used as the excellent carrier of the desired gene(s) to transter.

Allopolyploid wheat, Triticum aesticum L.has genetic mechanisms which ensure its meiotic regularity. Among these, the most efficient is perhaps the one that makes use of wheat mutants (Sear 1977) for the gene on chromosome 5B which is the main responsible for homocologous pairing suppression, i.e.Phl.

The high–pairing mutant (ph 1b / ph1b HPM) which is assumed to be a deficiency for the Ph1 locus on the long arm of chromosome 5B was found to be more significantly to promote homoeologous pairing than other corresponding gene. Since the Ph1 gene mainly restricts pairing between homologous chromosome, whereas the HPM (ph1b / ph1b) first to weaken this restriction, that is, not only to restrict homologous pairing, but also to have homoeologous chromosome paring, in all cases, favoured by the lack of the respective homologous partner was evidently not so "exclusive" between members of less related genomes. They (promoting genes of wheat) promote homoeologous pairing between wheat genomes and wheat with related alien genomes.

Regular meiotic behaviour in wheat dpends on the balanced effect of genes that suppress and promote homoeclogous chromosome pairing. Although the chromosomes can be classified as suppressors or promotors according to their global

effects on homoeologous paring, but that genes with different effects can be located on the different arms of the same chromosome, due to phlb mutant upsets the effect of Ph1 gene on 5BL arm, in facts, to strengthen the promotor gene effect on 5BS arm; meanwhile as only a locus deficiency of Ph1 gene, which ensure both the fertility gene on 5BL arm, and the integrity of 5B chromosome, so that using the phlb mutant resulted in progeny showing less aneuploid and sterility than does the Nullisomic 5B systems (Kibirige Sebunya and Knott 1983).

Telocentric chromosomes involving wheat or related alien chromosome can result from univalent and misdivision instead of normal longitudinal division simultaneously at meiosis. First telocentric chromosomes possess exposed centromere and thus largely to enhance its activity, which is to be useful for homoeologous chromosome pairing. It has been known, Robertsonian translocations involing wheat and alien chromosomes only can result from misdivision and centric fusion when a wheat and an alien chromosom both present as univalent at meiosis (Sears 1973). Second, the use of the telocentrics instead of the complete chromosome, as in Sears (1981), limited the amount of alien chromatin to be induced to recombine and also worked as a cytological marker for more precise pairing freguency assessment.

The aim of this paper is to further analyse the effect on the transter of alien genetic materials into wheat chromosomes by phlb mutant and DDT lines, as well as to further exploit them.

Materials and methods

The phlb mutant of T. aestivumL.cv. Chinese Spring produced by Sears (1977). The seeds of this line and other double ditelocentrics all were kindlly provided by Dr.ER Sears (1981). T. aestivum cv Varieties and Spring rye all are local varieties. Agrotriticum is an octoploid from variety Resources Research Institute, Chinese academy of Agricultural Science.

For observation of mitotic chromosomes, root tips were pretreated with iced water at 0℃ for over 24 hours and fixed in 1 acetic acid : 3 alcohol solution and stained by aceto–carmine or corbol pinkish–red. The spike for observation on meiosis of pollen mather cells (PMCs) were fixed by the Carnoy's solution. Preparation of root tip cells and PMCs were made on acetocarmine squash method as usual.

About over fifty PMCs and thirty root tip cells per plant were analysed from the synchronus MI slide. The paired chromosome complement was calculated.

Isozymes electrophoretic techniques and its straining methods as Tuleen & Hart's system to more or less modify.

Results and Discussion

A11 F_1 or F2 hybrid progenies of (phlb mutant x Agrotriticum) have higher total protein amount than that of chinese spring x Agrotriticum or another lower content parent, Agrotriticum seeds possess abundant protein (table 1).

These results indicated that the promoted effect of homoeologous pairing by phlb mutant is critical for alien gene recombination with wheat chromosomes.

Table 1 Biological characters and protein amount of F1, F_2 hybrid of (phlb × Agrotriticum), (CS × Agrotriticum) and its parersts

F_1, F_2 hybrid and its parents	height of plant (cm)	weight of 1000 grains (g)	rate of setting seeds	Total protein%
Chinese spring (CS)	94.0	15.1	* 2.55	17.26
F_1 CSX Agrotriticum(AT)	134.0	31.5	* 2.12	17.51
ATXCS	104.3	33.5	–	–
AT	100.7	26.4	* 2.52	20.98
F_1 phlbx AT	127.6	33.7	* 2.20	18.59
ATX phlb	106.0	–	–	–
phlb matant	80.9	12.4	–	17.56
F_2 phlbx AT	106.8 ± 13.8	–	48.1 ± 33	17.62
F_1 Durumx (ph1b × AT)	90.0	–	41.6	17.2
Durum	–	–	–	16.18

* each spikelet setted seeds

As esterase isozyme analysis using non–mature seeds and Anthers of Agrotriticam, phlb, CS. and its F_1 hybrids indicated that there wasn't obviously different in Zymogram E_1 region (i.e. fast mobility region). However, in slower region some differences has seen clearly, as compared the relative activity and mobility of E_3 region showed that there were two particular Zymogram bands in Agrotriticum anthers, which, in phlb or CS anther, could not be detected, It is worth while noting that the one of slower Zymogram band was only found in phlb F_1 hgbrids, but in F_1 hybrid of CSx Agrotriticum reciprocal cross the zymogram bands were not detected. It is clear that a Est–subunit gene has been transferred from Agrotriticum into wheat using phlb as one of parents.

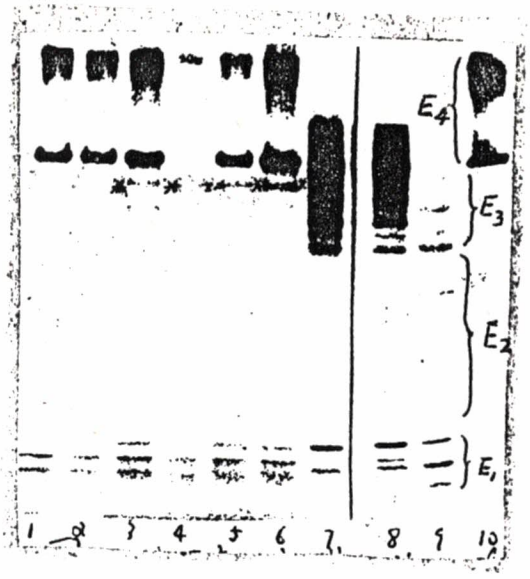

Fig.1 Zymogram phenotypes expressed by the anthers and the seeds of Agrotriticum, and phlb, CS and its hybrids for the esterase lsoenzyme.

1. AT×CS 2. CS×AT 3. AT×phlb
4. phlb 5. phlb×AT 6. AT Anthers
*7. AT non matural seeds 8. same as 7.
9. winter wheat seeds 10. CS anthers.

Table 2 shows the frequency and pattern paired at MI in the combination of containing phlb parent. if only phlb mutant exists where the multivalents and beteromorphic bivalents always can be detected (table 2) At metaphas I. And either Anaphase I or II the lagging chromosomes were always less than seven chromosomes, especially more few in Anaphase II, about three or four chromosomes to be seen. It is very appealing and suggestive, although no identification of chronsosomes was tried, that the minority of chromosomes might correspond to a genome set which would be that of E–genome. In the case, other non–lagging special chromosomes in Anaphase I and II which might be indeed take part in homoeologous chromosome pairing, and hence to obtain the desirable recombination products between the chromasome of wheat and the alien chromosomes.

Table 2 Frequency and pattern of chromosome pairing of F_1, F_2 hybrid contained phlb mutant

hybrids and parents	chromosome number of hybrid	number of PMCs tested	Univalents I	Bivalents Ring Ⅰ	Bivalents Rod Ⅱ	Total	Multivalents Ⅲ	Multivalents Ⅳ	Multivalents Ⅴ–Ⅵ	Anaphase (I) lagging chromosomes	Anaphase (I) bridges	Anaphase (II) lagging chromosomes	Anaphase (II) bridges
(phlbkAT)F_1	2n=46–51	110	5–10 (6.06)	0–13 (7.19)	6–10 (8.0)	15.19	0–3 (1.2)	0–3 (1.8)	0–2 (0.3)	0–6 (3.28)	0–3 (0.82)	0–3 (2.4)	0–2 (0.66)
(phlbxAT)F_2	40–49 (45)	53	1–3 (2.0)	6–9 (8.0)	7–8 (2.4)	15.4	0–1 (0.6)	1–1 (0.66)	0–1 (0.5)	–	–	–	–
Durumx (ATxphlb)F_1	38–43 (39.9)	56	4–5 (4.5)	8–10 (9)	8	17.0	0	0–1 (0.5)	0	9	–	0–7 (3.5)	–
Durumx (ATxphlb)F_2	39–44 (41.8)	89	0–2 (0.66)	13–14 (13.66)	3	16.66	0–1 (0.33)	0–2 (0.66)	0–2 (0.66)	–	multi–bridge	0	1

* ( ) indicates the average number

It is also worth emphasising that such phlb gene might be prevent "to exclud out" alien chromosomes when they transter into wheat chromosomes. However, certain chromosomal bridge features became apparent mostly in Anaphase I or II cells with deficient and or duplicate chromosomes, especially there were bsidge(s) to appear in anaphase II, where not only occured "breakage–fusion–bridge" circle, but also must take place complex chromosomal structural variation, such as inversion tandem duplication (Fig.2), tetra–or–triple strands double crossing–over (DCO) and etc.

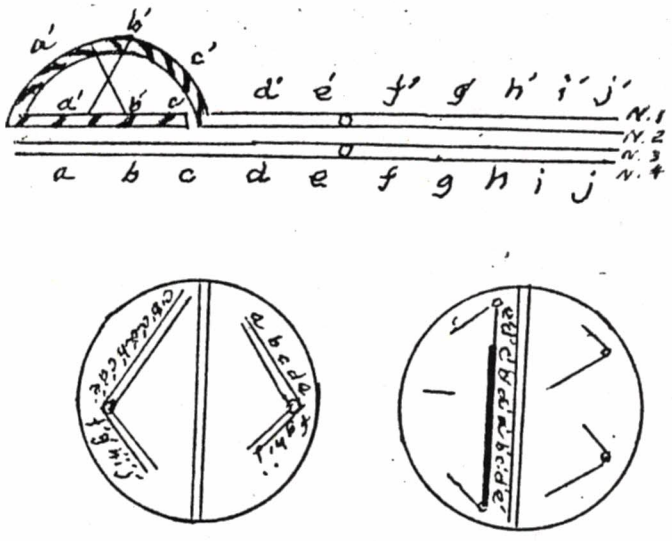

Fig.2 In Anaphase II chromosomal bridge formed from one of special cases by occurring inversion tandem duplication variation on the chromosome where should be due to a crossing–over between sister chromatids N.1 with N.2, and then to form a dicentric chromosomal bridge and a segment.

In addition, the erratic variation configurations At MI in the hybrid progenies of phlb mutant Fig 3–4 which showed the activity of phlb, where are many heteromorphie bivalent, but non–telocentric complement (Fig5a by arrow showed), i.e. always non–Robertsonian translocation instead of Robertsonian type, that was thus confirmed which wasn't resulted from misdivision and centric fusion, and it is probably just one of chracters of phlb mutant as a gene locus deficiency.

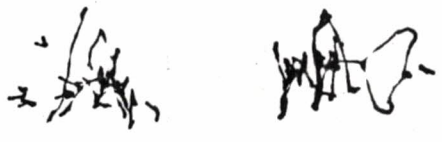

Fig 3 and 4 At MI, in the PMCs of the hybrid of (ph1b × Agrotriticun) many multivalents and hetero–morphic bivalents were detectees

Fig.5a At MI there are various heteomorphic bivalents, but non–Robertsonian translocation (arrows showed)

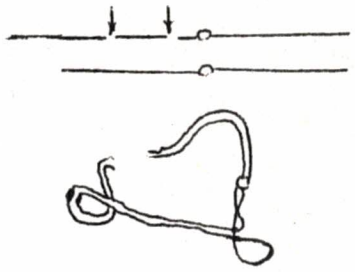

Fig.5b Diagram to illustrate the origin of interstitial deletion and its appearance at pachynema when paired in the heterozygows state, piring is relatively complete, with the other chromosome forming an unparied loop corresponding in size to the missing piece in the other chromosome. (cf. McClintock)

A final consideration of the higher frequencies of specific multivalent in MI configurations leads us to assume the possibility that the Ph1 gene locus may be involve the facultative heterochromation (or euchromatin's heterochromatinization). This could, to some extent, be compared to inactivation of X chromosome genes, i.e. Lyonization, both phenomena involve faculfative heterochromatinization, (cf. L.L. Qi, J. Sybenga stc, 1988) generally after its discovery, this chromosome will retain its heterochsomatic character for the remainder of its existence, and the structural genes in it will no longer be expressed phenotypically. (swanson 1981), as it were, when Ph1 gene should be inactivate, the phlb matant expressed apparently promoting homoeologous chromosome pairing, and thus formed multivalents were synapsis again by terminal to terminal each other, or more, all chromosomes formed a net work (Fig.6), this configuration was rare.

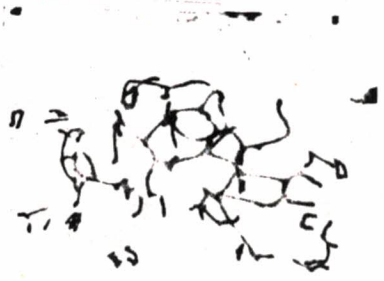

Fig.6 Maostly all chromosomes formed a net–work at MI in a PMC of (ph1b× Agrotriticum) progeny

It is of interest to note that the similarity and difference on meiotic behaviour from double ditelocentric (DDT) distant hybrid progenies with that of phlb mutant, those similarities as state above, the differences in which the DDT lines may be possessed the character forming inherent dicentric chromosomal bridge at anaphase I or II (wu et al. 1988) and then resulted the "breakage–fusion–bridge" circle, it is also advantageous to transter of alien chromosome or its fregment into wheat. such as the gene for resistance to wheat powdery mildew transterred from spring Rye into the(DD1B × SR)$_{F5}$ and the (DD7A × SR)$_{F5}$ hybrid progenies in our experiments.

It must also be added that the relative frequesscies of involving telocentric heteromorphic bivalents and multivalents showed an extensive pattern of Robertsonian translocation as another character.

As we have known, constitutive heterochromatin is present in mostly all centromere region of chromosome, where the variability in repetitive DNA overrule relatively the stability in coding DNA. Generally, constitutive heterochromatin is not convertible or revertible to a euchromatic status in a genetic sense, and hence there were rare Lyonization in DDT–lines hybrid. Cytologically, and in addition to its heteropycnosis, it reacts preferentially such as to the tendency to pair with other regions of similar character and in nonspecific and non–homologous manner, this has been termed "ectopic pairing" (Swanson 1981), and to breakage, to fusion, to inducing neocentric activity and so on. For example, In the hybrid progenies of (DD3D × SR)F_5, we still detected multivalent at MI, and when these hybrids were treatment by r–ray (20000 or 35000r) and checked (non–freat) using infrared spectra analysis which indicated that variations were apparently involving important radicals. Corresponding to the infraved spectra of thymina (Fig 7a and 7b), the original, 1696 cm^{-1} wavenumber is c=0 radical elastic vibrational peak (EVP), 2968cm^{-1} is C–H radical EVP, 3320 cm^{-1} is N–H radical EVP, As comparing with that of (DD3D × SR) progeny showed that 1696 cm^{-1} c=0 radical EVP mostly eliminated, it is suggestied that this C=0 radical chains were breaked seriously, especially using 20000 or 35000 r–ray treated, this C=0 radical EVP further crumble, and 2968cm^{-1} C–H radical, 3320cm^{-1} EVP appeared the same tendency. all indicated, some radical chains of (DD3D × SR) hybriol progeny were indeed broken to easy, (as known thymine also is a much more sensitive element of DNA), undoubted, this is an advantage for alien–genetic materials transterring to wheat chromosome.

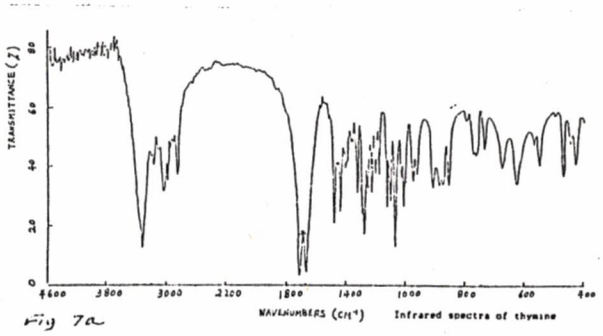

Fig.7a Wavenumbers (cm^{-1})
Infrared spectra of thymine

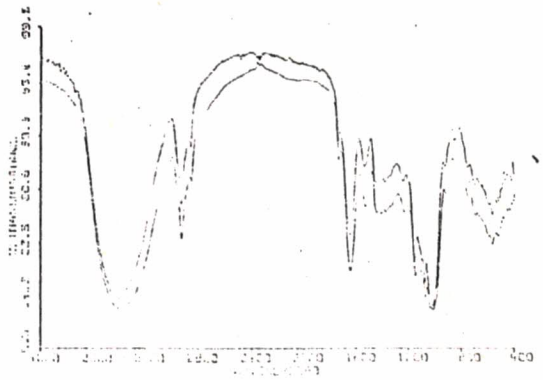

Fig.7b Infrared spectra of the progeny of (DD3D × SR)F_5.
Upper curve by 20000 r treatment
Lower curve showed check (non-treatment)

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