

Synthesis of the hexaploid club wheat and analysis about the homoeo-alleles of club gene *C*

Ohtsuka T and Ohtsuka I

Laboratory of Biology, Kanagawa University, Yokohama 221-8686, Japan

INTRODUCTION

The second author classified the tetraloid wheat (including wild strains, genome formulas are AABB and AAGG) into three groups^{1,2,3} as type-I(AB type), type-II(AG type) and type-III(AB' type) on the compatibility between the nuclear genes (gene *C_p* for plastid* development and gene *C_v* for mitochondrion** development or function) and cytoplasm (organelle having own DNA, plastid and mitochondrion, hereafter abbreviate as DNA-organelle) of *Aegilops squarrosa* (= *tauschii*) (genome formula is DD). Moreover, the second author classified the AB genome of hexaploid wheat of many sub-species of *Triticum aestivum* (genome formula are AABBDD) according to the criteria above-mentioned, and these results applied to the evolutionary pathway of polyploid wheats^{1,4}. Because, the compatibility between nuclear genes *C_p* (in case of plant) and/or *C_v* and these counter partner DNA-organelle is essential for genetically distinct strains (species or sub-species). At that evolutionary pathway, the club wheat (*T. aestivum* ssp. *compactum*, compact and dense spiked ssp., type-III-AB in AABBDD) was ranked as one of the old free-threshing hexaploid wheat, the other was the bread (common) wheat (*T. aestivum* ssp. *aestivum*). And primary hexaploid wheat (husked) were considered as di-phyletic origin, the Transcaucasia endemic macha wheat (*T. aestivum* ssp. *macha*, type-III-AB in AABBDD) and the European spelt wheat (*T. aestivum* ssp. *spelta*, type I-AB in AABBDD, the Germanic main crop). However, the second author supposed that the club wheat is an old free-threshing hexaploid wheat older than the bread wheat¹ by archaeobotanist's opinion⁵, and also supposed that the Iranian spelt wheat⁶ is the recent secondary origin by the cross between the bread wheat and husked tetraploid wheat (unpublished).

The second author already modified the evolutionary pathway partially of polyploid wheats as the European spelt wheat is secondary origin from the pentaploid hybrid between the club wheat and the emmer wheat (*T. turgidum* ssp. *dicoccum*, genome formula is AABB, type-I-AB, husked, one of the primary cultivated tetraploid wheat) based on the experimental result⁷ and archaeo-botanist's research⁸. This modification was reported by Zeller and his colleagues⁹. However, the origin of the club wheat in the evolutionary pathway is not complete yet. Because, the second author gathered¹ that the club wheat was originated from the macha wheat by mutation of the allele *q* to the allele *Q*, and the macha wheat originated from the ABD hybrid between Transcaucasia endemic husked tetraploid wheat of *T. turgidum* ssp. *plaeocolchicum* (= *georgicum*) (type-III-

AB, one of the primary cultivated tetraploid wheat, husked, compact and dense spiked ssp.) and *Ae. squarrosa* (DD) by the natural duplication. At that time^{1,4}, the second author supposed that the club (compact and dense spiked) gene *C* of the club wheat (locating on the second homoeologous group chromosome of D genome *i.e.* 2D chromosome¹⁰) was originated from 2A or 2B chromosome of AB genome of *T. turgidum* ssp. *plaeocolchicum*, and the gene *C* translocated to 2D chromosome from 2A or 2B chromosome at meiosis of the ABD hybrid. Nevertheless, there is no experimental evidence for the above supposition.

*in plant cell, develop to amiloplast, chloroplast or chromoplast. ***pl* = mitochondria.

SYNTHESIS OF THE HEXAPLOID CLUB WHEAT

The authors synthesized the club type (compact and dense spiked), free-threshing and hexaploid wheat strain (*i.e.* the synthetic club wheat, genome formula is AABBDD) with black glume from the self-pollinated progeny of the cross between the macha wheat (*T. aestivum* ssp. *macha*, Transcaucasia endemic husked hexaploid wheat, genome formula is AABBDD) and the black glume strain of tetraploid Persian wheat (*T. persicum*=*carthlicum*, carrying the allele *Q* and free-threshing, genome formula is AABB). The macha wheat is polymorphic crop including different spike types. The macha wheat strain used above cross as female was speltoid and semi-dense spike type. At 1993, we had occasion to cultivate (spring sowing) and analysis the F₂ line of the above cross in experimental farm of Washington State University by the aid from Prof. E. R. Allan, Prof. C. F. Konzak, Prof. S. S. Jones and Mr. J. Pritchett. In 928 individuals of the F₂, 92 individuals did not head (come up the spikes) or were very late and irregular heading (winter wheat type segregation at spring sowing cultivation, because the macha wheat is winter wheat). In 836 heading individuals (spring wheat type segregation, because the strain of *T. persicum* used above cross is spring wheat), one semi-dwarf individual (including I_{IV} at meiosis I) had awn-less club type spikes with black glume (29 individuals had sub-compact spikes). From this club type spiked individual's seed, we continued the cultivation in greenhouse in Hiratsuka (near Yokohama, Japan) by autumn sowing (controlled as short winter and long early summer condition), selecting the club type individual and self-pollinating (bagged). At early generation (F₄), all individuals were uniform and as same as old European club wheat, *i.e.*, tall and had the

awn-less*** club type spikes, but had black glume. At F₈ generation, we confirmed that all 15 individuals are 2n=42 (21_{II} at meiosis I of PMC). Hereafter, we name this line as the synthetic club wheat.

***The authors cultivate the experimental lines of wheat in greenhouse using 30cm diameter clay pot for each one individual, therefore, one individual put forth many shoot during long time than field cultivation. In this cultivate condition, the first shoots (about 10~20 shoots) of the synthetic club wheat heading awn-less spike, however, the delayed shoots heading awned spike.

ANALYSIS ABOUT THE HOMOEIO-ALLELES OF CLUB GENE C

Unrau, J. analyzed the club gene *C* of the club wheat (*T. aestivum* ssp. *compactum*, type-III) and concluded that the gene locating on the 2D chromosome¹⁰. However, there are tetraploid (without D genome) club type wheat, husked and AABB genome crop of *T. turgidum* ssp. *palaeocolchicum* (= *georgicum*) (type-III^{1,3,4}), husked and AAGG genome crop of *T. timopheevi* (type-II^{1,3,4}), and the ancient Egyptian free-threshing AABB genome crop of *T. turgidum* ssp. *pyramidale* (type-III^{1,3,4}, Harlan, J. R. named as “tetraploid club wheat”¹¹). These tetraploid club type wheat were considered to be older crops than the macaroni wheat (*T. turgidum* ssp. *durum*, type-I^{1,3,4})¹¹ and the rivet wheat (*T. turgidum* ssp. *turdidum*, type-I^{1,3,4}). The existence of these tetraploid club type wheat indicate to be the homoeio-alleles of the club gene *C* in A genome (locating on 2A chromosome?) and/or B genome (locating on 2B chromosome?). In addition, there is ancient hexaploid free-threshing club type wheat distinctive from the club wheat¹², the shot wheat, the ancient Indus Valley semi-dwarf wheat of *T. aestivum* ssp. *sphaerococcum*. The authors consider that the shot wheat is an old free-threshing hexaploid wheat, older than the bread wheat (*T. aestivum* ssp. *aestivum*), originated from the above-mentioned tetraploid (AABB) club type wheat (unpublished). From these facts and considerations, the analysis about the homoeio-alleles of club gene *C* is necessity for more complete understand of the evolutionary pathways of poliploid wheat.

(1) Control experiment

The authors crossed between the old European club wheat, the central European club wheat (maintained in Hokkaido University, Japan, from early time of 20th century as *T. compactum* No.44, and the late Dr. Hitoshi Kihara used this line in his lifework) and the Iberian club wheat (maintained in University of California, Davis, USA, as Big club 37). In this experiment, we cultivated 225 individuals of the F₂ line of above cross in greenhouse, as control experiment of the analysis about the homoeio-alleles of club gene *C*. Resultantly, all 225 individuals had normal club type spikes (awn-less).

(2) Experiment between the old European club wheat and the synthetic club wheat

The authors crossed between the old European club wheat (*T. compactum* No.44) and the synthetic club wheat. This time, we cultivated 226 individuals of the F₂ line of above cross in greenhouse. Resultantly, 194 individuals had the club type spikes and 32 individuals had the non-club type spikes. However, the club type spikes of the segregates were variable by individual and continuous *i.e.* semi-long club type (compactoid) spikes, normal club type spikes to extremely compact and dense type spikes, differed from the control experiment mentioned above. In this experiment, we determined on the sub-compactoid spikes of segregates into the non-club type spikes, because the different of the spike type were also continuous in this segregates (non-club) by individuals, however, about half the individuals had normal bread wheat type spikes. Moreover, awned vs. awn-less and black glume vs. yellow glume were segregated, independently from the compactness.

(3) Experiment between the old European club wheat and the shot wheat

The authors crossed between the old European club wheat (Big club 37) and the shot wheat (maintained in the former Kihara Institute for Biological Research, as *T. sphaerococcum* var. *rutundatam*). In this experiment, we cultivated 294 individuals of the F₂ line of above cross in greenhouse. Resultantly, 250 individuals had the club type spikes and 44 individuals had the non-club type spikes. However, all the non-club type spikes of the segregates were speltoid, differed from the non-club type spikes of the segregates of the above experiment (2) (all individuals of F₂ line, including the club type and the non-club type had gene *Q* type square head type spikes). In this experiment, the spikes among the club type category were variable by individual and continuous *i.e.* normal club type to extremely compact and dense type. Also, the non-club type category was continuous *i.e.* normal spelt type to extremely short spelt type. Moreover, individuals with awned spike were segregated as minority, independently from the compactness, nevertheless the Big club 37 and the shot wheat are awn-less strains.

DISCUSSION AND CONCLUSION

The synthetic club wheat had the homoeio-allele of club gene *C*, differed from the second author's supposition on the origin of the club wheat in evolutionary pathways of polyploid wheat^{1,4}. The homoeio-allele of club gene *C* (hereafter, abbreviate as the gene *C'*) of the synthetic club wheat should originated from A or B genome of the macha wheat, because AABB genome strain of first cross of the synthetic club wheat *i.e.* one strain of *T. turgidum* ssp. *persicum*=*cauthlicum* was very lax spike type, nevertheless having the gene *Q*. In that evolutionary pathways, the macha wheat is a primary husked hexaploid wheat (AABBDD) which originated from the cross between the primary cultivated (husked) tetraploid (AABB) of *T. turgidum* ssp. *paraeocochicum*

(compact and dense spiked) and *Ae. squarrosa*. Therefore, the gene *C'* should be originated from 2*A* or 2*B* chromosome of *ssp. palaeocolchicum*.

The shot wheat had the homoeo-allele of the club gene *C* (hereafter, abbreviate as the gene *C''*). We consider that the ancient Indus Valley main crop of the shot wheat is an old free-threshing hexaploid (AABBDD) wheat older than bread wheat, because the shot wheat is very modern type wheat than the European bread wheat of early time of 20th century, *i.e.* semi-dwarf and having stand type broad leaf. The gene *C''* was considered as another homoeo-allele, differed from the gene *C'*, because the non-club segregates appeared among F₂ generation of the cross between the old European (Iberian) club wheat and the shot wheat was all spelt wheat type (including long to extremely short type). This indicated that the shot wheat have the spelt gene *q* and the gene *C''* is not required the gene *Q* differed from the gene *C* of the club wheat and the gene *C'* of the synthetic club wheat⁷.

The club type segregates in two experiments (2) and (3) were variable by individual and continuous *i.e.* semi-long club type**** to extremely compact and dense club type. These results indicated that the club gene *C* and the homoeo-alleles have dosage effect as same as the gene *q* or *Q*.

****in the experiment (3), semi-long club type was classified into non-club type category, because these semi-long club types are not square head types, rather extremely short spelt types.

For the more exact conclusion, the homoeo-allele test between the synthetic club wheat and the shot wheat is necessity. Moreover, the analysis of DNA sequences of the gene *q* (or gene *Q*) of the shot wheat is necessity for determine the status of the shot wheat in the evolutionary pathways of polyploid wheat.

REFERENCES

- Ohtsuka, I. (1983). Classification of tetraploid wheat based on response to *Aegilops squarrosa* cytoplasm and origin of Dinkel wheat. *In*: Sakamoto, S. (ed.) Proc. 6th Int. Wheat Genet. Symposium, Kyoto, Japan, (1983): 993~1001.
- Ohtsuka, I. (1986). Genetic analysis of the compatible relationship between nuclear genes and cytoplasmic factors wheats and their wild relatives. *Wheat Inform. Serv.* No.63: 50~52.
- Ohtsuka, I. (1991). Genetic analysis of the compatible relation between tetraploid wheat genomes and *Aegilops squarrosa* cytoplasm. *In*: Sasakuma T. and Kinoshita T. (ed.) Nuclear and organellar genomes of wheat species. (Proc. Dr. H. Kihara Memorial Int. Symp. on Cytoplasmic Engineering in Wheat): 238~244.
- Ohtsuka, I. (1991). Genetic differentiation in wheat nuclear genomes in relation to compatibility with *Aegilops squarrosa* cytoplasm and application to phylogeny of poliploi wheat. *Jour. Fac. Agr. Hokkaido University*, Vol. 65, Pt. 2:127~198. (PDF version: AGRIS record, Record number:JP9302336 AGRIS 2006 – FAO of United Nation, key words: Ohtsuka Ichiro wheat)
- Hosono, H. (1954). Classification and distribution of wheat. *In*: Kihara, H. (ed.) Studies of wheat. (Yokendo, Tokyo, Japan, 1954): 5~132.(in Japanese)
- Kuckuck, H. and Schiemann, E. (1957). Uber das Vorkommen von Spelz und Emmer (*Triticum spelta* L. und *Tr. dicoccum* Schubl.) im Iran. *Z. Pflanzenzucht.* 38: 383~386.
- Ohtsuka, I. (1998). Origin of the central European spelt wheat. *In*: Slinkard A. E. (ed.) Proc. 9th Int. Wheat Genet. Symposium, University of Saskatchewan, Canada, (1998), 2: 303~305.
- Schiemann, E. (1951). New results on the history of cultivated cereals. *Heredity* 5: 305~320.
- Yan, Y., Hsam, S. L. K., Yu, J. Z., Jiang, Y., Ohtsuka, I. and Zeller, F. J. (2003). HMW and LMW glutenin alleles among putative tetraploid and hexploid European spelt wheat (*Triticum spelta* L.) progenitors. *Theor. Appl. Genet.* 107: 1321~1330.
- Unrau, J. (1950). The use of monosomes and nullisomes in cytogenetic studies of common wheat. *Sci. Agric.* 30: 66~89.
- Harlan, J. R. (1981). The early history of wheat: earliest traces to the sack of Rome. *In*: Evans L. T. and Peacock W. J. (ed.) *Wheat science-today and tomorrow*. Cambridge University Press.,1981
- Percival, J. (1921). *The wheat plant* : pp. 463, Duckworth and Co., London, 1921