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

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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 *Cp* for plastid* development and gene *Cv* 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 *Cp* (in case of plant) and/or *Cv* 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 hexaloid wheat, the other was the bread (common) wheat (*T. aesitivum* 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 wheat[1] by archaeo-botanist's opinion[5], and also supposed that the Iranian spelt wheat[6] is the recent secondary origin by the cross between the bread wheat and husked tetraploid wheat (unpublished).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 result[7] and archaeo-botanist's research[8,]. This modification was sported by Zeller and his colleagues[9]. However, the origin of the club wheat in the evolutionary pathway is not complete yet. Because, the second author gathered[1] 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 homoeologus group chromosome of D genome *i.e. 2D* chromosome[10]) 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 F_2 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. Pritcheff. In 928 individuals of the F_2, 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 1_{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 (F_4), 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 F_8 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 HOMOEO-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* chromosome[10]. 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 tetraloid 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 homoeo-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 wheat[12], 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 homoeo-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 F_2 line of above cross in greenhouse, as control experiment of the analysis about the homoeo-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 F_2 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 F_2 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 F_2 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 compactoness, nevertheless the Big club 37 and the shot wheat are awn-less strains.

DISCUSSION AND CONCLUSION

The synthetic club wheat had the homoeo-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 homoeo-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 *2A* or *2B* 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 F_2 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 wheat[7].

The club type segregates in two experiments (2) and (3) were variable by individual and continuos *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.

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