DNA CONTENT PER NUCLEUS IN RELATION TO PHYLOGENY OF WHEAT AND ITS RELATIVES¹⁾

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It is a well known fact that polyploidy plays an important role in the evolution of higher plants. The genus *Triticum* is divided into four groups according to morphological and cytogenetical features, that is, Einkorn group ($n=7$, genome constitution A), Emmer group ($n=14$, AB), Timopheevi group ($n=14$, AG) and Dinkel group ($n=21$, ABD). After Kihara (1944) and McFadden and Sears (1944, 1946) the D genome of Dinkel group was contributed by *Aegilops squarrosa* ($n=14$, D). On the other hand, *Ae. speltoides* has been considered to be the B genome donor to Emmer group (Sarkar and Stebbins 1956; Riley *et al.* 1958). However, the derivation of the G genome is not known yet.

Recently microspectrophotometry has been greatly improved and was applied to the cytological studies by many investigators. In *Triticum*, phylogenetical relationships have been discussed based on the DNA content per nucleus of related species since the beginning of the present decade.

Pai *et al.* (1961) reported that the DNA content per nucleus in common wheat was reduced by about one third of the sum of the DNA contents of its three analyzers. They thought that diminution of nuclear DNA was one of the diploidizing mechanisms which must have taken place in the evolutionary process of polyploid wheat. Upadhyaya and Swaminathan (1965) also recognized DNA diminution, but to a lesser extent than Pai *et al.* reported. Whereas, Rees (1963) and Rees and Walters (1965) could not detect the nuclear DNA diminution as reported by Pai *et al.* and reached the conclusion that polyploid wheat had a nuclear DNA content equal to the sum of DNA of its ancestors. Nishikawa and Furuta (1968) found no difference in DNA content per nucleus among 25 Japanese and U.S. varieties of common wheat.

As described above, there are discrepancies among the authors with respect to nuclear DNA content of wheat and its relatives. In order to throw new light upon this subject, the present investigation was carried out using artificially synthesized 6x wheat and its parental species in addition to diploid analyzers and common wheat.

MATERIALS AND METHODS

Species or strains used in the present study are listed in Table 1.

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Table 1. Species of *Triticum*, its relatives and synthesized 6x wheat used in the present study

Species	2n	Genome
<i>T. boeoticum</i> BOISS.	14	AA
<i>T. monococcum</i> L. var. <i>vulgare</i> KÖRN. (Early)	14	AA
<i>Ae. speltoides</i> TAUSCH	14	SS
<i>Ae. squarrosa</i> L. var. <i>typica</i> ZHUK. No. 2	14	DD
ssp. <i>stragulata</i>	14	DD
<i>T. dicoccoides</i> KÖRN. var. <i>spontaneo-nigrum</i> FLAKSB.	28	AABB
<i>T. dicoccum</i> SCHÜBL. cultivar Vernal	28	AABB
<i>T. aestivum</i> (L.) HAYEK. ssp. <i>vulgare</i> (VILL.) MK cultivar Chinese Spring	42	AABBDD
ssp. <i>spelta</i> (L.) THELL. var. <i>duhamelianum</i> KÖRN.	42	AABBDD
ssp. <i>macha</i> (DEK. et MEN.) MK var. <i>subletschchumicum</i>	42	AABBDD
ABD No. 1: amphidiploid derived from <i>T. dicoccoides</i> <i>spontaneo-nigrum</i> × <i>Ae. squarrosa</i> var. <i>typica</i> 2	42	AABBDD
ABD No. 13: amphidiploid derived from <i>T. dicoccum</i> (Vernal) × <i>Ae. squarrosa</i> ssp. <i>stragulata</i>	42	AABBDD

Pollen tetrads are extremely suitable for microspectrophotometry because they provide a large number of synchronized cells which can be easily squeezed out from the anther onto cover slips (24×32 mm) and contain spherical nuclei with homogeneously distributed chromatin. The young anthers with pollen tetrads were fixed with acetic-alcohol (1:3) for three days, soaked in 95% ethanol for three hours and stored in 75% ethanol in a refrigerator.

Cover slips on which pollen tetrads had been smeared were immersed in cold N-HCl for 5 minutes, then hydrolyzed with N-HCl at 60°C for five minutes and stained with Schiff's reagent according to Sibatani and Naora (1952). After washing in three changes of SO₂ water, 15 minutes in each, permanent preparations were made by the routine method.

Measurement of DNA content per nucleus was exclusively made by the two wave length method (500 and 570 mμ), using Olympus microspectrophotometer (MSP A-IV), and the relative DNA value in arbitrary units was read from Mendelsohn's table (Mendelsohn 1958). Measurement was carried out in three replications; ten nuclei were selected for measurement for each species (or variety) per replication.

In order to minimize the errors, the two species (or varieties) that were compared were, if possible, smeared on the same cover slip, or a common standard species (or variety) was smeared side by side with the species (or variety) to be compared on every cover slip.

RESULTS

1. DNA content per nucleus in diploid analyzers

The variance analysis of DNA content per nucleus in three analyzers is given in

Table 2. Variance analysis and test of significance of mean DNA content per nucleus in three analyzers of wheat

(a) Variance analysis

Source	DF	SS	MS
species	4	487347	121837**
replicates	10	69507	6951*
error	135	321559	2382

(b) Test of significance

Species	Genome	Mean	—558	—584	—673	—688
<i>T. monococcum</i>	A	695	137*	111*	22	7
<i>T. boeoticum</i>	A	688	130*	104*	15	
<i>Ae. speltooides</i>	S(B)	673	115*	89*		
<i>Ae. squarrosa</i>						
<i>strangulata</i>	D	584	26*			
<i>typica</i> No. 2	D	558				

* Significant at 5% level, ** Significant at 1% level

Table 3. DNA content per nucleus in common wheat (Chinese Spring), two strains of artificially synthesized 6x wheat and their parents

Series No. 1		Series No. 13	
Species	Mean±SD	Species	Mean±SD
<i>T. aestivum</i>	243.8±18.1	<i>T. aestivum</i>	613.7±54.1
ABD No. 1	209.8±19.2	ABD No. 13	618.7±39.7
<i>T. dicoccoides</i>	142.1±13.9	<i>T. dicoccum</i>	420.6±21.2
<i>Ae. squarrosa typica</i>	71.4± 4.3	<i>Ae. squarrosa strangulata</i>	175.7±13.0

Table 2. The significant difference between replications may be due to differences between the separately treated slides as to the color enhancement of Feulgen staining.

No difference was found not only between two species of the Einkorn group but also between Einkorn and *Ae. speltooides*. Whereas, DNA content of *Ae. squarrosa* (D genome analyzer) was evidently lower than that of the A and B genome analyzers. Moreover, it should be noticed that *Ae. squarrosa* ssp. *strangulata* contains a significantly higher amount of nuclear DNA than var. *typica* of the same species although this difference is not very large.

2. DNA content per nucleus in common wheat, artificially synthesized 6x wheat and its parents

Table 3 represents the mean values of nuclear DNA and standard deviations in common wheat, two strains of artificially synthesized 6x wheat and their parents. The direct comparison should not be made between series No. 1 and series No. 13, because these two series of experiments were carried out separately.

If an additive relationship of nuclear DNA content is assumed between a given amphidiploid and its parental species, the expected DNA value would be 213.5 for ABD No. 1 and 596.3 for ABD No. 13, respectively. The observed values, 209.8 for the for-

Table 4. Variance analysis of DNA content per nucleus of common wheat (Chinese Spring) and ABD No. 1

Source	DF	SS	MS
Strains (S)	1	17304	17304
Replicates (R)	2	261943	130972
S×R	2	10251	5126
Error	54	114084	2113

Table 5. Variance analysis of DNA content per nucleus in three subspecies of common wheat

Source	<i>spelta-vulgare</i>		<i>spelta-macha</i>	
	DF	MS	DF	MS
Subspecies (S)	1	11055	1	2100
Replicates (R)	1	130530	2	38181
S×R	1	11427	2	23474
Error	36	3008	54	3903

mer and 618.7 for the latter are closely near to the expectation, strongly supporting the additive relationship. The comparison of DNA content between common wheat and recently synthesized 6x wheat would be most critical in deciding whether diminution of nuclear DNA had taken place or not in the evolutionary process of polyploid wheat species. DNA content per nucleus in ABD No. 13 is extremely close to that of a variety of common wheat, Chinese Spring, but that of ABD No. 1 is rather lower than in Chinese Spring. However, the experiment in series No. 1 did not allow a direct comparison between ABD No. 1 and Chinese Spring, because the Emmer parent of ABD No. 1, *T. dicoccoides* was used as a standard species.

For direct comparison between ABD No. 1 and Chinese Spring, another series of experiments was carried out using the preparations in which pollen tetrads of ABD No. 1 and Chinese Spring were smeared side by side. The result (Table 4) was that Chinese Spring had higher amount of DNA than ABD No. 1, but the difference was not statistically significant.

3. DNA content per nucleus in Dinkel wheat

Variance analysis, the mean DNA contents and their standard deviations in three subspecies of Dinkel group are given in Table 5. The comparisons between ssp. *spelta* and ssp. *vulgare* (Chinese Spring) as well as between ssp. *spelta* and ssp. *macha* indicate that there was no significant difference among these three subspecies in relation to DNA content per nucleus.

DISCUSSION

Investigators who studied nuclear DNA amount in wheat and its relatives agree that diploid species differ in their DNA content depending upon the genomes they carry.

Table 6. DNA content per nucleus in wheat and its relatives reported in the previous and present papers

Species	Genome	Pai <i>et al.</i>	Rees	Upadhy <i>et al.</i>	Rees and Walters	Present data	
<i>T. monococcum</i>	A	1.07	1.30	1.09	1.31	1.22	
<i>T. boeoticum</i>	A	—	—	1.11	1.36	1.20	
<i>Ae. speltooides</i>	S	1.25	1.08	1.07	1.14	1.18	
<i>Ae. squarrosa</i>	D	1.00	1.00	1.00	1.00	1.00*	1.00**
<i>T. dicoccoides</i>	AB	—	—	2.12	2.40	1.99	—
<i>T. dicoccum</i>	AB	1.55	—	2.17	2.36	—	2.39
<i>T. durum</i>	AB	—	2.25	2.37	2.37	—	—
<i>T. aestivum</i>	ABD	2.06	3.29	2.92	3.40	3.41	3.49
ABD No. 1	ABD	—	—	—	—	2.94	—
ABD No. 13	ABD	—	—	—	—	—	3.52

* Series No. 1, ** Series No. 13, (see Table 3)

The results of previous workers are summarized along with those of the present study in Table 6. Among the three genomes of wheat, genome D contains the lowest DNA, while A has the highest and B (or S) a little lower content than A, but not significantly different between the last two.

The present genomes of wheat and its relatives must have evolved from a common primitive genome through the accumulation of structural changes of chromosomes. Some of chromosome aberrations must have been accompanied by increase or decrease of chromatin in the course of genome differentiation. This might have resulted in various amounts of DNA specific for the genome concerned. A variable amount of nuclear DNA in diploid species was also reported in some genera other than *Triticum*. Rees and Jones (1967) presented evidence that in *Lolium* the evolution of diploid species was associated with variation of nuclear DNA content, namely, the DNA content of three inbreeding species was about 35% larger than that of three outbreeding species tested. Rothfels *et al.* (1966), in the study on chromosome size and DNA content in species of *Anemone* and related genera, found considerable differences between genera but small within a genus. Southern (1967) studied the species relationship in *Tulipa*, and reported that in relation to DNA content per nucleus a continuous variation in diploid species was detected.

The DNA ratio in Einkorn (diploid), Emmer (tetraploid) and Dinkel (hexaploid) wheat is not 1:2:3; Bhaskaran and Swaminathan (1960) gave the ratio of 1:1.5:2.0, Upadhy and Swaminathan (1963) 1:1.9:2.8, Rees (1963) 1:1.7:2.5, Rees and Walters (1965) 1:1.8:2.5 and the present result was 1:2.0:2.8. This can be easily explained by the difference in DNA content among the three wheat genomes. As is evident from Table 6, the result of Pai *et al.* is unique in that *T. dicoccum* has a much lower DNA content than the sum of the DNA contents of *T. monococcum* and *Ae. speltooides*, and that for *T. aestivum* is also lower than the sum of those for *T. dicoccum* and *Ae. squarrosa*. They explained these results by assuming that polyploid wheat diminished its nuclear DNA in the course of evolution and emphasized the importance of DNA diminution as a mechanism of functional diploidization. Upadhy and Swaminathan (1963) recognized the diminution of

nuclear DNA in polyploid wheat, but to a lesser extent. However, Rees (1963) and Rees and Walters (1965) obtained no evidence that DNA amount appreciably diminished in polyploid wheat. They found that species or varieties with the same genome constitution have approximately the same amounts of nuclear DNA, and suggested the possibility that phylogenetical relationship can be traced by analyzing the DNA amount in related species.

The present investigation confirmed that DNA content per nucleus of a cultivated variety of common wheat is approximately equal to that of the two ABD strains of recently synthesized 6x wheat, especially No. 13. Moreover, there was no DNA difference among three subspecies of *T. aestivum* (Dinkel group). Those may suggest that the DNA content per nucleus of common wheat did not appreciably change since its origin several thousand years ago. The situation seems likely to be the same with Emmer wheat. Nishikawa and Furuta (1968) reported that they found no difference in DNA content per nucleus among 25 Japanese and U.S. varieties of common wheat. In addition, among species and varieties of Emmer wheat no differences were detected (Nishikawa and Sawai, unpublished). Taking these results into consideration, it may be concluded that the amount of DNA has been appreciably stable in polyploid wheat. In genus *Oryza*, Katayama (1967) reported that there could not be found any significant DNA differences among species with the same genome constitution. Though statistical analysis did not prove a significant difference between ABD No. 1 and ABD No. 13, the latter should be expected to contain a higher DNA amount than the former, because Emmer parents of ABD No. 1 and ABD No. 13 contain equal amounts of DNA and the D genome parent of the latter has a higher DNA content than that of the former. Further investigations will be presented elsewhere.

Finally, according to Rees and Walters (1965) the G genome analyzer, if it exists, must have a lower DNA content than the B analyzer. Thus, it is desirable to determine DNA amounts in diploid species of Triticineae in order to complete the whole picture of the phylogeny of wheat.

SUMMARY

1. Comparisons were made of DNA content per nucleus in common wheat, artificially synthesized 6x wheat, its parental species and three analyzers.
2. DNA content per nucleus in the D genome analyzer was the lowest, B genome had a little lower DNA content than A genome, but the difference between the last two was not significant.
3. Ssp. *strangulata* had a significantly higher DNA value than var. *typica* of the same species, *Ae. squarrosa*.
4. Two strains of synthesized 6x wheat, ABD No. 1 and ABD No. 13, have nuclear DNA equal to the sum of the DNA contents of their respective parents. Nuclear DNA content of cultivated common wheat (Chinese Spring) is quite the same as that of synthesized 6x wheat, especially ABD No. 13.
5. In three subspecies of *T. aestivum*, *vulgare*, *spelta* and *macha* no significant

difference was found in DNA content per nucleus.

6. Based on the above facts, the following conclusion may be reasonably drawn as far as wheat and its relatives are concerned:

a) Appreciable changes of DNA content might have resulted from chromosome aberrations accumulated in the course of genome differentiation of a common primitive genome at the diploid level.

b) The three different genomes, once established, have been appreciably stable and kept the amount of DNA constant either in diploid or polyploid condition.

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