タルホコムギにおける核当りDNA量の種内変異

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INTRASPECIFIC VARIATION OF NUCLEAR DNA CONTENT IN *AEGILOPS SQUARROSA* L.[1)]

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Aegilops squarrosa (n=7) is the donor of the third or D genome to common wheat and also the donor of the pivotal genome to five polyploid species of section Vertebrata of the genus *Aegilops*. This diploid species grows as a predominantly autogamous wild grass or weed in the Middle East and is classified by ear morphology into two subspecies, ssp. *eu-squarrosa* and ssp. *strangulata*. The former includes three varieties, *typica*, *anathera* and *meyeri* and the latter only *strangulata*. A comprehensive investigation in respect to morphology, genetics, cytology, physiology, susceptibility to rust and distribu tion of this species was reported by Kihara *et al.* (1965). *Ae. squarrosa* as well as *Triticum monococcum* and *Ae. speltoides* constitute a large unexplored gene pool for wheat breeding (Zohary *et al.* 1969). The practical method of introducing agriculturally important genetic variability into common wheat from *Ae. squarrosa* has already been established (Kerber and Dyck 1969).

Recently, Furuta *et al.* (1974) confirmed a considerable intraspecific difference in nuclear DNA content among four varieties of *Ae. squarrosa*. In order to estimate the amount of genetic variation within species in relation to phylogeny of polyploid wheat, we carried out cytophotometrical masurements of nuclear DNA content with 27 strains of *Ae. squarrosa*.

MATERIALS AND METHODS

Varieties and sources of 27 strains used in this study are listed in Table 1, and the collection sites are shown in Fig. 1. Twenty five strains were collected by Kyoto University Scientific Expedition to the Karakoram and Hindukush (KUSE in abbreviation) in 1955 and the remaining two strains, *typica* No. 1 and No. 2 have been kept as laboratory strains in Kyoto University.

Young spikes at the meiotic stage were fixed with modified Carnoy's fluid (glacial acetic acid 1: chloroform 3: 95% ethanol 3) for three days and preserved in 75% ethanol in a refrigerator. Anthers at the pollen tetrad stage of the standard (KUSE 2135-3) and of four other strains were smeared side by side on the cover slips. Subsequently, the pollen tetrads were dried, hydrolyzed with 1N HCl at 60°C for five minutes and stained by Schiff's reagent (Furuta 1975). Ten nuclei of each strain were

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Table 1. Varieties, sources and nuclear DNA content of 27 strains of *Aegilops squarrosa* studied

KUSE No.	Variety or type	Source	DNA content		
			$\bar{X}$±S.E.	C. V.*	relative value**
2001-3	var. *typica* L.	Pakistan	474.6± 7.7	9	1.12
2003-4	var. *anathera* Eig	〃	485.7±10.0	11	1.15
2033-1	var. *typica* L.	Afghanistan	424.8± 5.6	7	1.00
2042-2	〃	〃	426.1± 5.8	7	1.00
2058-3	intermediate type***	〃	404.6± 6.3	9	0.95
2095-2	var. *anathera* Eig	〃	443.6± 7.0	9	1.05
2106-2	var. *typica* L.	Iran	431.7± 7.9	10	1.02
2111-7	var. *strangulata* Eig	〃	490.9± 6.7	8	1.16
2112-3	〃	〃	465.9± 8.2	10	1.10
2112-4	〃	〃	453.2± 6.8	8	1.07
2118-1	〃	〃	449.0± 7.0	9	1.06
2119-1	〃	〃	484.3± 7.8	9	1.14
2124	〃	〃	469.6± 5.5	6	1.11
2128-4	var. *typica* L.	〃	415.2± 7.6	10	0.98
2129-1	〃	〃	445.6± 9.1	11	1.05
2130-3	intermediate type	〃	468.8± 9.4	11	1.11
2132-9	var. *typica* L.	〃	459.2± 5.3	6	1.08
2135-3	var. *strangulata* Eig	〃	467.5± 6.7	8	1.10
2144-2	var. *meyeri* Griseb.	〃	469.1± 4.8	6	1.11
2157-2	〃	〃	424.0± 7.0	9	1.00
2159-2	var. *typica* L.	〃	399.0± 8.0	11	0.94
2170-1	〃	〃	400.9± 7.3	10	0.95
2171-6	intermediate type	〃	437.1± 7.8	10	1.03
2172-3	〃	〃	464.2± 5.0	6	1.09
2182	〃	〃	478.2± 7.4	9	1.13
No. 1	var. *typica* L.	?	489.5± 5.6	12	1.15
No. 2	〃	?	424.0± 5.8	8	1.00

* Coefficient of variance (%).
** The relative value of each strain to *Ae. squarrosa var. typica* No. 2.
*** Intermediate type between var. *typica* and var. *anathera*.

measured in each of three replicated preparations.

RESULTS

The mean nuclear DNA value, standard error, coefficient of variation and value relative to var. *typica* No. 2 of each strain measured are shown in Table 1. Using the relative values, it is possible to compare nuclear DNA content of these strains with that of other species of *Aegilops* (Furuta 1975). The coefficient of variation of these strains varied from 6 % to 12 % with an average of 8.85 %. DNA values varied

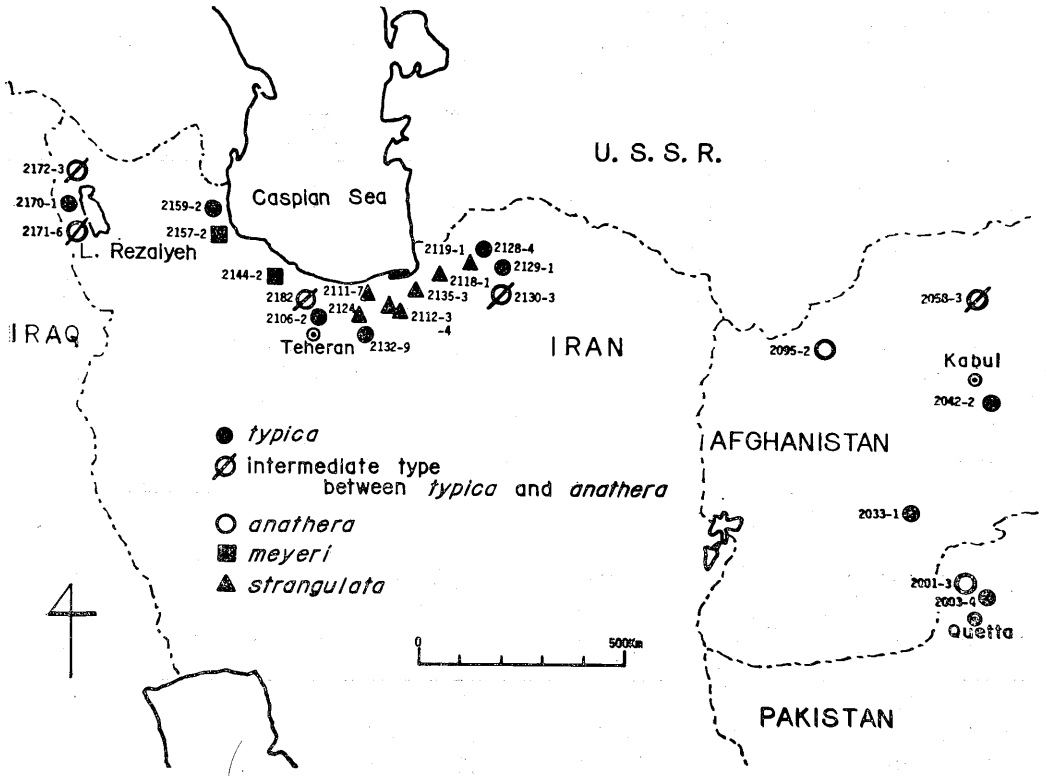

Fig. 1. Collection sites of 25 strains (KUSE) of *Aegilops squarrosa* used in the present study.

from 490.9 (*strangulata* KUSE 2111-7) to 399.0 (*typica* KUSE 2159-2), the former being 1.23 times greater than the latter.

In the analysis of variance (Table 2-a), it became apparent that there were significant differences among varieties, among strains within a variety and among replications. As shown in Table 2-b, intervarietal differences were found to be significant, between *strangulata* (468.6) and *anathera* (464.7) on the one hand and *typica* (435.5) on the other. Moreover, five intermediate types between *typica* and *anathera* were scattered between both varieties as to nuclear DNA content (Fig. 2). It should be noted here that the variation in DNA content of *Ae. squarrosa* is remarkably continuous throughout the whole species, though the value of *typica* is generally lower than that of others. This coincides with continuous variation of morphological traits in the species (Kihara *et al.* 1965). It is interesting, however, to note that no intravarietal differences were found among seven strains of *strangulata* (Table 2-b). These results confirm previous investigations in which only a few strains were involved (Nishikawa and Furuta 1969, Furuta *et al.* 1974). Tanaka and Matsumoto (1965) found no karyotypical differences among *typica* No. 1, *meyeri* KUSE 2144-2 and *strangulata* KUSE 2135-3, which did not differ significantly in nuclear DNA content in the present study.

In regard to relationships between DNA content and geographical distribution, five strains from Afghanistan and three from the southwest corner of the Caspian Sea—

Table 2. Variance analysis and test of significance for nuclear DNA content in 27 strains of *Aegilops squarrosa*

(a) Variance analysis

Source	D. F.	S. S.	M. S.	F-value
Strains	26	613,389	23,592	5.86**
var. *typica*	10	252,016	25,202	6.26**
intermediate type	4	107,231	26,808	6.66**
var. *anathera*	1	26,606	26,606	6.61*
var. *meyeri*	1	30,465	30,465	7.56**
var. *strangulata*	6	41,210	6,868	1.71
variety and type	4	155,861	38,965	9.67**
Replicates	54	217,486	4,028	3.02**
Error	729	973,466	1,335	
Total	809	2,417,730		

* and ** significant at 5% and 1%, respectively

(b) Test of significance on the mean differences between five varieties or type

Variety or type	No. of strains	Mean	*typica*	*meyeri*	inter-mediate	*anathera*
strangulata	7	468.6	33.1*	22.0	18.0	3.9
anathera	2	464.7	29.2*	18.1	14.1	
intermediate	5	450.6	15.1	4.0		
meyeri	2	446.6	11.1			
typica	11	435.5				

Lake Rezaiyeh region had low DNA contents.

DISCUSSION

Ae. squarrosa is very distinctive in its morphology, barrel type of disarticulation, genome, karyotype and geographical distribution in relation to the ten diploid species of *Aegilops* and *Triticum* and has been isolated almost completely from other diploid species (Kihara 1954, Chennaveeraiah 1960, Zohary *et al.* 1969). In addition to this, it has played an important role in the occurrence and differentiation of polyploid species of these genera (Kihara 1944, McFadden and Sears 1944, Kihara 1954, Zohary and Feldman 1962). The incorporation of this genome into hexaploid wheat or common wheat makes it a valuable species. Moreover, *Ae. squarrosa*, as one of diploid progenitors of common wheat, stores a large unexplored gene pool for wheat breeding. In respect to nuclear DNA content, *Ae. squarrosa* has the lowest among three diploid ancestors of common wheat (Rees 1963, Nishikawa and Furuta 1969) and furthermore has the lowest DNA content among nine diploid species of the genus (Furuta 1975). So far there exists no extensive review of variability of DNA in this species, except that of Furuta *et al.* (1974) who analyzed seven strains and found intraspecific varia-

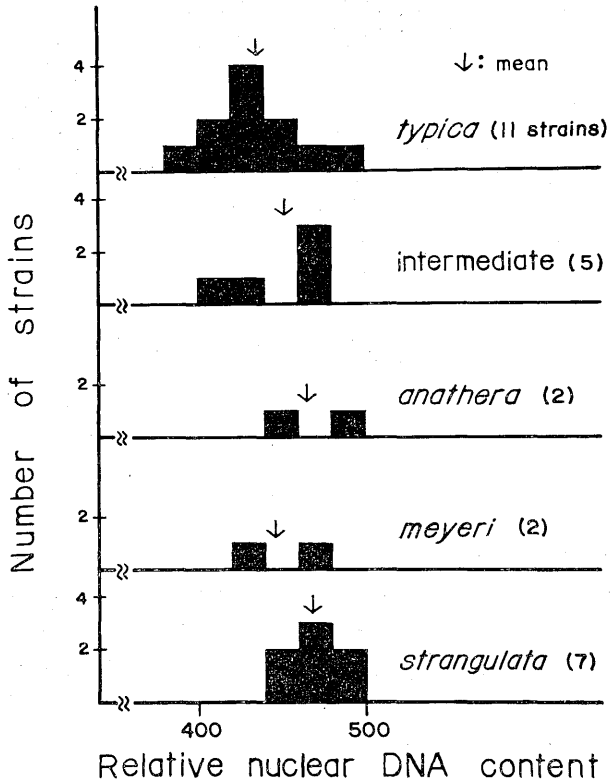

Fig. 2. Frequency distribution of nuclear DNA content in 27 starins of *Aegilops sequarrosa*.

tion. The present study revealed remarkable, continuous variation of nuclear DNA content among 27 strains of *Ae. squarrosa*. Moreover, Zohary *et al.* (1969) described *Ae. squarrosa* as having greater morphological diversity and ecological amplitude than any other diploid species of *Aegilops* and *Triticum*. Likewise, Johnson (1967) found electrophoretical variation in seed protein among 17 strains of *Ae. squarrosa*. It is evident from these facts that this diploid species is very polymorphic for many characters and can preserve a number of genes desirable for wheat breeding.

Of course, spontaneous aneuploids have never been reported in *Ae. squarrosa* and intervarietal or regional hybrids have good pairing and fertility characteristics as a whole. In only a single case, simple reciprocal translocation has been found (Kihara *et al.* 1965). Moreover, Chennaveeraiah (1960) emphasized that little difference in karyotype occurred among four strains analyzed of *Ae. squarrosa*. Considering these facts and the continuous variation in nuclear DNA content, the difference in DNA content of nucleus may be due to lengthwise duplication and/or deficiency of small chromosome segments as discussed in *Allium* (Rees 1972). These duplications or deficiencies would not be restricted to a specific chromosome(s), but would be distributed over whole chromosome complements.

Few studies on intraspecific variation in DNA content have been carried out in

higher plants to date. Katayama (1967) and Bennett and Smith (1971) concluded that no intraspecific variation is evident in rice or in barley, respectively. It is of interest that variation between different genome types was observed in the rice and uniformity between species as well as within species was found in the barley. On the other hand, some species of *Picea* and *Pinus* (Miksche 1968, 1971, Dhir and Miksche 1974) and *Vicia* (Chooi 1971) showed variability in DNA content. Except for the studies of conifer cited above, insufficient numbers of varieties or strains were measured in order to estimate intraspecific variation. Accordingly we are confirming the results found with other diploid species of *Triticum* and *Aegilops*. Intraspecific variation found in *Ae. squarrosa* occurs in at least some of the other species of section Sitopsis of genus *Aegilops* as well. In any case, speciation in diploid *Aegilops* is associated with change in nuclear DNA content (Furuta 1975) and this situation may prevail within species also.

SUMMARY

Feulgen-cytophotometrical measurement of nuclear DNA content in 27 strains of *Ae. squarrosa* revealed intraspecific variation, which was continuous, the highest value being 1.23 times greater than the lowest. Mean DNA values in an increasing order were var. *typica* (435.5), var. *meyeri* (446.6), var. *anathera* (464.7) and var. *strangulata* (468.6) and an intermediate type between *typica* and *anathera* was between the two in mean DNA value, also.

Intravarietal differences were significant in all varieties measured except var. *stragulata*. Variation in nuclear DNA content of *Ae. squarrosa* may be caused by lengthwise duplications or deficiencies of small segments of almost all chromosomes.

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LITERATURE CITED

Bennett, M. D., and J. B. Smith, 1971 The 4C nuclear DNA content of several *Hordeum* genotypes. Can. J. Genet. Cytol. **13**: 607–611.

Chennaveeraiah, M. S., 1960 Karyomorphologic and cytotaxonomic studies in *Aegilops*. Acta Horti Gotoburgensis **13**: 85–186.

Chooi, W. Y., 1971 Variation in nuclear DNA content in the genus *Vicia*. Genetics **68**: 195–211.

Dhir, N. K., and J. P. Miksche, 1974 Intraspecific variation of nuclear DNA content in *Pinus resinosa* Ait. Can. J. Genet. Cytol. **16**: 77–83.

Furuta, Y., 1975 Quantitative variation of nuclear DNA in genus *Aegilops*. Japan. J. Genet. **50** (in press).

Furuta, Y., K. Nishikawa, and T. Tanino, 1974 Stability in DNA content of AB genome com-

ponent of common wheat during the past seven thousand years. Japan. J. Genet. **49**: 179–187.

Johnson, B. L., 1967 Confirmation of the genome donors of *Aegilops cylindrica*. Nature **216**: 859–862.

Katayama, T., 1967 Relative amounts of deoxyribonucleic acid (DNA) per nucleus of diploid species in *Oryza*. Japan. J. Breed. **17**: 177–181.

Kerber, E. R., and P. L. Dyck, 1969 Inheritance in hexaploid wheat of leaf rust resistance and other characters derived from *Aegilops squarrosa*. Can. J. Genet. Cytol. **11**: 639–647.

Kihara, H., 1944 Discovery of the DD-analyser, one of the ancestors of *Triticum vulgare* (in Japanese). Agr. Hort. Japan **19**: 889–890.

Kihara, H., 1954 Consideration on the evolution and distribution physiological, of *Aegilops* species based on the analyser-method. Cytologia **19**: 336–357.

Kihara, H., K. Yamashita, and M. Tanaka, 1965 Morphological, physiological, genetical and cytological studies in *Aegilops* and *Triticum* collected from Pakistan, Afghanistan and Iran. Results of The Kyoto University Scientific Expedition to The Karakoram and Hindukush, 1955 **1**: 1–118.

McFadden, E. S., and E. R. Sears, 1944 The artificial synthesis of *Triticum spelta*. Genetics. **30**: 14.

Miksche, J. P., 1968 Quantitative study of intraspecific variation of DNA per cell in *Picea glauca* and *Pinus banksiana*. Can. J. Genet. Cytol. **10**: 590–600.

Miksche, J. P., 1971 Intraspecific variation of DNA per cell between *Picea sitchensis* (Bong.) Carr. provenances. Chromosoma **32**: 343–352.

Nishikawa, K., and Y. Furuta, 1969 DNA content per nucleus in relation to phylogeny of wheat and its relatives. Japan. J. Genet. **44**: 23–29.

Rees, H., 1963 Deoxyribonucleic acid and the ancestry of wheat. Nature **193**: 108–109.

Rees, H., 1972 DNA in higher plants. In Evolution of Genetic System (Ed. by Smith H. H., Brookhaven Symposia in Biology No. 23): 394–418.

Tanaka, S., and K. Matsumoto, 1965 Karyotype analysis in the genus *Aegilops*. I. Karyotypes of C, C^u and D genomes. Mem. Osaka Univ. Lib. Arts Educ., B, **14**: 212–219.

Zohary, D., and M. Feldman, 1962 Hybridization between amphidiploids and the evolution of polyploids in the wheat (*Aegilops-Triticum*) group. Evolution **16**: 44–61.

Zohary, D., J. R. Harlan, and A. Vardi, 1969 The wild diploid progenitors of wheat and their breeding value. Euphytica **18**: 58–65.