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SPECIES RELATIONSHIP OF WHEAT AND ITS PUTATIVE ANCESTORS AS VIEWED FROM ISOZYME VARIATION*

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SUMMARY

4x and 6x wheat and its putative ancestors were surveyed for variation in α- and β-maylase isozymes of dry and germinating seed to elucidate their species relationship. Lineage formulated based on the results obtained is summarized as follows. Cultivated form of Einkorn wheat, *monococcum* was derived from its wild form, *aegilopoides* or *thaoudar*. Emmer group received its genome A from *urartu* and its genome B as a repatterned genome that comprised at least $6S^l$ from *Aegilops longissima* and 7S from *Ae. speltoides*. On the other hand, genome A of Timopheevi group descended from *urartu* and its genome G from *Ae. speltoides*. The isoyzme markers which were expected in the donor of genome D to AABBDD hexaploid were found in *Ae. squarrosa* collected from the area stretching over Transcaucasus, northern Iran and south coast of Caspian Sea.

The genetical analysis of various kinds of isozymes is of prime importance for approaches to species relationship. Because the isozyme is the direct product of a particular gene and serves as its marker, species relationship would be described in more detail by means of tracing back the isozyme markers along the presently established course of evolution. Here will be presented the results of survey in wheat and its putative ancestors for isozyme variation in either α-amylase of germinating seed and β-amylase of dry and germinating seed analyzed by diso-isoelectric focusing, and thereby considered their species relationship.

Variation in Dinkel wheat

There were total seventeen bands of α-amylase isozyme detected in forty-three strains. They were grouped into thirteen patterns according to the zymogram. Fig. 1-a shows one of the pattern which occurred most frequently. The α-amylase isozyme band no. 2 (there-

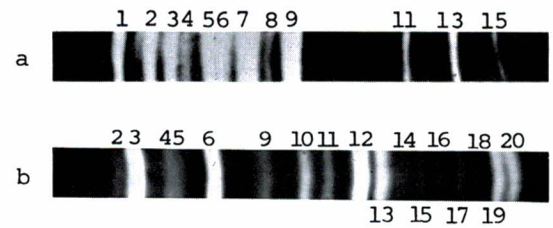

Fig. 1. Zymograms of α- and β-amylase in Chinese Spring
a: α-amylase, b: β-amylase.

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after shortened to the figure attached to α or β-, as α-2) and -13, α-3, -10 and -15, and α-1, -7, and -11 have been proved to be conditioned by the genes of genomes A, B and D, respectively (Nishikawa *et al.* 1981). With respect to β-amylase isozymes total twenty bands detected, sixteen of these being variable. Twenty strains examined were assorted into five patterns, one of which is shown in Fig. 1-b. β-3, -4, -9, -12 and -13 were conditioned by genome A and β-5, -6, -8, and -14 through -20 by genome D.

Variation in tetraploid wheat

Variation in α-amylase isozymes has already been reported in some detail (Nishikawa *et al.* 1979). At least twenty-two bands of α-amylase isozyme were detected in seventy strains of Emmer group and thirteen different patterns of zymogram were recognized.

Contrarily, Timopheevi group was extremely less variable and distinctly different from Emmer group in that two major bands, α-2 and -3 were totally absent. Forty-four strains of *araraticum* were grouped into two patterns. Eleven strains of *timopheevi* showed another pattern a little different from *araraticum*.

Twenty-three isozyme bands of β-amylase were detected in eighty-one strains of Emmer group. Fifteen bands of these were variable and twenty-seven different patterns of zymogram were temporarily distinguished. β-3, -4, -9, -12 and -13, the bands conditioned by 4Aβ were present together in every strains tested.

In fifty-six strains of Timopheevi group, seventeen isozyme bands were detected. There were eight patterns recognized. Seven of these contained forty-five strains of *araraticum* and the remaining one eleven strains of *timopheevi*. Here again β-3, -4, -9, -12 and -13 were present as common bands.

Variation in einkorn wheat

Sixty-two strains tested contained seventeen different isozyme bands of α-amylase. Five strains of *monococcum* invariably showed a particular zymogram that occurred most frequently in two types of wild form, *aegilopoides* and *thaoudar*. In addition to this, four different zymogram patterns were detected in these two types. α-2 and -13 conditioned by genome A of polyploid wheat have never been found in these patterns. Whereas they were detected in all but one in twenty-three strains of *urartu*.

There were total fourteen different isozyme bands of β-amylase detected in eighty strains and seven patterns of zymogram were distinguished. In two patterns which contained three strains of *urartu* and two of *thaoudar*, β-1 and -3 were clearly identified, but never detected in the other patterns. Those isozymes known as conditioned by the gene or genes on 4Aβ were identified together in these patterns.

Variation in section Sitopsis of Aegilops

speltoides: Twenty-two strains were surveyed for variation of α-amylase isozymes. There were fifteen different bands recognized. α-15 occurred in all strains, but α-10 moderately. However, α-3, one of the three which were known to be conditioned by genome B in polyploid wheat has never been identified. β-amylase zymogram of thirty-two strains showed total sixteen different bands and were grouped into eleven patterns.

longissima: Sixteen strains were surveyed for α-amylase isozyme. Four patterns of zymogram were recognized. α-3 was detected by sixty percent, and α-10 rarely, but α-15

was not. There were sixteen bands of β–amylase isozyme detected in eighteen strains tested. Out of these, only four bands were variable. Five zymogram patterns were recognized.

sharonensis: Total thirteen different bands of α–amylase isozyme were detected in thirteen strains tested. Six of these were variable. α–3 occurred in most of strains, but α–15 in no strain. Fifteen isozyme bands of β–amylase were detected in thirteen strains, four of these being variable.

searsii: The zymogram of α–amylase was totally different from other species of this section, comprising a fewer isozyme bands of strong activity. Four strains contained the active band which was specific to this species. Two patterns of zymogram of β–amylase were distinguished in five strains, which were somewhat different from each other in activity of some of eleven bands detected. Unlike α–amylase zymogram, *searsii* was not much different from others in β–amylase zymogram.

bicornis: Ten strains examined showed eleven different bands of α–amylase isozyme and six of these were variable. On the other hand, there were two patterns of β–amylase zymogram, the one comprising ten bands and the other two more bands in addition.

Variation in Aegilops spuarrosa

Sixty strains were examined for α–amylase isozymes (Nishikawa *et al.* 1980). There were total ten different bands recognized and seven zymogram patterns were distinguished. Those bands which were identified in Dinkel group as conditioned by genome D, α–1, –7 and –11 were detected together in one of these patterns. Thirty-one strains were surveyed for variation of β–amylase isozyme. Total twenty-one bands occurred and seven zymoram patterns were recognized. In two patterns of these, those bands conditioned by genome D, β–5, –6, –8 and –14 through –20 were detected.

Consideration of species relationship

Isozyme bands whose gene location is definitely known serve as the marker for tracing back evolutionary course. The frequencies with which those isozyme bands occurred are represented in Table 1. Either α–2 and –13, conditioned by genome A, was found in Emmer group, while in Timopheevi group only α–13 was detected. Whereas, in the genome A carrier or Einkorn wheat, *monococcum*, *aegilopoides* and *thaoudar* were similar to one another and carried neither α–2 nor –13. Contrarily, another type of wild form, *urartu* contained both α–2 and –13. In tetraploid wheat including Emmer group and Timopheevi group, all strains tested contained β–3, –4, –9, –12 and –13, which were known to be conditioned by chromosome 4A. Out of these five bands, β–3 was absent in *monococcum* and *aegilopoides*, but present in *thaoudar* and *urartu*. Based on these findings it is evident that genome A of Emmer group and therefore of Dinkel group carried the genes descended from *urartu*, and that the same is the case in Timpopheevi group, because it contain α–13. Derivation of genome A from *urartu* to polyploid wheat has been suggested before (Nagayoshi 1978; Caldwell and Kasarda 1978; Konarev *et al.* 1979).

On the other hand, α–3, –10 and –15 were conditioned by genome B of Emmer group and Dinkel group, while in Timopheevi group there were α–10 and –15 recognized. Among six species of section Sitopsis as the candidate of genome B donor, α–3 was present in *longissima*, *sharonensis* and *bicornis*, α–10 in *speltoides* and *longissima*, and α–15 in only *speltoides*.

Table 1. The isozyme markers and their frequencies (%) in wheat and its putative ancestors.

	α-amylase								β-amylase												
	1	2	3	7	10	11	13	15	3	4	5	6	8	9	14	15	16	17	18	19	20
	6DL*	6AL	6BL	6DL	6BL	7DL	7AL	7BL	4Aβ			4DL		4Aβ				4DL			
Dinkel	93	100	74	100	63	98	100	100	85	85	75	100	95	100	85	80	80	80	80	75	75
Emmer	0	98	65	0	52	0	99	93	100	100	49	100	16	100	91	79	79	1	1	0	0
Timopheevi	0	0	0	0	5	0	100	100	100	100	100	79	75	100	100	96	96	0	0	0	0
Einkorn																					
monococcum																					
aegilopoides	0	0	0	0	0	0	0	0	0	100	0	100	0	100	100	82	0	0	0	0	0
thaoudar									5	100	0	100	0	100	100	95	0	0	0	0	0
urartu	0	96	0	0	0	0	100	0	13	100	0	83	0	100	83	0	0	0	0	0	0
Ae. speltoides	3	0	0	0	26	0	0	100	66	88	0	0	100	9	100	100	94	84	16	0	0
Ae. longissima	0	58	60	0	9	100	100	0	100	100	0	11	100	0	100	100	100	100	0	0	0
Ae. sharonensis	0	72	83	0	0	100	100	0	100	92	0	8	100	8	100	100	100	85	0	0	0
Ae. bicornis	0	70	95	0	0	100	100	0	100	0	0	0	0	0	100	50	50	0	0	0	0
Ae. searsii	0	20	0	0	0	60	0	0	0	100	0	0	0	0	100	100	100	100	0	0	0
Ae. squarrosa	37	0	0	98	0	80	20	0	100	72	97	3	100	100	100	100	100	97	100	83	79

* Chromosome arm where the gene concerned locates.

Taking these findings together with data in different aspects (Nishikawa and Furuta 1978; Ogihara and Tsunewaki 1982 and others) into consideration, *longissima* is most likely to be the donor of the gene for α-3, and probably the gene for α–10, both of which locate on 6BL. α–15 as conditioned by 7BL must have been derived exclusively from *speltoides*. This implies that the present-day genome B is the reformed genome that has included at least either $6S^1$ and 7S in whole or part. Kimber and Athwal (1972) suggested considerable repatterning of genome B by hybridization before or after amphiploidization. While it is evident that *speloides* can fulfil by itself the requirement of the donor of the second genome (G) of Timopheevi group.

α–1, –7 and –11 were conditioned by the respective genes on 6D and 7D in Dinkel group and these were detected together in vars. *typica* and *strangulata* of *Ae. squarrosa*, especially in the latter (Nishikawa *et al.* 1980). With respect to β-amylase isozymes, all the bands known to be conditioned by genome D of Dinkel group were identified in the strains collected in northern Iran. Considering the results of α- and β–amylase isozyme together it can be infered that the donor of genome D distributes in the area stretching over Transcaucasus, through northern Iran to southeast coast of Caspian Sea.

REFERENCES

Caldwell, K. A. and D. D. Kasarda 1978. Assessment of genomic and species relationship in *Triticum* and *Aegilops* by PAGE and by differential staining of seed albumins and globulins. Theor. Appl. Genet. **52**: 273–280.

Kimber, G. and R. S. Athwal 1972. A reassessment of the course of evolution of wheat. Proc. Natl. Acad. Sci. USA **69**: 912–915.

Konarev, V. G., I. P. Gavrilyuk, N. K. Gubareva and T. I. Peneva 1979. Seed proteins in genome analysis, cultivar identification and documentation of cereal genetic resources; A review. Cereal Chem. **56**: 272–278.

Nagayoshi, T. 1978. Studies on amylase isozymes of *Triticeae*. III. Phylogenetic relationship of *Triticum urartu* in *Triticinae*. Sci. Rep. Fac. Agr. Kobe Univ. **13**: 89–92.

Nishikawa K. and Y. Furuta 1978. DNA content of nucleus and individual chromosomes and its evolutionary significance. Proc. 5th Int. Wheat Genet. Symp. (Indian Soc. Genet. Plant Breed., New Delhi): 133–138.

Nishikawa, K., Y. Furuta, Y. Hina and T. Yamada 1981. Genetic studies of α-amylase isozymes in wheat. IV. Genetic analyses in hexaploid wheat. Jpn. J. Genet. **56**: 385–395.

Nishikawa, K., Y. Furuta, S. Kudo and K. Ujihara 1979. Differentiation of tetraploid wheat in relation to DNA content of nucleus and alpha-amylase isozymes. Rep. Plant Germ-plasm Inst. Kyoto Univ. **4**: 30–38.

Ogihara Y. and K. Tsunewaki 1982. Molecular basis of the genetic diversity of the cytoplasm in *Triticum* and *Aegilops*. I. Diversity of the chloroplast genome and its lineage revealed by the restriction pattern of ct-DNA. Jpn. J. Genet. **57**: 371–396.