Secondary Association in *Eleusine coracana* (Finger Millet)

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Secondary association, as distinct from the primary (chiasma-making) association, is an attribute of genetic homoeology between the chromosomes of common descent notwithstanding their remote ancestry. Recent evidences from the work on *Triticum aestivum* (Riley 1960, Kempanna and Riley 1964) support this statement. Generally, investigations on the genetic basis of secondary association are less easy in materials other than *T. aestivum*, for the telocentric chromosomes, which, as cytological markers, were instrumental in the latter, are not available in others. Nevertheless, a nonrandom pattern with which the metaphase bivalents organize themselves constantly into a certain number of groups, as seen from the polar view, is presumed to be an implication of the secondary association principle. Of course, two techniques cannot be compared with each other in the matter of their efficacy. But the latter's wider application in varieties of plants (Darlington 1928, Lawrence 1931, Gustafsson 1934, Nandi 1936, Richaria 1937), and the relatively reliable conclusions that can be drawn therefrom should suggest it to be a better alternative if not equal.

Eleusine coracana Gaertn, commonly referred to as *Ragi* or finger millet is a member of the cultivated minor millets. Its 36 diploid chromosomes invariably form 18 bivalents (Fig. 1), but the latter are seen to orientate at the post-synaptic stage in the manner not consistent with random expectation. The apparent non-random component that influences metaphase orientation is supposed here to be the secondary affinity that exists between the homoeologous chromosomes. It was with the intention of collecting more evidence, therefore, on the pattern of metaphase grouping, and also for considering the probable phylogenetic significance of such a pattern of grouping this study was undertaken.

Material and methods

Anthers were fixed for 3 hours in Carnoy's fixative made up of 6 parts absolute alcohol, 3 parts chloroform and one part glacial acetic acid. Fuelgen stained anthers were squashed in propiocarmine. The latter was intended to reinforce staining of chromosomes. Slides were made permanent by following the normal butyl-alcohol method and mounting in euperol. All observations were made from permanent slides.

An assumption was made that, if orientation of bivalents was purely random, all the 18 bivalents of a PMC should assemble into one group or 18 groups or anyone of the groups in between with equal probability. The distorted ratios should suggest the interference by a non-random component which in the present instance is regarded as secondary affinity.

Results and discussion

Metaphase cells, in polar view, were scored for the number of bivalent groups in them, so that class frequencies were developed. Two hundred PMCs thus scored distributed themselves between 7 classes only, i.e., between

5-bivalent-group and 11-bivalent-group classes, with the predominance of 7, 8 and 9 group classes (Figs. 2, 3 and 4, Table 1).

Figs. 1-4. 1, I metaphase plate showing 18 bivalents in *Eleusine coracana*. The figure to its down represents the Indian Ink drawing of the same plate. 2, 3, and 4, I metaphase plates as observed from polar view. 2, represents the association of 18 bivalents into 7 groups. 3, shows the association into 8 groups and 4, shows the association into 9 groups. Figures to the down of each one of them are the Indian Ink drawings of the respective plates.

Apparent heterogeneity between the classes is revealed by the data through the significant χ^2 ($P = < 0.001$). This indicates that the bivalents assort themselves into certain number of groups more frequently than they do into

Table 1. Showing PMCs frequencies under different classes from 5-groups class to 11-groups class

	No. of bivalent groups in each cell							Total
	5	6	7	8	9	10	11	
Observed	6	16	36	35	78	17	12	200
Expected	28.6	28.6	28.6	28.6	28.6	28.6	28.6	200

$$\chi^2(6) = 126.39, P = < 0.001$$

others much against the random principle. Further, much of the value to the significant χ^2 seems to arise from one particular class, i.e., the class with 9-bivalent groups. This was statistically verified by comparing the cell frequency of this group against the pooled cell frequency of the rest of the classes (Table 2).

Table 2. Comparison between the cell frequency of 9-groups-class and the pooled frequency of the rest

	9-groups class	Pooled frequency of the rest	Total	$\chi^2_{(1)}$
Observed	78.00	122.00	200	99.54
Expected	28.60	171.40	200	P = < 0.001

Significant $\chi^2(P = < 0.001)$ thus establishes the overwhelming majority of the frequency under 9-groups-class, and so evidences its major impact on distorting the interclass ratios. The non-random distribution of cells between classes, which is apparently due to the predominance of the 9-groups-class, has resulted from the preferential spacing of bivalents, obviously caused by the residual chromosomal homologies. Thus evidence has been produced of the occurrence of secondary association in *Eleusine coracana*.

This indicates the polyploid nature of the species with 9 as the possible basic chromosome number. Coleman (1920) supposes it to be an allopolyploid with *E. indica*, $2n=18$, as one of the probable progenitors. But lack of sufficient cytogenetical knowledge does not lend itself to the further consideration of this hypothesis. Nevertheless, it can be argued that, if it were to be an autotetraploid, mere doubling of 18-diploid chromosome complement of *E. indica*, would account for the origin of the tetraploid *E. coracana*. By contrast, if it were to be considered an allotetraploid, and *E. indica*, as suggested by Coleman (*loc. cit.*), as one of the donor species, it will be necessary that the source of the coprogenitor whose genomic composition has to be similar to *E. indica* is established. Genomic similarity between the two so-called putative diploid parents must be a prerequisite in view of the fact that secondary association reflects the close intergenomic proximity.

Considering the cytogenetical situation of *E. coracana* either from the

auto- or from the allopolyploid point of view, multivalents ought to have occurred at meiosis. Their total absence and generally perfect meiotic regularity only with 18 bivalents characterised by the species strongly support the view that the species might have acquired cytological diploidization through a potential genetic mechanism analogous to polyploid wheats (Riley and Chapman 1958, Riley and Kempanna 1963). This extreme step appeared necessary to cause genomic isolation to ensure fertility through genetic and physiological stability. On such measure only depended the successful evolution of the polyploid *E. coracana*. However, this hypothesis needs to be verified through species hybrids.

Summary

Evidence has been gathered of the occurrence of secondary association in *E. coracana* through the scoring of PMCs based on the number of bivalent groups they had in, as observed from polar view.

Secondary association results indicate at the polyploid nature of the species with 9 as the possible basic chromosome number. The data available at present preclude, however, the further consideration of the problem concerning the auto- or allopolyploid nature of the species.

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