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# ESTIMATION OF THE NUMBER OF INITIAL CELLS IN PANICLE PRIMORDIA OF THE RICE EMBRYO*

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A technique of selection that will enable a substantial reduction in the size of the segregating population without any reduction in the chances of recovering all mutations induced will be highly useful in mutation breeding. Among the factors controlling the survival of an induced mutation, the characters of the seed such as the stage of differentiation of the embryo and the number Of primordial cells involved in the origin of each panicle are very important. Hence a knowledge of these factors will provide a dependable basis for the selection of $M_1$ material to be carried forward to the $M_2$.

Estimation of the number of initial cells involved in the origin of the various spike categories or branches has been made in crops such as barley, wheat, pea and flax. The multicellular nature of the rice embryo and its ability to develop mutually exclusive mutant sectors have already been reported (Nair, 1972). An attempt is made to estimate the number of initial cells in the primordia of various categories of rice panicles such as apical, primary and secondary ones.

## Materials and Methods

The rice variety Rohini treated with ethyl methane sulphonate and gamma rays formed the biological material. The apical, primary and secondary tillers on $M_1$ plants were marked with respect to node position and the ears on maturity were collected separately. The $M_2$ generation was raised as $M_1$ ear progenies. $M_2$ progenies segregating for chlorophyll and other seedling mutants were marked and segregation ratios for each were worked out.

Normal $M_2$ seedlings from progenies segregating with a deficit of recessive mutants (less than 20%) were transplanted in singles. Each $M_2$ plant was harvested separately. The $M_3$ generation was raised on $M_2$ plant progeny basis. The seedlings were examined at intervals of 3 to 4 days and the progenies segregating for seedling mutants were separately marked. The type of mutant, the number of mutants and the number of normal plants were recorded in each of the segregating progenies.

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The data from segregating $M_2$ plant progenies derived from the same $M_1$ ear were pooled and the mean $M_3$ segregation ratio for each progeny was calculated.

## Results and Discussion

### *a) Segregation ratio*

The segregation ratios calculated as percentage of recessive mutants in segregating progenies in the $M_2$ and $M_3$ generations are presented in Table 1. The $M_3$ ratios were generally higher than the corresponding $M_2$ ratios and ranged from 3,44 to 26.76%. Fujii (1960) reported that the segregation ratios were approximately normal in generations later than the $M_2$. Beard (1970) however observed that the $M_3$ ratios varied from 8 to 28% with the average at 20% in flax. Moh and Smith (1951) reported that the average ratio of chlorophyll mutants in wheat and barley was 20%.

### *b) Size of the mutated sector and number of initial cells*

The mean segregation ratio in the $M_3$ generation for each progeny was taken as the genuine ratio for the particular mutated effect. The size of the mutated sector of the $M_1$ panicle was therefore estimated by dividing the segregation ratio of mutants in the $M_2$ generation in each progeny by the corresponding $M_3$ ratio. Kawai and Sato (1965) in rice and Beard (1970) in flax have adopted similar procedures. On the other hand, Gaul (1961) in barley, D'Amato *et al*. (1962) in *durum* wheat and Osone (1963) in rice have assumed the $M_3$ ratio as 20% based on the report of Moh and Smith (1 951) that the average ratio of chlorophyll mutants in wheat and barley was 20%. Siddiq (1968) calculated the size of the mutated sector in rice by dividing the observed $M_2$ segregation ratio by the expected ratio of 25%. In the present study, the $M_3$ segregation ratios deviated from the expected value of 25% in most of the cases indicating that the actual segregation ratio in the $M_3$ generation of a progeny alone will serve as a reliable criterion for estimating the size of the mutated sector of the $M_1$ ear.

The number of initial (primordial) cells corresponding to the different panicle categories was calculated as the reciprocal of the size of the mutated sector because the size of the sector is inversely proportional to the number of initial cells from which the $M_1$ ear develops. This estimation is based on the assumption that the cells carrying the mutation has no developmental disadvantage in comparison to normal cells. If the cells with the mutation divide less frequently than the normal cells, the result would be an over estimation of the number of initial cells. Frydenberg *et al.* (1964) in barley and Muller (1965) in *Arabidopsis* reported that the mutated sector had no developmental advantage or disadvantage in comparison with the non-mutated sector. Lindgren *et al,* (1970) also found that diplontic selection did not exert any great influence on the size of the mutated sector.

Table 1
Segregation ratios, size of the mutated sector and number of initial cells

| Panicle category | Mutant type | Segregation ratio (% of mutants in segregating progenies) in the $M_2$ generation | Segregation ratio (% of mutants in segregating progenies) in the $M_3$ generation | Size of the mutated sector in the $M_1$ panicle ($M_2/M_3$) | No. of initial cells ($M_3/M_2$) |
|---|---|---|---|---|---|
| Apical A | Albina | 3.39 | 21.10 | 0.16 | 6 |
| | ,, | 8.93 | 19.08 | 0.45 | 2 |
| | ,, | 1.71 | 21.15 | 0.08 | 12 |
| | Chlorina | 4.00 | 15.40 | 0.26 | 4 |
| | Striata | 5.00 | 11.11 | 0.45 | 2 |
| | Albo–Viridis | 7.41 | 26.76 | 0.28 | 4 |
| Primary 2 | Chlorina | 5.26 | 13.04 | 0.40 | 2 |
| ,, 3 | Aibina | 11.11 | 12.21 | 0.91 | 1 |
| | ,, | 8.00 | 20.43 | 0,39 | 3 |
| | Striata | 3.45 | 10.28 | 0.33 | 3 |
| ,, 4 | Albina | 8.00 | 16.67 | 0.48 | 2 |
| | Striata | 15.62 | 25.00 | 0.62 | 2 |
| | Atbo-Viridis | 11.94 | 24.63 | 0.48 | 2 |
| | ,, | 18,18 | 21.96 | 0.83 | 1 |
| | Dwarf | 7.69 | 7.69 | 1.00 | 1 |
| ,, 5 | Aibina | 12.70 | 14.28 | 0.89 | 1 |
| | ,, | 14.29 | 17.14 | 0.83 | 1 |
| ,, 6 | Aibina | 16.52 | 19.88 | 0.83 | 1 |
| | ,, | 8.57 | 24.09 | 0.36 | 3 |
| ,, 7 | Aibina | 6.25 | 10.00 | 0,62 | 2 |
| ,, 8 | Albo–Viridis | 15.38 | 16.66 | 0.92 | 1 |
| ,, 9 | Aibina | 9.09 | 14.70 | 0.62 | 2 |
| | Striata | 6.67 | 10.00 | 0.66 | 2 |
| ,, 10 | Aibina | 11.11 | 24.38 | 0.45 | 2 |
| Secondary 2-2 | Aibina | 9.52 | 12.11 | 0.79 | 1 |
| ,, 2-3 | Albo–Viridis | 15,79 | 20.53 | 0.77 | 1 |
| ,, 2-4 | Lethal | 13.91 | 13.39 | 1.00 | 1 |
| ,, 3-2 | Aibina | 19.35 | 23.41 | 0.83 | 1 |
| ,, 3-3 | ,, | 12.50 | 21.72 | 0.58 | 2 |
| ,, 3-4 | Albo–Viridis | 14.55 | 17.64 | 0.82 | 1 |
| ,, 3-5 | Viridis | 12.50 | 13.87 | 0.90 | 1 |
| ,, 4-2 | Aibina | 16.67 | 17.06 | 0.98 | 1 |
| ,, 4-3 | Striata | 7.69 | 13.92 | 0.55 | 2 |
| ,, ,, | Minute | 9.09 | 14.28 | 0.64 | 2 |
| ,, 4-4 | Viridis | 10.34 | 13.27 | 0.78 | 1 |
| ,, 4-5 | Chlorina | 2.44 | 3.44 | 0,71 | 1 |

The estimated size of the mutated sector and number of initial cells in the panicles are also presented in Table 1. The different panicle progenies analysed within each category exhibited variation in the size of the mutated sector and number of initial celts. The variation in size in respect of the apica! ear was from 0.08 to 0.45. The estimated number of initial cells thereby ranged from 2 to 12. Osone (1963) reported that the number of initial cells contributing to the apical ear in rice is 5 to 6 and Nair (1979) estimated the maximum number as 16. The estimated number of initial cells for the apical ear was 4 in barley (Gaul, 1964), 7 or 8 in maize (Anderson *et al.*, 1949), 4 in *durum* wheat (D'Amato *et al.*, 1962) and 2 in sorghum (Kaukis and Reitz, 1955).

The size of the mutated sector of primary ears was comparatively larger (0.33 to 1.00) than that of the apical ear. Correspondingly the number of initial cells was smaller (1 to 3). Osone (1963) has aiso made similar observations in rice. Mericle and Mericle (1 961) reported that in the developing barley embryo after the 8 celled stage, more than one cell usually give rise to germinal tissue of each of the first five spikes. Jacobsen (1966) found 1 or 2 functional initial cells for each of the first 6 spikes in barley. Beard (1970) has also reported that each of branches in flax is derived from 1 to 3 initial cells.

The mutated sector size and number of initial cells for the secondary panicles indicate that they have originated from 1 to 2 initial cells. The primary and secondary ears of a primary group might have originated from the same set of initial cells. Osone (1963) reported that the corpus cell group leading to secondary tillers consisted part of the corpus cells leading to their mother ones. It can therefore be assumed that the differentiation into a primary and the secondaries of that primary group takes place at a later stage.

*c) Deficit of recessive mutants*

Some of the $M_2$ plant progenies showed deficit (less than 20%) of mutants in the $M_3$ generation also. Deficit was found in ail categories of panicles ie., apical, primaries and secondaries.

Mutated genes are expected to segregate in a 3:1 ratio in the $M_3$ and fate generations giving 25% mutants in segregating progenies. However, deficit of recessive mutants in later generations as observed in the present study has been reported by Yamaguchi (1962) and Kawai and Sato (1965) in rice, Moh and Smith (1951) and Gaul (1964) in barley and Beard (1970) in flax. This deficit has been attributed to haplontic elimination of gametes carrying the mutant gene or diplontic elimination of zygote or embryo carrying the gene in the homozygous condition. Gaul (1964) stated that the low competitive ability of gametes containing the mutation leads to haplontic elimination.

## Summary

An attempt is made to estimate the size of the mutated sector in $M_1$ panicles and number of initial cells constituting the various panicle primordia in the rice embryo. Normal plants from $M_2$ progenies segregating with a deficit on recessive mutants were carried forward to the $M_3$ generation of plant progeny basis. The data from segregating $M_3$ progenies derived from the same $M_1$ panicle were pooled and the mean $M_3$ ratio for each progeny was calculated.

The $M_3$ ratios were generally higher than the corresponding M, ratios. The size of the mutated sector of the $M_1$ panicle was estimated by dividing the $M_2$ ratio in each progeny by the corresponding $M_3$ ratio. The number of initial cells was obtained as the reciprocal of the sector size. Variation in the size of the mutated sector and number of initial cells was exhibited by panicles of the categories. For the apical ear the size of the sector was from 0.08 to 0.45 and number of initial cells from 2 to 12. The sector size in the primary ears ranged from 0.33 to 1 .00 and the number of initial cells from 1 to 3. The secondaries of primaries 2, 3 and 4 have originated from 1 to 2 initial cells. The primary and secondary ears of a primary group might have originated from the same set of initial cells in the embryo.

Deficit of recessive mutant was found in the $M_3$ generation of all categories of panicles. This deficit in a generation later than the $M_2$ is attributed to genetic or zygotic eliminations. Since the apical panicle has more number of initial cells and consequently smaller mutated sector, it is necessary to grow a large progeny to recover a mutation induced in its primordium.

## സംഗ്രഹം

നെൽവിത്തിലെ ഭ്രൂണത്തിൽ അടങ്ങിയിട്ടുള്ള പ്രാരംഭിക കോശങ്ങളുടെ എണ്ണം M_, $M_3$ തലമുറകളിലെ ഉൽപരിവർത്തിത ശതമാനത്തിൽനിന്ന് തിട്ടപ്പെടുത്തി. $M_2$ ശതമാനം പലപ്പോഴും 25ൽ കുറവായും $M_3$ ശതമാനം സാധാരണയായി $M_2$വിനേക്കാൾ അധികമായും കണ്ടു. ഓരോ കതിരിനേറയും $M_$ / $M_2$ ശതമാന flroro^nj ാതത്തി fijScnlന്ന് അതിന് ആധാരമായ ഭ്രൂണ പ്രാരംഭിക കോശങ്ങളുടെ എണ്ണം കണക്കാക്കി. ശീർഷസ്ഥ കതിര് 2 മുതൽ 12 വരെ കോശങ്ങളിൽനിന്ന് . പ്രാഥമിക കതിരുകൾക്ക് 1 ffl^roitrl 3 വരെ പ്രാരംഭിക കോശങ്ങളും ദ്വിതീയ കതിരുകൾക്ക് ഒന്നോ രണ്ടോ കോശങ്ങളും ആധാരമാണ്. പ്രാഥമിക, ദ്വിതീയ കതിരുകൾ ഒരേ സംഘം കോശങ്ങളിൽ നിന്ന് ഉത്ഭവിക്കുവാനും സാധ്യതയുണ്ട്.


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