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MUTATIONAL ANALYSIS OF EMBRYO DIFFERENTIATION IN RICE

N. Krishnankutty Nair and V. Gopinathan Nair
College of Agriculture, Vellayani-695 522, Trivandrum, Kerala

The main problem in mutation research is the screening of large populations following mutagenic treatments. A selection procedure in the M_1 generation which will reduce the size of the M_2 population without any reduction in the recovery of mutations will be of great value in mutation breeding. Knowledge on the differentiation of the embryo in the seed will enable the selection of suitable M_1 material for raising the M_2 and later generations.

Demarcation of mutated regions in the embryo of barley has been successfully attempted by Jacobsen (1966). The multicellular nature of the rice embryo and its ability to develop mutually exclusive mutant sectors are quite well known. But detailed information on the structure of the embryo is not yet available. The present investigation attempts to explore the nature of differentiation of the rice embryo.

Materials and Methods

Seeds of the rice variety 'Rohini' were treated with 2 doses each of ethyl methane sulphonate (EMS) and gamma rays. Seeds presoaked for 20 hours were treated with aqueous solution of EMS for 8 hours. Gamma irradiation was done on dry seeds using the Co^{60} gamma chamber 900 at the Sugarcane Breeding Institute, Coimbatore.

Sprouted seeds were sown on soil surface in pots. Irrigation was so regulated that tillering from the lower nodes is promoted. Tillers were classified according to the system adopted by Jacobsen (1966) in barley based on their ontogenic relationship. The pattern of tiller development is presented in Fig. 1. The main culm is marked 'A' and the coleoptile is marked 'C'. The primary and secondary tillers were classified according to their node position on the main and axillary culms respectively. The first primary tiller was marked '1' and the subsequent ones as 2, 3, etc., in numerical sequence. The secondary tillers were marked separately in each of the primaries. All surviving plants were labelled according to this pattern.

The M_2 generation was raised on M_1 ear progeny basis. The seedlings were scored for chlorophyll and other seedling mutants and the segregating progenies were separately marked. The type of mutant, the number of mutants and the number of normal seedlings were recorded in each of the segregating progenies.

Results and Discussion

Data on the emergence of primary tillers are presented in Table 1.

Table 1

Emergence of primary tillers

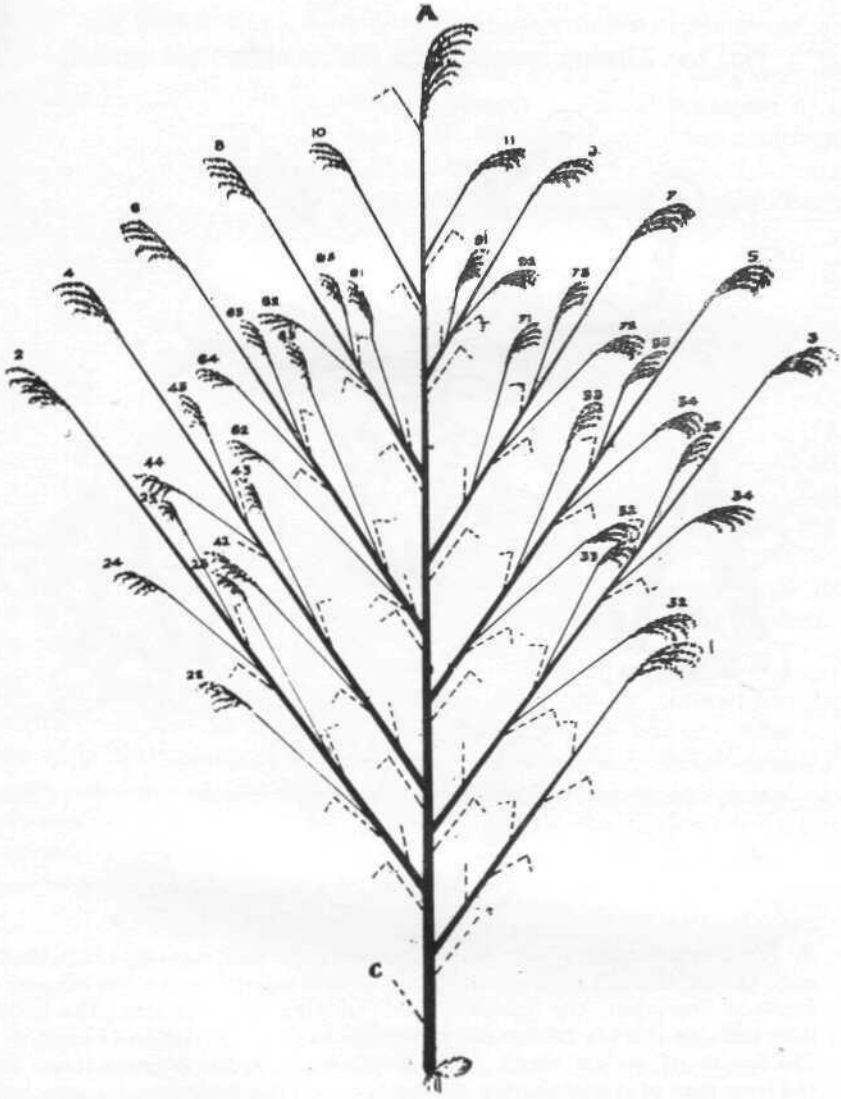
Category of tiller	No. of plants studied	No. of plants in which the tiller is present	Percentage of plants in which the tiller is present
C	450	0	0.00
1	"	1	0.22
2	"	151	33.56
3	"	377	83.78
4	"	416	92.44
5	"	318	70.67
6	"	196	43.56
7	"	129	28.67
8	"	131	29.11
9	"	165	36.67
10	"	124	27.56
11	"	12	2.67

The frequencies of emergence for the various categories of tillers were different. Tillers did not normally arise from the axils of the coleoptile and the first leaf. Mullenax (1972) stated that the rice embryo did not have a coleoptilar bud, but contains a meristem in the axil of the first leaf. The frequencies of emergence increased upto the fourth primary and thereafter gradually decreased.

The frequency with which two adjacent ears segregated for identical mutants was analysed. A comparison between two ears was possible when at least one of them segregated for a mutation. In comparison between two primary groups, all ears in one group were compared with all ears in the other group. The number of cases in which the same type of mutant appeared in both the ears was counted and the frequencies of cluster sharing estimated as the percentage of cases with the same mutation in both of the compared ears to the number of cases with a mutation in at least one of the two compared ears. The data are presented in Table 2.

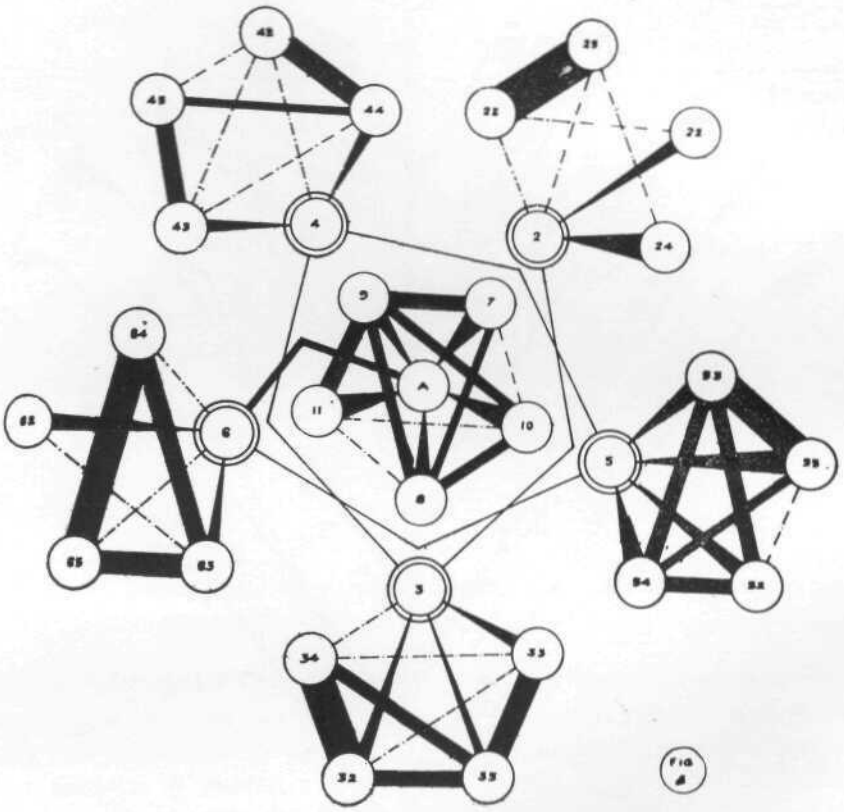
The cluster sharing frequencies of the comparisons of primary groups 2 with 3, 3 with 4, 4 with 5 and 5 with 6 were relatively low. The-frequencies of cluster sharing are higher for comparisons of ears belonging to group 6 onwards, between

Fig. 1. Tillering pattern in rice



'A' represents the apical ear. The numbers represent the category of tillers. Only primaries and secondaries are shown. 'C' represents the coleoptile. Dashed line represents the primary leaves. Dashed line bent at right angle represents ordinary leaves.

Fig. 2. Tillering system in rice indicating mutant clusters



'A' represents the apical ear. Numbered small circles represent individual ears. The outer circles represent all ears in each primary group. The connecting lines represent the mutant cluster sharing frequencies. The solid lines indicate that the compared ear categories have a mutation in common. The length of an ear circle arc supporting a solid line is proportional to the frequency of cluster sharing. The broken lines represent zero frequencies.

Table 2

Cluster sharing frequencies - comparison between primary groups of ears

Comparison within single plant	No. of cases with a mutation in at least one of the two compared ears	Cases with the same mutation in both of the compared ears	
		Number	Percentage
2 group with 3 group	27	1	3.70
3 " 4 "	106	4	3.77
4 " 5 "	151	6	3.97
5 " 6 "	82	3	3.66
6 " 7 "	26	3	11.54
7 " 8 "	18	2	11.11
8 " 9 "	16	3	18.75
9 " 10 "	13	2	15.38
10 " 11 "	2	0	0.00
11 " A "	4	2	50.00

themselves and to the apical (Table 3) indicating that the primary buds from the sixth onwards have not differentiated from the main shoot in the embyo. They differentiate subsequent to germination.

The low frequency of cluster sharing observed in comparison between adjacent primary groups up to the sixth indicates that the primary tiller primordia as far as the fifth have already differentiated from the main shoot primordium in a mature rice embryo. Eventhough the first axillary meristem rarely develops it can be considered to constitute a prospective independent mutant cluster. Based on these evidences the following six mutually exclusive mutant sectors can be distinguished in a mature rice embryo.

Ear A +
Group 11+10+9+8+7+6

Group 5

Group 4

Group 3

Group 2

Group 1

Table 3

Cluster sharing frequencies—comparisons within the apical group of ears

Comparison within single plant	No. of cases with a mutation in at least one of the two comparedears	Cases with the same mutation in both of the compared ears	
		Number	Percentage
A ear with 11 ear	4	2	50.00
A „ 10 „	11	5	45,45
A „ 9 „	18	5	27.78
A „ 8 „	11	3	27.27
A „ 7 „	9	4	44.44
A „ 6 „	41	6	14.63
11 „ 10 „	2	0	0.00
11 „ 9 „	3	1	33.33
11 „ 8 „	6	0	0.00
11 „ 7 „	0	0	0.00
11 „ 6 ,	4	0	0.00
10 „ 9 „	13	2	15.38
10 „ 8 „	13	3	23.08
10 „ 7 „	7	1	14.30
10 „ 6 „	20	7	2333
9 „ 8 „	16	3	18.75
9 „ 7 „	11	4	36.36
9 „ 6 „	27	2	7.41
8 „ 7 „	18	2	11.11
8 „ 6 „	19	0	0.00
7 „ 6 „	26	3	11.54

The analysis of cluster sharing frequencies for mutations thus indicates that a mature rice embryo contains a minimum of six mutually exclusive mutant sectors. This estimate approaches the suggestion of Swaminathan (1966) that the dormant rice embryo contains 8 to 10 initials which would give rise to tillers, Matsuo *et af.* (1958), Kawai (1962) and Osone (1963) while studying the independence in occurrence of mutations in different tiller groups have also indicated that ears of the lower primary tillers might mutate independent of the ear of the main culm. In the barley embryo, Jacobson (1966) proved anatomically and

mutationally the existence of independent mutant sectors as separate shoct primordia on a meristematic tissue area separated from the apex by leaf primordia.

Within each primary group, comparisons were made between the primary ear and its secondaries and between the different secondaries. The frequencies of cluster sharing were generally higher within the primary groups. Secondary tillers yield the same kind of mutation as the primary tillers from which they arise indicating that there are no independent mutant sectors within primary groups. Osone (1963) reported that secondary tillers gave the same kind of mutation as the primary ones and concluded that the corpus cell groups leading to the secondary tiller consisted part of the corpus cells leading to their mother ones.

A diagramatic representation of the tillering system in rice indicating mutant clusters is given in Fig. 2. The circle marked 'A' represents the apical ear and the numbered small circles represent the individual primary ears according to the tillering pattern in Fig. 1. The outer large circles symbolise all ears collectively in each primary group. The lines connecting rhe circles represent the co mparisons between individual ears and primary groups of different categories. A solid line indicates that the compared ear categories share a mutant in common. The lines connecting primary groups 2 with 3, 3 with 4, 4 with 5 and 5 with 6 are drawn thin because the cluster sharing frequencies in these comparisons are negligibly small (Table 2). The solid connections between individual ears are drawn in such a way that the lengths of the arcs which support the lines are proportional -to the cluster sharing frequencies recorded. The broken lines represent zero frequencies because of low number comparisons.

Several factors bias the cluster sharing frequency estimate, the most important being the size of the segregating ear. Comparison of ears of different sizes often under estimate the cluster sharing frequencies because mutations readily detected in a large ear may not be deected in a small one. Varying amounts of sterility further complicate the situation. Simultaneous occurrence of similar mutations independently in two of the compared ears will increase the observed frequency for cluster sharing.

The study of cluster sharing frequencies of mutation thus indicates that in rice the main shoot and the primary tillers up to the fifth mutate independently becauses their meristematic initials are well differentiated in the embryo. The possibility of incidence of a mutation in any one of these meristematic regions appears to be the same. But the frequencies with which the lower primary tillers emerge show considerable variation, the fourth primary having the highest frequency. Therefore, from the point of view of recovery of mutations it is necessary to provide optimum conditions to facilitate the development of all

primary ears upto the fifth in the M_1 generation. It is also important to collect separately all the primary ears upto the fifth besides the main ear in selecting M_1 material to be carried forward to the M_2 in a mutation breeding programme.

Summary

Rice seeds were treated with two doses each of EMS and gamma rays. The tillers in the M_1 generation were classified according to their ontogenic relationship. The M_2 generation was raised as M_1 *ear* progenies. Seedlings were scored for chlorophyll and other seedling mutants. The cluster sharing frequencies for mutations i. e., the frequency with which two M_1 ears segregated for identical mutations were determined by pair wise comparisons. The following conclusions were drawn.

The frequencies of emergence of various categories of tillers are different. Tillers in the axils of the coleoptile and the first primary leaf do not normally develop. The frequencies for primary tillers increase upto the fourth and thereafter gradually decrease.

Analysis of the frequencies of cluster sharing reveals that the primary tiller primordia upto the fifth have already differentiated from the main shoot primordium in the rice embryo. Thus there are at least six mutally exclusive mutant sectors which do not share a mutation in common. Independent mutant sectors are not detected within primary groups.

A diagramatic representation of the tillering system in rice indicating mutant clusters is presented. The diagram provides information on cluster sharing frequencies of mutations and gives an idea of the degree of differentiation in the dormant rice embryo.

The present study indicates that from the point of view of recovery of mutations, it is necessary to provide optimum conditions to facilitate the development of the lower primary ears in the M_1 plants. It is also important to collect all the primary ears up to the fifth besides the main ear from the M_1 plants for raising the M_2 generation in order to recover all the mutations induced.

സംഗ്രഹം

നെൽച്ചെടിയിൽ സാധാരണയായി കോളിയോപ്റ്റയിൽ, പ്രാഥമിക പത്രം എന്നിവയോടനുബന്ധിച്ച് ചിനപ്പുകൾ ഉത്ഭവിക്കാറില്ല. നാലാമത്തെ പ്രാഥമിക ചിനപ്പാണ് ഏറ്റവും കൂടുതൽ ചെടികളിൽ ആവിർഭവിക്കുന്നത്.

നെൽവിത്തിലെ ഭ്രൂണത്തിൽ അഞ്ചാമത്തെ പ്രാഥമിക മുലികം വരെ ശീർഷസ്ഥ മുലികത്തിൽ നിന്ന് വിഭേദിത സ്ഥിതിയിലാണ്. ഒരേ പ്രാഥമിക മുലികത്തിൽ വ്യത്യ

സ്ഥ വിഭാഗങ്ങളം ഉള്ളതായി കാണുന്നില്ല. അപ്രകാരം ഒരു ഭൂണത്തിൽ ഏറ്റവും കുറ ഞ്ഞത് ആറ് വ്യത്യസ്ഥ മൗലികങ്ങളം ഉണ്ടെന്നും ഇവയിൽ ഓരോന്നിലും വിഭിന്ന മ്യൂട്ടേഷ നുകളം ആവിർഭവിക്കുവാൻ സാദ്ധ്യത ഉണ്ടെന്നും അനുമാനിക്കാം.

നെല്ലിലെ മ്യൂട്ടേഷൻ പ്രജനന പ്രക്രിയയിൽ പ്രധാന ചിനപ്പിലേയും അടിയി ലുള്ള അഞ്ചുവരെ പ്രാഥമിക ചിനപ്പുകളിലേയും മണികളം ശേഖരിക്കേണ്ടതിന്റെ ആവ ശ്യം ഇതിൽനിന്ന് വ്യക്തമാണ്. എല്ലാ മ്യൂട്ടേഷനുകളും പ്രകടമാക്കുന്നതിനും അവയെ വേർതിരിച്ചെടുക്കുന്നതിനും ഇത് അത്യന്താപേക്ഷിതമാണ്.

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