

Latin-American Study Group Meeting on Induced
Mutations and Crop Improvement

MUTATIONAL RECONSTRUCTION OF CROP IDEOTYPES

By

M.S. SWAMINATHAN,
Indian Agricultural Research Institute,
New Delhi, India.

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I. INTRODUCTION:

The exploitation of heterosis in the form of commercial hybrids, the enhancement of the effects of additive gene action through synthetics and composites and the development of the concept of a dwarf plant type suited to good agronomy have all helped to raise the ceiling to yield in many crop plants to a level not considered possible before. Based on an understanding of the factors governing productivity, conceptual models or "ideotypes" (1) of crop varieties can be constructed, which if realised through breeding, would help to achieve still higher yields. The following are some of the desirable attributes of crop ideotypes, particularly in the tropics and subtropics where crop growth is possible throughout the year:

- a) Superior population performance.
- b) High productivity per day
- c) High photosynthetic ability.
- d) Low photo-respiration.
- e) Photo and thermo-insensitivity.
- f) High response to nutrients
- g) High productivity per unit of water
- h) Multiple resistance to pests
- i) Better protein quantity and quality
- j) Crop canopies that can retain and fix a maximum of CO_2 .

It is such a shift in selection criteria that have provided special scope for deploying mutation breeding techniques very

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effectively in the development of crop varieties suited for present-day needs (2,3). As a result, several new crop varieties developed by mutation breeding have been released in recent years for commercial cultivation in India (Table 1.) The results of some recent research at the Indian Agricultural Research Institute on the practical realisation of desirable ideotypes through mutation breeding are summarised in this paper:

2. BREEDING NEW IDEOTYPES OF WHEAT FOR UNIRRIGATED AREAS:

Nearly 66% of the wheat area of about 14 million hectares in India depends for crop growth only on rainfall. Asana (4) has developed an ideotype for such areas, which is designed to achieve a maximisation of the yield of the main tiller thereby compensating for poor tillering and upon the dissociation of undesirable associations such as earliness and poor root growth and earliness and a low number of spikelets per ear. This ideotype (Fig.1.) has the following attributes:

- a) A large number of spikelets and grains on the main ear through adventitious branching in the ear.
- b) A long peduncle which forms a substantial proportion of the length of the stem, thereby increasing photosynthesis in the peduncle and flag leaf.
- c) A semi-dwarf plant habit which helps to avoid lodging in fields supplied with fertilizer
- d) About 7 leaves on the main axis with a large flag leaf; the leaves preferably being arranged horizontally so as to permit better retention of dew and also better light interception.
- e) A good growth of roots in the 3rd and 4th feet of soil depth.
- f) Emergence of ears at such a time that will permit grains to develop at least for 5 weeks at a mean maximum temperature of about 25°C.

A study was initiated by Mehta and Swaminathan (unpublished) in 1967 to ascertain the possibility of isolating genotypes with the above attributes. The material for the study consisted of an induced

branched ear mutant of Triticum aestivum var. N P 797 (5) and tetraploid branched ear material derived from crosses between T. durum and T. turgidum var. mirabile. The material was treated with both gamma rays (20 Kr and 30 Kr), Ethylmethane Sulphonate (0.2%) and N-Nitroso-N-Methyl Urea } 0.02% }. Recurrent selection was practised for the desirable characters in M₂ and later generations and the extent of progress made so far in achieving the desirable ideotype is summarised in Table 2. The data indicate that the chances for realising Asana's ideotype for unirrigated wheat are very high. The most promising selections are being subjected to a further cycle of mutagen treatment, selection and recombination.

3. ALTERING LEAF CHARACTERS IN RICE TO PROMOTE BETTER LIGHT INTERCEPTION:

The introduction of the dwarfing gene in Oryza sativa sub sp. indica has helped to increase the yield of indica rices substantially through fertilizer application. In many high-yielding dwarf indicas, the total biological yield does not appear to be higher than in the tall indicas. The higher grain yield really arises from a higher harvest index (i.e., grain/straw ratio) The data from a study conducted by Asana and Salunke (unpublished) in Taichung Native 1 (a dwarf indica from Taiwan), Basmati 370 (a tall indica from India with long slender grains) and Jamuna (a dwarf, fine-grain variety developed at the I.A.R.I. by crossing Taichung Native 1 with Basmati 370 followed by four backcrosses to the Basmati parent) are given in Table 3. A detailed analysis of the production physiological processes in these 3 varieties by Asana and Salunke indicated that the net assimilation rate decreases with a drop in light transmission about 30 days after transplanting. The rapid fall in light intensity after 30 days appeared to affect adversely tillering and the realisation of the full sink capacity. A smaller leaf size and a lower number of leaves hence appeared to be desirable attributes for promoting a higher net assimilation rate in the population as a whole.

In order to examine the influence of leaf characters such as number, size, orientation and senescence pattern on percentage light transmission and net assimilation rate, the rice varieties Taichung Native 1 and Tainan 3 were treated with a wide range of mutagens including gamma rays, ethylmethane sulphonate and nitrosomethyl urea. Mutants with altered leaf characteristic were isolated in M₂, M₃ and later generations (Siddiq, Singh and Swaminathan, unpublished). There was considerable variation in the size, number, alignment and ageing pattern of leaves in the mutants (Fig. 2.). Some of these mutants had associated changes in panicle characters, such as density and fertility. Mutants with normal panicles but altered leaf characters are currently being used in studies of light transmission and dry matter production.

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Qualset et al. (6) have recently described a mutant in bread wheat which influences the structure of the crop canopy and thereby affords an opportunity to evaluate light interception and the physiological aspects of crop productivity. Evans and Dunstone (7) have concluded from a study of physiological changes in the evolution of yield in Triticum species that so far evolution has progressed through changes in grain and leaf size and in the proportion of dry weight mobilized to the grain. They however, feel that while photosynthetic rate has not limited the evolution of yield in wheat up to the present, it may well limit further evolution. The proceedings of a recent Symposium on "Physiological aspects of crop yield" (8) also make it clear that the next major break-through in increasing productivity may come from the development of plant types which possess an inherently greater potential for biological yield, since the advantage conferred by alterations in the harvest index has already been exploited to a great extent.

4. INDUCING PHOTO-INSENSITIVITY IN GOSSYPIUM HIRSUTUM:

Several varieties of G hirsutum have been developed in South India which possess a good yield potential and excellent fibre properties. These strains when grown at Delhi and other parts of North India start flowering only when the day length goes below 12 hours. With the onset of short days, the temperature also goes down and the yield of the varieties is poor. In order to isolate photo-insensitive mutants, Jain and Raut (unpublished) treated the long staple variety MCU-5 with different doses of gamma rays. The M_2 populations were screened for flowering habit and several mutants combining good yield and staple length with an early flowering habit were isolated (Table 4). These data suggest that in addition to selection in segregating generations being carried out under diverse environments as suggested by Borlaug (9) and Finlay (10), selection in mutagen-treated populations may also prove valuable for breaking the barriers to wide adaptation based on photo- and thermal-sensitivity.

5. IMPROVEMENT OF GRAIN SETTING IN TRITICALE:

Recent research has revealed that in hexaploid ($2n = 42$) Triticales, seed fertility can be improved greatly by selection, thereby indicating that sterility is under genetic control. There is evidence from the work of Dr. R. Riley and his co-workers at the Cambridge Plant Breeding Institute that in addition to disturbances in the breeding system, timing imbalance in meiotic stages leading to pairing and disjunctional

abnormalities may be responsible for sterility in the amphidiploids between Triticum durum and Secale cereale. Timing imbalance is known to be under genetic control and hence can be remedied by selecting appropriate gene combinations.

Some promising Triticales derived from crosses between T. durum and S. cereale were treated in 1967 by Sharma and Swaminathan (unpublished) with different doses of γ -rays and EMS. Observations were recorded on the number of grains per spike, number of spikelets per spike and the yield of the main spike in the M_2 and M_3 populations. The variability in the number of grains per spike was much greater in the mutagen treated populations as compared to the control (Table 5). The population has responded positively to selection.

Apart from variability for seed fertility, the M_2 and M_3 populations contained plants with plump grains, early maturity and dwarf stature. The seeds of these mutants are being multiplied for trials.

6. INDUCED VARIATION FOR PROTEIN PROPERTIES:

a) Bread Wheat: The development of rapid screening techniques such as the dye-binding-capacity (DBC) method for estimating the content of protein and basic amino acids, has made the examination of large populations of mutagen-treated material possible. Studies by Anand Kumar (unpublished) in the bread wheat variety Sharbati Sonora have shown that the variation for D.B.C. values is considerably enlarged by both γ -ray and EMS treatments (Fig. 3). It is also of interest that some induced mutants, such as Pusa Lerma, an amber-grain mutant of the Mexican semi-dwarf and red-grain variety, Lerma Rojo, show a striking enhancement in protein content with fertilizer application (Table 6). The data in Table 6 indicate that varieties differ in their ability to produce more protein with increased fertilizer application.

Pusa Lerma, a gamma-ray induced amber-grain mutant of Lerma Rojo, has given high yields in national trials conducted under the All-India Co-ordinated Wheat Improvement Programme in Central and Peninsular India. Its performance as compared to the best check, Kalyan Sona, is given in Table 7. Monosomic analysis has revealed that chromosome 3A of Lerma Rojo carries genes both for grain colour and protein content (11).

b) Barley: Munck et al. (12) have studied the genetics of spontaneously occurring high protein and high protein-cum-high lysine (Hiproly) character in barley. It is clear from their study that these traits are under simple genetic control. During a study of the M_2 and M_3 populations of a 6-rowed spring barley variety N.P.113 treated with γ -rays, Fast neutrons, EMS and NMU, several dwarf and early mutants were observed (Bansal, unpublished). Some of these mutants had a good yield potential and one early mutant, B.M. 20, had a protein content of over 17% (Table 8). This high protein mutant had normal fertility and grain development and was isolated in the treatment with 0.3% EMS (13).

In the M_2 progeny of EMS (0.3%) treated N.P. 113, a mutant characterised by a depression on the dorsal side of the grain was isolated. This "Notched grain" mutant was late by 8 to 10 days, had normal fertility and a 30% lower 1000-kernel weight. This mutant had over 17% protein content and it has been crossed with the parent strain to ascertain whether the notched grain character can be separated from the high protein trait (Table 9). The mutant had a lysine content of 3.49 gm per 100 gm protein and this was only slightly lower than that of the parent.

c) Panicum miliaceum: This millet has excellent protein properties (Table 10), but its yield potential is low. Hence, seeds of a few cultivars of this millet have been treated with mutagens, in order to isolate dwarf and fertilizer-responsive strains.

7. INDUCTION OF MUTATIONS AT THE HAPLOID LEVEL:

With the standardisation of a technique for the raising of haploids from anther cultures by Guha and Maheshwari (14) a way is now open for the large scale production of haploids and their use in mutation breeding. While the original finding was made in Datura innoxia, subsequent research has shown that haploids of Nicotiana tabacum (15,16,17,18) and of Oryza sativa (19,20), can be produced by in vitro anther culture. Nitsch and Pereau-Leroy (21) have used mutagens in culture media for inducing mutations at the haploid level in Nicotiana tabacum. Gamma rays were more effective than EMS in yielding mutants (22).

For using this technique successfully, it is necessary to standardise techniques for getting consistently a large number of haploids. Since genotypic differences in the ability of pollen to form embryoids seem to exist, Guha and Swaminathan (23) carried out a study with 20 different rice varieties. Since the techniques used may be of interest they are described in detail here.

Florets of twenty different varieties of rice were collected while still enclosed within the sheath. After sterilization in dilute chlorox solution, they were transferred to an inoculation chamber where the buds were dissected and five anthers were planted on a sterile nutrient medium. Blaydes' medium (24) for soybean callus cultures with 3% sucrose was used as the basal medium. Various growth substances such as indoleacetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, yeast extract and coconut milk were also added to the basal medium, when required. The media were sterilized by autoclaving at 15 lbs/in² for 15 minutes and solidified with 0.8% agar. The pH was adjusted to 5.8. Cultures were kept at 25°, for the initial 3-4 weeks in dark and then in light (warm white fluorescent, 1000 ft-c).

Anthers were planted at three stages of development - late meiotic and tetrad formation stage, uninucleate stage and on the day of anthesis. Young anthers implanted at the time of tetrad formation or earlier failed to grow on any media. When planted on the day of anthesis,

when pollen grains are mature, most of the anthers opened liberating the pollen grains which germinated giving rise to pollen tubes. Only anthers containing uninucleate pollen grains showed differentiation of pollen embryoids. Therefore, further experiments were carried out only with anthers containing uninucleate pollen grains. These results are similar to those reported for tobacco pollen.

In the basal medium without any growth adjuvants, anthers degenerated two to three weeks after inoculation. The addition of coconut milk (15%) and yeast extract (1000 ppm) stimulated many pollen grains to divide. The incorporation of 2 ppm IAA and 2 ppm 2,4-D and 1 ppm kinetin further stimulated division and, therefore, this medium was generally used for the induction of mitosis in pollen grains.

Acetocarmine squashes of the anthers were prepared at regular intervals throughout the culture period to assess the state of development of pollen. After one week in culture, some of the pollen grains started enlarging and attained two to three times their original volume. In these grains, the cytoplasm stained very densely. In another week, they underwent mitosis and became binucleate, where both the cells were morphologically alike. Soon, mitosis commenced in both the cells, which continued giving rise to multicellular pollen grains 3-4 weeks after planting. In older cultures, these multicellular masses (embryoids) burst out of their original wall and assumed a globular stage. If embryoids were dissected out at this stage and planted on a fresh medium containing coconut milk (15%) without auxin, they exhibited organized growth and developed into normal plantlets. Chromosome counts in root tips in several such seedlings revealed that they are haploids with $2n = 12$. However, if embryoids were allowed to grow in the original medium where they arose, divisions in the pollen grains continued in an irregular manner resulting in a mass of callus. It appeared that during the initial stages of development, 'pollen embryoids' get attached to the wall of the anthers by a suspensor-like outgrowth. It is very easy to miss this attachment, if anthers are squashed or subjected to even slight pressure. Careful dissections revealed this attachment, the anther wall apparently acting like a placenta. However, after some time, the embryoids get detached from the anther wall and grow independently.

There was a wide variation in the twenty varieties studied with regard to their potential for giving rise to androgenic haploids through this technique. Some primitive cultivars from Assam like Assam-5 gave an excellent response (Table 11). Tropical japonica varieties from Taiwan like Taichung 65 and Tainan-3 did not behave any differently than many of the indica varieties. The dwarf, high yielding indica strain, IR-8, developed at the International Rice Research Institute, the Phillipines, was found suitable for use in such studies. The promising varieties are now being used in studies on the induction of mutations at the haploid level.

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Table 1 : Commercial varieties released in India through mutation breeding

Crop	Name of the variety		Mutagen and year of release	Characteristics	Reference
	Parent	Mutant			
1. Wheat	N.P.799	N.P.836	X-rays, 1961	Fully awned and about 10% more yield	Jagathesan <u>et al.</u> (25)
	Sonora 64	Sharbati Sonora	UV + gamma rays, 1967	Amber grain, high protein	Varughese & Swaminathan(26)
2. Rice	T.141	Jagannath	X-rays, 1969	Fertilizer responsive, short stature with an average height of 95 cms in contrast to 140 cm of T.141	S.K.Sinha (Unpublished)
3. Castor	H.C.6	Aruna	Thermal Neutrons, 1968	120 days duration in contrast to 250 days in H.C.6; high yield	Bala Narasaiah & Kulkarni(27)
4. French Bean	Wax podded	Pusa Parvati	X-rays, 1969	Early, bush type with attractive light green pods.	Vishnu Swarup & Gill (28)
5. Tomato	Sioux	S.12	gamma rays,1969	Dwarf, 30% more yield than Sioux	K.S.Nandpuri (Unpublished)
	Meeruti	Pusa Lal Meeruti	X-rays, 1969	Uniform red colour	Jain, Sur and Raut (29)

Table 2: Progress made in the development of a branched-ear wheat strain

Plant part	Characteristics of the initial material in 1967	Characteristics of selections in mutagen-treated populations in 1970
Ear	Erratic branching, low spikelet fertility and low 1000-kernel weight	Good branching with as many as 19 adventitious branches per spike, good fertility with as many as 207 grains per spike, good 1000-kernel weight.
Peduncle	Poor peduncle exertion	Peduncle index high, promoting greater photosynthesis in the peduncle.
Plant height	Semi-dwarf to dwarf (less than 100 cms)	Semi-dwarf to dwarf (less than 100 cms)
Leaves	Leafy types with 14 to 15 leaves on the main stem	Two single leaf and several reduced leaf number mutants were isolated.
Roots	Variable depths	Deep root system, reaching the 3rd and 4th feet of soil depth
Ear	Very late, taking about 117 to 120 days from sowing to earing	Early mutants taking about 105 days from sowing to earing were isolated

Table 3 : Yield characteristics in dwarf and tall indica rice varieties

Character	V A R I E T Y		
	Taichung Native-1	Basmati-370	Jamuna
<u>FIELD</u>			
Grain yield (Q/ha)	74.4	31.1	52.8
Straw yield (Q/ha)	73.3	121.0	63.7
Total biological yield (Q/ha)	147.7	152.1	116.5
<u>POT</u>			
Grain yield (gm/plant)	91.0	72.5	84.7
Straw yield (gm/plant)	73.4	116.4	91.2
Harvest index	55.4	38.4	48.2

Table 4 : Characteristics of some induced mutants in Gossypium hirsutum var. MCU-5.

Material	gamma-ray dose	days to flower	yield in gms per plot	Staple length (mm)
M.C.U.5	-	120	25	29.0
Mutant No.1	5 Kr.	66	165	26.0
" 2	5 Kr.	62	300	27.5
" 4	20 Kr.	64	250	28.7
" 6	20 Kr.	69	200	31.6
" 8	30 Kr.	69	150	29.0
" 9	40 Kr.	66	120	26.0
" 10	50 Kr.	63	240	26.0

Table 5

Variation for grain fertility in the M_3 generation
of mutagen - treated Triticale

Strain	Treatment	No. of Plants studied	No. of seeds per spike	
			Range	Mean
6456-3-1	Control	1000	0-70	40.08
	EMS (0.2%)	1000	0-90	45.52
6450-2-1	Control	200	25-73	50.54
	20 Kr. gamma rays	1000	0-50	36.76
	EMS (0.2%)	1000	0-86	37.47
5027	Control	200	8-78	47.87
	20 Kr. gamma rays	1000	0-101	48.36

Table 6

All India Co-ordinated National Trial, 1969-70
Percentage of protein (on oven dry basis)*

Variety	Treatment		
	M_0	M_1	M_2
Kalyan sona	11.30	12.11	12.38
HD 1941	12.23	12.61	14.13
HD 1944	12.32	12.61	12.70
HD 1949	12.02	13.00	13.20
HD 1539	11.94	13.33	15.06
HD 1674	11.52	13.16	13.25
Pusa Lerma	11.77	14.85	16.45
EA 222-1	11.73	12.36	12.78
D.2117	12.91	13.08	13.75
WL 202	13.63	13.79	14.09
NI 5645	12.32	13.46	14.26
UP 301	12.87	13.92	14.55

M_0 - No fertilizer

M_1 - N 135 + P 67 + K 34 Kg/ha.

M_2 - N 200 + P 100 + K 50 Kg/ha.

* Data of A. Austin, H.D. Singh and V.K. Hanslas.

Table 7

Yield of Pusa Lerma (in Q./Ha.) in National Trials (1969-70)

Central Zone										Peninsular Zone										
M. Pradesh				Gujarat				Zonal Mean		Maharashtra				Zonal Mean						
M ₁		M ₂		M ₁		M ₂		M ₁		M ₂		M ₁		M ₂		M ₁		M ₂		
Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	
Pusa Lerma	38.5	3	41.0	1	27.5	2	30.8	1	35.8	2	38.5	1	43.1	1	41.7	1	43.1	1	41.7	1
Kalyan Sona	38.2	4	40.5	3	27.6	1	23.7	5	35.6	3	36.3	3	37.6	3	33.7	7	37.6	3	33.7	7

Y: Yield
R: Rank

M₁: N 135 + P 67 + K 34 Kg/ha

M₂: N 200 + P100 + K 50 Kg/ha

Table 8

Characteristics of some barley mutants

Material	Grain yield (Q/ha.)	Days to Maturity	1000-kernel weight(gms)	Protein content(%)
N.P.113(control)	54.1	120	42.0	11.2
B.M. 10	52.6	100	37.5	11.2
B.M. 20	43.9	95	36.3	17.45
B.M. 21	55.5	96	38.4	14.4

Table 9

Protein content of Notched grain Mutant in Barley var. N.P.113

Material	Protein(%)	DBC Value
N.P.113(control)	(a) 10.23	0.21
	(b) 11.24	0.22
Notched Mutant	(a) 18.29	0.305
	(b) 17.45	0.30

Table 10

Protein properties of some minor millets

Species and variety	Protein (%)	Lysine (Gm/100 gm Protein)	Tryptophan (Gm/100 gm protein)
<u>Eleusine corocana</u>			
I.E. 903	6.23	2.13	1.44
I.E. 901	7.97	2.63	1.36
<u>Setaria italica</u>			
I.S. 711	10.02	2.29	0.81
I.S. 263	11.56	2.58	1.05
<u>Panicum miliaceum</u>			
I. PM 1640	11.60	4.35	1.10
I. PM 1639	12.19	4.15	1.18
<u>Paspalum</u>			
<u>Scrobiculatum</u>			
I.Ps. 261	11.56	3.55	0.92
I.Ps. 19	12.62	3.78	1.14

Table 11 : Variation in embryoid formation in rice varieties

Sl. No.	Strain	No. of anthers inoculated	Response
<u>Indica strains</u>			
1.	Assam 271	160	Some callusing of pollen grain, but no embryoid formation.
2.	Assam 10456	130	No response
3.	Assam 10303	80	Some callusing of the pollen but no embryoid formation
4.	Assam Local	100	No response
5.	Assam 5955	1735	11% anthers produced pollen embryoid
6.	Assam 5	172	26% anthers produced pollen embryoid
7.	Tripura 2-5744	186	No response
8.	Assam culture	100	No response
9.	Soft grain type of West Bengal	245	Callusing
10.	Sabarmati	230	No response
11.	IR-8	1576	0.8% of the anthers showed pollen embryoids
12.	IR-532	100	No response
13.	Taichung Native-1	360	Pollen grains enlarged but no division
14.	N.P.130	600	No response
15.	Basmati-370	1735	0.3% anthers produced pollen embryoids
16.	Kala-Basmati	20	No response
17.	Sigadis	100	No response
18.	Sigadis IR-8 hybrid	148	No response
<u>Japonica strains</u>			
19.	Taichung 65	720	Slight callusing but no embryoid formation
20.	Tainan 3	480	No response

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- a) Superior population performance.
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effectively in the development of crop varieties suited for present-day needs (2,3). As a result, several new crop varieties developed by mutation breeding have been released in recent years for commercial cultivation in India (Table 1.) The results of some recent research at the Indian Agricultural Research Institute on the practical realisation of desirable ideotypes through mutation breeding are summarised in this paper:

2. BREEDING NEW IDEOTYPES OF WHEAT FOR UNIRRIGATED AREAS:

Nearly 66% of the wheat area of about 14 million hectares in India depends for crop growth only on rainfall. Asana (4) has developed an ideotype for such areas, which is designed to achieve a maximisation of the yield of the main tiller thereby compensating for poor tillering and upon the dissociation of undesirable associations such as earliness and poor root growth and earliness and a low number of spikelets per ear. This ideotype (Fig.1.) has the following attributes:

- a) A large number of spikelets and grains on the main ear through adventitious branching in the ear.
- b) A long peduncle which forms a substantial proportion of the length of the stem, thereby increasing photosynthesis in the peduncle and flag leaf.
- c) A semi-dwarf plant habit which helps to avoid lodging in fields supplied with fertilizer
- d) About 7 leaves on the main axis with a large flag leaf; the leaves preferably being arranged horizontally so as to permit better retention of dew and also better light interception.
- e) A good growth of roots in the 3rd and 4th feet of soil depth.
- f) Emergence of ears at such a time that will permit grains to develop at least for 5 weeks at a mean maximum temperature of about 25°C.

A study was initiated by Mehta and Swaminathan (unpublished) in 1967 to ascertain the possibility of isolating genotypes with the above attributes. The material for the study consisted of an induced

branched ear mutant of Triticum aestivum var. N P 797 (5) and tetraploid branched ear material derived from crosses between T. durum and T. turgidum var. mirabile. The material was treated with both gamma rays (20 Kr and 30 Kr), Ethylmethane Sulphonate (0.2%) and N-Nitroso-N-Methyl Urea (0.02%). Recurrent selection was practised for the desirable characters in M₂ and later generations and the extent of progress made so far in achieving the desirable ideotype is summarised in Table 2. The data indicate that the chances for realising Asana's ideotype for unirrigated wheat are very high. The most promising selections are being subjected to a further cycle of mutagen treatment, selection and recombination.

3. ALTERING LEAF CHARACTERS IN RICE TO PROMOTE BETTER LIGHT INTERCEPTION:

The introduction of the dwarfing gene in Oryza sativa sub sp. indica has helped to increase the yield of indica rices substantially through fertilizer application. In many high-yielding dwarf indicas, the total biological yield does not appear to be higher than in the tall indicas. The higher grain yield really arises from a higher harvest index (i.e., grain/straw ratio) The data from a study conducted by Asana and Salunke (unpublished) in Taichung Native 1 (a dwarf indica from Taiwan), Basmati 370 (a tall indica from India with long slender grains) and Jamuna (a dwarf, fine-grain variety developed at the I.A.R.I. by crossing Taichung Native 1 with Basmati 370 followed by four backcrosses to the Basmati parent) are given in Table 3. A detailed analysis of the production physiological processes in these 3 varieties by Asana and Salunke indicated that the net assimilation rate decreases with a drop in light transmission about 30 days after transplanting. The rapid fall in light intensity after 30 days appeared to affect adversely tillering and the realisation of the full sink capacity. A smaller leaf size and a lower number of leaves hence appeared to be desirable attributes for promoting a higher net assimilation rate in the population as a whole.

In order to examine the influence of leaf characters such as number, size, orientation and senescence pattern on percentage light transmission and net assimilation rate, the rice varieties Taichung Native 1 and Tainan 3 were treated with a wide range of mutagens including gamma rays, ethylmethane sulphonate and nitrosomethyl urea. Mutants with altered leaf characteristic were isolated in M₂, M₃ and later generations (Siddiq, Singh and Swaminathan, unpublished). There was considerable variation in the size, number, alignment and ageing pattern of leaves in the mutants (Fig. 2.). Some of these mutants had associated changes in panicle characters, such as density and fertility. Mutants with normal panicles but altered leaf characters are currently being used in studies of light transmission and dry matter production.

Qualset et al. (6) have recently described a mutant in bread wheat which influences the structure of the crop canopy and thereby affords an opportunity to evaluate light interception and the physiological aspects of crop productivity. Evans and Dunstone (7) have concluded from a study of physiological changes in the evolution of yield in Triticum species that so far evolution has progressed through changes in grain and leaf size and in the proportion of dry weight mobilized to the grain. They however, feel that while photosynthetic rate has not limited the evolution of yield in wheat up to the present, it may well limit further evolution. The proceedings of a recent Symposium on "Physiological aspects of crop yield" (8) also make it clear that the next major break-through in increasing productivity may come from the development of plant types which possess an inherently greater potential for biological yield, since the advantage conferred by alterations in the harvest index has already been exploited to a great extent.

4. INDUCING PHOTO-INSENSITIVITY IN GOSSYPIUM HIRSUTUM:

Several varieties of G. hirsutum have been developed in South India which possess a good yield potential and excellent fibre properties. These strains when grown at Delhi and other parts of North India start flowering only when the day length goes below 12 hours. With the onset of short days, the temperature also goes down and the yield of the varieties is poor. In order to isolate photo-insensitive mutants, Jain and Raut (unpublished) treated the long staple variety MCU-5 with different doses of gamma rays. The M₂ populations were screened for flowering habit and several mutants combining good yield and staple length with an early flowering habit were isolated (Table 4). These data suggest that in addition to selection in segregating generations being carried out under diverse environments as suggested by Borlaug (9) and Finlay (10), selection in mutagen-treated populations may also prove valuable for breaking the barriers to wide adaptation based on photo- and thermal-sensitivity.

5. IMPROVEMENT OF GRAIN SETTING IN TRITICALE:

Recent research has revealed that in hexaploid ($2n = 42$) Triticales, seed fertility can be improved greatly by selection, thereby indicating that sterility is under genetic control. There is evidence from the work of Dr. R. Riley and his co-workers at the Cambridge Plant Breeding Institute that in addition to disturbances in the breeding system, timing imbalance in meiotic stages leading to pairing and disjunctional

abnormalities may be responsible for sterility in the amphidiploids between Triticum durum and Secale cereale. Timing imbalance is known to be under genetic control and hence can be remedied by selecting appropriate gene combinations.

Some promising Triticales derived from crosses between T. durum and S. cereale were treated in 1967 by Sharma and Swaminathan (unpublished) with different doses of γ -rays and EMS. Observations were recorded on the number of grains per spike, number of spikelets per spike and the yield of the main spike in the M_2 and M_3 populations. The variability in the number of grains per spike was much greater in the mutagen treated populations as compared to the control (Table 5). The population has responded positively to selection.

Apart from variability for seed fertility, the M_2 and M_3 populations contained plants with plump grains, early maturity and dwarf stature. The seeds of these mutants are being multiplied for trials.

6. INDUCED VARIATION FOR PROTEIN PROPERTIES:

a) Bread Wheat: The development of rapid screening techniques such as the dye-binding-capacity (DRC) method for estimating the content of protein and basic amino acids, has made the examination of large populations of mutagen-treated material possible. Studies by Anand Kumar (unpublished) in the bread wheat variety Sharbati Sonora have shown that the variation for D.B.C. values is considerably enlarged by both γ -ray and EMS treatments (Fig. 3). It is also of interest that some induced mutants, such as Pusa Lerma, an amber-grain mutant of the Mexican semi-dwarf and red-grain variety, Lerma Rojo, show a striking enhancement in protein content with fertilizer application (Table 6). The data in Table 6 indicate that varieties differ in their ability to produce more protein with increased fertilizer application.

Pusa Lerma, a gamma-ray induced amber-grain mutant of Lerma Rojo, has given high yields in national trials conducted under the All-India Co-ordinated Wheat Improvement Programme in Central and Peninsular India. Its performance as compared to the best check, Kalyan Sona, is given in Table 7. Monosomic analysis has revealed that chromosome 3A of Lerma Rojo carries genes both for grain colour and protein content (11).

b) Barley: Munck et al. (12) have studied the genetics of spontaneously occurring high protein and high protein-cum-high lysine (Hiproly) character in barley. It is clear from their study that these traits are under simple genetic control. During a study of the M_2 and M_3 populations of a 6-rowed spring barley variety N.P.113 treated with γ -rays, Fast neutrons, EMS and NMJ, several dwarf and early mutants were observed (Bansal, unpublished). Some of these mutants had a good yield potential and one early mutant, B.M. 20, had a protein content of over 17% (Table 8). This high protein mutant had normal fertility and grain development and was isolated in the treatment with 0.3% EMS (13).

In the M_2 progeny of EMS (0.3%) treated N.P. 113, a mutant characterised by a depression on the dorsal side of the grain was isolated. This "Notched grain" mutant was late by 8 to 10 days, had normal fertility and a 30% lower 1000-kernel weight. This mutant had over 17% protein content and it has been crossed with the parent strain to ascertain whether the notched grain character can be separated from the high protein trait (Table 9). The mutant had a lysine content of 3.49 gm per 100 gm protein and this was only slightly lower than that of the parent.

c) Panicum miliaceum: This millet has excellent protein properties (Table 10), but its yield potential is low. Hence, seeds of a few cultivars of this millet have been treated with mutagens, in order to isolate dwarf and fertilizer-responsive strains.

7. INDUCTION OF MUTATIONS AT THE HAPLOID LEVEL:

With the standardisation of a technique for the raising of haploids from anther cultures by Guha and Maheshwari (14) a way is now open for the large scale production of haploids and their use in mutation breeding. While the original finding was made in Datura innoxia, subsequent research has shown that haploids of Nicotiana tabacum (15,16,17,18) and of Oryza sativa (19,20), can be produced by in vitro anther culture. Nitsch and Pereau-Leroy (21) have used mutagens in culture media for inducing mutations at the haploid level in Nicotiana tabacum. Gamma rays were more effective than EMS in yielding mutants (22).

For using this technique successfully, it is necessary to standardise techniques for getting consistently a large number of haploids. Since genotypic differences in the ability of pollen to form embryoids seem to exist, Guha and Swaminathan (23) carried out a study with 20 different rice varieties. Since the techniques used may be of interest they are described in detail here.

Florets of twenty different varieties of rice were collected while still enclosed within the sheath. After sterilization in dilute chlorox solution, they were transferred to an inoculation chamber where the buds were dissected and five anthers were planted on a sterile nutrient medium. Blaydes' medium (24) for soybean callus cultures with 3% sucrose was used as the basal medium. Various growth substances such as indoleacetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), kinetin, yeast extract and coconut milk were also added to the basal medium, when required. The media were sterilized by autoclaving at 15 lbs/in² for 15 minutes and solidified with 0.8% agar. The pH was adjusted to 5.8. Cultures were kept at 25°, for the initial 3-4 weeks in dark and then in light (warm white fluorescent, 1000 ft-c).

Anthers were planted at three stages of development - late meiotic and tetrad formation stage, uninucleate stage and on the day of anthesis. Young anthers implanted at the time of tetrad formation or earlier failed to grow on any media. When planted on the day of anthesis,

when pollen grains are mature, most of the anthers opened liberating the pollen grains which germinated giving rise to pollen tubes. Only anthers containing uninucleate pollen grains showed differentiation of pollen embryoids. Therefore, further experiments were carried out only with anthers containing uninucleate pollen grains. These results are similar to those reported for tobacco pollen.

In the basal medium without any growth adjuvants, anthers degenerated two to three weeks after inoculation. The addition of coconut milk (15%) and yeast extract (1000 ppm) stimulated many pollen grains to divide. The incorporation of 2 ppm IAA and 2 ppm 2,4-D and 1 ppm kinetin further stimulated division and, therefore, this medium was generally used for the induction of mitosis in pollen grains.

Acetocarmine squashes of the anthers were prepared at regular intervals throughout the culture period to assess the state of development of pollen. After one week in culture, some of the pollen grains started enlarging and attained two to three times their original volume. In these grains, the cytoplasm stained very densely. In another week, they underwent mitosis and became binucleate, where both the cells were morphologically alike. Soon, mitosis commenced in both the cells, which continued giving rise to multicellular pollen grains 3-4 weeks after planting. In older cultures, these multicellular masses (embryoids) burst out of their original wall and assumed a globular stage. If embryoids were dissected out at this stage and planted on a fresh medium containing coconut milk (15%) without auxin, they exhibited organized growth and developed into normal planlets. Chromosome counts in root tips in several such seedlings revealed that they are haploids with $2n = 12$. However, if embryoids were allowed to grow in the original medium where they arose, divisions in the pollen grains continued in an irregular manner resulting in a mass of callus. It appeared that during the initial stages of development, 'pollen embryoids' get attached to the wall of the anthers by a suspensor-like outgrowth. It is very easy to miss this attachment, if anthers are squashed or subjected to even slight pressure. Careful dissections revealed this attachment, the anther wall apparently acting like a placenta. However, after some time, the embryoids get detached from the anther wall and grow independently.

There was a wide variation in the twenty varieties studied with regard to their potential for giving rise to androgenic haploids through this technique. Some primitive cultivars from Assam like Assam-5 gave an excellent response (Table 11). Tropical japonica varieties from Taiwan like Taichung 65 and Tainan-3 did not behave any differently than many of the indica varieties. The dwarf, high yielding indica strain, IR-8, developed at the International Rice Research Institute, the Philippines, was found suitable for use in such studies. The promising varieties are now being used in studies on the induction of mutations at the haploid level.

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Table 1 : Commercial varieties released in India through mutation breeding

Crop	Name of the variety		Mutagen and year of release	Characteristics	Reference
	Parent	Mutant			
1. Wheat	N.P.799	N.P.836	X-rays, 1961	Fully awned and about 10% more yield	Jagathesan <u>et al.</u> (25)
	Sonora 64	Sharbati Sonora	UV + gamma rays, 1967	Amber grain, high protein	Varughese & Swaminathan(26)
2. Rice	T.141	Jagannath	X-rays, 1969	Fertilizer responsive, short stature with an average height of 95 cms in contrast to 140 cm of T.141	S.K.Sinha (Unpublished)
3. Castor	H.C.6	Aruna	Thermal Neutrons, 1968	120 days duration in contrast to 250 days in H.C.6; high yield	Bala Narasaiah & Kulkarni(27)
4. French Bean	Wax podded	Pusa Parvati	X-rays, 1969	Early, bush type with attractive light green pods.	Vishnu Swarup & Gill (28)
5. Tomato	Sioux	S.12	gamma rays, 1969	Dwarf, 30% more yield than Sioux	K.S.Nandpuri (Unpublished)
	Meeruti	Pusa Lal Meeruti	X-rays, 1969	Uniform red colour	Jain, Sur and Raut (29)

Table 2: Progress made in the development of a branched-ear wheat strain

Plant part	Characteristics of the initial material in 1967	Characteristics of selections in mutagen-treated populations in 1970
Ear	Erratic branching, low spikelet fertility and low 1000-kernel weight	Good branching with as many as 19 adventitious branches per spike, good fertility with as many as 207 grains per spike, good 1000-kernel weight.
Peduncle	Poor peduncle exertion	Peduncle index high, promoting greater photosynthesis in the peduncle.
Plant height	Semi-dwarf to dwarf (less than 100 cms)	Semi-dwarf to dwarf (less than 100 cms)
Leaves	Leafy types with 14 to 15 leaves on the main stem	Two single leaf and several reduced leaf number mutants were isolated.
Roots	Variable depths	Deep root system, reaching the 3rd and 4th feet of soil depth
Ear	Very late, taking about 117 to 120 days from sowing to earing	Early mutants taking about 105 days from sowing to earing were isolated

Table 3 : Yield characteristics in dwarf and tall indica rice varieties

Character	V A R I E T Y		
	Taichung Native-1	Basmati-370	Jamuna
<u>FIELD</u>			
Grain yield (Q/ha)	74.4	31.1	52.8
Straw yield (Q/ha)	73.3	121.0	63.7
Total biological yield (Q/ha)	147.7	152.1	116.5
<u>POT</u>			
Grain yield (gm/plant)	91.0	72.5	84.7
Straw yield (gm/plant)	73.4	116.4	91.2
Harvest index	55.4	38.4	48.2

Table 4 : Characteristics of some induced mutants in Gossypium hirsutum var. MCU-5.

Material	gamma-ray dose	days to flower	yield in gms per plot	Staple length (mm)
M.C.U.5	-	120	25	29.0
Mutant No.1	5 Kr.	66	165	26.0
" 2	5 Kr.	62	300	27.5
" 4	20 Kr.	64	250	28.7
" 6	20 Kr.	69	200	31.6
" 8	30 Kr.	69	150	29.0
" 9	40 Kr.	66	120	26.0
" 10	50 Kr.	63	240	26.0

Table 5

Variation for grain fertility in the M_3 generation
of mutagen - treated Triticale

Strain	Treatment	No. of Plants studied	No. of seeds per spike	
			Range	Mean
6456-3-1	Control	1000	0-70	40.08
	EMS (0.2%)	1000	0-90	45.52
6450-2-1	Control	200	25-73	50.54
	20 Kr. gamma rays	1000	0-80	36.76
	EMS (0.2%)	1000	0-86	37.47
5027	Control	200	8-78	47.87
	20 Kr. gamma rays	1000	0-101	48.36

Table 6

All India Co-ordinated National Trial, 1969-70
Percentage of protein (on oven dry basis)*

Variety	Treatment		
	M_0	M_1	M_2
Kalyan sona	11.30	12.11	12.38
HD 1941	12.23	12.61	14.13
HD 1944	12.32	12.61	12.70
HD 1949	12.02	13.00	13.20
HD 1539	11.94	13.33	15.06
HD 1674	11.52	13.16	13.25
Pusa Lerma	11.77	14.85	16.45
EA 222-1	11.73	12.36	12.78
D.2117	12.91	13.08	13.75
WL 202	13.63	13.79	14.09
NI 5645	12.32	13.46	14.26
UP 301	12.87	13.92	14.55

M_0 - No fertilizer

M_1 - N 135 + P 67 + K 34 Kg/ha.

M_2 - N 200 + P 100 + K 50 Kg/ha.

* Data of A. Austin, H.D. Singh and V.K. Hanslas.

Table 7

Yield of Pusa Lerma (in Q/Ha.) in National Trials (1969-70)

		Central Zone								Peninsular Zone											
		M. Pradesh				Gujarat				Zonal Mean				Maharashtra				Zonal Mean			
		M ₁		M ₂		M ₁		M ₂		M ₁		M ₂		M ₁		M ₂		M ₁		M ₂	
		Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R	Y	R
Pusa Lerma		38.5	3	41.0	1	27.5	2	30.8	1	35.8	2	38.5	1	43.1	1	41.7	1	43.1	1	41.7	1
Kalyan Sona		38.2	4	40.5	3	27.6	1	23.7	5	35.6	3	36.3	3	37.6	3	33.7	7	37.6	3	33.7	7

Y: Yield
R: Rank

M₁: N 135 + P 67 + K 34 Kg/ha

M₂: N 200 + P100 + K 50 Kg/ha

Table 8

Characteristics of some barley mutants

Material	Grain yield (Q/ha)	Days to Maturity	1000-kernel weight(gms)	Protein content(%)
N.P.113(control)	54.1	120	42.0	11.2
B.M. 10	52.6	100	37.5	11.2
B.M. 20	43.9	95	36.3	17.5
B.M. 21	55.5	96	38.4	14.4

Table 9

Protein content of Notched grain Mutant in Barley var. N.P.113

Material	Protein(%)	DBC Value
N.P.113(control)	(a) 10.23	0.21
	(b) 11.24	0.22
Notched Mutant	(a) 18.29	0.305
	(b) 17.45	0.30

Table 10

Protein properties of some minor millets

Species and variety	Protein (%)	Lysine (Gm/100 gm Protein)	Tryptophan (Gm/100 gm protein)
<u>Eleusine corocana</u>			
I.E. 903	6.23	2.13	1.44
I.E. 901	7.97	2.63	1.36
<u>Setaria italica</u>			
I.S. 711	10.02	2.29	0.81
I.S. 263	11.56	2.58	1.05
<u>Panicum miliaceum</u>			
I. PM 1640	11.60	4.35	1.10
I. PM 1639	12.19	4.15	1.18
<u>Paspalum</u>			
<u>Scrobiculatum</u>			
I.Ps. 261	11.56	3.55	0.92
I.Ps. 19	12.62	3.78	1.14

Table 11 : Variation in embryoid formation in rice varieties

Sl. No.	Strain	No. of anthers inoculated	Response
<u>Indica strains</u>			
1.	Assam 271	160	Some callusing of pollen grain, but no embryoid formation.
2.	Assam 10456	130	No response
3.	Assam 10303	80	Some callusing of the pollen but no embryoid formation
4.	Assam Local	100	No response
5.	Assam 5955	1735	11% anthers produced pollen embryoid
6.	Assam 5	172	26% anthers produced pollen embryoid
7.	Tripura 2-5744	186	No response
8.	Assam culture	100	No response
9.	Soft grain type of West Bengal	245	Callusing
10.	Sabarmati	230	No response
11.	IR-8	1576	0.8% of the anthers showed pollen embryoids
12.	IR-532	100	No response
13.	Taichung Native-1	360	Pollen grains enlarged but no division
14.	N.P.130	600	No response
15.	Basmati-370	1735	0.3% anthers produced pollen embryoids
16.	Kala-Basmati	20	No response
17.	Sigadis	100	No response
18.	Sigadis IR-8 hybrid	148	No response
<u>Japonica strains</u>			
19.	Taichung 65	720	Slight callusing but no embryoid formation
20.	Tainan 3	480	No response