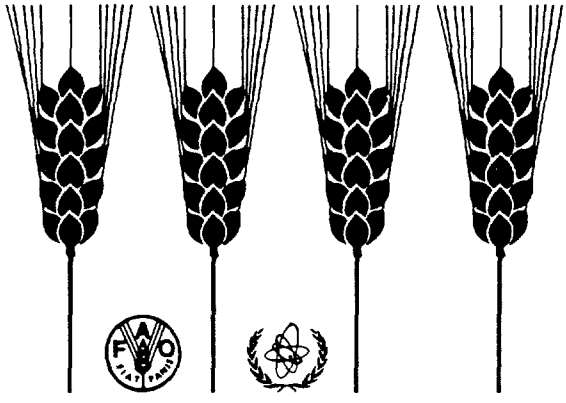

Mutation Breeding Newsletter

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A high yielding mungbean variety through mutation breeding

Dry seeds of yellow mosaic virus (MYMV) susceptible mungbean varieties (Vigna radiata (L.) Wilczek), T-44 and ML 26 were exposed to ^{60}Co gamma rays (10, 50 kR) at the Indian Agricultural Research Institute, New Delhi. M_1 seeds were sown in the kharif (wet) season 1976 along with the unirradiated controls. Each M_1 plant was bagged to ensure selfing. The M_2 and M_3 generations were grown in the kharif seasons 1977 and 1978, respectively, and were handled as pedigree method of breeding. As a standard, the blackgram cultivar UL-2 (also highly susceptible to MYMV) was replicated after each four rows in M_2 and after six rows in M_3. In visual selection, emphasis was on high number of pods plant and/or seeds per pod. 11 promising M_4 lines (eight from ML 26 and three from T-44) were tested in the kharif season 1979 in randomized block design with three replications for yield, yield components and resistance to MYMV.Line ML 26/10/3 with moderate resistance to MYMV, significantly out-yielded the parent variety ML 26. It was derived from the 10 kR dose.

This line was evaluated in multi-location trials in 1980, 1981 and 1982 kharif seasons and in 1981 and 1982 zaid (dry) seasons in comparison with Pant Moong 1 (a newly released variety for U.P.), T-44 and K 851 (older varieties for U.P.), and ML 5 (the national check). The yield and days to maturity are presented in Table 1 and 2. ML26/10/3 has been released as "Pant Moong 2" to the farmers of Uttar Pradesh province in 1982.

Table 1. Yield (kg/ha) of Pant Moong 2 (ML26/10/3) vs. check varieties in trials conducted in Uttar Pradesh (India)

	Varieties				
Year	Pant Moong 2	Pant Moong 1	K 851	T-44	ML 5
Kharif					
1980	825.5	780.0	477.7	532.5	544.0
1981	947.2	910.1	529.2	437.8	864.0
1982	733.3	766.9	499.1	397.0	898.4
Mean	835.3	819.0	502.0	455.8	768.8
Zaid					
1981	523.2	472.8	400.5	443.6	--
1982	686.4	654.4	600.4	499.0	--
Mean	604.8	563.6	500.5	471.3	--

Table 2: Days to maturity of Pant Moong 2 vs. check varieties

	Varieties				
Year	Pant Moong 2	Pant Moong 1	K 851	T-44	ML 5
Kharif					
1980	78	82	74	70	--
1981	67	66	67	62	76
1982	75	78	73	68	88
Mean	73	75	71	67	82
Zaid					
1981	67	70	68	69	--
1982	77	79	77	78	--
Mean	72	74	72	73	--

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Promising performance of chickpea mutants

In MBNL No. 20 (July 1982), a brief account was given of high yielding micro-mutants being tested under the All-India Co-ordinated Pulses Improvement Programme since 1979-80 . The performance of these chickpea mutants is shown in Table 1. On account of its stable yield performance over the last three years and resistance to wilt, Ascochyta blight and stunt virus, BGM-405 entered the Co-ordinated Trial for 4th year evaluation during rabi 1982-83. In Vidaribha Region of Maharashtra State, amongst the 13 common varieties tried for the last 2-3 years on an average of six locations, BGM-405 gave the highest yield (1556 kg/ha) as compared with the best local check var. Warangal and Chaffa (1444 and 1218 kg/ha), respectively.

The results of CVT Desi for the rabi season 1981-82 show that mutant BGM-408 (2134 kg/ha) has again out-yielded the best check (1859 kg/ha) occupying second rank on the basis of All-India average as well as in the zonal average. Besides yield, BGM-408 showed resistance to severe attack of Ascochyta blight. Similarly, the other mutant BGM-413 also showed higher yield and resistance to wilt, blight and stunt virus. Both these mutant strains undergo third year evaluation in CVT during the rabi season 1982-83.

The five Desi mutants (BGM-416, 417, 418, 419, 421) entered in Co-ordinated trials in 1981-82 have shown promising performance for grain yield as well as high resistance for a number of diseases like wilt (BGM-416, 417, 419), root rot (BGM-417, 418, 419, 421), collar rot (BGM-416, 417, 418, 419, 421), foot rot (BGM-417, 418). All these mutants undergo second year evaluation during the rabi season 1982-83.

On account of their high disease resistance during 1981-82, mutant strains BGM-408, BGM-413 and BGM-416 entered a Special Trial during 1982-83, which includes only selected high yielding and disease resistant varieties available in the country.

Out of our 100 lines tested by ICRISAT pathologists, nine mutant lines showed less than 20% mortality as against 100% in control in the multiple disease sick plots at ICRISAT Hyderabad during 1979-80. One of these nine strains, MCK-43 appears to be highly resistant as it again showed only 9.2% and 7.3% mortality compared to 100% of control during 1980-81 and 1981-82 seasons, respectively, at ICRISAT.

In the Kabuli group, the mutant strain BGM-415 (1545 kg/ha) out-yielded the best check L-550 (1351 kg/ha) since last two years in

Table 1. Performance of chickpea mutant strains in the All-India Coordinated Trials (grain yield kg/ha).

Year	Variety/Mutant	North plains West zone	North plains East zone	Central zone	Peninsular zone	Mean
	Desi mutants					
1979-80	Best check	2121	1996	1712	1122	1737
	BGM-401	2256	1929	1740	1312	1809
	BGM-402	2253	2175	1897	1091	1854
	BGM-403	2165	2393	1913	1089	1890
	BGM-404	2224	2143	2039	1083	1872
	BGM-405	2316	2126	1731	1413	1896
	BGM-406	-	-	1921	1234	1577
1980-81	Best check	2493	1673	-	-	2083
	BGM-406	2502	1884	-	-	2193
	BGM-407	2476	1833	-	-	2154
	BGM-408	2761	1828	-	-	2294
	BGM-413	2528	2118	-	-	2323
1981-82	Best check	1848		1626		1737
	BGM-416	1785		1755		1770
	BGM-417	1964		1738		1851
	BGM-418	1674		1708		1691
	BGM-419	1955		1629		1792
	BGM-421	-		1701		1701

Kabuli mutants	Year 1980-81	81-82	Mean
Best check	1757	985	1371
BGM-415	1985	1105	1545
BGM-424	-	1121	

- = Not tested

Table 2. Performance of some new chickpea mutant strains in M_3 and M_4 generation institute trials

Mutant strain	Seed yield (kg/ha)	Harvest (index (%)
M_3 generation (Average of two locations)		
C-235 (check)	1886	38.20
MJ - 9	2257	38.40
MJ - 11	2270	39.03
MJ - 31	2384	37.89
MP - 27	2371	39.00
MR - 30	2344	41.46
MT - 46	2214	40.15
CD at 5%	263	
M_4 generation (average of three locations)		
C-235 (check)	2256	36.08
MD - 1	2595	36.89
MG - 6	2659	38.47
MA - 20	2710	39.28
MB - 41	2718	36.73
MB - 43	2609	37.15
MB - 45	2762	38.95
CD at 5%	244	

the Coordinated Trials and undergoes third year testing during 1982-83. One more mutant BGM-424 out-yielded the best check in Kabuli CVT during 1981-82.

Four more Desi mutant strains (BGM-425, 426, 427, 428) and two more Kabuli mutants (BGM-429, 430) entered Co-ordinated Trials in 1982-83.

In addition to the above strains, which are already under Co-ordinated Trials, new mutants of Desi type have been compared for their yield potential against the best national check C-235 (Table 2) in the Institute Trial. Six strains in M_3 and another six in M_4 showed convincing superiority over the best check variety. Besides high yield some of the new chickpea mutants have a higher harvest index (ca 40%) than the check C-235 (ca 37%). Genotypes with high harvest index can be cultivated under high density.

References

KHARKWAL, M.C. Induced micromutations in chickpea. Pulse Crops Newsletter, 1: 1(1981) 17-18.

KHARKWAL, M.C. Applied mutagenesis in chickpea. FAO/IAEA Mutation Breeding Newsletter No. 20 (July 1982) 14-15.

SHARMA, B. and KHARKWAL, M.C. Mutation studies and mutation breeding in grainlegumes. Proc. Third FAO/IAEA Research Co-ordination Meeting, 1982, Seoul, Republic of Korea. (IAEA-TECDOC, in press)

ANONYMOUS, Proceedings of the Workshop on Rabi Pulses (All-India Coordinated Research Project for Improvement of Pulses), ICAR, New Delhi (1979-82).

(Contributed by Kharkwal, M.C., Division of Genetics, Indian Agricultural Research Institute, New Delhi 110-012, India)

Sesame mutants

Sesame (Sesamum indicum L.) is an important edible oil crop in India. Mutants were induced in the cultivar N62-32. Details have been reported before (1,2). 25 mutant lines (M8) with their main identifying features are listed below:

Multilocular capsules (S1, S2 and S3): Capsules with 8 locules, rarely with 12, 16 and 20 locules. Vegetative and floral parts fasciated in S1 and S2.

Multicapsule (S6, S7 and S8): Two or three capsules in each leaf axil. S6 and S7: Early maturing with reduced plant height. In S7, all the three capsules are of the same size. S8: Late maturing with dark green foliage and light brown seed coat colour.

Large capsule (S13): Capsules with greater width and bigger seed size.

Short capsule (S15 and S19): Reduced capsule size with less seed number. S19, open plant type with longer petioles and deeply serrated leaf lamina.

Small capsule (S17): Capsules smaller but many, globular, densely hairy with less shattering and lower number of large seeds compared to the parent (2).

Long capsule (S40 and S43): Capsules long and appressed, long petioles and pedicles, branches fasciated, plants tend to lodge in rainy season.

Hairy capsule (S47): All the plant parts and capsules covered with dense hairs.

More capsules (S27, S30, S31, S34, S36, S53, S56, S149 and S337): Morphologically similar to the parent but with significantly increased number of capsules per plant and yields higher than the parent.

Yellow green (S22): Plant parts yellowish green all throughout. Just before maturity, stem turns shiny yellow while capsules turn dark yellow.

Less branched S23): Normally unbranched, occasionally with two primary branches. It grows taller than the parent in rainy season.

Split corolla (S64): In the flower, the five corolla lobes free except near the base with fertile pollen and sterile gynoecium.

From among 48 mutant lines that were studied for earliness, seed yield and yield components eleven promising lines with a high number of capsules/plant were further evaluated in replicated yield trials during four crop seasons (two crops per year). Two lines, S36-10 and S337-1, showed a significantly higher seed yield than the parent (768 and 708) kg/ha against 500 kg/ha). Five promising mutant lines are being evaluated at the Agricultural University, Akola, Maharashtra State.

In the M_4, mutant lines were also screened for oil content and ten promising lines were tested further during three more crop seasons. In general, oil percentage was found to be higher in the rainy season, compared to winter. There was a significant increase in oil content over the parent in all ten mutant lines in rainy season but only in four mutant lines (S1-7, S1-8, S22-2 and S34-3) in the winter. The highest oil percentage (mean of two seasons in 1980) was 51.8% in mutant lines S34-3, compared to 48.1% in the parent.

References

(1) Murty, G.S.S., Promising sesame mutants. FAO/IAEA Mutation Breeding Newsletter 16: (1980) 14-15.

(2) Murty, G.S.S., Radiation induced small capsule mutant in sesame (Sesamum indicum L.). Curr. Sci. 49: (18) (1980) 717-719.

(Contributed by G.S.S. Murty and C.R. Bhatia, Biology and Agriculture Division, Bhabha Atomic Energy Centre, Trombay, Bombay - 400 085, India

A pea mutant with symbiotic nitrogen fixation in the presence of nitrate

The inhibiting effect of nitrate on nodulation and acetylene reduction of N_2-fixing nodules is a well known phenomenon in legumes. By mutagenic treatment of pea (Pisum sativum) seeds of cv. Rondo, variability was induced and in the M_2-generation a mutant, efficiently nodulating in the presence of 15 mM KNO_3, was selected. The mutant trait appeares to be monogenic and recessive and the gene was designated as nod3. Nodulation of mutant nod3 and wild type cv. Rondo was investigated in absence and presence of KNO_3 in the medium; the mutant showed much better nodulation under both culture conditions. The acetylene reduction per plant of mutant nod3, nodulated on nitrogen-free medium, appeared to be much higher than that in cv. Rondo.

Summary of a paper to be published in Plant Science Letters.
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Increased nitrogen fixation in an induced mungbean mutant

Growth analysis of induced mutants selected from mungbean cultivar S-8 revealed that the mutant M.76 had a significantly increased leaf area higher phytomass, and a rapid rate of dry matter increase in comparison to the parent (Thakare et al., 1981). Plant nitrogen in the above ground parts was estimated at 15, 30, 45 and 60 days after sowing (DAS). Maximum N/plant was at 60 DAS. At this stage, M.76 and S-8 had 282 $\pm$ 40 and 229 $\pm$ 17 mg N/plant, respectively. The number of nodules/plant and the nitrogen fixing capacity of the nodules was estimated by the acetylene reduction technique (Wacek and Brill, 1976) using gas chromatography.

Nodules were sampled each time from 18 plants grown in the field in 6 replications at 10, 20, 30, 40, 50, 60 and 80 DAS. Both in the parent and the mutant the number of nodules/plant increased up to 40 DAS, remained the same up to 60 DAS and declined thereafter. However, the number of nodules was higher in M.76 (Table 1).

Table 1. Number of nodules per plant

Variety/Mutant	Days after sowing 40	50	60
S-8 (parent)	23.6 + 3.0	26.6 + 3.8	23.5 + 2.8
M.76 (mutant)	30.6 + 3.2	30.2 + 2.6	27.7 + 3.9

Table 2. Nitrogen fixation

Variety/Mutant	Days after sowing 40	50	60
		nM C_2H_4/nodule/h	
S-8 (parent)	0.36	2.77	1.28
M.76 (mutant)	0.36	15.16	2.50
		nM C_2H_4/plant/h	
S-8 (parent)	9.10	73.77	30.23
M.76 (mutant)	11.20	457.90	69.49

Nitrogen fixation measured as nM C_2H_2 reduced per hour was highest at 50 DAS (Table 2). At this stage, M.76 showed nearly six fold increase over the parent. Experiments are in progress to investigate parameters contributing to N-fixation, but also to check N-fixation and yield of lines selected after crossing M.76 to the parent and other agronomically valuable cultivars.

References

1. THAKARE, R.G., PAWAR, S.E., JOSHUA, D.C., MITRA, R. and BHATIA, C.R. Variation in some physiological components of yield in induced mutants of mungbean. In: Induced Mutations - A Tool in Plant Breeding, IAEA Vienna (1981), pp. 213-226.
2. WACEK, T.J. and BRILL J. Simple rapid assay for screening nitrogen fixing ability in Soybean. Crop Science 16: 519-522 (1976).

(Contributed by Thakare, R.G., David, K.A.V., Thomas, J. and Bhatia, C.R., Biology and Agriculture Division, Bhabha Atomic Research Centre, Trombay, Bombay - 400 085, India).

Productive salt tolerant and fine quality mutant of IR-8

IR-8 is the most popular rice variety through N. India because of its short stature, early maturity, synchronous tillering, high yield, etc. But it has coarse bold grains with translucent kernels having a white belly. Moreover, it is salt susceptible. This has limited its extensive cultivation in the areas with high soil salinity. By treating IR-8 seeds for 6 hours with 1.5% EMS, an early maturing, high yielding, protein rich, transparent and fine grained mutant IRm6 was obtained (Table 1). It exhibits high salt tolerance as revealed by seed germination and seedling survival in hydroculture tests using NaCl, Na_2SO_4 and $CaCl_2 2H_2O$ singly and in combination in various proportions up to 15 mM/cm3. The slightly increased plant height of IRm6 is not detrimental because the mutant is still resistant to lodging and matures much earlier than IR-8. This early, productive, salt tolerant, fine grained mutant of IR-8 appears to be an important achievement in rice improvement

Table 1. Performance of IR-8 and its mutant IRm6. (Mean value of 40 observations)

Characteristics	IR-8	IRm6
Plant height (cm)	90.5 ± 0.74	95.9 ± 0.63
Productive tiller number	8.6 ± 0.49	11.7 ± 0.35
Days to maturity	150.6 ± 0.51	130.8 ± 0.37
Grain length (mm)	6.4 ± 0.02	6.3 ± 0.01
Grain breadth (mm)	2.7 ± 0.01	2.4 ± 0.01
Grain yield (t/ha)	5.3 ± 0.20	6.5 ± 0.24
Seed protein (%)	7.1 ± 0.04	8.7 ± 0.05
*Photosynthetic pigments (mg/g)	1.3 ± 0.01	1.9 ± 0.03
Harvest index	4.9 ± 0.01	5.5 ± 0.02

* mean value of 5 observations
± values represent standard error

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Variability induction in chickpea through sodium azide treatment

Chickpea (Cicer arietinum L.) is one of the important grain legume crops of Bangladesh. There are only four recommended varieties of this crop [1] [2]. To create more variability for crop improvement, we undertook to induce mutations in Hyprosola by using sodium azide (NaN_3). Two preliminary experiments were conducted to determine the effective doses of the chemical for mutagenesis studies.

In two experiments, 1.00 mM doses of NaN_3 were used on 50 seeds for each treatment. Germination percentage and length of root and shoot of the seedlings were recorded. No seedling survived at 1.00 mM dose. Following 0.20 and 0.40 mM doses, the shoot length in 8-day old seedlings compared to the controls were 16 and 68% reduced root lengths reductions were 28 and 5%, respectively. A 50% reduction in seedling size may be obtained at a dose in between 0.20 and 0.40 mM. Therefore, these concentrations of NaN_3 were considered to be recommendably effective doses for chickpea.

In 1981, Hyprosola seeds were treated with 0.2, 0./3 and 0.4 mM concentrations of NaN_3 for induction of mutations. Five hundred seeds were used for each treatment. The M_1 plants did not show major variability. In total 754 M_1 plants survived but only 255 reached maturity. Single plant progenies were grown in M_2, and a lot of variation for qualitative and quantitative characters was recorded. 1.3% of 5080 M_2 plants were variant types, consisting of sterile, broadleaf, white flower, erect, dwarf, bushy, early and chlorophyll-deficient types. The white-flowered mutants were found to be vigorous and reasonably free from diseases. The bold-seeded Kabuli type chickpea generally has white flowers [3], but these white-flowered mutants did not produce bolder seeds. Sixty M_2 plants produced higher seed yield/plant than the control. All these variants will be subjected to further selection and possibly used in crossing.

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REFERENCES

[1] ANONYMOUS, An Inventory of Improved Crop Varieties/Strains in Bangladesh Agricultural Research Council, Farmgate, Airport Road, Dhaka (1975; p. 28

[2] SHAIKH, M.A.Q., AHMED, Z.U., MAJID, M.A. and WADUD, M.A., A high-yielding and high-protein mutant of chickpea (Cicer arietinum L.) derived through mutation breeding. Environ. and Exptl. Bot. 22:4 (1982) 483-489.

[3] VAN DER MASEN, L.J.G., Cicer L., A monograph of the genus with special reference to the chickpea (Cicer arietinum L.), its ecology and cultivation. Mededelingen Landhouwhoge School, Wageningen, Netherlands (1972).

(Contributed by M.A.Q. Shaikh, M.R.I. Khan, K.M. Shamsuzzaman, and A.D. Bhuiya, Plant Genetics Division, Institute of Nuclear Agriculture, P.O. Box No. 4, Mymensingh, Bangladesh).

Mutation induction in blackgram for higher seed yield and disease resistance

There are only two recommended varieties of blackgram (*Vigna mungo* L.) in Bangladesh but both are not high yielding. Therefore, to create variability, seeds of two accessions (B-10 and B-23) of blackgram were treated with ^{60}Co gamma rays in the year 1978. In the M_2 generation, genotypes with higher seed yields, erect plant types and disease resistance were selected. True-breeding lines were isolated in the M_3 generation. Preliminary yield assessments were made in both the M_3 and M_4 generations.

Finally, in the M_5 generation the mutants were put into a microplot yield trial along with the mother varieties. Five mutants were superior in respect of seed yield and other agronomic characteristics (Table). The mutant B-10/M-25 is a dwarf type and moderately resistant to Yellow Mosaic Virus and Cercospora leaf spot diseases. This mutant gave the highest seed yield among all the genotypes tested. The high yield may be due to a high number of pods per plant which has been found in black gram to have the highest correlation with seed yield [1].

The mutant B-10/M-23 is erect, determinate in plant type and bears upright pods compared to downward/horizontally borne pods of the existing cultivars [2]. It offers scope for accommodating an increased number of plants per unit area of land and thus may give much higher yield. The mutants M-18, M-58, M-36 and M-63 also produced higher seed yields than the mother varieties. The yield potential of all these mutants will be determined in the M_6 and M_7 generations by multilocational agronomic trials.

Table: Seed yield and other agronomic characters of blackgram mutants and their parents in M_5 generation.

Variety or mutant line	Plant height (cm)	No. of inflores-cences/ plant	No. of pods/ plant	No. of seeds/ pod	100-seed weight (g)	Seed yield/ plot (g)
M-25	13.4	6.9	20.0	6.0	3.7	600.8
M-18	15.6	6.4	19.1	6.0	3.5	510.3
M-58	14.8	6.1	15.3	6.3	3.6	494.1
M-36	16.4	7.4	19.3	6.6	3.5	467.3
M-63	15.2	7.1	21.5	5.9	3.3	446.6
M-67	16.0	5.8	15.3	6.5	3.8	356.1
M-23	16.6	4.7	12.9	6.0	4.9	341.9
M-59	16.1	4.9	12.1	5.9	4.4	303.7
B-10	14.8	7.0	19.2	6.2	3.7	387.8
(Mother variety of mutants M-23, M-25, M-36, M-58, M-59, M-63, M-67)						
B-2	16.1	6.5	18.4	5.6	2.7	288.0
(Mother variety of mutant M-18)						

(This work is supported by IAEA under Research Contract No. 1921/RB)

REFERENCES

[1] MAJID, M.A., KHANUM, S., SHAIKH, M.A.Q. and BHUIYA, A.D. 1982. Genetic variability and correlation studies in blackgram. Bangladesh J. of Agric. 7(3-4): 98 - 102.

[2] SHAIKH, M.A.Q. and MAJID, M.A. 1982. Altered plant architecture in a blackgram mutant. Mut. Breed. Newsletter, No. 19, p. 11, IAEA, Vienna.

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In vitro regeneration of plants from cotyledons in grain legumes

Earlier reports [1,2] of growing plants from pieces of groundnut cotyledons, led us to explore the possibilities of obtaining plants from cotyledons of groundnut, mungbean and pigeon pea. In mutation experiments such a technique could provide a possibility to avoid or, at least, reduce "diplontic selection" due to intrasomatic competition in mutagen induced chimeras resulting from seeds treated with mutagens.

When groundnut, mung bean and pigeon pea cotyledons were kept on moist filter paper in petri dishes, callus growth was observed in all three species at the proximal end of the cotyledons, where they were separated from the rest of the embryo. The number of cotyledons forming callus was highest in groundnut and smallest in pigeon pea. Groundnut cotyledons produced shoots and roots from the initial callus and resulting plants produced flowers and fruits. Under such non-aseptic conditions, infection rate was too high in mung bean and pigeon pea and it was not possible to obtain plants from cotyledons of these species with this simple procedure.

The following procedure seems to be better: Mungbean seeds soaked in 0.1% $HgCl_2$ for about 15 minutes were thoroughly washed in water and then germinated either in water or 1% sucrose solidified with 0.8% agar. Within 24-30 hours, the two cotyledons were separated from the rest of the embryo with a needle. Cotyledons were cultured on MS basal medium [3] at 25 $\pm$ 2°C under continuous illumination. Within 25-30 days, the proximal end of about 50% of the cotyledons gave rise to plants with shoot and roots. These plants were similar to those obtained from the normal embryo. Two cultivars, ML-5 and S-8, were tested and both were found to produce cotyledonary plants without the addition of any exogenous hormones to the medium. These plants were initially transferred to autoclaved soil in paper cups and after about a week, transplanted into non-sterile pots in the field. They produced fruits with viable seeds. The progeny of such plants was similar to the parental type.

Now we have produced cotyledonary plants from gamma irradiated seeds to study: (1) the frequency and spectrum of mutations observed in the M_2 generation from cotyledonary M_1 plants compared with M_1 plants grown from irradiated seeds in the usual way. (2) The extent of chimerism in cotyledonary plants to infer if the adventitious shoots originate from one or several cells. These experiments are in progress.

REFERENCES

[1] ILLINGWORTH, J.E. Peanut plants from single de-embryonated cotyledons. Hortscience 3 (1968) 238-276.

[2] ILLINGWORTH, J.E. Peanut plants from single de-embryonated cotyledons or cotyledonary fragments. Hortscience 9 (1974) 462.

[3] MURASHIGE, T. and SKOOG, F. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiologia Plantarum 15 (1962) 473-497.

(Contributed by Mathews, Helena and Bhatia, C.R., Bio-Organic Division and Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay - 400 085, India)

Experimental mutagenesis of in-vitro cultured plant cells and protoplasts

One of the promising developments in induced mutation research are in-vitro techniques for induction and isolation of mutants. The advantages of the in-vitro methods over the conventional techniques of seed, bud or pollen treatment are many:

- A large number of cells can be handled.
- Different screening methods can be used for selection mutants.
- Mutants are mostly non-chimeric.
- Procedure could be very rapid.

In the literature, there are a large number of reports describing isolation of mutant cell lines from various species. However, for the utilization of such mutants in plant breeding programmes, it is essential that:

1. Plants are regenerated from the mutant cell lines.
2. The regenerated plants and their seeds (or vegetative progeny in the case of vegetatively propagated plants) express the mutant phenotype for which the cell lines are selected.
3. The mutant character is transferred to other genotypes after hybridization.
4. The mutants have no alterations or a low number of them at 'non-target' loci.

Further, from the viewpoint of plant breeding, it is important to know whether there are differences in the genetic variability induced in in vitro cell and protoplast cultures with and without mutagen treatment in comparison with the "conventional" mutation experiments. As a result of intensive mutation research in important crop plants during the last three decades, the mutation rates to be expected after seed treatment in relation to the mutagen dose are known reasonably accurately. The more frequent mutant types that can be identified under field and/or glass-house environment visually without using any special screening procedures, are rather well documented for important crop plants.

In order to pave the ground for in-vitro mutation breeding, we surveyed the literature for in-vitro mutagenic treatments and mutation rates. Our findings are compiled in the Table, with the hope that they would be useful for others in planning in-vitro mutation experiments.

TABLE 1. Mutagenic treatments and mutation rates in vitro

Object	Mutagen and Dose	Mutant obtained (Resistance to)	Mutation Rate	Regen. achd.	Ref.
TOBACCO					
Cells	NT	Valine	2.2 E-6	Yes	5
	NT	Ethionine (200 µM)	5.5 E-8		13
	NT	5-methyl tryptophan (44 µM)	1.5 E-6		41
	NT	Selenocystine and seleno-methionine	4.3 E-8		15
	NT	Isonicotinic acid hydrazide	22 lines	Yes	44
	NT	Chlorate	6.6 E-8		29
	NT	Cycloheximide	E-6		26
	NT	Chlorate	none		30
	NT	Chlorate	1.5 E-9 (nitrate reductase)	Yes	30
			5.5 E-9 (leaky mutants)		
	NT	5-BUdR (100 mg/l)	8 lines (17 regenerated plants retained resistance)	Yes	24
	NT	Threonine	1 line		25
		Methionine	2 lines		"
		Valine	5 lines	Yes	"
	NT	Glycine hydroxamate (1 mM)	7 lines	Yes	21
	NT	Picloram, Hydroxyurea (100 - 500 µM)	0-100%	Yes	10
	NT	Picloram (500 µM)	2 E-5	Yes	11
	UV 1000 ergs/mm²	Valine	1.4 E-5	Yes	5
	UV 3.3 E 5 erg/cm²/min, 2h	Isonicotinic acid hydrazide 1 mM	20 lines	Yes	4
	UV 330 J/m2	Carboxin 1 mM	1 line	Yes	34
	EMS 0.3%, 1h, 25°C	Cycloheximide	E-6		26
	EMS 0.25%, 3h,	Amino ethyl-L-cysteine 0.5 mM and delta hydroxy-lysine (0.6 mM)	5 E-7		43
	EMS	Toxin (0.05-0.25%)	2 plants	Yes	9
	NEU 25 mM	Chlorate	2.0 E-7		29
	NEU 0.25 mM or NMU	Chlorate	4.5 E-7	Yes	30
			2.5 E-7 (nitrate reductase)		
	5BUdR 1 E-5	Temperature sensitive	2.0 E-7	Yes	27
	5BUdR 1 E-5	Auxotrophs requiring amino-acids, vitamins, purines	6	Yes	8
Proto-plasts	NT	Valine	none		7
	X-rays 5 E03 r	Nutritional mutants	none		1
	EMS 0.2%	Isopropyl N-phenyl carbamate	one mutant	Yes	2
	EMS 200 mg/ml	Valine	none		7
	MNNG 4 µg/ml, 6 h	Valine 2.5 mM	2.0 E-5		7
POTATO					
Cells	NT	Resistance to culture filtrates of *Phytophthora infestans*	8.8 E-4	Yes	3
	X-rays 1000 R	Same as above	5.0 E-3		3

continued on next page

TABLE 1 - (continued)

Object	Mutagen and Dose	Mutant obtained (Resistance to)	Mutation Rate	Regen. achd.	Ref.
POTATO (continued)					
Proto-plasts	NT	Morphological and physiological traits		all 65 protoclones	36
TOMATO					
Cells	NT	Aluminium 200 µm		2.03 E-7 8.32 E-6	28
CARROT					
Cells	NT	5-methyl tryptophan 220 µM		3.00 E-7	42
	NT	--ditto-- 0.5 mM		E-6 to E-7	39
	NT	Cycloheximide		5.40 E-8	38
	NT	Azetidine-2 carboxylic acid 1.00 E-3 M		1 cell line	31
	NT	Ethionine 0.5 mM and hydroxyproline 1.5 mM		none	43
	EMS 2.5 %, 2h	Cycloheximide		7.70 E-7	38
	EMS 0.25%, 4h	Ethionine 0.5 mM and hydroxy proline 1.5 mM		E-7	43
	MNNG 100 µg/ml	Cycloheximide		4.40 E-6	38
	MNNG 2.8 mM, 2h	Altered pigmentation		4 lines	32
	Colch 2.50 E-4 M	Colchicine Streptomycin 1 mg/ml N-methyl alanine 10 mg/ml		1.00 E-3	45
SOYA BEAN					
Cells	NT	Azetidine-2-carboxylic acid 100 µM		4.00 E-8	13
	EMS 0.16%	8-azaguanine		4.20 E-7	40
Proto-plasts	MNNG 50 µg/ml 16 h,28 C	5-BUdR 20 µg/ml		4.00 E-5	33
RAPE					
Cells	EMS 1-2%, 2.5-5 h OR MNNG 20-100 µg/ml, 2.5-5 h	Resistance to pathogen Phoma lingam		1.00 E-9 to 3.00 E-9	Yes 35

continued next page

TABLE 1 - (continued)

Object	Mutagen and Dose	Mutant obtained (Resistance to)	Mutation Rate	Regen. achd.	Ref.
SUGAR CANE					
Cells	NT	Eye spot disease toxin	15 - 20 %	Yes	17
	NT	Fiji disease	1.9 %		"
	NT	Smut	5.00 E-2	Yes	22
	NT	Superior yield	2 out of 11	Yes	23
	NT	Eye spot disease toxin	10 - 11 %	Yes	18
	MMS 50 mg/l	ditto	3 - 13 %	Yes	"
	Ion- 3 kr izing rad	ditto	12 %	Yes	"
	Gamma rays 500-2000 r	Large number of mutants		Yes	17
	Colch, 8-eth, EMS, MMS, MNNG 5-4000 ppm	Wide range of mutants		Yes	17
MAIZE					
Cells	NT	Endosperm and seedling mutants	64A - 1.2 S65 - 0.8	Yes	14
	NT	LT 2.5 mM	3 independ-lines		20
	NT	Pathotoxin	all resist- ant after 5th cycle	Yes	16
	NT	Pathotoxin *Drechslera maydis*	All plants from Texas male sterile cultures exposed to toxin were resistant	Yes	6
	Sodium-azide	Lysine and threonine 2 mM each	1 stable line	Yes	19
RICE					
Cells	NT	5-methyl tryptophan	2 plants	Yes	12
	NT	Sterile	14.6 %		37
		Tetraploids	3.4 %		37
		Stripes, open spikelets	0.9 %		"
		Chlorophyl mutants	8.8 %		"

Abbreviations used in table:

2.0 E-5 Exponential notation, indicates 2.0×10^{-5}
5MT 5 Methyl tryptophan
8-eth 8-ethoxycaffeine
BUdR Bromodeoxyyridine
Colch Colchicine
EMS Ethyl methane sulfonate
MMS Methyl methane sulfonate
NT No treatment
NMMG Nitroso methyl guanidine
NEU Nitroso ethyl urea
NMU Nitroso methyl urea
Reg. achd. Regeneration achieved

REFERENCES

[1] AVIV, D. and GALUN, E., Pl. Sci. Lett. 8 (1977a), 299-304

[2] AVIV, D. and GALUN, E., Pflanzenphysiol. 83 (1977b), 267-274.

[3] BEHNKE, M., Theor. Appl. Genet. 55 (1979), 69-71

[4] BERLYN, M.B., Theor. Appl. Genet. 58 (1980), 19-26

[5] BOURGIN, J.P., Mol. Gen. Genet. 161 (1978), 225-230

[6] BRETTELL, R.I.S., THOMAS, E. and INGRAM, D.S., Theor. Appl. Genet 58 (1980) 55-58

[7] CABOCHE, M., and MULLER, J.F., in: Plant Cell Cultures: Results and Perspective. Eds. Sala, F., Parisi, B., Cella, R. and Ciferri, O. Elsevier/North-Holland Biomedical Press. pp. 133-138

[8] CARLSON, P.S., Science 168 (1970), 487-489

[9] CARLSON, P.S., Science 180 (1973), 1366-1368

[10] CHALEFF, R.F., and KEIL, R.L., Mol. Gen. Genet 181 (1981), 254-258

[11] CHALEFF, R.S., and PARSONS, M.F., Proc. Natl. Adad. Sci. USA 75 (1978) 5104-5107

[12] CHEN, C.C., and CHEN, C.M., Natl. Sci. Counc. Monthly, ROC 8(8) (1980), 697 - 702

[13] DAS, P.K. and WIDHOLM, J.M., in Proc. of the Int. Sym. on Plant Cell Culture Crop Improvement held Dec. 6-10, 1981. Calcutta, pp. 411-417

[14] EDALLO, S., ZUCCHINALLI, D., PERENZIN, M. and SALAMINI, F., Maydica 26 (1981) 39-56

[15] FLASHMAN, S.M. and FILNER, P., Pl. Sci. Lett. 13 (1978), 219-229.

[16] GENGENBACH, B.G., GREEN, C.E. and DONOVAN, C.M., Proc. Natl. Acad. Sci. USA 74 (1977), 5113-5117.

[17] HEINZ, D.J., in: Induced Mutations in Vegetatively Propagated Plants IAEA, Vienna (1973), 53-59.

[18] HEINZ, D.J., KRISHNAMURTHI, M. NICKELL, L.G. and MARETZKI, A. in Plant Cell, Tissue, and Organ Culture. Eds. Reinert, J. and Bajaj, Y.P.S. Springer-Verlag, Berlin Heidelberg New York (1977), pp 3-16

[19] HIBBERD, K.A. and GREEN, C.E., Proc. Natl. Acad. Sci. USA. 79 (1982), 559-563

[20] HIBBERD, K.A., WALTER, T., GREEN, C.E. and GENGENBACH, B.G., Plants 148 (1980), 83-187.

[21] LAWYER, A.L., Berlyn, M.B. and ZELITCH, I., Pl. Physiol. 66 (1980), 334-341.

[22] LIU, M.C., in: Plant Tissue Culture - Methods and Application in Agriculture, 1981, pp. 299-323

[23] LIU, M.C. and CHEN, W.H. Euphytica 27 (1978), 273-282

[24] MAISURIAN, A.N., KHADEYEVA, N.V. and POGOSOV, V.Z., Acad. Sci. USSR 3 (1981a), 555-561

[25] MAISURIAN, A.N., KHADEYEVA, N.V. and POGOSOV, V. Z., Acad. Sci. USSR. 4 (1981b), 774-777.

[26] MALIGA, P., LAZAR, G., SUAB, Z. and NAGY, F., Molec gen. Genet. 149 (1976), 267-271

[27] MALMBERG, R.L., Genetics 92 (1979), 215-221

[28] MEREDITH, C.P., Pl. Sci. Lett. 12 (1978), 25-34

[29] MULLER, A.J. and GRAFE, R., Molec. Gen. Genet. 161 (1978), 67-76

[30] ULLER, A.J. GRAFE, R., MENDEL, R.R. and SAELBACH, I., in: Proc. Int. Symp. Experimental mutagenesis in plants. Eds. Filev, K., Daskaloff, S., Mehandjiev, A., and Vassileva, M., (1978),pp 301-308

[31] NIELSEN, E., AMILENI, A.R., RONCHI, S. and PARISI, B., in: Plant Cell Cultures: Results and Perspectives. Eds. Sala, F., Parisi, B., Cella, R. and Ciferri, O., (1980), pp 151-156

[32] NISHI, A., YOSHIDA, A., MORI, M. and SUGARO, N., Photochemistry 13 (1974), 1653-1656

[33] OHYAMA, K., Expl. Cell. Res. 89 (1974), 31-38

[34] POLACCO, J.C. and POLACCO, M.L., Ann. New York Acad. Sci. 287 (1977), 385-400

[35] SACRISTAN, M.D., Theor. Appl. Genet. 61 (1982), 193-200

[36] SECOR, G.A. and SHEPARD, J.F., Crop Sci. 21 (1981), 102-105

[37] SUENAGA, K., ABRIGO, E.M. and Yoshida, S., IRRI Research Paper Series No. 79, August 1982, Part I, 1-12

[38] SUNG, Z.R., Genetics 84 (1976), 51-57

[39] SUNG, Z.R., Planta 145 (1979), 339-345

[40] WEBER, G. and LARK, K.G., Theor. and Appl. Gen. 55 (1979), 81-86

[41] WILHOLM, J.M., Biochem. Biophys. Acta 261 (1972 a), 52-58

[42] WIDHOLM, J.M., Can. J. Bot. 54 (1976), 1523-1529.

[43] ZELITCH, Z. and BERLYN, M.B., Pl. Physiol. 69 (1982), 198-204

[44] ZRYD, J.P., Experientia 35 (1979), 1168-1169

(Contributed by Helena Mathews and C.R. Bhatia, Bio-Organic Division and Biology and Agriculture Division, Bhabha Atomic Research Centre, Bombay 400 085, India.)

Spectrum of wheat cultivars in Hungary

The total wheat acreage in Hungary is ca 1.3 million hectares.

The spectrum of wheat varieties 1978-1982 shows a steep increase in popularity of the mutant variety MV-8 reaching about 1/3 of the wheat acreage only 3 years after release.

Variety	1978	1979	1980	1981	1982
Jubilejnaja	23.9	19.7	19.7	21.2	19.0
Libellula	18.3	10.6	8.8	6.7	2.3
Szava	11.5	9.1	8.6	6.5	1.0
Mv-4	10.5	10.4	15.6	15.6	15.0
Mv-5	8.0	3.7	3.5	1.5	0.4
GK-3	4.8	3.6	2.1	0.5	-
GKF-2	3.7	2.5	0.9	0.1	-
Partizanka	3 6	7.9	8.1	7.8	2.0
Bezostaja	3.2	0.6	0.1	-	-
Rana 2	2.7	12.1	13.0	11.0	7.9
Rana 1	2.1	7.0	7.5	6.0	0.8
Rana 3	0.7	2.4	2.6	2.2	-
GK-Tiszataj	0.6	1.2	2.4	5.1	2.0
GK-Szeged	-	1.0	4.0	3.2	4.6
Mv-8 (mutant variety)	-	-	1.0	10.0	31.4
Super Zlatna	-	-	-	0.2	4.2
Others	6.4	2.2	2.7	2.3	10.8
	100	100	100	100	100

REFERENCES

Mutation Breeding Newsletter No. 12, p. 2; No. 16, p. 4 and 17

New Books

Induced Mutations in Plant Breeding
W. Gottschalk, G. Wolff
Monographs on Theoretical and Applied Genetics 7
Springer-Verlag Berlin 1983

Seed Proteins-Biochemistry, Genetics, Nutritive Value
W. Gottschalk, H.P. Müller (editors)
Martinus Nijhoff/Dr. W. Junk Publishers 1983
Advances in Agricultural Biotechnology

Chimerism in irradiated dicotyledonous plants
Report of a FAO/IAEA Consultants Meeting 1981
IAEA-TECDOC-289 1983

Plant Breeding Solutions for Environmental Stress Problems in Crop Production
(1982 Plant Science Lectures, covering mineral stress, drought, salinity, mineral nutrition), Iowa State Journal of Research
May 1983. $7.-
Copies can be obtained through M.J. Vivian, Agronomy Department
Iowa State University, Ames, Iowa, 50011, USA.

Future events

1984

16th Stadler Genetics Symposium on Gene Manipulation in Plant Improvement
Columbia, Missouri, 19-21 March 1984
Contact: J.P. Gustafson, Curtis Hall, University of Missouri, Columbia, Missouri, 65211, USA

Second Mediterranean Conference of Genetics, Cairo, 27-30 March 1984
Contact: Egypt Society of Genetics, c/o Department of Genetics, Faculty of Agriculture, University of Cairo, Giza, Egypt

FAO/IAEA Training Course on the Induction and Use of Induced Mutations in Plant Breeding
FAO/IAEA Biotechnology Laboratory, Seibersdorf, 3 April - 18 May 1984

World Soybean Research Conference III, Ames, Iowa, 12-17 August 1984
Contact: W.R. Fehr, Department of Agronomy, Iowa State University, Ames IA 50011, USA

International Symposium on Plant Tissue and Cell Culture - Application to Crop Improvement
Olomouc, Czechoslovakia, 4-10 September 1984
Contact: Dr. F.J. Novak, Institute of Experimental Botany
Sokolovaka 6 CS-77200 Olomouc (CSSR)

International Symposium on Genetic Manipulation in Crops
Beijing, People's Republic of China, 22-26 October 1984
Contact: Dr. Hu Han Shao Qiquan, Institute of Genetics, Academia Sinica, Beijing, P.R. of China

1985

Second International Oat Research Workshop
Aberystwyth (UK) 10-15 July 1985
Contact: Dr. Dudley Lawes
Welsh Plant Breeding Station
Plas Gogerddan, Aberystwyth, Dyfed, (UK)

LAST BUT NOT LEAST

Please submit your contributions to the Newsletter by 1 June and 1 December of each year.

Authors are kindly requested to take into account that the readers want to learn about new findings and new methods but would also like to see the most relevant data on which statement and conclusions are based. Conclusions should be precise and distinguish facts from speculation. The length of contributions should not exceed 2-3 typewritten pages, including tables. References to publications containing a more detailed description of methods or evaluation of findings are welcome but should generally be limited to one or two.

Alexander MICKE

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