FNCA／MEXT
Technical Material

Achievement
Sub-Project on
Drought Tolerance in Sorghum and Soybean
(2002 – 2006)

Mutation Breeding Project

Forum for Nuclear Cooperation in Asia (FNCA)

March, 2009

Foreword

Two of the most urgent applications of mutation breeding are the generation of superior cultivars meeting the nutritious needs of the growing populations in Asia and development of superior genotypes having adaptation to global changes and expanding dry lands. The Mutation Breeding Project focused on the improvement of drought tolerance in soybean and sorghum represents one project developed by the Forum for Nuclear Cooperation in Asia (FNCA) in 2002. This project was established with the collaboration of member countries such as Indonesia, Malaysia, The Philippines and Vietnam for soybean breeding, and China and Indonesia for sorghum breeding. Soybean and Sorghum were selected as the target crop because soybean is one of the most important legume crops in tropical, subtropical, temperate zones as well as most Asian countries. Sorghum was selected because it is utilized in the drought-prone regions of Asia and is fifth in importance as a cereal food crop when compared to rice, wheat, maize and barley. In addition, prior evaluation of soybean and sorghum germplasm resources did not successfully identify accessions exhibiting level of extreme drought tolerance. As such, mutation breeding is a very useful method to induce favorable changes in germplasm when required breeding materials are not otherwise available.

Activities of this project focused on a wide array of important agronomic topics; of which the following were deemed of highest importance: (1) exchange of breeding materials and promising mutants; (2) information regarding efficiency of screening methods for drought-tolerance; (3) breeding techniques such as F_1 hybrid breeding of sorghum; and (4) information regarding various unique mutations such as super-nodulation in soybean. Each year, results and encountered problems within this project are discussed at the FNCA Workshop held in the member countries. In this project, promising lines both of soybean and sorghum have been identified and evaluation tests for the registration are being conducted. Since the cooperative research investigations fulfilled the project goals, the project was terminated in 2006. However, additional years of work are required for the plant breeders to develop and release new cultivars to the farmers. Following the identification of promising lines, yield trials at several locations, spanning at least 3 growing seasons, are required for cultivar registration.

It is our hope that the promising lines developed in this project will benefit the farmers of the drought-prone regions of Asia. Editors also hope that this book will be useful not only for the breeders interested in gamma ray induced mutation breeding, but also for the breeders of soybean and sorghum.

We are grateful to the Ministry of Education, Culture, Sports, Science and Technology-Japan for their financial support and the management assistance they provided to the project.

I would like to state my appreciation to the contributing authors for their achievements in this project and the submission of their final reports.

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March 2009

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1-1. China

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As per the general plan of the Multilateral Research Program (MRP-1) under the framework of FNCA Agriculture Project (Mutation Breeding), we have exchanged sorghum mutant germplasms with Indonesia, and have done multi-location evaluation for further use, popularized new mutant varieties, and used introduced germplasm in breeding program. This paper will give an outline summary on the researches during project years (2002-2006) as follows.

1 Goal of the MRP

This project aims to form an Asia regional cooperation network of drought tolerance improvement in sorghum by using mutation induction technique and related biotechnology.

(1) To collect, identify and utilize useful germplasms of drought tolerance for regional and national sorghum breeding programs in agricultural institutions and extend planting and utilization of the improved cultivars.
(2) To exchange the improvement techniques for raising efficiency of screening for drought tolerance in sorghum.
(3) To form Asia regional co-operation network of drought tolerance improvement in sorghum by holding meetings, inviting experts to scientific visits and exchange sorghum plant materials and information.

2 Project Progress

2.1 Exchange useful sorghum germplasms of drought tolerance

Totally China has provided Indonesia with 4 mutant or hybrid sorghum: Yuanza 1(Hybrid), Zhenzhu, Yuan8002, SP84002; Indonesia has provided China with 5 mutant and their initial material: ME/30/9, ET/20/477, DU/30/95B, DU/20/3E and JA/30/58 and Durra .

2.2 Observation on Sorghum mutant germplasm from Indonesia

5 sorghum mutants introduced from Indonesia were planted in IAAE's experimental farm in 2002. Experimental results are showed as in the table1.

Table 1. Performance of sorghum mutants introduced from Indonesia

	ME/30/9	ET/20/477	DU/30/95B	DU/20/3E	JA/30/58
Growth period (d)	125	>160	159	159	95
Plant height (cm)	310	350	250	250	220
Stem diameter (cm)	0.6	2.5	1.7	1.7	1.5
Number of tillers	1.0	2.3	1.0	1.5	2.0
Panicle length (cm)	23	30	20	23	38
Seed wt/panicle (g)	70	-	60	80	40
1000-seed weight(g)	36.5	-	28.0	21.0	18.0
Bx (%)	-	5.0	10.0	8.0	-
Type	Grain	Forage	Grain	Grain	Broom

Among the 5 mutants, JA/30/58 was very early mature, with short stalks and big spikes. It could be used as a good cultivar of broom sorghum. DU/30/95B has a bright red seed color, very beautiful, could be used for decoration. ME/30/9 and DU/20/3E have bright white color, shows good market quality. ET/20/447 was multi-tillering. It had thick and strong stalks (not sweet) and high biomass yield. It might be utilized as a good cultivar of forage sorghum or be used as a high biomass parent in energy crop sweet sorghum breeding. But it cannot get mature enough to propagation in Beijing.

2.3 Multi-location Evaluation of Sorghum Mutants and Parents

In 2005, a total number of seven varieties included four mutants and one parent from Indonesia (Du/30/95B, Du/20/100B, DU/20/3E, ET/20/477and Durra), one mutant and one parent from China (Yuanyu 8002 and SP4-8002) were used to do multi-location trial to evaluate their possible uses for forage or grain production in Beijing, Gansu and Heilongjiang, respectively. Randomized complete block design with 3 replications, row length 6 meters with 3 rows in one plot, row spacing 60cm and plant-to-plant space 50cm. The sowing date was 20 April 2005 in Beijing, 25 April 2005 in Gansu and 29 April 2005 in Heilongjiang.

Among the mutants and parents, Yuan8002 and SP4-8002 belong to grain sorghum but Du/30/95B, Du/20/100B, DU/20/3E, ET/20/477and Durra forage usage according to their agronomic performance.

Yuan8002 and SP4-8002 can be used as restoring lines of cytoplasmic male sterility of sorghum, with short stalks and growth period. SP4-8002 was about 5 days delay in heading date than its parent Yuan8002, which will be helpful to hybrid seed production.

Durra, DU/30/95B,Du/20/100B and ET/20/477 produced high biological yield with very high plant height but too long growth period in the above three locations.

Performance comparison of these varieties in Beijing was showed as Table2.

Table 2. Performance of sorghum mutants and parents with drought tolerance in Beijing in 2004

	1	2	3	4	5	6	7
Growth period (d)	110	115	183	182	183	159	>190
Plant height (cm)	138	122	248	251	262	250	350
Stem diameter(cm)	1.41	1.42	1.51	1.53	1.55	1.70	2.50
Number of tillers	1.20	1.10	1.65	1.80	1.75	1.50	2.30
Panicle length(cm)	25.8	27.1	23.5	23.1	23.2	23.0	30.0
Seed wt/panicle(g)	54.6	55.1	60.3	62.6	61.5	80.0	-
1000-seed weight(g)	25.0	24.8	25.8	26.3	26.1	21.0	-
Bx(%)	3.0	3.0	8.0	10.0	9.0	8.0	5.0

Note:1. Yuan8002 (P); 2. SP4-8002; 3. Dura (P); 4. DU/30/95B; 5. Du/20/100B; 6. DU/20/3E; 7. ET/20/477

2.4 Use of introduced mutant germplasm in breeding program

Among 5mutant from Indonesia, ET/20/447 is a useful germplasm with very high biomass yield, but it can't get mature enough for reproduction. In order to use its useful genes, in 2003 we treated its seedlings after three leaves shoot in short sunlight (9 hr/d) for 32 days. It head 95day after sowing and short sunlight treatment. Then emasculated and pollinated with sweet sorghum CV Rio, Rio/BJk156-1-3-1 and Roma. Finally 26 seeds from cross [ET/20/477]/Rio, 50 seeds from [ET/20/477]/Roma and 50 seeds from [ET/20/477]/[Rio/BJK156 -1-3-1].

F_1 generation

In 2004 all these seeds were planted in the experiment field, and F_2 seeds were obtained. ET/20/477 is 350 cm, Rio/156-1-3-1 is an advanced lines, juicy and sweet (brix 16-18%), 360-380cm high. F_1 plants were 5.0-5.3m tall and 3-4wm thick (see Fig.1), showing very strong heterosis.

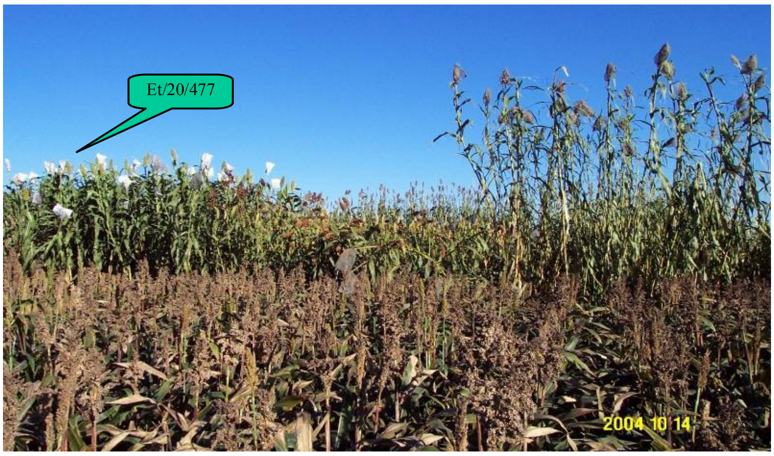

Fig1. F_1 populations of [ET/20/477]/Roma, [ET/20/477]/[Rio/156-1-3-1 and [ET/20/477]/Rio

F_2 generation

In 2005 F_2 populations (Fig2) were planted in the experimental field for selection of single plant with high biomass potential, sweet or non-sweet. From 300 F_2 population of [ET/20/477]/Roma, 35 plants were selected; from 240 F_2 of [ET/20/477]/[Rio/156-1-3-1, 46 were selected; 240 from F_2 of [ET/20/477]/Rio, 56 plants were selected. Plants with ET/20/477 characters and early mature have been recovered.

Distributions of some important characters were analyzed. Take [ET/20/477]/ [Rio/156-1-3-1] as an example, 64 F_2 plants were examined, the distributions of plant heights (Fig.3), stem types (dry or juicy)(Fig.4), Maturity (showed as mature state when frosting, i.e. waxy to full mature, milk or flowering)(Fig.5). For juicy plants, juice Brix was measured with a hand refractometer. And Brix distribution was showed as Fig 6.

Plants height

ET/20/477 is 350cm high. Rio/156-1-3-1 is an advanced line, 360-380cm high. Among 64 F_2 plants examined, 13 plants were higher than 5m, 19 were between 4.6-5m, 17 were shorter than two parents. It showed plants with ET/20/477 height character could be recovered.

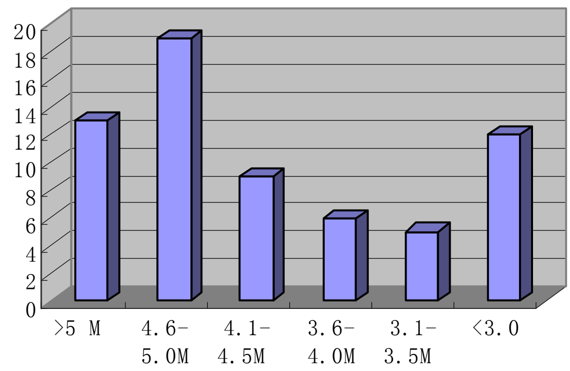

Fig2 Distribution of Plant height in F_2[ET/20/477]/ [Rio/156-1-3-1]

Stem types

ET/20/477 is a dry type, and Rio/156-1-3-1 is a juicy type, with brix 16-18%. Among 64 F_2 plants observed, there were two types of stems, 12.5% juicy and 87.5% dry, which showed juicy is controlled by recessive gene. It is easy to get a dry type with high biomass yield.

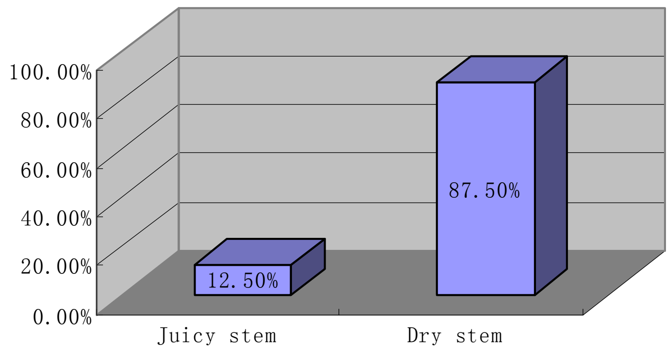

Fig3 Distribution of Plant types in F2[ET/20/477]/ [Rio/BJK156-1-3-1]

Maturity

ET/20/477 is very late mature, and could not mature enough for further reproduction. That is the key reason to treat it and cross with early varieties so as to keep its useful genes. Among 64 F_2 plants, 54.7% could develop to waxy to full ripe, enough to reproduction, 45.3% flowering to milk, difficult to reproduce.

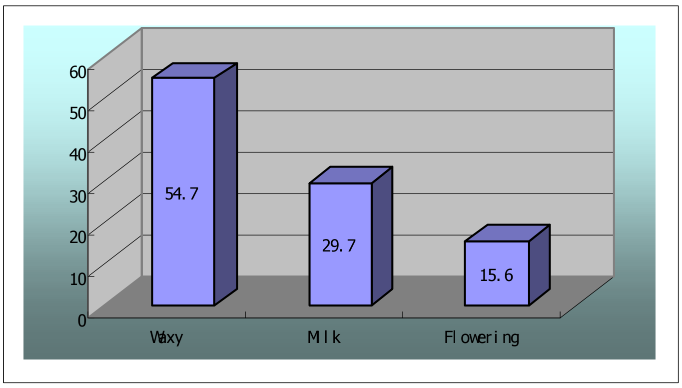

Fig4 Distribution of maturity in F_2 [ET/20/477]/ [Rio/BJK156-1-3-1]

Juice Brix

Among 64 F_2 plants, only 8 or 12.5% were sweet. Brix were from 10-18%, 3 plants between 10-13%, 4 plants 13-16%, 1 plant 18%. Generally sweet type is later mature. It needs a large population to select for a sweet type with high biomass yield.

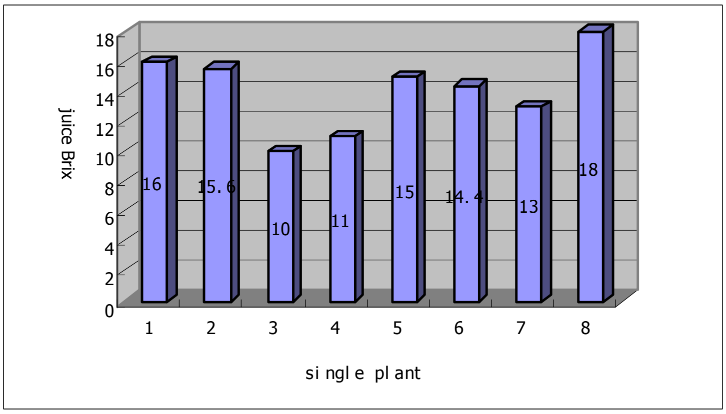

Fig5 Distribution of Brix in F_2 [ET/20/477]/ [Rio/156-1-3-1]

F_3 generation

F_3 generation is growing as Fig. 6. The result has yet to obtain.

Fig 6 F_3 generation of [ET/20/477]/ [Rio/156-1-3-1]

2.5 Mutation technology in sorghum improvement

2.5.1 Production of M_1 and M_2 population after gamma radiation

Seeds of grain sorghum variety "*Zhenzhu*" and sweet sorghum "*Sart*" were irradiated by gamma rays, 100Gy, 150Gy, 200Gy, 250Gy, 350Gy and 400Gy, respectively. The results of the germination test and the field planting showed that 200Gy～250Gy could be the appropriate radiation dose, while very lower survival plants in 350 Gy treatment and no seeds in 400 Gy in M_1 generation. M_2 seeds from 100Gy to 350Gy were obtained and will be used to laboratory test for drought tolerance by PEG6000 and field planting in next year.

2.5.2 Mutational effects of new mutagens on sorghum

Seeds of sorghum cv. *Y030* and *Yuan8002* were irradiated by proton, with the energy of 3Mev and dosage of 300Gy, and synchrotron (ultra-soft X rays, 5～20λ), respectively. The result showed that the biological effects of proton and synchrotron irradiation on the current generation of treated sorghum seeds are very similar. The sorghum seedlings in 1～3 leaflets stage from the treated seeds showed variations of stripe chlorophyll deletion, with the deletion rate near 100%. However, no such variation was found in the treatments with 200Gy gamma rays irradiation. The chlorophyll deletion was also not reported in literature.

Electron beam was used to irradiate the seeds of four sorghum cultivars, i.e.,*Wusawu, Jinwu*, *Aisi* and *Yuan2B*. The preliminary results from bioeffect analysis showed the proper doses of electron beam to treat sorghum seeds were to be 30～50 Gy for white grain and 100～150 Gy for red grain, respectively. This dose is significantly lower than that of gamma rays, indicating a high relative biological effect.

2.6 New varieties

Yuantian No 1 a sweet type showed as Fig 7, released in 2002. It was developed from Rio as initial material by induced mutation with gamma ray. It is grown ca. 1000 hectares.

Fig7 Plants, stem and spike of *Yuantian No 1*

Hybrid variety *Yuanza 502*(*Yuan2/ Sp8002*) In the variety comparison experiment, 25 new crosses made with mutants Cms lines or restorer lines were evaluated, Yuan2/ Sp8002 showed more than 20% higher in grain yield than original Yuan2/ Yuan8002. Sp8002 is a mutant developed by space breeding. The performance of this cross shows as Fig 8.

Fig.7 Field performance of new hybrid variety *Yuan2/ Sp8002*

3 Discussion

Through this project, participating countries exchange their research experience, mutant share achievement, which is conducive to all participating experts and also good for enhancing application of atomic energy in a wider research field.

Induced mutation has become a conventional breeding approach, it become less and less an opportunity to get support as an independent project. So it is suggested that the future FNCA project be combined with other related domestic project. It may be better to organize project group as end-up use, not as single crop, such could attack more experts to participate in FNCA activity.

1-2. Indonesia

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Introduction

Sorghum (*Sorghum bicolor* L.) is a cereal crop that is usually grown under hot and dry conditions. According to House (1985) sorghum might be originated from the headwaters of the Niger River in Africa. Archaeological evidence suggested that the practice of sorghum domestication was introduced from Ethiopia to Egypt about 3000 B.C. Now about 80 % of sorghum cultivation is found in the Africa and Asian regions, however, the world sorghum production is still dominated by the USA, India, Nigeria, China, Mexico, Sudan and Argentina.

In many countries sorghum is generally used as food source, animal feed, and raw materials for industry. According to ICRISAT/FAO (1996), as global food source sorghum ranks the fifth after wheat, rice, corn, and barley. In many countries, sorghum grains and stovers are mainly used for animal feed. Sorghum grains are also used for industry such as ethanol, bear, wine, syrup, paint, glue, and modified starch. Related to energy source, countries like USA, India and China, have developed sweet sorghum as raw materials of bioethanol (biofuel) production. In the USA sorghum can produce up to 10,000 while in China 7,000 and in India 3,000-4,000 liters ethanol/ha/year. In India an improved, pressurized, multifuel (kerosene, ethanol or diesel) mantle lantern producing light output of 1,250-1,300 lumens (equivalent to that from a 100 W light bulb) called "Noorie" was developed (Reference). A pressurized alcohol stove with a heating capacity of 3 kilowatts for 85% (v/v) ethanol concentration with a thermal efficiency of 30-50% was also created (RAJVANSHI and NIMBKAR, 2004).

Sorghum has a high yield potential, comparable to those of rice, wheat, and maize. On a field basis, grain yields have exceeded 11 ton/ha (in Japan, total (seed + stems and leaves) dry yield are higher than 26t/ha), with above average yields ranging from 7-9 ton/ha where moisture is not a limiting factor in Indonesia. In those dry areas where sorghum is commonly grown, yields of 3-4 ton/ha are obtained under normal condition (Hause, 1985). Sorghum is also known to have wide adaptability, ranging from lowland, medium and highland altitude. Highest yields are usually obtained from varieties maturing in 100-120 days. Late-maturing varieties tend to be appropriate for forage crop.

Sorghum is beleived to have high tolerance to adverse conditions such as drought. Compare to maize, sorghum has a more extensive and fibrous root system. The plant roots penetrate a greater volume of soil to obtain moisture. Fertilizer, even under low rainfall conditions, encourages root development, hence the root are able to extract moisture from a greater volume of soil. Sorghum requires less moisture for growth than some other cereal crops. A study shows that sorghum requires 332 kg of water per kg of accumulated dry matter, while maize requires 368 kg of water, barley 434 kg, and wheat 514 kg. Compared to maize, sorghum is also more tolerant to water lodging, salinity, and aluminum toxicity (RANA and RAO, 2000).

Based on form of its spike and basic spikelet, sorghum is classified into 5 races namely *Bicolor*, *Guenia*, *Caudatum*, *Kafir*, and *Durra*. The characteristic of basic spikelet for each race is shown in Figure 1. Race Durra having white color of grains is the one that is commonly cultivated as grain sorghum and used as food source. Among race Durra, there is a variety having high sugar content in its stalk, a type what so called sweet sorghum. In many countries, sweet sorghum is used for syrup, sugar (*jaggery*), and/or ethanol industry (RAJVANSHI and NIMBKAR, 2004; UNDERSANDER et al., 1990).

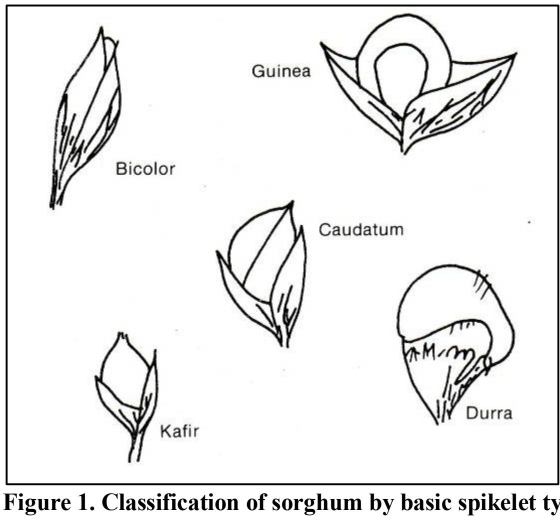

Figure 1. Classification of sorghum by basic spikelet type

Because sorghum is not Indonesian origin, so its genetic variability found in Indonesia is still limited. Some sorghum genotypes have been introduced from abroad e.g. from the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT) in India. Through plant breeding programs, the Ministry of Agriculture has released some sorghum varieties such as *UPCA*, *Keris*, *Mandau*, *Higari*, *Badik*, *Gadam*, *Sangkur*, *Lumbu* and *Kawali*. These national varieties have a big potential to be cultivated and developed in the arable land in Indonesia especially during wet seasons. Further research on sorghum breeding is needed especially to search for genotypes that can be grown and cultivated during dry seasons.

FNCA Mutation Breeding Project

Under FNCA plant mutation breeding project, the first meeting of the sub-project entitled "Drought Tolerance in sorghum and soybean" was held in Indonesia on 25 February - 01 March 2002. The participating countries included China, Indonesia, Japan, the Philippine, and Vietnam. The goal of this sub-project is to provide a broad base of common interests and to strengthen research collaboration among participating countries in the field of mutation breeding. Two kinds of crop species i.e. sorghum and soybean have been selected as targets for indication of drought tolerant crops. China and Indonesia took part in breeding sorghum, while the Philippine and Vietnam were in soybean. Exchange of breeding materials was agreed among the participating countries, which necessarily intended only for breeding purposes. (This part will be written in the preface of the report (book).)

For sorghum, the sub-project has been implemented accordingly. Exchange of sorghum breeding materials (sorghum seeds) has been made between counterparts of Indonesia and China. The list of the exchanged materials is presented in Table 1 below.

Table 1. List of exchanged sorghum breeding materials between counterparts of Indonesia and China

From Indonesia to China	From China to Indonesia
ET/20/477	*Zhengzhu*
DU/20/100-B	*Yuan 002*
DU/20/3E	*Yuantianza*
DU/30/95-B	*Yuanyu 8002*
Durra	*SP4-8002*

Sorghum Breeding For Drought Tolerance

Drought problem is one of limiting factors in agriculture development in Indonesia especially in Java and eastern part of the country. The problem is that annual dry season in some regions sometime happens quite long i.e. for about 6 months, starting from May up to October. During dry season most arable land usually becomes fallow, so farmers harvest almost nothing from

their land. For example, in the district of Gunungkidul in Yogyakarta Province, during dry season farmers attempt to import corn leaves in a huge amount from other Districts or Provinces in order to suffice their ruminant animal feeds.
For the areas having drought problem such as that in Gunung Kidul District, selection of crop species indicating high water use efficiency would be of valuable to be introduced. Sorghum plant (*Sorghum bicolor* L.) is thought to have high tolerance to drought (ICRISAT/FAO, 1996). Sorghum breeding to improve its yield in drought prone areas can be of importance for increasing the overall agricultural production in the region. Its biomass may be used for animal feed while the grains may be used as an alternative food during dry seasons.

The Indonesian farmers have long known sorghum landraces but its improvement and development seem to be neglected if compared to the other food crops. By being able to grow sorghum during dry seasons, it is hoped that the fallow land at least can produce biomass for feeding the animals. If good quality of sorghum variety is available, it may also provide food source to the local people so that it will prevent a hunger. As a food source, sorghum has been reported to have good nutrition values (DEPKES RI. 1992). Thus, sorghum has a big potential to help farmers increase their income, stimulate economic growth in the rural areas, and its cultivation will also promote soil improvement to support sustainable agriculture development.

Sorghum is actually not Indonesian origin but it has a big potential to be grown and cultivated in this country owing to its wide adaptability. However, sorghum is not as popular as other cereal crops and it is still insignificantly grown by very limited farmers. Available genetic variability of sorghum plant is also low, thus, plant breeding program is badly required in order to support sorghum development in the country. The breeding objective is to search for superior genotypes to help improve sorghum production with good quality according to its use either as food, animal feed or material for industry. Any plant breeding method such as that of selection, introduction, hybridization, mutation and/or other related biotechnology may be of appropriate to be applied in sorghum improvement program.

With regard to drought tolerance, indirect and direct selection methods have been implemented in sorghum breeding (SOERANTO, 2003). These methods are briefly described below:

A. Indirect selection:

- **Using 25% Polyethilene Glycol (PEG Method).** This method is used in seedling stage. It can reduce water potential equivalent to natural drought condition so that water absorption by roots is affected.
- **Using 0.3 % solution of potassium iodide.** This method is used in seedling stage. It can simulate leaf senescence and abscission equivalent to natural drought condition (Singh and Chaudhary, 1998).

B. Direct selection:

The plants are grown directly in drought prone areas during dry season. Sowing time is usually adjusted by the end of the rainy season. Production of total biomass, yield and its components can be used as criteria for selection.

The Use of Mutation Techniques

A. Gamma Irradiation

Research on sorghum mutation breeding was carried out at the Center for Research and Development of Isotope and Radiation Technology, National Nuclear Energy Agency (BATAN). Through research collaboration with the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), a number of precious breeding materials were obtained (as listed in Table 1; only exchanged materials are listed in Table 1). In our previous mutation breeding program, Durra variety from ICRISAT was used as starting breeding material owing to its high yielding and white grain type with good quality to be used for food and animal feed source.

The seeds were irradiated with Gamma rays emitted from Cobalt-60 source which is installed in the Gamma Chamber 4000A model. The dose levels 0-1000 Gy with increment of 100 Gy was used in order to study the optimal dose estimated around LD-50. Best fitting software was used to calculate the relationship between the doses and growth rates and to estimate the optimal doses. For example, Figure 2 shows the relationship between doses of Gamma irradiation doses (Gy) and survival rates (%) of sorghum Durra variety. It was found that the fit function is as follows:

3rd degree Polynomial Fit: $y=a+bx+cx^2+dx^3$

Coefficient Data: a = 95.984; b = -0.098; c = -7.615e-005; d = 7.966e-008

R = 0.99326615

LD-50 = 395.88 Gy

Gamma irradiation had significantly increased sorghum genetic variation in the M_2 (SOERANTO et al., 2001). Plant selections based on phenotypic variation were started in the M_2 generation, focused on the improved agronomic characters compared to that of the control plants. Through selection processes, a number of mutant lines with various desirable agronomic characteristics had been identified (SOERANTO and NAKANISHI, 2003). Some mutant lines had been tested for drought tolerance in Gunungkidul and Bantul Districts of Yogyakarta Province during dry season 2001 (M_3), 2002 (M_4), 2003 (M_5), and 2004 (M_6). The drought tolerance lines had been obtained namely B-68, B-69, B-72, B-75, B-83, B-90, B-92, B-94, B-95, and B-100. Biomass production and grain yields of these mutant lines (3-4 ton/ha) were significantly higher compared to that of original variety Durra and the national check variety (UPCA and Higari) which yielded only 1-2 ton/ha in dry seasons. Visual performances of some mutant lines are presented in Figure 3.

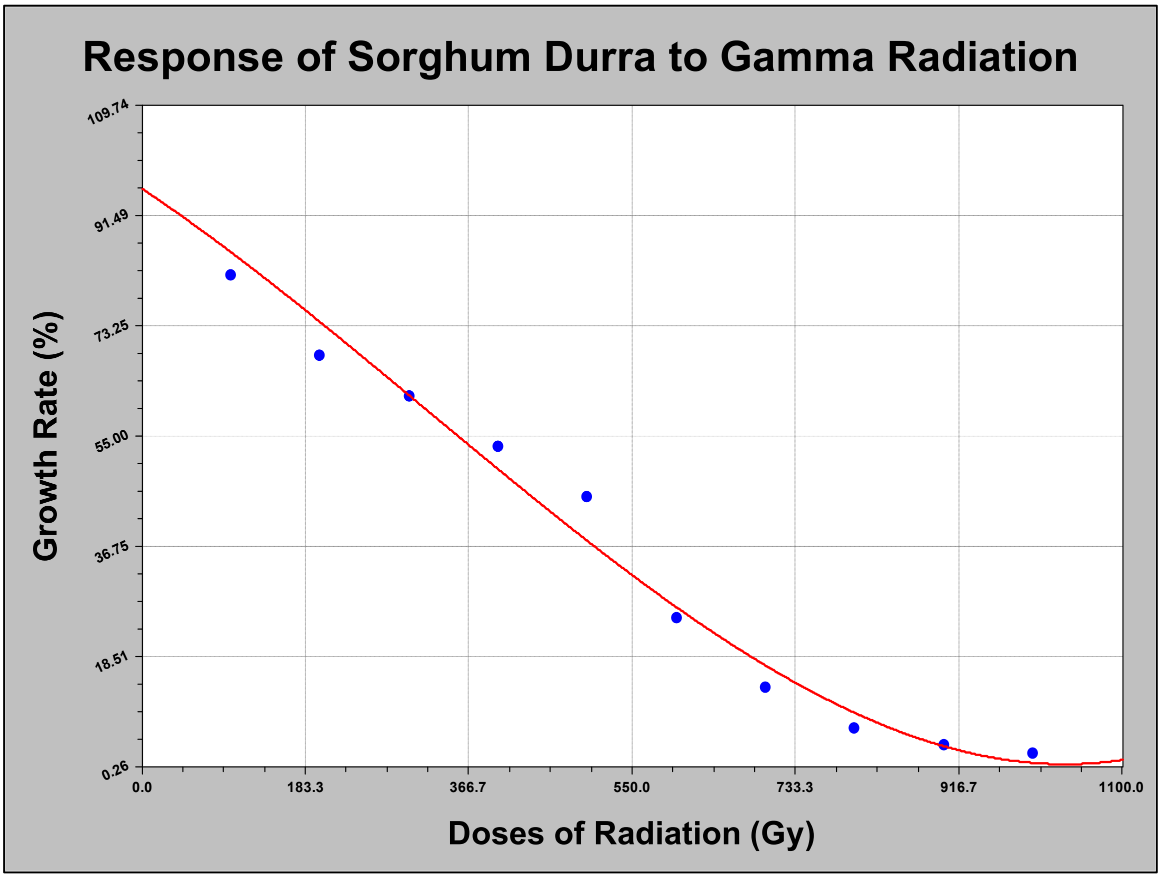

Figure 2. Response curve of sorghum Durra variety to Gamma irradiation in the M_1 generation

B. Ion-beam (C-ion) Irradiation

Mutation breeding in sorghum using Gamma irradiation was just started in 2005. Through FNCA Mutation Breeding Project framework, the utilization of ion beam (C-ion) for inducing mutation in sorghum was implemented to sorghum variety *Durra* and *Zhengzu*. Seed irradiation was conducted at TIARA facility of Japan Atomic Energy Agency (JAEA) with the dose levels of 0 (control), 50, 100, 150, 200 and 300 Gy. The irradiated seeds were evaluated in Indonesia. Response of sorghum to ion-beam was studied in the M_1 generation by its germination rate and growth. Relationship between doses and germination rate was studied using best fitting curve software. The curve functions were calculated and the LD-50 values were estimated. It seemed that each variety gave different response to ion-beam.

Within the ion-beam treatment, *Durra* and *Zhengzu* gave different response curves and the LD-50 values were found to be 376.30 Gy and 292.15 Gy, respectively. Meanwhile, the LD-50 value for Gamma radiation on sorghum Durra was 395.88 Gy. Comparing the ion beam and Gamma radiation, it seemed that LD-50 value for Gamma radiation was higher than ion beam radiation. The differences might be due to the highest dose levels used in the treatments. The highest dose level used in ion beam radiation treatment was 300 Gy while in Gamma radiation treatment was 1000 Gy. Results might suggest increasing the dose levels in ion beam radiation treatment since the LD-50 values were about the same or slightly higher than the highest dose levels used. The effects of ion beam treatments to plant genetic variability are measured in the M_2 generation by estimating genetic variance component of some agronomic characters.

In 2006, *Zhengzu* variety was irradiated again with ion-beam (C-ion, 320 MeV) at TIARA facility of Japan Atomic Energy Agency (JAEA) with the dose levels of 40 and 50 Gy. Meanwhile, sorghum variety *Durra* and *Zhengzu* were also irradiated again with ion-beam (C-ion) at RIKEN facility in Japan with the dose levels of 25, 50, 75, 150 and 200 Gy, respectively. All of these ion-beam irradiated materials are now being studied and evaluated in the field experiments in Indonesia. Until now, there is not yet found any desirable and useful sorghum mutants resulted from the ion-beam irradiation.

Highlight Results

1. A number of 10 drought tolerance mutant lines of sorghum have been obtained. In dry seasons, these lines have biomass production and grain yields significantly higher than that of original variety Durra and the national check variety (UPCA and Higari).
2. Our sorghum germplasm collections have been enhanced with these mutant lines, local and national sorghum varieties, exchanged breeding materials from China (through FNCA framework), and introduced genotypes from ICRISAT (Table of the data).
3. Some laboratory equipments for sorghum research have been provided by IAEA through

TC-Project INS/05/030.

4. Good sorghum research collaboration with some national and international counterparts and the end users has been established.
5. A private company (LIPPO Enterprises) has identified some of our sorghum mutant lines to be good for food, animal feed, starch and ethanol industry. (Will you write this collaboration more in detail with picture and data)

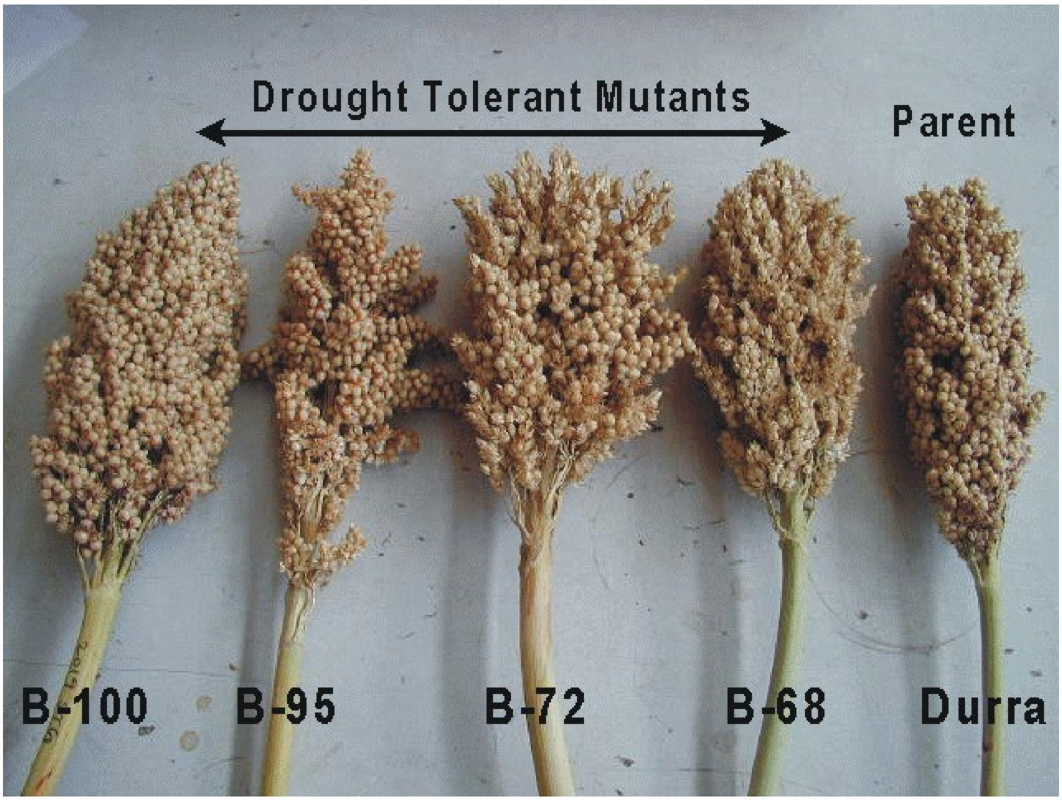

Figure 3. Variation of sorghum head form and size resulted from Gamma irradiation treatments

Other Supports

- LIPPO Enterprises (MOU with BATAN was signed on 12 April 2005) (as menteined abave.)
- International Atomic Energy Agency (through TC-Project INS/5/030)
- International Crop Research Institute for the Semi-Arid Tropics (ICRISAT)
- Japan Society for the Promotion of Science (JSPS) through the Core University Program of Tokyo University

Future Plan

- Multi-location trials of sorghum mutant lines, at least in 12 locations, before being submitted for official releases as new sorghum varieties to the Ministry of Agriculture.
- Continuing mutation breeding to improve yields and quality of sorghum used as food, animal feed, and material for industry. The development of sorghum cultivation will be directed to make optimal use of the marginal land, especially in drought prone areas, so that it will not compete with the other crops in term of land utilization.
- Continuing and strengthening sorghum research collaboration with national and international counterparts
- Integrating Molecular Assisted Selection (MAS) and TILLING technology in pyramiding and characterization of the mutated gene(s) in sorghum.

Conclusions

Mutation breeding program using Gamma radiation in sorghum in Indonesia had resulted 10 promising mutant lines. These lines indicated drought tolerance and were promising to be developed further in Indonesia. Through FNCA Mutation Breeding for Drought Tolerance project, exchange of sorghum seeds had been implemented between counterparts of China and Indonesia. All sorghum from China could grow and perform well in Indonesia. *Zhengzu* has been included in our mutation-breeding program owing to its indication of drought tolerance and high yielding. Induced mutation using ion-beam irradiation is being studied on sorghum *Durra* and *Zhengzu*. Sorghum research in Indonesia is supported by some national and international counterparts.

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2. Soybean

2-1. Indonesia

2-2. Malaysia

2-3. The Philippines

2-4. Vietnam

2-1. Indonesia

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Introduction

In Indonesia, soybean (*Glycine max* (L.) Merril) has become the second main food crop after rice and has been included in the National Food Program since 1984 for increasing nutritious food in human diet. It is one of the major low-cost sources of protein and edible vegetable oil. Mostly, soybean is consumed as processed food products, such as tempe (fermented bean), bean curd (tofu), kecap (soy sauce) and cooking oil (Damardjati *et al*., 1996). Soybean is also used as animal feed stock and raw material for industry.

Not only the consumption of soybean has been increased from 2,270,764 ton in 1998 to 2,278,000 ton in 2000 (CBS, 2006), but also the production of soybean has been decreased from 1,547,263 ton in 1998 to 797,135 ton in 2005 and the average yield of soybean is around 1.2 to 1.3 ton ha^{-1} (CBS, 2005). However, as yet it has been impossible to meet the increasing consumption and hence approximately 50% of the domestic production of soybean has to be imported. Therefore, to supply the domestic demand, it is important to increase domestic production.

The domestic production of soybean could increase by intensification and extensification (expansion growth area). Both of methods need suitable varieties. Intensifying production by planting pattern and integrated cultivation technology has been done in rice field and dry land in Java. However, the fertile soil in Java is always decreasing approximately 20,000 hectares every year by non-agricultural purposes (Notohadiprawiro, 1996). Extensification by expansion of the soybean area is only possible in outside Java which usually infertile viz. acid, drought, saline etc. A total potential area of soybean production is 55.2 million ha in Sumatra, Kalimantan, Sulawesi and Papua. These lands are dry and acid lands (Adhi, 1987). These lands could be suitable for soybean production after major improvement of soil fertility and irrigation. It is make uneconomic cost production. This constrain might be overcome among others by use of adapted varieties.

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FNCA Soybean Mutation Breeding Project

Indonesia joined the FNCA Soybean Mutation Breeding sub-project in 2003. Exchange of breeding materials (soybean seeds) has been made in 2004 between counterparts of Indonesia, Vietnam, the Philippines, Korea and Malaysia. The list of the exchanged materials is presented in Table 1 below.

Table 1. List of exchanged soybean breeding materials between counterparts of Indonesia, Vietnam, Korea, Thailand, and the Philippines.

No.	Genotypes	Country of Origin
1	5-MBB	Indonesia
2	25A-MBB	Indonesia
3	29-MBB	Indonesia
4	47-MBB	Indonesia
5	55-MBB	Indonesia
6	60-MBB	Indonesia
7	I-209	Indonesia
8	M-220	Indonesia
9	GH-7 (Rajabasa)	Indonesia
10	M.103	Vietnam
11	DT.84	Vietnam
12	Bangsakong	Korea
13	CM60-10kr-71PS-21	Thailand
14	CM60	Thailand
15	PSB-Sy-4	Philippines
16	NSIC-Sy-8	Philippines
17	PCB-Sy-5	Philippines
18	BPI-Sy-4	Philippines
19	Tanggamus,	Local check (Indonesia)
20	Willis)	Local check (Indonesia)

Cultivar Guntur was used as breeding material in this sub-project to increase variability, and then 2 selected mutant lines (No. 9 and 23) were crossed. The F_1 hybrid of cv. Muria x PI-48025 was irradiated by gamma rays. The method has been established, which the optimum dose was 150 Gy, and the rate was 750 $Gy.h^{-1}$. Some promising mutant lines have been developed and then tested in multi-locations in 2003. The GH-7 mutant line was proposed to the National Seed Board for evaluation in 2004.

The genetic materials from participating countries tested at The Citayam Research Station in the year 2004 and 2005. In the dry season 2006 the experiment was carried out to investigate the performance of twenty-soybean genotypes (including mutant lines and local check) at Cikemeuh Research Station. Elevation of these locations is 75 m above sea level. The soil type is red-yellow phodzolic. The soil was dry and no irrigation. Natural drought climate was used to evaluate the mutant lines. Twenty genotypes mutant lines from Indonesia (9 mutants), Vietnam (2 genotypes), Thailand (2 genotypes), the Philippines (4 genotypes), and Korea (1 genotype), and 2 national control varieties had been used. The treatments were applied in a Randomized Complete Block Design with three replications. Data on yield and agronomic characters i.e. dry seed weight, mature plant height, number of branches per plant, number of nodes per plant, number of pods per plant, weigh of 100 seeds, and days to maturity were observed.

Results and Discussion

The result of 2003 multi-location trials showed that the GH-7 mutant line had excellent performance. It had high yielding (22.4 $qu.ha^{-1}$) due to having medium seed size (15 g/100 seeds), and plenty of pod number per plant. The GH-7 line was tolerant enough to acid soil and drought. It was named cultivar Rajabasa.

Three-year data (2004-2006) showed that the yield average of the 60-MBB mutant line (13.84 $qu.ha^{-1}$) and 47-MBB (12.7 $qu.ha^{-1}$) was significantly higher than Tanggamus cv. (10.43 $qu.ha^{-1}$), Rajabasa cv. (9.4 $qu.ha^{-1}$) and Willis cv. (9.1 $qu.ha^{-1}$) as a local checks (P=5%). High yield of 60-MBB and 47-MBB mutant lines were due to the bigger seed size, high number of pods, high number of branches, and high number of nodes per plant. There was no different between M-220 and 60-MBB on dry seed weight per plant at P=5%. The CM.60 line from Thailand had the lowest yield (2.65 $qu.ha^{-1}$) due to low pod number per plant, and short stature.

The data showed that the average of seed weight per plant of Bangsakong variety from Korea was significantly lower than other genotypes (1.26 g). This was due to less number of pods (16.63), number of nods (14.10), and short plant (30.63 cm). The 60-MBB, 25-A-MBB, 47-MBB, 5-MBB, 55-MBB and 29-MBB mutant lines had big seed size. The 100-seed weights were ranging from 22.0 to 27.2 g.

There was no significant difference between NSIC-SY-5 (Phil) and DT.84 (Viet) but significantly lower than control varieties Tanggamus and Rajabasa cv.

Eight genotypes with pure line selection for 6 generation of local mutant lines with drought tolerance have been selected. Four mutant lines (GH-7, I-209, M-220 and 60-MBB) were already distributed to field test in Malaysia and Philippines. Two students of the Sriwijaya and Bogor Agricultural Universities had used mutant lines to study the characters of mutant and field test. The GH-7 mutant line was released as cv. Rajabasa, and then 2.2 ton certified seed of cv. Rajabasa has been distributed in South Sumatra and Jambi Provinces. All mutant lines had been evaluated by the committee. In the mid 2007, M-220 line will be released as Mitani cv.

Conclusion

Guntur cv. was breeding material to increase variability for this project, and then two selected mutant lines (no. 9 and 23) were crossed. F_1 of Muria cv. x PI-48025 were irradiated by gamma rays. Some mutant lines were promising to be released. The method has been established, which the optimum dose was 150 Gy, and the rate was 750 $Gy.h^{-1}$. The methods to increased variation were by combination of crossing and irradiation. Natural drought climate was used to evaluate the mutant lines. Eight lines with pure line selection for 6 generation of the mutants with drought tolerance have been developed. Availability to share promising mutant lines among the participating countries and to use them as parental lines for developing new varieties. Four mutant lines were already distributed to field test in Malaysia and Philippines as participating countries. Two students of the Sriwijaya and Bogor Agricultural Universities had used mutant lines to study the characters of mutant and field test. The GH-7 mutant line was released as cv. Rajabasa. 2.2 ton certified seed of cv. Rajabasa has been distributed in South Sumatra and Jambi Province. All mutant lines had been evaluated by the committee. In the mid 2007, M-220 line will be released as Mitani cv.

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Annex

Table 1. Agronomic Characters of eleven Mutant Lines and three National Varieties of Soybean at Cikemeuh in dry season 2006.

No.	Genotypes	Yield qu.ha^{-1}	Dry seed weight, g/plant	Weight of 100 seeds g	Plant Height Cm	Number of nodes/ plant	Number of Branches / plant	Number of pods/ plant
1	60-MBB	13.85	6.34	27.2	56.97	12.83	2.0	27.23
2	47-MBB	12.71	5.55	25.2	53.53	13.00	1.6	23.80
3	5-MBB	12.11	5.43	24.5	52.20	12.33	1.5	25.47
4	55-MBB	11.55	5.19	22.0	56.00	12.43	2.2	23.00
5	29-MBB	10.92	4.93	24.5	47.13	11.20	1.7	20.90
6	Tanggamus	10.43	4.69	8.5	61.17	16.83	2.1	34.70
7	Rajabasa	9.42	4.77	13.5	66.27	15.67	2.1	33.47
8	Willis	9.07	4.22	9.0	70.43	17.30	1.8	29.07
9	25-A-MBB	8.96	6.05	26.0	54.40	13.33	2.2	29.07
10	NSIC-SY-8	7.20	3.42	12.5	59.63	16.13	0.7	15.63
11	DT.84	7.19	4.89	15.5	57.87	12.53	0.6	17.90
12	I-209	6.95	4.25	12.5	66.47	16.50	2.6	32.77
13	PCB-SY-5	6.95	3.11	13.0	70.17	15.57	0.9	20.07
14	M-220	6.51	4.96	12.0	53.8	15.63	2.6	29.37
15	M.103	6.22	3.81	15.0	56.20	13.63	2.0	26.53
16	PSB-SY-4	5.84	3.21	14.0	52.77	13.60	1.8	20.27
17	CM.60-KR	5.44	4.76	14.5	50.63	14.10	1.4	25.23
18	BPI-SY-4	3.77	3.43	15.0	53.27	13.47	1.7	20.33
19	Bangsakong	3.24	1.26	13.0	30.63	10.60	1.5	16.93
20	CM.60	2.64	4.76	14.5	50.63	14.10	1.6	15.63
	LSD (5%)	2.00	1.68		7.6	1.37	0.8	10.31
	CV (%)	15.03	22.95		8.19	5.87	19.5	15.03

2-2. Malaysia

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Introduction

Mutation breeding is considered as an additional tool in augmenting other breeding techniques, even though many important crops in Malaysia already have elaborate improvement programmes. The use of induced mutations in crop improvement was initiated in the 1970's, and involved crops such as soybean, rice, banana and roselle.

Gauged on the achievements made over the last couples of decades, only few induced mutations have met with varying success The early work on mutation breeding of legumes was initiated at UKM in 1976, where soybean and winged bean were used (Jalani & Zakri 1980). The research on induced mutation of soybean was carried out with the support of the IAEA at UKM, and the main objective was to breed for high yield and good adaptation to the warm humid tropical environments (Jalani & Zakri, 1980; Mak & Funnah, 1983; Mak & Yap, 1986; Yap, 1986; Zakri & Jalani, 1986; Zakri, 1991; Mohamad & Mohd. Nazir, 1997; Mohamad et al., 1999). More recently, drought tolerance traits have become sought after to increase productivity in tropical environments (Barrida *et al.* 2005; Masrizal *et al.*, 2005).

In soybean mutation breeding, ethylmethanesulphonate (EMS) and gamma irradiation were used, and following several selections, two high yielding and well-adapted mutants were recovered. They were designated as A15 and A5. A15 is a large seeded mutant with a high pod number, while A5 is an early maturing dwarf with a good yield potential. The two mutants flowered in 35 and 32 days respectively, compared with Palmetto, which flowered after 34 days. The maturation periods for the three were 98, 84 and 97 days, respectively.

However, even though reported yields from experiments for the three were high, the field trials did not show up the same performance. In fact, Palmetto is still the leading control variety with moderately low yield levels of 1.0-2.0 t/ha. Based on an introduction of nine soybean varieties from USA in 1996, field evaluations showed that two of them, namely Ottootan and Bossier, had potentially good performance. The black-seeded variety Ottootan was engaged in induced mutations, but the mutants produced were not able to improve significantly the soybean productivity. The objective of this paper is to briefly report on the performance of the three potential soybean mutant lines accessed through FNCA network under local conditions.

Introduction of Drought-Tolerant Mutant Lines

As a result of the FNCA 2003 Workshop on Mutation Breeding in Cavite and Manila, it was suggested that four soybean mutant lines be made available by soybean breeder from Philippine Nuclear Research Institute (PNRI) for field testing in Malaysia. Barrida *et al.* (2005) reported that three soybean mutant lines, namely BPI-SY-4, PSB-SY-4 and PSB-SY-5 showed some degree of drought tolerance under Philippine conditions. Four samples containing 20 seeds of each line were received by the breeder at UKM in late 2003 (*pers. comm.* Paul). One of the seed samples did not germinate completely.

Seed Increase Plots

The remaining three accessions, PSB-SY-4, BPI-SY-4 and PSB-SY-5 went through seed increase in 2004 as single hills. Each entry had six replications. Some morphological and agronomic data on the three mutant lines were taken. They were also compared with Ottootan, A5, and A15. Morphological and agronomic characteristics included plant heights at flowering and maturity, 100-seed weight, number of seeds per pod, number pods per plant, pod length and width, leaf length and width, seed weight per plant, and days to flowering and to maturity (Norliyana, 2005; Mohamad *et al.*, 2005; Loo, 2006).

The accession BPI-SY-4 had the best morpho-agronomic assessment. This was because it had the highest seed weight per plant (49.4 g), the shortest days to maturity (82 days) as well as the highest 100-seed weight (23.1 g). All accessions were observed to have little seed dormancy.

Replicated Field Trial

In mid-August 2005, the three mutant lines PSB-SY-4, PSB-SY-5, BPI-SY-4 were planted in field plots to assess their field performance. The field experiment used Palmetto, A15 and Ottootan as control varieties. However, Palmetto suffered severe germination problems and was excluded from statistical analyses. Ottootan is black small-seeded variety introduced from US. A15 is a mutant line developed by Universiti Kebangsaan Malaysia (UKM) from Palmetto through mutation breeding (Zakri 1991). In the past, Palmetto and A15 had been reported to produce yields of between 1,000 and 2,000 kg/ha (Anon., 1978; Yap & Lee, 1975).

The experiment was conducted at UKM Bangi using a split plot design with three replications. Each entry is planted as a 4-row plot comprising 26 plants. Each row is 5m in length, and the plants were planted in 50 x 20 cm spacing. The main plots were assigned to two irrigation treatments, namely with irrigation and without irrigation. With irrigation, experimental plots were irrigated throughout the crop season; without irrigation, experimental plots were only given irrigation until 30 days after seed germination. The remaining part of the crop season was only subject to natural rainfall, essentially to simulate less water conditions. Harvesting was completed in late November 2005.

Analysis of variance showed all morphological and yield components' characteristics did not differ significantly ($p>0.05$) between the irrigation treatments (Tables 1 & 2). However, the number of pods per plant showed a significant interaction between irrigation and soybean accessions (Figure 1). The soybean accessions showed significant differences ($p<0.05$) for all characteristics. Tukey test showed that PSB-SY-4 was the earliest to flower and mature, in 32 and 83 days, respectively. Plant heights at flowering for A15 and Ottootan were 1.3 and 3.2 cm higher than the other accessions, but plant heights at maturity for BPI-SY-4, PSB-SY-5 and Ottootan were 29.9 to 53.5 cm higher than A15 and PSB-SY-4.

Conclusion

The mutant line PSB-SY-4 was slightly earlier than the control A15. The field trial showed that the accession BPI-SY-4 recorded the highest for the number of pods per plant (153), 100-seed weight (22.2 g), seed weight per plant (48.3 g) and seed yield per plot (3.83 kg) compared with other accessions. However, despite its good field performance and yielding capacity, the experiment was not able to compare PSB-SY-4 with Palmetto, which represents the key control variety under our local conditions.

Acknowledgements

The authors would like to thank UKM, MINT, FNCA, PNRI and Mr. Paul for the materials and support in this research. They are also grateful to Ms. Norliyana and Ms. Loo for their data and information discussed in this paper.

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Table 1: Analysis of variance for morpho-agronomic traits of soybean accessions in the field trial

Source of variation	Degrees of freedom (df)	Days to flowering		Plant height at flowering (cm)		Maturity (days)		Plant height at harvest (cm)	
		SS	MS	SS	MS	SS	MS	SS	MS
Block	2	0.47	0.23	141.42	70.71**	1.40	0.70	4987.34	2493.67**
Irrigation	1	0.83	0.83	7.40	7.40	0.30	0.30	209.09	209.09
Error (a)	4	2.07	1.03	435.80	217.90	2.60	1.30	4025.68	2012.84
Accessions	4	76.87	19.21**	277.99	69.50**	868.47	217.12**	77444.41	19361.10**
Irrigation x Acc	2	0.33	0.08	92.40	23.10	11.53	2.88	491.74	122.94
Error (b)	16	6.80	0.43	2038.90	12.28	38.00	2.38	24268.98	146.20
Total	29								
C.V. (%)			1.89		9.32		1.67		16.26

* p<0.05, ** p<0.01

Table 1: Analysis of variance for morpho-agronomic traits of soybean accessions in the field trial (cont.)

Source of variation	Degrees of freedom (df)	Number of pods per plant		100-seed weight (g)		Seed yield per plant (g)		Yield (kg/ha)	
		SS	MS	SS	MS	SS		SS	MS
Block	2	8830.04	4415.02**	13.24	6.62	28.09	14.05	0.56	0.28
Irrigation	1	12086.81	12086.81	18.55	18.55	25.19	25.19	0.07	0.07
Error (a)	4	10240.59	5120.29	12.98	6.49	133.95	66.98	0.53	0.26
Accessions	4	24720.81	61800.45**	736.85	184.21**	775.40	193.85*	2.78	0.70*
Irrigation x Acc	2	23084.61	5771.15**	18.51	4.63	73.00	18.25	0.60	0.15
Error (b)	16	135445.54	815.84	166.63	10.41	466.80	28.18	30.7	0.19
Total	29								
C.V. (%)			20.90		13.77		17.44		13.43

* p<0.05, ** p<0.01

Table 2: Means of morpho-agronomic traits of soybean accessions in the field trial

Factor	Treatments	Days to flowering	Plant height at flowering (cm)	Maturity (days)	Plant height at harvest (cm)
Irrigation	With irrigation	35 a	37.4 a	92 a	75.4 a
	Without irrigation	34 a	37.8 a	92 a	73.3 a
Accession	BPI-SY-4	36 a	37.2 b	99 a	95.3 a
	PSB-SY-4	32 d	36.5 b	83 c	41.8 c
	PSB-SY-5	33 c	36.3 b	98 a	88.5 a
	Ottootan (control)	36 ab	38.5 ab	91 b	88.0 a
	A15 (control)	35 b	39.5 a	90 b	58.1 b

Within a column, means with same letters are not significantly different at p<0.05.

Table 2: Means of morpho-agronomic traits of soybean accessions in the field trial (cont.)

Factor	Treatments	Number of pods per plant	100-seed weight (g)	Seed yield per plant (g)	Yield (kg/ha)
Irrigation	With irrigation	144.9 a	19.3 a	40.2 a	3.31 a
	Without irrigation	128.5 b	17.7 a	38.3 a	3.21 a
Accessions	BPI-SY-4	153.0 b	22.2 a	48.3 a	3.83 a
	PSB-SY-4	88.5 d	24.4 a	32.5 b	2.99 b
	PSB-SY-5	138.2 b	19.6 ab	38.5 b	3.08 ab
	Ottootan (control)	195.6 a	10.3 c	39.1 ab	3.32 ab
	A15 (control)	108.2 c	16.1 b	37.9 b	3.09ab

Within a column, means with same letters are not significantly different at p<0.05.

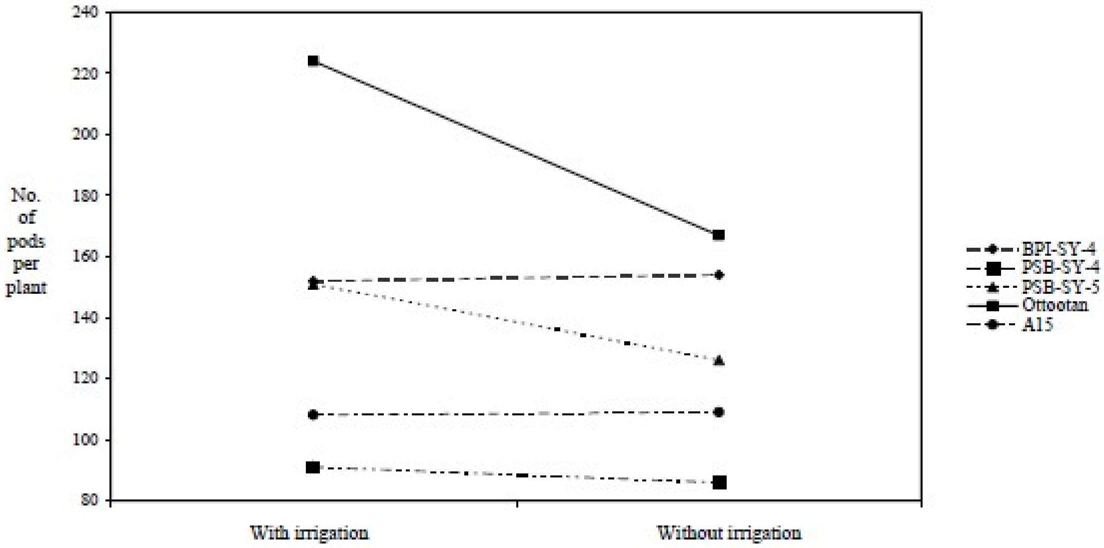

Figure 1: Interaction effects between irrigation treatments and soybean accessions in the field trial

2-3. The Philippines

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Introduction

Soybean (*Glycine max* L. Merr.) is one of the most important sources of protein and oil. It contributes about 60% of the world protein consumption and 53% of the oil need. In the Philippines, soybean is grown both for animal feed and human food. As animal feed, it is the major component in the formulation of mixed livestock and poultry feed. As human food, it is used as substitute for cow's milk and other food preparation like "tokwa", "miso", "tahuri", "taho", etc. It has also many commercial applications especially in the manufacturing industries having a high oil content of 20%. Its production, however, lags behind demand because of the convergence of causes like lack of price and production support, slow development of adapted and high-yielding varieties particularly those that have drought tolerance.

Drought is one of the most debilitating conditions farmers have to face especially if irrigation water is not available. This is true to crops that do not posses deep and extensive root system, like soybean because even a short dry spell can damage the crop. Measures to circumvent dry spell are sometimes implemented like timing of planting to coincide with rainfall pattern but it cannot be relied on because of the unpredictable weather especially with the occurrence of the "El Nino" phenomenon.

Thus, prolong dry spell devastate the crop. This is the main reason why the good results in the experimental fields are not attained in the farmer's fields. Developing varieties that are high yielding and drought tolerant are the main breeding objectives. At present, yield of the newly developed varieties is only a little over 2 tons per hectare which is below the world standard. Because of the low yield, less than 1000ha are planted to soybean in 2001 and as a consequence, the Philippines is importing more than 96% of our requirements valued at P 70 million a year, draining part of our foreign exchange. To increase the yield of soybean requires

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new lines that are adapted to the tropical condition. Since the source of breeding materials come mainly from the temperate countries, the use of induced mutations to generate the desired characters is needed. Based on the FAO Mutant Variety Database more than 1,800 developed varieties including soybean have been officially released.

In 2002, the Philippines, through the Philippine Nuclear Research Institute (PNRI) started participating in the Forum for Nuclear Cooperation in Asia (FNCA) Multilateral Research Program on "Mutation Breeding for Drought Tolerance in Soybeans" in collaboration with Vietnam and Indonesia. This project includes exchange of soybean germplasm developed by different breeding centers in Asia. Development of high yielding varieties that are drought tolerant will encourage farmers to grow more of these crops and would result in increased productivity, poverty alleviation and improved nutrition. This would also mean saving due to shorter breeding time in the development of improved soybean varieties.

The accomplishments (2002-2007) of this project on "Mutation Breeding for Drought Tolerance in Soybeans" are summarized in this report.

Objectives

To develop soybean varieties with good agronomic characteristics such as short stature, early maturing, high yielding and drought tolerant.

Methodology

Research studies conducted for the attainment of the project objectives were as follows.

1. Collection of different varieties of soybean to be used for mutation induction.
2. Determination of radiosensitivity of different soybean varieties to gamma radiation.
3. Screening for drought tolerance and identification of putative mutants in the M_2 generation.
4. Screening for drought tolerance in the M_3 generation of the introduced varieties from Vietnam and the M_4 generation of the local soybean varieties
5. Growing and selection of mutants in the M_4 of the introduced and M_5 generations of local soybean varieties.
6. Further selection of mutants for drought tolerance and other agronomic traits in the M_5 generation of the introduced varieties from Vietnam and M_6 generation of the local soybean varieties.

Collection of different varieties of soybean to be used for mutation induction

Four local soybean varieties (BPI-Sy 4, PSB-Sy 4, PSB-Sy 5 and NSIC-Sy 8) were obtained from the Bureau of Plant Industry, Economic Garden, Los Banos, Laguna. These varieties were sent to Vietnam in exchange of four genetic materials of soybean (AKO-6, DT 84, DT 95 and DT 96) as per agreement with participating countries involved in the Forum for Nuclear Cooperation in Asia (FNCA) Multilateral Research Program on Mutation Breeding for Drought Tolerance in soybeans. Eight varieties of soybean were used for irradiation using gamma rays to induce mutation.

Determination of radiosensitivity of different soybean varieties to gamma radiation

Dry seeds of four local soybean varieties (BPI-Sy 4, PSB-Sy 4, PSB-Sy 5 and NSIC Sy 8) and four varieties from Vietnam (AKO-6, DT 84, DT 95 and DT 96) were irradiated at doses of gamma radiation ranging from 100 to 500Gy using Gamma Cell-220 irradiator of the Philippine Nuclear Research Institute (PNRI). Irradiated and non-irradiated local and introduced varieties were planted at the PNRI experimental field and at Bulacan National Agricultural College (BNAC). Data on percentage germination, seedling height, mature plant height, days to flower and number of pods were taken in the M_1 generation. Based on the results of this study, the optimum dose was determined. Processing of the seeds were done and planted as M_2 generation. Data on chlorophyll mutations were collected and putative mutants were likewise identified and selected. Another batch of irradiation was done using the optimum dose of 200 and 250Gy. Data on the different growth parameters mentioned above were also taken to determine the radiosensitivity of different soybean varieties.

Screening for drought tolerance and identification of putative mutants in the M_2 generation

Identification of the mutants was done in the M_2 generation. For drought tolerance test, eight varieties were simultaneously planted in a well prepared soil on March 4, 2003. The distance of planting is 60cm between rows and 10cm between hills with 1 seed per hill. The rate of fertilization used was 40 kg/ha NPK (14-14-14).

The treatment consisted of withdrawing irrigation water 20, 30, 40 and 50 days after planting. The control was regularly irrigated until maturity. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Data on days to flower, mature plant height, weight of harvested seeds from 2-meter sample length were taken. Routine cultural practices (i.e. watering, weeding, fertilization, hilling up and spraying with insecticides) were done for the optimum growth of the plants.

Screening for drought tolerance in the M_3 generation of the introduced varieties from Vietnam and the M_4 generation of the local soybean varieties

Seeds of the local soybean varieties (PSB-Sy 4, PSB-Sy 5, NSIC-Sy 8 and BPI-Sy 4) and the introduced varieties from Vietnam (AKO-6, DT 84 and DT 95) selected in the previous generations that exhibited desirable agronomic characteristics (drought tolerant, early maturing, short stature, high yielding) were planted at the experimental field of PNRI, Quezon City and at the cooperator's field at the Bulacan Agricultural State College (BASC), San Ildefonso, Bulacan, during the dry season. The experiment was laid in Randomized Complete Block Design (RCBD) with three replications. The same distance of planting and experimental design used the previous study was also used in the M_3 and in the succeeding generations. Data on number of days to flower, plant height at maturity, number of pods per plant, number of seeds per plant and 100-seed weight were gathered. During the whole growing season, plants that exhibited earliness, short stature, compact growth and tolerance to drought were tagged and selected. Methods to identify the drought tolerant mutants have been established under greenhouse and field experiments. Under greenhouse pot experiment, withdrawal of irrigation at various developmental stages of the plant was conducted. Roots were examined and plants that have long and ramified root system were selected for drought tolerance. Plants that have thickened leaves that remain green at maturity were selected since these plant characters are presumed to be associated with drought tolerance. Under field conditions, plants with thickened leaves that are green in color at maturity were also selected as putative mutants for drought tolerance.

Growing and selection of mutants in the M_4 of the introduced varieties and M_5 generations of local soybean varieties

The growing of the M_4 generation of four local soybean varieties and three introduced varieties from Vietnam mentioned above were also conducted at the experimental field of BNAC and PNRI. These were planted in a well prepared soil. The distance of planting and the experimental design used were the same as in the previous studies. In the screening for drought tolerance in the M_4 generation the test materials used were the introduced varieties from Vietnam that have been irradiated with 200 and 250Gy and subjected to withdrawal of irrigation 30 days after planting. Another study was undertaken to select mutants fro drought tolerance in the M_5 generation of four local soybean varieties with varying days of irrigation withdrawals (30, 40, and 50 days after seeding). Various growth parameters and yield components were taken. Percentage drought tolerant lines, as well as the number of early maturing plants were also recorded.

Further selection of mutants for drought tolerance and other desirable agronomic traits in the M_5 of the introduced varieties from Vietnam and M_6 generation of the local soybean varieties

The growing of the M_5 generation of three introduced varieties (AKO-6, DT 84 and DT 95) from Vietnam and the M_6 generation of three local varieties (PSB-Sy 4, BPI-Sy 4 and PSB-Sy 5) were conducted at the experimental field of PNRI, Quezon City on February, 2006. These were planted in a well-prepared soil and the experimental lay-out used were the same as in the previous studies. The plants were fertilized with complete fertilizer (14-14-14) at the rate of 40 kg/ha. Routinary cultural practices were undertaken for the optimum growth of the plants.

In the screening for drought tolerance in the M_5 generation the test materials used were the introduced varieties from Vietnam that have been irradiated with 200 and 250Gy and subjected to withdrawal of irrigation 20 days after planting. In the M_6 generation selection of mutants for drought tolerance and other desirable agronomic traits was done with varying irrigation withdrawals of 20 and 30 days after planting. The same data on the different growth parameters, yield and drought tolerance were gathered.

Results and Discussions

Collection of different varieties of soybean to be used for mutation induction

Four local varieties (PSB-Sy 4, PSB-Sy 5, BPI-Sy 4, NSIC-Sy 8) and four varieties from ietnam (AKO 6, DT 84, DT 95 and DT 96) were used as breeding materials for mutation induction. Exchanged of genetic materials between Vietnam and the Philippines (as greed upon in the 2002 Formulation Meeting) was undertaken. Production of pure lines of these varieties was done.

Determination of radiosensitivity of different soybean varieties to gamma radiation

The method of irradiation was established. Seeds of the local and introduced varieties from Vietnam were irradiated with gamma rays ranging from 100 to 300Gy and the optimum dose was determined to be at 200 and 250Gy.

The effects of gamma radiation in the M_1 generation of four local soybean varieties were assessed and shown in Table 1. Percentage germination was not affected by the two doses of 200 and 250Gy gamma radiation in the four varieties used. Based on the seedling height, varieties BPI-Sy 4, PSB-Sy 4 and PSB-Sy 5 have similar sensitivity. No significant differences were observed in the 200Gy treatment and the control except for the 250Gy. In NSIC-Sy 8 variety however, doses of 200, 250Gy and the control were not significantly different.

In mature plant height, variety BPI- Sy 4, PSB-Sy 5 and NSIC- Sy 8 had the same response. PSB-Sy 4 was the least sensitive to gamma radiation of 200 and 250Gy. The number of days to flower for all varieties was not affected by the treatment. Likewise, the maturities of all varieties were not affected by gamma radiation.

The effects of gamma radiation in the M_1 generation of four soybean varieties obtained from Vietnam are presented in Table 2. In the introduced varieties, doses of 200 and 250Gy did not affect the percentage germination of four soybean varieties (AKO 6, DT 84, DT 95 and DT 96). Significant differences in seedling height were observed in doses of 200, 250Gy and the control except for the variety AKO 6. There were significant differences in plant height of mature plants between the control and the treatment dose of 250Gy in varieties DT 95 and AKO 6. However, no significant differences were observed between the control and the irradiated ones in varieties DT 84 and DT 96.

The number of days to flower was not affected by gamma irradiation. No significant difference was noted between the control and the irradiated ones (200 and 250Gy) in all varieties used.

Data on number of pods per plant is presented in Table 3. Significant differences between the control and 200Gy treatment in variety DT 96 were observed. Likewise, significant differences were noted between the control and 250Gy treatment of soybean variety AKO 6, DT 84 and DT 95.

The different types of chlorophyll mutations in the M_2 generations of four local soybean varieties are shown in Table 3. The predominant type of chlorophyll mutation is the chlorina, followed by striata and spotted yellow for all the varieties used. The greatest number of chlorina mutant was observed at 250Gy for varieties PSB-Sy 4 followed by PSB-Sy 5 and then NSIC-Sy 8; and at treatment dose of 200Gy for PSB-Sy 5, NSIC-Sy 8 and BPI-Sy 4. Spotted yellow mutant was noted only at 200Gy for NSIC-Sy 8 and at 250Gy for variety PSB-Sy 4. No albino mutant was induced in all of the four varieties used in the experiment.

The highest frequency of chlorophyll mutation was at treatment dose of 250Gy for varieties PSB-Sy 4, BPI-Sy 4 and NSIC-Sy 8 and at 200Gy for PSB-Sy 5 (Table 3a).

The frequency and type of chlorophyll mutations in the M_2 generation of soybean varieties obtained from Vietnam are shown in Tables 3b and 3c. Only chlorina mutant was induced at 200 and 250Gy treatment in four (4) varieties. (AKO 6, DT 84, DT 95 and DT 96). Sixteen (16) striata were induced at 200Gy treatments in variety DT 84 and only one (1) striata was found in variety DT 96 formerly exposed to 200Gy Co-60 gamma rays.

The treatment with the highest frequency of chlorophyll mutation was at 250Gy in varieties DT 84, DT 96 and DT 95 (Table 3c).

Desirable mutant lines selected in the M_2 generation are presented in Table 4. The highest number of early and high yielding mutants was selected at 250 Gy for varieties BPI-Sy 4 and at 200Gy for PSB-Sy 4.

Screening for drought tolerance and identification of putative mutants in the M_2 generation

Results of the drought tolerance tests are presented in Tables 5-9. The number of days to flowering of the eight soybean varieties was not affected by the treatments (irrigation withdrawals 20, 30, 40 and 50 days after planting) and the control (Table 5). On the other hand mature plant height was affected by irrigation withdrawals 20 and 30 days after planting including the control (Table 6). No significant differences were noted on plant height of 40 and 50 days irrigation withdrawals in all the eight varieties.

The number of pods of all the different varieties of soybean as affected by different duration of irrigation is shown in Table 7. No significant differences were observed among the treatments except for the control.

The seed weight from the 2m sampled length showed that differences were noted only in the 20 and 30 days irrigation withdrawal treatments (Table 8). The 40 and 50 days irrigation withdrawal treatments including the control were not significantly different indicating that the most critical period for irrigation in soybean varieties tested is in the first 30 days. The yield from the 40 and 50 day irrigation withdrawal treatments were lower than the control but the differences were not statistically significant (Table 8).

When the effects of the different treatments were analyzed on a per variety basis, some varieties were not affected at all showing that they were tolerant to drought (Table 9). For example, the yields of variety DT 84, DT 96, BPI-Sy 4, PSB-Sy 4, PSB-Sy 5 and NSIC-Sy 8 were not significantly affected although the highest yield was obtained in the control of AKO 6 followed by NSIC-Sy 8. Even in the other treatments, the same varieties yielded the highest indicating their good performance.

Screening for drought tolerance in the M_3 generation of the introduced varieties from Vietnam and the M_4 generation of the local soybean varieties

The effects of gamma irradiation on different soybean varieties in the M_3 and M_4 generations are shown in Tables 10 and 11. The number of days to flower in the local varieties did not vary much; same result was obtained from Vietnamese varieties. With regards to plant height at maturity, it can be noticed that a gradual reduction in height resulted as the dose was increased in both the M_3 and M_4 generations. Doses of 200 and 250Gy were not significantly different from each other in both varieties in the M_4 generation. As for the number of pods per plant in the M_4 generation, the control plants had significantly higher yield than the treated population; same results was obtained in the number of seeds per plant. However, variety PSB-Sy 4 treated with 250Gy produced higher number of pods and seeds per plant than those treated with 200Gy. For BPI-Sy 4, the control was significantly different from the treated plants as far as the number of pods is concerned.

Considering the weight of 100-seed, insignificant differences were obtained among the varieties and treatments used in both the Philippine and Vietnamese varieties (Tables 12 and 13). Results of test for drought tolerance showed that varieties from Vietnam were quite adapted to Philippine conditions because they survived even without watering from flowering stage until maturity. The only problem is their climbing characteristic which needs some trellises for support. Irradiation of these varieties was done to produce shorter and non-lodging plants. Short plants were already selected in the M_3 generation of Vietnamese varieties. Both Philippine varieties, treated with 200Gy showed some degree of tolerance to drought. Some morphological mutations observed in the M_3 and M_4 generations were: early maturing, short stature and compact growth habit (Table 14).

Growing and selection of mutants with desirable agronomic traits in the M_4 of the introduced and M_5 generations of local soybean varieties

The effect of different doses of gamma radiation on the plant height at maturity of four local soybean varieties in the M_4 generation is presented in Table 15a. Analysis of variance showed insignificant difference between the treatments and the varieties used (Table 15b).

However, interaction between varieties and gamma radiation significantly affect the length of roots (Tables 16a and 16b). PSB-Sy 4 at doses of 100 and 200Gy and PSB-Sy 5 at 300Gy gave significantly longer roots than BPI-Sy 4 and NSIC- Sy 8 at all levels of radiation.

With regards to the number of lateral roots no significant difference was observed among the varieties and gamma radiation used (Tables 17a and Table 17b).

The number of pods per plant in the M_4 generation of four local varieties was not affected by different levels of gamma radiation (Tables 18a and 18b). Similar result was obtained in the weight of 100 seeds (Table 19a and 19b).

The yield in the M_4 generation was not affected by different levels of gamma irradiation (Table 20a). Analysis of variance showed insignificant result between the varieties and treatments (Table 20b).

The number of drought tolerant plants is presented in Table 21a and 21b. Significant difference was obtained among the varieties (Table 21b). BPI-Sy 4 had the highest average number of tolerant plants followed by NSIC-Sy 8, PSB-Sy 5 and PSB-Sy 4.

Growing and selection of desirable mutants in the M_4 generation of introduced varieties from Vietnam

Mature plant height of varieties from Vietnam in the M_4 generation as affected by withdrawal of irrigation 30 days after planting is shown in Table 22. Irradiation at 200Gy with variety DT 95 increased in height by about 52.9% and only 3.2 % with DT 84. Increasing the dose to 250Gy reduced plant height in both varieties. However, no change in height was observed in variety AKO 6 irradiated with 200Gy as compared with the control.

The number of days to flowering as affected by withdrawal of irrigation 30 days after planting is shown in Table 23. It was found that irradiation did not affect the number of days to flowering. However, AKO 6 flowered earlier followed by DT 84 and DT 95 regardless of dosage used.

The number of pods per plant revealed that DT 95 and DT 84 obtained the highest number at 200Gy (Table 24). AKO 6 irradiated with 200Gy showed no difference in the number of pods as compared with the control. Weight of 100 seeds was not affected by the variety and dose of gamma radiation used (Table 25).

The increase in the number of seeds with irradiation at 200Gy in varieties DT 95 and DT 84 resulted in the increase in the number of seeds per plant. Such increase was about 15.6% with variety DT 95 and as high as 49.9% with variety DT 84. A reduction in number of seeds was obtained at 250Gy in both varieties (Table 26).

The varieties DT 95, DT 84 and AKO 6 (Table 27) were also adapted to Philippine condition as shown on the % of drought tolerant lines selected. However, the plants selected were still considered to be putative mutants. Confirmation and evaluation will be done in the succeeding generations.

DT 95 irradiated with 200Gy gave the highest number of early maturing plants as compared with the control (Table 28). This was followed by 250Gy of the same variety.

Plant height of the control local varieties (PSB-Sy 4, PSB-SY 5 and BPI-Sy 4) in the M_5 generation consistently increased with increasing days of irrigation withdrawn at 30, 40 and 50 days after seeding (Table 29). Irrigation withdrawn at 30 days in PSB-Sy 5 at 200 to 250Gy has comparable height with those at the doses of radiation with irrigation withdrawn at 50 days after seeding. In PSB-Sy 4, only the highest of 250Gy consistently resulted in plant height comparable with those at irrigation withdrawn at 30, 40 and 50 days after seeding respectively. Slight stimulation of plant height was noted in the variety BPI-Sy 4 at 200 and 250Gy as compared with the control.

Number of days to flowering in all varieties was not affected by irrigation withdrawals and irradiation treatments (Table 30).

Number of pods in all the control (unirradiated) increased with irrigation withdrawn at 30, 40 and 50 days after seeding (Table 31). Irradiation treatment of PSB-Sy 4 at 200 and 250Gy decreased the number of pods when irrigation was withdrawn at 40 and 50 days after seeding. Increase in number of pods of 22.4% and 18.6% was obtained in the same variety at 200 and 250Gy over the control even if irrigation was withdrawn 30 days after seeding. Increases in the number of pods in PSB-Sy 4 at 200Gy with irrigation withdrawn 30 days after seeding were 34.6 and 33.7% as compared with the same dose at 40 and 50 days withdrawal treatment, respectively. On the other hand, irradiation dose of 250Gy at 30 days irrigation withdrawal increased the number of pods by about 30.8% and 37.6% over 40 and 50 days irrigation treatments at the same irradiation dose, respectively. BPI-Sy 4 at 200Gy resulted in a slight increase in the number of pods when irrigation was withdrawn at 50 days after seeding. Both irrigation withdrawn at 40 and 50 days after seeding and irradiation treatments were detrimental to the production of pods in the variety PSB-Sy 5. However, at 30 days irrigation withdrawal, the number of pods of PSB-Sy 5 at 200 and 250Gy increased by 12.8% and 27.4% as compared with control, respectively.

The effects of irradiation and varying days of irrigation withdrawals after seeding on the number of seeds per plant followed the same trend with those of the number of pods per plant (Table 32). Based on seed production per plant PSB-Sy 4 at 200Gy can tolerate drought conditions with production increases of 17.2, 12.9 and 7.8% over the control with irrigation withdrawn at 30, 40 and 50 days after seeding, respectively. Increase in the number of seeds per plant at 250Gy with irrigation withdrawn at 30 days after seeding were 23.5 and 25.6% over the same dose at 40 and 50 days irrigation withdrawal, respectively. Both irradiation and irrigation withdrawn at 30 and 40 days after seeding did not affect the number of seeds of BPI-Sy 4. However, at 250Gy of the same variety with irrigation withdrawn up to 50 days after seeding increased the number of seeds by about 32.2% over the control.

In the variety PSB-Sy 5, none of the irradiation and irrigation treatments affected the number of seeds produced per plant. Likewise, weight of 100 seeds was not affected by irradiation and irrigation treatments in the varieties tested (Table 33).

The number of mutants selected in the M_5 generation that were early maturing and drought tolerant (plants with green leaves at maturity, Fig. 1) that were subjected to irrigation withdrawal 30 days after seeding is presented in Table 34. The number of early maturing plants ranges from 9-151. PSB-Sy 4 that was irradiated with 250Gy have the most number of early maturing mutants (151) followed by 125 in the variety BPI-Sy 4 that were exposed to the same dose of gamma radiation. Likewise, similar trend was noted with regards to the number of plants that have green leaves at maturity are presumed to be drought tolerant.

Further selection of mutants for drought tolerance and other desirable agronomic traits in the M_5 of the introduced varieties from Vietnam and M_6 generation of the local soybean varieties

Plant height at maturity was not significantly different among gamma radiation of 200, 250Gy and the control (0 Gy) in variety DT 95 with irrigation withdrawn 20 days after planting (Table 35). The selected mutant lines at 200 and 250Gy were significantly taller than DT 84 and AKO 6 receiving the same dose of gamma radiation. AKO 6 at 200Gy was the shortest with a reduction in plant height of about 13.3 % over that of the control (0Gy).

The number of days to flower of the selected mutant lines from the introduced soybean varieties from Vietnam and their control was not significantly different from each other at irrigation withdrawal 20 days after planting (Table 36). Flowering ranged from 39-41 days after planting.

The number of pods in all selected mutant lines at 200 and 250Gy was not significantly different from the control (0Gy) within variety (Table 37). However, significant differences were noted at 200 and 250Gy in variety DT 95 and DT 84 at the same dose of gamma radiation.

This increase in the number of pods in DT 95 mutant lines ranges from 41.9 to 58.6 % as compared with DT 84 (control) and at 200 and 250Gy, respectively.

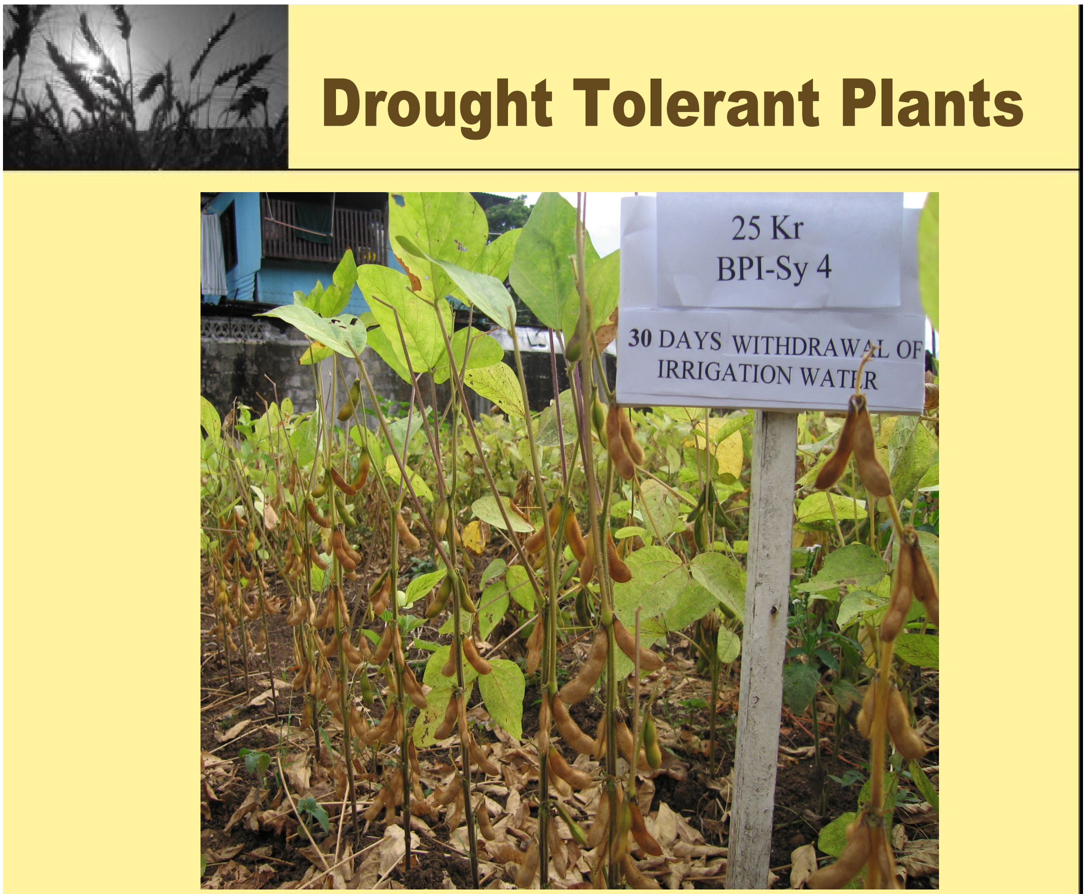

Fig. 1. Drought tolerant mutants with green leaves at maturity obtained in 250 Gy with irrigation withdrawan 30 days after planting.

Withdrawal of irrigation at 20 days after planting did not significantly affect the number of seeds of mutant lines at 200 and 250Gy and the control in three varieties tested. Although not significant DT 84 at 200 and 250Gy increased the number of seeds per plant by about 14.8 and 22.4% over the control, while DT 95 at 250Gy gave an increase of 10.2 % as compared with the control. DT 95 at 250Gy has the most number of seeds per plant (Table 38).

The weight of 100 seeds was not significantly different between the control varieties and selected mutants at 200 and 250Gy (Table 39). DT 84 at 200Gy although not significant gave a slight increase at 14.8 % and 7.3 % in seed weight over the control and DT 84 at 250Gy, respectively.

Shortly before harvest heavy rain occurred causing depressed grain yield (Table 40). Inspite of the low yield, significant effect of the stress condition was still manifested on the different mutant selections at 200 and 250Gy and the control. DT 84 at 200 and 250Gy gave comparable yields but significant increase in grain yields by as much as 51.6 and 35.4 % over the control, respectively. On the other hand AKO 6 at 200Gy significantly out-yielded its control by about 113.6 %.

Selection of mutants for drought tolerance and other desirable agronomic traits in the M_6 generation of three local soybean varieties

PSB-Sy 5 was the tallest among the varieties tested regardless of the irrigation treatments imposed (Table 41). Plant height at 200 and 250Gy was not significantly different from the unirradiated control in varieties PSB-Sy 4 and PSB-Sy 5 subjected to irrigation withdrawn at 20 and 30 days after planting, respectively. BPI-Sy 4 at 250Gy was significantly taller than 200Gy and the control at irrigation withdrawn 20 days after planting.

The number of days to flower was not significantly affected by irrigation treatments imposed in all the three varieties tested and mutants selected at 200 and 250Gy (Table 42). Flowering ranged from 35 to 37 days after planting.

The number of pods was not significantly different from the selected mutant lines in all 3 varieties and their respective control at irrigation withdrawn at 20 days after planting (Table 43). In BPI-Sy 4 at 250Gy significantly increased the number of pods by as much as 41.8 % over the control and 29.9 % over the mutant lines at 200Gy subjected to irrigation withdrawn 30 days after planting. The number of pods at 250Gy in BPI-Sy 4 showed significant increase of 47.8, 46.2 and 42.9 % over the control and at 200 and 250Gy, respectively at irrigation withdrawn 30 days after planting.

The number of seeds per plant among the selected mutants at 20 days irrigation withdrawal was not significantly different with each other and their respective control in all the varieties (PSB-Sy 4, BPI-Sy 4 and PSB-Sy 5). In irrigation withdrawal at 30 days after planting, BPI-Sy 4 at 250 significantly increased the number of seed per plant by about 35.3 and 20.2 % over the control and at 200Gy, respectively. The same mutant selection showed significant increase in the number of seeds of about 35.5, 41.4 and 42.2% over PSB-Sy 4 control and PSB-Sy at 200 and 250Gy, respectively. PSB-Sy 5 at 200Gy has the most number of seeds followed by BPI-Sy 4 at 250Gy (Table 44).

In irrigation withdrawal at 20 and 30 days after planting, the weight of 100 seeds at 200 and 250Gy of the three varieties (PSB-Sy 4, BPI-Sy 4 and PSB-Sy 5) and their respective control was not significantly different with each other (Table 45).

Grain yields at 200 and 250Gy in all the varieties tested were not significantly different with each other in all the treatment imposed (Table 46). In the withdrawal of irrigation water, 20 days after planting, PSB-Sy 4 at 200Gy increased grain yield (although not significant) by about 11.1% over the control, while BPI-Sy 4 at 200Gy gave an increase of 14.8% over the control. At 30 day withdrawal of irrigation water, PSB-Sy 5 at 250Gy increased yield by as much as 23.2% over PSB-Sy 4 control and only 17.2% with PSB-Sy 4 at 200Gy.

Conclusion

Promising mutant lines for drought tolerance, earliness and increase yield have been obtained at 200 and 250 Gy gamma radiation in both the local and introduced varieties from Vietnam. Multiplication of these mutant lines is presently being undertaken. Further selection of drought tolerant lines in the M_6 and M_7 generations will be done to confirm and evaluate previous results. Yield performance test will be conducted in multi-location trial.

Contributory factors for the attainment of the project objectives are as follows:

1. Support from PNRI-GIA.
2. Collaboration with Bulacan Agricultural State College (BASC), San Ildefonso, Bulacan.
3. Participation in the FNCA Multilateral Research Program on the Mutation Breeding for Drought Tolerance in Soybean in collaboration with Vietnam, Indonesia and Malaysia.

Publication of Results

Paper Published:

Asencion, A.B., A.G. Lapade, A.O. Grafia, A.C. Barrida, A.M. Veluz and L.J. Marbella. 2004. Induced mutations for the improvement of soybean. *(Glycine max L.)* in The Philippines. Philippine Nuclear Journal 14: 12-25.

Paper presented:

Lapade, A.G., A.C. Barrida and A.O. Grafia. 2006. Status Report on FNCA MRP-1 On Drought Tolerance of Soybean *(Glycine max L.)* in the Philippines. Paper Presented in 2006 FNCA Mutation Breeding Workshop, September 3-7, 2006, Takasaki, Japan.

Barrida, A.C., A.G. Lapade, A.O. Grafia and J.C. Mananguit, 2005. Status Report on FNCA MRP-1 on Drought Tolerance of Soybean *(Glycine max L.)* in the Philippines. Paper presented in the 2005 FNCA Mutation Breeding Workshop, December 5-9, 2005, Kuala Lumpur, Malaysia.

Barrida, A.C., A.O. Grafia, A.G. Lapade, A.B. Asencion and E. Costmiano. 2004. Status

Report on FNCA MRP-1 on Drought Tolerance of Soybean *(Glycine max L.)* in The Philippines. Paper presented in the 2004 Workshop on Mutation Breeding. August 30 to September 4, 2004. Yogyakarta, Indonesia.

Asencion, A.B., A.G. Lapade, A.C. Barrida, A.M. Veluz, and L.J. Marbella. 2003. Status Report on FNCA MRP-1 on Drought Tolerance of Soybean *(Glycine max L.)* in the Philippines. Paper presented in Mutation Breeding Workshop, September 22-26, 2003. Southwoods, Manila, Philippines.

Lapade, A.G. 2002. Status of Mutation Breeding in the Philippines. Paper presented in the Formulation Meeting MRP-1 Drought Tolerance of Sorghum and Soybean, Jakarta, Indonesia.

Technical Progress Report on "Soybean Drought Tolerance in the Philippines". 2002-2005 PNRI, Diliman, Quezon City, Philippines.

Other results and riffle effects of the project:

Two students from the De La Salle University conducted their thesis related to this project. The Bachelor of Science Degree in Agriculture thesis is entitled "The effects of different doses of gamma radiation and various water regimes on soybean". The Master of Science in Agriculture (major in Agronomy) thesis is on the "Effects of different water regimes on the growth and yield of some drought tolerant varieties of soybean *(Glycine max L.)"*. Mrs. Avelina G. Lapade, the Project leader acted as co-adviser to these students.

The project on mutation breeding for drought tolerance was funded by PNRI-GIA in collaboration with Dr. Josefina C. Mananguit and her researcher from Bulacan Agricultural State College (BASC) San Indefonso, Bulacan. This institution provided experimental area for large population to screen our developed mutants. Through this project, researchers and students were informed of the potential of mutation breeding in the improvement of soybean and other crops.

Table 1. Effects of gamma irradiation on four (4) Philippine soybean varieties in the M_1 generation

Variety	Treatment	% Germination	Seedling ht. cm.	Mature plant Height (cm)	Days to flower	No. of pods
BPI-Sy 4	Control	94.20	14.27 a	75.6 a	34.0	8.5
	200	93.35	14.43 a	70.6 ab	34.0	7.1
	250	93.35	11.00 b	63.5 b	34.0	4.1
						n.s.
PSB-Sy 4	Control	90.00	13.00 a	63.1	35.3	16.4 a
	200	93.20	12.13 a	62.3	35.7	8.5 b
	250	93.50	8.70 b	61.8	34.3	6.1 b
				n.s.		
PSB-Sy 5	Control	90.00	14.00 a	67.3 a	36.7	15.7 a
	200	93.35	14.07 a	64.8 b	36.0	9.0 b
	250	86.65	10.23 b	60.6 b	36.0	7.2 b
NSIC Sy-8	Control	86.00	10.40	68.6 a	37.0	12.6 a
	200	85.00	10.70	63.2 b	36.3	9.6 b
	250	85.00	10.23	58.0 b	38.7	5.1 b

1/ In a column, means with the same letters are not significantly different at 5% level by DMRT.

Table 2. Effects of gamma radiation in the M_1 generation of four soybean varieties obtained from Vietnam 1/

Varieties	Treatments (Gy)	% Germination	Seedling ht. cm.	Mature plant height	Days to flower	No. of pods Per plant
AKO 6	Control	88.3	12.5	69.9 a	41.0	13.8 a
	200	88.3	9.5	61.3 ab	41.0	9.2 a
	250	90.0	8.7	55.7 b	41.3	5.3 b
			Ns			
DT 84	Control	86.7	10.6 a	56.0	40.7	11.4 a
	200	90.0	9.1 b	49.7	42.0	9.6 a
	250	88.3	8.4 c	43.3	42.0	4.4 b
				ns		
DT 95	Control	88.3	9.6 a	49.7 a	39.3	9.6 a
	200	88.3	9.2 b	48.4 a	41.0	8.4 a
	250	88.3	6.5 c	38.5 b	40.7	5.8 b
DT 96	Control	95.0	14.9 a	50.4	42.7	10.5 a
	200	93.3	12.4 b	47.8	42.0	7.2 b
	250	82.3	8.9 c	40.5	43.3	4.9 b

1/ In a column, means with the same letters are not significantly different at 5% level by DMRT

Table 3. Types of chlorophyll mutation observed in the M_2 generation of Philippine soybean varieties

Varieties	Treatments Gy	Types of chlorophyll mutations			
		Striata	Chlorina	Spotted yellow	Albino
BPI-Sy 4	Control	0	0	0	0
	200	4	24	0	0
	250	4	24	0	0
PSB-Sy 4	Control	0	0	0	0
	200	0	12	0	0
	250	4	52	4	0
PSB-Sy 5	Control	0	0	0	0
	200	0	64	0	0
	250	0	48	0	0
NSIC-Sy 8	Control	0	0	0	0
	200	4	56	3	0
	250	0	44	0	0

Table 3a. Frequency of chlorophyll mutations in the M_2 generation of Philippine soybean varieties after treatment with gamma rays

Varieties	Treatments	Total M_2 Seedlings	No. of M_2 Mutants	% Chlorophyll Mutations
BPI Sy-4	Control	2,028	0	0
	200	4,284	28	0.65
	250	1,820	28	1.54
PSBSy-4	Control	3,760	0	0
	200	1,984	12	0.60
	250	3,868	60	1.55
PSB Sy-5	Control	5,380	0	0
	200	4,924	64	1.30
	250	4,344	48	1.11
NSIC Sy-8	Control	8,440	0	0
	200	9,192	63	0.63
	250	3,452	44	1.27

Table 3b. Types of chlorophyll mutation in the M_2 generation of Vietnamese varieties

Variety	Treatment (Gy)	Type of chlorophyll mutations			
		Striata	Chlorina	Spotted Yellow	Albino
AKO 6	Control	0	0	0	0
	200	0	47	0	0
	250	0	28	0	0
DT 84	Control	0	0	0	0
	200	16	0	0	0
	250	0	37	0	0
DT 95	Control	0	0	0	0
	200	0	26	0	0
	250	0	29	0	0
DT 96	Control	0	0	0	0
	200	1	23	0	0
	250	0	30	0	0

Table 3c. Frequency of chlorophyll mutation in the Vietnamese soybean varieties

Variety	Treatment (Gy)	Total M_2 Seedlings	No. of M_2 Mutant Seedlings	% Chlorophyll mutation rate
AKO 6	Control	3570	0	0
	200	3657	47	1.28
	250	2122	28	1.32
DT 84	Control	2620	0	0
	200	1923	16	0.83
	250	1992	37	1.86
DT 95	Control	2707	0	0
	200	2823	26	0.92
	250	1986	29	1.4
DT 96	Control	2950	0	0
	200	1833	24	1.30
	250	1963	30	1.53

Table 4. Number of mutants selected in the M_2 generation and their desirable traits

Variety	Treatment	No. selected	Characteristics
PSB Sy-4	Control	-	-
	200	14	early, high yielding
	250	5	Early
BPI Sy-4	Control	-	-
	200	12	early, high yield
	250	33	early, high yield
PSB SY-5	Control	-	-
	200	8	early, high yield
	250	6	early high yield
NSIC Sy-8	Control	-	-
	200	4	Early
	250	4	Early

Table 5. Days to flower of eight soybean varieties subjected to varying irrigation withdrawals

Variety	Control	20 days after seeding	30 days after seeding	40 days after seeding	50 days after seeding
AKO 6	35.5	35.7	35.7	36.0	35.0
DT 84	34.7	35.0	35.3	34.7	34.7
DT 95	39.3	39.7	39.7	39.7	39.3
DT 96	38.0	39.0	40.7	40.7	40.3
BPI Sy-4	36.0	36.3	36.0	36.3	36.3
PSB Sy-4	34.0	34.3	34.3	43.7	34.3
PSB Sy-5	34.7	35.0	35.3	35.3	35.0
NSIC Sy-9	43.3	44.0	44.3	44.6	43.6

Table 6. Mature plant heights (cm) of eight soybean varieties subjected to varying irrigation withdrawals

Variety	Control	20 days	30 days	40 days	50 days
AKO 6	34.1ab	26.5b	26.56c	27.3	29.0
DT 84	30.0abc	26.2b	29.9b	26.9	27.4
DT 95	37.5a	32.9a	34.3a	32.7	33.3
DT 96	26.4bc	22.1bc	22.6d	24.0	25.0
BPI Sy-4	27.5bc	25.5b	26.9c	18.2	27.7
PSB Sy-4	28.7bc	24.8b	25.8c	26.9	27.3
PSB Sy-5	23.5c	18.6c	20.5c	22.0	24.5
NSIC Sy-8	28 bc	22.5bc	26.4c	27.0ns	27.5 ns

1/ In a column, means with the same letters are not significantly different at 5% level by DMRT.
ns = non-significant

Table 7. Number of pods of eight soybean varieties subjected to varying irrigation withdrawals

Variety	Control	Irrigation Withdrawals			
		20 days after seeding	30 days after seeding	40 days after seeding	50 days after seeding
AKO 6	13.9a	6.6	56.3	8.1	8.5
DT 84	9.5b	4.1	5.2	5.0	5.4
DT 95	6.3b	3.6	4.4	4.8	5.6
DT 96	7.3b	5.1	5.6	6.1	6.6
BPI-Sy 4	7.0b	4.9	5.4	5.4	5.7
PSB-Sy 4	7.7b	5.6	6.2	6.7	6.7
PSB-Sy 5	6.4b	4.4	5.1	5.6	7.3
NSIC Sy-8	7.2b	4.9 ns	5.9 ns	6.6 ns	6.9 ns

1/ In a column, means with the same letters are not significantly different at 5% level by DMRT.

Table 8. Seed weights (gm) from the 2m sampled length of eighth soybean varieties subjected to varying irrigation withdrawals

Variety	Control	Irrigation withdrawals			
		20 days after seeding	30 days after seeding	40 days after seeding	50 days after seeding
AKO 6	32.48	11.48b	13.28c	16.63	14.56
DT 84	17.84	8.69d	9.93e	15.06	13.44
DT 95	21.25	6.95c	10.75d	16.25	18.11
DT 96	22.55	8.43d	13.17c	11.75	11.68
BPI Sy-4	14.76	6.70e	7.22f	10.31	10.66
PSB Sy-4	16.32	9.52c	6.78g	10.93	7.60
PSB Sy-5	14.86	9.55c	13.82b	13.64	14.58
NSIC Sy-8	27.45 ns	17.21a	16.22a	19.05 ns	21.08 ns

Table 9. Seed weights from 2-m sampled length of eight soybean varieties subjected to varying length of irrigation withdrawals

Treatments	Varieties							
	AKO 6	DT 84	DT 95	DT 96	BPI Sy-4	PSB y-4	PSB Sy-5	NSIC Sy-8
Control	32.48a	17.84	21.25a	22.55	14.76	16.32	14.86	27.45
20 days	11.48b	8.69d	6.95c	8.43d	6.7e	9.52c	9.55c	17.21a
30 days	13.28b	9.93c	10.75b	13.17c	7.22f	6.78g	13.82b	16.32a
40 days	16.63b	15.06	16.26	11.75	10.31	10.93	13.64	19.05
50 days	14.56	13.44 ns	18.11	11.68 ns	10.66 ns	7.60 ns	14.58 ns	21.08 ns

[1]/ In a column, means with the same letters are not significantly different at 5% level by DMRT.

Table 10. Effects of gamma irradiation on Philippine soybean varieties in the M_3 generation

Variety	Treatment	Days to flower	Mature plt. ht. (cm)	No. of pods per plant	No. of seed per plant
PSB St-4	Control	35.3	63.10	70.00	120.50
	200 Gy	35.7	32.23	22.17	39.32
	250 Gy	34.3	33.42	15.25	27.45
BPI-Sy 4	Control	34.0	75.60	80.50	170.50
	200 Gy	34.0	34.43	19.06	31.41
	250 Gy	34.0	58.90	30.00	55.30

Table 11. Effects of gamma irradiation on different soybean varieties in the M_4 generation

Variety	Treatment	Days to Flowering	Mature plt. ht. (cm.)	No. of seeds per pod	Nos. of pods per plant
PSB-Sy 4	Control	45	31.85	70.30	122.50
	200 Gy	45	25.90	35.80	70.80
	250 Gy	45	26.05	47.00	81.40
BPI-Sy 4	Control	45	38.25	85.50	175.50
	200 Gy	45	28.95	62.30	113.30
	250 Gy	45	31.00	60.00	127.50

Table 12. 100-seed weight in grams of Philippine soybean varieties as affected by gamma irradiation in the M_4 generation

Varieties	Treatment	Weight (gms.)
PSB-Sy 4	Control	20.75
	200 Gy	19.62
	250 Gy	20.72
BPI-Sy 4	Control	21.55
	200 Gy	21.21
	250 Gy	19.72

Table 13. Effects of gamma irradiation on Vietnamese varieties in the M_3 generation

Varieties	Treatment	Days to flowering	100-seed wt. in grams
DT- 84	Control	40	18.00
	200 Gy	39	18.65
	250 Gy	39	18.14
DT-95	Control	39	17.51
	200 Gy	39	17.71
	250 Gy	39	17.72
AKO 6	Control	41	16.15
	200 gy	39	15.00
	250 gy	41	17.18

Table 14. Number of mutants selected and their characteristics

Variety	Treatment	No. Selected	Characteristic
SB-Sy 4	Control	-	-
	200 Gy	15	early, high yield
		2	compact, high yield
		3	drought tolerant
	250 Gy	4	early, short
BPI-Sy 4	Control	-	-
	200 Gy	16	early, high yield drought tolerant
	250 Gy	6	early, short

Table 15a. The effects of different doses of gamma radiation on the height of plants at maturity of four local soybean varieties (M_4 generation) grown at BASC.

Varieties	Dose (Gy)	Replications			
		Rep 1	Rep 2	Rep 3	Mean
PSB Sy	0	70	54.1	58.3	60.8
	200	72.1	73.6	77.6	74.4
	200	67.8	78.4	74.6	73.6
	300	64.5	70.9	68.5	68.0
BPI Sy 4	0	66.8	58.7	62.3	62.6
	100	70.2	72	78.7	73.6
	200	63.2	69.5	69.9	67.5
	300	72	68.1	68.9	69.7
NSIC-Sy 8	0	75.1	75.8	65.2	72.0
	100	76.6	81.2	72.2	76.7
	200	69.3	58.8	71.3	66.5
	300	72.1	59.9	69.8	67.3
PSB Sy 5	0	66.4	55.3	70.1	63.9
	100	69.7	71.4	77.2	72.8
	200	61.6	74.9	68.4	68.3
	300	73.9	78.4	55.8	69.4

Table 15b. ANOVA on plant height at maturity

Source Of Variation	Degree Of Freedom	Sum Of Squares	Mean Square	Computed F[b]		Tabular F 5%	Tabular F 1%
Replication	2	3.61	1.80				
Gamma Radiation (A)	3	554.72	184.91	3.57	ns	4.76	9.78
Error (a)	6	310.88	51.81				
Variety (B)	3	36.74	12.25	< 1		3.01	4.72
A x B	9	312.50	34.72	1.00	ns	2.3	3.25
Error (b)	24	835.09	34.80				
Total	47	2053.54					

[a] *cv* (a) =10.40% *cv* (b) = 8.53%

[b] *ns = not significant*

Table 16a. The effects of different doses of gamma radiation on the length of roots (cm) of four local soybean varieties (M_4 generation) grown at BASC.

Varieties	Dose (Gy)	Replications Rep 1	Rep 2	Rep 3	Mean 1/
PSB Sy	0	11	12	9.8	10.9de
	100	13.5	13.2	12.9	13.2ab
	200	14.6	14.7	13.5	14.3a
	300	11.9	12.1	10	11.3cde
BPI Sy 4	0	12.8	10	9.8	10.9de
	100	12.4	11.9	11	11.8cde
	200	10.4	9.1	10.1	9.9e
	300	12	11.8	11.5	11.8cde
NSIC-Sy 8	0	11	13.5	12.7	12.4bcd
	100	10.5	9.4	9.8	9.9e
	200	11.7	10.8	12.8	11.8cde
	300	11.8	10.8	11.2	11.3cde
PSB Sy 5	0	11.8	11	9.8	10.9de
	100	11.8	9.8	12.3	11.3cde
	200	11	10.5	9.7	10.4e
	300	13.6	11.9	13.2	12.9abc

1/ In a column, means with the same letters are not significantly different at 5% level by DMRT.

Table 16b. ANOVA on length of roots (M_4 generation)[a]

Source Of Variation	Degree of Freedom	Sum Of Squares	Mean Square	Computed F^b		Tabular F	
						5%	1%
Replication	2	4.77	2.39				
Gamma Radiation (A)	3	1.82	0.61	1.25	*ns*	4.76	9.78
Error (a)	6	2.91	0.49				
Variety (B)	3	13.13	4.38	5.11	**	3.01	4.72
A x B	9	48.56	5.40	6.30	**	2.3	3.25
Error (b)	24	20.54	0.86				
Total	47	91.74					
[a]*cv* (a) =	6.03%	*cv* (b) =	8.01%				

[b] *ns = not significant, ** = highly significant*

Table 17a. The effects of different doses of gamma radiation on the number of lateral roots of four local soybean varieties (M_4 generation) grown at BASC

Varieties	Dose (Gy)	Replications			
		Rep 1	Rep 2	Rep 3	Mean
PSB Sy 4	0	12.4	10.3	7.2	10.0
	100	14.4	6.4	5.9	8.9
	200	11	6.8	5.9	7.9
	300	7.7	6	3.7	5.8
BPI Sy 4	0	6.8	5.8	8.4	7.0
	100	8.8	6.9	10.3	8.7
	200	9.3	5.2	5	6.5
	300	8.7	9.9	5.9	8.2
NSIC-Sy 8	0	7.9	6.5	6	6.8
	100	7.2	7.2	4.9	6.4
	200	14.11	6	6.4	8.8
	300	8.6	6.4	5.5	6.8
PSB Sy 5	0	12.2	6.5	4.1	7.6
	100	11.2	6.1	7.2	8.2
	200	15.6	7.9	3.9	9.1
	300	8.4	6.2	8.7	7.8

Table 17b. ANOVA on the number of lateral roots[a]

Source Of Variation	Degree Of Freedom	Sum Of Squares	Mean Square	Computed F[b]		Tabular F 5%	Tabular F 1%
Replication	2	152.65	76.33				
Gamma Radiation (A)	3	6.93	2.31	< 1		4.76	9.78
Error (a)	6	37.10	6.18				
Variety (B)	3	7.51	2.50	< 1		3.01	4.72
A x B	9	45.42	5.05	1.24	*ns*	2.3	3.25
Error (b)	24	97.60	4.07				
Total	47	347.21					

[a] *cv* (a) = 31.96% *cv* (b) = 25.92%

[b] *ns = not significant*

Table 18a. The effects of different doses of gamma radiation on the number of pods per plant of four local soybean varieties (M_4 generation) grown at BASC

Treatments	Varieties	Replications Rep 1	Rep 2	Rep 3	Mean
PSB Sy 4	0	23.7	33.1	22.4	26.4
	100	38.4	28.3	31.1	32.6
	200	26.9	26.5	24.3	25.9
	300	30.9	36.1	16.2	27.7
BPI Sy 4	0	61.1	28.8	24.9	38.3
	100	24.8	26.2	26.1	25.7
	200	24.3	25.8	18.8	23.0
	300	24.3	28.2	26.7	26.4
NSIC-Sy 8	0	54.2	15.8	29.1	33.0
	100	24.4	30.7	20.2	25.1
	200	38.1	25.5	25.8	29.8
	300	45.8	22.1	22.4	30.1
PSB Sy 5	0	29.2	22.5	18.4	23.4
	100	31.7	27.3	36.7	31.9
	200	24.7	27.2	13.5	21.8
	300	31.3	22.6	20.7	24.9

Table 19a. The effects of different doses of gamma radiation on the weight of 100 seed (in grams) of four local soybean varieties (M_4 generation) grown at BASC

Varieties	Dose (Gy)	Replications			
		Rep 1	Rep 2	Rep 3	Mean
			(gram)		
PSB Sy 4	0	100	40	70	70.0
	100	60	30	50	46.7
	200	50	40	40	43.3
	300	40	60	40	46.7
BPI Sy 4	0	120	30	40	63.3
	100	40	40	30	36.7
	200	40	40	40	40.0
	300	40	40	40	40.0
NSIC-Sy 8	0	100	40	50	63.3
	100	30	40	20	30.0
	200	40	40	40	40.0
	300	100	60	30	63.3
PSB Sy 5	0	70	30	50	50.0
	100	80	30	50	53.3
	200	40	40	50	43.3
	300	60	40	50	50.0

Table 19b. ANOVA on weight of 100 seeds (M_4 generation)[a]

Source Of Variation	Degree Of Freedom	Sum Of Squares	Mean Square	Computed F[b]		Tabular F	
						5%	1%
Replication	2	5037.50	2518.75				
Gamma Radiation (A)	3	3225.00	1075.00	1.34	*ns*	4.76	9.78
Error (a)	6	4812.50	802.08				
Variety (B)	3	275.00	91.67	< 1		3.01	4.72
A x B	9	2225.00	247.22	1.25	*ns*	2.3	3.25
Error (b)	24	4750.00	197.92				
Total	47	20325.00					

[a] *cv* (a) = 58.09% *cv* (b) = 28.86%

[b] *ns = not significant*

Table 20a. The effects of different doses of gamma radiation on the yield in kilograms per plot of four local soybean varieties (M_4 generation) at BASC

Varieties	Dose (Gy)	Replications			
		Rep 1	Rep 2	Rep 3	Mean
PSB Sy 4	0	1.4	1.25	1	1.2
	100	1	1	1.1	1.0
	200	1	1.1	1	1.0
	300	0.4	1.3	.85	0.9
BPI Sy 4	0	1.2	0.5	1	0.9
	100	.75	1.25	1.25	1.1
	200	1	1	1	1.0
	300	1.5	1.4	1.1	1.3
NSIC-Sy 8	0	1.2	1	0.9	1.0
	100	1.3	0.75	0.9	1.0
	200	1.25	0.8	0.5	0.9
	300	1.1	1	1.5	1.2
PSB Sy 5	0	1	0.9	1	1.0
	100	0.75	0.5	1	0.8
	200	1	0.9	0.8	0.9
	300	1.3	1.3	1.25	1.3

Table 20b. ANOVA on yield (M_4 generation)[a]

Source Of Variation	Degrees Of Freedom	Sum Of Squares	Mean Square	Computed		Tabular F	
				F[b]		5%	1%
Replication	2	0.05167	0.02583				
Gamma Radiation (A)	3	0.36307	0.12102	1.93	*ns*	4.76	9.78
Error (a)	6	0.37708	0.06285				
Variety (B)	3	0.06682	0.02227	< 1		3.01	4.72
A x B	9	0.79005	0.08778	1.54	*ns*	2.3	3.25
Error (b)	24	1.37125	0.05714				
Total	47	3.01995					
[a]*cv* (a) =	24.43%	*cv* (b) =	23.30%				

[a]*cv* (a) = 24.43% *cv* (b) = 23.30%

[b] *ns = not significant*

Table 21a. The effects of different doses of gamma radiation on the number of drought tolerant plants of four local soybean varieties (M_4 generation) grown at BASC

Varieties	Treatments	Replications			
		Rep 1	Rep 2	Rep 3	Mean
PSB Sy-4	0	-	-	-	-
	100	10	15	20	15.0
	200	14	20	18	17.3
	300	20	25	20	21.7
BPI Sy 4	0	-	-	-	-
	100	20	25	28	24.3
	200	28	30	26	28.0
	300	35	20	24	26.3
NSIC-Sy 8	0	-	-	-	-
	100	18	28	30	25.3
	200	10	15	22	15.7
	300	28	25	20	24.3
PSB Sy 5	0	-	-	-	-
	100	12	28	25	21.7
	200	10	20	19	16.3
	300	15	24	30	23.0

Table 21b. ANOVA on number of drought tolerant Plants[a]

Source Of Variation	Degree Of Freedom	Sum Of Squares	Mean Square	Computed F[b]		Tabular F	
						5%	1%
Replication	2	192.17	96.08				
Gamma Radiation (A)	2	121.50	60.75	[c]			
Error (a)	4	164.83	41.21				
Variety (B)	3	323.64	107.88	4.89	*	3.16	5.09
A x B	6	211.61	35.27	1.60	ns	3.66	4.01
Error (b)	18	397.00	22.06				
Total	35	1410.75					

[a]*cv* (a) = 29.74% *cv* (b) = 21.76%, [b] * = significant at 5% level [ns] = not significant
[c]Error (b) d.f. not adequate for valid test of significance.

Table 22. Mature plant height in the M_4 generation of three soybean varieties from Vietnam as affected by irrigation withdrawal 30 days after planting

Varieties	Treatments (Gy)	Plant height (cms.)
DT 95	Control	60.50
	200 Gy	62.45
	250 Gy	58.70
DT 84	Control	42.90
	200 Gy	65.60
	250 Gy	39.80
AKO 6	Control	45.40
	200 Gy	45.57

Table 23. Number of days to flowering (M_4 generation) of three soybean varieties from Vietnam as affected by irrigation withdrawal 30 days after planting

Varieties	Treatments(Gy)	Days to flower
DT 95	Control	38
	200 Gy	38
	250 Gy	38
DT 84	Control	36
	200 Gy	36
	250 Gy	36
AKO 6	Control	35
	200 Gy	35

Table 24. Number of pods per plant (M_4 generation) of three soybean varieties from Vietnam as affected by irrigation withdrawal 30 days after planting

Varieties	Treatments (Gy)	Pods per plant
DT 95	Control	17.92
	200 Gy	24.05
	250 Gy	20.10
DT 84	Control	21.40
	200 Gy	28.70
	250 Gy	15.00
AKO 6	Control	16.80
	200 Gy	17.85

Table 25. Weight of 100 seeds (in the M_4 generation) of three soybean varieties from Vietnam as affected by irrigation withdrawal 30 days after planting

Varieties	Treatments (Gy)	Weight in grams
DT 95	Control	13.62
	200 Gy	12.60
	250 Gy	12.00
DT 84	Control	14.12
	200 Gy	13.45
	250 Gy	13.65
AKO 6	Control	11.64
	200 Gy	12.32

Table 26. Number of seeds per plant (M_4 generation) of three soybean varieties from Vietnam as affected by irrigation withdrawal 30 days after planting

Varieties	Treatment (Gy)	Seeds per plant
DT 95	Control	54.66
	200 Gy	63.20
	250 Gy	52.30
DT 84	Control	46.70
	200 Gy	70.00
	250 Gy	34.50
AKO 6	Control	34.30
	200 Gy	35.71

Table 27. Number of drought tolerant lines selected in the M_4 generation of three soybean varieties from Vietnam as affected by irrigation withdrawal 30 days after planting

Varieties	Treatments (Gy)	No.	% of drought tolerant
DT 95	Control	26	4.20
	200 Gy	36	5.81
	250 Gy	50	8.07
DT 84	Control	27	4.36
	200 Gy	33	5.33
	250 Gy	25	4.04
AKO 6	Control	25	4.04
	200 Gy	37	5.98

Table 28. Number of early maturing plants selected in the M_4 generation of three soybean varieties from Vietnam as affected by irrigation withdrawal 30 days after planting

Varieties	Treatments (Gy)	Number of early maturing plants
DT 95	Control	46
	200 Gy	81
	250 Gy	75
DT 84	Control	40
	200 Gy	19
	250 Gy	-
AKO 6	Control	-
	200 Gy	-

Table 29. Mature plant height (in centimeters) of local soybean varieties in the M_5 generation as affected with varying days of irrigation withdrawals

Varieties	Treatments (Gy)	Irrigation Withdrawals		
		30 days after seeding	40 days after seeding	50 days after seeding
PSB-Sy 4	Control	50.05	50.30	53.70
	200	49.75	51.70	55.70
	250	50.25	43.30	51.40
BPI-Sy 4	Control	49.15	50.10	56.65
	200	42.25	46.35	59.73
	250	51.00	54.40	59.10
PSB-Sy 5	Control	58.45	67.45	62.30
	200	66.25	74.70	66.25
	250	68.80	71.73	65.05

Table 30. Number of days to flower of local soybean varieties in the M_5 generation as affected with varying days of irrigation withdrawals

Varieties	Treatments(Gy)	Irrigation Withdrawals		
		30 days after seeding	40 days after seeding	50 days after seeding
PSB-Sy 4	Control	35	35	35
	200	35	35	35
	250	35	35	35
BPI-Sy 4	Control	35	36	35
	200	35	36	35
	250	35	36	35
PSB-Sy 5	Control	35	35	35
	200	35	35	35

Table 31. Number of pods per plant of local soybean varieties in the M_5 generation as affected with varying days of irrigation withdrawals

Varieties	Treatments(Gy)	Irrigation Withdrawals		
		30 days after seeding	40 days after seeding	50 days after Seeding
PSB-Sy 4	Control	23.80	24.35	25.75
	200	29.15	21.65	21.80
	250	28.22	21.57	20.50
BPI-Sy 4	Control	27.82	27.59	20.25
	200	19.90	26.57	25.06
	250	23.47	23.10	18.22
PSB-Sy 5	Control	16.00	17.37	25.95
	200	18.25	15.95	23.80
	250	19.32	15.22	23.35

Table 32. Number of seeds per plant of different soybean varieties in the M_5 generation as affected with varying days of irrigation withdrawals

Varieties	Treatments(Gy)	Irrigation Withdrawals		
		30 days after seeding	40 days after seeding	50 days after Seeding
PSB-Sy 4	Control	45.80	46.70	49.77
	200	53.67	38.57	42.45
	250	48.42	39.22	38.50
BPI-Sy 4	Control	48.80	48.32	39.75
	200	37.32	48.55	52.53
	250	47.62	47.05	34.62
PSB-Sy 5	Control	31.10	34.22	53.35
	200	35.07	29.79	48.85
	250	39.62	30.70	49.90

Table 33. Weight (grams) of 100 seeds of local soybean varieties in the M_5 generation as affected with varying days of irrigation withdrawals

Varieties	Treatments(Gy)	Irrigation Withdrawals		
		30 days after seeding	40 days after seeding	50 days after seeding
PSB-Sy 4	Control	16.20	16.17	15.52
	200	15.91	14.91	16.66
	250	15.68	16.28	14.40
BPI-Sy 4	Control	14.06	14.75	14.18
	200	15.51	14.46	13.40
	250	13.12	13.42	13.77
PSB-Sy 5	Control	15.31	15.51	14.67
	200	16.00	15.82	16.91
	250	17.22	14.76	14.15

Table 34. Number of mutants selected in the M_5 generation that were early maturing and plants with green leaves at maturity that were subjected to irrigation withdrawal 30 days after seeding

Varieties	Radiation Treatments (Gy)	Number of plants selected:	
		Early-maturing	with green leaves at maturity
PSB-Sy 4	Control	9	3
	200	86	38
	250	52	16
BPI-Sy 4	Control	-	33
	200	32	47
	250	125	92
PSB-Sy 5	Control	18	60
	200	86	81
	250	151	99

Table 35. Height (cm) of selected drought tolerant mutant lines in the M_5 generation of three soybean varieties from Vietnam as affected by irrigation withdrawal 20 days after planting

Varieties	Dose (Gy)	Means a/
DT 95	0	44.65 a
	200	42.6 a
	250	40.86 ab
DT 84	0	30.3 cd
	200	38.25 b
	250	31.05 cd
AKO 6	0	33.45 c

a/ In a column, means with the same letters are not significantly different at 5 % level based on DMRT.

Table 36. Number of days to flower of selected drought tolerant mutant lines in the M_5 generation of three soybean varieties from Vietnam as affected by irrigation withdrawal 20 days after planting

Varieties	Dose (Gy)	Means ns/
DT 95	0	40
	200	39
	250	39
DT 84	0	39
	200	39
	250	39
AKO 6	0	41
	250	40

ns = not significant

Table 37. Number of pods per plant of selected drought tolerant mutant lines in the M_5 generation of three soybean varieties from Vietnam as affected by irrigation withdrawal 20 days after planting

Varieties	Dose (Gy)	Means a/
DT 95	0	16.65 abc
	200	19.45 a
	250	19.6 a
DT 84	0	12.4 c
	200	13.7 bc
	250	13.7 bc
AKO 6	0	17.25 abc
	200	18.35 ab

a/ In a column, means with the same letters are not significantly different at 5 % level based on DMRT.

Table 38. Number of seeds per plant of selected drought tolerant mutant lines in the M_5 generation of three soybean varieties from Vietnam as affected by irrigation withdrawal 20 days after planting

Varieties	Dose (Gy)	ns/ Means
DT 95	0	41.65
	200	43.35
	250	45.9
DT 84	0	26.9
	200	32.95
	250	30.9
AKO 6	0	39.95
	200	36.1

ns – Not significant

Table 39. Weight of 100 seeds in grams of selected drought tolerant mutant lines in the M_5 generation of three soybean varieties from Vietnam as affected by irrigation withdrawal 20 days after planting

Varieties	Dose (Gy)	ns/ Means
DT 95	0	13.22
	200	13.56
	250	3.71
DT 84	0	13.04
	200	14.98
	250	13.96
AKO 6	0	12.15
	200	12.80

ns = not significant

Table 40. Yield in tons per hectare of selected drought tolerant mutant lines in the M_5 generation of three soybean varieties from Vietnam as affected by irrigation

Varieties	Dose (Gy)	Means
DT 95	0	0.39 ab
	200	0.44 a
	250	0.47 a
DT 84	0	0.31 bc
	200	0.47 a
	250	0.42 a
AKO 6	0	0.22 c
	200	0.47 a

a/ In a column, means with the same letters are not significantly different at 5 % level based on DMRT.

Table 41. Plant height (cm) at maturity of selected mutant lines in the M_6 generation of three local soybean varieties as affected by the different irrigation treatments

Local Varieties	Irrigation Withdrawn		
	Dose (Gy)	20 DAP	30 DAP
PSB-Sy 4	0	a/ 43.8 c	a/ 43.36 bc
	200	41.9 c	45.1 c
	250	40.53 c	42.5 c
BPI-Sy 4	0	43.2 c	48.13 bc
	200	41.66 c	40.66 c
	250	53.0 b	54.33 b
PSB-Sy 5	0	75.96 a	76.4 a
	200	77.1 a	78.56 a
	250	74.06 a	80.93 a

a/ In a column, means with the same letters are not significantly different at 5 % level based on DMRT.
DAP – Days after planting

Table 42. Number of days to flower of selected mutant lines in the M_6 generation of three local soybean varieties as affected by the different irrigation treatments

Local varieties	Dose (Gy)	20 DAP	30 DAP
PB Sy-4	0	ns/ 36	ns/ 35
	200	35	35
	250	35	35
BPI Sy-4	0	35	35
	200	35	35
	250	36	37
PB Sy-5	0	37	37
	200	36	37
	250	36	36

ns/ Not significant
DAP – Days after planting

Table 43. Number of pods per plant of selected mutant lines in the M_6 generation of three local soybean varieties as affected by the different irrigation treatments

Local varieties	Dose (Gy)	Irrigation withdrawals	
		20 DAP	30 DAP
PSB Sy-4	0	59.5	56.66 d
	200	56.96	57.3 d
	250	53.4	58.63 cd
BPI sy-4	0	45.56	59.06 cd
	200	54.66	65.5 bcd
	250	49.9	83.8 a
PSB Sy-5	0	53.1	75.33 ab
	200	52.9	85.2 a
	250	56.66	72.23 abc

a/ In a column, means with the same letters are not significantly different at 5 % level based on DMRT
ns/ Not significant
DAP – Days after planting

Table 44. Number of seeds per plant of selected mutant lines in the M_6 generation of three local soybean varieties as affected by the different irrigation treatments

Local Varieties	Dose (Gy)	20 DAP	30 DAP
PSB Sy-4	0	a/ 120.5 a	a/ 111.53 cd
	200	107.46 ab	106.9 d
	250	104.73 ab	106.1
BPI Sy-4	0	89.20 b	111.67 cd
	200	103.46 ab	125.73 bcd
	250	91.9 b	151.14 ab
PSB SY-5	0	106.76 ab	152.83 ab
	200	102.7 ab	169.1 a
	250	116.73 a	139.73 abc

a/ In a column, means with the same letters are not significantly different at 5 % level based on DMRT.
DAP – Days after planting

Table 45. Weight of 100 seeds in grams of selected mutant lines in the M_6 generation of three local soybean varieties as affected by the different irrigation treatments

Local Varieties	Dose (Gy)	20 DAP	30 DAP
PSB Sy-4	0	ns/ 17.95	ns/ 18.14
	200	19.11	18.46
	250	19.26	17.15
BPI Sy-4	0	18.01	19.03
	200	18.80	18.97
	250	18.67	16.26
PSB Sy-5	0	18.52	17.43
	200	19.21	19.12
	250	18.45	18.88

ns – Not significant
DAP – Days after planting

Table 46. Total yield in tons per hectare of selected mutant lines in the M_6 generation of three local soybean varieties as affected by the different irrigation treatments

Local Varieties	Dose (Gy)	20 DAP*	30 DAP
PSB Sy-4	0	1.26	1.46
	200	1.40	1.63
	250	1.37	1.64
BPI Sy-4	0	1.22	1.46
	200	1.40	1.48
	250	1.02	1.14
PSB Sy-5	0	1.18	1.38
	200	1.24	1.45
	250	1.27	1.70

ns/ Not significant
DAP – Days after planting

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2-4. Vietnam

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This report summarized some results of soybean mutation breeding program at AGI and through FNCA network. Starting materials including imported varieties, national selected varieties, mutants and hybrid lines were used and treated by using radiation as mutagens with suitable doses, treatment methods to repair, improve the variety defects and isolate mutant lines.

1. Introduction

Because of long stretching on the North Latitude of 15^0, Vietnam has diversified weather and complicated topography. The weather is divided into two distinct patterns: the rainy season (from May to November) and the dry one. The cultivated land is 9.6 million ha of which 2.5 can be accessed to water and used for food production including rice and maize. The 75% of cultivated land must be depended on natural rainfall which leads to the need of releasing varieties resistant to drought including soybean.

In Vietnam, soybean (Glycine max (L.) Merr.), an important food and industrial crop, provides the protein need and oil for human being, the food for animals and the materials for industry. Although spreading of soybean cultivated area in Vietnam still has a large potency, but it increases quite slowly. In 2005, the soybean planted area was reached only to 203,000 ha with the yield of 1.43 ton/ha (66,5% average world yield), and the soybean produce was 290,290 thousand tons, meanwhile in 2005, Vietnam had to import 1.0 million tons (equivalent of dried seed) from foreign countries. Up to 2015, Vietnam intends to import 1.5 – 2.0 million tons/year of soybean.

Thus the problem, which made by the fact of production and market to Vietnamese soybean breeders was selecting and creating soybean varieties with short growth duration (75-100 days), high yield (1.5-3.5 tons/ha), good seed quality, tolerant to drought, resistant to diseases, adapted to crop pattern and ecological regions in the whole country.

Since 1960 it has been shown that soybean was an autogamous species having narrow genetic

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base, thus induced mutation seems to be the most suitable technique for increasing variation effect in soybean breeding (Conger et al, 1976). Nowadays, by induced mutation, many authors have formed several soybean varieties, with tolerance to diseases (Tulmann Neto et al, 1988), with high protein and oil content (Qui and Gao, 1988) and with many agronomic desired traits (Micke, 1993; D' Surhey et al, 1993; Bhatnagar and Tiwari, 1991).

In Vietnam, Tran Dinh Long (1990) selected M-103 soybean variety by using EI. At AGI, the researcher group, leaded by Mai Quang Vinh (1985, 1987, 1990, 1995, 1998, 2000, 2002, 2004), has been carrying out the research on the application of induced mutation and bred 10 mutant soybean varieties and tens other promising varieties and lines.

2. Selection of soybean variety DT96 with high-yielding and drought tolerance

2.1. Material, method and results:

2.1.1. The selection of starting breeding materials

Two mutant soybean varieties DT90 (Original from F_2 hybrid line (K.7002 x Coc chum, radiated by Gamma ray- Co^{60}/180 Gy) and DT84 (Original from DT80 x DT 76, F_3 hybrid line: D.3/033 used as starting breeding materials were radiated by Gamma ray- Co^{60}/180 Gy) were used for interbreeding. They were created by Agricultural Genetics Institute (AGI) and adopted as national varieties. Both of them have high seed yield (2.5 – 3.5 tons/ha), short growth duration (85 – 95 days) and rather poor tolerance to drought. Besides, the variety DT90 has good seed quality, resistance to diseases and high tolerance to low temperature, however has low tolerance to high temperature. On the contrary, the variety DT84 has high tolerance to high temperature but has low tolerance to low temperature.

2.1.2. Selection of hybrid lines with drought tolerance

- The Single-seed descent method (SSDM) was applied on the hybrid populations at the beginning of F_4 generation and promising lines with drought tolerance were selected.
- Drought tolerance lines were selected by testing the rate of seed germination in sugar solution as introduced by VIR (1982) in the laboratory.
- The promising lines were evaluated basically with two control varieties DT90 and DT84 at the field in a continuous assessment of 3 crops/year. They were planted under growth condition with and without supplement of water, and were observed directly the level of flag leaves under the hot and dry weather condition of summer noon in North of Vietnam.

2.1.3. Development of promising lines by using the local adaptability test

5 lines were developed by pure line selection of the promising lines with drought tolerance for 9 generations. The results of local adaptability test at 5 stations showed that the line D.96 (adopted as national variety in 2004 and named DT96) was tolerant to drought and high yielding (Table 1).

Table 1. Assessment results of seed germination level in sugar solution 10.8% (equivalent of 9 at)

Varieties and lines	Variety DT90	Variety DT84	Line D.45	Line D.57	Line D.86	Line D.87	Line D.96
The rate of germination (%)	62	65	50	53	62	67	70

The result of Table 1 showed that D.96 proved germination ability in sugar solution 10.8%, among 5 lines when being compared with the parent lines (DT90 and DT84), D.96 had highest germination level (70%) which was significantly higher than that of the parent lines (62% and 65%). This characteristic is also interrelating to its drought tolerance ability as shown in Table 2.

The assessment result of the drought tolerance ability at three different growth periods: I-Shooting period; II- Blooming period; III- Firm-ripe period (Table 2) showed that D.96 had highest level (4-5) while its parents had lower levels (3-4). D.96 also proved hot tolerance ability similar to that of DT84 and higher than that of DT90. Assessment result of seed yielding/ a plant showed that D.96 also had highest yield (9.6 gr/ a plant), significantly higher than that of it parents DT90 (8.86 gr/ a plant) and DT84 (7.34 gr/ a plant). The line D.96 was adopted by Vietnamese Agricultural Ministry as a new national soybean variety in 2004 and named officially as DT96.

Table 2 Drought tolerance characteristics of lines (In Spring 2002)

Characteristics / Varieties and lines	Days to maturity	Drought tolerance level (*)			Hot tolerance level (**)	Seed yield /plant (g)
		I	II	III		
Variety DT90	90	3	4	4	5	8.86
Variety DT84	95	4	4	4	2	7.34
Line D.45	90	3	3	4	3	5.46
Line D.57	89	3	3	3	4	6.89
Line D.86	96	4	3	3	4	7.45
Line D.87	85	3	3	4	3	6.98
Line D.96	96	4	5	5	5	9.42

Table legend:

(*) – Drought tolerance levels were marked with the point 1 – 5: 1 – 2 - Low; 3 - Average; 4 – Rather high; 5 – High; I – shooting period; II – blooming period; III – firm-ripe stage

(**) – Hot tolerance levels were marked with the same point as those of drought tolerance

Table 3 Evaluation result of basic characteristics of examined varieties with the control variety DT84

Crops	Varieties	Days to maturity (days)	Plant height (cm)	Pods/ plant	1000-grain weight (g)	Potential yield (tons/ha)	Field yield (tons/ha)	Comparison level with the control (%)
In the Spring crop 1996 (with supplementary of water)z								
	DT-84 (I)	92	44.0	18.6	185	2.41	1.85	100.0
	DT90 (II)	99	48.6	21.1	200	2.96	2.28	122.8
	DT-96	97	53.3	21.3	192	2.87	2.21	119.4
	LSD.0.05					0.05	0.04	
	CV (%)					6.45	5.34	
In the Spring crop 1996 (without supplementary of water)								
	DT-84(I)	93	43.2	16.9	182	2.19	1.68	100.0
	DT90(II)	100	46.5	19.1	189	2.69	2.05	122.0
	DT-96	98	52.2	20.3	191	2.79	2.10	125.0
	LSD.0.05					0.16	0.14	
	CV (%)					5.34	4.98	
In the Summer crop 1996 (with supplementary of water)								
	DT-84(1)	90	59.1	29.9	184	3.31	2.54	100.0
	DT90(II)	97	42.5	23.4	200	2.81	2.16	85.0
	DT-96	95	50.8	26.6	205	3.28	2.52	99.2
	LSD.0.05					0.17	0.16	
	CV (%)					7.22	6.34	
In the Summer crop 1996 (without supplementary of water)								
	DT-84(I)	89	56.2	26.4	180	2.87	2.01	100.0
	DT90(II)	95	40.6	20.5	185	2.44	1.71	85.0
	DT-96	95	49.3	25.6	200	3.12	2.40	119.4
	LSD.0.05					0.14	0.09	
	CV (%)					6.18	5.22	
In the Winter crop 1996 (with supplementary of water)								
	DT-84(I)	85	35.3	15.3	195	2.21	1.70	100.0
	DT90(II)	90	38.3	17.9	206	2.45	1.88	110.6
	DT-96	89	41.2	16.3	211	2.53	1.96	115.3
	LSD.0.05					0.16	0.13	
	CV (%)					6.28	5.58	
In the Winter crop 1996 (without supplementary of water)								
	DT-84(I)	81	29.4	11.7	150	1.70	1.30	100.0
	DT90(II)	85	31.9	13.7	158	1.87	1.46	112.3
	DT-96	87	33.4	13.6	165	2.11	1.69	130.0
	LSD.0.05					0.18	0.16	
	CV (%)					6.25	5.35	

Table 4. National assessment results of seed yield at 5 stations in spring 2002 (quintals/ha)

Stations / Varieties	Vinh Phuc	Phu Tho	Bac Giang	Hai Duong	Thanh Hoa	Quang Ngai	Average	
							Seed yield	Comparison yield with the control variety (%)
V74(Control variety)	29.28	19.5	13.80	27.71	16.70	18.3	20.88	0
T21	26.19	16.6	19.00	28.33	14.26	13.3	19.61	-6.10
DT2000	32.38	20.4	20.03	28.90	16.05	9.1	21.14	+1.20
DT99.2	24.76	17.6	21.30	20.18	12.96	9.0	17.63	-15.60
D2101	29.28	18.1	18.13	27.71	16.21	10.7	20.03	-4.07
D2102	32.38	21.9	18.27	29.61	17.22	14.9	22.37	+7.10
MA97	34.76	20.4	18.60	25.33	16.63	16.0	21.95	+5.10
912	31.43	18.0	20.87	23.47	17.31	11.9	20.50	-1.80
DT-96	***35.23***	***19.0***	***18.07***	***30.33***	***16.60***	***18.6***	***22.98***	***+10.10***
CV (%)	3.0	4.3	11.4	5.8	8.8	6.0		
$LSD_{0.05}$	1.62	1.41	3.68	2.68	2.43	1.40		

2.2. Application of the gamma-Co^{60} to improving 3 high-yielding Vietnamese soybean varieties with new characters of short growth duration and drought tolerance

2.2.1. Material and Method

Treatment Methods

The field experiment was conducted at the Hanoi Research Station in Summer Season 2005.

Starting Materials: DT96, DT2001 and D.158.

Treatment method and time: radiating Gamma Co^{60} on dry seeds in the late June 2005.

Dose treatments: 150, 200, 250 Gy

Table 5 Improvement targets of variety characteristic

Variety characteristics	DT96		DT2001		D.158	
	Origin	Target	Origin	Target	Origin	Target
Growth duration (days)	100	90 - 92	100	90 - 92	100	90 - 92
Yield (quintal/ha)	22 –35	22-35	23-40	22-38	22-38	21-36
Drough tolerance	Rather high	High	Medium	Rather high	Rather high	High
Hot tolerance	High	High	High	High	Rather high	High

Research methods :

- M_1 plants: Examining mitosis index, spectrum and frequency of chromosomal aberration of parenchyma cell of root-tip, rate of germination in room and field condition, rate of survival in field, variation rule of morphological characteristics and occurring dominant mutant.
- M_2, M_3 plants: Examining variation frequency and spectrum, the genetic rule of M_1 variations, variation coefficient, determining optimal dose and concentration of each mutagen to each variety.
- M_4 and so on generations: selecting, evaluating obtained mutant collection according to breeding standard, drawing the influence rule of mutagens to genotype of each kind of varieties, finding out the optimal condition and treatment method.
- Field tests procedure and data statistics were carried out after general principle (Rokiski, 1973, Dospekhov 1979)...
- Using method of anaphase analysis (Youssev, Poukhalski 1968). Temporary preparation made after modified method of Iutmann, Topinskaia et al 1975.
- Assessment of drought tolerance: the germination in solution of sacharose 10.8% (9 atmosphe according to VIR guideline, 1982), the germination in hot condition of 44^0C (5 h, according to Volcova, Kozusco, 1984); research on criteria of water regime of soybean leaves during growth duration (according to Gazunova, 1984); the drought tolerance can be discovered by level of drying leaves. The trial data research of soybean varieties in mountainous area where watering depends on natural rainfall in two provinces: Son La and Ha Giang;
- Data treatment on computer according to IRRISTAT program.

2. 2.2. Results and discussion

a) Variation types appeared in M_1 generation

During observation on the field we found that due to different effect of mutagen doses, many types of variation were appeared resulting in rather large variation spectrums among the experimental soybean lines. The variation manifested through the change of the shape, the size, and the color of vegetative organs (root, stem, leaf) or reproductive organs (flower, pod and also seed). Besides, there were variation types which caused the increase in number of leaf, number of branch or the change of leaf distribution in a plant, and variation types which caused the sterile in the whole plant or in the half part of the plant etc. In addition using radiation as mutagen can result in a mutation population containing many useful mutations. They are maybe dominant mutations (expressing immediately right at M_1 generation) or recessive mutations (only expressing later since M_2 generation obtained from self-pollination of M_1). For example: Useful dominant mutations increasing the yield of variety and having a high economic value (the variations cause the increase in pod number of plant or in the number of four seed-containing pod, or high seed weight/ plant, or the increase in seed size.. etc.). Obtained variations are the results from the effect of different doses of γ Co^{60} mutagen on three soybean experiment varieties DT96, DT2001, D.158. Those variations can appear singly in each plant or many different variations can appear simultaneously in a plant.

However in the research scope for M_1 generation we just concerned about some usually occurred variation types such as flattened stem, split stem, curved stem, early ramified, shrink top. The research results were shown in the Table 6 and Chart 1.

Table 6. Morphologic modifications on stems of the treated plants at M_1

Treatment	**Flattened Stem**		**Split Stem**		**Curved Stem**		**Early Ramified**		**Shrink Top**	
	Plants	%	Plants	%	Plants	%	Plants	%	Plants	%
DT96										
Control	0		0		0		0		0	
150 Gy	14	4.6	28	9.2	58	19.1	0	0	4	1.3
200 Gy	32	10.3	45	14.4	47	15.1	0	0	5	1.6
250 Gy	38	13.9	51	18.7	40	14.7	0	0	7	2.6
DT2001										
Control	0		0		0		4		0	
150 Gy	6	2.0	15	5.1	25	8.4	0	0	4	1.3
200 Gy	6	2.4	23	9.0	37	14.5	9	3.5	3	1.2
250 Gy	4	1.6	30	12.3	33	13.5	5	2	3	1.2
D.158										
Control	0		0		0		8		0	
150 Gy	1	0.3	21	6.3	35	10.6	4	1.2	4	1.2
200 Gy	3	1.0	32	10.2	33	10.5	8	2.5	6	1.9
250 Gy	4	1.5	47	17.5	32	11.9	0	0	5	1.9

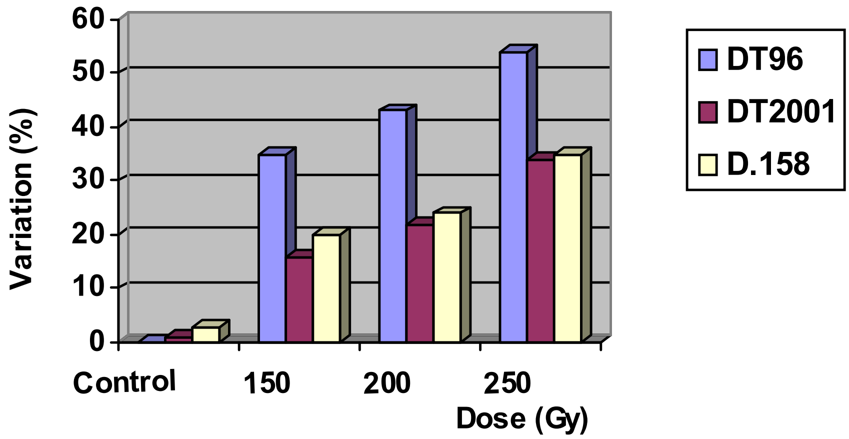

Chart 1. Variation of mutagen treated plants at M_1

The result of Table 6 shows that the effects of γ Co^{60} mutagen doses on mutant rates of five mutation types are different: The variety DT2001 and the line D.158 are over sensitive than the variety DT96. Early ramified variation type did not appear in DT96 at all treated formulas, revealing that this variety has a narrower mutation spectrum.

Appearance of the variation types in treated formulas here followed a rule is that as stronger mutagen dose was treated as higher the variation frequency was increased. Variation frequency was calculated on harvested plants by the ratio (%) of variation having plants number to plant total number. For example variation frequency of the control formula of DT96 was 0%, in the formulas treated with gradually increased doses of 150 Gy, 200 Gy, 250 Gy, variation frequencies were also increased corresponding to 35%, 43% and 54%. In the case of the line D.158, variation frequency of the control was 3%, in the treated formulas it was increased from 20% at the treatment of 200 Gy to 35% at the treatment of 250 Gy.

Thus in all examined soybean varieties and line DT96, DT2001 and D.158, different variation types were appeared after treated the plants with the mutagen and variation frequency was increased when the mutagen dose was increased.

b) Appearance of the mutants having economic values in M_2-M_4 generations

+ Generation M_2

Soybean is self-pollinate plant. Thus recessive mutant usually appears one generation later than dominant mutant does, which means that some mutants just appear since M_2.

We found that radiation treated formulas had variation frequencies higher than that of the control. Table 7 showed that the dependence of variation frequency on treating dose in M_2 was not similar with that in M_1. In M_1 generation variation frequency was increased accompanying with the increase of treated dose (150 Gy – 200 Gy - 250 Gy). However in M_2, variation frequency was highest at the 200 Gy treating dose. For example, DT96 had variation frequency of 0.033% at treated dose of 200 Gy, while at treating doses of 150 Gy and 250 Gy the variation frequencies were 0.024% and 0.019 %, respectively. DT2001 had variation frequency of 0.033% at treating dose of 200 Gy, which was higher than those at the treating doses of 150 Gy (0.028%) and of 250 Gy (0.026%). This character was also similar with what appeared in treated soybean line D.158.

+ Generation M_3, M_4

The target of inducing experimental mutation on the plant is to make new varieties of high yield, early harvest and drought tolerance. Thus after mutagen treatment on starting materials, during observation of the growth and development of the soybean plant at M_2 generation we also interested in looking for useful variations for breed selection. In M_2 generation, the appeared variation types having economical values were the plants bearing high branch number, high pod number especially of the pod containing three seeds, big and firm seed etc. Those variation types appeared in M_2 generation much more than in M_1. At the treating dose of 200 Gy, useful variation types appeared much more than those of 150 Gy and 250 Gy. For example, from the mutagen treated D.158 we obtained 1 plant having 50 pods among of that 31 three seed-pods, 5 four seed-pods; 3 plants having the heights of 35- 36 cm and bearing 5- 6 branches on each plant. From the 150 Gy treated formula of DT2001 we obtained 2 plants which had P1000 of the seed were 192 gr and 192.6 gr, 9.4 and 10 gr respectively higher than that of the control. Especially some plants obtained from this treated formula had many useful variation characters in the same plant such as having short stem, strongly branched, having high pod number, bearing big and good shape seeds. These might be variation types having prospect of using for breed selection in the future. On M_4 grown at the summer 2006 crop (Table 8), examination result of the mutant lines bearing the stable characteristics which were inherited from M_3 showed that some lines taking short time to be mature but they still could get high yield as the origin varieties did (D.96/26, D.01/58, D.58/224) or even could get higher yield due to bearing bigger seed or higher pod number in each plant (D.96/150, D.01/245).

3. Conclusions and suggestions

3.1. Conclusions

From the research result of mutagen treatment on three experimental objects DT96, DT2001 and D.158 during summer and winter crops in 2005, we come to the conclusions as following:

1. In M_1 generation variation frequencies in all three experiment objects were increased according to the increase of mutagen doses 150 Gy-200 Gy-250 Gy. However in M_2 generation highest variation frequency was of treatment at 200 Gy dose.
2. By observation from M_1 to M_2, we found that the treating dose of 200 Gy induced much useful variation types having economical values more than that at other treating doses. Many variation types were appeared in M_2 indicating that variation spectrum of this generation was higher than of M_1.
3. Treatment by γCo^{60} mutagen could change some characteristics, among of those there were quite a lot useful variation types. Especially shortening growth time and increasing in the yield, improving drought tolerance for the plant are realizable.

3.2 Suggestions

By continuing observation on variation types of M_1 and M_2 generation, especially the types having economical value such as short stem having high yield, shortened growth time, drought tolerance, we can hope to obtain mutant lines as our desire, suitable with selection target for drought tolerance, high yield, short growth time and being widely adapt with the environment.

Table 7 Variation of generation M_2 after γ-Co^{60} treatment

No	Type of variation	DT96				DT2001				D.158			
		Control	150 Gy	200 Gy	250 Gy	Control	150 Gy	200 Gy	250 Gy	Control	150 Gy	200 Gy	250 Gy
1.	Dwarf	0	+	+	+	0	+	+	+	0	+	+	+
2.	Tall	0	+	+	+	0	+	+	+	0	+	+	+
3.	Curved	0	+	+	+	0	+	+	+	0	+	+	+
4.	Dwart and early ramified	0	+	+	+	0	+	+	+	0	+	+	+
5.	Multiply ramified	0	+	+	+	0	+	+	+	0	+	+	+
6.	Non ramified	0	+	+	+	0	+	+	+	0	+	+	+
7.	Thick leaf on stem	+	0	+	+	0	+	0	+	0	+	+	+
8.	Leaf shape	0	+	+	+	+	+	+	+	0	+	+	+
9.	Leaflet	0	+	+	+	0	+	+	+	0	+	+	+
10.	Leaf colour	0	+	+	+	0	+	+	+	0	+	+	+
11.	Sterile	0	+	+	+	0	+	+	+	0	+	+	+
12.	Pod with 4-seed	0	0	+	+	0	0	+	0	0	0	0	0
13.	Seed colour	0	0	0	+	0	0	0	+	0	0	+	0
14.	Rareripe	0	+	+	+	+	0	+	+	+	0	+	+
15.	Late reripe	+	+	+	0	+	+	+	+	+	0	0	+
16.	Total of variation type	2	13	15	15	3	13	14	15	2	12	14	14
17.	Total of variation plants	2	19	26	13	3	24	25	18	2	15	27	22
18.	Fre-cy of variation (%)	0.002	0.024	0.033	0.019	0.003	0.028	0.033	0.026	0.002	0.019	0.037	0.032

Remark: + : yes; 0 : no

Table 8. Performance of generation M_4 of some promising mutant lines at summer (2006)

No	variety, Mutant Lines	Treatment	Days to maturity	Plant Height (cm)	Number of Branches/ plant	Number of Nodes/ plant	Number of Pods/ plant	Weight of 100 seeds, g	Dry seed weight, g/plant
1	***DT96***	***Origin***	***96***	***57.2***	***5.7***	***10.4***	***25.6***	***195.6***	***10.7***
2	96/26	150 Gy	91	55.3	4.6	9.9	27.5	193.8	11.2
3	96/52	200 Gy	96	58.4	5.9	11.6	28.3	200.6	11.9
4	96/150	150 Gy	98	59.5	6.4	11.8	30.5	186.0	125
5	***DT2001***	***Origin***	***95***	***55.8***	***4.7***	***11.3***	***34.7***	***162.0***	***12.4***
6	01/58	150 Gy	90	53.8	5.6	10.2	32.7	164.9	11.8
7	01/245	200 Gy	98	58.9	5.8	11.3	34.5	170.5	12.9
8	***D.158***	***Origin***	***98***	***60.5***	***6.8***	***12.5***	***40.7***	***190.3***	***16.2***
9	58/224	200 Gy	92	56.4	6.0	10.7	37.9	195.3	16.3

This report was prepared by the Nuclear Safety Research Association as a part of activities on the MEXT Forum for Nuclear Cooperation in Asia (FNCA).