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*Sunflower mutant lines developed by gamma rays mutagenesis. A decorative mutant line Decor 110 (Photo courtesy of Dr. M. Christov) – For more information, see paper on Mutation Breeding in Bulgaria, Page 4*

## To Our Readers

Breeding a new variety is far more complex and takes much more time than performing a laboratory experiment in well controlled conditions. Further, breeding information is often not published in scientific journals, and is sometimes kept as a trade secret. Therefore, it is not an easy job to collect and analyse relevant information and write a paper to review the achievements in plant breeding. As in many other countries, induced mutations have played an important role in crop breeding in Bulgaria. In this issue, Dr. N. Tomlekova presents an excellent paper on this subject. She has succeeded in portraying a comprehensive picture of research and application of mutation breeding in Bulgaria: about 80 mutant varieties of 14 different plant species; leading mutant varieties are covering about 50% of maize growing area and almost 100% of durum wheat area; novel mutations have not only played a role in improving resistance/tolerance to biotic/abiotic stresses, quality and nutrition traits, but also in facilitating hybrid seed production and enabling adaptation to mechanization of crop production; thousands of mutant lines have been generated and preserved as germplasm collections and used in breeding programmes. The great success in hybrid maize breeding may surprise most readers since it is widely believed that out-crossing crops like maize have sufficient genetic variability, and that induced mutations have limited roles. Such perceptions should be re-assessed against the great success of maize mutation breeding in Bulgaria.



**IAEA**  
International Atomic Energy Agency

Chronic radiation enables continuous mutagenic treatment of living organisms. This requires special facilities such as: gamma phytotrons, gamma greenhouses or gamma fields. Brief introductions of such facilities, currently available, are included in this issue.

It has been a pleasure being the editor of Plant Mutation Reports over the past five years and I am very pleased that we have made significant progress on this journal in terms of the quality of published papers. Further progress will

continue to take place with the establishment of an online manuscript submission and review system as well as a new editorial team in the coming years, if not months. Since I am finishing my contract with the IAEA soon, I would like to take this opportunity to thank you (authors, reviewers and readers) for the support you have given.

*Qingyao Shu*

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## Review

**Induced Mutagenesis for Crop Improvement in Bulgaria**

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**Abstract**

Experimental mutagenesis has been investigated and applied in crop breeding in various Bulgarian agricultural research institutes during the last half century. In this paper some major accomplishments achieved in Bulgaria are highlighted. Both, physical mutagens (mainly gamma rays) and chemical mutagens (mainly EMS, NMU, NEU), have been used and their proper doses have been established. According to the information available to the author, there are more than 76 new cultivars developed using induced mutants in Bulgaria, namely: barley (5), wheat (5), durum wheat (9), maize (26), sunflower (3), lentil (4), bean (2), pea (1), chickpea and vetch (2), soybean (5), tomato (6), pepper (4), cotton (2), tobacco (2). Some of the mutant cultivars such as maize hybrid Kneja 509 and durum wheat cultivar Gergana have become leading cultivars occupying up to 50% of the growing area of the crop concerned. In durum wheat, mutant cultivars have not only covered almost all the growing areas but also doubled the yield in the past 30 years. The achievements in mutation breeding programmes have also had a significant impact on the progress of genetic research by elucidating the underlying mechanisms of induced mutations and the training of many young researchers and university students through their involvement in various research projects. A number of mutant lines with novel characteristics and mutant cultivars of economical importance together with relevant techniques used in the development and characterization of those mutant lines/cultivars are described in this paper.

**Keywords:** Mutation breeding, induced mutagenesis; physical mutagen, chemical mutagen, cereals, legumes, vegetable crops

**Introduction**

In the late 1920s, it was discovered that genetic make-up is amenable to change by induced mutagenesis. This has since been applied as an established method for increasing genetic variability in many crop plants. In Bulgaria, mutation breeding has been widely used since the 1950s. Initial mutagenesis studies were mainly directed to identify the optimum mutagen and dose (or combination of different mutagens) to achieve high frequencies and broad ranges of induced mutations. Breeding lines and cultivars were developed with the establishment of proper protocols of physical and chemical mutagenesis in various crop species, such as: cereals, forage, fibre, vegetable crops, tobacco, etc. Such investigations and breeding

work have been pursued in several research institutes, notably, in universities, supported by national research programmes, as well as several IAEA projects, including a number of coordinated research projects and technical cooperation projects.

A large range of chemical and physical mutagens have been investigated for their use in crop improvement and Table 1 shows the most commonly used ones. X rays, easy to access and apply, were frequently used as sources of ionizing radiation. Gamma rays were used for both, intact plants and parts of plants as well as seeds. Fast neutrons, known to be strongly harmful to chromosomes, were used for seed treatment. UV-light – causing formation of T-T dimers and lasers has been used recently. Nowadays, with the permission of the Nuclear Regulatory Agency, 18 gamma irradiation facilities, using either cobalt-60 or caesium-137, are in operation in Bulgaria for either industrial or scientific (7) or for medical purposes (11). A number of chemical mutagens were also frequently used.

To date, more than 76 mutant cultivars have been developed in Bulgaria (Table 1). In addition, a large number of mutant lines are maintained as working germplasm collections in respective institutes, where they were developed, or at the National Seed Genebank in Sadovo. There is an ongoing assessment on the potential use of mutants in agriculture. Considering the fact that induced mutations are generally deleterious, such large number of mutant cultivars and lines released in major crops is impressive. It is relevant to emphasize here that in all cases the mutant cultivars have emerged as superior to other entries prior to being approved for commercial release. The lines and cultivars developed demonstrated that gene mutations can be useful for obtaining genotypes with higher yield potential, altered plant architecture, characteristics suitable for mechanical harvesting, reduced lodging, early ripening, higher levels of protein and  $\beta$ -carotene content, disease and pest resistance and drought tolerance, wide ecological adaptability (Table 1).

This article aims at summarizing some of the main and recent achievements of induced mutagenesis in major crops in Bulgaria. It also provides some perspectives of future mutation breeding.



Table 1. Mutagenesis and mutant cultivars of various crops registered in Bulgaria

Crops species	Commonly used mutagens and range of doses	Number of mutant cultivars developed**	Main mutant characteristics reported
Barley	$^{60}\text{Co}$ $\gamma$ rays (20-100 Gy); UV-C light (0.5-5 J/cm <sup>2</sup> )	3 (2)	Increased productivity; improved grain characteristics; spike fertility; short and lodging resistant stalks; short vegetation period; high hardness to dip; high cold tolerance, resistance to powdery mildew and to brown, black and stem rust
Common wheat	$^{60}\text{Co}$ $\gamma$ rays (50 Gy); $\text{NaN}_3$ (0.1, 1-10 mM); EMS (0.1-0.3%)	4 (1)	High grain yield; high and stable productivity; ecological adaptability, drought and cold tolerance; improved tolerance to lodging and shedding; resistance to brown rust; quality index - softening of dough and energy for dough deformation
Durum wheat	$^{60}\text{Co}$ $\gamma$ rays (50-200 Gy); EMS (0.1-3.5%)	8 (1)	High yield, high productivity; high cold tolerance; short stem; awnlessness; new leaf shape - spherococcum, erectum, compactum, spread rosette
Maize	NEU (0.001-0.01%); NMU (0.001-0.01%); Dioxane (<1%); DMS (0.1-0.3%); DES (0.1-0.3%); EMS (0.1-0.3%)	(26)	High grain yield and productivity; tolerance to dense sowing; early ripening; drought tolerance; high protein content; high biomass dry matter; shifts in the flowering time; white-colour grain; strong stem; altered ear length; increased number of rows
Sunflower	$^{60}\text{Co}$ $\gamma$ rays (20-500 Gy); $^{137}\text{Cs}$ $\gamma$ rays (8-540 Gy); Ultrasound (0.5-25.5 w/cm <sup>2</sup> ); EMS (0.2 - 0.8%)	(3)	Higher seed oil content; higher plants; larger leaves; greater number of branches; larger diameter of stem and plant head; altered size, position, shape, colour of leaves, seeds and inflorescence; increased seed weight; shortened vegetation period; small stalk height; increased oil contents; improved oil seed composition; cytoplasmic male sterility; better combining ability; resistance to <i>Orobanche cumana</i>
Lentil	$^{60}\text{Co}$ $\gamma$ rays (30-40 Gy); $^{137}\text{Cs}$ $\gamma$ rays (30-40 Gy); FN (10 Gy); EMS (0.05-0.3%); DES (0.05-0.25%); MMS (0.05-0.2%); $\text{NaN}_3$ (0.001-0.1%); NMU (0.0065-0.025%); NEK (0.0065-0.025%); $^{60}\text{Co}$ $\gamma$ rays (20-40 Gy) + EMS (0.05-0.1%)	4	Higher yield; early ripening; optimization of bush shape; increased grain size; resistances to: <i>Ascochyta</i> blight, <i>Fusarium</i> , <i>Anthraxnose</i> , <i>Stemphylium</i> and viruses; non-lodging stem; low tannin levels; changed leaf colour and shape; increased grain protein content plant: short, tall, dwarf-like, with different degree of lodging; Grain shattering resistance; grain mass; changed cotyledon colour and seed coat
Bean	$^{60}\text{Co}$ $\gamma$ rays (80-100 Gy); Colchicine (0.1%)	2	Long narrow leaves; sickle-shaped pods; flowers with three ovaries compact habit; early ripening; simultaneous pod ripening; dark-green corrugated leaves; increased pod weight; changed pod shape; unifoliate; chlorophyll mutations – albina, xanta, chlorine, chloroviridis, chlorotica; resistance to: CBB, HB, BCMV, CMV, CIYVV, Rust, <i>Anthraxnose</i> , <i>Sclerotinia</i>
Pea	$^{60}\text{Co}$ $\gamma$ rays (40 Gy); FN (2.5 - 15 Gy); EMS (0.1-0.6%); EI (0.2%, 0.01-0.06%, 0.005%); DES (0.05-0.6%); NMU (0.001%); DMS (0.2%); $^{60}\text{Co}$ $\gamma$ rays (2.5-5 Gy) or FN (0.25-0.50 Gy) + EMS (0.2%) or DES (0.2%); $^{60}\text{Co}$ $\gamma$ rays (80Gy) + EMS (0.05%); $^{60}\text{Co}$ $\gamma$ rays (40 Gy) + EMS (0.2%)	1	strong and non lodging stem – ‘alfa’ type; increased number of pods and seeds; early ripening; drought tolerance; great number of fertile branches; increased protein levels; resistances to <i>Ascochyta</i> blight, <i>Erysiphe poligoni</i> , SCCV virus; tolerance to <i>Brushus pisi</i>
Chickpea; vetch	$^{60}\text{Co}$ $\gamma$ rays (50-350 Gy); EMS (0.05-0.2%); $^{60}\text{Co}$ $\gamma$ rays (50 Gy) + EMS (0.05-0.1%)	(2)	Morphological mutations

Crops species	Commonly used mutagens and range of doses	Number of mutant cultivars developed**	Main mutant characteristics reported
Grass - Sorghum sudanense	DMS (0.1-0.3%); DES (0.0005-0.01%); NMU (0.0005-0.01%); NEU (0.0005-0.01%)		Mutations in reproductive organs; shorter corn-flower
Soybean	<sup>60</sup> Co γ rays (25-150 Gy); FN (0.025-10Gy); EMS (0.05-0.2 M) EMS (0.1%); NMU (0.001 - 0.1%); NaN <sub>3</sub> (0.001M); MMS (0.001-0.1%); <sup>60</sup> Co γ rays (40-50 Gy) + EMS (0.1%); <sup>60</sup> Co γ rays (50 Gy) + MMS (0.1%); <sup>60</sup> Co γ rays (50 Gy) + NMU (0.05%); FN (40 Gy) + EMS (0.05 M)	5	Drought tolerance; improved architecture of shrub; early-ripening; increased crude protein in grain; resistances to charcoal rot, stem cancer, bacterial blight, bacteriosis and mildew; increased protein content; dwarf type; vertically arranged leaves; altered leaflet number
Pepper	<sup>60</sup> Co γ rays (80-120 Gy); X rays (120 Gy); EMS (0.5-0.7%)	2 (2)	Male-sterility: nuclear (ms <sub>3</sub> , ms <sub>8</sub> ); CMS (ms <sub>5</sub> ); resistance to powdery mildew; sulfur-white (sw); high β-carotene content; dwarfism (dw, dw-2); short conic fruits; anthocyaninless (al); marbled leaves; yellow cotyledons; light green leaves; high dry matter content; early ripening
Cotton	<sup>60</sup> Co γ rays (100-150 Gy)	2	High yield; length uniformity; high maturity coefficient; shorter vegetative period; high fibre strength
Tobacco	DES (0.01-0.2 M); FN (90-150 Gy)	2	Male sterility, shorter vegetation period, resistance to: Peronospora tabacina, Erysiphe cicoracearum, Agrotis ipsilon

\* Abbreviations: NaN<sub>3</sub>: sodium azide; NMU: N-nitroso-N-methyl-urea; NEU: N-nitroso-N-ethyl-uUrea; NEK: N-nitroso-N-ethyl-carbamide; DMS: dimethylsulphate; DES: diethylsulphate; EMS: ethyl methane sulfonate; El: ethylene imine; FN: fast neutron.

\*\* The numbers are presented as 'mutant cultivars directly using induced mutants (mutant cultivars developed using one or more induced mutants in cross breeding)'

## Cereals

### Barley

Induced mutagenesis in barley was first investigated in 1967 at the Institute of Barley, Karnobat, now known as the Institute of Agriculture (Stefanov, 1995). Through extensive investigations, several mutants were developed with valuable biological and economic characteristics, including increased productivity and number of fertile spikes, short and lodging resistant stalks, short vegetation period, increased cold tolerance, etc. (Table 1; Gramatikova *et al.*, 1996; 2005a; 2005b; Dyulgerova, 2008).

Some mutants have been used as gene resources in barley breeding. Two cultivars were developed through gamma rays mutagenesis: **Markeli 5** (1976) and **Diana** (1983). Two other cultivars: **Krasi 2** (1983) and **Jubiley** (1982) were developed through hybridization with mutant lines. A new feed barley cultivar **IZ Bori** (Kt3026) was released in 2009; it was developed by sodium azide mutagenesis of a breeding line 280-7 and belongs to *Hordeum sativum* Jess, subsp. *Vulgare*, var. *paralelim*. IZ Bori is a winter type cultivar, highly tolerant to dip with good tolerance to cold temperatures (ranked Group III for cold tolerance similar to the control cultivar Odeskiy 31). IZ Bori shows good to very good resistance to powdery

mildew, as well as to brown, black and stem rust. The strict self-pollination habit of IZ Bori is favorable for maintaining its homogeneity. Its grain yield exceeds the control cultivar by 15-17% and its grain protein and lysine content is higher than standard cultivars, hence its grains are of improved nutritional value. IZ Bori is well-adapted to all kinds of growing conditions and can consequently be grown throughout the country.

Barley has also been used for basic research of DNA damage and repair in Bulgaria. Gecheff *et al.*, (1994) and Georgieva *et al.*, (2008) demonstrated that the heterogeneity of DNA damage induction and repair is dependent on the chromatin organization, transcriptional activity and nature of individual DNA sequences. Mutagenic treatments using ionizing radiation, UV-C light and bleomycin were applied in barley. Selective induction and differential repair in individual genes and defined DNA sequences were investigated. However, results indicated that there was no noticeable relationship between the transcriptional activity of rRNA genes and their repair potential when bleomycin was applied (Manova *et al.*, 2003; 2006; 2009).

## Wheat

### Common wheat

Two types of wheat cultivars are grown in Bulgaria: soft (common wheat) (*Triticum aestivum* L.) and hard (durum wheat) (*T. durum* Desf.). *T. aestivum* is the most widely spread wheat type worldwide and the only improved cultivar grown in Bulgaria. The main common wheat breeding centres in Bulgaria are the Dobrudza Agriculture Institute (DAI) in General Toshevo, the Institute of Plant Genetic Resources (IPGR), Sadovo, and until recently the Institute of Agriculture (IA) in Karnobat. Experimental mutagenesis of common wheat was also carried out at the Institute of Genetics, Sofia (Savov, 1976).

Since the 1970s, gamma rays, sodium azide and EMS, used in combination or alone, has been used for mutation induction and breeding in wheat (Georgiev, 1977; Rachovska, 1996) (Table 1). In addition, the effectiveness of mutagenic treatment of hybrid materials was investigated. For example, F<sub>2</sub> seeds were treated with sodium azide for selecting mutants (Rachovska *et al.*, 2002a, 2002b, 2003; Mangova *et al.*, 2004). Mutation breeding has produced the following mutant cultivars:

**Ideal** was developed as a strong wheat cultivar with improved dough properties (Dzhelepov, 1982). **Myriana** is a winter bread wheat cultivar developed through crossing with a mutant line at the IA, **Karnobat**, and released in 1991. It is a high yielding cultivar that outyielded the standard by 10-12%. It has high winter hardness, good resistance to diseases and good quality grain. **Myriana** has also proven to be suitable for growing in Turkey, Northern Greece, Italy, Southern France, Spain, Portugal, Albania and Serbia. Two other cultivars **Avanguard** and **Guinness/1322** were registered in 2005 by the Bulgarian Executive Agency for Variety Testing, Field Inspection and Seed Control (IASAS) (Rachovska *et al.*, 2005). Guinness/1322 was included in the Bulgarian Official Catalogue of Varieties of Agriculture and Vegetable Plant Species (Bulgarian Official Catalogue of Varieties). Guinness/1322 is a new winter common wheat cultivar that was developed from the cultivar Katya by <sup>60</sup>Co gamma rays irradiation at a dose of 50 Gy. High productivity, ecological adaptability and drought tolerance are its distinctive advantages compared to other cultivars. It is distinguished from its parental cultivar Katya by improved resistance to lodging and shedding. **Fermer** is the newest Bulgarian winter cultivar registered in 2009 (Fig. 1). It was developed by G. Rachovska in the IPGR by gamma irradiation (50 Gy) of dry seeds of the cultivar Pobeda. According to the grain quality parameters, Fermer belongs to the Group B - medium to high power wheat, suitable for self-baking of bread wheat. Fermer is considered as a significant breeding achievement with the following distinguished characteristics: its stable and

high productivity; excellent drought and cold tolerance (ranked as the highest cold tolerance) and resistance to the brown rust (race 10R). These characteristics make it suitable to be grown in various ecological environments. It attains basic yield with limited input of nitrogen and produces additional grains with the increase of nitrogen input, which makes it economically effective. This is the newest direction of the breeding programme in the IPGR, Sadovo. Fermer has all characteristics needed for widespread growing in the country.



Figure 1. The newest Bulgarian winter wheat cultivar Fermer (Photo courtesy of Dr. G. Rachovska)

Six new candidate cultivars, all developed by crossing with mutants, are pending for registration. A characteristics-based cultivar collection, comprising of 129 consolidated mutant lines of common wheat with useful properties have been developed in the IPGR (Rachovska, 1990). Under the law of protection of new plant cultivars and animal breeds and in agreement with the Convention on Biological Diversity, this collection was registered as original germplasm. In 2003, the collection was deposited for long-term storage at the National Gene Bank of Bulgaria and can solely be used for scientific purposes.

### Durum wheat

Durum wheat originates from the Mediterranean region. The main breeding centre of the crop in Bulgaria is the Institute of Cotton and Durum Wheat (ICDW) in Chirpan. Mutation techniques have been successfully deployed in durum wheat improvement in Bulgaria since the 1980s. Different frequencies of induced mutations were reported for <sup>60</sup>Co gamma rays and EMS mutagenesis, the highest frequency was achieved by 2h treatment of 0.5 – 3.5% EMS (Table 1).

A total of 8 cultivars have been developed by the combined use of mutation induction and cross breeding at the ICDW and listed in the Bulgarian Official Catalogue of Varieties. The use of those mutant cultivars has substantially and continuously increased the yield level, cold tolerance and grain quality of durum wheat in Bulgaria. **Gergana**, released in 1984, was developed by crossing a durum wheat cultivar and a mutant line. The F<sub>2</sub> hybrid seeds were further irradiated with 100 Gy gamma rays. It possesses characteristics such as high yield and good cold tolerance. It had covered 40-45% of the durum wheat area in Bulgaria until 1990 (Yanev, 1987). **Progres**, released in 1990, was developed from a cross between two mutant lines. Presently, it is the prevailing cultivar due to its high quality and productivity, covering about 80% of the durum wheat area in the country and was notified as the cultivar with the highest yield, 8340 kg/ha in 1988. **Beloslava**, released in 1997, was also developed from a cross between two mutant lines. It is the Bulgarian cultivar that has the highest quality with grains of about 40-42% wet gluten (standard requirement is 28%) and 18-19% raw protein content (standard requirement is 14.5-15%). The highest yield reached 8500 kg/ha.

**Vazhod**, released in 1999, was developed from a cross between Gergana and Zagorka. The F<sub>2</sub> hybrid seeds were irradiated with 50 Gy gamma rays. It is currently the cultivar with highest yield (>8500 kg/ha) and covers 20% of the durum wheat area in Bulgaria. It is resistant to *Puccinia graminis* and *Puccinie recondite tritice* and highly resistant to other diseases. It also has high cold tolerance, which makes it suitable for the northern areas of the country. **Deyana** belongs to the Group B – medium-strong durum wheat cultivars. In the testing period its average yield reached 5530 kg/ha (5160 kg/ha for standard cultivars Progress and Saturn 1). It is medium early-ripening cultivar and forms ears 3 to 8 days later than the average standard cultivars and demonstrates good winter hardness. **Zvezditsa** is medium early-ripening cultivar, with medium ear height. It belongs to the Group B – medium-strong durum wheat cultivars. The average yield for the testing period is 5470 kg/ha (5160 kg/ha for standard cultivars Progress and Saturn 1). Over the years of testing this cultivar demonstrated lower winter-tolerance. **Yavor** was developed from a cross between a mutant line and the cultivar Zagorka. The F<sub>2</sub> hybrid seeds were irradiated with 100 Gy gamma rays. **Impuls** was developed by crossing another mutant line with the cultivar Zagorka and its F<sub>2</sub> seeds were irradiated with 100 Gy gamma rays. Its stem height is 90-100 cm with good lodging hardness. The cultivars Yavor and Impuls are in the process of forced multiplication and appear to have high grain productivity and quality. Yavor is resistant to *Puccinia graminis* and *Puccinie recondite tritice*.

It is worthwhile mentioning that old cultivars grown till 1980, had stalks longer than 1.5 m while mutant cultivars, grown presently, are around 85-90 cm. Mutant cultivars currently cover almost the whole durum wheat area in the country. A big part of the durum wheat is exported. High quality mutant characteristics are preferred for the production of pasta. The use of mutant cultivars has significantly increased the productivity of durum wheat. Nowadays these durum wheat cultivars attain a grain yield almost equal to common wheat.

### Maize

Although maize was only introduced a few centuries ago to Bulgaria, it has attained a great social-economic importance (Todorovska *et al.*, 2008). The main breeding centre in Bulgaria is the Maize Research Institute (MRI), in Kneja. In 1973, a research programme on induced mutagenesis for accelerating breeding by inducing additional genetic variation in elite lines and hybrids was initiated at the MRI, in Kneja (Christov *et al.*, 1995), with the first maize mutant hybrid cultivar being developed in 1980 (Hristov *et al.*, 1987a; 1987b). The results of studies on the choice of mutagen and optimum doses for inducing mutants with high productivity and combining ability were reported in a number of publications (Christova *et al.*, 1993; 1995; Genov *et al.*, 1987; Genov, 1990; Hristov, 1985; Hristov *et al.*, 1989; Hristova *et al.*, 1991). As a major output of these studies, a principal scheme known as **Recurrent Reciprocal Mutation Breeding (RRMB)** was developed (Hristova *et al.*, 1984a; 1984b; 1987a; 1987b). However, the details of this technology are not fully reported; it is essentially an improved version of the traditional Reciprocal Recurrent Breeding method (Christov, 1977; Christova *et al.*, 1987; Christova, 1988; Hristova *et al.*, 1987b). The RRMB scheme was especially designed for fixing chemically induced mutant genes controlling quantitative traits and combining ability by selecting highly competitive hybrid combinations. An essential peculiarity of the breeding in Kneja is that low concentrations of chemical mutagens are used for treatment of seeds at a 24-h exposure with Dioxane as the medium solvent.

**Choice of mutagen and dose.** A number of chemical mutagens have been assessed for inducing mutants in maize (Tables 1 and 2). The highest number of mutations was generated by using dimethyl sulfate (DMS) and diethyl sulfate (DES) at 0.15-0.2% concentrations. According to studies conducted in the MRI, in Kneja, nitroso diethyl or dimethyl carbamides and diazo ketones are considered to be the most efficient mutagens. The mutagens frequently used are N-nitroso-N-ethyl-urea (NEU) and N-nitroso-N-methyl-urea (NMU) at 0.001% concentration. In a minor scale 1,4-bis-diazo-acetyl butane (DAB) is used.



Maize seeds usually have 4 initial germ cells, which grow into a plant. In mutagenesis these four initial cells should all be mutated. The results showed that after 22-24 hours, the seed absorbs water equal to its weight, indicating cell initiation. For this reason, low concentrations of DNA alkylating mutagens are applied with prolonged exposure, often in combination with other solvents. Dioxane is one of the intermediary solvents that is used, leading to improved efficiency of mutagenic treatment. Applied in concentration <1% the Dioxane does not affect the mutation effect. Otherwise, it probably acts like antidote, which reduces the harmful effect of the chemical mutagen and consequently the mutagen can be applied at higher doses. Dioxane achieves high quality emulsions and provides the best possible oil-like mixture of mutagen and water. It increases the penetration of chemical mutagens across cellular and intracellular membranes and enables the mutagen to reach the target initial cells and target components (DNA).

**Initial materials for mutagenic treatment.** Mutagenic treatment is often conducted for elite inbred lines for expanding its genetic basis. The best hybrid AxB with the highest yield is selected in a competitive examination. If the yield of the hybrid AxB significantly exceeds others, it indicates that classical recurrent selection has reached this peak potential and the chance for further improvement through cross and backcross programmes is small, while induced mutagenesis may have a chance. The mutagenesis for increasing the genetic variation of the inbred line is conducted at this stage and an effective way for selecting mutants without many test crosses is developed. Mutant lines are selected after testing and included in the list of parent lines for developing new hybrids. Mutation breeding is conducted in parallel for the inbred lines, the parents of hybrid AxB. For mutants of line A, the tester is the B and vice versa and hybrid AxB is always used as a control.

Table 2. Mutant lines, parents of released maize hybrids

No.	Hybrid cultivar *	Release year	Maturity group	Main characteristics	Combination **	Procedure of mutant inbred development ***
1	Kneja HP 633	1980	600	High protein	B-37 × <i>XM 532</i>	OH-43, seeds, DMS (0.2%) + methanol
2	Kneja HP 556	1982	500	High protein, drought tolerant	A-632 × <i>XM 532</i>	OH-43, seeds, DMS (0.2%) + methanol
3	Kneja 510	1983	500	High yield, mid-late	A-632 × <i>XM 521</i>	OH-43, seeds, NEU (0.0001%) + methanol
4	Kneja 641	1983	600	High yield in irrigation, stable, grown in acid soils	B-73C × <i>XM 568-1</i>	<i>XM 568</i> , seeds, NMU (0.001%) + dioxane (1%) (second cycle)
5	Kneja 712	1987	600	High yield, stable, drought tolerant	B-73 × <i>XM 552</i>	OH-40b, seeds, NMU (0.001%) + dioxane (1%)
6	Kneja 666	1987	600	Three-linear corn type, very high yield, silage type, tolerant to very high dip sowing	B-78 × <i>XM 199</i>	multicorn population, seeds, NMU (0.001%) + DAB (0.05%) + dioxane (1%)
7	Kneja 674	1989	600	Silage type, for silage and grain; very drought tolerant	(B-73 × B-84) × <i>XM 199</i>	multicorn population, seeds, NMU (0.001%) + DAB (0.05%) + dioxane (1%)
8	Kneja 570	1992	500	Mid-late, for silage and grain drought tolerant,	(B-579 × B-84) × <i>XM 552</i>	OH-40b, seeds, NMU (0.001%) + dioxane (1%)
9	Kneja 509	1993	500	Mid-late, for grain, very drought tolerant	<i>XM 87-136</i> × Mo17	B-37, seeds, DMS (0.2%) + DAB (0.05%) + dioxane (1%)
10	Kneja 682	1994	600	Late, high yield, for grain, drought tolerant	<i>XM 88-113</i> × Mo17	B-84, seeds, NMU (0.001%) + dioxane (1%)
11	Kneja 596	1995	500	Mid-late, high yield, for grain	<i>XM 8451</i> × H-108	B-73, seeds, NEU (0.0001%) + dioxane (1%)
12	Kneja 419	1997	400	Mid-early, for grain	<i>XM 85-105</i> × <i>XM 93-295</i>	P-3737, seeds, NMU (0.001%) + dioxane (1%)
13	Kneja 423	1997	400	Mid-early, for grain	<i>XM 92 470</i> × <i>XM-93 295</i>	P-3737, seeds, NMU (0.001%) + DAB (0.05%) + dioxane (1%)

No.	Hybrid cultivar *	Release year	Maturity group	Main characteristics	Combination **	Procedure of mutant inbred development ***
14	Kneja 590	1994	500	Mid-late, for grain and silage	<b>K 4640A</b> × H-108	B-73, germs, colchicine (0.05%)
15	Kneja 683	1994	600	Late, high-yield, for grain and silage	<b>K 4640B</b> × Mo17	B-73, germs, colchicine (0.05%)
16	Kneja 594	1995	500	Mid-late, for grain and silage	<b>K 4640A</b> × H-109	B-73, germs, colchicine (0.05%)
17	Kneja 689W	1995	600	White grain	527/44/77 × <b>PXM 4699</b>	Mo 17, seeds, AA 2d + NMU (0.001%)
18	Kneja 598	1997	500	Mid-late, for grain	<b>PM 4547</b> × Mo17	A-634, seeds, AA 2d
19	Kneja 512	1998	500	Mid-late, high yield, for grain and silage	<b>XM 4544</b> × Mo17	Sirena, seeds, DMS (0.15%)
20	Kneja 698W	1998	600	White grain	<b>PCM 4699</b> × <b>PM 4662</b>	Mo 17, seeds, AA 2d + NMU (0.001%); B-73, seeds, γ ray (80 Gy)
21	Kneja 634	1999	600	Late, for grain	<b>K 4640A</b> × <b>K 4651</b>	B-73, germs, 5d (2x2h per day) colchicine (0.05%) + vacuum Mo 17, germs, 8h colchicine (0.05%)
22	Kneja 515	2003	500	Mid-late, for grain	<b>XM 4521</b> × <b>PCM 4550</b>	B-73, seeds, AA 4d + NEU (0.001%)
23	Kneja 435	2005	400	Mid-early, high yield, for grain, resistant to virus and fungus diseases	<b>XM 4418</b> × <b>K 4652</b>	Mirna, seeds, NEU (0.001%) in alcohol; Mo17, germs, colchicine (0.05%)
24	Kneja 621	2007	600	Late, for grain and silage, resistant to main diseases	KC-4647 × <b>K 4640B</b>	B-73, germs, colchicine (0.05%)
25	Kneja 546	2009	500	Mid-late, high yield, for grain and silage	<b>XM 4527</b> × H-108	P-3737, seeds, NMU (0.001%)
26	Kneja 627	2009	600	Late, for grain	<b>PCM 4658</b> × Mo 17	(522xB-84), seeds, AA+DAB (0.1%)

\* Names with HP are high protein cultivars and with W are of white grains;

\*\* The mutant inbred lines are shown in bold; inbred lines starting with 'XM' are developed by chemical mutagenesis, with 'PM' by physical mutagenesis, and with 'PCM' by combined treatment of physical and chemical mutagen;

\*\*\* Treatments of maize seeds or germs with chemical mutagen were conducted at room temperature at the ratio of seed weight: dH<sub>2</sub>O = 1:5; pH of distilled water is 4.8-5. Exposition for treatment 24 h, solvent was changed after 12 h. Dioxane (methanol) was used as medium solvent in all treatments. AA 2 – Accelerating aging of seeds used as a method for obtaining mutations; Most of treatments are conducted in inbred lines; hybrid seeds are: P-3737, Sirena of the company Pioneer (USA) and the cross (UC 522 × B 84); Hybrid P 3737, known Panonia, is used as control.

A total number of 92 maize hybrids were developed by the MRI in Kneja in the period 1966-2009. Four thousand hybrids developed by mutagenic selection were competitively tested on a yearly basis in Kneja, and 26 hybrid cultivars were developed through experimental mutagenesis starting from 1980 (Table 2).

There are no statistical data of acreage for each hybrid, but, it is known that the mutant hybrid **Kneja 509** was the most widely grown cultivar on 40-50% of the maize area in Bulgaria till recently. It is now being replaced by **Kneja 683A** (Genov *et al.*, 2005a; 2005b). Since 1994, despite the entering of numerous companies from the

European Union (EU), the United States of America (USA) and Israel, the main maize hybrid in Bulgaria has been Kneja 509, a medium late-season cultivar. Kneja 509 is also known to have the best drought tolerance. Kneja 509 is grown in desert conditions in Morocco, and also gives a high yield by only 2 irrigations (it usually requires 4 irrigations). The high protein mutant hybrid **Kneja 556** has been grown mainly for silage. By 1990, it has contributed significantly to the maintenance of the milk-corn belt of the field of Sofia. At present **Kneja 435** is much preferred (Genov *et al.*, 2005b). The pedigree of some inbred lines, used in commercial hybrid maize pro-

duction, is shown on a diagram (Fig. 2). Since 1980's, high-protein hybrids **Kneja HP 633**, **Kneja HP 556** and **Kneja MHP 556** have been widely cultivated in Bulgaria for silage. The first is a late-season hybrid (maturity group FAO600), while the other two are medium late-season (FAO500). Their protein grain content is about 12% as compared to a maximum of 9-10% of common maize hybrids. **Kneja 510** and **Kneja 641** are not only

released in Bulgaria but also in Greece. **Kneja 666** is a mutant hybrid officially registered in 1987 as silage maize. It was developed by crossing mutant inbred line XM 199Rf3Rf4 induced by chemical mutagen (EMS+DAB). The main improved attributes of this hybrid are high grain yield (up to 15 t/ha), high protein (10%), with a biomass dry matter (up to 32 t/ha) and with 3.9% protein.

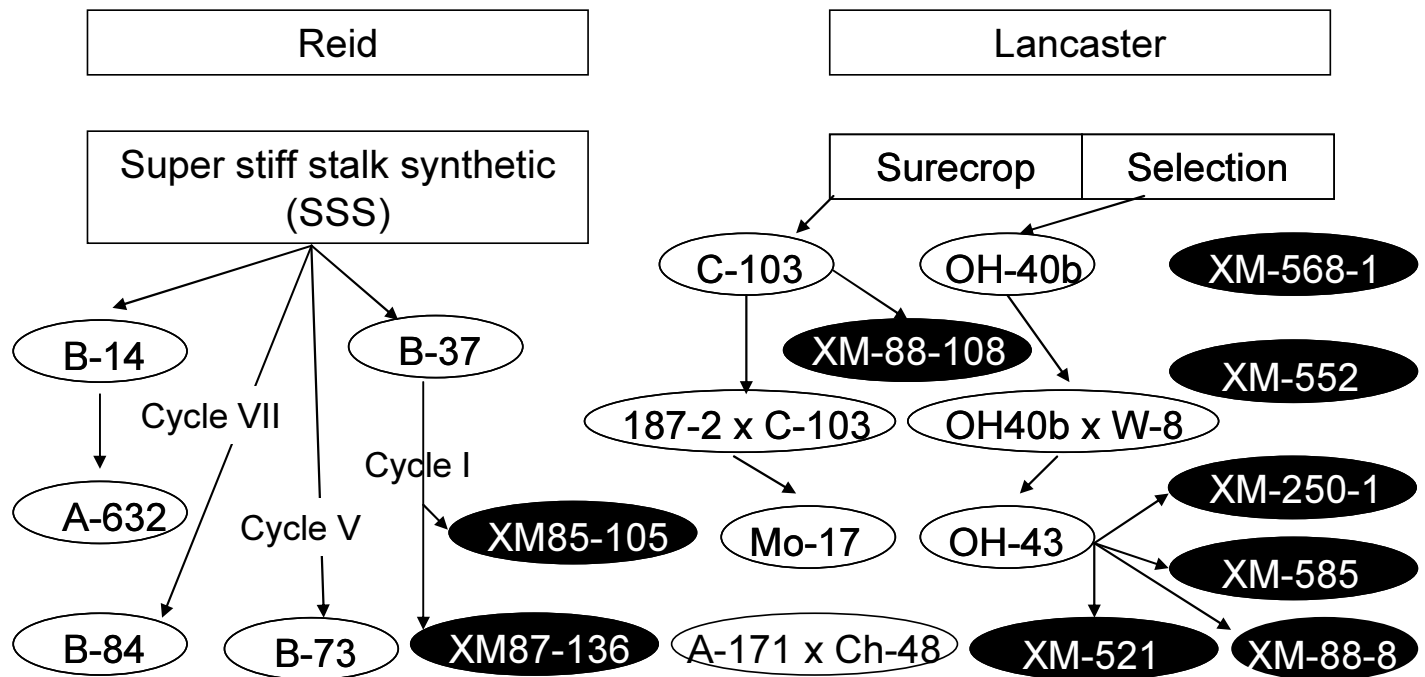


Figure 2. Evolutionary links of classical and mutant maize inbreds. Mutant inbred lines are in black background. (Photo adapted from Kostova *et al.*, 2006 - courtesy of Dr. N. Christov)

It should be noted that due to confidentiality reasons, lines and hybrids developed by mutagenesis, and some information on maize breeding processes are not published. It was not before the Symposium on Experimental Mutagenesis, which took place in Plovdiv in 1987, that the first world-wide mutant maize hybrids, released by an

official state authority, were registered, on the request of A. Micke of the IAEA. The main information about mutant hybrid cultivars, released in Bulgaria, is given in Table 2; some mutant inbred lines and hybrid cultivars are shown in Fig. 3. Some important maize hybrid cultivars are highlighted below.

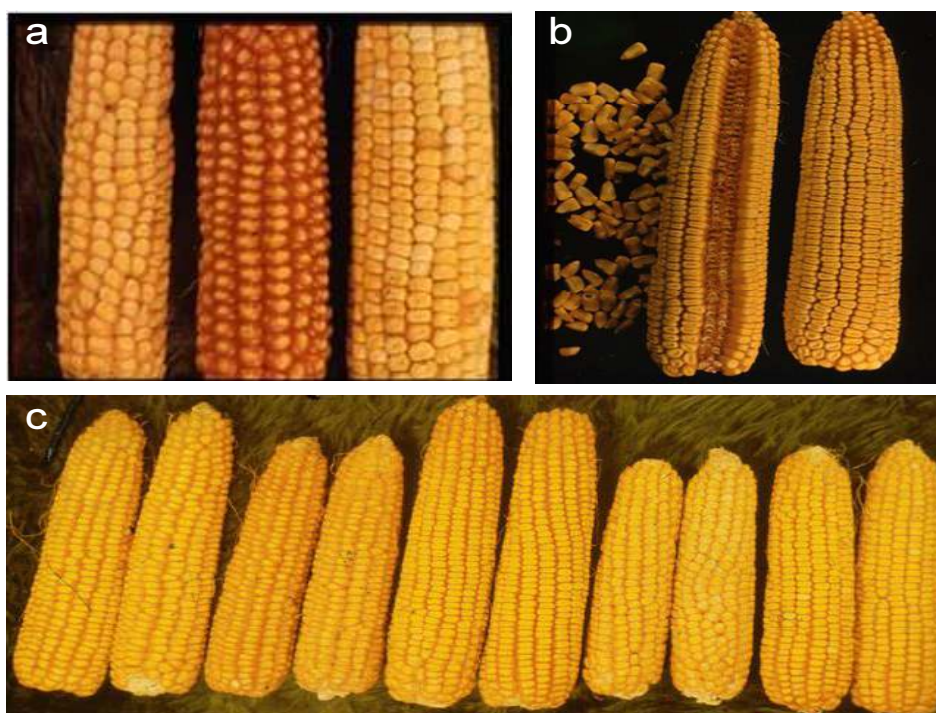


Figure 3. Mutant inbred lines and hybrid cultivars developed in Bulgaria. **Panel a** (from left to right): Inbred line OH-40b, its mutant line CM-552, and high-lysine mutant line (homozygous for opaque-2 and one of the parents of hybrid cultivars Kneja-712 and Kneja-570); **Panel b**: Mutant hybrid cultivar Kneja-641; **Panel c** (from left to right, two corn-cobs for each genotype): OH-43/CM-532//CM-532; CM-532; OH-43/CM-532; OH-43; OH-43/CM-532//OH-43. (Photo courtesy of Dr. K. Christov)

Kneja 689W, Kneja 698W and Kneja 634 are new maize hybrids, which have been recently released. **Kneja 689W** is a late maize hybrid for grain (Genov *et al.*, 2009). It is a representative of maize hybrids with special qualities. In the food processing industry, white grain maize is used for production of fine bread stuffs, and the population all together uses it for making boiled maize, hominy and other meals. In three-year tests the average yield of Kneja 689W was 7.9% higher than the control cultivar H 708. The moisture content in the harvesting time was 2% more than that of the standard, which is a characteristic of white maize. Kneja 689W possesses good hardness to lodging. **Kneja 698W** is also a grain hybrid; its white colour was a recessive mutation of the gene Y (Yellow), induced by chemical and physical mutagens. Kneja 698W showed resistance to most of widespread maize diseases in field conditions in Bulgaria. **Kneja 634** is a late maize hybrid for grain (Genov *et al.*, 2009). In 2005 the hybrid was registered and included in List A of the Bulgarian Official Catalogue of Varieties and in 2006 it was protected by a certificate of the Patent Agency. In the state cultivar testing in 4 different stations, Kneja 634 exceeded the standards H 708, Ivana and Kneja 530 by 128%, 116% and 130% grain yield.

Making use of the means and methods of experimental cytogenetics and genetics, a study was initiated at adopt-

ing intergeneric hybridization (Maize  $\times$  Teosinte), chemical and physical mutagenesis, accelerating aging of seeds and their combined use into maize breeding (Christov, 1977; Genov, 1988).

The mutant inbred lines, developed through experimental mutagenesis, were chosen for molecular characterization because in addition to the improved grain yield, proven by their predominance in the Bulgarian breeding programs, they showed drought tolerance, shifts in the flowering time as compared to the initial inbreds: two of B37 and four of OH-43 (Kostova *et al.*, 2006a; 2007). They contribute to generate diversity in the elite maize germplasm. Some of the variations may affect the major quantitative trait loci (QTLs). Simple Sequence Repeat (SSR) and single nucleotide polymorphisms (SNP) polymorphisms were observed in the major candidate genes involved in the flowering time. Polymorphic SSRs were identified between mutant inbreds and their parents (Christov *et al.*, 2004). SNPs and indels were detected in the region flanking the SH2 domain of *dwarf5* gene in some of the mutant inbreds as a result of single strand chain polymorphism (SSCP) and sequencing analyses (Kostova *et al.*, 2006b).



A working collection of 6985 inbred lines is available at the MRI, Kneja developed by the application of different mutagenic agents. Of these inbred lines, 654 are stabilized lines that are used in various directions of breeding and production. Some of them are parents of some released hybrids. Breeding of the other stabilized lines is directed to find suitable components for developing hybrids with high yield combined with other characters. Seven of the recently released hybrids were developed from mutant lines. A total of 6333 M<sub>4</sub> mutant lines are currently being utilized in breeding. Selected lines will be used to develop new hybrids, which will be tested in IASAS for registration in the Bulgarian Official Catalogue of Varieties.

### Sunflower

The application of biotechnologies in sunflower (*Helianthus annuus*) breeding was conducted in the DZI, General Toshevo (Ivanov *et al.*, 1995). Gamma rays (both <sup>60</sup>Co and <sup>137</sup>Cs), ultrasonic treatment and EMS have been used in sunflower mutagenesis in Bulgaria (Table 1; Christov, 1990; 1995; 2002; Christov *et al.*, 1996). In most cases, seeds are used for treatment and are sown in the field on the same day, together with untreated seeds as control (10% of the total number of treated seeds). In a few cases, shoots are treated with gamma rays.

During the vegetation period of M<sub>1</sub> plants, phenological observations were conducted and biometric measurements were taken. Parts of the plants were isolated by paper or cloth bags to enable self-pollination for the production of M<sub>2</sub> seeds. M<sub>2</sub> seeds from each head were sown as a separate progeny. Selection in M<sub>2</sub> was carried out twice – before flowering and following the harvesting of ripe heads. M<sub>3</sub> seeds were evaluated for characteristics such as the mass of 1000 seeds, seed oil content and kernel: shell proportion. Female fertility was determined by the amount of seeds obtained in free pollination, while self-fertility was determined by the amount of seeds by self-pollination in isolation. The selected M<sub>3</sub> seeds from each head were sown as separate progenies. The same procedure was applied in the next generations. The stability of the mutated traits was confirmed in M<sub>3</sub>, M<sub>4</sub> and advanced generations if needed. The mutant lines were registered in the collection of DZI in General Toshevo. The following parents have been used in sunflower mutagenesis: 24 inbred cultivars developed in Bulgaria and Russia; 4 hybrid cultivars developed in Bulgaria and Serbia; 17 breeding lines developed in Bulgaria and the USA and 6 mutant lines developed in DZI, General Toshevo. Several mutants have been isolated for the following morphological and biological characters: size, position, shape and colouration of leaves and of the seeds; position and shape of the inflorescence; seed weight; vegetation period; stalk height and appearance of branching plants with male sterile flowers of the inflores-

cence (Christov, 1990; 1995; 1996; 1999; 2002; Christov *et al.*, 1996; 2009); increased oil content (Christov, 1990; 2002; Christov *et al.*, 1996); improved seed oil composition (Ivanov *et al.*, 1985; Christov *et al.*, 1996); cytoplasmic male sterility (Christov, 1993; Christov, 1999); high combining ability, resistance to diseases and to parasite *Orobanche cumana*. Genetic analysis showed that a part of mutated morphological traits were controlled by recessive genes while the resistance to *Orobanche cumana* is controlled by dominant genes. The mutant line IN1, with altered petiole, leaf shape, shape of the inflorescence (Fig. 4), was developed from cultivar VINK8931 using 150 Gy <sup>60</sup>Co gamma rays (Christov, 1996); it also carries a recessive mutation that renders resistance to *Orobanche cumana*. The decorative sunflower mutant Decor 110 (Fig. 4) was also developed using 70 Gy <sup>60</sup>Co gamma rays (Christov, personal communication). Two of the mutant lines have been used in sunflower breeding programmes.



Figure 4. Sunflower mutant lines developed by gamma rays mutagenesis. *Top*: A morphological mutant IN1 showing altered shape of petiole, leaf and florescence. *Bottom*: A decorative mutant line Decor 110 (Photo courtesy of Dr. M. Christov)

Over 100 mutant lines were developed with important economic characters; some are especially promising for sunflower breeding. Mutant lines 222, 223, 226 and 596, originating from the cultivar **Start**, are high in both oleic

(42.8 to 46.5%) and stearic acid content (10.9 to 12.4%), their iodine numbers - a characteristic reflecting the level of non-saturated fatty acids, are in the range from 104.8 to 110.0 U, which is 15.0-20.2 U lower than **Start**, and 26.1-31.3 U lower than cultivar **Peredovik**. These lines can be used for developing new cultivars with even lower iodine numbers, which are desired for basic raw material in the manufacturing of margarine – hydrogenated sunflower oil. New sources of cytoplasmic male sterility (4 lines) were developed after gamma ray irradiation of seeds of cultivars **Peredovik** (2), **Stadion** and **Hemus** (1 each). Nineteen mutant restorer lines (R lines) were developed with good combining ability, having medium-sized seeds and high seed oil content, and all having branching stalks and being resistant to downy mildew.

*In vitro* techniques have also been used in sunflower mutagenesis and a number of mutant lines with useful characteristics for breeding have been developed (Encheva *et al.*, 1993; 2002; 2003a; 2003c; 2004a; 2004b). The first mutant line R 12,002 was developed by irradiation of immature embryos with 8 Gy  $^{137}\text{Cs}$  gamma rays. R 12,002 has a great mass of 1000 seeds and is used as the male parent of hybrid sunflower cultivar **Rada**, which was registered in 2006. The second mutant line R 12,003 was also developed by *in vitro* mutagenesis. Immature embryos were treated by ultrasounds at a dose of 25.5 w/cm<sup>2</sup> for 1 minute. R 12,003 possesses high seed oil content and has a large number of seeds per head and is used as the male parent of the hybrid cultivar **Yana**. Yana was initially known as hybrid 80 and was officially registered in 2009.

**Madan** is a sunflower hybrid released in 2008 (Christov, personal communication). Its female parent is a mutant line (6127) that was developed from the breeding line 7515R after 120 Gy  $^{60}\text{Co}$  gamma irradiation. Madan has large seeds with improved characters suitable for food (oil content ~29% and protein content >22%).

## Legumes and forage crops

### Lentil

In Bulgaria, studies on radiation-induced mutagenesis in lentil date back to 1980 in the DAI-General Toshevo and IG-Sofia. Gamma rays ( $^{60}\text{Co}$  and  $^{137}\text{Cs}$ ) and, to a lesser extent, neutrons and laser beams, as well as chemical mutagens such as EMS, MMS and  $\text{NaN}_3$  have been used for mutagenesis (Table 1). The lethal effect of MMS was stronger than that of EMS and  $\text{NaN}_3$  in lentil (Mihov, 1994). Mutant lines of valuable economic characters have enriched lentil germplasm and have been used in breeding for new cultivars.

The mutant lines have been assessed mainly for their usefulness in increasing production potential, tolerance to abiotic and biotic stress, and improving biochemical and cooking qualities (Stoyanova *et al.*, 1994). The chimeric

structure of  $M_1$  plants was also studied (Mihov *et al.*, 1990a; 1990b; 1995). Mutant lines resistant to diseases with economic importance in lentil caused by *C. truncation*, *A. lentis* were developed. A number of high yielding lentil mutant lines were produced and also had a good resistance to some economically important diseases: *Fusarium*, *Anthracnose*, *Stemphylium* and viruses (Mihov *et al.*, 1995). Mutant lines with non-lodging stem and low content of antinutrition components (tannins) were produced (Mihov *et al.*, 1999).

Up to now, four lentil cultivars have been developed through experimental mutagenesis in the DAI and registered by the IASAS. Having higher production potential and a number of new valuable economic and biological qualities, producers have shown considerable interest in these cultivars. **Mutant 17 MM**, released in 1999, differs from its parent cultivar by its larger leaflets, pods and seeds, seed pod colour, better resistance to diseases Anthracnose, Stemohylium, and viruses. It is high yielding, with larger seeds and very good culinary quality and organoleptic properties. It also demonstrates better drought tolerance (Mihov *et al.*, 1999; 2001). **Zornitsa**, released in 2000, was developed by treating seeds of Tadjikskaya 95 with 0.1% of EMS. It is medium-ripe, has larger leaflets, pods and seeds. It has yellow-green seed pods and red to yellow cotyledons, which are different from the grey colour of Tadjikskaya 95. The cultivar is highly productive and highly resistant to Anthracnose (*Colletotrichum truncatum*), Ascochyta blight (*A. lentis*) and viruses. It is characterized by high protein content (28.7%) and very good culinary and organoleptic properties (Mihov *et al.*, 2001). **Djudje** (Dwarf), released in 2000, was developed by treating dried seeds of Tadjikskaya 95 with 30 Gy gamma rays in 1988. It significantly differs from Tadjikskaya 95 by its low, bushy and non-lodging stem, non-shattering pods and its better resistance to *Fusarium* and *Botrytis*. It has better productivity and higher protein content (27.9%). It is a suitable parental cultivar in cross breeding for improving plant architecture for mechanized harvesting ('bushy' type) (Mihov *et al.*, 2001). **Elitsa**, released in 2001, was developed by irradiation of Tadjikskaya 95 seeds with 40 Gy  $^{60}\text{Co}$  gamma rays. It is distinguished as being highly productive (2454 kg/ha, which is 34.4% higher than average standards) and resistant to main lentil diseases (Mihov *et al.*, 2005).

### Beans

A high frequency of spontaneous mutations that exceeds other plant species was described in common bean, *Phaseolus vulgaris* L. (Rukmanski 2005). It was observed that most morphological characters could be mutated in common bean after applying gamma rays or chemical mutagens. In 1979, a breeding programme with the application of induced mutagenesis in French bean

started at the Vegetable Crops Research Institute (Maritsa VCRI) in Plovdiv.

**Zarya** is a cultivar suitable for canning industry and is susceptible to common bacterial blight (CBB) (*Xanthomonas axanopodis* pv. *phaseoli*). Consequently, for the first time a mutation breeding project in common bean was initiated using gamma irradiation of dry seeds. The lethal dose (LD<sub>100</sub>) of this cultivar was found to be 160 Gy and the semi-lethal one (LD<sub>50</sub>) - 120 Gy, therefore, doses of 80 and 100 Gy were used in the mutagenesis breeding programme (Zagorcheva *et al.*, 1983a). Several mutants were selected and showed resistance to CBB (Fig. 5; Table 1).



Figure 5. Common bean cultivar *Zarya* (top, susceptible) and its mutant line A-8-40 (bottom, resistant) showing infection of common bacterial blight after artificial inoculation (Photo courtesy of Dr. I. Poryazov)

The application of induced mutagenesis in common bean in the Agricultural University, Plovdiv, resulted the development of two cultivars with high importance to the rural economics – both, **Plovdiv 11M** and **Plovdiv 15**, were developed through EMS treatment ( $1.25 \cdot 10^{-2}$  M) of cultivar Dobrudzhanski 2 (Delchev *et al.*, 1983; Petkova *et al.*, 1983a; 1983b; Svetleva, 1983; Svetleva *et al.*, 1983).

Gamma rays (80 Gy <sup>60</sup>Co) were also applied in garden bean for inducing mutants of the following characters: (1) flowers with three ovaries (Zagorcheva *et al.*, 1987); (2) long narrow leaves and sickle-shaped pods (Zagorcheva *et al.*, 1988); (3) six days earlier maturing and simultaneously ripening pods; (4) compact growth habit with archi-

ture suitable for mechanical harvesting and dark-green corrugated leaves; (5) increased pod weight and changed pod shape; (6) unifoliolate; (7) chlorophyll mutations – albina, xanta, chlorine, chloroviridis, chlorotica (Zagorcheva *et al.*, 1983b); (8) higher resistance to halo blight (HB) (*Pseudomonas savastanoi* var. *phaseolicola* (Gardan), race 6 and productivity (Rodrigues *et al.*, 1988).

Meiosis of pollen mother cells (PMCs) of CBB-resistant mutants was studied. Distributed bivalent pairing was found, as well as formation of univalent and multivalent, fragmentations of chromosomes and early division of chromosomes into chromatides. As a result, genetically irregular gametes were formed (Nikolova *et al.*, 1991). These meiotic disturbances suggested that the gamma rays induced chromosome aberrations – translocation between two non-homologous chromosomes and inversions. One accessory chromosome was present in the narrow leaves bean mutation (Nikolova *et al.*, 1986).

CBB resistant mutants were advanced up to M<sub>5</sub> and six of them were crossed with elite bean genotypes aiming at transferring the resistant gene into local germplasm and combining it with other economically valuable characters (Nikolova *et al.*, 1991; Sofkova *et al.*, 2004a). In parallel homogeneous recombinant inbred lines were tested for resistance to some economically important diseases in Bulgaria, including CBB, HB, bean common mosaic virus (BCMV), cucumber mosaic virus (CMV), clover yellow vein virus (CIYVV), Rust (*Uromyces appendiculatus*), Anthracnose (*C. lindemuthianum*) and Sclerotinia (*S. sclerotiorum*) using different scoring systems (Sofkova *et al.*, 2004b; 2005).

### Pea

Sine 1966, mutagenesis studies on pea have been carried out in the IG, Sofia and later in the DAI, General Toshevo and the Experimental Station of Soybean (ESS) in Pleven. Different physical and chemical mutagens were tested alone or in combination with *in vitro* techniques (Table 1; Mehandjiev *et al.*, 1975; Todorova, 2009; Vassileva, 1978; 1980). Additive mutagenic effects of combined treatment of 40 Gy gamma rays and 0.2% EMS were observed.

A great variability of germplasm was created with useful economical characters for direct and indirect utilization in breeding: strong and non-lodging stem, increased number of pods and seeds, earliness and drought tolerance, mutants with fertile branching as well as increased protein content (Mehandjiev *et al.*, 1998; 2001a; 2001b; Vassileva, 1977). The use of ‘alfa’ type mutants has resulted in significant improvement of tolerance to lodging. With regard to increasing the adaptability of peas, 10 early-maturing and two ultra-early-maturing lines were produced – among which 190 and 198 maturing 15 days earlier. During the two typically dry years, the entire genetic



resource was evaluated and lines with good drought tolerance were selected. A high productive mutant spring forage pea was selected in M<sub>3</sub> having smaller seeds than the initial cultivar Kristal.

**Sredetz**, an officially released mutant cultivar, and the mutant line **Celeste**, have been developed through the combined treatment of gamma-irradiation and EMS. **Champion**, a mutant line, was developed by using 10 Gy gamma rays. The above mentioned, garden pea cultivar and lines, possess increased productivity and improved quality indices. In yield trials (1998-2000), the yield of Sredetz exceeded the control (Pleven-4) by 139.3%, attaining 5760 kg/ha similar to Champion and Celeste (5800 kg/ha). On the basis of the phenolic content in grain and damages caused by *Bruchus pisi*, hybrids tolerant to this pest such as (1) **Diktron** × **Finale**, (2) **Diktron** × **Finale** and (3) **Tirkis** × **A6**, were released, in which the mother is a mutant line, obtained by 80 Gy gamma rays+0.05% EMS. Cultivar Sredetz, line Celeste and hybrid **Diktron** × **Finale** showed good resistance to *Ascochyta pisi*. Cultivar Ballet was used for gamma rays irradiation and lines 33, 66 and 68 were developed, which demonstrated tolerance to *Erysiphe poligoni*. A number of mutant lines with resistance to SCCV were also registered, developed by gamma irradiation of pea cultivars Kristal and Drujba (Mehandjiev *et al.*, 2002a; 2002b).

### Chickpea

During the last twenty years, breeding of cool grain legume crops has been carried out at the DAI in General Toshevo (Mihov *et al.*, 2002). Two chickpea cultivars (Obraztsov chiflik and Steпноj) with contrasting features were treated either with <sup>60</sup>Co gamma rays or EMS or both (Table 1; Atanasova *et al.*, 2004a; 2004b). The results showed that there is a significant genotypic effect on the frequency of induced mutations.

As a result of crossing with a mutant line, one cultivar of the crop **bitter vetch (Borina)** and **pea vine (Strandja)** were developed and registered.

In 2001, new genotypes of plant species chickpea and vetch were presented for testing and registration. Seed production of the main cultivars was organized to meet the demands of the producers in the country. Mutant lines with different morphological characters of great interest to breeding were developed, which enriched the germplasm of chickpea. Some of them are directly used in breeding new cultivars and others are used as parents in cross-breeding programmes.

### Soybean

From 1980 to 2009, experimental mutagenesis was applied as a breeding method for enhancing genetic diversity in soybean (*Glycine max* L. Merrill), which is considered as a forage crop in Bulgaria. The Institute of Ge-

netics in Sofia (IG) and the Experimental Station of Soybean (ESS) in Pleven are the main institutes working on soybean experimental mutagenesis in Bulgaria. A large number of experiments have been performed on soybean mutagenesis using physical mutagens, such as <sup>60</sup>Co or <sup>137</sup>Cs gamma rays (Alexieva *et al.*, 1990; 1991; Alexieva, 1992; Georgiev *et al.*, 2000; Goranova *et al.*, 1987; Todorova, 2002; Todorova *et al.*, 2001) and fast neutrons (Goranova *et al.*, 1986), as well as chemical mutagens – EMS, MMS, NMU and sodium azide (Georgiev *et al.*, 2000; Todorova, 2002; 2005), or in combination (Aleksieva *et al.*, 2000; Todorova, 2002; 2005) (Table 1).

Three mutant lines (B22, B115, B206), resistant to bacterial blight, have been developed from cultivars Ravnika and Bachka through gamma irradiation or treatment of chemical mutagens EMS and NMU, or their combination (Georgiev *et al.*, 2000). Mutant lines, resistant to stem cancer and charcoal rot were identified from progenies of cultivars Hadson and Daniela and irradiated with gamma rays (Todorova *et al.*, 2001; Todorova, 2002). Mutant lines with the following characteristics were also developed: high productivity potential under irrigated and non-irrigated conditions, improved architecture of shrub, early-ripening and increased content of crude grain protein. Furthermore, mutant lines with the following traits were registered: increased protein content, dwarf type, vertically arranged leaves, altered number of the compound leaflets (from 3 to 4-5), resistance to diseases - charcoal rot, stem cancer (Aleksieva *et al.*, 1991; 1995; 1999; 2000; 2003).

Seven mutant soybean cultivars have been developed. The old ones including **Bisser**, **Zarya**, **Boryana** and **Zvezda**, developed in IG, Sofia, are no more registered in the Bulgarian Official Catalogue of Varieties. The following three mutant cultivars were newly developed in the ESS, Pleven. **Rosa** was tested in the system of IASAS in 2006-2008. It was developed by irradiation of seeds of the cultivar Hodson with 80 Gy gamma rays. It attained in cultivar competition trials, high yield under both - irrigated and nonirrigated conditions - and showed resistance to diseases of bacteriosis and mildew (Todorova, 2010). **Mira 96** was developed through gamma irradiation (50 Gy) and registered in 2001 (Aleksieva *et al.*, 2003). **Srebrina** was developed through hybridization of the mutant line LN2 (from Asgrow 2440 by 25 Gy <sup>60</sup>Co gamma rays) with the mutant cultivar Zarya. Srebrina was registered in 2004 (Aleksieva *et al.*, 2005).

### Sorghum sudanense

The Experimental Station of Soybean, in Pleven, is the main institute working on the grass *Sorghum sudanense* (Piper), a forage crop, related to the application of experimental mutagenesis (Golubinova *et al.*, 2007). Chemical mutagens DMS, DES were applied in concen-



trations of 0.1%; 0.15% and 0.3%, and NMU, NEU - in concentrations of 0.0005%; 0.001% and 0.01% (Table 1). Dose response and mutation frequency of two accessions, Voronezhskaya 9 and Vercors, were investigated from 2005 to 2006. The mutation frequency varied from 0.46 to 3.95%, Voronezhskaya 9 having a higher mutation frequency compared to Vercors. Mutations of reproductive traits and some other biological traits that are of interest to breeding were developed.

## Vegetable crops

### Tomato

Extensive investigations on mutant genes controlling male sterility in tomato, aimed at developing a system facilitating hybrid seed production, have been carried out at the Institute of Genetics in Sofia (Georgiev, 1991; Atanassova, 1991; 2007; Atanassova *et al.*, 2002). Cultivars Balkan, Kom, Elina, and Odysseus were developed in the IG, Sofia and in the company Geosem Select, Sofia. They all possess functional male sterility gene *ps-2*. Balkan and Kom were released in the early 1990s and are still widely grown in Bulgaria. **Balkan** is a very early semi determinate hybrid with high total productivity and is recommended for both - protected cropping (plastic tunnels) and open field production. Its fruits are smooth and uniform, round in shape, with 100-120 g standard weight. Green shoulders disappear totally at ripening. It is resistant to *Tomato mosaic virus (Tm-2)*, *Verticillium dahliae (V)*, *Fusarium oxysporum* f. sp. *lycopersicy* race 0 (*F*). **Odysseus** is an indeterminate, medium early hybrid cultivar, recommended for open field production. It is high yielding with fruit of 100-120g standard weight.

Odysseus produces fruits of flattened globe shape, with intensive red skin and a nice internal colour. Green shoulders disappear totally at ripening. It possesses a good transport and storage quality and is resistant to *Tomato mosaic virus (Tm-2)*, *Verticillium dahliae (V)*, *Fusarium oxysporum* f. sp. *lycopersicy* race 0, *Fusarium radici lycopersici (FORL)*. **Elina** is a medium early, semi determinate hybrid for both - protected cropping (plastic tunnels) and open field production. The plant is vigorous with upright leaves and has an easy fruit setting ability under low temperature conditions. Its fruits are round and firm, about 130-150 g standard weight, with an excellent quality and extended shelf life. Green shoulders disappear totally at ripening. It is resistant to *Tomato mosaic virus (Tm-2)*, *Verticillium dahliae (V)*, *Fusarium oxysporum* f. sp. *lycopersicy* race 0 (*F*). **Reyana** is a medium early; indeterminate hybrid recommended for open field production and protected cropping (plastic tunnels). Its fruits are firm, smooth and tasty, having 170-190 g standard weight and an extended shelf life. It is resistant to *Tomato mosaic virus (Tm-2)*, *Verticillium dahliae (V)*, *Fusarium oxysporum* f. sp. *lycopersicy* race 0 (*F*). **Rosalina Rossa** is a medium early, indeterminate hybrid recommended for open field production and protected cropping (plastic tunnels). It produces firm, smooth and tasty pink fruits of 160-180 g standard weight, which are greatly appreciated by Bulgarian consumers (Fig. 6). It is resistant to *Tomato mosaic virus (Tm-2)*, *Verticillium dahliae (V)*, *Fusarium oxysporum* f. sp. *lycopersicy* race 0 (*F*). The seed parent *ps 2* of Rosalina Rossa hybrid is shown in Fig. 6 possessing *potato leaf (c)* that enables easy and rapid determination of hybrid seed purity.



Figure 6. Tomato hybrid cultivar Rosalina Rossa. Left: Its pink fruits and; right: its seed parent *ps 2* showing potato leaves. (Photos courtesy of Dr. B. Atanassova)

Several criteria must be met before male sterile plants can be used in hybrid seed production; however, it was found

that each type of male sterility exhibited both - advantages and disadvantages (Atanassova, 2007; Atanassova

*et al.*, 2007). It was demonstrated that the functional male sterility, controlled by the gene *ps 2* (positional sterile 2), was the most suitable one for practical application (Atanassova *et al.* 1997a; Atanassova, 2000). Different morphological markers such as potato leaf (*c*), anthocyaninless of Hoffmann (*ah*), anthocyanin without (*aw*) etc. were studied for their usefulness in hybrid seed production (Atanassova *et al.*, 1997b; 2001) and have been introduced into *ps 2* seed parents, that are used for a number of commercial hybrids. These marker traits permitted testing the purity of hybrid seeds at germination or seedling stage. During the last decade, about 80% of tomato hybrid cultivars included in the Bulgarian Official Catalogue of Varieties possess *ps 2* sterile seed parents (Atanassova, 2007; Atanassova *et al.*, 2007). Therefore, the use of the *ps 2* - sterile seed parents in tomato hybrid seed production is no more a theory, but a practice.

Comparative studies on the time needed for the emasculation of floral buds - as practiced when using fertile seed parent, and of flowers at anthesis - as practiced when using a *ps 2*-line as seed parent, indicated that the latter was easier and almost two times faster than the former. On the other hand, hybrid seed yield, achieved using *ps 2* lines, was also 1.5-2.0 times higher (depending on the seed parent genotype) than that of fertile lines (Atanassova, 1999; 2000).

### Pepper

A significant contribution to improvement of sweet pepper (*Capsicum annuum* L.) was made in the Institute of Genetics in Sofia. The mutagenic effect and optimum doses of <sup>60</sup>Co gamma rays (80-120 Gy), X rays (120 Gy) and EMS (0.5-0.7%) were systematically investigated (Table 1; Daskalov 1968; 1971; 1972; 1973a; 1973b; 1974; 1977; 1986; Daskalov *et al.*, 1987). As a result of mutation experiments, a great number of mutants useful for pepper breeding have been developed and described. The gene symbols as well as a short description of the mutants are given in the gene list (Daskalov, 1973a).

Nuclear and nuclear-cytoplasmic male sterile genotypes in pepper were developed by treatment of X rays and gamma rays; the nuclear male sterility mutations were denoted as *ms-3*, *ms-4*, *ms-6*, *ms-7* and *ms-8* (Daskalov, 1968; 1973b). The inheritance and stability of male sterility mutations and their possible use in breeding programmes and in hybrid seed production were investigated (Daskalov, 1971; 1976), including cytological studies of mutants with recessive genes *ms<sub>3</sub>* and *ms<sub>8</sub>* and with the CMS genotype *Sms-5ms-5* (Daskalov, 1991). No essential differences were detected between nuclear male sterile and CMS plants in the timing and mechanisms of degeneration and lethality of the meiocytes. Abnormal function of the tapetum in both types of male sterility and a sterilizing effect of S cytoplasm of the CMS plants was

found. Daskalov *et al.*, (1988) proposed the use of female sterile mutants as pollenizer in the hybrid seed production with a recessive conditional lethal gene. The genes *ms-3* and *ms-8* have been used for development of a new technology of hybrid seed production and for development of 9 hybrid cultivars.

Todorova and Daskalov (1979) generated mutant plants resistant to powdery mildew using gamma rays, fast neutrons and EMS. After subsequent selection, 8 resistant lines were developed. A mutant with sulfur-white immature colour (gene mutation of the allele series *sw*) was reported by Daskalov (1986). Daskalov (1974) developed a mutant with orange mature fruits by treating dry seeds with X rays. The fruits are characterized by increased levels of  $\beta$ -carotene (provitamin A) (Tomlekova *et al.*, 2008) (Fig. 7). A number of new breeding lines are currently being evaluated and a biochemical selection is being performed. The mutation has also been introduced into other breeding programmes in the country, such as in the Maritsa Vegetable Crops Research Institute in Plovdiv (Todorova *et al.*, 2009), and in Moldova (Tomlekova *et al.*, 2009b). The efforts to combine the high- $\beta$ -carotene mutation with other mutations (fruit morphology, male-sterility mutations) are on-going in the Maritsa Vegetable Crops Research Institute. A molecular marker of the high- $\beta$ -carotene mutation was assessed for exploiting different mutant lines by marker-assisted selection towards increased fruit nutritive quality (Tomlekova *et al.*, 2009a).



Figure 7. Wild type pepper (upper) and mutant line 35 with increased  $\beta$ -carotene content (bottom)

Daskalov (1973a; 1974) developed two dwarf mutant denoted as *dw* and *dw-2* by gamma irradiation. Tomlekova *et al.*, (2007) induced a compact type mutant with determinate growth pattern by 0.7% EMS. Mutants are suitable for mechanized cultivation and for one-time harvest. Another possible use of such mutants is to serve as a tool in genetic and mutation research (Daskalov, 1981). After irradiating dry seeds with X rays, a mutant with short conic fruits was developed by Daskalov (1972). A number of mutants with altered fruit morphology of the cultivar **Hebar** were induced (Tomlekova *et al.*, 2007). Easily recognizable markers are very useful in hybrid seed production, mutation breeding and genetics studies. As such, some of these mutant phenotypes have been developed, e.g. three anthocyaninless (*al*) mutants, lack of blue stain on the hypocotyl, nodes, fruit and anthers, mutants with marbled leaves, yellow cotyledons, light green leaves, etc. (Daskalov, 1972; 1974; 1975). In addition, mutant lines with increased fruit dry matter content and early ripening were developed by X rays mutagenesis (Daskalov, unpublished data).

The following mutant cultivars have already been released in pepper. **Albena** is an early ripening cultivar, has high yielding potential with attractive fruit and excellent flavour (Daskalov, 1975). **Krichimski ran** is an early and high yielding hybrid cultivar (Daskalov and Milkova, 1976, cited by Daskalov, 1986). **Ljulin** is an early and high yielding hybrid (Milkova and Daskalov, 1983, cited by Daskalov, 1986). **Pirin** is a cultivar resistant to powdery mildew cultivar (Todorova and Daskalov, unpublished). **Oranzeva kapiya** is a cultivar with high content of  $\beta$ -carotene (Provitamin A) (Daskalov, 1991).

## Industrial crops

### Cotton

Cotton mutation breeding has been performed in the Cotton and Durum Wheat Research Institute, Chirpan, with the aim to improve fibre quality, increase yield potential and attain earliness. Mutant genotypes of cotton were developed by application of gamma rays (Table 1). Two new mutant cotton cultivars were registered in the system of IASAS, Approbation and Seed Control). **Trakia** is a mutant cultivar that was registered in 2007 (Fig. 8) (Stoilova *et al.*, 2009; Valkova, 2010). The mutant line was induced by irradiating hybrid seeds of Ogosta  $\times$  76223 (*G. hirsutum*) with 100 Gy gamma rays in 1993. It achieved an average yield of 2530 kg/ha cotton-gin, exceeding standard cultivars Chirpan 539 and Avangard 264 by about 8.5%. It also has better length uniformity and ripeness degree than the standards. The cultivar is featured by a shorter vegetative period (119 days), which gives a high yield ( $\sim$ 1540 kg/ha) for September harvests.

**Helius** is another mutant cultivar that was registered in 2007 (Fig. 8) (Stoilova *et al.*, 2009; Valkova, 2010). The mutant was induced by irradiating seeds of the Uzbek cultivar C-6530 with 150 Gy gamma rays in 1994. During yield trials, its average yield reached 2810 kg/ha cotton-gin, exceeding control cultivars by 8.4%. The average production in September was 1500.3 kg/ha, compared to 1300 kg/ha for Chirpan 539 and 1300.2 kg/ha for Avangard 264. Its high pre-winter harvest in September is mainly due to its short vegetation period ( $\sim$ 130 days). However, Helius is susceptible to verticillium wilt. The technological traits of these two cultivars are shown in Table 3.



Figure 8. Cotton mutant cultivars released in Bulgaria (Photo courtesy of Dr. N. Valkova)



Table 3. Technological traits of cotton cultivars Trakia and Helius

Cultivar	Strength (g)	Metric number (m)	Modal length (mm)	Staple length (mm)
1999 – 2002				
Trakia	4.15	5065	27.59	29.12
Chirpan-539 (control)	4.05	5382	27.92	30.61
2001 – 2004				
Helius	4.32	5761	27.15	29.70
Chirpan-539 (control)	3.91	6025	27.35	30.17

A number of recently developed mutant lines were evaluated for stability of five most important economic traits under diverse environments (years) using Shukla and Kang's stability parameters (Valkova *et al.*, 2006; Dechev *et al.*, 2007). The following mutant lines were proven to have high stability: ML-364, ML-362, ML-288, ML-240, ML-220 and ML-191. Over the past years these mutant lines have been subjected to state testing for biological and economic properties at three different stations in the country and a few of them may become new cotton cultivars.

### Tobacco

Induced mutagenesis is applied in tobacco (*Nicotiana tabacum* L.) breeding in the Institute of Tobacco and Tobacco Products, Plovdiv, with the use of diethyl sulfate and fast neutrons. The effective doses of irradiation for fast neutrons and concentrations and duration for diethyl sulfate treatment were established. A number of useful mutations were induced by irradiation with 90 Gy and 120 Gy fast neutrons and 0.1 M diethyl sulphate solution (Nikolov *et al.*, 1979).

So far, two new tobacco cultivars, **Virdzinia Mutant 890** and **Virdzinia Mutant 891**, with shorter vegetation period, resistance to diseases (*Peronospora tabacina*, *Erysiphe cicoracearum*, *Agrotis ipsilon*) and excellent technological indices have been developed in Bulgaria (Stoyanov, 1980).

### Conclusions

The success of a breeding programme depends heavily on the availability of germplasm with desired traits. The usefulness of induced mutagenesis for generating novel germplasm and breeding new cultivars of crops of importance to Bulgaria is demonstrated by the great achievements deliberated in this paper. The new cultivars developed by experimental mutagenesis have been attaining a special place in Bulgarian agriculture because of their high productivity, wide ecological adaptability and suitability for mechanized growing and harvesting, as well as

good resistance to diseases and pests and high quality/nutritional parameters.

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### References

- Alexieva A. (1991) Dwarf soy-bean mutative strain with vertically arranged leaves and increased protein content. *Comptes Rendus de l'Académie Bulgare des Sciences*, 44(9):93-95.
- Aleksieva A. (1992) Utilization of gamma-radiation to create soybean genetic diversity and bacterial blight resistant forms. Ph.D. Dissertation.
- Aleksieva, A. (1995) Economical characterization of soya mutant lines created by irradiation mutagenesis. *Plant Science*, 32(1-2):140-142 (in Bulgarian).
- Alexieva A. and Nikolov Ch. (1990) Effect of gamma-radiation on some radiobiological and genetic plant reactions in four soybean cultivars. *Genetics and Breeding*, 23(5):416-423.
- Alexieva A. and Nikolov Ch. (1991) Effect of gamma rays on the radiosensitivity and mutability of soybean cultivars. *Genetics and Breeding*, 24(4):249-255.
- Aleksieva A., Georgiev I. and Mehandzhiev A. (1999) Creation of mutant soybean forms resistant to stem cancer and charcoal rot diseases by using the experimental mutagenesis method. *Plant Science*, 36:369-372 (in Bulgarian).



- Aleksieva A. and Todorova R. (2000) Study on the biological effect of gamma-rays and EMS applied separately or in cross on four soybean cultivars. *Plant Science*, 37:571-575 (in Bulgarian).
- Aleksieva A. (2000) Utilization of experimental mutagenesis for developing high productive soybean lines. *Plant Science*, 37:576-579 (in Bulgarian).
- Aleksieva A., Ivanova K. and Dimitrov St. (2003) A new mutant cultivar – Mira-96. *Plant Science*, 40:180-183 (in Bulgarian).
- Atanasova D. and Mihov M. (2004a) Investigation on frequency and spectrum of mutations induced by different mutagenic treatments on chickpea (*Cicer arietinum* L.), Res. Commun. of U.S.B., branch Dobrich, 6(1):67-71.
- Atanasova D. and Mihov M. (2004b) Using experimental mutagenesis on chickpea (*Cicer arietinum* L.) J. of Field Crops Studies, I(2):233-238.
- Aleksieva A. and Sabev V. (2005) A new high-yielding Bulgarian soybean cultivar. *Plant Science*, 42:476-480, (in Bulgarian).
- Atanassova B. (1991) Linkage studies of the “positional sterility-2” mutant in tomato. *J. Genet. & Breed.*, 45:293-296.
- Atanassova B., Shtereva L. and Molle E. (1997a) Effect of three anthocyaninless genes on germination in tomato (*Lycopersicon esculentum* Mill.) I. Seed germination under optimal conditions. *Euphytica*, 95:89-98.
- Atanassova B., Shtereva L. and Molle E. (1997b) Effect of three anthocyaninless genes on germination in tomato (*Lycopersicon esculentum* Mill.) II. Seed germination under stress conditions. *Euphytica*, 97:31-38.
- Atanassova B. (1999) Functional male sterility (*ps2*) in tomato (*Lycopersicon esculentum* Mill.) and its application in breeding and hybrid seed production. *Euphytica*, 107(1):13-21.
- Atanassova B. (2000) Functional male sterility in tomato (*Lycopersicon esculentum* Mill.) and its application in hybrid seed production. *Acta Physiol. Plantarum*, 22(3):221-225.
- Atanassova B. (2007) Genic male sterility and its application in tomato (*Lycopersicon esculentum* Mill.). Hybrid Breeding and Hybrid Seed Production. *Acta Horticulturae*, 729:45-51.
- Atanassova B., Daskalov S., Shtereva L. and Balatcheva E. (2001) Anthocyaninless mutations improving tomato and pepper tolerance to adverse climatic conditions. *Euphytica*, 120:357-365.
- Atanassova B. and Georgiev Hr. (2002) Using genic male sterility in improving hybrid seed production in tomato (*Lycopersicon esculentum* Mill.). *Acta Horticulturae*, 579:45-48.
- Atanassova B. and Georgiev Hr. (2007) Expression of heterosis by hybridization. Genetic improvement of solanaceous, V. 2. Tomato, Sci. Publishers, USA, 113-151.
- Christov K. (1977) Cytogenetic studies of some new methods for the development of initial for maize material breeding. Ph.D. Dissertation.
- Christov K. and Christova P. (1995) Mutation breeding of maize - method and achievements. *Plant Sci. Bg. J.*, 1-2:40-43.
- Christov M. (1990) Use of gamma rays for production of new sunflower forms. 4<sup>th</sup> national conference “Ionization radiation and lasers in agriculture and food industry”, Varna, September 20-23 1990.
- Christov M. (1993) Gamma ray and ultrasound induced male sterility in sunflower. *Mutation Breeding Newsletter*, IAEA, Vienna, 15-16.
- Christov M. (1995) Development of new sunflower forms by treating seeds with gamma rays. The First Balkan Symposium on Breeding and Cultivation of Wheat, Sunflower and Legume Crops, June 26-29, Albena, Bulgaria.
- Christov M. (1996) A new sunflower mutant form. *Helia*, 19: 24, 39-46.
- Christov M. (1999) Production of new CMS sources in sunflower. *Helia*, 22: 31, 1-12.
- Christov M. (2002) Results from the use of distant hybridization and experimental mutagenesis for improvement of sunflower. Jubilee Session on the 50<sup>th</sup> anniversary of Dobrudzha Agricultural Institute, I, 315-335.
- Christov M. (2008) Changes in sunflower morphology as a result of applying of the experimental mutagenesis. Proceedings of the International Conference “Conventional and Molecular Breeding of Field and Vegetable Crops”, Novi Sad, Serbia, November 24-27, 2008, 184-186.
- Christov M. and Nikolova V. (1996) Increasing of the Sunflower Genetic Diversity by Mutagenesis. In: Proceedings of 14<sup>th</sup> International Sunflower Conference, Beijing/Shenyang, China, 19-30.

- Christov M., Piskov Al., Encheva J., Valkova D., Drumeva M., Nenova N., Nikolova V., Encheva J., Shindrova P., Petrov P. and Georgiev G. 2009. Developing sunflower hybrid cultivars with increased productive potential, resistant to economic important for the country diseases and parasite broomrape using classical and biotechnological methods. *In: Modern scientific problems of developing cultivars and hybrids of oil crops and technologies of its growing. Proceeding of International Conference, August 4-6, 2009, Zaporozhie, Ukraine, 80-82.*
- Christov N., Todorovska E., Fasoula D., Ioannides I., Atanassov A. and Hristov K. (2004) Molecular characterization of chemical mutagenesis induced diversity in elite maize germplasm. *Genetika, 36(1):47-60.*
- Christova P. (1988) Genetics and breeding studies in relation to the improvement of some methods for heterosis selection and achievements in the selection of mid-late and late hybrids of maize. Dr. Sc. Dissertation.
- Christova P. and Christov K. (1987) The mutant inbred lines and hybrids developed by recurrent mutation selections in maize. II. International symposium on Experimental Mutagenesis in plants, 26-30 October, 1987, Plovdiv.
- Christova P., Christov I., Christov N. and Christov K. (1993) Chemical mutagenesis affects some quantitative traits and the zein composition of maize inbred lines. *In: A. Bianchi, E. Lupotto and M. Mollo (eds.) Breeding and molecular biology. Accomplishments and future promise. Proceeding of the 16<sup>th</sup> Conference of the Eucarpia, Maize and Sorghum Section, Bergamo, Italy, 166-173.*
- Christova P. and Christov K. (1995) Achievements of maize breeding and genetics in maize. Maize breeding, production and utilization 50 years of maize research institute, Zemun pole, Beograd, Zemun pole, Yugoslavia.
- Daskalov S. (1968) A male sterile pepper (*C. annuum* L.) mutant. *TAG, 38:370.*
- Daskalov S. (1971) Two new male sterile pepper (*C. annuum* L.) mutants. *C. R. Acad. Sci. Agr. Bulg., 4:291.*
- Daskalov S. (1972) Investigations on induced mutations in pepper (*C. annuum* L.). Morphological characteristics of mutants in the cultivar P. kapiya 794. *Genet. and Breed., 5:221.*
- Daskalov S. (1973a) Gene list for the pepper. *Genet. and Breed., 6:401.*
- Daskalov S. (1973b) Three new male sterile mutants in pepper (*C. annuum* L.). *C.R. Acad. Agr. Bulg., 6:39.*
- Daskalov S. (1974) Investigations on induced mutations in sweet pepper (*C. annuum* L.). *In: EUCARPIA Genetics and Breeding of Capsicum, Budapest, 81.*
- Daskalov S. (1975) Albena: a new mutant cultivar in sweet pepper for early and mid-early field production. *Horticulture, 12:22.*
- Daskalov S. (1976) Seed setting of male sterile mutants in connection with heterosis breeding in pepper (*C. annuum* L.). *Genetica Agraria, 30:407.*
- Daskalov S. (1977) Induced mutations in sweet pepper (*C. annuum* L.). *In: C. R. 3-me Congress EUCARPIA Piment, 155.*
- Daskalov S. (1981) Value of dwarf or compact type mutants as a tool in breeding programmes, exemplified with *C. annuum* L. *In: Induced Mut. Tool in Plant Research, IAEA, 501.*
- Daskalov S. (1986) Mutation breeding in pepper. *Mutation Breeding Review, IAEA/FAO, 4, pp. 25.*
- Daskalov S. (1991) Experimental mutagenesis and mutation breeding in pepper. *Capsicum Newsletter, 10:13-20 (Invited paper).*
- Daskalov S. and Mihailov L. (1983) A new method of pepper hybrid seed production based on mutant genes. *In: Proc. 5th EUCARPIA Meeting of the Capsicum and Eggplant Working Group: 131.*
- Daskalov S. and Mihailov L. (1988) A new method for hybrid seed production based on CMS combined with a lethal gene and a female sterile pollenizer in *C. annuum* L., *TAG, 76:530.*
- Daskalov S. and Milkova L. (1976) Krichimski ran: a new heterosis cultivar in sweet pepper. *Horticulture, 10:22.*
- Daskalov S., Milkova L. and Mihailov L. (1987) Use of mutations in heterosis breeding (*Capsicum annuum*). *Experimental mutagenesis in plants, II International Symposium, October, 26-30, 1987, Plovdiv, 152-156.*
- Delchev I. and Svetleva D. (1983) Frequency and spectrum of morphological mutations induced from the action of gamma-radiation and alkylating agents in *Phaseolus vulgaris* L. (Savi). *Proceedings of the First national conference of genetics and selection, Razgrad, 19-21 November, 1983, 28.*

- Dechev D. and Valkova N. (2007) Phenotype stability evaluation of mutant cotton genotypes by some traits. International scientific conference, Stara Zagora, Vol. 1. Plant-growing, 353-356.
- Dyulgerova B. (2008) Biological and economic characters of multi-row mutant lines of winter barley. Proceedings of International Scientific Conference, Stara Zagora, 5-6 June, 2008.
- Dzhelepov K. (1982) Spontaneous and experimental mutagenesis and its employment in breeding for new wheat cultivars. Ph.D. Abstract of dissertation, p. 34.
- Encheva J., Ivanov P., Tsvetkova F. and Nikolova V. (1993) Development of a new initial breeding material in sunflower (*Helianthus annuus* L.) using direct organogenesis and somatic embryogenesis, *Euphytica*, 68:181-185.
- Encheva J., Tsvetkova F. and Ivanov P. (2002) Creating genetic variability in sunflower through the direct organogenesis method, independently and in cross with gamma irradiation, *Helia*, 25(37):85-92.
- Encheva J., Christov M., Nenov N., Ivanov P. and Encheva V. (2003a) Genetic variability of sunflower (*Helianthus annuus* L.) created by cross of polycross hybridization and ultrasonic or gamma irradiation. *BJAS*, 9(3):321-327.
- Encheva J., Christov M., Nenov N., Tsvetkova F., Ivanov P., Shindrova P. and Encheva V. (2003b) Developing genetic variability in sunflower (*Helianthus annuus* L.) by combined use of hybridization with gamma radiation or ultrasound. *Helia*, 26(38):99-108.
- Encheva J., Tsvetkova F. and Ivanov P. (2003c) Comparison between somaclonal variation and induced mutagenesis in tissue culture of sunflower line RHA-857 (*Helianthus annuus* L.), *BJAS*, 9(2):179-184.
- Encheva J., Christov M., Ivanov P. (2004a) Developing of B lines in sunflower (*Helianthus annuus* L.) by combined use of polycross method with ultrasound and embryo culture method, *BJAS*, 10(3):281-290.
- Encheva J., Nenov N., Christov M., Tsvetkova F., Shindrova P., Encheva V. and Ivanov P. (2004b) Two new fertility restorers of sunflower (*Helianthus annuus* L.) created by combined use of interlinear hybridization, gamma irradiation and embryo culture method. *Plant Sciences*, 41(2):112-118.
- Gecheff K., Chvarleva T., Georgiev S., Wilkes T. and Karp A. (1994) Cytological and molecular evidence of deletion of ribosomal RNA genes in chromosome 6 of barley (*Hordeum vulgare* L.). *Genome*, 37:419-425.
- Genov M. (1988) Genetic studies on diploid and tetraploid maize in conjunction with the heterosis and mutation variation. Dissertation, Kneja 1988.
- Genov M. (1990) Opportunities to obtain tetraploid lines in maize by mutagenic effects. *Journal Genetics and Breeding*, 23(6):430-439.
- Genov, M. and Genova I. (1987) Results of Experimental Mutagenesis Applied to Maize Inbreds. Proceeding of the Second International Symposium on Experimental Mutagenesis in Plants, Plovdiv, 184-188.
- Genov M. and Genova I. (2005a) The hybrid Kneja 683A – a new achievement of contemporaneous Bulgarian maize breeding. *Plant Science*, 42(4):303-307.
- Genov M. and Genova I. (2005b) A new maize hybrid. *Plant Science*, 42(3):209-211.
- Genov M., Genova I. and Petrovska N. (2005b) Kneja 435 - a new maize hybrid from the mid early group. *Plant Science*, 46(3): 230-232.
- Genov M., Genova I. and Vulchinkov St. (2009) The hybrid Kneja 689W – late maize with white grain. *Plant Science*, 46(2):126-129.
- Georgiev S. (1977) Investigation of the effect of some chemical mutagens in *Triticum* species with different ploidy level. Dissertation, Sofia.
- Georgiev Hr. (1991) Heterosis in tomato breeding *In*: G. Kallou [ed.] Genetic improvement of tomato. Springer-Verlag, Berlin, 83-98.
- Georgiev I., Mehandjiev A., Goranova K., Aleksieva A. and Todorova R. (2000) Creation of genetic diversity for breeding soybean resistant to diseases. *Plant Science*, 37:587-591.
- Georgieva M. and Stoilov L. (2008) Assessment of DNA double-strand breaks produced by bleomycin in barley genome by the comet assay. *Env. Mol. Mutagen.*, 49:381-387.
- Golubinova I. and Alexiev I. (2007) Study of susceptibility and mutability of *Sorghum sudanense* (Piper.) Stapf. under the effect of some chemical mutagens. International Scientific Conference “Plant genetics stock – The basis of agriculture of today”, 13–14 June, Plovdiv.
- Goranova K. and Aleksieva A. (1986) Application of Experimental Mutagenesis in Soybean. *Plant Science*, 23(12):3-8, (in Bulgarian).
- Goranova K. and Alexieva A. (1987) Application of experimental mutagenesis in soybean. *In*: Experi-



- mental Mutagenesis in Plants. II International Symposium, Plovdiv, October, 26-30, 1987, 189-191.
- Gramatikova M. and Dyulgerova B. (2005a) Double-row mutant lines of barley. Proceedings "Balkan Scientific Conference, Karnobat", 138-141.
- Gramatikova M., Dyulgerova B. and Dimova A. (2005b) Mutations in genetic and breeding program in Karnobat. Proceedings "Balkan Scientific Conference, Karnobat", 80-84.
- Gramatikova M., Rachovska G. and Burgazova Y. (2003) Increasing genetic diversity through experimental mutagenesis. AU-Plovdiv, The 100<sup>th</sup> anniversary of P. Popov, Scientific works, 27(2):119-124.
- Gramatikova M. and Todorov Y. (1996) Mutagenic specificity recording micro and macro mutants induced. Genetic Newsletter, 27:68-72.
- Hristov K. (1985) Intensification of breeding with maize. Agric. Sci. Bg., 4:38-42.
- Hristov K. and Hristova P. (1987a) Cytoplasmic male sterility in maize (Review), pp. 90.
- Hristov K. and Hristova P. (1987b) Achievements of mutation genetics in maize. The 14<sup>th</sup> meeting of the maize and sorghum section of EUCARPIA, September, 6-13, 1987, Nitra, Czechoslovakia.
- Hristov K. and Ivanova M. (1989) Some aspects of mutation and tissue technology in maize breeding. First National Scientific Conference: Application of *in vitro* techniques in agricultural plants, Plovdiv, Bulgaria.
- Hristova P. and Hristov K. (1984a) Mutation breeding in maize I. Genetic approach in the selection of objects for mutagen treatment and goals of mutation breeding. Genetics and Plant Breeding, 17(4):237-244.
- Hristova P. and Hristov K. (1984b) Mutation breeding in maize II. Chemical mutagenesis - application in maize breeding and principles. Genetics and Plant Breeding, 17(6):407-417.
- Hristova P. and Hristov K. (1987a) Achievements of mutation breeding in maize. The 14<sup>th</sup> meeting of the maize and sorghum section of EUCARPIA, 6-13 September, 1987, Nitra, Czechoslovakia.
- Hristova P. and Hristov K. (1987b) The mutant inbred lines and hybrids developed in recurrent mutation breeding in maize. II. International Symposium of Experimental Mutagenesis in Plants, October, 26-30, 1987, Plovdiv, Bulgaria.
- Hristova P., Hristov K., Genova I. and Genov M. (1991) Transition from classical to new genetic methods in hybrid maize breeding. Agric. Sci. Bg., 1-3:91-100.
- Ivanov P. and Ivanov I.G. (1985) A mutant sunflower line with high content of palmitic acid. Genetics and Breeding, 2, p. 123-127.
- Ivanov P., Belchev I., Milkova V., Encheva J., Todorova M., Nenova N., Ivanova I. and Nikolova V. (1995) Application of Biotechnology in wheat and sunflower breeding program. Breeding and cultivation of wheat, sunflower and legume crops in the Balkan countries, 26-29 June, Albena, Bulgaria, 1-6.
- Kostova A., Todorovska E., Christov N. and Atanasov A. (2006a) Assessment of genetic variability induced by chemical mutagenesis in elite maize germplasm via SSR markers. Journal of Crop Improvement, 16(1/2)(31/32):37-48.
- Kostova A., Todorovska E., Christov N., Vulchinkova P., Genov M. and Atanasov A. (2006b) Investigation of maize lines controlling drought tolerance by molecular markers. Agricultural Science, XXXIX(5):50-55.
- Kostova A., Todorovska E., Christov N., Sevov V., Genov M., Vulchinkov S. and Atanasov A. (2007) Assessing the genetic diversity of Bulgarian maize germplasm using microsatellite markers. Maydica, 52:251-255.
- Mangova M. and Rachovska G. (2004) Technological characteristics of newly developed mutant common winter wheat lines. Plant Soil Environ., 50(2):84-87.
- Manova V., Gecheff K. and Stoilov L. (2006) Efficient repair of bleomycin-induced double-strand breaks in barley ribosomal genes. Mutat. Res., 601:179-190.
- Manova V., Georgieva M., Borisov B., Stoilova B., Gecheff K. and Stoilov L. (2009) Genomic and gene-specific induction and repair of DNA damage in barley. Q.Y. Shu (ed.), Induced Plant Mutations in the Genomics Era, FAO of the UN, Rome, 133-136.
- Manova V. and Stoilov L. (2003) Induction and recovery of double-strand breaks in barley ribosomal DNA. DNA Repair (Amst.), 2:983-990.
- Mehandjiev A., Kosturkova G. and Mihov M. (2001a) Enrichment of *Pisum sativum* gene resources through combined use of physical and chemical mutagens. Israel Journal of Plant Sciences, 49:279-284.

- Mehandjiev A. and Mihov M. (2001b) Contribution of Experimental Mutagenesis for Genetic Improvement of Pea (*P. sativum*). Agricultural University-Plovdiv, Bulgaria, Scientific Works, Jubilee Scientific Session "80<sup>th</sup> Anniversary of the Higher Agricultural Education in Bulgaria" XLVI(3):27-33.
- Mehandjiev A., Mihov M., Kosturkova G. and Radeva R. (2002a) Problems and Achievements in Genetic Improvement of Peas. 50 years Dobrudza Agricultural Institute – Jubilee Scientific Session "Breeding and Agrotechnique of Field Crops", 287-298.
- Mehandjiev A., Mihov M., Kosturkova G. and Radeva R. (2002b) Genetic improvement of peas using experimental mutagenesis. Production. Session II.4.3-Use of genetic variability and Breeding methods, 201.
- Mehandjiev A., Todorova Y., Mihov M. and Stoyanova M. (1998) Enrichment of *Pisum sativum* genetic variation through experimental mutagenesis. Plant Science, 35:180-182.
- Mehandjiev A. and Vassileva M. (1975) Mutagenic effect of gamma-rays and fast neutrons on *Pisum sativum*. C. r. de l'Acad. bulgare des sciences, 8(4):27-33.
- Mihov M. (1994) Lentil improvement – a strategy to increase its production in Bulgaria. International Symposium on Poises Research, April 2-6, 1994, New Delhi.
- Mihov M. and Mehandzhiev A. (1990a) Investigation of mutation variability in lentil (*Lens culinaris* Medic.). International Symposium on the Contribution of Plant Mutation Breeding to Crop Improvement, June 18-22, 1990, Vienna, 117-118.
- Mihov M. and Mehandjiev A. (1990b) Influence of different doses of gamma radiation (Co<sup>60</sup>) on the elements of lentil productivity. Report "Breeding, seed production and agrotechnology of field crops", Ruse, 152-158.
- Mihov M. and Mehandzhiev A. (1995) Application of experimental mutagenesis in lentil (*L. culinaris* Medic.) 2<sup>nd</sup> European Conference on Grain Legumes July 9-13, 1995 – Copenhagen, Denmark. Production - Session II.4.3. Use of Genetic Variability and Breeding Methods, 250.
- Mihov M. and Mehandzhiev A. (1995) Some results of application of experimental mutagenesis on lentil (*Lens culinaris* Medic.). Higher Institute of Agriculture - Plovdiv, Jubilee Scientific Session' Abstracts and papers, October 1995, IV(1):153-156.
- Mihov M., Mehandjiev A. and Stoyanova M. (2002) Achievement in the Breeding of Cool Grain Legume Crops. 50 years Dobrudza Agricultural Institute – Jubilee Scientific Session "Breeding and agrotechnique of field crops", 283-286.
- Mihov M., Stoyanova M., Atanasova D. and Mehandjiev A. (1999) Enrichment of lentil's germplasm (*L. culinaris* Medic.). Agrarian Sciences Res. Commun. Of USB, branch Dobrich, 1:57-60.
- Mihov M., Stoyanova M. and Mehandjiev A. (2001) Some results of application of experimental mutagenesis on lentil (*Lens culinaris* Medic.). Jubilee Scientific Session "80<sup>th</sup> Anniversary of the Higher Agricultural Education in Bulgaria". Agricultural University-Plovdiv, Bulgaria, Scientific Works, XLVI(2):397-400.
- Mihov M., Stoyanova M., Atanasova D. and Mehandjiev, A. (2005) Elitsa - new high productive lentil cultivar (*Lens culinaris* Medic.), News of the Union of Scientists – Ruse, Series: Agrarian and veterinarian and medical sciences, 5:93-95.
- Milkova L. and Daskalov S. (1983) Lyulin: a hybrid pepper cultivar for early field production. In: Proc. 5<sup>th</sup> EUCARPIA Meeting of the Capsicum and Eggplant working group, 202.
- Nikolov H. and Stoyanov B. (1979) Possibilities of applying induced mutagenesis in tobacco breeding. Bulgarian tobacco, 11.
- Nikolova V. and Poryazov I. (1991) Pollen mother cell meiosis in mutants of *Phaseolus vulgaris* L. resistant to *Xanthomonas campestris* var. *phaseoli* (Smith) Dye. Genetics and Breeding, 24(6):422-426.
- Nikolova V., Zagorcheva L. and Poryazov I. (1986) Induction of polyploid forms in the species *Phaseolus vulgaris* L., *Phaseolus acutifolius* A. Gray and *Phaseolus coccineus* L. Genetics and Breeding, 19(2):132-137.
- Petkova S., Djondjurov L. and Svetleva D. (1983a) Determining parameters of the mitotic cycle in *Phaseolus vulgaris* L. (Savi), C. r. de l'Acad. bulgare des sciences, 36:7.
- Petkova S. and Svetleva D. (1983b) Influence of the phase of mitotic cycle on the effect of gamma radiation and alkylating agents in *Phaseolus vulgaris* L. (Savi). Proceedings of the First national conference of genetics and selection, Razgrad, 19-21 November, 1983, 28.

- Rachovska G. (1990) Influence and mutagenic effectiveness of sodium azide and gamma rays in soft winter wheat. Proceedings of International Symposium "Wheat Breeding – Prospects and Future Approaches", Bulgaria, 321-324.
- Rachovska G. (1996) Investigation on effects of sodium azide, gamma rays and their combine action in bread winter wheat. 5<sup>th</sup> International Wheat Conference, June 10-14, Ankara, Turkey, 75.
- Rachovska G. and Dimova D. (2002a) Evaluation of Selection Effectiveness for Productivity in Mutant Populations of Common Bread Wheat, *BJAS*, 8:473-478.
- Rachoska G. and Rachovski G. (2002b) A comparison of breeding effectiveness for productivity in hybrid and hybrid-mutant populations of common bread wheat, Scientific Works, Agr. Univ., Plovdiv, XLVII(1):197-202.
- Rachovska G. and Rachovski G. (2005) Winter common wheat cultivar Guinness/1322. *Field Crops Studies*, 2(2):187-191.
- Rachovska G., Dimova D., Bojinov B. and Rachovski G. (2003) A study on the biological and economic traits of newly developed mutant common winter wheat lines. *Bulg. J. Agric. Sci.*, 9:607–614.
- Rodrigues F., Rukmanski G. and Poryazov I. (1988) Study on halo blight (*Pseudomonas phaseolicola* (Burk) Dows) sensitivity of some bean lines developed through hybridization and mutagenesis. Jubilee Scientific Session, SU "Kliment Ohridski", Biological Faculty, Sofia.
- Rukmanski G. (2005) Role of the mutations in the living nature. pp. 128.
- Savov P. (1976) Some results from the irradiation of hybrid wheat seeds. *In: Experimental mutagenesis in plants* C. BAS, 159–165.
- Sofkova S. and Poryazov I. (2004 a) Study of the reaction of recombinant inbred lines snap bean (*Phaseolus vulgaris* L.) to common bacterial blight (*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye). International Conference on Horticulture Postgraduate (PhD) "Study System and Condition in Europe", November 17–19, 2004, Lednice, Czech Republic, 206-211.
- Sofkova S. and Poryazov I. (2004 b) Study of the reaction of recombinant inbred lines snap bean (*Phaseolus vulgaris* L.) to halo blight (*Pseudomonas syringae* pv. *phaseolicola* (Burkh.). "Field Crops Research", Doroudzha Agricultural Institute, General Toshevo, 2004, 1-2:342-348.
- Sofkova S. (2005) Study of architecture, productivity and disease resistance of cultivars and recombinant inbred lines garden bean. Ph.D. Dissertation. Agricultural University, Plovdiv.
- Sofkova S., Kostova D. and Ivanova I. (2005) Susceptibility of *Phaseolus vulgaris* cultivars and lines to *Cucumber Mosaic Virus* (CMV). *Plant Science*, 42:355-362.
- Stefanov T. (1995) Application of the experimental mutagenesis in the winter barley breeding. *Plant Science*, 32(1-2):27-29.
- Stoilova A. and Valkova N. (2009) Natural Fibres – Their Attractiveness in Multi-Directional Applications, Monograph, Gdynia.
- Stoyanov, B. (1970) Use of ionizing radiations for inducing mutations in tobacco. Third scientific and technical conference –September9-10, 1970, Plovdiv.
- Stoyanov B. (1980) Possibilities of applying induced mutagenesis in tobacco breeding. Ph.D. Dissertation.
- Stoyanova M. and Mihov M. (1994) Study on lentil mutant forms produced in irradiation by gamma rays. EUCARPIA, Oil and protein crops section, September22-24, 1994, Albena, Bulgaria, 284-288.
- Svetleva D. (1983) Effect of gamma-radiation and of some alkylating agents of *Phaseolus vulgaris* L. (Savi), Ph.D. Dissertation, Plovdiv.
- Svetleva D., Petkova S. and Delchev I. (1983) Cytogenetic effect from the individual and combined influence with different chemical mutagenic factors and gamma-radiation during the third phase of the mitotic cycle of *Phaseolus vulgaris* L. (Savi). Proceedings of the XII Symposium of young scientists, 19-21 May, 1983, 68-69.
- Todorova J. and Daskalov S. (1979) Possibilities for the utilization of some mutagenic factors in changing sweet pepper susceptibility to powdery mildew (*Leveillula solanacearum* Gol. f. *capsici* Berg.). *Genet. and Breed.*, 12:174.
- Todorova R. (2002) Study and development of new soybean genotypes resistant to abiotic stress. Ph.D. Dissertation.
- Todorova R. (2005) Study of biological effect of application of physical and chemical mutagens in two new soybean cultivars. Jubilee Conference Breeding and technological aspects in the production and processing of soybean and other legume crops, September 8, 2005, Pavlikeni, 86-93.



- Todorova R. (2010) Some results of studies on genetic improvement of soybean. Crop Science. Presented at the Conference in 2007, Stara Zagora.
- Todorova R. (2009) Study of biological effect of chemical mutagen application in two new pea cultivars. International Scientific Conference Plant Genetic Resources - The Basis of Agriculture of Today, June 13-14, 2007, IPGR, Sadovo, 111-114.
- Todorova R., Georgiev I., Vladova R. and Mehandjiev A. (2001) Some possibilities of using irradiation mutagenesis for obtaining soybean lines resistant to stem cancer. Comptes rendus de l'Academie des Sciences, 54:1, Biologie Genetique, 89-92.
- Todorova V. and Tomlekova N. (2009) Morphological assessment of pepper mutant lines and influence of variation factors on variability of studied characters. Acta Horticulturae, 297-303.
- Todorovska E., Kostova A., Ilchovska M., Guenov M., Guenova I. and Christov N. (2008) Impact of breeding methods on the genetic diversity of Bulgarian maize germplasm. Plant gem Albena, plant genomics European meeting. September 24-27, 2008, Albena, Bulgaria.
- Tomlekova N., Todorova V. and Daskalov S. (2007) Creating variation in pepper (*Capsicum annuum* L.) Through induced mutagenesis. Plant Sciences, 44:44-47.
- Tomlekova N., Yancheva S., Todorova V., Panchev I., Marinova D., Baudoin J.-P., and Daskalov S. (2008) Biochemical and molecular characterization of high carotene sweet pepper mutants assessed their biological value. The 15<sup>th</sup> International Symposium on Carotenoids, 22-28 June 2008, Carotenoid Science, V(12):141.
- Tomlekova N., Todorova V., Petkova V., Yancheva S., Nikolova V., Panchev I. and Penchev E. (2009a) Creation and evaluation of induced mutants for pepper breeding programmes. Proceedings of FAO/IAEA International Symposium on Induced Mutations in Plants. August 12-15, 2009, Vienna, Austria, 72:209-214.
- Tomlekova N., Timina O.O., Timin and O.Y. (2009b) Achievements and perspectives of sweet pepper breeding towards high beta-carotene. Acta Horticulturae, I:205-209.
- Tsvetkova F. (1970) Production of initial material in sunflower by gamma-ray irradiation. Genetics and breeding, 3: 3.
- Yanev S. (1987) Induced mutagenesis in plant breeding of durum wheat – achievements and problems. Experimental mutagenesis in plants. II International Symposium, October, 26-30, 1987, Plovdiv, 93-98.
- Zagorcheva L. and Poryazov I. (1983a) Induction of mutation in green beans by gamma rays. Ann. Rep. of the Bean Imp. Coop., 26:89-90.
- Zagorcheva L. and Poryazov I. (1983b) Results of applying experimental mutagenesis in garden beans. Genetics and plant breeding, 16(2):132-138.
- Zagorcheva L., Poryazov I. and Nikolova V. (1987) Chromosome mutations in some vegetable crops. Proceedings of the second international symposium on experimental mutagenesis in plants, Plovdiv, October 26-30, 1987, 367-369.
- Zagorcheva L., Poryazov I. and Nikolova V. (1988) Developing initial breeding lines through experimental mutagenesis and interspecies hybridization. Genetics and breeding, 21(5):385-390.
- Valkova N. (2010) Trakia and Helios – new Bulgarian cotton cultivars. Proceedings of Plenary Meeting of the Inter-Regional Research Network on Cotton. 28.09.–01.10.2008, Alexandroupoli, Greece.
- Valkova N. and Dechev D. (2006) Phenotype stability of mutant cotton lines for some quality traits of fibre. International scientific conference, Stara Zagora, Vol. 1 Plant-growing, 109-111.
- Vassileva M. (1977) New mutant forms in *Pisum sativum*. C. r. de l'Academie bulgare des sciences, 30:6.
- Vassileva M. (1978) Induced genetic variability in *Pisum sativum*. Experimental mutagenesis in plants (Sofia), 440-447.
- Vassileva M. (1980) Study on the sensitivity to different physical and chemical mutagenic factors and the mutation process in peas (*Pisum L.*), D.B.Sc. Dissertation, Sofia.

## Research Article

**A Study on EMS and Gamma Mutagenesis of Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub]**

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**Abstract**

Dry and pure seeds of two clusterbean cultivars (RGC 936 and HGS 365) were treated with gamma rays (10, 20, 30 and 40 kR) and EMS (0.1, 0.2, 0.3 and 0.4 per cent). Gradual reduction in germination and subsequent survival of the treated population were observed with the increased mutagen dose in both cultivars. Four different types of morphological mutants were observed with the maximum mutation frequency of spreading types in case of higher doses of gamma rays. Positive association was observed between mutagen dose and mutation frequency of morphological traits in  $M_2$  generation. Three different types of chlorophyll mutant viz. *chlorina*, *xantha* and *albina* were observed: chlorophyll mutants induced with gamma rays had higher frequency than EMS induced mutants. Fifty best performing progenies, identified in  $M_3$  on the basis of earliness and higher seed yield, were further tested and fifteen of them were selected in  $M_4$  generation.

**Introduction**

Clusterbean (*Cyamopsis tetragonoloba* L. Taub) is a main arid legume crop, traditionally grown as a system sustaining crop under poorly endowed conditions with minimal management. It is grown over an area of 3.0 million hectares in India, mostly in the northwestern parts of the country, such as the states of Rajasthan (80% of the total acreage), Haryana and Gujarat. However, it is of great concern that the total area of cultivation and productivity levels have been fluctuating over the years (area: 1.5-3.0 million hectare and productivity: 50-500 kg), since it is grown as a rainfed crop and the distribution of rainfall (less than 100 mm to more than 500 mm) is so erratic and uneven in this region.

Mutagenesis is rewarding in situations where naturally existing genetic variability is low (either over all diversity or variability for specific traits) and where simply inherited defects need to be rectified in an otherwise agronomically superior cultivar (Chopra and Sharma, 1985). Mutation breeding is a potentially powerful tool for clusterbean improvement, since it has very limited exploitable and useful genetic variation. The creation of variability through hybridization is very difficult and cumbersome owing to very small and delicate flower structures, which often result in very poor seeds setting in the manually hybridized buds and higher frequency of flower drop during and after crossing (Arora and Pahuja, 2008). There is also limited variability for characters of

economic importance in existing cultivars. In this study, chemical and physical mutagenesis was employed for creation of variability in two widely grown clusterbean cultivars.

**Materials and methods****Mutagenic treatment**

The seeds of two clusterbean cultivars, RGC 936 and HG 365, were treated with gamma rays and ethyl methane sulphonate (EMS). Gamma irradiation was made for dry seeds at doses of 10, 20, 30 and 40 kR in the Indian Agricultural Research Institute, New Delhi, with a  $^{60}\text{Co}$  source. The EMS treatment was performed in freshly prepared phosphate buffer (pH 7.0) after pre-soaking the seeds for 6 h at doses of 0.1, 0.2, 0.3 and 0.4 per cent for 6 h followed by post-treatment washing of 1 h under gentle flow of running tap water.

**Handling of  $M_1$  and  $M_2$  plants**

The data on germination of  $M_1$  seeds and subsequent survival of the  $M_1$  plants till maturity were recorded.  $M_1$  plants were individually harvested to raise the  $M_2$  generation following the plant to row method. The  $M_2$  generation was screened for morphological and chlorophyll mutations. Chlorophyll mutants were determined and grouped by their type in  $M_2$  generation using classifications of Priilin *et al.*, (1976). Growth pattern of  $M_2$  plants were compared with their respective parental cultivars for the selection of morphological mutations, i.e. spreading, dwarf, mating and vigorous plant types, together with agronomic and yield related mutants for early maturity, higher yield, more number of branches, clusters and pods, plant height, number of seeds per pod and pod length. Mutation frequency was calculated on the basis of 100  $M_2$  plant rows according to Gaul's (1964) method.  $M_2$  plants with superior performance were selected and their performances were assessed in  $M_3$  and  $M_4$  generations.

**Assessment of  $M_3$  and  $M_4$  lines**

Fifty  $M_3$  progenies were selected on the basis of their *per se* performance for certain characteristics of economic importance. In  $M_4$  generation the 50  $M_3$  progenies were further evaluated to assess the stability of the promising mutants. These progenies were sown in 4 rows of 4 m length each spaced 30 cm apart along with checks.

Proper agronomic practices were followed for raising a health crop in all the generations ( $M_1$   $M_4$ ) and grown strictly under rainfed conditions. The observations were recorded on 5 randomly selected plants in each progeny. The statistical analysis was performed as per the standard statistical procedures.

## Results

### Effect of mutagenic treatment on germination and survival

In  $M_1$  generation the initial damage caused by the mutagenic treatments is judged by the reduction in germination and further survival of the plants up to maturity. Gradual reduction in germination and subsequent survival of the treated population was observed with the increase of mutagen dose in both cultivars (Fig. 1). Higher dose (40 kR) gamma rays caused the maximum reduction of germination in cultivar HGS 365 while the damage was comparable at lower doses in both cultivars. The decline in survival rate was minimum at lower dose (10 kR) of gamma rays in both the cultivars, while higher doses were much more detrimental. Lower concentration of EMS reduced germination more drastically in RGC 936 while at higher concentration was detrimental to HGS 365, but at 0.3% concentration, the damage was comparable in both cultivars. The survival rate of the germinated individuals was lower in HGS 365 than RGC 936.

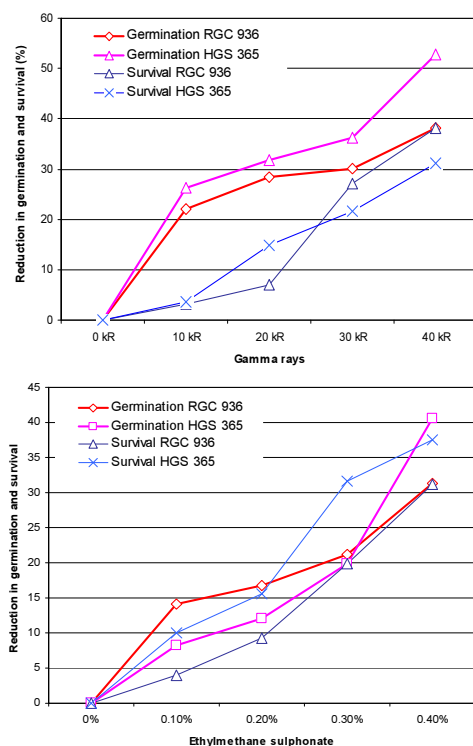


Figure 1. Effect of gamma rays (upper) and EMS (bottom) on germination and survival of clusterbean.

### Mutation frequency in the $M_2$ generation

#### Chlorophyll deficiency

Three different types of chlorophyll deficiency mutant viz. *chlorina*, *xantha* and *albino* were observed. The mutation frequency of *albino* type was always the highest in all treatments, while those of *xantha* and *chlorina* varied between cultivars and among doses (Table 1). In general, the mutation frequency of *albino* type induced by gamma rays was higher than by EMS in both cultivars while no definite pattern was observed for *chlorina* and *xantha* (Table 1). The cultivars HGS 365 (21.8% & 23.5%) had higher overall chlorophyll mutation frequencies than RGC 936 (16.6% & 12.2%) treated either with gamma rays or EMS (Table 1).

#### Morphological and agronomic traits

Frequencies of various morphological mutations were also computed and presented in Table 1. Four different types of morphological trait, namely spreading (branched from base with upright behaviour), dwarf, mating (branches spread on ground like turf) and vigorous, were observed. Spreading and vigorous mutants were more frequently observed with gamma treatments in both cultivars than EMS, but this trend was not observed for dwarf and mating mutants (Table 1). Except for the spreading traits, HGS 365 had higher mutation frequencies than RGC 936 in both gamma and EMS mutagenesis, which is consistent with the chlorophyll deficiency mutations (Table 1).

#### Performance of $M_3$ and $M_4$ progenies

In  $M_3$  generation fifty progenies were selected on the basis of their *per se* performance for agronomic traits (Table 2). Twenty promising progenies were found superior for earliness, twenty for grain yield per plant, five for plant height, nine for pods per plant and ten for seeds per pod over the respective parent (control). Some of the progenies had shown superiority for more than one trait (yield, pods per plant and seeds per pod). These progenies were evaluated in  $M_4$  generation for their yield performance and other agronomic traits (Table 3). Mutant progeny RGC 936-30-2 matured in just 65 days with higher grain yield (8.02 g) and maximum number of seed bearing pods (9.7) closely followed by RGC 936-1-17, which matured in 68 days with almost the same yield level (7.93 g) with maximum branches (5.9), clusters per plant (12.9), pods per plant (27.1) and seeds per pod (9.7). This mutant progeny showed superiority for all the agronomic traits except maturity with almost the same yield level (7.93 g) than that of the best yielder. The other mutant progeny of RGC 936 also showed superiority for more than one trait.



Eight promising mutants were identified in cultivar HGS 365 on the basis of their agronomic performance and superiority over parental control. Mutant progeny HGS 365-15-5 was found superior with higher yield per plant

(9.95g), branches per plant (6.3), clusters per plant (13.7), pods per plant (34.8) and seeds per pod (9.1). Besides yield, other mutants of HGS 365 had also shown superiority for other agronomic traits.

Table 1. Frequency of morphological and chlorophyll mutations in clusterbean

Cultivars/ Treatment	Morphological mutations (%)					Chlorophyll mutations (%)			
	Spreading	Dwarf	Mating	Vigorous	Total	Albino	Chlorina	Xantha	Total
RGC 936, Gamma rays									
Control	-	-	-	-	-	-	-	-	-
10 kR	0.87	-	0.43	0.40	1.70	1.14	0.57	0.85	2.56
20 kR	2.42	0.85	1.30	0.76	5.33	1.78	0.88	1.78	4.44
30 kR	3.65	4.56	5.87	2.05	16.13	3.23	1.29	0.00	4.52
40 kR	16.72	8.18	3.09	4.21	32.20	3.39	1.69	0.00	5.08
RGC 936, EMS									
0.1%	-	0.62	0.43	-	1.05	0.85	0.42	0.40	1.67
0.2%	2.15	0.62	0.40	0.25	3.42	1.03	0.68	0.65	2.36
0.3%	4.54	2.12	2.02	0.46	9.14	1.71	1.14	1.15	4.00
0.4%	6.77	3.42	5.84	1.21	17.24	2.75	1.38	0.00	4.13
HGS 365, Gamma rays									
Control	-	-	-	-	-	-	-	-	-
10 kR	1.16	0.28	0.57	-	2.01	1.34	0.67	1.34	3.35
20 kR	2.39	1.92	1.14	1.58	7.03	2.70	1.08	1.08	4.86
30 kR	4.66	3.72	10.31	4.47	23.16	2.86	2.10	2.11	7.07
40 kR	15.18	8.96	7.34	16.76	48.24	5.88	0.00	2.35	8.23
HGS 365, EMS									
0.1%	0.36	0.28	0.67	0.59	1.90	1.09	0.54	0.82	2.45
0.2%	1.71	0.80	1.26	2.23	6.00	2.50	1.00	1.50	5.00
0.3%	3.28	1.74	10.65	2.42	18.09	2.86	1.90	1.90	6.66
0.4%	4.76	16.35	8.98	4.39	34.48	5.13	0.00	2.56	7.69

Table 2. Selected promising progenies for agronomic traits in M<sub>3</sub> generation

SN	Agronomic trait	RGC 936		HGS 365	
		Mutants*	Parent	Mutants*	Parent
1	Early maturity (days)	< 66 (10)	72	< 65 (10)	73
2	High yielding (g/plant)	> 6.0 (10)	5.23	> 6.5 (10)	5.58
3	Plant height (cm)	> 45.0 (2)	38.0	> 50.0 (3)	42.0
4	Number of pods per plant	> 24.0 (5)	18.0	> 29.0 (4)	19.0
5	Number of seeds per pod	> 9.0 (4)	8.2	> 9.0 (6)	8.4

\* Figures in parentheses show the number of progenies selected for the trait. These numbers exceed fifty because some progenies were common for more than one trait.

Table 3. Agronomic performance of promising M<sub>4</sub> progenies

Mutants	Yield per plant (g)	Days to maturity	Plant height (cm)	Branches per plant	Clusters per plant	Pods per plant	Seeds per pod
RGC 936-1-5	6.89	67.5	42.7	5.7	12.3	25.5	9.2
RGC 936-1-17	7.93	68.0	46.0	5.9	12.9	27.1	9.7
RGC 936-5-4	7.90	65.5	43.7	5.9	12.7	24.5	8.8
RGC 936-12-11	6.81	66.5	43.4	5.6	11.8	22.6	8.5
RGC 936-16-2	7.54	66.5	44.8	5.8	12.2	23.8	9.0
RGC 936-20-22	7.48	68.5	41.9	5.7	11.9	22.2	8.9
RGC 936-30-2	8.02	65.0	43.0	5.5	12.6	22.9	9.7
<b>RGC 936 (C)</b>	<b>4.26</b>	<b>71.5</b>	<b>38.1</b>	<b>4.5</b>	<b>9.2</b>	<b>18.1</b>	<b>7.9</b>
HGS 365-2-7	7.82	67.5	44.6	5.4	11.9	24.6	8.8
HGS 365-3-10	7.35	68.0	44.3	5.4	12.0	24.6	9.0
HGS 365-7-5	7.74	69.0	50.0	5.5	11.8	25.1	8.8
HGS 365-10-2	7.84	65.0	49.3	4.8	10.2	25.8	9.1
HGS 365-12-6	9.16	67.0	46.7	4.7	12.1	33.6	8.5
HGS 365-15-2	8.47	68.0	49.0	4.9	13.4	28.2	9.0
HGS 365-15-5	9.95	69.0	48.8	6.3	13.7	34.8	9.1
HGS 365-18-4	9.50	63.5	45.9	6.2	10.4	30.6	8.8
<b>HGS 365 (C)</b>	<b>4.55</b>	<b>72.3</b>	<b>36.3</b>	<b>4.4</b>	<b>9.2</b>	<b>18.3</b>	<b>8.3</b>

## Discussion

In the present study, gradual reduction in germination and subsequent survival of treated population were observed with corresponding increase in concentration/dose of both mutagens. These findings are consistent with the earlier findings of Rao and Rao (1982) in clusterbean. However, Singh and Chowdhary (1972) did not observe significant effect up to 40 kR on germination, yet there was a considerable effect at 100 and 150 kR doses of gamma irradiation in clusterbean. Frequencies of morphological and chlorophyll mutations were observed in

M<sub>2</sub> generation since all the variants are expected to be expressed in M<sub>2</sub> generation. Higher frequency of certain plant types at higher doses might be due to considerable changes at DNA level by the mutagens while lack of any pattern of certain morpho-types or chlorophyll mutants might be due to random nature of mutations. It was observed that both the cultivars manifested differential reactions to the treatment with gamma rays and EMS. Differential behaviour of various mutagens and selective response of cultivars might be due to the mode of action of the mutagens employed and genetic variability among the

cultivars used in the study. These findings are consistent with the findings of Badami and Bhalla (1992) in clusterbean and others in pulses (Kothekar 1989, Waghmare and Mehra 2001 and Gaikwad and Kothekar 2004).

On the basis of mean performance and variance, selected M<sub>2</sub> plants were advanced to M<sub>3</sub> generation and agronomically superior mutant progenies, and were tested for their stability in M<sub>4</sub> generation. The purpose of the present study was to isolate early maturing and high yielding mutant lines. Fifteen mutants, seven derived from RGC 936 and eight from HGS 365 were found to be superior for earliness as well as higher grain yield. Yadav *et al.*, (2004) had also isolated mutants in M<sub>3</sub> generation from clusterbean cultivar RGC 197 through gamma irradiation.

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#### References

- Arora R.N. and Pahuja S.K. (2008). Mutagenesis in guar (*Cyamopsis tetragonoloba* L. Taub). Plant Mutation Reports 2 (1): 7-9.
- Badami P.S. and Bhalla J.K. (1992). Mutagenic effectiveness and efficiency of gamma rays, magnetic field and sodium azide in clusterbean. Ad. Plant Sci. 5(2): 534-541.
- Chopra V.L. and Sharma, R.P. 1985. Induced mutations in crop improvement. (Ed. V.L. Chopra). Genetic Manipulations in Crop Plants. Oxford & IBH Publishing Company, New Delhi. Pp. 22-48.
- Gaikwad N.B. and Kothekar V.S. 2004. Mutagenic effectiveness and efficiency of ethyl methane sulphate and sodium azide in lentil (*Lens culinaris* Medik.). Indian J. Genet. 64: 73-74.
- Gaul H. 1964. Mutations in Plant Breeding. Radiation Botany 4(3): 155-237.
- Kothekar V.S. 1989. Differential radiosensitivity in mothbean. Curr. Sci., 58: 758-760.
- Priilin O., Shneider T. and Orav. 1976. Study on chemical mutagenesis in agricultural plants. Ed. Valgus, Tallin, p. 146-151.
- Rao S. and Rao D. 1982. Studies on the effect of X- irradiation on *Cyamopsis tetragonoloba*. Proc. Indian Nat. Sci. Acad. (Biological Sciences). 48: 410-415.
- Singh B.P. and Chowdhary J.B. 1972. Studies on irradiated guar. Nucleus. 15: 16-22.
- Waghmare V.N. and Mehra R.B. 2001. Induced chlorophyll mutations, mutagenic effectiveness and efficiency in *Lathyrus sativus* L. Indian J. Genet., 61: 53-56.
- Yadav S.L., Singh V.V. and Ramkrishana K. 2004. Evaluation of promising M<sub>3</sub> progenies in guar [*Cyamopsis tetragonoloba* (L.) Taub]. Indian J. Genet. 64 (1): 75-76.



## Production of Doubled Haploids in Tunisian Durum Wheat (*Triticum durum* Desf.) Cultivars through unpollinated Ovary Culture

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### Abstract

The use of doubled haploid technology improves the efficiency of cultivar development and enables homozygous genotypes to be obtained in one generation. The major problem with this approach is the low efficiency of green plant regeneration particularly for durum wheat (*Triticum durum* Desf.). In order to improve the efficiency of green doubled haploid gynogenesis, we have analysed the effect of spike pretreatments. Cold treatment is the most efficient and the most genotype-independent for ovary response and calli development. Mannitol treatment is also interesting for its practicality and has quite comparable results to the cold treatment. PEG based treatments were shown to be inefficient for callus induction.

For induction and differentiation media, high 2,4-D, vitamins, glutamine content, and maltose based medium was more efficient for inducing the ovary response and a high rate of callus development. Using the optimised protocol, gynogenesis was performed on three Tunisian genotypes and green fertile doubled haploid plants were obtained. A strong genotypic variation in the gynogenesis response was observed. The Azizi genotype was unresponsive to gynogenesis while the Khiar genotype was the best performing genotype.

**Keywords:** *In vitro* gynogenesis; doubled haploid; durum wheat; pretreatment.

**Abbreviations:** DH- doubled haploid plant; 2,4-D- 2,4 Dichlorophenoxyacetic acid; 2ip- 6-( $\gamma$ ,  $\gamma$ - Dimethylamino)purine; NAA- Naphtaleneacetic acid.

### Introduction

Doubled haploid production can accelerate cultivar development by removing the long and slow process of increasing homozygosity by self pollination. This strategy offers the possibility to obtain in one generation perfectly homozygous plants. Doubled haploid lines can be obtained by androgenesis (De Buyser *et al.*, 2002; Zheng *et al.*, 2003; Patel *et al.*, 2004; Soriano *et al.*, 2008), gynogenesis (Mdarhri-Alaoui *et al.*, 1998 ; Sibi *et al.*, 2001; Slama-Ayed and Slim-Amara, 2007), and inter specific or inter generic crosses (Kasha *et al.*, 2003; Szarejko, 2003). Durum wheat is considered as a recalcitrant species to androgenesis as regeneration often results in sterile plantlets (Cistué *et al.*, 2006; Slama-Ayed *et al.*, 2006; Labbani *et al.*, 2007). It was shown that many cultivars produce exclusively albino plantlets (Labbani *et al.*, 2005).

Gynogenesis has been suggested as an alternative to androgenesis in durum wheat as albino plantlets are not produced. Gynogenesis was shown to be an efficient method for regenerating green haploid plants (Mdarhri-Alaoui *et al.*, 1998; Sibi *et al.*, 2001). In sugar beet, it was shown to be the most successful method for doubled haploid plant production (Gürel *et al.*, 2000). In gynogenesis, the embryogenesis is indirect starting with a callus development from the unpollinated ovary followed by regeneration of haploid plantlets. In fact, this technique can be divided into three important steps: (i) the pretreatment, commonly applied in order to switch the gametophytic pathway into a sporophytic development to induce embryogenesis (Touarev *et al.*, 2000); (ii) the induction and differentiation phases, which results in the callus formation. Step two is strongly dependent on culture conditions and is the most delicate phase of the process (Slim-Amara, 2000). Finally, (iii) the regeneration phase with development of haploid plantlets.

The overall efficiency of gynogenesis in developing doubled haploids is quite low and many attempts were undertaken in order to improve it. The only pretreatment tested so far is the cold treatment of the spikes. It was shown that the best response was observed when the spikes were placed in water at 4°C for 5 to 15 days, before ovary extraction (Mdarhri-Alaoui *et al.*, 1998; Sibi *et al.*, 2001). In order to further improve the rate of doubled haploid production through gynogenesis, we have studied the effect of cold pretreatment associated with or without mannitol and/or PEG. In solution, mannitol and PEG are used to induce an osmotic stress. This pretreatment creates more negative water potential, facilitating a water-restricted environment (Ilić-Grubor *et al.*, 1998). We also compared the efficiency of two different induction and differentiation media for ovary response and callus development. Finally, using the best protocol identified, we have developed doubled haploid plants by unpollinated ovary culture from three different durum wheat genotypes.

### Materials and methods

#### Donor

Three Tunisian durum wheat cultivars were used in this study: Hmira, Azizi and Khiar. Plants were grown in the

experimental fields of the National Agronomic Institute of Tunisia. Spikes were collected when the microspores were at the late uninucleate or binucleate stage and stored in the different pre-treatment solutions in the dark at 4°C.

#### Culture media

Spikes were collected and subject to two pretreatments T1 and T2 consisting in 14 days in the dark at 4°C and 0.3M Mannitol for 7 days at 4°C in the dark respectively. Twenty ovaries of 1 to 1.5 mm length were cultivated on 5.5 cm Petri dishes sealed with parafilm, containing the different induction media in dark at 27°C for 5 to 6

weeks (Sibi and Fakiri, 1994; Mdarhri-Alaoui *et al.*, 1998). Responding ovaries, whose size had increased, were transferred to the differentiation media for 6 weeks at 25°C with a 16-hour photoperiod at a light intensity of 80-100  $\mu\text{E m}^{-2} \text{s}^{-1}$  to induce the callus development.

We have used the Sibi *et al.*, (2001) induction (Ind M<sub>1</sub>) and differentiation (Diff M<sub>1</sub>) media, and the modified Mdarhri-Alaoui *et al.*, (1998) induction (IndM<sub>2</sub>) and differentiation (Diff M<sub>2</sub>) media. The compositions of media are presented in Table 1.

Table 1. Composition of media for induction, differentiation and development

Components	Ind M <sub>1</sub>	Ind M <sub>2</sub>	Diff M <sub>1</sub>	Diff M <sub>2</sub>	Dev M
<b>Macroelements g/l</b>					
NH <sub>4</sub> NO <sub>3</sub>	0.160	0.825	0.160	1.650	0.160
CaCl <sub>2</sub> , 4H <sub>2</sub> O	0.440	0.220	0.440	0.440	0.440
MgSO <sub>4</sub> , 7H <sub>2</sub> O	0.370	0.185	0.370	0.370	0.370
KH <sub>2</sub> PO <sub>4</sub>	0.170	0.085	0.170	0.170	0.170
KNO <sub>3</sub>	1.900	0.950	1.900	1.900	1.900
FeEDTA	0.040	0.060	0.040	0.060	0.040
<b>Microelements mg/l</b>					
KI	0.83	0.83	0.83	0.83	0.83
H <sub>3</sub> BO <sub>3</sub>	6.20	6.20	6.20	6.20	6.20
MnSO <sub>4</sub> , 2H <sub>2</sub> O	22.30	16.88	22.30	16.88	22.30
ZnSO <sub>4</sub> , 2H <sub>2</sub> O	8.60	8.60	8.60	8.60	8.60
Na <sub>2</sub> MO <sub>4</sub> , 4H <sub>2</sub> O	0.25	0.25	0.25	0.25	0.25
CuSO <sub>4</sub> , 5H <sub>2</sub> O	0.025	0.025	0.025	0.025	0.025
CoCl <sub>2</sub> , 6H <sub>2</sub> O	0.025	0.025	0.025	0.025	0.025
<b>Vitamins mg/l</b>					
Nicotinic acid	1	0.5	0.5	0.5	0.5
Pyridoxine HCL	1	0.5	0.5	0.5	0.5
Thiamine HCL	1	0.1	0.1	0.1	0.1
Pyruvate Na					5.0
<b>Aminoacids mg/l</b>					
Glutamine	750	-	146	-	146
Glycine	-	2	2.25	2	2.25
L-asparagine	-	150	-	150	-
Myo-Inositol	100	100	100	100	100
<b>Growth regulators mg/l</b>					
2,4-D	2	1	1	-	-
NAA	-	-	1	-	-
Kinetin	0.5	1	-	-	-
2iPA	-	-	0.1	-	-
<b>g/l</b>					
Maltose	60	-	-	-	-
Saccharose	-	30	30	30	30
Purified Agar	7	7	7	7	7
pH	5.8	5.8	5.8	5.8	5.8

Ind M<sub>1</sub> and Ind M<sub>2</sub>: Induction media; Diff M<sub>1</sub> and Diff M<sub>2</sub>: Differentiation media; Dev: Development medium.

### Spike Pretreatments

Spikes were subject to four pretreatments T1: 14 days in the dark at 4°C; T2: 0.3M Mannitol for 7 days at 4° in the dark. T3 consisted in a solution of PEG<sub>4000</sub> 1% at 4°C for 5 days in the dark and T<sub>4</sub> in a solution of PEG<sub>4000</sub> 1% at 4°C for 10 days.

### Ovary culture, plantlets regeneration and chromosome doubling

One thousand ovaries from the pretreated spikes per genotype were isolated and subject to the *in vitro* culture as described above using the IndM<sub>1</sub> and the Diff M<sub>1</sub> media. The percentage of responding ovaries and developed calli were then calculated. The ovaries that had developed callus were transferred to development medium and kept in the same conditions for regeneration. The composition of the development media is indicated in Table 1. The ploidy level of the developed plantlets was determined before and after colchicine treatment for all regenerated plantlets using the chromosome count protocol of Jahier *et al.*, (1992).

Colchicine treatment was performed by placing the plantlets at the three-leaf stage in 0.1% colchicine solution

(Barnabàs and Szakàcs, 2000), then rinsed carefully for 0.5-1h before plants were transferred to soil.

### Data Analysis

Analysis of variance (ANOVA) was conducted using SAS computer software (1988). The data were analyzed as two factors for culture media and pretreatment and as one factor for pretreatment and for genotype.

### Results and discussion

#### Effect of pretreatment and media

Two major reports have described doubled haploid development using gynogenesis for durum wheat (Mdarhri-Alaoui *et al.*, 1998 and Sibi *et al.*, 2001). The protocols use different induction and differentiation media. In order to compare the efficiency of these media and their interaction with different pretreatments, 1600 ovaries were assayed from the three genotypes and were subjected to the previously described T1 and T2 pretreatment and cultivated using the different induction and differentiation media. Table 2 refers to the percentage of responding ovaries and the percentage of developed calli.

Table 2. The response and calli formation of durum ovaries cultured on different media after different pretreatment

Culture media	Responding ovaries (%)		Calli (%)	
	Cold treatment at 4°C for 14 d	Cold treatment at 4°C for 7d in 0,3M Mannitol	Cold treatment at 4°C for 14 d	Cold treatment at 4°C for 7d in 0,3M Mannitol
Ind M1 / Diff M1	68.37 <sub>a</sub>	61.67 <sub>b</sub>	63.88 <sub>a</sub>	60.14 <sub>a</sub>
Ind M2 /Diff M2	63.93 <sub>ab</sub>	61.94 <sub>b</sub>	45.97 <sub>b</sub>	41.71 <sub>b</sub>

Culture media Ind M1, Diff M1, Ind M2 and Diff M2 see table 1; Data with different letters are significantly different at  $p \leq 0.05$  (Newman and Keuls test).

For induction, the T1 treatment (14 days cold) gave better results than the T2 treatment (mannitol 0.3M) especially using the Ind M<sub>1</sub> media, while there was no sharp difference between the two induction media. However, callus development was significantly higher using the Differentiation media M<sub>1</sub> than the Differentiation M<sub>2</sub>. Diff M<sub>1</sub> is characterized by the presence of high organic nitrogen (glutamine), 2,4-D content and maltose rather than sucrose. Based on these results, a 14 days cold treatment coupled with Ind M<sub>1</sub> and Diff M<sub>1</sub> media were the most efficient conditions for ovary responsiveness and calli development of the studied durum wheat genotypes. Based on these results, we chose the M<sub>1</sub> (Sibi *et al.*, 2001) media (induction and differentiation) for the following experiments and preselected the pretreatment: 14 days cold at 4°C.

#### Effect of pretreatment

In order to improve the overall efficiency of gynogenesis on durum wheat and identify the most efficient treatment, we have attempted to analyze the effect of different spike pretreatments on the ovary response to the *in vitro* culture and on the callus development.

Spikes were subject to four pretreatments before ovary extraction. T1 was a 14 days cold treatment which has been used in other experiments (Mdarhri-Alaoui *et al.*, 1998; Sibi *et al.*, 2001). T2 consisted of a 0.3M mannitol treatment at 4°C during 7 days. T3 and T4 are PEG treatments at 4°C for 5 and 10 days respectively.

Our results showed that induction and differentiation phases were significantly different using the diverse pre-

treatments for the three genotypes (Fig. 1). It was shown that pretreatment was used to trigger ovary development from gametophytic to sporophytic pathways. For gynogenesis, only cold treatments were used so far. Sibi *et al.*, (2001) used cold treatments for 7 to 14 days and showed that some genotypes exhibit the highest rate of ovary response at 7 days while others at 11 days. Mdarhri-Alaoui *et al.*, 1998 showed that the best response was obtained with 5 to 15 days pretreatments. In our study, 14 days cold treatment was effective in inducing the ovary response and the callus development in all three studied genotypes.

Mannitol was showed to be an efficient pretreatment in androgenesis (Cistué *et al.*, 2006). In gynogenesis, our results showed that, except in ovary response in the Khيار

genotype, mannitol pre-treatment is as effective as the cold treatment. However, mannitol treatment is efficient as it only requires 7 days while T1 is a 14 days long treatment. PEG can induce an osmotic stress and was tested for the first time as a pretreatment in this study. We have shown that the T3 ovary response was comparable, for all genotypes to the cold treatment. However, the number of calli developed with T3 is significantly lower than T1 for Khيار and Hmira. T4 pretreatment is a 10 days cold treatment in PEG while T3 lasts only five days. It appears that the long PEG based pretreatments can improve calli production but only in some genotypes such as Khيار. PEG based pretreatments are not only less efficient but also more genotype-dependent than mannitol based and cold treatments.

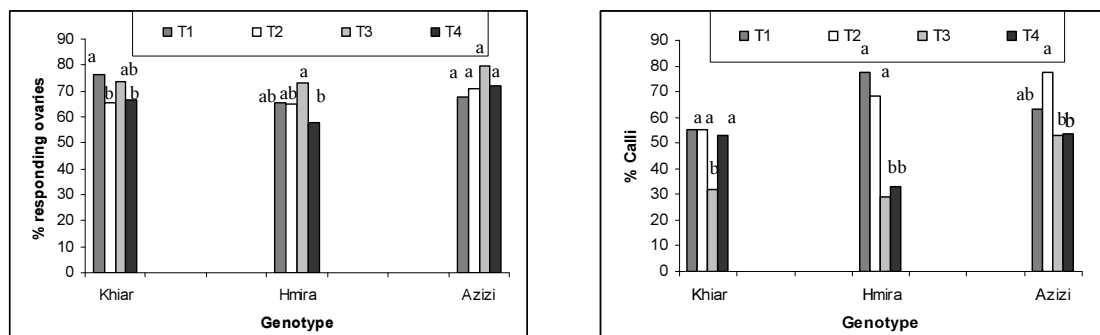


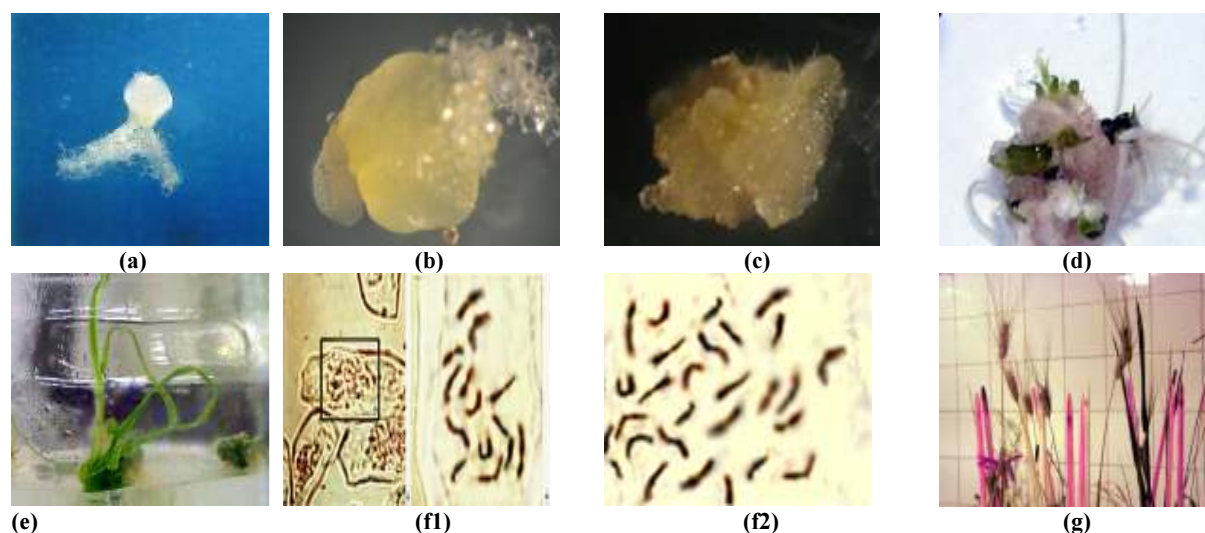
Figure 1. Effect of pretreatments on gynogenesis in durum wheat in terms of ovary response (left panel) and calli formation (right panel). Pretreatment of ovaries was performed (T1) in water at 4°C for 14 days, or (T2) in 0.3 M Mannitol at 4°C for 7 days, or (T3) in 1%PEG<sub>4000</sub> at 4°C for 5 days, or (T4) in 1% PEG<sub>4000</sub> at 4°C for 10 days. Data with different letters are significantly different at  $p \leq 0.05$  (Newman and Keuls test).

### Durum wheat doubled haploid production

The optimized protocol indicated above was used to develop doubled haploid plants using three durum wheat genotypes as donor plants. Unpollinated ovaries (Fig. 2-a) were cultivated on the Ind M<sub>1</sub> medium to obtain responding ovaries (swelling ovaries) (Fig. 2-b). These were transferred on the Diff M<sub>1</sub> medium and exposed to light and lead to calli differentiation Fig. 2-c). These calli

were transferred to development medium and developed green shoots (Fig. 2-d), and the regenerated plantlets (Fig. 2-e) were subject to chromosome counting to assess their ploidy level (Fig. 2-f1). Haploid plants were treated with colchicine 0.1% to obtain fertile doubled haploid plants (Fig. 2-f2 and Fig. 2-g).





**Figure 2.** Production of fertile doubled haploid plants from ovary culture. (a) Cultivated unpollinated ovary; (b) Ovary after 2 weeks in induction medium in the dark at 27°C; (c) Callus obtained after 3 weeks of *in vitro* culture in differentiation medium; (d) Callus with green shoots obtained after 3 weeks in development medium with a 16-hour photoperiod at 25°C; (e) Green plantlets regenerated after 4 weeks in development medium with a 16-hour photoperiod at 25°C; (f1) Chromosomes of regenerated haploid plantlet; (f2) Chromosomes of doubled haploid plantlets obtained through colchicines treatment; (g) Fertile doubled haploid plants.

### Genotypic variation in the gynogenesis response

More than 5000 unpollinated ovaries were cultured from the three genotypes of durum wheat (Azizi, Khiar and Hmira) using the same procedure described above. Gynogenesis response is dependent on the donor parent genotype. The Azizi genotype was not responsive to gynogenesis as no haploid plant could be obtained. Khiar and Hmira expressed high gynogenetic capacity with respectively 21 and 39 regenerated plantlets. Chromosome counting showed that all these plantlets were haploid,

indicating that the regeneration was obtained from unpollinated ovaries and not from diploid ovary tissues. Haploid plantlets were recovered from Khiar at a percentage of 3.1% and from Hmira at a percentage of 3.5% of the responding ovaries (Table 3). In our study, the rate of haploid plants regenerated was comparatively good according to previous studies with durum wheat (Sibi *et al.*, 2001 and Mdarhri-Alaoui *et al.*, 1998). The 60 regenerated haploid plants were all green.

**Table 3.** Gynogenic response in three durum wheat Tunisian genotypes: Azizi, Khiar and Hmira

Cultivar	Cultured ovaries number	Responsive ovaries number	Calli number	Green shoots formed number	Haploid plants number	Doubled haploid plants number
Azizi	1600	988	61	34	0	0
Khlar	1790	680	74	38	21	6
Hmira	2000	1100	269	126	39	5

The Number presented is the means of six replicates.

Six doubled haploid out of 21 Khlar haploid plants and five out of 39 Hmira haploid plants could be obtained using colchicine chromosome doubling (Table 3). This low frequency can be accounted for the toxic effect of the colchicines as reported by Barnabàs and Szakács (2000).

However, these plants did not show any abnormality in the chromosome number or in the morphology of the plant. Moreover, all the doubled haploid plants obtained were green and fertile. In fact, anther and microspore cul-

ture has been long used to develop doubled haploid plants in cereals (Jaiti *et al.*, 2000; Dogramaci-Altunetpe *et al.*, 2001; Picard and De Buyser, 2002; Liu *et al.*, 2002; Jacquard *et al.*, 2006). But in most cases, the high rate of albino plants is considered as a major limitation of this technique.

This study reports an efficient protocol for developing green and fertile doubled haploid plants using gynogene-

sis of some genotypes of durum wheat. This protocol can be implemented and applied in breeding programs.

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### References

- Barnabàs B. and Szakàcs E. (2000) Improved strains of cereals by anther culture technology. Paper presented at COST 824 final meeting on the Biotechnological Approaches for Utilization of Gametic Cells, Slovenia 1-5 July 2000.
- Cistué L., Soriano M., Castillo A.M., Vallés M.P., Sanz J.M. and Echàvarri B. (2006) Production of doubled haploids in durum wheat (*Triticum turgidum* L.) through isolated microspore culture. *Plant cell rep* 25 (4): 257-264.
- De Buyser J., Touraine P. Jaiti F. and Picard E. (2002) Haplodiploïdisation par culture de microspores isolées de blé *in vitro*. Biotechnologies végétales : Techniques de laboratoire. In Haïcour R. (ed) Pref. Georges Ducreux. Paris. Tec et Doc Montréal, AUF, pp: 257-273.
- Dogramaci-Altunetpe M., Peterson T.S. and Jauhar P.P. (2001) Anther culture derived regenerants of durum wheat and their cytological characterization. *The journal of Heredity* 92:56-64.
- Gürel S., Gürel E. and Kaya Z. (2000) Doubled haploid plant production from unpollinated ovules of sugar beet (*Beta vulgaris* L.) *Plant cell rep* 19: 1155-1159
- Ilić-Grubor K., Attree S.M. and Fowke L.C. (1998) Induction of microspore-derived embryos of *Brassica napus* L. with polyethylene glycol (PEG) as osmoticum in a low sucrose medium. *Plant Cell Reports* 17: 329-333.
- Jacquard C., Asakaviciute R., Hamalian A.M., Sangwan R.S., Devaux P. and Clément C. (2006) Effects of annual cycle and spike position on microspore embryogenesis and albinism. *Plant Cell Reports* 25: 375-381.
- Jahier J., Chevre A.M., Eber F., Delourme R. and Tanguy A.M. (1992) Techniques de cytogénétique végétale. France.
- Jaiti F., El Jaafari S., El Hadrami I., Nachit M., Baum M., De Buyser J. and Picard E. (2000) Problématique de la culture de microspores chez le blé dur *Triticum turgidum* L. var. durum *L'amélioration du blé dur dans la région méditerranéennes, nouveau défis*, série A (40): 149-152.
- Kasha K.J., Simion E., Miner M., Letarte J. and Hu T.C. (2003) Haploid wheat isolated microspore culture protocol. Doubled Haploid production in crop plants, edited by Maluszynski M., Kasha K.J., Forster B.P. and Szarejko I., Kluwer Academic Publishers, pp 77-83.
- Labani Z., Richard N., De Buyser J. and Picard E. (2005) Plantes chlorophylliennes de blé dur obtenues par culture de microspores isolées: importance des prétraitements. *C. R. Biologies* 328: 713-723.
- Labani Z., de Buyser J. and Picard. E. (2007) Effect of mannitol pretreatment to improve green plant regeneration on isolated microspore culture in *Triticum turgidum* ssp. durum cv. 'Jennah Khetifa'. *Plant Breeding* 126: 6, 565-568.
- Liu W., Zheng M.Y. and Konzak C.F. (2002) Improving green plant production via isolated microspore culture in bread wheat (*Triticum aestivum* L.). *Plant Cell Reports* 20: 821-824.
- Mdarhri-Alaoui M., Saidi N., Chlayah A. and Chlayah H. (1998) Obtention par gynogenèse *in vitro* de plantes haploïdes chlorophylliennes chez le blé dur. *C R Acad Sci Paris, Sciences de la vie/ Life Sciences* 321: 25-30.
- Patel M. Norman L.D., Donald R.M. and James O.B. (2004) Optimization of culture conditions for improved plant regeneration efficiency from wheat microspore culture. *Euphytica* Vol.140 (3): 197-204.
- Picard E. and De Buyser J. (2002) Haplodiploïdisation par culture d'anthères en milieu liquide chez le blé. Biotechnologies végétales In Haicour R (ed) Techniques de laboratoire. Tec et Doc Montréal, AUF, pp 243-256.
- SAS (1988) SAS Institute SAS/STAT users guide. Release 6.03 SAS Inst, Cary, NC (ed) 1988.
- Slama-Ayed O., Slim-Amara H., De Buyser J. and Picard E. (2006) Effet du prétraitement des épis sur l'aptitude de la régénération *in vitro* des microspores isolées chez une variété tunisienne de blé dur (*Triticum durum* Desf). *Revue de L'INAT*: Vol. 21 n°1: 123-132.
- Slama-Ayed O. and Slim-Amara H. (2007) Production of doubled haploids in durum wheat (*Triticum durum* Desf.) through culture of unpollinated ovaries, *Plant Cell, Tissue & Organ Culture* volume 91, N°2: 125-133.

- Slim Amara H. (2000) L'Haplodiploidisation par androgenèse *in vitro* et son déterminisme génétique chez le blé dur *Triticum turgidum* L. subsp. *Durum* Desf. Thèse de Doctorat d'Etat en Sciences Agronomiques. Institut National Agronomique de Tunisie 152p.
- Sibi M. and Fakiri M. (1994) Gynogenèse chez des génotypes marocains d'orge (*Hordeum vulgare*) In: AUPELF-UREF Libbey Eurotext J (ed) Quel avenir pour l'amélioration des plantes? 1994, pp 337-344.
- Sibi M., Kobaissi A. and Shakafandeh A. (2001) Green haploid plants from unpollinated ovary culture in tetraploid wheat (*Triticum durum* Desf.). *Euphytica* 122 (2): 351-359.
- Soriano M., Cistué L. and Castillo A.M. (2008) Enhanced induction of microspore embryogenesis after *n* - butanol treatment in wheat (*Triticum aestivum* L.) anther culture. *Plant cell Reports* 27 (5): 805-811.
- Szarejko I. (2003) Anther culture for doubled haploid production in barley (*Hordeum vulgare*). In Maluszynski M., Kasha K.J., Forster B.P. (ed) Doubled Haploid Production in Crop Plant. Szarejko I Kluwer Acad Publ, pp 35-42.
- Touraev A., Tashpulatov A., Indrianto A., Barinova I., Katholnigg H., Akimcheva S., Ribarits A., Voronin V., Zhexsembekova M. and Heberle-Bors E. (2000) Fundamental aspects of microspore embryogenesis. Biotechnological approaches for utilisation of gametic cells. Cost 824final meeting, Bled, Slovenia, 1 to 5 July 2000, 205-214.
- Zheng M.Y., Liu W., Weng Y., Polle E. And Konzak. C.F. (2003) Production of doubled haploids in Wheat (*Triticum aestivum* L.) through Microspore Embryogenesis triggered by inducer chemicals. In M. Maluszynski *et al.*, (eds.), Doubled Haploid production in crop plants, pp 83-94.

## Article

**Genetic Improvement of an Indigenous Aromatic Variety 'Jajai 77' through Mutagenesis**

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*Nuclear Institute of Agriculture, Tando Jam, Pakistan**\*E-mail: azeem\_nia@yahoo.com***Abstract**

The mutant Jajai-25/A has been developed from an indigenous aromatic rice variety Jajai 77 through radiation by gamma rays (250 Gy). The mutant Jajai-25/A was initially selected on the basis of short plant stature, lodging resistance and improved yield components. After confirmation, the performance was evaluated in preliminary and advanced yield trials. The analysis of variance of different trials revealed significant differences among all the mutants, parents and check varieties. This mutant was significantly better than its mother variety Jajai 77 in respect of all the yield and yield contributing traits. It has shown consistency in better paddy yield than all the mutant lines, including its parent and local check in preliminary and advanced yield varietal trials. Jajai-25/A produced 57% higher paddy yield than its parent Jajai-77 and 33% higher than the check variety Super Basmati. The mutant maintained its yield superiority in the zonal trials in Sindh province and produced 3911 kg/ha in comparison to Super Basmati (2941kg/ha). In the national trials, it exhibited yield superiority and ranked first (4372 kg/ha) at the national level (NURYT-2006).

**Keywords:** Rice, landrace, aroma, quality, mutagenesis

**Introduction**

Rice feeds more people than any other food crops in the world. In Pakistan it is grown on an area of about 2.581 million hectares, producing 5.438 million tonnes with an average yield of 2107 kg per hectare. Rice has emerged as an important commodity for earning foreign exchange (Anonymous 2006-07). Each year, about 1.8 million tonnes of non-scented rice is being produced in Sindh for export. High yielding, non-aromatic rice varieties are cultivated on about 85% of the total arable area in Sindh, while the remaining 15% of the area is covered by traditional tall, scented (Sugdasi) varieties like Sohneri Sugdasi, Sada Gulab, Kangni and Jajai 77, as well as other non-scented varieties.

Mutation breeding provides the means for enhancing the genetic variation and genetic variability may also be created by modulating the effects of mutagens. Mutation techniques have played an important role in the evolution of high yielding varieties of crops, particularly rice (Maluszynski, *et al.*, 1986). Genetic variability induced in rice through gamma rays for selecting new genotypes with improved grain quality and high yield potentials have been documented (Rutger, 1983; McKenzie and Rutger, 1986; Liu *et al.*, 2004). Being indigenous aromatic rice, Jajai-77 possesses good aroma and well

is adapted in Sindh, however, due to tall plant habit, it frequently lodges, has poor grain set and produces low yield. Therefore, the present study was conducted to induce genetic variability through gamma rays in Jajai-77, for the development of new genotypes with relatively shorter plant structure, tolerant to lodging, and having high grain yield potential with in-built good grain quality.

**Materials and methods**

During the year 1997, three different doses (150, 200, 250 Gy of <sup>60</sup>Co source) of gamma rays were used to irradiate pure and dry seeds (14% moisture content) of an indigenous aromatic rice variety, Jajai-77, and popular variety Basmati 370. Irradiated and non-irradiated seeds were sown in nursery beds separately. Seedlings (25 - 30d old) were transplanted in the standing water in the field at a uniform distance of 20 × 20 cm between plant to plant and row to row. The mother panicle of each M<sub>1</sub> plant was harvested. M<sub>2</sub> generation was grown from each panicle separately and mutants with productive traits such as synchrony in flowering, with more tillers per plant, increased panicle fertility percentage, panicle size, thousand grain weight and reduced plant height were selected. Mutants that were confirmed for the mutated attributes in M<sub>3</sub> and M<sub>4</sub> generations were retained and data was statistically analyzed (Steel and Torrie, 1986; Duncun, 1955). Finally, a mutant line Jajai 25/A was selected and tested with other promising mutants of Basmati 370 and Sonehri Sugdasi in replicated fashion in micro, preliminary and station varietal trials at NIA experimental farm, Tando Jam, during the years 2001, 2002 and 2003, respectively.

Further evaluation was done in zonal varietal trials for two years (2004 and 2005) over eight different sites in Sindh for adaptability testing. These trials were designed in a randomized complete block design with three replications at each site in each year with a plot size of 5m × 3m. Data on days to 50% flowering, plant height, number of productive tillers per plant, panicle length, fertile grains per panicle, thousand grain weight and grain yield (kg/ha) were recorded at Tando Jam site. Data for paddy yield (kg/ha) and mean yield performance of genotypes at individual sites was recorded. The data on paddy yield was statistically analyzed to determine the significant differences among the genotypes. On the basis of better yield performance, Jajai 25/A was promoted in national trials during 2006.



## Results and Discussion

### Selection of mutant lines

The mean performance of the mutants evaluated in M<sub>4</sub> generation is shown in Table 1. There were significant differences among the mutants for all the characters except the panicle length. The minimum height was observed in Bas.15-22 (109.0 cm) followed by Jajai 25/A (110.3 cm) and Sada Gulab 15-4 (118.3 cm). This indicates that radiation has induced shortness in plant height. Short stature plants have the advantage to utilize more inputs and possess resistance to lodging as compared to tall ones, hence produce better yield (Baloch *et al.*, 1999; Hu, 1991). The maximum number of productive tillers per plant was found in the mutant Bas.15-22 (17) fol-

lowed by the mutant Jajai 25/A (16), which was significantly more than in the check variety (14). There was no significant difference in panicle length among all the genotypes. Higher numbers of fertile grains per panicle and panicle fertility percentage was observed in the mutant Jajai 25/A, while thousand grain weight was found higher in the mutant Bas.15-5 (23.2 g). Jajai 25/A showed 10-15 days (50% heading) earliness compared to its mother and check varieties. The better yield components and earliness in maturity contributed significantly, and mutant line Jajai 25/A yielded better than all other contesting mutants, parent and check variety, with a yield of 25.7g per plant, followed by Bas.15-22 (23.4g).

Table 1. Agronomic performance of different mutant lines in M<sub>4</sub> generations

Mutants/Varieties	Plant height (cm)	No. of Tillers/plant	Panicle length (cm)	Fertile grains per panicle	Fertility %age	1000 grain weight (g)	50% flowering date	Grain yield/plant (g)
Basmati 370 (P)	162.3 a	12 d	27.8 a	67 e	63 bc	19.6 d	118 a	15.7 gh
Bas-15-5	132.0 b	15 bc	27.5 a	71 de	71 b	23.2 ab	107 bcd	17.5 fg
Bas-15-22	109.0 d	17 a	28.8 a	134 b	81 a	18.9 d	95 e	23.4 b
Bas-213	153.0 a	14 c	27.5 a	71 de	84 a	22.2 bc	114 ab	14.8 h
Jajai 77 (Parent)	158.7 a	12 d	28.8 a	88 c	68 b	22.2 bc	111 abc	18.4 ef
Jajai 77-25/A	110.3 d	16 ab	28.7 a	150 a	89 a	21.9 c	86 f	25.7 a
Sada Gulab (P)	151.3 a	12 d	28.5 a	71 de	57 c	24.0 a	101 de	19.9 de
S.G-15-4	118.3 cd	15 abc	28.4 a	93 c	64 bc	22.3 bc	96 e	22.4 bc
Super Bas (Check)	130.3 bc	14 bc	28.0 a	77 d	81 a	21.4 c	105 cd	21.0 cd

### Micro and zonal yield trials

In the micro yield trial (Table 2) Jajai 25/A produced 4912 kg/ha paddy yield which was more than double than its mother variety (2387 Kg/ha) and 62% higher than check variety Super Basmati (3037 Kg/ha). In a preliminary yield trial, Jajai 25/A yielded 54% and 97% higher

than its mother variety and check variety, respectively. The performance of Jajai 25/A was again outstanding in station yield trials (Table 2). The three years data of the trials revealed that the yield of Jajai 25/A was 182.4% higher than the mother variety and 64.1% than the check variety (Table 2).

Table 2. Performance of different rice mutant lines for paddy yield (Kg/ha) in different varietal trials conducted during 2001, 2002, 2003

Varieties/Mutants	Micro Yield Trial 2001	Preliminary Yield Trial 2002	Station Varietal Trial 2003	Average over three years	% Increase over mother variety	% Increase over check
Basmati 370 (P)	2437	3043	2366	2615		
Bas-15-5	4050	3240	4523	3938	51.6	25.8
Bas-15-22	2137	3433	3948	3172	21.3	1.3
Bas-213	2200	3920	2249	2789	6.7	-10.9
Jajai 77 (P)	2387	3627	1724	1820		
Jajai 77-25/A	4912	5587	4919	5139	182.4	64.1
Sada Gulab (P)	2187	2837	1683	2236		
S.G-15-4	4925	2897	3707	3843	71.9	22.7
Super Bas (C)	3037	2833	3524	3131		

The Sindh province has diversified ecological zones; therefore, genotypes with wide adaptability are required to be identified. Similarly, crop stability is the ability of a crop to exhibit minimum interaction with both, predictable and unpredictable environments (Qayyum *et al.*, 2000; Akram *et al.*, 1999). Varieties that have low  $g \times e$  interaction and have high stable yields are desirable to crop breeders. Therefore, evolution of rice varieties with better stability performance over a wide range of environments will ensure long term plentiful harvests (Fan *et al.*, 2000; Fehr, 1987; Hulmel and Lecomte, 2003). Jajai 25/A along with other advanced mutants, their parents and check variety were evaluated at eight different sites of Sindh in replicated zonal varietal trials during 2004 and 2005 (Table 3). Jajai 25/A maintained its yield over a range of environments in Sindh province and produced 3980 kg/ha, 27.5% and 38% more yield than its parent

and the check variety at Tando Jam site. At Badin site it produced 76% more yield than the mother variety and comparatively similar yield to the check variety. Likewise, the mutant Jajai 25/A produced 154% and 19% more yield than both, the parent and check varieties at Thatta in both years, 2004 and 2005. At Sanghar, Dadu and Larkana sites the mutant Jajai 25/A produced higher yield than the mother and check varieties. At Shikarpur and Jacobabad, Jajai 25/A was significantly better than the mother variety in paddy yield but not found significantly different from the check variety. On average of all the sites, Jajai 25/A ranked first and yielded better with a paddy yield of 3249 kg/ha, which was 114% and 11.84 more than the parent and the check variety, respectively, followed by Basmati 15-14 and Basmati 15-5.

Table 3. Average performance of different rice mutant lines for paddy yield (Kg/ha) in zonal varietal trials conducted during 2004 and 2005

Mutants/ varieties	T.jam	Badin	Thatta	Sanghar	Dadu	Larkana	Shikarpur	Jacobabad	Mean over Genotypes	Rank
Basmati - 370	1827 f	1567 e	1133 f	1353 f	1947 e	2307 d	1013 d	1940 f	1636	10
Basmati -15-5	3247 b	3313 b	2407 cde	2726 c	3033 b	3227 b	2553 ab	2740 c	2906	3
Basmati -15-14	3527 b	3700 a	2720 b	3000 b	3387 a	3420 a	2780 a	3073 b	3201	2
Basmati - 30-2	2427 d	2793 c	2547 bcd	2720 c	2700 c	2813 c	2427 b	2353 de	2598	7
Basmati -1.5-16	2807 c	3167 b	2613 bcd	3253 a	2553 d	3233 b	1940 c	2613 cd	2772	6
Basmati -2.0-11	2080 ef	2660 c	2347 de	2360 d	2080 e	2600 c	2047 c	2280 e	2307	9
Jajai-77-P	1060 g	1880 d	1107 f	1240 f	1567 f	2033 d	1160 d	2093 ef	1518	11
Jajai-15/B	3320 b	3240 b	2653 bc	1927 e	2800 c	3213 b	2573 ab	3220 a	2868	5
Jajai – 25/A	3980 a	3313 b	2816 a	3013 b	3420 a	3527 a	2613 ab	3307 a	3249	1
Jajai-30-2	2173 de	2618 c	2120 e	2580 cd	2767 c	3253 b	2100 c	2320 de	2491	8
S. Basmati (c)	2880 c	3360 b	2373 cde	2747 c	2727 c	3433 a	2480 ab	3240 a	2905	4

Means followed by the same letters are not significantly different from each other at 5% level

### National yield trials

The ecological zones where rice is cultivated differ widely in temperature, rainfall and other environmental factors. Such diversified environments demand the extensive testing of newly evolved varieties in all the rice growing zones (Lafitte and Courtois, 2002; Singh and Payasi, 1999; Yang *et al.*, 2001). The multi-environmental National Uniform Yield Trials (NUYT) are a vital link between genetic improvements and the production environments. Candidate lines contributed by rice breeders of the country are tested for their yield, adaptability, disease and insect pest reaction in different rice growing ecologies. The data generated from these trials serves as the basis for recommendations of the Variety Evaluation Committee (VEC) to provincial and national seed councils, regarding the approval and release

of new varieties. Jajai 25/A was sent to NUYT to evaluate its yield performance and adaptability testing in different rice growing ecologies of the country. Results revealed that Jajai 25/A produced better yield than all the mutants at all locations. It yielded 54.5% more yield than the check variety at Jamra followed by 42.3%, 38.7% and 18.0% at Bhimber, Larkana and Kala Shah Kaku, respectively. The mean yield over all the sites indicated that the Jajai 25/A has produced a yield of 4372 kg/ha and ranked first among all the contesting candidates and check varieties in the NUYT (Table 4).

### Conclusion

The high yielding rice mutant Jajai 25/A with fine grain has been developed from a land race Jajai 77 through gamma rays (250 Gy). It has delivered consistently better

yield as compared to its parent and other promising mutants and the commercial check variety Basmati Super. Thus mutant Jajai 25/A has potential for release as a new variety for general cultivation. It will significantly con-

tribute towards national agriculture production and improve the socio-economic conditions of the farming community of Pakistan, especially in Sindh.

Table 4. Performance of aromatic candidate rice varieties in National Uniform Rice Yield Trials - 2006

Designation	Mingora	Bhimber	Sialkot	Gwl	KSK	Faroqabad	Fsd	D.I.Khan	Bwp	Osta M.	Dokri	Larkana	Jamra	T.Jam	Average
PARC-278	X	3045 b	4967 b	6067	3685 abc	3460 abc	5769 ab	1715 bc	3580 f	4522	5944 a	3611 abcde	4571 cd	4324 c	4250
PARC-279	4427 a	3360 b	3967 cd	5640	3234 abc	3453 abc	5279 abc	1619 bcd	4320 a	5255	5722 a	3066 cde	5729 ab	4011 cde	4220
PARC-280	X	4075 a	5833 a	4973	3627 abc	2900 c	6366 ab	1593 bcd	4210 ab	4688	4944 ab	3111 cde	2112 f	4153 cde	4045
PARC-281	X	3135 b	4400 cd	4240	2509 e	3060 bc	3879 c	678 e	3970 cd	5100	4916 ab	4575 a	5092 abc	3384 f	3764
PARC-282	5382 a	3112 b	5033 b	4440	1483 f	2578 c	4806 bc	2715 a	4074 bc	5178	5639 a	4377 a	4861 bc	3931 de	4114
PARC-283	X	2913 bc	3900 cd	5667	3774 ab	3067 bc	5278 abc	863 de	3977 cd	5233	5444 a	2944 de	5208 abc	4231 cde	4038
PARC-284	2191 a	3191 b	5433 ab	4300	3160 bc	X	6399 ab	2334 ab	3810 e	4893	5227 a	4222 ab	5034 abc	4700 b	4222
<b>PARC-285 (Jajai 25/A)</b>	<b>X</b>	<b>3191 b</b>	<b>4123 cd</b>	<b>5080</b>	<b>3667 abc</b>	<b>2973 bc</b>	<b>6694 a</b>	<b>1108 cde</b>	<b>3892 de</b>	<b>5033</b>	<b>5833 a</b>	<b>4144 abc</b>	<b>5902 a</b>	<b>5202 a</b>	<b>4372</b>
PARC-286	X	2203 cd	5883 a	5387	3873 a	4073 ab	5740 ab	1481 cde	4147 bc	5199	5388 a	3244 bcde	3877 de	4946 ab	4264
PARC-287	4422 a	2735 bcd	4117 cd	3473	3694 abc	4193 a	5878 ab	1278 cde	4106 bc	4666	5472 a	3700 abcde	3761 de	3880 e	3955
PARC-288	4338 a	2122 d	3683 d	4327	2227 e	4360 a	5268 abc	1593 bcd	3570 f	4674	4122 b	4011 abcd	3819 de	3980 de	3721
PARC-289	4145 a	2801 bcd	4307 cd	3140	2554 de	3013 bc	5989 ab	1696 bcd	3320 g	4400	5694 a	2833 e	3108 e	4822 b	3701
PARC-290 S. Basmati	X	2243 cd	5817 a	4887	3107 cd	2880 c	6418 ab	1874 bc	4220 ab	5022	5666 a	2988 de	3819 de	4995 ab	4148

Figures followed by the same letters are not significantly different at 5% level by DMR test.

X = Not matured

## References

- Akram M., Ali S.S. and Abassi F.M. 1999. Genotype-environment interaction and stability parameters for paddy yield. Pak. J. Sci. and Ind. Res., 42(5): 279-281.
- Anonymous. 2006-07. Agricultural Statistics of Pakistan, Ministry of Food, Agriculture and Livestock, Govt. of Pakistan, Islamabad.
- Baloch A.W., Soomro A.M., Mustafa G., Bughio M.S. and Bughio H.R. 1999. Mutagenesis for reduced plant height and high grain yield in Jajai 77, an aromatic rice (*Oryza sativa* L.) variety. Pak. J. Bot., 31: 469-474.
- Duncan D.B. 1955. Multiple range and multiple F test. Biometrics 11:1-42.
- Fan L.J., Ming H.B. and Xing X.D. 2000. A method for selecting locations in rice cultivar trials. Chinese J. Rice Sci., 14(1):58-60.
- Fehr W.R. 1987. Genotype x environment interaction. In: Principles of Cultivar Development, Theory and Technique (vol.1). Macmillan Publishing Company, New York, pp. 247-258.
- Hu C.H. 1991. Use of induced semi-dwarfing gene to alter the rice plant type and cultural breeding practices for sustainable agriculture. In: Plant Mutation Breeding for Crop Improvement. Vol.1, IAEA, Vienna, pp. 167-172.
- Huilmel M.B. and Lecomte C. 2003. Effect of environmental varieties on genotype x environment interaction of winter wheat. Crop Sci., 43:608-617.
- Lafitte H.R. and Courtois B. 2002. Interpreting cultivar x environment interactions for yield in upland rice. Crop Sci., 42:1409-1420.
- Liu L., van Zanten. L.Q.Y., Shu Q.Y. and Maluszynski M. 2004. Officially released mutant varieties in China. Mutation Breeding Review 14:26-41.
- Maluszynski M., Micke A. and Donini B. 1986. Genes for semi-dwarfism in rice induced by mutagenesis. In: Rice Genetics, IRRI, Manila, Pp: 729-737.

- McKenzie K.S. and Rutger J.N. 1986. A new semi-dwarf mutant in a long grain rice cultivar. *Crop Sci.*, 26: 81-84.
- Qayyum A., Mufti M.U. and Rabbani S.A. 2000. Evaluation of different genotypes for stability in yield performance. *Pak. J. Sci. and Ind. Res.*, 43(3):188-190.
- Rutger J.N. 1983. Applications of induced and spontaneous mutation in rice breeding and genetics. *Advances in Agronomy*, 36: 383-413.
- Singh A.K. and Payasi S.K. 1999. Stability assessment in early duration genotypes of rice. *Crop Res.*, 18(3): 433-436.
- Steel R.G.D. and Torrie J.H. 1986. *Principles and Procedures of Statistics*. 2<sup>nd</sup> ed. McGraw-Hill, New York.
- Yang C.I., Jun Y.S., Pyeong J.Y., Chune C.H., Boum S.Y. and Yang C.I. 2001. Genotype x environment interaction of rice yield in multi-location trials. *Korean J. Crop Sci.*, 46(6): 453-458.



*Mutant Varieties***Binachinabadam-4: A High Yielding Mutant Variety of Groundnut with Medium Pod Size**

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**Abstract**

With a view to develop a high yielding variety of groundnut with medium sized pod and kernel, 1000 seeds of the local popular cultivar Dacca-1 with smaller pod size were irradiated with 200 Gy gamma rays in 1997. At maturity, all M<sub>1</sub> plants were harvested and pods were kept separately. During M<sub>2</sub>– M<sub>4</sub> generations, selection was conducted following high mean and high/low variances compared to the check Dacca-1. Fourteen true breeding lines were selected in a preliminary yield trial at M<sub>5</sub> generation, based on pod size and yield performance. Based on the same criteria, in an advance yield trial, only 8 mutant families were further selected. From zonal yield trials, 3 top yielding mutant lines were selected. Of these three mutant lines, D<sub>1</sub>/20/3M-30 produced the highest average yield with pod size significantly bigger than the parent variety but smaller than Binachinabadam-2, a variety with bold pod size, found in both, farmer's field and on-station trials. D<sub>1</sub>/20/3M-30 has been registered as Binachinabadam-4 for commercial cultivation in 2008.

**Keywords:** groundnut; Binachinabadam-4; mutant variety; Gamma ray irradiation; yield trial

**Introduction**

Groundnut (*Arachis hypogaea* L.) is the second most important oil seed crop in Bangladesh following mustard/rapeseed, with an annual production of 130,000 metric tons (AIS, 2009). Its seeds contain 45-56% high quality edible oil, 25-30% proteins (Savage *et al.*, 1994) and 20% carbohydrate together with vitamins E and B. A pound of peanuts provides food energy that is equivalent to 2 pounds of beef, 9 pints of milk or 36 medium sized eggs (Woodroof, 1983). Being a multipurpose crop, it can help alleviate vegetable oil and protein shortages in the country. Apart from its use as manure, its oil cake is excellent cattle feed. Groundnut can also fix atmospheric N<sub>2</sub> @ 80-160 kg/ha through its root nodule bacteria and helps to maintain sustainable crop production and friendly environment (Lee, 1998). Recently, it was discovered that its oil is free from cholesterol. Groundnut varieties with bold sized pod and kernel take longer periods to mature, need consequently more seeds per unit area and have lower shell out percentage than the smaller one. Thus, this study was initiated to develop high yielding varieties of groundnut with medium sized pod. Genetic improvement of any yield attributes, both qualitative and quantitative in nature, has been successful

through this technique (Shaikh, 1991; Mia *et al.*, 1996; Begum *et al.*, 1997; Das *et al.*, 1995; Shamsuzzaman *et al.*, 1998; Hamid *et al.*, 2006). The present efforts of mutation application on groundnut were therefore undertaken with a view to developing high yielding groundnut variety with medium sized pods.

**Materials and methods**

One thousand seeds of a local popular cultivar Dacca-1 were irradiated with 200 Gy gamma rays. Treated seeds of Dacca-1 were immediately sown at BINA farm, Mymensingh, in November 1997. At maturity, M<sub>1</sub> plants were harvested and dry pods were kept separately for growing M<sub>2</sub> population. M<sub>2</sub> progenies were evaluated following augmented design. In this generation, ten plants with competitive yields from each and every M<sub>2</sub> progenies, including the check varieties, were recorded. Sixty-two progenies that produced significantly higher numbers of pods and pod yield than the checks were selected. The exact same procedure of selection was practiced in M<sub>3</sub> generation. A preliminary yield trial was carried out with 24 M<sub>5</sub> true breeding mutant families, of which 14 were selected. These true breeding M<sub>6</sub> mutant families were then put into advanced yield trials in 2002-2003 at BINA farm, Mymensingh, and BINA sub-station farm, Ishurdi, Pabna. Based on yield performance and earliness, 8 top yielding mutant families were further selected for zonal yield trials the following year. From the zonal trials, three top yielding mutant lines were selected for on-station and on-farm trials. In all following yield trials, spacing, fertilizer doses, cultural and intercultural operations, and data collection methods were the same. Spacing between plants and rows were 15cm and 30cm, respectively. Fertilizers were applied following the Fertilizer Recommendation Guide (BARC, 2005) during final land preparation. Recommended cultural and intercultural operations were also followed. Data on plant height, number of primary branch and pod, pod yield per plant and 100-pod weight were gathered after harvested from 10 randomly selected competitive plants.

**Preliminary yield trial**

This experiment was carried out with 23 M<sub>5</sub> mutant families and their parent Dacca-1 at BINA headquarters farm, Mymensingh, and Ishurdi sub-station farm, during 2001-2002, following RCB design with three replications. A unit plot size was 2m × 1m. Pod yield was recorded from

an area of 1.26 m<sup>2</sup> from each of the plots and weighed after being properly sun dried. It was then converted to pod yield per hectare. Finally, all the recorded data underwent proper statistical analysis and are presented in Table 1.

#### *Advanced yield trial*

This experiment was carried out with 12 M<sub>6</sub> mutants and 2 check varieties at BINA headquarters and Ishurdi sub-

station farms during 2002-2003, following RCB design with 3 replications, and set on 28 and 21 November 2002, respectively. A unit plot comprised 11 rows of 1.8m length and was separated from each other by 50cm. Pod yield was recorded from an area of 1m<sup>2</sup> from each of the plots and weighed after being properly sun dried. Then it was converted to pod yield per hectare. Finally, all the recorded data underwent proper statistical analysis and is presented in Table 2.

Table 1. Means of pod yield attributes of 23 M<sub>5</sub> mutants of groundnut

Mutants/check	Plant height (cm)	Primary branch/plant (no.)	Pod/plant (no.)	Pod weight/plant (g)	100-pod weight (g)	Pod yield/ha (kg)
D <sub>1</sub> /20/3M-5	34.10	6.90	30.53	22.12	72.74	2657
D <sub>1</sub> /20/3M-8	37.13	6.03	31.00	28.07	90.81	3210
D <sub>1</sub> /20/3M-9	42.73	5.93	29.04	19.07	65.89	2322
D <sub>1</sub> /20/3M-13	38.27	6.50	28.33	19.05	67.25	2284
D <sub>1</sub> /20/3M-17	37.70	5.80	32.60	20.40	62.63	2532
D <sub>1</sub> /20/3M-22	38.80	6.77	37.70	23.36	62.18	2753
D <sub>1</sub> /20/3M-24	32.87	5.73	32.70	22.55	68.96	2871
D <sub>1</sub> /20/3M-30	37.03	6.57	39.10	28.18	72.08	3335
D <sub>1</sub> /20/3M-31	40.63	5.93	32.30	21.89	67.79	2455
D <sub>1</sub> /20/3M-33	38.60	6.33	35.47	22.21	62.64	2815
D <sub>1</sub> /20/4M-37	36.63	6.17	31.53	19.44	61.68	2458
D <sub>1</sub> /20/3M-38	39.20	6.27	30.47	21.16	69.55	2944
D <sub>1</sub> /20/3M-43	39.17	6.23	30.80	24.55	79.71	2871
D <sub>1</sub> /20/4M-9(1)	38.73	6.60	32.63	18.56	56.99	2288
D <sub>1</sub> /20/4M-37(1)	36.70	5.73	28.93	18.08	62.53	1995
D <sub>1</sub> /20/4M-37(2)	35.80	5.90	32.30	18.09	55.95	2249
D <sub>1</sub> /20/4M-116(1)	35.67	5.80	29.13	19.41	66.61	2136
D <sub>1</sub> /20/4M-132(1)	29.53	5.10	26.33	18.45	70.11	2067
D <sub>1</sub> /20/4M-315(1)	33.87	5.30	27.17	18.88	69.30	2196
D <sub>1</sub> /20/4M-35(1)	36.93	5.70	35.70	21.06	59.13	2025
D <sub>1</sub> /20/4M-41	36.17	5.57	28.17	21.28	75.60	2567
D <sub>1</sub> /20/4M-116	37.33	6.23	26.63	18.94	71.04	2664
D <sub>1</sub> /20/4M-117	37.57	6.07	33.90	20.37	60.13	2013
Dacca-1(Check)	38.40	6.50	30.07	18.85	62.68	2003
LSD <sub>(0.05)</sub>	4.50	NS	2.22	2.48	9.06	182

Table 2. Means of pod yield and yield attributes of 14 M<sub>6</sub> mutants of groundnut

Mutants/check	Plant height (cm)	Primary branches/plant (no.)	Pods/plant (no.)	Pod weight/plant (g)	100-pod weight (g)	Pod yield/ha (kg)
D <sub>1</sub> /20/3M-8	44.5	4.5	13.6	8.1	69.5	1749
D <sub>1</sub> /20/3M-13	42.6	4.8	15.9	9.1	67.1	1948
D <sub>1</sub> /20/3M-22	43.9	4.5	16.6	9.1	62.0	1496
D <sub>1</sub> /20/3M-24	40.7	4.8	16.7	9.7	61.3	1820
D <sub>1</sub> /20/3M-30	41.2	4.9	17.4	10.3	60.6	1962
D <sub>1</sub> /20/3M-31	45.4	4.8	16.4	9.5	62.0	1629
D <sub>1</sub> /20/3M-33	42.6	4.8	19.4	11.1	63.0	2089
D <sub>1</sub> /20/3M-38	43.8	4.8	18.9	10.7	62.1	2133
D <sub>1</sub> /20/3M-43	43.4	4.9	15.9	9.4	68.5	1976
D <sub>1</sub> /20/4M-9(1)	44.5	4.6	17.2	10.5	57.3	1787
D <sub>1</sub> /20/4M-41	46.6	4.8	19.4	11.8	66.1	2182
D <sub>1</sub> /20/4M-116	43.8	4.7	17.1	10.1	61.5	2034
Binachinabadam-2 (Check)	30.8	4.7	14.8	9.5	73.1	2013
Dacca-1 (Check)	46.1	4.6	15.8	10.0	62.1	1695
LSD <sub>(0.05)</sub>	4.0	NS	4.1	2.5	6.8	479.4

### Zonal yield trial

This experiment was carried out with eight M<sub>7</sub> mutant lines and two check varieties at three locations for selecting high yielding, disease/insect-pest resistant/tolerant mutants. Unit plot sizes of 4.0m × 3.0m were used in each location. Seeds were sown on 17 and 24 December 2003 and 1 January 2004 at BINA farm, Mymensingh,

Ishurdi and Magura, respectively. Pod yield was recorded from an area of 5m<sup>2</sup> from each plot after being properly sun dried and finally converted to yield per hectare. All the recorded data underwent proper statistical analysis and is presented in Table 3.

Table 3. Means of pod yield and yield attributes of 14 M<sub>6</sub> mutants of groundnut

Mutants/check	Plant height (cm)	Primary branch/plant (no.)	Pod/plant (no.)	Pod weight/plant (g)	100-pod weight (g)	Pod yield/ ha (kg)		
						Mymensingh	Ishurdi	Magura
D <sub>1</sub> /20/4M-41	61.08	4.35	23.15	16.27	70.83	2394	2865	2668
D <sub>1</sub> /20/3M-38	60.82	4.05	19.17	13.55	70.29	2163	2485	2947
D <sub>1</sub> /20/3M-33	58.37	4.37	22.57	14.92	65.49	2459	2928	3105
D <sub>1</sub> /20/4M-116	62.82	4.47	20.52	15.25	76.67	2512	2977	2631
D <sub>1</sub> /20/3M-43	61.10	4.60	25.85	18.16	70.53	3159	2528	2394
D <sub>1</sub> /20/3M-30	57.98	4.22	24.83	16.77	66.88	2088	3497	3400
D <sub>1</sub> /20/3M-13	61.72	4.62	22.83	17.46	75.40	2059	2946	3047
D <sub>1</sub> /15/62-30	67.22	4.40	24.53	15.90	64.25	2405	3197	2335
Dacca-1	59.13	4.40	23.32	16.66	71.26	2398	3109	3018
Binachinabadam-2	39.70	4.58	16.80	13.29	79.48	2808	2138	3008
LSD <sub>(0.05)</sub>	5.47	NS	2.24	1.29	6.70	216	216	518

### ***On-station and farmer's field trial with M<sub>8</sub> mutants of groundnut***

This experiment was performed with two M<sub>8</sub> mutant lines and an earlier developed mutant, D<sub>1</sub>/15/62-30 at four locations. The cultivar, Dacca-1, and Binachinabadam-2 were also included in this experiment as check varieties. The on-station trials were set at BINA headquarters farm, Mymensingh, and at BINA substation farm, Ishurdi, on 23 November and 11 December 2004, respectively.

Apart from these, two Farmer's Field Trials were set at Haybatpur, Natore and Boda, Panchagar, on 31 January and 12 February 2005, respectively. All the trials followed RCB designs with three replications. A unit plot size was 5.0m × 4.0m. Pod yield was recorded from an area of 6 m<sup>2</sup> from each plot of each location after proper sun drying and finally converted to yield per hectare. All the recorded data underwent proper statistical analysis and is presented in Table 4.

Table 4. Means of yield and yield attributes of M<sub>8</sub> groundnut mutants, averaged over all the locations

Mutants/ Checks	Plant height (cm)	Primary branch/ plant (no.)	Pod/ Plant (no.)	Pod yield/ plant (g)	100 -pod weight (g)	Yield (kg/ha)			
						Mymensingh	Ishurdi	Natore	Boda
D <sub>1</sub> /20/3M-43	48.78	3.94	14.9 8	10.66	71.78	1495	1499	2352	1623
D <sub>1</sub> /20/3M-30	45.56	4.05	18.2 8	13.38	72.47	1055	2134	2528	2745
D <sub>1</sub> /15/62-30	47.44	4.05	18.0 5	12.37	67.92	1538	1617	2410	2166
Dacca-1	45.42	4.13	15.3 3	9.65	62.43	1936	1411	1940	1822
Binachinabadam-2	32.21	3.91	13.5 4	10.28	76.84	1435	1587	2352	1940
LSD(0.05)	2.96	Non- significant	1.50	0.80	8.02		318		

## **Results and discussion**

### ***Performance in preliminary yield trial***

Three mutants D<sub>1</sub>/20/3M-24, D<sub>1</sub>/20/4M-132(1) and D<sub>1</sub>/20/4M-315(1) had significantly shorter plant height than the parent variety Dacca-1. This means that it is possible to generate a dwarf mutant of groundnut by treating its seeds with 200Gy gamma rays. This result is in conformity with that of Hamid *et al.*, (2006), who also developed two dwarf varieties of groundnut by irradiating the seeds of a tall mutant of groundnut with 200 Gy doses of gamma ray. It appears that the number of primary branches/plant did not differ significantly from that of its parent in preliminary, advance, zonal and even in the on-farm and on-station trials (Tables 1, 2 and 3). This means that the primary branch number is the most non-mutated trait to gamma rays, while pod number and pod yield/plant, pod size and pod yield/ha were the most mutated traits, particularly in the preliminary yield trial, although this higher rate of mutation did not appear in the later generations (Tables 1, 2 and 3).

Finally, twelve mutant families, including one dwarf mutant D<sub>1</sub>/20/3M-24 that produced significantly higher pod yield than the check Dacca-1, were selected for advanced yield trial in the following year.

### ***Performance in advanced yield trial***

Two mutant lines, D<sub>1</sub>/20/3M-24 and D<sub>1</sub>/20/3M-30, had significantly shorter height than their parent Dacca-1 in this trial, although the latter had significantly taller height in M<sub>5</sub> and later generations (Tables 1 and 3). This means that with change of location the mutant D<sub>1</sub>/20/3M-30 had no consistency in height. None of the 12 mutants had significantly higher numbers of branches, pods or higher pod yield/plant than the parent, except mutant D<sub>1</sub>/20/3M-8, which had significantly higher pod size, expressed here as 100-pod weight (Table 2). 10 mutant families had produced considerably higher yield/ha than the parent, including D<sub>1</sub>/20/4M-41. Only this mutant had produced significantly higher yield/ha. Seven mutant families were selected for the zonal yield trial in the following year. These were: D<sub>1</sub>/20/4M-41, D<sub>1</sub>/20/3M-38, D<sub>1</sub>/20/3M-33, D<sub>1</sub>/20/4M-116, D<sub>1</sub>/20/3M-43, D<sub>1</sub>/20/3M-30, and D<sub>1</sub>/20/3M-13.

### ***Performance in zonal yield trial***

The mutant D<sub>1</sub>/20/3M-43 had produced the highest yield, followed by D<sub>1</sub>/15/62-30 and D<sub>1</sub>/20/3M-30, sharing equal rank among them. Dacca-1 had a statistically similar rank to most of the mutants, except Binachinabadam-2 and D<sub>1</sub>/20/3M-38. The mutant D<sub>1</sub>/20/3M-38 produced the lowest yield of all the mutants and checks.



At Mymensingh, the mutant D<sub>1</sub>/20/3M-43 had produced the highest pod yield/ha followed by Binachinabadam-2 and D<sub>1</sub>/20/3M-33. However, at Ishurdi, the mutant D<sub>1</sub>/20/3M-30 had produced the highest yield, followed by D<sub>1</sub>/15/62-30.

#### **Performance in on-station and farmer's field trial**

This experiment was performed with two M<sub>8</sub> mutant lines and an earlier developed mutant, D<sub>1</sub>/15/62-30, at four locations. The cultivar, Dacca-1, and Binachinabadam-2 were also included in this experiment as check varieties. The on-station trials were set at BINA headquarters farm, Mymensingh, and at BINA substation farm, Ishurdi, on 23 November and 11 December 2004, respectively. Apart from these, two farmer's field trials were set at Haybatpur, Natore, and Boda, Panchagar, on 31 January and 12 February 2005, respectively. All the trials followed RCB designs with three replications. A unit plot size was 5.0m × 4.0m. Pod yield was recorded from an area of 6m<sup>2</sup> from each plot of each location after being properly sun dried and finally converted to yield per hectare. All the recorded data underwent proper statistical analysis and are presented in Table 4.

Mean yield over all locations was the highest in D<sub>1</sub>/20/3M-30, not significantly different from D<sub>1</sub>/15/62-30. Moreover, this mutant line had significantly bigger pod size than its parent Dacca-1, but smaller than Binachinabadam-2, a variety with bold size pods. The check variety, Dacca-1, had produced the lowest yield. Locations, means of yield and yield attributes are presented in Table 6. The mutant lines/check varieties had shown different responses at different locations for pod number, pod yield/plant and yield/ha. The mutant D<sub>1</sub>/20/3M-30 showed the highest scores for all these three traits at all the locations except in Mymensingh.

#### **Registration of new variety**

The National Seed Board of Bangladesh has registered D<sub>1</sub>/20/3M-30 as Binachinabadam-4 for commercial cultivation by the farmers of Bangladesh in 2008.

#### **References**

- AIS, 2009. Bangladesh Agricultural Information Service, Krishi Diary.
- Begum S.N., Begum S., Ahmed Z.U. and Razzaque M.A. 1997. Selection of promising mutants of mungbean through gamma irradiation. *Bangladesh J. Nuclear Agric.*13:79-82.
- Das M.L., Rahman A., Malek M.A. and Pathan A.J. 1995. Stability and fatty acid analyses or selecting promising mutants of rapeseed. *Bangladesh J. Nuclear Agric.*11:1-8.
- Hamid M.A., Azad M.A.K and Howlader M.A.R. 2006. Development of three groundnut varieties with improved quantitative and qualitative traits through induced mutation. *Plant Mutation Reports* 1(2):14-16.
- Lee H.S. and Lee S. 1998. Introduction to development and characterization of super nodulating soybean mutant, nitrate inhibition of nodulation and nitrogen fixation in super nodulating soyabean mutant *Korean J. Crop Sci.*43(1) :23-27.
- Mia M.F.U., Sheikh M.A.Q. and Ahmad Q.N. 1996. Induction of bold podded and seeded mutants in groundnut (*Arachis hypogaea* L.) and their yield performance. *Bangladesh J. Nuclear Agric.*12:61-71.
- Savage G.P and Keenan J.I. 1994. Relationship between seed character, plant growth and yield parameters of *Phaseolus vulgaris*. *J.Agron. Crop Sci.* 163:105-108.
- Shaikh M.A.Q. 1991. Pulses improvement through nuclear and conventional techniques, In: *Advances in pulse Research in Bangladesh.* (J. Kumar ed.) Proc. 2<sup>nd</sup> Natl. Workshop on Pulses, 6-8 June, 1989, BARI, Joydebpur . pp. 33-41.
- Shamsuzzaman K.M., Hamid M.A., Azad M.A.K. and Shaikh M.A.Q. 1998. Development of early maturing and high yielding cotton genotypes through induced mutation. *Bangladesh J. Nuclear Agric.*14:1-8.
- Woodroof L.G. 1983. *Peanuts Processing and Products* (3<sup>rd</sup> Ed.) AVI publishing, Connecticut.

*Short Communication***Gamma Phytotron: A New Chronic Gamma Irradiation Facility**

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Genetic improvement by chronic irradiation is another important option of mutation breeding technique, especially when a wide array of mutants and minimal growth arrest are needed. Therefore, a chronic irradiation of living plant materials has been favored to induce useful mutants in mutation breeding. Unfortunately, these kinds of facilities are scarce and only a few Asian countries including Japan, Malaysia, and Thailand have operational chronic irradiation facilities such as gamma field, gamma greenhouse, and gamma phytotron, respectively. There are still many factors to consider when operating these types of facilities such as security and management issues. For this purpose, we constructed a new gamma phytotron (Fig. 1) in the Advanced Radiation Technology Institute (ARTI), Korea Atomic Energy Research Institute (KAERI), Jeongeup, Jeonbuk, Rep. of Korea.



Figure 1. The front view of the gamma phytotron

The gamma phytotron in ARTI can be occupied with living pot plants or cultured callus during long periods of chronic irradiation at lower doses (Fig. 2). The ionizing source is  $^{60}\text{Co}$  with the radioactivity strength of about 400 curies. The facility consists of an irradiation room, a non-irradiation room, a glasshouse for acclimation, an operating room, and an office. The total area of the irradiation room is about 104.16m<sup>2</sup>. The target plant materials for a gamma ray irradiation can be arranged from 2m (612.9 mGy/h) to 7m (60.1 mGy/h) from the  $^{60}\text{Co}$  source at present. For safety reasons, the building, where the  $^{60}\text{Co}$  source is located, is surrounded by concrete walls with

1.2m depth and a twofold lead shielded door between the control room and the irradiation room. Moreover, the irradiation room is equipped with two CCD camera systems, which enable an inner situation check of the control room. The irradiation room and non-irradiation control room have automatic control systems for various ranges of temperature (15~35°C), humidity (50~80%) and light condition (maximum 30,000lux), which can be finely set up according to the growth conditions of targeted plants (Fig. 3). The difference of mutagenic effects of the acute and chronic irradiation can be compared using the same treatment dose. To date, we have finished the chronic irradiation of Arabidopsis, rice, tobacco and many ornamental plants to examine the biological effect. Also, microarray experiments were designed to compare expression shift of target genes responding to the acute and chronic irradiation in Arabidopsis and rice. It is expected that the heavy applications of the chronic gamma phytotron in ARTI will be extended to other crops and plants, which are eventually provided to domestic and global communities for mutation breeding and fundamental research.



Figure 2. The inner part of irradiation room in gamma phytotron. Light source can be controlled up and down for the optimum light intensity.

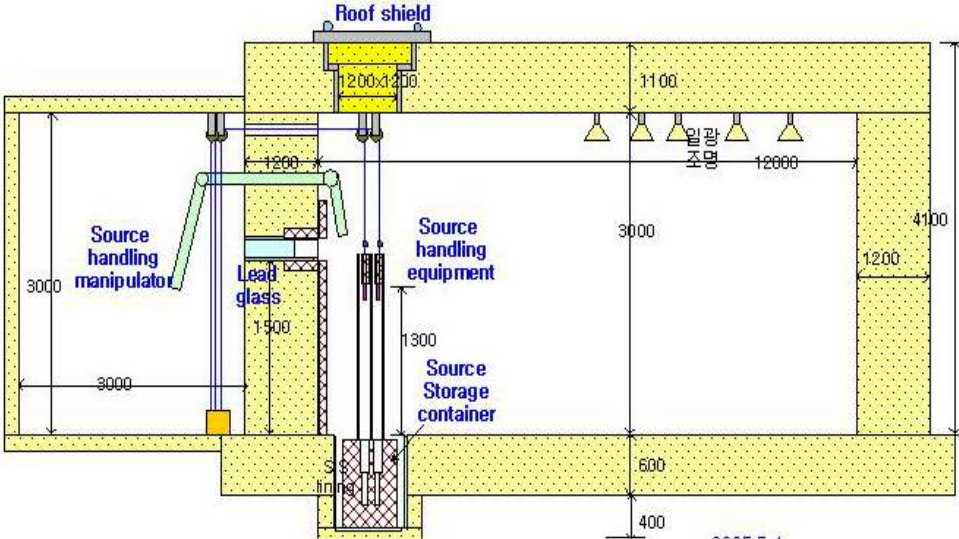


Figure 3. The simplified design of the gamma ray source and manipulation apparatus



*Short Communication***Malaysian Nuclear Agency Gamma Greenhouse**

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The irradiation facility is located in a slope valley and is surrounded by areas classified as alert zone, safe zone and clear zone (Fig. 1a, b). The facility comprises of an open topped irradiation area 30 meters in diameter (Fig. 1c). The central portion is covered with a translucent polycarbonate roof with no biological shielding effect. The irradiator is centrally located and is fitted with a sky shine shield directly above the exposed source. The sky

shine hood incorporates a water deluge system, which may be connected to the fire detection system. The 30-meter diameter irradiation area is protected by a partial concrete wall with an entry maze and the site topography. This irradiation area is protected by a sophisticated interlock system, which only allows the source to be exposed when all the prerequisite safety conditions are met, and automatically returns the source to the safe storage position if any safety device is compromised.



Figure 1. Malaysian nuclear agency gamma greenhouse a) Schematic view of the facility with different safety levels, b) birds view of the facility and surrounding area, c) a full view of the gamma greenhouse.



The irradiator utilizes lead encased in steel for the biological shield when in the safe storage position. The steel jacket is designed to maintain the integrity of the lead in the event of a severe fire. The main irradiation area is further protected by a 300-metre diameter exclusion zone, which is also protected by the safety interlock circuit. The source is only exposed when the entire 300-meter diameter site is free from personnel. The irradiation source is double-encapsulated 800 Ci RSL6050 Caesium-137 Source Pencil. The entry exit maze is fitted with an override system, which allows a person trapped within the maze to open the gate. This action automatically breaks the safety interlock circuit causing the source to automatically return to the safe storage position. The facility also incorporates an emergency refuge area close to the normal entry exit gate at the 15-meter diameter point. This provides a safe area for any person trapped in the facility.

### **Description and assessment of dose rate**

Dose rate assessments have been carried out around the shielding wall and above the sky shine shield. Dose rates have also been calculated at the nearest access point to the source in the exposed position, which is at the fence situated 150 meters from the source. The design of the irradiation facility, known as PPS/Cs800/021 is shown in the Fig. 1. The design is an open topped facility with the irradiator positioned centrally. The nearest access point, when the source is in exposed position, is 150 meters from the source. Dose assessments are carried out for three positions: 1) on the outer face of the concrete biological shield, 2) immediately above the sky shine hood, and 3) at the 150-metre point, which is the closest access point when the source is in the exposed position. The dose assessment is calculated at 2 meters above ground level. The biological shield was designed to ensure the safety of personnel at the 150-meter barrier fence. The design will ensure the radiation dose level at the 150-meter fence and in all areas that are normally occupied during operation that this panoramic irradiator will not exceed 0.02 millisievert (2 millirems) per hour.

Calculation 1: Dose rate on the outside face of the concrete biological shield has been calculated to <math><0.011734</math> micro-sieverts per hour.

Calculation 2: Dose rate immediately above the sky shine hood been calculated to be <math><0.004813</math> micro-sieverts per hour.

Calculation 3: Dose rate at the 150-meter diameter fence has been calculated to be <math><0.000718</math> micro-sieverts per hour.

### **Main function and applications**

#### *Function of the facility*

The facility can be utilized 1) to conduct research activities in mutation techniques using chronic irradiation for crop improvement, especially in producing new mutant varieties with desired characters such as high yield, resistance to abiotic and biotic stress, and improved quality traits, which are highly potential to be commercialized; 2) to establish a centre for the collection and storage of database of radiation response of the tropical and subtropical crops, with special references to fruit trees, ornamentals, vegetables, landscaping plants, herbal and medicinal plants, forest trees, mushrooms and also microorganisms such as bacteria and fungi; 3) to be the Training Center for Nuclear Technology in Agriculture in Malaysia to enable plant breeders and scientists to understand radiation technology and mutation techniques and to be able to apply these techniques successfully in their plant breeding programs; 4) to establish Nuclear Malaysia as a 'One-Stop Irradiation Centre' for providing gamma irradiation services to plant breeders and scientists in Malaysia as well as in the ASEAN Region.

The ultimate goals of the Center are to transfer the technology, disseminate useful information and release beneficial new mutant plant varieties for the farmers and end-users.

#### *Practical Application*

In order to achieve high rate of mutations, it is proposed that agriculture samples be exposed to chronic gamma irradiation for 20h/day. For this facility, irradiation exposure will start from 12.00 pm until 08.00 am the following day. The source will be shut off for 4 hours (from 08.00 am to 12.00 pm) for sample preparation before irradiation and maintenance work. This irradiation facility will be used for chronic irradiation of a wide variety of agricultural and biological samples such as seeds, bulbs, cuttings, pot plants, tissue culture materials and also other samples such as bacteria and fungi. In such cases, samples will be exposed to low dose gamma radiation over long periods of time (hours, weeks, months or even years), depending on the nature and sensitivity of the samples. Exposures that are continued over long periods of time (usually weeks, months or years) are referred to as chronic. Exposures delivered in minutes or a few hours are referred to as acute.

***Radiation sensitivity of plants towards chronic gamma irradiation***

Crop species are listed according from the least sensitive to the most sensitive:

- Grass family: such as rice, corns, etc (placed close to the source)
- Broad-leaf ornamental plants
- Broad-leaf tree plants
- Forest species (placed furthest from the source)

Crop plants with big genome are the most sensitive and plants with small chromosomes are less sensitive to-

wards gamma irradiation. Example, Lily plant (ornamental) has big genome, therefore most sensitive and Chrysanthemums are considered as moderately sensitive. In addition, plants with high ploidy level have high tolerance towards irradiation. Types of tissue culture materials to be irradiated usually depend on the types of characteristics to be improved. For example, if the colour of the flower is to be changed, then flower parts (petals) should be tissue cultured and irradiated and the same principle applies to leaves.

## Short Communication

## Gamma Radiation Facilities at the Institute of Radiation Breeding

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## Introduction

It was estimated that more than 20 gamma fields had been established all over the world including the Brookhaven National Laboratory, the largest one in the USA (Kawara, 1969). In Japan in 1959, the construction of gamma field was launched and the Committee for the Establishment of the Institute of Radiation Breeding was put together to discuss the building location site, structure and the irradiation source, etc. The committee members consisted of university professors, researchers from the National Institute of Agricultural Science, Riken and the National Institute of Genetics. The committee also included researchers from the Architecture Research Institute and the Japan Physico-Chemistry Institute, who considered the structure's safety against strong earthquakes estimating the amount of scattered radiation of the Gamma Field by conducting field experiments. As a result, Japan's Gamma Field structure, irradiation mechanism, shape of the source, is different from those of the other countries with respect to the changes of exposure rates and energy of gamma irradiation (Kawara, 1969).

The Institute of Radiation Breeding (IRB) has been engaged in the development of new crop strains through mutation induction, and has been conducting research into more efficient methods for inducing mutation. While working on contribution towards breeding of new varieties of various seed-propagated, vegetatively propagated and woody crops, the IRB is also involved in the development of mutant resources for genomic analysis and new technologies for plant breeding, including the elucidation of gene expression mechanisms in mutants. The IRB provides irradiation services and cooperative research at the request of universities, private industries, prefectural experiment stations, and incorporated agencies of the Ministry of Agriculture, Forestry and Fisheries in Japan.

## Irradiation facilities

The following radiation sources exist in the IRB: Gamma Field and Gamma Greenhouse for chronic irradiation for growing plants, and Gamma Room for acute irradiation.

*Gamma field*

It is now the world's largest radiation field for plant breeding (Fig. 1): 100 m radius (31,400 m<sup>2</sup>) with 88.8 TBq <sup>60</sup>Co source at the center and surrounded by a shield-

ing dike 8 m high, so that primary radiation can be cut-off to the outside of the field (Fig. 2). The facility is utilized for the chronic irradiation (8 hours a day - Monday to Friday night) for various crops, such as fruit trees, mulberry trees, tea trees, flowers, upland crops, rice etc. The facility is mostly useful for the irradiation of large plants.

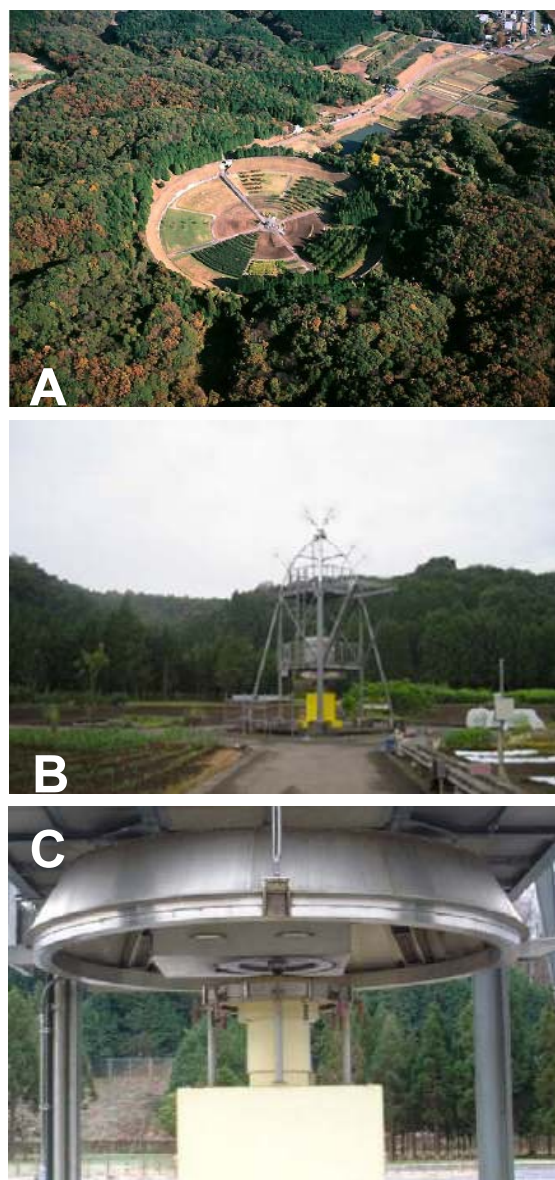


Figure 1. The gamma field in the Radiation Breeding Institute, Japan. A) The location of the gamma field B) Irradiation tower of the gamma field C) Its shielded box of <sup>60</sup>Co





Figure 2. A) A building of gamma room for acute irradiation. B) Irradiation to seeds C) to *in vitro* materials D) and to plants. (Photos courtesy of Toshikazu Morishita, IRB, NIAS)

Since 1962, the  $^{60}\text{Co}$  source has been renewed every 2-years. At the outset of the operation, a daily irradiation of 20 hours (from 12 a.m to 8 a.m. of the following day) was conducted. Currently, the daily irradiation time has been reduced to 8 hours (from 12 a.m. to 8 p.m.). Furthermore and as a result of the recommendation of the International Commission on Radiological Protection (ICRP), which was made in 2001, restricted areas have been extended. Now, the irradiation dose of the gamma ray at the nearest point of the field (10m from the  $^{60}\text{Co}$  source) is about 2 Gy/day, which is almost 300,000 times to the natural condition. This means the total dose for 1 000 years under natural condition is applied to the plants in one day. And the furthest point (100 m from the center) is approximately 2 000 times to the natural condition.

#### **Gamma room**

The Gamma Room, a square room of 54 m<sup>2</sup> (6m × 9m), is located in a shielded building with a 44.4 TBq  $^{60}\text{Co}$  source (Fig. 2). It was constructed in 1966 and used for acute irradiation of seeds, bulbs, tubers scions, small plants and *in vitro* materials. As it is a shielded building, one can walk around the building during irradiation. Its  $^{60}\text{Co}$  source has been renewed every 4-years.

#### **Gamma greenhouse**

The Gamma Greenhouse is an octagonal greenhouse (150 m<sup>2</sup>) of 7 m radius with a 4.81 TBq  $^{137}\text{Cs}$  source (Fig. 3).

It was constructed in 1964 and has been used for the chronic irradiation of tropical and subtropical crops, such as sugarcane, pineapple and banana etc., throughout the year as the temperature inside of the greenhouse is controlled at ca. 20°C in winter.



Figure 3. Gamma greenhouse

#### **References**

Kawara K. (1969) Structural characteristics and the exposure rates in the Japan's Gamma Field, Gamma Field Symposia, 6: 1-18.



# New FAO/IAEA Database of Mutant Varieties and Genetic Stocks

Welcome to our new FAO/IAEA Database of Mutant Varieties and Genetic Stocks! At the moment, we just completed construction of the part for Mutant Variety Database, which is still in the process of information updating. We will add the other part for Mutant Genetic Stocks in due time. The new database has improved over

the FAO/IAEA Mutant Variety Database in many ways. We are working to make the new database as the global information source of mutant varieties and mutant genetic stocks, as well as activities and events related to plant mutation breeding and research.



The key feature of the database is that you can register your mutant varieties from your desktop. For this purpose, you need first register an account; then you will be authorized to submit or edit a mutant variety.

We would greatly appreciate your support by registering your mutant variety in our database. Once the variety is registered, it will have its own 'homepage' (see below). Therefore, you can use it as an important platform to

showcase your new varieties (The introduction of this variety may be shown in local language).

Please visit the website <http://mvgs.iaea.org> and send us your valuable suggestions and comments regarding the structure and content of this database. Please also send us other information, related to plant mutation breeding and mutant varieties, genetic stocks; we may post them on the website.



**YOU MAY STILL SEND US INFORMATION ON YOUR MUTANT VARIETY AND WE WILL UPLOAD THEM INTO THE SYSTEM, IF IT IS DIFFICULT FOR YOU TO DO SO.**

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Plant Mutation Reports (PMRs) publishes (mini) reviews, short communications and complete research papers in all areas of plant mutation research which focuses on mutagenesis, mutation induction, mutant characterization, and mutant applications. It also publishes description papers on mutant germplasm and mutant varieties. Papers on social-economic impact analysis of induced mutations and mutant varieties are also accepted.

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- Review articles may be organized according to their specific requirements.
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#### Acknowledgements

- Acknowledgements of grants, support etc, should follow the text and precede the references.

#### References

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- Periodicals: Shamsuzzaman K.M. and Shaikh M.A.Q. (1991) Early maturing and high seed yielding chickpea mutant. *Mut Breed Newslett* 37: 4-5.
- Books (edited by someone other than author of article): Maluszynski M. (1990) Gene manipulation in plant improvement. In: Gustafsson J.P. (ed), *Induced Mutations in Plant Improvement*. Plenum press, New York. Pp239-250.
- Books (identical author and editor) van Harten A.M. (1998) *Mutation Breeding, Theory and Practice*. Cambridge University Press, Cambridge, U.K. pp. 237-240.

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- All tables and figures, i.e., photographs, graphs and diagrams should be referred to as either 'Table' or 'Fig.' and be numbered consecutively (1, 2, etc.) in the text.
- In tables, footnotes are preferred over long explanatory material in the heading or table body. Such explanatory footnotes, identified by superscript letters, should be placed immediately below the table.
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#### Units and symbols

The standard SI units and symbols should be used throughout ([www.scenta.co.uk/tcaep/science/siunit/index.htm](http://www.scenta.co.uk/tcaep/science/siunit/index.htm)).

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