CONCURRENT SESSION 9

Induced Mutations in Seed Crop Breeding (1)

Developing Herbicide-Tolerant Crops From Mutations

S Tan* & S J Bowe

Abstract

Several herbicide-tolerant crops have been developed and commercialized from herbicide-tolerant mutants obtained through chemical mutagenesis followed by herbicide selection or direct herbicide selection of spontaneous mutations. All mutations used in commercial herbicide-tolerant crops are derived from a single nucleotide substitution of genes that encode enzymes or proteins targeted by herbicides. The alleles of all commercial herbicide-tolerant mutations are incompletely-dominant except for the triazine-tolerant mutation.

Introduction

Herbicide-tolerant crops in combination with their corresponding herbicides are able to control many weeds that cannot be or are less effectively controlled with other means [1]. Commercial herbicide-tolerant crops developed from herbicide-tolerant mutants include imidazolinone-tolerant maize, rice, wheat, oilseed rape, sunflower, and lentil; sulfonylurea-tolerant soybean and sunflower; cyclohexanedione-tolerant maize; and triazine-tolerant oilseed rape [2].

Development of herbicide-tolerant mutants

Most of the herbicide-tolerant mutants were developed through chemical mutagenesis followed by herbicide selection [1]. Among the chemical mutagens, EMS was the most popular one. Several herbicide-tolerant mutants were also discovered through direct herbicide selection of spontaneous mutations [1]. Although gamma irradiation was also attempted in mutagenesis for herbicide tolerance, no commercial herbicide-tolerance trait has been developed by using this method [3].

Characterization of herbicide-tolerant mutations

All mutations used in commercial herbicide-tolerant crops are derived from a single nucleotide substitution of genes that encode enzymes or proteins targeted by herbicides. Imidazolinone-tolerant maize, rice, wheat, and oilseed rape have a gene variant encoding an altered acetohydroxyacid synthase (AHAS) with the S653N amino acid substitution [1]. Additionally, imidazolinone-tolerant maize and oilseed rape have an AHAS with the W574L amino acid substitution [1]. Imidazolinone-tolerant sunflower has been developed from the A205V AHAS gene mutation [1]. In contrast, sulfonylurea-tolerant sunflower selected from a farm field has an AHAS enzyme variant with the P197L amino acid substitution [4]. Similarly, sulfonylurea-tolerant soybean has a P197S AHAS gene mutation [5]. Sulfonylurea-tolerant sunflower from seed mutagenesis and imidazolinone-tolerant lentil are also derived from AHAS gene mutations [6, 7]. Cyclohexanedione-tolerant maize has an altered acetyl-CoA carboxylase with the I1781L amino acid substitution [8]. Triazine-tolerant oil seed rape possesses a *psbA* gene variant that encodes the D1 protein of photosynthesis with the S264G amino acid substitution [9].

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Incorporation of herbicide-tolerant traits into elite varieties

To confer commercial tolerance to herbicides, some herbicide-tolerance alleles can be heterozygous, others need to be homozygous, and the rest must be stacked with another tolerance gene. The alleles of all commercial herbicide-tolerant mutations are incompletely-dominant and not pleiotropic except for the triazine-tolerant mutation which is inherited maternally and linked with several agronomic traits. The herticide-tolerant trait can be incorporated in elite varieties through crossing of the elite variety with a trait donor.

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Marker-assisted Backcrossing to Incorporate Two Low Phytate Alleles Into the Tennessee Soybean Cultivar 5601T

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Abstract

Development of low phytate soybean is favorable to the environment by reducing phosphorous loads to agricultural lands and surface waters. The trait also provides enhanced nutrition and metabolism for poultry and swine. Our source for the trait was a low phytate germplasm (CX1834-1-2) developed by the USDA and Purdue University through ethyl methanesulfonate (EMS) mutagenesis. The objective of this project was to develop a commercially acceptable, superior quality, high-yielding soybean cultivar with low seed phytate. In order to incorporate the low phytate trait into our regionally adapted soybean cultivar '5601T', we have combined a series of backcrosses with marker-assisted selection (MAS) at each backcross stage. Simple sequence repeat (SSR) markers have enabled us to i) transfer two recessive alleles governing the low phytate trait and ii) identify which specific individual backcross plants had DNA of the greatest commonality with the genome of the recurrent parent 5601T. We utilized two low-phytate SSR markers Satt237 (linkage group N) and Satt561 (linkage group L) for dual marker assisted selection for gene transfer of the low phytate trait. Molecular markers dispersed across the genome proved to be effective for facilitating genome recovery of the high-yielding 5601T recurrent parent every backcross generation. Chemical analyses confirmed that the low phytate trait was inherited in concert with the molecular markers. Thirty three lines homozygous for both low phytate recessive alleles have been planted in 2008 in a yield trial at the East Tennessee Research and Education Center in Knoxville, TN for field evaluation and seed production.

Introduction

The phosphorus in soybean seed is stored primarily as phytic acid or phytate [1]. Development of new cultivars with low phytate is important because phytate is of nutritional and environmental concern. Phytic acid can be anti-nutritional because it is a strong chelator of mineral nutrients and reduces the availability of divalent cations such as calcium, manganese, iron, magnesium and zinc. The phosphorus in phytate is non-bioavailable to monogastric animals such as poultry and swine [2].

Wilcox, *et al.* [3] isolated mutants with high inorganic phosphous (Pi) and low phytic acid (lpa) phosphorus through ethyl methanesulfonate (EMS) mutagenesis. The germplasm CX1834-1-2 was developed from these mutant populations. Through segregation analysis, Oltmans, *et al.* [4] determined the inheritance of low-phytate phosphorous in soybean is controlled by recessive alleles *pha1* and *pha2* with duplicate dominant epistasis; both alleles must be present to obtain low-phytate seed.

Research by Walker, *et al.* [5] indicated that the simple sequence repeat (SSR) markers Satt237 on soybean linkage group N and Satt561 on linkage group L are associated with quantitative trait loci (QTL) for phytate level in soybean. Satt237 was linked to a major locus associated with seed phytate and Satt561 had a smaller effect on seed phytate concentration.

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Our objective was to utilize these markers to facilitate *lpa* trait introgression from the non agronomic germplasm CX1834-1-2 into our regionally adapted and productive cultivar '5601T'.

5601T is a conventional (Maturity Group V) soybean cultivar developed by the Tennessee Agricultural Experiment Station and released in 2001 [6]. It was released because of its high yield throughout broad geographic regions of southern USA. It was the highest yielding line in the USDA Maturity Group V Regional tests for Tennessee and Kentucky for the years 2005, 2006, and 2007. It is currently used as a USDA check cultivar in the Southern Regional Uniform Tests. Because of its yield performance along with its somewhat higher protein content, it was an ideal line for transfer of the low phytate characteristic.

Materials and Methods

DNA Extractions

Three to five leaves were sampled from each plant. One leaf from each sample was pressed onto Whatman FTA® plant cards (Brentford, Middlesex, UK) and the remaining leaves were stored at 80°C. In order to identify double heterozygotes, we extracted samples from FTA cards according to Whatman instructions. Once we had identified the double heterozygotes we used Qiagen Plant DNeasy (Qiagen, Hilden, Germany) extractions for the corresponding stored leaf tissue to obtain a larger quantity of DNA from those individuals which would be submitted to analysis with a larger number of markers.

Polymerase Chain Reaction (PCR) amplification with SSR markers

Song, *et al.* [7] have made linkage analysis and more than 1,000 SSRs available to the soybean research community. These DNA markers serve as genetic landmarks interspersed throughout the 50,000 or more genes contained by the 20 chromosomes or linkage groups of the soybean genome. The markers on this linkage map can be used for marker-assisted selection.

PCR amplification was achieved with a ThermoHybaid multi-block system (Franklin, MA) with the following amplification conditions:

1 cycle	94°C for 5min
35 cycles	94°C denaturation 25sec, 48°C annealing 30sec, and 72°C extension for 30sec
1 cycle	72°C extension for 5min

Reverse primers were synthesized by Sigma Genosys custsvc@sial.com. Forward primers were synthesized by Sigma Proligo oligosupport-us@proligo.com and labeled with WellRed Dye. Our PCR protocol used HotMasterMix containing HotMaster Taq DNA Polymerase (5 Prime, Inc. Gaithersburg, MD)

Capillary electrophoresis

The Beckman CEQ™ 8800 Genetic Analysis System (Beckman Coulter, Fullerton, CA) was used to separate PCR products by capillary electrophoresis. Electropherograms are shown in **Figure 1**.

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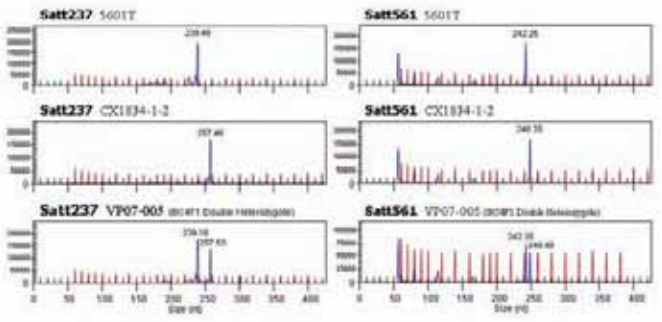

Figure 1 Separation of PCR products by size for the two markers associated with low phytate. The BC4F1 plant 005 is heterozygous for both Satt237 and Satt561.

Table 1. Molecular genetic characterization reveals the underlying genotype at two loci among BC2F1 individuals targeting low phytate soybean development

AXB 2005 BC2F1 PLANT	Satt237 239nt = 5601T 257nt = CX1834-1-2	MSatt561 259nt = 5601T 265nt = CX1834-1-2	GENOTYPE 'A' = pha1 'B' = pha2
18 Plants	5601T	5601T	AABB
17 Plants	5601T	HETEROZYGOTE	AABb
22 Plants	HETEROZYGOTE	5601T	AaBB
19 Plants	HETEROZYGOTE	HETEROZYGOTE	AaBb

Colorometric assays for inorganic phosphate (Pi)

In the low phytate soybean lines developed by Wilcox, *et al.* [3], low phytic acid levels are inversely associated with an elevated level of Pi, which is easier to measure. We use a method of analysis adapted from Chen, *et al.* [8] and described by Raboy [9]. "Chen's reagent" reacts with Pi to produce a blue color, and the intensity of the blue indicates the concentration of Pi in the extract solution. Therefore, samples which produce a darker blue color are from low-phytate seed.

Marker-assisted backcrossing to introgress the low phytate trait

Our backcrossing strategy was to build upon the elite qualities of the recurrent parent 5601T by adding the low phytate trait. CX1834-1-2 was used as the pollen donor in our initial cross. The CX1834-1-2 germplasm was derived from a cross of 'Athow' (normal phytic acid levels) to M153-1-4-6-14, a selection from the *lpa* M153 mutant line by Wilcox, *et al.* [3].

First generation hybrid (BC1F1) plants were grown in a winter nursery in Isabela, Puerto Rico during winter/spring season of 2005. DNA from individual plants was collected and analyzed by capillary electrophoresis with the Beckman CEQ8800. BC_1F_1 individuals were identified from DNA selections to utilize for pollen transfer. Each selected donor contained the low phytate type alleles at both the molecular linkage group (MLG) N Satt237 locus and at the MLG L Satt561 locus in heterozygous form, thereby enabling dual marker assisted selection for gene transfer of the low phytate trait. A colorimetric assay, developed by Raboy, *et al.* [9] was used to measure inorganic phosphorous content among BC1F2 individuals in Knoxville, TN. Results of those tests confirmed that indeed the low phytate trait was inherited in concert with the molecular markers. This was important proof of concept that molecular markers tightly linked to the underlying QTL governing soybean seed phytate can be successfully utilized with a marker-assisted selection approach.

BC_2F_1 hybrids were grown in Knoxville, TN during summer 2005. DNA from each BC_2F_1 individual was isolated and analyzed at the Satt237 and Satt561 loci to identify low phytate genetic transmission. Seventy-six BC_2F_1 plants exhibited allelic segregation patterns at the Satt237 and Satt561 loci consistent with Chi-square expectations for a 1:1:1:1 inheritance pattern of gamete transmission from double-heterozygote parents (**Table 1**). Thus we verified that when molecular marker selection was employed, genetic transfer of the low phytate loci continued to the BC_2 generation.

BC_2F_1 individuals which proved to exist in the double-heterozygous state were screened for an array of polymorphic SSR markers spanning the soybean genome, in order to identify those individuals with greatest recurrent parent genome similarity.

The five best BC_2F_1 individuals, whose genetic similarities with the recurrent parent ranged from 87% to 93% were identified, and used in hybridizations with the recurrent parent to form BC_3F_1 hybrid seeds. Each selected donor contained one copy of the low phytate type allele at both the MLG N Satt237 locus and at the MLG L Satt561 locus, enabling dual marker assisted selection for gene transfer of the low phytate trait to proceed.

DNA analyses of putative BC_3F_1 seeds grown in Knoxville TN in 2006 revealed that a fourth backcross would be needed to recover a strong recurrent parent genome. The four best individuals which were confirmed double heterozygotes for the two low phytate loci and which molecular markers showed simultaneously had the greatest DNA homology with 5601T (recurrent parent) were selected as donors to form the BC_4F_1 generation.

Seeds of soybean plants from earlier generations (BC_1, BC_2, BC_3) of this project were analyzed for phosphorous content in order to identify those that expressed the low phytate trait, following DNA molecular marker assisted trait selection. All generation lines were planted in rows at the East Tennessee Research and Education Center in Knoxville, TN for field evaluation and seed production. Chemical analyses confirmed that the low phytate trait was inherited in concert with the molecular markers.

Table 2. DNA selections identified as heterozygous BC_4F_1 individuals at the Satt237 and Satt561 loci

LINKAGE GROUPS			N	L	A2	D2	F	G	K	L	N	O	O	SCORE
♀	♂	BC4F1	Satt 237	Satt 237	Satt 237	Satt 237	Satt 237	Satt 237	Satt 237	Satt 237	Satt 237	Satt 237	Satt 237	
5601T	91-10	001	HET	HET	0	0	1	0	0	0	0	0	0	1
5601T	91-10	002	HET	HET	0	.	1	1	0	0	.	0	0	2
5601T	91-10	005	HET	HET	0	0	0	0	0	0	0	0	0	0
5601T	91-10	009	HET	HET	0	0	1	0	0	0	.	0	0	1
5601T	91-12	014	HET	HET	0	0	1	0	0	1	0	0	0	2
5601T	91-12	017	HET	HET	1	0	0	0	0	.	0	0	0	1
5601T	91-21	029	HET	HET	0	0	0	0	0	0	0	0	0	0
5601T	91-24	030	HET	HET	0	0	0	0	0	0	1	1	0	2
5601T	90-19	052	HET	HET	1	0	0	.	1	0	0	0	0	2
5601T	90-19	054	HET	HET	.	0	0	0	.	0	0	1	0	1
5601T	90-22	062	HET	HET	0	0	0	0	1	0	0	1	0	2
5601T	90-22	063	HET	HET	0	0	0	0	0	0	0	1	0	1
5601T	90-22	064	HET	HET	0	0	0	0	1	0	0	1	0	2
5601T	90-23	072	HET	HET	0	0	0	.	1	0	0	1	1	3
5601T	90-23	073	HET	HET	1	.	0	0	.	0	0	1	0	2

SCORE: summation over linkage groups, where 1=Heterozygote, 0=5601T allele. We expect plants with the lowest score sum to have retained more of the 5601T genome

Newly created BC_4F_1 hybrid seeds were grown in a lighted nursery in Puerto Rico during the winter of 2006-2007. Leaf tissue was collected from each BC_4F_1 individual plant and DNA extracted to confirm true double-hybrids at the low-phytate loci, using molecular markers Satt237 and Satt561 via capillary gel electrophoresis on our Beckman-Coulter CEQ 8800 genetic analysis system. This enabled identification

of 15 double heterozyotes in the 5601T genetic background. Then, SSR markers (dispersed throughout the genome) which were polymorphic between the recurrent parent and the donor were screened to determine genomic similarity with the 5601T genome (**Table 2**). Two BC_4F_1 plants (Plant 005 from BC_3F_1 pollen donor 91-10 and Plant 029 from BC_3F_1 pollen donor 91-21) showed perfect genetic identity with the recurrent parent genome.

Summary

All 15 BC_4F_1 plants, from the 2006-2007 Puerto Rico winter nursery which were double heterozygotes for the two SSR markers Satt237 and Satt561, were advanced in BC_4F_2 rows grown in Knoxville the summer of 2007. Single plants were pulled from all progeny rows originating from plant 005 and 029 and $BC_4F_{2:3}$ seed was harvested. Three to five seed from each plant was analyzed for phytate by the colorimetric assay. Seeds with high inorganic P and low phytate produced a solution that was dark blue in color. Seeds from these lines were sent to our winter nursery in Homestead Florida for seed increase in the 2007-2008 season. Seed was returned to us as $BC_4F_{2:4}$ lines which are currently in a yield trial at the East Tennessee Research and Education Center in Knoxville, TN.

In summary, the molecular markers Satt237 and Satt561 proved to be effective for dual marker assisted selection for gene transfer of two recessive genes which collectively confer the low phytate trait, as evidenced by chemical analysis of seed phosphorous in selected progeny. The original donor line (CX1834-1-2) is poorly agronomic: it suffers from significant losses in seed germination, flowers and sets pods too early in southern USA latitudes, and produces low seed yields. A strategy was needed to rapidly transfer the donor's low phytate trait to a high-yielding genetic background. Molecular markers dispersed across the genome proved to be effective for facilitating genome recovery of the high-yielding 5601T recurrent parent every backcross generation. By the BC_4 stage we have fully captured the recurrent parent genome while simultaneously confirming the presence of both low phytate loci.

ACKNOWLEDGMENTS

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The Improvement of TAEK-Sagel Chickpea (*Cicer arietinum* L.) Mutant Variety in Turkey

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Abstract

This research is aimed to improve chickpea varieties that are well-adapted to chickpea growing areas, resistant to cold, suitable to machinery harvest type, exhibit high yield and high protein content, bigger seed size, resistance to antracnose and other diseases and pests, and improved quality characteristics. This chickpea breeding project was started with ILC 482, Akçin-91 and AK 71114 parental varieties and eight different gamma radiation dose rates between 50-400Gy were used. After following mutation breeding steps, location experiments started for testing yield and quality characteristics in 2004. According to the results of these experiments two outstanding mutant lines were given for registration. One of them was registrated TAEK Sagel in 2006. In this paper, the yield and quality characteristics of 'TAEK-Sagel' mutant chickpea variety are discussed. It was found that this mutant has 186 kg/da average yield with 23% seed protein content. In addition, its cooking time was shorter than the others (37 minutes).

Introduction

Chickpea is an important legume in Turkey. Turkey is one of the most important legume gene centers in the world [1]. The most widely known characteristics of chickpea are that they are an important vegetable protein source used in human and animal nutrition. The dry seeds of chickpea, have two to three times more protein than traditional wheat. In addition, it has a high carbohydrate content and is valued as a important energy source. Chickpea is also very rich in some vitamins and minerals. In the plant breeding, mutation induction has become an effective way of supplementing existing germplasm and improving cultivars. Many successful examples of mutation induction have proved that mutation breeding is an effective and important approach to legume improvement. The induced mutation technique in chickpea has proved successful and good results have been attained. Realizing the potential of induced mutations, a mutation-breeding programme was initiated at the Nuclear Agriculture Section of the Saraykoy Nuclear Research and Training Center in 1994. The purpose of the study was to obtain high-yielding chickpea varieties with large seeds, good cooking quality and high protein content.

Materials and Methods

Seeds of the local chickpea varieties (Ak-71114, Akcin and ILC482) were irradiated with 0 (Control), 50, 100, 150, 200, 250, 300, 350 and 400Gy of Gamma -rays by using of ^{60}Co source [2-3]. One thousand seeds per treatment were sown in the field for the M_1 generation. At maturity, 3,500 single plants were harvested and 20 seeds taken from each M_1 plant and planted in the following season. During plant growth, mutants of the desired traits (earliness, yield per plant, plant height, first pot height and Ascochyta blight (*Ascochyta rabie*) resistance) were identified and isolated. Two thousand, five hundred and twenty desirable M_2 mutants were selected and grown in progeny rows as the M_3 generation. The protein content was analyzed for the M_3 and M_4 seeds. In the M_5 generation, preliminary yield trials were conducted and after field observation, quality criteria (such as grain size, grain type, cooking time and protein content) were analyzed, and 12 mutant lines were selected. The mutant and control varieties were performed at two locations (Saraykoy and Haymana) for two years (M_7, M_8). Randomized complete block design (RCBD) with three replications was used for field trials. All data of investigated characteristics such as seed yield, grain size, grain type, first pot height, Ascochyta blight (*Ascochyta rabie*), cooking and protein content were analyzed statistically [4]. After these experiments, 2 promising mutant lines were selected and given to the Directorate of Seed Registration and Certification Center for official registration. These two mutants were tested for two years (2004 and 2005), in five different locations of Turkey (Ankara, Esenboga, Haymana, Konya and Eskişehir) by the Seed Registration and Certification Center.

Results and Discussion

Laboratory experiments

The effects of different doses of gamma radiation on seedling height in M_1 are presented **Table 1** and **Fig. 1**.

As compared to the control (untreated), seedling height was reduced in the irradiated material. Depending on the increasing dose rates, the seedling height was reduced from 15,7 cm to 27 cm for ILC482 and from 15,8 cm to 4,7 cm for AK71114. Laboratory experiments showed that the seedling height decreased when treated with increasing radiation doses [5]. The growth reduction doses (GR-50) were determined as 210Gy and 240Gy for ILC 482 and AK71114, respectively [6].

Field experiments

During plant growth in the field, mutants with the desired traits (earliness, yield per plant, plant height, first pot height and Ascochyta blight (*Ascochyta rabie*) resistance) were identified and isolated. About 2,520 desirable M_2 mutants were selected and grown in progeny rows as the M_3 generation. The protein content was analyzed for the M_3 and M_4 seeds. In M_5 generation, preliminary yield trials were conducted and after field observation, and quality criteria (grain size, grain type, cooking, protein) analyzed, and a total of 12 mutant lines were selected [7].

M_7 and M_8 generation were conducted with five promising mutant lines and control varieties (standard), at two locations (Saraykoy and Haymana). The results of these experiments were presented in **Table 2**. TAEK Sağel and TAEK Sağel-10 mutants gave the highest seed yield (about 220 kg/da) in both locations.

Morphological, agronomic and quality characteristics of the chickpea mutant lines are shown in **Table 3** and **Table 4**.

Registration experiments

Seed yield and seed quality results from Directorate of Seed Registration and Certification Center, are given in **Table 5**, **Table 6** and **Table 7**.

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TAEK Sağel and TAEK Sağel-10 mutant lines gave a good performance in Ankara, Konya and Eskişehir. The average seed yield was 186 kg/da for these mutants (**Table 5**).

TAEK Sağel mutant line had 23.2 % protein content. This mutant has 41g dry weight, 85g wet weight, 45g/seed water uptake capacity, 1.15 % water uptake index, 81ml dry volume, 0.47ml/seed swelling capacity and 2.52 % swelling index. Cooking time was lower (37 minutes) than the others (**Table 6**).

In addition, the TAEK Sağel mutant line had a good stability as yield performance in different locations. Error mean square for this mutant was 171.2 and was much lower than the others (**Table 7**).

After two years of registration experiments, one of outstanding mutants was officially released as mutant chickpea variety under the name TAEK SAGEL in 2006.

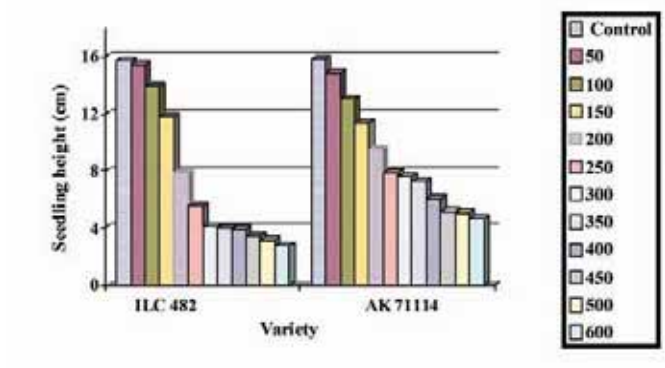

Figure 1 Effect of gamma radiation on seedling height in M_1.

Table 1. Effect of gamma radiation on seedling height in M_1

Chickpea variety	Dose(Gray)											
	Control	50	100	150	200	250	300	350	400	450	500	600
ILC 482	15,7	15,5	13,9	11,8	7,9	5,5	4,1	3,9	3,9	3,4	3,1	2,7
AK 71114	15,8	14,9	13,0	11,46	9,5	7,8	7,6	7,2	6,1	5,1	5,0	4,6

Table 2. Chickpea yield results in two locations (2002-2003)

Variety	Dose (Gray)	Saraykoy (2002)	Saraykoy (2003)	Haymana (2003)	Average yield (kg/da)
TAEK SAGEL	150	228.2 ab	239.6 a	193.2 a	220.3
TAEK SAGEL 10	100	236.8 a	231.4 ab	191.1 a	219.8
TAEK 8	200	202.9 cd	226.4 b	178.6 a	202,6
TAEK 7	400	171.3 f	213.2 c	175.6 b	186.7
TAEK 6	250	189.9 de	207.8 c	173.6 b	190.4
AK-71114 (ST)	St	176.3 ef	212.5 c	175.0 b	187.9
AKÇ N-91 (ST)	St	166.7 f	212.8 c	176.8 b	185.4
ILC 482 (ST)	St	217.1 bc	177.5 d	188.2 b	194.3
F		**	**	**	
CV (%)		13.73	8.87	4.46	
LCD		4.46	14.92	7.75	

*In a column, means followed by a common letter are not significantly different at the 0.05 P.

Table 3. Morphological characteristics of chickpea (2003)

Variety	Dose (Gy)	% 50 flower (days)	Maturity (days)	Plant height (cm)	First pot height (cm)	No of branches (plant)	No of pods (plant)	No of seeds (plant)	Growth habit (1-3)	Ascochyta blight (1-9)	100 Seed weight (g)	Yield (kg/da)
TAEK SAGEL	150	69	96	46,3	16,9	2-4	33,2	31,8	1	3	47,2	239,6 a
TAEK SAGEL-10	100	68	98	45,5	16,3	2-4	32,9	32,3	1	3	46,4	231,4 ab
TAEK-8	200	67	96	44,9	16,7	2-4	32,0	30,1	1	4	44,5	226,4 b
TAEK-7	400	68	97	44,4	17,1	2-4	29,6	28,8	1	5	44,0	213,2 c
TAEK-6	250	69	99	44,9	16,9	2-3	31,0	30,5	1	5	40,3	207,8 c
AK-71114 (ST)	St	66	98	44,4	16,2	2-3	30,0	29,0	1	5	40,9	212,5 c
AKÇIN 91 (ST)	St	65	95	44,7	15,9	3-4	33,1	32,3	1	5	40,1	212,8 c
ILC-482 (ST)	St	63	97	45,8	18,1	3-5	42,2	37,4	2	5	32,3	177,5 d

*In a column, means followed by a common letter are not significantly different at the 0.05 P.

Table 4. Quality characteristics of the chickpea mutants (2003)

Variety	Dry weight (g)	Wet weight (g)	Water uptake capacity (g/seed)	Water uptake index (%)	Dry volume (ml)	Wet volume (ml)	Swelling capacity (ml/seed)	Swelling index (%)	Cooking time (min)	Protein (%)
TAEK SAGEL	46,4	97,3	0.51	1.10	90	195	0.55	2.37	37	23.2
TAEK SAGEL-10	48,4	100,1	0.52	1.07	89	187	0.48	2.24	45	23.6
TAEK-8	46,1	95,8	0.49	1.08	85	188	0.53	2.50	38	22.9
TAEK-7	44,5	92,5	0.48	1.08	84	188	0.54	2.60	39	21.2
TAEK-6	40,1	84,4	0.44	1.11	80	175	0.45	2.51	39	21.7
AK-71114 (st)	43,1	88,8	0.46	1.06	81	182	0.50	2.58	50	23.0
AKCIN 91 (st)	41,4	84,3	0.43	1.04	83	183	0.50	2.53	45	21.4
ILC482 (st)	32,8	68,3	0,35	1.08	76	168	0.43	2.66	55	22.3
GÖKÇE (st)	44,2	89,5	0.45	1.03	83	188	0.55	2.65	51	21,3

Table 5. Chickpea registration experiment results (2004-2005)

Variety	Ankara 2004	Esenboga 2005	Haymana 2005	Konya		Eskisehir		Average Yield (kg/da)
				2004	2005	2004	2005	
TAEK SAGEL	213.3	196.7	204.6	166.9	154.9	212.2	154.5	186.7
TAEK SAGEL -10	200,6	194.7	249.8	170.1	149.3	213.3	125.2	186.2
AKN 291	157.4	157.4	197.3	145.8	108.4	167.6	94.9	147.0
Uzunlu 99 (st)	163.2	118.7	136.0	102.4	92.4	163.4	108.1	126.3
Gökçe (st)	213.4	199.0	168.2	167.7	125.6	189.4	156.5	174.3
Akçin 91 (st)	179.2	173.1	275.9	137.8	115.4	160.6	132.9	167.8
Canıtez 87 (st)	200.2	159.3	203.9	89.2	115.8	183.3	159.7	158.8
							F	**
							CV (%)	10.7
							LSD	9.3

Table 6. Quality characteristics of the chickpea mutants

Variety	Dry weight (g)	Wet weight (g)	Water uptake capacity (g/seed)	Water uptake index (%)	Dry volume (ml)	Wet volume (ml)	Swelling capacity (ml/seed)	Swelling index (%)	Cooking time (min)
TAEK SAGEL	41	85	0.45	1.15	81	178	0.47	2.52	37
TAEK SAGEL-10	43	90	0.47	1.13	82	181	0.49	2.53	48
AKN 291	46	99	0.53	1.14	86	189	0.53	2.47	48
Uzunlu 99 (st)	43	91	0.48	1.16	82	182	0.50	2.56	45
Gökçe (st)	40	84	0.44	1.09	81	175	0.44	2.42	54
Akçin 91 (st)	44	91	0.47	1.11	83	182	0.49	2.48	53
Canıtez 87 (st)	47	97	0.50	1.04	86	185	0.49	2.36	55

Table 7. Chickpea registration experiment some stability parameters

Variety	Yield (kg/da)	%	b		a	R^2	%CV	Error mean square
TAEK SAGEL	186.7	118.1	0.997	0.150	8.5	0.89	7.9	171.2
TAEK SAGEL-10	182.2	115.2	1.424	0.220	-60.3	0.89	11.2	340.9
AKN 291	147.0		0.924	0.208	0.9	0.80	11.7	298.9
Uzunlu 99 (st)	126.3		0.665	0.234	21.1	0.62	15.4	377.8
Gökçe (st)	174.3		0.604	0.276	78.9	0.49	13.2	525.5
Akçin 91 (st)	167.8		1.379	0.326	-50.2	0.78	16.1	730.7
Canıtez 87 (st)	158.8		1.069	0.302	-10.4	0.71	15.8	631.7
Std. Average Yield	156.8							
Gen. Average Yield	158.1	100.0						

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Development of *B. Napus* Canola Quality Varieties Suitable for Indian Agro-climatic Conditions by Induced Mutations

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Abstract

In mutation experiments, dry seed and presoaked seeds of canola quality 'Westar' were used with different doses / concentrations of Gamma-rays and chemical mutagens EMS and SA. Among several morphological mutants, 11 early maturing mutants were identified. The maturity of these mutants ranged from 90-15 days as against 169 days of Westar in Central India. The maturity of mutants NUDB-38 and NUDB-26-11 was 107 and 105 days respectively. These mutants were further evaluated for their yield and agronomical characters along with non-canola quality *B. napus* checks GSL-1 and *B. juncea* varieties Pusa Bold and Varuna. NUDB-38 has shown 33% superior yield performance over check variety GSL-1 (non-canola quality) and the oil yield was comparable with B. juncea national check variety Varuna in Zone II. Where as NUDB-26-11 was comparable (2% higher) with gobhi sarson check GSL-1 and it has given 24.6% higher yield over *B. juncea* check variety Varuna in Zone I under different agroclimatic conditions in the national testing (Indian Council for Agriculture Research) where *B. napus* is grown. Mutant NUDB-38 has been granted US patent (patent No. US 6706953 B2 dated March 16th, 2004) for its early maturity and high yield potential. Mutant NUDB-26-11 was identified for release for Zone I by the All India Coordinated Research Project on Rapeseed Mustard during 2007.

Introduction

Brassica napus (L) commonly known as rapeseed (or 'gobhi sarson' in India), is grown in Europe, Canada, China, Australia, northwestern parts of the USA, and a few other countries. The development of canola quality *B. napus* varieties in early 1980's, revolutionized the Brassica seed industries thus making available nutritionally superior quality oil for human consumption and quality seed meal for animals. 'Tower' was first canola quality *B. napus* variety released in Canada [1]. The cultivation of *B. napus* in India is restricted to few states in northwestern region, which has a longer winter. The exotic *B. napus* varieties introduced in the country were not commercially successful due to their late maturity and poor seed yield. Later, non-canola quality variety GSL-1 was released in Punjab during 1984-85, which is used as national check in coordinated national trials. Fourteen new Brassica varieties have been developed using Gamma-rays, X-rays, EMS, DMS, and MNH for early maturity, high yield, cold tolerance, height, disease resistance, oil content and altered fatty acid composition [2]. The present investigation was initiated at RTM Nagpur University by Late Dr. A. S. Khalatkar on induction of mutations in *B. napus* in cv Westar (canola quality), with the objective of inducing early maturing mutants with high yield potential suitable for commercial cultivation for Indian agro-climatic conditions.

Materials and Methods

In mutation experiments, dry and presoaked seeds were used with different doses / concentrations of Gamma-rays and chemical mutagens EMS and SA. The effectiveness and efficiency of these mutagens were studied in M_1 generation using different parameters. Several mutations affecting different morphological and biochemical characters were identified. Among these, 11 early flowering mutants (38-49 days) as compared to variety Westar (75 days) were identified. The maturity of these mutants ranged from 90 -115 days, as opposed to 169 days of Westar in Central India.

These mutants were further evaluated for their yield and agronomical characters along with non canola quality *B. napus* checks GSL-1 and *B. juncea* varieties Pusa Bold and Varuna under different agroclimatic conditions in the national testing (Indian Council for Agriculture Research) programme in Zone II and Zone I where *B. napus* is grown.

Results and Discussion

Induction of earliness has been most important objective of mutation breeding in several crops. Early maturing mutants in rice [3], castor [4], and soybean [5] have been released as varieties for commercial cultivation worldwide. Early maturing mung bean mutant variety TAP-7 has helped bring a larger area under multiple cropping systems in specific ecological condition [6].

In the present study, out of the 11 early flowering and maturing mutants, in M_2, three mutants were obtained after being presoaked in water for 12 hours and 6 hours in 0.06% SA treatment. It flowered in 40-51 days. The oil and seed quality of these mutants was similar to that of Westar. Their breeding behavior was studied in M_3 and M_4. The data presented in **Table 1** shows the ancillary and biochemical characters of these three selected mutants in M_5 against canola quality *B. napus* check HPN-3 and non-canola quality *B. juncea* checks Pusa Bold. Mutant 69 was earliest to flower but yielded less than other two mutants. Mutant 38 and Mutant 26-11 gave 65% and 55% higher yield as compared to canola quality check HPN-3 respectively. These two mutants were later renamed as NUDB-38 and NUDB-26-11 respectively.

Table 1. Ancillary and biochemical characters of mutants in initial trial in M_5 generation at Nagpur (Central India).

Selection/ Variety	Plant height	50% Flowering (Days)	Maturity (Days)	100 seed wt (g)	Oil content (%)	Glucosinolate By Tes-tape	Erucic acid (%)	Yield (Kg/ha)
Mutant-69	133.2 ± 1.95	40.25 ± 0.75	102.75 ± 1.31	0.368 ± 0.01	41.87 ± 0.31	(-)	0	659.16
Mutant-38	141.3 ± 3.07	50.75 ± 0.62	115.25 ± 0.85	0.409 ± 0.01	40.81 ± 0.39	(-)	0	850.74
Mutant-26-11	151.6 ± 2.82	51.25 ± 1.03	112.00 ± 1.29	0.367 ± 0.006	42.06 ± 0.34	(-)	0	799.81
HPN-3 (C)	162.9 ± 2.96	62.25 ± 0.47	127.25 ± 1.10	0.312 ± 0.01	40.23 ± 0.74	(-)	0	515.2
Pusa Bold (C)	175.7 ± 3.44	53.00 ± 1.08	112.25 ± 1.43	0.527 ± 0.02	37.68 ± 0.37	(4+)	46.7	1079.8
Varuna (C)	168.8 ± 2.92	52.75 ± 0.85	112.75 ± 0.62	0.493 ± 0.01	37.65 ± 0.33	(4+)	47.97	907.7

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Yield evaluation of NUDB-38

Mutant NUDB-38 was evaluated in the All India Coordinated Research Project on rapeseed Mustard in Zone II during 2000-01 to 2002-03. The maturity of NUDB-38 in Zone II was 149 days, which was comparable to non-canola quality *B. napus* national check GSL 1 (153 days). It has shown 33% superior yield performance over check variety GSL-1 (**Table 2**) and the oil yield was comparable with *B. juncea* national check variety Varuna.

US patent: Mutant NUDB-38 has been granted US patent (patent No. US 6706953 B2; dated March 16th, 2004) for its canola quality, early maturity and high yield potential.

Table 2. Mean performance of mutant NUDB-38 in the All India Coordinated Research Project on rapeseed mustard in Zone II during 2000-01 and 2002-03

Variety/ Strain	Yield (Kg/ ha)	% Increase over GSL-1	% Increase over Varuna	Oil yield (Kg/ha)	Oil content (%)	Maturity (days)	Erucic acid (%)	Glucosinolate (µmole/g defatted meal)
NUDB-38	1323 (13)	32.96	-2.6	521	39.2	149	0.9	21.96
GSL-1	995 (9)			372	38.6	153	40.13	193.90
Varuna (C)	1358 (13)			512	38.5	149	49.9	

*Figures in parenthesis are total nº. of locations

Yield evaluation of NUDB-26-11

Mutant NUDB-26-11 was also evaluated in the All India Coordinated Research Project on Rapeseed Mustard in Zone I during 2001-02 to 2005-06. The maturity of NUDB-26-11 was 154 days in Zone I, which was comparable to national check GSL 1 (157 days) (Table No. 3). The yield performance of NUDB-26-11 was comparable (2% higher) with non canola quality gobhi sarson check GSL-1 and it has given 24.6% higher yield over B. juncea check variety Varuna in Zone I.

Release of variety NUDB-26-11: Mutant NUDB-26-11 has been identified for release for Zone I by the All India Coordinated Research Project on Rapeseed Mustard during 2007. The release proposal has been submitted for notification.

Table 3. Mean performance of mutant NUDB-26-11 in the All India Coordinated Research Project on rapeseed mustard in Zone I during 2001-02 and 2005-06

Variety/Strain	Yield (Kg/ha)	% Increase	Oil content (%)	Days to maturity	Erucic acid (%)	Glucosinolate (µmole/g defatted meal)
NUDB-26.11	1145 (9)	-	38.7	154	1.11	25.53
GSL-1	1120 (9)	2.2	40.7	157	42.06	68.27
Varuna (C)	863(8)	24.6	39.5	153	49.90	85.07

*Figures in parenthesis are total no. of locations

Conclusion

These studies have indicated that mutation experiments with specific objectives can result in developing varieties suitable for specific regions with high yield potential. Early maturing mutant NUDB-38 has been granted US patent (patent No. US 6706953 B2; dated March 16th, 2004) and NUDB-26-11 has been identified for release for Zone I by the All India Coordinated Research Project on Rapeseed Mustard during 2007. Both these mutants have given superior/ equal yield as compared to non-canola quality check GSL-1.

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