

## Mutagenesis as a tool for improvement of traits in grasspea (*Lathyrus sativus* L.)

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### Introduction

Grasspea (*Lathyrus sativus* L.) is produced as major crop in Bangladesh, China, India and Pakistan, and to a lesser extent in many countries of Europe, the Middle East, northern Africa, as well as in South America. It is extensively cultivated and naturalised in the Middle East countries of Iraq, Iran, Afghanistan, Syria and Lebanon and in Ethiopia, Egypt, Morocco, Algeria and Libya in northern Africa<sup>(1)</sup>. In Europe grasspea was cultivated in the Balkans by around 8000 BC<sup>(4)</sup>. Foods prepared from the seeds have been popular in Europe and are still being used locally in Spain, France, Germany and Bulgaria.

According to Milczak<sup>(5)</sup> grasspea was introduced to Poland (Podlasie Region) in the 17<sup>th</sup> century, probably with arrival and settling of the Tatars. Now grasspea is grown on a marginal scale in Eastern Poland, it is cultivated as a vegetable and is locally known as "soczewica podlaska" (Podlaska lentil). The first plant material for breeding purposes in 1991 constituted a local population from the village of Derewiczna. As results of breeding work in the following few years, in cooperation with the Breeding Station for Vegetables in Nochowo, the first Polish cultivars: Derek and Krab were released<sup>(5)</sup>.

The main advantages of grasspea are: resistance to diseases and environmental stress conditions, high yielding potential (at the level of pea) and favourable nutritional composition of seed. Grasspea is very tolerant to drought, due to a very hardy and penetrating root system and can be grown on a wide range of soil types, including very poor soils. Undesirable features such as prostrate plant habit, indeterminate growth, late maturity, pod shattering and neurotoxin content (ODAP) are the most important factors limiting the broader introduction of grasspea for growing in different environmental conditions.

Many crop improvement programs are presently addressing research on different aspects of grasspea,<sup>(2,3)</sup> and mutagenesis and mutation breeding can be a valuable supplement to conventional breeding methods. It can be used to create additional genetic variability that may be utilised by plant breeders in the

development of cultivars for specific purposes or with specific adaptations. To create new genetic variability of traits and mutations of possible economic interest the seed of two Polish cultivars were treated with chemomutagens.

### Material and Methods

A composite of 1640 seeds was assembled by two Polish cultivars of *Lathyrus sativus* - Derek and Krab being treated with chemical mutagens: N-nitroso-N-methyl urea (NMU) and sodium azide (NaN<sub>3</sub>). Samples of dry seeds were soaked for 12 h in distilled water at room temperature (24°C). After soaking the seeds were treated for 3 h with one of eight different freshly prepared aqueous solutions of NMU (0.5, 0.8, 1.1 and 1.4 mM) or NaN<sub>3</sub> (2.0, 4.0, 6.0 and 8.0 mM). The seeds were then washed with running tap-water to remove the mutagens from the seed surface. One hundred treated seeds of each treatment were sown in a randomised-block design with 3 replications at an experimental field in Cerekwica with spacing 50 x 20 cm, immediately after the treatment. The untreated seeds were sown as control. Data were recorded on plant height, pod number per plant, pod length, seeds number and weight per main stem and seed number and weight per plant. The obtained data in M<sub>1</sub> (biological injury level) were expressed stimulation (+) or reduction (-) calculated as a percentage of control value.

The seeds of M<sub>1</sub> plants were harvested and sown in field conditions. In the M<sub>2</sub> progeny of the treated and control material was screened for chlorophyll and morphological mutations. From M<sub>2</sub> progeny 82 viable mutants were selected in respect to plant habit, maturity, stem shape, leaf size, flower colour, pod size, seed size and colour according to systems suggested by Swaminathan<sup>(11)</sup> and Nerkar<sup>(6)</sup>.

All chosen genotypes and initial cultivars were analysed for morphological traits and yield components. The results allowed estimates of mean values and ranges of analysed traits. The plants in M<sub>2</sub> were grown in conditions of environmental stress caused by very a strong spring drought.

## Results and Discussion

In the  $M_1$  progeny the effect of stimulation or reduction of analysed traits as compared to initial cultivars was observed (Tables 1 and 2). In the case of  $NaN_3$  a small stimulation effect appeared only for low dose of mutagen and cv. Derek. In other cases different level of reduction was noticed. As compared to  $NaN_3$ , NMU showed a markedly stronger influence on the grade of biological injuries. All doses of mutagen decreased the plant height, pod length and parameters of yield structure. This reduction was more visible for NMU (Table 1) than  $NaN_3$  (Table 2) for used doses and both cultivars. The level of biological injuries expressed by reduction of traits value increase with use of higher doses of chemomutagens. Similar effects in *Lens culinaris* were observed for gamma rays, EMS and NMU<sup>(8)</sup>. Particularly useful traits for estimation of susceptibility of plants to the chemomutagen constituted the traits directly connected with fertility: number and weight of seeds per pod and plant. The highest dose of NMU (1.4 mM) reduced the number and weight of seeds per

plant by about 70% for both cultivars. According to Singh and Chaturvedi<sup>(10)</sup> who used gamma rays and EMS, seedling height, pollen fertility and plant survival were very sensitive indicators for estimation of biological injury level in  $M_1$  progeny. Independently of dose the susceptibility of cultivars to mutagens are genetically specific<sup>(6)</sup>. In our experiment no distinct differences in susceptibility between both cultivars were observed. It was particularly true for higher doses of NMU and  $NaN_3$  and yield contributing parameters.

Genetically effect of mutagens was analysed in  $M_2$  in conditions of strong spring drought (Table 3). A wide spectrum of chlorophyll mutations, *albina*, *chlorina*, *chlorina-virescens*, *chlorotica*, and *chlorotica-virescens* were observed. There was an increase in the frequency of mutation with an increase in the dosage of NMU and  $NaN_3$ . A higher frequency of chlorophyll mutations was noticed for NMU than for  $NaN_3$  and in cv. Krab compared to Derek (Tables 1 and 2).

**Table 1. Stimulation (+) or reduction (-)\* of analysed traits of grasspea in  $M_1$  progeny and frequency of chlorophyll mutations in  $M_2$  after use of NMU.**

NMU dose (mM)	Plant height	Pods/plant	Pod length	Seeds/pod	Seed weight/pod on main stem	Seeds/plant on main stem	Seed weight/plant	Chlorophyll mutation (%)
<b>Cultivar Derek</b>								
0.5	+1.0	+18.2	-1.4	-19.5	-6.5	-23.4	-8.8	1.85
0.8	-0.5	-16.3	-4.8	-38.9	-22.6	-82.4	-50.6	2.74
1.1	-7.0	-25.3	-11.6	-50.0	-30.7	-76.9	-66.8	3.18
1.4	-4.8	-68.6	-21.4	-52.8	-50.8	-79.8	-75.3	4.31
<b>Cultivar Krab</b>								
0.5	-8.2	-19.5	-0.9	-16.1	+2.5	-49.7	-37.9	2.57
0.8	-19.3	-42.0	-6.0	-32.2	-14.8	-64.5	-53.9	3.01
1.1	-25.1	-32.5	-11.0	-41.9	-22.4	-67.1	-62.9	4.55
1.4	-26.2	-46.7	-2.4	-41.9	-25.0	-76.8	-71.3	5.08

\*stimulation and reduction level % of control value

**Table 2. Stimulation (+) or reduction (-)\* of analysed traits of grasspea in  $M_1$  progeny and frequency of chlorophyll mutations in  $M_2$  after use of  $NaN_3$ .**

$NaN_3$ dose (mM)	Plant height	Pods/plant	Pod length	Seeds/pod	Seed weight/pod on main stem	Seeds/plant on main stem	Seed weight/plant	Chlorophyll mutation (%)
<b>Cultivar Derek</b>								
2.0	+17.1	+6.9	+1.2	-20.0	-8.0	-15.1	-3.6	0.90
4.0	+4.5	+7.6	+1.8	-22.5	-8.8	-24.9	-7.2	1.41
6.0	-6.8	+0.1	-0.1	-35.0	-14.0	-33.4	-19.2	1.95
8.0	-2.9	-0.1	-2.8	-27.5	-21.6	-32.0	-35.5	3.27
<b>Cultivar Krab</b>								
2.0	-6.0	+5.8	-0.3	-3.3	-6.4	+0.4	-0.3	1.08
4.0	-23.8	-7.0	-5.6	-6.7	-0.1	-20.8	-27.3	1.50
6.0	-26.8	-13.5	-5.3	-10.0	-2.4	-28.9	-22.6	2.76
8.0	-28.8	-23.7	-8.4	-19.4	-4.1	-47.8	-38.1	3.77

\* stimulation and reduction level was calculated as percent of control value

The higher frequency of chlorophyll mutations observed for cv. Krab was positively correlated with genetic variability of visible mutants according to the system suggested by Swaminathan<sup>(11)</sup> (Table 4). From M<sub>2</sub> plants of cv. Krab 68 and cv. Derek 14 morphological mutants were selected. The mean value and range of traits of chosen plants are presented in Table 5. A wide spectrum of viable mutants were observed affecting plant habit, branching, maturity and parameters of yield structure. The chemomutagens were particularly efficient in inducing plant habit variability as dwarfism, semi-dwarfism, erectness and low and high branching forms, which confirm data obtained by Prasad and Das<sup>(7)</sup>. The mean value of plant height for cv. Krab and Derek was respectively 75.3 and 69.2 cm and chosen M<sub>2</sub> plants 54.0 and 55.6 cm. The range of these traits for M<sub>2</sub> plants were

respectively: 24-85 cm and 32-80 cm and for initial forms: 66-85 cm and 61-74 cm. Gamma rays and EMS altered polygenic variability in M<sub>2</sub>; plant height ranged between 26-120 cm with mean 71.9 cm, and number of primary branches per plant: range 2-14 with mean 4.95<sup>(13)</sup>. The number of all branches per M<sub>2</sub> plants ranged between 1-44 for cv. Krab and 9-32 for cv. Derek. The grasspea showed indeterminate growth with inclination to produce a high number of branches and biomass and in many cases decreased seed yield, seed size and prolonged maturity period. The semi-dwarf forms with suitable first legume height, lower branch number and improved earliness and stable yield ability seem to be interesting initial material for breeding purposes. Such forms were selected in M<sub>2</sub> progeny.

**Table 3. The temperature and rainfall in spring 2000 for region of Wielkopolska (data from Breeding Station Szelejewo).**

	March			April			May			June		
	1-10	11-20	21-31	1-10	11-20	21-30	1-10	11-20	21-31	1-10	11-20	21-30
<b>Temp (°C)</b>	4.1	3.6	5.3	10.3	15.2	20.2	18.3	16.0	15.9	19.3	21.8	19.7
<b>Rainfall (mm)</b>	41.7	28.9	34.9	0	17.1	0	0	14.4	36.7	10.2	1.7	7.5

**Table 4. Classification of viable mutations found (after Swaminathan, 1965).**

Character	Visible mutations	Macromutations	Systematic mutations
<b>Plant habit</b>	Erect, spreading, subnormal	Small plant type, stunted, minutum (miniature size)	Grassy
<b>Maturity</b>	Early flowering synchronous maturity	-	-
<b>Branching</b>	Non-branching, shy branching	-	-
<b>Stem</b>	-	Brittle stem	Afila (non-winged stem)
<b>Leaf</b>	Leaflets-broad, narrow, long, short, obovate, wiry, three and four leaflets	Coriacea (leathery leaf), abnormal leaflets, curly leaf, circinnata (rolled leaf)	-
<b>Stipule</b>	-	-	Serrated stipule
<b>Flower</b>	Albus (white), roseus (crimson), cyaneus (light blue)	Half-split keel	Extra whorl of corolla, epicalyx, bracteolata, extended peduncle
<b>Pod</b>	Large poded, small poded, tereta (cylindrical pod), declinata (curved pod)	-	-
<b>Seed</b>	Coloured seed coat	Small seeded	-

Particularly wide variability was observed for number of pods per plant, number and weight of seeds per pod and plant as well as for 100 seed weight (Table 5). One of the most important traits influencing yielding ability is pods number per plant<sup>(9,12)</sup>. The value of this trait for M<sub>2</sub> plants Derek und Krab ranged respectively between 19-168 and 8-222. Studies using gamma rays and EMS showed this trait in M<sub>2</sub> ranged between 6-560<sup>(13)</sup>. In many cases the M<sub>2</sub> plants produced a high number of pods per plant and were characterised by a decrease in 100 seed weight and increased production of sterile pods. The 100 seed weight in M<sub>2</sub> for Krab and Derek ranged respectively between: 4.80-19.41 g and 5.1-10.8 g; for initial cultivars: 12.2- 13.9 and 8.3-9.9 g. After use of gamma rays and EMS the 100 seed weight in M<sub>2</sub> range was 6.1-22.4 g<sup>(13)</sup>.

In Table 6 are presented the most promising mutants for breeding purposes obtained in M<sub>2</sub> progeny. The analysis of M<sub>3</sub> and M<sub>4</sub> may confirm that some mutants have economic interest with the aim of using this to produce new Polish cultivars adapted to our climatic conditions.

As mentioned, the M<sub>2</sub> progeny were grown in conditions of very strong spring drought (Table 3). This environmental stress had no negative influence on the growth and development of grasspea, however, for other crops nearby the stress reduced growth. This confirms the high tolerance of this crop to drought observed by other investigators<sup>(2,3,4,5)</sup>.

**Table 5. Induced polygenic variability in M<sub>2</sub>.**

Traits	Cultivar Krab				Cultivar Derek			
	Initial form		M <sub>2</sub> plants		Initial form		M <sub>2</sub> plants	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Plant height (cm)	75.3	66-85	54.0	24-82	69.2	61-74	55.6	32-80
First legume height (cm)	17.2	15-20	11.9	7-25	13.3	10-15	10.8	5-15
No. stem/plant	12.6	10-14	17.6	1-44	15.6	13-18	15.7	9-32
No. pods/plant	68.1	61-76	63.7	8-222	77.4	64-80	61.5	19-168
No. fertile pods/plant	65.0	59-64	55.8	7-196	75.1	74-76	53.9	14-163
No. sterile pods/plant	3.1	0-5	7.9	0-36	2.2	0-9	7.7	0-20
Pod length (cm)	3.6	3.2-3.9	3.44	2.42-4.17	3.36	3.01-3.54	3.10	1.88-3.67
Pod width (cm)	1.28	1.11-1.34	1.25	1.03-1.52	1.22	1.18-1.29	1.21	1.08-1.37
No. seeds/pods/main stem	2.9	2.7-3.3	2.68	1.6-3.9	3.20	2.9-3.5	2.91	2.2-3.6
Wt. seed/pod/main stem (g)	0.32	0.29-0.34	0.29	0.11-0.49	0.20	0.17-0.24	0.21	0.13-0.25
No. seeds/plant	125.2	116-135	125.4	16-361	150.2	141-168	131.3	26-220
Wt. seeds/plant (g)	16.9	15.4-17.3	13.55	0.95-43.86	13.5	12.7-13.9	9.85	1.33-33.57
100 seed wt. (g)	13.5	12.2-13.9	10.80	4.80-19.41	9.3	8.3-9.9	7.45	51-10.8

**Table 6. Mutants means for analyzed traits of chosen forms.**

Initial form (in italics) and mutants	Plant height (cm)	First legume height (cm)	No. stems per plant	No. pods per plant	No. fertile pods/plant	No. sterile pods/plant	Pod length (cm)	Pod width (cm)	Seeds/pod on main stem	Seed wt/pod main stem (g)	No. seeds per plant	Seed wt per plant (g)	100 seed weight (g)	Days to maturity
<i>cv Krab</i>	75.3	17.2	12.6	68.1	65.0	3.1	3.61	1.28	2.9	0.32	125.2	16.91	13.5	118
16/K	61.1	12.3	32.0	146.0	108.0	38.0	3.88	1.71	2.3	0.37	195.0	32.12	16.5	115
18/K	55.0	13.1	14.0	52.0	36.0	16.0	3.72	1.47	1.9	0.31	43.0	6.23	14.4	114
21/K	44.1	7.0	15.0	48.0	48.0	0	3.97	1.37	2.9	0.42	109.0	15.13	13.9	115
34/K	56.3	11.4	25.0	61.0	61.0	0	4.09	1.43	3.2	0.46	170.0	24.03	14.1	111
53/K	54.4	13.7	22.0	102.0	93.0	9.0	4.05	1.46	3.3	0.40	213.0	26.89	12.6	118
55/K	56.4	13.6	20.0	86.0	86.9	0	3.89	1.28	3.2	0.40	210.0	25.62	12.2	111
63/K	44.2	11.0	25.0	66.0	61.0	6	2.99	1.35	2.7	0.39	119.0	17.78	14.9	115
70/K	45.5	18.1	20.0	65.0	63.0	2	3.22	1.26	2.1	0.28	125.0	16.72	13.4	120
72/K	24.1	9.2	9.0	8.0	8.0	0	2.42	1.05	1.8	0.14	18.0	1.45	8.0	109
<i>cv Derek</i>	69.2	13.3	15.6	77.4	75.1	2.2	3.36	1.22	3.2	0.20	150.2	13.51	9.30	120
13/D	56.3	11.5	32.0	168.0	163.0	5.0	3.22	1.13	3.0	0.22	415.0	33.57	8.11	120
19/D	57.2	9.1	18.0	105.0	92.0	12.0	3.13	1.23	2.5	0.19	177.0	13.81	7.82	116
21/D	52.4	18.9	12.0	81.0	71.0	10.0	3.37	1.21	3.6	0.24	220.0	14.34	6.51	112

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