Curly Stem – an Induced Mutation in Flax (*Linum usitatissimum* L.)

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Abstract: After ethyl methane sulfonate (EMS) treatment of two flax lines, curly stem mutations appeared in both, besides of other mutations. Genetic analysis of one CS mutant line confirmed a monogenic inheritance of the changed stem shape. The curly stem allele is partially dominant over the wild type allele for straight stem. Homozygotic mutants have a curly stem, heterozygotic plants have a flexuous stem, while the stem of homozygotic recessive plants is straight. The expression of the curly stem character is affected by factors influencing plant growth. The utilisation of this mutation for ornamental and other purposes is considered.

Keywords: *Linum usitatissimum* L.; flax; induced mutation; ethyl methanesulfonate; curly stem; inheritance

Induced mutagenesis has rarely been used to increase genetic variability in flax breeding (GILL 1987). Extreme low α-linolenic acid content in linseed oil is one of the most important mutations utilised in breeding of new linseed varieties (GREEN & MARSHALL 1984; ROWLAND 1990), resulting in oil of quite different quality. In both cited papers the mutations were induced by EMS treatment of mature seeds. In our laboratory we also use EMS for flax mutagenesis (TEJKLOVÁ 1995). Different morphological deviations were found in the M_2 generation, apart of curly stem (abbreviated further as "CS"). The CS mutation emerged in five independent M_1 progenies of two EMS-treated lines. A similar or same mutation was described by BIANU *et al.* (1972) who named it "flexuous stem". Flexuous stem was labelled *Fx* and described as a single-gene controlled character dominant over straight stem *fx*.

MATERIALS AND METHODS

In 1991 two flax lines, Te 93/13 and Su 45/85, further referred to as "SS-lines", were treated with EMS. 2000 seeds of each line were shaken for 18 hours in 100 ml 0.4% EMS in covered vessels. The treated seeds were rinsed with distilled water and then washed for 10 minutes in running tap water. The seeds of each line were then suspended in 2 l of water and poured in the field into nine 1-m furrows, spaced 10 cm. As a control, 1000 seeds of each line were treated with distilled water instead of EMS.

In M_1 emergence and the number of fertile plants were recorded. Mature M_1 plants were harvested individually and numbered. All M_2 seeds were sown progeny by progeny in 1-m rows, spaced 10 cm, 4–7 progenies in one row representing approximately 100 seeds. The M_2 generation was screened for morphological deviations.

M_2 plants with CS were individually harvested. From them non-segregating CS-lines were derived during the 3rd to the 5th generation. In the 6th generation one of the non-segregating CS lines (Su 216/353/61a/227) was reciprocally crossed with two SS lines (Su K7/98/76 and Te 60/100/652/9/58). The stem morphology of F_1 plants was recorded. Segregation ratios of stem phenotypes were counted in the F_2 generation and tested using the χ^2 test. Segregation ratios within individual progenies were counted in the F_3 generation.

RESULTS

Emergence of treated seeds was about 50% in the M_1 generation (Fig. 1), compared with 100% of non-treated seeds. During ontogenesis, lethal mutations (e.g. yellow and white chlorophyll mutations), plant sterility and increased sensitivity of some plants to diseases appeared in the treated material, so that the seeds from only 439 plants of line Te 93/13 and 485 plants of line Su 45/85 could be harvested (Table 1). Many different morphological deviations were observed in the M_2 generation, CS among them (Fig. 2). The CS mutation was found in progenies of three Te 93/13-M_1 plants (Te 140, Te 235, Te 246)

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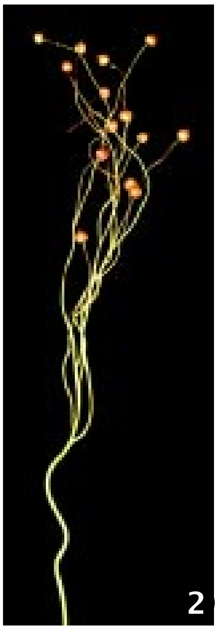
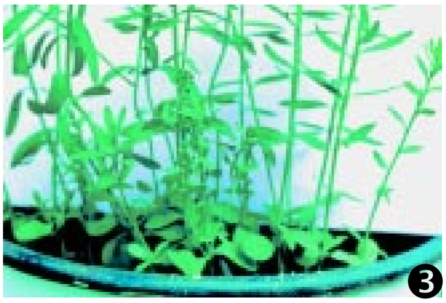
Fig. 1. Field plots with Te 93/13. M_1 generation after EMS treatment (on the left) and control plants (on the right)





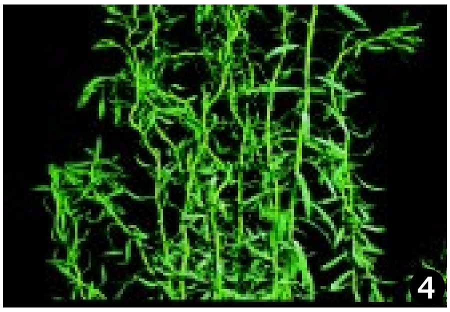


Fig. 2. Plant of M_2 generation – Su 216/354 with curly stem and branches

Fig. 3. Progeny of plant Su 216/289k (M_3 generation) at the beginning of the fast growth stage. Curvature of stems is already visible in mutant carriers

Fig. 4. Typical curly shape of stems in plants of line Su 216/354/15/34. M_5 generation, ontogenetic stage: flower-bud formation

Fig. 5. Twining behaviour of mutant stem. Stems growing closely side by side twine around each other

Fig. 6. Line Su 216/354/15/411 with curly stem (M_5 generation). Non segregating line without flexuous and straight stems

and of two Su 45/85-M_1 plants (Su 96, Su 216). The CS mutation frequency, calculated as frequency of M_2 progenies with CS mutations, was 0,68% in the line Te 93/13 and 0,41% in the line Su 45/85 (Table 1). Plants with curly stems were fertile

Progenies of selected CS plants were monitored from germination to maturity. The CS phenotype was already slightly expressed at the beginning of the fast growth period (Fig. 3). The typical curly shape of stems could be observed at the flower-bud formation stage (Fig. 4). CS

Fig. 7. Cross of Su K7/98/76 (mother line on the left, straight stems) and Su 216/353/61a/227 (father line on the right, curly stems), F1 hybrids (flexuous stems) in the middle

Fig. 8. Segregation ratio in F_2 generation after crossing Su K7/98/76 with Su 216/353/61a/227: 1 straight stems : 2 flexuous stems : 1 curly stems

Fig. 9. Graceful curves of curly stem impress very aesthetically

Authors of photographs: E. Jahoda – 1, 3; M. Griga – 2, 6, 9 and E. Tejklová – 4, 5, 7, 8

plants, when growing closely, tended to twine around each other like twining plants (Fig. 5). Not only the stem was curly, but also branches of the inflorescence exhibited this character (Fig. 2). Curly stems were more fragile compared to straight stems.

Non-segregating lines with curly stems were selected during three generations. One of these lines, Su 216/354/15/411 is shown in Fig. 6. Results of crossing of the CS line Su 216/353/61a/227 and the SS line Su K7/98/76 are shown in Fig. 7. The same results were obtained with the cross Su 216/353/61a/227 × Te 60/100/652/9/58. Stems of all F_1 plants were curly but less than in the parental line Su 216/353/61a/227, therefore this stem phenotype was labelled "flexuous stem" (FS). Progenies of reciprocal crosses did not phenotypically differ in F_1. In the F_2 generation, all three stem phenotypes occurred together (Fig. 8). The ratio of CS:FS:SS did not differ significantly from 1:2:1.

Offsprings of F_2 plants with the SS phenotype were all SS and only rarely at about 2% FS. Offsprings of CS plants were all CS and only rarely, up to 1%, FS. Progenies of FS plants segregated in the same way as the F_2 generation:1 CS:2 FS:1 SS.

CS plants were shorter and produced less capsules than parental SS lines or heterozygotic FS plants (Figs 7 and 8).

DISCUSSION

Since the plants were cultivated at relatively high density (about 450 plants per 0.9 m^2 in M_1 and about 900 plants per 1 m^2 in the following generations), acci-

Table 1. Effect of EMS on two lines of flax; M_1 generation

Line	Treatment	No. sown seeds	Germination* (%)	No. fertile plants	Fertile plants frequency* (%)	No. M_2 progenies with mutation "curly stem"	"Curly stem" mutation frequency** (%)
Te 93/13	EMS	2000	56.45	439	21.95	3	0.68
	Control	1000	100	978	97.80	0	0
Su 45/85	EMS	2000	50.85	485	24.25	2	0.41
	Control	1000	100	985	98.50	0	0

*related to sown seeds, **related to fertile plants dental outcrossing due to insects or to mutual touching of flowers during anthesis could not be excluded. *Linum usitatissimum* is considered an autogamous species, but some authors observed 0.3–3.4% outcrossing, depending on variety, season, plant distance and insect visit (GILL 1987). Therefore it can not be excluded, that the mutation frequency for "curly stem" was in fact lower than estimated and that some curly plants detected in the M_2 generation originated from accidental crosspollination with a chimeric curly mutant sector of a M_1 plant. Progenies, in which CS plants appeared, were derived from chimeric M_1 plants, and could therefore involve in the M_2 generation SS offsprings from genetically SS sectors. It therefore did not make much sense to count the ratio of CS to SS types in this generation. In the first three generations after mutagenesis we did not distinguish between CS and FS. The stem phenotypes were distinguished and segregation ratio was reliably estimated only after controlled reciprocal crossing.

The segregation ratios in F_2 suggest, that the stem phenotype is controlled by two alleles of one gene. The CS allele is partially dominant over the SS allele. Dominant homozygotes are CS, while heterozygotes are FS and recessive homozygotes are SS. This is slightly different from BIANU *et al*. (1972) who have found full dominance of flexuous stem (in our terminology CS) over straight stem (SS). Our hypothesis of partial dominance was confirmed by segregation ratios in the F_3 generation. Rare occurrence of FS plants in the progenies of CS or SS plants was probably due to accidental outcrossing in the previous generation.

The recognition of the stem phenotype was sometimes difficult. In some cases, especially in lodged or small, not fully developed plants, it was difficult to recognize if the stem was curly, flexuous or even straight. The CS expression might therefore depend on some factors influencing plant growth, as for example diseases, drought and nutrition.

CS plants often look very aesthetically (Fig. 9). Flax with curly stem can be used for decorative purposes like curly forms of other plant species, e.g., of willow, hazel or birch. The fragility of curly stems is also of potential use. This feature, introduced into linseed cultivars, could avoid problems with winding up of stems onto harvester mechanisms.

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Abstract

TEJKLOVÁ E. (2002): **Mutace zkadeřený stonek u lnu** (*Linum usitatissimum* L.). Czech J. Genet. Plant Breed., **38**: 125–128.

Po ovlivnění semen etylmetansulfonátem byly ve dvou liniích přadného lnu indukovány mutace, mezi jinými také zkadeřený stonek. Genetickou analýzou bylo zjištěno, že tvar stonku je určen dvěma alelami jednoho alelového páru, z nichž alela pro zkadeřený stonek je neúplně dominantní nad alelou pro rovný stonek. U dominantního homozygota je stonek zkadeřený, u heterozygota je zvlněný a u recesívního homozygota je rovný. Exprese znaku zkadeřený stonek závisí na faktorech, které ovlivňují růst rostlin.

Klíčová slova: *Linum usitatissimum* L.; len; indukovaná mutace; etylmetansulfonát; zkadeřený stonek; dědičnost

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