DE GRUYTER
OPEN

herba polonica
Vol. 61 No. 1 2015

DOI: 10.1515/hepo-2015-0007

EXPERIMENTAL PAPER

Analysis of accumulation of cadmium in seeds of selected breeding linseed (*Linum usitatissimum* L.) genotypes cultivated for medicinal purposes

MARCIN PRACZYK*, KRZYSZTOF HELLER, GRAŻYNA SILSKA, PRZEMYSŁAW BARANIECKI

Institute of Natural Fibres and Medicinal Plants
Wojska Polskiego 71B
60-630 Poznań, Poland

*corresponding author: phone: +4861 8455 854; e-mail: marcin.praczyk@iwnirz.pl

S u m m a r y

The objective of the study was genetic and breeding screening of selected varieties and hybrids of oil flax for in-seed cadmium fixing ability. Seven parental varieties as well as nineteen F_1 and F_2 hybrids were analyzed. The varieties were crossed in di-allelic scheme, according to the Griffing method II. Significant differences were found in the activity of fixing cadmium from the soil between analysed flax parental forms and hybrids. High variability of tested genotypes, calculated by the variance coefficient, was observed in F_1 generation. The variability of tested genotypes in the F_2 generation was two times lower.
The analysis of general combining ability (GCA) showed that Chinese cultivar Shanxi reduced significantly the fixing cadmium from the soil in combinations of F_1 generation crossing. However, the effects of the specific combining ability (SCA) of tested hybrids to reduce the cadmium content in seeds were statistically insignificant. The analysis of genetic components variance showed no significance both in domination and cumulative genes which suggests a complex inheritance pattern of the tested trait.

Key words: *Linum usitatissimum, cadmium accumulation, breeding*

INTRODUCTION

Cadmium is a very toxic element, causing damage mainly to kidneys, liver and blood circulatory system. It can be found naturally in soil, usually at low amounts. The content of cadmium in farmland soils usually is lower than 0.5 mg/kg. Thus, the majority of soils can be considered as not contaminated or contaminated to a low extent. However, this element can be additionally introduced to the soil with fertilizers, sewage sediments or transport. Cadmium is not an indispensable element for plant growth and reproduction, however, it can be accumulated in relatively high amounts with no negative effect on ontogenesis [1]. Hence, some plant species that are especially efficient in fixing cadmium can accumulate it in the amounts exceeding safe thresholds set in the standards [1]. Kuboi et al. [2] classified selected plant families according to ability of cadmium accumulation on specified groups: low cadmium accumulation ability (legumes), average ability (grasses, *Liliaceae*, *Cucurbitaceae*, *Apiaceae*) and high ability (*Chenopodiaceae*, *Brassicaceae* and *Compositeae*). Flax is one of species of high ability to accumulate cadmium from soil [3]. For centuries flax has been known for its health beneficial effect and nowadays it becomes a diet component of increasing importance. According to Zając et al. [4], the oil flax cultivars (lineseed) can be used for food purposes due to its chemical composition. They investigated the seed chemical composition of two lineseed cultivars (Opal and Hungarian Gold) and showed the high content of ester extract (460 g kg^{-1} DM), neutral detergent fibre (415 g kg^{-1} DM), total protein (225 g kg^{-1} DM) and nitrogen free extract (186 g kg^{-1} DM) in dry matter (DM). Linseed is a traditional source of mucilage with protective effect on alimentary tract. It protects the mucous membranes, oesophagus, stomach and duodenum, shielding them from harmful substances. In addition to protecting and coating properties, linseed also shows mild laxative action.

Currently, a lot of research focuses on beneficial effect of lignans on human health, of which the linseed is an especially rich source [5]. The results of the latest studies confirm the effectiveness of lignans in controlling the division of cancer cells of lung tumours [6]. Excessive content of cadmium in linseed can hamper the industrial use of this feedstock.

The process of genetic extraction of cadmium by plants from soil is not well understood yet. Nevertheless, the research on genotypes with low cadmium accumulation ability is an important part of breeding programmes [7].

The objective of presented study was the selection of perspective lines for breeding oil flax cultivars with low ability to accumulate cadmium in seeds.

MATERIALS AND METHODS

The experiment consisted of tests on seven cultivars of oil flax from Polish *Linum* collection crossed in a di-allelic scheme, according to the Griffing method II. As a result, 21 crossing combinations were obtained. Plants in two hybrids produced no seeds, therefore, the study covered seven parental cultivars and 19 hybrids (tab. 1).

Table 1.

List of tested linseed genotypes cultivated in soil with different content of cadmium

No.	Tested linseed genotype
1	Abby
2	Shanxi
3	Alessandra
4	La Estanzuela 117
5	AC MC Duff
6	Lindor
7	Dufferin
8	Abby x Shanxi
9	Abby x Alessandra
10	Abby x Estanzuela 117
11	Abby x AC MC Duff
12	Abby x Lindor
13	Abby x Dufferin
14	Shanxi x Alessandra
15	Shanxi x Estanzuela 117
16	Shanxi x AC MC Duff
17	Shanxi x Lindor
18	Shanxi x Dufferin
19	Alessandra x Estanzuela 117
20	Alessandra x AC MC Duff
21	Alessandra x Lindor
22	Alessandra x Dufferin
23	Estanzuela 117 x AC MC Duff
24	AC MC Duff x Lindor
25	AC MC Duff x Dufferin
26	Lindor x Dufferin

The study was conducted in 2011–2013. It consisted of crossing of parental cultivars and experiments with parental cultivars as well as their F_1 and F_2 generations. The F_2 generation was obtained as a result of self-pollination of the F_1. The experiment was set in pots, in vegetation hall of the Institute of Natural Fibres and Medicinal Plants Research Station in Pętkowo (wielkopolskie district). In the experiment with F_1 and F_2 hybrid generations, two cadmium concentrations in soil were used: 2 mg of Cd/kg and 4 mg of Cd/kg of soil. The soil was collected from the farmland of the Research Station in Pętkowo and was characterized by neutral reaction, trace content of heavy metals (cadmium content in soil before experiment was 0.1 mg/kg). The soil was of IVa class. Flax seeds of analyzed genotypes (30 per pot) were sown in three replications for both cadmium concentrations in soil by randomized method.

The content of cadmium in the seed was determined by the method of electrothermal atomic absorption (ET-AAS) followed by the breeding analysis of cadmium accumulation ability from the soil.

The evaluation of flax genotypes variability according to tested trait was carried out by mean, range and variance coefficients. The analysis of variance was also carried out by determination of the significance of observed differences in accumulation of cadmium in seeds. Then, the general combining ability of parental cultivars (GCA), specific combining ability of hybrids (SCA), hybrid vigour in relation to average in parents and variance analysis of genetic components was conducted. No genetic charactersitics' were determined (Mather genetic parameters, heritability in narrow and broad sense, average degree of domination) as the error-born variability was higher than variability of parents. Calculations were done using the DGH2 software [8].

RESULTS AND DISCUSSION

Analysis of variance revealed the significant differences in the cadmium content in seeds of flax genotypes studied in the experiments, both in F_1 and F_2 generations (tab. 2). Information on the occurrence of varietal differences in terms of cadmium binding ability from the soil can be found in the reports of numerous authors and is reported for different plant species [1, 2, 9]. In the experiment on parental cultivars and F_1 hybrids, the average content of cadmium in flax genotypes seeds, analyzed at cadmium concentration in soil of 2 mg Cd/kg was 0.53 mg, while at concentration of 4 mg/kg was 0.77 mg (tab. 3). The average content of cadmium in seeds of studied genotypes of flax, calculated for both concentrations of cadmium in soil was 0.65 mg Cd/kg in the experiment with parental cultivars and F_1 hybrids.

The F_2 generation was obtained by self-pollination of the F_1 plants. Therefore, the seed of F_2 plants contained specific amounts of cadmium, as given in table 2. Reintroduction of cadmium ions in soil increased the amount of this element in seeds of F_2 generation plants (tab. 2). The average content of cadmium in seed at a concentration of cadmium of 2 mg Cd/kg of soil was 1.34 mg Cd/kg, and at the concentrations of cadmium in soil of 4 mg/kg was 2.21 mg (tab. 3). The average content of cadmium in seeds of flax genotypes studied, calculated both for concentrations of cadmium in soil in the experiment with parental cultivars and F_2 hybrids was 1.77 mg Cd/kg.

Table 2.

Average value of cadmium accumulation in seeds for parental varieties and F_1,F_2 generation of the breeding hybrids of flax (mean of 2 years)

Genotype	Cd content in seeds					
	F_1 Cd content in soil 2 mg/kg	F_2 Cd content in soil 2 mg/kg	F-statistic value	F_1 Cd content in soil 4 mg/kg	F_2 Cd content in soil 4 mg/kg	F-statistic value
Abby	0.3	1.46	89.1*	0.3	1.91	123.6*
Shanxi	0.43	1.44		0.3	2.85	
Alessandra	1.51	1.78		1.1	3.04	
La Estanzuela 117	0.37	1.46		0.59	2.52	
AC MC Duff	1.4	1.67		0.54	1.81	
Lindor	0.35	1.59		0.63	2.25	
Dufferin	0.52	1.32		1.1	1.47	
Abby x Shanxi	0.22	0.94		0.51	1.41	
Abby x Alessandra	0.28	1.62		0.85	1.72	
Abby x Estanzuela 117	0.2	1.65		0.7	1.96	
Abby x AC EC Duff	0.21	1.63		0.36	2.27	
Abby x Lindor	0.43	1.56		1.22	2.55	
Abby x Dufferin	1.47	1.81		0.43	1.74	
Shanxi x Alessandra	0.6	1.35		0.4	2.41	
Shanxi x Estanzuela 117	0.41	1.25		0.69	2.45	
Shanxi x AC MC Duff	0.17	0.96		0.41	1.83	
Shanxi x Lindor	0.31	1.3		0.7	1.75	
Shanxi x Dufferin	0.2	1.58		0.6	1.88	
Alessandra x Estanzuela 117	0.39	1.29		1.98	3.14	
Alessandra x AC MC Duff	0.75	1.72		0.85	3.12	
Alessandra x Lindor	0.52	1.03		0.66	3.71	
Alessandra x Dufferin	0.75	1.28		1.21	2.16	
Estanzuela 117 x AC MC Duff	0.3	0.93		0.55	1.95	
AC MC Duff x Lindor	0.31	0.97		0.87	1.7	
AC MC Duff x Dufferin	0.24	0.49		0.76	1.76	
Lindor x Dufferin	0.91	0.99		1.72	2.1	

Critical value for simultaneous testing $F_{0.05} = 4.03$

* Significant at the level of 0.05

Table 3.

Variability analysis of cadmium accumulation in flax seeds (F_1 and F_2 generation) by univariate statistical methods (mean of 2 years)

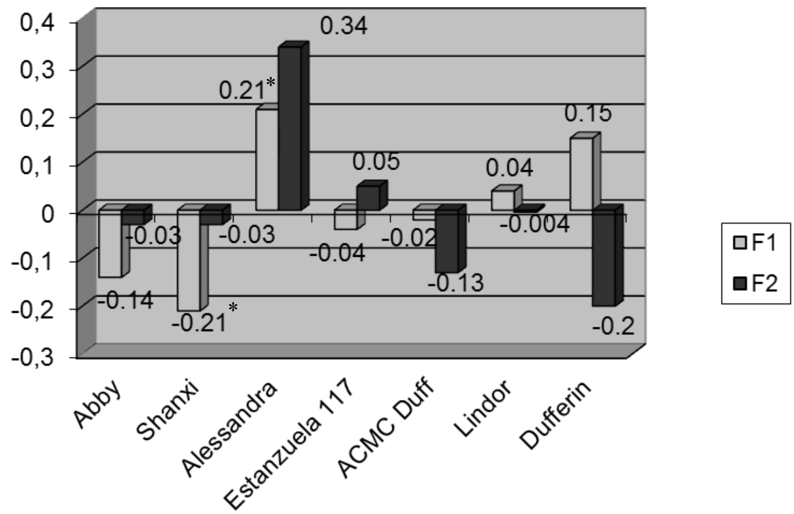
Trait	Generation	Mean		Range between lowest and highest value		Variability coefficient [%]	
Cd content in seeds		Cd content in soil 2 mg/kg	Cd content in soil 4 mg/kg	Cd content in soil 2 mg/kg	Cd content in soil 4 mg/kg	Cd content in soil 2 mg/kg	Cd content in soil 4 mg/kg
	F_1	0.53	0.77	1.47	1.68	79.2	53.2
	F_2	1.34	2.21	1.32	2.30	23.9	26.2

In the experiment with parental cultivars and the F_1 hybrids at the concentration of cadmium in soil of 2 mg/kg, the hybrids showing the lowest ability of seeds to accumulate cadmium were Abby x La Estanzuela 117 and Shanxi x Dufferin (tab. 2), while the most active variants were parental AC MC Duff and Alessandra (tab. 2). At a concentration of 4 mg cadmium per 1 kg of soil, the lowest accumulation activity was found in the seeds of the Abby x Shanxi hybrid, whereas the highest activity in seeds of La Estanzuela Alessandra x 117 hybrid (tab. 2).

A high variation of cadmium uptake from the soil was observed, especially at the concentration of the cadmium in the soil of 2 mg/kg. In the F_2 this variation was low. The calculated coefficient of variation received far less value in the F_2 generation, as compared with the F_1 generation (tab. 3). The effect of low variation characteristics in the experiment was due to relatively low content of cadmium in soil. Reports in the literature indicate that the variation calculated for the binding activity of cadmium from the soil is increased by growing content of this element in the soil. Bjelkova et al. [10] found significant differences in the content of cadmium in seed between four tested cultivars of oil flax at high concentrations of cadmium in the soil (60 mg/kg and 100 mg/kg). The analysis of the soil from our study at the Agricultural Chemical Station in Poznań showed that the amounts of cadmium was fed significantly lower, which are found in most soils used in agriculture.

The low activity of binding cadmium from the soil should be one of the directions of breeding, in developing lineseed cultivars of high economic value for medicinal use [1]. Such studies were initiated in Canada. In this study, a genetic and breeding screening of tested genotypes for accumulation of cadmium in seeds was performed. The GCA analysis showed that the Shanxi cultivar significantly influenced the content of cadmium in seeds of F_1 hybrids. The impact of other varieties on the lowering of cadmium accumulation in seeds of hybrids was statistically insignificant (fig. 1). In the F_2 generation, none of tested parent varieties significantly reduced the cadmium content in the seeds of hybrids (fig. 1). In turn, the Alessandra variety significantly increased cadmium content in the seeds of hybrids, both in the F_1 and F_2

generations (fig. 1.). The results show the suitability of the Shanxi variety to obtain varieties with reduced activity of accumulation of cadmium in seeds.



* GCA effect significant at the level of 0.05

Figure 1.

GCA effect of tested parental varieties for cadmium accumulation in seeds of F_1 and F_2 generation linseed plants (mean from 2 years)

No positive results were obtained while testing the hybrids. No significant adverse SCA effects for the binding activity of cadmium from the soil in the case of any tested hybrid were found, both in F_1 and F_2 generations (tab. 4). There was a significant positive SCA effect for the combination of Lindor x Dufferin hybrid (tab. 4). Therefore, none of the analyzed hybrids can be recommended for breeding varieties with low binding activity of cadmium from the soil.

There were also no statistically significant heterosis effects of flax hybrids examined in terms of the binding activity of cadmium from the soil, calculated for the average of the parental varieties. No significance of both, positive and negative effects of heterosis, occurred in the F_1 and F_2 (tab. 5). In the literature there are found reports regarding the effect of heterosis in terms of accumulation of cadmium in seeds focus on open-pollinated species, where the use of heterosis effect is an important part of breeding programs. Li et al. [11] studied 36 F_1 hybrids of sunflower of which 27 showed a significant negative heterosis effect for the binding of cadmium from the soil. In the case of flax, the use of heterosis effect in breeding varieties with low cadmium-binding activity from the soil is not recommended. The same is with other economically important traits of flax, as reported by many authors [11-13].

It was not possible to determine the genetic characteristics in discussed experiments due to very low variability associated with the error. Only the variance analysis of genetic components for the binding activity of cadmium from the soil of the tested flax genotypes was carried out. The analysis results showed significant role both of cumulative and dominant alleles (tab. 6). The reports of authors investigating the genetic binding of cadmium from the soil by other species, however, indicate that the uptake of cadmium by plants is controlled by the additive effects of genes [14, 15]. This demonstrates that it is possible to conduct an effective selection for low activity of cadmium uptake from the soil in the segregating hybrid F_2 generations.

Table 4.

SCA effect of tested hybrids for cadmium accumulation in linseed seeds. Results for F_1 and F_2 generation (mean of 2 years)

No.	Combination of crossing	SCA effect	
		F_1	F_2
1	Abby x Shanxi	0.06	–0.53
2	Abby x Alessandra	–0.17	–0.39
3	Abby x Estanzuela 117	–0.03	0.02
4	Abby x AC MC Duff	–0.21	0.35
5	Abby x Lindor	0.26	0.33
6	Abby x Dufferin	0.28	0.25
7	Shanxi x Alessandra	–0.16	–0.19
8	Shanxi x Estanzuela 117	0.14	0.06
9	Shanxi x AC MC Duff	–0.13	–0.21
10	Shanxi x Lindor	0.02	–0.2
11	Shanxi x Dufferin	–0.19	0.19
12	Alessandra x Estanzuela 117	0.35	0.06
13	Alessandra x AC MC Duff	–0.06	0.46
14	Alessandra x Lindor	–0.33	0.28
15	Alessandra x Dufferin	–0.04	–0.17
16	Estanzuela 117 x AC MC Duff	–0.17	–0.24
17	AC MC Duff x Lindor	–0.09	–0.29
18	AC MC Duff x Dufferin	–0.29	–0.29
19	Lindor x Dufferin	0.65*	–0.19

*SCA effect significant at the level of 0.05

Table 5.

Heterosis effect in respect to cadmium accumulation in linssed seeds versus mean of parental varieties for F_1 and F_2 generation (mean of 2 years)

No.	Combination of crossing	Heterosis effect	
		F_1	F_2
1	Abby x Shanxi	0.03	–0.74
2	Abby x Alessandra	–0.23	–0.37
3	Abby x Estanzuela 117	0.06	–0.03
4	Abby x AC MC Duff	–0.41	0.30
5	Abby x Lindor	0.43	0.25
6	Abby x Dufferin	0.39	0.23
7	Shanxi x Alessandra	–0.33	–0.39
8	Shanxi x Estanzuela 117	0.12	–0.21
9	Shanxi x AC MC Duff	–0.44	–0.48
10	Shanxi x Lindor	0.07	–0.50
11	Shanxi x Dufferin	–0.18	–0.04
12	Alessandra x Estanzuela 117	0.29	0.01
13	Alessandra x AC MC Duff	–0.40	0.41
14	Alessandra x Lindor	–0.30	0.20
15	Alessandra x Dufferin	–0.07	–0.18
16	Estanzuela 117 x AC MC Duff	–0.36	–0.35
17	AC MC Duff x Lindor	–0.20	–0.42
18	AC MC Duff x Dufferin	–0.45	–0.37
19	Lindor x Dufferin	0.85	–0.30

*Non-effect of heterosis significant at the level 0.05

Table 6.

Variance analysis of genetic components for cadmium accumulation in F_1 and F_2 generation (mean of 2 years)

Variation source	Sum of squares	Mean square	F statistic value	Probability P ($F>F_0$)
Dominance	1.81	0.09	0.34	.9927
One direction	0.13	0.13	0.53	.4727
Remainder	1.68			
Additivity	1.58	0.26	1.05	.4120

CONCLUSIONS

1. Analysis of variance showed significant differences in the accumulation of cadmium in seeds between the analyzed varieties and hybrids of oil flax.
2. Analysis of parental varieties GCA effects showed that the Shanxi variety significantly decreased the cadmium content in the seeds of F_1 hybrids. This suggests the use of this cultivar in the breeding programme of lines characterized by decreased binding activity of cadmium bonding from the soil to be used for medical purposes.
3. No significant adverse SCA effects of analyzed crossing combinations for the ability of binding cadmium from the soil were observed. The resulting hybrids consist no valuable starting materials for breeding varieties with reduced ability to accumulate cadmium in seeds.
4. A slight negative heterosis effect in relation to the average of the parents of surveyed F_1 hybrids for the binding of cadmium from the soil. None of the analyzed hybrids showed a statistically significant heterosis effect.
5. Analysis of genetic variance showed no significant influence of both cumulative and non-cumulative gene activity on the uptake of cadmium from soil by flax plants. The results of other studies indicate, however, the advantage of cumulative gene effect, which demonstrates the possibility to conduct an effective selection for low activity of cadmium uptake from the soil in the segregating F_2 generation hybrids.

REFERENCES

1. Grant CA, Buckley WT, Bailey LD, Selles F. Cadmium accumulation in crops. Can. J. Plant Sci. 1997; 78:1-17.
2. Kuboi T, Noguchi A, Yzaki J. Family-dependent cadmium accumulation characteristics in higher plants. Plant Soil 1997; 92:405-415.
3. Wielebski F. Zawartość substancji szkodliwych w nasionach i słomie lnu oleistego (*Linum usitatissimum* L.) uprawianego w bezpośrednim sąsiedztwie szlaków komunikacyjnych. Rośliny Oleiste – Oilseed Crops 2012; 33:113-126.
4. Zając T, Borowiec F, Micek P. Porównanie produktywności, składu chemicznego i profilu kwasów tłuszczowych żółtych i brązowych nasion lnu oleistego. Rośliny Oleiste – Oilseed Crops 2001; 22:441-453.
5. Englen G. A survey of lignan content in commercially available flax supplements. Proceedings of the 65th Flax Institute of the United States, Fargo, ND 2014: 28-33.
6. Chikara S, Dhillon H, Lindsey K, Reindl KM. Suppression of lung cancer cell proliferation and migration in vitro by flaxseed-derived lignans. Proceedings of the 65th Flax Institute of the United States, Fargo, ND 2014: 55-60.
7. Gaudchau M, Schneider M. Investigation of heavy metal accumulation in various medicinal plants and linseed. Beitr. Zuchtungsforsch 1996; 2:381-384.
8. Kala R, Chudzik H, Dobek A, Kiełczewska H. DGH 1.0 – statistical analysis system for genetic and breeding experiments. Agric Univ, Poznań, Poland 1994.

9. Singh P, Srivastava R, Narain V, Dubey S. Combining ability and heterosis for seed, yield and oil content in linseed *(Linum usitatissimum* L.*).* Indian J Agric Sci 2009; 79:229-232.
10. Bjelkova M, Gencurova V, Griga M. Accumulation of cadmium by flax and linseed cultivars in field – simulated conditions: A potential for phytoremediation of Cd-contaminated soils. Ind Cr Prod 2011; 33:761-774.
11. Li YM, Chaney RL, Schneiter AA, Miller JF. Combining ability and heterosis estimates for cadmium level in sunflower. Crop Sc. 1995; 35(4):1015-1019.
12. Tyson H. Genetic control of seed weight in flax (*Linum usitatissimum* L.) and possible implications. Theor Appl Genet 1989; 77:260-270.
13. Singh P, Srivastava R, Narain V, Dubey S. Combining ability and heterosis for seed, yield and oil content in linseed *(Linum usitatissimum* L.*).* Indian J Agric Sci 2009; 79:229-232.
14. Induri BR, Ellis DR, Slavov GT, Yin T, Zhang X, Muchero W, Tuskan GA, DiFazio SP. Identification of quantitative trait loci and candidate genes for cadmium tolerance in *Populus*. Three Physiol 2012; 32(5):626-638.
15. Shehata A, Comstock V. Heterosis and combining ability estimates in F_2 flax populations as influenced by plant density. Crop Sci 2010; 11(4): 534-536.

ANALIZA GENETYCZNO-HODOWLANA WIĄZANIA KADMU W WYBRANYCH GENOTYPACH LNU OLEISTEGO UPRAWIANYCH DLA CELÓW LECZNICZYCH

MARCIN PRACZYK*, KRZYSZTOF HELLER, GRAŻYNA SILSKA, PRZEMYSŁAW BARANIECKI

Instytut Włókien Naturalnych i Roślin Zielarskich
Wojska Polskiego 71B
60-630 Poznań

*autor, do którego należy kierować korespondencję: tel.: +4861 8455 854;
e-mail: marcin.praczyk@iwnirz.pl

S t r e s z c z e n i e

Celem badań była analiza genetyczno-hodowlana wybranych odmian i kombinacji krzyżowania lnu oleistego pod względem wiązania kadmu z gleby w nasionach. Analizowano siedem odmian rodzicielskich i dziewiętnaście mieszańców pokolenia F_1 i F_2. Odmiany krzyżowano ze sobą w układzie diallelicznym, według II metody Griffinga.
Stwierdzono występowanie istotnych różnic w aktywności wiązania kadmu z gleby pomiędzy analizowanymi odmianami rodzicielskimi i mieszańcami lnu. Wysoką zmienność badanych genotypów, obliczoną za pomocą współczynnika zmienności obserwowano w pokoleniu $F_{1.}$ Zmienność badanych genotypów w pokoleniu F_2 była dwukrotnie mniejsza. Analiza efektów ogólnej zdolności kombinacyjnej wykazała, że chińska odmiana Sha-

nxi powodowała istotne obniżenie aktywności wiązania kadmu z gleby w kombinacjach krzyżowania w pokoleniu F_1, jednakowoż efekty specyficznej zdolności kombinacyjnej badanych mieszańców, pod względem obniżenia zawartości kadmu w nasionach, były już nieistotne statystycznie. Analiza wariancji komponentów genetycznych wykazała brak istotności zarówno dominowania, jak i addytywności, co sugeruje skomplikowany charakter dziedziczenia badanej cechy.

Słowa kluczowe: *Linum usitatissimum, wiązanie kadmu, hodowla*