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Agronomic performance and oil quality of crambe as affected by genotype and environment

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(With 1 figure)

Summary

Crambe (*Crambe abyssinica* Hochst. Ex. R. E. Fries) is a new industrial oilseed crop with a high content of erucic acid and a considerable agronomic potential. A set of eleven accessions of crambe was subjected to field trials from 1988 to 1991 at Gross Enzersdorf and from 1990 to 1991 at Gleisdorf, Austria. Both the agronomic performance as well as seed composition were investigated as influenced by genetic and environmental factors. Seed yields in the broad range of 972 to 3328 kg/ha were observed across all genotypes and environments. Variation in seed yield was predominantly due to environmental effects whereas broad sense heritability estimated among genetic entries was low. Oil content of pods (seed + pericarp) was in the range of 22.6 to 38.4 % of pod dry matter and erucic acid content made up 53.9 to 63.1 % of seed oil. Erucic acid content was significantly affected by genotype and location, highest contents of erucic acid were found in BGRC 34311 and BelAnn. Generally, variation in erucic acid content was lower than in other fatty acids such as oleic, linoleic or linolenic acid, which were significantly influenced by temperature during the period of seed development.

Key-words: *Crambe abyssinica*, yield, oil composition, erucic acid.

Ertragsleistungen und Ölqualität von Crambe in Abhängigkeit von Genotyp und Umwelt

Zusammenfassung

Crambe (*Crambe abyssinica* Hochst. Ex. R. E. Fries) gilt als eine neue Industrieölpflanze aus der Familie der Brassicaceae, die sich durch hohe Erucasäuregehalte im Samenöl sowie ein beachtliches Ertragspotential auszeichnet. Elf Crambe-Genotypen wurden von 1988 bis 1991 in Groß-Enzersdorf und von 1990 bis 1991 in Gleisdorf in Feldversuchen geprüft, wobei sowohl die Ertragsleistungen als auch die Zusammensetzung des Erntegutes in Abhängigkeit von Genotyp und Umweltbedingungen untersucht wurden. Die Samenerträge schwankten — über alle Genotypen und Umwelten betrachtet — in dem sehr weiten Bereich von 972 bis 3328 kg/ha; diese Variation im Ertrag konnte primär auf Umwelteinflüsse zurückgeführt werden, wogegen die Heritabilität des Merkmals Ertrag innerhalb des geprüften Sortimentes eher gering war. Der Ölgehalt des aus Samen und Fruchtwand (Pericarp) zusammengesetzten Erntegutes lag zwischen 22,6 und 38,4 % der Samentrockenmasse, wobei Erucasäure einen Anteil von 53,9 bis 63,1 % der Fettsäurezusammensetzung einnahm. Der Erucasäuregehalt wurde sowohl durch genetische als auch durch Standortbedingungen beeinflußt, die höchsten Gehalte wurden an den Genotypen BGRC 34311 und BelAnn festgestellt. Im allgemeinen variierte der Erucasäuregehalt weit weniger stark als der Gehalt anderer Fettsäuren wie etwa Öl-, Linol- oder Linolensäure, welche durch die Temperaturverhältnisse während der Phase der Samenentwicklung stärker beeinflußt wurden.

Schlüsselworte: *Crambe abyssinica,* Ertrag, Fettsäurezusammensetzung, Erucasäure.

1. Introduction

Crambe (*Crambe abyssinica* Hochst. Ex. R. E. Fries) is considered to be an appropriate source of high erucic acid seed oil, which has a promising range of applications in oleochemical industry (Leonard 1993). Although crambe is a relatively new crop, its agronomic potential as an annual oilseed crop is well-known since several decades (Lessman and Meier 1972, McGregor et al. 1961). In recent agronomic investigations, crambe has been found to be flea beetle resistant (Anderson et al. 1992), moderately tolerant to salinity (Francois and Kleiman 1990, Fowler 1991) and tolerant to frequently applied herbicides (Stougaard and Moomaw 1991).

So far, crambe has not been subjected to extensive and continuous breeding work and the availability of genetically diverse germplasm is meager; nevertheless, crambe is remarkably competitive to other oilseed crops in both erucic acid content of seed oil and yield of erucic acid per unit of area (Leppik and White 1975, Seehuber 1987, van Soest et al. 1993). Recently, crambe seed oil has been characterized to be highly symmetrical with respect to the 1- and 3-position of erucic acid in seed triacylglycerols, which enables the use of 1,3-specific lipases for oil hydrolysis in order to obtain erucic acid at higher concentrations (Muuse et al. 1992). Variation in erucic acid content of crambe seed oil has frequently been reported to be in the range of 51 to 60 %. The selection of lines with an improved erucic acid content has been demonstrated by Campbell et al. (1986 a). However, a significant enhancement of erucic acid percentage seems to be difficult because of the rather low genetic variability present in this character (Appelqvist and Jönsson 1970). Limited variation in erucic acid content of crambe may also be due to environmental effects (Earle et al. 1966), stage of seed development (McKillican 1966) or planting date (Massey and Jellum 1973).

The objective of this research was to determine both the agronomic performance and seed composition of a set of different crambe genotypes grown in two contrasting environments in Austria. A special emphasis has been devoted to investigate the variation of erucic acid content in seed oil.

2. Materials and Methods

Trial entries

Crambe genotypes Indy, Prophet, BelAnn, BelEnzian, C-22, C-29, C-37, NU 52865, BGRC 34305, BGRC 34311 and BGRC 34312 were used in the present study. Seed samples of different crambe accessions were kindly provided by

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Field trial locations and agronomic parameters

Field trials were conducted at Gross Enzersdorf (located near Vienna, Austria) from 1988 to 1991 on a tchernosem-like, medium heavy soil with a pH of 7.5 and at Gleisdorf (in the south of Austria) in 1990 and 1991 on a heavy pseudogley with pH of 6.0. The climate of Gross Enzersdorf is continental being characterized by warm and dry summers (annual mean precipitation = 572 mm, annual mean temperature = 9.8 °C), whereas Gleisdorf is in the transition zone between continental and Mediterranean conditions with warm and moist summers (highest rates of rainfall between June and August, annual mean precipitation = 831 mm, annual mean temperature = 8.6 °C). Monthly mean temperature and precipitation during the growing period of crambe are given in table 1 for the respective environments.

Table 1

Monthly mean temperature (° C) and precipitation (mm) during growing period at the different environments (Gross Enzersdorf = GE 1988—1991, Gleisdorf = GL 1990—1991)

Month	Mean temperature (°C)						Precipitation (mm)					
	GE 1988	GE 1989	GE 1990	GE 1991	GL 1990	GL 1991	GE 1988	GE 1989	GE 1990	GE 1991	GL 1990	GL 1991
April	9.4	10.6	8.8	8.7	7.9	7.3	19.3	47.1	80.0	14.0	90.2	44.2
May	15.7	14.2	15.5	11.9	14.4	11.0	34.7	68.3	23.4	131.5	87.6	107.1
June	17.2	16.1	17.9	17.2	16.4	16.5	42.4	77.0	59.5	61.9	154.1	174.0
July	20.6	20.4	19.5	21.0	18.0	19.6	38.8	54.0	38.2	65.7	117.0	134.8

Field trials were planted during the first week of April in each year. A mineral fertilizer was applied prior to sowing at rates of 70 kg/ha N, 70 kg/ha P_2O_5, and 140 kg/ha K_2O. Crambe seeds with pericarp were sown at a depth of 2—3 cm and at a rate of 200 seeds per m^2 to six row plots of 10 m^2 size. Experiments were arranged as randomized complete block designs with three to five replications. Experimental plots were combine harvested during the second half of July in each year.

Determination of qualitative characters

In order to measure the seed percentage of crambe pods, about 10 g of fruits (seed + pericarp) were dehulled manually, and clean seed and pericarp fractions were weighed.

The oil content of crambe seed samples ground with pericarp was determined gravimetrically after a Soxhlet extraction of the oil with petroleum ether. Triacylglycerols extracted from seeds were saponified in potassium hydroxide and liberated fatty acids were methylated in methanol. Subsequently, fatty acid composition was measured by analysis of methyl esters using gas-liquid chromatography (GLC). The total content of glucosinolates of crambe seeds with hulls was analyzed from samples harvested in Gross Enzersdorf 1991 by quantitative determination of enzymatically released glucose (FIEBIG et al. 1988).

Data collection and statistical analysis

Seed yields of hulled seeds are given in kg/ha on a 13 % moisture basis, data on oil contents are provided on a dry weight basis of hulled seed. Oil content and fatty acid composition were determined from mixed samples taken from all replications of a particular genotype at one location.

Analyses of variance of yield data were calculated for each single trial and across the total of 6 environments. Oil content and fatty acid data of different genotypes were subjected to an ANOVA combined over two years and two locations (Gross Enzersdorf and Gleisdorf 1990—1991 = orthogonal set of data). Genotypes, years and locations were considered to be fixed effects. Genotype × year × location interactions were used as the error term in F-tests.

3. Results

Crambe seed yield performance in each of the single trials is summarized in table 2. Yields ranging from 972 to 3328 kg/ha have been observed. The strong influence of environmental conditions on seed yield is obvious from the mean yields of single trials. The low seed yields at Gross Enzersdorf 1989 and Gleisdorf 1990 were mainly due to low plant density and poor development of stands because of heavy rainfalls and low temperatures after sowing. In a combined analysis of variance over locations and years (results not shown), environmental effects were highly significant whereas differences between genotypes were not significant. Generally, broad sense heritability of seed yield based on single trial data calculated from components of variance was medium to low among the

Table 2

Variation in seed yield (kg/ha) of different crambe genotypes as influenced by location and year
(Gross Enzersdorf= GE 1988—1991, Gleisdorf= GL 1990—1991)

	GE 1988	GE 1989	GE 1990	GE 1991	GL 1990	GL 1991
Minimum yield	2066	972	2106	2360	1057	2530
Mean yield	2321	1331	2330	2644	1398	2976
Maximum yield	2802	2050	2632	2857	1688	3328
Number of entries	5	11	11	11	11	11
Coefficient of variation (%)	13.6	15.7	10.2	7.6	13.4	10.9
F-Test (difference between entries)	n. s.	***	*	*	*	n. s.
Broad sense heritability (%)	36	69	24	19	38	22

(n. s. = not significant, ***, * significant at the 0.1 and 5% level, respectively)

Table 3

Overall variation of quality parameters in different crambe entries across 5 environments

Parameter	Minimum	Mean	Maximum	Sample size	Std. dev.	C. V. (%)
Oil content of pod (%)	22.6	31.3	38.4	49	4.56	14.6
Palmitic acid C 16:0	1.8	2.6	4.5	49	0.50	19.5
Stearic acid C 18:0	0.0	0.8	1.3	49	0.21	26.1
Oleic acid C 18:1	14.0	17.3	21.0	49	1.52	8.8
Linoleic acid C 18:2	6.7	9.3	14.1	49	1.45	15.6
Linolenic acid C 18:3	6.6	8.5	11.4	49	1.30	15.4
Eicosenic acid C 20:1	0.4	2.0	3.4	49	0.73	35.9
Erucic acid C 22:1	53.9	58.3	63.1	49	2.03	3.5
Glucosinolate content (µmol/g)[a]	74	86	103	11	7.79	9.1
Thousand pod weight (g)[b]	5.4	6.1	6.9	33	0.37	6.1
Seed % of pod[b]	59.1	69.4	70.7	33	0.05	7.8

([a, b]: data from 1 or 3 environments only) entries examined. Broad sense heritability estimated from the combined analysis of all trials was less than 5 %. Other agronomic characters such as plant height and date of flowering were significantly affected by both genotype and environment, whereas differences in lodging and seed shattering were due to environmental effects only (results not shown).

An overview of the total variation in seed quality parameters is given in table 3. High variation was detected in both eicosenic and stearic acids which are present at low concentrations in crambe seed oil. Erucic acid content of seed oil was in the range of 53.9 to 63.1 % showing a rather low coefficient of variation. Low variation was also found in thousand pod weight, seed percentage of pod weight and glucosinolate content of pod. Oil content of crambe pods (seed + hull) was within the broad range of 22.6 to 38.4 %. Assuming an average seed content of pod of 69.4 %, oil content of dehulled seed can be calculated to be in the range of 32.6 to 55.3 (mean = 45.1) %. Generally, fatty acid composition was significantly affected by year, location, genotype and year by location type interactions, as indicated in table 4. However, erucic acid content was influenced neither by year nor by interaction effects. This was observed also for the thousand pod weight and seed percentage of pod weight (results not presented in the table).

Table 4

Influence of genotypes, years, locations and interaction effects on oil content and fatty acid composition of crambe seed

Character	Sources of variance according to the ANOVA model: Main effects			Interactions		
	Years	Locations	Genotypes	Y×L	Y×G	L×G
Oil content	n.s.	n.s.	n.s.	**	n.s.	n.s.
C 16:0	n.s.	+	n.s.	n.s.	n.s.	n.s.
C 18:0	*	n.s.	n.s.	n.s.	n.s.	n.s.
C 18:1	n.s.	***	**	***	n.s.	n.s.
C 18:2	**	***	**	+	n.s.	n.s.
C 18:3	n.s.	***	*	***	n.s.	n.s.
C 20:1	***	***	***	***	n.s.	n.s.
C 22:1	n.s.	*	*	n.s.	n.s.	n.s.

(n.s. = not significant; ***, **, *, +: significant at the 0.1, 1, 5 and 10 % level, respectively)

Table 5

Coefficients of correlation between seed yield, oil and fatty acid contents of a total of 49 crambe samples (data pooled across genotypes and environments)

	Seed yield	Oil content	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3	C 20:1
Oil content	0.107	–						
C 16:0	–0.337*	0.011	–					
C 18:0	0.037	–0.226	–0.103	–				
C 18:1	0.751**	0.054	–0.212	0.165	–			
C 18:2	–0.662**	–0.124	0.235	0.194	–0.360	–		
C 18:3	–0.525**	–0.348*	0.306*	–0.257	–0.578**	0.142	–	
C 20:1	0.432**	0.376**	–0.198	0.419**	0.494**	–0.239	–0.752**	–
C 22:1	0.311*	0.194	–0.386**	–0.433**	–0.112	–0.742**	–0.128	–0.018

Phenotypic coefficients of correlation between seed yield, oil content and fatty acid composition are shown in table 5. Seed yield was not correlated with oil content. No significant relationship was found also between erucic acid (as well as most of the other fatty acids) and oil content. Erucic acid content was negatively correlated with palmitic, stearic and linoleic acids. The influence of temperature (monthly mean temperatures during vegetation period) on oil and fatty acid con-

Table 6

Coefficients of correlation between oil and fatty acid contents (n = 49 samples) and monthly mean temperatures during vegetation period

Month	Oil %	C 16:0	C 18:0	C 18:1	C 18:2	C 18:3	C 20:1	C 22:1
April	−0.469**	0.123	−0.177	−0.343*	0.316*	0.551**	−0.814**	−0.165
May	−0.311*	0.132	0.141	−0.338*	−0.141	0.451**	−0.088	0.076
June	0.021	−0.218	0.186	0.535**	−0.717**	−0.452**	0.582**	0.345*
July	−0.227	−0.162	−0.283	0.317*	−0.216	0.005	−0.410**	0.147

Table 7

Erucic acid content (%) in seed oils of crambe genotypes (mean values across four environments)

Rank	Genotype	Mean*	% of total mean
1	BGRC 34311	59.8 [a]	103.4
2	BelAnn	59.4 [a]	102.8
3	BGRC 34312	58.6 [ab]	101.4
4	Indy	58.6 [ab]	101.4
5	Prophet	58.3 [ab]	100.9
6	BGRC 34305	57.7 [ab]	99.8
7	C-29	57.5 [ab]	99.5
8	C-37	57.2 [ab]	99.0
9	NU 52865	56.9 [ab]	98.5
10	BelEnzian	55.9 [b]	96.7
11	C-22	55.8 [b]	96.6
	Total mean	57.8	100.0
	LSD 5 %	1.95	3.37

(* Values which are followed by the same letter are not significantly different at the 5 % level according to a Bonferroni-Holm test)

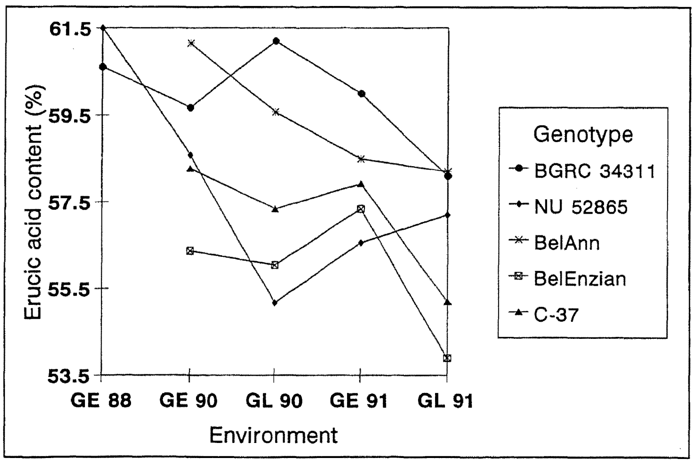

Fig. 1: Erucic acid content of different crambe genotypes in five environments

tents is demonstrated in table 6. Oleic, linoleic, linolenic and eicosenic acid content were significantly influenced by temperature during June, which roughly represents the flowering and seed development period. Erucic acid content was affected by temperature to a minor extent, whereas contents of saturated fatty acids were not at all altered due to temperature influences.

Erucic acid contents of individual crambe genotypes are listed in table 7; highest contents were found in BelAnn, Indy, Prophet and BGRC lines. Erucic acid contents of BGRC 34311 and BelAnn were more stable across different locations/years than those of NU 52865 and other lines, as demonstrated in figure 1.

4. Discussion

The considerable yielding ability of crambe grown in Middle Europe (Seehuber 1987, van Soest et al. 1993) has been confirmed by the present study. Although seed yields of comparable sets of genotypes tended to be higher in Europe than in other areas of production (e. g. Campbell et al. 1986 a, b, Francois and Kleiman 1990), a substantial variation in yield had to be noted, which was attributable to environmental effects. This reflects the current need to optimize different agronomic parameters in order to stabilize yield performance. Significant differences in yield of particular genotypes were found in some of the single trials (table 2), but a reproducible ranking of genotypes across trials could not be obtained, which indicates the rather low genetic variability among the accessions available at present. A low heritability in characters such as seed yield, oil content and maturity has also been reported from progenies of different crosses between *C. abyssinica* and *C. hispanica* types (Meier and Lessman 1973).

The total content of glucosinolates in crambe, as communicated in table 3, roughly estimates the magnitude of the amount present in both seed and hull; special plant breeding investigations will be necessary in order to be able to reduce the relatively high content of this antinutritional compound. Crambe meal contains a considerable proportion of protein with a very well-balanced amino acid profile useful as an animal feed (Liu Yong-Gang et al. 1993). Therefore, a genetic reduction of glucosinolate content will probably be the most crucial point towards utilizing crambe processing residues without the need of a costly procedure to inactivate thioglucosidase activity (Lessman and Kirleis 1979, Lessman and McCaslin 1987).

Erucic acid is being considered the main compound of interest in crambe seed oil. Total variation in erucic acid percentage was relatively low as compared to the variation in other fatty acids such as stearic, linoleic or eicosenic acid (table 3). The influence of years and locations on erucic acid content (table 4) as well as the effect of temperature during the seed development period (table 6) were small. A similar pattern of low environmental variation of erucic acid as compared to other fatty acids was also found in high erucic acid cultivars of spring rapeseed (Schuster and Taghizadeh 1980). This type of compositional stability is of special importance for the production of oleochemical raw materials. The high erucic acid content of BelAnn was confirmed by the present study (table 7), whereas erucic acid performance of BelEnzian was lower than expected from the results of Campbell et al. (1986 a). Erucic, eicosenic and oleic acid were enhanced by higher temperatures during the seed filling period, whereas concentrations of linoleic and linolenic acids were increased by lower temperatures. This type of temperature interference on fatty acid content has been found in several oil crop species and is due to increased desaturase activity at low temperature because of higher oxygen solubility (Stumpf 1989).

Genetic improvement of erucic acid content beyond the limit of 66.6 % is impossible in cruciferous crops using conventional breeding techniques. This is due to the characteristic of acyltransferases positioning erucic acid at the 1- and 3-positions of the triglyceride but not at the 2-position (WOLTER et al. 1991). In addition, it has recently been shown that eicosenic acid is also mainly esterified to the 1- and 3-positions thereby reducing potential binding sites of erucic acid (MUUSE et al. 1992). According to the results presented, an alternative approach to increase the erucic acid yield per unit area — besides selection for higher seed yield — is to improve oil content: This could be accomplished by selection for enhanced oil content of seed itself or by reducing the hull proportion of crambe pods. As shown in table 3, seeds make up between 60 and 70 % of total pod mass. Although a significant genetic variation in seed percentage of pod weight has not been detected so far, reduction of hulls (e.g. by using a mutation breeding approach) would also be desirable in order to improve test weight of the harvest product and to reduce crude fibre content of crambe meal, which is largely made up of the pericarp section (LIU YONG-GANG et al. 1993).

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