



On heterosis, inbreeding depression and general combining ability in annual caraway (*Carum carvi*)

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Abstract Caraway (*Carum carvi*) is a medicinal and aromatic plant of the Apiaceae family with a long history of cultivation. To this day, improvements in yield and essential oil content are desirable. In the past, line breeding was used to increase essential oil content with the final intention of combining inbred lines to a synthetic variety by outcrossing. Outcrossing should overcome inbreeding depression and exploit heterosis vice versa. In this study, we wanted to detect whether and to what extent heterosis can be exploited in caraway. In a randomized complete block design with two years of growing and four repetitions per year and genotype, we compared 18 inbred lines with 18

corresponding F_1 populations produced in a polycross. In addition to yield, we estimated the beginning of flowering, the end of flowering, maturity, height, thousand-grain weight, stalk attachment rate, shattering rate and essential oil content. Linear mixed models were used to compute variance components, heritability and best linear unbiased estimates. As major result, we detected the existence of better parent heterosis in caraway. To summarize, outcrossing led to a significant increase in yield, thousand-grain weight and height and to an earlier beginning of flowering, end of flowering and maturity. In two-year data, no effect of outcrossing on the essential oil content was observed, but single year data revealed slight effects. We found strong negative correlations between developmental traits and yield. Hence, selection of early developing genotypes seems highly recommendable. Results make us confident that improved annual varieties can be introduced soon.

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Introduction

Caraway (*Carum carvi* L., $2n = 2x = 20$) is a medicinal and aromatic plant of the Apiaceae family (Pank 2012). Seeds are used as spice and pharmaceutical to ease gastrointestinal afflictions. They contain essential

oil, which mainly consists of limonene and carvone (Ruszkowska 1998). For pharmaceutical use, the European Pharmacopeia (Ph. Eur.) demands a minimum essential oil content of 30 ml/kg (Ph. Eur. 2020a, b). Therefore, essential oil content is the major quality trait in caraway breeding. Nevertheless, breeders and farmers focus on appropriate seed yields.

There is evidence for caraway cultivation in Europe from the medieval period until today. Cultivation and breeding in Europe were restricted to biennial varieties until annual varieties were introduced in the 1990s (Németh 1998). Annual growing of caraway can potentially decrease production costs and allows better integration into crop rotation. However, first annual varieties had a low essential oil content (Pank 2012) and seed yields were poor. 'Sprinter' was the first annual variety that reached an essential oil content of above 30 ml/kg in most growing seasons. Seed yields were improved as well. Nonetheless, annual varieties are yet behind biennial varieties (Erpenbach 2012). Hence, farmers still desire annual varieties with higher seed yields and higher essential oil content.

In many crops, yields were substantially improved by exploiting heterosis. To our knowledge, systematic heterosis breeding was conducted or initialized in the Apiaceae family for carrot (Simon 2019), celery and celeriac (Bruznican et al. 2020), coriander (Gholizadeh et al. 2018) and fennel (Palumbo et al. 2018). Concerning yield, heterosis can be defined as the yield increment of a hybrid compared to the average of parents (mid parent heterosis). A breeder might be more interested in a hybrid whose yield is even higher than the yield of the better parent (better parent heterosis or syn. high parent heterosis, Kaeppler 2012). Heterosis is usually strong in outcrossing species (Liu et al. 2020). Recently, we revealed a mixed mating system with predominant outcrossing within a set of caraway inbred lines, which were placed in a polycross. The outcrossing rate was 66.5% on average and ranged from 51.6 to 82.0% (von Maydell et al. 2020). Predominant outcrossing suggests that heterosis can be exploited in caraway.

Hybrid breeding is the most accurate breeding method to exploit heterosis systematically. However, an efficient system to control pollination (e.g. cytoplasmic male sterility, CMS), which would be necessary for hybrid breeding, is not available for caraway. As alternative method to exploit heterosis in caraway, Pank et al. (2007) suggested breeding of synthetic

varieties, a specialized method of population breeding. Briefly, breeding material (e.g. a set of inbred lines) is composed to a population by random outcrossing. Either seeds from just one step of outcrossing (Syn_1) or further propagated seeds (Syn_{2-x}) are sold as a synthetic variety. Beforehand, the breeding material is selected based on the general combining ability (GCA).

In the year 2018, we conducted a polycross based on a set of 18 breeding lines of the fourth and fifth inbred generation. This polycross had already been used to investigate the outcrossing rate (von Maydell et al. 2020). In this study as a consecutive step, we wanted to detect whether and to what extent heterosis can be exploited in caraway. Neither knowledge on heterosis, nor on inbreeding depression in caraway is available, so far. Therefore, our investigations should add valuable new insights to caraway breeding research. Furthermore, we intended to select breeding lines to compose a synthetic variety with high seed yields and exceptional essential oil content. In case of success, improved pre-bred annual material could be available soon. Additionally, we aimed to investigate correlations between traits and heritability of traits. Statistics on correlations and heritability are scarcely available for caraway. Our findings could support selection decisions in future breeding processes.

Material and methods

Material and field trials

The development of the breeding material used in this study was described in von Maydell et al. (2020). Briefly, we used inbred material in the fourth and fifth inbred generation that originated from crossings between biennial and annual material. The material was selected for annual flowering and high essential oil content. In 2018, 18 inbred lines were outcrossed using the neighbor-balanced polycross design from Olesen (1976), implemented by Varghese et al. (2015), to produce 18 corresponding F_1 populations for GCA testing.

The GCA test was carried out at the JKI location in Quedlinburg in 2019 and in 2020. As integral part of these trials, we also tested the performance of the inbred lines per se. Four cultivars or selections were added as standards so that in total 40 genotypes were

tested. An overview of the material used in the field trials is provided in Table 1. We used a randomized complete block design with four repetitions per genotype and year. Each block was divided in halves by a path for the irrigation device and harvester. A single plot was 8 m in length and 1.5 m in breadth (Fig. 1). Seeds were sown in six rows per plot with 100 germinable seeds per m² on April 1, 2019 and April 6, 2020. The herbicide Bandur (Bayer) was applied before germination. Irrigation was carried out over three weeks after sowing. All investigated traits are described in Table 2. We estimated three different quantiles of the beginning of flowering of a plot to reveal potential heterogeneity within genotypes. Plots were harvested soon after reaching maturity using a combine harvester (individual harvesting dates). Harvested seeds were purified before measuring yield and seed traits.

Statistics

In general, statistical analysis was carried out using R software (R Core Team 2018), and the package ggplot2 (Wickam 2016) was used for graphical visualization of results.

To compute broad-sense heritabilities (h^2) for traits across years we estimated variance components using the lme4 package (Bates et al. 2015). All effects were set as random. Variance components were extracted from the model using the function VarCorr.

The heritability was computed based on following formula: $h^2 = \frac{V_G}{V_G + \frac{V_{GY}}{y} + \frac{V_R}{yr}}$, where V_G , V_{GY} and V_R stand

for the variance components genotype, genotype-year interaction and residual error, respectively, and y and r represent the number of years and repetitions, respectively.

Best linear unbiased estimates (BLUEs) per genotype were drawn from the model with the function coef. For this purpose, genotype was set as a fixed effect. In this case, BLUEs were identical with arithmetic means. We computed the GCA for each inbred line as the difference between the yield of the corresponding F₁ population and the average yield of all F₁ populations based on BLUEs.

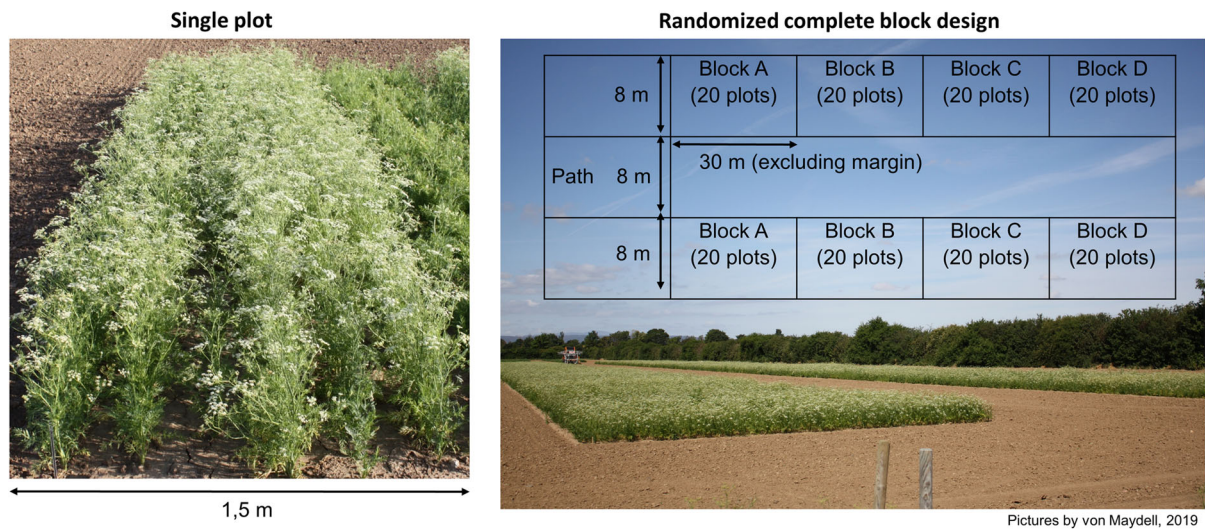
Normal distribution was tested using the Shapiro–Wilk test. Spearman correlation coefficients (r) and associated p-values were computed using the function rcorr from the package Hmisc (Harrell 2020). The correlation matrix was visualized using the package corrplot (Wei and Simko 2017). We implemented the default hierarchical clustering for ordering traits. We further used the function chart.Correlation from the package Performance Analytics (Peterson et al. 2014).

We compared inbred lines and corresponding F₁ populations across traits and tested for significant differences using a paired Wilcoxon test. Furthermore, we analyzed how far variation between F₁ populations could be explained by variation between inbred lines: We conducted a linear regression analysis using the function lm and we computed the coefficients of determination (R^2) and associated p-values using the function rcorr (Harrell 2020).

Table 1 An overview of the naming and status of the caraway material tested in the field trials at the JKI location in Quedlinburg in 2019 and 2020. L01 and F01 exemplify the

relation between inbred line with the prefix L and the corresponding F₁ population with the prefix F

Name	Status	Name	Status
L01	Inbred line	F01	F ₁ population from line L01 produced in the polycross of 2018
L02– L18	Inbred lines	F02–F18	Corresponding F ₁ populations from respective inbred lines produced in the polycross of 2018
‘Aprim’	Cultivar bred for winter-annual growing by Agritec Ltd. (Šumperk, Czech Republic), standard for low essential oil content	‘Sprinter’	Cultivar bred by Chrestensen GmbH (Erfurt, Germany), standard for seed yield with medium essential oil content
‘Ines’	Selection/cultivar bred by Dr. Junghanns GmbH (Ascherleben, Germany), most used cultivar in Saxony-Anhalt, standard for seed yield with medium essential oil content	‘Selka’	Selection developed by Julius Kuehn Institute (Quedlinburg, Germany), standard for high essential oil content



Pictures by von Maydell, 2019

Fig. 1 Pictures from a single plot (here a margin plot) and the field trial at the JKI location in Quedlinburg in 2019, including a sketch of the randomized complete block design

Results

Summary data and correlations between traits

Summary data (based on BLUEs), variance components and heritability (both based on single plot data) for all investigated traits are listed in Table 3. Single plot data can be retrieved from Table S1 and BLUEs can be found in Table S2. Figure 2 displays Spearman correlation coefficients for correlations between traits. The traits are ordered by a hierarchical clustering. We use the retrieved clusters to structure the following description of results in this chapter. We chose Spearman correlation coefficients because data distribution deviated from normal distribution. Moreover, in several cases the relationship between correlated traits might be monotonic rather than linear across the entire range of values (see Fig. S1). Additional correlation tables for two-year and single year data can be found in Table S3. This table also comprises correlation tables for inbred lines only, to remove potential effects from grouping factors. Most correlations described below for the entire dataset were found for this reduced dataset as well.

Developmental traits

In general, we found a broad range across genotypes for all developmental traits. The difference between

the earliest and the latest genotype was lowest for the beginning of flowering (B10) with about 18 days and highest for maturity with about 27 days. The effect of the genotype clearly dominates in the analysis of variance components. The heritability ranged from 0.96 for the beginning of flowering (B10) to 0.78 for maturity (Table 3). All developmental stages were positively correlated. Correlations between maturity and the other developmental traits were a bit lower. All developmental traits were negatively correlated with yield, thousand-grain weight and height, but correlations with height failed to be significant. Here, the correlation coefficients decreased from the beginning of flowering (B10) towards maturity (Fig. 2).

Yield, thousand-grain weight and height

Estimated values ranged from 8.13 to 135.99 g/m², from 1.37 to 2.38 g and from 49.33 to 73.44 cm for yield, thousand-grain weight and height, respectively. For thousand-grain weight and height, we observed that most genotypes had rather high values within the given range, whereas yield values were widely distributed (Fig. S1). For yield, we found a dominating effect of the genotype in the analysis of variance components and a high heritability (0.91; Table 3). For thousand-grain weight and height, we found a considerable residual error and for thousand-grain weight, in addition, a considerable genotype-year interaction

Table 2 List of evaluated traits for 40 caraway genotypes at the JKI location in Quedlinburg in 2019 and 2020. Abbr = abbreviation

Abbr	Trait	Unit	Method of measurement
B10	Time to beginning of flowering of 10% of plants of a plot	Days	Time after sowing when about 10% of plants of a plot had begun flowering, defined as the opening of the first umbel
B50	Time to beginning of flowering of 50% of plants of a plot	Days	Time after sowing when about 50% of plants of a plot had begun flowering, defined as the opening of the first umbel
B90	Time to beginning of flowering of 90% of plants of a plot	Days	Time after sowing when about 90% of plants of a plot had begun flowering, defined as the opening of the first umbel
EOF	Time to end of flowering of 90% of plants of a plot	Days	Time after sowing when about 90% of plants of a plot had finished flowering, defined as the decay of petals in the last flowering umbels
HGT	Plant height	cm	Average height of ten erected plants following each other in a middle row of a plot (average SD between replicates = 6.4)
MAT	Time to maturity of a plot	days	Time after sowing when a plot reached brown maturity, i.e. was ready for harvesting
SHT	Shattering rate	%	When mature, ten different umbellets per plots were pulled gently. The number of cases in which seeds break loose from the umbellets times 10 was defined as the shattering rate. Three repetitions per plot (average SD between replicates = 4.55)
YLD	Seed yield	g/m ²	Weight of purified seeds of a plot divided by 12
TGW	Thousand grain weight	g	Weight of 100 randomly chosen purified seeds times 10. Double achenes were defined as two seeds. Three repetitions per plot (average SD between replicates = 0.14)
STA	Stalk attachment rate	%	Number of seeds from 100 randomly chosen purified seeds with an attached pedicel (flower stalk) of a minimum length of 1 cm. Double achenes with attached pedicel were defined as two seeds, but one pedicel. Three repetitions per plot (average SD between replicates = 1.5)
OIL	Essential oil content	ml/ 100 g	Essential oil content measured by distillation using the device according to Ph.Eur. (2020a, b). 6 g of purified seeds were ground for 20 s using IKA mill A10. 5 g of ground tissue were filled in a 250 ml flask and distilled with 75 ml distilled water for 90 min. 24.1% of measurements were duplicated (average SD between replicates = 0.156)

effect. Heritability was yet high for height (0.88), but lower for thousand-grain weight (0.66; Table 3). The three traits were positively correlated (Fig. 2). Correlations with developmental traits are described above.

Shattering rate and stalk attachment rate

Most genotypes had a high shattering rate and a low stalk attachment rate. By name, the cultivar 'Aprim' had the lowest shattering rate (41.25%) and merely two more genotypes (inbred lines) had a shattering rate below 60% (Table S2). The effect of the genotype was slightly higher than the residual error in the analysis of variance components of the shattering rate. The heritability of the shattering rate was 0.7. The range of the stalk attachment rate was rather narrow (from 0.5 to 7.38%). The residual error dominates in the

analysis of variance components and the heritability was 0.69 for the stalk attachment rate (Table 3). Both traits were negatively correlated ($r = -0.59$, Fig. 2).

Essential oil content

The essential oil content ranged from 2.13 to 5.14 ml/100 g (BLUEs, Table 3). Most genotypes showed an essential oil content between 3 and 4 ml/100 g (Table 3, Table S2). The effects of genotype, genotype-year interaction and residual error were approximately equally high, which resulted in a heritability of 0.62 (Table 3). The high effect of genotype-year interaction corresponds with contradictory observations on correlations comparing both years of growing. In 2019, essential oil content was positively correlated with yield and negatively correlated with the

Table 3 (1) Summary data, (2) variance components and heritability for all investigated traits based on (1) 40 BLUEs or (2) 320 observations (plots) per trait. Both from 40 genotypes grown in two years with four repetitions per year. Mean = arithmetic mean, SD = standard deviation,

CV = coefficient of variance, Min = minimum, Max = maximum, V = variance component [G = genotype, GY = genotype-year interaction, Y = year, R = residual error], h^2 = broad-sense heritability. Trait abbreviations and units in Table 2

Trait	(1) Mean	SD	CV	Min	Max	(2) V_G	V_{GY}	V_Y	V_R	h^2
B10	87.56	4.41	0.05	80.50	98.63	18.58	1.36	7.13	1.29	0.96
B50	91.82	5.23	0.06	84.62	105.25	24.30	5.11	8.10	3.98	0.89
B90	96.71	5.99	0.06	88.00	113.87	30.40	10.46	6.16	2.38	0.85
EOF	129.64	4.48	0.03	120.25	140.38	18.17	3.18	4.35	2.55	0.91
HGT	63.99	5.82	0.09	49.33	73.44	29.88	1.43	0.04	26.68	0.88
MAT	166.67	4.83	0.03	153.75	180.50	18.28	7.82	1.02	9.25	0.78
SHT	86.38	13.46	0.16	41.25	100.00	126.65	78.43	0.00	121.87	0.70
YLD	87.39	35.81	0.41	8.13	135.99	1173.14	139.71	0.00	315.66	0.91
TGW	1.98	0.19	0.10	1.37	2.38	0.02	0.02	0.00	0.02	0.66
STA	1.88	1.41	0.75	0.50	7.38	1.37	0.39	1.41	3.29	0.69
OIL	3.57	0.60	0.17	2.13	5.14	0.22	0.22	0.00	0.20	0.62

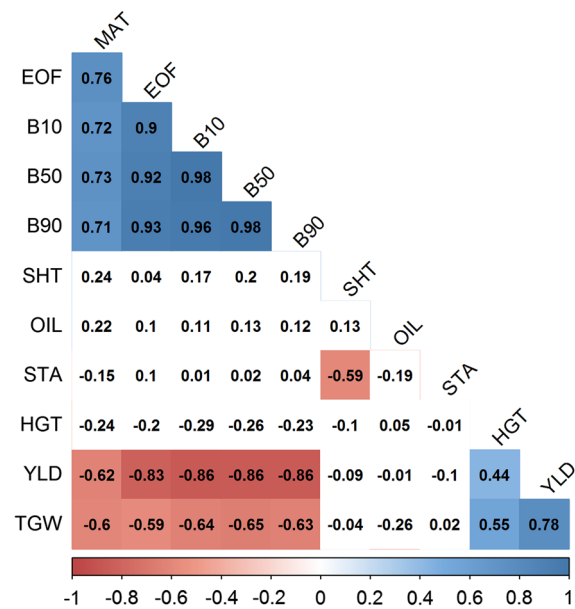


Fig. 2 Spearman correlation coefficients for correlations between traits. Negative correlations are highlighted by a red color scale and positive correlations by a blue color scale. In both cases, darker colors indicate stronger correlations. Correlations with r nearby zero and correlations with a p -value < 0.05 have a white background. A hierarchical clustering was used for ordering traits. Based on BLUEs from 40 genotypes grown in two years with four repetitions per year. Trait abbreviations and units in Table 2

beginning of flowering and the end of flowering. In 2020, essential oil content was negatively correlated with yield and positively correlated with the beginning of flowering and the end of flowering (Table S3). Across both years, no correlation was detectable (Fig. 2).

Comparison between inbred lines and F₁ populations & GCA

Yield and GCA

Figure 3 displays the average seed yield of all genotypes across both years and allows comparing each inbred line with the respective F₁ population (e.g. L01 with F01). We observed a broad range within inbred lines from 8.13 (L15) to 109.77 g/m² (L16) and an average seed yield of 59.33 g/m². In each case, the inbred line was outperformed by the corresponding F₁ population. The average seed yield of the F₁ populations was 108.97 g/m². The difference between inbred lines and F₁ populations was significant (Table 4). The inbred lines did not reach the yield level of the standard population cultivars 'Sprinter' and 'Ines', whereas several F₁ populations reached this level. However, no F₁ population considerably outperformed 'Sprinter'.

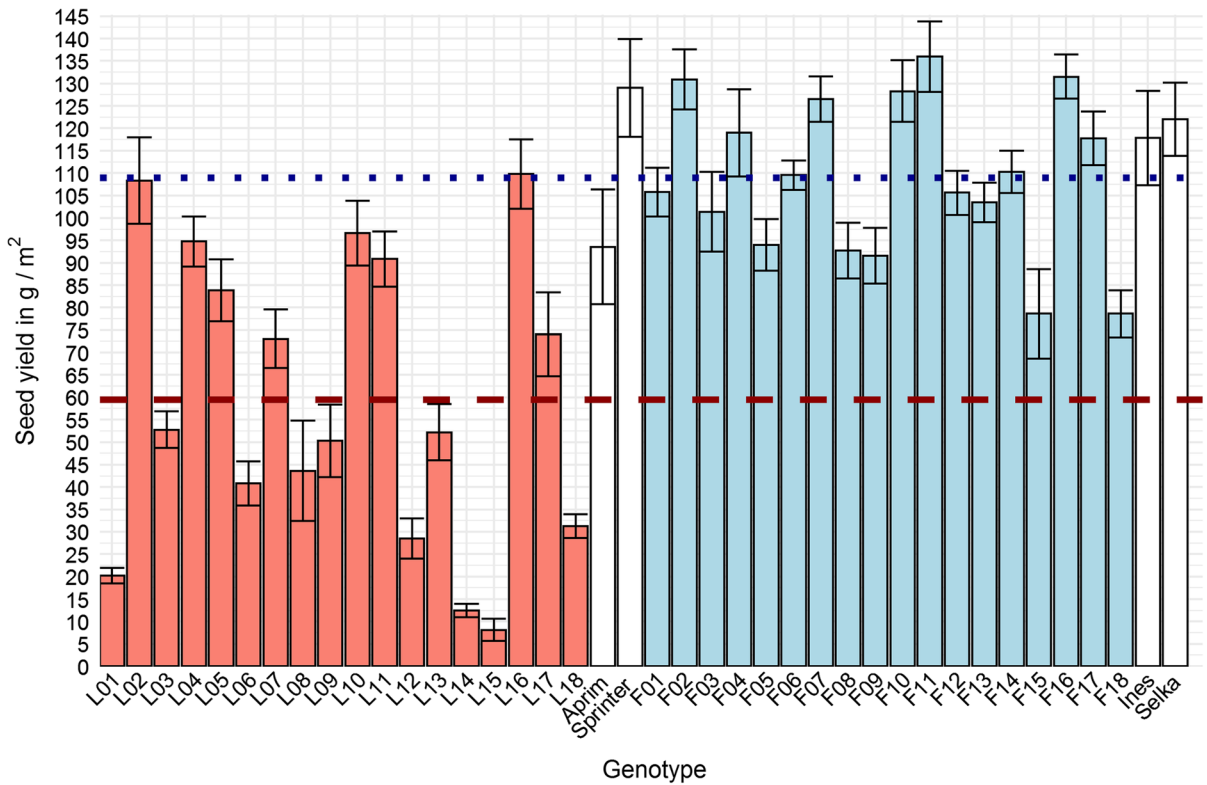


Fig. 3 Average seed yields of 40 genotypes grown in two years with four repetitions per year. Red colored bars stand for inbred lines, blue colored bars for F₁ populations and white colored bars for standards. Error bars = standard errors. Dashed red

line = average seed yield of all 18 inbred lines (L01-L18), dotted blue line = average seed yield of all 18 F₁ populations (F01-F18)

Table 4 (1) Results from paired Wilcoxon tests and (2) values associated with linear regression analysis. Both from pairwise comparisons between inbred lines and corresponding F₁ populations within investigated traits. Based on 18 pairwise comparisons between BLUEs from 18 inbred lines and 18 corresponding F₁ populations grown in two years with four

repetitions per year. Trait abbreviations and units in Table 2. Mean (I) = average of all inbred lines. Mean (F₁) = average of all F₁ populations. DIF (F₁-I) = difference between Mean (F₁) and Mean (I). RMSE = Root Mean Squared Error. R² = coefficient of determination

Trait	(1) Mean (I)	Mean (F ₁)	DIF (F ₁ -I)	P value	(2) RMSE	R ²	P value
B10	90.32	86.06	- 4.26	< 0.001	1.00	0.64	< 0.001
B50	95.06	89.91	- 5.15	< 0.001	1.04	0.64	< 0.001
B90	100.20	94.78	- 5.42	< 0.001	1.66	0.55	< 0.001
EOF	131.56	129.47	- 2.09	0.019	1.39	0.63	< 0.001
HGT	59.78	67.67	7.89	< 0.001	2.77	0.06	0.332
MAT	168.77	166.01	- 2.76	0.005	1.80	0.48	0.002
SHT	85.21	89.05	3.84	0.417	3.03	0.78	< 0.001
YLD	59.53	108.97	49.44	< 0.001	11.81	0.53	< 0.001
TGW	1.84	2.08	0.24	< 0.001	0.04	0.49	0.001
STA	2.20	1.68	- 0.52	0.196	0.92	0.26	0.031
OIL	3.60	3.56	- 0.04	0.962	0.25	0.35	0.01

GCA estimates can already be retrieved from Fig. 3 by comparing the individual seed yield of F_1 populations to their overall mean. In Fig. 4, GCA estimates per se are shown for easier comparison. Linear regression revealed that 53% of variation between F_1 populations could be explained by variation between inbred lines. The yield of the maternal lines significantly correlated with the yield of the corresponding F_1 populations (Fig. 5, Table 4).

Essential oil content

Figure 6 adds information on the average essential oil content of genotypes as most important quality trait for later selection decisions. Again, we observed a broad range within inbred lines from 2.13 (L11) to 4.94 ml/100 g (L04). The essential oil content of inbred lines averaged 3.6 ml/100 g. This is nearly identical with the average essential oil content of F_1 populations (3.56 ml/100 g). As a difference, we observed a narrower range for F_1 populations from 2.99 (F03) to 4.16 ml/100 g (F18). Single year observations were

contradictory: In 2019, the average essential oil content of F_1 populations was slightly higher than the average of inbred lines (3.85 vs. 3.41 ml/100 g), but in 2020, it was slightly lower (3.38 vs. 3.79 ml/100 g). The coefficient of determination shows that 35% of variation between F_1 populations could be explained by variation between inbred lines. The correlation was significant (Table 4).

Other traits

Apart from yield, we found significant differences between inbred lines and F_1 populations for all developmental traits, height and thousand-grain weight. Summarized, F_1 populations were earlier in development, plants grew higher and seeds were heavier in weight (Table 4). We found a significant correlation between values of inbred lines and corresponding F_1 populations for all traits except height. The coefficient of determination ranged from 0.26 (stalk attachment rate) to 0.78 (shattering rate, Table 4).

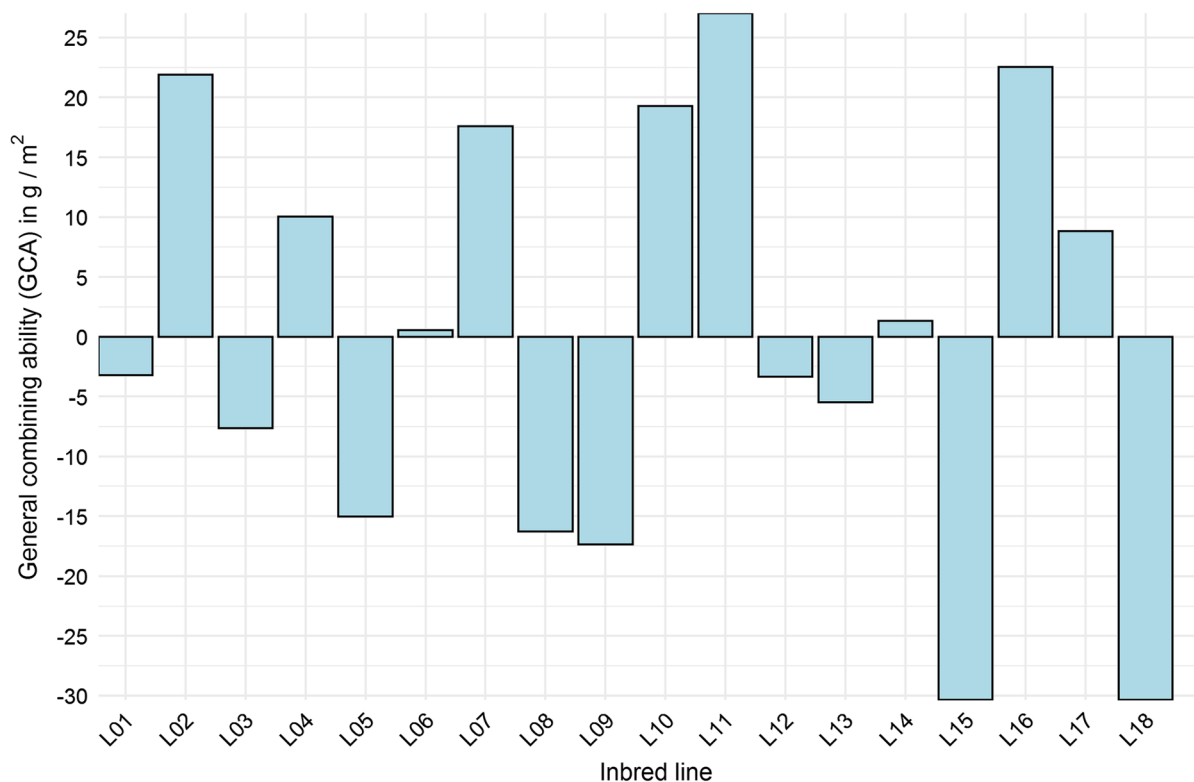


Fig. 4 General combining ability (GCA) of 18 inbred lines. Based on BLUEs from 18 corresponding F_1 populations grown in two years with four repetitions per year

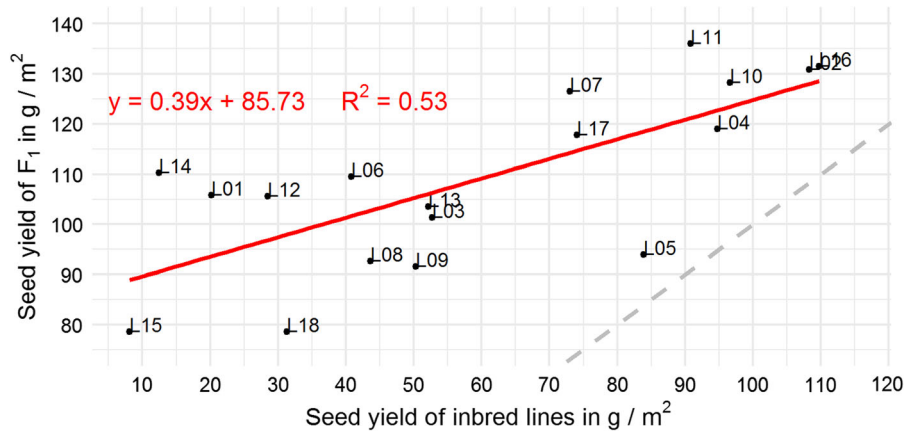


Fig. 5 Linear regression between seed yield of 18 inbred lines and seed yield of 18 corresponding F₁ populations grown in 2 years with four repetitions per year. R² = coefficient of determination. Red line = regression line, dashed grey line = bisector

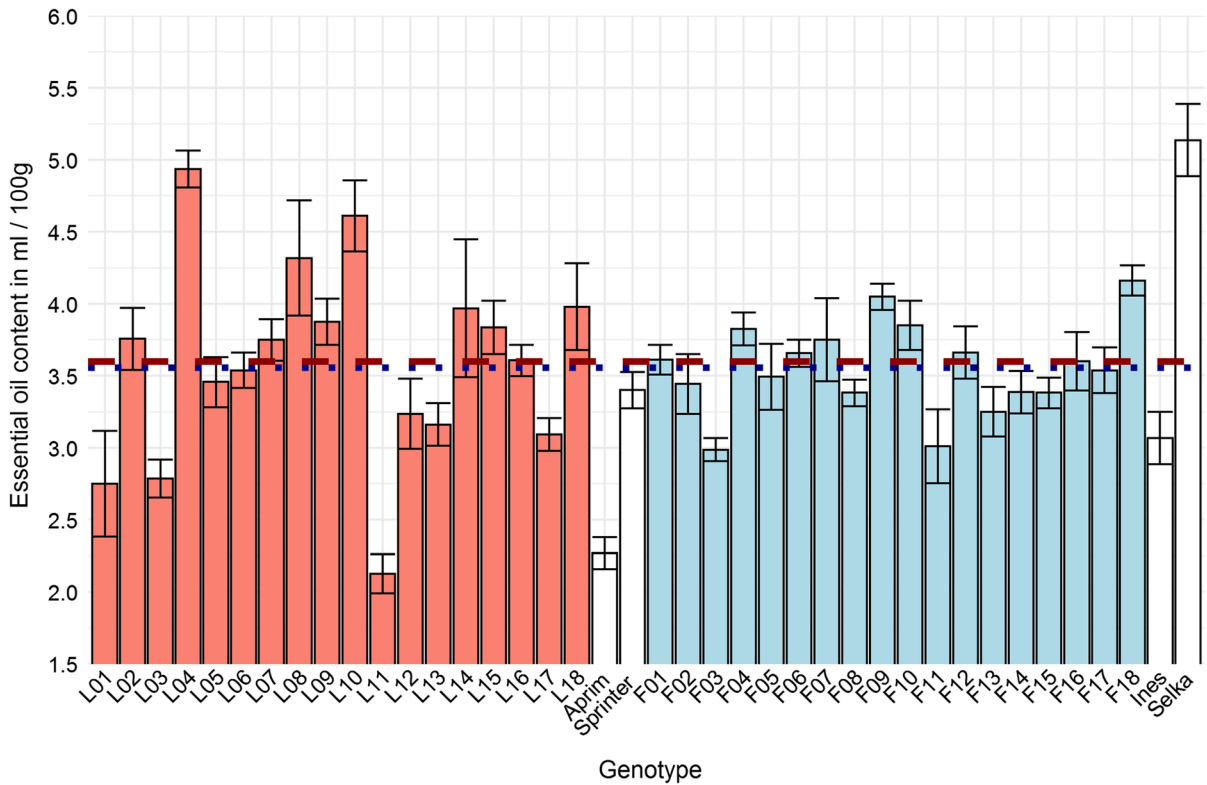


Fig. 6 Average essential oil content measured by distillation of 40 genotypes grown in two years with four repetitions per year. Red colored bars stand for inbred lines, blue colored bars for F₁ populations and white colored bars for standards. Error bars = standard errors. Dashed red line = average essential oil content of all 18 inbred lines (L01–L18), dotted blue line = average essential oil content of all 18 F₁ populations (F01–F18)

Discussion

Heterosis and inbreeding depression

Caraway breeders expected that inbreeding would cause inbreeding depression. They suggested sticking to population breeding (Toxopeus 1998). To the best of our knowledge, we showed now for the first time that strong inbreeding depression indeed occurs in caraway after inbreeding. We found some very low yielding inbred lines that can be described as highly inbreeding depressive lines. All inbred lines failed to reach the yield level of the standard population cultivar 'Sprinter'. A yield test of JKI breeding lines in the third inbred generation and of corresponding F_1 populations did not yet reveal strong inbreeding depression and heterosis (Pank et al. 2007). Possibly, inbreeding depression was mainly generated in the subsequent step/steps of inbreeding. Finally, we can argue that the existence of inbreeding depression as reverse side of heterosis (Mackay et al. 2021) can be considered evident, if we can provide evidence for heterosis.

All and, importantly, even the best yielding inbred lines showed a yield increase after outcrossing in the polycross. Therefore, we can state that heterosis as better parent heterosis can be exploited in caraway. Whereas we can decidedly make this general statement, a statement on heterosis for each inbred line is more difficult. The prior evaluation of the polycross revealed that we could not assume completely random pollination in the polycross. Furthermore, inbred lines had different outcrossing rates (von Maydell et al. 2020). Without knowledge of the paternal side of the F_1 populations, we can compute neither mid-parent heterosis, nor better-parent heterosis for each line. Nevertheless, such computations might be of scientific interest rather than important for the breeding progress. Not the heterosis, but the GCA is used to select components for a synthetic variety.

General combining ability & performance of lines per se

In theory, the GCA should comprise most information necessary to select components for a synthetic variety. The GCA should be constituted of the performance of a line per se and the heterosis generated by outcrossing. Based on this assumption, the performance of a

line per se could be ignored in selection decisions. Wright (1973) and further authors discussed using the general varietal ability (GVA) instead of GCA, which gives additional emphasis on the performance of the inbred lines per se, in particular, if the number of components is low. Essentially, this should account for decreasing heterozygosity according to Hardy–Weinberg equilibrium in Syn_2 or later generations. However, again we have to keep the mixed mating system of caraway in mind. Since outcrossing is likely to be incomplete in Syn_1 generation, we rather expect that outcrossing proceeds and heterozygosity increases in Syn_2 or later generations. In this case, the significance of the performance of the inbred lines per se for the later synthetic variety might actually be slightly overestimated within the GCA, determined in our study. The estimation of the specific combining ability (SCA) would provide information on non-additive effects, but the necessary procedure would be too laborious without an efficient system to produce F_1 seeds in multiple crossings.

For breeding synthetics, it seems advisable to focus on inbred lines with a high yield per se. We found a strong correlation of the yield of inbred lines with the yield of the corresponding F_1 populations and hence with GCA. In the end, selection based on GCA might make no large difference to selection based on performance of inbred lines per se in our dataset. Selection of high yielding lines should also be an advantage from a long-term perspective. Research in many species revealed that heterosis might be widely explained by additive dominance effects, whereas overdominance or epistasis merely play additional roles. (Kaepler 2012; Mackay et al. 2021; Zhou et al. 2018). By recurrent selection within the gene pool of high yielding caraway breeding material, favorable dominant alleles could be accumulated. As alternative to single crossings, a synthetic population can provide the genetic basis for future breeding programs.

If we take into account that performance of lines per se strongly correlates with GCA and that the mixed mating system causes some statistical noise, one could question whether costly GCA testing is necessary, after all. It might be more cost efficient to test a few different combinations of high yielding inbred lines directly after a preselection of lines. This notion certainly applies to the present dataset of just about five valuable inbred lines. In the near future, the pool of valuable inbred lines might be considerably

extended. In a larger pool of high yielding inbred lines, the effect of the performance of lines per se should decrease and other factors like genetic distance might increase in importance.

Besides, it did not surprise that the yield of inbred lines per se correlated with the yield of the F_1 populations and that we found such correlations for most other traits. First, the maternal line genetically contributes 50% to a F_1 population. Second, this proportion increases due to incomplete outcrossing. On average, about one third of individuals of an F_1 population should genetically be identical with the maternal line. On the other side, we were surprised that the plots of F_1 populations visually seemed to be rather uniform, i.e. they showed a homogeneous flowering, ripening and plant horizon. We suspect that inbreeding depressive plants were suppressed within the plot. Should this assumption be true, this could have an impact on our expectations for Syn_1 , Syn_2 and later synthetic generations. First, more heterosis might already be exploited in Syn_1 than we argued before, if outcrossed individuals occupy most of the space in a plot. Second, a synthetic population might virtually improve itself and favor outcrossing over generations. Synthetic populations should be investigated over several generations to gather evidence for such questions.

Selection for higher essential oil content

Next to yield, the essential oil content has to be considered as most important quality trait. The heritability of the essential oil content was only medium, partially due to a high genotype-year interaction effect. We assume this corresponds with the contradictory findings on correlations between essential oil content and other traits comparing both years of growing. We observed that most early flowering genotypes had a lower essential oil content in 2020 than in 2019, whereas most late flowering genotypes had a higher essential oil content in 2020. We assume that the essential oil content to a considerable degree depends on weather conditions at a certain stage of development. Literature indicates that environmental conditions in the ripening period play a major role (Bouwmeester et al. 1995; Toxopeus and Bouwmeester 1992).

Although high genotype-year interaction effects are detrimental to the breeding progress, we should not

neglect the equally high genotype effect. Most importantly, we found inbred lines that combine acceptable yields with high essential oil content. Hence, we can select for higher essential oil content within the given gene pool. Usually, quality traits do not show strong inbreeding depression and heterosis (Mackay et al. 2021). Our results are in accordance with this general observation because across both years we found no significant difference between inbred lines and corresponding F_1 populations. Yet, F_1 populations are earlier in development, which can affect essential oil content in both directions probably depending on weather conditions. This should explain the contradictory effects of outcrossing comparing both years of growing.

Significance of other traits for selection decisions

Developmental traits

The beginning and the end of flowering showed a very strong correlation with yield, a high heritability and can easily be estimated. They could be valuable traits to select for higher yield particularly in breeding stages that do not allow reliable yield estimations. The strong correlations with yield were already detected within the polycross data (von Maydell et al. 2020). Probably, late developing genotypes will not play any role in future breeding processes because of the low seed yields of these genotypes. Besides, in a selected pool of early flowering genotypes, genotype-year interaction effects of the essential oil content, as discussed above, might be reduced. Maturity showed a lower correlation with yield and a lower heritability. Hence, this trait might be a bit less suitable for selections. Pank (2012) mentioned early ripening as breeding goal for annual caraway. Early flowering and ripening might be advantageous due to better water availability, better pollination conditions or avoidance of pathogens like powdery mildew. However, we do not claim a definite causal relationship, i.e. that early development per se leads to increasing yield. Some genotypes might just have a higher 'general vitality', e.g. due to a more efficient production of metabolites, that goes along with faster vegetative growth, earlier generative development and higher yields. This might also be the likeliest explanation for the earlier development of F_1 populations compared to inbred lines.

Thousand-grain weight and height

It does not surprise that yield was positively correlated with thousand-grain weight. Acimovic et al. (2015) found a strong positive correlation between thousand-grain weight and harvest index for annual caraway. Thousand-grain weight might be less suitable for selection processes than the beginning or the end of flowering, because correlation with yield and heritability were lower and estimation is more laborious. From experience, we know that usually the early flowering umbels within plants dominate yield formation in caraway, whereas late flowering umbels produce only few additional seeds. This goes together with a reduction of female organs in later umbels (Pank 2012). Hence, selection towards higher thousand-grain weight might be a better option than selecting for higher numbers of umbels and seeds per plant.

Results for height showed a higher heritability than thousand-grain weight, but a lower correlation with yield. Although estimation of height is easy, it might be no preferable trait for selection processes. We found a large residual error in the analysis of variance components that was connected to a high variance within plots. For both, thousand-grain weight and height, we found a heterosis effect that goes along with the positive correlation with yield.

Shattering rate and stalk attachment rate

Selection of non-shattering genotypes is a general breeding goal for caraway (Pank 2012). Toxopeus and Lubberts (1994) detected severe yield losses after too late harvesting of shattering biennial varieties, whereas yield of non-shattering varieties was unaffected by late harvesting. In our study, only the cultivar Aprim and two inbred lines showed a clearly reduced shattering rate. Here, it must be considered that the shattering rate, as we measured it, was neither a measure of the real nor a measure of the potential loss of seeds before or during harvest, but an approximate measure of the firmness with which mature seeds are connected to the umbel. Unlike Toxopeus and Lubberts (1994) we found no negative correlation between shattering rate and yield. This might be due to the unbalanced dataset or the method of measurement, but probably we also harvested the plots in time so that severe losses by shattering were

avoided. It remains to be seen in future practical trials with standard harvesting machines whether shattering can be a problem within the selected breeding material.

Some farmers stated that strong non-shattering genotypes might tend to higher stalk attachment rates. High stalk attachment rates are detrimental to marketing. Since we found a negative correlation between shattering rate and stalk attachment rate, that statement might be true. We measured a maximum stalk attachment rate of 7.38% that would yet be no issue for marketing. However, the stalk attachment rate can strongly depend on harvesting and post-harvesting techniques (Pank 2012) and could be a problem in practical growing.

Conclusions

For the first time, we highlighted the importance of heterosis to gain acceptable yields in caraway. Summarized, outcrossing led to a significant increase in yield, thousand-grain weight and height and to earlier beginning of flowering, end of flowering and maturity. We assume that outcrossing can slightly affect the essential oil content in both directions depending on weather conditions in certain developmental stages. It remains to be seen whether the composition of the best lines to a synthetic variety can exceed standard population cultivars considerably, at last. Nonetheless, results make confident that breeding of synthetic varieties will be the method of choice for future caraway breeding as long as hybrid breeding is not feasible. The fact that caraway exhibits a mixed mating system makes it difficult to predict potential yields of synthetic populations based on GCA testing alone. It will be necessary to evaluate the consecutive steps of the breeding method thoroughly. Additionally, we provided insights into correlations between traits. Most conspicuously, we found strong negative correlations between yield and all developmental traits. Therefore, we can recommend selecting for early developing genotypes in breeding programs, at least under growing conditions in Germany.

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Author contributions DvM designed and conducted the trial. DvM performed statistical analyses, supported by AMS and MRG. DvM investigated literature and wrote the original draft. AMS, AEM, MRG, UH, FM and WJ revised the draft. FM and WJ were responsible for project planning, administration and funding acquisition. All authors read and approved the submitted version.

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Availability of data and material Data used for this study are included in the manuscript or in supplementary material.

Code availability No specialized code was used for this study.

Declarations

Conflict of interest We disclosed all entities that supported the funding of the project.

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References

Acimovic M, Filipovic V, Stankovic J, Cvetkovic M, Đukanović L (2015) The influence of environmental conditions on *Carum carvi* L. var. *annuum* seed quality. *Field Veg Crops Res* 52:91–96. <https://doi.org/10.5937/ratpov52-8022>

Bates DM, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. <https://doi.org/10.18637/jss.v067.i01>

Bouwmeester HJ, Davies JAR, Smid HG, Welten RSA (1995) Physiological limitations to carvone yield in caraway (*Carum-carvi* L.). *Ind Crops Prod* 4:39–51. [https://doi.org/10.1016/0926-6690\(95\)00009-2](https://doi.org/10.1016/0926-6690(95)00009-2)

Bruznican S, De Clercq H, Eeckhaut T, Van Huylenbroeck J, Geelen D (2020) Celery and celeriac: a critical view on present and future breeding. *Front Plant Sci* 10:12. <https://doi.org/10.3389/fpls.2019.01699>

Erpenbach JT (2012) Pflanzenbauliche und züchterische Beurteilung von ein- und zweijährigen Kümmelsorten und -herkünften (*Carum carvi* L.). diploma thesis, University of Bonn

Gholizadeh A, Dehghani H, Khodadadi M, Gulick PJ (2018) Genetic combining ability of coriander genotypes for agronomic and phytochemical traits in response to contrasting irrigation regimes. *PLoS ONE* 13:15. <https://doi.org/10.1371/journal.pone.0199630>

Harrell FE (2020) Hmisc: Harrell Miscellaneous. R package version 4.3–1.

Kaepler S (2012) Heterosis: Many Genes, Many Mechanisms—End The Search For An Undiscovered Unifying Theory. *ISRN Botany* 2012:682824. <https://doi.org/10.5402/2012/682824>

Liu J, Li MJ, Zhang Q, Wei X, Huang XH (2020) Exploring the molecular basis of heterosis for plant breeding. *J Integr Plant Biol* 62:287–298. <https://doi.org/10.1111/jipb.12804>

Mackay IJ, Cockram J, Howell P, Powell W (2021) Understanding the classics: the unifying concepts of transgressive segregation, inbreeding depression and heterosis and their central relevance for crop breeding. *Plant Biotechnol J* 19:26–34. <https://doi.org/10.1111/pbi.13481>

Németh É (1998) Introduction. In: Németh E (ed) Caraway the genus *carum*, vol 7. Medicinal and aromatic plants—industrial profiles. Harwood Academic Publisher, Amsterdam, pp 1–8

Olesen K (1976) A completely balanced polycross design. *Euphytica* 25:485–488. <https://doi.org/10.1007/BF00041582>

Palumbo F, Galla G, Vitulo N, Barcaccia G (2018) First draft genome sequencing of fennel (*Foeniculum vulgare* Mill.): identification of simple sequence repeats and their application in marker-assisted breeding. *Mol Breed* 38:17. <https://doi.org/10.1007/s11032-018-0884-0>

Pank F (2012) Kümmel (*Carum carvi* L.). In: Hoppe B (ed) *Handbuch des Arznei- und Gewürzpflanzenbaus*, vol 4. Verein für Arznei- und Gewürzpflanzen SALUPLANTA e.V., Bernburg, pp 728–762

Pank F, Kruger H, Quilitzsch R (2007) Results of a polycross-test with annual caraway (*Carum carvi* L. var. *annuum* hort). *Zeitschrift Für Arznei- & Gewürzpflanzen* 12:127–135

Peterson BG, Carl P, Boudt K, Bennett R, Ulrich J, Zivot E (2014) PerformanceAnalytics: econometric tools for performance and risk analysis. *J: R Package Version* 1(3)

Ph. Eur. (2020) Kümmel Carvi Fructus. In: *Europäisches Arzneibuch*, vol 10, p 2270

Ph. Eur. (2020b) Ätherische Öle in pflanzlichen Drogen. *Europäisches Arzneibuch* 10:430–432

R Core Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing.

- Ruszkowska J (1998) Main chemical constituents of Carum. In: Nemeth E (ed) Caraway the genus carum. Medicinal and Aromatic Plants - Industrial Profiles, Amsterdam, pp 35–54
- Simon PW (2019) Classical and molecular carrot breeding. In: Simon P, Iorizzo M, Grzebelus D, Baranski R (eds) Carrot genome. Compendium of plant genomes. Springer, Cham, pp 137–147. https://doi.org/10.1007/978-3-030-03389-7_9
- Toxopeus H (1998) A century of breeding caraway in the Netherlands. In: Németh É (ed) Caraway the genus carum, vol 7. Hardwood Academic Publishers, Amsterdam, pp 105–128
- Toxopeus H, Bouwmeester HJ (1992) Improvement of caraway essential oil and carvone production in The Netherlands. Ind Crops Prod 1:295–301. [https://doi.org/10.1016/0926-6690\(92\)90031-P](https://doi.org/10.1016/0926-6690(92)90031-P)
- Toxopeus H, Lubberts HJ (1994) Effect of genotype and environment on carvone yield and yield components of winter-caraway in the Netherlands. Ind Crops Prod 3:37–42. [https://doi.org/10.1016/0926-6690\(94\)90076-0](https://doi.org/10.1016/0926-6690(94)90076-0)
- Varghese C, Varghese E, Jaggi S, Bhowmik A (2015) Experimental designs for open pollination in polycross trials. J Appl Stat 42:2478–2484. <https://doi.org/10.1080/02664763.2015.1043860>
- von Maydell D, Brandes J, Lehnert H, Junghanns W, Marthe F (2020) Breeding synthetic varieties in annual caraway: observations on the outcrossing rate in a polycross using a high-throughput genotyping system. Euphytica 217:1. <https://doi.org/10.1007/s10681-020-02732-5>
- Wei T, Simko V (2017) R package “corrplot”: Visualization of a Correlation Matrix (Version 0.84).
- Wickam H (2016) ggplot2: elegant graphics for data analysis. Springer, New York
- Wright AJ (1973) The selection of parents for synthetic varieties of outbreeding diploid crops. Theor Appl Genet 43:79–82. <https://doi.org/10.1007/BF00274961>
- Zhou Z et al (2018) Dissecting the genetic basis underlying combining ability of plant height related traits in maize. Front Plant Sci. <https://doi.org/10.3389/fpls.2018.01117>

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