Optimization of agricultural practices for crambe in Europe

Published Version:
Berzuini S., Zanetti F., Christou M., Alexopoulou E., Krzyzaniak M., Stolarski M.J., et al. (2021). Optimization of agricultural practices for crambe in Europe. INDUSTRIAL CROPS AND PRODUCTS, 171(1 November 2021), 1-9 [10.1016/j.indcrop.2021.113880].

Published:
DOI: http://doi.org/10.1016/j.indcrop.2021.113880

Sara Berzuini[a], Federica Zanetti[a*], Myrsini Christou[b], Efthymia Alexopoulou[b], Michał Krzyżaniak[c], Mariusz J. Stolarski[c], FedericoFerioli[e], Andrea Monti[a]

Optimization of agricultural practices for crambe in Europe

which has been published in final form in

Industrial Crops and Products Volume 171, 1 November 2021, n. 113880

The final published version is available online at:
https://doi.org/10.1016/j.indcrop.2021.113880

Optimization of agricultural practices for crambe in Europe

Sara Berzuini[a], Federica Zanetti[a*], Myrsini Christou[b], Efthymia Alexopoulou[b], Michał Krzyżaniak[c], Mariusz J. Stolarski[c], FedericoFerioli[e], Andrea Monti[a]

[a]Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum–Università di Bologna, Viale Fanin 44, 40127 Bologna, Italy

[b]Centre for Renewable Energy Sources and Saving,19th km Marathonos Avenue, 19009, Pikermi, Greece

[c]University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Genetics, Plant Breeding and Bioresource Engineering, Plac Łodzki 3,10-724 Olsztyn, Poland

[e]Department of Agricultural and Food Sciences (DISTAL), Alma Mater Studiorum–Università di Bologna, Food Science University Campus, Piazza Goidanich 60, 47521, Cesena, Italy

[*]Corresponding author: federica.zanetti5@unibo.it

ABSTRACT

Crambe (*Crambe abyssinica* Hochst R.E. Fries) has recently attracted a renewed interest by the bio-based industry due to its high seed oil content (up to 57%), particularly erucic acid (up to 65% of total fatty acids), short growing cycle, and high drought tolerance. A field trial was conducted during four consecutive growing seasons (2016-19) in Greece, Poland, and Italy. The commercial crambe variety (Galactica) was sown in early, intermediate, and late sowing dates in spring at two seeding rates (LD: 100 seeds m^{-2}, and HD: 200 seeds m^{-2}) in a factorial design at each test location. Mean crambe seed yields exceeded 1.5 Mg ha^{-1} across all years and locations. Italy and Greece were the most productive sites, with average seed yields of 2.11 Mg ha^{-1} and 1.97 Mg ha^{-1}, respectively. Oil yield, which was only determined in Italy and Poland, was about 30% greater in the southern environment (Italy). Nevertheless, 1000-seed weight (TKW) was greater in Poland (6.49 g) than Italy (6.12 g), revealing that lower temperatures during seed filling resulted in heavier seeds. In conclusion, sowing date played a key role in crambe productivity, with the earliest sowings resulting in greatest yields across all locations.

Key-words: Non-food crop; *Crambe abyssinica*; sowing date; seeding rate; seed yield; seed weight.

1. Introduction

Recently, the concept of bioeconomy has been introduced as a new paradigm to create, build up, and modernize economic systems based on a sustainable use of renewable biological resources (Aguilar et al., 2019), aimed at decoupling economic growth and exploitation of energy from fossil resources (Golembiewski et al., 2015). In this scenario, agriculture has been identified as a key sector in Europe for providing biobased feedstocks, like vegetable oils for biofuels and biobased materials to replace fossil resources. In particular, the industrial sector is searching for new raw materials to significantly replace fossil-based materials, which are constantly increasing in cost and a source of greenhouse gases (Carlsson et al., 2011). As a biobased feedstock, erucic acid is a long chain fatty acid (C22:1) with specific and well-established industrial applications, such as the production of erucamide (a slip agent for polyethylene production, Zorn et al., 2019), biolubricants, additives, and material for fibers (Zhu, 2016). Commonly, erucic acid is obtained from vegetable oils, in particular high erucic acid rapeseed (HEAR) (*Brassica napus* var. oleifera). However, cross-pollination problems of HEAR with canola quality rapeseed, which is cultivated worldwide, has raised concerns about the future large-scale production of HEAR without applying restrictions to prevent its gene flow (Samarappuli et al., 2020). Furthermore, because the seeds of HEAR and canola are physically identical, it is impossible to separate them during processing, causing problems with contamination since erucic acid is not legislated for food uses in both Europe (Regulation (EU) 2015/2284) and the USA (Abbott et al., 2003).

Crambe is a 1-m-tall herbaceous annual species belonging to *Brassicaceae* family and native to the Mediterranean. It can be an interesting source of erucic acid as an alternative to HEAR, due to its lower environmental impact (Krzyżaniak et al., 2013), higher erucic acid content, and morphologically different seeds than HEAR, thus ensuring that rapeseed seed can be contaminated. In addition, crambe is characterized by a relatively short growing cycle (90-110 d, Zanetti et al., 2016), needing about 1300-1500 GDD (Growing Degree Days, with a base temperature of 5°C) from sowing to harvest (Meijer and Mathijssen, 1996), that makes it well fitting into conventional European crop rotations. It can be also easily harvested with common harvest machines (Pari et al., 2020). Crambe is also recognized for its high tolerance to drought (Pitol et al., 2010) and soil salinity (Ionov et al., 2013), wide adaptability to different environmental conditions (Von Cossel et al., 2019), limited seed shattering, and resistance to diseases and pests (Machado et al., 2007; Ropelewska et al., 2020). In Brazil, recent studies identified crambe as a suitable break crop in rotation with soybean or as a summer cover crop (Bordin et al., 2020; Secco et al., 2021). Furthermore, in a controlled environment study, Acharya et al. (2019) showed that crambe is a poor host for soybean cyst nematodes. Taken together, these characteristics make crambe cultivation possible even in marginal land, avoiding the competition for arable land currently used for food production (Von Cossel et al., 2019). Despite its adaptability, crambe seed and oil yield can be highly influenced by environmental conditions (Reginato et al., 2013; Zorzenoni et al., 2019), thus limiting the potential scale up of this oilseed crop. As reported by Falasca et al. (2010) frost damage can occur at seedling stage when temperature falls below -5°C. Another limiting factor is waterlogging (Zhu, 2016) that can cause sudden plant death and the occurrence of several diseases (Glaser, 1996; Viana et al., 2015). Furthermore, in a Mediterranean climate, high temperatures at the end of the growing cycle, in particular during seed filling phase, significantly reduced crambe seed yield (Zanetti et al., 2016). Row seeding can be performed by a small grain mechanic or pneumatic seeder, or by a cultipacker seeder (Oplinger et al., 1991). Row spacing can vary from 0.12 and 0.90 m, but spacings, narrower than ≈0.30 m, usually improve seed yield reducing weed presence and promoting uniform maturity (Zoz et al., 2018). Seeding depth also deeply influences crambe performances, in particular sowing depths between 9-19 mm are recommended (Brandão et al., 2014), but in dry condition deeper sowing, up to ≈ 25 mm, promotes a better establishment (Oplinger et al., 1991).

Seed yields can vary greatly depending on growing environment; literature shows a seed yield from 0.97 to 2.95 Mg ha^{-1} (dry matter basis) across different European countries (see Righini et al., 2016 and references therein). Seed oil content also greatly varies between 35-60% (Pitol et al., 2010), but the most recurrent values are in the range of 35 to 45%, the main differences in oil content are due to seeding time or tested varieties (Samarappuli et al., 2020). Crambe meal, obtained after oil extraction, has a high protein content (>50% crude protein in hulled seeds, Samarappuli et al., 2020) and presents a digestibility similar to soybean meal. However, the presence of glucosinolates (70–150 μmol g^{-1} in the seed), in particular *epi*-progoitrin, is toxic to monogastric animals (Tripathi and Mishra, 2007); its use in food/feed applications, therefore, can only be upon detoxifying the meal (Zhu, 2016). Additional uses of crambe oil can be for the production of jet-fuel or biodiesel (Cajamarca et al., 2018) as crambe oil being characterized by higher calorific value and oxidative stability compared with soybean (Wazilewski et al., 2013).

For those reasons, more knowledge is needed to make decisions about best sowing date, seeding rate, and the optimal growing location to improve crambe agronomic productivity in Europe. Thus, the scope of this study was to determine the effect of sowing date, seeding rate, and climate on the agronomic performance of crambe across a wide range of environments spanning Italy, Greece, and Poland.

2. Material and Methods

2.1 Experimental layout and agronomic management

The commercial crambe variety, Galactica (supplied by Wageningen University and Research, Wageningen, The Netherland), was evaluated in Cadriano (Italy), Aliartos (Greece) and Łężany (Poland) during multiple seasons (2016-2019). The specific soil and historical climatic characterizations of each location are reported in Table 1. While Cadriano (Italy) and Aliartos (Greece) are both located in the Mediterranean region, but are characterized by two different types of climate, namely Mediterranean north (Cadriano) and Mediterranean south (Aliartos), Łężany (Poland) has a continental climate (Metzger et al., 2005).

Galactica was sown in three consecutive spring sowing dates (SD1, SD2, and SD3) at each test location in three or four consecutive years from 2016 to 2019 (Table 2). Adverse meteorological conditions in 2018 in Italy only permitted establishment of SD3, thus the trial was repeated in 2019. The sowing dates were defined as early (SD1), intermediate (SD2) and late (SD3) considering the environmental specificities of each test location and the growth needs of crambe (Samarappuli et al., 2020). Two different seeding rates were also compared at all locations and defined as: LD (= 110 seed m^{-2} corresponding to 0.26 m interrow distance) vs. HD (= 220 seeds m^{-2} corresponding to 0.13 m in Italy and Greece and 0.15 m in Poland interrow distance). The trials were arranged in a split-plot design with three or four replicates (depending on the location), with seeding time as the main plot and seeding rate as sub-plots. Individual sub-plot size was 1.7 m in Italy and Greece and 1.5 m in Poland width (corresponding to 12 rows in the HD and 6 rows in the LD plot) and 6 m long. In Italy and Poland sowing was carried out mechanically using a plot seeder, whereas in Greece sowing was done manually. NPK fertilization was optimized at each location depending on soil type and nutrient availability aiming at maintaining crambe under non-limiting conditions, as reported in Table 1. All the trials were rainfed, apart from Aliartos (Greece) in 2016, where about 60 mm of water was applied by a sprinkler irrigation system to promote crambe establishment and early growth. Weed control was performed manually, and pest and disease control were never needed. Otherwise in Poland chemical treatments (Indoxacarb at a rate of 25.5 g ha^{-1}), against diamondback moth (*Plutella xilostella*) were applied only in 2016.

2.2 Meteorological data

The main meteorological data, i.e. air temperature (minimum and maximum), daily precipitation, and number of rainy days were collected throughout the growing season by weather stations located nearby each experimental location (Table 2). GDD (Growing Degree days) were calculated for each location, seeding time, and growing season, from sowing to harvest using the following equation:

$$GDD = \sum[(T_{max}+T_{min})/2 - T_{base}] \qquad \text{Eq. (1)}$$

Where T_{max} and T_{min} are the maximum and minimum air temperature, respectively, and T_{base} for crambe was defined as 5°C (Meijer and Mathijssen, 1996).

Additional meteorological variables were calculated to further explain variations in crambe productivity across locations using the following equations:

$$\text{Precipitation/rainy d} = \frac{\text{cumulative precipitation from sowing to harvesr}}{\text{number of rainy days from sowing to harvest}} \qquad \text{Eq. (2)}$$

$$\text{GDD/rainy d} = \frac{\sum[(\text{Tmax}+\text{Tmin})/2 - \text{Tbase}]}{\text{number of rainy d from sowing to harvest}} \qquad \text{Eq. (3)}$$

2.3 Plant measurements

At all locations, harvesting was carried out in the same way. Before harvest, plant height was measured as the mean height of the crambe stand in each plot. When crambe plants reached maturity (i.e., residual seed moisture content < ~12%) the central portion of each plot, corresponding to 6 m^2, was manually cut at soil level and then threshed. Seed and straw (i.e., plant residue after removing seed) were individually weighted, and representative subsamples were oven dried at 105°C until constant weight to determine residual moisture. Straw and seed yields were adjusted to dry matter (DM). After harvest, only in Italy and Poland, the number of plants in two central 1-m-long rows of each plot were counted to determine plant density. Also, in Italy and Poland on representative sub-samples of seeds from individual plots, the thousand kernel weight (TKW) was determined by averaging the weight of 3 replicates of 1000 seeds each.

Oil content was determined in crambe seed samples from Italy and Poland only for the HD plots, adopting the following methodology. About 30 g of crambe seeds (including silicles) were ground in a coffee grinder for 40 s. An aliquot corresponding to 1.5 g of ground material was exactly weighed in a cellulose extraction thimble (22 × 80 mm), which was then inserted in a 30 mL glass extractor. Lipid extraction was performed in an in-line extraction unit for Soxhlet extraction (mod. R 306) from Behr Labor-Technik (Düsseldorf, Germany), using 60 mL of n-hexane as organic solvent. Soxhlet extraction was carried out for 2 h from the start of solvent siphoning into the round bottom flask placed on the heating element. Some pieces of pumice stone were added to the distillation flask to avoid bumping of liquid as the temperature increased. The extract containing the lipidic fraction was dried for 90 min over anhydrous sodium sulphate at 4°C, occasionally shaking. The organic solvent was then filtered over sodium sulphate in a 100 mL flat bottom flask and removed under reduced pressure at 30 °C in a rotary evaporator. The residual oil was further dried under nitrogen flow for 5 min keeping the flask in a water bath (50-55°C) and exactly weighed. Crambe oil yield was calculated multiplying the dry seed yield by the seed oil content. Fatty acid methyl esters (FAME) of crambe seed oil were quantified using gas chromatography according to the methods of Christopherson and Glass (1969), only in the samples from Italy.

2.4 Statistical analysis

Prior to ANOVA, the homoscedasticity of variance was verified with Bartlett's Test for $P \leq 0.05$. A 3-way ANOVA was adopted to test the effect of location, seeding time, and seeding rate on crambe straw yield, seed yield, and final plant height. For final plant density, seed weight (TKW) and oil yield, only data from Italy and Poland were compared in a 3-way ANOVA to test the effect of seeding time, seeding rate, and location. When ANOVA revealed statistically different means, the LSD, Fisher's test, was used to separate means ($P \leq 0.05$). Linear regression was used to understand the effect of different meteorological variables on the surveyed agronomic parameters. When the regression was found significant for $P \leq 0.05$ the coefficient of determination (r^2) has been reported. All the statistical analyses were carried using the Statgraphics Centurion 18 software (ver. 18.1.13, Statgraphics Technologies Inc., Virginia, USA).

3. Results

3.1 Meteorological data analysis and crop development

The meteorological conditions surveyed in the study were consistent with the typical climate of each test location during crambe growth cycle. The locations differed more in terms of precipitation than temperatures (Table 2). Aliartos (Greece) was the driest site, with on average 84 mm of precipitation from sowing to harvest, and in 2016, seeding time SD2 and SD3 received less than 30 mm of precipitation, thus requiring irrigation. Cadriano (Italy) had on average about 200 mm of precipitation from sowing to harvest, but in 2017 this amount was only half as much. Łężany (Poland) was the wettest site, but again there was great variation across years with cumulative precipitation ranging from 134 mm for SD2 in 2018, up to 405 mm for SD1 in 2016. Despite the variability in seasonal precipitation crambe developed well in all the test locations.

The mean Galactica thermal time from sowing to harvest was 1265 GDD, considering the different seeding time and locations (Table 2). Greece reported the lowest (965 GDD in 2017 SD1) and the highest values (1461 GDD in 2018 SD1) for GDD, presumably in relation to greater temperature variation across years and seeding time accompanied with extreme precipitation variability. In Italy and Poland, GDD were less variable, with a mean value of 1265 GDD in Cadriano (Italy) and 1352 GDD in Łężany (Poland), corresponding to at least ~100 d with a maximum of 130 d in Łężany (Poland) in 2016 (SD1) from sowing to harvest. Crambe crop cycle (Table 2) was always longer in the earlier seeding time (SD1) in Italy and Poland, while in Greece there was little variation across years and seeding time, with Galactica maturity averaging 88 d.

3.2 Crambe crop performance

All crop productivity parameters were significantly ($P≤0.05$) influenced by location, while seeding time affected only plant height at harvest, seed yield, and 1000-seed weight (TKW) (Table 3). Seeding rate had a significant effect only on seed yield and final plant density (Table 3). A significant location x seeding time interaction ($P≤0.05$) was detected for seed yield (Table 3).

Straw yields were significantly greater ($P≤0.05$) in Greece and Italy, averaging 3.79 Mg ha^{-1} DM, than at Poland, which averaged 2.20 Mg ha^{-1} DM (Fig. 1): a 42% reduction in comparison. Crambe seed yield was the most variable parameter across test locations, years, seeding rates, and sowing dates, showing a high variation coefficient (CV=0.34). Seed yield was the highest in Italy (2.11 Mg ha^{-1} DM), but not different than in Greece, where the mean value was 1.97 Mg ha^{-1} DM. Again, Poland was the least productive site reporting a mean seed yield of 1.73 Mg ha^{-1} DM. Seed yield also varied significantly among sowing dates and seeding rates (Table 3). In particular, the delay of sowing caused a linear decrease in the seed yield ($P≤0.05$). The highest seed yield was achieved in the earliest sowing date (SD1) with a mean of 2.25 Mg ha^{-1} DM. When sowing was delayed, there was a 15% decline in yield between SD1 and SD2 and again from SD2 to SD3. Seeding rate played a role in crambe seed yield and higher rate significantly increased seed yields (2.02 vs. 1.83 Mg ha^{-1} DM in HD and LD respectively, $P≤0.05$, Fig. 2A). The interaction between location and seeding time significantly affected crambe seed yield (Fig. 2B). There were no significant differences among sowing dates in Italy; while in Poland there was a difference between SD1 and SD3. In Greece crambe seed yield steadily declined from SD1 to SD3, and the early sowing in Greece (SD1) had the highest yield across all locations and sowing date, averaging 2.74 Mg ha^{-1} DM ($P≤0.05$). Final plant density, which was only measured in Italy and Poland, confirmed that higher seeding rate promoted greater plant density at harvest (Fig. 3). However, in Italy, crambe stands had more than double the number of plants (75 plants m^{-2}) than in Poland (35 plants m^{-2}) (Fig. 3), and this might partially explain the higher seed yield surveyed in Italy compared with Poland. Crambe plant height at harvest was affected by both location and seeding time (Table 3). In Italy, crambe produced the tallest plants (1.05 m), while in Greece they were the shortest (0.75 m, $P≤0.05$). When considering the effect of sowing date, SD3 showed significantly taller plants compared with SD1 and SD2, but the differences was minimal (i.e. +6% and +8% SD1 compared with SD2 and SD3, respectively). Location and sowing date significantly affected TKW ($P≤0.05$) (Fig. 4). Galactica produced heavier seeds in Poland than in Italy (6.49 vs. 6.12 g TKW, in Poland and Italy, respectively $P≤0.05$). The effect of seeding time on TKW was similar to that found for seed yield. A linear decrease (~10%) was measured in TKW between SD1 and SD2, and between SD2 and SD3 ($P≤0.05$). Furthermore, crambe oil yield in Italy was found to be 32% greater than in Poland (Fig. 5).

Significant linear relationships were measured for crambe final plant density, plant height at harvest, TKW, and oil yield when regressed against specific meteorological variables (Fig. 6). However, for seed yield, none of the considered meteorological variables showed a significant relationship. In particular, final plant density (plants m^{-2}) was significantly and positively related with the ratio precipitation/rainy d (r^2= 0.38, $P<0.01$), showing that plant density increased when precipitation exceeded a certain amount (~13 mm) for each rain event. Plant height at harvest showed a negative relation with the ratio GDD/rainy d ($r^2 = 0.29$, $P<0.01$), indicating that height declined with both higher GDD and low precipitation availability. Crambe TKW was negatively related to mean maximum temperature from sowing to harvest ($r^2 = 0.34$, $P<0.01$), indicating that higher temperatures induced crambe to form lighter seeds. Finally, crambe oil yield showed a positive relation with GDD ($r^2 = 0.37$, $P<0.01$), demonstrating that longer thermal time from sowing to harvest promoted greater oil yield.

4. Discussion

For many years crambe has been studied for its feasible introduction into European cropping systems as a source of high erucic seed oil. Despite growing interest by the biobased industry, crambe cultivation has never taken off in Europe, and only sporadically worldwide. Crambe oil is characterized by high compositional stability (Sokólski et al., 2020), and so has great potential as an ideal feedstock for the biobased industry. In the present study, this quality trait of crambe was confirmed. Seed oil composition was analyzed only in Italy (data not presented), and a mean erucic acid content of 54% DM was measured, irrespective of growing seasons and seeding time, a value in line with other studies in similar environments (Fanigliulo et al., 2021; Zanetti et al., 2016). As far as authors know, this is the first study in which the same crambe variety, was grown under very different environmental conditions (Greece, Italy, and Poland). The present results confirmed the broad environmental suitability of crambe to European climates, as previously reported by several authors (Righini et al., 2016; Stolarski et al., 2018; Von Cossel et al., 2019). Galactica was able to produce adequate seed yields (>1.5 Mg ha^{-1} DM) even when precipitation was very limited (<50 mm, from sowing to harvest) and its growth cycle short (~80 d). Moreover, Galactica's growth cycle was found stable across the different locations and seeding time, with values similar to that previously reported (Meijer et al., 1999). This trait might be of interest for crambe inclusion in European rotations, since farmers appreciate having clear and fixed windows for harvesting new crops to better manage farm labor and equipment.

In the present study, higher seed yields were achieved in Greece and Italy (southern sites), rather than Poland (northern site), while Zanetti et al. (2016), when comparing two locations in Italy, observed lower production in southern Italy (i.e. Sicily), where higher temperatures at the end of the growing cycle may have impeded crambe growth and productivity. In the present study, average growing season temperatures were similar across locations (Table 2). Because seeding time were optimized to local conditions, the lower productivity in Poland might have been associated with excessive and uneven precipitation. This may have interfered with germination, flowering, or seed formation phases. Crambe has been shown to have tolerance to water limitation (Machado et al., 2007), and is negatively impacted by excessively wet conditions (Sammarapuli et al., 2020; Wang et al., 2000). The suitability of crambe to milder and drier conditions was indicated by the relationship between final plant height and the GDD/rainy d ratio (Fig. 7), demonstrating that crambe preferred environments with higher temperatures and precipitation concentrated in single events rather than spread along the cycle. Seed yield in Poland (1.73 Mg ha^{-1} DM) is similar to values observed by Sokólski et al. (2020), and slightly higher than those found by Krzyżaniak and Stolarski (2019), who reported a maximum value of 1.51 Mg ha^{-1} DM in large scale trials (>1 ha). Crambe seed yields in Italy (grand mean 2.11 Mg ha^{-1} DM) are considerably higher than those previously reported by Laghetti et al. (1995), possibly due to improved genetics of Galactica compared with older accessions. Other Italian studies (Fontana et al., 1998; Zanetti et al., 2016) reported that crambe could exceed 3 Mg ha^{-1} DM in northern Italy, indicating high variability for seed yield. In agreement with Meijer et al. (1999), a compensation effect between seed yield and seed weight was observed in the present study, in fact in Poland, despite lower yields, crambe seed weight was significantly higher than in Italy. Also, oil yield was promoted with increased GDD accumulation (Fig. 6), confirming the higher suitability of crambe to northern Italy compared with Poland. The reported oil yields - ranging from 0.58 to 0.76 Mg ha^{-1} DM in Poland and Italy, respectively - are similar to other European reports (Fontana et al., 1998; Zanetti et al., 2016) and are also consistent with recent studies in Brazil (Secco et al., 2021). Oil yield values above 0.3 Mg ha^{-1} DM could be considered a reliable breakeven yield for calculating the profitability of crambe as demonstrated by Stolarski et al. (2018). Thus, the reported oil yields could assure a net income to farmers, particularly when crambe is sown in early spring or grown in southern European regions. Therefore, the definition of the optimal seeding time remains a strategic point for the future scale up of crambe. In particular, early sowing assured significantly higher seed yields in Greece, while in Italy and Poland the differences among seeding time was less variable (Fig. 2, bottom graph). As reported by Zanetti et al. (2016), in the southern Mediterranean climate the identification of optimal sowing time for crambe to achieve high yields needs to consider sowing early enough to avoid high temperatures (>35°C) during seed maturation. Different than in previous studies (Laghetti et al., 1995; Zanetti et al., 2016), in the present trials, crambe was sown in autumn in Greece because previous unpublished tests showed susceptibility to frost damage, making autumn sowing very challenging and with yield results lower than in spring sowing (A. Alexopoulou, personal communication).

Crambe seed weight (TKW) was also influenced by seeding time. In contrast with the study by Adamsen and Coffelt (2005), which reported no significant differences between seeding time, in the present study the earliest seeding time resulted in significantly higher TKW, and a progressive reduction was observed when delaying seeding time. This demonstrated that increased temperature during seed filling can reduce seed weight (Zanetti et al., 2016). Furthermore, the regression analysis (Fig. 6) highlighted that the higher maximum temperatures caused the formation of lighter seeds in the warmer conditions, i.e. southern environment and/or delayed seeding time.

For alternative crops, like crambe, for which selective herbicides are not yet available, the identification of the optimal seeding rate results a crucial aspect for their agronomic management. In the present study, seeding rate significantly affected only final plant density and seed yield, which were higher under the highest density (HD). Thus, a seeding rate of 220 seeds m^{-2} was identified as appropriate to achieve sustained seed yield, irrespectively of the growing environment nor the seeding time. The tested HD seeding rate assured a better soil coverage, in accordance with the results by Viana et al. (2015). Nevertheless, a doubling of the seeding rate (HD) only increased final plant stand by 38%, presumably in relation to increased competition for available resources (i.e., space, nutrients, water, etc.). Thus, further studies are needed for the fine tuning of crambe seeding rate, in particular when using pneumatic precision seeders, as in the case of rapeseed.

5. Conclusions

The commercial crambe variety Galactica was successfully cultivated in Europe across different locations and climates, but sowing date needed to be tailored to local conditions to maximize yield. In the Mediterranean region, early sowing between mid-February and early March allowed achieving the best productivity, likely by avoiding drought and extreme high temperatures during anthesis and seed development. In Northern Europe, spring sowing should be performed in early to mid-April to avoid low temperature and/or freezing injure. The adoption of higher seeding rate promoted seed yield and soil coverage, thus representing the best option for establishing crambe in Europe, irrespectively of seeding time. In the present study the southern sites (i.e., Italy or Greece) resulted more suitable for crambe cultivation than the northern one (i.e., Poland), but the productive performance of crambe was always above the economic threshold for farmers' profitability.

6. Acknowledgments

We specially thank Dr. Russ Gesch (USTA-ARS) for helping to review and edit the manuscript. Authors want to acknowledge for the technical supervision of the trials in Italy Angela Vecchi and Giuseppe Di Girolamo, and the LARAS laboratory staff (UNIBO) for helping in seed processing, cleaning and TKW determination. In Poland the results were obtained as part of a comprehensive study financed by University of Warmia and Mazury in Olsztyn, Faculty of Agriculture and Forestry, Department of Genetics, Plant Breeding and Bioresource Engineering (grant No. 30.610.007-110)

CRediT authorship contribution statement

Sara Berzuini: Data curation; Formal analysis; Writing - original draft; **Federica Zanetti**: Conceptualization; Data curation; Methodology; Software, Formal analysis; Writing - original draft; **Myrsini Christou**: Writing - review & editing; **Efthymia Alexopoulou**: Data curation; Writing - review & editing; **Michal Krzyżaniak**: Data curation; Writing - review & editing; **Mariusz J. Stolarski**: Writing - review & editing; **Federico Ferioli**: Data curation; Formal analysis; Writing - review & editing; **Andrea Monti**: Conceptualization; Funding acquisition; Supervision; Writing - review & editing

7. References

Abbott, P., Baines, J., Fox, P., Graf, L., Kelly, L., Stanley, G., Tomaska, L., 2003. Review of the regulations for contaminants and natural toxicants. Food Control. 14(6), 383–389. https://doi.org/10.1016/S0956-7135(03)00040-9.

Acharya, K., Yana, G., Berti, M., 2019. Can winter camelina, crambe, and brown mustard reduce soybean cyst nematode populations? Ind. Crop. Prod. 140, 111637. https://doi.org/10.1016/j.indcrop.2019.111637

Adamsen, F.J., Coffelt, T.A., 2005. Planting date effects on flowering, seed yield, and oil content of rape and crambe cultivars. Ind. Crop. Prod. 21(3), 293-307. https://doi.org/10.1016/j.indcrop.2004.04.012

Aguilar, A., Twardowski, T., Wohlgemuth, R., 2019. Bioeconomy for Sustainable Development. Biotechnol. J., 14: 1800638. https://doi.org/10.1002/biot.201800638

Bordin, I., Dos Santos Silva, N., Da Silva, T.R.B., Dos Santos, J.B., Gil, L.G., Dos Santos Canalli, L.B., Hojo, R.H., Llanillo, R.F., 2020. Soybean cropping systems on sandy soil of the Caiuá Sandstone formation in Northwestern Paraná, Brasil. Semin., Ciênc. Agrár. 41(5), 2061-2070. 10.5433/1679-0359.2020v41n5Supl1p206

Brandão, A.G., Silva, T.R.B., Henrique, L.A.V., Santos, J.S., Gonçalves, F.M.G., Kohatsu, D.S., Goncalves, A.C. Jr., 2014. Initial development of crambe due to sowing in different depths. Afr. J. Agric.Res. 10, 927–930.

Cajamarca, F.A., Lancheros, A.F., Araújo, P.M., Mizubuti, I.Y., Simonelli, S.M., Ida, E.I., Guimarães, M.F., 2018. Evaluation of various species of winter oleaginous plants for the production of biodiesel in the state of parana, brazil. Ind. Crop. Prod. 126, 113-118. https://doi.org/10.1016/j.indcrop.2018.10.003https://doi.org/10.1016/j.indcrop.2018.10.003

Carlsson, A.S., Yilmaz, J.L., Green, A.G., Stymne, S., Hofvander, P., 2011. Replacing fossil oil with fresh oil - with what and for what? Eur. J. Lipid Sci Tech. 113(7), 812-831. https://doi.org/10.1002/ejlt.201100032

Christopherson, S.W., Glass R.L., 1969. Preparation of milk fat methyl esters by alcoholysis in an essentially non-alcoholic solution. J. Dairy Sci. 52, 1289-1290. https://doi.org/10.3168/jds.S0022-0302(69)86739-1

European Parliament, Council of the European Union, 2015. Regulation (EU) 2015/2284 of the European Parliament and of the Council of 25 November 2015. OJ L 327, p. 23–24.

Falasca, S.L., Flores, N., Lamas, M.C., Carballo, S.M., Anschau, A., 2010. *Crambe abyssinica*: An almost unknown crop with a promissory future to produce biodiesel in Argentina. Int. J. Hydrog. Energy 35(11), 5808-5812. https://doi.org/10.1016/j.ijhydene.2010.02.095

Fanigliulo, R., Pochi, D., Bondioli, P., Grilli, R., Fornaciari, L., Folegatti, L., Malaguti, L., Matteo, R., Ugolini, L., Lazzeri, L., 2021. Semi-refined *Crambe abyssinica* (Hochst. EX R.E.Fr.) oil as a biobased hydraulic fluid for agricultural applications. Biomass Conv. Bioref. 1-13. https://doi.org/10.1007/s13399-020-01213-y

Fontana, F., Lazzeri, L., Malaguti, L., Galletti, S., 1998. Agronomic characterization of some *Crambe abyssinica* genotypes in a locality of the Po Valley. Eur. J. Agron. 9, 117–126. https://doi.org/10.1016/S1161-0301(98)00037-9

Glaser, L.K., 1996 Prepared by the Economic Research Service for the Risk Management Agency, Federal Crop Insurance Corporation.

Golembiewski, B., Sick, N., Bröring, S., 2015. The emerging research landscape on bioeconomy: What has been done so far and what is essential from a technology and innovation management perspective? Innov. Food Sci. Emerg. Technol. 29, 308-317. doi:10.1016/j.ifset.2015.03.006

Ionov, M., Yuldasheva, N., Ulchenko, N., Glushenkova, A.I., Heuer, B., 2013. Growth, Development and Yield of *Crambe Abyssinica* Under Saline Irrigation in the Greenhouse. J. Agro. Crop Sci. 199: 331-339. https://doi.org/10.1111/jac.12027

Krzyżaniak, M., Stolarski, M., Śnieg, M., Christou, M., Alexopoulou, E., 2013. Life cycle assessment of *Crambe abyssinica* production for an integrated multi-product biorefinery. Environ. Biotechnol. 9(2), 72-80. 10.14799/ebms220

Krzyżaniak, M., Stolarski, M.J., 2019. Life cycle assessment of camelina and crambe production for biorefinery and energy purposes. J. Clean. Prod. 237, 117755. https://doi.org/10.1016/j.jclepro.2019.117755

Laghetti, G., Piergiovanni, A.R., Perrino, P., 1995. Yield and oil quality in selected lines of *Crambe abyssinica* hochst. ex R.E. fries and *C. hispanica* L. grown in Italy. Ind. Crop. Prod. 4(3), 203-212. https://doi.org/10.1016/0926-6690(95)00033-9

Machado, M.F., Brasil, A.N., Oliveira, L.S., Nunes, D.L., 2007. Estudo do crambe (*Crambe abyssinica*) como fonte de óleo para produção de biodiesel. Itaúna/MG–UFMG.

Meijer, W.J.M., Mathijssen, E.W.J.M., 1996. Analysis of crop performance in research on inulin, fibre and oilseed crops. Ind. Crop. Prod. 5, 253-264. https://doi.org/10.1016/S0926-6690(97)82785-9

Meijer, W.J.M., Mathijssen, E.W.J.M., Kreuzer, A.D., 1999. Low pod numbers and inefficient use of radiation are major constraints to high productivity in crambe crops. Ind. Crop. Prod. 9(3), 221-233. https://doi.org/10.1016/S0926-6690(98)00035-1

Metzger, M.J., Bunce, R.G.H., Jongman, R.H.G., Mücher, C.A., Watkins, J.W., 2005. A climatic stratification of the environment of Europe. Glob. Ecol. Biogeogr. 14: 549-563. https://doi.org/10.1111/j.1466-822X.2005.00190.x

Oplinger, E.S., Oelke, E.A., Kaminski, A.R., Putnam, D.H., Teynor, T.M., Doll, J.D., Kelling, K.A., Durgan, B.R., Noetzel, D.M., 1991. Crambe. In Alternative Field Crops Manual; University of Wisconsin–Extension, Cooperative: Madison, WI, USA.

Pari, L., Latterini, F., Stefanoni, W., 2020. Herbaceous Oil Crops, a Review on Mechanical Harvesting State of the Art. Agriculture 10, 309. https://doi.org/10.3390/agriculture10080309

Pitol, C., Broch, D.L., Roscoe, R., 2010. Tecnologia e producão: crambe. Maracajú,Fundacão MS.

Reginato, P., Alves de Souza, C.M., da Silva, C.J., Leyva Rafull, L.Z., 2013. Agronomic performance and seed quality of crambe at different sowing dates and depths. Pesq. agropec. Bras. 48(10), 1410-1413. https://doi.org/10.1590/S0100-204X2013001000013.

Righini, D., Zanetti, F., Monti, A., 2016. The bio-based economy can serve as the springboard for camelina and crambe to quit the limbo. OCL 23(5), D504. https://doi.org/10.1051/ocl/2016021

Ropelewska, E., Jankowski, K.J., 2020. Effect of sulfur fertilization on the physical and chemical properties of crambe (*Crambe abyssinica* Hochst ex RE Fries) seeds. OCL 27, 18. https://doi.org/10.1051/ocl/2020008

Samarappuli, D., Zanetti, F., Berzuini, S., Berti, M.T., 2020. Crambe (*Crambe abyssinica* Hochst): A non-food oilseed crop with great potential: A review. Agronomy (Basel) 10(9), 1380. https://doi.org/10.3390/agronomy10091380

Secco, D., Bassegio, D., De Villa, B., Ciotti de Marins, A., Zanão Junior, L.A., Benetoli da Silva, T.R., Melegari de Souza, S.N., 2021. Crambe oil yield and soil physical properties responses to no-tillage, cover crops and chiseling. Ind. Crop. Prod. 161, 113174. https://doi.org/10.1016/j.indcrop.2020.113174

Sokólski, M., Załuski, D., Jankowski, K., 2020. Crambe: Seed yield and quality in response to nitrogen and sulfur — A case study in north-eastern Poland. Agronomy 10(9), 1436. https://doi.org/10.3390/agronomy10091436

Stolarski, M.J, Krzyzaniak, M., Kwiatkowski, J., Tworkowski, J., Szczukowski, S., 2018. Energy and economic efficiency of camelina and crambe biomass production on a large-scale farm in north-eastern Poland. Energy 150, 770-780. https://doi.org/10.1016/j.energy.2018.03.021

Stolarski, M.J., Krzyżaniak, M., Tworkowski, J., Załuski, D., Kwiatkowski, J., Szczukowski, S., 2019. Camelina and crambe production–Energy efficiency indices depending on nitrogen fertilizer application. Ind. Crop. Prod. 137, 386-395. https://doi.org/10.1016/j.indcrop.2019.05.047

Tripathi, M.K., Mishra, A.S., 2007. Glucosinolates in animal nutrition: A review. Anim. Feed Sci. Technol. 132(1), 1-27. https://doi.org/10.1016/j.anifeedsci.2006.03.003

Viana, O.H., Santos, R.F., de Oliveira, R.C., Secco, D., de Souza, S.N.M., Tokura, L.K., Gurgacz, F., 2015. Crambe (*'Crambe abyssinica'* H.) development and productivity under different sowing densities. Aust. J. Crop Sci., 9(8), 690. https://search.informit.org/doi/10.3316/informit.502255870680512

Von Cossel, M., Lewandowski, I., Elbersen, B., Staritsky, I., Van Eupen, M., Iqbal, Y., Mantel, S., Scordia, D, Testa, G., Cosentino, S.L., Maliarenko, O., Eleftheriadis, I., Zanetti, F., Monti, A., Lazdina, D., Neimane, S., Lamy, I., Ciadamidaro, L., Sanz, M., Carrasco, J.E., Ciria, P., McCallum, I., Trindade, L.M., Van Loo, E.N., Elbersen, W., Fernando, A.L., Papazoglou, E.G., Alexopoulou, E., 2019. Marginal agricultural land low-input systems for biomass production. Energies 12(16), 3123. https://doi.org/10.3390/en12163123

Wang, Y.P., Tang, J.S., Chu, C.Q., Tian, J., 2000. A preliminary study on the introduction and cultivation of *Crambe abyssinica* in China, an oil plant for industrial uses. Ind. Crop. Prod.12(1), 47-52. https://doi.org/10.1016/S0926-6690(99)00066-7

Wazilewski, W.T., Bariccatti, R.A., Martins, G.I., Secco, D., de Souza, S.N.M., Rosa, H.A., Chaves, L.I., 2013. Study of the methyl crambe (*Crambe abyssinica* Hochst) and soybean biodiesel oxidative stability. Ind. Crop. Prod. 43, 207-212. 10.1016/j.indcrop.2012.07.046

Zanetti, F., Scordia, D., Vamerali, T., Copani, V., Dal Cortivo, C., Mosca, G., 2016. *Crambe abyssinica* a non-food crop with potential for the Mediterranean climate: Insights on productive performances and root growth. Ind. Crop. Prod. 90, 152-160. https://doi.org/10.1016/j.indcrop.2016.06.023

Zhu, L., 2016. Chapter 7 - Crambe (*Crambe abyssinica*), in: McKeon, T., A., Hayes, D., G., Hildebrand, D., F., Weselake, R., J. (Eds.) Industrial oil crops (pp. 195-205) Elsevier Inc. https://doi.org/10.1016/B978-1-893997-98-1.00007-5

Zorn, K., Oroz-Guinea, I., Bornscheuer, U.T., 2019. Strategies for enriching erucic acid from *Crambe abyssinica* oil by improved Candida antarctica lipase A variants. Process Biochemistry, 79, 65-73. https://doi.org/10.1016/j.procbio.2018.12.022

Zorzenoni, T.O., Andrade, A.P. d., Higashibara, L.R., Cajamarca, F.A., Okumura, R.S., Prete, C.C., 2019. Sowing date and fungicide application in the agronomic performance of oleaginous brassica for the biodiesel production. Revista Ceres, 66(4), 257-264. https://doi.org/10.1590/0034-737x201966040003.

Zoz, T., Steiner, F., Zoz, A., Castagnara, D.D., Witt, T., Zanotto, M., Auld, D., 2018. Effect of row spacing and plant density on grain yield and yield components of *Crambe abyssinica* Hochst. Semin Cienc Agrar, 39, 393-402. http://dx.doi.org/10.5433/1679-0359.2018v39n1p393

Figure 1. Crambe straw yield (Mg DM ha^{-1}) in the multi-location and multi-year trial in response to the main effect location (Italy vs. Greece vs. Poland). Different letters: significant different means for $P \leq 0.05$ (LSD's Fisher test).

Figure 2. A: Crambe seed yield (Mg DM ha^{-1}) in the multi-location and multi-year trial in response to the main effect seeding rate (HD 220 seeds m^{-2} vs. LD 110 seeds m^{-2}). Different letters: significant different means for $P \leq 0.05$ (LSD Fisher's test). B: Crambe seed yield (Mg DM ha^{-1}) in the multi-location and multi-year trial in response in response to the interaction between location and sowing date. Different letters: significant different means for $P \leq 0.05$ (LSD's Fisher's test).

Figure 3 Final crambe plant density (pp m^{-2}) at harvest in the multi-location and multi-year trial in response to the main effects: location (Italy vs. Poland) and seeding rate (HD 220 seeds m^{-2} vs. LD 110 seeds m^{-2}). Different letters: significant different means for $P \leq 0.05$ (LSD Fisher's test) within the same main effect.

Figure 4. Crambe thousand seed weight (TKW, g) in the multi-location and multi-year trial in response to the main effects: location (Italy vs. Poland) and sowing date (SD1 vs. SD2 vs. SD3). Different letters: significant different means for $P \leq 0.05$ (LSD Fisher's test) within the same main effect.

Figure 5. Crambe oil yield (Mg DM ha^{-1}) in the multi-location and multi-year trial in response to the main effect: location (Italy vs. Poland). Different letters: significant different means for $P \leq 0.05$ (LSD Fisher's test).

Figure 6. Significant linear regressions between crambe final plant density, plant height, TKW, and oil yield and meteorological variables for data from Italy and Poland. r^2= Coefficient of determination. ** = significant for $P \leq 0.01$.

Table 1. Geographical coordinates, soil type, historical climatic data (20-years), soil tillage and NPK fertilization adopted in the crambe trials are reported.

Country Location	Italy Bologna	Greece Aliartos	Poland Łężany
Coordinates	44° 33’ N, 11° 23’ E	38° 22’ N, 23° 06’ E	53° 58’ N, 21° 08’ E
Soil Type	Clay Loam	Sandy Loam	Sandy Loam
Mean annual precipitation (mm)	613	485	683
Mean annual temperature (°C)	13.4	16.7	8.0
Tillage for seedbed preparation	Ploughing + rotary tilling	Ploughing + rotary tilling	Ploughing +rotary tilling + harrowing + rolling
NPK fertilization (kg ha^{-1})	50+0+0	83+45+45	120+0+0

Table 2. Sowing and harvesting dates, Growing Degree Days (= GDD), cycle length (d) from sowing to harvest, and main meteorological parameters surveyed in the crambe multi-year (2016-2019) trial grown across three locations in Europe. GDD = growing degree days from sowing to harvest; Prec = cumulative precipitation from sowing to harvest; T_{min} = mean minimum temperature from sowing to harvest; T_{max} = mean maximum temperature from sowing to harvest.

Country	Year	ST	Sowing	Harvest	GDD*	Cycle	Rain.	T_{min}	T_{max}
(Location)			date			(d)	(mm)		°C
Greece	2016	SD1	Mar 08	Jun 15	1237	104	88	10.5	23.3
(Aliartos)		SD2	Mar 21	Jun 18	1203	90	25.5	11.5	25.2
		SD3	Apr 05	Jun 23	1209	80	9.9	13.1	27.1
	2017	SD1	Feb 28	May 26	964	88	164	8.4	21.1
		SD2	Mar 19	Jun 12	1157	86	133.3	10.6	23.4
		SD3	Apr 03	Jun 18	1146	77	132.7	11.9	24.6
	2018	SD1	Mar 03	Jun 10	1461	100	61.2	9.5	22.2
		SD2	Mar 19	Jun 15	1416	89	58.7	9.6	22.8
		SD3	Apr 03	Jun 20	1378	79	65.2	10.4	24.4
Average					1241	88	82	10.3	23.8
Italy	2016	SD1	Feb 12	Jun 20	1158	128	383.2	8.9	19.0
(Bologna)		SD2	Mar 15	Jun 30	1355	108	226.6	11.1	22.5
		SD3	Mar 30	Jul 17	1457	106	247.8	13.1	24.5
	2017	SD1	Feb 17	Jun 14	1169	118	106.8	8.3	21.5
		SD2	Mar 01	Jun 14	1147	106	105.6	9.0	22.5
		SD3	Mar 15	Jun 21	1219	99	103.6	10.5	23.9
	2018	SD3	Mar 27	Jul 05	1341	101	127.6	13.2	25.3
	2019	SD1	Feb 13	Jun 26	1253	134	229.8	7.8	20.8

		SD2	Feb 28	Jun 26	1228	119	229.6	8.9	21.5
		SD3	Mar 14	Jul 04	1329	113	231.0	10.3	22.9
Average					1206	113	199.2	10.1	22.4
Poland	2016	SD1	Apr 05	Aug 08	1339	130	405.3	10.4	20.7
(Łężany)		SD2	Apr 15	Aug 12	1286	120	391.0	10.7	21.2
		SD3	Apr 25	Aug 21	1365	119	404.2	11.4	22.0
	2017	SD1	Apr 10	Aug 16	1281	129	237.4	8.5	20.9
		SD2	Apr 20	Aug 16	1270	119	212.4	9.1	21.8
		SD3	Apr 28	Aug 25	1325	120	212.2	9.9	22.6
	2018	SD1	Apr 16	Aug 03	1430	110	145.2	10.8	24.6
		SD2	Apr 26	Aug 07	1416	104	134.4	11.4	25.4
		SD3	Apr 28	Aug 16	1459	102	165.4	12.3	25.9
Average					1352	144	256.4	10.5	22.8

*base temperature for GDD calculation was 5°C (Meijer and Mathijssen. 1996)

Table 3. ANOVA results (F-values and Statistical significance) for the surveyed parameters (Straw yield, seed yield, plant height, final plant density, oil yield and TKW) in the multi-year multi-location trails on crambe. Loc = location (Greece vs. Italy vs. Poland); SD = sowing date (SD1 vs. SD2 vs. SD3); SR = seeding rate (HD 220 seeds m^{-2} vs. LD 110 seeds m^{-2}).

Source of Variation	Straw yield	Seed yield	Plant height	Final plant density[1]	Oil yield[2]	TKW[1]
Loc	61.26 **	8.45**	79.12**	60.13**	21.11**	7.94**
SD	2.48 *ns*	24.79***	3.35*	1.58 *ns*	2.29 *ns*	33.06**
SR	2.68 *ns*	4.41*	2.19 *ns*	11.67**	-	0.04 *ns*
Loc x SD	1.13 *ns*	9.15**	2.02 *ns*	2.40 *ns*	0.61 *ns*	1.06 *ns*
Loc X SR	2.48 *ns*	1.78 *ns*	0.85 *ns*	3.28 *ns*	-	0.01 *ns*
SD X SR	0.13 *ns*	0.06 *ns*	0.04 *ns*	0.24 *ns*	-	0.43 *ns*
Loc X SD X SR	1.39 *ns*	0.15 *ns*	0.87 *ns*	0.82 *ns*	-	0.14 *ns*

*, ** Significant at the 0.05, 0.01probability levels, respectively (LSD Fishers' test); *ns*=not significant.

[1] only data from Italy and Poland are included in the ANOVA

[2] only data from Italy and Poland from only HD plots are included in the ANOVA

Figure 1. Crambe straw yield (Mg DM ha^{-1}) in the multi-location and multi-year trial in response to the main effect location (Italy vs. Greece vs. Poland). Vertical bars: standard error. Different letters: significant different means for $P \leq 0.05$ (LSD's Fisher test).

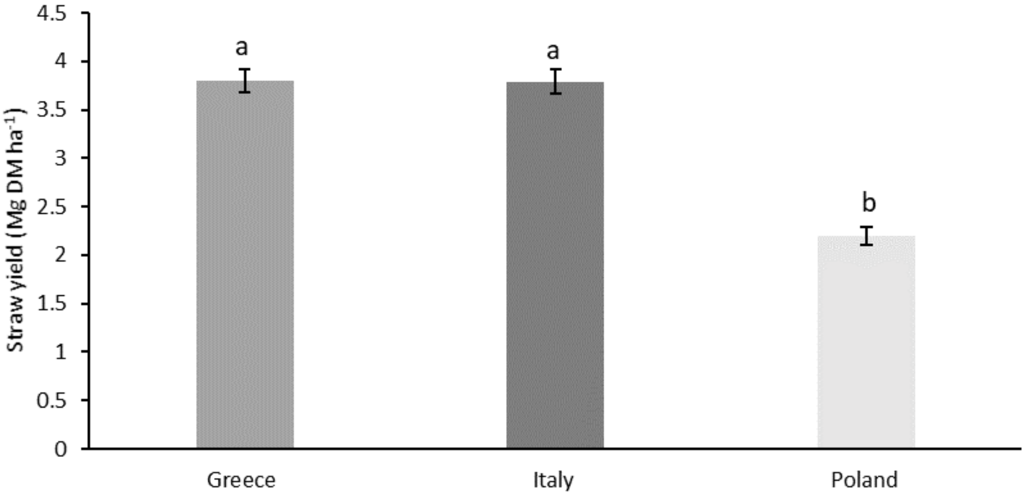

Figure 2. A: Crambe seed yield (Mg DM ha^{-1}) in the multi-location and multi-year trial in response to the main effect seeding rate (HD 220 seeds m^{-2} vs. LD 110 seeds m^{-2}). Different letters: significant different means for P≤0.05 (LSD Fisher's test). B: Crambe seed yield (Mg DM ha^{-1}) in the multi-location and multi-year trial in response in response to the interaction between location and sowing date. Vertical bars: standard error. Different letters: significant different means for P≤0.05 (LSD's Fisher's test).

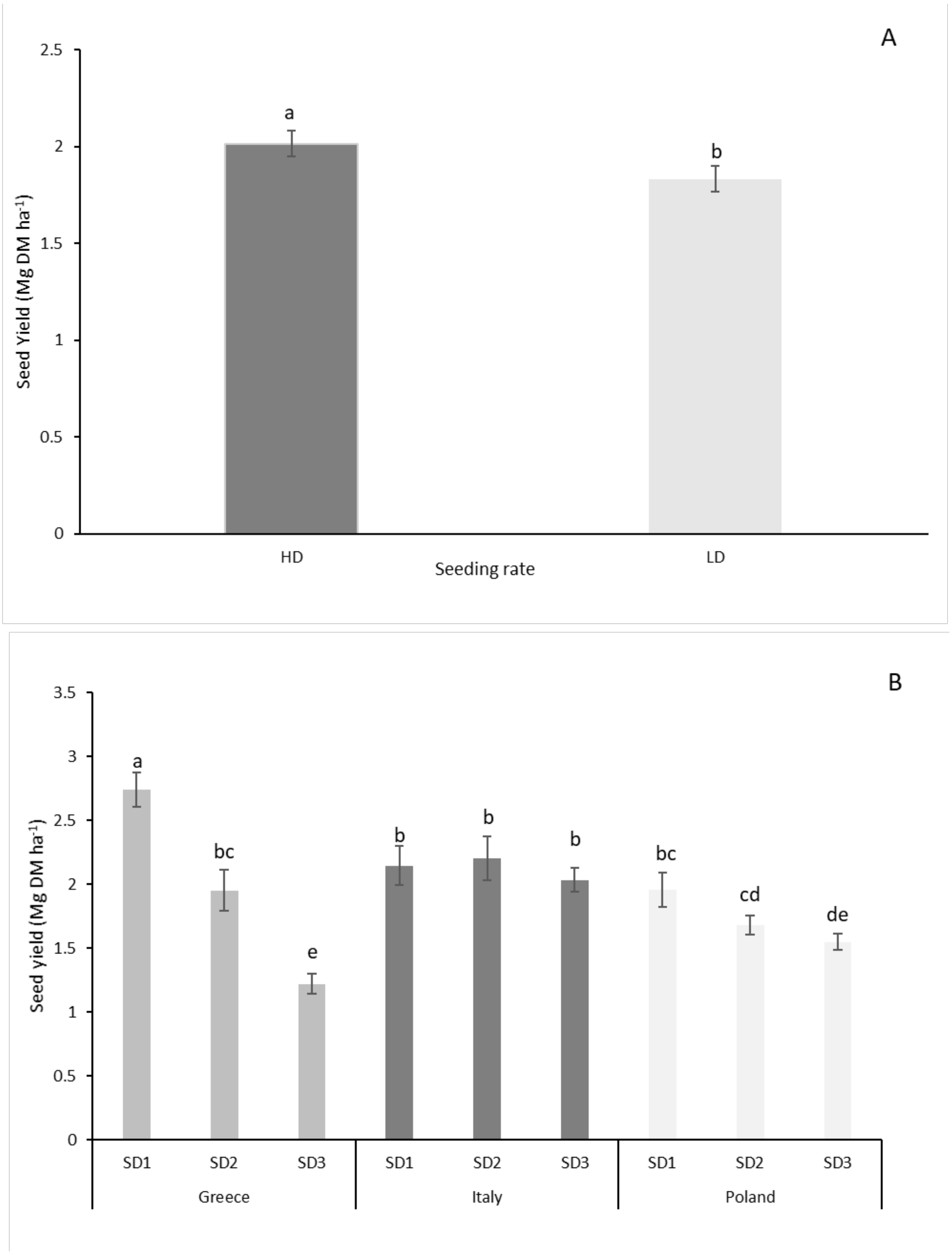

Figure 3. Final crambe plant density (pp m^{-2}) at harvest in the multi-location and multi-year trial in response to the main effects: location (Italy vs. Poland) and seeding rate (HD 220 seeds m^{-2} vs. LD 110 seeds m^{-2}). Vertical bars: standard error. Different letters: significant different means for *P*≤0.05 (LSD Fisher's test) within the same main effect.

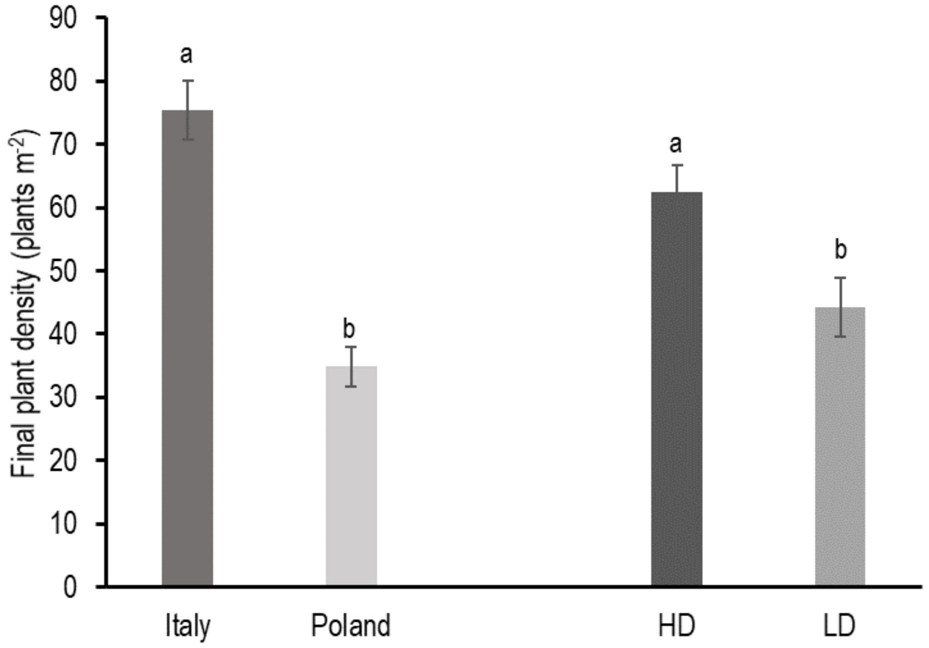

Figure 4. Crambe thousand seed weight (TKW, g) in the multi-location and multi-year trial in response to the main effects: location (Italy vs. Poland) and sowing date (SD1 vs. SD2 vs. SD3). Vertical bars: standard error. Different letters: significant different means for *P*≤0.05 (LSD Fisher's test) within the same main effect.

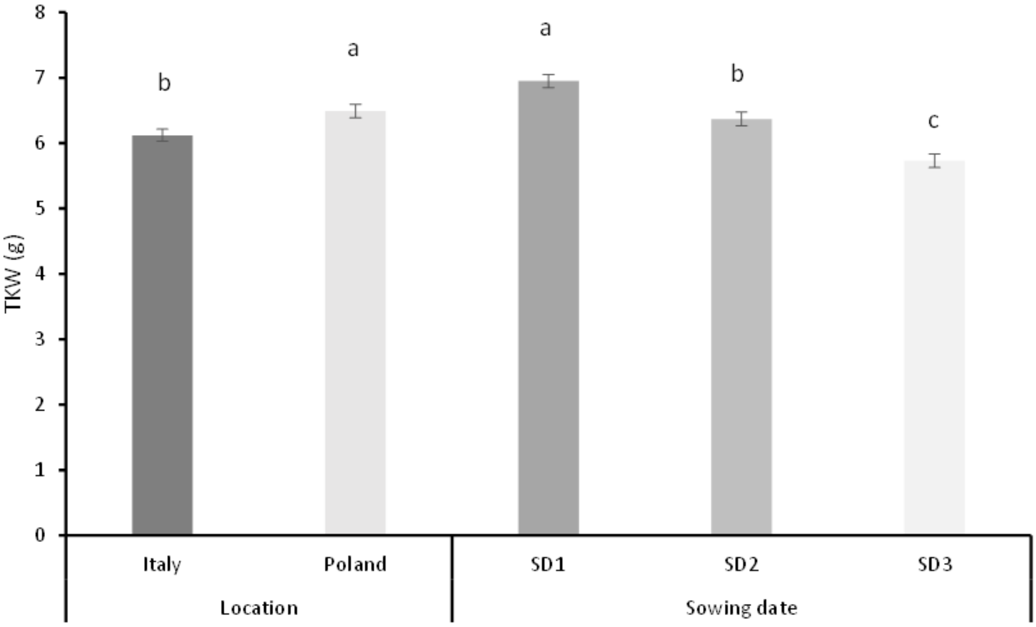

Figure 5. Crambe oil yield (Mg DM ha^{-1}) in the multi-location and multi-year trial in response to the main effect: location (Italy vs. Poland). Vertical bars: standard error. Different letters: significant different means for *P*≤0.05 (LSD Fisher's test).

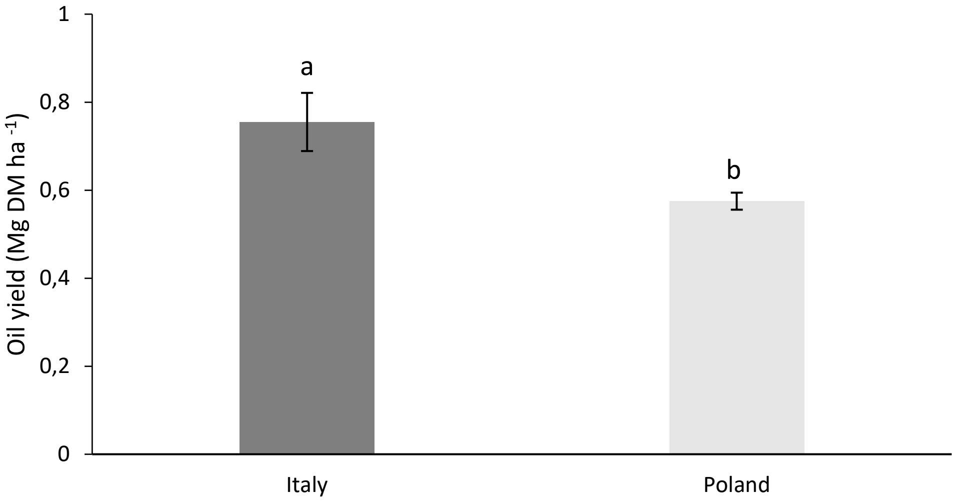

Figure 6. Significant linear regressions between crambe final plant density, plant height, TKW, and oil yield and meteorological variables for data from Italy and Poland. r^2= Coefficient of determination. ** = significant for $P \leq 0.01$.