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Linseed as a dual-purpose crop: evaluation of cultivar suitability and analysis of yield determinants

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Abstract

Linseed is enjoying renewed popularity worldwide thanks to emerging market opportunities for raw material derived from seeds and stems. The dual-purpose cultivation is particularly attractive to growers for its capacity to yield both seed/oil and straw/fibre; however, field-based research is necessary to identify suitable cultivars to meet modern production goals. Under these premises, a 3-year experimental campaign was conducted to evaluate 18 linseed cultivars potentially interesting for dual-purpose cultivation in Northern Italy. Cultivar performance was evaluated in terms of seed, straw and fibre yield, oil composition and stem fibre content. Inter-annual weather variability explained the largest portion of the total variance. However, the 'cultivar × year' interaction was not significant except for seed oil content and composition. Stability analysis showed that at least half of the cultivars were unstable for oil content and α-linolenic acid fraction. A Structural Equation Model was developed to investigate causal relationships between the productive performance and factors such as environmental variables, phenological traits, plant size and density. Rainfall was beneficial to seed yield, both before and after flowering, whereas higher post-flowering air temperature had a depressive effect. A higher oil content was favoured by pre-flowering rainfall. Plant height was negatively associated with seed yield and oil content, but it was positively associated with straw and fibre yield. Plant density was critical for fibre yield since below 500 plants/m^2 the increased plant branching makes it difficult to extract fibre. Together with plant density, plant height could be used to manipulate the seed/straw ratio according to production goals.

Introduction

The cultivation of *Linum usitatissimum* (flax/linseed) has long been abandoned in Italy, where, during its maximum expansion, between 1850 and 1870, it covered a surface of about 50.000 ha (Donà Dalle Rose 1951). Aside from two massive relaunching efforts made between 1936 and 1950, when the dedicated area reached 20 000 ha (Mameli-Calvino 1969), and in the second half of the 1970s, when it peaked at 10 000 ha (Venturi *et al.* 1994), this crop disappeared steadily and progressively from the whole national territory, now surviving in only about 200 ha dedicated to local niche seed production, concentrated in central-northern regions (Istituto Nazionale di Statistica 2016).

As in other traditional linen-producing countries, this irreversible decline was essentially due to the advent of cotton and synthetic fibres. In Italy, the process was further accelerated by the use of obsolete agrotechniques and varieties, underdeveloped mechanization and overall difficulty of obtaining high-grade textile fibre (Sessa 1934). Another problem which continues to discourage flax cultivation is the typical dry summer climate, which hinders post-harvest dew-retting of stems. Fibre can only be extracted by water-retting in energy-consuming facilities, which raises costs and makes yarn production economically uncompetitive.

Opportunities for the re-introduction of flax/linseed in Italy may come from the recently renewed interest for this crop driven by new market opportunities, which appear particularly promising for seed/oil-based materials. Beyond traditional non-food uses, which remain of topical interest (Zanetti *et al.* 2013), the demand for seed/oil-based products is growing fast for functional food for human nutrition. Many health benefits associated with seed and oil consumption have been reported, thanks to the high content of α-linolenic acid, lignans, proteins and dietary fibre in the seed, which are beneficial in combating cardiovascular diseases, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders (for extensive reviews, see Jhala & Hall 2010; Singh *et al.* 2011; Goyal *et al.* 2014).

Functional properties of seeds are also appreciated for livestock feeding, to improve animal health and growth performance (Singh *et al.* 2011), as well as to enrich and optimize animal-derived products with n-3 fatty acid content and proteins (Corino *et al.* 2014).

Since linseed fibre and shives can be utilized in manifold non-textile industrial applications (Pillin *et al.* 2011; Zuk *et al.* 2015), linseed has potential as a dual-purpose crop, with seed/oil as the main product and fibre/shives as by-products (Rennebaum *et al.* 2002). This is not a new idea, as studies investigating linseed-based dual-purpose cultivation have already appeared in recent times (Foster *et al.* 1997, 1998; Sankari 2000*a*, *b*; Rennebaum *et al.* 2002; Diederichsen & Ulrich 2009; Irvine *et al.* 2010). Without needing to satisfy textile quality standards, fibre yield as a by-product could integrate with linseed cultivation income, thus making the crop more attractive to Italian growers.

The crop also would be greatly welcomed in Italy for improving agro-ecosystem sustainability (Casa *et al.* 1999), as linseed has proved to be potentially interesting for its low water and chemical input requirements (Hocking *et al.* 1987; Cremaschi *et al.* 1995; Casa *et al.* 2000) and because it would allow widening of crop rotations, especially in Southern Italy, to break durum wheat monoculture (Cereti *et al.* 1994; D'Antuono & Rossini 1994, 2006).

However, after being abandoned for such a long time, the reintroduction of this crop is not straightforward. As no plant breeding has been done locally for decades, new cultivation would necessarily depend on ancient autochthonous or foreign cultivars. One priority is, therefore, to identify suitable cultivars according to evaluation criteria targeted at modern production systems and diversified end uses.

Cultivar evaluation should also adequately characterize the response to climatic factors, which can play a major role in the success of cultivation (D'Antuono & Rossini 1995; Zając *et al.* 2012). Field-based research conducted over the last two decades has demonstrated that linseed yield is strongly affected by environmental factors, causing high inter-annual yield variations (Cremaschi *et al.* 1995; Casa *et al.* 1999; Hassan & Leitch 2001; Adugna & Labuschagne 2002, 2003; Zając *et al.* 2012). Agronomic guidelines also need to be reviewed and updated in relation to the new production goals.

The main motivation of the present study is the idea that the increasing demand for linseed-derived raw materials represents an opportunity for re-launching this crop in Italy, and that dual-purpose cultivation could take better advantage of the wide range of market outlets, given their capacity for providing both seeds and straw materials in a single cultivation cycle. Pioneering studies addressing linseed as a dual-purpose crop in Italy have started to appear (Tavarini *et al.* 2016), as field research is needed to fill the knowledge gap that has accumulated over the years regarding genotype performance and agronomic guidelines.

In consideration of this, a 3-year field trial was undertaken to evaluate the productive characteristics of 18 linseed/flax cultivars, potentially interesting for quality-oriented dual-purpose cultivation. The accessions were selected from a flax/linseed germplasm collection for which routine observations for the main phenological and productive traits (seed and straw yield) were conducted over 23 years. The work aimed at assessing the quantitative and qualitative productive potential for seed/oil as well as fibre/straw as a by-product, and the effect and relative importance of agro-environmental factors on the productive response to evaluate environmental suitability.

Materials and methods

Plant material

Eighteen linseed or flax accessions were chosen from a germplasm collection, set up and maintained at the CREA – Research Centre for Cereal and Industrial Crops (CREA-CI) since 1989. The collection contained 350 accessions (of flax and linseed), including varieties and Italian landraces. The seedstock at the Centre experimental farm, located near Bologna, Italy (44°31′ N, 11°29′ E, 29 m a.s.l.), is refreshed routinely. During plant growth, morpho-phenological traits, as well as seed/straw production, were observed and recorded. Oil content and fatty acid composition of the accessions were also characterized. These observations were conducted on small plots, 1.5 m^2 in size without repetition, so measurements of quantitative traits were not completely reliable. They were, however, considered informative, whenever multi-year observations were available, for the selection of promising cultivars to be validated in field trials at appropriate agronomic scale. The accessions chosen for the present evaluation had previously been observed for at least 3 years.

The majority of cultivars were of 'linseed' type in the traditional sense, except for one fibre type (Afghanistan) and one dual-purpose type (Oregon). They were chosen for their good seed yield and oil content, but other traits were also considered: seed weight, which is correlated with oil content (Green & Marshall 1981; Diederichsen & Raney 2006), and α-linolenic seed content, which was the oil component of major interest. Mikael was chosen as the reference cultivar for oil yield, as it is very well-known across Europe, and has been tested previously in Italy (Casa *et al.* 1999), where it showed high-yielding capacity (Cremaschi *et al.* 1995). In order to enhance straw/fibre production, some accessions were also chosen for tall plant height, for its direct quantitative association with straw/fibre production (Diederichsen & Ulrich 2009). Another selection criterion was represented by the length of the 'technical stem', i.e. the portion of the main stem between the root collar and the first branch insertion. Long technical stems produce long high-quality fibre.

The selected group included Claudia and Credo, two Italian cultivars released by CREA-CI (formerly Research Institute for Industrial Crops) in 1995, as mass selections of the Italian landraces Ragusano and Buseto Palizzolo. Since the multi-year selection process was conducted at the same experimental farm as the trials reported here, these cultivars had the additional interest of representing a benchmark for environment suitability.

Field trials

The experiments were carried out from 2011 to 2013 at the aforementioned location, on a silt-loam soil. Sowing was conducted on 23 March in 2011 and 2013, and on 19 March in 2012, using a cereal plot drill (Wintersteiger, Austria). The trials were arranged in a randomized block design with three replicates. Single plot size was 18 m^2. The crop was planted in rows spaced 15 cm apart, with a target plant density of 500 plants/m^2, following usual recommendations for the location (Cremaschi *et al.* 1995). The seed amount to be used for obtaining the target seed rate was calculated from laboratory-assessed *in vitro* germinability, increased by 20% to allow for possible emergence losses (Casa *et al.* 1999). Pre-sowing fertilization was applied with 70 kg nitrogen (N) as ammonium sulphate. No irrigation was provided; weeds were controlled with bentazon, sodium salt (Basagran® SG; BASF, Germany), at 0.8 kg/ha of a commercial product.

During growth, phenological observations were carried out. The emergence date was recorded when the number of visible seedlings covered one half of the plot area. The beginning and end of flowering were recorded when the proportion of plants showing at least one opened flower was 0.30, and when the proportion of plants with no more flowers was 0.90, respectively. The crop was harvested at full seed maturity (hereafter 'maturity'),

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growth stage (GS) 89 in the scale proposed by Smith & Froment (1998), as the seed was the intended main product. The site was equipped with an automated weather station for daily measurement of air temperature and precipitation, which were recorded since 1977.

At harvest, the following observations were made: plant density, plant height, thousand seed weight (TSW), number of bolls per plant (BPP) and number of seeds per boll (SPB). The measurements were taken on three samples, chosen randomly within each plot, each one made up of three-row segments, 50 cm in length.

After sample collection, the whole plot seed production was harvested with a plot combine (Wintersteiger, Austria). The dry weight of seed and stem was measured after oven drying at 105 °C for 16 h and at 85 °C for 48 h, respectively.

Post-harvest fibre assessments

Stem fibre content was determined in 2012 and 2013 only. The assays were performed on the same plant samples taken before harvest, after boll removal. Stems were cut from roots, weighed and put in cylindrical steel containers, where they were plunged in water with a 1 : 20 stem:water weight ratio, and kept for 5 days at 30 °C in a stove, to decompose the pectin that binds fibres to the woody stem.

After open-air drying, stems were processed with a laboratory scutching machine, based on that described by Bargale (1990) to extract the raw fibre. The raw fibre bundles were brushed manually to remove fibres shorter than 30 cm, together with impurities and shives. Fibre content was then calculated as the proportion of the remaining long fibre fraction on the total straw weight.

Post-harvest seed oil extraction and analysis

The fatty acids were extracted from 150 mg of freshly milled seeds from each replicated plot by hexane and trans-methylated in 2 N potassium hydroxide (KOH) methanolic solution according to Conte *et al.* (1989). Fatty acid methyl esters (FAMEs) were analysed by a gas chromatography-flame ionization detector (FID; Carlo Erba HRGC 5300 Mega Series, Thermo Fischer Scientific, Waltham, MA, USA) on a capillary column Restek RT × 2330 (30 m × 0.25 mm × 0.2 μm) with oven temperature programming (170 °C initial temperature for 12 min, followed by a gradient of 20 °C/min to 240 °C, for 3 min), helium as carrier gas at 1 ml/min and split mode 40 : 1. The detector and injector temperature was 260 °C.

Chemical standards were used for identification of individual fatty acids. The internal normalization method (ISO 1998) was used to determine the fatty acid composition. Residual oil content was determined by the standard Soxhlet extraction method using hexane as solvent.

The oil fatty acids were abbreviated as follows: ALA, α-linolenic acid; LA, linoleic acid; OA, oleic acid; PA, palmitic acid; SA, stearic acid.

Production performance assessment

Evaluation of the production performance was based on multiple variables assessed after harvest and post-harvest analysis, listed and abbreviated as follows: (i) seed yield (t/ha, SEED_Y); (ii) oil yield (t/ha, OIL_Y); (iii) seed oil content (proportion of seed weight, OIL_C); (iv) straw yield (t/ha, STRAW_Y); (v) fibre yield (t/ha, FIBRE_Y); (vi) stem fibre content (proportion of stem weight, FIBRE_C).

Data analysis

The results were analysed using two-way mixed ANOVA, with type-III sum of squares (which assesses the contribution of each predictor over and above all others), including cultivar and year as fixed and random factors, respectively. The cultivar by year interaction was likely affected by some variability in plant density among cultivars and across years, so this variable was included as a covariate in the analysis. Plant density is known to have little effect on seed and oil yield (Albrechtsen & Dybing 1973; Casa *et al.* 1999), but it was expected to have an influence on straw and fibre yield, which were of great interest for the present study (Easson & Molloy 2000).

The R software v. 3.1.3 (R Core Team 2015) was used to fit the ANOVA model to data and calculate variance component [lm() function, standard package, https://stat.ethz.ch/R-manual/R-devel/library/stats/html/lm.html]. Univariate *F*-tests for the main factors and interaction effects were performed after calculating appropriate *F*-ratios in the ANOVA from the expected mean squares (MS): the fixed factor was tested against the interaction MS, while the random factor and the interaction were tested against the overall error MS (Zar 2010). The means were compared using the least significant difference test at $P < 0.05$ (LSD0.05).

A stability analysis was performed for the variables where the genotype × year interaction was significant, by means of the ecovalence (W_i) method according to Wricke (1962). Ecovalence quantifies the contribution of each genotype to the genotype × year interaction and was computed as

$$W_i = \sum_j (x_{ij} - \bar{x}_i - \bar{x}_j + \bar{x})^2$$

where for each variable to be analysed, x_{ij} is the mean of the *i*th genotype in the *j*th year, $\bar{x}_i$ is the mean of the *i*th genotype over the years, $\bar{x}_j$ is the mean of the *j*th year over genotypes, and $\bar{x}$ the overall mean.

Stable genotypes have low ecovalence values. The hypothesis that W_i differs from 0, i.e. that it contributed significantly to the interaction, was tested by an *F*-test (Shukla 1972; Sierts *et al.* 1987; Fernandez 1991; Diepenbrock *et al.* 1995).

Structural equation modelling

Understanding how cultivars respond to environmental conditions requires a methodology capable of analysing the highest complexity underlying genotype × environment interactions, which involve multiple driving factors and many sequential biological processes.

Structural equation modelling (S.E.M.) is a multivariate statistical analysis technique which is well suited for analysing hypotheses consisting of complex networks of interrelated variables (Shipley 2000; Grace 2006; Kline 2011; Lamb *et al.* 2011). It may be considered an evolution of path analysis, with an emphasis on estimating causal effects through the study of path relations, thanks to its capacity for testing direct and indirect relationships. By this approach, researchers are able to investigate how different processes interact and how their effects propagate through the systems under study.

Hypotheses about how a system responds to driving variables are modelled through a network of directional paths connecting

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variables under study; direct effects describe simple cause-and-effect relationships between two variables, whereas indirect effects are mediated by other variables. Of particular relevance is the capacity for quantifying the relative importance of different effects, even if expressed in different units, by standardized coefficients as indicators of the magnitude of the various paths in the model. Another attractive feature of S.E.M. which distinguishes it from other statistical methods is that the whole model structure is tested together with its specific parameters in a single analysis process.

In view of this, a structural equation model was constructed to test causal relationships between productive results and a set of environmental, morphological, phenological and stand factors. The model considered two types of effects:

- *Direct effects*: those linking driving factors directly to the dependent variables (SEED_Y, OIL_C, STRAW_Y). The following driving factors were considered: average temperature and cumulative rainfall from emergence to flowering; average temperature and cumulative rainfall from flowering to maturity; number of days from emergence to flowering and from emergence to maturity; plant density and plant height.
- *Indirect effects*: those linking driving factors to dependent variables as above, but via the yield components BPP, SPB, TSW as intermediate variables.

OIL_Y was not considered in the analysis because this variable was not independent, as it was derived mathematically from SEED_Y and OIL_C. Oil composition data were not included either, to limit the number of variables. FIBRE_Y and FIBRE_C were also excluded due to lack of data. The model was formalized and fitted to data using the lavaan package of R software (Rosseel 2012).

Results

Weather conditions

Total rainfall from sowing to maturity was 123.2, 174.9 and 317.8 mm in 2011, 2012 and 2013, respectively (Fig. 1*a*). Only 2012 was close to the historical mean of the location, which was 211.6 mm for the 1977–2013 period, whilst the other 2 years were above and below it by about 100 mm. In the last 2 years, the largest rainfall fraction fell before flowering, whilst in 2011 this latter phase was characterized by very low rainfall. The highest post-flowering rainfall was recorded in 2011 and the lowest in 2012, which amounted to only 4.4 mm.

Concerning the growing season average air temperature (Fig. 1*b*), 2013 was the coolest year at 16.9 °C; 2011 and 2012 were rather similar, with 17.7 °C and 17.6 °C, respectively. The mean air temperature for the 3 years varied between 14.1 °C (in 2012) and 14.8 °C (in 2011) before flowering; the variation during flowering was between 18.7 °C (in 2013) and 20.9 °C (in 2011), whereas after flowering until plant maturity it was between 22.4 °C (in 2011) and 24.4 °C (in 2012). Overall, mean temperatures were all above the historical means for each phenophase and for the whole growing period. The only exceptions were mean temperature during flowering in 2013 (18.9 °C *v.* 19.0 °C) and after flowering in 2011 (21.8 °C *v.* 22.1 °C).

Crop development

The average length of the emergence-maturity period was 87.6 days, with little within-cultivar variation, as the minimum–maximum difference was only 3 days (Fig. 2*a*). A higher variability was observed in the time interval from emergence to the beginning of flowering, where the difference between the earliest cultivar (Ocean, 37.9 days) and the latest (Olinette, 44.7 days) was 6.8 days. An intermediate min–max difference of 4.7 days was observed for the beginning to the end of the flowering interval.

On a by-year basis (Fig. 2*b*), the average emergence-maturity period in 2011 was close to 2013, with 88.6 and 91.6 days, respectively, whilst it was sensibly lower in 2012, with 82.1 days. Flowering duration showed a high variability among the years. This was particularly due to prolonged flowering in 2013, when it lasted 24.3 days, compared with 16 days in 2012 and 10.6 days in 2011. This variation was inversely related to that of the seed maturation phase, which decreased from 34.9 days in 2011 to 29.2 days in 2013.

Agronomic behaviour

Crop establishment was regular and uniform in all 3 years. Although plant density was fixed by the experimental protocol, a certain variability could not be avoided: at harvest time the mean actual plant densities (plants/m^2) were 435.8, 549.3 and 411.6 in 2011, 2012 and 2013, respectively.

The cultivars under study showed significant differences in the traits, as the cultivar effect was always significant ($P < 0.001$, except FIBRE_C with $P < 0.05$). The year effect was also always significant ($P < 0.001$), with the exception of FIBRE_Y (Table 1).

The year factor explained the largest part of the variation, in terms of MS, with the exception of FIBRE_Y, where the cultivar effect was higher than the year effect. The interaction term was always very low with respect to the two main factors and was statistically significant only for OIL_C ($P < 0.001$).

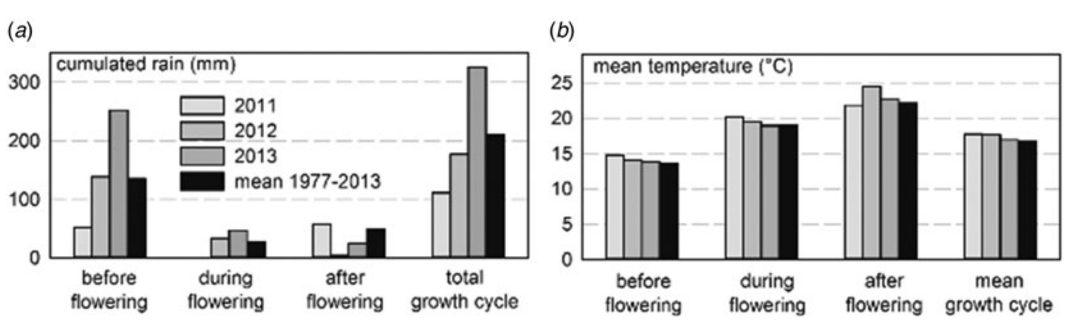

Fig. 1. Cumulated rainfall and mean air temperature during the main phenophases for each year of the experimental campaign and for the long-term mean (1977–2013).

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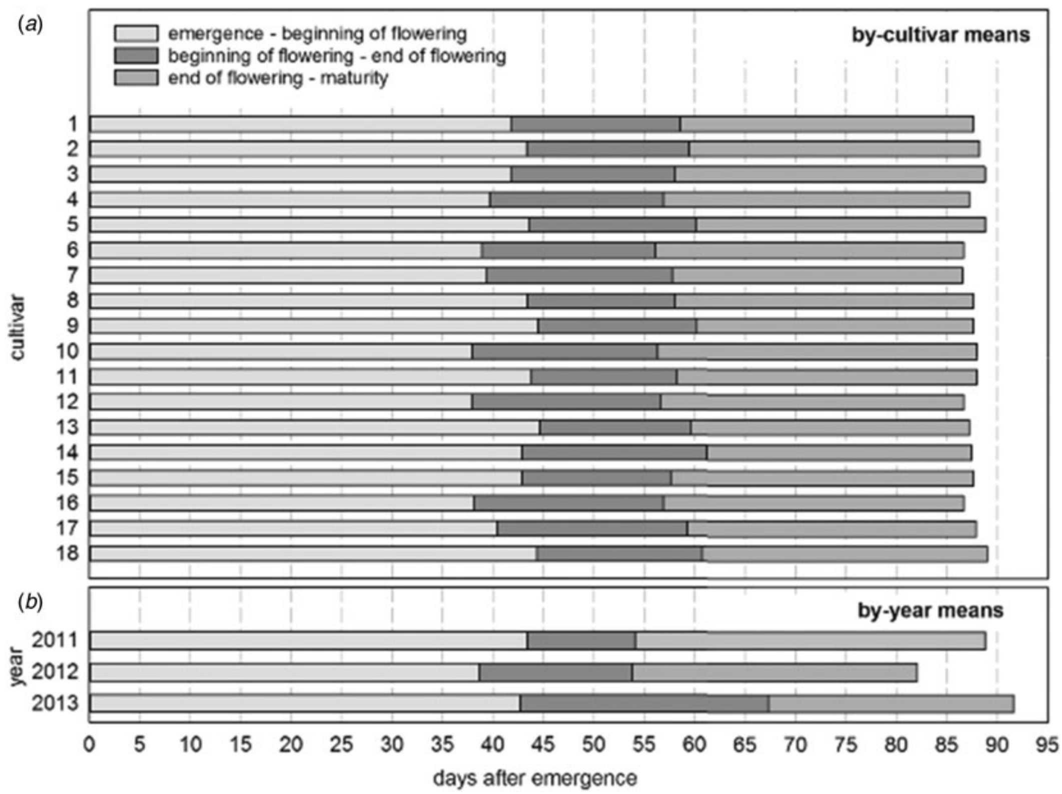

Fig. 2. Duration of phenological phases (days after emergence), averaged over the 3 years for each cultivar (upper panel) and across cultivars for each year (lower panel).

Seed yield

The highest average SEED_Y was obtained from the reference cultivar Mikael (2.34 t/ha). Overall, 14 cultivars produced more than 2 t/ha and differentiated well from the remaining lowest yielding ones (Camporeale, Hella, Norlin, Siracusano), which yielded between 1.49 and 1.75 t/ha (Table 2). Year was the highest source of variation, having MS more than fivefold higher than cultivar (Table 1). The highest yields were seen in 2013, with an average of 2.18 t/ha, only slightly higher than 2.13 t/ha obtained in 2011. The lowest average yield, recorded in 2012, was 1.76 t/ha (Table 2). However, some cultivars still yielded about 2.0 t/ha in 2012: Afghanistan (2.05 t/ha), Mikael (2.04 t/ha), Olinette (2.03 t/ha) and Camporeale (1.99 t/ha; not shown).

Yield components

Results for seed yield components (Table 3) show that the cultivar effect was significant ($P < 0.001$) for SPB and for TSW but not for BPP, whilst the year effect was significant ($P < 0.001$) for all yield components. For BPP and SPB the year effect was more than five times higher than the cultivar effect in terms of MS. All the yield

Table 1. Two-way ANOVA results (mean squares) for seed yield (SEED_Y), oil yield (OIL_Y), seed oil content (OIL_C), straw yield (STRAW_Y), fibre yield (FIBRE_Y) and stem fibre content (FIBRE_C)

Source of variation	SEED_Y	OIL_Y	OIL_C	STRAW_Y	FIBRE_Y	FIBRE_C
Main factors						
Cultivar	0.541	0.110	18.31	1.95	0.096	0.002
	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	($P < 0.05$)
Year	2.869	0.781	186.20	44.20	0.012	0.066
	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)		($P < 0.001$)
Cultivar × year	0.052	0.010	2.56	0.10	0.009	0.001
			($P < 0.001$)			
Residuals	0.036	0.061	0.28	0.15	0.006	0.001
Covariate						
Plant density	0.024	0.0003	0.11	1.07	0.129	0.005
				($P < 0.01$)	($P < 0.001$)	($P < 0.01$)

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Table 2. Post-harvest results.

	SEED_Y (t/ha)	OIL_C (g/kg)	OIL_Y (t/ha)	STRAW_Y (t/ha)	FIBRE_C (g/kg)	FIBRE_Y (t/ha)
Cultivar						
Afghanistan	2.25 ± 0.094	392 ± 9.7	0.88 ± 0.046	2.8 ± 0.29	101 ± 4.7	0.32 ± 0.041
Atalante	2.03 ± 0.091	409 ± 5.4	0.83 ± 0.039	3.1 ± 0.36	118 ± 14.5	0.38 ± 0.032
Bison	2.10 ± 0.092	412 ± 4.6	0.87 ± 0.036	3.1 ± 0.20	125 ± 9.4	0.41 ± 0.028
Buenos Aires	2.20 ± 0.120	401 ± 3.8	0.89 ± 0.053	2.5 ± 0.25	133 ± 28.6	0.29 ± 0.029
Camporeale	1.49 ± 0.077	378 ± 9.6	0.56 ± 0.031	4.2 ± 0.29	166 ± 27.0	0.75 ± 0.96
Claudia	2.32 ± 0.118	432 ± 3.7	1.01 ± 0.058	2.9 ± 0.26	98 ± 9.2	0.30 ± 0.015
Credo	2.22 ± 0.095	402 ± 7.0	0.89 ± 0.039	2.5 ± 0.20	108 ± 11.1	0.27 ± 0.016
Hella	1.75 ± 0.065	386 ± 8.1	0.68 ± 0.032	3.3 ± 0.25	118 ± 16.0	0.40 ± 0.034
Iduna	2.09 ± 0.100	415 ± 4.9	0.87 ± 0.047	2.9 ± 0.33	114 ± 9.1	0.36 ± 0.022
Mikael	2.34 ± 0.085	420 ± 4.2	0.98 ± 0.042	2.3 ± 0.27	116 ± 16.6	0.28 ± 0.032
Norlin	1.64 ± 0.095	397 ± 5.5	0.66 ± 0.047	3.5 ± 0.24	173 ± 17.6	0.63 ± 0.030
Ocean	2.07 ± 0.116	401 ± 5.0	0.83 ± 0.055	2.2 ± 0.29	130 ± 26.6	0.27 ± 0.031
Olinette	2.07 ± 0.057	404 ± 5.5	0.84 ± 0.033	2.8 ± 0.32	140 ± 14.0	0.44 ± 0.047
Oregon	2.07 ± 0.087	386 ± 8.4	0.80 ± 0.034	3.1 ± 0.29	108 ± 6.5	0.37 ± 0.026
Pacific	2.01 ± 0.066	401 ± 6.2	0.81 ± 0.032	3.0 ± 0.33	118 ± 8.5	0.38 ± 0.029
Pugliese	2.10 ± 0.117	429 ± 3.9	0.90 ± 0.056	2.5 ± 0.22	115 ± 18.0	0.28 ± 0.017
Quarandi	2.16 ± 0.064	410 ± 4.6	0.89 ± 0.027	2.7 ± 0.25	130 ± 8.5	0.39 ± 0.034
Siracusano	1.60 ± 0.122	397 ± 5.5	0.75 ± 0.078	3.0 ± 0.43	127 ± 13.0	0.43 ± 0.030
Average	*2.03 ± 0.028*	*404 ± 1.7*	*0.83 ± 0.013*	*2.9 ± 0.07*	*123 ± 3.8*	*0.39 ± 0.014*
Year						
2011	2.13 ± 0.043	388 ± 22.1	0.8 ± 0.02	2.3 ± 0.49	–	–
2012	1.76 ± 0.038	399 ± 13.4	0.71 ± 0.017	2.5 ± 0.58	148 ± 5.2	0.37 ± 0.168
2013	2.18 ± 0.044	425 ± 10.2	0.95 ± 0.018	3.9 ± 0.65	99 ± 3.1	0.39 ± 0.132
$LSD_{0.05}$						
Cultivar	0.22	15.26	0.01	0.33	42.97	0.14
Year	0.07	2.05	0.006	0.15	9.08	NS
Cultivar × year	NS	8.69	NS	NS	NS	NS

The 'cultivar' frame reports the 3-year averages for each single cultivar; the 'year' frame reports the average of all cultivars for each year of the experimental campaign (average ± S.E.M.)

components were influenced significantly ($P < 0.001$) by plant density.

TSW was the only component where the cultivar effect was higher than the year effect. The cultivar × year interaction was significant ($P < 0.001$) for BPP and for TSW only. The TSW by-year average was quite stable, varying between 6.4 and 6.8 g. However, the slightly higher value in 2013 was significantly different ($P < 0.05$) from the previous 2 years (Table 4).

Seed oil content and yield

The cultivar factor showed significant differences ($P < 0.001$) for OIL_C and OIL_Y (Table 1). Claudia had the highest average OIL_C at 432 g/kg, and the highest OIL_Y at 1.01 t/ha, while the lowest values were found in Camporeale, with an average OIL_C of 378 g/kg and OIL_Y of 0.56 t/ha (Table 2).

Year had a significant effect ($P < 0.001$) on both OIL_C and OIL_Y, and it was much higher than the cultivar effect (Table 1). The highest OIL_C was recorded in 2013, while similar values were obtained in the previous 2 years. The OIL_Y by-year variation was similar to that observed in SEED_Y, with the lowest production recorded in 2012 and the highest in 2013, with an intermediate value in 2011 (Table 2).

The cultivar × year interaction effect was significant ($P < 0.001$) only for OIL_C (Table 1), so a stability analysis was performed, showing that 11 cultivars had significant ecovalence values, thus this trait was unstable (Table 5).

Oil composition

The cultivar effect was significant ($P < 0.001$) for all fatty acids (Table 6). The year effect was significant ($P < 0.001$) for all fatty acids except for OA, which was the only one where the cultivar-dependent variation was higher than the year-associated variation. The cultivar × year interaction was significant ($P < 0.001$, except OA with $P < 0.01$) for all fatty acids except for PA. Plant density had a significant effect ($P < 0.01$) on unsaturated fatty acids; however, results were quantitatively negligible; for an increase of 100

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Table 3. Two-way ANOVA results (mean squares) for the yield components: number of bolls per plant (BPP), number of seeds per boll (SPB), thousand seed weight (TSW)

Source of variation	BPP	SPB	TSW
Main effects			
Cultivar	608	2.247	9.697
		($P<0.001$)	($P<0.001$)
Year	3415	15.718	2.050
	($P<0.001$)	($P<0.001$)	($P<0.001$)
Cultivar × year	388	0.218	0.488
	($P<0.001$)		($P<0.001$)
Residuals	23	0.158	0.044
Covariate			
Plant density	3235	1.518	2.804
	($P<0.001$)	($P<0.01$)	($P<0.001$)

plants/m^2, OA fraction increased by 0.03 g/kg and LA by 0.002 g/kg, while ALA decreased by −0.029 g/kg (not shown).

The fatty acid profiles, averaged by cultivar and by year are reported in Table 7. The cultivar with the highest ALA content was Buenos Aires, which reached a 3-year average of 577 g/kg. Overall, eight cultivars had an average ALA >500 g/kg. The absolute highest value was 598 g/kg recorded on Buenos Aires in 2011 (not shown).

On a by-year basis, OA was the only component to stay unchanged; PA and SA had the lowest values in 2011, while in 2012 and 2013 they had higher and similar values. Contents of LA and ALA were significantly different ($P<0.05$) in each of the 3 years, with opposing variation patterns: LA increased from 2011 to 2013, while ALA decreased (Table 7).

The ALA content was also the most unstable, as half of the cultivars presented significant ($P<0.05$ or lower) W_i values for this trait (Table 5). Instability was, however, concentrated mostly on Atalante, Siracusano and Camporeale. Buenos Aires, characterized by the highest ALA content, had one of the lowest ecovalence values for this trait.

Straw and fibre

The cultivar effect was significant for STRAW_Y as well as for FIBRE_Y ($P<0.001$) and FIBRE_C ($P<0.05$) (Table 1). The year factor was higher than the cultivar factor except for FIBRE_Y, where it was non-significant. A strong effect of plant density was present: in FIBRE_Y, plant density was the highest source of variation, whilst in FIBRE_C its effect was higher than that of the cultivar.

The average STRAW_Y varied between 4.2 t/ha (Camporeale) and 2.2 t/ha (Ocean). STRAW_Y was higher in 2013, whilst in the first 2 years it was only slightly different and almost halved with respect to 2013 (Table 2).

Camporeale and Norlin produced the highest FIBRE_C, reaching the values of 166 and 173 g/kg, and the highest FIBRE_Y with values of 0.75 and 0.63 t/ha. The by-year average of FIBRE_Y was the same in the 2 years it was measured, whereas FIBRE_C in 2013 was lower compared with 2012 (Table 2).

In order to explore the causes of reduced FIBRE_C in 2013, tow proportion after fibre extraction was plotted both against plant density and the average number of branches per plant, reporting both variables in logarithmic scale as the variation patterns were non-linear (Fig. 3). It is clear from the graphs that the tow discarded by the mechanical process increased at the lower plant density. It can also be appreciated that tow variation was more strongly related to the number of branches per plant than to plant density, as demonstrated by the higher R^2 value.

Table 4. Number of bolls per plant (BPP), number of seeds per boll (SPB) and thousand seed weight (TSW) recorded at harvest

	BPP	SPB	TSW
Cultivar			
Afghanistan	10.6 ± 0.90	7.3 ± 0.14	5.9 ± 0.32
Atalante	20.0 ± 3.46	7.9 ± 0.24	5.7 ± 0.04
Bison	9.9 ± 0.96	7.5 ± 0.24	6.1 ± 0.08
Buenos Aires	14.9 ± 2.71	6.3 ± 0.18	6.9 ± 0.08
Camporeale	6.8 ± 1.27	7.4 ± 0.33	5.3 ± 0.07
Claudia	8.6 ± 0.95	7.1 ± 0.14	7.3 ± 0.16
Credo	12.7 ± 2.80	6.8 ± 0.16	8.5 ± 0.14
Hella	16.7 ± 4.52	6.6 ± 0.24	7.0 ± 0.10
Iduna	40.8 ± 12.21	7.0 ± 0.20	6.1 ± 0.10
Mikael	16.2 ± 3.23	6.9 ± 0.18	7.1 ± 0.09
Norlin	13.9 ± 4.53	8.1 ± 0.17	4.8 ± 0.08
Ocean	17.6 ± 4.13	6.8 ± 0.16	8.5 ± 0.07
Olinette	29.3 ± 9.53	7.1 ± 0.29	6.0 ± 0.16
Oregon	12.4 ± 1.34	6.6 ± 0.27	5.6 ± 0.12
Pacific	12.1 ± 1.63	7.5 ± 0.20	6.8 ± 0.12
Pugliese	11.8 ± 1.54	7.0 ± 0.17	7.2 ± 0.12
Quarandi	8.1 ± 1.00	6.2 ± 0.15	7.4 ± 0.07
Siracusano	11.2 ± 3.01	6.9 ± 0.21	5.4 ± 0.33
Average	*15.2 ± 1.17*	*7.0 ± 0.06*	*6.5 ± 0.086*
Year			
2011	23 ± 3.0	7.5 ± 0.09	6.5 ± 0.14
2012	6.9 ± 0.27	6.5 ± 0.09	6.4 ± 0.16
2013	16 ± 1.0	7.2 ± 0.08	6.8 ± 0.15
$LSD_{0.05}$			
Cultivar	NS	0.49	0.72
Year	1.88	0.15	0.08
Cultivar × year	7.98	NS	0.34

The 'cultivar' frame reports the 3-year averages for each single cultivar; the 'year' frame reports the average of all cultivars for each year of the experimental campaign (average ± S.E.M.).

Structural equation modelling

The model as it was originally conceived failed to converge. After a number of trials and errors, it was decided to drop SPB, keeping only BPP and TSW as intermediate variables. After this modification, the model converged with a chi-square of 3.514 and a P-value of 0.319, with three degrees of freedom. Since the P-value associated with the chi-square was well above the

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Table 5. Ecovalence values calculated for seed oil content (OIL_C) and for seed oil fatty acid fractions (PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; ALA, α-linolenic acid)

		Oil composition				
Cultivar	OIL_C	PA	SA	OA	LA	ALA
Atalante	6.25	0.47	0.003	10.85	0.737	18.205
	(P < 0.001)			(P < 0.01)	(P < 0.05)	(P < 0.001)
Afghanistan	0.2	0.165	0.06	4.619	0.197	2.843
						(P < 0.05)
Bison	0.24	0.052	0.013	0.441	0.243	0.682
Buenos Aires	3.02	0.108	0.018	0.268	0.23	0.257
	(P < 0.001)					
Camporeale	5.52	0.109	0.022	4.085	0.601	8.056
	(P < 0.001)					(P < 0.001)
Claudia	1.64	0.362	0.037	0.585	0.34	0.255
	(P < 0.001)					
Credo	0.68	0.471	0.115	1.243	0.007	1.982
	(P < 0.05)		(P < 0.05)			(P < 0.05)
Hella	1.67	0.098	0.008	0.843	0.718	4.136
	(P < 0.001)				(P < 0.05)	(P < 0.01)
Iduna	0.32	0.286	0.016	2.089	0.071	1.164
Mikael	0.97	0.018	0.004	4.081	0.29	2.301
	(P < 0.01)					(P < 0.05)
Norlin	0.38	0.46	0.119	0.884	0.111	1.111
			(P < 0.01)			
Ocean	0.18	0.197	0.378	2.632	0.294	0.754
Olinette	2.07	1.47	0.003	0.163	1.62	0.476
	(P < 0.001)	(P < 0.01)			(P < 0.01)	
Oregon	1.94	0.386	0.016	0.834	0.697	1.8
	(P < 0.001)				(P < 0.05)	
Pacific	0.13	0.134	0.288	0.593	0.143	0.816
			(P < 0.001)			
Pugliese	2.72	0.612	0.006	0.391	0.615	4.258
	(P < 0.001)					(P < 0.01)
Quarandi	0.8	0.197	0.049	0.681	0.238	2.038
	(P < 0.05)					(P < 0.05)
Siracusano	0.03	0.194	0.148	56.946	5.847	28.857
			(P < 0.01)	(P < 0.001)	(P < 0.001)	(P < 0.001)

conventional critical threshold of 0.05, the structure of the data did not differ significantly from the hypothesized model.

The fitted model is illustrated graphically in Fig. 4, which displays the relationships that were found to be statistically significant. The first thing to highlight is that only direct effects were found to be significant. Indirect associations via yield components were not detected, even though BPP and especially TSW were strongly affected by various factors under evaluation. Thousand seed weight, in particular, showed a more complex determinism than BPP, as it was negatively affected by air temperature before and after flowering, by days to flowering and days to maturity, plant height and density, whereas rain had a positive effect both

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Table 6. Two-way ANOVA results (mean squares) for seed oil fatty acid fractions (PA, palmitic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; ALA, α-linolenic acid)

Source of variation	PA	SA	OA	LA	ALA
Main effects					
Cultivar	2.402	2.394	58.11	14.98	91.76
	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)	($P < 0.001$)
Year	3.816	12.098	6.36	33.06	218.57
	($P < 0.001$)	($P < 0.001$)		($P < 0.001$)	($P < 0.001$)
Cultivar × year	0.507	0.117	7.45	1.08	6.88
		($P < 0.001$)	($P < 0.01$)	($P < 0.001$)	($P < 0.001$)
Residuals	0.376	0.039	3.35	0.36	0.96
Covariate					
Plant density	0.664	0.001	23.80	2.51	6.13
			($P < 0.01$)	($P < 0.01$)	($P < 0.01$)

before and after full flowering. BPP was sensitive to only two factors, the strongest being plant density, with a negative effect, and air temperature from emergence to the beginning of flowering, with a positive effect.

Rainfall before and after flowering was highly beneficial to SEED_Y; plant height and post-flowering air temperature had instead a depressive effect. Seed oil content was affected positively by rainfall occurring before flowering, and similarly to SEED_Y, it was associated negatively with plant height. The straw yield was favoured by plant height, plant density and rainfall before flowering.

The relationship between SEED_Y and plant height was further analysed with respect to the cultivar and year factors. If within-cultivar variation was considered, plant height was associated positively with SEED_Y, but if considering within-year variation, a negative correlation was found (Table 8).

The same by-cultivar and by-year variation patterns were observed for OIL_C, but here within-cultivar positive correlation was significant only for Olinette ($P < 0.01$) and Siracusano ($P < 0.05$), whilst within-year negative correlation was significant for all years ($P < 0.05$ or lower) (not shown).

Results obtained for STRAW_Y can partly surrogate indications for FIBRE_C and FIBRE_Y, which were not included in the model due to lack of data. As shown in Table 9, plant density and height were correlated significantly ($P < 0.001$) and positively with FIBRE_Y, while FIBRE_C was correlated only with plant density ($P < 0.001$).

Discussion

The productive behaviour of the tested cultivars was different, but the highest source of variation for most characters was the year, which can be interpreted as the combination of the season-long action of multiple climate factors. The cultivar × year interaction was almost never significant; when it was, it was quantitatively less relevant than the simple effects. This simplifies the evaluation of cultivars, as they tend to maintain their relative ranking across the years. The need to evaluate cultivar performance also in terms of yield stability to better orient growers' choices has been already outlined (Adugna & Labuschagne 2003; Lafond *et al.* 2008), especially when the interaction depends on unpredictable factors, such as inter-annual climate fluctuations, in which stable varieties are to be preferred (Adugna & Labuschagne 2002). Rennebaum *et al.* (2002) also reported a more prominent role of year for oil and straw yield, with a negligible genotype × year interaction, whilst in regard to OIL_C, they found the cultivar effect to be higher than the year effect.

Crop development

Only small differences among cultivars were observed in the timing of phenological events. Here it must be emphasized that phenological features were not considered in the selection of cultivars under study. Since phenology traits are key features for adaptation of a genotype to a given environment (Chmielewsky 2013), it is to be expected that genotypes that adapt well within a single environment show similar phenology patterns.

With respect to 2011, the longer duration of flowering time in 2012 and especially in 2013 is likely to have been caused by lower temperatures and more abundant rainfall before and during flowering, which stimulated the production of new branches and flowers (Gabiana *et al.* 2005). This behaviour was confirmed by previous observations on various cultivars at the same location, where, on average, flowering lasted from 10 to 20 days. Since the germplasm collection was started, flowering durations comparable with those recorded in 2013 only occurred in 1995, with time lengths ranging from 17 to 28 days, and again in 1999, with durations between 12 and 26 days (not shown) (Maestrini C., unpublished data). Both 1995 and 1999 were characterized by intense precipitation during flowering, i.e. 129.5 and 71.5 mm, respectively (not shown). The lower air temperature recorded during this phase in 2013 could have enhanced the effect of rain. The flowering period is known to be longer under cooler conditions, which may occur as an effect, for example, of early sowing date and time of emergence as found by Casa *et al.* (1999), who reported flowering durations of up to 1 month.

Seed yield, oil production and composition

The lowest average SEED_Y was harvested in 2012, the year with the lowest post-flowering precipitation and the highest post-flowering temperature. All the same, some cultivars were able to

Table 7. Average content (g/kg), of palmitic (PA), stearic (SA), oleic (OA), linoleic (LA) and α-linolenic acid (ALA) in seed oil

	PA	SA	OA	LA	ALA
Cultivar					
Afghanistan	67 ± 2.9	43 ± 1.4	240 ± 7.8	139 ± 4.0	511 ± 14.6
Atalante	67 ± 2.1	44 ± 1.5	195 ± 5.5	142 ± 2.3	552 ± 9.5
Bison	64 ± 2.1	37 ± 1.4	243 ± 2.4	157 ± 3.1	498 ± 7.1
Buenos Aires	66 ± 2.8	37 ± 1.5	183 ± 1.3	134 ± 2.9	577 ± 5.8
Camporeale	60 ± 1.0	44 ± 1.6	244 ± 3.4	169 ± 2.5	484 ± 3.0
Claudia	68 ± 1.7	51 ± 1.9	274 ± 2.3	135 ± 3.6	472 ± 6.2
Credo	70 ± 2.5	56 ± 0.9	249 ± 2.0	149 ± 2.6	476 ± 3.3
Hella	61 ± 1.5	47 ± 1.5	206 ± 2.8	156 ± 2.5	530 ± 6.9
Iduna	66 ± 1.2	48 ± 1.7	215 ± 4.2	164 ± 2.3	507 ± 7.0
Mikael	69 ± 2.2	48 ± 1.4	250 ± 5.2	138 ± 2.3	494 ± 8.9
Norlin	66 ± 2.1	47 ± 2.1	252 ± 3.2	169 ± 2.8	466 ± 7.4
Ocean	74 ± 2.4	46 ± 0.6	242 ± 4.6	148 ± 3.6	489 ± 7.2
Olinette	75 ± 3.6	53 ± 1.3	202 ± 1.9	131 ± 7.8	538 ± 10.1
Oregon	69 ± 2.4	47 ± 1.3	240 ± 2.0	160 ± 2.5	483 ± 4.7
Pacific	52 ± 1.7	55 ± 2.5	219 ± 3.0	147 ± 6.6	527 ± 10.1
Pugliese	66 ± 2.2	50 ± 1.6	273 ± 2.1	133 ± 3.7	477 ± 7.4
Quarandi	64 ± 2.1	48 ± 1.6	248 ± 2.4	150 ± 3.3	490 ± 7.6
Siracusano	63 ± 1.3	44 ± 1.4	215 ± 3.9	129 ± 2.9	550 ± 6.6
Average	*66 ± 0.6*	*47 ± 0.5*	*235 ± 2.5*	*147 ± 1.2*	*507 ± 0.3*
Year					
2011	63 ± 1.0	42 ± 0.7	225 ± 3.9	139 ± 1.5	531 ± 4.5
2012	66 ± 1.1	50 ± 0.7	236 ± 3.3	147 ± 2.2	500 ± 4.1
2013	68 ± 1.1	50 ± 0.8	236 ± 3.8	155 ± 1.9	491 ± 5.2
$LSD_{0.05}$					
Cultivar	6.85	3.25	27.33	10.25	25.45
Year	2.34	0.75	NS	2.28	3.73
Cultivar × year	NS	3.19	29.50	9.69	15.81

The 'cultivar' frame reports the 3-year averages for each single cultivar; the 'year' frame reports the average of all cultivars for each year of the experimental campaign (average ± S.E.M.)

provide satisfactory yields between 2.0 and 1.9 t/ha. The year factor also had the highest impact on OIL_C and OIL_Y, though a significant cultivar-dependent variability was found, in agreement with Green & Marshall (1981) and Gallardo *et al.* (2014). As in Zhang *et al.* (2016), a significant cultivar × year interaction was evident for OIL_C. As ecovalence analysis showed, the interaction effect was attributable only to some of the cultivars, i.e. those having significant W_i values ($P < 0.05$ or lower).

As far as oil composition is concerned, both cultivar and year were effective on the relative fractions of oil fatty acids. The mono-unsaturated OA was conditioned only by the cultivar effect during seed development, as already reported in Dybing & Zimmerman (1966) and Zhang *et al.* (2016), and by the cultivar × year interaction, for which ecovalence value was significant only in two cultivars. With regard to the unsaturated fatty acid oil components, it was shown that the number of unstable cultivars is higher for LA and ALA.

The only fatty acid which decreased over the 3 years was ALA, with the lowest average value observed in 2013. Upon first glance, this result appears problematic, as it contrasts with the well-documented effect of high temperatures during seed maturation, when a decrease in polyunsaturated fatty acids and a corresponding increase in OA is generally observed (Canvin 1965; Dybing & Zimmerman 1966; Green 1986). External temperature was already known to modulate important transcriptional and post-transcriptional regulation steps of unsaturated fatty acid biosynthesis (Fofana *et al.* 2006; Baud & Lepiniec 2010). Therefore, the lower ALA level observed in 2012 compared with 2011 is consistent with this knowledge, as in 2012 post-flowering temperatures were higher than in 2011. However, in 2013, with temperatures during seed maturation comparable with those in 2011, a similar ALA content would also have been expected, whereas, to the contrary, it was lower than in 2012, when post-flowering temperatures were much higher.

To advance another possible explanation for this by-year variability, it must first be noted that in terms of thermal time, the whole reproductive cycle from the beginning of flowering to maturity had a similar duration in each of the 3 years.

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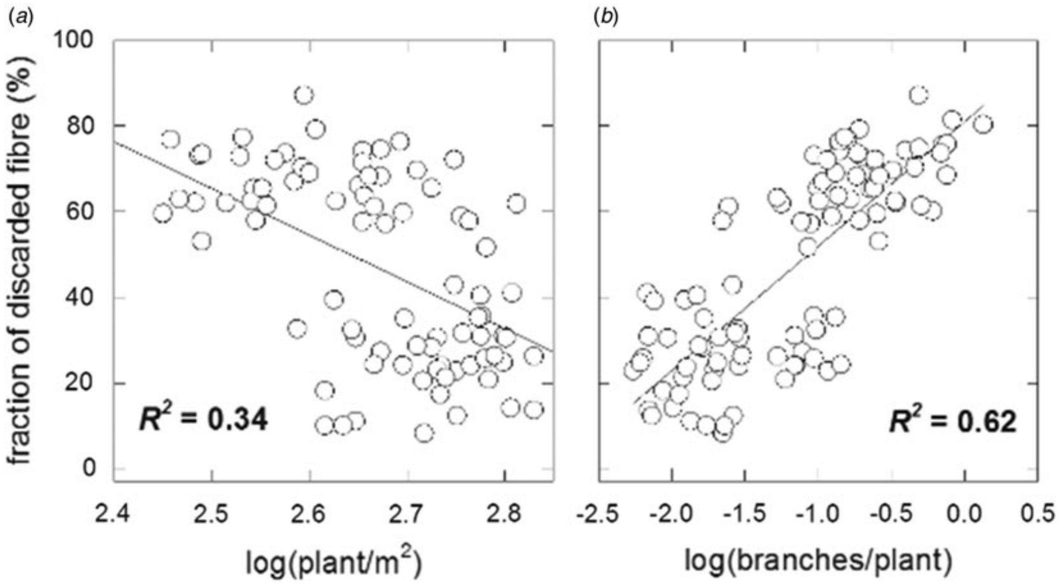

Fig. 3. Dependency of the discarded fibre fraction (%) on plant density (left) and on a number of branches per plant (right). Each point is a single measurement pooling cultivars and years (2012 and 2013).

Assuming a base temperature of 2 °C (Angus *et al.* 1981; D'Antuono & Rossini 1995) the accumulated growing degree days were on average 907.5, 926.7 and 932.3 °C d in 2011, 2012 and 2013, respectively. Given the stability of the reproductive cycle time, an increased flowering duration brings about a shortening of ripening time. Indeed, the time interval from the end of flowering to maturity decreased over the 3 years, meaning that a larger fraction of developing seeds had less time to ripen in 2013 with respect to 2011 and 2012. As a consequence, since the creation of ALA is the last step of the desaturation chain, generated by the conversion of LA into ALA, which accumulates during the last phase of seed maturation (Dybing & Zimmerman 1966), there was less time available for ALA synthesis. The same effect could have contributed to a lower ALA seed content in 2012 compared with 2011, as in 2012 flowering lasted 6 days longer.

When LA and ALA content were plotted against the duration of the period between the end of flowering and maturity, a significant linear relationship was observed for both fatty acids (Fig. 5).

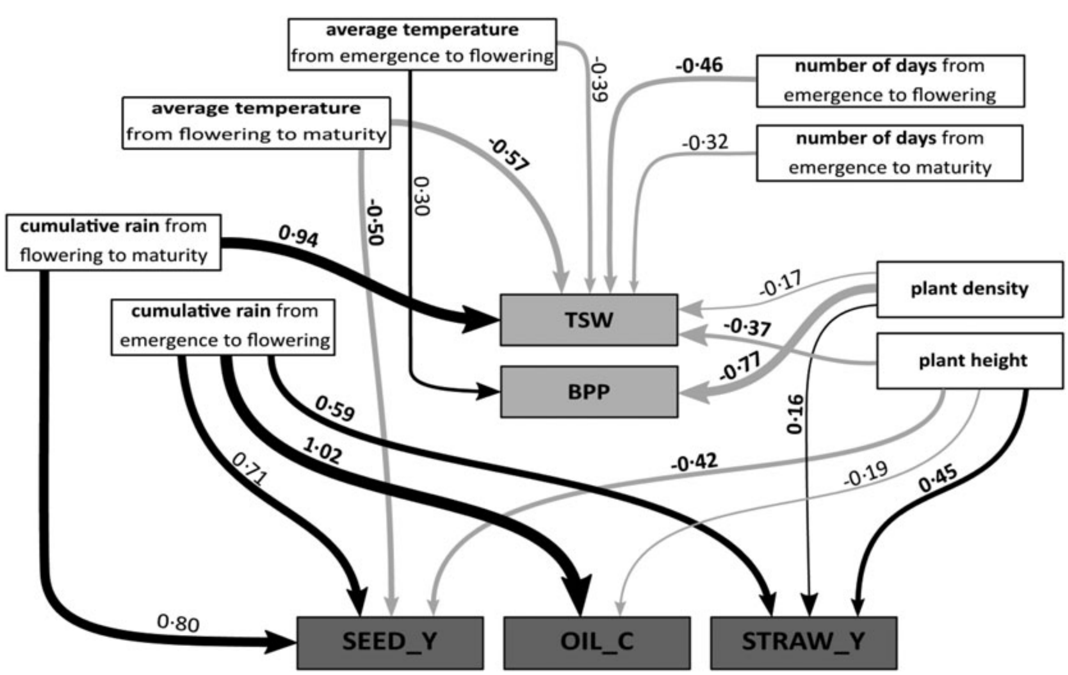

Fig. 4. Structural equation model representing causal relationships among independent variables (white boxes), intermediate variables (light grey boxes) and dependent variables (grey boxes). Significant paths are represented as black (positive relationships) or grey arrows (negative relationships). Arrow thickness is proportional to standardized path coefficients (showed beside arrows). TSW, thousand seeds weight; BPP, number of bolls per plant; SEED_Y, seed yield; OIL_C, oil content; STRAW_Y, straw yield. Path coefficients are significant for $P < 0.05$ (normal characters) or $P < 0.01$ (bold characters).

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Table 8. Pearson's correlation coefficient (*r*) calculated for SEED_Y *v.* plant height on a by-cultivar and on a by-year basis. *P* was calculated with *t*-test

By-cultivar SEED_Y *v.* plant height correlation		
Cultivar	*r*	*P*
Afghanistan	0.39	NS
Atalante	0.81	<0.01
Bison	0.64	NS
Buenos Aires	0.96	<0.001
Camporeale	0.79	<0.05
Claudia	0.62	NS
Credo	0.32	NS
Hella	0.75	<0.05
Iduna	0.64	NS
Mikael	0.85	<0.01
Norlin	0.83	<0.01
Ocean	0.50	NS
Olinette	0.51	NS
Oregon	0.86	<0.01
Pacific	0.85	<0.01
Pugliese	0.56	NS
Quarandi	0.59	NS
Siracusano	0.22	NS
By-year SEED_Y *v.* plant height correlation		
Year	*r*	*P*
2013	−0.620	<0.001
2011	−0.560	<0.001
2012	−0.490	<0.001

According to this relationship, during seed maturation ALA increased by 35.9 g/kg in 10 days, demonstrating that, under normal and stressful conditions, ALA accumulates progressively in the seed. It can be concluded that ALA content is sensitive to environmental conditions that have an influence on the length of the maturation period, particularly those prolonging flowering or accelerating maturation.

No clear indications emerged about the effect of plant density on oil composition: ALA decreased with plant density in 2011, whilst in 2012 and 2013 it showed no response (not shown). Gubbels & Kenaschuk (1989) reported the behaviour of

Table 9. Correlation matrix (*r*) for fibre yield (FIBRE_Y) and stem fibre content (FIBRE_C) with respect to plant density and height

	FIBRE_Y	FIBRE_C
Plant density	0.45	0.55
	($P < 0.001$)	($P < 0.001$)
Plant height	0.57	−0.15.
	($P < 0.001$)	NS

Statistical significance was assessed by *t*-test.

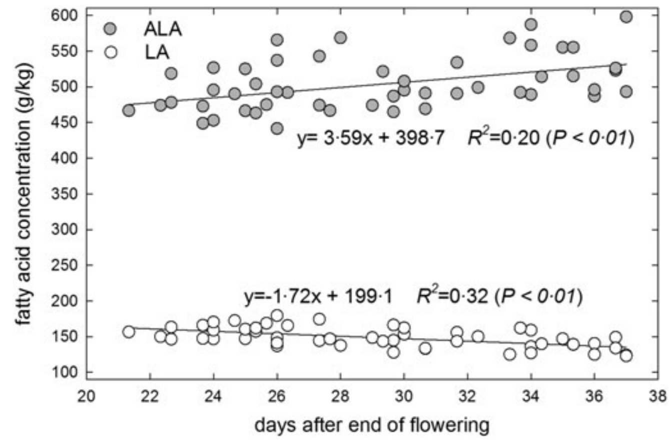

Fig. 5. Relationship between the relative content of linoleic (LA), α-linolenic acid (ALA) and the length of maturity period (end of flowering to maturity). Each point is the mean of three replicates for each cultivar and year combination. Error bars (S.E.M.) are smaller than symbols.

unsaturated flax oil fraction similar to that observed in the present study for ALA in 2011.

This complex behaviour probably arises from the presence of multiple mechanisms which can modulate the impact of plant density on seed maturation. For instance, plant density impacts on leaf persistence and it strongly affects branching, leading to uneven seed formation and ripening. Interaction of these effects with other factors, such as the climatic-dependence of flowering length, further contributes to oil composition variability.

Straw and fibre yield

The highest STRAW_Y and FIBRE_Y were obtained with Camporeale landrace and Norlin, two accessions selected for their tall stature, which, however, showed poor SEED_Y and OIL_Y. This is similar to flax genotypes characterized by longer stems with respect to linseed genotypes (Diederichsen & Ulrich 2009). The straw yield was strongly influenced by year, as cool and wet conditions in 2013 favoured vegetative development.

The FIBRE_C values obtained varied from 98 to 173 g/kg, with Norlin reaching the highest value. Diederichsen & Ulrich (2009) reported higher FIBRE_C values, from 163 to 273 g/kg for the linseed genotypes tested, among which Norlin was the lowest ranking one with fibre content very close to the value reported in the present study. Zuk *et al.* (2015), however, report FIBRE_C content around 150 g/kg for linseed, near to the value reported in the present study for 2012.

The current results demonstrated a significant dependence of FIBRE_C on plant density, which, as other authors report, stimulates an increment of secondary branching (Albrechtsen & Dybing 1973; Diepenbrock & Pörksen 1992; Casa *et al.* 1999). A higher branch number makes mechanical fibre extraction more difficult, thus explaining FIBRE_C decrease in 2013, when the lowest plant density was registered: in 2012 tow proportion on the total fibre was 255 g/kg, while in 2013 it was 688 g/kg. The difference in FIBRE_C between 2012 and 2013 caused FIBRE_Y to be almost equal in the 2 years, despite the high STRAW_Y variation.

Although the cultivar effect on fibre was found to be significant, it is nonetheless not very pronounced, especially for FIBRE_Y which, aside for the two top-ranking cultivars, showed

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no significant difference among cultivars. This is consistent with findings reported by Rennebaum *et al.* (2002), who also found a lower cultivar effect for fibre, with respect to the year effect.

Structural equation modelling analysis

Only direct effects were detected by S.E.M. as no significant associations were found between the dependent variables and the yield components. Concerning BPP, this finding can be partly explained by BPP dependence on plant density, which showed sufficient variability to impact on BPP and STRAW_Y. This variation, however, did not affect SEED_Y because of the presence of compensatory effects: a higher plant density decreases BPP, but it increases the number of bolls/m^2. Within a certain density interval, this effect keeps SEED_Y unchanged (Albrechtsen & Dybing 1973; Casa *et al.* 1999). It is more difficult to explain the absence of an association between SEED_Y and TSW. One possibility is the existence of an inverse relationship between TSW and SPB (not shown), so any effect favouring TSW is partially compensated by a decrease in SPB. However, TSW was characterized by a more complex determinism with respect to SEED_Y, as it responded to a higher number of factors, which may have raised variability, weakening the correlation between SEED_Y and TSW. The current results, therefore, do not confirm the validity of TSW and BPP as selection criteria for yield, as reported by other authors (Adugna & Labuschagne 2003; Diederichsen & Raney 2006; Zając *et al.* 2012).

As far as the direct effects are concerned, rainfall was beneficial for SEED_Y throughout the whole growth cycle. However, rainfall does not appear critical in the pre-flowering phase, since good production, above 2.0 t/ha, were also possible in 2011, when rainfall was well below the historical mean registered for this phase. More important are post-flowering conditions, which were very dry in 2012, the year when the lowest average yield was recorded. The fact that some cultivars were able to provide good yield results supports previous studies highlighting that linseed has moderate water requirements (Casa *et al.* 2000), but it also confirms that scarcity of rainfall during the seed filling stage is one of the principal causes of linseed yield loss, together with heat stress (Lühs & Friedt 1994; Adugna & Labuschagne 2002). Pre-flowering rainfall had a strong effect on OIL_C: the values in 2011 and 2012 were practically the same, despite the lowest post-flowering precipitation in 2012. Seed oil content reached the highest value in 2013, the year with the highest rainfall in the pre-flowering phase. Rainfall in this phase was also very important for STRAW_Y, as it promoted vegetative growth.

A high air temperature during seed ripening showed a depressive effect on SEED_Y, which agrees with previous findings indicating high temperature as a harmful factor for seed yield, oil content and composition, and identified it as one of the principal causes of yield loss (Dybing & Zimmerman 1965, 1966; Kenaschuk 1975; Green 1986; Adugna & Labuschagne 2002, 2003). According to Casa *et al.* (1999), the negative effect of high temperature results from plant development acceleration, which shortens the growth period. Other authors have shown that late sowing raises the probability of exposing the crop to higher temperatures during ripening and indicated boll number per plant as the most affected character (Ford & Zimmerman 1964; Taylor & Morrice 1991; D'Antuono & Rossini 2006; Shaikh *et al.* 2009; Pageau & Lajeunesse 2011). Detection of a temperature effect in these trials is noteworthy in view of forthcoming climate change, as it is to be expected that linseed cultivation may be further challenged by temperature increase in the short to medium term. The problem could be faced by anticipating sowing, so escaping the excessive heat in the last cycle phases, as suggested by Casa *et al.* (1999) for linseed cultivation in central Italy. This strategy will demand further work on breeding and cultivar evaluation to improve adaptation, possibly exploring the potential of local landraces (D'Antuono & Rossini 2006).

According to S.E.M. analysis, plant height was associated negatively with SEED_Y, but the relationship was positive if within-cultivar variation was considered. This result is coherent with findings reported by Adugna & Labuschagne (2003), and it is intuitively explained by the fact that plant growth is enhanced by favourable growing conditions, which often coincide with high seed production. The relationship under within-year variation is instead negative, due to the disruptive selection that over the history of this crop separated and distinguished seed from fibre-types (Soto-Cerda *et al.* 2013). Unsurprisingly, because of their direct association with straw biomass, plant height and density were key factors for straw production. Stand density was fixed by experimental protocol, but a certain variability was nevertheless obtained, without impacting on SEED_Y nor OIL_C, similarly to what has been reported by Albrechtsen & Dybing (1973) and Gubbels & Kenaschuk (1989), who found seed and oil yield were not affected by range stand density between 100 and 700 plants/m^2. Moreover, trials conducted both in Northern Italy (Cremaschi *et al.* 1995) and in Central Italy (D'Antuono & Rossini 1994; Casa *et al.* 1999) evidenced very little effect on yield with plant densities under 800 plants/m^2. Stand density was sufficient, instead, to impact on STRAW_Y.

This result suggests the possibility of using density to manipulate agronomic behaviour, modifying the seed/straw ratio according to cultivation goals. Unlike conventional oil-based cultivation, dual-purpose linseed can, therefore, be grown at higher plant densities, as this appears to be an efficient way to increase straw yield, even within narrow density variation intervals. It is, however, interesting to note that in the present study the effect of height on STRAW_Y is stronger than stand density, suggesting that the right seed/straw balance is not only to be sought through seeding rate but also by plant height, which is partly under genetic control.

Conclusions

The 3-year field trial reported in the present paper has demonstrated that with a proper choice of linseed cultivars within available accessions, it is possible to obtain a seed yield above 2 t/ha with an oil content of at least 400 g/kg and an ALA content above 500 g/kg in a Po Valley location, in Northern Italy. The group of cultivars satisfying all these evaluation criteria were Olinette, Iduna, Buenos Aires, Pacific and Atalante. In addition to these main products, the same cultivars could provide similar amounts of straw (2.5–3.0 t/ha) and fibre (0.3–0.44 t/ha). Secondary branching is a critical factor for a satisfactory fibre production and should be limited by avoiding low-density population, i.e. below 500 plants/m^2. If this condition is met, fibre content of 150 g/kg can be obtained.

It is therefore fundamental, in a dual-purpose linseed cultivation, to achieve the correct plant density to find the best compromise between good seed and oil yield and the right height, promoting vegetative growth and straw yield. Depending on the cultivation purpose, cultivar choice could be different. If good seed and oil yields are to be achieved, the best cultivars are

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Mikael and Claudia. If the target is seed with high ALA content, in which a trade-off between yield and quality must be sought, then Buenos Aires is the best choice. The development cycle takes around 87 days from emergence to harvest. Flowering duration was highly variable from year to year, thus impacting on the subsequent maturation length, which in turn impacted on oil quality.

Structural equation modelling analysis highlighted that rainfall was beneficial for SEED_Y, especially in the post-flowering phase, whilst pre-flowering rainfall was more critical for OIL_C. Straw production was favoured by pre-flowering rainfall, plant size and density. Air temperature after flowering had a negative impact on SEED_Y.

Overall, the results reported in the present paper confirm that linseed can perform satisfactorily as a dual-purpose crop; it has low water requirements, but it is sensitive to temperature. In view of upcoming climate change, linseed cultivation protocols should explore anticipated sowing, to escape the warmest period in the late cycle phases, a strategy which demands further work on breeding and cultivar evaluation to improve adaptation to warmer climates.

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