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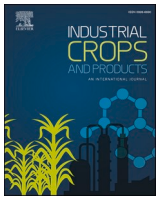

Seed growth and oil accumulation in two different varieties of industrial hemp (*Cannabis sativa* L.)

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ABSTRACT

Nowadays, the main hemp foods products derive from hemp seed (seed, dehulled seed, meal and oil) and an uncertain economic sustainability of the hemp food supply chain remains to be resolved. The cause is often to be found in unsatisfactory quantitative-qualitative seed yield. Following the above, an experiment was conducted utilizing Futura and Zenit varieties with different crop cycle duration, cultivated in two locations (S. Osvaldo and Verzegnis), for two years (2019 and 2020) and with two sowing times (conventional and delayed). A total number of five-nine seed samples of ten seeds each, depending on location and variety, were collected from the middle of inflorescence of five plants for each of the three replications, starting from 50 Growing Degree Days (GDDs) after flowering until physiological maturity. The following characteristics were determined on each seed: whole seed weight, kernel weight, pericarp weight, whole seed oil content, oil weight, kernel oil content, kernel percentage, hull to kernel ratio and oil to kernel ratio. The achievement of seed with high oil content and high weight at maturity is mainly due to the accumulation rate intensity rather than length of the seed-filling period. The accumulation rate for seed growth and oil accumulation is mainly dependent on the genotype, but also affected by daily mean temperatures above 23°C recorded during the first 20 days after flowering that resulted the sub-phase most susceptible to temperature. The use of the character oil weight per seed, instead of whole seed oil content commonly utilized in breeding activity with the aim of increasing the oil content in oil crops, could simultaneously improve the seed and kernel weight; both parameters highly appreciated in the hemp food chain.

1. Introduction

Industrial Hemp or Hemp (*Cannabis sativa* L.) is an herbaceous plant belonging to the Cannabaceae family and genus *Cannabis*. Until the early 2000s the main product of industrial hemp was the short fibre and shives utilized mainly as litter, in construction and as industrial technical materials, while recently, interest has considerably increased in the seeds for food purposes, as evidenced by about 20 thousands of tons of hemp seeds produced in the EU in 2018 (Mirizzi and Troyano, 2018). Since 2017 the demand for hemp-based agri-food products has grown by 500% (Sorrentino, 2021) and in 2021 the food industry held more than 25% of the industrial hemp market share (Prescient & Strategic Intelligence Report, 2022). The main hemp food products derive from hemp seed as it is or as gluten-free seed meal characterized mainly by a protein (globulin) rich in essential amino acids (Docimo et al., 2014). The other product is hemp oil, which is rich in polyphenolic compounds (Andre et al., 2016) with a 3:1 ratio between omega-6 and omega-3, considered the best proportion for humans by medical research and the most advanced theories in the field of nutrition (Montserrat-de La Paz et al., 2014; Siano et al., 2018; Sorrentino, 2021; Zhang and Tsao, 2016). To date hemp seed oil has been a product of a niche market; it is mainly produced on-farm, using just a screw press, followed by a mechanical filtration without any purification or refining process and marketed directly by farmers, by health-food shops, local markets or via the internet (Giupponi et al., 2020). The seeds obtained from field cultivation often show a very heterogeneous quality, with mature and full seeds together with green unripe and empty or partially-filled seeds with a very low oil content. This can affect the oil yield and quality, with unsatisfactory characteristics relating to colour, aroma and flavour (Matthäus and Brühl, 2008; Tura et al., 2022). All this determines an uncertain economic sustainability of the hemp food supply chain, although the price of hemp oil is higher (Personal Communication,

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Table 1
Field trials planning.

Cultivar	Location	Date of Sowing	Year	Environmental Code
	S. Osvaldo	I	2019	2019_I_SO
	S. Osvaldo	II	2019	2019_II_SO
Futura	Verzegnis	II	2019	2019_VE
	S. Osvaldo	I	2020	2020_I_SO
	S. Osvaldo	II	2020	2020_II_SO
	S. Osvaldo	I	2019	2019_I_SO
	S. Osvaldo	II	2019	2019_II_SO
Zenit	Verzegnis	II	2019	2019_VE
	S. Osvaldo	I	2020	2020_I_SO
	S. Osvaldo	II	2020	2020_II_SO

2023) than the average price of extra virgin olive oil and many other seed oils (sunflower, rapeseed, etc.).

Hemp fruit, usually known as seed, is an achene, formed mainly by a thin two-layered pericarp (hull) covering an endosperm and two cotyledons (kernel). An achene contains about 20–25% of protein, 20–30% of carbohydrates, 10–15% of insoluble fibre and 20–35% of oil (Pate, 1999). The oil accumulates almost completely in the kernel and its amount, concentration and fatty acid composition depends on both genotype and environment (Eržen et al., 2023; Ferfuia et al., 2021; Irakli et al., 2019; Tsaliki et al., 2021). It is well known that the oil content of hemp seed at maturity varies significantly among cultivars (Abdollahi et al., 2020; Baldini et al., 2018; Eržen et al., 2023; Ferfuia et al., 2021). Conversely, less or no information is available about the effect of environmental factors and their interaction with genotypes (GEI) on seed weight and seed oil concentration at maturity, with the exception of preliminary studies about temperature effects conducted by Baldini et al. (2020). Moreover until today, no information has been available in the literature about the dynamics and rates of seed growth and oil accumulation during seed filling period, contrary to other oil crops such as sunflower, where the above parameters are well-described (Mantese et al., 2006; Rondanini et al., 2006, 2003). Among environmental factors, temperature during seed filling has been identified as one of the main drivers of seed weight, seed oil content and fatty acid composition in several oil crops such as soybean (Tamagno et al., 2020; Veas et al., 2022; Zuil et al., 2012), sunflower (Aguirrezábal et al., 2003; Angeloni et al., 2021; Izquierdo and Aguirrezábal, 2008; Mantese et al., 2006; Rondanini et al., 2006, 2003) and safflower (Mohammadi et al., 2018). Current trends towards increased global temperature (Easterling et al., 1997) may increase the probability of occurrence of heat stress in many regions of the world (Conroy et al., 1994). The projected average increase of 3.2°C towards the end of this century (Calvin et al., 2023), in addition to recent increases in the area cropped with hemp (European Commission, 2023) in warmer areas highlights the risks of heat stress to which the crop will be subjected in the coming years. Following the above, this study was conducted with the aim of evaluating the effect of different environments and temperatures during seed-filling period on several parameters related to seed growth and oil accumulation of two monoecious hemp varieties with different crop cycle duration.

Table 2
ANOVA statistical analysis results performed on characters detected at seed maturity stage.

Source of Variation	Seed Weight (mg)	Oil Weight per seed (mg)	Seed Oil Content (%)	Kernel (%)	Kernel weight (mg)	Hull weight (mg)	Hull/ kernel ratio[a]	Oil/ kernel ratio[a]
Block	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cultivar (*C*)	*	***	***	*	**	n.s.	n.s.	*
Environment(*E*)	***	***	***	***	***	*	***	***
CxE	n.s.	n.s.	***	*	**	**	***	n.s.

[a] dimensionless; *, ** and *** Significant at the $p < 0.05$, 0.01 and 0.001 levels, respectively. n.s.= not significant.

Table 3
Minimum, mean and maximum values of the main seed parameters analysed in the seed maturity stage trial.

	Seed weight (mg)	Oil weight per seed (mg)	Seed Oil Content (%)	Kernel (%)	Kernel weight (mg)	Hull weight (mg)	Hull/ kernel ratio[a]	Oil/ kernel ratio[a]
Minimum	9.00	1.69	10.8	57.9	5.60	1.82	0.11	0.18
Mean	17.54	3.55	20.4	69.4	12.36	5.18	0.45	0.30
Maximum	25.50	4.88	28.9	83.2	16.68	10.30	1.05	0.44

[a] dimensionless.

Table 4
Duration of the main phenological phases in days and related GDDs (Values are means ± S.E.).

Cultivar	Location	Year	Sowing time	Emergency-Flowering		F-PM[a]	
				days	GDDs	days	GDDs
	SO[b]	2019	I	94±2	1102±43	28±3	454±23
	SO	2019	II	66±3	978±47	27±2	449±22
Futura	SO	2020	I	94±2	941±41	33±1	475±24
	SO	2020	II	72±2	864±33	25±2	352±13
	VE[c]	2019	II	52±2	705±37	25±2	195±33
	Mean			76±2	918±53	28±2	385±44
	SO	2019	I	75±2	811±63	34±2	528±25
	SO	2019	II	53±5	817±61	34±2	526±32
Zenit	SO	2020	I	73±2	565±53	33±2	498±31
	SO	2020	II	52±2	570±54	32±2	497±31
	VE	2019	II	33±2	474±43	35±3	398±24
	Mean			57±5	647±62	34±3	489±20

[a] F-PM: from Flowering to Physiological Maturity; [b]SO = Sant'Osvaldo; [c]VE = Verzegnis.

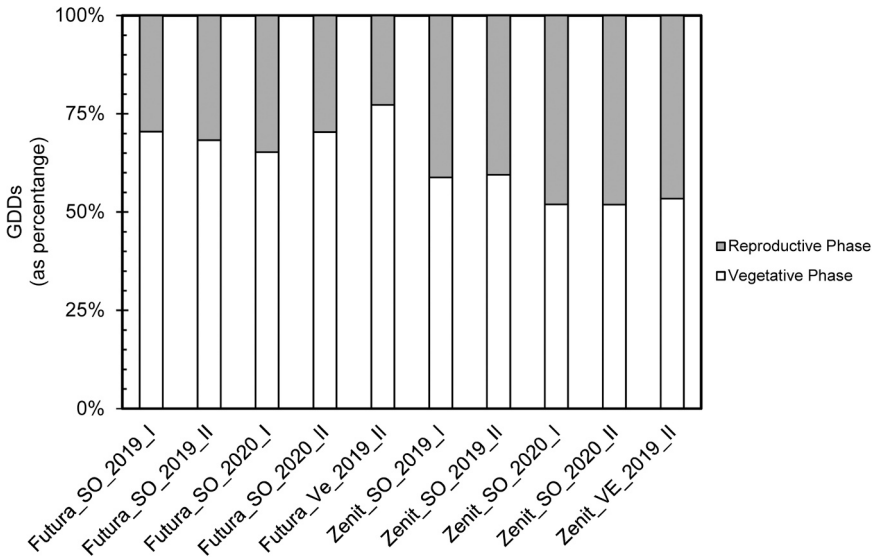

Fig. 1. GDDs accumulation in hemp cultivars during vegetative and reproductive stages as percentage.

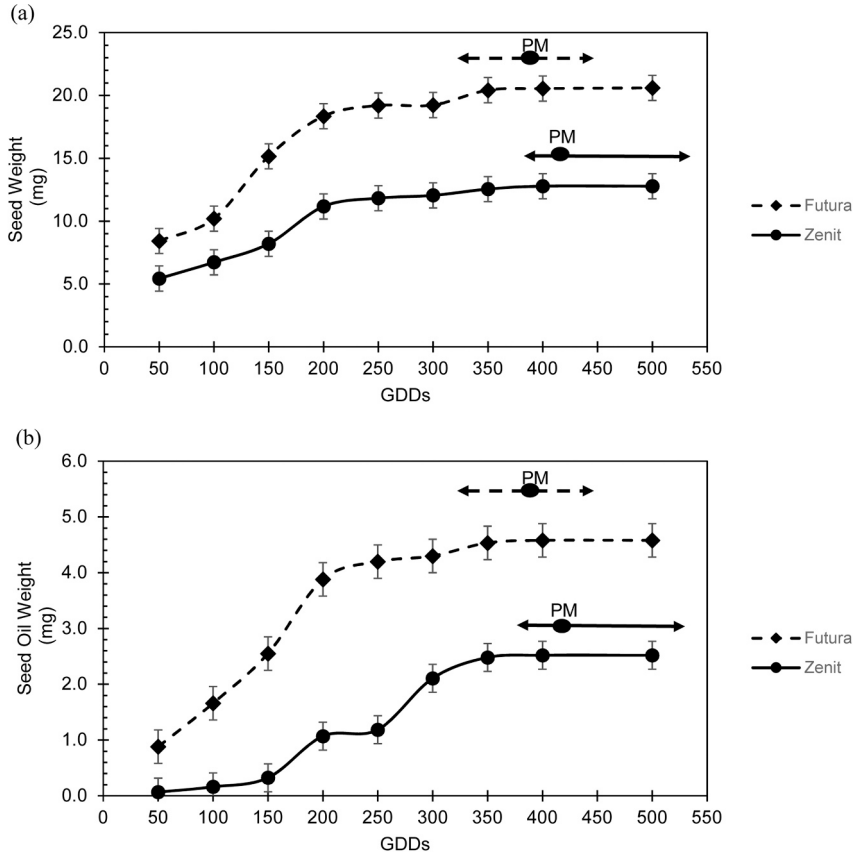

Fig. 2. Seed weight variation (a) and dynamics of oil accumulation (b) during seed filling period in hemp. Sampling started at 50 GDDs after flowering until Physiological Maturity (PM). Solid and dotted lines with arrows represent the range of PM for the two cultivars and the symbol PM (●) is the PM average across environments. Data are means ± SE.

2. Materials and methods

2.1. Plant materials

Two monoecious cultivars, namely Futura 75 and Zenit, were evaluated under five different environments. The main characteristics of these monoecious genotypes are reported in Supplementary (Table S.1). These cultivars, with a different cycle duration under field trial environmental conditions, are listed in the EU database of registered hemp varieties and normally used in several Italian and European field trials (Tang et al., 2016).

Table 5
Average accumulation rates as mg GDD^{-1} or as mg day^{-1} (in brackets) during the Flowering-PM phase.

GDD	Futura		Zenit	
	Seed filling	Oil accumulation	Seed filling	Oil accumulation
	mg GDD^{-1}			
Early (0–50)	0.17	0.018	0.11	0.001
Medium (50–200)	0.10	0.018	0.06	0.015
End of cycle (200–400)	0.02	0.006	0.01	0.007
Mean cycle[a]	0.05 (0.60)	0.011 (0.13)	0.03 (0.46)	0.006 (0.09)

[a] From flowering to physiological maturity.

2.2. Field Trials

Field trials were planned as reported in Table 1. Two locations (S. Osvaldo, SO; Verzegnis, VE) in 2019 and one location (SO) in 2020 were considered. Two sowing periods were carried out in each location and year: normal (I), considered usual for the environment and delayed (II) by about more than 30 days with respect to the normal one. The two sowing dates determined a significant difference in climatic conditions in correspondence to the flowering-maturity period of the two varieties, so that these can be considered as two distinct locations. In Verzegnis, sowing date I was missed because of adverse weather conditions, so only data from the last date of sowing (II) are available. Following the above, the five environments considered were: SO2019I; SO2019II; VE2019II; SO2020I and SO2020II.

The two locations are characterized by different altitude and pedo-climatic conditions. The first one (SO) is located on the plain with a gravel-rich sandy soil, while the second (VE) is on a hillside, it has a very shallow soil (about 35 cm) with a silty loam texture with 17% gravel and a sub-alkaline pH. Details of the different trial locations, the main soil physical-chemical characteristics and main climatic parameters recorded during each hemp crop cycle compared with the previous 28-year period (1992–2019) are reported in Supplementary (Table S.2, Figure S.1, S.2 and S.3).

2.3. Phenological phases duration

The duration of the several physiological phases during hemp crop development was computed as calendar days (days) and Growing Degree Days (GDDs). The accumulation of GDDs above a base temperature of 10°C (Tb) (Faux et al., 2013) was calculated using the following formula:

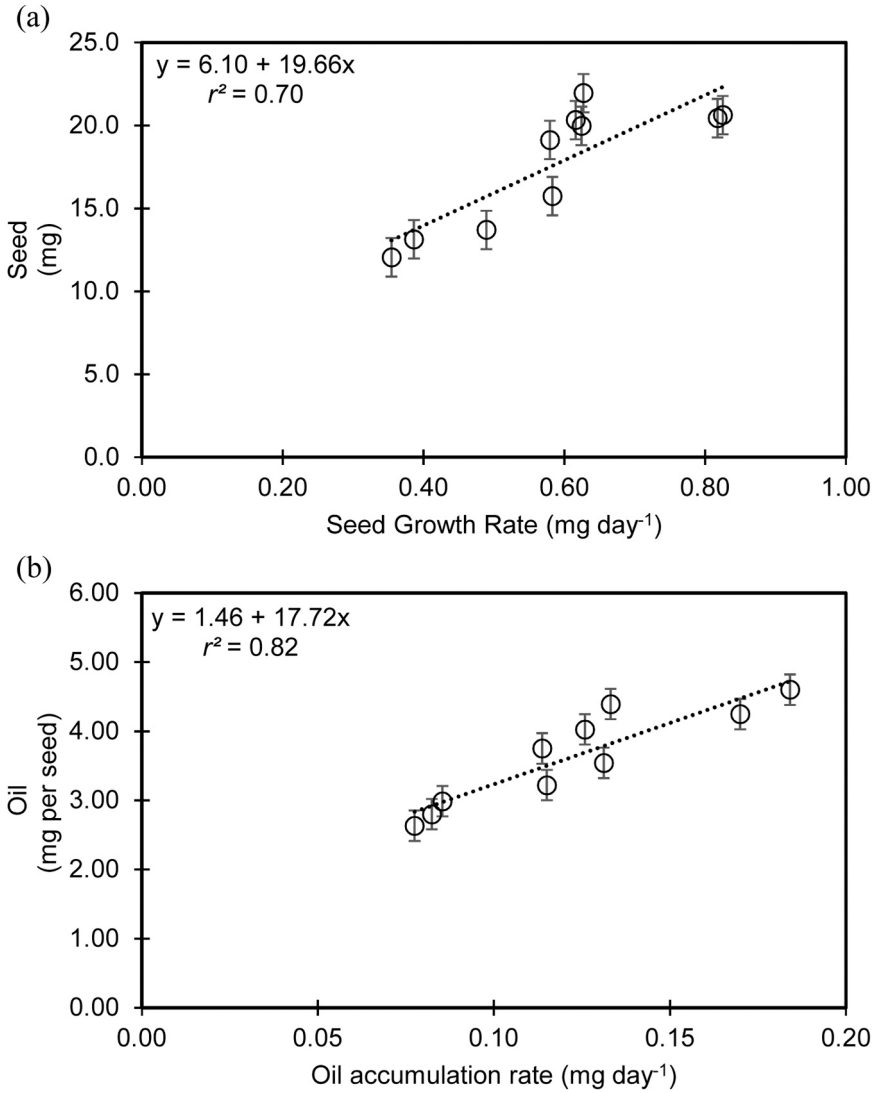

Fig. 3. Relationship between (a) seed weight (mg) and seed growth rate (mg day^{-1}; p-value < 0.001) and (b) oil weight per seed (mg) and oil accumulation rate (mg day^{-1}; p-value < 0.001).

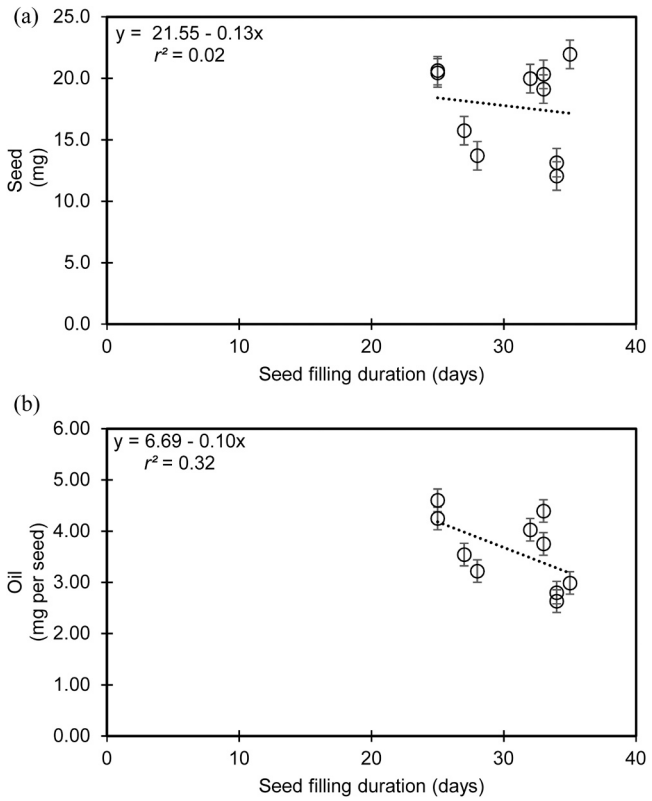

Fig. 4. Relationships between seed filling duration (days) and (a) seed weight (mg per seed; *p*-value = 0.71) and (b) oil weight (oil mg per seed; *p*-value = 0.59).

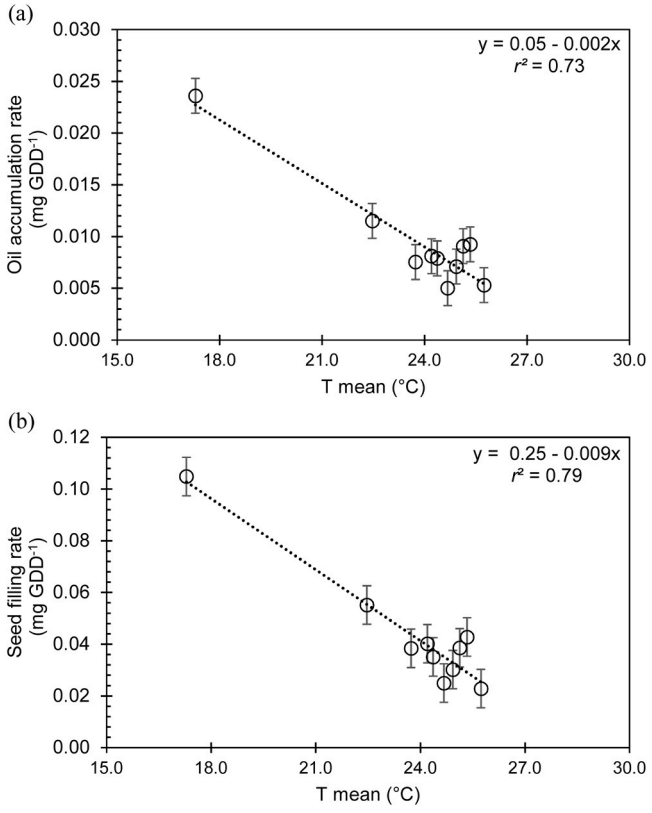

Fig. 5. Relationship between daily mean temperature and (a) oil accumulation rate (*p*-value <0.001) or (b) seed filling rate (*p*-value <0.001).

$$GDD = \Sigma\ [(Tmax + Tmin)/2] - Tb \quad (1)$$

where Tmax and Tmin are the daily maximum and minimum temperatures in °C, respectively.

2.4. Experimental design and seed characteristics determination

The experimental scheme adopted was a randomized block design with four replications, each experimental unit being 30 m^2. To study the dynamics of seed growth and oil accumulation until physiological maturity, a micro-plot (5 m^2) was randomly located in each block, where five plants at the same development phase were selected.

During the entire seed-filling period, samples of seeds were collected starting from 50 GDDs after flowering until physiological maturity (calculated by method of seed constant weight). The hemp inflorescence of each plant was previously divided into three sections (top, middle and bottom) based on the female flower at beginning of flowering (code 2303 in Mediavilla et al., 1998). Ten seeds from each sampled plant were collected at 50 GDD intervals from the middle section on the hemp inflorescence, for a total number of 5–9 samplings, depending on location and cultivar. Thus, all the seeds collected on the same date were at the same development stage. As the same inflorescence was repeatedly sampled, after a sampling, the neighbouring seeds were not collected, to avoid alterations due to compensatory growth of the remaining seeds. Afterwards, the seeds were dried in a ventilated oven at 60°C for 72 hours and cooled on silica gel before weighing. Physiological maturity (PM) was determined as code 2307 (Mediavilla et al., 1998) and as days after flowering and GDD in correspondence to the first sampling of three consecutive samplings with constant seed weight.

At physiological maturity, all plants in each block were harvested, except from the micro-plot area. Seeds were separated from the inflorescence by means of a threshing machine, cleaned and the empty seeds removed. The remaining seeds were weighed and dried in a ventilated oven at 100°C for 1 hour and used for further determinations.

For each seed sample collected during the seed-filling period and at seed maturity, the following characteristics were determined: whole seed weight (SW, mg); kernel weight (KW, mg), obtained after dissection from the pericarp; pericarp weight (PW, mg), determined as difference between whole seed and kernel weight; whole seed oil content (SOC, as % on seed dry matter); oil weight (SOW, mg oil/seed); kernel oil content (KOC, as % on kernel dry matter); kernel percentage (KP, % kernel on seed dry matter); hull to kernel ratio (H:K) and oil to kernel ratio (O:K).

2.5. GC analysis and seed oil content determination

Analyses of whole seed oil content were performed on the same seeds used to determine seed dry weight dynamics. The usual methods for lipid content determination (e.g. Soxhlet) could not be used in this study due to the small sample size. Total fat content was determined according to AOAC official method 996.06 (AOAC, 2003) with some modifications. *n*-Hexane was used as the extraction solvent and fatty acids were converted into Fatty Acid Methyl Esters (FAMEs) by transesterification with a methanolic potassium hydroxide solution (2 N). Tridecanoic acid methyl ester was used as internal standard, then the FAMEs composition of hemp seeds was determined using GC. Five microlitres of sample were injected in split mode into a GC equipped with a Flame Ionization Detector (FID) with the following specifications. Capillary column HP-88 (60 m × 0.25 mm, id 0.20 μm) and helium as carrier gas were used for the analyses. The injector and detector temperatures were 270 and 250°C, respectively, the oven temperature was initially kept at 150°C for 10 min and increased to 180°C at the rate of 3°C per minute and maintained at 180°C for 10 min. Afterwards, the oven temperature was increased to 240°C at the rate of 5°C per minute and maintained at 240°C for 10 min. Identification and quantitative evaluation of fatty acids was determined confronting retention times and areas with those

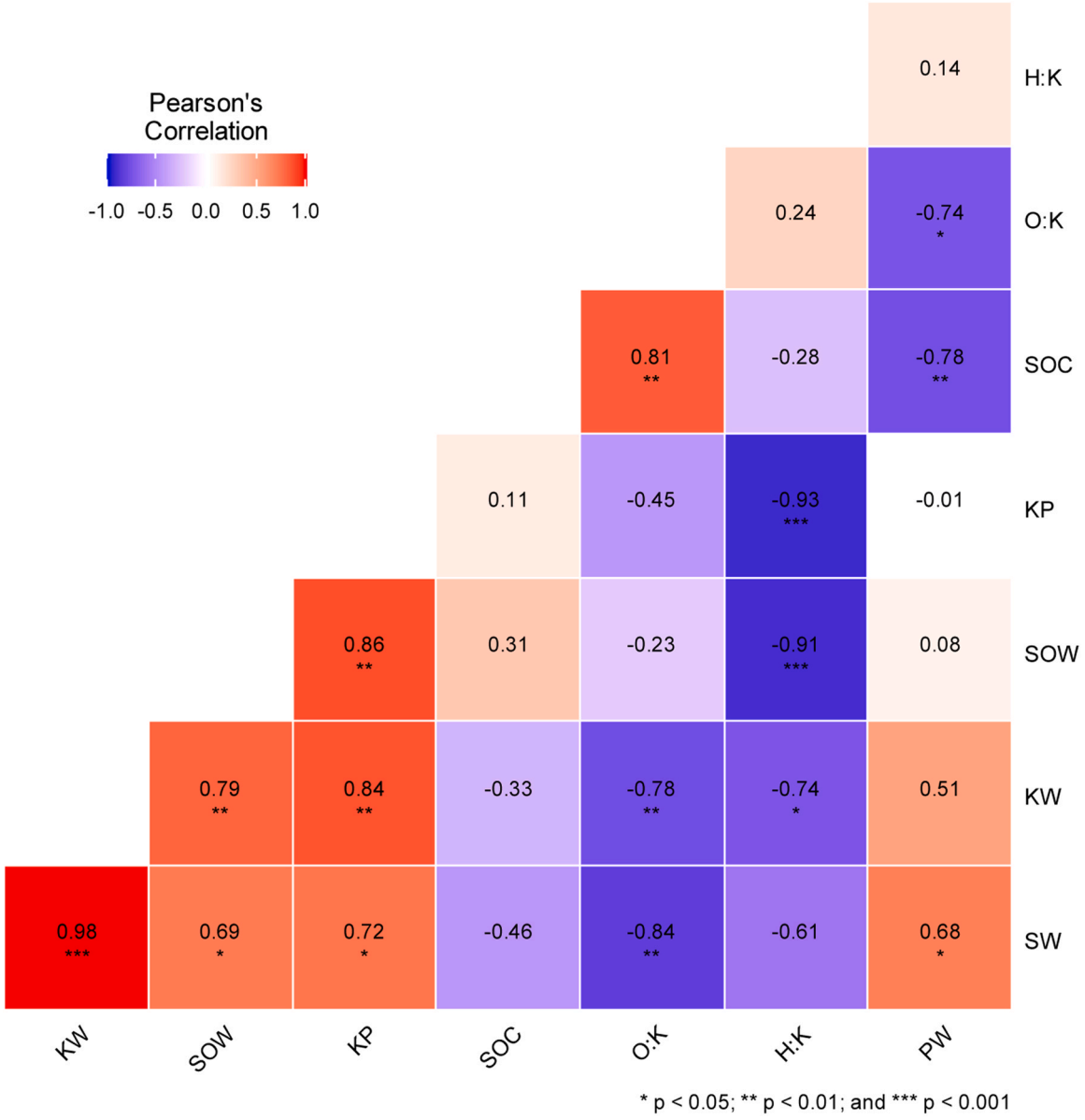

Fig. 6. Heatmap of the correlation analysis (Pearson correlation coefficient) between seed-related traits from two cultivar and five environment. Whole seed weight (SW); kernel weight (KW); oil weight per seed (SOW); kernel percentage (KP); whole seed oil content (SOC); oil to kernel ratio (O:K); hull to kernel ratio (H:K); pericarp weight (PW).

of standard mixes FAMEs. Total fat was calculated as the sum of individual fatty acids expressed as triglyceride equivalents. All analyses were performed in duplicate.

2.6. Statistical analysis

Statistical analysis was performed using R version 4.0.2 (R Core Team, 2020). Shapiro–Wilk normality test was performed to test normality condition. The homogeneity of variance was tested by Levene's test. A two-way Analysis of Variance (ANOVA) was performed as fixed-effect model with cultivars and environment as source of variation for seed characteristics at physiological maturity. Significance of each source of variation was evaluated by *F*-test. When the *F*-ratio revealed significant differences, means were compared by the Duncan test at $p \leq 0.05$. For the effect of temperatures during seed-filling period, a bootstrap analysis was performed to select the most critical period and the best temperature predictor (t mean, max or min) for several seed characteristics (seed oil accumulation rate, seed weight, etc.). One thousand random samplings with replacement were performed in R (Bootstrap Resampling, boot package) and an adjustment was obtained for each sample. Adjustments were evaluated by comparing the distribution of the 1000 r^2 values. Pearson correlation coefficients were also computed to measure the strength of linear association among investigated seed traits. Correlation matrix was displayed as Heatmap in R.

3. Results and discussion

Table 2 shows the results obtained from the ANOVA analysis about the average effect of the main treatments (Cultivars and Environments) and their interaction (*CxE*) on the seed characteristics measured at maturity stage. The average effect of the Environment (*E*) factor was particularly significant for all characteristics considered, whereas the average effect of Cultivar (*C*) was highly significant for seed, oil and kernel weight. While the interaction effect (*CxE*) significantly influenced the expression of oil content, hull/kernel ratio, kernel and hull weight.

Table 3 shows the minimum, mean and maximum values of the seed

characteristics at maturity stage obtained in this experiment. Note the large differences between minimum and maximum values found for all the parameters measured, confirming the great difference between the varieties analysed, but especially the wide environmental variability (Table 1) that characterized the experiment.

3.1. Duration of the main phenological phases

Table 4 indicates (as expected) that Futura exhibits a significantly longer vegetative phase than Zenit, at each location, regardless of whether expressed in days or GDDs, whereas when the reproductive phase is considered, the contrary occurs. Indeed, the average duration of the seed filling period was approximately 27.6 ± 1.9 and 33.6 ± 3.3 days, for Futura and Zenit, respectively (Table 4), similar durations to those previously reported for these genotypes in similar environments (Baldini et al., 2020, 2018; Ferfuia et al., 2021). The differences between cultivars persisted when seed-filling duration was expressed in GDDs rather than calendar time. The average seed-filling duration was ca. 385 GDDs for Futura and ca. 489 GDDs for Zenit (Table 4). Futura reduces the average duration of the seed-filling phase across the environments to 33 ± 2% of the entire crop cycle, unlike Zenit that reduces the same period duration to 45 ± 3% (Fig. 1). This result confirms that, in this environment, the earliness or lateness of the hemp varieties is due exclusively to the different duration of the vegetative phase, caused by the specific genotype characteristics. On the contrary, the duration of the seed-filling period is apparently almost exclusively dependent on climatic conditions, as a consequence of the lower temperatures, shorter day-length and lower light intensities that are experienced during seed development of late-flowering cultivars in several species (Baldini et al., 2018, 2020; Mohammadi et al., 2018; Rondanini et al., 2003, 2006; Veas et al., 2022; Zuil et al., 2012). Physiological maturity expressed as constant seed weight took place on average five days earlier with respect to identifying it as phenological code (Mediavilla et al., 1998). This is comparable to other crops such as sunflower (Rondanini et al., 2006, 2003).

3.2. Dynamics of seed growth and seed oil accumulation

The dynamics of seed weight and oil weight accumulation per seed were fitted to the duration of the seed-filling period expressed as GDDs, to compare the two genotypes across different environments (Fig. 2a, b). Both varieties show a significant increase in seed weight up to about 200 GDDs (about 18 days after flowering, as average across environments), after which the weight increases become marginal until physiological maturity (Fig. 2a). The higher weight of the Futura seed is already evident in correspondence with the first sampling (50 GDDs corresponding to approximately six days after flowering, on average). This difference increases until a maximum at about 200 GDDs, and then remains almost constant until maturity. This difference between the two varieties is clearly due to the higher seed-filling rate of Futura, which showed an average value of 0.05 ± 0.0015 mg GDD^{-1} across environments (corresponding to 0.60 mg day^{-1}) compared with 0.03 ± 0.0012 mg GDD^{-1}, (corresponding to 0.46 mg day^{-1}) of Zenit (Table 5). The maximum peak of seed growth rate was observed within 0–50 GDDs after flowering in both cultivars, with maximum values of 0.17 and 0.11 mg GDD^{-1}, for Futura and Zenit, respectively. The seed-filling rate then progressively decreased in both varieties from 200 GDDs, until almost reaching 0 at physiological maturity, corresponding to about 350 GDDs after flowering for Futura and 400 for Zenit (Table 5 and Fig. 2a). Also considering oil weight accumulation in the seed, Futura already showed a statistically significantly higher value compared with Zenit in correspondence with the first sampling at 50 GDDs after flowering. Oil accumulation in Futura, as mean across environments, started with a first phase of rapid synthesis until 200 GDDs after flowering, followed by a second phase characterized by a very slow rate that lasted until about 350 GGDs (Fig. 2b). Zenit, on the contrary, showed a slow oil accumulation in the early stages up to 150 GDDs after flowering, then a rapid synthesis up to 300 GDDs, and again a slowdown up to 350 GDDs, at the maximum oil accumulation in the seed. Both cultivars reach their maximum oil accumulation at almost physiological seed maturity.

Again in this case, the better performance of Futura is due to an oil accumulation rate of 0.011 mg GDD^{-1} (0.13 mg day^{-1}), as average of the whole seed filling period, significantly higher than the value of 0.006 (0.09) shown by Zenit (Table 5). Futura showed a maximum oil accumulation rate of 0.018 mg GDD^{-1} recorded during the period 0–200 GDDs (a period of about 18 days from flowering) (Table 5 and Fig. 3). Conversely, the highest oil accumulation rate value of Zenit was 0.015 mg $GDDs^{-1}$ and occurred for a shorter period (50–200 GDDs) with respect to Futura (Table 5), suggesting a different sensitivity of the fat biosynthetic system to the climatic-environmental conditions of these two cultivars.

The significantly higher seed weight and seed oil content at maturity of Futura with respect to Zenit (Figs. 2 and 3) appear to be influenced by a higher seed growth rate and oil accumulation rate, as average values throughout the cycle and as maximum values, with significant differences already evident at 50 GDDs (Figs. 2 and 3). As a confirmation of this hypothesis, the positive and highly significant relationship between seed weight and seed oil content at maturity and the respective growth rates expressed as mg per day are reported (Fig. 3a, b). In particular, the variations in seed weight and seed oil content, as mean among environments and cultivars, are explained more than 70 and 82% respectively, by seed growth rate and oil accumulation rate (Fig. 3), similarly to results already obtained in other oil crops (González Belo et al., 2017; López Pereira et al., 2000; Satorre, 2010). The absence of a relationship between seed growth rate and oil accumulation rate with seed-filling duration, is clearly observed in hemp (Fig. 4a and b), as already reported in soybean (Veas et al., 2022) but contrary to what was observed in sunflower (Mantese et al., 2006; Rondanini et al., 2006, 2003), where a longer duration of the seed-filling period is often responsible for a heavier seed and higher seed oil content.

Furthermore, the relationship was investigated between three temperatures (min, mean, max) at ten different sub-phases of the seed filling period and seed growth rate and oil accumulation rate, as described in materials and methods (Bootstrap study) and Supplementary (Table S.4 and S.5). The sub-phase 150 GDDs after flowering resulted as being the most susceptible to temperature and then utilized for the study. Indeed, the relationships between temperature and seed growth rate and oil accumulation rate, analysed through Bootstrap, showed more or less close and significant relationships (different r^2) depending on the temperature analysed (Tmin, Tmean or Tmax) and the sub-phase considered during seed filling period. For both characteristics, the r^2 of the relationships were maximum with the mean temperature at 150 GDDs (Table S.4 and S.5 in the Supplementary). Both accumulation rates are affected by an increasing of mean temperature during the first 150 GDDs after flowering (corresponding to a period of about 15 days after flowering, as mean across environments and varieties) along the temperature range explored (about 17–26°C) (Fig. 5). In hemp, this result highlights a rate reduction for both characteristics certainly with temperature up to about 23°C (Fig. 5). Quite similar results were found in sunflower, where the seed oil concentration decreased with temperature up to about 25°C (Rondanini et al., 2003) and slightly different in soybean, where oil concentration increased with the temperature (daily or minimum) with an optimum of 25–28°C (Canvin, 1965; Piper and Boote, 1999; Wolf et al., 1982).

The characteristic high SOC has often been chosen in breeding programmes to increase oil production in many oilseed crop varieties (Alfonso, 2020; Liu et al., 2022; Mantese et al., 2006). In many cases this objective has been achieved through a simultaneous reduction in PW (due to the absence of oil in the seed pericarp), exploiting the highly negative relationship between the two characteristics, as also observed in hemp (Fig. 6). However, the adoption of the same breeding strategy in hemp, in which SOC is negatively linked to both SW (-0.46) and KW

(-0.33), could also lead to obtaining small and light seeds, which are undesirable especially for seed for food use or processing. On the contrary, the utilization of the SOW parameter instead of SOC, in addition to increasing the seed oil content, would also improve other interesting seed traits, such as SW, KW and KP due to their high positive relationship (+ 0.69, + 0.79 and + 0.86, respectively) (Fig. 6).

4. Conclusions

In hemp, the achievement of seeds with high oil content and high weight at maturity is mainly due to the daily accumulation rate rather than length of the seed-filling period, as instead observed in other oil crops. The accumulation rate of both characteristics depends mainly on the genetic characteristics and thus on the variety, but has also been shown to be sensitive to temperature. This allows the farmer to be guided in the choice of cultivar and sowing time of hemp cultivated for seed yield, according to the specific environment characterized by high temperatures at the seed-filling stage. In particular, it would be necessary to avoid that mean temperatures above 23°C occur during about the first 20 days after the end of flowering. Indeed, this sub-phase proved to be the most sensitive to temperatures in hemp for the accumulation rate of oil and dry matter in the seed. Moreover, the characteristic high oil contents in the kernel, conversely to seed oil percentage, would result in heavier and larger seeds and kernels, traits much appreciated for seed for direct (dehulled) or processed food use (oil and flour). This information might be of assistance to breeding activities, recently oriented towards increasing the oil content in hemp seed, in exploiting the genetic variability of the species, with the aim of improving the sustainability of the agri-food chain.

CRediT authorship contribution statement

Claudio Ferfuia: Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Barbara Piani:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Nicolò Fantin:** Writing – original draft, Investigation, Formal analysis. **Fabio Zuliani:** Investigation, Data curation. **Mario Baldini:** Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.118723.

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