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Fibre and Seed Productivity of Industrial Hemp (*Cannabis sativa* L.) Varieties under Mediterranean Conditions

Eleni Tsaliki [1,*], Apostolos Kalivas [1], Zofija Jankauskiene [2], Maria Irakli [1], Catherine Cook [1], Ioannis Grigoriadis [1], Ioannis Panoras [1], Ioannis Vasilakoglou [3] and Kitsios Dhima [4]

[1] Institute of Plant Breeding and Genetic Resources, Hellenic Agricultural Organization—DEMETER, 57001 Thessaloniki, Greece; kalyvas@ipgrb.gr (A.K.); irakli@cerealinstitute.gr (M.I.); cook@bio.auth.gr (C.C.); y.gregoras@gmail.com (I.G.); y.panoras@gmail.com (I.P.)

[2] Lithuanian Research Centre of Agriculture and Forestry, Institute of Agriculture, Instituto al. 1, LT-58344 Kėdainių r, Lithuania; sofija.jankauskien@lammc.lt

[3] Department of Agriculture—Agrotechnology, University of Thessaly, Geopolis of Larissa, 41500 Larissa, Greece; vasilakoglou@uth.gr

[4] Department of Agriculture, International Hellenic University, 57400 Echedoros, Greece; dimas@ihu.gr

* Correspondence: tsaliki@ipgrb.gr; Tel.: +30-231-0471-544

Abstract: Farmers' interest in renewable raw materials such as hemp (*Cannabis sativa* L.) fibres has recently increased, but hemp productivity is strongly affected by genotype and environment conditions. A 3-year field experiment was conducted under Mediterranean environment in northern Greece to evaluate the productivity (regarding fibres and seeds) of six monoecious hemp varieties. The vars. Futura 75 and Bialobrzeskie provided the greatest ($p < 0.01$) fibre productivity (4.57 and 4.27 t ha^{-1}, respectively), which were 77.1% and 65.5%, respectively, greater than that of the least productive var. Fedora 17. However, the vars. Santhica 27, Tygra and Bialobrzeskie provided the highest ($p < 0.05$) seed yield (2.7, 2.9 and 2.6 t ha^{-1}, respectively), which were 28.6%, 38.1% and 23.8%, respectively, greater than that of the least productive var. Futura 75. Hemp fibre yield was strongly positively correlated with total biomass (R^2 = 0.8612) and stem biomass yield (R^2 = 0.9742), while it was inversely correlated with fibre strength (R^2 = 0.424). Hemp seed yield was not correlated with the hemp plant density, height, total biomass or stem biomass yield. The six hemp genotypes evaluated in the study had Δ9-tetrahydrocannabinol (THC) content lower than 0.2% satisfying the European legislation requirements for industrial hemp varieties. The results of the study indicated that, under Mediterranean conditions (northern Greece), the var. Bialobrzeskie showed high productivity, as averaged across years, for both fibres and seeds. This result is very helpful for farmers which should prefer hemp varieties of dual-purpose production (stems and inflorescences or stems and seeds) adapted best to their local environment.

Keywords: hemp (*Cannabis sativa* L.); fibre; seed; biomass; variety evaluation; cannabinoids

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1. Introduction

Hemp (*Cannabis sativa* L.) belongs to the oldest and most known versatile plants. It was cultivated all over the world, due to its fast growth and low-demand in resources, until its ban in the 1930s [1]. However, demand for renewable raw materials has recently increased and hemp cultivation has returned as a sustainable and high yielding crop that can be grown for a multitude of products and industrial applications [2,3]. Hemp has also participated in many agro-industrial fields such as agriculture, textiles, bio-composite, paper-making, automotive, construction, bio-fuel, functional food, oil, cosmetics, personal care and pharmaceutical industry [4], because of the high-quality cellulose in its stems, the high-quality oil in seeds and valuable resins in the inflorescence. In addition, industrial hemp varieties (cultivated for stem fibres and seeds) registered in the European Catalogue have to contain less than 0.2% Δ9-tetrahydrocannabinol (THC) according to EC regulation 809/2014 [5].

Although weather conditions in southern Europe are favourable for hemp growth there is little background knowledge regarding the productivity of the recently registered varieties [3] due to the interruption of hemp production in the second half of the last century. Nevertheless, many farmers are interested in the reintroduction of hemp cultivation in Southern Europe and, according to the European Industrial Hemp Association (EIHA), in 2018 the hemp cultivation area in Europe was 50,081 ha [6]. The main problems faced by the renewal of industrial hemp cultivation in Europe include i. the selection of the most appropriate variety to European conditions, ii. the lack regarding the agronomic data to incorporate into Mediterranean farming systems and practices [7], iii. the end use of the final product and iv. the negative attitude towards hemp cultivation due to the THC content.

The germplasm used is crucial for the success of the cultivation, because of its environmental adaptability and production potential, as well as in terms of final quality and end-use [8]. Hemp is cultivated mainly for its fibrous stem but the most profitable practice seems to be the combination of utilizing both fibre and seed in multiple uses [9,10] and available varieties may present noticeably divergent characteristics, making them more suitable for different end products.

The planting season of industrial hemp in the Northern hemisphere is in spring, from the second half of April [11] to mid-May [12]. These sowing times allow the period of vegetative growth to coincide with the optimal growing temperatures and longest days necessary to delay flowering and maximize stem growth [13]. Earlier or later plantings in the Northern hemisphere limit crop growth and yield, mainly in response to low temperatures, inadequate solar radiation, and short-day lengths [11]. The crop response in the field is the result of an interaction between genotype, environment, and management, with plant density, mineral nutrition, and irrigation regime being the main factors involved in the final yield and its quality [14]. Cannabis plants grow well under temperate conditions. Rapid growth is achieved on well-drained, fertile, medium-heavy soils and the crop should be established in a well worked seedbed free of compaction. Early flowering can be related to a reduction in final yield because, once flowering occurs, the plant responds by suppressing its vegetative development, thereby ending plant (stem) elongation [12]. When hemp crops are healthy and under no limiting factors (water or nutrients), biomass production is directly related to the amount of light received by the crop [15].

Agriculturally, hemp is a relatively high-yielding crop, with low or no pesticide requirement, and modest demands for fertilizer. Due to these features, its introduction into the intensive Mediterranean farming systems could be constitute a long-term strategy in maintaining farming systems and practices that are particularly favorable to environmental and climate policy goals [8].

Based on the above mentioned, the aim of the study was the evaluation of the productivity of six well adapted industrial hemp varieties, registered in the European Catalogue and commercially available to farmers, in northern Greece under Mediterranean conditions. This study provides agronomic and productivity data for industrial hemp in order to incorporate its cultivation into Mediterranean farming systems and practices. In European countries such as Greece, the interruption of hemp production happened in the second half of the last century and it was only in 2016, that the reintroduction of hemp cultivation was decided with almost no background knowledge and production evaluation. There is thus, a need to compare under a particular environment, varieties of different origin, with different characteristics and establish optimal local agronomic practices for industrial hemp production.

2. Materials and Methods

2.1. Experimental Design and Plant Materials

A field experiment was conducted in 2016 (yr 1) and was repeated in 2017 (yr 2) and 2018 (yr 3) at the fields of the Plant Breeding and Genetic Resources Institute (Hellenic Agricultural Organization—DEMETER) in Thessaloniki, northern Greece (longitude

230°00′13″ E, latitude 400°32′12″ N, elevation 0–1 m). The experiment was established in a sandy loam soil (Typic Xeropsamment), common in alluvial plains [16] like Thermi, Thessaloniki, with clay at 192 g kg^{-1}, silt at 336 g kg^{-1}, sand at 472 g kg^{-1}, organic C content at 0.4%, $CaCO_3$ at 1.5%, pH (1:2 H_2O) at 7.7 and electrical conductivity at 0.275 dS m^{-1}. Pre-seeding soil analysis (at 0–30 cm soil depth) indicated that the initial nitrate, phosphorus (Olsen) and potassium contents were 4.15, 2.81 and 71.0 mg kg^{-1}, respectively. The previous crop was durum wheat harvested in mid-June. Wheat straw was removed as bales and then the experimental area was ploughed.

A randomized complete block design was employed, with six monoecious hemp varieties replicated four times. The evaluated varieties were originated from France (vars. Santhica 27, Fedora 17, Felina 32 and Futura 75; supplied by the Cooperative Centrale des Producteurs de Semensces de Chanvre) or from Poland (vars. Tygra and Bialobrzeskie; supplied by the Institute of Natural Fibers and Medicinal Plants). These varieties were selected not only for their origin and availability but also on the basis of their THC content (as required by EC regulation) and of their growing period (early to late) [17]. The hemp varieties were sown by hand on 18 May 2016, 3 April 2017 and 12 April 2018. The seed rate was 50 kg ha^{-1} [8], reflecting also the farmers' practices for fibre and seed production. The same seed lot of each variety was used for all the years and the germination percentages of the seeds were >85%, measured annually, as described by [18]. Nitrogen, phosphorus and potassium at 22, 12 and 24 kg ha^{-1}, respectively, were broadcast applied 2 days before hemp sowing, according to the soil analysis and the conventional crop nutrition recommendations, as reported by [1]. The experimental area was cultivated with a rotavator to prepare the hemp seedbed and to incorporate the fertilisers. Nitrogen at 60 kg ha^{-1} was also applied manually, as ammonium nitrate at 40 days after seeding (DAS) and incorporated into the soil the same day using rakes. Plot size was 8 × 2.5 m including 14 rows of hemp with a distance between rows of 17 cm. Alleys of 2 m wide were used for plots separation. The experimental area was sprinkle-irrigated five times during each growing period. No pesticides were applied during crop establishment and growth, while the emerged weed species were hand-removed, as needed.

2.2. Growth Conditions

The climate in the experimental area is characterized as the typical Mediterranean with warm dry summer and cool humid winter. The mean monthly temperature and total monthly rainfall data recorded near the experimental area (over a distance of approximately 1000 m) are shown in Figure 1a. The Growing Degree Days (GDD) for the crop was calculated as [11]:

$$GDD = \int \{[(Tmax + Tmin)/2] - Tbase\}dt \quad (1)$$

where Tmax and Tmin were the daily maximum and minimum temperature, respectively. The base temperature for hemp (Tbase) was equal to 0 °C for the period between sowing and emergence, while it was equal to 2 °C during the hemp growing period. The total water supply (rainfall plus irrigation) and the GDD are shown in Figure 1b.

2.3. Hemp Development

The plant density was evaluated 4 weeks after emergence in a representative marked square meter in the centre of each plot. The whole hemp plants were hand-harvesting by hand trimmer and the number of plants were counted. Plant height and diameter of the main stem of the six varieties were also recorded at the male flowering (growing stage code 2303 to 2304) [19]. All the varieties reached flowering almost at the same period.

2.4. Hemp Fibre and Seed Yields

At harvest for fibres (growing stage code 2305, beginning of seed maturity), all hemp plants growing in two of the centre 8 m long rows of each plot were hand-harvested leaving stubble of about 3–5 cm. Harvest dates were 30 August 2016, 12 July 2017 and 16 July

2018. The total fresh biomass of hemp plants was determined in each plot. Afterwards, the hemp stems were prepared by cutting away the top part of the plant containing panicle and leaves. The stem weight was recorded and hemp stalk samples (of one kg each) were water retted for 7 days in bulk with clean water, according to the method reported by [20]. After retting the fibres separated manually from the core and then allowed to dry in ambient temperature. The obtained air-dried fibres was weighed and fibre content (Fc) was calculated by the equation:

$$Fc\ (\%) = (DW_{fibre} \times 100)/DW_{total} \quad (2)$$

where DW_{fibre} was the weight of the dried fibres and DW_{total} was the total dried weight (fibres plus shives). The fibre yield for each replicated was calculated by multiplying the stem yield by the fibre content.

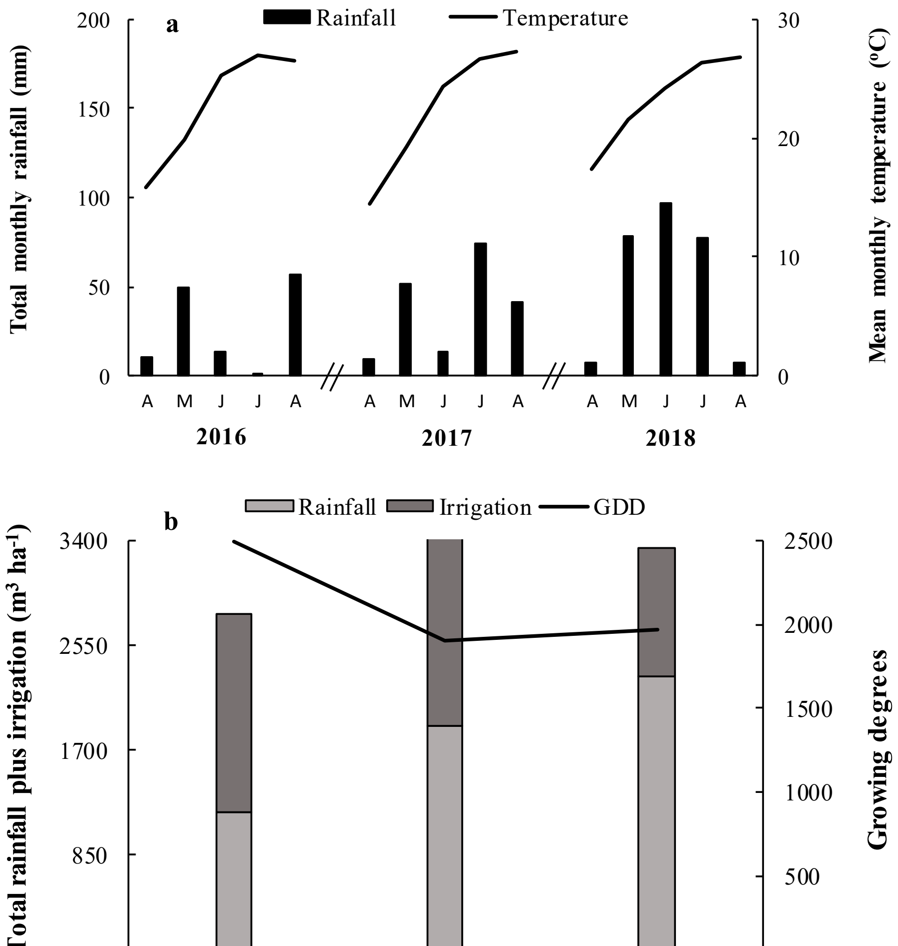

Figure 1. Total monthly rainfall and mean monthly temperature (**a**) and total water inputs and Growth Degree Days (**b**) during the three hemp growing seasons.

At the end of seed maturity (growing stage code 2307), hemp plants growing in two of the centre 8 m long rows of each plot were harvested and air-dried. Harvest dates were 23 September 2016, 22 August 2017 or 20 August 2018. The seeds were separated from the leaves and stems with shivers and an aspirator (model Selecta Zig Zag, PETKUS Selecta, Hem, The Netherlands), weighted and the 1000-seed weight was evaluated.

2.5. Fibre Physical Parameters

The physical parameters indicating the quality of the hemp fibres were evaluated at the Upyte Experimental Station of Lithuanian Research Centre of Agriculture and Forestry according to the methodology reported by [20,21]. Especially, the flexibility (in mm) of ten fibre samples for each replicate sample was evaluated by a G-2 device (VNIIL—All-Russian Flax Research Institute, Torzhok, Russia) and the fibre strength n newton (N) was evaluated by a dynamometer (model DKV-60, CNII—Central Research Institute, St. Petersburg, Russia). For each replicate, the means of the ten measurements were used for the statistical analysis.

2.6. Cannabinoid Analysis

The Δ9-tetrahydrocannabinol (THC) and the cannabidiol (CBD) concentrations of hemp spikes of 50 randomly selected plants from each plot were determined by gas chromatography according to EC regulation (No 809/2014) [5]. Dried powdered plant material (0.1 g) was extracted twice in a sonication bath with 5 mL hexane containing 0.035% *w*/*v* squalene at 20 min, followed by centrifugation at 3000 rpm for 5 min. The obtained extract has been passed through a 0.22 µm PTFE syringe filter before GC analysis. A TRACE™ Gas Chromatograph (Thermo Scientific, Fitchburg, MA, USA, formerly Thermo Electron, Milan, Italy) equipped with an AS3000 Autosampler and a Flame Ionisation Detector (FID) was used for the analyses. The Rtx-5, RESTEK non-polar capillary column with 5% diphenyl, 95% dimethylpolysiloxane (30 m length, 0.25 mm inner diameter, and 0.25 µm film thickness) was used. The carrier gas was helium at a constant flow rate (1.0 mL min^{-1}). Samples (1 µL) were injected into the column in split mode (1:10). Analysis was performed using a column temperature program that began 120 °C for 5 min, which was then increased to 300 °C at a rate of 20 °C min^{-1} and then held constant for a further 4 min. The injection port and detector (FID) temperature was 300 °C. The total analysis time was 20 min.

The ChromQuest V4.0 (Thermo Scientific, Fitchburg, MA, USA, formerly Thermo Electron Corporation) software package for GC-FID was used for equipment control, data acquisition and chromatogram analysis. The concentrations of THC and CBD were quantified using calibration curves obtained by graphing the ratio of the peak area of the standards to the peak area of the internal standard (squalene) versus the standard concentration [22].

2.7. Statistical Analyses

All the measured hemp parameters (plant density, stem height and thickness, fresh biomass, yield components, physical and chemical parameters) were analysed by a combined-across year analysis of variance (ANOVA). Bartlett's test was used for the examination of the homogeneity of variances, while the MSTAT program [23] was used to conduct the ANOVAs. The Fisher's Least Significant Difference test procedures were used to detect and separate mean treatment differences at $p = 0.05$.

Linear regression analyses were also conducted by the SPSS program [24] to determine the relationship between hemp fibre yield or seed yield with the hemp plant density stem height, stem thickness, total biomass, stem weight, fibre content, fibre flexibility or fibre strength.

3. Results

3.1. Growth Conditions

In yr 1, the growing degrees days (GDD) of the hemp growing period were greater (2492.5) than those in yr 2 (1898.3) or yr 3 (1965.5) (Figure 1b). The rainfall recorded from May-to July in yr 1 was lower (63.1 mm) than that recorded during the same period in yr 2 (139.2 mm) or yr 3 (251.8 mm) (Figure 1a). The total water (rainfall plus irrigation) applied on hemp varieties during the growing seasons in yr 1, yr 2 and yr 3 was 280 mm, 399 mm and 334 mm, respectively (Figure 1b).

3.2. Hemp Development

The conducted ANOVAs indicated that the plant density, height and main stem diameter in most cases were affected by year ($p < 0.001$), hemp variety ($p < 0.001$) and their interaction ($p < 0.001$). The year × hemp variety interaction means are presented in Table 1 and Figure 2. In the same figures, the variety main effect is also presented to facilitate varieties comparison.

Table 1. Hemp plant density (plants m^{-2}) at 4 weeks after seeding of six industrial hemp varieties.

Hemp Variety	Hemp Plant m^{-2}			
	Yr 1	**Yr 2**	**Yr 3**	**Over Year**
Santhica 27	110	148	90	116
Futura 75	68	78	98	81
Felina 32	66	106	135	102
Tygra	72	112	118	101
Bialobrzeskie	115	152	158	142
Fedora 17	63	177	128	123
$LSD_{0.05}$		33		19
Over variety	82	129	121	
$LSD_{0.05}$		12		

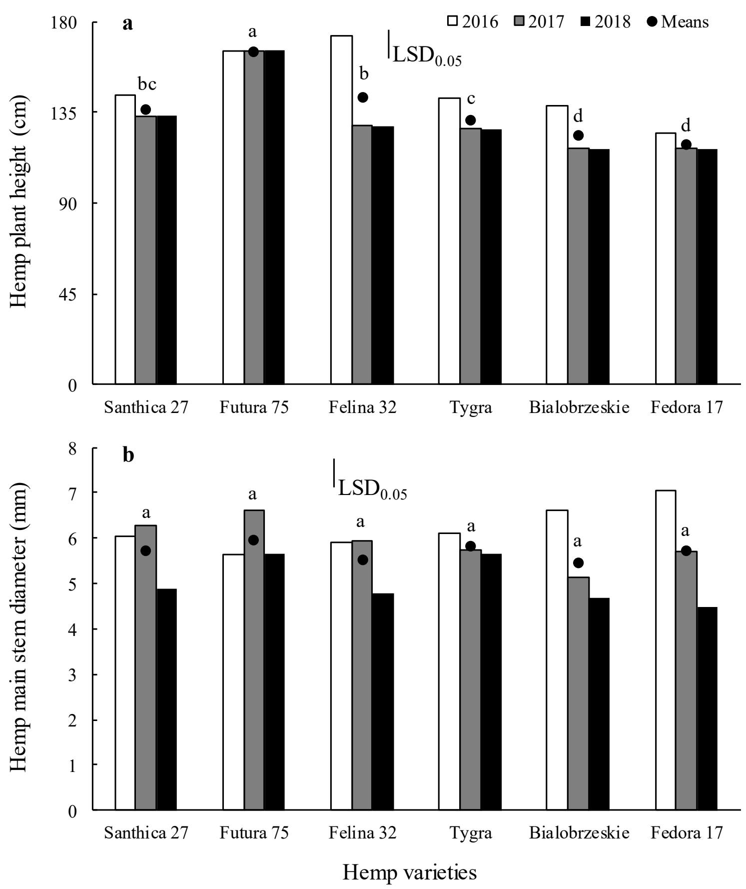

Figure 2. Stem height (**a**) and diameter (**b**) of six industrial hemp varieties. For the year × variety interaction, columns with height differences lower than the LSD height did not significantly differ according to the Fisher's Protected Least Significant Difference (LSD) test at $p = 0.05$. For the variety effect, points with the same letter did not significantly differ according to the Fisher's Protected Least Significant Difference test at $p = 0.05$.

For each year, the beginning of hemp germination was observed at 8 DAS. A few days later the hemp had fully emerged and the seedlings exhibited the first pair of the true leaves. Visually no differences among seedlings of the six varieties were noticed. In yr 1, the vars. Santhica 27 and Bialobrzeskie emerged in greater density (110 and 115 plants m^{-2}, respectively) than the other varieties (Table 1). In yr 2, the var. Fedora 17 provided the greatest emergence (177 plants m^{-2}), while the vars. Santhica 27 and Bialobrzeskie emerged in slightly lower density. However, in yr 3, the vars. Felina 32, Bialobrzeskie and Fedora 17 emerged in greater density (135, 158 and 128 plants m^{-2}, respectively) than the other varieties. Averaged across years, the var. Futura 75 provided the lowest emergence (81 plants m^{-2}), while the var. Bialobrzeskie the greatest (141 plants m^{-2}).

Averaged across years, the height of the var. Futura 75 was greater (165 cm) than that of the other varieties (Figure 2a). In yr 1, the height of hemp varieties was in most cases slightly greater than that recorded in yr 2 and yr 3 (Table 2), with the exception of var. Felina 32, for which the height in yr 1 was significantly greater (34%) than its height in yr 2 and yr 3.

Table 2. Year main effects for the hemp characteristics evaluated during the experiment.

Year Main Effect						
	Plant Height * (cm)	**Stem Diameter (mm)**	**Biomass (t ha^{-1})**	**Stem Yield (t ha^{-1})**	**Fibres (%)**	**Fibre Strength (N)**
Year 1	147.9 a	6.2 a	8.25 a	25.27 a	24.63 b	11.04 c
Year 2	131.2 b	5.9 a	1.83 c	6.85 c	26.02 a	12.84 a
Year 3	131.2 b	5.0 b	3.39 b	11.58 b	24.41 b	11.96 b
	Fibre Flexibility (mm)	**Fibre Yield (t ha^{-1})**	**Seed Yield (t ha^{-1})**	**1000-seeds Weight (g)**	**THC (%)**	**CBD (%)**
Year 1	27.88 a	6.22 a	2.70 a	6.38 b	0.133 a	1.165 a
Year 2	26.43 a	1.78 c	2.32 a	6.17 b	0.101 b	1.137 b
Year 3	27.35 a	2.79 b	2.60 a	8.99 a	0.103 b	0.870 c

* In each characteristic, values followed by the same letter did not significantly differ according to the Fisher's Protected Least Significant Difference test at $p = 0.05$.

The diameter of the main stem of the six hemp varieties ranged from 4.49 mm to 7.05 mm (Figure 2b). For most hemp varieties, the stem diameter was lower in yr 3 than in yr 1 and yr 2. In yr 1, the greatest stem diameter was observed in the vars. Fedora 17 and Bialobrzeskie. However, in yr 2, the greatest stem diameter was observed for the vars. Futura 75 and Santhica 27, while in yr 3 the greatest stem diameter was provided by the vars. Futura 75 and Tygra. Averaged across years, little differences were recorded among hemp varieties regarding the main stem diameter (Table 2).

3.3. Hemp Fibre and Seed Yields

According to ANOVA results, fibre content was significantly affected by variety ($p < 0.001$), while fresh biomass, stem yield and fibre yield were affected by year ($p < 0.001$), hemp variety ($p < 0.001$) and their interaction ($p < 0.001$). Hemp seed yield was affected by hemp variety ($p < 0.05$) and year × variety interaction ($p < 0.001$), while the 1000 seeds yield was affected by year ($p < 0.001$), hemp variety ($p < 0.001$) and their interaction ($p < 0.001$). So, the year × hemp variety interaction means are presented in Figures 4–6, while variety main effect is also presented to facilitate varieties comparison.

In yr 1, all hemp varieties produced greater total fresh biomass (Table 2) and stem yield than those provided in yr 2 and yr 3 (Figure 3). In yr 1, the greatest total biomass was provided by the vars. Bialobrzeskie (106.5 t ha^{-1}) followed by Felina 32 (97.5 t ha^{-1}) (Figure 3a). In yr 2, no significant total biomass differences were observed among hemp varieties, while in yr 3 the greatest total biomass was provided by the var. Futura 75 (46.5 t ha^{-1}). Averaged across years, the lowest total biomass was observed in the var.

Santhica 27 (35.0 t ha^{-1}). In yr 1, the greatest stem yield was provided by the var. Bialobrzeskie (35.0 t ha^{-1}), while in yr 3 the greatest stem weight was provided by the var. Futura 75 (19.8 t ha^{-1}) (Figure 3b). Averaged across years, the vars. Futura 75 followed by Bialobrzeskie achieved the greater stem yield (18.6 and 16.6 t ha^{-1}, respectively) than the other varieties.

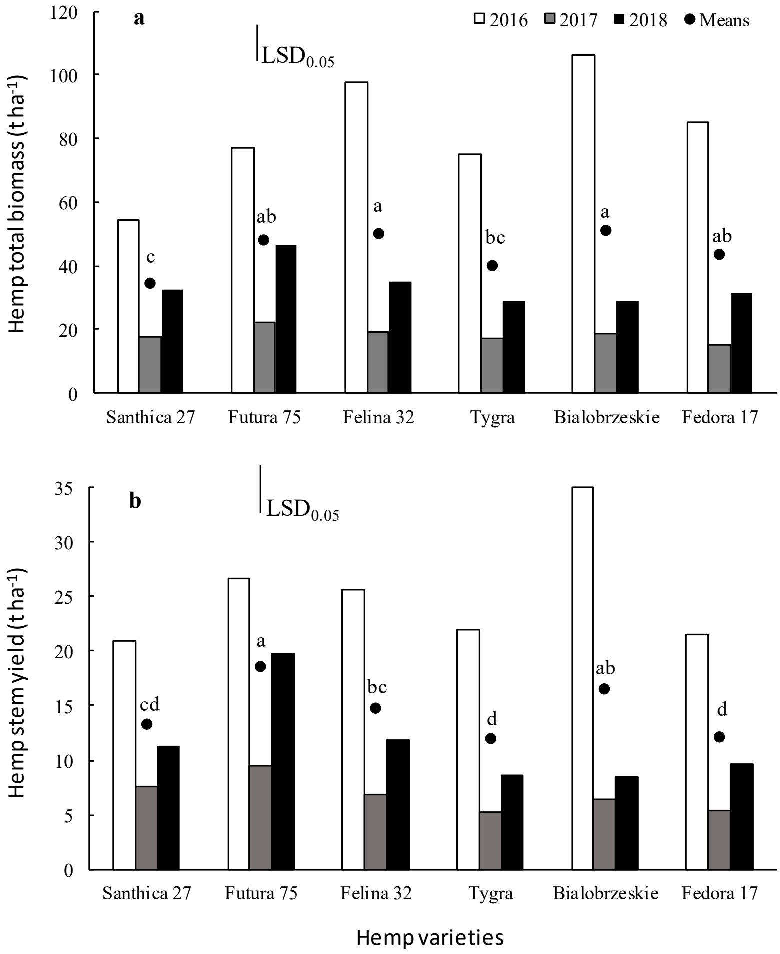

Figure 3. Total (**a**) and stem (**b**) biomass of six industrial hemp varieties. For the year × variety interaction, columns with height differences lower than the LSD height did not significantly differ according to the Fisher's Protected Least Significant Difference (LSD) test at $p = 0.05$. For the variety effect, points with the same letter did not significantly differ according to the Fisher's Protected Least Significant Difference test at $p = 0.05$.

Regarding the hemp fibre content, no significant differences were observed among years for each variety (Figure 4a). Averaged across years, the greatest fibre content was observed for the vars. Santhica 27 (27.3%), Tygra (26.5%) and Bialobrzeskie (27.1%). Regarding the hemp yield in fibres, in yr 1, the greatest fibre yield was observed in the vars. Futura 75 (6.78 t ha^{-1}) and Bialobrzeskie (8.65 t ha^{-1}) (Figure 4b). In yr 2, no differences were observed among hemp varieties regarding the fibre yield, while in yr 3 the greatest fibre yield was provided by the vars. Futura 75 (4.53 t ha^{-1}) and Santhica 27 (2.90 t ha^{-1}). Averaged across years, the greatest fibre yield was provided by the vars. Futura 75 (4.57 t ha^{-1}) and Bialobrzeskie (4.28 t ha^{-1}). Hemp fibre yield was strongly positively correlated with total biomass and stem yield (Figure 5a,b), while it was negatively correlated with fibre strength (Figure 5h).

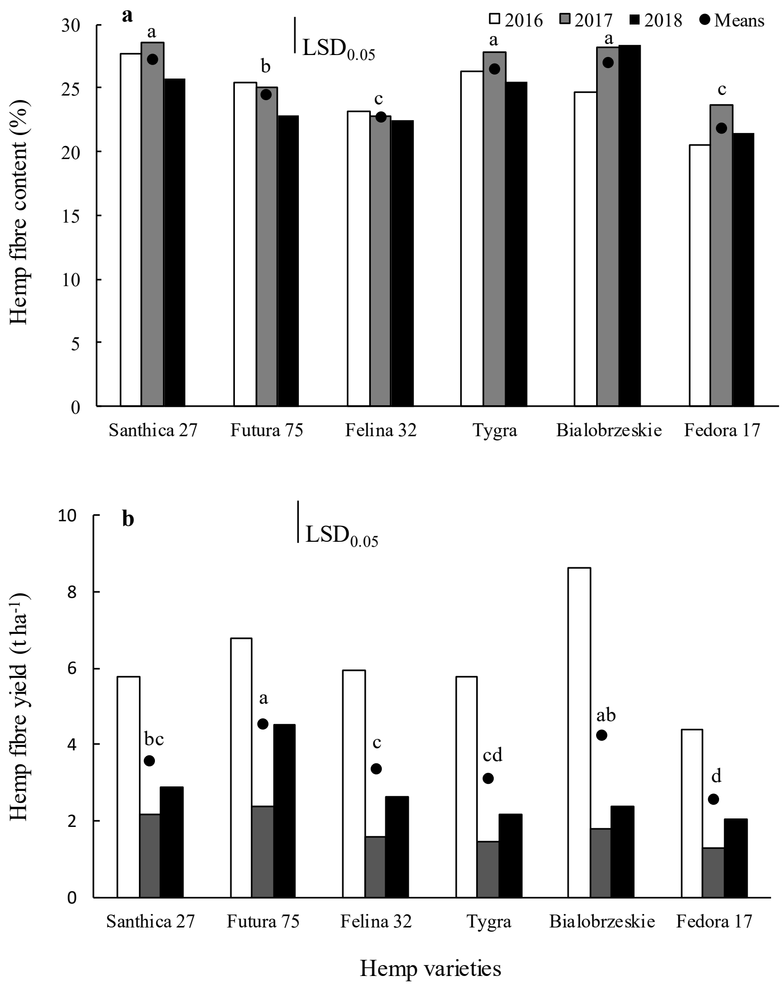

Figure 4. Fibre percentage (**a**) and fibre yield (**b**) of six industrial hemp varieties. For the year × variety interaction, columns with height differences lower than the LSD height did not significantly differ according to the Fisher's Protected Least Significant Difference (LSD) test at $p = 0.05$. For the variety effect, points with the same letter did not significantly differ according to the Fisher's Protected Least Significant Difference test at $p = 0.05$.

Average across years, the vars. Tygra, Santhica 27 and Bialobrzeskie provided slightly greater seed yield (2.9, 2.7 and 2.5 t ha^{-1}, respectively) than the other varieties (Figure 6a). It is worth to notice that, in yr 1, the greatest seed yield was provided by the var. Tygra (3.99 t ha^{-1}), while the lowest by the var. Fedora 17 (1.79 t ha^{-1}). In yr 2, the var. Santhica 27 provided greater seed yield than the other varieties, except for the var. Fedora 17, while in yr 3 the var. Futura 75 provided slightly lower seed yield (2.11 t ha^{-1}) than the other varieties. All varieties had a greater 1000-seed weight in yr 3 than in yr 1 and yr 2 (Figure 6b). The var. Fedora 17 provided the greatest 1000-seed weight (10.9 g). Averaged across years, the vars. Futura 75 and Fedora 17 provided greater 1000-seed weight (7.9 and 8.3 g, respectively) than the vars. Santhica 27 (6.4 g) and Bialobrzeskie (6.1 g). Hemp seed yield was not significantly correlated with the other hemp characteristics (Figure 7).

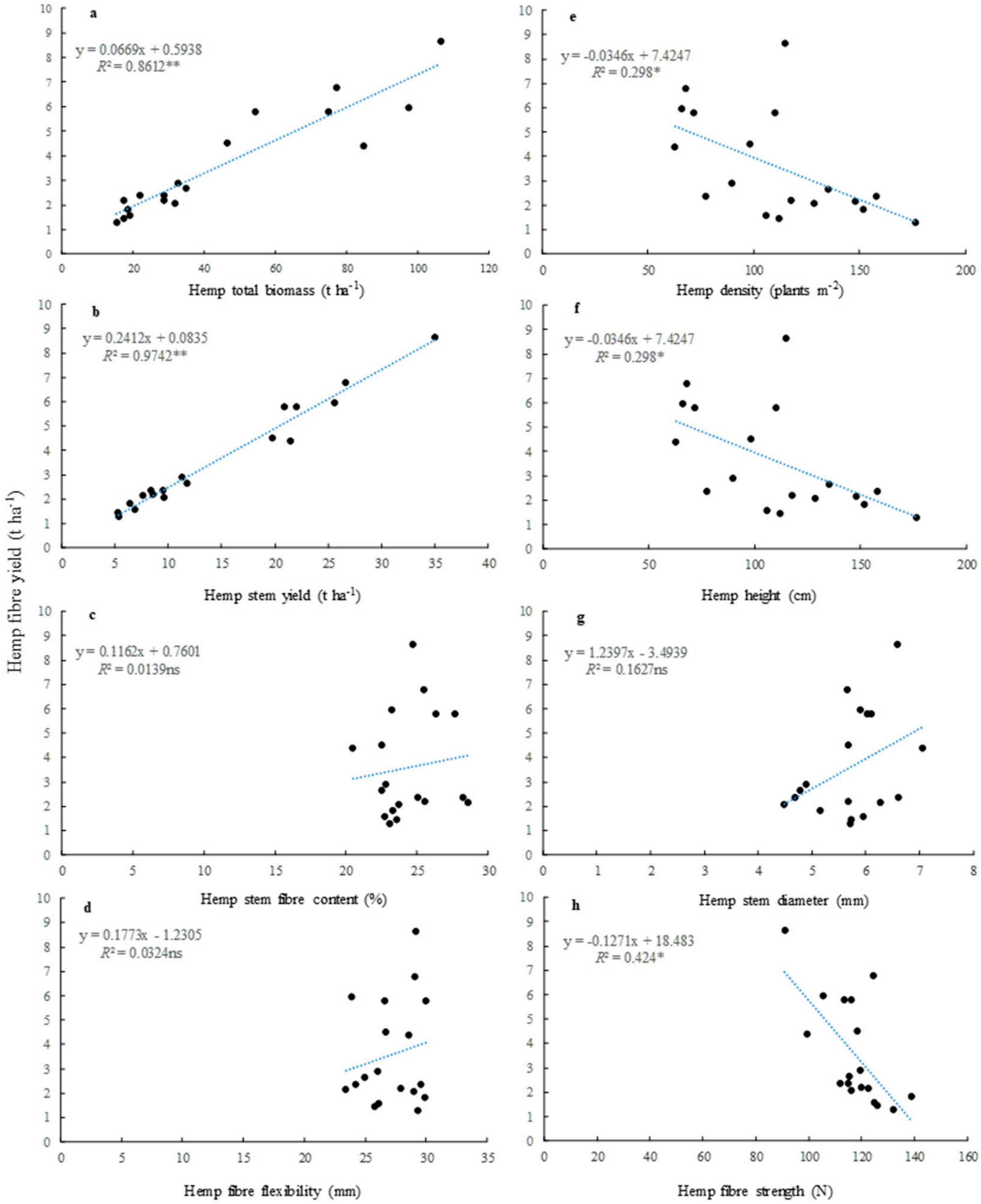

Figure 5. Correlations (linear regressions) between hemp fibre yield and (**a**) total biomass, (**b**) stem yield, (**c**) stem fibre content, (**d**) fibre flexibility, (**e**) plant density, (**f**) height, (**g**) stem diameter and (**h**) fibre strength of six hemp varieties. * $p < 0.05$, ** $p < 0.01$ and ns = no significance difference.

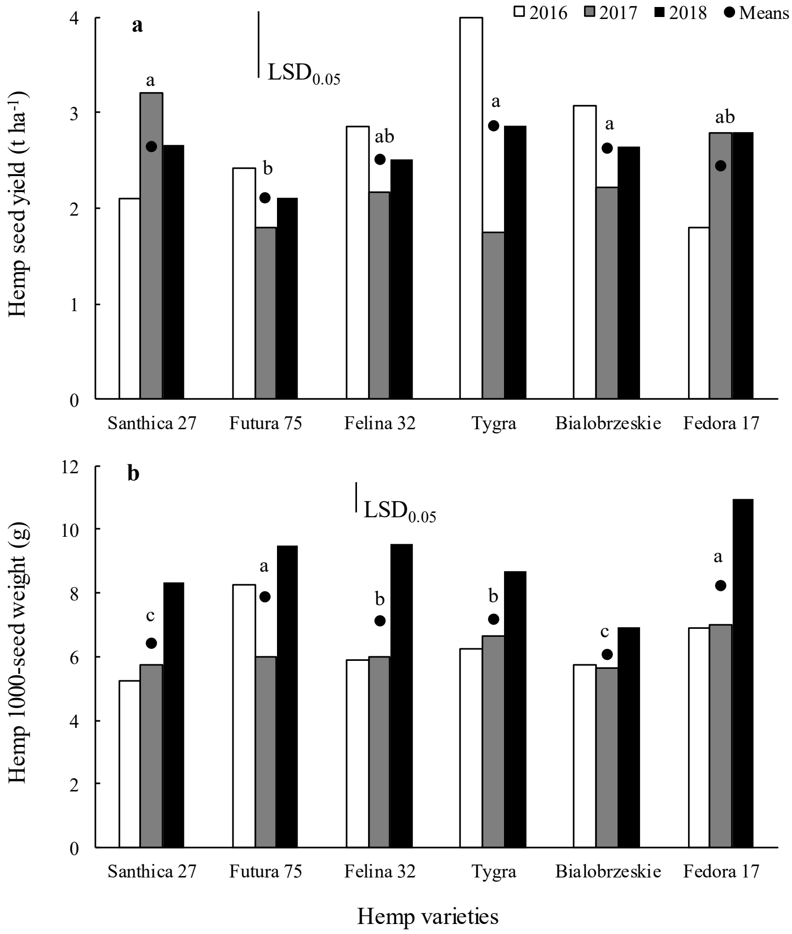

Figure 6. Seed yield (**a**) and 1000-seed weight (**b**) of six industrial hemp varieties. For the year × variety interaction, columns with height differences lower than the LSD height did not significantly differ according to the Fisher's Protected Least Significant Difference (LSD) test at p = 0.05. For the variety effect, points with the same letter did not significantly differ according to the Fisher's Protected Least Significant Difference test at p = 0.05.

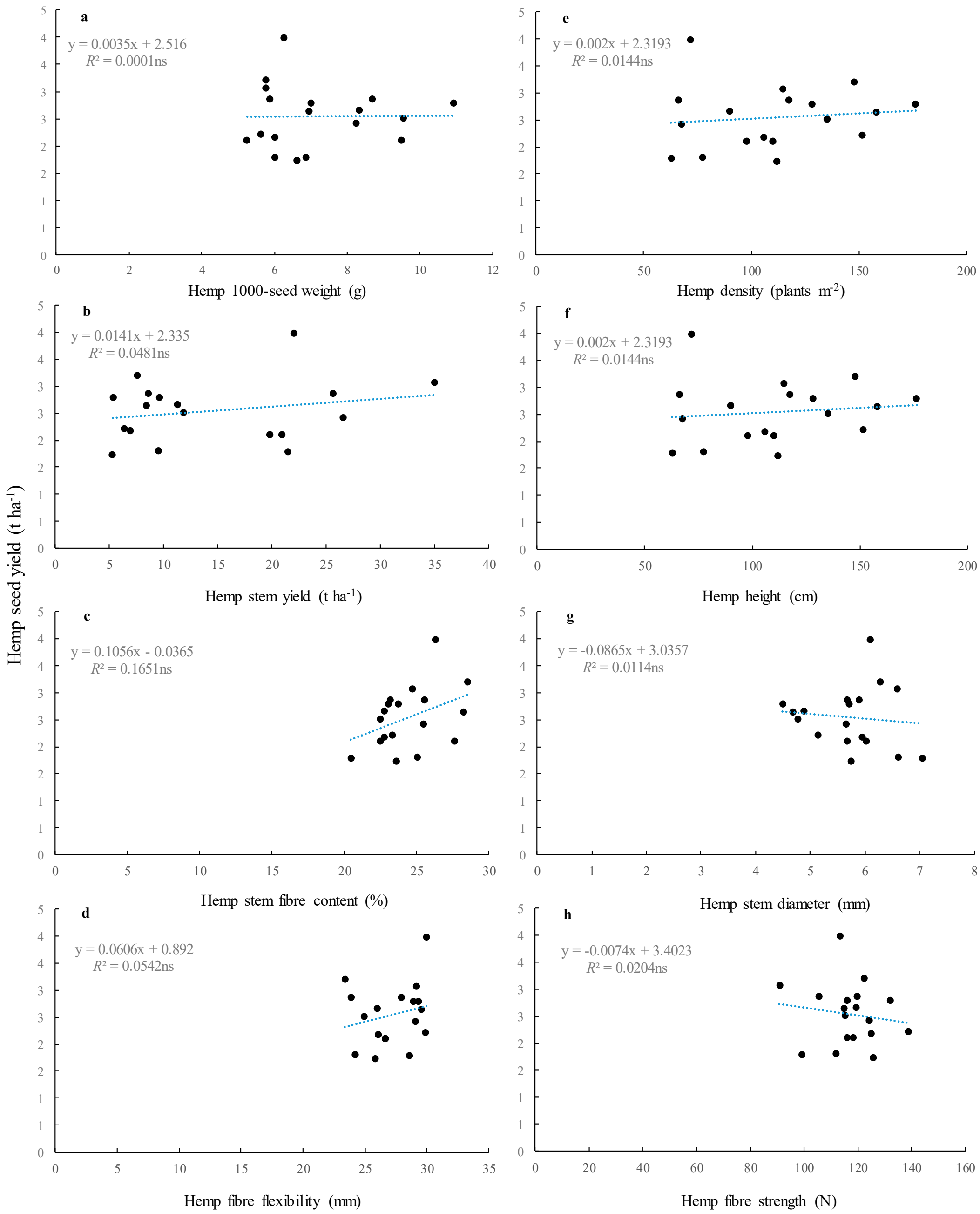

Figure 7. Correlations (linear regressions) between hemp seed yield and (**a**) 1000-seed weight, (**b**) stem yield, (**c**) stem fibre content, (**d**) fibre flexibility, (**e**) plant density, (**f**) height, (**g**) stem diameter and (**h**) fibre strength of six hemp varieties. ns = no significance difference.

3.4. Fibre Physical Parameters

The flexibility of hemp fibres was affected by hemp variety ($p < 0.001$), while the fibre strength was affected by year ($p < 0.001$) and year × variety interaction ($p < 0.001$). In particular, the fibre flexibility of the vars. Bialobrzeskie and Fedora 17 was greater (29.5 and 28.9 mm, respectively) than that of the vars. Santhica 27 (25.4 mm) and Felina 32 (25 mm) (Figure 8a). For most varieties, the fibre strength was slightly lower in yr 1 than in yr 2 and yr 3 (Figure 8b). Averaged across years, no differences were observed among varieties regarding the fibre strength which ranged from 114.9 N to 119.7 N.

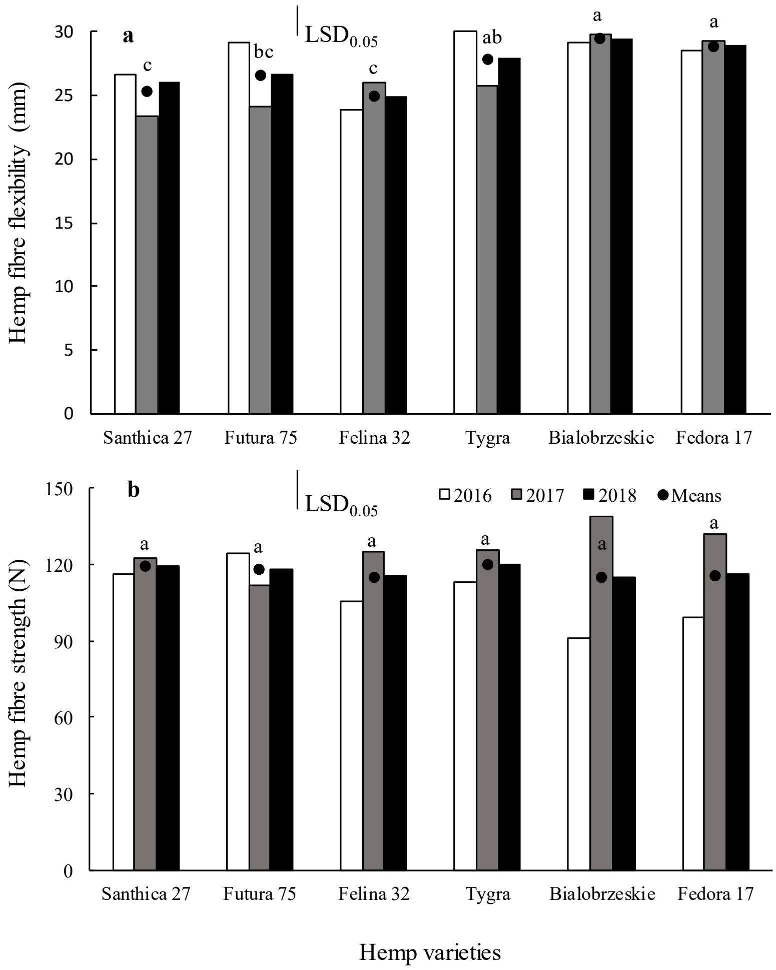

Figure 8. Fibre flexibility (**a**) and fibre strength (**b**) of six industrial hemp varieties. For the year × variety interaction, columns with height differences lower than the LSD height did not significantly differ according to the Fisher's Protected Least Significant Difference test at $p = 0.05$. For the variety effect, points with the same letter did not significantly differ according to the Fisher's Protected Least Significant Difference test at $p = 0.05$.

3.5. Cannabinoid Content

Both THC and CBD contents were significantly affected by year ($p < 0.001$), hemp variety ($p < 0.001$) and their interaction ($p < 0.001$). So, the year × hemp variety interaction means are presented in Figure 9 facilitating varieties comparison by the variety main effect presented. All hemp varieties had THC content lower than 0.2%, which is the highest allowable limit according to the European Regulation (Figure 9a). In all years, the var. Santhica 27 indicated the lowest THC (0%) and CBD (0.023%) contents (Figure 9). Average across years, the var. Tygra had the highest THC content (0.172%) (Figure 9a), while the var. Futura 75 had the highest CBD content (1.464%) (Figure 9b). For the vars. Futura 75, Felina 32 and Fedora 17, the lowest THC content was observed in yr 3, while for the vars. Tygra and Bialobrzeskie in yr 2.

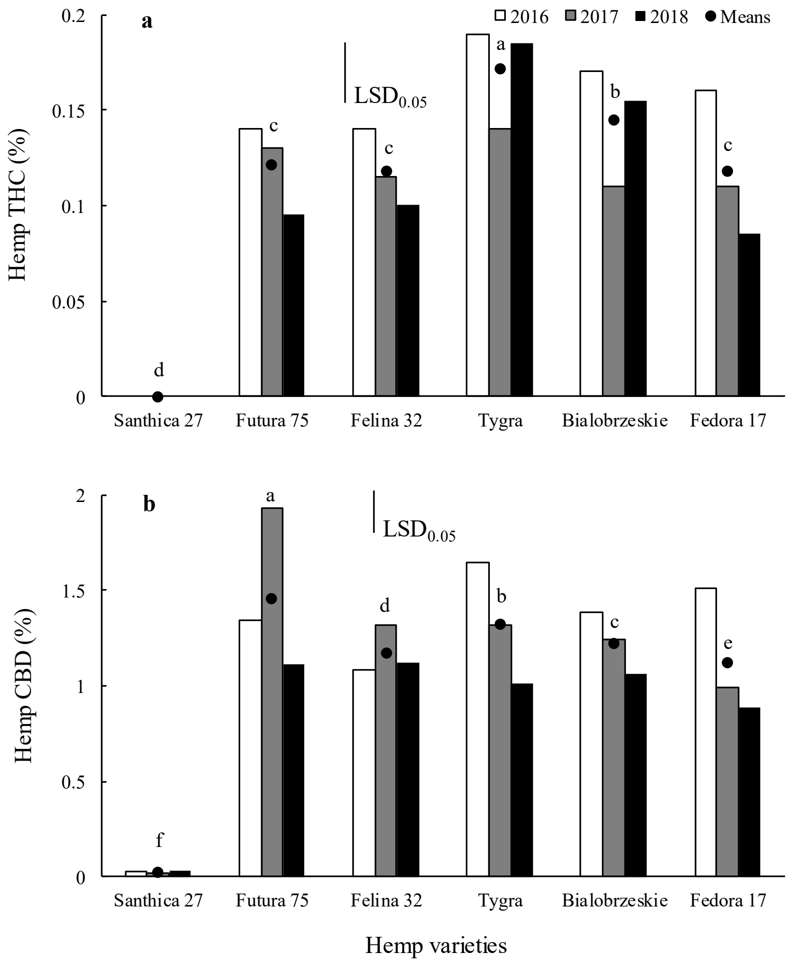

Figure 9. Tetrahydrocannabinol (THC) (**a**) and cannabidiol (CBD) (**b**) content of six industrial hemp varieties. For the year × variety interaction, columns with height differences lower than the LSD height did not significantly differ according to the Fisher's Protected Least Significant Difference (LSD) test at $p = 0.05$. For the variety effect, points with the same letter did not significantly differ according to the Fisher's Protected Least Significant Difference test at $p = 0.05$.

4. Discussion

4.1. Growth Conditions

The total water input received by the six hemp varieties in the 3-year experiment ranged from 280 mm to 330 mm. Early genotypes of hemp monoecious varieties cultivated for fibre production needed about 250 mm of water, as reported by [12].

The duration from sowing to flowering of the hemp varieties ranged from 95 days (yr 3) to 104 (yr 1) days requiring a heat quantity which ranged from 1966 GDD to 2493 GDD. Ref. [3] found that the hemp varieties in Italy had growth duration from 55 (earliest variety) to 107 (latest variety) days. The lower GDD for hemp varieties recorded in yr 2 and yr 3, as compared with those in yr 1, could be attributed to earlier sowing in yr 2 and yr 3 which resulted in hemp growth occurring at lower temperatures. Industrial hemp plants required a total heat quantity over the growing period of 1900 to 2000 GDD from germination to technical maturity [14]. In Italy, [1] also found that the total heat quantity over the growing period of hemp ranged from 2459 GDD to 3328 GDD.

4.2. Hemp Development

Industrial hemp is usually sown at high seed density to obtain a higher competitive ability against weeds. Additionally, plant density significantly affects stem biometrics and fibre quality because hemp plants grown at high densities are usually taller with thinner stems as compared with plants grown at low densities [25]. The lower hemp plant density recorded for most varieties in yr 1 compared to those in yr 2 and yr 3 could be associated with the later date of sowing resulting in unfavourable emergence conditions for the hemp plants. The lower plant density in yr 1 could have contributed to the slightly greater plant height observed in yr 1. Ref. [26] found that the optimum temperature for hemp germination ranged from 27.3 °C to 29.8 °C, and that at greater temperatures hemp emergence was reducedThe tallest hemp plants were recorded at the lowest plant density and reached the reproductive stage later according to [7] findings. Similarly, the stem diameter was inversely correlated with plant density. However, in the current experiment, the differences in plant densities were not strongly correlated with differences in stem diameter. It is reported [3] that the tallest hemp variety (var. CS) had a height of 279 to 290 cm, while the shortest (var. Uso-31) had a height of 161 to 201 cm. Furthermore, [27], evaluating 14 hemp varieties, found that the stem diameter ranged from 3.9 mm to 6.3 and that, for the monoecious varieties, the fibre content did not strongly correlate with stem diameter. Ref. [28] found that the height of five monoecious varieties ranged from 179 cm to 201 cm, while their stem diameter ranged from 6.4 mm to 7.1 mm.

4.3. Hemp Fibre and Seed Yields

The average total biomass of the six varieties in yr 1 was 82.6 t ha^{-1} followed by those of 2018 (33.9 t ha^{-1}) and 2017 (18.3 t ha^{-1}). This greater hemp total biomass recorded in yr 1 might be explained by the slightly longer growing season in yr 1, as well as to the greater GDD received by the hemp varieties from seeding to maturity [28]. On the contrary, Ref. [8] found that the fibre and seed yields decreased when the hemp seeding date was postponed from April to June. High hemp total biomass has been reported under Mediterranean conditions (Greece) by [17], while the hemp stem yield provided in yr 2 and yr 3 in this study is in accordance with the results of [28] hemp cultivated under Mediterranean conditions in Italy where the total hemp biomass ranged from 28.82 t ha^{-1} to 31.21 t ha^{-1}. Ref. [9], evaluating 14 hemp varieties in four environments, found that the stem yield ranged from 3.7 t ha^{-1} to 22.7 t ha^{-1}. Ref. [7] reported hemp dry stem yield which ranged from 3.48 t ha^{-1} to 8.30 t ha^{-1}. The total biomass of six hemp varieties cultivated in Italy varied from 32.5 t ha^{-1} to 45.5 t ha^{-1} [29], indicating that the cultivation of varieties bred in cooler northern environments results in completely different productivity in southern environments. Ref. [30] found that, in Denmark, the stem and fibre yields ranged from 6.2 t ha^{-1} to 10.5 t ha^{-1} and 1.7 t ha^{-1} to 3.1 t ha^{-1}, respectively. Furthermore, [9] found, in Belgium, that the fibre yield of the vars. Fedora 17, Santhica 27 and Felina 32 ranged from 7.21 t ha^{-1} to 9.04 t ha^{-1}, 8.33 t ha^{-1} to 10.39 t ha^{-1} and 7.77 t ha^{-1} to 9.21 t ha^{-1}, respectively.

In this study hemp fibre content ranged from 20.5% to 28.6%. The vars. Santhica 27, Bialobrzeskie and Tygra provided the highest fibre content (27.3%, 27.1% and 26.5% respectively, as averaged across year). Similarly, [21] reported the fibre content of the var. Bialobrzeskie cultivated in Lithuania ranged from 25.1% to 30.8%. Ref. [10] found that the fibre content of 14 hemp varieties ranged from 21% to 43%. All hemp varieties provided greater fibre yield in yr 1 (ranged from 4.40 t ha^{-1} to 8.65 t ha^{-1}) than in yr 2 (ranged from 1.28 t ha^{-1} to 2.38 t ha^{-1}) and yr 3 (ranged from 2.06 t ha^{-1} to 4.53 t ha^{-1}) due to the greater biomass yield achieved in yr 1. It was also found that the stem yield of six hemp varieties ranged from 4.0 t ha^{-1} to 14.0 t ha^{-1}, and that the var. Bialobrzeskie was one of the most productive varieties [3]. Furthermore, [10] reported fibre yield which ranged from 1.3 t ha^{-1} to 7.4 t ha^{-1}.

Hemp fibre yield was strongly correlated with total and stem biomass, while it was inversely correlated with crop density, plant height and fibre strength. Similarly, Ref. [7] reported that hemp density negatively affected stem biomass, as well as plant height and stem diameter.

Averaged across years, the seed yield of the six hemp varieties ranged from 2.1 t ha^{-1} to 2.9 t ha^{-1}. Relatively few studies have provided information on the seed productivity of hemp cultivars in a wide range of European environments. Ref. [3] reported that the seed yield of the vars. Bialobrzeskie and Fedora ranged from 0.61 t ha^{-1} to 0.65 t ha^{-1} and from 0.92 t ha^{-1} to 1.00 t ha^{-1}, respectively. Ref. [10] also concluded that there was a wide range of seed productivity (0.3-to 2.4 t ha^{-1}) for 14 commercial hemp cultivars when they were compared in four contrasting environmental conditions in Europe and the highest seed yield (2.4 t ha^{-1}) and seed harvest index were found for the earliest maturing cultivar. Ref. [9] found that, in Belgium, the seed yield of the vars. Fedora 17, Santhica 27 and Felina 32 ranged from 0.29 t ha^{-1} to 1.75 t ha^{-1}, 0.19 t ha^{-1} to 1.56 t ha^{-1} and 0.26 t ha^{-1} to 1.75 t ha^{-1}, respectively. Ref. [30] found that the seed yield of hemp grown in Denmark ranged from 0.43 t ha^{-1} to 0.68 t ha^{-1}.

The fact that hemp seed yield was not correlated with the hemp plant density, height, total biomass or stem biomass indicates that seed yield maybe affected by other parameters. The hemp seed yield was negatively correlated with the growing period from emergence to full flowering, according to previous study [10]. In addition, the hemp fibre yield was not affected when the seeding rate increased from 16 kg ha^{-1} to 64 kg ha^{-1}, while the seed yield was decreased by about 25% as found by [30].

4.4. Fibre Physical Parameters

Averaged across years, little differences were recorded among the six hemp varieties regarding the fibre flexibility (ranged from 25 to 29.5 mm) and the fibre strength (ranged from 114.9 N to 119.7 N). Ref. [21] found that, in Lithuania, the flexibility of the var. Bialobrzeskie ranged from 20.4 mm to 29.9 mm, while its fibre strength ranged from 52.6 N to 139.3 N.

4.5. Cannabinoid Content

All hemp varieties tested had a THC content lower than 0.2% corresponding to the European requirements for industrial hemp varieties. This fact indicates that the six evaluated varieties are suitable for cultivation as industrial hemp varieties under Mediterranean conditions. Furthermore, [28] found that the THC content in the dust of hemp was not increased (because of possible accumulation) by the mechanical treatment of hemp stems and it was averaged 0.0386%. It is notable that var. Santhica 27 had zero THC, whereas var. Tygra the highest in all of the growing years studied. The var. Santhica 27, according to [31] has zero THC, because it was selected from a single plant with high cannabigerol (CBG) and no THC content.

The responses of the studied hemp varieties to growth conditions in northern Greece, was good with respect sense to CBD production. The varieties Futura 75 and Tygra had the highest CBD contents and the values in whole inflorescences were found to be comparable to reported values as [32] also observed. Furthermore, Santhica 27 was not found to be appropriate for CBD production since only trace amounts of CBD were detected [32].

The 0.2% THC content barrier has specifically proved to be a hindrance to European CBD producers because in hemp there is a close correlation between potential CBD: THC concentrations. In a previous study [33] the average potential CBD: THC concentrations was about 20:1. European scientists and researchers have therefore not been incentivized to develop the high-yield seed varieties and high-CBD strains, that are now in great demand, because if such strains accumulate CBD contents greater than 4%, THC content will exceed the 0.2% limit [33].

5. Conclusions

The six hemp genotypes evaluated in this study indicated that they could be adapted well to the Mediterranean environment, while their THC content was lower than 0.2% satisfying the European requirements for industrial hemp varieties. There was great variability in hemp productivity among the three years. However, under the Mediterranean conditions of northern Greece, the vars. Futura 75 and Bialobrzeskie showed the greatest fibre productivity, as averaged across years, while the vars. Santhica 27, Tygra and Bialobrzeskie shown the greatest seed yield. Many factors, including minimum and maximum temperature during the growing season, total water inputs and plant density, affect the productivity of each hemp variety. Therefore, since hemp yield is related to the choice of genotype it will be beneficial for hemp producers to choose hemp varieties suitable for dual-purpose production (stems and inflorescences or stems and seeds) and those which are best adapted best to their local environment.

By comparing the response of hemp varieties, that display differing characteristics, to the Mediterranean environment of Northern Greece, this study provides valuable information concerning the success of reintroducing hemp cultivation in Greece, as not many studies are yet available. Concerning further research, this could be focused on adding more genotypes in different experimentation areas and developing a strategic plan to identify the potential areas and optimum agronomic practices for hemp cultivation in Greece.

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