PLANT DENSITY AND NITROGEN FERTILIZATION AFFECT AGRONOMIC PERFORMANCE OF INDUSTRIAL HEMP (*Cannabis sativa* L.) IN MEDITERRANEAN ENVIRONMENT

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ABSTRACT

The renewed interest in industrial hemp (*Cannabis sativa* L.) is due to its large number of applications and for the wide range of agro-environmental conditions under which it can be cultivated. Two-year field experiments (2007 and 2008 growing seasons) were carried out in a Mediterranean environment of central Italy with the aim of assessing the impact of genotype, plant density and N fertilization on hemp yield, in terms of stems, inflorescences and seeds. The treatments consisted in: (a) seven genotypes (Epsilon68, Fedora17, Felina32, Ferimon, Futura75, Santhica27, and Uso31); three plant density (40, 80, and 120 plants m^{-2}); two N fertilization levels (50 and 100 kg of N ha^{-1}). Physiological parameters, plant height, stem weight and diameter, inflorescence yield, seed yield and the characteristics of hemp and weed aboveground biomass were recorded. High plant density resulted in shorter plant height compared with low plant density (-41%) as the hemp plants tended to reach the reproductive stage early at high density. At full flowering, stem yield ranged from 3.4 to 8.0 t ha^{-1} of dry matter and was positively correlated with the duration of vegetative phase, which tended to be high in the intermediate flowering genotypes (Epsilon68, Futura75 and Santhica27). Stem diameter was inversely correlated with plant density (6.7, 5.8 and 5.2 mm at 40, 80 and 120 plants m^{-2}, respectively). Conversely to stem yield, inflorescence and seed production proved to be higher in the early flowering genotypes (Fedora17, Felina32, Ferimon and Uso31) and increased as plant density increased. High N fertilization level had a positive impact on stems rather than inflorescence and seed yields (on average +28%, +17% and +4% in 100 kg of N ha^{-1} compared with 50 kg of N ha^{-1} fertilization level, respectively). Farmers should consider making a dual-purpose production of stems and inflorescences or stems and seeds, even if it is clear that yield is related to the choice of genotype. Further research should be carried out to find various genotypes as well as flexible agronomical practices that are able to improve both traditional (stems) and innovative (inflorescences and seeds) hemp yields under Mediterranean conditions.

KEYWORDS

Hemp; Stems; Inflorescences; Seeds

1. INTRODUCTION

Industrial hemp (*Cannabis sativa* L.) is an annual herbaceous crop of Asian origin considered to be one of the oldest crops known to man (Yang, 1991) and it is traditionally grown in many regions of Europe for its fiber production (Amaducci et al., 2015). The versatility of hemp lends itself to the development of numerous products that can be made from the high-quality cellulose contained in the stems, from valuable essential oils and resins found in the inflorescences and from the high-quality oil and proteins contained in the seeds (Carus et al., 2013). In fact, hemp can be used in numerous agro-industrial fields such as agriculture, textiles, bio-composites, papermaking, construction, bio-fuel, functional foods, personal care and cosmetics (Salentijn et al., 2015). Although industrial hemp can be used in a wide range of industrial applications, the growing popularity of synthetic fibers and the increase in labor costs are probably responsible for the continual drop in hemp cultivation worldwide since the 19th century (Allavena, 1962). Furthermore, in many States hemp production has been forbidden due to its δ-9-tetrahydrocannabinol (THC) content, which is a phyto-chemical drug component. From an agronomical point of view, hemp is considered to be a high-yielding crop that requires few technical inputs (Amaducci et al., 2015) and therefore does not negatively affect the environment (Finnan and Styles, 2013). Moreover, industrial hemp is an excellent break crop that can improve soil structure due to its extensive root system (Amaducci et al., 2008a), reduce weed pressure and increase the yield of the subsequent crop (Bosca and Karus, 1997). Due to its numerous crop characteristics, hemp has great potential as an alternative rotation crop and could improve the agronomic and economic sustainability of farmers (Finnan and Styles, 2013). Currently, industrial hemp is considered to be a niche crop cultivated on less than 15,000 ha in Europe (Carus et al., 2013). Consequently, it has not been subjected to the intensive agronomical and breeding programs for its development and improvement like other major crops during the last decades (Salentijn et al., 2015). Furthermore, some of the germoplasm of old hemp varieties has been lost and the development of suitable machinery for hemp cultivation, harvesting and processing has been halted (Cappelletto et al., 2001). The cultivation of appropriate genotypes for a specific end use adapted to a given environment is essential for achieving an advantageous hemp crop (Tang et al., 2016). Moreover, it is important to evaluate hemp phenology in order to provide farmers with decision support information concerning cultivation practices. In fact, hemp phenology is associated with photoperiod and different yields can be obtained in a specific environment due to the sensitivity of various genotypes (Amaducci et al., 2015).

Nowadays, environmental concerns and multi-purpose production have brought renewed interest in industrial hemp, however there is little agronomical information to support hemp cultivation (Tang et al., 2016). Besides hemp fiber production, there is growing interest in cultivating industrial hemp for other purposes such as using its inflorescence for extracting essential oils (Bertoli et al., 2010) and its seeds for alimentary oil and flour production (Mihoc et al., 2012). However, few studies have compared the performance of the current commercial genotypes of industrial hemp (Tang et al., 2016) and there is little available information regarding agronomical practices (Blade, 1998). Therefore, considering the lack of information on hemp genotypes, it is difficult for Mediterranean farmers to select the most suitable genotype for different kinds of utilization (Westerhuis et al., 2009). This study hypothesized that appropriate hemp genotypes and agronomical practices are required for obtaining the various products of hemp such as stems, inflorescences and seeds. The main aim of this study was to assess the impact of the combined effect of hemp genotypes, plant density and N fertilization on the yields of stems, inflorescences and seeds, under the Mediterranean agro-climatic conditions of central Italy. The specific objectives were: (1) to investigate the adaptability of different hemp genotypes under the Mediterranean conditions of central Italy; (2) to assess the range effect of plant density and N fertilization of stem, inflorescence and seed yield; (3) to assess the quality of hemp seeds in terms of oil and protein content.

2. MATERIALS AND METHODS

2.1.Site and experimental design description

Field experiments were carried out in the 2007 and 2008 growing seasons at the Experimental Farm "Nello Lupori" of the University of Tuscia in Viterbo (latitude 42°25' N, longitude 12°04' E and altitude 310 m a.s.l.). The climate of the experimental site located in Central Italy is typically Mediterranean with a mean annual temperature of 14.5°C, minimum and maximum temperatures observed in February and July respectively, and approximately 750 mm total annual rainfall which falls mainly in the period October – May. The soil is volcanic, classified as *Typic Xerofluvent* with the following average characteristics in the 0 – 30 cm soil layer: 10.4 % clay, 13.3 % silt, 76.3 % sand; pH 6.9 (water, 1:2.5); organic matter 1.3 % (Lotti methods); total N 0.94 g kg^{-1} of dry soil (Kjeldahl); available P_2O_5 33 mg kg^{-1}; and exchangeable K_2O 575 mg kg^{-1}.

The field trials were carried out in two adjacent fields on the same site previously cropped with durum wheat (*Triticum durum* Desf.). The following experimental treatments were applied: (i) seven hemp genotypes [Epsilon 68 (E68), Fedora 17 (F17), Felina 32 (F32), Ferimon Fe), Futura 75 (F75), Santhica 27 (S27) and Uso 31 (U31)]; (ii) three plant density (40, 80, and 120 plants m^{-2} of initial density); and (iii) two levels of N fertilization (50 and 100 kg of N ha^{-1}). For both years the

experimental design was a split-split-plot, where the main plots were represented by the hemp genotypes, the sub-plots were the plant density, and the sub-sub-plots were the N fertilization levels. The main plot size was 90 m^2 (9 m x 10 m), the subplot size was 30 m^2 (3 m x 10 m), and the sub-sub-plot size was 15 m^2 (3 m x 5 m). The treatments were replicated three times for a total of 126 plots for each year. The plot size adopted is the size commonly used when conducting hemp field trials (Bertoli et al., 2010; Cosentino et al., 2012; Hall et al., 2013). In both years, the main plots in the field trial were separated by a 5 m wide alleys for equipment operation during the experimentation.

2.2.Field experiment description

In the autumn of both years, following durum wheat harvesting, the soil was plowed in at depth of 30 cm and then left bare throughout the winter season by means of disc harrow cultivation whenever necessary, in order to eliminate weed seedlings. Two weeks before hemp sowing, the field was fertilized with 100 kg ha^{-1} of P_2O_5 as triple superphosphate. After applying the fertilizer, the soil was disked twice for seedbed preparation. Hemp genotypes were sown with an experimental sowing machine (Wintersteiger, Ried im Innkreis, Austria) on 28 March 2007 and on 10 April 2008 planting the seeds in rows at 0.5 m interrow spacing. A wider interrow (0.5 m) compared with common practice was adopted in the experiment in order to carry out N fertilization and mechanical weed control in the early stage of hemp growth. The hemp seed rate was 7, 14 and 28 kg ha^{-1}. Ten days after the emergence, the hemp seedlings were thinned manually in order to reach the target densities of 40, 80, and 120 plants m^{-2} (hereafter called D1, D2, and D3). The range of plant density adopted in this experiment is in accordance with the range used in similar environments in recent studies on hemp cultivation (Zatta et al., 2012; Amaducci et al., 2015). Nitrogen fertilization was applied at a ratio of 50 and 100 kg N ha^{-1} (hereafter called N50 and N100, respectively), as ammonium nitrate, divided into two doses applied at 15 and 30 days after crop emergence (50% of the total amount for each application). Considering that N fertilization has a positive response to hemp yield (Amaducci et al., 2015), the N fertilization levels tested in this experiment represent two plausible rates that could be adopted under farm conditions. Although K plays an important role in hemp fiber production (Amaducci et al., 2015), K fertilizer was not applied due to the high levels present in the soil (low level of K ranges from 0 to 80 mg kg^{-1}). Following each N fertilization, a rotary hoe was applied in order to incorporate the N fertilizer and to control inter-row weeds when the hemp seedlings were very sensitive to weed competition (Bhattarai and Midmore, 2014). Driving speed and implement settings were kept constant for each set of experiments (plant density and N fertilization level). The hemp genotypes were harvested twice: (i) at flowering for stem and inflorescence yield determination; and (ii) at seed maturity for seed yield determination.

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138 *2.3.Field measurements and sample preparation*

139 Immediately after sowing, a 1.0 m central row was randomly selected and from the appearance of the
140 first plant until full emergence the number of plantlets was counted every other day for a 2-week
141 period. The emergence date for each genotype was set as the date when 50% of the hemp seedlings
142 had emerged. Similarly, 10 representative plants in the same plant rows were selected and marked in
143 order to carry out full flowering observations (50% of the plants that reached this stage, Amaducci et
144 al., 2008b). Flowering observations were carried out twice a week when the flowering stage was
145 approaching, in order to determine the full flowering date precisely. Plant height was measured from
146 the base of the plant to the growing tip 30 and 90 days after emergence during the vegetative growth
147 of the hemp genotype.

148 The first crop harvesting was carried out within 10 days from the full flowering of each genotype,
149 while the second hemp harvesting was performed at seed maturity. At each harvesting time, all of the
150 plants in the 4 middle rows of each sub-sub-plot, a set of 1 m^2 area, were cut above soil surface (10
151 cm from the soil surface). At the first hemp harvesting, ten representative plants were then sub-
152 sampled and divided into two fractions: stems and inflorescence (by cutting the 30 upper part of the
153 stem). In the inflorescence sub-samples, flowers and leaves were included as they are generally used
154 for determining the chemical compounds of biomedical interest (Bertoli et al., 2010). Plants samples
155 were oven dried at 70°C until constant weight in order to evaluate the aboveground biomass. The
156 dried plant samples were then weighed to determine biomass. At the second hemp harvesting, the
157 seed samples were air dried at low temperatures (< 25°C) for 48 h in order to bring seed water below
158 100 g kg^{-1}, and then cleaned, the empty seeds were removed, and weighed for seed yield
159 determination. Natural weeds were sampled at the second hemp harvesting in all plots. Weeds were
160 sampled using a 1 m^2 rectangular quadrat (50 cm x 200 cm) placed randomly over the same area
161 where hemp plants were sampled by hand-clipping the weeds at the soil surface. The total weed
162 aboveground biomass was oven dried at 70°C until constant weight. A sub sample of seeds (100 g)
163 of the most productive hemp genotypes was ground and used for oil extraction using hexane (46 g of
164 sample in 150 ml of hexane), as described by Oomah et al. (2002). Protein content percentage was
165 determined by generic combustion analysis (LECO FP-528) on 0.5 g samples of dried seed (105°C,
166 16 h) using a 6.25 N to protein conversion factor. The meteorological data, including temperatures,
167 rainfall and potential evapo-transpiration, were collected from an automatic meteorological station
168 located approximately 150 m from the experimental site. The Cd, which is the sum of the degrees
169 since hemp sowing to seedling emergence with base temperature 0°C, was also calculated.

170

171 *2.4.Statistical analysis*

172 All data were analyzed with the analysis of variance (ANOVA) using JMP statistical software
173 package version 4.0 (SAS, 1996). ANOVA was performed for the 2-year period, considering the year
174 as repeated measure across time. Following the Bartlett test, the percentage data was transformed into
175 angular transformation before analysis in order to homogenize the variance (Gomez & Gomez, 1984).
176 The data reported in the tables were back transformed. A split-split-split plot experimental design
177 was used for analyzing: physiological response (time-span of sowing – emergence and emergence –
178 flowering) on plant height at 30 and 90 days after sowing, stem yield, stem diameter, seed yield, oil
179 and protein content of hemp seeds and weed biomass, where the year was considered as the main
180 factor, the hemp genotype as the split factor, the hemp plant density as the split-split factor and the N
181 fertilization level as the split-split-split factor. Fisher's protected least significant difference at 5%
182 level of probability (LSD 0.05) was used to compare the main effect and interaction means.
183
184 **3. RESULTS**
185 *3.1.Weather conditions*

186 The distribution of daily minimum and maximum air temperatures, rainfall and evapo-transpiration
187 recorded during the 2007 and 2008 growing seasons are shown in Fig. 1. Moderate mean temperatures
188 were recorded during the hemp growing seasons from March to September in both experimental
189 years, which gradually increased towards crop maturity. However, the data showed considerably
190 different trends between the experimental years. In fact, the air temperature was higher in 2007
191 compared with 2008 (on average 17.4 *vs*. 16.7°C, respectively). The minimum temperature during
192 the crop period ranged from 3°C in April 2008 to 21°C in August 2007, while the maximum
193 temperature in the same period varied from 10°C in April 2008 to 36°C in August 2007 (Fig. 1). Total
194 rainfall data, from sowing until harvesting of hemp crop, showed that the 2007 growing season was
195 drier than the 2008 growing season (338 *vs*. 520 mm, respectively, Fig. 1), while similar values were
196 observed for evapo-transpiration in both growing seasons (on average 861 mm).
197
198 *3.2.Phenological response and growth*

199 The time-spans from sowing to emergence and from emergence to flowering were significant for
200 genotype ($P \leq 0.001$), plant density ($P \leq 0.05$ and 0.001, respectively), year ($P \leq 0.001$) and the
201 interaction of year by genotype ($P \leq 0.01$ and 0.05, respectively). In addition, the time-span from
202 emergence to flowering was significant for N fertilization as main effect ($P < 0.001$) and the
203 interactions between N fertilization by genotype, N fertilization by plant density and N fertilization
204 by genotype and plant density ($P \leq 0.05$). The hemp seedlings generally emerged from 6 to 11 days

after hemp sowing depending on genotypes, in fact it occurred earlier in E68 and S27 compared with the other genotypes (on average 7 *vs*. 9 d, respectively, Table 1). Regarding plant density, the seedling emergence values tended to be higher in D1 than D2 and D3.

The duration of the vegetative phase, from emergence to full flowering, ranged from a minimum of 58 d in F17 in D3 with N50 to a maximum of 81 d in F75 in D1 with N50. Among the genotypes, the lowest period of vegetative phase duration was observed in F17, while the highest was observed in F75 (Table 1). The vegetative phase of the N50 fertilization level was generally longer than that of N100 (on average 69 *vs*. 67 d, respectively). In N50 fertilization level, the duration of the vegetative phase was similar in E, F17, Fe, and U31 regardless plant density, while in F32, F75, and S27 it was higher in D1 and D2 than D3 plant density (Table 1). In N100, all genotypes showed higher values of the duration of the vegetative phase in D1 and D2 compared with D3 plant density (on average 69 *vs*. 65 d, respectively).

Plant height was measured at 30 and 90 days after emergence (DAE) during vegetative growth and the values are reported in Table 2. The analysis shows that plant height at 30 and 90 DAE was significant for genotype ($P \leq 0.05$ and 0.001, respectively), plant density ($P \leq 0.001$) and year ($P \leq 0.001$). Moreover, at 90 DAE it was affected by N fertilization ($P \leq 0.001$) and the interactions between genotype by plant density ($P \leq 0.01$) and N fertilization by genotype ($P \leq 0.05$). At 30 DAE, similar plant height values were observed for all hemp genotypes (on average 38.8 cm), except for F32 and U31, which showed low plant height values (on average 37.2 cm). At 90 DAE, the highest plants were observed in E68 and the lowest in U31 (on average 280.5 and 145.7 cm, respectively) and the plants were generally higher in N100 than in N50 treatments. The hemp plant density values were high in D1, intermediate in D2, and low in D3 (on average 239.5, 218.9 and 193.9 cm, respectively, Table 2)

3.3.Hemp stem yield, its characteristics and inflorescence yield

The stem yield was significant for genotype, plant density, N fertilization and year as main effects ($P \leq 0.001$) and for the interactions genotype by plant density, N fertilization by genotype, N fertilization by genotype and plant density ($P \leq 0.05$). The stem yield was generally higher in N100 than in N50 (on average 5.84 *vs*. 4.86 t ha^{-1} of DM, respectively, Table 3). The stem yield of the N50 treatments ranged from 3.40 in F32 in D1 to 7.19 t ha^{-1} of DM in S27 in D3, and tended to be higher in D3, intermediate in D2 and lower in D1 plant density, except for the F75 and U31 genotypes for which the stem yield was higher in D3 and D2 than D1 plant density (Table 3). The stem yield of the N100 treatments ranged from 3.92 in F17 in D1 to 8.30 t ha^{-1} of DM in S27 in D3 and showed a similar trend to that observed in N50 (Table 3).

The stem diameter was significant for genotype, N fertilization, year ($P \leq 0.001$) and plant density ($P \leq 0.01$) as main effects, moreover there were interactions between N fertilization by genotype, N fertilization by plant density ($P \leq 0.05$). In both years, the largest stem diameter was generally observed in E68 and D1 (on average 6.3 and 6.5 mm, respectively, Table 4). Similarly, in regards to the N fertilization level, the stem diameter in N100 was greater than N50 (on average 6.1 *vs*. 5.4 mm, respectively), except in U31 and D3 where it was similar for both N100 and N50 fertilization level. In general, cultivating hemp in high plant density conditions produced plants that had a lighter stem weight, a smaller stem diameter and a shorter plant height than in low plant density conditions (Fig. 2).

The analysis of stem inflorescence showed significant effects for genotype, plant density, N fertilization and year ($P \leq 0.001$) and the interactions concerning genotype by plant density, N fertilization by genotype, N fertilization by genotype and plant density ($P \leq 0.05$). Hemp inflorescence yield ranged from 1.11 in F75 in D1 with N50 to 2.94 t ha^{-1} of DM in F32 in D3 with N100 and it was generally higher in N100 compared with N50 (2.04 *vs*. 1.79 t ha^{-1} of DM, respectively, Table 5), even if at both N fertilization levels, inflorescence tended to be higher in D3 and D2 (on average 2.09 t ha^{-1} of DM) than in D1 (on average 1.56 t ha^{-1} of DM), except for S27 where the inflorescence weight was similar among plant density. Among the genotypes F17, F32, Fe, and U31 generally showed higher inflorescence weight than E, F75, and S27 (Table 5).

3.4.Hemp seed yield and its characteristics

In general, the hemp seed yield was affected by all main effects ($P \leq 0.001$) and the interactions genotype by plant density, N fertilization by genotype, N fertilization by plant density, N fertilization by genotype and plant density ($P \leq 0.05$). Hemp seed yield was generally higher in N100 compared with N50 (0.99 *vs*. 0.86 t ha^{-1} of DM, respectively, Table 6). In the N50 treatments, seed yield ranged from 0.01 in S27 in D1 to 2.44 t ha^{-1} of DM in Fe in D3, and tended to be higher in D3 and D2 and lower in D1, except for E, F75, and S27. Among the hemp genotypes, seed yield was high in Fe, F17, F32, and U31, while similar, low values were observed in E, F75, and S27 regardless plant density (Table 6). In N100, seed yield followed a similar trend to N50 as it generally ranged from 0.11 in E68 in D2 to 2.27 t ha^{-1} of DM in Fe in D3 and was highest in Fe (Table 6). Thousand seed weight of the hemp genotypes was affected by genotype x plant density and genotype x N fertilization interactions (Fig. 3). The thousand seed weight (TGW) ranged from 19.7 g in F17 in D1 to 5.8 g in S27 in D3 and was generally higher in D1 and lower in D2 and D3 plant densities (on average 14.6 *vs*. 12.9 g, respectively, Fig. 3). Moreover, the seed weight tended to be higher in N100 than in N50 fertilization level (on average 14.2 *vs*. 13.5 g, respectively), except for F32 and S27 which showed similar values

between the N fertilizations (Fig. 3). The oil and protein content, only measured for the most seed productive genotypes, were affected by genotype x plant density and genotype x N fertilization interactions, respectively (Fig. 4). Oil content ranged from 310 g kg^{-1} in Fe in D3 to 263 g kg^{-1} in U31 in D1 and tended to rise as plant density increased, while the protein content ranged from 260 g kg^{-1} in F17 with N100 to 238 g kg^{-1} in U31 with N50 and tended to rise when N fertilization was increased (Fig. 4).

3.5. Weed biomass

The weed aboveground biomass was significant for genotype ($P \leq 0.001$), plant density and year ($P \leq 0.01$) N fertilization ($P \leq 0.05$), furthermore it was affected by the interactions between genotype by plant density and N fertilization by genotype ($P \leq 0.05$). Weed aboveground biomass ranged from 90.7 in F17 in D1 to 39.3 g m^{-2} of DM in E68 in D3, and it tended to be higher in D1 than D2 and D3 (on average 73.0 *vs*. 50.0 g m^{-2} of DM, respectively), even if similar values were observed in the F32 genotype regardless plant density (Table 7). As expected N fertilization affected the weed aboveground biomass, which was higher in N100 (on average 61.55 g m^{-2} of DM) and lower in N50 (on average 57.3 g m^{-2} of DM), except for E, Fe, and S27 which showed similar weed aboveground biomass values in both the N fertilization levels (Table 7).

4. DISCUSSION

The hemp genotypes tested in this study proved to adapt well to the Mediterranean environment of Central Italy. The emergence of each hemp genotype was uniform and the establishment was regular in both experimental years. The faster seedling emergence observed in 2008 compared with 2007 was probably due to better weather conditions (on average 99.7 *vs*. 93.6 °Cd, respectively), which stimulated hemp seed emergence (van der Werf et al., 1995). The significant variations were observed among hemp genotypes during the sowing – emergence period may be due to the genetic differences and seed quality variation as already noted for other crops (Tang et al., 2016). After emergence, the hemp plants grew regularly throughout both growing seasons. Furthermore, no plant mortality was observed throughout both growing seasons indicating that all plant densities adopted in this study were suitable for hemp cultivation in order to avoid self-thinning (Struik et al., 2000). Moreover, increasing the rates of N did not have a detrimental effect on plant density in both years (Vera et al., 2004). Although all plant densities were under a threshold to determine self-thinning, they played a crucial role in determining plant shape. In fact, plant height seemed to be affected by inter-specific competition, and it was higher in 120 than 80 and 40 plants m^{-2} in the early stage of growth (60 DAE), probably due to light scarcity, which determined stem elongation in high plant density plots in order

307 to avoid the shade (Burczyk et al., 2009). Similarly, increasing the rate of N fertilizer led to an increase
308 in plant height (Vera et al., 2004). Consequently, canopy development in the early growing stage was
309 more rapid at higher plant density (120 plants m^{-2}) and N fertilization level (100 kg N ha^{-1}) compared
310 with the other treatments (data not shown). Although there was a favorable initial growth trend at 120
311 plant m^{-2} (D3), it changed to favor the D1 plant density (40 plants m^{-2}) at full flowering stage,
312 probably due to the fact that hemp plants grown in highly competitive environments tend to reach the
313 reproductive stage earlier than plants cultivated in non-competitive conditions (Amaducci et al.,
314 2008c; Westerhuis et al.,2009). Similarly, plant diameter and aboveground biomass per plant were
315 strongly affected by plant density and showed a clear inverse relationship with plant density, as
316 suggested by previous Authors (Bhattarai and Midmore, 2014). At 120 plants m^{-2} the hemp plants
317 were thinner and accumulated less biomass than at 80 and 40 plants m^{-2}, and probably had lower
318 cortical surface with a higher surface – volume ratio (Cromack, 1998). These effects in hemp are
319 considered positive as they determine high quality fibers (Amaducci et al., 2015). In fact, fiber cell
320 diameter tends to decrease with increasing plant density due to intra-specific competition that
321 increases with higher crop plant density. Under these conditions, the plants have finer stems with
322 elongated basal internodes characterized with longer fibers (Hall et al., 2013). Consequently, it is
323 assumed that better quality fibers are obtained for textile applications under high plant density.
324 Furthermore, the mechanical harvesting of hemp plants requires less energy to process thinner stems
325 at high plant density (Chen et al., 2014). However, the hot, dry weather conditions observed in
326 summer 2007 compared with summer 2008 seemed to accelerate development in all of the genotypes
327 tested, except for Fedora17 that flowered at the same time in both growing seasons. Due to early
328 flowering, stem yield in 2007 was lower than in 2008. The accuracy of the identification of the
329 flowering time based on agronomical factors is essential as a decision support system for the various
330 hemp productions. According to Amaducci et al. (2015), the flowering stage in hemp is deemed to be
331 the most important event in hemp cultivation, as it affects production in terms of stems, inflorescences
332 and seeds. In our study, the duration of the vegetative phase (from emergence to flowering) varied
333 greatly among the genotypes and was proportional to the stem yield, probably due to the fact that
334 once flowering starts, dry matter accumulation drops rapidly (Struik et al., 2000). Indeed, in early
335 flowering genotypes such as Fedora17, Felina32, Ferimon and Uso31, the time from emergence to
336 flowering was around 60 days from seedling emergence and was characterized by short plant stature
337 (Bhattarai and Midmore, 2014). Consequently, the highest stem yield was observed for the late
338 flowering genotypes, such as Epsilon68, Futura75 and Santhica27, while the lowest stem yield was
339 observed for early flowering genotypes. For these genotypes, Faux et al. (2013) suggests anticipating
340 sowing time in order to lengthen the crop cycle, even if there is the risk of cold damage in the northern

Mediterranean North zone when the soil temperature drops under 10°C (Cosentino et al., 2012). However, highly variable stem yields were observed for all genotypes and densities, which ranged from 3.4 t ha^{-1} of DM at the lowest plant density in Felina32 to 8.2 t ha^{-1} of DM at the highest plant density in Futura75. The genotypes tested in this study were monoecious varieties which are characterized by early flowering and lower stem height compared with dioecious genotypes, meaning that higher stem yields could be obtained in the same environment by adopting dioecious varieties (Berenji et al., 2013; Bertoli et al., 2010). Plant density also significantly affected the production of stems, even if the plant density levels used in this experiment seemed lower compared with those suggested in previous studies that indicated a range of 150 - 200 plants m^{-2} at crop emergence for reaching optimum stem yield (Amaducci et al., 2015, Faux 2013). Although stem yields tended to be higher in the late-flowering genotypes, inflorescence and seed production were higher in the early flowering genotypes (Fedora17, Felina32, Ferimon and Uso31). In particular, the higher seed yield production of the early flowering genotypes was probably due to higher temperatures, longer day length and higher light intensities during their seed development compared with late flowering genotypes. This finding is in agreement with Höppner and Menge-Hartmann (2007) who determined that late-flowering genotypes produced significantly lower seed yields than early genotypes, which is in contrast with the dual-purpose of stem and seed production proposed by Tang et al. (2016), while it could better fit the dual-purpose in terms of stem and inflorescence, considering that hemp could be grown for producing essential oils of biomedical interest from inflorescence (Bertoli et al., 2010). In fact, variations in crop management may be necessary for dual-purpose production (stems and seeds) since seed maturity is essential for seed production (Faux et al., 2013), while fiber production and quality decrease due to senescence (Mediavilla et al., 2001). Furthermore, dual-purpose hemp cultivation (fibers and seeds) should consider that stem yield is generally lower compared with when hemp is grown for fiber production, even if the top third of the stem does not account for much biomass and thus could be used for other purposes (Mediavilla et al., 2001). From an agronomical point of view, hemp cultivation is often followed by a winter cereal, therefore it is essential to harvest hemp early in order to prepare the soil for the following crop (Amaducci et al., 2015). Consequently, stem and inflorescence productions seem to fit better than stem and seed productions in order to avoid unfavorable conditions for the seedbed preparation of the following crop in the Mediterranean environment.

Among the early flowering genotypes, a plant density of 120 plants m^{-2} resulted in the highest seed yield and oil content thus proving to be suitable for cultivating hemp for this purpose, even if the thousand grain weight tended to be low. Although it is proved that high doses of N fertilization stimulated the photosynthetic performance of hemp plants at all plant densities (Maļceva et al., 2011),

this study showed that higher N fertilizer level in hemp has a positive impact mainly on stem yield than for inflorescence and seed yields (on average +28%, +17% and +4% at 100 kg of N ha^{-1} than at 50 kg of N ha^{-1}, respectively). In this study, N fertilizer was applied twice before canopy closure yet it is possible that dividing the N fertilizer into several applications throughout the growing season may help to improve inflorescence and seed yields. However, it is interesting to note that N fertilization levels affected the protein content of the seeds which tended to be higher at 100 kg N ha^{-1}, according to the results reported by Vera et al. (2004).

The results of this study confirmed that hemp is an effective weed-suppressive crop mainly because of its high competitiveness for limited resources (Bhattarai et al., 2014) and for the release of allelochemicals (Pudełko et al., 2014), even if weed growth was affected differently according to variety. Generally, Epsilon68, Futura75 and Santhica27 resulted in low weed biomass at seed harvesting compared with the other genotypes. These genotypes grew rapidly and probably overshadowed the soil quickly thus impeding weed development and reducing emergence (Ranalli, 1999). However, the initial slow growing rate of hemp plants makes it essential to control the weeds in order to avoid strong effects of competition. For this reason the hoeing cultivation applied twice at 15 and 30 days after crop emergence proved to be useful for managing the weeds in the hemp crop. The results suggest that a high seeding rate has the potential to reduce weed infestation (Weiner et al., 2010) and is therefore a point in favor of hemp cultivation as it reflects the aim of the Sustainable Use of Pesticide Directive (2009/128/EC) by reducing the amount of herbicide required for its cultivation.

5. CONCLUSIONS

Considering that there is growing interest in worldwide hemp cultivation due the multitude of end products, and that its cultivation is expected to rise notably over the next years, it is essential to identify how main agronomical factors influence the quantity and the quality of hemp products. In this study genotypes and plant density proved to be relevant for determining the quality and yield of the crop. In the Northern Mediterranean environment it seems that it is advisable to use intermediate or late flowering genotypes for stem production, while early flowering genotypes produce more inflorescences and seeds. However, farmers should consider making a dual-purpose production of stems and inflorescences or stems and seeds, even if it is clear that yield is related to the choice of genotype. The results suggest that in Central Italy, 120 plants m^{-2}, with an interrow spacing of 0.5 m, is a suitable plant density for obtaining high yields of stem, inflorescence and seeds, which prevents undesirable self-thinning due to inter-specific competition. However, since in this study stem, inflorescence and seed yield increased with the increase in plant density, further research should be

409 carried out to determine whether plant densities higher than 120 plants m^{-2} could be used to improve
410 hemp yields, in terms of both quantity and quality. As expected the high N fertilization level strongly
411 effected stem yield, while inflorescence and seed production was less affected. Further research
412 should be carried out on different genotypes and to find flexible agronomical practices that are able
413 to improve innovative hemp yields, such as the production of inflorescence for medicinal use and
414 seeds for oil and flour, grown under Mediterranean conditions.
415
416 **ACKNOWLEDGEMENT**
417 This research was funded by the University of Tuscia. The authors wish to thank Claudio Stefanoni
418 and Fulvia Gatti for their technical assistance and the CanapaLive associations for participating in the
419 research activities.
420
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Table 1. The main effect of genotype on the time-span of the period plant – emergence and the interaction effect of genotype x plant density x N fertilization level on the time-span of the period emergence – flowering.

Genotype	Sowing – Emergence (days)	Emergence – Flowering (days)					
		N_{50}			N_{100}		
		D_1	D_2	D_3	D_1	D_2	D_3
E68	6.6 c	75.9 bA	73.1 bA	72.8 bA	73.5 bA	72.5 bAB	70.5 bB
F17	8.2 b	60.6 dA	61.3 dA	58.3 dA	61.9 dA	58.9 eA	55.7 eB
F32	10.2 a	67.3 cA	66.8 cA	64.4 cB	66.1 cA	63.9 dA	60.1 dB
F75	7.2 bc	81.4 aA	79.4 aAB	77.2 aB	79.1 aA	79.1 aA	76.1 aB
Fe	9.6 a	68.0 cA	67.4 cA	67.2 cA	69.5 bA	67.1 cA	63.4 cB
S27	6.6 c	74.4 bA	74.1 bAB	71.6 bB	70.6 bA	71.1 bA	66.2 cB
U31	9.9 a	65.0 cA	66.5 cA	64.7 cA	64.4 cdA	61.6 cdB	60.6 dB

Values belonging to the same characteristic with different letters in rows for plant density (upper case letter) and in columns for hemp genotype (lower case letter) are statistically different according to LSD (0.05). E68 = Epsilon68; F17 = Fedora17; F32 = Felina32; F75 = Futura75; Fe = Ferimon; S27 = Santhica27; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.

Table 2. The main effect of genotype on plant height at 30 days after emergence (DAE) and the interaction effects of genotype x N fertilization level and genotype x plant density on plant height at 90 DAE.

Genotype	Plant height at 30 DAE (cm)	Plant height at 90 DAE (cm)				
		N_{50}	N_{100}	D_1	D_2	D_3
E68	39.6 a	272.6 aA	288.3 aA	306.4 aA	286.2 aB	248.9 aC
F17	39.4 a	186.8 cB	214.9 dA	225.1 dA	201.2 deB	176.2 dC
F32	37.2 b	198.0 cA	215.9 dA	227.0 dA	210.4 dB	183.5 dC
F75	39.6 a	228.3 bB	251.6 cA	263.4 cA	238.3 cB	218.2 cC
Fe	39.1 a	182.0 cB	202.0 dA	217.0 dA	197.0 eB	162.2 eC
S27	39.7 a	242.1 bB	269.2 bA	283.0 bA	253.7 bB	231.4 bC
U31	37.2 b	139.4 dA	152.0 eA	154.9 eA	145.4 fAB	136.8 fB

Values belonging to the same characteristic with different letters in rows for N fertilization level or plant density (upper case letter) and in columns for hemp genotype (lower case letter) are statistically different according to LSD (0.05). E68 = Epsilon68; F17 = Fedora17; F32 = Felina32; F75 = Futura75; Fe = Ferimon; S27 = Santhica27; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.

Table 3. The interaction effects of genotype x plant density x N fertilization level on hemp stem yield.

Genotype	Stem yield (t ha^{-1} of DM)											
	----------N_{50}----------						----------N_{100}----------					
	D_1		D_2		D_3		D_1		D_2		D_3	
E68	3.85	abC	4.98	abB	6.53	abA	5.21	aB	5.96	aB	7.72	aA
F17	3.66	abC	4.74	abB	5.83	bA	3.92	bC	5.63	abB	7.49	bA
F32	3.40	bC	4.45	bB	5.66	bcA	4.26	bC	5.15	bB	6.40	cA
F75	4.23	aB	5.38	aA	6.02	bA	4.30	bC	6.14	aB	8.20	aA
Fe	3.57	abC	4.61	abB	5.60	bcA	4.21	bC	5.70	abB	7.35	bA
S27	3.97	abC	5.14	abB	7.19	aA	4.65	abC	6.01	aB	8.30	aA
U31	3.48	bB	4.77	abA	4.99	cA	4.02	bB	5.67	abA	6.33	cA

Values belonging to the same characteristic with different letters in rows for plant density (within N fertilization level) and in columns for hemp genotype (lower case letter) are statistically different according to LSD (0.05). E68 = Epsilon68; F17 = Fedora17; F32 = Felina32; F75 = Futura75; Fe = Ferimon; S27 = Santhica27; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.

Table 4. The interaction effects of genotype x N fertilization and plant density x N fertilization level on stem diameter at the first hemp harvesting.

Genotype	Stem diameter (mm)	
	N_{50}	N_{100}
E68	5.9 aB	6.7 aA
F17	5.4 abB	6.0 bA
F32	5.1 bA	5.6 bA
F75	5.5 abB	6.4 abA
Fe	5.1 bB	5.8 bA
S27	5.5 abB	6.4 abA
U31	5.0 bA	5.6 bA
Plant density		
D1	6.0 aB	6.9 aA
D2	5.4 aB	6.3 aA
D3	4.6 bA	5.1 cA

Values belonging to the same characteristic with different letters in rows for N fertilization level (upper case letter) and in columns for hemp genotype or plant density (lower case letter) are statistically different according to LSD (0.05). E68 = Epsilon68; F17 = Fedora17; F32 = Felina32; F75 = Futura75; Fe = Ferimon; S27 = Santhica27; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.

Table 5. The interaction effects of genotype x plant density x N fertilization level on hemp inflorescence yield.

Genotype	Inflorescence yield (t ha^{-1} of FM)											
	----------N_{50}----------						----------N_{100}----------					
	D_1		D_2		D_3		D_1		D_2		D_3	
E68	1.25	bB	1.58	bAB	1.86	bA	1.34	bB	1.71	bAB	1.90	cA
F17	1.48	abB	2.07	aA	2.19	abA	1.91	aB	2.32	aA	2.63	abA
F32	1.69	abB	2.13	aA	2.31	aA	2.12	aB	2.52	aB	2.94	aA
F75	1.11	bB	1.64	bA	1.79	bA	1.32	bB	1.83	bA	1.91	cA
Fe	1.73	aB	2.16	aAB	2.29	abA	1.81	abB	2.38	aA	2.45	bA
S27	1.29	bA	1.43	bA	1.63	bA	1.43	bB	1.85	bA	1.77	cA
U31	1.61	abB	2.06	aA	2.23	abA	1.71	abB	2.30	aA	2.64	abA

Values belonging to the same characteristic with different letters in rows for plant density (within N fertilization level) and in columns for hemp genotype (lower case letter) are statistically different according to LSD (0.05). E68 = Epsilon68; F17 = Fedora17; F32 = Felina32; F75 = Futura75; Fe = Ferimon; S27 = Santhica27; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.

Table 6. The interaction effects of genotype x plant density x N fertilization level on hemp seed yield.

Genotype	Seed yield (t ha^{-1} of DM)											
	----------N_{50}----------						----------N_{100}----------					
	D_1		D_2		D_3		D_1		D_2		D_3	
E68	0.02	cA	0.02	cA	0.03	dA	0.12	cA	0.11	cA	0.12	cA
F17	1.06	bC	1.61	aB	2.02	abA	1.04	bB	1.68	bA	1.93	abA
F32	1.01	bC	1.36	abB	1.92	bA	1.13	bB	1.69	bA	2.00	abA
F75	0.03	cA	0.04	cA	0.04	dA	0.13	cA	0.15	cA	0.14	cA
Fe	1.20	aC	1.75	aB	2.44	aA	1.60	aB	2.26	aA	2.27	aA
S27	0.01	cA	0.02	cA	0.02	dA	0.12	cA	0.13	cA	0.12	cA
U31	1.02	bB	1.14	bAB	1.39	cA	0.81	bB	1.44	bA	1.82	bA

Values belonging to the same characteristic with different letters in rows for plant density (within N fertilization level) and in columns for hemp genotype (lower case letter) are statistically different according to LSD (0.05). E68 = Epsilon68; F17 = Fedora17; F32 = Felina32; F75 = Futura75; Fe = Ferimon; S27 = Santhica27; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.

Table 7. The interaction effects of genotype x plant density and genotype x N fertilization level on weed biomass at the second hemp harvesting.

Genotype	Weed biomass (g m^{-2} of DM)				
	D_1	D_2	D_3	N_{50}	N_{100}
E68	55.73 cA	45.33 bAB	39.25 bB	46.16 dA	47.38 eA
F17	90.67 aA	64.85 abB	42.86 bC	61.36 bB	70.89 bA
F32	74.12 bA	64.00 abA	62.19 abA	63.70 bB	69.84 bA
F75	65.03 bcA	47.03 bB	39.73 bB	47.15 dB	54.04 cdA
Fe	72.00 bA	53.84 bB	42.83 bB	55.86 cA	56.59 cA
S27	64.98 bcA	48.99 bB	40.62 bB	51.78 cdA	51.28 dA
U31	88.50 aA	76.78 aB	68.21 aB	74.82 aB	80.84 aA

Values belonging to the same characteristic with different letters in rows for plant density or N fertilization level (upper case letter) and in columns for hemp genotype (lower case letter) are statistically different according to LSD (0.05). E68 = Epsilon68; F17 = Fedora17; F32 = Felina32; F75 = Futura75; Fe = Ferimon; S27 = Santhica27; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.

Figure 1. Daily minimum and maximum temperatures, rainfall and evapo-transpiration throughout the periods of study in 2007 and 2008 growing seasons.

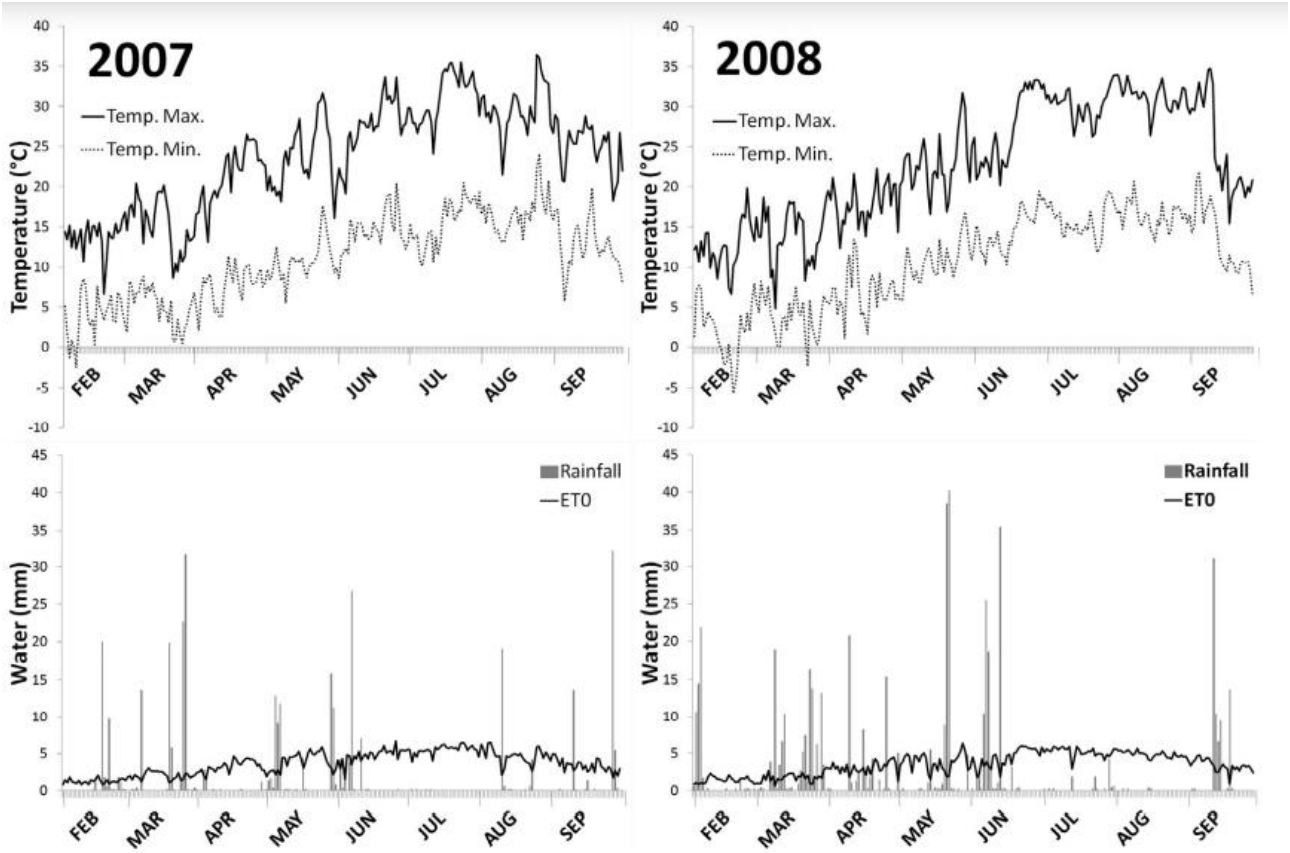

Figure 2. The effect of plant density on stem dry weight, stem diameter and plant height at 90 DAE of hemp at both N50 and N100 fertilization level.

Error bars represent ± standard error from mean (n = 42). The significance level is (***), (**), and (*) significant at $P \leq 0.001$, $P \leq 0.01$, and $P \leq 0.05$, respectively.

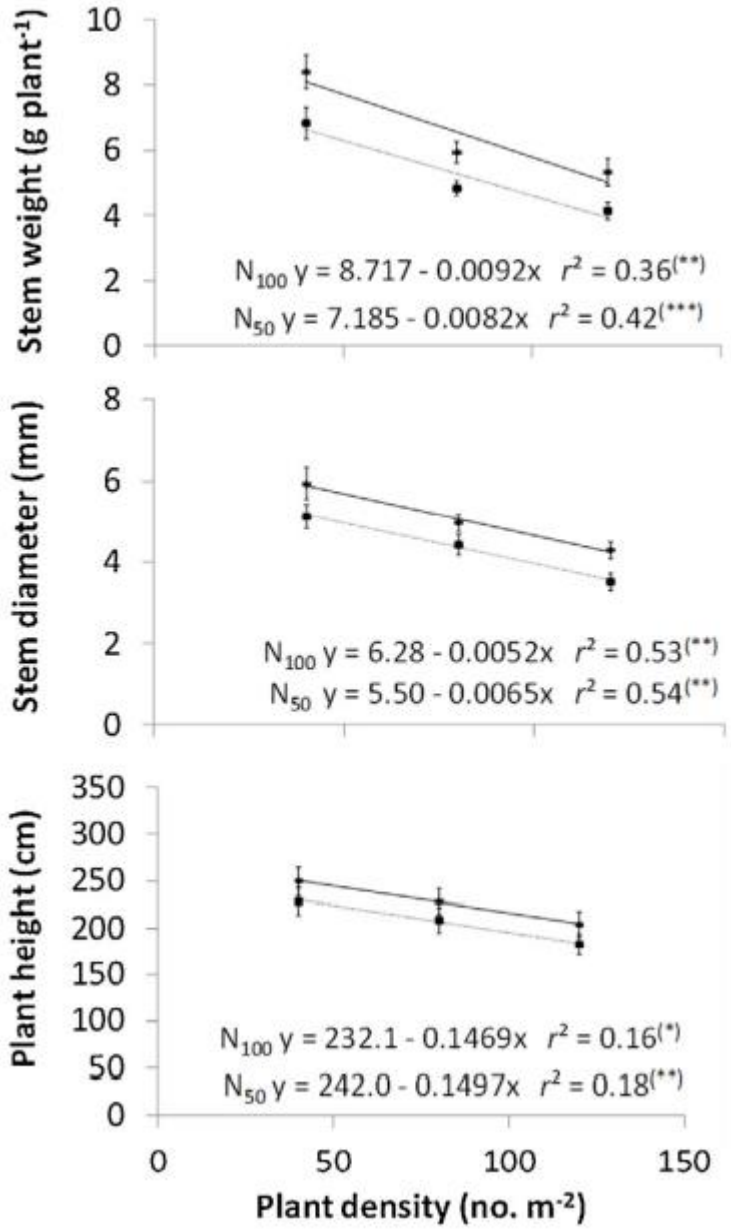

Figure 3. The interaction effect of genotype x plant density and genotype x N fertilization level on hemp thousand grain weight (TGW).

Values belonging to the same characteristic followed by the same letter are not significantly different according to LSD (0.05). E68 = Epsilon68; F17 = Fedora17; F32 = Felina32; F75 = Futura75; Fe = Ferimon; S27 = Santhica27; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.

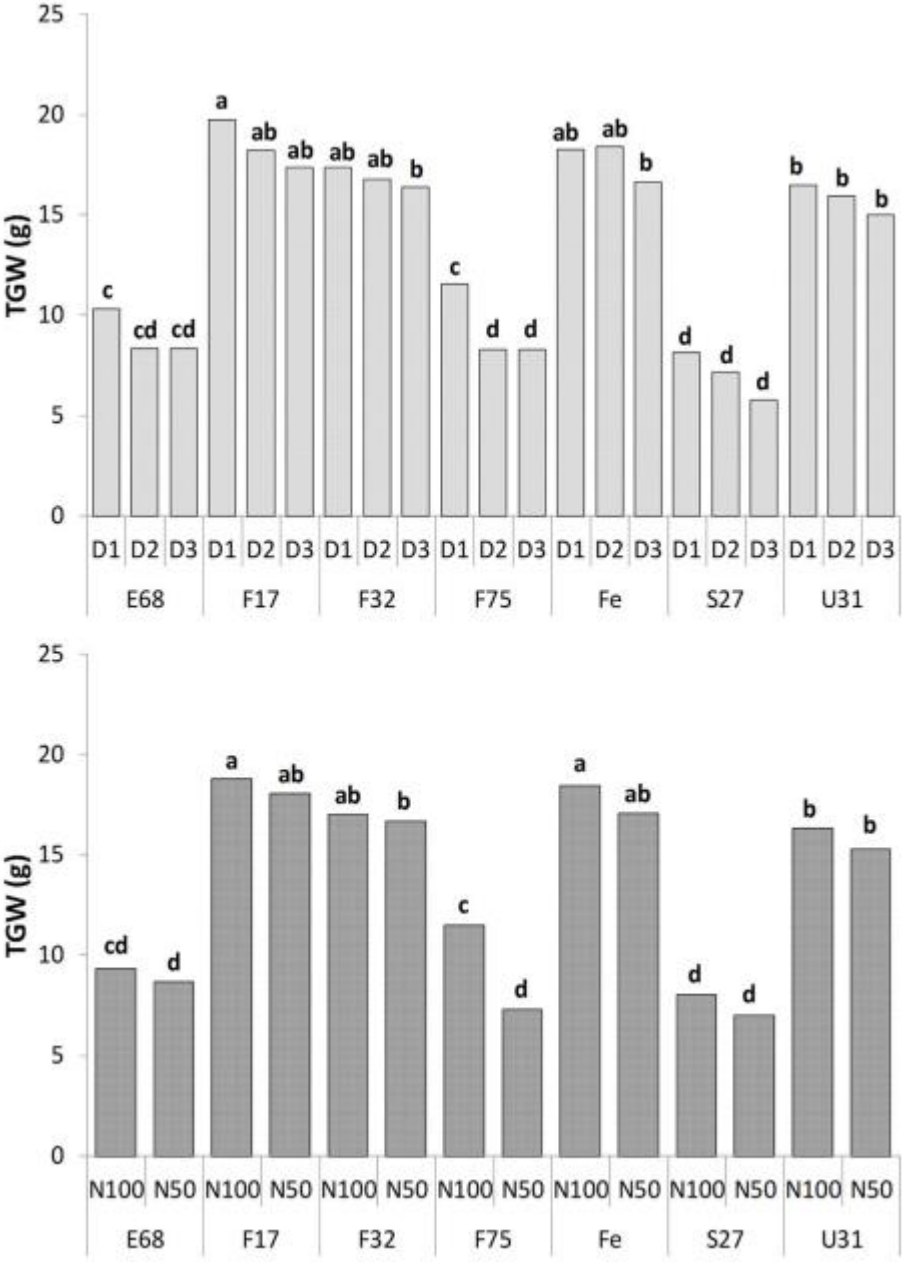

Figure 4. The interaction effect of genotype x plant density and genotype x N fertilization level on oil content and protein content, respectively, of hemp seeds.

Values belonging to the same characteristic followed by the same letter are not significantly different according to LSD (0.05). F17 = Fedora17; F32 = Felina32; Fe = Ferimon; U31 = Uso31; D1, D2, and D3 = 40, 80, and 120 plants m^{-2}; N100 and N50 = 100 and 50 kg of N ha^{-1}.