

Plant density contribution to seed oil content: the responses of contrasting sunflower genotypes grown in multi-environmental network

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ABSTRACT

- **Background and Aims:** The use of crop models for simulating sunflower performance should be based on solid knowledge of determining factors and the processes by which they impact on final outputs. Conflicting results have led to a left-open concern about planting density effect on oil content. This study aimed at clarifying the contribution of planting density to final oil content by identifying putative processes that could be likely involved in this relationship.
- **Methods:** We collected data from multi-location experiments and survey carried out in France. The first dataset (2009) involved 2 genotypes (LG 5450 and Vellox) submitted to 6 levels of plant density (3 to 8 plants/m²) in a split-plot design. The second dataset (2007 to 2009) was obtained from supplying basins investigation where 3 genotypes were grown (NK Countri, NK Ferti and PR64H32) under a narrower range of planting densities (2 to 6 plants/m²). We explored correlations between plant density and oil content with the following variables: hull content (% hull), mean seed weight, maximum leaf area index (LAI_{max}) and leaf area duration (LAD). Contributions of genotype, soil, density and their interactions were evaluated using ANOVA.
- **Key Results:** Significant genotype ($p=5,5e-14$), soil ($p=0,008$), density x soil ($p=0,06$) and genotype x soil ($p=0,07$) interaction effects on oil content were detected in experimental plots, and genotype effect in supplying basins network ($p=8,8e-13$), hence correlations study was conducted per genotype and/or soil type. Plant density contribution to oil content was 3 times less than genotype effect and 2 times less than soil effect. In all genotypes, increasing planting density always led to decreases in mean seed weight, that did not necessarily led to hull and oil contents variations.
- **Conclusion and key points of discussion:** 3 categories of genotypes behavior could be distinguished: (1) density impacted oil content through changes in mean seed weight/hull content (LG 5450, NK Countri); (2) density impacted oil content through changes in mean seed weight without changes in hull content (Vellox); (3) oil content strongly correlated to hull content but not in response to plant density variations (NK Ferti, PR64H32). Other possible determining factors (kernel weight, kernel oil content, water stress...) of final oil content are discussed.
- **Contribution to research:** These promising results should be the starting point for evaluating sunflower genotypes response to plant density under a larger and more well-characterized range of environments, for identifying pathways linking plant density to oil content, and integrating them in SUNFLO crop simulation model.

Key words: hull content, individual seed weight, path analysis, plant density, seed oil content

INTRODUCTION

Crop management is of major importance in sunflower because of its determining effect on yield components. Plant density indeed influences sunflower performance through changes in the amount of radiation intercepted by the leaves and consequent dry matter produced. A too low number of plants per unit area penalizes yield because of lack of competition, but a too high one leads to a risk of lodging and yield loss (Merrien, 1992). Anyway, sunflower can compensate stand reduction by increases in seed number per head in spite of decreases in mean seed weight (Robinson, 1978). Still, inconsistent results were reported on the effect of plant density on final oil content (Gubbels and Dedio, 1986; Rizzardi et al., 1992; Diepenbrock et al., 2001).

Oil content [amount of oil (mg) per grain dry weight (mg)] is determined genetically (Fick, 1997), but also modulated by environment and crop management (Connor and Hall, 1997). Oil content was described to whether increase with increasing plant density, or to not significantly vary despite large plant density ranges. It turns out that certain genotypes are more or less sensitive to plant density than others (Rizzardi, 1992). In limiting conditions, other factors such as water stress could have reduced leaf area duration and thus final oil content, given that lipogenesis mainly relies on photosynthetic activity during grain filling (Merrien, 1992). Anyway most studies report that oil content is only little affected by plant density variations, but the weight of plant density effect on oil content has never been clearly quantified, even when confronted with other factors.

Besides, the underlying processes by which plant density could affect final oil content remain unstated. Clearly, the effect of plant density on oil content might involve some other intermediate variables, such as 1000-seed weight (Diepenbrock and Feil, 2001) or hull:kernel ratio (Rizzardi et al., 1992). We assume that a common pathway should likely take place whatever genotype or environment; the diagnosis of limiting factors could help to understand unexpected variations of oil content in response to plant density. Starting from solid reports of plant density effect, we propose the following pathway:

From one hand, increasing plant density results in decreasing seed weight. This involves changes in hull weight as described by Rizzardi et al. (1992). On the other hand, negative relationships between hull content/seed weight and oil content have been reported by Denis and Vear (1996).

Higher plant density → lower seed weight
⇒ Lower hull weight } higher oil content
⇒ Higher seed number }

The objectives of this study are: (1) to quantify the effect of plant density on oil content in limiting and non-limiting conditions; (2) to validate/refute/adjust the putative path of underlying processes by confronting it to experimental data.

2. MATERIALS AND METHODS

2.1 Experimental trials and agricultural survey networks- Experimental trials were carried out in 2009 with 2 genotypes (LG 5450 and Vellox). These were sown on May, 11th, on a deep clay soil (En Crambade location) and a shallow sandy clay loam soil (Montmaur location). Soil available water at sowing was estimated to be 250 mm for the 150 cm-soil depth (deep soil) and 80 mm for the 90cm-soil depth (shallow).

After emergence, stands were thinned to the respective plant densities of 3 (D1), 4 (D2), 5 (D3), 6 (D4), 7 (D5) and 8 (D6) plants per m² with a row spacing of 50 cm. Four replicates per modality were arranged in a split plot design in each location. Plots were fertilized as needed and protected against weeds, but not irrigated.

An agricultural survey (Champolivier et al., 2011) was conducted in two supplying basins from 2007 to 2009 as part of a collaborative project with South-West French cooperatives, whose objective was to make a diagnosis of yield and quality determinants. Field networks (about 100 plots per year) were built most sparingly as possible under contrasting crop management practices. Complete data were available for 3 genotypes (NK Countri, NK Ferti and PR64H32).

2.2. Agronomic traits measurements - Assuming the previous putative pathway of plant density effect on oil content, we considered 5 variables: plant density, oil content, hull content (available only at D1, D3 and D6 for experimental trials), mean seed weight and seed number per m². Maximum leaf area index (LAI_{max}) and leaf area duration (LAD) were determined in experimental trials. Seed oil content was measured using RMN. LAI_{max} was obtained from LAI-2000 measurements, and LAD corresponds to cumulated green LAI from last anthesis to physiological maturity.

2.3. Statistical tools - Correlation matrixes were built by including all previously chosen variables for each trial type. Significant relationships were tested using Pearson p-value criterion (with R.1.3 software package). The effect of planting density, genotype and planting density x genotype interactions on the variables were tested using ANOVA. For the agricultural survey data, we subdivided the observed planting densities values into classes and attributed modalities equally to each genotype in order to test planting density and genotype effects by using ANOVA. Year effect on the variables was tested only in NK Countri since it was the only genotype that was grown over the 3 years. Level of acceptable significance was set to less than 10% error.

3. RESULTS

3.1. Experimental trials

No significant effect of plant density could be detected on oil content when considering the whole dataset ($p=0,57$, table 1), but strong genotype ($p= 5,57e-14$), soil ($p= 0,008$), density x soil ($p=0,06$) and genotype x soil ($p=0,07$) interactions affected significantly oil content. For further statistical investigation, we then decided to consider separately the genotypes grown on each soil type. Notations will be LG_deep_soil and Vellox_deep_soil for LG 5450 and Vellox grown in En Crambade; otherwise, these will be noted LG_superf_soil and Vellox_superf_soil for Montmaur.

Table 1. p-values (ANOVA on experimental trials) corresponding to genotype, plant density, soil type and their interactions effects on oil content, grain number per m², mean seed weight, LAImax, LAD and hull content.

	oil content	grain number per m ²	mean seed weight	LAImax	LAD	hul content
genotype	5.573e-14	<2.2e-16	8.964e-12	<2.2e-16	<2.2e-16	0.1020488
plant_density	0.577929	8.998e-09	<2.2e-16	0.0082499	0.4174	0.0004081
soil_type	0.008457	<2.2e-16	<2.2e-16	<2.2e-16	<2.2e-16	0.0025580
genotype:plant_density	0.109654	0.735417	0.4910	0.8340894	0.4824	0.0572068
genotype:soil type	0.071606	0.031523	0.5860	0.0002952	1.721e-12	0.5320325
plant_density:soil type	0.061609	0.002144	8.644e-08	0.0026229	0.1687	0.0185942
genotype:plant_density:soil type	0.891114	0.882197	0.1215	0.7146013	0.7759	0.1901064

3.1.1. LG 5450 genotype in deep and superficial soils (Fig.1)

There was a significant and positive relationship between plant density and oil content ($r=0,81$; $p=0,007$) in LG_deep_soil. Oil content also positively correlated to grain number per m² ($r=0,93$; $p=0,0002$), and negatively with mean seed weight ($r=-0,83$; $p=0,006$) and hull content ($r=-0,64$; $p=0,06$). Grain number per m² and mean seed weight negatively correlated ($r=-0,88$; $p=0,001$)

On the other side, plant density affected mean seed weight ($r=-0,99$; $p<0,001$), grain number per m² ($r=0,87$; $p=0,002$), LAImax ($r=0,62$; $p=0,07$) and hull content ($r=-0,62$; $p=0,07$). LAD and LAImax were negatively associated with hull content ($r=-0,68$; $p=0,04$ and $r=-0,62$; $p=0,07$); the latter positively related to mean seed weight ($r=0,64$; $p=0,06$) but negatively with oil content ($r=-0,64$; $p=0,06$). In contrast with LG_deep_soil, no correlation was found between oil content and plant density ($r=0,16$; $p=0,68$) when LG 5450 was grown on shallow soil. None of the studied variables could be significantly correlated to oil content.

Considering plant density correlates, both mean seed weight ($r=-0,89$; $p=0,001$) and hull content ($r=-0,70$; $p=0,03$) were negatively associated with plant density, as observed in LG_deep_soil. Both were also positively related to each other ($r=0,63$; $p=0,07$). No significant relationship was found between grain number and mean seed weight, but grain number per m² positively correlated to LAD ($r=0,61$; $p=0,08$). Only density effect on mean seed weight and hull content were found to be significant in variance analysis ($p=0,0001$ and $p=0,01$ respectively).

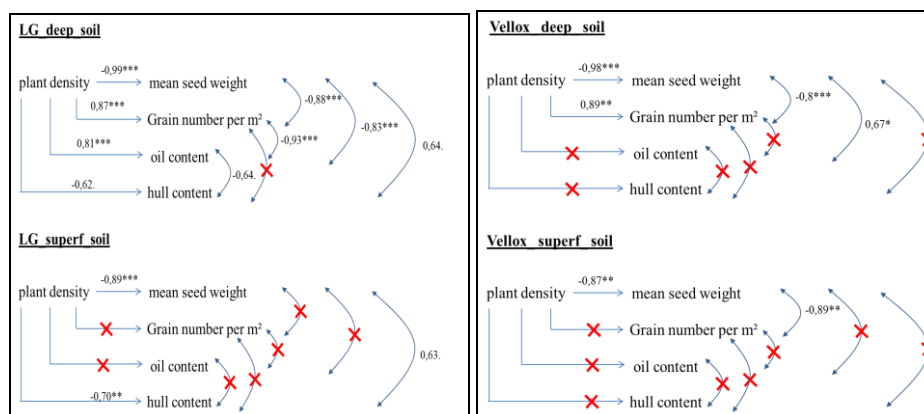


Fig.1. Illustrative path diagrams in LG 5450 and Vellox genotypes, linking plant density and oil content with 4 other variables: mean seed weight, grain number per m², oil content and hull content. Significant correlation coefficients are indicated next to the arrows. Non-significant relationships are marked by a cross.

3.1.2. Vellox genotype in deep and superficial soils (Fig.1)

Whether on deep or shallow soil, no significant correlation could be detected between plant density and oil content in Vellox. On deep soil, oil content correlated only to mean seed weight ($r=0,67$; $p=0,06$); this relationship was not detected on shallow soil ($r=0,31$; $p=0,41$). In addition, plant density was negatively related to mean seed weight ($r=-0,98$; $p<0,001$) and LAImax ($r=0,69$; $p=0,06$), as observed in the case of LG_deep_soil. Plant density correlated positively to grain number per m² ($r=0,89$; $p=0,003$) only on deep soil. These effects of plant density were also detected by the means of ANOVA ($p=1,25e-08$, 0,003 and 0,03 respectively, table not shown). In Vellox_superf_soil, grain number did not relate to mean seed weight ($r=0,05$ $p=0,89$). No other significant relationships were detected for hull content, grain number and LAD.

3.2. Agricultural survey networks.

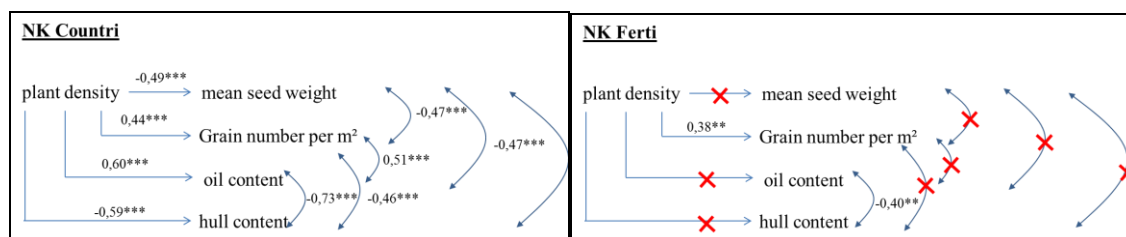
Based on whole dataset from supplying basin networks, oil content significantly correlated with plant density ($r=0,48$; $p<0,001$), hull content ($r=-0,79$; $p<0,001$), mean seed weight ($r=-0,34$; $p<0,001$) and grain number per m² ($r=0,32$; $p<0,001$). Besides, plant density negatively correlated to hull content ($r=-0,49$; $p<0,001$) and mean seed weight ($r=-0,32$; $p<0,001$) and positively to grain number per m² ($r=0,39$; $p<0,001$). Although small, mean seed weight was positively related to hull content ($r=0,14$; $p=0,05$).

We used a similar approach as for experimental trials, and separated data for each genotype.

In NK Countri genotype, oil content significantly correlated to plant density ($r=0,60$; $p<0,001$). Negative relationships were found between plant density and hull content ($r=-0,73$; $p<0,001$) and mean seed weight ($r=-0,47$; $p<0,001$); correlation was positive with grain number per m² ($r=0,51$; $p<0,001$). Mean seed weight was positively related to hull content ($r=0,22$; $p=0,02$) and negatively to grain number per m² ($r=-0,47$; $p<0,001$).

In NK Ferti genotype, oil content was not related to plant density ($r=0,10$; $p=0,56$), but only to hull content ($r=-0,40$; $p=0,01$). Plant density was correlated only to grain number per m² ($r=0,38$; $p=0,01$). Mean seed weight could not be related to grain number per m², plant density and hull content.

In PR64H32 genotype, oil content was significantly related to plant density ($r=0,30$; $p=0,04$), and strongly to hull content ($r=-0,78$; $p<0,001$). Plant density negatively correlated to mean seed weight ($r=-0,42$; $p=0,004$) and grain number per m² ($r=0,28$; $p=0,07$). Negative relationship was found between mean seed weight and grain number per m² ($r=-0,51$; $p<0,001$).



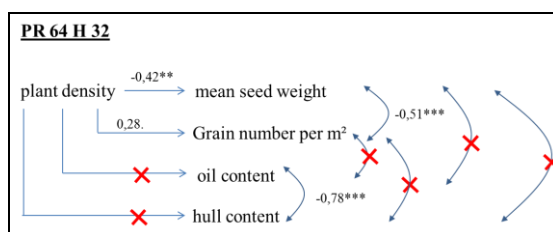


Fig.2. Illustrative path diagrams in NK Countri, NK Ferti, and PR64H32 genotypes, linking plant density and oil content with 4 other variables: mean seed weight, grain number per m², oil content and hull content. Significant correlation coefficients are indicated next to the arrows. Non-significant relationships are marked by a cross.

Observed values of plant densities were categorized into 5 classes (means for D1: 3; D2: 3,6; D3: 4,5; D4: 5,4 and D5: 6 plants per m²); the modalities the 3 genotypes shared were equally distributed in order to launch an ANOVA test. These were ranging from 3 to 5,4 plants per m² on average.

No significant effect of interactions between plant density and genotype were found on any variable (table 2); genotypic effect was dominant on oil content ($p=8,7e-13$), hull content ($p=2,7e-16$), grain number per m² ($p=0,002$) and mean seed weight ($p=9,8e-10$). Plant density effect was significant on mean seed weight ($p=0,003$) and to a lesser extent, on oil content ($p=0,06$).

Table 2. p-values (ANOVA on NK Countri, NK Ferti and PR64H32) corresponding to plant density, genotype and their interactions effect on oil content, grain number, mean seed weight, and hull proportion.

	oil content	grain number	mean seed weight	hull proportion
plant_density	0.06751	0.117507	0.003318	0.9194
genotype	8.794e-13	0.002097	9.837e-10	2.748e-16
plant_density:genotype	0.47513	0.984301	0.147309	0.6267

DISCUSSION

Plant density contribution to final oil content

Focus will be done on experimental trials first in order to comment, assess and compare the effects of plant density on final oil content, assuming all other factors being equal per trial. Taking data as a whole, we detected significant genotype, soil type, genotype x soil type and density x soil type interactions effects on seed oil content. The average amplitudes of oil content variations governed by genotype, soil type, and plant density were 3 points, 2 and 1 point respectively. This contribution of plant density could be seen as quite low compared to other determining factors. This is a actually lower but still in line with oil content variations as found in Gubbels and Dedio (1986) or Diepenbrock et al. (2001) with similar plant densities range tested (amplitude varied from 1 to 2,3 oil points depending on the genotype and the climatic year). Contribution of genotype effect on oil content was also variable (from 0,7 to 5,5 point variation). Moreover, the behavior of each genotype was not uniform across plant densities; LG 5450 increased its oil content from lowest to highest density whereas oil content in Vellox tended to decrease with increasing plant density. This highlights the need to better understand underlying processes leading to such variations of oil content.

Putative pathway of plant density effect on oil content

In this study we assumed that density effect on final oil content was mediated by chosen intermediate variables, known to respond to plant density variations and linked to oil content. The hypothesis was that establishing correlations from both sides should lead to the deduction of a common pathway linking plant density, LAD/LAI_{max}, mean seed weight, seed number, hull content and oil content. Despite varying degrees of associations, strong relationships were always found between plant density and seed weight (Robinson, 1978) and plant density and maximum leaf area index (Ferreira and Abreu, 2001). We did not find any significant relationship neither between density and leaf area duration after flowering, in contrast with Barros et al. (2004), nor between leaf area duration and oil content; this is quite surprising since leaf area duration, i.e the capacity of the crop to maintaining functional green leaves after flowering, conditions lipogenesis (Merrien, 1996).

The decrease of seed weight in response to increasing plant density is often cited in literature. Head diameter is described to be reduced under high plant densities; this would likely involve more competition for space and assimilates between seeds on the receptacle (Miller et al., 1984).

Alternatively, plant density effect could be likened at least partially to pre- and post-anthesis shadings, since it involves leaves overlapping and changes in intercepted radiation (Ferreira and Abreu, 2001). Lindström et al. (2006 ; 2007) demonstrated that both shading periods produced thinner and lighter pericarps due to a reduction in number of pericarp middle layer strata and changes in cell wall thickness. If higher plant density leads to lower pericarp and mean seed weights, it should result in higher oil content, knowing that oil mostly accumulates in the kernel part (Connor et Hall, 1997).

Unfortunately, we were unable to validate our putative processing scheme for all genotypes and environments. It is obvious that our putative scheme worked only with LG 5450 genotype in non-limiting conditions, and NK Countri genotype. The relationship between hull content and mean seed weight was not systematic; hull content could not be associated neither with plant density, nor with mean seed weight in Vellox, NK Ferti and PR64H32. Strong negative correlations between plant density and hull content were found only in LG 5450 (whatever soil type) and NK Countri. In Vellox, plant density affected mean seed weight and the latter was linked to oil content variations, but not through hull content variations. In NK Ferti and PR64H32, oil content was mainly determined by hull content, but no relationship with seed weight could be established. It would mean that factors other than plant density regulated hull content and its impact in both genotypes; these could be water stress, reported to promote higher hull content (Denis and Vear, 1994) or nitrogen status (hull contents in Vellox were highly correlated with NNI_F1 values, data not shown).

To conclude, the attempt to identify a common pathway for plant density effect on final oil content was compromised by strong genotype, soil, genotype x environment and density x environment interactions effects, resulting in a wide range of correlation degree at different steps of the putative pathway.

Hope is though permitted since we have succeeded in establishing path fragments shared by some genotypes, throughout experimental trials and supplying basin data. A better characterization of environments where genotypes are grown as well as a multi-environmental evaluation of genotypes response to plant density could help to improve our understanding of plant density contribution to oil content. Identified processes could further be used in sunflower crop models to forecast the effect of crop management on yield components, including oil content.

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