



# Source and sink indicators for determining nitrogen, plant density and genotype effects on oil and protein contents in sunflower achenes



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## ABSTRACT

Given the diversification of oilseed-based products, sunflower is a competitive crop for obtaining high oil and protein concentrations; however, both are subject to genotypic and environmental variability. We analyzed individual and interaction effects of nitrogen (N), plant density (D) and genotype (G) in a 2-year field experiment. A set of “sink” (oil, protein, and hull concentrations and quantities) and “source” (leaf area duration, nitrogen uptake, nitrogen and biomass remobilizations) indicators were measured at harvest in a split-split-plot design with contrasting nitrogen (N+: 150 kg ha<sup>-1</sup>; N-: no fertilization), plant density (D1: 3 and D2: 4.5 plants m<sup>-2</sup>) and genotype (cv. LG5451HO in 2011, cv. Olledy in 2012 and cv. Kerbel in both years) treatments. We found that nitrogen had a significant positive effect on protein concentration and plant density had a positive effect on nitrogen uptake after flowering. Oil concentration was not related to oil weight but was related to plant dry matter at flowering and biomass remobilization. Protein concentration was related to protein weight and nitrogen nutrition index at flowering and to nitrogen uptake and leaf area duration after flowering. Significant interaction effects were identified on sink (N × D, D × G) and source (N × G) indicators in the 2012 experiment, which was only partly explained by differences in initial states at flowering. In this study, the genotype that maximized oil concentration depended on nitrogen and plant density conditions, while the genotype that maximized protein concentration was the same regardless of cropping conditions. We highlight the importance of analyzing effects of determining factors on oil accumulation during grain filling.

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## 1. Introduction

Grain oil concentration is a major economic criterion for oilseeds production. The current context of high oleic variety production combined with the growing worldwide demand for proteins for human and animal consumption have encouraged farmers to add value to their oilseed productions by targeting varieties with high oil and high protein concentrations (Terres Inovia, 2015). Sunflower (*Helianthus annuus* L.) is a good compromise since its seeds are rich in oil (the highest oil concentration, ~50%, among oilseeds; Berger

et al., 2010), and its protein concentration can compete with that of rapeseed (~35%) through new dehulling methods (Peyronnet et al., 2014). However, obtaining a high oil concentration depends not only on the choice of genotype, but also on the soil and weather conditions in which sunflower is grown. From the comparison of two contrasting varieties in a multi-site study, Champolivier et al. (2011) demonstrated that variations in oil concentration related to environmental factors were greater than those related to genotypic differences. Andrianasolo et al. (2014) found that 50% of oil concentration variability was explained by the genotypic potential for oil concentration and 20% by environmental indicators, indicating that 30% of oil variability remained unexplained. They suggested that interactions between genotype, environment and management factors (G × E × M) could be included in the unexplained variability. Statistical methods have been proposed to analyze G × E × M in a wide range of species, such as wheat (Yan and Hunt, 2001), barley (Van Oosterom et al., 1993), soybean (Sudarić et al., 2006), maize

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(Kang and Gorman, 1989) or oilseed rape (Shafii and Price, 1992). De la Vega and Chapman (2001) analyzed such interactions in sunflower for grain and oil yield in Argentina. However, only a few authors have studied the influence of  $G \times E \times M$  interactions on oil and proteins concentrations in sunflower, even though both genetic (Fick and Miller, 1997; Ebrahimi et al., 2008, 2009) and environmental determinants (Bauchot and Merrien, 1988; Merrien, 1992) are well established. For protein concentration in particular, little is known except that it is inversely proportional to oil concentration (Connor and Hall, 1997). Based on this well-known antagonistic relationship, we assume that  $G \times E \times M$  interactions exist, directly or indirectly, for protein concentration. This is important for providing the most suitable  $G \times E \times M$  advice to maximize either of these concentrations.

To achieve this objective, the first step is to determine and understand the effects of individual factors, identify the most influential ones and quantify their effects and potential interactions. Usually studies deal with the analysis and the ranking of contributing factors and their potential interactions, but do not end up with rankings of genotypes or rankings of best  $G \times E \times M$  combinations, such as in maize (Hirel et al., 2001) or wheat (Barracough et al., 2014). When moving to more complex statistical analysis, nitrogen or plant density effects could not be separated from global “environmental” effects (De la Vega and Chapman, 2001).

To our knowledge, these factors' effects have not been extensively assessed in sunflower. Most studies focus on one factor at a time (Dosio et al., 2000; Aguirrezábal et al., 2003; Lindström et al., 2006; Mantese et al., 2006), or at most two factors without any attempt to quantify the effect of each factor (Angeloni et al., 2012; Echarte et al., 2013). The difficulty is that factors can influence many parts of plant organs, such as grain components, and/or related canopy functioning (carbon assimilation and remobilization). Sources, i.e. organs that provide assimilates and nitrogen for the organs demanding them – sinks – (Dordas, 2012) may both be affected.

For instance, the influence of genotype can be found at both sink and source levels. In maize, genotypic differences could be found on embryo-kernel ratio or embryo oil concentration (Tanaka and Maddonni, 2009), but also on differential leaf senescence and shoot nitrogen concentration (Pommel et al., 2006) at “source” level. In sunflower, genotype effect was found on the sink level through differences in potential oil concentrations, hull/kernel ratios (Mantese et al., 2006) and/or potential kernel oil concentrations (Aguirrezábal et al., 2009), and at the source level through differences in stay-green capacity and leaf senescence dynamics (De la Vega et al., 2011).

Another often-reported driver of oil concentration is solar radiation (Dosio et al., 2000; Aguirrezábal et al., 2003), which is partly or totally influenced by variations in plant density. Lindström et al. (2006) demonstrated direct effects of radiation availability on the relative contributions of hulls and kernels, while Rizzardì et al. (1992) demonstrated that, depending on the genotype, the influence of plant density on oil concentration was either neutral or positive. Plant density also acts on leaf area duration (Ferreira and Abreu, 2001; Barros et al., 2004), as evidenced too in maize (Borrás et al., 2003) and potentially on the availability of assimilates for grain components development.

Among factors that can be managed, the influence of nitrogen on oil concentration was rarely studied in sunflower. The effects of this factor was largely studied in other species such as wheat (Martre et al., 2003) or oilseed rape (Colnenne et al., 2002). In sunflower, nitrogen is known to increase protein concentration and to indirectly decrease oil concentration (Merrien, 1992; Connor and Hall, 1997). However, the mechanism leading to this antagonism is not clear. Čupina et al. (1992) suggested that nitrogen inhibited sugar production, while Connor and Sadras (1992) advocated for a

**Table 1**

Monthly meteorological data (global radiation, total rainfall and mean temperatures) in the 2011 and 2012 experiments.

Year	Climatic variable	April	May	June	July	August
2011	Global radiation (MJ m <sup>-2</sup> )	20.5	22.0	19.8	20.9	20.1
	Rainfall (mm)	26.0	38.5	41.0	86.5	21.5
	Mean temperature (T°C)	15.5	18.2	18.7	20.2	22.5
2012	Global radiation (MJ m <sup>-2</sup> )	13.8	21.2	22.4	22.9	20.9
	Rainfall (mm)	69.0	75.5	53.5	58.0	48.5
	Mean temperature (T°C)	11.4	16.7	20.5	21.1	23.5

diluting effect of oil concentration. At the source scale, nitrogen helps to increase green leaf surface area at flowering (Ferreira and Abreu, 2001) and leaf area duration (LAD), indicating a greater availability of assimilates for oil production. N was demonstrated to display similar effects on leaf area index (LAI) and LAD of sorghum (Borrell et al., 2001). Nitrogen's influence on source and sink components in sunflower merits further investigation.

Consequently, it appears that to determine  $G \times E \times M$  interactions, it is necessary to analyze the main factors' effects at both source and sink levels. Three scenarios are possible: (1) factors influence sources of assimilation, (2) factors influence sources of remobilization, and (3) factors directly influence the sinks themselves. The third scenario requires determining the concentrations (%) in oil and/or protein weights (g m<sup>-2</sup>) and total seed weights, assuming that a given factor can have a variable intensity and a positive or negative influence depending on the grain component considered.  $G \times E \times M$  interactions could also arise from initial differences at flowering, leading to more marked differences at harvest. For instance, Sadras et al. (1993) demonstrated that differences in genotype architecture at flowering led to differences in ability to remobilize carbohydrates during grain filling. Plants with higher biomass at flowering would probably have more potential for remobilization. It is a matter of identifying agronomic indicators that evolve in the same way as oil and protein concentrations. Such indicators at flowering are potential predictors of oil and/or protein concentrations at harvest.

The objectives of this study were to (1) identify which indicators were influenced by genotype, nitrogen and plant density (2) characterize and quantify these effects at source and sink levels through relevant indicators; and (3) identify potential early predictors of oil and protein concentrations.

## 2. Materials and methods

### 2.1. Site and experimental design

#### 2.1.1. Site characteristics

Two field experiments were conducted in 2011 and 2012 at the INRA station in Auzeville, southwestern France (43°31'41.8"N, 1°29'58.6"E). Sunflower was grown in a deep loamy soil (accessible root depth >100 cm) with a potential available water reserve of 180 mm and little or no stoniness. The crop was preceded by maize in 2010 and sorghum in 2011; residual N before sowing was 48 and 33 kg N ha<sup>-1</sup> in 2011 and 2012 respectively. Three commercial hybrids were used: cv. Kerbel (in both years), cv. LG5451HO (2011) and cv. Olledy (2012). Cultivars differed in achene oil concentration (cv. Kerbel: high-oil; cv. Olledy: medium-oil; cv. LG5451HO: low-oil) and hull/kernel ratio (higher for cv. Kerbel). Daily weather data, i.e. global radiation, rainfall, minimum and maximum temperature and potential evapotranspiration, were collected locally at a height of 2 m (Table 1).

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