Agriculture, Ecosystems and Environment 247 (2017) 225–235

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Leaf gas exchange physiology and ion homeostasis of oilseed rape (*Brassica napus* L.) under Mediterranean conditions: Associations with seed yield and quality

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ARTICLE INFO

Keywords:
Calcium
Carbon isotope discrimination
Cation ratios
Magnesium
Osmoregulation
Sodium

ABSTRACT

For two growth seasons, four oilseed rape cultivars were tested in two locations under Mediterranean conditions in order to identify leaf physiological [gas exchange and related traits, chlorophyll content (assessed by SPAD), carbon isotope discrimination (Δ), canopy area index] and elemental traits (K, Na, Ca, Mg, their sum and ratios) related to yield and quality. Determinations took place at one vegetative (stem elongation-BBCH 33 stage) and two reproductive stages (50% flowering of the central inflorescence-BBCH 65 stage and end of flowering-BBCH 69 stage). Yield and seed quality were strongly affected by growth season and location; cultivars differed only in quality traits [seed oil concentration (Oil), protein concentration (Prot), glucosinolates in seed meal (Glu), and erucic acid concentration (Eru)]. Exempting SPAD, cultivars did not also differ in physiological traits and this is a possible reason for the lack of significant differentiation in yield. Cultivars that excluded Na more effectively were those with higher SPAD. Across growth seasons and locations, heavy-textured soil, lower temperatures and high precipitation in winter resulted in higher yield and quality. Larger canopy area at BBCH 65 stage led to higher oil yield (OY). In contrast, higher CO_2 assimilation rate (at BBCH 65 stage) and SPAD (BBCH 65 and 69 stages) were indicative of stressful conditions (high temperatures and low rainfall) since these two traits were negatively correlated with OY. Moreover, SPAD at BBCH 65 stage was also negatively correlated with Oil. CO_2 assimilation rate was strongly controlled by stomata and was almost the half of that previously reported for temperate environments. In accordance with previous works, K was the most effective osmoticum accumulated in oilseed rape leaves under stressful conditions. As a result, leaf K concentration was correlated negatively with Oil and positively with protein concentration; these two quality traits are inversely affected by stressful conditions. Exclusion of Na by K (higher leaf K/Na ratio) at the reproductive stages was related to lower Oil in oilseed rape, which is a salinity tolerant species. A negative correlation found between leaf K and Ca concentrations can be indicative of a partial substitution of K by Ca, in its osmotic role, in oilseed rape grown on calcareous soils under semi-arid conditions.

1. Introduction

Oilseed rape (*Brassica napus* L.) is a major source of oil and feed protein worldwide (Berry and Spink, 2006). It is better adapted to temperate environments while is considered as a “risky” crop, in terms of low seed yield and oil content, at semi-arid regions (Del Gatto et al., 2015; Farré et al., 2007).

The poor adaptability of oilseed rape in semi-arid environments like Mediterranean is ascribed to low rainfall and/or high temperatures during growth season (Farré et al., 2007; Gunasekera et al., 2006; Robertson and Holland, 2004). Often, water shortage and thermal stress take place concurrently in semi-arid environments and are possibly flanked by salinity stress framing crops within a complex of stresses (Munns, 2002).

Thermal and water stresses affect drastically oilseed rape yield and quality when occur during a critical period, which has been set from early flowering to grain filling (Kutcher et al., 2010; Ma et al., 2006). This period is an energy-demanding growth stage for the crop due to the high energy cost of lipid biosynthesis. Factors that affect negatively CO_2 assimilation and photosynthetically active area minimize the energy budget available for seed filling and oil accumulation. A large photosynthetically active area, for as long as possible during the critical period, increase nitrogen utilization by oilseed rape contributing to higher yields (Dreccer et al., 2000; Habekotté, 1997; Kappen et al.,

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http://dx.doi.org/10.1016/j.agee.2017.06.036
Received 22 February 2017; Received in revised form 11 June 2017; Accepted 24 June 2017
0167-8809/

1998; Papantoniou et al., 2013). After anthesis, photosynthetic area is mainly consisted of growing pods, which underperform photosynthetically compared to leaves and thus, they cannot fill the photosynthetic gap of receding leaves (Gammelvind et al., 1996; Jensen et al., 1998). Additionally, drought and high temperatures alter leaf lipid metabolism affecting fatty acid composition (oleic, linolenic and erucic acids), seed protein and glucosinolates in meal, which are toxic at $>$ 20 μmol/g (Andersen et al., 1996; Benhassaine-Kesri et al., 2002; Bouchereau et al., 1996; Jensen et al., 1996; Wilmer et al., 1996).

The negative effects of water and thermal stress are more pronounced on light-textured soils (Farré et al., 2007) but it is regarded that oilseed rape can recover, with affordable losses on yield and quality, when stresses occur at early vegetative growth stages (Gan et al., 2004; Ma et al., 2006; Müller et al., 2010).

The risk of growing oilseed rape under semi-arid conditions can be mitigated by screening and selecting tolerant cultivars. To date, traits such as leaf chlorophyll content, transpirational cooling and osmoregulation (the maintenance of cell turgor by the accumulation of solutes) have been recognized as contributors to water and drought stress tolerance (Kumar and Singh, 1998; Ma et al., 2004; Majidi et al., 2015; Niknam et al., 2003; Turner and Asseng, 2005; Wright et al., 1997). These traits are strongly interconnected; organic (e.g. soluble sugars, proline) and inorganic (e.g. K^+, nitrate) solutes accumulate in guard cells maintaining turgor and keeping stomata open thus securing transpiration, which drops leaf temperature and cools canopy. As a result, CO_2 assimilation persists since photosynthetic machinery is healthy and chlorophyll remains undamaged. Moreover, *Brassica* species showed phenotypic plasticity for drought avoidance by earlier flowering, which was related to leaf-level water use efficiency (WUE, the ratio of CO_2 assimilation to water transpiration), as assessed by its long-term proxy, carbon isotope discrimination (Δ, a measure of the $^{13}C/^{12}C$ ratio in plant tissues compared with the air) (Franks, 2011). Increased WUE is considered as an important trait for drought tolerance and was found to relate with high photosynthesis and low stomatal conductance in *Brassica rapa* lines; a promising finding regarding crop improvement for drought adaptation (Edwards et al., 2011, 2012).

There is evidence that the accumulation of organic solutes, which contribute to osmoregulation, is triggered and affected by inorganic cations (K^+, Na^+, Ca^{2+}, Mg^{2+}) and reflected to Δ values (Gao et al., 2006; Tsialtas and Maslaris, 2006; Tsialtas et al., 2010). However, in oilseed rape, only K^+ was yet recognized as important osmotic factor whereas Na^+, Ca^{2+} and Mg^{2+} were designated as insignificant contributors to osmoregulation (Ma et al., 2004).

It is long known that this quartet of chemically relative metals exhibit facilitation and/or antagonism in plants; they share common mechanisms of uptake and transportation, show partial substitution in roles and actions, the excess of one can downregulate the concentration of the others, while one's deprivation can release a cascade of signaling and reactions as a countermeasure to the imbalance (Benito et al., 2014; Hermans et al., 2006; Karley and White, 2009; Maathuis, 2009; Peiter, 2011; Subbarao et al., 2003).

The complex relationship between K and its kin cations was mainly studied in works regarding *Brassica* species tolerance to salinity. *Brassica napus* is characterized as the most tolerant to salinity among the Brassicas, which, in general, tolerate salinity more efficiently than cereals and legumes (Cutforth et al., 2009; Steppuhn et al., 2001). Effective Na exclusion, maintenance of high K/Na and Ca/Na ratios and higher retention of K^+ in shoots and roots are mechanisms contributing to salinity tolerance in *Brassica* species (Ahsraf and McNeilly, 2004; Chakraborty et al., 2016a, 2016b; Rezaei et al., 2006). Since element concentration in plant tissues is a highly heritable trait (Broadley et al., 2004, 2008), screening for genotypes with improved ion homeostasis (K^+/Na^+, Ca^{2+}/Na^+, Mg^{2+}/Na^+) or modifying growing conditions (e.g. element additions) could enhance oilseed rape response to salinity (Badr et al., 2010; Collins et al., 2008; Liu et al., 2014; Rezaei et al., 2006). Yet, cation interactions and homeostasis were also found to play an important role in plant performance under non-saline, semi-arid conditions on calcareous soils. There, the high availability of soil Ca seemed to affect significantly plant responses due to a partial substitution of K role by Ca (Tsialtas et al., 2016, 2017).

Oilseed rape is a new crop in Greece and Mediterranean region as the winter type of the crop was introduced a decade ago to provide oil for biodiesel production. Thus, there is a deep lack of field data on crop performance in regard to leaf gas exchange physiology and cation homeostasis under the semi-arid, non-saline Mediterranean conditions. In the present study, four oilseed rape cultivars were tested over two growth seasons at two locations of contrasting productivity. A suite of physiological assessments [leaf chlorophyll content (SPAD), canopy area index (CAI), CO_2 assimilation rate (A), transpiration rate (E), intercellular CO_2 concentration (c_i), stomatal conductance (g_s), air-to-leaf temperature difference ($\Delta T = T_{air}$-T_{leaf}), intrinsic water use efficiency (A/g_s) and carbon isotope discrimination (Δ)] along with leaf cation measurements (K, Na, Ca, Mg) at three growth stages (stem elongation, mid-flowering, end of flowering) were employed. The aim of the study was to figure out any significant correlation between leaf physiological and elemental traits with oilseed rape yield and quality and identify the critical stages for crop performance.

2. Materials and methods

2.1. Site and experiment set up

The experimentation took place at central (Larissa; 39°41′N, 22°27′E, 70 m) and northern Greece (Xalastra; 40°38′N, 22°45′E, 6 m) during 2005–2006 and 2006–2007 growing seasons (hereafter 2006 and 2007, respectively). Certain soil characteristics and climatic conditions (monthly rainfall and mean temperature) are presented in Table 1 and Fig. 1, respectively. Both locations had alkaline, calcareous, non-saline soils with that at Larissa being of lighter texture compared to the soil at Xalastra. In 2007, winter (January and February) was milder with higher mean monthly temperatures and lower precipitation in both locations.

Three hybrids (Exact, Excalibur, Royal) and an inbred line (Fortis) were sown by hand on 21 October at Larissa and 23 October 2005 and 27 October 2006 at Xalastra. The experimental design was a randomized complete block (RCB) with four replications. Each 8 m long plot was consisted of five rows, 0.3 m apart and seeds were sown on the row

Table 1

Certain soil properties (0–30 cm depth) of the two locations before the establishment of the experiments in 2005–2006 growth season. Determinations followed Bigham (1996).

	Larissa	Xalastra
Sand (%)[a]	54	32
Silt (%)[a]	22	48
Clay (%)[a]	24	20
Texture	Sandy clay loam	Loam
pH (1:1 in H_2O)[b]	7.72	7.70
$CaCO_3$ (%)[c]	8.57	3.80
Organic matter (%)[d]	1.2	1.6
N-NO_3 (mg kg^{-1})[e]	20.8	16.9
P-Olsen (mg kg^{-1})[f]	7.36	12.7
K (mg kg^{-1})[g]	330	150
Na (mg kg^{-1})[g]	174	44
Ca (mg kg^{-1})[g]	9125	2680
Mg (mg kg^{-1})[g]	515	505
EC (mS cm^{-1})[h]	0.89	0.45

[a] Hydrometer method.
[b] By pHmeter.
[c] Volumetrically.
[d] Wet oxidation method.
[e] Extraction with KCl 2 M.
[f] Olsen method.
[g] Extraction with 1 N CH_3COONH_4.
[h] In the saturation extract by conductance meter.