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Dynamics of oil and tocopherol accumulation in sunflower grains and its impact on final oil quality

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ABSTRACT

Tocopherols are one of the most important bioactive compounds in vegetable oils. It is known that these antioxidants present a dilution like relationship with oil weight per grain but the mechanism underlying this relationship are unknown. The aim of this work was to analyze the dynamics of tocopherol accumulation in sunflower grains, its relationship with oil accumulation and its effects on final oil quality in genotypes with different fatty acid composition. Three field experiments were conducted with genotypes with different potential fatty acid composition (a traditional, a high oleic and a high stearic – high oleic) and treatments with different source (intercepted solar radiation) or sink (grains) during grain filling to obtain varied grain filling conditions and grains with different oil concentration and oil unsaturation. Intercepted solar radiation modified oil per grain but did not affect tocopherol per grain. The rate of accumulation explained 79% and 74% of the oil and tocopherol per grain variation, respectively. When intercepted solar radiation increased, the duration of the period of oil and tocopherols accumulation increased, being the first the most responsive. These differences in the duration of accumulation periods are reflected in a larger relative increase in oil than tocopherols per grain and thus a dilution of the latter in the oil. These differences in the dynamics of oil and tocopherol accumulation are common to genotypes with different level of unsaturation. These results help to understand the mechanism associated with the dilution curve of oil tocopherol concentration reported in the literature.

1. Introduction

Bioactive compounds, present in several foods, have a health benefit for human, being the tocopherols one of the most important ones in vegetable oils. These compounds protect oil from peroxidation by scavenging lipid peroxyl radicals (AOCS, 2014 Falk and Munné-Bosch, 2010; Fisk et al., 2008; Kamal-Eldin and Andersson, 1997; Sattler et al., 2003). In humans, tocopherols play an important role as vitamin E, contributing to maintaining the immune system and delaying the pathogenesis of a variety of degenerative diseases (Bramley et al., 2000). An intake of at least 0.6 mg tocopherol equivalents (tocopherols adjusted to the biological activity of α – tocopherol) per gram of polyunsaturated fatty acid is recommended for average adult humans (Belitz et al., 2009; Valk and Hornstra, 2000). In a context where adding value in origin has acquired importance for maintaining and exploring new markets, identifying, characterizing and quantifying the variability of these compounds brings knowledge to the oil industry to increase the value of edible oils. Knowing the factors that affect the synthesis of tocopherols and its relationship with other constituents of the grain could serve as a tool to select grains or oils with high concentration of these bioactive compounds and design management strategies to obtain them.

Oil tocopherols concentration presents inter – and intra – species variability. For example, Gunstone et al. (1994) reported variations in tocopherols concentration from 271 to 2188 μg/g of oil among species. Differences among genotypes were also reported (Velasco et al., 2002) and within a genotype grown under different environmental conditions (Ali et al., 2009; Anastasi et al., 2010; Velasco and Fernández-Martínez, 2012). For example, Nolasco et al. (2006) reported variation from 708 to 936 μg/g of oil for sunflower genotypes grown in a trial including 7

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locations. Izquierdo et al. (2011) observed variability in tocopherol concentration in soybean, corn and rapeseed (e.g. 1304–2732, 1304–2732 and 520–940 μg/g of oil, respectively) depending on the environment where grains were grown. It is not known the reasons that cause such variation in antioxidant oil concentration, even within genotype.

Some of the variability in oil tocopherol concentration could be explained by the source available for synthesis during grain filling (e.g. intercepted solar radiation, ISR), and its relationship with oil synthesis. An increase in ISR per plant increased the amount of tocopherol per grain (Izquierdo et al., 2011) but reduced its concentration in the oil. Therefore, there is a dilution − like response between the tocopherols concentration in sunflower oil and the amount of oil in the grain (Nolasco et al., 2004). Oil and tocopherols are synthesized from Acetyl − CoA, like an intermediary for the degradation of glucose from photosynthesis (Almeida et al., 2011; Merah et al., 2012). It is known that reductions in ISR per plant reduce oil accumulation mainly by a reduction in the duration of the accumulation period (Izquierdo et al., 2008). It is unknown however if the duration of tocopherol accumulation period is also reduced, or there is an effect of ISR on the rate of tocopherol accumulation. Knowing the effect of ISR on tocopherol accumulation and its relation with oil accumulation during grain filling could help to identify the underlying mechanisms of the dilution − like response between the tocopherols concentration in the oil and the amount of oil in the grain reported by Nolasco et al. (2004).

In sunflower, there are nowadays genotypes with different fatty acid composition (e.g. traditional, high oleic, high stearic − high oleic). These varied compositions imply different oil oxidative stability via variations in the amount of unsaturated fatty acids. For example, linoleic acid concentration vary from 32.5 to 71.9% among traditional and 0.8–20.4% among high oleic genotypes (Angeloni et al., 2016 Izquierdo and Aguirrezábal, 2008). Several authors reported associations between tocopherols and fatty acid composition in vegetables oils. For example, Gotor et al. (2015) reported in sunflower correlations of −0.70, −0.21 and 0.40, between oil tocopherol concentration and the percentage of palmitic, stearic and oleic acid, respectively. In soybean oil with low linolenic acid content, Whent et al. (2009) reported correlations of −0.38 and 0.49 between oil tocopherol concentration and the percentages of palmitic and oleic acid, respectively. The association between tocopherols and fatty acid compositions were also reported by other authors (Dolde et al., 1999; Kamal-Eldin and Andersson, 1997). Taking into account the effect of tocopherols as oil antioxidants, it is necessary to understand the mechanism while plants adjust tocopherol content in relation to the oil stability. The aim of this work is to analyze the dynamics of tocopherol accumulation in sunflower seeds and its relationship with oil accumulation and its effects on final oil tocopherols concentration in genotypes with different fatty acid composition.

2. Materials and methods

In order to analyze the dynamics of tocopherols accumulation during grain filling we performed field experiments with genotypes with different potential fatty acid composition to obtain different oil unsaturation degree (Table 1). Treatments to modify the source or sink were applied to vary grain filling conditions obtaining grains with different oil concentration (Aguirrezábal et al., 2003; Izquierdo et al., 2008) and unsaturation degree within each genotype (Izquierdo et al., 2009).

The experiments were performed in Balcarce (37°S 58°W). Sowing dates were: 9 − nov (Exp 1), 30 − oct (Exp 2) and 22 − oct (Exp 3, Table 1). In Exp 1 and 2, three genotypes were sown: a traditional (Macon, Syngenta), a high oleic (HO, Olisun 2, Advanta Seeds SAIC) and a high stearic − high oleic (HSHO, HS05, Advanta Seeds SAIC). Traditional and HSHO genotypes were also sown in Exp 3. The experiments were conducted as a split plots base on randomized complete block design with three replications, genotypes were assigned to the main plot and treatments to modify the source or sink (S − S) were assigned to the sub − plot. Each sub − plot consisted of six rows, 9 m long and 0.70 m apart, with a seed density of 7 pl/m^2. Source or sink treatments (S − S) were applied at the beginning of grain filling (R_6, Schneiter and Miller, 1981). Treatments were i) removal of 66% ($D_{66\%}$), 75% ($D_{75\%}$), 80% ($D_{80\%}$) or 100% ($D_{100\%}$) of the leaves, ii) removal of 50% of the grains ($R_{50\%}$) and iii) control (T) (Table 1). Plants phenology was recorded as Schneiter and Miller (1981). Capitula were covered with polyamide bags, before flowering, to prevent cross − pollination and accordingly to preserve the fatty acid composition of each genotype. Weeds and pests were controlled and water and nutritional stress was prevented by irrigation and fertilization. In all experiments, temperature and intercepted solar radiation were measured. The photosynthetic active radiation (PAR) intercepted per plant was measured according to Izquierdo et al. (2011). PAR intercepted per plant was accumulated from R_6 to physiological maturity. A wide range of ISR per plant was explored among treatments and experiments (Table 1).

Capitula samples were collected during grain filling, every 3–4 days. Yield components (weight per grain and grain number), oil content (Robertson and Morrison, 1979) and tocopherols content and composition (AOCS, 1998a; IUPAC, 1992) were determined in all samples. Iodine index was calculated according to (AOCS, 1998b) (Table 1) and it was being used as a measure of the degree of fatty acid unsaturation. Data were analyzed with analysis of variance (ANOVA). Differences among treatments were evaluated with Tukeýs test. Linear adjustments were performed to compare the rate and duration of accumulation of oil and tocopherols. Analyses were carried out with the R statistical package (R Core Team, 2012).

3. Results

3.1. Oil and tocopherol content per grain

Oil content per grain varied between 5.5–36.0, 13.0–30.0 and 15.0–31.7 mg for Exp 1, 2 and 3, respectively. In all experiments, oil content increased as ISR per plant increased (Fig. 1a), being $R_{50\%}$ and $D_{100\%}$ the treatments with the highest and lowest values, respectively. The HSHO had the lowest grain oil content in all experiments, (Fig. 1a) due to their lower grain weight and grain oil concentration (data not shown). Tocopherols content per grain varied between 9.4–22.1, 16.4–25.6 and 22.4–30.2 μg for Exp 1, 2 and 3, respectively. The main tocopherol was α − tocopherol followed by β − tocopherol, which represented, in average, 97% and 2% of total tocopherols, respectively. Treatments with a severe defoliation ($D_{100\%}$ and $D_{75\%}$) reduced tocopherol compared to other treatments. However, ISR per plant did not account for the variations in tocopherol per grain among treatments and experiments (Fig. 1b).

3.2. Dynamics of oil and tocopherol content per grain accumulation

Data of the dynamics of oil and total tocopherols content per grain were fitted to linear − plateau models to compare accumulation rates and the duration of accumulation period among genotypes and S − S treatments. For example, the dynamics of oil, total tocopherols, α and β tocopherol per grain for control treatment of the traditional and HO genotypes from Exp 2 are shown in Fig. 2. The accumulation of α − tocopherol accounted for 99% of the variation of total tocopherol in all genotypes evaluated (Fig. 2 b and d).

Even the rate of oil accumulation and the duration of the accumulation period widely varied among treatments, genotypes and experiments (Table 2). Defoliation treatments presented the lowest accumulation rates in all experiments. In general, the traditional genotype showed higher oil accumulation rates than HSHO in all treatments evaluated in Exp 1. The duration of oil accumulation period varied between 21 and 39 day among treatments, genotypes and experiments.

Table 1
Sowing date, name and type of hybrids, flowering date, treatment and range of ISR during grain filling (MJ/pl) and iodine index (g of iodine/100 g oil) for each experiment.

Exp	Sowing date	Hybrid		Flowering date	Treatments	ISR	Iodine index
1	11/9/09	Macon	Traditional	01/18/2010	$D_{100\%}$	7.0–46.4	119–135
		Olisun 2	HO	01/24/2010	$D_{66\%}$	11.0–47.5	83–87
		HS05	HSHO	01/18/2010	Control	3.3–37.8	68–76
					$R_{50\%}$		
2	10/30/12	Macon	Traditional	01/08/2013	$D_{75\%}$	9.9–31.3	106–134
		Olisun 2	HO	01/15/2013	Control	8.3–29.5	78–86
		HS05	HSHO	01/08/2013	$R_{50\%}$	5.0–21.8	69–76
3	10/22/14	Macon	Traditional	01/05/2015	$D_{80\%}$	7.8–20.8	127–135
		HS05	HSHO	01/04/2015	Control	7.7–21.6	74–78

As for the rate of accumulation, defoliation treatments decreased the duration of oil accumulation period in all experiments. Traditional and HSHO presented the highest and lowest duration of oil accumulation period, respectively.

The dynamics of tocopherols accumulation also varied among treatments, genotypes and experiments. The rate of tocopherol accumulation in grains varied between 0.54 and 1.48 μg/day (Table 2). Tocopherol accumulation rates were modified by S−S treatments only in HO genotype in Exp 2, were $D_{75\%}$ presented lower rate than $R_{50\%}$. HO genotype presented the lowest rate of tocopherol accumulaion in Exp 1, except in control treatment, whereas HSHO showed the lowest value. Genotypes did not differ in the tocopherol accumulation rate in Exp 2 and 3. The duration of tocopherol accumulation period varied between 23 and 37 day. In all genotypes, $D_{100\%}$ and $D_{75\%}$ treatments decreased the duration of this period. Differences in the duration of tocopherol accumulation period among genotypes were only observed in Exp 2, where the traditional hybrid in general was the one with the longest period. There were not differences between rate or duration of total tocopherol and α−tocopherol. S−S treatment did not affect the rate and duration of β−tocopherol (data not shown).

When data from all experiments were analyzed toghether, the variation in oil content per grain among treatments and experiments was mostly explained by the rate of accumulation ($p < 0.0001$, Fig. 3). In this figure, it can be seen that only slight variations in oil content were explained by changes in duration of the oil accumulation period, where points of similar accumulation rate present different oil content. For example, in HSHO hybrid, decreases in oil accumulation rate were partially counterbalanced by an increase in duration of accumulation period. In regard to tocopherol per grain, both rate and duration of the accumulation period significantly explained the variations among treatments, genotypes and experiments ($p < 0.0001$). The accumulation rate explained 75% of tocopherol variation while duration, only 12%. In both components, variations in the duration of the accumulation period were mainly observed in those treatments with a severe defoliation ($D_{100\%}$) which induced a premature senescence (points indicated with arrows in Fig. 3).

When oil and tocopherol accumulation rates were analyzed toghether, different responses among genotypes were observed (Fig. 4). Traditional hybrid did not present any relationship between both rates. However, HO and HSHO genotypes increased tocopherol accumulation rate as oil accumulation rate increased. HSHO presented lower rate of oil accumulation than the other hybrids, but similar rates of tocopherol accumulation. In all genotypes, the durations of oil and tocopherols accumulation were positively associated. The longer the period of oil accumulation the longer the period of tocopherols accumulation. However, this relationship was not 1:1, since in general the duration of the period of oil accumulation increased more than that of tocopherols accumulation.

The difference in the duration of oil and tocopherol accumulation periods increased as ISR increased (Fig. 5). Intercepted solar radiation explained about 70% of the variation in the difference in the length of both accumulation periods for the traditional and HO hybrids. The regressions between these genotypes were coincident ($p > 0.8422$). No association was found in HSHO genotype ($p > 0.2174$). This no association could be explained because this hybrid counterbalances the increase in accumulation rate decreasing the duration (see triangles close to 15d line in Fig. 3a).

This Figure shows that at low levels of ISR per plant, the duration of the periods of oil and tocopherols accumulation are similar, but when ISR increases the period of oil accumulation also increases while the period of tocopherol accumulation does not increase. This longer oil accumulation period together with its higher accumulation rate compare to those of tocopherol accumulation results in the dilution of the tocopherols in the oil (Fig. 6).

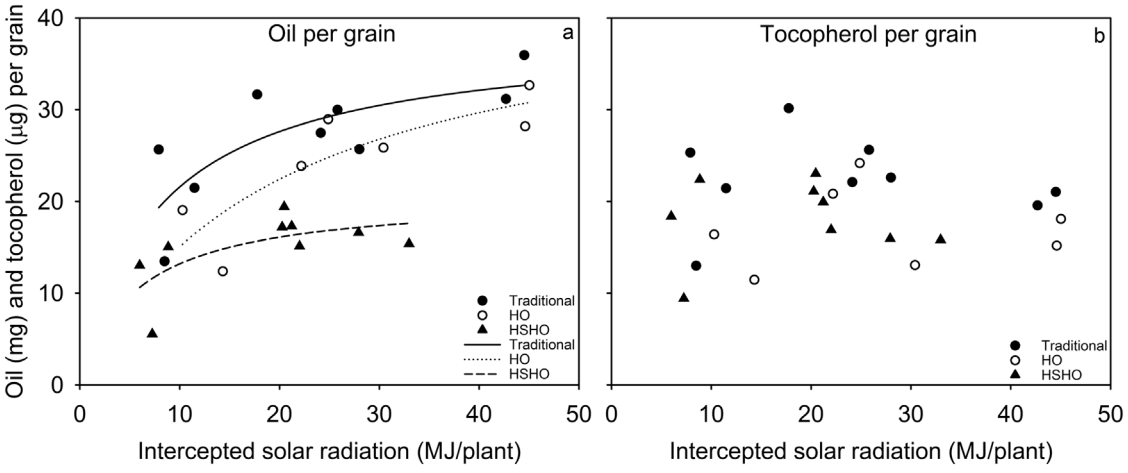

Fig. 1. Oil per grain (mg, a) and tocopherols per grain (μg, b) related to intercepted solar radiation (MJ/plant), for a traditional, a high oleic (HO) and a high stearic−high oleic (HSHO) sunflower genotype from the three experiments.

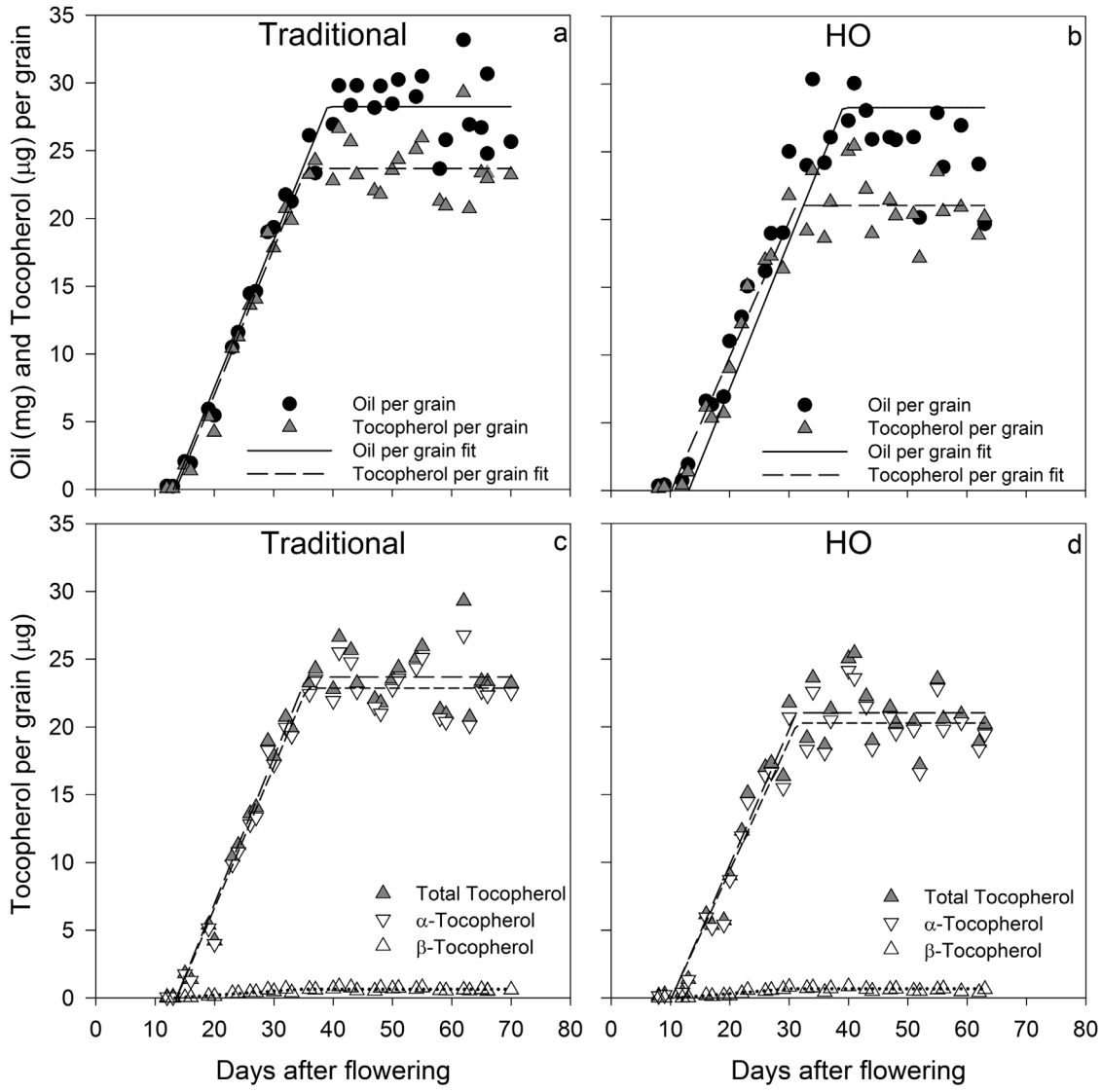

Fig. 2. Dynamics of oil and tocopherol content per grain (a and b) and tocopherols composition (c and d) for control treatment of the traditional and high oleic (HO) genotypes of Exp 2.

Table 2
Ranges of rates and durations of the periods of oil and tocopherols accumulation in sunflower seeds. The effects of source−sink treatments (S−S) and genotype are presented as * ($p < 0.05$) or NS ($p > 0.05$).

Exp		Oil per grain		Tocopherol per grain	
		Rate (mg/day)	Duration (day)	Rate (µg/day)	Duration (day)
1	Range	0.39–1.41	21–37	0.54–1.13	23–32
	S−S	*	*	*ns*	*
	Genotype	*	*	*	*ns*
2	Range	0.67–1.21	28–39	0.82–1.19	28–36
	S−S	*	*	*	*
	Genotype	*	*	*ns*	*
3	Range	0.83–1.27	30–38	0.91–1.48	30–37
	S−S	*ns*	*	*ns*	*ns*
	Genotype	*	*	*ns*	*ns*

These results could help to explain the decrease in tocopherols concentration to increases in ISR per plant and therefore the dilution ratio reported by Nolasco et al. (2004) and Izquierdo et al. (2011). As ISR increases, the duration of oil accumulation increases more than the duration of tocopherols accumulation. Thus, the amount of oil increases in a higher proportion than tocopherols, causing a dilution of this last in oil.

4. Discussion

The analyzed experiments allowed to explore very varied conditions during grain filling evidenced by the wide range of ISR per plant (3.3–47.5 MJ/plant) and oil per grain obtained (5.5–36 mg/grain) through the S−S treatments, genotypes and experiments. Under these conditions, a wide range of tocopherols per grain was observed (9.4–30.3 µg/grain). These quantities of tocopherols per grain are within the ranges reported by Anastasi et al. (2010) and Velasco and Ruiz-Méndez (2015). The variations in oil per grain were related to ISR during grain filling as reported in the literature (Andrade and Ferreiro, 1996; Dosio et al., 2000; Izquierdo et al., 2008; Ruiz and Maddonni, 2006). However, the variations in tocopherols per grain were not related to ISR per plant during grain filling. Although a severe defoliation reduced the amount of tocopherols per grain, similar to the effect of shading plants reported by Nolasco et al. (2004) and Izquierdo et al. (2011), ISR did not account for the variability in tocopherols per grain among treatments, genotypes and experiments. So, factors other than ISR or temperature (which was similar among treatments and experiments, data not shown) may account for the variability in tocopherols per grain.

The dynamics of oil and tocopherol accumulation were similar to those reported in the literature (Dong et al., 2007; Izquierdo et al.,

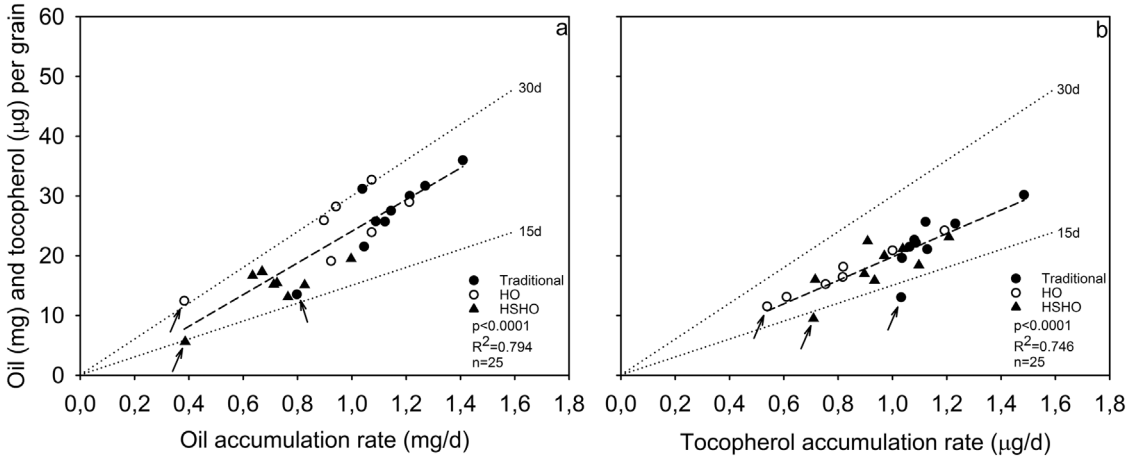

Fig. 3. Oil per grain (a) or tocopherols per grain (b) as a function of its rates of accumulation for all genotypes, treatments and experiments. Linear regressions were significant at $p < 0.05$. Dotted lines represent equal seed component accumulation durations (15 or 30 days). The arrows indicate data from $D_{100\%}$ treatment.

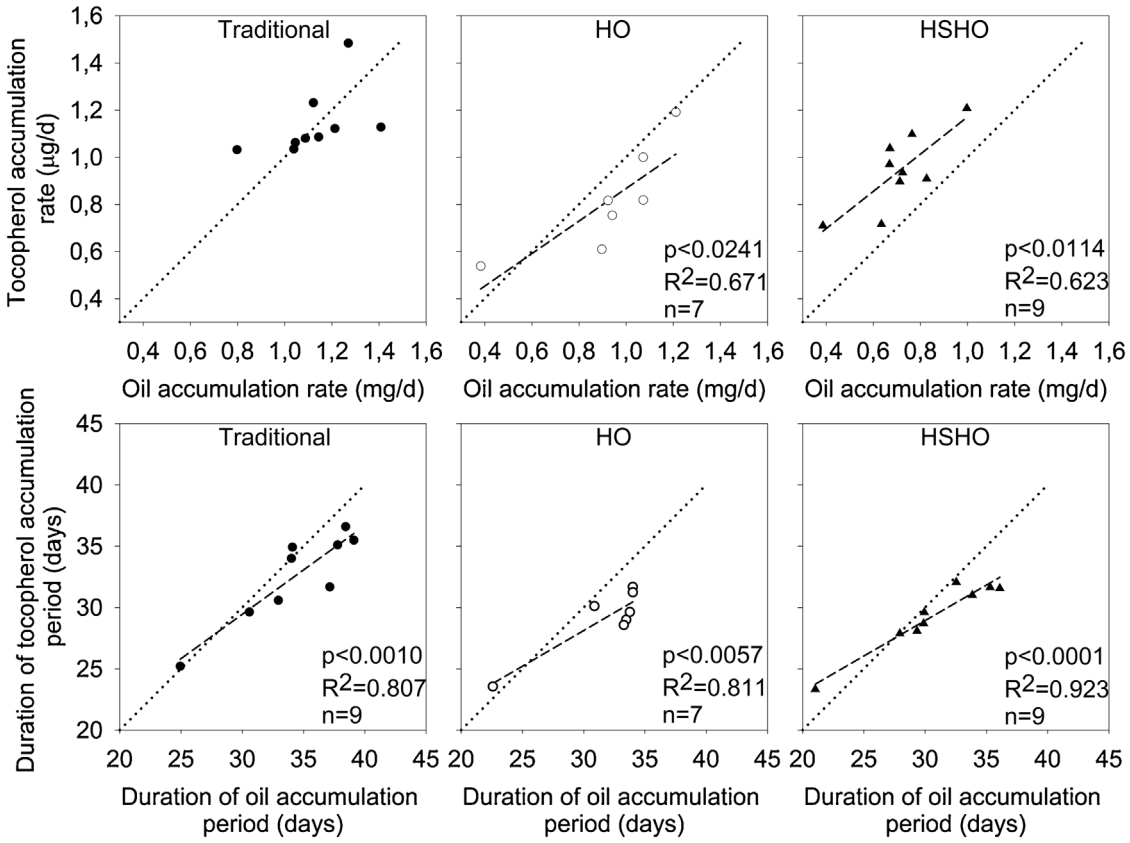

Fig. 4. Relationship between oil and tocopherol accumulation rates (upper graphs) and between the durations of the accumulation periods of oil and tocopherols (lower graphs) for the genotypes evaluated in all experiments: a traditional, a high oleic (HO) and a high stearic – high oleic (HSHO). Dotted lines represent the theoretical slopes of 1.

2008; Mantese et al., 2006; Ruiz and Maddonni, 2006). The rate of oil accumuation and the duration of the accumulation period ranged between 0.39–1.41 mg/day and 21–39 days considering all genotypes, S – S treatments and experiments. The duration of this period was similar to those reported in the literature (Rondanini et al., 2003; Ruiz and Maddonni, 2006). The oil accumulation rate in this work was higher than that reported by Rondanini et al. (2003) (close to 0.5 mg/day), probably because these authors worked with inbred lines, which have lower potential yield than the hybrids used in our experiments. In relation to the accumulation of tocopherols, results from this work agree with those from Dong et al. (2007), who observed that the period of greatest accumulation was between 12 and 33 days after anthesis, remaining constant afterwards. The effects of S – S treatments on oil accumulation dynamic were similar to those reported in the literature for this species (Izquierdo et al., 2008), but the litte or lack of effect of S – S treatments on tocopherol accumulation dynamics is novel. Research on the rate and duration of accumulation period is scarce and no studies have found a relationship between tocopherol and oil accumulation dynamics.

In several genotypes of sunflower it was reported that increasing source (e.g. ISR per plant) increased mainly the rate of grain weight accumulation (Andrade and Ferreiro, 1996; Izquierdo et al., 2008). As oil is the major reserve component of sunflower grains, consequently, the rate of oil accumulation would increase with higher ISR per plant.

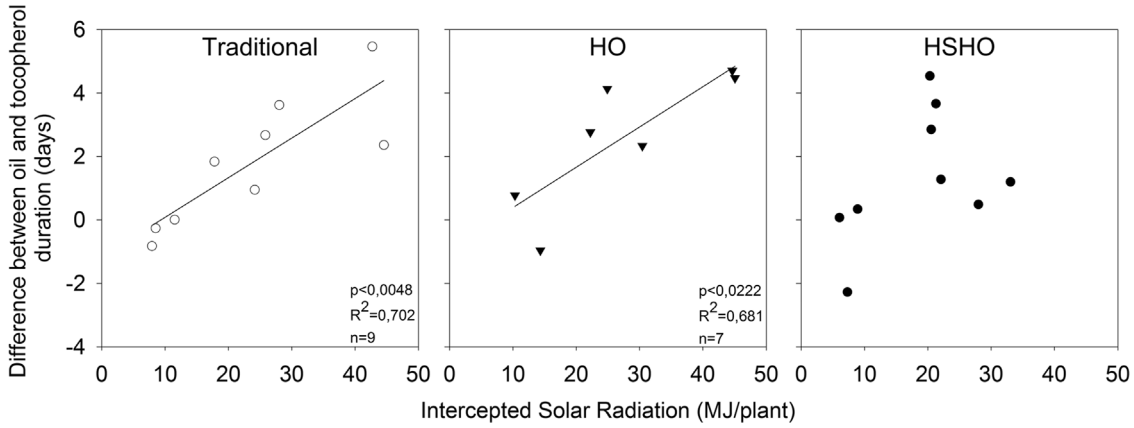

Fig. 5. Difference in the duration of the accumulation period of oil and tocopherol as a function of intercepted solar radiation per plant during grain filling for the traditional, high oleic (HO) and high stearic – high oleic (HSHO) genotypes in all experiments.

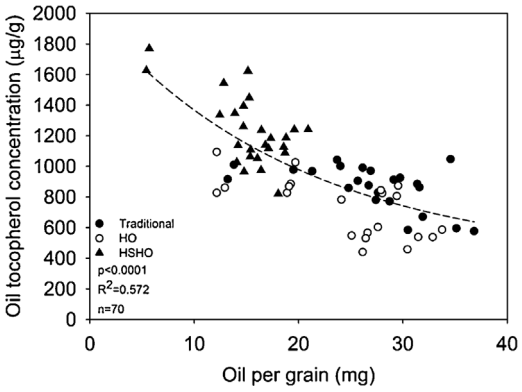

Fig. 6. Oil tocopherol concentration as a function of oil content per grain for a traditional, a high oleic (HO) and a high stearic – high oleic (HSHO) sunflower genotype in all experiments.

In this sense, our experiments show that the oil accumulation rate explained more than 79% of the variation in oil per grain, in agreement with the literature (López Pereira et al., 1999; Satorre et al., 2010). These results are in accordance also with those reported for soybean by Rotundo et al. (2011) where the accumulation rate explained 74% of oil content variation. As was mentioned by these authors, a slight counterbalance of duration could occur when accumulation rate decreased (Fig. 3a). Similarly to oil accumulation, the rate of tocopherol accumulation explained more than 74% of the variation of tocopherol content per grain. Duration of tocopherol accumulation only decreased with a severe restriction of source as a total leaf removal ($D_{100\%}$). If that extreme treatment is removed from the analysis, accumulation rate would explain 85% of tocopherol per grain variations.

The dynamics of oil and tocopherol accumulation were related. When oil accumulation rate increased, the rate of tocopherol accumulation also increased. However, an increase in the duration of oil accumulation was not accompanied by a similar increase in the duration of tocopherols accumulation. The difference in the duration of both accumulation periods was greater when ISR per plant increased. It is known that ISR per plant increases the duration of oil accumulation (Andrade and Ferreiro, 1996; Izquierdo et al., 2008), but its little effect on the duration of the period of tocopherol accumulation is a novel result. Those differences in the duration of both accumulation periods can be explained considering that oil is mainly a storage compound while tocopherols are structural components of the cells (Hussain et al., 2013; Li et al., 2008). In oil seeds, it is known that tocopherols are intrinsic components of oil bodies, the specialized structures where lipids are stored as reported by Fisk et al. (2006). These authors reported that 38% tocopherol content in sunflower seeds was associated with oil bodies. In soybean, γ – tocopherol, the tocopherol isoform with greatest concentration in this specie, was found more closely associated with oil bodies than the other tocopherols isoforms (Fisk and Gray, 2011).

When the amount of assimilation increases (e.g. ISR per plant), the oil accumulation increases more than tocopherol and therefore, the oil tocopherol concentration decreases (Izquierdo et al., 2011; Nolasco et al., 2004). This dilution – like relationship between oil content and tocopherol concentration was also reported in soybean and rape (Izquierdo et al., 2011). It would be interesting to analyze the dynamics of oil and tocopherol accumulation in these species to confirm that the mechanisms that produce this dilution effect are similar to those observed in sunflower.

The traditional and high oleic genotypes present higher oil yield potential than the high stearic – high oleic one (Martínez et al., 2012), which in general did not present lower tocopherol per grain than the others (Fig. 1b). Also, the three genotypes present very different oil fatty acid composition (iodine index ranging from 68 to 135 g of iodine/100 g of oil) and consequently different susceptibility to oxidation. Given that tocopherols are amphiphilic and are therefore typically located at membrane interfaces (i.e. oil body), these compounds will increase oxidative stability due to their spatial location close to the point of initiation of oxidation (the oil/water interface), thereby reducing oil body – to – oil body propagation (Fisk and Gray, 2011). So, it is possible that tocopherol content is more related to cell size or size and number of oil body than the amount of assimilation during grain filling. This possible association between tocopherols and grain anatomy needs further research.

The effect of different management practices (e.g. sowing date, row spacing, fertilization, irrigation, etc.) on tocopherols in soybean was reported (Carrera and Seguin, 2016; Seguin et al., 2010). According to our results crop management practices will also affect tocopherols in sunflower but mainly via variations in oil synthesis. It is widely known that crop management practices as sowing date, plant density and row spacing, fertilization, irrigation, among other decisions, affect oil synthesis and the crop yield (Andrade 1996; Aguirrezábal et al., 2003Andrade 1996; Aguirrezábal et al., 2003), mainly by affecting leaf development and solar radiation interception (Ruiz and Maddonni, 2006), the main source for oil synthesis. This increase in radiation benefits more the oil synthesis than the tocopherols synthesis, producing the dilution of these compounds in the oil. High yield and high tocopherol concentration could only be obtained by increasing grain number per plant as proposed by Aguirrezábal et al. (2009) and Peper et al. (2007). Sunflower grains from low productivity environments may be of interest for industry as their oil will present high tocopherol concentration (Nolasco et al., 2004) representing an added value in origin.

5. Conclusion

The variability in tocopherols per grain was mainly explained by variations in the rate of accumulation rather than by the duration of the accumulation period. Source (ISR) increase had an effect in increasing the duration of the period of oil and tocopherols accumulation, being the first the most responsive. These differences in the duration of accumulation are reflected in a larger relative increase in oil than in tocopherols, which results in a dilution of the latter in the extracted oil. Those mechanisms of tocopherol accumulation are common to genotypes with different iodine value. These results help to understand the mechanism associated to the dilution curve of oil tocopherol concentration reported in the literature.

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