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Germination responses to temperature and water potential as affected by seed oil composition in sunflower

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ABSTRACT

Sunflower genotypes with increased concentration of stearic and oleic acids have been developed to fulfil industrial specific requirements. Fatty acid composition of membranes has been widely described to affect seed germination, but the effect of composition of reserve lipids on germination is still unknown. Two experiments were conducted including: traditional (high linoleic), high stearic–high oleic, high stearic–high linoleic and high oleic genotypes. Thermal and hydrotime analyses were performed on germination data obtained from the incubation of seeds at different temperatures and water potentials. Fatty acid composition affected the parameters values of thermal time model. Base temperature was inversely related to linoleic acid concentration. Genotypes with high linoleic acid concentration germinated earlier at low temperatures, while no significant differences among genotypes at temperatures above 15 °C were found. High stearic–high oleic genotypes showed lower thermal time requirement for 50% germination than traditional and high stearic–high linoleic genotypes. The parameters of hydrotime model were not affected by fatty acid composition, but at low water potentials and high temperatures thermo-dormancy was induced and strongly reduced final seed germination in all genotypes studied. In conclusion, breeding for higher oleic acid and lower linoleic acid concentration in sunflower oil may affect seed germination performance at low temperatures.

1. Introduction

As a consequence of genetic breeding for improving oil quality for different uses in industry, sunflower genotypes exist with different oil fatty acid composition. These genotypes have mutations that modify the expression of enzymes involved in fatty acid synthesis (Tarrago-Trani et al., 2006). Examples of these genotypes are high oleic (HO) and high stearic–high linoleic (HSHL) genotypes with >80% and 25–30% of oleic and stearic acids, respectively (Fernandez-Moya et al., 2000) and the high stearic–high oleic (HSHO) genotypes, with 15% and 60% of stearic and oleic acids, respectively (CAA, 2011). In general, genotypes with those mutations store oil with a lower unsaturation level than traditional ones (Fernandez-Moya et al., 2000; Pleite et al., 2006). This lower unsaturation occurs by increasing stearic (saturated) or oleic (monounsaturated) fatty acid concentrations and by reducing the concentration of linoleic acid (polyunsaturated).

Germination is one of the most important events in crop production. A uniform stand of plants is essential to achieve high yields. Temperature (T) and water potential (Ψ) are the most important external factors that affect seed germination rate and germination percentage (Alvarado and Bradford, 2002; Bewley and Black, 1994). To describe the response of seed lots germination to temperature and water potential, Garcia-Huidobro et al. (1982) and Gummerson (1986) developed the thermal time and hydrotime models, respectively. The thermal time model defines two parameters: base temperature (T_b) and thermal time ($\theta_{T(g)}$). Base temperature is the minimum temperature that allows seeds to germinate. Thermal time is the cumulative temperature (°Cd), above

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T_b, required for germination to achieve percentile g of a population (Eq. (1)). This model allows characterizing the germination time course curve for a seed population through Eq. (2).

$$\theta_{T(g)} = (T - T_b)t_g \qquad \frac{1}{t_{(g)}} = \frac{T - T_g}{\theta_{T(g)}} \tag{1}$$

$$probit(g) = K + \frac{(T - T_b)t_g}{\sigma} \tag{2}$$

where K is an intercept constant when thermal time is zero, t_g is the time to reach percentile g, and σ is the standard deviation of thermal time (Ellis et al., 1986). This model is applied in the suboptimal temperature range, between T_b and T_o (T_o is the temperature where the germination rate is maximum), where germination rate linearly increases with temperature.

The hydrotime model also defines two parameters: base water potential ($\Psi_{b(g)}$) and hydrotime (θ_H). The base water potential is the theoretical threshold that will prevent germination of fraction g. Hydrotime is the cumulative water potential (MPad) above $\Psi_{b(g)}$, required for germination (Eq. (3)). The time course curve can be characterized by a probit transformation (Eq. (4))

$$\theta = [\Psi - \Psi_{b(g)}]t_g \qquad \frac{1}{t_g} = \frac{\Psi - \Psi_{b(g)}}{\theta_H} \tag{3}$$

$$probit(g) = \frac{(\Psi - \theta_H/t_g) - \Psi_{b(50)}}{\sigma_{\Psi b}} \tag{4}$$

where $\Psi_{b(50)}$ is the mean base water potential and $\sigma_{\Psi b}$ is the standard deviation of $\Psi_{b(50)}$. The parameters of these models are commonly used to characterize and compare seed germination of different species and genotypes within a species (Alvarado and Bradford, 2002; Bradford, 2002; Cheng and Bradford, 1999; Windauer et al., 2012).

The ability of seeds to germinate at different temperatures or water potentials depends on their composition (Bewley and Black, 1994). Fatty acid composition of cell membranes may affect their functionality, and ultimately seed germination. At low temperature, a high proportion of polyunsaturated fatty acids maintain membranes fluidity, thus allowing sustained activity of membrane-bound enzymes (Nishida and Murata, 1996). However, while the effects of membrane composition on seed germination are well known (e.g. Nishida and Murata, 1996; Steim et al., 1969; Wolfe, 1978), possible effects of fatty acid composition of the reserve lipids on seed germination at different temperatures remain almost unexplored. This is particularly relevant for oleaginous seeds such as sunflower, in which reserve lipids can reach concentrations as high as 38–53% (de la Vega et al., 2007; Izquierdo et al., 2008).

In sunflower, available studies about the effect of oil fatty acid composition on germination ability are scarce and contradictory. A negative linear relationship between mean time to germination and linoleic acid concentration was observed in seeds incubated at 4 °C (Downes, 1985), but Murcia et al. (2006) did not find any relationship between oleic and linoleic acid concentrations and mean germination time. Also, there are no studies linking fatty acid composition of sunflower seeds with germination response to water potential and possible interactions with temperature. Given the large variability of unsaturation of reserve lipids in sunflower seeds, it is necessary to know the effects of fatty composition of these reserves on seed germination. The aim of this work was to evaluate the effect of variations in sunflower seed reserve lipids composition on its ability to germinate under different temperatures and water potentials by analysing how changes in the concentration of different fatty acids modify parameters values of thermal time and hydrotime models of seed germination.

2. Materials and methods

Two experiments were carried out using genotypes with modified oil fatty acid composition. A high stearic–high oleic hybrid and a line (HSHO – H_1 and HSHO – L_1) and a high stearic–high linoleic hybrid and two lines (HSHL – H_2, HSHL – L_2 and HSHL – L_3) were used in Experiment 1. A HSHO (HSHO – H_3) and a high oleic (HO – H_4) hybrid were used in Experiment 2 (Table 1). The high oleic and the high stearic traits are given by the Pervenets and CAS 3 mutations, respectively (Osorio et al., 1995; Soldatov, 1976). Traditional genotypes (with no mutations) were also included in both experiments (Traditional – T_1 and Traditional – T_2). All genotypes were provided by Advanta Semillas SAIC, except for the Traditional – T_1 that was provided by Asociación de Cooperativas Argentinas. Oil fatty acid composition of seeds was determined by gas chromatography using a Shimadzu GC-2014 (Kyoto, Japan) chromatograph coupled with FID detector. Oil extraction and methylation were performed following the technique proposed by Sukhija and Palmquist (1988). Each fatty acid was expressed as a percentage of the total fatty acids identified in the oil (Table 1).

In both experiments, seed germination was evaluated with three replications in darkness, placing 25 seeds in a 9 cm diameter Petri dish containing four layers of tissue paper. Prior to each experiment, seeds were cleaned with 2% sodium hypochlorite solution for 30–60 s and washed with distilled water five times. Seeds in Experiment 1 were incubated at 5, 10, 13, 16, 22, 27, 30, 34, 38 and 40 °C and moistened with 11 mL distilled water (water potential: 0 MPa). Seeds in Experiment 2 were incubated at 5, 10, 18, 21, 24, 30, 34, 38 and 40 °C and moistened with 11 mL of distilled water or solutions of polyethylene glycol (PEG 6000) of −0.3, −0.6, −0.9 and −1.2 MPa (Michel and Kaufmann, 1973). All dishes were periodically re-irrigated to avoid desiccation. Germination was recorded at the protrusion of the radicle (3 mm) and it was monitored every 12 h at the beginning of incubation, or every 24 h

Table 1
Oil fatty acid composition of traditional, high stearic–high oleic (HSHO), high stearic–high linoleic (HSHL) and high oleic (HO) genotypes.

Exp.	ID	Genotype	Type	Fatty acid concentration (%)			
				Palmitic	Stearic	Oleic	Linoleic
1	Traditional – T_1	ACA 885	Traditional	7.1	2.9	17.1	72.9
	HSHO – H_1	E0 801	HSHO	6.1	21.4	65.6	5.1
	HSHO – L_1	ADV 3816	HSHO	6.5	20.5	62.5	8.6
	HSHL – H_2	ADV 1504/ADV 3512	HSHL	7.2	22.3	11	58.3
	HSHL – L_2	ADV 3512	HSHL	6.2	25.1	10.1	56.9
	HSHL – L_3	ADV 1504	HSHL	9	25.3	9.1	54.8
2	Traditional – T_2	VDH 487	Traditional	4.6	6.2	42.2	47
	HSHO – H_3	HS05	HSHO	4.4	16.2	76.4	3
	HO – H_4	Olisun 2	HO	3.2	2.5	90.8	3.5

when the germination percentage was approaching the plateau (i.e. 80% germination in most cases). Each treatment was finished when the number of germinated seeds remained constant. Tetrazolium test was performed at the end of the experiments to assess the viability of non-germinated seeds (International Seed Testing Association, 1999). The germination percentage was calculated for the viable fraction of the seed population. Sigmoidal curves were fitted to obtain the time needed to achieve 50% of maximum germination of viable seeds (t_{50}). The germination rate was calculated as $1/t_{50}$.

The values of thermal time model parameters, T_b and $\theta_{T(50)}$, were determined using repeated probit regression analysis as described by Ellis et al. (1986), for Experiment 1 and Experiment 2 at 0 MPa. The values of hydrotime model parameters, θ_H and $\Psi_{b(50)}$,were fitted using repeated probit regression (Bradford, 1990), at each temperature, for all data of Experiment 2. The effect of fatty acid composition on thermal time and hydrotime parameters was tested by ANOVA and the means were compared by Tukey test (R CORE TEAM, 2012). Association between parameters and fatty acid composition was tested by Pearson correlation.

3. Results

At 0 MPa, final seed germination ranged between 82% and 100%, and this variation was not associated with temperature within the range 5–38 °C (Figs. 1 and 2A–C). No germination was observed at temperatures above 38 °C. At lower water potentials, high temperatures (≥34 °C) strongly reduced final seed germination and this effect was stronger when water potential was lower. Under these conditions, the traditional and the HO genotypes were the most and least affected, respectively (Fig. 2). It is important to notice that the HO genotype was the only one that germinated at 34 °C/−1.2 MPa and 38 °C/−0.9 MPa. At 38 °C/−1.2 MPa no germination was observed in any genotype.

The optimum temperature for rate of germination (T_o) varied among the nine genotypes. In HSHL – H_2, Traditional – T_2, HSHO – H_3 and HO – H_4, T_o was clearly defined and had values very close to 27 °C (Fig. 3C) and 34 °C (Fig. 3E–G), with no evidence of substantial variations in these optimal values among the seeds within each population. In Traditional – T_1 and HSHO – H_1, however, T_o was more difficult to define. In Traditional – T_1, T_o was between 16 and 27 °C (Fig. 3A) and for HSHO – H_1, the germination rate increased for 30% and 40% fractions but decreased for others (Fig. 3B). At 38 °C, an unexpected high germination rate was observed in a few seed (Fig. 3A–C).

Time needed to achieve 50% of maximum germination (t_{50}) decreased as temperature increased in both experiments (Fig. 4). This decrease was pronounced between 5 and 16 °C. Genotype × temperature interaction was observed for t_{50} in Experiment 1 ($p < 0.0001$) and genotype × temperature × water potential interaction was observed in Experiment 2 ($p < 0.0001$). The greatest differences among genotypes were observed at 5 °C, where traditional genotypes showed the lowest t_{50} and thus, the fastest germination rate (Fig. 4). When water potential decreased, t_{50} sharply increased, especially when temperature was lower than 18 °C (data not shown). Time needed to achieve 50% of maximum germination of the traditional and HO genotypes was the most and least affected by low temperature and water potential, respectively.

The parameters of thermal time and hydrotime models presented statistical differences among genotypes. The ranges of T_b were different between experiments (0.8–2.7 °C and 2.4–3.3 °C for Experiment 1 and 2, respectively). In both experiments, genotypes with modified fatty acid composition presented higher T_b than traditional ones ($p < 0.001$, Fig. 5A). However, it must be noted that T_b of traditional genotypes substantially differed (0.9 °C vs. 2.4 °C for Traditional – T_1 and Traditional – T_2, respectively). Small differences in T_b were observed among genotypes with modified fatty acid composition in Experiment 1 but no differences were found in Experiment 2. Thermal time requirement for 50% germination differed among genotypes ($p < 0.0257$) and ranged between 38.5 and 54.7 °Cd and between 32.3 and 37.7 °Cd for Experiment 1 and 2, respectively (Fig. 5C). Traditional genotypes had slightly higher $\theta_{T(50)}$ than HSHO and HO genotypes but similar or lower $\theta_{T(50)}$ than HSHL genotypes.

The base water potential of genotypes Traditional – T_2 and HSHO – H_3 was fairly constant within 5–30 °C temperature range, and varied between −1.44 and −1.61 MPa (Fig. 5B). The base water potential of HO – H_4 genotype presented a larger variation, particularly at low temperature (i.e. −2.71 MPa at 5 °C vs. −1.58 MPa at 10 °C). The three genotypes increased their base water potential at temperatures higher than 30 °C. The θ_H decreased as temperature increased in all genotypes. This decrease was more pronounced at low temperature. Only at 5 °C genotypes presented different θ_H where the HO doubled the values of the other genotypes (Fig. 5D).

When T_b was plotted against oil components, a negative linear correlation was observed with palmitic or linoleic acid concentration, while a positive linear correlation was obtained with oleic acid concentration (Fig. 6). These relationships explain the differences in T_b among traditional and modified genotypes and between traditional genotypes of both experiments. No correlation was found between T_b and stearic acid concentration (not shown).

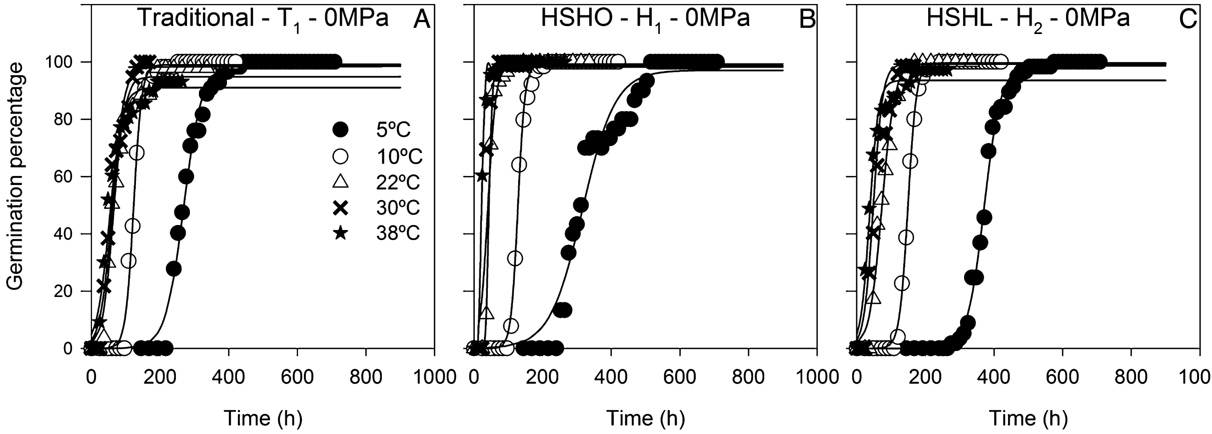

Fig. 1. Germination time course for different temperatures for the Traditional – T_1, HSHO – H_1, and HSHL – H_2, hybrids from Experiment 1 at 0 MPa. Lines correspond to sigmoidal model adjusted to experimental data and the horizontal lines are the extrapolation of the plateau.

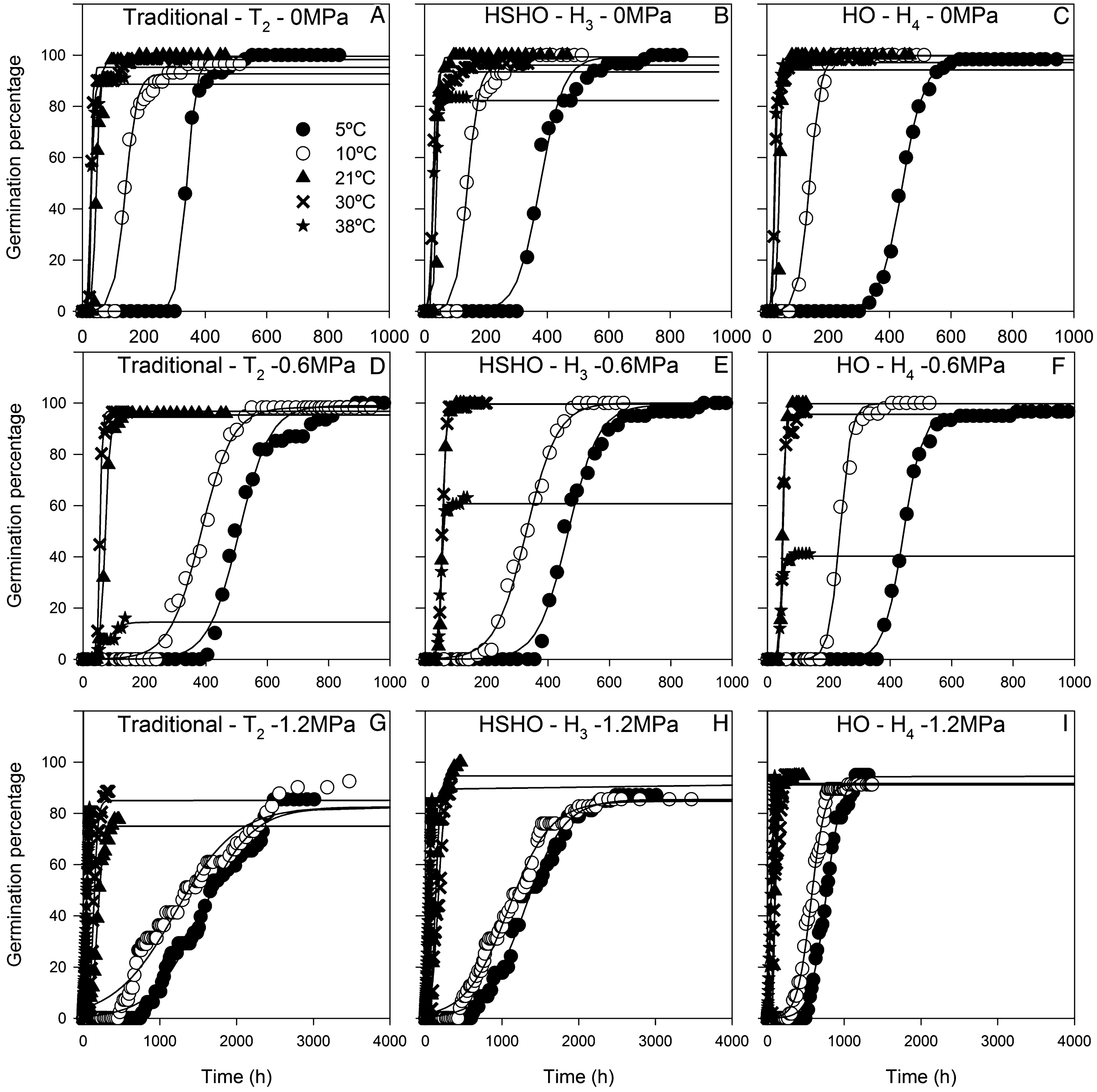

Fig. 2. Germination time course for different temperatures and water potentials for Traditional – T_2, HSHO – H_3 and HO – H_4 genotypes from Experiment 2 at 0, −0.6 and −1.2 MPa. At −1.2 MPa, Traditional – T_2 and HSHO – H_3 genotypes at 34 °C and −1.2 MPa and all genotypes at 38 °C did not germinate. Note the different scale in *x*-axis at −1.2 MPa. Lines correspond to sigmoidal model adjusted to experimental data and the horizontal lines are the extrapolation of the plateau.

The correlations between $\theta_{T(50)}$ and fatty acid concentrations were inverse to those described for T_b ($p < 0.0001$, not shown). The variations in hydrotime or base water potential were not associated with the concentration of any fatty acid.

4. Discussion

Under full water availability (0 MPa), high final germination values were obtained within the temperature range 5–38 °C, indicating lack of dormancy. However, no germination was observed at temperatures above 38 °C. This effect was not due to loss of viability, since germination percentage was expressed as the fraction of viable seeds, but was likely due to the induction of thermodormancy. Such effect was reported before for sunflower seeds by Corbineau et al. (1990, 1988), in potato seeds by Alvarado and Bradford (2002) and in *Jatropha curcas* seeds by Windauer et al. (2012).

In agreement with the thermal time model, a bilinear response of seed germination rate to temperature was observed (Alvarado and Bradford, 2002; Covell et al., 1986; Ellis et al., 1986; Garcia-Huidobro et al., 1982). Base temperature ranged between 0.7 and 3.3 °C depending on genotype. These T_b values are lower than those reported by Connor and Hall (1997) and Trudgill et al. (2000) for this species (between 3.0 and 6.9 °C). On the other hand, the T_o values observed in this work (27–34 °C) were slightly higher than that reported by Gay et al. (1991), about 25 °C, but similar to those reported by Khalifa et al. (2000) for traditional and high oleic genotypes (30–36 °C). Only Traditional – T_1 seems to present a range for T_o, similar to those found for other species (Berti and Johnson, 2008; Ellis et al., 1986). In accordance with the maximum

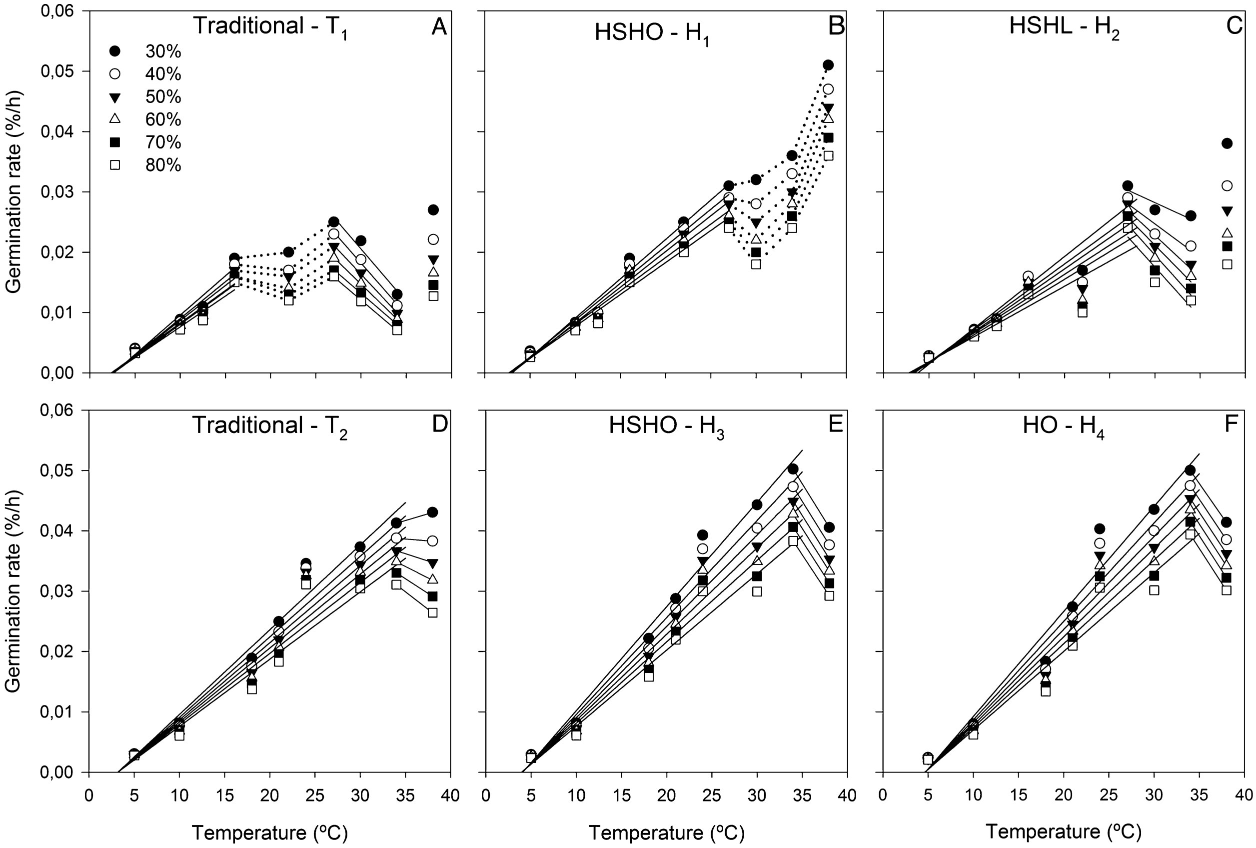

Fig. 3. Relationship between the rate of progress of seed germination to 30–80% vs. temperature for hybrids of Experiment 1 (A–C) and Experiment 2 at 0 MPa (D–F). The dotted lines correspond to the range of nonlinear response. The values at 38 °C of A and C were not taken into account because they were considered out of the range of analysis.

temperatures reported by Connor and Hall (1997) seeds incubated at 40 °C did not germinate.

Under restricted water availability, final seed germination percentage was reduced as water potential decreased, and this effect was more pronounced at higher temperatures. The reduction in final seed germination due to low water availability is in agreement with results reported by Kaya et al. (2006) in a traditional sunflower genotype. In general, the base water potential ($\Psi_{b(50)}$) was fairly constant at temperatures between 5 and 30 °C, ranging from −2.71 MPa to −1.44 MPa, but it increased at temperatures higher than 30 °C evidencing the induction of thermo-dormancy as mentioned above or the expression of dormancy (Windauer et al., 2012). Indeed, a displacement of $\Psi_{b(50)}$ towards less negative values with increases in the incubation temperature may be regarded as either expression of dormancy (Batlla et al., 2009; Windauer et al., 2012) or induction into secondary dormancy (Windauer et al., 2012), depending on the persistency of the displacement: a permanent displacement should be regarded as induction into secondary dormancy while, if it is transitory (i.e. $\Psi_{b(50)}$ comes back to its original value when incubation is performed at temperatures

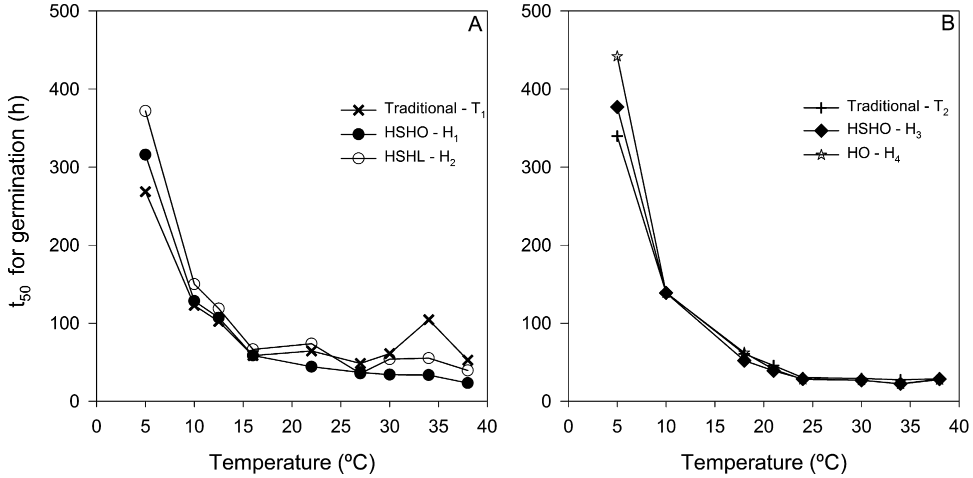

Fig. 4. Relationship between time needed to achieve 50% of maximum germination of viable seeds (t_{50}) vs. temperature for hybrids of Experiment 1 (A) and Experiment 2 at 0 MPa (B).

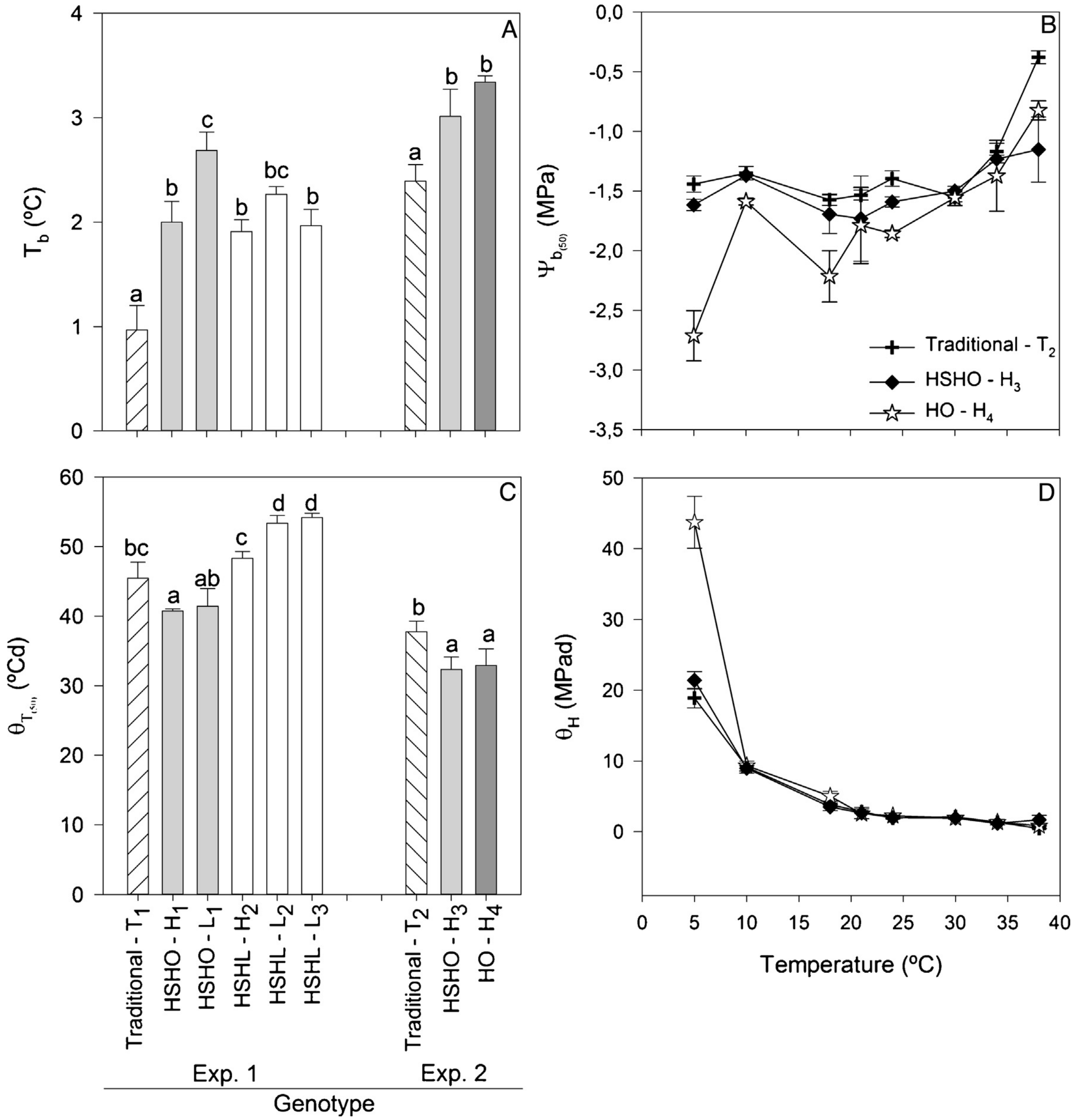

Fig. 5. (A) Base temperature (T_b) and (C) thermal time ($\theta_{T(50)}$) for genotypes in Experiment 1 and 2. (B) Base water potential ($\Psi_{b(50)}$) and (D) hydrotime (θ_H) vs. temperature for genotypes in Experiment 2. Error bars indicate SD. For each experiment, same letters indicate no significant difference (Tukey<0.05).

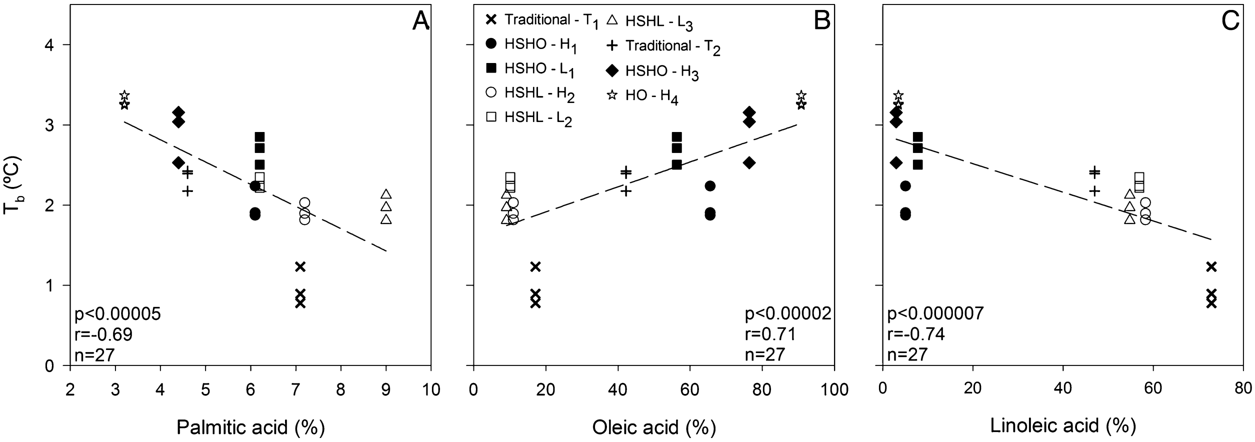

Fig. 6. Base temperature (T_b) of genotypes from Experiment 1 and 2 vs. the concentration of (A) palmitic, (B) oleic and (C) linoleic acid.

at which final germination percentage is not reduced), it should be regarded as dormancy expression (Windauer et al., 2012). We did not carry out the experiments to assess this difference.

Although general trends in the response of germination to temperature and water potential are common to all genotypes studied, significant differences were observed among them in the parameters of thermal time and hydrotime models. The variability in T_b (including that between traditional genotypes in Experiment 1 and 2) could be partially accounted for by the differences in concentration of oleic and linoleic acids (Fig. 6B and C). This correlation between T_b and reserve fatty acid composition was not been previously reported. The possible mechanisms involved in these responses include variations in membrane functionality and reserve lipids breakdown during germination.

First, it is well known that a phase transition of membrane lipids, from crystalline-liquid to solid-gel takes place when temperature decreases (Nishida and Murata, 1996; Steim et al., 1969), and this process affects membrane permeability and enzyme functionality (Wolfe, 1978). The temperature at which this change occurs depends on the lipid composition, being lower with higher lipids unsaturation. Sunflower genotypes with modified fatty acid composition have shown to present variations in both reserve and membrane lipids (Fernandez-Moya et al., 2000). Therefore, better membrane functionality at low temperatures in genotypes bearing high percentage of polyunsaturated fatty acids might be expected and this might explain the fact that T_b was inversely related to linoleic acid concentration. It should be noted that T_b also decreased when palmitic acid concentration increased. This association is unlikely to represent a mechanistic link, but may rather be an indirect consequence of the effect of linoleic acid on T_b, since both fatty acids are correlated (Izquierdo et al., 2006).

The second possibility concerns oil reserve breakdown in germinating seeds. During the early phases, reserves located near the embryo are the most important ones to provide energy to the germinating seed (Bewley and Black, 1994; Nonogaki et al., 2010; Sanchez-Linares et al., 2012). These reserves become accessible via a hydrolization catalysed by a triacylglycerol (TAG) lipase and then a β-oxidation of the free fatty acids occurs. For TAGs with linoleic acid, there is a specific linoleate 13-lipoxigenase enzyme that is capable of oxygenating linoleate still sterified to the TAG, without the preceding action of a lipid hydrolysing enzyme (Feussner et al., 1997). The oxygenated fatty acid fraction is preferentially released from the lipid bodies to undergo β-oxidation (Feussner et al., 2001). Therefore, genotypes with high concentration of linoleic acid would have an advantage during germination by presenting this preferential pathway of lipid breakdown.

The HO genotype was the only one that kept its ability to germinate even under extreme temperature and water potential conditions. The $\Psi_{b(50)}$ of this genotype was strongly reduced at 5 °C, when the other genotypes kept this parameter constant in a wide range of temperatures. This reduction in $\Psi_{b(50)}$ at low temperature was also observed by Ni and Bradford (1992) in tomato and these authors attributed such response to an accumulation of solutes that allowed seed water potential to decrease and therefore allowed the seed to be imbibed under low water availability. This could be a physiological response of some genotypes that would allow them to adapt to unfavourable conditions such as extreme temperatures (<10 °C or >30 °C) and low water availability (water potential below −0.3 MPa).

5. Conclusions

The present study shows that fatty acid composition of reserve lipids influences sunflower seed germination by affecting T_b, which was found to be inversely correlated with linoleic acid concentration. Because of this relationship, genetic improvement leading to a decrease in the unsaturation of sunflower oil may have practical consequences on seedling establishment, particularly in early sowing dates at which soil temperatures are low, and especially if combined with reduced water availability. Under these conditions, traditional genotypes would present advantages compared to less unsaturated genotypes. Modifications in fatty acid composition of reserve lipids of many oil crops have been developed by breeding (e.g. Spasibionek, 2006; McVetty and Scarth, 2002 in rapeseed; Hammond and Fehr, 1984; Wilcox et al., 1984 in soybean). The possibility that the present findings may be extrapolated to such species requires further investigation.

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