Europ. J. Agronomy 73 (2016) 25–33

Contents lists available at ScienceDirect

European Journal of Agronomy

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

A new sunflower high oleic mutation confers stable oil grain fatty acid composition across environments

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ARTICLE INFO

Article history:
Received 5 May 2015
Received in revised form 4 October 2015
Accepted 11 October 2015
Available online 11 November 2015

Keywords:
Ultra-high oleic mutant
Oil composition
Temperature
Grain filling

ABSTRACT

The oil industry demands sunflower oils with high oleic acid content. New varieties producing high oleic oils independently of the growing environment are needed as growers could receive an extra prime for offering them. Oil fatty acid composition of high oleic sunflower hybrids currently available carrying the Pervenets mutation could however be affected by the temperature during the grain filling period. A new high oleic mutation has been obtained to attain oils with ultra-high oleic levels (>90%oleic acid content). This new structural mutation would be able to reduce the variation in oleic acid percentage to changes in the minimum night temperature (MNT). The aim of this work was to assess the response of oil fatty acid composition of the new high oleic mutation to MNT compared to traditional and Pervenets genotypes. Field experiments in different sowing dates and locations and one growth chambers experiment were performed to explore a wide range of temperatures (11.8–23.2 °C) during grain filling. The oleic acid percentage in traditional and high oleic Pervenets genotypes varied between 15.0–50.9% and 87.4–91.2%, respectively, while the new mutation genotype presented values of oleic acid between 91.3 and 92.5%. Moreover, the oleic acid percentage of traditional and Pervenets genotypes showed a linear and positive response to temperature (slopes 2.95 and 0.28%oleic acid °C^{-1}, respectively). No response to temperature was detected in the new mutation genotype. The ultra-high oleic quality from the new high oleic sunflower mutant could be obtained in a wide range of environments as the fatty acid composition was not affected by temperature during grain filling, representing an advantage over the high oleic Pervenets and traditional genotypes.

1. Introduction

Fatty acid composition is the main determinant of oil quality. Final ideal sunflower oil fatty acid composition depends on its end-use (Rondanini et al., 2003). High oleic sunflower hybrids have been developed through conventional breeding with oils that approach up to 89% of oleic acid percentage (Dorrel and Vick, 1997) as compared to traditional hybrids containing less than 50% of oleic acid (Sadras and Villalobos, 1996). High oleic hybrids have been released to the seed market since 1996 to satisfy an industry demand of seed oil with high stability and nutritional value. The area sown with high oleic hybrids has been increased being nowadays about two million hectares, representing about 11% of the total sunflower area around the world (Labalette et al., 2012).

The environmental conditions, the genotype and its interaction determine oil fatty acid composition (Echarte et al., 2010). Temperature is the main factor affecting fatty acid composition in sunflower oil (Izquierdo et al., 2002; Rochester and Silver, 1983; Harris et al., 1978). Particularly, minimum night temperature (MNT) between 100 and 300° days after flowering (ddaf) was identified as the best oleic acid percentage predictor in traditional and high oleic sunflowers (Izquierdo et al., 2006; Izquierdo et al., 2002). Literature suggests that the response of the oleic acid percentage to temperature highly depends on its interaction with the genotype. All the commercial high oleic hybrids currently available carry the high oleic mutation Pervenets (Soldatov, 1976) which decreases but does not eliminate the response of oleic acid percentage to MNT

Abbreviations: bp, base pairs; ddaf, degree day after flowering; ODS, microsomal oleoyl phosphatidylcholine desaturase enzyme.

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http://dx.doi.org/10.1016/j.eja.2015.10.003
1161-0301/

compared to traditional hybrids (e.g. Izquierdo and Aguirrezábal, 2008). Oleic acid percentage of several Pervenets high oleic genotypes was not stable when grown in different environments (e.g. different locations (Van der Merwe et al., 2013) and sowing dates (Flagella et al., 2002)). Interestingly, temperature showed higher effect in oil fatty acid composition of high oleic maize and soybean genotypes than in traditional ones (Zuil et al., 2012) while no effect was found in traditional and high oleic canola (Alberio et al., 2013). Fatty acid composition in sunflower oils was also affected by other environmental factors, although in lesser extent compared to the temperature (e.g. water regime (Flagella et al., 2002), soil moisture (Baldini et al., 2003) and intercepted solar radiation (Echarte et al., 2013, 2010)). Moreover, temperature and intercepted solar radiation additively affected oil fatty acid composition in sunflower (Echarte et al., 2012) maize and soybean (Zuil et al., 2012).

Demands of oil industry with food destinations in terms of product quality are in constant increase (Labalette et al., 2012). On the one hand, ultra-high oleic sunflower oils (over 90% of oleic acid) are being demanded because of the flavour of fried products produced by these oils and its thermo-oxidative stability. These types of oils would be highly valuable for food (e.g. fried products, bakery and margarines), cosmetic (emulsions) and oleo chemic industries (e.g. ecological lubricants and plastic; Garcés, personal communication). On the other hand, since a prime over the regular price is paid to oils with an oleic acid percentage above a threshold, growers are interested in crops that reach the standard of high oleic oils and are stable in their oleic acid percentage across the growing environment (e.g. regions, sowing dates, etc.). Therefore sunflower breeders are engaged in creating genotypes producing stable ultra-high oleic oils (more than 90% with linoleic acid percentage lower than 2.5%).

A new sunflower high oleic mutation, identified as the mutant 29,066 and hereinafter called NM1, has been recently described by León et al. (2013). It involves in a gene insertion of 4872 bp at nucleotide position 201 (Zambelli et al., 2015). The consequence of the insertion was the generation of a premature stop codon producing a truncated oleate desaturase protein (ODS), accounting for the accumulation of oleic acid in grain oil at the expense of linoleic acid. Due to the nature of the mutation, a low response to MNT could be expected in genotypes carrying this mutation. On the other hand, in varieties carrying the mutation Pervenets, the high oleic acid percentage is due to a duplication affecting the 3′-end of oleoyl-phosphatidyl choline desaturase-1 (FAD2-1) gene that produces a transcription silencing and therefore an increase of oleic acid percentage when compared to traditional hybrids (Lacombe et al., 2009; Martínez-Rivas et al., 2001). The inhibitory effect of this mutation may be reversible, which could probably account for the variation of oleic acid percentage when temperature changes described for high oleic sunflower hybrids (Izquierdo and Aguirrezábal, 2008; Izquierdo et al., 2002; Triboï-Blondel et al., 2000).

The aim of this work was to assess the response of oil fatty acid composition of the new high oleic mutation NM1 to MNT as compared to traditional and Pervenets genotypes. To attain this goal, three sunflowers near isogenic lines carrying the NM1 mutation, the Pervenets mutation and a wild type were exposed to different MNT during grain filling by growing them in the field in different locations and sowing dates and in a growth chamber experiment.

2. Materials and methods

2.1. Plant material and description

Three near isogenic lines were used: one traditional and two high oleic provided by Advanta Semillas S.A.I.C. The traditional genotype was ADV 060 (T) and the high oleic genotypes were ADV 363 (P, carrying the Pervenets mutation) and ADV 032 (NM1, carrying the new high oleic mutation). These high oleic genotypes were developed by backcross conversion of the T line using as donor sunflower materials bearing Pervenets and NM1 high oleic mutations. Pervenets was developed by treating seeds from the wild type population with dimethyl-sulfate (DMS), inducing a 3′-end duplication on the oleate desaturase-1 gene (ODS-1) (Soldatov, 1976) interfering with the regulation of the transcription of the seed-specific FAD2 gene selecting for increased oleic acid sunflower (Martínez-Rivas et al., 2001). The NM1 genotype was developed by the treatment of seeds with X-rays obtaining a premature stop codon producing a truncated ODS protein and selecting for increased oleic acid sunflower (León et al., 2013). T and P genotypes were stabilized after at least six successive self-pollinated generations. The NM1 genotype was stabilized after five or six self-pollinated generations according to the growing season, 2012–13 and 2013–14, respectively.

2.2. Field and growth chambers experiments

Twelve field experiments and one under controlled condition in growth chambers (GC) were performed (Table 1).

The field experiments were conducted at the Experimental Stations of Instituto Nacional de Tecnología Agropecuaria Balcarce (UIB), Advanta Semillas S.A.I.C. Balcarce (ADV), Advanta Semillas S.A.I.C. Venado Tuerto (VT) and Instituto Nacional de Tecnología Agropecuaria Reconquista (R). In Balcarce four sowing dates were conducted at each growing season (Table 1). In all experiments, the three near isogenic lines were sown by hand with a complete randomized blocks design with two replicates. The plant density was 6 pl m^{-2}.

Nutrient and water availability were maintained by fertilizing and irrigation. Nutrient availability was measured by collecting soil samples from two depths, 0–20 cm and 20–40 cm. In Table 1 data corresponding to 0–20 cm are presented. Organic matter was measured through oxidation using the chromic acid method (Walkley and Black, 1934). Nitrate was determined according to Echeverría et al. (2000) and available phosphorous (P-Bray) was determined according to Bray and Kurtz (1945). According to Díaz-Zorita (2014) soil analysis indicated that N fertilization was not necessary in Reconquista, Venado Tuerto and Balcarce (Table 1). In the first growing season of Balcarce (UIB, 2012–13) urea (120 kg ha^{-1}) was added fifteen days after sowing. Also P concentration was high enough for the adequate development of sunflower plants in all locations and growing seasons (Table 1). Differences between replicates in each location and sowing date were not significant ($p > 0.05$, data not shown). Pest and diseases controls were applied when necessary.

Flowering of a plot was registered when 95% of the plants were at R5.1 (Schneiter and Miller, 1981). Before flowering capitula were covered with self-pollination bags. Each plant was hand-pollinated every day during five days. Ten plants per plot were harvested at physiological maturity that was determined by the brown color of bracts in the capitula (Farizo et al., 1982).

Air temperature in Balcarce was measured with Cu constantan thermocouples (Termoquar, Buenos Aires, Argentina) and data were recorded with data loggers (Cavadevices, Buenos Aires, Argentina). Air temperature from Venado Tuerto and Reconquista were obtained from meteorological stations, placed near the field experiments (<200 m). The night temperature was calculated as the average of MNT during the 100–300 ddaf period.

The GC experiment was performed to study the effect of temperature keeping constant other environmental factors. The same three genotypes were sown in pots filled with sandy-loam soil and supplied with water and nutrients as described by Izquierdo

Table 1
Location, identification of the experiment (ID), latitude (°S), genotypes (T: traditional; P: high oleic Pervenets; NM1: high oleic new mutation), sowing dates, flowering dates, mean of minimum night temperatures (MNT) during the 100–300 ddaf period and soil characteristics (organic matter, nitrate, phosphorus and type of soil from samples collected at 20 cm depth) at field experimental sites and GC experiment. T1, T2 and T3 corresponded to the treatments that were carried out in GC. N/A means "not accounted".

Location	ID	Latitude °S	Genotype	Sowing date	Flowering date	Mean of MNT during 100–300 ddaf period (°C)			Organic matter (%)	Nitrate (ppm)	Phosphorus (ppm)	Type of soil
Reconquista	R-1	29	T	09/11/12	11/19/12	19.1			2.2	11.3	39.4	Aquic Argiudol
			P		11/20/12	19.0						
			NM1		11/28/12	18.4						
Venado Tuerto	VT-1	33	T	10/19/12	12/27/12	17.5			N/A	N/A	N/A	Typic Argiudoll
			P		12/29/12	18.7						
			NM1		12/30/12	18.5						
Balcarce	ADV-1	37	T	10/25/12	1/9/13	14.7			3.8	21.7	32.5	Typic Argiudoll
			P		1/9/13	14.8						
			NM1		1/9/13	14.7						
Balcarce	UIB-1	37	T	10/30/12	1/9/13	14.7			5.8	25.4	29.7	Typic Argiudoll
			P		1/9/13	14.7						
			NM1		1/9/13	14.7						
Balcarce	ADV-2	37	T	11/28/12	02/15/13	11.9			3.8	28.0	40.7	Typic Argiudoll
			P		02/15/13	11.8						
			NM1		02/14/13	11.8						
Balcarce	UIB-2	37	T	12/11/12	2/7/13	14.8			5.8	25.4	29.7	Typic Argiudoll
			P		2/7/13	14.8						
			NM1		2/7/13	14.0						
Reconquista	R-2	29	T	09/05/13	11/21/13	19.3			2.3	10.5	14.8	Aquic Argiudoll
			P		11/22/13	19.7						
			NM1		11/26/13	19.4						
Venado Tuerto	VT-2	33	T	10/14/13	12/20/13	22.1			2.8	41.0	72.4	Typic Argiudoll
			P		12/22/13	20.9						
			NM1		12/22/13	20.9						
Balcarce	ADV-3	37	T	10/15/13	01/11/14	16.5			3.7	24.8	36.6	Typic Argiudoll
			P		02/14/14	15.7						
			NM1		02/13/14	15.7						
Balcarce	UIB-3	37	T	09/05/13	01/17/14	16.2			4.7	102.6	20.1	Typic Argiudoll
			P		01/20/14	15.9						
			NM1		01/20/14	15.9						
Balcarce	ADV-4	37	T	12/09/13	02/15/14	13.5			3.7	24.8	36.6	Typic
			P		02/16/14	13.5						Argiudoll
			NM1		02/16/14	13.5						
Balcarce	UIB-4	37	T	12/18/13	02/23/14	12.4			4.7	102.6	20.1	Typic Argiudoll
			P		02/23/14	12.4						
			NM1		02/23/14	12.4						
						T1	T2	T3				
GC			T	09/22/12	11/24/13							Sandy-loam
			P		11/23/13	13.1	17.1	23.2	8.5	N/A	69.7	Sandy-loam
			NM1		11/23/13							Sandy-loam

et al. (2002). Three seeds per pot were sown and after seedling emergence plants were thinned to one per pot. Plants were maintained in good water and nutritional conditions and were kept in a greenhouse until treatment initiation. Flowering was determined as in field experiments and self-pollination bags were also used before it. Flowering occurred the 23rd or 24th November, depending on the genotype. At flowering plants were moved to three different GCs where they were exposed to three different temperatures during the whole grain filling period. Seven plants per genotype were exposed to different day/night temperature regimes. The applied treatments were: T1 = 15/13 °C, T2 = 19/17 °C and T3 = 25/23 °C (day/night temperatures). Because the night temperature for each treatment remained constant throughout the grain filling period, it was considered the night temperature as the average of MNT during the 100–300 ddaf period for fatty acid composition calculation. Each temperature regime was achieved using GC (Refrimax S.R.L., Mar del Plata, Argentina) with 12-h photoperiod and incident photosynthetically active radiation at the top of the plants of 690 ± 75 μmol m^{-2} s^{-1} (Izquierdo et al., 2006, 2002). Although the temperature was settled by each growth chamber as described here above, temperature was also measured every 60 s and the hourly average was registered in datalogers (Cavadevices, Buenos Aires, Argentina). MNT was calculated from these data. Capitula were harvested at physiological maturity that was visually determined by the brown color of bracts in the capitula (Farizo et al., 1982).

2.3. Sample processing and chemical determinations

Oil extraction and analysis of fatty acid composition were performed as described by Ruiz-López et al. (2003). Oil fatty acid composition from seed oil was determined by gas–liquid chromatography with an Agilent 6890 gas chromatograph with FID detector (Agilent Technologies Inc., Palo Alto, CA, USA). The column used was a Supelco SP-2380 fused silica capillary column (30 m length, 0.25 mm i.d., 0.20 mm film thickness: Bellefonte, PA, USA), and hydrogen was used as the carrier gas at 28 cm s^{-1}. The detector and injector temperature was 200 °C, and the column temperature was kept at 170 °C.

2.4. Data analysis

Fatty acid composition data were analyzed by using variance procedures included in Infostat (2008). Residuals of fatty acid percentages were homogeneously distributed around zero (0) so data were not transformed. When statistical differences were detected between genotypes or locations the highest p value was presented. Treatment means were compared by Tukey test ($p < 0.05$). The different fatty acids percentages were related to temperature using linear regression. Minimum night temperature during the 100–300 ddaf period was used as independent variable to analyze the variations in oleic acid percentage in the sunflower genotypes, using a base temperature of 6 °C (Kiniry et al., 1992).

3. Results

3.1. Fatty acid composition

In field experiments the oleic acid percentage of the T genotype ranged from 15.0 to 50.9% while the average of the oleic acid percentage in P and NM1 high oleic genotypes varied from 87.4 to 91.2% and from 91.3 to 92.5%, respectively (Fig. 1), depending on the location, sowing date and growing season. Oleic acid percentage varied significantly between genotypes ($p < 0.01$) in each experiment. In T and P genotypes, oleic acid percentage differed significantly between locations in each growing season ($p < 0.001$; Fig. 1), while in NM1 genotype oleic acid percentage remained constant except at ADV-1, VT-2 and the second sowing date at Balcarce (ADV-4 and UIB-4), where the oleic acid percentage decreased compares to the second growing season ($p < 0.05$; Fig. 1). The CV of oleic acid percentage for each genotype across locations and sowing dates were 6.9% for T, 0.6% for P and 0.4% for NM1 high oleic genotypes. In the T genotype the highest oleic acid percentage was registered at Reconquista and the lowest at Balcarce in both growing seasons (UIB-2 and UIB-4). Oleic acid percentage decreased at Reconquista, Venado Tuerto and UIB-3 and increased at ADV-3, ADV-4 and UIB-4 in the second growing season (2013–14) compared to the first one ($p < 0.01$; Fig. 1). In P genotype, the highest oleic acid percentage was registered at Reconquista in the first growing season and at ADV-3 in the second one. The lowest oleic acid percentage was registered at ADV-2 and ADV-4 in the first and the second growing seasons, respectively. Oleic acid percentage decreased at Reconquista, Venado Tuerto and at UIB-4, increased at Balcarce (ADV-3 and UIB-3, $p < 0.01$; Fig. 1) and did not vary significantly at ADV-2 and ADV-4 in the second growing season compared to the first one. In the NM1 genotype, the highest oleic acid values were obtained at Venado Tuerto and UIB-3 in the first and the second growing season, respectively. The lowest oleic acid percentage was registered at ADV-1 and VT-2, respectively. Oleic acid percentage decreased at Reconquista and Venado Tuerto, increased at Balcarce (ADV-3 and UIB-3, $p < 0.01$; Fig. 1) and did not differ significantly at Balcarce (ADV-4 and UIB-4) in the second growing season compared to the first one. Despite this decline in the second growing season, oleic acid percentage in NM1 remained always above 90%.

Linoleic acid percentage in field experiments varied from 43.7 to 74.4% for T genotype, while for P and NM1 high oleic genotypes ranged between 1.9–5.1% and 1.1–1.9%, respectively (Table 2). Linoleic acid percentage differed significantly between genotypes in some experiments ($p < 0.01$, Table 2). In T genotype linoleic acid percentage differed significantly among locations in both growing season ($p < 0.001$). In P genotype the same trend was observed in both growing seasons, while in the second one, only the second sowing date from Balcarce differed significantly from the others ($p < 0.001$). In NM1 genotype linoleic acid percentage differed significantly between Balcarce and both locations Reconquista and Venado Tuerto in both growing seasons ($p < 0.05$). In T genotype, the highest and the lowest linoleic acid percentage were registered at Balcarce and Reconquista in both growing season, respectively. Linoleic acid percentage increased at Reconquista, ADV-3 and UIB-3 ($p < 0.01$), decreased at ADV-4 and UIB-4 ($p < 0.01$) and did not differed significantly at Venado Tuerto in the second growing season compared to the first one. In P genotype, the highest linoleic acid percentage was registered at ADV-4 and the lowest at VT-1. Linoleic acid percentage increased at ADV-4 and UIB-4 ($p < 0.01$), decreased at ADV-3 and UIB-3 ($p < 0.01$) and did not differed significantly at Reconquista and Venado Tuerto in the second growing season compared to the first one. In NM1 genotype, the highest linoleic acid percentage was registered at UIB-4 and the lowest at VT-1. Linoleic acid percentage increased at Venado Tuerto, ADV-4 and UIB-4 ($p < 0.01$) and did not differed significantly at Reconquista ADV-3 and UIB-3 in the second growing season compared to the first one.

Palmitic and stearic acid percentages in field experiments ranged between 3.8–4.6% and 4.1–5.7% for T, between 2.4–3.3% and 3.7–4.8% for P and between 2.3–3.7% and 2.6–3.6% for NM1, respectively (Table 2). Palmitic and stearic acids differed between locations in both growing season ($p < 0.05$) in T, P and NM1 genotypes. Palmitic acid percentage in T genotype varied significantly from P and NM1 genotypes ($p < 0.01$, Table 2). It also differed significantly between P and NM1 genotypes in Reconquista, Venado Tuerto, ADV-3, UIB-2 and UIB-3 ($p < 0.01$, Table 2). Stearic acid

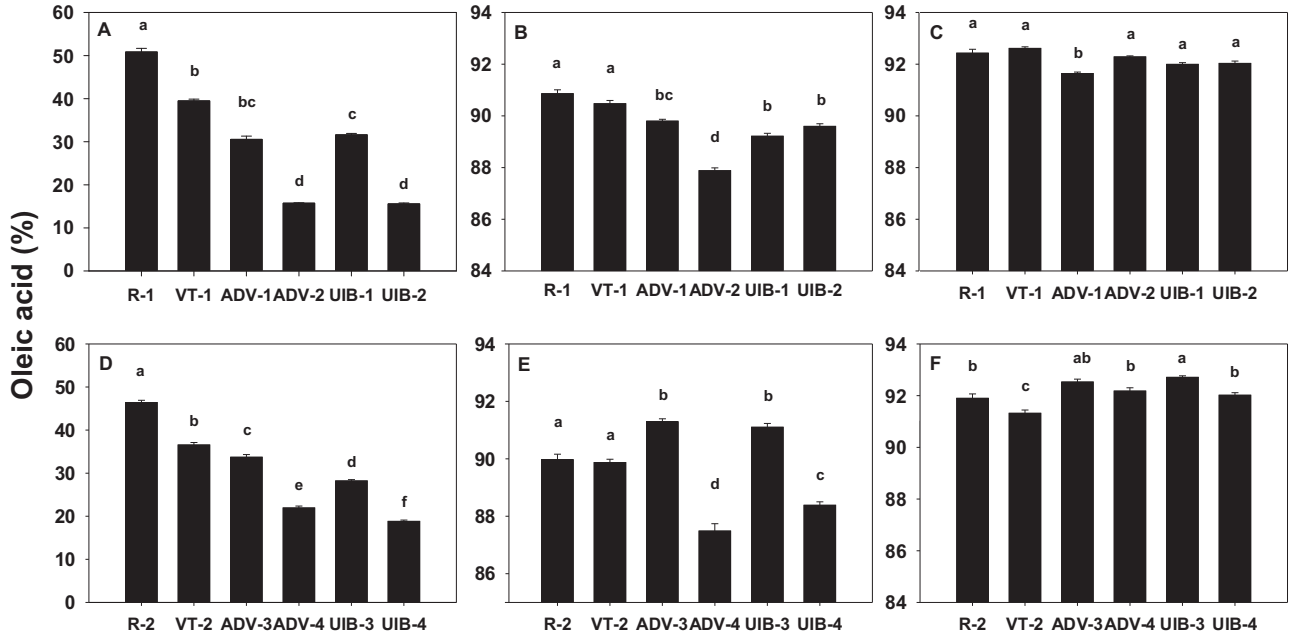

Fig. 1. Oleic acid percentage of the three sunflower genotypes, T (A and D), P (B and E) and NM1 (C and F) sown in the field in different locations, sowing dates and years. Each column represents the percentage of oleic acid in each location and/or sowing date in the first growing season (2012–13 upper pains) and in the second growing season (2013–14 bottom pains). References: Reconquista: R-1 and R-2; Venado Tuerto: VT-1 and VT-2; Balcarce: ADV-1, ADV-2, ADV-3, ADV-4, UIB-1, UIB-2, UIB-3, UIB-4 (see Section 2). Data are average of 10 plants per block (two blocks). The bars represent the standard error of the mean. For each genotype, different letters over the bars indicate significant differences ($p < 0.05$) between locations or sowing date (ANOVA and Tukey test).

Table 2
Palmitic, stearic and linoleic acid percentages of three sunflower genotypes: traditional (T), high oleic Pervenets (P) and high oleic new mutation (NM1) from the field trials for three locations and twelve different sowing dates (Reconquista: R-1 and R-2; Venado Tuerto: VT-1 and VT-2; Balcarce: ADV-1, ADV-2, ADV-3, ADV-4, UIB-1, UIB-2, UIB-3, UIB-4). Data are average of 10 plants per block (two blocks) ± SD. Different letters indicate significant differences between genotypes.

Location	ID	Sowing date	Genotype	Palmitic acid (%)	Stearic acid (%)	Linoleic acid (%)
Reconquista	R-1	09-	T	4.1 ± 0.3a	4.1 ± 0.3b	44.4 ± 3.4a
	R-1	11-	P	2.8 ± 0.2b	4.3 ± 0.4b	1.9 ± 0.3b
	R-1	2012	NM1	3.2 ± 0.1c	2.6 ± 0.3a	1.4 ± 0.2b
	R-2	09-	T	4.4 ± 0.2a	4.9 ± 0.4a	43.7 ± 2.4a
	R-2	05-	P	3.4 ± 0.2b	4.3 ± 0.5b	1.9 ± 0.1b
	R-2	2013	NM1	3.6 ± 0.1c	2.8 ± 0.4c	1.2 ± 0.5b
Venado	VT-1	10-	T	3.8 ± 0.3a	4.8 ± 0.2a	51.4 ± 2.0a
Tuerto	VT-1	19-	P	2.7 ± 0.1b	3.9 ± 0.3b	2.0 ± 0.2b
	VT-1	2012	NM1	2.9 ± 0.2c	3.0 ± 0.2c	1.1 ± 0.1b
	VT-2	10-	T	4.5 ± 0.2a	5.2 ± 0.3a	53.2 ± 2.4a
	VT-2	14-	P	3.1 ± 0.1b	4.4 ± 0.2b	2.2 ± 0.3b
	VT-2	2013	NM1	3.4 ± 0.1c	3.4 ± 0.2c	1.4 ± 0.3c
Balcarce	ADV-1	10-	T	4.1 ± 0.3a	5.1 ± 0.4a	59.9 ± 5.2a
	ADV-1	25-	P	2.5 ± 0.1b	4.1 ± 0.3b	3.1 ± 0.4b
	ADV-1	2012	NM1	3.2 ± 0.1c	3.2 ± 0.2c	1.5 ± 0.3c
	ADV-2	11-	T	4.1 ± 0.2a	5.6 ± 0.4a	74.4 ± 0.8a
	ADV-2	28-	P	2.5 ± 0.1b	4.1 ± 0.2b	3.5 ± 0.3b
	ADV-2	2012	NM1	2.6 ± 0.1b	3.6 ± 0.3c	1.4 ± 0.1c
	ADV-3	10-	T	3.8 ± 0.2a	4.5 ± 0.5a	57.6 ± 2.3a
	ADV-3	15-	P	2.4 ± 0.1b	3.7 ± 0.3b	2.1 ± 0.2b
	ADV-3	2013	NM1	2.8 ± 0.2c	2.9 ± 0.2c	1.3 ± 0.3b
	ADV-4	12-	T	4.5 ± 0.4a	5.4 ± 0.4a	67.7 ± 1.8a
	ADV-4	09-	P	2.8 ± 0.7b	4.2 ± 0.4b	5.1 ± 0.7b
	ADV-4	2013	NM1	2.7 ± 0.2b	2.9 ± 0.4c	1.8 ± 0.4c
	UIB-1	10-	T	4.2 ± 0.2a	5.6 ± 0.3a	58.1 ± 1.4a
	UIB-1	30-	P	2.9 ± 0.2b	4.8 ± 0.4b	2.6 ± 0.2b
	UIB-1	2012	NM1	2.9 ± 0.1b	3.5 ± 0.3c	1.2 ± 0.1c
	UIB-2	12-	T	4.1 ± 0.1a	5.7 ± 0.5a	74.1 ± 0.9a
	UIB-2	11-	P	2.4 ± 0.2b	4.7 ± 0.5b	4.6 ± 0.4b
	UIB-2	2012	NM1	2.3 ± 0.1c	3.5 ± 0.3c	1.6 ± 0.1c
	UIB-3	11-	T	4.1 ± 0.1a	5.0 ± 0.3a	62.2 ± 1.2a
	UIB-3	05-	P	2.7 ± 0.1b	3.6 ± 0.3b	2.2 ± 0.2b
	UIB-3	2013	NM1	2.9 ± 0.1c	2.8 ± 0.2c	1.2 ± 0.2c
	UIB-4	12-	T	4.3 ± 0.2a	5.4 ± 0.3a	71.0 ± 1.1a
	UIB-4	18-	P	2.6 ± 0.1b	3.9 ± 0.3b	4.7 ± 0.4b
	UIB-4	2013	NM1	2.6 ± 0.1b	3.1 ± 0.2c	1.9 ± 0.2c

Table 3
Palmitic, stearic and linoleic acid percentages of the three sunflower near isogenic lines: traditional (T), high oleic Pervenets (P) and a new high oleic genotype (NM1) for three treatments: T1 = 15/13 °C; T2 = 19/17 °C and; T3 = 23/21 °C (day/night temperature) from the GC experiments. Data are average of 8/9 plants per treatment ± SD. For each genotype and fatty acid, means follow by different letters are significantly different ($p < 0.05$) between temperature treatments (ANOVA and Tukey test).

		Fatty acid concentration (%)		
Genotype	Treatment	Palmitic acid	Stearic acid	Linoleic acid
Tl	15/13 °C	3.4 ± 0.3a	5.2 ± 0.5a	55.6 ± 4.2a
	19/17 °C	3.8 ± 0.2ab	4.9 ± 0.5a	33.5 ± 2.5b
	23/21 °C	3.9 ± 0.3b	3.8 ± 0.3b	39.0 ± 9.5b
P	15/13 °C	2.3 ± 0.2a	3.5 ± 0.4a	3.2 ± 0.4a
	19/17 °C	2.7 ± 0.1b	3.2 ± 0.2ab	2.3 ± 0.3b
	23/21 °C	2.7 ± 0.1b	2.8 ± 0.2b	1.5 ± 0.2c
NM1	15/13 °C	2.5 ± 0.2a	3.1 ± 0.5a	2.4 ± 0.5a
	19/17 °C	3.0 ± 0.1b	3.2 ± 0.7a	2.3 ± 0.4a
	23/21 °C	3.2 ± 0.3b	2.5 ± 0.1b	1.6 ± 0.3b

percentage in T genotypes differed from both high oleic genotypes ($p < 0.01$, Table 2) in every experiment except at R-1. In T genotype, palmitic acid increased at Reconquista, Venado Tuerto and ADV-4 ($p < 0.01$), decreased at ADV-3 ($p < 0.01$) and did not differed significantly at UIB-3 and UIB-4 in the second growing season compared to the first one. Stearic acid increased at Reconquista and Venado Tuerto and decreased at Balcarce ($p < 0.01$) and did not differ significantly at UIB-4 in the second growing season compared to the first one. In the P genotype, palmitic acid increased at Reconquista, Venado Tuerto and ADV-4 ($p < 0.01$), decreased at UIB-3 ($p < 0.01$) and did not differ at ADV-3 and UIB-4 in the second growing season compared to the first one. Stearic acid decreased at Balcarce ($p < 0.01$) and did not differ at Reconquista and Venado Tuerto in the second growing season compared to the first one. In the NM1 genotype, palmitic acid increased at Reconquista, Venado Tuerto and ADV-4 ($p < 0.01$), decreased at ADV-3 ($p < 0.01$) and did not differ at UIB-3 and UIB-4 in the second growing season compared to the first one. Stearic acid increased at Reconquista and Venado Tuerto ($p < 0.01$) and decreased at Balcarce ($p < 0.01$) in the second growing season compared to the first one.

In the GC experiment, oleic acid percentage ranged from 35.3 to 57.3% and from 90.6 to 92.4% in T and P genotypes, respectively, whereas oleic acid percentage in the NM1 genotype ranged from 91.7 to 92.4%. Differences between treatments were registered in T and P genotypes ($p < 0.001$; Fig. 2-A and B), increasing oleic acid percentage when the temperature increased. Oleic acid percentage in NM1 genotype remained constant among treatments (Fig. 2-C). The P genotype reached the same oleic acid percentage as the NM1 genotype at the highest MNT. The coefficients of variation (CV) of the oleic acid percentage for each genotype between treatments in GC were 24.0%, 1.1% and 0.7%, for the T, P and NM1 genotypes, respectively.

Other fatty acids than the oleic one were also affected by MNT (Table 3). Linoleic acid percentage varied between 33.5 and 55.6% in T genotype, while in P and NM1 genotypes varied between 1.5 and 3.2% and between 1.6 and 2.4%, respectively. Linoleic acid percentage in T and P genotypes decreased as the MNT increased ($p < 0.05$; Table 3), while in NM1 the linoleic acid differed significantly between treatments by decreasing it at the lowest MNT ($p < 0.05$; Table 3). Palmitic acid percentage increased at higher MNT in all genotypes ($p < 0.05$; Table 3) while stearic acid percentage decreased at the highest MNT treatment in all genotypes ($p < 0.01$; Table 3).

3.2. Relationships between fatty acids percentages and temperature

The minimum night temperature in the field experiments varied between 11.8 and 22.1 °C depending on the location, growing season and sowing date; in the GC experiments this temperature varied between 13.1 and 23.2 °C (Table 1).

Relationships between the oleic or linoleic acids percentages and the MNT were established for each genotype (Fig. 3). In the T and P genotypes oleic acid percentage and MNT were linearly and positively related. In these genotypes, increasing MNT increased oleic acid percentage ($p < 0.001$; Fig. 3A and C). In NM1 the oleic acid percentage was almost constant as temperature changed. The relationship between the oleic acid percentage and the MNT was not significant ($p = 0.51$; Fig. 3E). The slopes of the relations in T and P genotypes were 2.95 and 0.28 %oleic acid °C^{-1}, respectively. The inverse trend was observed for linoleic acid percentage in T and P genotypes ($p < 0.001$; Fig. 3B and D) with slopes of −2.85 and −0.29 %linoleic acid °C^{-1}. Saturated fatty acids were also affected by MNT. In the T genotype, increasing MNT decreased stearic acid percentage ($p < 0.01$, $r^2 = 0.43$), with no effect on the palmitic acid. In the P genotype increasing MNT increased palmitic acid percentage ($p < 0.05$, $r^2 = 0.28$) but did not affect stearic acid. In the NM1 genotype increasing MNT resulted in a decrease of the stearic acid and an increase in palmitic acid ($p < 0.05$, $r^2 = 0.19$ and $p < 0.001$, $r^2 = 0.57$).

Oleic and linoleic acid percentages were linearly and negatively related to each other in the three genotypes but with different slopes. The slopes for each relationship were −1.03, −1.01 and −0.54 %oleic%linoleic^{-1} acids in T ($p < 0.0001$), P ($p < 0.0001$) and NM1 ($p < 0.05$), respectively. In T and P genotypes the relationship indicates that an increase in the oleic acid percentage corresponded to a decrease of similar magnitude in linoleic acid percentage. In the NM1 genotype, the effect is reduced by half, indicating that an increase in the oleic acid percentage corresponded to a decrease of half a point of linoleic acid. The other half a point was accounted for by the decrease of total saturated fatty acids when the oleic acid increased ($p < 0.0001$; $r^2 = 0.53$; slope = −0.78 %oleic%total saturated fatty acids^{-1}).

4. Discussion

The new high oleic mutation (NM1) showed a stable oleic acid percentage in its oil independently of changes in MNT. Variations in temperature of up to 12 °C (from 11.8 to 23.2 °C) did not change the oleic acid percentage in NM1, maintaining a high oleic acid level (>91.3%). MNT affected oleic acid percentage during grain filling in T and P genotypes as reported by Izquierdo et al. (2002) for other traditional genotypes and one Pervenets genotype. The P genotype attained the same oleic acid percentage that NM1 genotype only at the highest temperatures tested, since this fatty acid decreased when the MNT decreased as it was observed by several authors for other high oleic sunflower genotypes (Grunvald et al., 2013; Izquierdo and Aguirrezábal, 2008; Flagella et al., 2002). The difference in the response of the oleic acid percentage to MNT between the P and NM1 genotypes could be attributed to an inhibition of the ODS activity associated to each high oleic mutation. Probably, the fact that NM1 mutation produces a non-active protein makes the oleic acid desaturation to be more dramatically affected by temperature than in P genotypes, where maybe some slight gene transcription can be induced as MNT changes.

The T and P genotypes had a similar response pattern to that already described for traditional hybrids (Izquierdo et al., 2006) and a high oleic genotype (Izquierdo and Aguirrezábal, 2008) in the range of temperatures explored in this work. The slope of

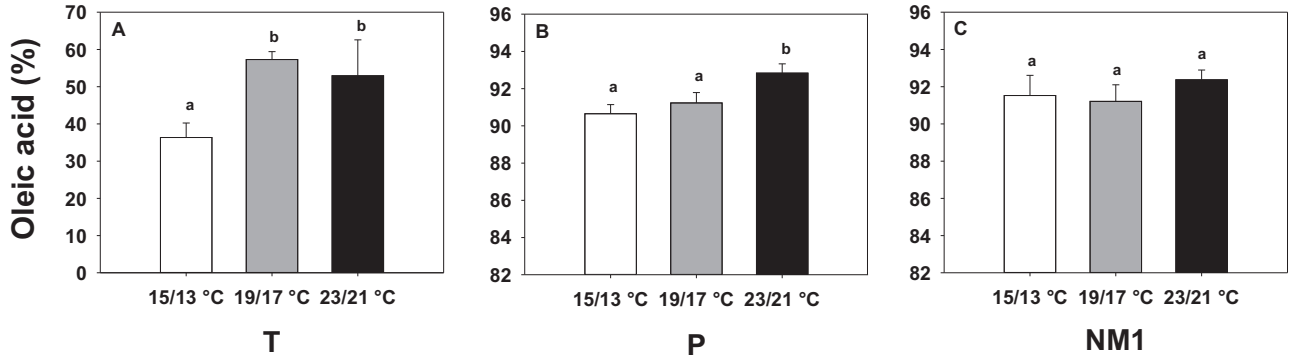

Fig. 2. Oleic acid percentage of the three sunflower near isogenic lines, (A) T and (B) P and (C) NM1 for T1 (15/13 °C day/night, white bars), T2 (19/17 °C day/night, grey bars) and T3 (23/21 °C, day/night, black bars) from the GC experiment. Data are average of 8/9 plants per growth chamber ± SE. For each genotype, different letters over the bars (a and b) indicate significant differences ($p < 0.05$) between temperature treatments (ANOVA and Tukey test).

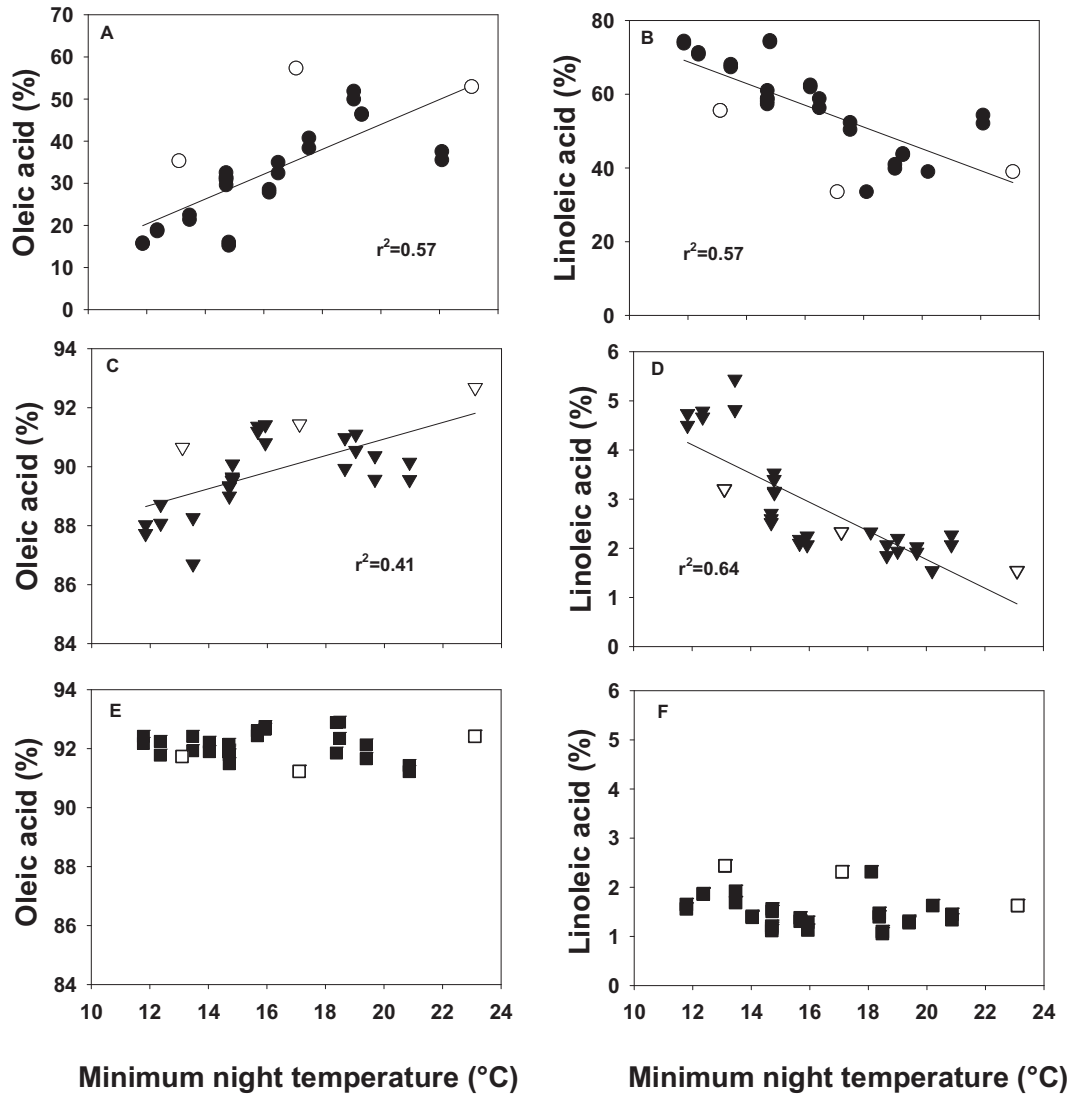

Fig. 3. Oleic and linoleic acid percentage as a function of the minimum night temperature during the 100–300 ddaf period for the traditional genotype (A and B), the P genotype (C and D) and the NM1 genotype (E and F) for GC and field experiments. Symbols correspond to Field-T (●), GC-T (○), Field-P (▼), GC-P (▽), Field-NM1 (■), GC-NM1 (□). Solid lines show the linear regression between the oleic or linoleic acid percentages and the minimum night temperature (simple linear regression, $p < 0.001$).

the relation between oleic acid and MNT in the T genotype was similar to that registered by Izquierdo et al., (2006) in the range of temperatures between 13.0 and 22.0 °C (MNT). In the case of the high oleic genotype, although a sigmoidal response of the oleic acid percentage to MNT during the 100–300 ddaf period was described (Izquierdo and Aguirrezábal, 2008), a reanalysis of the data obtained by these authors for the range of temperatures studied in this work showed a significant and linear relationship as

the one observed in our study. The NM1 genotype remained stable in the range of temperatures explored in this work, contrary to that reported by several authors in other high oleic genotypes (Grunvald et al., 2013; Van der Merwe et al., 2013; Zuil et al., 2012; Izquierdo and Aguirrezábal, 2008; Triboï-Blondel et al., 2000). Although the high oleic genotype carrying the Pervenets mutation was less sensitive to temperature than the traditional genotype, our results confirmed that this mutation is not stable. Conversely the genotype carrying the new high oleic mutation was not affected by temperature changes.

In this work near isogenic lines were used to compare both high oleic genotypes, P and NM1, independently of the genetic background. The advantage of using near isogenic lines is the purity of the genetic pool. So it can avoid the presence of genes that might generate epistasis and confuse the effects. Genetic background can interact with the mutation, via the modifiers genes, affecting the response of oleic acid to temperature (Schuppert et al., 2006). It would be interesting to test the response of sunflower genotypes from different genetic backgrounds carrying the NM1 mutation to MNT prove if stable oleic acid percentage conferred by the NM1 mutation also depends on its interaction with the genetic background.

Experiments were performed in field conditions including different sowing dates, seasons and latitudes where many environmental factors, others than temperature, are interacting. A GC experiment was also carried out varying temperature and maintaining constant the other factors. Obtaining results first under a wide variable combination of different environmental factors (field trials) and then under controlled conditions confirm the idea that the temperature was the most important environmental factor in determining fatty acid composition in all genotypes. It was possible to analyse together field and GC data using the MNT as the best descriptor of oleic acid percentage (Izquierdo et al., 2006). Interestingly, even though the low variability registered in oleic acid in both high oleic genotypes, it was possible not only to establish relationships between oleic or linoleic acids and MNT, but also to characterize different responses among genotypes.

In GC experiment all genotypes presented the same trend as in field experiments. In addition, oleic acid percentage in T and P genotypes was related to MNT. The fact that P genotype attained higher oleic percentage than the expected in the controlled conditions, suggest the idea of an interaction between the temperature and other environmental factors, like different rainfall (Pritchard et al., 2000), photoperiod and radiation (Echarte et al., 2010; Izquierdo et al., 2009; Santalla et al., 1995), contributing to obtain a lesser response of oleic acid percentage in field trials than in GC. The high stability of the oleic acid percentage in NM1 genotype was observed in controlled conditions (where only temperature varies) but also in field conditions where other environmental factors can interact with the temperature to determine the oleic acid percentage.

It is well known that the oleic/linoleic ratio is environmentally dependent (Fernández-Martínez et al., 2004). Linoleic acid also showed a different response to temperature depending on the genotype. The oleic/linoleic stoichiometry relation in T and P genotypes was around 1:1, that is, an increase of 1 point of oleic acid approximately corresponds with a decrease of 1 point of linoleic acid. The same trend was shown by several authors in traditional and high oleic genotypes (Izquierdo et al., 2013; Van der Merwe et al., 2013; Echarte et al., 2010; Izquierdo and Aguirrezábal, 2008; Rolletschek et al., 2007; Izquierdo et al., 2006; Rondanini et al., 2003; Izquierdo et al., 2002; Triboï-Blondel et al., 2000; Garcés et al., 1992; Lajara et al., 1990). In the NM1 genotype, due to the small range of variation of the oleic and linoleic acid percentage (91.2–92.5%, and 1.1–2.4%, respectively), it would have expected no relationship between these fatty acids. However, the oleic/linoleic stoichiometry relation registered in NM1 was about 1:0.5 (1 point of oleic acid approximately corresponds with the decrease of half a point of linoleic acid). This could only be explained by two specific data points that determine the significance of the relationship. These data points corresponded to T1 and T2 treatments where the values of oleic acid percentage were lower than in T3 (91.7, 91.2 and 92.4% for T1, T2 and T3, respectively from the growth chambers experiment) and the values of linoleic acid percentage were higher than in the T3 (2.4, 2.3 and 1.6%, respectively). If these two points are not taken into account, the relationship between oleic and linoleic acid would not be significant ($p = 0.255$). In fact the low variation observed in oleic acid percentage was accounted for by the decrease of the total saturated fatty acids, as it was reported by Martínez-Force et al. (1998). However, palmitic and stearic acid presented a different response to changes in MNT. In some cases temperature increased saturated fatty acids, in others decreased as reported by Izquierdo and Aguirrezábal (2008). This could be due to an effect of the temperature on some enzyme different from the ODS (e.g. the fatty acid synthase I (FAS I), FAS II or the steaoryl-ACP desaturase described by Serrano-Vega et al. (2005)).

5. Conclusion

The oil market currently demands better quality products. In such context, this work presents new insights to obtain sunflower oil with a higher oleic acid and a lower linoleic acid percentage, which could be preferred for several food and industrial uses compared to the oil quality obtained by sowing the currently cultivated high oleic hybrids. Moreover, the results presented in this work suggest that such oil could be produced in a wide range of sowing dates and regions around the world as the fatty acid composition of the genotype carrying the new mutation was not affected by temperature during grain filling. Despite the higher stability of the oleic acid percentage face to temperature changes compared to this observed in the traditional hybrid, the high oleic hybrid carrying the currently used Pervenets mutation was affected by temperature. To incorporate the new mutation in sunflower hybrids with good performance for other agronomic traits (e.g. yield, drought and disease tolerance) seems then promising for high oleic hybrids breeding programs. To attain this goal, further investigation could be useful to better understand the interactions among the new mutation, the genetic backgrounds and the minimum night temperature.

Acknowledgements

Constanza Alberio holds a scholarship from CONICET, Dr. Luis A.N. Aguirrezábal and Dr. Natalia Izquierdo are her Director and Co-director of both, scholarship and PhD thesis, respectively. This work is part of C. A PhD thesis at Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Mar del Plata. This research was supported by Agencia Nacional de Promoción Científica y Tecnológica PICT 0941, PIP 0362Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Universidad Nacional de Mar del Plata (UNMdP). Authors wish to thank Luis Méndez for technical assistance.

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