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# Genetic control of physiological traits associated to low temperature growth in sunflower under early sowing conditions

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#### Keywords: Sunflower Early sowing Cold tolerance Physiological traits Genetic variability OTL mapping

#### ABSTRACT

This study was conducted to identify physiological traits associated with cold tolerance in sunflower and to identify the genomic regions involved in their variation. A population of 98 recombinant inbred lines (RILs) and their two parents were sown in the field as usual sowing date (control) and one or two months earlier (long-term low temperature treatments). A trait commonly used to underlying cold tolerance related to the degree of membrane damage, as well as traits associated with growth capacity (chlorophyll content, potential photochemical efficiency of photosystem II and plant dry weight) and finally those reflecting acclimation mechanism to stress conditions (osmotic potential at full turgor, and specific leaf area) have been investigated at early development stages. Significant differences were observed among the three sowing dates for all traits. Chlorophyll content and specific leaf area are genetically associated with cold tolerance. Genetic gains were observed for chlorophyll content and specific lot rouge of a cold tolerance. Genetic gains were observed for chlorophyll content and specific leaf area are genetically associated with cold tolerance. Genetic gains were observed for cold tolerance and osmotic potential traits in some of early sowing dates, which suggest that they could be used for cold tolerance in breeding programs. QTL analyses show that several putative genomic regions are involved in the variation of the physiological traits studied under low temperature. Major QTLs for cold tolerance associated with SSR markers such as *ORS331\_2* for the cell membrane stability should be checked in several environments to see if they can be used in marker-assisted selection programs.

### 1. Introduction

Sunflower is one of the most important oil crops worldwide. This summer crop is mainly cultivated under rather high temperature. Water deficit stress which can take place during critical periods of flowering and grain filling induces yield decline [1]. Two main strategies have been studied to maximize the sunflower production under drought stress conditions. The first way is to improve the drought tolerance of sunflower cultivars [2,3]. Early sowing is the second way to avoid the critical water stress period [4]. The effect of early sowing and winter planting has been studied in sunflower in several Mediterranean countries [5,6]. Authors have shown that early sowings improve the water availability [7] and increase the yield of the crop [8,6]. In temperate regions, early sowing compared with conventional sowing is associated with long-term low temperature exposure during first stages of development in sunflower.

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Low temperature exposure has consequences for most biological processes. It initiates a number of physiological changes which lead the plant to be more cold tolerant [9]. Among the numerous metabolic changes, photosynthesis is the main physiological process studied under low temperature condition in many species including Arabidopsis [10], alfalfa [11], rice [12], maize [13], wheat [14] and barley [15]. Photosynthetic modifications are notably characterized by changes in photochemical efficiency [16,17] in response to photooxidation [18], photoprotection [13] and photoinhibition [14,19]. Accumulation of metabolites and low molecular weight solutes such as carbohydrates into the cytoplasm constitute another major metabolic change observed in plants after exposure to low temperature [20]. These solutes contribute to decrease the osmotic potential which leads to decrease the cytoplasmic freezing point and prevent dehydration in cells [21,22]. However, low temperature exposure can induced cell structural alterations as observed in several warm-season crop species [23] due to membrane lipid degradation [24].

The genetic dissection of the quantitative traits controlling the adaptive response of crops to abiotic stress is a prerequisite to allow cost-effective applications of genomics-based approaches to breeding programs [25]. Sunflower QTL mapping was conducted for agronomical traits [26–28], and for photosynthesis and water status traits under water stress condition [29,30]. QTLs for cold

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tolerance during the first stages of development has been identified in rice [31,12], winter wheat [32], maize [33,17] and sorghum [34]. As far as we know, no study in the literature refers to genetic analysis of physiological traits associated with cold tolerance in sunflower.

The objectives of this research are to study a set of physiological traits associated with cold tolerance, to understand which are affected in the first development stage in sunflower subjected to early sowing associated with long-term low temperature exposure and to analyze the genetic basis of low temperature tolerance in sunflower. Identifying physiological traits associated with molecular markers involved in low temperature tolerance would be useful in breeding programs.

#### 2. Materials and methods

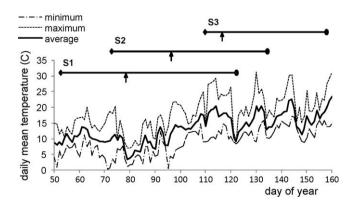
#### 2.1. Plant material and experimental conditions

A population of 98 RILs of sunflower (*Helianthus annuus*) and their parents RHA266 and PAC2 were sown in the field to investigate cold tolerance response under early sowing condition. Recombinant inbred lines were sown at three dates: usual sowing date as control (S3 in April) and one or two months earlier (S2 in March and S1 in February). For the last 10 years the mean temperature registered during April was 12.7 °C ranging from 7.9 °C to 17.5 °C, the mean temperature during March was 10.4 °C ranging from 5.6 °C to 15.2 °C and during February 7.5 °C ranging from 3.7 °C to 11.3 °C. Air temperature was recorded daily at 2 m above the soil surface close to the experimental site.

For each sowing date three replications per genotype were performed. The replications were two rows of 3 m long with 50 cm between rows and 25 cm between plants in rows. Each replication per genotype consisted of an experimental unit of 24 plants. Plants were exposed to periods of low temperature depending on the sowing dates (Fig. 1). Date of emergence was determined for each experimental unit when 50% of emergence was reached [35]. Sampling was realized at 800 °Cd in each sowing date. Cumulative degree days (°Cd) was calculated as the sum of the average daily temperature. Each sample consisted of the aerial part of a single plant per experimental unit. Samples have been placed 24 h at 4 °C before physiological traits measurement.

#### 2.2. Trait measurements

Long-term low temperature exposure effects were determined using two photosynthetic, three non-photosynthetic traits and the plant dry weight measurement. The photosynthetic traits are chlorophyll fluorescence and chlorophyll content. Chlorophyll



**Fig. 1.** Daily mean temperature during the growing season. The three sowing dates are indicated S1, S2 and S3. The dates of emergence are indicated by vertical arrows and the sampling date for each sowing date is marked by the black circle.

fluorescence was performed with a pulse-amplitude modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany) for the younger fully expanded leaves after keeping 1 h under dark condition. The minimum fluorescence (Fo) and the maximum fluorescence (Fm) following a saturating light pulse ( $8000 \mu mol m^{-2} s^{-1}$ ) were measured. The variable fluorescence (Fv = Fm – Fo) and the ratio of variable maximum fluorescence (Fv/Fm) were calculated. The Fv/Fm ratio following a saturating light pulse represents a measure of the potential photochemical efficiency of photosystem II electron transport [36]. Chlorophyll content (CC) was determined with a portable SPAD-502 chlorophyll meter (Konica Minolta, Osaka, Japan). Measurements were performed for three samples through the middle section of the younger fully expanded leaves.

Non-photosynthetic traits studied are osmotic potential, relative electrolyte leakage and specific leaf area. Osmotic potential at full turgor (OP<sub>FT</sub>) was measured on expressed sap of frozen and thawed leaves using 10 mL aliquots placed in an osmometer (Wescor Model 5520, Logan, Utah, USA) calibrated with manufacturer solutions. The relative electrolyte leakage (REL) was performed according to the protocol of Campos et al. [24] using leaf discs (2 cm diameter) from young fully expanded leaves which were rinsed three times with demineralised water then placed in tube with 10 mL of demineralised water. Electrolyte leakage (EL) was measured with conductimeter (WTW LF 95, sonde TetraCon 96, Germany) after 24 h of floating at room temperature. Then, tubes were autoclaved for 15 min (121 °C) to kill the leaf tissue and release the total electrolytes (TE). Results were expressed as relative electrolyte content (REL) calculated as (EL/TE)  $\times$  100 (%). The specific leaf area (SLA) was determined with discs (2 cm diameter) cut on the third fully expanded leaves and dried (48 h, 80 °C). SLA was calculated as leaf area/leaf dry weight  $(m^2 kg^{-1})$ . The total aerial part of three plants per genotype per sowing date per replication was dried at 80 °C for 48 h and the plant dry weight (PDW) was determined.

#### 2.3. Statistical analysis

Means comparison between the three sowing dates for each studied trait was tested with the Kruskal–Wallis test [37]. The normality of the traits distribution was tested with the Shapiro–Wilk test. For each sowing date, a mixed model with genotypes (RILs and parents) and replication effects, was used for analysis of the experimental data. Statistical analyses were performed with SPSS for Windows (15. 0). Genetic gain for each trait was calculated as the differences between the mean of the top 10% selected RILs and the mean of the parents.

#### 2.4. QTL analysis

The sunflower reference map recently constructed in our department by Poormohammad Kiani et al. [30] was used for detection of QTLs. The mapping population was developed through single seed descent from F2 plants derived from a cross between 'PAC2' and 'RHA266' [38]. RHA266 was obtained from a cross between wild *H. annuus* and Peredovik by USDA and PAC2 is an INRA-France inbred line from a cross between *H. petiolaris* and "HA61" [39]. RHA266 compared to PAC2 have a higher values for yield, leaf area at flowering, and lower values for plant height and total dry matter [39–41]. Under water stress conditions the two parental lines differed significantly for leaf area at flowering, leaf area duration, plant height, total dry matter, head weight and seed quality traits [42,41]. This map consisted of 495 markers (304 AFLP and 191 SSR), placed in 17 linkage groups with a mean density of one locus for 3.7 cM. Each linkage group presumably corresponds

#### Table 1

Analysis of variance for physiological traits in a population of sunflower recombinant inbred lines (RILs) and their two parents grown in three sowing dates: one control sowing date (S3) and two early sowing dates associated with low temperature (S1 and S2).

Sowing date		Fv/Fm	CC	SLA	REL	OP <sub>FT</sub>	PDW
S3	Mean	0.830 a	31.7 a	26.6 a	21.6 a	-0.64 a	3.84 a
	Range	0.711/0.862	21.2/45.2	17.9/36.9	13.6/37.5	-0.92/-0.44	0.74/18.21
	MS <sub>G</sub>	0.001***	41.085 <sup>***</sup>	15.182 <sup>NS</sup>	17.519 <sup>NS</sup>	0.011 <sup>NS</sup>	28.348***
S2	Mean	0.812 b	29.4 b	24.9 b	23.6 b	-0.74 b	2.38 b
	Range	0.634/0.855	18.5/41.0	16.2/33.7	15.8/34.1	-1.10/-0.56	0.34/10.80
	MS <sub>G</sub>	0.001***	26.195 <sup>***</sup>	12.919 <sup>****</sup>	13.865 <sup>**</sup>	0.006**	9.476***
S1	Mean	0.792 c	27.9 c	25.6 b	25.7 c	-0.76 c	0.67 c
	Range	0.594/0.858	19.5/35.0	18.0/34.5	16.4/38.1	-0.99/-0.58	0.05/2.42
	MS <sub>G</sub>	0.002***	15.180 <sup>***</sup>	13.221 <sup>***</sup>	28.862 <sup>***</sup>	0.006***	0.150 <sup>NS</sup>

Fv/Fm: potential photochemical efficiency of photosystem II; CC: chlorophyll content (SPAD values); SLA: specific leaf area ( $m^2/kg$ ); REL: relative electrolyte leakage (%); OP<sub>FT</sub>: osmotic potential at full turgor (MPa); PDW: plant dry weight (g); MSG: genotype mean square; NS: non-significant. Values with a common letter in the same column are not significantly different at *p* = 0.05 (Kruskal–Wallis test)

\* Significant at 0.01 probability level.

Significant at 0.001 probability level.

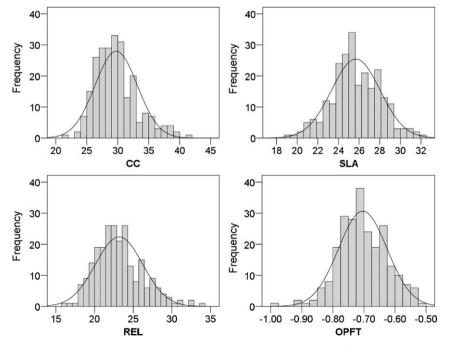
to one of the 17 chromosomes in the haploid sunflower genome (x = 17) [30]. The QTL location was estimated with the composite interval mapping method of QTL Cartographer version 2.5 software [43,44] using mean values of the three replicates for each RIL in each sowing date. The control marker number and the window size were 15 and 15 cM, respectively. The LOD score criterion for QTL significance was estimated by mean of a permutation test with 1000 permutations [45]. Mapchart 2.1 was used for graphical presentation of linkage groups and map positions of the SSR and AFLP markers.

#### 3. Results

Contrasted thermal conditions are observed for the three sowing dates (Fig. 1). The mean temperatures from sowing to sampling are lower in the two early sowing than in control with 11.7 °C for S1, 12.9 °C for S2 and 16.4 °C for S3. The mean temperature from sowing to emergence is lower in S1 (10.3 °C) and S2 (8.4 °C) compared with S3 (17.8 °C), whereas temperatures

recorded from emergence to sampling period are lower in S1 (12.4  $^{\circ}$ C) compared with S2 (15.6  $^{\circ}$ C) and S3 (16.2  $^{\circ}$ C).

The means comparison between the three sowing dates for the studied traits shows that sowing date has significant effect on all physiological traits (Table 1). A normal distribution was observed for the specific leaf area SLA and the osmotic potential at full turgor (OP<sub>FT</sub>) as presented in Fig. 2. According to the Shapiro-Wilk test the distribution of some other traits deviate from normality. As normalizing data through transformation may misrepresent differences among individuals by pulling skewed tails toward the centre of the distribution [45], all phenotypic analyses were performed on untransformed data. Significant differences are observed between S1, S2 and S3 for all the studied traits except for SLA, in which means of S1 and S2 are not significantly different. The mean values of the potential photochemical efficiency of photosystem II (Fv/Fm), chlorophyll content (CC), SLA, (OP<sub>FT</sub>) and the plant dry weight (PDW) are lower in early sowing dates (S1 and S2) compared with the control S3 (Table 1). On the contrary, the mean value of REL is higher in S1 and S2 than in S3 (Table 2).



**Fig. 2.** Distribution for physiological traits: chlorophyll content—CC (SPAD values), the specific leaf area—SLA ( $m^2/kg$ ), the relative electrolyte leakage—REL (%) and the osmotic potential at full turgor—OPFT (MPa) in a population of sunflower recombinant inbred lines (RILs) and their two parents grown in three sowing dates.

#### Table 2

Genetic gain for physiological traits in a population of sunflower recombinant inbred lines (RILs) and their two parents grown in three sowing dates: one control sowing date (S3) and two early sowing dates associated with low temperature (S1 and S2).

Sowing date		Fv/Fm	CC	SLA	REL	OP <sub>FT</sub>	PDW
S3	PAC2 (P1)	0.839	30.9	24.4	18.5	-0.65	2.15
	RHA266 (P2)	0.818	28.0	26.5	21.3	-0.68	2.15
	P1-P2	0.021 <sup>NS</sup>	2.88 <sup>NS</sup>	-2.0 <sup>NS</sup>	-2.8 <sup>NS</sup>	0.03 <sup>NS</sup>	0.00 <sup>NS</sup>
	$X_{\rm P}$	0.828	29.4	25.5	19.9	-0.66	2.15
	$\bar{X}_{RIL}$	0.830	31.8	26.6	21.6	-0.64	3.87
	$\bar{X}_{10\% \text{ best RIL}}$	0.852	38.9	30.8	17.8	-0.75	11.28
	$ar{X_{ ext{RIL}}} - ar{X_{ ext{P}}}$	0.002 <sup>NS</sup>	2.4 <sup>NS</sup>	1.2 <sup>NS</sup>	1.7 <sup>NS</sup>	0.02 <sup>NS</sup>	1.72 <sup>NS</sup>
	$GG10\% = \bar{X_{10\%bestRIL}} - \bar{X_P}$	0.023 <sup>NS</sup>	9.4***	5.3 <sup>NS</sup>	-2.1 <sup>NS</sup>	-0.09 <sup>NS</sup>	9.13
S2	PAC2 (P1)	0.801	29.3	24.7	21.3	-0.71	0.91
	RHA266 (P2)	0.752	27.5	23.6	26.0	-0.72	1.43
	P1-P2	0.050**	1.78 <sup>NS</sup>	1.0 <sup>NS</sup>	-4.7 <sup>NS</sup>	0.00 <sup>NS</sup>	-0.52 <sup>NS</sup>
	$X_{ m P}$	0.777	28.4	24.1	23.7	-0.71	1.17
	X <sub>RIL</sub>	0.813	29.4	24.9	23.6	-0.74	2.40
	$\bar{X}_{10\% \text{ best RIL}}$	2.006	34.5	28.3	20.4	-0.85	6.70
	$ar{X_{ ext{RIL}}} - ar{X_{ ext{P}}}$	0.036*	1.0 <sup>NS</sup>	0.8 <sup>NS</sup>	0.0 <sup>NS</sup>	-0.03 <sup>NS</sup>	1.23 <sup>NS</sup>
	$\text{GG10\%} = \bar{X}_{10\%\text{best RIL}} - \bar{X}_{\text{P}}$	1.229***	<b>6.1</b> <sup>***</sup>	4.1 <sup>NS</sup>	-3.3 <sup>NS</sup>	- <b>0.14</b>	5.53
S1	PAC2 (P1)	0.838	29.8	24.8	23.3	-0.75	0.89
	RHA266 (P2)	0.793	28.9	26.6	25.7	-0.72	0.90
	P1-P2	0.045 <sup>NS</sup>	0.91 <sup>NS</sup>	-1.9 <sup>NS</sup>	-2.4 <sup>NS</sup>	-0.03 <sup>NS</sup>	$-0.01^{NS}$
	$\bar{X_{\mathrm{P}}}$	0.815	29.3	25.7	24.5	-0.74	0.89
	$\bar{X}_{RIL}$	0.791	27.9	25.6	25.7	-0.76	0.67
	$\bar{X}_{10\%}$ best RIL	0.831	31.7	29.3	20.7	-0.85	1.10
	$\bar{X}_{\text{RIL}} - \bar{X}_{\text{P}}$	$-0.024^{NS}$	$-1.4^{NS}$	-0.2 <sup>NS</sup>	1.2 <sup>NS</sup>	$-0.02^{NS}$	-0.22 <sup>NS</sup>
	$GG10\% = \bar{X}_{10\% \text{ best RIL}} - \bar{X}_{\text{P}}$	0.016 <sup>NS</sup>	2.4 <sup>NS</sup>	3.6 <sup>NS</sup>	-3.8 <sup>NS</sup>	- <b>0.12</b> ***	0.21 <sup>NS</sup>

Fv/Fm: potential photochemical efficiency of photosystem II; CC: chlorophyll content (SPAD values); SLA: specific leaf area ( $m^2/kg$ ); REL: relative electrolyte leakage (%); OP<sub>FT</sub>: osmotic potential at full turgor (MPa); PDW: plant dry weight (g). The significant differences are presented as bold-face. 'PAC2' (P1) and 'RHA266' (P2): parental lines;  $\bar{X}_{P}$ : mean of two parental lines;  $\bar{X}_{RIL}$ : mean of recombinant inbred lines (RILs);  $\bar{X}_{10\% \text{ best RIL}}$ : the mean of the top 10% selected RILs; GG10%: genetic gain when the mean of the top 10% selected RILs is compared with the mean of the parents. NS: non-significant.

\* Significant at 0.05 probability level.

Significant at 0.01 probability level.

"" Significant at 0.001 probability level.

Analysis of variance of the 98 recombinant inbred lines and their parents ('PAC2' and 'RHA266') is summarized in Table 1. Photosynthetic traits (Fv/Fm and CC) present significant differences between genotypes for each sowing date. Non-photosynthetic traits SLA, REL and  $OP_{FT}$ , show significant variability only for S1 and S2. Concerning the plant dry weight (PDW), significant genotypic variability is observed in S2 and S3. The difference between the two parents is significant only for Fv/Fm in S2 (Table 2), in spite of the existence of genetic variability in RILs for all traits (Table 1). Differences for the mean value of all traits between RILs (X<sub>RIL</sub>) and their parents ( $X_P$ ) are not significant, except for Fv/Fm in S2 (Table 2). Genetic gain, as the difference between the mean of 10% selected RILs ( $X_{10\% best RIL}$ ) and the mean of parents ( $X_P$ ), is significant for  $OP_{FT}$  in S1 and S2, for Fv/Fm in S2 and for CC and PDW in S3.

The map position and the characteristics of QTLs associated with the studied traits for the three sowing dates are presented in Table 3. For an easier overview of overlapping QTLs between the traits and the sowing dates, an image of all QTL regions is presented in Fig. 3. Three to nine QTLs are identified depending to the traits and sowing dates. QTLs explain from 4.6% to 23% of the phenotypic variance of the traits  $(R^2)$ . Additive effects present positive or negative values showing that both parental lines contribute to the expression of the different traits. The largest amount of phenotypic variance explains by a QTL detected for Fv/Fm is 22% (Fv/Fm-S3-16.2). This QTL is co-located with two overlapping QTLs detected for CC in S2 and S3 conditions. The most important QTL for CC is identified on linkage group 9 (ORS805) and explains 19% of the total phenotypic variance. The major QTL for SLA (SLA-S3-16.1) is located on the linkage group 16 and explain 22% of the phenotypic variance. The most important QTL detected for REL on the linkage group 7 is associated with the SSR marker ORS331\_2 and explain 23% of the phenotypic variance. This QTL is identified in the two early sowing dates (S1 and S2). The major QTL for PDW (*PDW-S3*-5.1) explains 20% of phenotypic variance in S3 and is overlapped with QTL detected for PDW in S2. Among the identified QTLs, one is stable for SLA across the three sowing dates on linkage group 12 (Table 3). Two QTLs are common between S1 and S2 for REL on linkage group 7 and for OP<sub>FT</sub> on linkage group 10. Eight QTLs are common between S2 and S3 for Fv/Fm (linkage group 9), for CC (linkage groups 2, 12 and 16) and for PDW (linkage groups 5, 15 and 17).

Overlapping QTLs between traits are observed mainly in control sowing date. In S3, QTL for plant dry weight (PDW) is co-located with the QTL detected for REL on linkage group 4 and with the QTL detected for Fv/Fm on the linkage group 15. Two QTLs detected for Fv/Fm are co-located with QTLs identified for CC in linkage groups 15 and 16. Overlapping QTLs for CC, OP<sub>FT</sub> and SLA are also detected in the linkage group 9. In the early sowing S1, QTLs for SLA and REL are co-located in linkage group 4.

#### 4. Discussion

Physiological changes induced by low temperature affected all the traits studied. Non-significant differences were found for all traits studied between the means of the RILs and the mean of the parents (Table 2). This indicates that the RILs are representative of possible genotypic combinations of the two parents for the studied traits, as it was also previously reported by Poormohammad Kiani et al. [30], for the water status and the osmotic adjustment of sunflower under two water treatments. Genetic gain was significant for the chlorophyll content (CC) and plant dry weight (PDW) in S2 and S3 and for the osmotic potential at full turgor (OP<sub>FT</sub>) in S1 and S2 (Table 2). This might be due to positive transgressive segregation resulting from the accumulation of favorable alleles in some RILs. Transgressive segregation has

#### Table 3

Map position and effect of QTLs for potential photochemical efficiency of photosystem II (Fv/Fm), chlorophyll content (CC), specific leaf area (SLA), relative electrolyte leakage (REL), osmotic potential at full turgor (OP<sub>FT</sub>) and plant dry weight (PDW) detected in RILs under three sowing conditions: one control sowing date (S3) and two early sowing dates associated with low temperature (S1 and S2). The threshold level of the LOD score for each trait was estimated by means of a permutation test with 1000 permutations.

S1         Prim. Prim. Prim.S3-10.1         9         MM7.         4.28         3.0         0.12         -0.007           Prim.S3-10.1         15         ESS01         00.0         3.6         0.11         -0.007           Prim.S3-16.1         15         ESS02         00.0         3.6         0.11         -0.007           Prim.S3-16.1         15         ESS02         10.0         3.6         0.11         -0.007           Prim.S3-16.1         15         ESS02         10.5         7.1         0.07         -0.007           CC3.5.1         6         DETMEL, 1         88.4         6.6         0.022         -0.017           CC3.5.12.1         12         DESS02         10.5         7.1         0.06         -2.038           CC3.5.12.1         12         DESS02         2.1.1         3.2         0.07         1.072           CC3.5.12.1         12         DESS02         3.3         0.08         0.01         0.16         0.039           SLA         SLA5.5.2.1         12         DESS02         0.03         0.00         0.01         0.05         0.01         0.016         0.039         0.01         0.016         0.039         0.01         -0.055<	Sowing date	Trait	QTL	Linkage group	Nearest marker	Position cM <sup>a</sup>	LOD score	$R^{2b}$	Additive effect
Part         Part <th< td=""><td>S3</td><td>Fv/Fm</td><td>Fv/Fm-S3-9.1</td><td>9</td><td>HA477</td><td>42.8</td><td>3.9</td><td>0.12</td><td>-0.007</td></th<>	S3	Fv/Fm	Fv/Fm-S3-9.1	9	HA477	42.8	3.9	0.12	-0.007
S2         PyBm-S3-141         14         OBS01         103.0         3.6         0.11         0.007           hyBm-S3-161         16         E270847_10         76.0         3.6         0.11         -0.007           CC         CC3321         2         E81080_12         11.5         4.1         0.007         -0.009           CC         CC3321         2         E81080_12         11.5         4.1         0.01         -1.399           CC         CC3371         10         D07         1.021         1.03         0.19         -1.211           CC337.610         16         D06407_2         2.1         3.2         0.07         1.072           CC33.76.2         11         D07         D07         0.073         0.07         0.073           SIA         SA5121         12         E61007         2.06.3         3.0         0.07         0.073           SIA-S121         12         E61007         2.06.3         7.7         0.02         -1.133           SIA-S121         12         E61007         2.06.4         3.0         0.04         -0.75           SIA-S121         13         E61007         2.06.4         3.2         0.01         <		,	Fv/Fm-S3-10.1	10	E35M61 11	41.7		0.07	-0.005
Pylm 57:15.1         15         F15484.4         86.4         5.6         0.11         0.007           Pylm 57:162         16         E7706.1         89.4         6.6         0.22         -0.009           CC         CC53:21         2         E1106.12         115         4.1         0.02         -0.009           CC37:51         1         0         08719.2         135         4.1         0.11         -0.039           CC37:52:1         1         0         08719.2         131         7.3         0.07         1.073           CC37:52:2         16         E7704.2         82         3.5         0.07         1.059           CC37:51:2         1         E7306.2         279         3.7         0.07         1.059           SA3.10:1         13         E7806.2         279         3.7         0.08         -0.08           SA3.10:1         13         E7806.2         103         3.4         0.06         -0.755           SA3.10:1         13         E7806.2         103         4.3         0.16         -0.04           SA3.10:1         13         E7806.2         0.14         -0.055         0.14         -0.055           S			,						
Prime-32-16.2         16         E7/M47_20         76.0         5.0         1.07         -0.000           CC         CCS1-2.1         2         FAIMS0,12         11.4         4.1         0.07         -1.010           CC         CCS1-2.1         2         FAIMS0,12         11.5         4.1         0.16         -2.038           CCS3-3.1         10         0.05805         2.0         7.3         0.10         -1.231           CCS3-11         10         0.05805         2.0         7.3         0.10         -1.232           SIA         SA521         2         ESM60,4         88.8         5.1         0.02         -0.75           SIA-57.1         12         EMM50,9         2.0         3.0         0.00         -0.75           SIA-57.1         12         EMM50,9         2.0         3.0         0.00         -0.75           SIA-57.1         13         HA120         3.7         0.02         -1.168         0.00         -0.75           SIA-57.1         10         ESM60,5         0.10         1.0         0.00         -0.75           SIA-57.1         1         EMM52,9         1.03         1.0         0.00         0.01 <t< td=""><td></td><td></td><td>,</td><td></td><td></td><td></td><td></td><td></td><td></td></t<>			,						
Feynesch 62         66         F27661_1         884         66         622         -0.00           CC         CC53261         6         F11650_12         115         44         0.06         -2.238           CC53761         9         OSS805         2.0         7.3         0.18         -1.721           CC537101         16         OSS805         2.0         7.3         0.07         1.072           CC537102         16         DSM6412         3.3         5.0         0.07         1.085           SAS 7711         7         ESM67_2         2.7         3.7         0.07         0.073           SAS 7711         7         ESM67_2         2.0         3.9         0.00         0.073           SAS 7711         7         ESM67_2         2.0         3.9         0.00         0.073           SAS 7714         13         HATS2         1.052         2.0         3.9         0.00         0.073           SAS 7714         13         ESM652         2.0         3.9         0.01         1.03           SAS 7714         14         ESM652         1.03         2.01         1.23         0.01         1.23           REL         <			'		—				
CC         CC-321         2         FMMS0.2         11.5         4.1         0.07         1.388           CC3-3-11         9         008305         2.0         7.3         0.19         -1.721           CC3-711         12         008305         2.0         7.3         0.07         1.062           CC3-16.2         16         67.742         8.2         3.3         0.07         1.063           CC3-16.2         16         67.742         8.6         3.5         0.08         -0.726           SLA-37.1         7         67.399.1         9.081009         9.6         6.0         0.16         0.995           SLA-37.1         11         EMMS0.9         3.4         0.06         -0.726           SLA-37.11         13         EMMS0.9         3.4         0.06         -0.714           SLA-37.11         13         EMMS0.9         3.4         0.10         -1.739           SLA-37.11         13         EMMS0.9         1.04         0.17         -1.238           SLA-37.11         13         EMMS0.9         1.05         0.01         -1.339           SLA-37.11         13         EMMS0.9         1.04         0.07         0.06			'						
CC33-6.1         6         64/M48.2         18.5         7.4         0.16        2.038           CC33-12.1         13         00507.2         21.1         3.2         0.07         1.027           CC31-12.1         13         00507.2         21.1         3.2         0.07         1.027           CC31-12.1         16         077447.5         968.4         5.6         0.06         -0.755           SLA         SLA52.1         12         64050.9         20.3         3.0         0.06         -0.755           SLA-52.1.1         13         64050.9         2.0         3.9         0.09         -0.715           SLA-53.1.1         13         64050.9         2.0         3.9         0.01         -0.718           SLA-53.1.1         13         64050.9         1.06.2         3.16.2         1.012         1.208           REL         REL-53-1.4         4         1.0492.9         1.024         1.012         1.208           REL-33.10         15         1.0370.3         6.04         1.1         0.12         1.028           REL-35.13         15         1.04052.9         1.024         1.040         0.022           PW         0975.5.1.7 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
CC 53-31         9         OKS805         2.0         7.3         0.19         -1.721           CC 53-16.2         16         OKS67L2         211         3.2         0.07         1.095           CC 53-16.2         16         DSM67L2         86.8         3.1         0.07         1.095           SLA         SLAS17.1         2         DSM67L2         27.9         3.7         0.08         -0.735           SLAS17.1         11         DK1000         3.0         6.0         0.08         -0.735           SLAS3-11         13         DK1000         3.0         6.0         0.09         0.0         0.00         0.00         0.0         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00		CC							
22         CC 3-12.1         12         OBS07.2         21.1         3.2         0.07         1.072           CC 33-16.2         16         DEXM2.5         86.8         5.1         0.12         -1.923           SIA         SLAS-S2.1         2         DESM0.4         98.6         3.6         0.08         -1.726           SLAS.53.9.1         9         DESK0.9         2.0         3.9         0.07         -1.923           SLAS.54.1         14         HEMS0.9         2.0         3.9         0.08         -1.735           SLAS.54.1         16         DESK0.9         2.0         3.9         0.04         -1.735           SLAS.54.1         16         DESK0.9         2.0         3.9         0.04         -1.735           SLAS.54.1         16         DESK0.9         2.0         3.9         0.01         1.022         -1.168           BLS.54.1         16         DESK0.9         5.01         1.02         0.014         1.03         -1.269           BLS.54.1         10         DESK0.2         DESK0.2         0.01         1.02         -2.254           BLS.54.1         10         DESK0.2         DESK0.2         0.01         1.0 <t< td=""><td></td><td></td><td></td><td></td><td>—</td><td></td><td></td><td></td><td></td></t<>					—				
CC 53-16.2         16         05548.2         8.2         3.5         0.07         1.085           SIA         SIA-S3-2.1         2         ESM69.4         96.6         3.6         0.08         -0.725           SIAS-S3-51.1         9         0051009         9.6         0.016         0.996         0.016         0.996           SIAS-S1-12.1         12         E40050.9         2.0         3.9         0.08         -0.715           SIAS-S1-12.1         13         1403720         37.4         3.4         0.08         -0.715           SIAS-S1-11         13         1403720         37.4         3.4         0.08         -0.715           SIAS-S1-13         13         15005.5         0.04         7.1         0.12         1.209           BEL         5.51.2         1         10         E55062.5         1128         110         0.23         -2254           BEL-S3-12.1         13         E9047.21         0.0         4.3         0.10         0.002           OPr         0975.35.1         5         E41062.7         0.0         4.3         0.10         0.002           OPr-S3-7.1         7         095391.2         0.0         3.1         <									
CC-3-162         16         E7M#7.5         86.8         5.1         0.12         -1-23           SIA         SIAS3-21         7         E3M50.2         27.9         3.7         0.07         0.076           SIAS3-31         10         E3M50.2         27.9         3.7         0.07         0.075           SIAS3-31         11         H0330         2.0         3.9         0.09         -0.755           SIAS3-131         11         H0330         2.16.2         3.7         0.09         -0.714           SIAS3-161         16         0.0302.2         105.9         7.7         0.02         -1.183           REL         RELS3-1         4         H0991         34.2         48         0.07         -2.857           RELS3-1         10         E35M02.3         9.16         40         0.07         -0.857           RELS3-1         10         E35M02.5         9.011         0.022         0.011         0.022           OFm         OFF3-5.71         7         D5M2.2         0.00         43         0.10         0.022           OFm - 0775-5.81         9         05312.2         0.00         43         0.01         0.007			CC-S3-12.1		ORS671_2				
SLA         SLA-S3-71         2         ESM00-1         996         36         0.08         -0.765           SLAS3-21         9         OK1000         96         6.0         0.05         0.057           SLAS3-21         13         EM050_9         2.0         3.9         0.00         -0.755           SLAS3-21         13         EM050_9         2.0         3.9         0.00         -0.755           SLAS3-21         13         EM050_9         2.0         3.9         0.00         -0.755           SLAS3-21         13         EM160_9         2.0         3.7         0.02         -1.714           SLAS3-21         14         EM050_9         6.04         7.1         0.12         -1.268           RELS3-11         10         E3740_2.5         67.6         8.0         0.11         0.902           RELS3-10         10         E3740_2.5         61.3         3.7         0.08         -0.072           RELS3-12         2         EM047_2.1         0.0         4.3         0.10         0.020           OPT         OPT-35-3-1         7         OKSB02_6         61.3         3.7         0.08         -0.097           RELS3-11			CC-S3-16.1	16		8.2	3.5	0.07	1.095
SLA-53-71         7         E33(5)2         2.7.9         3.7         0.07         0.673           SLA-53-71.1         12         E40(85).9         2.0         3.9         0.08         -0.755           SLA-53-71.1         13         H43320         77.7         0.02         -0.714           SLA-53-71.1         14         41(M320)         1022         7.7         0.02         -0.714           SLA-53-71.1         4         H6951         54.2         4.7         0.00         -0.714           SLA-53-71.1         4         H6951         54.2         4.7         0.00         -0.714           SLA-53-71.1         4         H6951         54.2         0.07         -0.857         1.0         1.0         1.0         1.0         1.0         0.0         0.14         1.558         1.0         0.00         0.00         0.01         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00         0.00			CC-S3-16.2	16	E37M47_5	86.8	5.1	0.12	-1.523
SLA-53-71         7         ESS(5)/2         2.2.9         3.7         0.07         6.673           SLA-53-71.1         12         EX0(850.9         2.0         3.9         0.08         -0.755           SLA-53-71.1         13         H43320         37.4         3.4         0.08         -0.715           SLA-53-71.1         14         4140320         1262.2         3.7         0.00         -0.714           SLA-53-71.1         4         H49310         342.2         4.8         0.10         -1.209           REL         REL-53-41         4         H4991         5.6         6.0         4.7         1.02         1.368           RES-31.0         10         E37049.5         91.9         4.2         0.07         -0.857           RES-35.1.1         12         E20047.2         1.0         4.3         0.10         0.022           POW         POFT.53.7.1         7         OK531.2         0.0         4.3         0.10         0.022           POW         POWS-32.1         2         ES805.26         6.1.3         3.7         0.08         -0.997           POWS-31.1         13         H42000         70.4         4.5         0.11 <t< td=""><td></td><td>SLA</td><td>SLA-53-21</td><td>2</td><td>E35M60_4</td><td>98.6</td><td>3.6</td><td>0.08</td><td>-0.726</td></t<>		SLA	SLA-53-21	2	E35M60_4	98.6	3.6	0.08	-0.726
SLA-33-21         9         ORS.NOD0         9.6         6.0         0.16         0.996           SLA-53-12.1         12         EMMSD         2.1         3.3         0.09         -0.755           SLA-53-14.1         13         H43320         37.4         3.4         0.08         -0.715           SLA-53-16.1         16         06303.2         105.3         7.7         0.22         -1.168           REL         REL53-4.1         4         H4001         3.4.2         4.6         0.01         -1.209           REL-53-1.0         10         E7M49.5         67.6         8.0         0.01         15.99           REL-53-1.1         13         E4MM7.21         0.0         4.3         0.010         0022           OPr         OPFr.35.7.1         7         00331.2         0.0         4.3         0.010         0022           OPr/1007Fr.35.1.1         2         E1M82.7         0.0         4.3         0.010         0.022           OPr/1007Fr.35.1.1         3         H40305         6.4.4         50         0.09         1.203           PDW-75.7.1         7         ORS505         0.0         7.1         0.06         1.075		ULL I			—				
SL-53-L1         12         E4005(2.9)         2.0         3.9         0.08         -0.755           SLA3-14.1         14         E1106(2.9)         128.2         3.7         0.08         -0.715           SLA3-14.1         14         E1106(2.9)         128.2         3.7         0.08         -0.714           SLA3-14.1         14         H091         3.42         4.8         0.10         -1.289           REL         REL-53-12         4         H091         3.42         4.8         0.10         -1.289           REL-33-10         10         E37049.5         67.6         6.0         0.14         1.599           RE-33-10.2         10         E35042.9         1.12.8         1.19         0.02         -0.072           RE-33-10.2         10         E35042.9         0.0         4.3         0.10         0.022           RPM         P0Fr53-5.1         5         FMM42.7         0.0         4.3         0.10         0.022           QFFT         QFFT-33-7.1         7         Q68312.2         0.0         4.3         0.10         0.020           QFW-31-1.1         14         F40059.2         6.64.4         50         0.09         1.203					—				
SL-53-131         13         H03320         37.4         34.4         0.08         -0.715           REL         RE-53-61         16         06509.2         105.9         7.7         0.22         -1.168           REL-32-41         H991         32.2         48         0.00         -1.299         -1.168           RE-53-61         10         E53062.5         61.4         7.1         0.12         -1.289           RE-53-10.1         10         E53062.9         61.6         3.0         0.01         -1.299           RE-53-13.1         13         E00047.21         0.0         43         0.10         0.022           0Pr         0PF7.35.71         7         06331.2         0.0         43         0.10         0.022           0PW         P0W-35.21         2         E30062.9         61.3         3.7         0.08         -0.007           0PW         P0W-35.21         2         E30062.9         64.4         0.10         -0.027           0PW         P0W-35.1         13         104208         7.7         3.8         0.00         -1.203           PDW-35.51         15         E30062.9         64.4         4.5         0.01         -1.21									
SLAS3-16.1         16         608503.2         125.2         3.7         0.09         -0.714           REL         REL3S-16.1         4         H091         34.2         4.8         0.00         -1.209           RELS3-10.1         10         E0055.5         67.6         8.0         0.01         -2.209           REL3S-10.2         10         E35404.5         67.6         8.0         0.01         -5.374           RELS3-10.2         10         E35402.9         112.8         1.19         0.23         -2.254           RELS3-13.1         13         E40M47.21         0.0         4.3         0.10         0.002           0Pr         0975.53.1         5         E11M62.7         0.0         4.3         0.10         0.022           0Pr         0975.53.1         5         E10M62.7         0.0         4.3         0.10         0.022           0Pr         0975.53.1         5         H04050.6         64.4         5.0         0.01         1.208           P0W-33-51.1         4         E40459.12         2.5         4.5         0.11         1.306         1.477           P0W-53-17.1         17         E31M48.28         100.2         4.5					—				
SLAS-3-6.1         16         ORS02,2         105.9         7.7         0.22         -1.188           REL         RELS3-4.1         4         MOSD 5         66.3         7.1         0.12         0.12           REL 33-0.1         0         EX3060 5         67.5         8.0         0.14         -1.209           REL 35-10.1         10         EX3062,5         17.8         8.0         0.14         -0.224           REL 35-10.1         10         EX3062,5         17.8         8.0         0.01         0.002           OPr,         OPT53-5.1         5         EMM02,7         0.0         4.3         0.10         0.002           OPW         OPT53-5.1         5         EMM02,7         0.0         4.3         0.10         0.002           OPW         OPT53-5.1         5         HM052,6         61.3         3.7         0.06         -0.997           DW-33-1.1         4         EMM052,6         61.4         5.0         0.09         -1.731           DW-33-1.1         13         HA700         7.4         8.9         0.20         -1.731           DW-33-1.1         14         MA700         7.4         8.9         0.00         -1.003 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
REL         REL 33.4.1         4 <i>H4991</i> 34.2         4.8         0.10         -1.209           REL33.4.2         4 <i>EX0M05.5</i> 91.9         4.2         0.07         -0.857           REL33.10.1         10 <i>EX0M05.5</i> 91.9         4.2         0.07         -0.857           REL33.10.1         10 <i>EX0M05.5</i> 91.5         8.0         0.14         1.359           REL33.1.1         13 <i>EX0M07.21</i> 0.0         4.3         0.10         0.0022           OPm         OPT35.5.1         5 <i>EX1M02.7</i> 0.0         4.3         0.10         0.0022           PDW         PDW53.4.1         4 <i>EX0M52.6</i> 61.3         3.7         0.08         -0.907           PDW53.1.1         5 <i>HA7006</i> 7.04         8.9         0.00         -1.036           PDW53.5.1         15 <i>EX0M52.6</i> 61.3         3.7         0.08         -0.907           PDW53.5.1         15 <i>EX0M52.6</i> 64.4         50         0.01         -1.306           PDW53.5.1         15 <i>EX0M52.6</i> 64.4         4.9         0.13					—				
RE: 53-42         4         FAMMSD_6         60.4         7.1         0.12         1.288           RE: 53-10.1         10         E37M40_5         67.6         8.0         0.14         1.559           RE: 53-10.1         10         E35M62_9         11.28         11.9         0.23         -2.254           RE: 53-13.1         13         FA0M47_21         0.0         5.9         0.11         0.902           0Prr         0PT535-17         7         0PS31_2         0.0         4.8         0.15         -0.027           0PT535-11         9         0S8805         0.0         3.1         0.10         0.020           PDW         FDW-53-2.1         2         ESM50_26         6.13         3.7         0.08         -0.097           PDW-33-2.1         2         ESM50_26         6.44         5.0         0.01         1.010         1.00           PDW-35-13.1         15         ESM48_4         0.44         4.0         1.0         1.0         1.0         1.1         1.1         1.0         1.1         1.1         1.0         1.5         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1         1.1 <td< td=""><td></td><td></td><td>561-55-10.1</td><td>10</td><td>010505_2</td><td>105.5</td><td>7.7</td><td>0.22</td><td>-1.100</td></td<>			561-55-10.1	10	010505_2	105.5	7.7	0.22	-1.100
RE: 53-01         9         E33M60_5         91,9         42         0.07         -0.857           RE: 53-10.2         10         E35M62_9         112.8         0.19         0.23         -2.24           RE: 53-13.1         13         E40M52_7         10.0         5.9         0.11         0.092           OPr         OPF: 53-7.1         7         0.06311_2         0.0         4.3         0.10         0.022           PDW         PDW:33-4.1         4         E40M52_12         0.0         4.3         0.10         0.022           PDW         PDW:33-4.1         4         E40M59_0         6.13         3.7         0.08         -0.997           PDW:33-4.1         4         E40M59_0         6.44         5.0         0.09         1.203           PDW:33-4.1         14         E40M59_0         6.44         4.9         0.01         -1.214           PDW:33-4.1         14         OF1728         0.0         7.1         0.16         1.417           PDW:33-1.1         17         E40M59_0         10.2         3.5         0.07         -1.033           PDW:33-1.1         17         E45M42_4         844         4.9         0.10         -1.214		REL							
REL-S3-10.1         10         E77M40_5         67.6         8.0         0.14         1.559           REL-S3-10.2         10         E55M6_2         112.8         11.9         0.23         -2.254           REL-S3-10.2         10         E55M6_2         11.28         10.0         5.9         0.11         0.092           OPr         OPFT-S3-7.1         7         005805         0.0         3.1         0.10         0.022           OPW         OPT-S3-9.1         9         E8805.0         6.13         3.7         0.08         -0.997           PDW-S3-2.1         2         E8805.0         6.64         5.0         0.01         -1.306           PDW-S3-1.5         HA700         70.4         8.9         0.20         -1.711           PDW-S3-1.1         11         HA208         7.27         3.8         0.09         1.026           PDW-S3-1.7.1         17         E51M42.4         8.44         4.9         0.13         -1.214           PDW-S3-1.7.1         17         E51M42.8         110.2         4.5         0.10         -1.214           PDW-S3-1.7.1         17         E51M42.9         7.81         3.7         0.07         -0.006			REL-S3-4.2		E40M59_6	60.4			1.268
REL-35-10.2         10         ESM62.9         112.8         11.9         0.23        2.254           0PrT         0PfT-35-5.1         5         F41M62.7         0.0         4.3         0.10         0.022           0PT-30-7.1         7         0K331.2         0.0         4.8         0.15         -0.027           0PT-35-7.1         7         0K3805         0.0         3.1         0.10         0.022           PDW         PDW-53-2.1         2         ESM50.26         61.3         3.7         0.06         -0.097           PDW-53-1.1         4         F40M52.5         64.4         5.0         0.09         1.203           PDW-53-1.1         5         H43700         7.04         8.9         0.20         -1.751           PDW-53-1.1         13         H44208         7.2         3.8         0.09         1.026           PDW-53-1.1         17         F41M48.3         10.2         3.5         0.07         -1.043           PDW-53-7.1         7         H41448         25.6         3.4         0.10         -0.007           PV/Fm-52-8.1         13         0.7         -0.633         3.1         0.06         0.005			REL-S3-9.1	9	E33M60_5	91.9	4.2	0.07	-0.857
REL-S3-13.1         13         F40M47_21         0.0         5.9         0.11         0.902           0PT         0PFT-53-5.1         5         F41M62_7         0.0         4.3         0.10         0.022           0PFTS3-7.1         7         0K5805         0.0         3.1         0.10         0.020           PDW         0PFTS3-7.1         2         E58N50_26         61.3         3.7         0.08         -0.997           PDW-33-4.1         4         F40M59_12         25.6         4.5         0.11         -1.306           PDW-33-5.1         5         HA3700         70.4         8.9         0.20         -1.731           PDW-53-1.1         13         HA4208         72.7         3.8         0.09         1.3         -1.530           PDW-53-17.1         17         E51M62_8         110.2         3.5         0.07         -1.063           S2         Fv/Fm         Fv/Fm-S2-7.1         7         NR531_1         12.5         6.2         0.13         -0.008           PV/M-52-11         5         E57M44_9         3.1         0.06         -0.007         -0.006         -0.005         -1.214         -1.214         -1.214         -1.214         -1.21			REL-S3-10.1	10	E37M49_5	67.6	8.0	0.14	1.559
OPrr OPFT-32-5.1         OPFT-53-5.1         5         F41M62_7         0.0         4.3         0.10         0.022           PDW         PDW-53-2.1         2         OKS31,2         0.0         4.8         0.15         -0.027           PDW         PDW-53-2.1         2         ESM50,26         61.3         3.7         0.08         -0.027           PDW-53-4.2         4         EAM50,26         64.4         5.0         0.0997         -1.36           PDW-53-1.1         5         H47006         7.04         8.9         0.20         -1.751           PDW-53-1.1         13         H4208         7.27         3.8         0.09         -1.026           PDW-53-1.1         15         ESM42.4         84.4         4.9         0.13         -1.026           PDW-53-1.7         17         ESM42.8         10.2         3.5         0.07         -1.063           PU/Fm32.9.1         15         ESM42.8         110.2         3.5         0.07         -1.063           PU/Fm32.9.1         15         ESM42.8         110.2         3.5         0.07         -0.006           CC         CC-2.9.1         1         EAM50.18         20.1         3.5         0.07<			REL-S3-10.2	10	E35M62_9	112.8	11.9	0.23	-2.254
$S2 \qquad Prime Prime Size 1 & 0 (Size 1 & 0 $			REL-S3-13.1	13	E40M47_21	0.0	5.9	0.11	0.902
S2         Pv/Fn S3-71         7         ORS31_2         00         48         0.15         -0.027           PDW         PDV-S3-21         2         E3N55_26         61.3         3.7         0.08         -0.097           PDWS-S3-21         2         E3N55_26         61.3         3.7         0.08         -0.097           PDW-S3-24         4         E40M59_12         25.6         4.5         0.11         -1.306           PDW-S3-51.3         5         HA3700         704         8.9         0.20         -1.751           PDW-S3-13.1         13         HA4208         72.7         3.8         0.09         1.026           PDW-S3-17.1         17         E4M48_3         102.4         4.5         0.10         -1.214           PDW-S3-17.2         17         E3M42_9         8.1         10.2         3.5         0.07         -1.063           S2         Pv/Fm-S2-7.2         7         H1446_3         102.4         4.5         0.10         -1.214           PDW-S3-15.1         15         E7M49_9         7.4         3.7         0.07         -0.005           Pv/Fm-S2-2.1         7         H1546_2.1         1.1.5         4.0         0.6		OD		~	F41MC2 7	0.0	4.2	0.10	0.022
OPFT-S3-9.1         9         ORS805         0.0         3.1         0.10         0.020           PDW         PDW-33-2.1         2         E38M50.26         61.3         3.7         0.08         -0.997           PDW-33-4.1         4         E40M59.12         25.6         4.5         0.11         -1.306           PDW-33-4.2         4         E40M59.2         25.6         64.4         5.0         0.09         1.203           PDW-33-4.1         14         0651128         0.0         7.1         0.16         1.417           PDW-33-17.1         15         E35M48.4         84.4         4.9         0.13         -1.530           PDW-33-17.1         17         E41M48.2         100.2         4.5         0.10         -1.214           PDW-33-17.2         17         E35M62.8         110.2         3.5         0.07         -1.063           S2         Fv/Fm.S2-7.1         7         HA1848         25.6         3.4         0.10         -0.006           Fv/Fm.S2-15.1         15         E27M49.9         78.1         3.7         0.07         -9.064           CC C-22-21.1         1         E40M50.18         20.1         3.5         0.07		OP <sub>FT</sub>			—				
$ \begin{array}{c cccc} PDW & PDW-S3-2.1 & 2 & E38M50_{-26} & 61.3 & 3.7 & 0.08 & -0.997 \\ PDW-S3-4.1 & 4 & E40M59_{-12} & 25.6 & 4.5 & 0.11 & -1.306 \\ PDW-S3-5.1 & 5 & HA3700 & 70.4 & 8.9 & 0.20 & -1.751 \\ PDW-S3-5.1 & 15 & HA4208 & 72.7 & 3.8 & 0.09 & 1.026 \\ PDW-S3-17.1 & 14 & 0051128 & 0.0 & 7.1 & 0.16 & 1.417 \\ PDW-S3-17.1 & 17 & E41M48_{$					_				
PDW-S3-4.1         4         E40M59_12         25.6         4.5         0.11         -1.306           PDW-S3-5.1         5         H43700         70.4         8.9         0.20         -1.731           PDW-S3-14.1         14         0RS1128         0.0         7.1         0.16         1.417           PDW-S3-15.1         15         E35M42.4         8.4.4         4.9         0.13         -1.330           PDW-S3-17.1         17         E41M42.3         10.2.4         4.5         0.07         -1.063           PDW-S3-17.2         17         CRS31.1         1.2.5         6.2         0.13         -0.008           PV/Fm-S2-7.2         7         HA1848         25.6         3.4         0.10         -0.007           Fv/Fm-S2-7.2         7         HA1848         25.6         3.4         0.16         -0.007           Fv/Fm-S2-8.1         8         SSU217         7.2         8.1         0.16         -0.006           CC         CCS2-1.1         1         E40M50_18         2.011         3.5         0.07         -0.006           CC         CCS2-1.1         1         E40M50_17         1.8         7.4         0.04         1.1414			0PF1-53-9.1	9	083805	0.0	3.1	0.10	0.020
PDW-53-42         4         E400459_6         64.4         5.0         0.09         1.203           PDW-53-13.1         13         HA4208         7.2.7         3.8         0.09         1.026           PDW-53-13.1         13         HA4208         7.2.7         3.8         0.09         1.026           PDW-53-15.1         15         E550484.4         84.4         4.9         0.13         -1.530           PDW-53-17.2         17         E410482.3         102.4         4.5         0.00         -1.066           PDW-53-17.2         17         E410482.3         102.4         4.5         0.00         -1.066           S2         Fv/Fm         Py/Fm-52-7.1         7         OR5331_1         12.5         6.2         0.13         -0.008           PV/Fm-52-7.2         7         HA1848         25.6         3.4         0.10         -0.007           PV/Fm-52-7.1         7         E410450_18         20.1         3.5         0.07         0.954           CC         CC-52-1.1         1         E400450_18         20.1         3.5         0.07         0.954           CC (C-22-1.1         1         E40450_173         51.4         3.7         0.086		PDW	PDW-S3-2.1	2	E38M50_26	61.3	3.7	0.08	-0.997
PDW-S3-5.1         5         HA3200         70.4         8.9         0.20         -1.751           PDW-S3-14.1         14         06S1128         0.0         7.1         0.16         1.417           PDW-S3-14.1         14         06S1128         0.0         7.1         0.16         1.417           PDW-S3-17.1         17         E3SM42         84.4         4.9         0.10         -1.530           PDW-S3-17.2         17         E3SM62_8         110.2         3.5         0.07         -1.063           S2         Fv/Fm         Pr/fm.S2-7.7         7         HA1848         25.6         3.4         0.10         -0.007           Pv/fm.S2-8.1         8         SSU217         7.2         8.1         0.16         -0.007           Pv/fm.S2-8.1         15         E37M49_9         78.1         3.7         0.07         -0.006           CC 22-2.1         1         F44M50_18         20.1         3.5         0.07         0.954           CC 22-2.1         2         F41M50_18         20.1         3.5         0.07         0.954           CC 22-10.1         10         E35M61_2         11.5         40         0.06         0.970			PDW-S3-4.1	4	E40M59_12	25.6	4.5	0.11	-1.306
PDW-S3-5.1         5         HA3200         70.4         8.9         0.20         -1.751           PDW-S3-14.1         14         06S1128         0.0         7.1         0.16         1.417           PDW-S3-14.1         14         06S1128         0.0         7.1         0.16         1.417           PDW-S3-17.1         17         E3SM42         84.4         4.9         0.10         -1.530           PDW-S3-17.2         17         E3SM62_8         110.2         3.5         0.07         -1.063           S2         Fv/Fm         Pr/fm.S2-7.7         7         HA1848         25.6         3.4         0.10         -0.007           Pv/fm.S2-8.1         8         SSU217         7.2         8.1         0.16         -0.007           Pv/fm.S2-8.1         15         E37M49_9         78.1         3.7         0.07         -0.006           CC 22-2.1         1         F44M50_18         20.1         3.5         0.07         0.954           CC 22-2.1         2         F41M50_18         20.1         3.5         0.07         0.954           CC 22-10.1         10         E35M61_2         11.5         40         0.06         0.970			PDW-S3-4.2	4	E40M59_6	64.4	5.0	0.09	1.203
PDW-33-13.1         13         HA4208         72.7         3.8         0.09         1.026           PDW-33-15.1         15         E35M48.4         84.4         4.9         0.13         -1.530           PDW-33-15.1         17         E35M62.8         110.2         3.5         0.07         -1.063           S2         Fv/Fm         Py/Fm-32-7.1         7         CK5331_1         12.5         6.2         0.13         -0.008           Fv/Fm-22-7.2         17         E35M62.8         110.2         5.5         0.07         -1.063           S2         Fv/Fm-32-7.2         7         HA1848         25.6         3.4         0.10         -0.007           Fv/Fm-32-9.1         9         HA2053         43.9         3.1         0.06         -0.005           Fv/Fm-52-15.1         15         E37M49.9         78.1         3.7         0.07         -0.006           CC         CC         CC-52-1.1         1         E40M50.12         11.5         4.0         0.06         0.894           CC-52-2.1         1         16         E37M49.9         78.1         3.7         0.07         -0.005           CC-52-1.1         1         E40M50.12         11.5			PDW-S3-5.1	5	HA3700	70.4		0.20	
PDW-S3-14.1         14         OKS1/28         0.0         7.1         0.16         1.417           PDW-S3-17.1         17         E35M42, 4         8.4         4.9         0.13         -1.530           PDW-S3-17.2         17         E35M62, 8         110.2         3.5         0.07         -1.063           S2         Fv/Fm <s2-7.1< td="">         7         OKS31_1         12.5         6.2         0.13         -0.008           Fv/Fm-S2-8.1         8         SU217         7.2         8.1         0.16         0.010           Fv/Fm-S2-8.1         8         SU217         7.2         8.1         0.16         0.010           Fv/Fm-S2-8.1         9         HA2053         43.9         3.1         0.06         -0.007           Fv/Fm-S2-15.1         15         E37M49_9         78.1         3.7         0.07         0.954           CC-S2-2.1         1         E40M50_18         20.1         3.5         0.07         0.954           CC-S2-2.1         1         E40M50_17         2.3.1         4.4         0.06         0.899           CC-S2-1.1         1         E40M50_17.2         2.3.1         4.4         0.886         0.7         0.6.33         0.6</s2-7.1<>									
PDW-S3-15.1         15         F25M48.4         44.4         4.9         0.13         -1.530           S2         Fv/Fm         Fv/Fm-S2-7.1         7         F41M48.3         10.2         3.5         0.07         -1.063           S2         Fv/Fm         Fv/Fm-S2-7.2         7         HA1848         25.6         3.4         0.10         -0.008           Fv/Fm-S2-8.1         8         SSU217         7.2         8.1         0.16         0.010           Fv/fm-S2-9.1         9         HA2053         43.9         3.1         0.06         -0.005           Fv/fm-S2-15.1         15         EX7M49.9         78.1         3.7         0.07         -0.066           CC         CC-S2-1.1         1         E40M50.18         20.1         3.5         0.07         -0.066           CC-S2-2.1         2         E41M50.12         11.5         4.0         0.66         0.894           CC-S2-1.1         10         E35M61.5         134.1         3.6         0.06         0.970           CC-S2-1.2         11         10         E35M61.5         134.1         3.6         0.06         0.973           CC-S2-1.6         16         E37M47.5         86.8 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
PDW-S3-17.1         17         E4IM48_3         102.4         4.5         0.10         -1.124           S2         Fv/Fm         Fv/Fm-S2-7.1         7         RRS31_1         12.5         6.2         0.13         -0.008           Fv/Fm-S2-7.2         7         HA1848         25.6         3.4         0.10         -0.007           Fv/Fm-S2-8.1         8         SSU217         7.2         8.1         0.16         0.010           Fv/Fm-S2-9.1         9         H42053         43.9         3.1         0.06         -0.005           Fv/Fm-S2-15.1         15         E37M49_9         78.1         3.7         0.07         -0.006           CC         CC S2-1.1         1         E40M50_18         20.1         3.5         0.07         0.954           CC S2-1.1         1         E40M50_17         7.1.8         7.4         0.14         1.414           CC S2-1.1         1         E40M50_17         7.3.1         4.4         0.08         0.889           CC S2-1.1         1         E40M50_17         7.3.1         4.4         0.08         0.6971           CC S2-1.1         10         E35M61_6         13.4.1         3.6         0.06         0.7									
PDW-S3-17.2         17         E35M62_8         110.2         3.5         0.07         -1.063           S2         Fv/Fm         Fv/Fm-S2-7.1         7         PR331_1         12.5         6.2         0.13         -0.008           Fv/Fm-S2-7.2         7         HA1848         25.6         3.4         0.10         -0.007           Fv/Fm-S2-8.1         8         SSU217         7.2         8.1         0.16         0.010           Fv/Fm-S2-9.1         9         HA2053         43.9         3.1         0.06         -0.005           Fv/Fm-S2-15.1         15         E37M49_9         78.1         3.7         0.07         0.954           CC-S2-1.1         1         E40M50_18         20.1         3.5         0.07         0.954           CC-S2-1.1         1         E40M50_12         11.5         4.0         0.06         0.894           CC-S2-1.1         10         E35M62_1         71.8         7.4         0.14         1414           CC-S2-1.1         14         E32M62_1         71.8         3.4         0.06         0.773           CC-S2-1.6.1         16         E37M47_5         86.8         3.8         0.07         -0.887 <tr< td=""><td></td><td></td><td></td><td></td><td>—</td><td></td><td></td><td></td><td></td></tr<>					—				
Fv/fm-S2-72         7         HA1848         25.6         3.4         0.10        0.007           Fv/fm-S2-8.1         8         SSU217         7.2         8.1         0.16         0.010           Fv/fm-S2-9.1         9         HA2053         33.9         3.1         0.06        0.005           Fv/fm-S2-15.1         15         E37M45_9         78.1         3.7         0.07         -9.006           CC         CC-S2-11         1         E40M50_12         11.5         40         0.06         0.894           CC-S2-21         2         E41M50_12         11.5         40         0.66         0.970           CC-S2-10.1         10         E35M61_6         134.1         3.6         0.06         0.970           CC-S2-12.1         12         ORS671_2         23.1         4.4         0.889         0.6         0.773           CC-S2-16.1         16         E37M47_5         86.8         3.8         0.06         0.773           CC-S2-16.1         16         E37M47_19         40.2         3.5         0.08         -0.637           SLA-S2-S.1         5         E41M62_30         17.5         4.7         0.08         -0.637					—				
Fv/fm-S2-72         7         HA1848         25.6         3.4         0.10        0.007           Fv/fm-S2-8.1         8         SSU217         7.2         8.1         0.16         0.010           Fv/fm-S2-9.1         9         HA2053         33.9         3.1         0.06        0.005           Fv/fm-S2-15.1         15         E37M45_9         78.1         3.7         0.07         -9.006           CC         CC-S2-11         1         E40M50_12         11.5         40         0.06         0.894           CC-S2-21         2         E41M50_12         11.5         40         0.66         0.970           CC-S2-10.1         10         E35M61_6         134.1         3.6         0.06         0.970           CC-S2-12.1         12         ORS671_2         23.1         4.4         0.889         0.6         0.773           CC-S2-16.1         16         E37M47_5         86.8         3.8         0.06         0.773           CC-S2-16.1         16         E37M47_19         40.2         3.5         0.08         -0.637           SLA-S2-S.1         5         E41M62_30         17.5         4.7         0.08         -0.637									
Fv/Fm-S2-8.1         8         SSU217         7.2         8.1         0.16         0.010           Pv/Fm-S2-9.1         9         HA2053         43.9         3.1         0.06         -0.005           CC         CC-S2-1.1         15         E37M49_9         78.1         3.7         0.07         -0.006           CC         CC-S2-1.1         2         E41M50_12         11.5         4.0         0.06         0.894           CC-S2-1.1         4         E35M61_6         71.81         7.4         0.14         1.414           CC-S2-10.1         10         E35M61_6         134.1         3.6         0.06         0.894           CC-S2-12.1         12         ORS671_2         23.1         4.4         0.08         0.886           CC-S2-13.1         15         ORS677_2         63.3         40.06         0.773           CC-S2-16.1         16         E37M47_5         86.8         3.8         0.07         -0.887           SLA-S2-S1         5         E41M62_30         17.5         4.7         0.08         -0.637           SLA-S2-S1.1         1         E35M62_4         38.8         3.8         0.07         0.669           SLA-S2-10.1 </td <td>S2</td> <td>Fv/Fm</td> <td>Fv/Fm-S2-7.1</td> <td>7</td> <td>ORS331_1</td> <td>12.5</td> <td>6.2</td> <td>0.13</td> <td>-0.008</td>	S2	Fv/Fm	Fv/Fm-S2-7.1	7	ORS331_1	12.5	6.2	0.13	-0.008
Fy/Fm-S2-9.1       9       HA2053       43.9       3.1       0.06       -0.005         CC       CC-S2-1.1       1       E437M49_9       78.1       3.7       0.07       -0.006         CC       S2-1.1       1       E40M50_18       20.1       3.5       0.07       -0.096         CC-S2-2.1       2       E41M50_12       11.5       4.0       0.06       0.894         CC-S2-1.1       1       CC-S2-1.1       1.6       6.35M61_6       134.1       3.6       0.06       0.970         CC-S2-1.1       1       CC-S2-1.1       1       CC-S2-1.1       3.1       4.4       0.08       0.889         CC-S2-1.1       1       CC-S2-1.1       1       CC-S2-1.1       3.1       3.1       0.06       0.970         CC-S2-1.1       1       CC-S2-1.1       1       CC-S2-1.1       3.1       3.1       3.6       0.06       0.773         CC-S2-1.5.1       15       CRS687       66.3       3.4       0.06       0.773         CC-S2-1.6.1       16       E37M47_5       86.8       3.8       0.07       -0.689         SLA-S2-1.1       1       E35M62_4       3.8.8       3.8       0.07       0			Fv/Fm-S2-7.2	7	HA1848	25.6	3.4	0.10	-0.007
Fy/Fm-S2-9.1       9       HA2053       43.9       3.1       0.06       -0.005         CC       CC-S2-1.1       1       E437M49_9       78.1       3.7       0.07       -0.006         CC       S2-1.1       1       E40M50_18       20.1       3.5       0.07       -0.096         CC-S2-2.1       2       E41M50_12       11.5       4.0       0.06       0.894         CC-S2-1.1       1       CC-S2-1.1       1.6       6.35M61_6       134.1       3.6       0.06       0.970         CC-S2-1.1       1       CC-S2-1.1       1       CC-S2-1.1       3.1       4.4       0.08       0.889         CC-S2-1.1       1       CC-S2-1.1       1       CC-S2-1.1       3.1       3.1       0.06       0.970         CC-S2-1.1       1       CC-S2-1.1       1       CC-S2-1.1       3.1       3.1       3.6       0.06       0.773         CC-S2-1.5.1       15       CRS687       66.3       3.4       0.06       0.773         CC-S2-1.6.1       16       E37M47_5       86.8       3.8       0.07       -0.689         SLA-S2-1.1       1       E35M62_4       3.8.8       3.8       0.07       0			Fv/Fm-S2-8.1	8	SSU217	7.2	8.1	0.16	0.010
Fv/Fm-S2-15.1         15         E37M49_9         78.1         3.7         0.07         -0.006           CC         CC-S2-1.1         1         E40M50_18         20.1         3.5         0.07         0.954           CC-S2-2.1         2         E41M50_12         11.5         4.0         0.06         0.894           CC-S2-2.1         2         E41M50_12         11.5         4.0         0.06         0.894           CC-S2-10.1         10         E33M61_6         134.1         3.6         0.06         0.970           CC-S2-12.1         12         ORS671_2         23.1         4.4         0.08         0.889           CC-S2-15.1         15         ORS687         66.3         3.4         0.06         0.773           CC-S2-15.1         16         E37M47_5         86.8         3.8         0.07         -0.887           SLA         SLA-S2-8.1         8         SL30         26.9         7.7         0.14         -0.853           SLA-S2-8.1         8         SL30         26.9         7.7         0.14         -0.853           SLA-S2-8.1         8         E35M65_9         65.1         4.6         0.09         0.714 <t< td=""><td></td><td></td><td>Fv/Fm-S2-9.1</td><td></td><td>HA2053</td><td>43.9</td><td>3.1</td><td>0.06</td><td>-0.005</td></t<>			Fv/Fm-S2-9.1		HA2053	43.9	3.1	0.06	-0.005
CC-S2-2.1         2         E41M50_12         11.5         4.0         0.06         0.894           CC-S2-4.1         4         E35M62_1         71.8         7.4         0.14         1.414           CC-S2-4.1         10         E35M61_6         134.1         3.6         0.06         0.970           CC-S2-12.1         12         0R5671_2         23.1         4.4         0.08         0.889           CC-S2-15.1         15         0R5687         66.3         3.4         0.06         0.773           CC-S2-16.1         16         E37M47_5         86.8         3.8         0.07         -0.887           SLA         SLA-S2-8.1         5         E41M62_30         17.5         4.7         0.08         -0.637           SLA-S2-8.1         8         SSL30         26.9         7.7         0.14         -0.853           SLA-S2-8.1         8         SSL30         26.9         7.7         0.14         -0.853           SLA-S2-10.1         10         E35M62_4         3.8         3.8         0.07         0.669           SLA-S2-11.1         11         E36M59_9         65.1         4.6         0.09         -0.756           SLA-S2-11.1			Fv/Fm-S2-15.1		E37M49_9	78.1		0.07	-0.006
CC-S2-2.1         2         E41M50_12         11.5         4.0         0.06         0.894           CC-S2-4.1         4         E35M62_1         71.8         7.4         0.14         1.414           CC-S2-4.1         10         E35M61_6         134.1         3.6         0.06         0.970           CC-S2-12.1         12         0R5671_2         23.1         4.4         0.08         0.889           CC-S2-15.1         15         0R5687         66.3         3.4         0.06         0.773           CC-S2-16.1         16         E37M47_5         86.8         3.8         0.07         -0.887           SLA         SLA-S2-8.1         5         E41M62_30         17.5         4.7         0.08         -0.637           SLA-S2-8.1         8         SSL30         26.9         7.7         0.14         -0.853           SLA-S2-8.1         8         SSL30         26.9         7.7         0.14         -0.853           SLA-S2-10.1         10         E35M62_4         3.8         3.8         0.07         0.669           SLA-S2-11.1         11         E36M59_9         65.1         4.6         0.09         -0.756           SLA-S2-11.1		66	CC C2 1 1	1	F40ME0 10	20.1	25	0.07	0.054
CC-S2-4.1         4         E35M62_1         71.8         7.4         0.14         1.414           CC-S2-10.1         10         E35M61_6         134.1         3.6         0.06         0.970           CC-S2-12.1         12         ORS671_2         23.1         4.4         0.08         0.889           CC-S2-14.1         14         E32M61_13         51.4         3.7         0.08         0.886           CC-S2-15.1         15         ORS687         66.3         3.4         0.06         0.773           CC-S2-16.1         16         E37M47_5         86.8         3.8         0.07         -0.887           SLA         SLA-S2-5.1         5         E41M62_30         17.5         4.7         0.08         -0.637           SLA-S2-8.1         8         SSL30         26.9         7.7         0.14         -0.853           SLA-S2-10.1         10         E35M62_4         38.8         3.8         0.07         0.669           SLA-S2-11.1         11         E36M59_9         65.1         4.6         0.09         -0.714           SLA-S2-15.1         15         SU223         11.6         3.4         0.05         0.544           REL		u			—				
CC-52-10.1       10       E33M61_6       134.1       3.6       0.06       0.970         CC-52-12.1       12       0RS671_2       23.1       4.4       0.08       0.889         CC-52-12.1       12       0RS677       66.3       3.4       0.06       0.773         CC-52-15.1       15       0RS687       66.3       3.4       0.06       0.773         CC-52-16.1       16       E37M47_5       86.8       3.8       0.07       -0.887         SLA       SLA-S2-5.1       5       E41M62_30       17.5       4.7       0.08       -0.637         SLA-S2-8.2       8       SSL30       26.9       7.7       0.14       -0.853         SLA-S2-10.1       10       E35M62_4       38.8       3.8       0.07       0.669         SLA-S2-12.1       12       E40M50_9       0.0       3.4       0.06       -0.576         SLA-S2-13.1       14       ORS1128       0.0       3.3       0.07       0.595         SLA-S2-14.1       14       ORS1128       0.0       3.3       0.07       0.595         SLA-S2-15.1       15       SSU223       11.6       3.4       0.05       0.544         <									
CC-S2-12.1       12       ORS671_2       23.1       4.4       0.08       0.889         CC-S2-14.1       14       B22M61_13       51.4       3.7       0.08       0.886         CC-S2-15.1       15       ORS687       66.3       3.4       0.06       0.773         CC-S2-16.1       16       E37M47_5       86.8       3.8       0.07       -0.887         SLA       SLA-S2-5.1       5       E41M62_30       17.5       4.7       0.08       -0.637         SLA-S2-8.1       8       SSL30       26.9       7.7       0.14       -0.853         SLA-S2-10.1       10       E35M62_4       38.8       3.8       0.07       0.669         SLA-S2-11.1       11       E36M59_9       65.1       4.6       0.09       0.714         SLA-S2-12.1       12       E40M50_9       0.0       3.4       0.06       -0.576         SLA-S2-15.1       15       SSU223       11.6       3.4       0.05       0.544         REL       REL-S2-5.1       5       E35M49_6       48.4       6.0       0.21       1.168         REL       REL-S2-5.1       7       5       SU233       11.6       3.4       0.									
CC-S2-14.1       14       E32M61_13       51.4       3.7       0.08       0.886         CC-S2-15.1       15       ORS687       66.3       3.4       0.06       0.773         CC-S2-16.1       16       E37M47_5       86.8       3.8       0.07       -0.887         SLA       SLA-S2-5.1       5       E41M62_30       17.5       4.7       0.08       -0.637         SLA-S2-8.2       8       E37M47_19       40.2       3.5       0.09       -0.689         SLA-S2-8.2       8       E37M47_19       40.2       3.5       0.09       -0.689         SLA-S2-10.1       10       E35M62_4       38.8       3.8       0.07       0.669         SLA-S2-12.1       12       E40M50_9       0.0       3.4       0.06       -0.576         SLA-S2-14.1       14       ORS1128       0.0       3.3       0.07       0.595         SLA-S2-14.1       14       ORS1128       0.0       3.3       0.07       0.595         SLA-S2-14.1       14       ORS312       2.0       9.5       0.544         REL       REL-S2-5.1       5       E35M60_1       25.6       4.4       0.09       -0.752									
CC-S2-15.1         15         ORS687         66.3         3.4         0.06         0.773           CC-S2-16.1         16         E37M47_5         86.8         3.8         0.07         -0.887           SLA         SLA-S2-5.1         5         E41M62_30         17.5         4.7         0.08         -0.637           SLA-S2-8.1         8         SSL30         26.9         7.7         0.14         -0.853           SLA-S2-8.2         8         E37M47_19         40.2         3.5         0.09         -0.669           SLA-S2-10.1         10         E35M62_4         38.8         3.8         0.07         0.669           SLA-S2-11.1         11         E36M59_9         65.1         4.6         0.09         0.714           SLA-S2-12.1         12         E40M50_9         0.0         3.3         0.07         0.595           SLA-S2-15.1         15         SU223         11.6         3.4         0.05         0.544           REL         REL-S2-5.1         5         E35M60_1         25.6         4.4         0.09         -0.752           REL-S2-5.1         5         E35M49_6         48.4         6.0         0.21         1.168									
CC-S2-16.1       16       E37M47_5       86.8       3.8       0.07       -0.887         SLA       SLA-S2-5.1       5       E41M62_30       17.5       4.7       0.08       -0.637         SLA-S2-8.1       8       SSL30       26.9       7.7       0.14       -0.853         SLA-S2-8.2       8       E37M47_19       40.2       3.5       0.09       -0.689         SLA-S2-10.1       10       E35M62_4       38.8       3.8       0.07       0.669         SLA-S2-12.1       11       E36M59_9       65.1       4.6       0.09       -0.714         SLA-S2-12.1       12       E40M50_9       0.0       3.4       0.06       -0.576         SLA-S2-15.1       15       SSU223       11.6       3.4       0.05       0.544         REL       REL-S2-5.1       5       E35M60_1       25.6       4.4       0.09       -0.752         REL-S2-5.1       5       E35M49_6       48.4       6.0       0.21       1.168         REL-S2-5.1       6       E41M48_2       18.5       7.5       0.15       -1.212         REL-S2-5.2       5       E35M49_6       48.4       6.0       0.23       -1.192					—				
SLA       SLA-S2-5.1       5       E41M62_30       17.5       4.7       0.08       -0.637         SLA-S2-8.1       8       SSL30       26.9       7.7       0.14       -0.853         SLA-S2-8.2       8       E37M47_19       40.2       3.5       0.09       -0.689         SLA-S2-10.1       10       E35M62_4       38.8       3.8       0.07       0.669         SLA-S2-11.1       11       E36M59_9       65.1       4.6       0.09       0.714         SLA-S2-12.1       12       E40M50_9       0.0       3.4       0.06       -0.576         SLA-S2-15.1       15       SSU223       11.6       3.4       0.05       0.544         REL       REL-S2-5.1       5       E35M60_1       25.6       4.4       0.09       -0.752         REL-S2-5.2       5       E35M49_6       48.4       6.0       0.21       1.168         REL-S2-5.1       5       E35M49_2       18.5       7.5       0.15       -1.212         REL-S2-6.1       6       E41M48_2       18.5       7.5       0.15       -1.212         REL-S2-7.1       7       ORS331_2       2.0       9.5       0.23       -1.192									
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$			CC-52-16.1	16	E37M47_5	86.8	3.8	0.07	-0.887
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$		SLA	SLA-S2-5.1	5	E41M62_30	17.5	4.7	0.08	-0.637
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REL-S2-7.1         7         ORS331_2         2.0         9.5         0.23         -1.192           REL-S2-11.1         11         ORS354         11.4         4.1         0.08         0.688           REL-S2-13.1         13         E33M48_20         32.9         7.6         0.18         1.071           REL-S2-13.1         13         ORS316         60.7         5.2         0.11         -0.840           OP <sub>FT</sub> OPFT-S2-10.1         10         E32M61_7         59.2         4.9         0.11         -0.031					—				
REL-S2-11.1         11         ORS354         11.4         4.1         0.08         0.688           REL-S2-13.1         13         E33M48_20         32.9         7.6         0.18         1.071           REL-S2-13.1         13         ORS316         60.7         5.2         0.11         -0.840           OP <sub>FT</sub> OPFT-S2-10.1         10         E32M61_7         59.2         4.9         0.11         -0.031					—				
REL-S2-13.1         13         E33M48_20         32.9         7.6         0.18         1.071           REL-S2-13.1         13         ORS316         60.7         5.2         0.11         -0.840           OP <sub>FT</sub> OPFT-S2-10.1         10         E32M61_7         59.2         4.9         0.11         -0.031					_				
REL-S2-13.1         13         ORS316         60.7         5.2         0.11         -0.840           OP <sub>FT</sub> OPFT-S2-10.1         10         E32M61_7         59.2         4.9         0.11         -0.031									
OP <sub>FT</sub> OPFT-S2-10.1 10 E32M61_7 59.2 4.9 0.11 -0.031					—				
			REL-S2-13.1	13	ORS316	60.7	5.2	0.11	-0.840
		OP	OPFT_\$2_10.1	10	F32M61 7	59.2	49	0.11	_0.031
		OI FT	OPFT-S2-10.1 OPFT-S2-10.2	10	HA2579	166.5	3.3	0.08	0.022

Table 3	(Continued)
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Sowing date	Trait	QTL	Linkage group	Nearest marker	Position cM <sup>a</sup>	LOD score	R <sup>2b</sup>	Additive effect
		OPFT-S2-14.1	14	HA2714	21.6	3.5	0.10	0.023
		OPFT-S2-15.1	15	E38M60_2	20.1	3.8	0.07	0.018
		OPFT-S2-16.1	16	E37M47_26	22.2	4.8	0.11	-0.022
		OPFT-S2-16.2	16	E38M48_7	161.5	5.1	0.10	0.021
	PDW	PDW-S2-1.1	1	HA4090	66.4	3.6	0.09	0.529
		PDW-S2-5.1	5	HA3700	70.4	3.3	0.07	-0.499
		PDW-S2-10.1	10	SSL49	151.0	3.8	0.09	-0.603
		PDW-S2-10.2	10	ORS807	162.7	4.9	0.11	-0.647
		PDW-S2-15.1	15	E35M48_4	86.4	3.8	0.10	-0.692
		PDW-S2-17.1	17	ORS297	31.2	3.4	0.06	0.520
		PDW-S2-17.2	17	E41M48_3	102.4	5.9	0.13	-0.702
		PDW-S2-17.3	17	E35M62_8	110.2	3.0	0.07	-0.519
S1	Fv/Fm	Fv/Fm-S1-10.1	10	E35M61_6	131.0	6.0	0.13	-0.017
51	1 V/1 111	Fv/Fm-S1-14.1	14	ORS391	73.4	4.0	0.09	0.009
		Fv/Fm-S1-17.1	17	E41M48_3	102.4	4.4	0.10	-0.010
	СС	CC-S1-5.1	5	E41M62_30	19.5	4.9	0.12	-0.987
		CC-S1-14.1	14	HA3886	14.4	3.2	0.11	-0.789
		CC-S1-17.1	17	ORS1040	120.4	6.8	0.16	1.151
	SLA	SLA-S1-4.1	4	E37M47_8	17.8	5.2	0.13	0.934
		SLA-S1-12.1	12	E40M50_9	0.0	3.7	0.07	-0.598
		SLA-S1-13.1	13	ORS511	52.7	6.5	0.15	0.978
		SLA-S1-14.1	14	E41M48_12	115.6	3.9	0.08	0.743
	REL	REL-S1-2.1	2	E32M47 9	2.0	3.9	0.11	1.176
		REL-S1-4.1	4	E35M49_4	18.1	6.0	0.12	-1.914
		REL-S1-5.1	5	ORS533	80.9	4.3	0.11	-1.198
		REL-S1-7.1	7	ORS331_2	0.0	7.3	0.17	-1.549
		REL-S1-17.1	17	E38M48_1	128.9	3.5	0.11	-1.105
	OP <sub>FT</sub>	OPFT-S1-1.1	1	E33M48_2	29.3	5.4	0.12	-0.018
		OPFT-S1-5.1	5	E37M61_10	12.3	6.3	0.12	-0.021
		OPFT-S1-5.2	5	E41M59_10	31.9	3.1	0.10	0.016
		OPFT-S1-10.1	10	ORS1144	162.7	4.8	0.11	-0.017
		OPFT-S1-11.1	11	ORS1146	11.8	6.1	0.12	0.018
		OPFT-S1-13.1	13	ORS316	72.7	4.6	0.10	-0.016
		OPFT-S1-15.1	15	E35M48_4	82.4	3.9	0.07	-0.014
		OPFT-S1-16.1	16	E38M60_11	138.4	6.0	0.17	-0.021
		OPFT-S1-17.1	17	E35M62_5	40.6	3.4	0.07	0.014
	PDW	PDW-S1-9.1	9	ORS428_2	39.8	5.6	0.14	0.112
		PDW-S1-14.1	14	ORS1128	0.0	3.6	0.09	0.081
		PDW-S1-16.1	16	HA3582	13.9	4.8	0.13	-0.103
		PDW-S1-16.2	16	E32M47_1	44.5	4.8	0.17	0.148

<sup>a</sup> From the north of linkage group.

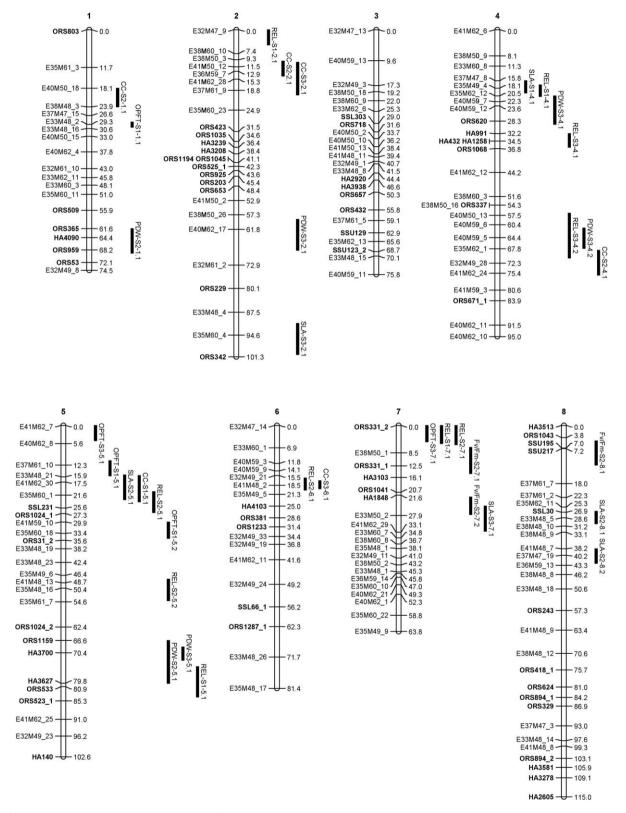
<sup>b</sup> Percentage of individual phenotypic variance explained.

already been observed for drought adaptive traits on the same RIL population [42,46]. QTLs identified in the present study showed that several putative genomic regions were involved in the expression of the physiological traits under the three sowing dates (Table 3). The positive and negative signs of additive effect at the different loci indicated the genetic contribution of both parental lines. This confirms the transgressive segregation observed at phenotypic level. Most of QTLs were detected only in one specific environmental condition and constitute adaptative QTLs [25]. The analysis of genes expression showed that 108 cDNA clones were found to be differentially expressed in response to low temperature in sunflower and about 90% of these genes were down regulated [47]. It has been established that the expression of hundreds of genes is altered in response to low temperature [48].

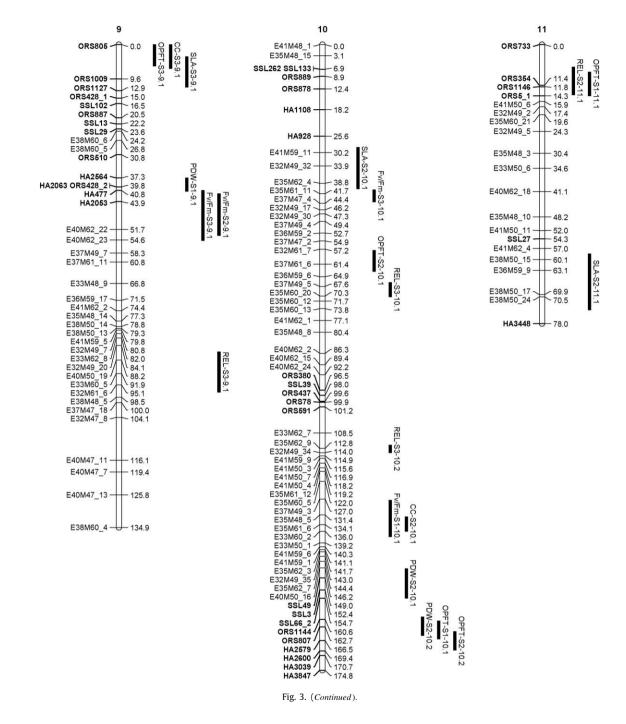
REL was higher after long-term low temperature exposure compared to control (S3) (Table 1) which shows that low temperature leads to a decrease in cell membrane stability. Higher cell membrane stability (which is associated with low values of REL) in response to cold stress after long-term low temperature exposure is considered as a cold acclimation process [10]. This phenomenon was reported in *Arabidopsis* and in rose clover [10]. In contrast, our results show that the cell membrane stability in RILs studied was lower after a long-term low temperature exposure indicating cold damage. This confirms results obtained by Hewezi et al. [47]. The authors have evaluated the frost tolerance of sunflower at -3.8 °C, -4.8 °C and -5.8 °C by measurements of electrolyte leakage. They found that after exposure to low temperature, no cold acclimation but cold damage was observed in the sunflower genotypes studied. In our study, the most important QTL for REL found on linkage group 7 linked to the SSR marker (*ORS331\_2*), was common between S1 and S2 (Table 3). These genomic regions should be more investigated to see if they present QTLs related to cold tolerance which are stable in many environments and also in other genetic backgrounds, then they should be used for marker-assisted selection programs.

The long-term low temperature exposure induced a reduction of growth capacity as indicated by the reduction of photosynthetic activity (CC and Fv/Fm) and a reduction of dry matter accumulation (Table 1). Common genomic regions were involved between S2 and S3 conditions for photosynthetic traits and dry matter accumulation on the linkage groups 2, 9, 12, and 15–17 (Fig. 3). Two regions in linkage groups 10 and 16 were detected where QTLs for photosynthetic traits (Fv/Fm and CC) were co-located. These two genomic regions should contain genes with pleiotropic effect which control at the same time efficiency of photosystem II and chlorophyll content. A strong reduction of the plant dry matter was observed in S1 compared to S2 and S3 (Table 1). Low temperatures did not occur during the same period of plant development for S1 and S2 (Fig. 1): plants in S1 were maintained at low temperature after emergence stage whereas in S2, the temperature after

emergence was close to control condition (daily mean temperature of 15.6 °C and 16.2 °C for S2 and S3, respectively). Photosynthetic apparatus has been activated for S2 and S3 under the same temperature conditions whereas in S1 temperatures were colder



**Fig. 3.** Sunflower genetic linkage map showing the position of QTL associated with physiological traits in three sowing dates: one control sowing date (S3) and two early sowing dates associated with low temperature (S1 and S2). The physiological traits investigated are the potential photochemical efficiency of photosystem II (Fv/Fm), the chlorophyll content (CC), the specific leaf area (SLA), the relative electrolyte leakage (REL), the osmotic potential at full turgor (OPFT) and the plant dry weight (PDW). The positions of the QTLs are represented on the right side of the linkage groups. Bars represent intervals associated with the QTLs.



on this stage. These low temperatures occurring in S1 have probably induced the detection of specific QTLs associated to low temperature adaptation of the photosynthetic apparatus. The effect of low temperature on photosynthetic efficiency has been reported in Arabidopsis thaliana [10], maize [49], rice [50], wheat [14] and barley [15] but as far as we know, it is not studied in sunflower. In our study, plants grown under low temperature conditions were characterized by a lower photochemical efficiency of photosystem II (Fv/Fm) compared with plants grown in control condition (S3). As shown in wheat, the decrease in the photochemical efficiency of photosystem II (Fv/Fm) can result from the photodamage of the D1 protein of PSII reaction centres and the increase of dissipating excitation energy in the PSII antennae as heat [14]. Low temperature exposure induced lower chlorophyll content compared with control (Table 1). This could reflect the photoprotective process due to the modification of the pigment composition to improve the ability to dissipate the excess light energy as heat via the xanthophyll cycle [51,52]. Photoprotective process leads to a decrease of the chlorophyll content and an increase of the zeaxanthin content as shown by Leipner et al. [53] in maize under low temperature condition. Specific QTLs detected for CC, Fv/Fm and PDW in S1 may be involved in these acclimation processes. These QTLs associated with SSRs marker as *ORS331\_1* for photochemical efficiency of photosystem II (Fv/Fm), *ORS1040* for the chlorophyll content (CC) and *ORS428\_2* for the plant dry weight (PDW) could be QTLs of interest for cold acclimation of the growth capacity. Co-located QTLs for CC and REL traits in S1 were detected in the linkage group 17. This genomic region may be implied in cold tolerance and growth acclimation to low temperature (Fig. 3).

Early sowing associated with low temperature induced a significant reduction of the specific leaf area (SLA). In our experiment, sunflower shows a significant genetic variability for

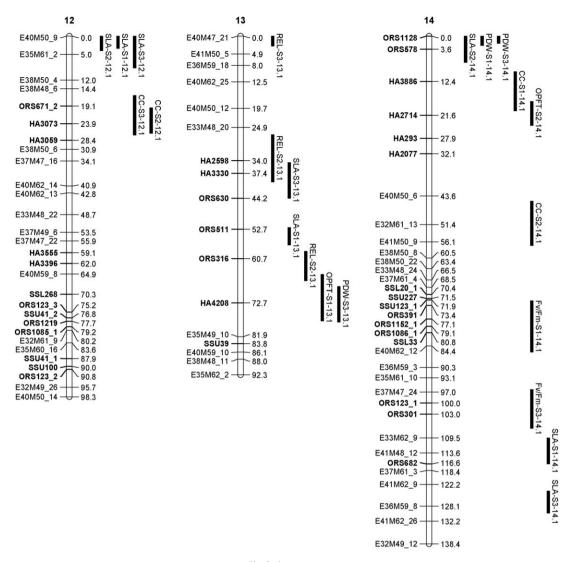


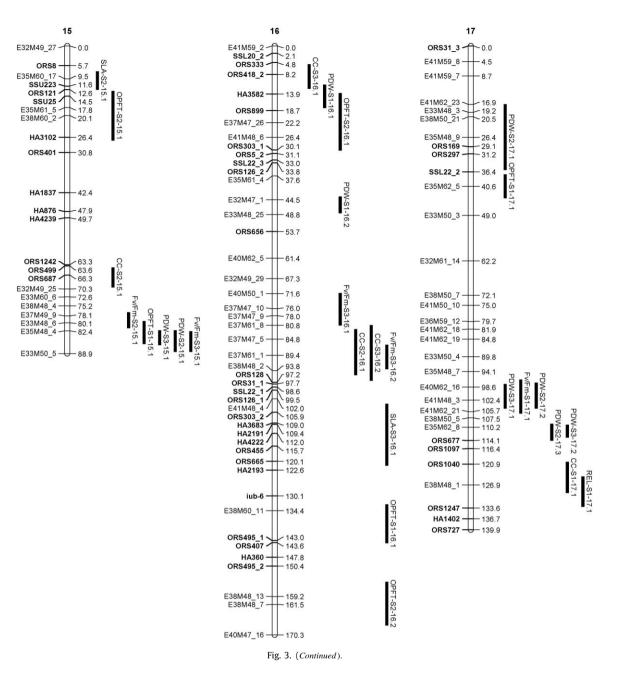
Fig. 3. (Continued).

SLA in response to low temperature. On linkage group 12, one region was detected where QTLs for SLA were co-located in all conditions indicated that this genomic region is stable for the control of the SLA (Fig. 3). Two overlapped QTLs between SLA and REL were detected on the linkage group 4 in S1 and on the linkage group 5 in S2, which show that SLA may be a component of cold tolerance. This phenomenon has been also reported in maize [54–56] and different annual legumes [57]. Verheul et al. [56] suggested that low SLA could arise from thicker leaves due to a thicker mesophyll and wax layers and a thicker cuticle. These morphological modifications are similar to xerophytic adaptation, and are commonly exhibited by freeze-tolerant plants [58].

Low temperature implied a decrease of the osmotic potential at full turgor ( $OP_{FT}$ ) (Table 1). This indicates an increase of the intracellular osmolyte concentration in sunflower genotypes in response to low temperature exposure. The osmotic potential presented a substantial genetic variability after low temperature exposure (Table 1), which shows that the osmotic potential is a useful trait to screen genotypes under low temperature treatment. This trait is a well-known indicator for cold acclimation in Citrus, Spinach and Petunia [22], white clover [59] and Puma Rey [21]. The locations of QTLs identified in the present study for osmotic potential at full turgor ( $OP_{FT}$ ) when compared with those controlling water status related traits reported by Poormohammad

Kiani et al. [30], showed overlapping on linkage groups 1, 5, 9, 16 and 17. On linkage group 16 we have identified the QTL, *OPF-S2-16.1* under low temperature condition which is overlapped with five QTLs identified by Poormohammad Kiani et al. [30] for relative water content, leaf water potential, turgor potential and osmotic potential at full turgor under water stress condition. This suggests that osmotic regulation observed in response to cold and drought stress were regulated by common genomic region. It was shown by Nakashima and Yamaguchi-Shinozaki [60] in *Arabidopsis* that the same genes (dehydration-responsive elements) were activated for osmotic regulation under cold and drought stress.

The whole results of our experiment do highlight that longterm low temperature exposure leads to a decrease in cell membrane stability associated with a decrease of the plant growth capacity (decrease of the plant dry weight, reduction of the chlorophyll content and the potential photochemical efficiency of photosystem II) and the osmotic potential at full turgor. We have also identified the traits associated with cold tolerance, as CC and SLA which are genetically related to REL. These traits would be more investigated to be used to screen genotypes of sunflower for cold tolerance improvement. Genomic regions presenting QTLs associated with SSR markers for REL are interesting for cold tolerance and should be more investigated for their stability across different environments.



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#### References

- [1] Z. Flagella, T. Rotunno, E. Tarantino, R. Di Caterina, A. De Caro, Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water regime, Eur. J. Agron. 17 (2002) 221–230.
- [2] P. Maury, F. Mojayad, M. Berger, C. Planchon, Photochemical response to drought acclimation in two sunflower genotypes, Physiol. Plant. 98 (1996) 57–66.
- [3] G.A. Pereyra-Irujo, L. Velázquez, C. Granier, L.A.N. Aguirrezábal, A method for drought tolerance screening in sunflower, Plant Breed. 126 (2007) 445–448.
- [4] V. Gimeno, J. Fernandez-Martinez, E. Fereres, Sunflower response to winter plantings in a mediterranean environment, Helia 8 (1985) 63–67.
- [5] A.J. de la Vega, A.J. Hall, Effects of planting date, genotype, and their interactions on sunflower yield. II. Components of oil yield, Crop Sci. 42 (2002) 1202–1210.
- [6] V. Gimeno, J. Fernandez-Martinez, E. Fereres, Winter planting as a means of drought escape in sunflower, Field Crop Res. 22 (1989) 307–316.

- [7] M.A. Soriano, F. Orgaz, F.J. Villalobos, E. Fereres, Efficiency of water use of early plantings of sunflower, Eur. J. Agron. 21 (2004) 465–476.
- [8] U. Anastasi, M. Cammarata, V. Abbate, Yield potential and oil quality of sunflower (oleic and standard) grown between autumn and summer, Ital. J. Agron. 4 (2000) 23–36.
- [9] J. Browse, Z. Xin, Temperature sensing and cold acclimation, Curr. Opin. Plant Biol. 4 (2001) 241–246.
- [10] M. Uemura, R.A. Joseph, P.L. Steponkus, Cold acclimation of Arabidopsis thaliana (effect on plasma membrane lipid composition and freeze-induced lesions), Plant Physiol. 109 (1995) 15–30.
- [11] T.R. Peoples, D.W. Koch, S.C. Smith, Relationship between chloroplast membrane fatty acid composition and photosynthetic response to a chilling temperature in four alfalfa cultivars, Plant Physiol. 61 (1978) 472–473.
- [12] Z. Zhi-Hong, S. Li, L. Wei, C. Wei, Z. Ying-Guo, A major QTL conferring cold tolerance at the early seedling stage using recombinant inbred lines of rice (*Oryza* sativa L.), Plant Sci. 168 (2005) 527–534.
- [13] M.J. Fryer, K. Oxborough, B. Martin, D.R. Ort, N.R. Baker, Factors associated with depression of photosynthetic quantum efficiency in maize at low growth temperature, Plant Physiol. 108 (1995) 761–767.
- [14] Q.J. Groom, N.R. Baker, Analysis of light-induced depressions of photosynthesis in leaves of a wheat crop during the winter, Plant Physiol. 100 (1992) 1217–1223.
- [15] D.H. Greer, G. Öqust, C. Ottander, Photoinhibition and recovery of photosynthesis in intact barley leaves at 5 °C and 20 °C, Physiol. Plant. 81 (1991) 203–210.

- [16] Y. Fracheboud, P. Haldimann, J. Leipner, P. Stamp, Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays L.*), J. Exp. Bot. 50 (1999) 1533–1540.
- [17] C. Jompuk, Y. Fracheboud, P. Stamp, J. Leipner, Mapping of quantitative trait loci associated with chilling tolerance in maize (*Zea mays L.*) seedlings grown under field conditions, J. Exp. Bot. 56 (2005) 1153–1163.
- [18] R.R. Wise, Chilling-enhanced photooxidation: the production, action and study of reactive oxygen species produced during chilling in the light, Photosynth. Res. 45 (1995) 79–97.
- [19] M.J. Verheul, P.R. Van Hassel, P. Stamp, Comparison of maize inbred lines differing in low temperature tolerance: effect of acclimation at suboptimal temperature on chloroplast functioning, Ann. Bot. 76 (1995) 7–14.
- [20] C.L. Guy, Cold acclimation and freezing stress tolerance: role of protein metabolism, Annu. Rev. Plant Physiol. Plant Mol. Biol. 41 (1990) 187–223.
- [21] K.L. Koster, D.V. Lynch, Solute accumulation and compartmentation during the cold acclimation of puma rye, Plant Physiol. 98 (1992) 108–113.
- [22] G. Yelenosky, C.L. Guy, Freezing tolerance of citrus, spinach, and petunia leaf tissue: osmotic adjustment and sensitivity to freeze induced cellular dehydration, Plant Physiol. 89 (1989) 444–451.
- [23] A.M. Ismail, A.E. Hall, Variation in traits associated with chilling tolerance during emergence in cowpea germplasm, Field Crop Res. 77 (2002) 99–113.
- [24] P.S. Campos, V. Quartin, J.C. Ramalho, M.A. Nunes, Electrolyte leakage and lipid degradation account for cold sensitivity in leaves of *Coffea* sp. plants, J. Plant Physiol. 160 (2003) 283–292.
- [25] N.C. Collins, F. Tardieu, R. Tuberosa, Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiol. 147 (2008) 469–486.
- [26] G.R. Al Chaarani, A. Roustaee, L. Gentzbittel, L. Mokrani, G. Barrault, G. Dechamp-Guillaume, A. Sarrafi, A QTL analysis of sunflower partial resistance to downy mildew (*Plasmopara halstedii*) and black stem (*Phoma macdonaldii*) by the use of recombinant inbred lines (RILs), Theor. Appl. Genet. 104 (2002) 490–496.
- [27] P.-F. Bert, G. Dechamp-Guillaume, F. Serre, I. Jouan, D.T. de Labrouhe, P. Nicolas, F. Vear, Comparative genetic analysis of quantitative traits in sunflower (*Helianthus annuus* L.), Theor. Appl. Genet. 109 (2004) 865–874.
- [28] L. Mokrani, L. Gentzbittel, F. Azanza, L. Fitamant, G. Al Chaarani, A. Sarrafi, Mapping and analysis of quantitative trait loci for grain oil content and agronomic traits using AFLP and SSR in sunflower (*Helianthus annuus* L.), Theor. Appl. Genet. 106 (2002) 149–156.
- [29] D. Herve, F. Fabre, E.F. Berrios, N. Leroux, G.A. Chaarani, C. Planchon, A. Sarrafi, L. Gentzbittel, QTL analysis of photosynthesis and water status traits in sunflower (*Helianthus annuus* L.) under greenhouse conditions, J. Exp. Bot. 52 (2001) 1857–1864.
- [30] S. Poormohammad Kiani, P. Talia, P. Maury, P. Grieu, R. Heinz, A. Perrault, V. Nishinakamasu, E. Hopp, L. Gentzbittel, N. Paniego, A. Sarrafi, Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments, Plant Sci. 172 (2007) 773–787.
- [31] Q. Lou, L. Chen, Z. Sun, Y. Xing, J. Li, X. Xu, H. Mei, L. Luo, A major QTL associated with cold tolerance at seedling stage in rice (*Oryza sativa* L.), Euphytica 158 (2007) 87–94.
- [32] M. Baga, S. Chodaparambil, A. Limin, M. Pecar, D. Fowler, R. Chibbar, Identification of quantitative trait loci and associated candidate genes for low-temperature tolerance in cold-hardy winter wheat, Funct. Integr. Genomics 7 (2007) 53–68.
- [33] Y. Fracheboud, C. Jompuk, J.M. Ribaut, P. Stamp, J. Leipner, Genetic analysis of cold-tolerance of photosynthesis in maize, Plant Mol. Biol. 56 (2004) 241–253.
- [34] J. Knoll, N. Gunaratna, G. Ejeta, QTL analysis of early-season cold tolerance in sorghum, Theor. Appl. Genet. 116 (2008) 577–587.
- [35] A.A. Schneiter, J.F. Miller, Description of sunflower growth stages, Crop Sci. 21 (1981) 901–903.
- [36] G.H. Krause, Photoinhibition of photosynthesis. An evaluation of damaging and protective mechanisms, Physiol. Plant. 74 (1988) 566–574.
- [37] P. Dagnelie, Statistique théorique et appliquée, Tome 2: Inférence statistique à une et deux dimention, De Boeck et Larcier s.a., Département De Boeck université, Paris, Bruxelles, 1998.
- [38] E. Flores Berrios, L. Gentzbittel, H. Kayyal, G. Alibert, A. Sarrafi, AFLP mapping of QTLs for in vitro organogenesis traits using recombinant inbred lines in sunflower (*Helianthus annuus* L.), Theor. Appl. Genet. 101 (2000) 1299–1306.

- [39] L. Gentzbittel, F. Vear, Y.X. Zhang, A. Bervillé, P. Nicolas, Development of a consensus linkage RFLP map of cultivated sunflower (*Helianthus annuus* L.), Theor. Appl. Genet. 90 (1995) 1079–1086.
- [40] G.R. Al Chaarani, L. Gentzbittel, X.Q. Huang, A. Sarrafi, Genotypic variation and identification of QTLs for agronomic traits, using AFLP and SSR markers in RILs of sunflower (*Helianthus annuus* L.), TAG Theor. Appl. Genet. 109 (2004) 1353– 1360.
- [41] S. Poormohammad Kiani, P. Maury, L. Nouri, P. Grieu, A. Sarrafi, QTL analysis of yield-related traits in sunflower under different water treatments, Plant Breed. (2009), doi:10.1111/j.1439-0523.2009.01628.x.
- [42] A. Ebrahimi, P. Maury, M. Berger, S. Poormohammad Kiani, A. Nabipour, P. Grieu, A. Sarrafi, QTL mapping of seed-quality traits in sunflower recombinant inbred lines under different water regimes, Genome 51 (2008) 599–615.
- [43] C. Basten, B. Weir, Z. Zeng, QTL Cartographer: A Reference Manual and Tutorial for QTL Mapping, North Carolina State University, USA, 2007.
- [44] Z.B. Zeng, Precision mapping of quantitative trait loci, Genetics 136 (1994) 1457– 1468.
- [45] G.A. Churchill, R.W. Doerge, Empirical threshold values for quantitative trait mapping, Genetics 138 (1994) 963–971.
- [46] S. Poormohammad Kiani, P. Grieu, P. Maury, T. Hewezi, L. Gentzbittel, A. Sarrafi, Genetic variability for physiological traits under drought conditions and differential expression of water stress-associated genes in sunflower (*Helianthus annuus* L), Theor. Appl. Genet. 114 (2007) 193–207.
- [47] T. Hewezi, M. Leger, W. El Kayal, L. Gentzbittel, Transcriptional profiling of sunflower plants growing under low temperatures reveals an extensive downregulation of gene expression associated with chilling sensitivity, J. Exp. Bot. 57 (2006) 3109–3122.
- [48] H. Van Buskirk, M. Thomashow, Arabidopsis transcription factors regulating cold acclimation, Physiol. Plant. 126 (2006) 72–80.
- [49] A.H. Kingston-Smith, J. Harbinson, J. Williams, C.H. Foyer, Effect of chilling on carbon assimilation, enzyme activation, and photosynthetic electron transport in the absence of photoinhibition in maize leaves, Plant Physiol. 114 (1997) 1039– 1046.
- [50] B.A. Moll, K.E. Steinback, Chilling sensitivity in *Oryza sativa*: the role of protein phosphorylation in protection against photoinhibition, Plant Physiol. 80 (1986) 420–423.
- [51] B. Demmig-Adams, W.W. Adams III, The role of xanthophyll cycle carotenoids in the protection of photosynthesis, Trends Plant Sci. 1 (1996) 21–26.
- [52] P. Haldimann, Low growth temperature-induced changes to pigment composition and photosynthesis in Zea mays genotypes differing in chilling sensitivity, Plant Cell Environ. 21 (1998) 200–208.
- [53] J. Leipner, Y. Fracheboud, P. Stamp, Effect of growing season on the photosynthetic apparatus and leaf antioxidative defenses in two maize genotypes of different chilling tolerance, Environ. Exp. Bot. 42 (1999) 129–139.
- [54] A. Hund, Y. Fracheboud, A. Soldati, E. Frascaroli, S. Salvi, P. Stamp, QTL controlling root and shoot traits of maize seedlings under cold stress, Theor. Appl. Genet. 109 (2004) 618–629.
- [55] A. Hund, E. Frascaroli, J. Leipner, C. Jompuk, P. Stamp, Y. Fracheboud, Cold tolerance of the photosynthetic apparatus: pleiotropic relationship between photosynthetic performance and specific leaf area of maize seedlings, Mol. Breed. 16 (2005) 321–331.
- [56] M.J. Verheul, C. Picatto, P. Stamp, Growth and development of maize (Zea mays L.) seedlings under chilling conditions in the field, Eur. J. Agron. 5 (1996) 31–43.
- [57] M. Hekneby, M.C. Antolín, M. Sánchez-Díaz, Frost resistance and biochemical changes during cold acclimation in different annual legumes, Environ. Exp. Bot. 55 (2006) 305–314.
- [58] D.J. Allen, D.R. Ort, Impacts of chilling temperatures on photosynthesis in warmclimate plants, Trends Plant Sci. 6 (2001) 36–42.
- [59] M.P. Guinchard, C. Robin, P. Grieu, A. Guckert, Cold acclimation in white clover subjected to chilling and frost: changes in water and carbohydrates status, Eur. J. Agron. 6 (1997) 225–233.
- [60] K. Nakashima, K. Yamaguchi-Shinozaki, Regulons involved in osmotic stressresponsive and cold stress-responsive gene expression in plants, Physiol. Plant. 126 (2006) 62–71.