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How Sowing Date Affects Development and Perfomance of Safflower Through Climate Variables

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ABSTRACT

Safflower (*Carthamus tinctorius* L.) has unrealized potential as an alternative crop in many semiarid regions including central Argentina. Our objective was to relate how temperature and

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/csc2.20548.

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precipitation conditions with fall (5 June 2012 and 23 April 2013) and winter sowing (13 August 2012 and 20 August 2013) affected phenology, yield, yield components, and oil percent in four winter and eight spring-type safflower accessions in the semiarid region of central Argentina. Fall sowing was associated with lower temperatures, higher precipitation, lower heat:moisture stress indices and precipitation deficits than winter sowing. Rosette period lasted 55 days longer, and stem elongation to anthesis period 30 days longer in fall than in winter sowing. However, anthesis was advanced only few days in fall sowing and duration of post-anthesis development was comparable between sowing regimes and years. Fall sowing plants averaged 3252 filled grains m^{-2} and a grain yield of 109.8 g m^{-2} while winter sowing plants 1443 filled grains m^{-2} and a grain yield of 49.3 g m^{-2}. Grain yield was 35% higher in winter than in spring-type accessions, but winter types had lower oil percent (22.0%) compared with spring-types (33.3%). In the semiarid region of central Argentina, we recommend fall sowing as it extended the growing season in terms of days pre-anthesis and presented favorable climatic conditions for safflower development.

Abbreviations:

BBCH: Biologische, Bundesanstalt, Bundessortenamt, Chemische Industrie.

FDR: false discovery rate procedure

GDD: growing degree days

H:M: heat:moisture indices

PET: potential evapotranspiration

R_s: solar radiation

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Tmax: daily maximum temperature

Tmean: mean daily temperature

Tmin: daily minimum temperature

INTRODUCTION

Safflower is an annual crop principally grown for its high-quality edible and industrial oil (Smith, 1996). It has been grown primarily for its colorful petals used as a food coloring and flavoring agent, for vegetable oil and for preparing textile dye in East, Central and North Asia, America, North Africa, Europe and Caucasia (Esendal, 2001).

Safflower develops a rosette after emergence when numerous prostrate leaves are formed (Li & Mündel, 1996). During the rosette stage (emergence to stem elongation), safflower is relatively resistant to cold temperatures (Yazdi-Samadi & Zali, 1979). This tolerance declines abruptly once the stem elongation stage begins (Landry, Fuchs, Bradley, & Johnson, 2017). Winter-type safflower is characterized by a longer rosette period (Ghanavati & Knowles, 1977), a more prostrate plant habit, and superior cold acclimation enhances its cold tolerance (Johnson & Li, 2008a).

Safflower fruits (grains) are normally white or cream in color with the hull representing 35-45% of the grain weight (Smith, 1996). Most safflower accessions have a seed oil fatty acid profile of about 6-8% palmitic acid, 2-3% stearic acid, 16-20% oleic acid, and 71-75% linoleic acid (Knowles, 1989). Safflower oil genetics and breeding has resulted in very high linoleic acid (87-89%), high oleic acid (75-80%), intermediate oleic acid (41-53%), and high stearic acid (4-11%) types (Hamdan, Pérez-Vich, Velasco & Fernandez-Martínez, 2009).

In general, sowing seasons have a large influence on crop yield (Andrade, Cirilo, Uhart, & Otegui, 1996; Barros, de Carvalho, & Basch, 2004). Where winters are severe, fall planting can

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increase production if there are cultivars with enough winter hardiness to survive. When fall planted, winter-types accessions survived better and yielded more than spring-types in Iran, and in the Inland Pacific Northwest of the United States, where minimum temperature ranged from −4.4 °C to –26 °C (Yazdi-Samadi & Zali, 1979; Johnson, Li, & Bradley, 2006; Johnson, Petrie, Franchini, & Evans, 2012). Yet winter-types are generally not available commercially owing to the need for high oleic acid content and improved oil percent. In central and southern Italy, where winters are relatively mild, fall sown spring-type safflower yielded more than when spring sown (Salera, 1997; Cazzato, Ventricelli, & Corleto, 1997; Corleto, Cazzato, & Annese, 2001). This was attributed to deeper root development allowing water uptake from deeper soil layers.

Sowing date and the accession may also have an important effect on oil percent and quality of safflower grains. Coşge, Gürbüz, & Kiralan (2007) observed higher grain oil percent in fall than in spring sowing independently of the accessions they tested. Gecgel, Demirci, Esendal, & Tasan (2007) reported that sowing date and accession entry interacted in such a way that oil percent in a high linoleic genotype was higher in fall sowing, but for a high oleic genotype it was higher in a spring sowing. Climatic conditions, particularly temperature during seed development can affect seed oil percent of safflower (Camas, Cirak, & Esendal, 2007; Coşge, Gürbüz, & Kiralan, 2007). Shabana, Mohsen, Gouda, & Hafez (2013) reported reduced oil percent in safflower with high temperature during the seed development. Also sowing date can affect oil quality. Roche, Mouloungui, Cerny, & Merah (2019) reported that a delayed sowing date reduced unsaturated fatty acids and changed sterol composition and content.

There have been several studies to evaluate the best safflower sowing dates in different world regions. Many studies have been completed under a Mediterranean precipitation pattern with mild winters (Corleto, Cazzato, & Annese, 2001; Yau, 2007) or

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extended or extreme winter freezing (Bergland, Riveland, & Bergman, 2007; Johnson, Petrie, Franchini, & Evans, 2012; Ghanbari-Odivi, Hashemzade, Bahrampour, & Saeidi, 2013).

Semi-arid central Argentina climatic conditions (Bon, Sanchez, Carrascal, & Romagnoli. (2014) are not comparable with studies from other regions. As far as we know, no studies on how climate variables within key growth periods are correlated with safflower production have been carried out.

In the semiarid region of central Argentina, spring-type safflower cultivars have been commercially available and are usually winter sown, with an average grain yield of 800-900 kg ha^{-1} (Franchini, Flemmer, & Lindström, 2012). The mentioned region is characterized by fall and spring rain periods, dry winters, and hot summers (Gabella, Zapperi, & Campos, 2010). Winters are relatively mild but freezing temperatures can threaten crop survival. With winter sowing, yield components often develop when temperatures are high and moisture increasingly limited. Thus, we hypothesized that in the semi-arid region of central Argentina fall sowing should allow crop establishment and development when temperature and precipitation conditions are more optimal.

Our objective was to relate how temperature and precipitation conditions with fall and winter sowing dates affected phenology, yield, yield components, and oil percent in winter and spring-type safflower accessions.

MATERIALS AND METHODS

Plant materials

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Winter and spring-type safflower was obtained from the United States Department of Agriculture, Agricultural Research Service, Western Regional Plant Introduction Station Pullman, Washington State, United States of America. The winter-types were all high linoleic acid types and included WSRC01 (PI 651878), WSRC02 (PI 651879), WSRC03 (PI 651880) (Johnson & Li, 2008b) and KN144-C3 (W6 39446). Spring-types included the high linoleic cultivars Gila (PI 537692) and Girard (PI 525457), and the high oleic acid cultivars Montola (PI 538025), Lesaf 496 (PI 603208), UC-1 (PI 572434), OLE (PI 537695) and Oleic Leed (PI 560177). The spring cultivar CW99 OL, one of the high oleic accessions cultivated in the study region, was also included.

Site and experimental design

All the plant accessions were grown at the Argentine Cooperatives Association experimental field, Cabildo, Buenos Aires, Argentina (38°36'7.2" S, 61° 58' 26.4" W). The soil was a typical sandy-loam, neutral, Petrocalcic Paleustoll with a calcareous hard-pan 75 cm deep (Soil Survey Staff, 1999). The ploughable soil depth is susceptible to wind and water erosion. The climate is temperate with an average annual rainfall of 638 mm, with useful precipitation for safflower typically concentrated in the fall and spring months (Aliaga, Ferrelli, & Piccolo, 2017).

The experiment consisted of two sowing regimes over two years. Fall sowing dates were on 5 June 2012 and 23 April 2013, and winter sowing dates were on 13 August 2012 and 20 August 2013. It was arranged in split plots based on a randomized complete block design with three replicates. Plant accessions and sowing regimes were randomly assigned to the main plots and to the subplots, respectively. Subplots were 1.5 m long and 1.40 m wide with four rows spaced 0.35 m apart and 10 cm between plants in each row resulting in a plant density of 29 plants m^{-2}. Seed were

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sown at 2.5 cm deep by hand seeding. Plants were grown under rainfed conditions and weeds were controlled. There was minimal damage from insects and diseases.

Plant sampling and measurement

Dates of emergence, stem elongation, anthesis and harvest maturity were recorded when at least 50% of the plants reached a given stage following the BBCH (Biologische, Bundesanstalt, Bundessortenamt, Chemische Industrie) phenological scale for safflower (Flemmer, Franchini, & Lindström, 2015). For each sowing regime, year, and accession combination, phenology data were used to define growth stage periods including rosette (emergence to elongation), elongation to anthesis, anthesis to harvest maturity and emergence to maturity.

Plants of the two internal rows were harvested at maturity and results were expressed on square meter area basis. Samples were dried to constant weight at 60 °C (72 h) and then capitula number was determined. The capitula were manually threshed, and aborted florets and incompletely developed grains discarded. Then, filled grain number and weight per unit area were determined.

In addition, capitula of 10 plants per subplot were collected at harvest maturity for oil percent determination. Capitula were air dried at room temperature and threshed to obtain 10 g of grains. Grain oil content was determined according to the IUPAC (International Union of Pure and Applied Chemistry, 1992) method 1.122 by an exhaustive extraction with the analytical reagent n-hexane (90%, bp 68-72 ºC) in a Soxhlet apparatus (Difffenbacher & Pocklington, 1992). Oil miscella was initially recovered by rotary evaporation at 50 °C under low pressure, and then by nitrogen displacement until constant weight was obtained. Total grain oil percent was expressed as a

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percentage of sample dry weight. Oil yield (g m^{-2}) was calculated as product of weight of filled grains (g m^{-2}) and grain oil percent.

Climate variables

Daily maximum (Tmax) and minimum (Tmin) temperatures and precipitation were collected by a weather station (EasyWeather, version 2.0) located at the experimental site and global solar radiation measured with a sensor 20 km from the experimental site (Davis Instruments, Hayward, CA, USA). Temperature and solar radiation were taken each day at half hour intervals. Solar radiation was summed for each growth period. As potential stress indicators, heat:moisture indices (H:M), potential evapotranspiration (PET) and the precipitation deficit were calculated for each growth period resulting from each sowing regime, year, and accession combination.

The H:M indices were calculated as H:M = (°C+10)/(precipitation/1000), where °C was either average (Tmean) or maximum temperature (Tmax). PET was calculated from the sum of solar radiation (R_s) as given by Hargreaves & Allen (2003), where PET=0.0135 R_s (Tmean + 17.8). The R_s was expressed as total kJ m^{-2} and converted to the equivalent mm of water using the heat of vaporization at 25 °C (J per kg H_2O) (Datt, 2011). The precipitation deficit was the fraction of precipitation relative to total PET for each growth period and calculated as (PET - precipitation)/PET (Hargreaves, 1975). Growing degree days (GDD) were calculated as given by McMaster & Wilhelm (1997) using daily Tmin and Tmax and a base temperature of 5ºC as:

$$\text{Accumulative GDD} = \sum_{\text{Start day}}^{\text{Current day}} [(\text{Tmax}+\text{Tmin})/2 - \text{Tb}]$$

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Where Tmax was the maximum daily temperature, Tmin the minimum daily temperature and Tb, the base temperature, which was taken as 5°C.

Statistical analyses

The experimental factors (accessions, sowing regimes, years) were assumed fixed. Data were subjected to analysis of variance and treatment means were compared using the least significant difference test ($P<0.05$) (Di Rienzo et al., 2014). When necessary, orthogonal contrasts were applied.

Linear correlation was used for determining associations between measured plant traits and climate variables. This was done for each accession mean within sowing date and year ($n=32$). Within each plant trait and growth period, a total of 36 correlations resulted, so false positives using traditional P-values were likely. For that reason, we used the Benjamini-Hochberg false discovery rate procedure (FDR) (Benjamini & Hochberg, 1995) as outlined by McDonald (2014). Calculations were completed using the spreadsheet provided by McDonald (2014) with P-values declared significant using a FDR of 0.05.

RESULTS

Climate variables

In the experimental year 2012, the lowest temperatures were in July with two consecutive nights of 7 to 12 hours under 0 °C, with -6 °C the lowest. However, no visible plant damage occurred

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because the fall sown plants were still in the cold tolerant rosette period (emergence to elongation) and the winter sowing had not yet been conducted.

In 2013, however, the lowest temperatures were recorded at the end of August, when three consecutive nights had 5 to 12 consecutive hours of temperatures under 0 °C, with -7 °C the lowest. By that time, winter sown plants had still not emerged, but some fall sown plants had started elongation. Frost mortality did occur with most plants of CW99 OL and all plants of UC-1. As a result, these two accessions were removed from the analysis.

None of the plants of KN144-C3 and Lesaf 496 survived in either experimental year. Their mortality was associated with twisted and broken stems. It is not clear why this occurred but Cerrota, Lindström, & Etchenique (2018) found that even though twisted stems were thicker, vascular tissue support was reduced and secondary cells walls were thinner than normal stems. As a result, KN144-C3 and Lesaf 496 were also not included in the analysis.

The highest temperatures were usually recorded during the anthesis to maturity period and were substantially higher in 2013 than 2012 (Table 1) with maximum daily temperatures often 38 to 40 °C in 2013 but never exceeded 34 °C in 2012.

Over the entire growing season precipitation was higher in 2012 than 2013, and higher in fall than in winter sowing (Table 1). Temperatures were also lower in the fall than in the winter sowing leading to less stress as measured by H:M indices, PET, and precipitation deficit (Table 1).

Phenology

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The duration of key phenological stages in days and the GDD were strongly affected by sowing date (Table 1 and 2). In the rosette stage and the elongation to anthesis stage, the year x sowing date interaction was also strong for days and GDD. For GDD from anthesis to maturity, no factors were significant except year (Table 2).

Duration in days and GDD for the rosette period and the stem elongation to anthesis period were longer ($P<0.05$) in the fall than in the winter sowing (Figure 1 A-D). In turn, the durations of those growth periods in both days and GDD were longer in 2013 than in 2012 for fall sowing, and mainly associated with the earlier sowing date in 2013 than 2012. This tendency was not clearly evident for winter sowing (Figure 1 A-D).

Elongation started earlier in the fall than in the winter sowing both years and for all accessions. In 2012, stem elongation started between 18 and 26 September in fall sowing and between 3 and 19 October in the winter sowing. In 2013, stem elongation started between 5 August and 2 September in fall sowing and between 16 and 30 October in the winter sowing.

By anthesis, differences between sowing dates were compressed and diminishing, with anthesis occurring between 17 and 23 November in fall sowing, and between 24 November and 7 December in the winter sowing both years, but with nearly equal days and GDD values (Figure 1 E, F).

Crop harvest variables

Analyses of variance for year, accession, and sowing date are presented in Table 3. Based on the very high and significant F values, the year effect was dominant for weight per grain and accession effects for grain oil percent. On the contrary, sowing date effects were very strong for

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capitula number, filled grain number, grain yield and oil yield. Interactions were most prevalent for weight per grain and grain oil percent.

For grain yield and filled grain number none of the interactions were significant so the means of main effects summarized the results (Table 4). Average yield was twice as high in 2012 than in 2013 and filled grain number was 55% higher in 2012. Likewise, average grain yield and filled grain number with fall sowing were more than twice that of the winter sowing.

Means among accessions for filled grain number and grain yield often overlapped (Table 4) and were thus weaker than year and sowing date effects. Filled grain number was similar among accessions (Table 5) but grain yield was generally higher in winter-types (94.8 g m^{-2}) than in spring-types (70.4 g m^{-2}) ($P<0.05$) (Table 4 and 5). This was evidenced by comparing both types using an orthogonal contrast (Table 5). In 2012, grain weight was in general similar for both sowing dates but in 2013, grain weight was in general higher in fall than in winter sowing (Figure 2A).

Among accessions, capitula number had a significant year x accession interaction (Table 3), primarily caused by a different response of the spring-types Gila, Montola, and Oleic Leed between years (data not presented). Capitula number also had a significant year x sowing date interaction (Table 3). The fall sowing in 2013 had more capitula (245 m^{-2}) than in 2012 (206 m^{-2}) but the winter sowing for 2012 and 2013 had nearly an equal capitula number (111 and 106 m^{-2}, respectively). Even with that higher capitula number in the fall 2013 sowing, grain yield was still higher in the 2012 than in the 2013 fall sowing (136 and 83 g m^{-2}), resulting from 32% more filled grain number and 23% higher weight per grain. Averaged over years, fall sowing had more than twice the capitula number than did winter sowing (225 and 109 m^{-2}, respectively).

Differences in grain oil percent were mainly among accessions (Table 3). As expected, spring-types, improved for oil content, had higher grain oil percent than the unimproved winter-types both

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years (Figure 2B). As with grain yield, oil yield was strongly affected by year, sowing date, and accession but unlike grain yield there was a significant accession x sowing date interaction (Table 3). For winter sowing in both years, no accession differed in oil yield but significant accession differences existed for fall sowing (Figure 3). Even though oil percent was lower in winter-types, it was offset by the higher yields, so oil yield of winter-types and spring types tended not to differ within sowing dates (Figure 3).

Correlations between yield, yield components and climatic variables

For the entire season, for each sowing date and year, grain yield and yield components (capitula number, filled grain number and weight per grain) were positively correlated with precipitation and negatively with Tmax and stress indices (n=32) (Table 6).

There were also significant correlations between yield and yield components and with climatic variables within other growth stage periods (Table 6). For example, capitula number correlated only with climatic variables during pre-anthesis development, especially the elongation to anthesis period.

Filled grain number negatively and strongly correlated with moisture and stress indices during the elongation to harvest maturity period. Grain per weight was negatively correlated with climatic variables mainly in the anthesis to maturity period.

All significant correlations for GDD except filled grain number from anthesis to maturity were positive; higher GDD was generally related to higher production values as lower temperatures in fall sowings extended the duration of pre-anthesis development. Correlations between grain oil percent

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and climate variables were infrequent, only occurring for GGD and the heat:moisture indices in the elongation to anthesis period. Correlations of oil yield with climatic variables were nearly identical to those for grain yield (Table 6). Indeed, oil yield and grain yield were strongly and positively correlated (r= 0.93**).

DISCUSSION

The different sowing date and year combinations resulted in four different growing environments with contrasting temperature and precipitation characteristics (Table 1). Those different environments substantially influenced phenology, yield components and grain production.

Grain yield was twice as high in the fall than in winter sowing, regardless of years and accessions. The more optimal temperature and precipitation regimes and a longer period of pre-anthesis growth in fall sowing would have resulted in a greater root development (Smith, 1996) and accumulation of photosynthetic assimilates (Koutroubas, Papakosta & Doitsinis, 2004). These, in turn, would have promoted the higher capitula and filled grain number, hence the higher grain yield, observed in our study. Corletto, Cazzato, & Annese (2001), Yau (2007), Ozturk (2019) and Johnson, Petrie, Franchini, & Evans (2012) also found that fall sowing of safflower yielded more than spring sowing. In these studies, plants in the more productive environments escaped much of the high temperature and precipitation deficits common with later sowing.

Noteworthy were the differences between sowing dates in GGD (Figure 1). For the rosette and the elongation to anthesis periods in 2013, all fall sown accessions grew under lower temperatures but accumulated more GGD than those under the higher temperatures in the winter

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sowing (Figure 1B). The same general pattern was observed for the anthesis to maturity period in 2013. In 2012, GDD was generally lower and accession differences less consistent than in 2013, but the pattern was similar. Thus, accumulated temperatures as GDD were only partly predictive of crop development. This could result from other environmental factors such as higher water deficits (Desclaux & Roumet, 1996) and/or differences in how accessions responded to the temperature differences caused by the sowing dates (Zhou & Wang, 2018).

Although potential grain number is set during pre-anthesis, environmental stresses during early post anthesis can reduce filled grain number due to flower or developing embryo abortion (Egli, 1998; Lindström, Pellegrini, Aguirrezabal & Hernández, 2006). Once filled grain number is fixed (Lindström & Hernández, 2015), adjustment in grain yield as consequence stress conditions could occurr only through variation in grain weight. In our work, the higher moisture and thermal stress indices during the emergence to maturity period in 2013 than in 2012 (Table 1) reduced grain yield, filled grain number, and to a lesser extent weight per grain (Figure 2A). In fact, grain yield and number were negatively correlated with precipitation deficit and thermal stress indices during this period (Table 6). So, differences in safflower performance between both years could be attributed to differences in climatic conditions.

As in other grain crops (Andrade & Ferreiro, 1996), filled grain number was the component that mostly explained differences in grain yield between years and sowing date (Table 4). Unlike other studies (Adams, 1967; Slafer, Savin & Sadras, 2014), we found no evidence for compensating effects between filled grain number and weight per grain (Table 4 and Figure 2A). Except for OLE and Oleic Leed, weight per grain was not affected by sowing date in 2012, and even with the high filled grain number with fall sowing in 2013, higher weight per grain was often observed. Thus, in the more optimal environments there were little or no compensating effects between grain number and weight per grain, a likely result of favorable growth conditions providing the required

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pre-anthesis assimilates for grain filling (Koutroubas, Papakosta, & Doitsinis, 2004). This was also supported by the negative correlations between weight per grain and precipitation deficit and the thermal stress indices during pre-anthesis periods (Table 6).

Accession effects were generally weaker than sowing date and year effects (Tables 2 and 3), and mean difference among accession for grain yield and filled grain number were often not significant (Table 3), except for grain oil content (Fig 2B). Higher grain oil content of spring types has resulted from breeding, for improved oil percent; winter-types have not yet been improved for oil content (Figure 2B).

With some exceptions, significant correlations between yield components and climate variables at the different growth periods (rosette, elongation to anthesis, and anthesis to maturity) were consistent with the growth stage when the different yield component are fixed (Flemmer, Franchini & Lindström, 2015). Fewer significant correlations were observed during the rosette period, especially for precipitation (Table 6), as safflower plants are smaller and temperatures usually lower during the rosette period. Thus, high temperature and lower precipitation is usually less limiting than during later periods. Capitula number, consistent with being fixed before anthesis (Flemmer, Franchini & Lindström, 2015), never correlated with climate variables during the anthesis to maturity period.

Correlations between oil content and climate variables occurred only in the elongation to anthesis period (Table 6). These correlations were positive for GDD and negative for the H:M stress indices; no significant correlations were found for oil content during the grain filling. Yet, Coşge, Gürbüz, & Kiralan (2007) did find an oil content response to temperature during the grain filling, Alessi, Power, & Zimmerman (1981) reported a positive correlation between oil content and GGD during grain filling and Coşge, Kiralan, & Hassanien (2015) showed that water deficits reduced oil

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content through oxidation of fatty acids. Nevertheless, in our study the pattern of correlation for GDD and stress indices for oil content was consistent with all yield components except weight per grain (Table 6).

Winter-types yielded more as a group than the spring-types. Since winter adaptation is advantageous with fall sowing, winter-types offer an opportunity to reduce freezing damage in regions with extended winter freezing (Johnson, Petrie, Franchini, & Evans, 2012), but also where winters are relatively mild but with potential freezing damage. This includes semi-arid central Argentina and much of the Mediterranean region. If oil percentage is increased in winter-types, and winter hardiness and high yield are maintained, fall-sowing will be possible in places where freezing is more common.

Further research evaluating the effect of sowing date (fall, winter, and spring) on safflower yield at diverse locations in Argentina could expand and refine our finding. Continued work on the dynamics of grain oil content and fatty acid composition would advance the understanding of oil quality factors important to marketing. The development of winter-type safflower with improved oil content and high in oleic acid could expand areas of safflower production in frost-prone regions in Argentina and other countries.

Conclusion

Fall sowing led to a substantial extension of the growing season in terms of days of pre-anthesis growth and development. The longer development in fall than in winter sowing coincided with more favorable climatic conditions resulting in higher safflower production. These results were observed in both the relatively productive 2012 season and in the 2013 season with higher stress conditions. The results have the potential to improve and expand safflower production in

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semi-arid central Argentina and areas with similar climates. The results support the potential improvement of safflower production in semi-arid central Argentina and areas with similar climates through fall instead of winter sowing.

CONFLICT OF INTEREST STATEMENT

Authors declare that there is no conflict of interest.

ACKNOWLEDGMENTS. This work was supported by grants from Oleaginosa Moreno Hermanos S.A. and the Secretaría General de Ciencia y Tecnología (Universidad Nacional del Sur), Argentina.

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FIGURE 1 Days and growing degree days (GDD) for the rosette period (emergence to stem elongation, **A-B**), stem elongation to anthesis beginning **(C-D)** and anthesis to harvest maturity **(E-F)** of eight safflower accessions grown for two years and sowing dates at the Argentine Cooperatives Association (Cabildo, Buenos Aires, Argentina). For each year and growing period, bars topped by different letters are significantly different (P<0.05).

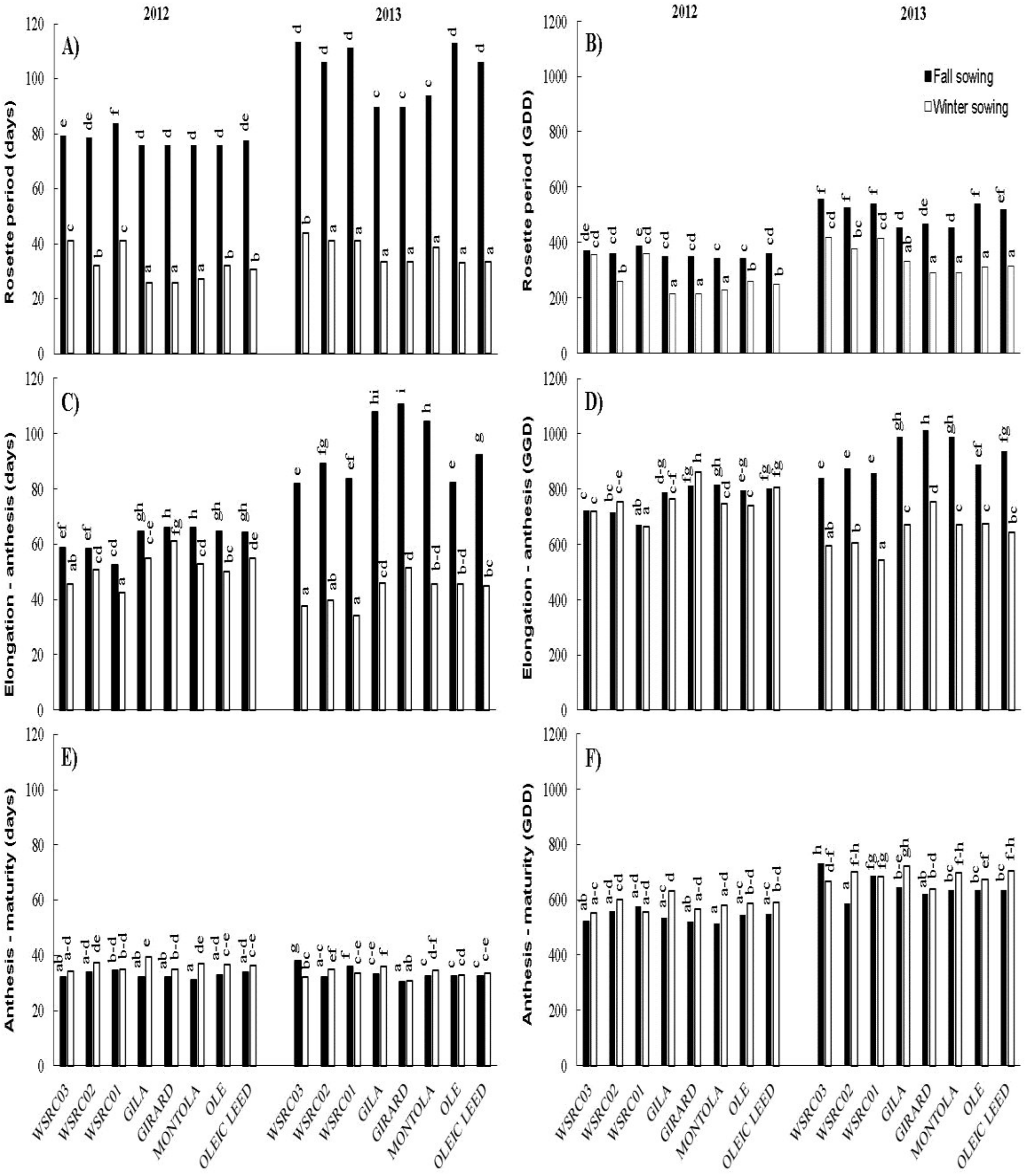

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FIGURE 2 Weight per grain **(GW,** mg grain^{-1}) **(A)** and grain oil content (%) **(B)** for eight safflower accessions grown for two years and sowing dates at the Argentine Cooperatives Association experimental field (Cabildo, Buenos Aires, Argentina). For each year and variable, bars topped by different letters are significantly different (P<0.05).

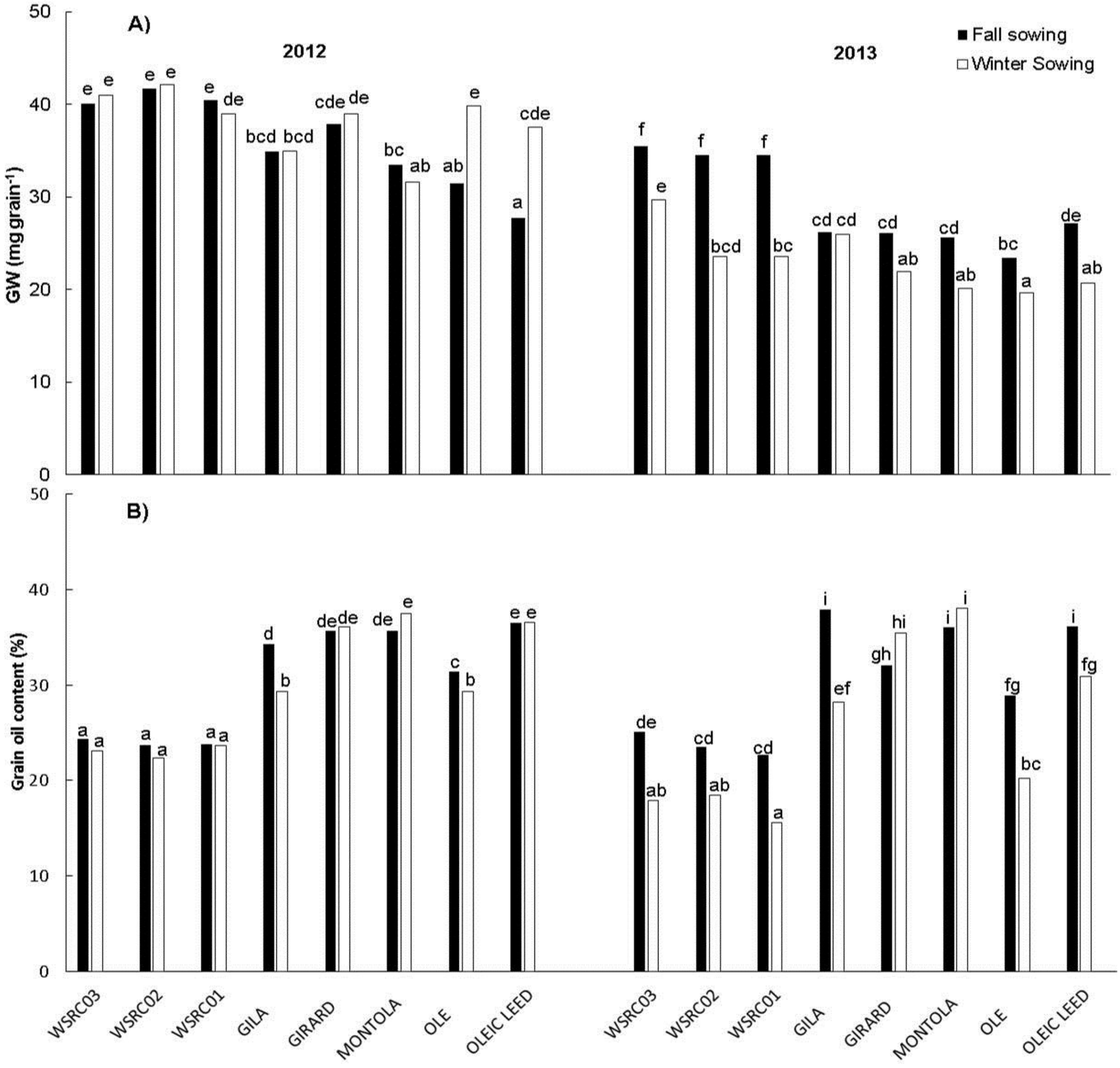

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FIGURE 3 Oil yield (g m^{-2}) for eight safflower accessions grown for two years and sowing dates at the Argentine Cooperatives Association experimental field (Cabildo, Buenos Aires, Argentina). For each year, bars topped by different letters are significantly different (P<0.05).

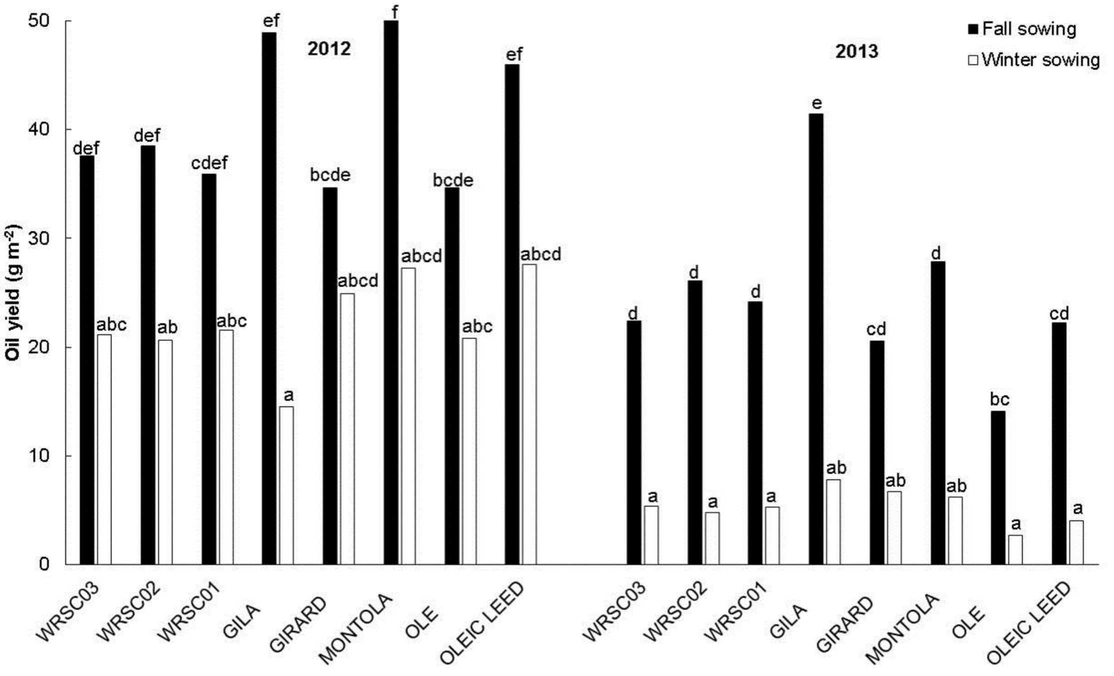

TABLE 1 Values of climate variables for the different growth stage periods of eight safflower accessions grown for two years and sowing dates at the Argentine Cooperatives Association experimental field (Cabildo, Buenos Aires, Argentina). The entire growing season, emergence to maturity, are shown in bold for each year and sowing date.

Year	Sowing dates	Growth stage periods†	days	Tmax§	Tmin	Tmean	Precipitation	GDD	Tmean H:M	Tmax H:M	PET per day	Precipitation deficit
2012	Fall	**Emerg to Mat**	**176.0**	**16.1**	**13.2**	**14.6**	**351.0**	**1723.0**	**42.1**	**46.2**	**2.84**	**0.36**
		Rosette	78.1	9.9	9.2	9.5	85.1	374.0	112.0	116.1	1.26	0.14
		Elon to Anth	61.5	19.8	14.8	17.3	128.9	764.6	134.4	153.8	3.82	0.45

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		Anth to Mat	36.4	23.2	19.1	21.2	136.8	603.9	155.1	170.2	5.41	0.30
2012	Winter	**Emerg to Mat**	**120.6**	**20.6**	**16.0**	**18.2**	**273.5**	**1608.4**	**67.5**	**76.1**	**4.28**	**0.51**
		Rosette	32.8	13.5	12.7	13.1	25.3	274.3	1464.3	1509.8	2.77	0.74
		Elon to Anth	50.3	23.5	15.6	19.5	133.2	742.6	147.7	177.5	4.52	0.41
		Anth to Mat	37.6	23.0	19.2	21.0	116.2	617.4	181.3	198.2	5.49	0.44
2013	Fall	**Emerg to Mat**	**230.6**	**21.4**	**6.6**	**13.1**	**315.1**	**1918.4**	**41.7**	**68.0**	**2.45**	**0.48**
		Rosette	102.8	16.9	2.8	9.3	76.6	465.8	121.5	220.3	1.20	0.37
		Elon to Anth	93.9	22.1	7.6	13.8	205.2	840.9	67.2	107.9	2.97	0.26
		Anth to Mat	34.0	33.4	15.1	23.1	33.5	632.6	688.4	997.4	5.88	0.83
2013	Winter	**Emerg to Mat**	**114.8**	**27.0**	**11.7**	**18.2**	**221.8**	**1534.7**	**82.3**	**122.0**	**4.47**	**0.60**
		Rosette	40.3	20.2	6.9	12.5	125.7	313.1	104.9	170.5	2.76	-0.12
		Elon to Anth	41.1	27.9	12.6	19.1	56.6	584.0	413.0	599.5	4.82	0.72
		Anth to Mat	33.4	34.2	16.2	24.1	39.1	657.2	615.1	874.0	6.53	0.82

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†, Emerg (emergence), Elon: stem elongation, Rosette: Emerg to Elon, Anth: anthesis, Mat: maturity.

§, T: temperature (ºC), GDD: growing degree days with a base temperature of 5 °C, H:M: heat:

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moisture index, PET: potential evapotranspiration, Precipitation deficit: (1 – precipitation)/PET

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TABLE 2 F values and probability levels of the analyses of variance for the effect of year, accession, sowing date, and their interactions for days and growing degree days (GDD) in three growth stage periods for eight safflower accessions grown for two years and sowing dates (fall and winter) at the Argentine Cooperatives Association experimental field (Cabildo, Buenos Aires, Argentina).

	Rosette†		**Elongation to anthesis**		**Anthesis to maturity**	
Source of variation	Days	GDD	Days	GDD	Days	GDD
Year (Y)	74.4**	75.9**	176.7**	4.6 NS	4.5 NS	94.7**
Accession (A)	7.5**	21.4**	47.6**	32.1**	4.6**	0.7 NS
Sowing date (SD)	5685.8**	667.4**	2372.7**	562.9**	0.8 NS	3.1 NS
YxA	1.5 NS	1.1 NS	4.6**	1.1 NS	3.4**	1.6 NS
YxSD	178.6**	58.2**	999.3 **	489.4**	23.6**	0.6 NS
AXSD	3.0*	5.8**	4.9**	3.1*	5.8**	0.8 NS
YxAXSD	8.1**	3.2*	8.3**	2.5*	1.6 NS	0.8 NS

*Significant at the .05 probability level. **Significant at the .01 probability level. NS, not significant (P>0.05).

†, emergence to stem elongation period.

TABLE 3 F values and probability level of the analyses of variance for yield components, grain yield, grain oil percent, and oil yield of eight safflower accessions grown for two years and sowing dates (fall and winter) at the Argentine Cooperatives Association experimental field (Cabildo, Buenos Aires, Argentina).

	Capitula	**FGN†**	**Weight per grain**	**Grain yield**	**Grain oil**	**Oil yield**
Source of variation	**m^{-2}**	**m^{-2}**	**mg**	**g m^{-2}**	**%**	**g m^{-2}**
Year (Y)	5.7 NS	31.5**	472.4**	96.8**	41.2**	65.4**
Accession (A)	9.4**	2.6*	25.4**	5.8**	235.4**	3.8**
Sowing date (SD)	335.0**	203.6**	15.0**	177.0**	70.5**	174.6**

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YxA	3.1*	2.1 NS	3.5**	1.1 NS	7.7**	2.2 NS
YxSD	12.3**	0.9 NS	67.6**	0.0 NS	31.1 **	0.1NS
AxSD	1.0 NS	1.5 NS	4.9**	2.0 NS	11.7**	2.9*
YxAxSD	1.7 NS	0.4 NS	3.4**	0.2 NS	3.4**	0.1 NS

*Significant at the .05 probability level. **Significant at the .01 probability level. NS, not significant (P>0.05).

†filled grain number

TABLE 4 Mean comparisons for grain yield and filled grain number of eight safflower accessions grown for two years and sowing dates at the Argentine Cooperatives Association experimental field (Cabildo, Buenos Aires, Argentina).

		Grain yield	**Filled grain number**
		g m^{-2}	**m^{-2}**
Year	2012	106.1a	2854.7a
	2013	53.0b	1840.3b
Sowing date	Fall	109.8a	3252.4a
	Winter	49.3b	1442.7b
Accession	WSRC03	91.1ab	2317.4ab
	WSRC02	97.9a	2494.0a
	WSRC01	95.3a	2553.5a
	Gila	82.9abc	2595.5a
	Girard	62.3d	1973.5b
	Montola	76.9bcd	2637.3a
	OLE	61.0d	1956.7b
	Oleic Leed	68.9cd	2252.1ab

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TABLE 6 Linear correlation coefficients between yield, yield components and climate variables for the different growth stage periods of eight safflower accessions grown for two years (2012 and 2013) and sowing dates (fall and winter) at the Argentine Cooperatives Association experimental field (Cabildo, Buenos Aires, Argentina). The entire growing season, emergence to maturity, are shown in bold for each factor.

Within year, sowing date or accession, means in a column followed by the same letter are not significantly different (P>0.05).

TABLE 5 Orthogonal contrast between three winter and five spring type accessions in grain yield and filled grain number averaged over accessions and for two years at the Argentine Cooperatives Association experimental field (Cabildo, Buenos Aires, Argentina).

	Grain yield	**Filled grain number**
Orthogonal contrast	**g m^{-2}**	**m^{-2}**
Winter versus spring	6.2*	0.4 NS

*Significant at the .05 probability level. NS, not significant (P>0.05).

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Variable	Growth stage period†	Tmax‡	Tmin	Tmean	Precip	GDD	Tmean H:M	Tmax H:M	PET per day	Precipitation deficit	Frequency
Grain yield	**Emerg to Mat**	**-0.90***	**0.09**	**-0.56***	**0.88***	**0.47***	**-0.78***	**-0.89***	**-0.64***	**-0.90***	**0.89**
	Rosette	-0.85*	0.15	-0.59*	-0.30	0.32	-0.12	-0.13	-0.67*	0.22	0.33
	Elon to Anth	-0.86*	0.18	-0.30	0.44*	0.51*	-0.57*	-0.62*	-0.47*	-0.51*	0.78
	Anth to Mat	-0.62*	0.46*	-0.69*	0.66*	-0.31	-0.57*	-0.56*	-0.80*	-0.68*	0.89
Capitula number	**Emerg to Mat**	**-0.48***	**-0.63***	**-0.90***	**0.76***	**0.87***	**-0.85***	**-0.63***	**-0.90***	**-0.59***	**1.00**
	Rosette	-0.28	-0.57*	-0.86*	0.05	0.78*	-0.33	-0.32	-0.87*	-0.01	0.44
	Elon to Anth	-0.61*	-0.55*	-0.80*	0.68*	0.59*	-0.54*	-0.51*	-0.82*	-0.63*	1.00
	Anth to Mat	0.10	-0.30	-0.06	-0.07	-0.10	0.18	0.20	-0.32	0.04	0.00
Filled grain	**Emerg to Mat**	**-0.87***	**-0.10**	**-0.71***	**0.92***	**0.62***	**-0.86***	**-0.90***	**-0.77***	**-0.90***	**0.89**

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number											
	Rosette	-0.77*	-0.03	-0.71*	-0.25	0.41*	-0.16	-0.18	-0.79*	0.17	0.67
	Elon to Anth	-0.90*	-0.03	-0.49*	0.57*	0.66*	-0.63*	-0.65*	-0.64*	-0.58*	0.89
	Anth to Mat	-0.48*	0.30	-0.58*	0.51*	-0.41*	-0.41*	-0.40*	-0.74*	-0.55*	0.89
Weight per grain	**Emerg to Mat**	**-0.69***	**0.49***	**-0.05**	**0.48***	**0.11**	**-0.36**	**-0.64***	**-0.14**	**-0.54***	**0.56**
	Rosette	-0.74*	0.56*	-0.03	-0.64*	-0.09	0.25	0.22	-0.12	0.61*	0.44
	Elon to Anth	-0.56*	0.49*	0.14	0.27	0.28	-0.47*	-0.54*	-0.05	-0.44*	0.56
	Anth to Mat	-0.77*	0.67*	-0.81*	0.74*	-0.15	-0.73*	-0.73*	-0.79*	-0.72*	0.89
Grain oil percent	**Emerg to Mat**	-0.29	-0.02	-0.22	0.29	0.22	-0.30	-0.32	-0.24	-0.27	0.00
	Rosette	-0.29	0.01	-0.24	-0.32	-0.18	0.15	0.15	-0.28	0.13	0.00

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	Elon to Anth	-0.40	-0.10	-0.28	0.39	0.60*	-0.53*	-0.53*	-0.34	-0.33	0.33
	Anth to Mat	-0.18	0.13	-0.21	0.15	-0.36	-0.14	-0.14	-0.24	-0.18	0.00
Oil yield	**Emerg to Mat**	-0.91*	0.08	-0.58*	0.88*	0.48*	-0.79*	-0.90*	-0.66*	-0.91*	0.89
	Rosette	-0.85*	0.14	-0.61*	-0.33	0.22	-0.07	-0.08	-0.69*	0.21	0.33
	Elon to Anth	-0.90*	0.14	-0.35	0.49*	0.65*	-0.63*	-0.67*	-0.52*	-0.52*	0.78
	Anth to Mat	-0.62*	0.46*	-0.69*	0.64*	-0.46*	-0.56*	-0.55*	-0.79*	-0.68*	1.00

*, Significant at the .05 probability level.

†, Emerg: emergence, Elon: stem elongation, Rosette: Emerg to Elon, Anth: anthesis, Mat: maturity.

‡, T: temperature (ºC), GDD: growing degree days with a base of 5 °C, H:M: heat:moisture index, PET: potential evapotranspiration, Precipitation deficit: (1 - precipitation)/PET

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