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Exploring Source-Sink Relationship for the Formation of Grain Yield in Sunflower

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ABSTRACT

Developing high-yielding varieties of sunflower as oilseed staple crops requires knowledge of physiologic and molecular mechanisms involved in yield formation. Source strength, sink demand, and their interactions play crucial roles in the yield formation of sunflowers. The persistence of assimilate flux to the developing grains mainly determines sink demand. There was no information on the molecular mechanism for assimilate flux to the sink organ of sunflowers. To shed light on molecular events engaging in assimilate flux to sink organs, two experiments were carried out on five sunflower inbred lines differing in their grain yields. Source-related parameters (such as leaf biomass, area, and number) and sink-associated attributes (such as floret number at the first anthesis and capitulum biomass and diameter, in addition to changes in biomass of capitulum and stem, at the first anthesis with those at physiological maturity) were evaluated across all the inbred lines. The *Invertase* gene expression level was measured on the receptacle base of three inbred lines, showing discrepancies in the source, sink, and grain yield performances. The results showed that while no significant correlation was found between source strength and sink demand with grain yield, higher grain yield was likely attributed to the persistence of assimilate flux to the capitulum base during grain filling. This phenomenon was discussed to be due to higher *Invertase* activity in the receptacle base.

Keywords: Grain yield, source-to-sink relations, sunflower

INTRODUCTION

Sunflower is staple oilseed crops with a kernel oil content of about 55%. Sunflower oil is the fourth most important vegetable oil globally (Grompone, 2005). The crop shows wide adaptability to various climate conditions, thus attracting a great deal of attention for breeders with the aim of yield development (Vear, 2016). Yield improvement in grain crops is mostly achieved through either higher biomass production or harvest index (HI) or both (Evans, 1996; Sharma and Smith, 1986), according to plant species.

Sunflower, a C3 crop, has a high photosynthetic capacity and rate (English et al., 1979), which is similar to many C4 species (Lloyd and Canvin, 1979; Rawson and Constable, 1980). Nevertheless, a high ratio of the transpiration rate to carbon fixation (Rawson and Constable, 1980), low HI (English et al., 1979), inefficient assimilation partitioning to grains, inefficient management in partitioning carbon assimilates to developing grain (English et al., 1979), low pre-anthesis reserved carbohydrate (Pereira et al., 2000) are among limiting factors proposed targets of yield improvements.

Biomass production is mostly influenced by source strength; nevertheless, HI is determined by sink-source relationships (Smith et al., 2018; Venkateswarlu and Visperas, 1987). Investigation of sunflower cultivars released in a period of 60 years in Argentina indicated that breeding programs had been directed toward increases in the partitioning of assimilates toward grain (Pereira et al., 2000), and increasing biomass production would be the method of choice for further increase in the yield (Smith et al., 2018). The formation of grain yield in crops, including sunflower, depends on reaching assimilates from sources to grain (as sink organ) (Sadras et al., 1993; Venkateswarlu and Visperas, 1987). Most fraction of the assimilates is supplied by current photosynthesis (Hall et al., 1995; Rafiei et al., 2013) and fewer ones from pre-anthesis stored carbohydrates (Hall et al., 1989; Hall et al., 1990; Pereira et al., 2008). Assimilates synthesized before anthesis are exported from the leaf and stored in the form of non-structural carbohydrate (NSC) in vegetative parts, such as stem nodes (e.g., wheat, soybean, and sunflower), pods, petioles (in soybean), and cub (in maize) (Kühbauch and Thome, 1989; Pereira et al., 2008; Seebauer et al., 2010; Streeter and Jeffers, 1979).

Managing assimilate production (source-related activities), storage (sink-related activities and NSC storing), and their partitioning make avenues of research for plant physiologists and breeders (Baker et al., 1984; Lee and Tollenaar, 2007; Lichthardt et al., 2020; Ludewig and Sonnewald, 2016). Increases in HI, which have been pointed out by the aforementioned researchers, are the key

point in developing varieties of higher grain yield. To achieve the goal, there must be a non-stop assimilate flux to filling grain from the first anthesis to physiological maturity. Identifying the cause of persisting flux requires knowing the molecular level of assimilate transportation. There are several lines of evidence mentioning the role of starch metabolizing enzymes in assimilate partitioning to developing seeds (Ishimaru et al., 2005; Saeedipour and Moradi, 2011; Yang et al., 2004). In sunflowers, no report was found on the role of the enzymes in grain filling. Nevertheless, it has been proven that invertase, hexokinase, and fructokinase are responsible for establishing the levels of soluble carbohydrates in sunflower seeds (Troncoso-Ponce et al., 2009). Assimilate fluxes from phloem into sunflower grains go through the receptacle base and capitulum as intermediate sink, even though there is no molecular data to support this claim. In this paper, physiological and molecular components affecting the variation of the grain yield of several sunflower inbred lines are discussed.

MATERIALS AND METHODS

Plant Genetic Materials

Five inbred lines of a sunflower, developed at the Oilseed Crop Research Department, Seed and Plant Improvement Institute (SPII), Karaj, Iran, were used in this investigation (Table 1).

Cultivation Systems and Treatments

The experiment was conducted in two environments: one in the experimental station of SPII in Karaj and the other in the experimental station of the Tarbiat Modares University (TMU) located on the Tehran-Karaj Freeway in the years 2017 and 2018, respectively. The location, soil, and climate characteristics are presented in Table 2.

Seeds were planted 25 cm apart with a row spacing of 60 cm. Each plot contained four rows of 4 m long each, with two external rows as borders and two internal rows serving as the experimental plants. The two internal rows were harvested after excluding one plant from each end of the rows. In each environment, experiments were conducted in a complete randomized block design with inbred lines as treatments and three replicates. The seeding date was chosen such that anthesis occurs when the temperature is nearly 30 °C, starting from mid-April and three later sowing dates with 10-day intervals.

The nitrogen-phosphorous-potassium-containing fertilizers (urea, 450 Kg/ha; superphosphate triple, 150 Kg; potassium sulfate, 220 Kg/ha) were applied based on the soil analysis. Weeds were

controlled manually. Plots were irrigated twice a week before anthesis and weekly from the anthesis to physiological maturity. To prevent bird injury, the capitulum was covered by newspapers after flowering.

Trait Measurement

In each plot, five competing plants were randomly selected and used for trait measurements. Leaf number, leaf area, and leaf biomass were measured at the first anthesis. Leaf area was measured on detached leaves of each plant using a digital leaf area meter device (Licor 3100, USA) and summed up to provide leaf area of the plant. Organ biomass (see below) was determined by oven-drying to a constant weight at 70 °C. Filled and empty grains of each capitulum were counted, and their biomass was considered grain yield per plant. Receptacle and capitulum biomass were determined at the first anthesis and physiological maturity, respectively. Non-seed biomass of capitulum was calculated by subtracting biomass of grains from capitulum biomass. NSC content was measured by the following formula:

$$NSC=(W_{ra}+W_{sa})-(W_{cfgm}+W_{sm})$$

where W_{ra}, Ws_a, W_{cfgm}, and W_{sm} are the biomass of receptacle at the first anthesis, biomass of the stem five uppermost nodes at the first anthesis, biomass of grain-free capitulum at physiological maturity, and biomass of the stem five uppermost nodes at physiological maturity.

The contribution of current photosynthesis to grain yield was estimated by subtracting NSC from grain yield per plant.

Capitulum radial diameter was measured by a digital caliper 10 days after physiologic maturity; also, it was used for calculating capitulum area. The average available area per grain was calculated by dividing the capitulum area by the number of grains per capitulum.

Pieces of 0.10 g tissues were sampled from the same region of the receptacle base and the five uppermost nodes of the stem. The specimen was flash-frozen in liquid nitrogen and kept under -80 °C for further analysis.

Starch Content Measurement and Gene Expression Analysis

Insoluble carbohydrates, also referred to as SC content (mg/g) of the frozen samples, were determined according to the method described by Sheligt (Sheligl, 1986) and glucose as standard. The data are presented as the mean of three replicates over the two environments.

Total RNA was extracted from 100 mg of the frozen samples using the TransZol Up Plus RNA Extraction kit (Beijing, China) according to the manufacturer's instructions, and its concentration and purity were evaluated by a spectrophotometer. Polymerase chain reaction (PCR) using primer pairs for *Invertase* and *Actin* (Table 3) was used to test the lack of any genomic DNA residual after treating the total RNA with RNase-free *DNase*I (Roche, Mannheim, Germany). The integrity of RNA was checked on a 1 % (w/v) agarose gel before and after *DNase*I treatment.

The first-strand cDNA synthesis was carried out using 2 µg of *RNase* I-treated RNA using the SuperScript III reverse transcriptase kit (Invitrogen, Karlsruhe, Germany) according to the manufacturer's instructions.

PCRs were conducted using the Roche System (Applied Biosystems, Darmstadt, Germany) and SYBR Green as a dye. Reactions with a final volume of 20 µL contained 4 µL of a template (cDNA or total RNA), 200 mM of each primer (1 µL of mixed forward and reverse primers with a concentration of 0.5 mM each, see Table 3), and 4 µL of a SYBR Green RealQ plus 2X master mix (Ampliqon). The applied thermal profile was 50 °C for 2 min and 95 ºC for 10 min, as well as 40 cycles of 95 °C for 15 s and 60 °C for 1 min. After 40 cycles, the specificity of the amplification was checked by heating from 60 °C to 95 °C with a ramp speed of 1.9 °C/min and producing the melting curves. The data are presented as the mean of three replications over the two environments.

The normalized expression level of *Invertase* based on *Actin* (Table 3) was shown as $40\text{-}\Delta C_t$, where C_t is the cycle number at which SYBR Green fluorescence in a PCR reaction reaches an arbitrary value during the exponential phase of DNA amplification, and ΔC_t is the C_t for *Invertase* normalized based on that of the *Actin* gene ($\Delta C_t = C_{t[Invertase]} - C_{t\,[Actin]}$).

Data Analysis

Combined analysis of variance was performed over the two environments by R4.3.1. Before analysis of variance, the data was evaluated for normality through performing Kolmogorov-Smirnov normality test by SPSS ver 27 and transformed up on deviation from normality. Mean comparisons was done through Duncan Multiple Range Test. Pearson correlation analysis was conducted on the raw data using SPSS ver 27.

RESULTS

Like all grain crops, yield improvement is archived through understanding determinant factors affecting the sink, source, and interrelationships. There are several lines of evidence showing the

effect of physiological cues on this interrelationship, but to the best of our knowledge, no data are available on the molecular physiology of events responsible for the interrelationships in the sunflower. In this study, however, we investigated the physiological aspects of grain yield formation in sunflower inbred lines and tried to use molecular knowledge to find the obscure part of the yield formation, which was pointed out in the previous studies.

Genotypes Has Significant Impacts on the Behavior of All Investigated Traits

A combined analysis of variance (ANOVA) over the two environments was conducted (Table S1). Effect of environment (location, year or both) was significant for most the traits except for leaf area per plant, receptacle dry weight at anthesis, stem dry weight at anthesis, grain yield, capitulum diameter, and current photosynthesis contribution to grain yield. Thus for, performing investigation in two environments, led to removing effects of environment on the mentioned traits. The analysis showed that the effect of genotype-by-environment interaction (G×Env) was not significant for all traits; thus, a pooling error comprising of environmental error and G× Env was estimated. Upon the pooling, genotypes appeared to have significant effects on all leaf-related traits, biomass of various organs, capitulum attributes, and contribution of remobilization to the grain yield at $\alpha \leq 0.05$. Nevertheless, contribution of current photosynthesis to grain yield was not significantly affected by genotype effect. Accordingly, we took the average of genotypes across environments for all the traits.

We investigated the appearance of the sunflower inbred lines regarding various source- and sink-related traits and combined the data with molecular data obtained from genotypes with differential grain yield to explain grain formation determinants in the sunflower. The details of the results are presented as follows:

Sunflower Inbred Lines Exhibit Variation in Source-Related Capacity

The lines were significantly different in total leaf area per plant at anthesis. Line BF1814 produced the largest leaf area (0.8 m^2), while inbred M-289 produced the smallest leaf area (0.2 m^2; Fig. 1a). The number of leaves per plant was also different, ranging from 21 for BF1814 to 25 for Bline1221 (Fig. 1b). Total dry mass of leaves at anthesis followed the same pattern as total leaf area, and BF1814 and M-289 showed the largest and smallest total leaf dry mass, respectively (Fig. 1c).

Pre-Anthesis Stored Photo-Assimilates is Differently Distributed between Stem and Receptacle base

Pre-anthesis stored biomass of the receptacle base and the upper part of the stem were significantly different among the lines. The biomass of the receptacle base for all lines was always greater than the biomass for the upper part of the stem (Fig. 2). This result indicates that before anthesis, more assimilates are translocated and stored in the receptacle base than in the upper part of the stem. According to the amount of biomass stored before anthesis, the lines were grouped into two classes: one class contained BF1814 and BF81-196 with the largest values, and the other included the rest of the lines showing similar values of the receptacle base biomass. Moreover, at anthesis, the lines showed similar classification patterns for the biomass of the upper part of the stem and leaves (Fig. 2). Because the assimilates stored in the receptacle base provide the energy required for developing florets, the amount of the assimilates stored before anthesis may be used to estimate energy supply for floret formation, indirectly affecting grain number at maturity.

Sink-Related Attributes are Responsible for Variation in Grain Yield across Inbred Lines

Grain yield per plant was measured at maturity by removing all achenes (filled and empty grains) from the capitulum and weighing the dried achenes thereafter.

Bline1221 had the largest grain yield per plant (33.5 g), followed by BF81-196 (29.1 g), BF1814 (20.2 g), Bline19 (18.4 g), and M-289 (14.9 g; Fig. 3a). All the lines used in this experiment were single-headed; thus, all the grain-targeted assimilates produced in source organs reached a single head. As a result, the single head (capitulum) was considered the only aboveground sink.

At physiological maturity, the biomass of the capitulum after removing all achenes was measured (non-seed capitulum biomass). This trait revealed how much of the allocated assimilate to the capitulum is not partitioned toward grains, thus called capitulum structural carbohydrates (CSC). Lines BF1221 and M-289 had the largest and smallest CSC content, respectively (Fig. 3b). High variation was found in CSC content among the lines (CV = 20%). Interestingly, a nearly constant ratio was observed between the total biomass of capitulum (CSC + grain yield) and grain yield (Fig. 3c and Table 4). Thus, it may conclude that irrespective of genotype, a general mechanism plays a role within the capitulum for allocating capitulum dry matter to grains.

The number of achenes per capitulum was used as the estimation of sink size. The inbred lines were classified into two categories according to the sink size (Fig. 4a). In one category, there exist BF1221, M-289, and BF81-196 with a similar number of achenes per capitulum and, in the other

one, Bline1814 and Bline19 with a significantly larger number of achenes per capitulum. Capitulum radial diameter (Fig. 4b) followed the same pattern as average space per achene on the capitulum (Fig. 4c): BF1814 and Bline19 had similarly the largest capitulum diameters and average available area per grain, followed by the rest of the lines with statistically the same values for the two traits.

Current Photosynthesis has a Higher Impact on Grain Yield Formation than Remobilization

Changes in the starch content of the receptacle base were monitored in four-day interval sampling dates starting from the first anthesis. The results of the starch content over the sampling dates are presented in Fig. 5.

The results showed that for all the inbred lines, starch content continued to rise in the receptacle base until the eighth day after the first anthesis and decline thereafter (Fig. 5). The same patterns in the dynamic of the starch content were observed in the five uppermost nodes of the stem (data are not shown). Thus, the eighth day after the first anthesis was considered the time for starting the remobilization of dissolved NSCs from the receptacle base and the upper part of the stem toward achenes.

Remobilization amount (RA) was calculated based on the differences in the total biomass of the achene-free capitulum and the five uppermost nodes in the stem at physiological maturity from the biomass of the organs on the eighth day after the first anthesis. Dividing the RA by the grain yield, the contribution of RA to grain yield (RAC) was estimated. Also, we subtracted the RAC from grain yield, which gave us an estimation of the current photosynthesis contribution to grain yield (CPC).

BF1814 and M-28 showed a maximum RAC of 33%, while its minimum was 22% for BF81-196 (Fig. 6a). Comparison between the contribution of current photosynthesis (Fig. 6b) and remobilization to grain yield revealed that the contribution was much larger for current photosynthesis than that of remobilization (from 64% for BF1814 and M-289 to about 80% for BF81-196; Fig. 6). As a result, we concluded that photosynthesis activity in the period after anthesis is more determinant in grain filing than that of pre-anthesis. Nevertheless, we will show that other factors play important roles in importing photosynthates to the capitulum.

The *Invertase* Gene Differentially Expressed over the Lines with Similar Sink Size

Invertase expression, normalized according to an internal control gene, *Actin*, was measured in three inbred lines, showing discrepancies between the source, sink power at anthesis, and grain yield (Fig. 7). According to Fig. 7, while Bline1221 and M-2890 showed similar values for the gene expression, BF1814 has significantly lower *Invertase* gene expression.

DISCUSSION

The source-sink formation and relationship is fundamental to understanding the crop growth and yield formation (White et al., 2016) and helps plant breeders to make efficient and better decisions for yield improvement.

Leaves produce assimilates and thus serve as a source organ. Source strength, as a product of source size and source activity, is associated with the total biomass of the source tissue and the specific rate of photosynthates biosynthesis (White et al., 2016). The 14th and upper leaves in sunflower have a high rate of net photosynthesis (English et al., 1979) or gross photosynthesis (Rawson and Constable, 1980), respectively, which is accounted for source activity. Total leaf area and average leaf size are commonly used to express source size in sunflowers (Pereira et al., 1999). By these definitions, in our study, BF1814 and M-289 showed the highest and lowest source strength, respectively.

Smith et al. (2018) and Rennie and Turgeon (2009) indicated that there were multiple mechanisms functioning in assimilate transportation in the same plant species. A strong correlation between the leaf biomass and receptacle and a weak correlation between the leaf biomass and the number of florets per capitulum were observed in this study (Table 5). These correlations may indicate a lack of considerable limitations in assimilate uploading in the leaves and the presence of limitations in the unloading of the assimilate at the receptacle base before anthesis. Meanwhile, these correlations highlight the presence of multiple mechanisms involved in assimilate uploading, unloading, and transportation in the in the evaluated sunflower lines.

Grain yield per plant of single-headed sunflower genotypes is determined as a product of the number of achenes (filled and empty) per capitulum and 1,000-grain weight (Ion et al., 2015; Villalobos et al., 1996), where the number of achenes plays a much greater role in yield formation (Connor and Sadras, 1992). Achenes are the strongest sink after starting anthesis (Connor and Sadras, 1992). The sink strength is defined as a product of sink size and sink activity (White et al., 2016). Sink size in sunflower has a close correlation with the floret number, which is determined

289 at the flowering time (Steer et al., 1988). Thus, a capitulum with a greater number of achenes and
290 thereby a bigger radial length is considered a stronger sink. Because floret formation and
291 development lead to a great demand for assimilate before anthesis (Alkio et al., 2002; Connor and
292 Sadras, 1992), genotypes with a greater number of achenes (i.e., BF1814 and Bline19) are expected
293 to have more receptacle dry matter at anthesis and vice versa—a relationship we did not observe
294 in Bline19 and BF81-196 (Figs. 3 and 4).
295 Grain filling is supported by assimilate flux to the grain, which is decomposed into current
296 photosynthesis (photo-assimilate) (Epila et al., 2018) and pre-anthesis NSC stored in stem
297 (Bihmidine et al., 2013; Evans, 1996; Streeter and Jeffers, 1979) and receptacle base (Baker et al.,
298 1984; English et al., 1979; Hall et al., 1989; Pereira et al., 2008; Pereira et al., 2000). In our study,
299 NSC could only contribute up to 33% of achene dry weight, indicating more contribution of current
300 photosynthesis to grain yield, which was less than the same figure (45%) reported by other
301 researchers under the same conditions (Pereira et al., 2008). Pereira et al. (2008) divided and
302 estimated the contribution of pre-anthesis NSC to grain filling into two origins: the receptacle and
303 stem. In the present investigation, all evaluated genotypes were indifferent in their contribution of
304 NSC and current photosynthesis to grain filling; therefore, it can be concluded that the limitation
305 in the remobilization of NSC and photo-assimilates may be a less deterministic factor for grain
306 yield of sunflower.
307 Development of achenes on the capitulum is limited mainly by three factors: a) limited space and
308 the physical pressure exerted by the neighboring achene on the capitulum (Hernández, 2015;
309 Lindström et al., 2006; Sinsawat and Steer, 1993), b) insufficient assimilates (Alkio and Grimm,
310 2003; Kühbauch and Thome, 1989), and c) competition among developing achene (Behbahanzadeh
311 et al., 2012; Sinsawat and Steer, 1993; Steer et al., 1988). The capitulum radial diameter is used as
312 a good estimation of the average available area for each achene (Sinsawat and Steer, 1993). Thus,
313 if sufficient assimilate is provided, the bigger the capitulum radial diameter, the more available
314 space per achene on the capitulum, and thus the greater potential sink size. Thus, in our experiment,
315 while BF1814 and BF81-196 had the biggest amount of pre-anthesis receptacle weight, BF1814
316 and Bline19 showed the biggest sink size.
317 Sufficient assimilate supply to the developing grains depends on two factors: physical resistance
318 in the assimilate flux route from the source to filling achenes and biochemical barriers against
319 reaching the assimilates in the phloem to the receptacle base and achenes thereafter. Every

floret/achene on the receptacle/capitulum receives photo-assimilate from three neighboring orthostichies (Alkio et al., 2002; Sinclair, 1994), and also there are lines of evidence for the lack of structural and functional deficiency in the vascular connection between the receptacle and all the grain on the capitulum (Alkio and Grimm, 2003). As a result, we conclude that biochemical barriers are thought to be determinant factors in reaching assimilates to filling achenes.

Summing up the above observations, lines BF1814 and Bline19 have the biggest sink size, and line BF1814 has the strongest source. However, line with the strongest source and sink (BF1814) could not produce the greatest grain yield, provided a counter-argument against current ideas on source-sink relationships and the source-limitation hypothesis of yield formation in sunflower under a normal cultivation system, as reported by Alkio et al. (2003) and Behbahanzadeh et al. (2012). The contribution of NSC and current photosynthesis to grain filling was nearly similar for all the evaluated genotypes (Fig. 6 and Table 4). Thus, we observed that sink size and source stringency, according to the current terminology (White et al., 2016), were unlikely to play a strong impact on grain yield.

While the contribution of the receptacle as temporary intermediate storage of current photosynthesis has been proposed (Hall et al., 1989; Pereira et al., 2008), the molecular mechanisms of this process remain to be elucidated. Sucrose is the major mobile form of assimilates in nearly all plant species (Farrar et al., 2000). Accumulation of unloaded assimilates in the sink organ creates negative feedback to the current assimilate toward the sink (Farrar and Minchin, 1991). Ideally, in the sink organ, the unloaded sucrose has to be rapidly hydrolyzed into glucose and fructose, a task that is accomplished by Invertases (Sturm, 1999). Thus, in the sink organ, sucrose released from the phloem is broken down by Invertase to minimize the negative feedback (Roitsch and Tanner, 1996) and maintain the assimilate flux to the sink.

We observed that M-289 and Bline1221 showed the lowest and highest grain yield, CSC, and total capitulum biomass at maturity, respectively—but interestingly, with the same level of *Invertase* gene expression in the receptacle base. BF1814, on the other hand, showed a large sink size and pre-anthesis stem and receptacle base biomass—but low capitulum total biomass and grain yield. The *Invertase* expression level was lower in BF1814 than in M-289 and Bline1221. In the current study, we observed a constant ratio between the total dry matter of the capitulum (CSC + grain yield) and grain yield across the lines (Table 4), indicating that the grain yield differences are probably more attributable to the total dry matter of capitulum than to the allocation of NSC of the

capitulum to developing grains, which was originally suggested by English et al. (1979). We think that the allocation of assimilates to the capitulum has a more prominent effect on yield than the re-distribution of assimilates to the developing grains. This finding is illustrated in a proposed model of yield formation (Fig. 8), where the Invertase activity in the receptacle base creates a negative gradient and maintains assimilate flux to the receptacle base as temporary storage of current photosynthates, which in turn, provides sufficient dry matter for more grain filling. Future studies can monitor the concentrations of two simple sugars, glucose and fructose movements, from the receptacle base into the filling grains to further verify this proposed model.

CONCLUSION

Observation over a limited number of sunflower inbred lines indicated the need for re-defining the sink strength concept in sunflowers. In other words, re-defining sink in sunflowers requires factoring in source activity, metabolic activity, and sink size (the number of florets per receptacle), which are the determinants of assimilate gradient down to filling grains. Our results suggest that the sucrose metabolism enzyme Invertase highly contributes to maintaining the assimilate flux down to the developing grains. The receptacle base (i) stores NSC before anthesis and (ii) maintains assimilate flux to the developing grain; thus, it plays a role as an intermediate sink. Our results also suggest that the overexpression of sucrose metabolism-related enzymes, in addition to higher HI and source stringency, is a prominent strategy for breeding high yielding varieties in sunflower.

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Table S1. Combined analysis of variance across the two experiments performed in five sunflower inbred lines.

		Mean square												
Source of Variation	df	Leaf area/plant	No leaf/plant	Leaf weight/plant	Rec. weight1Δ	Stem weight1 $	Grain yield	NSCB	Cap weight 2£	Achene per cap	Cap diameter	Space per achene	RAC	CPC
Environment (Env)	1	0.04	66.90	47.38	7.01	27.46	0.41	75.84	667.41	12200.83	1.32	0.02	3154.13	0.01
Replication/Env	4	0.00	4.53	0.68	8.64	13.90	1.45	2.11	3.79	55.27	2.60	0.00	198.70	1.19
Genotypes (G)	4	0.26**	24.22**	25.70**	27.76*	48.08**	347.28**	25.96**	254.17**	58734.42**	2.92 *	0.02**	222.32ns	352.12*
Pooled Error	20	0.001	2.57	1.54	4.11	1.68	2.68	1.32	59.82	237.40	1.06	0.002	111.75	2.72
CV (%)		9.76	5.36	14.34	8.7	6.71	6.27	6.62	7.12	0.85	7.6	8.05	38.92	6.34

* and ** represents significant differences at $\alpha \leq 0.05, \alpha \leq 0.01$, respectively. Ns stands for nonsignificant differences.

Δ: Receptacle biomass at first anthesis, $: biomass of upper part of stem at anthesis, £: Capitulum biomass at maturity (including achenes)

Rec, Cap, NSCB, RAC, CPC, FGWCW stands for receptacle base, capitulum, non-seed capitulum biomass, contribution of remobilization amount to grain yield, current photosynthesis contribution to grain yield, Fraction of Grain weight in capitulum weight respectively.

Cont. Table S1

		Mean square	
Source of Variation	df	FGWCW	FCSCWCW
Environment (Env)	1	0.01	0.3
Replication/Env	4	0.2	0.26
Genotypes (G)	4	0.56 *	0.089 ns
Pooled Error	20	0.112	0.1
CV (%)		6.45	4.86

.* and ** represents significant differences at $\alpha \leq 0.05, \alpha \leq 0.01$, respectively. Ns stands for nonsignificant differences

FGWCW and FCSCWCW stands for Fraction of Grain weight in capitulum weight and Fraction of CSC weight in the capitulum weight, respectively.

Table 1- Sunflower inbred lines used in this investigation

Genetic material	Characteristic
Bline19	B line, late maturity
M-289	Mutant inbred line, Donated kindly by Dr Ahmad Sarrafi
BF81-196	Hybrid mother line, B line
BF18141	Hybrid mother line, B line
Bline1221	Hybrid mother line, B line

Table 2- Soil, geographic, and climatologic parameters for the two environments.

Environment	Soil attributes		Longitude	Latitude	Height from sea level	Annual precipitation	Temperature			
	Soil texture	pH					June	Jul	Aug.	Sep.
SPII	Sandy Clay Loam	8	51° 6' East	35° 49' North	1313	243	26.2	34	30.4	23.5
TMU	Clay Loam	7.4	51° 10' East	35° 44' North	1353	247.3	23.4	28.2	22.9	18.1

Table 3. The primer pair sequences and accession number for *Invertase* and *Actin*

Gene name	Primer name	Primer sequence (5' – 3')	Accession number
Invertase	INV F*	CCAAAAACATATCGGACCC	XM_035988205.1
	INV R*	CCATAATCATACTGTAACC	
Actin	ACTIN F	CAGGCCGTGCTTTCCCTCTA	DY915068 (Ochogavía et al., 2017)
	ACTIN R	GGTCACGA CCAGCGAGATCA	

* F and R stand for forward and reverse, respectively.

Table 4. Means of capitulum dry weight components at physiological maturity

Genotype name	Grain yield (g)	Capitulum structural carbohydrates (CSC) (g)	Fraction of Grain weight in capitulum weight (g/g)	Fraction of CSC weight in the capitulum weight (g/g)
BF1814	20.16 c	47.94 c	0.30 a	0.71 a
BF81-196	29.14 b	58.3 b	0.33 a	0.66 a
M-289	14.88 d	42.79 d	0.26 ab	0.74 a
Bline19	18.39 c	52.76 c	0.26 b	0.75 a
Bline1221	33.55 a	71.31 a	0.32 a	0.69 a
CV (%)	33	20	11	5

Means with common letter are not significantly different at $\alpha \leq 0.05$. CV stands for coefficient of variation.

Table 5. Correlation coefficients between leaf biomass, receptacle base biomass, and number of floret per capitulum.

Traits	Receptacle base biomass	Number of florets per capitulum
Leaf biomass	0.94**	0.41ns

** represents significant differences at $,\alpha \leq 0.01$, respectively. Ns stands for nonsignificant differences

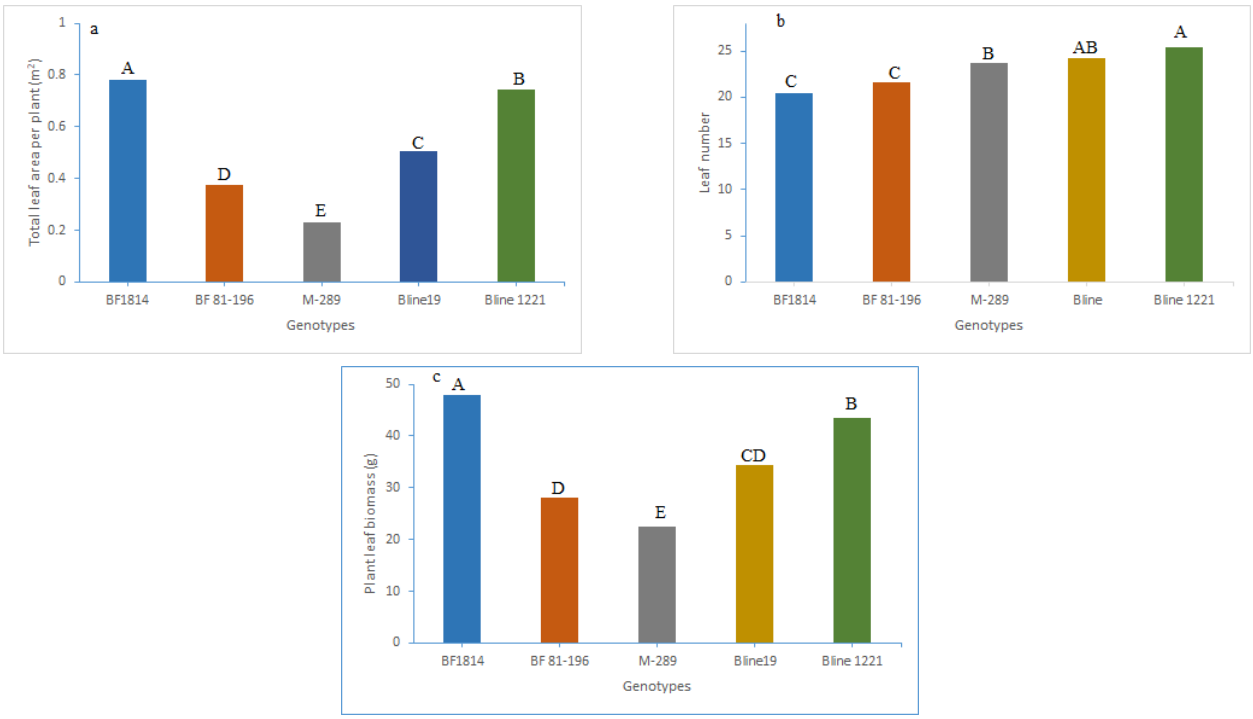


Fig. 1. Leaf characteristics of five sunflower inbred lines at the first anthesis. a) Total leaf area per plant. b) Number of leaves per plant. c) Total leaf weight per plant. In each panel, means having common letters are not significantly different at $\alpha \leq 0.05$.

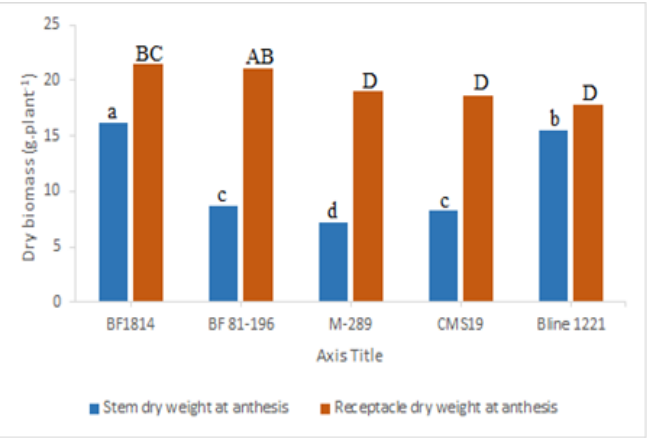


Fig. 2. The dry weight of the upper part of the stem (in cyan) and receptacle (in gray) of five sunflower inbred lines at the first anthesis. The small and capital letters are independently used to show significant differences for stem biomass and receptacle biomass, respectively. In each trait, means having common letters are not significantly different at $\alpha \leq 0.05$.

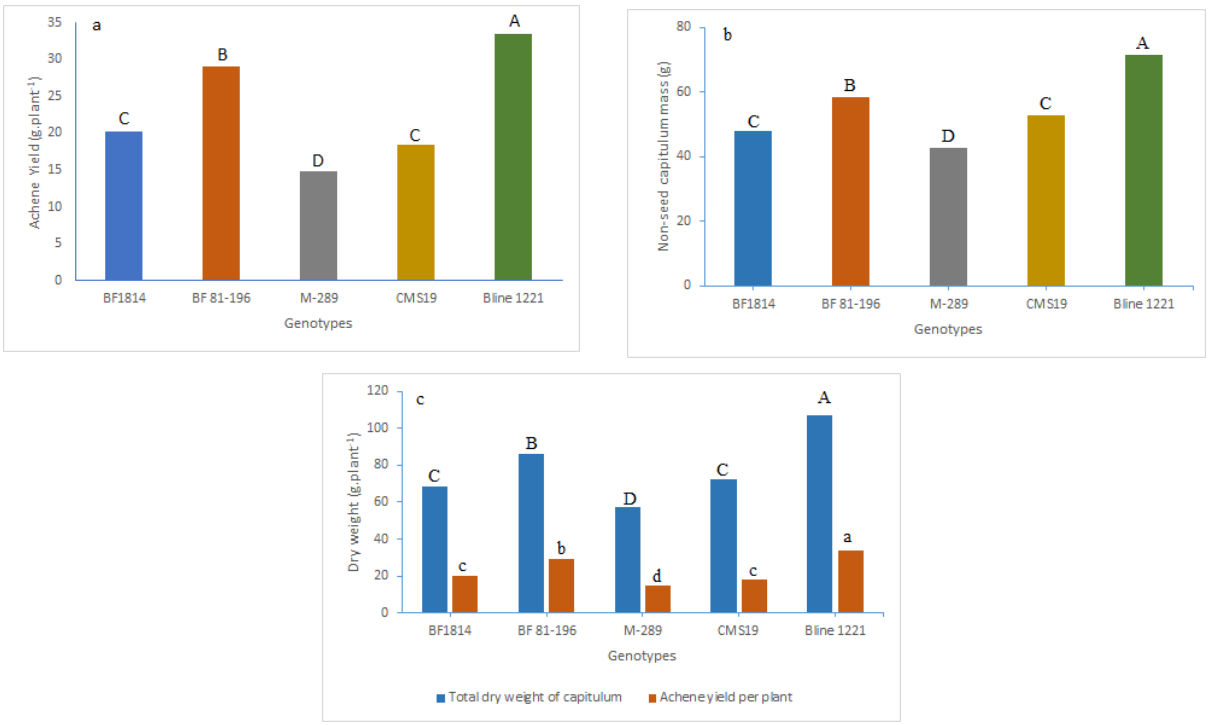
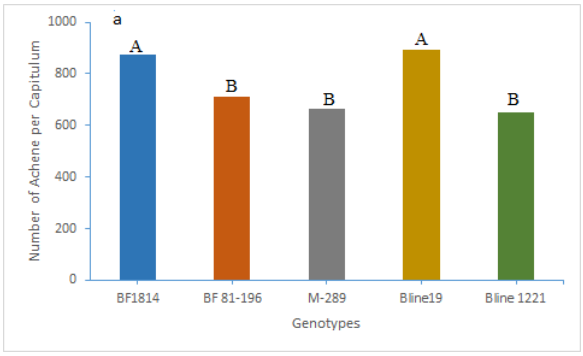
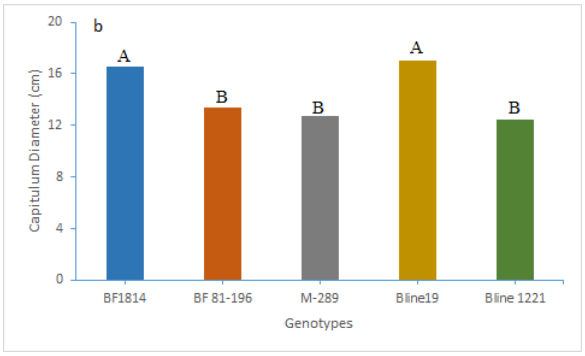


Fig. 3. The dry weight at physiological maturity of five sunflower inbred lines. a) Achene (grain) yield per plant. b) Capitulum weight after removing all achenes (capitulum structural carbohydrates, CSC). c) A panel showing both achenes yield and capitulum weight (capitulum body plus all the achenes). In each panel and c panel, means having common letters are not significantly different at $\alpha \leq 0.05$. In panel c, the small and capital letters are independently used to show significant differences at $\alpha \leq 0.05$ for achene and capitulum biomass, respectively.





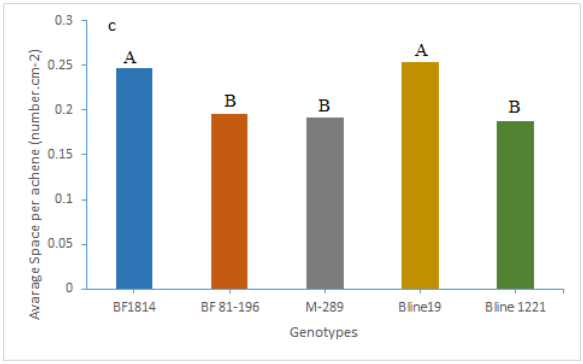


Fig 4. Sink size-related attributes of five sunflower inbred lines. a) number of achenes (filled plus empty) per capitulum, b) capitulum diameter, and c) average space per achenes on the capitulum. In each panel, means having common letters are not significantly different at $\alpha \leq 0.05$.

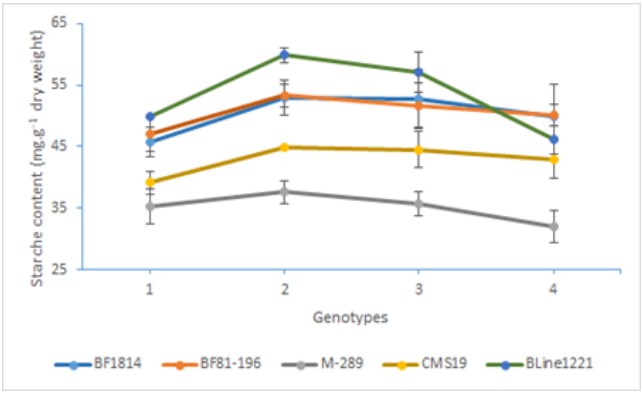


Fig 5. Trends of starch concentration on the receptacle base of five sunflower inbred lines in interval dates after anthesis. X axis units represent 4-days intervals. Data are shown as mean ± SD (n=6).

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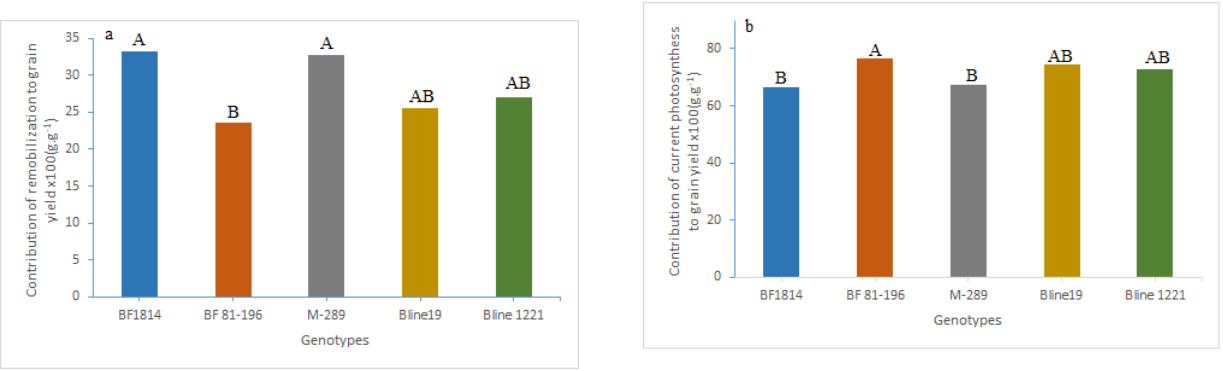
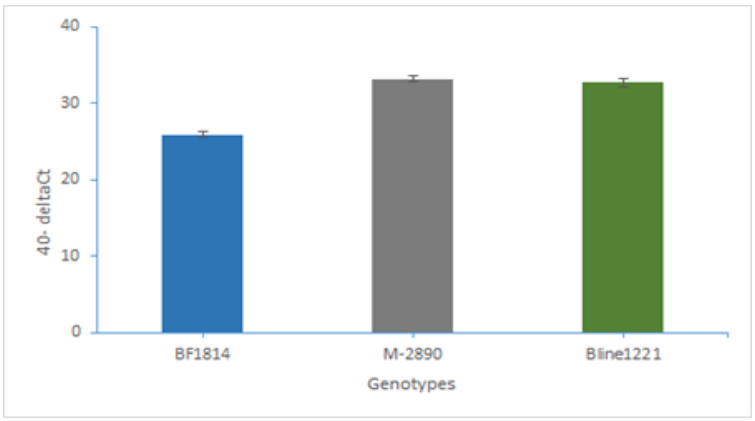


607

608 **Fig 6**. Contribution of non-structural carbohydrates (NSC) stored in receptacle base and upper
609 part of stem (a) and current photosynthesis (b) in grain yield of five sunflower inbred lines. In each
610 panel, means having common letters are not significantly different at $\alpha \leq 0.05$.
611



612

613 **Fig 7.** *Invertase* gene expression in receptacle base of three sunflower genotypes at anthesis.
614 Expression data were normalized according to *Actin*, as reference gene (ΔC_t) and subtracted from
615 40 to make the comparisons easier. Data are shown as mean ± SD (n=6).

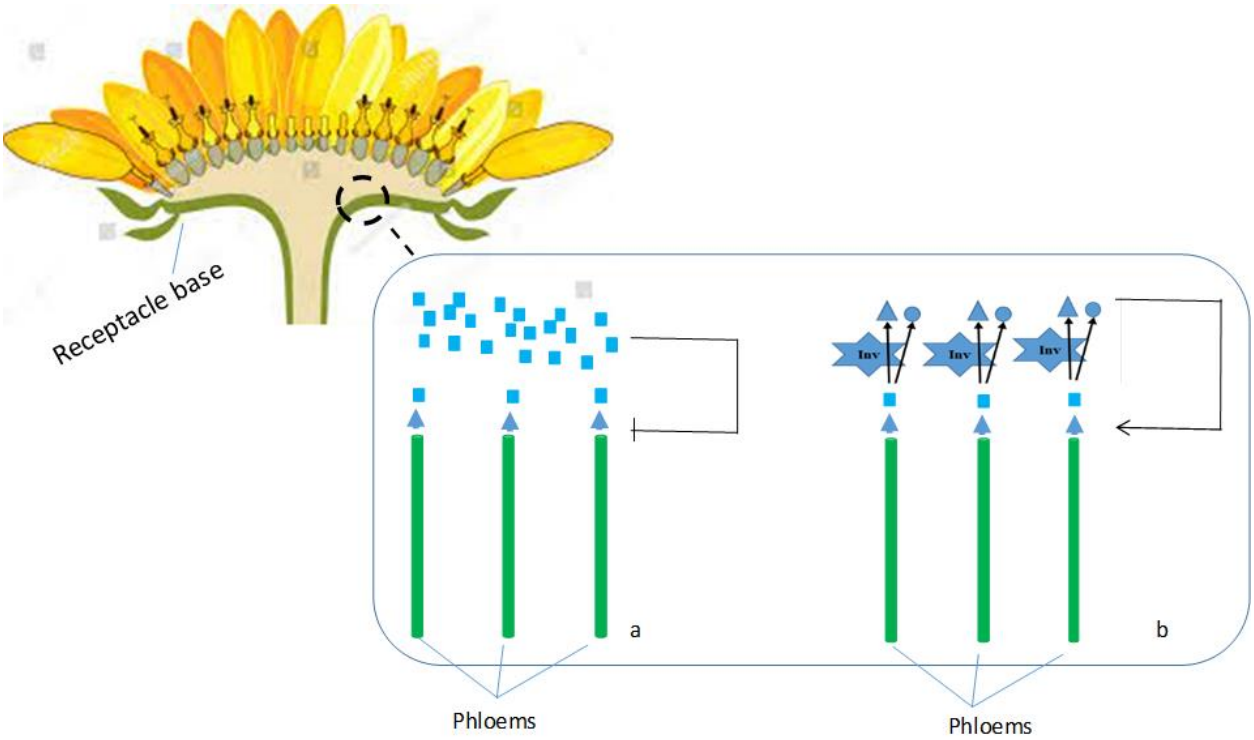


Fig. 8. Proposed model mentioning the role of the Invertase enzyme (Inv) presented in the receptacle base to hydrolyze the imported sucrose (squares) into glucose (triangles) and fructose (circles). In the low or lack of Inv activity, the unloaded sucrose from phloem is accumulated in the receptacle base as temporary base and results in negative feedback on unloading of sucrose (a). The unloading sucrose is broken down by Inv into simpler sugars, and thus, removes the negative feedback in the receptacle base and creates a negative gradient and maintains assimilate flux to the receptacle base.