Yield and Oil Improvements in Clearfield® Plus Sunflowers

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- The Clearfield® or imidazolinone tolerance trait, originally crossed from resistant wild sunflowers into cultivated sunflowers, is based on a single basepair mutation, *Ahasl1-1,* in the gene encoding the large subunit acetohydroxyacid synthase (AHAS) enzyme. To achieve commercial tolerance, both the *Ahasl1-1*gene and one or more enhancer genes are needed in the final Clearfield (ImiSun) hybrid. It has been shown that the *Ahasl1-1* gene was originally introgressed into the cultivated sunflower germplasm along with a 40 cM genomic region originating from the wild parental source. Since this genomic region includes some mapped quantitative trait loci (QTLs) for grain oil content and seed weight, it was suggested that the wild parental sunflower sequences linked to the *Ahasl1* locus in ImiSun sunflowers resulted in an agronomic drag for yield and oil content. In 2010, a novel herbicide tolerance trait in sunflowers, Clearfield Plus**,** was first launched in Argentina. This new herbicide tolerance trait is based on a different basepair mutation, *CLHA-Plus* or *Ahasl1-3,* in the same large subunit *Ahasl* gene and does not require any enhancers while conferring higher crop tolerance. As the mutation controlling the Clearfield Plus trait has no contribution from a wild source, it was hypothesized that hybrids based on this novel gene, *CLHA-Plus or Ahasl11-3*, may have better seed yield and oil content than ImiSun hybrids based on *Ahasl1-1*. It was also hypothesized that hybrids based on the *CLHA-Plus* mutation, or CLPlus hybrids, had similar yield and seed oil content to conventional hybrids.
- Conventional and CLPlus isohybrids were field tested over 4 years in randomized full plot field trials in Argentina, France, Hungary and the USA. ImiSun and CLPlus isohybrids were field tested over the last few years in Argentina and in 2011 in the EU and in North America. Entries were assessed for agronomic performance, yield and percent grain oil on a per plot basis. Preliminary data from one set of isogenic hybrids indicates that there are significant differences in grain oil content, yield and thousand kernel weight between ImiSun hybrids (*Ahasl1-1*/ *Ahasl1-1*) and the conventional or CLPlus hybrids (*Ahasl1-1/ Ahasl1-3* near-iso and *Ahasl1-3/ Ahasl1-3* isohybrids) when the environments are stressed or when the trials were treated with an imidazolinone herbicide formulation. In high stress or stringent environments, as measured by a low environmental oil content index, significant grain oil increases of up to 2% were observed in CLPlus hybrids over ImiSun isohybrids. No significant differences in grain yield, thousand kernel weight and oil content could be observed when comparing conventional hybrids to their CLPlus isogenic hybrid counterparts.
- The observed significant increase in grain yield, thousand kernel weight and seed oil content in CLPlus hybrids over ImiSun hybrids is believed to be due to a lack of linkage drag since there are no wild sunflower genomic sequences surrounding *Ahasl1-3* (*CLHA-Plus).* The absence of significant differences in yield and oil content between conventional and isogenic CLPlus hybrids indicates that there are no yield penalties and no oil content penalties associated with this novel trait.
- In this respect, conversion of parental lines to introduce this trait using a marker assisted backcross procedure is an efficient approach to developing CLPlus hybrids while maintaining the agronomic performance of the original genotype. Future studies will focus on broadening the current analysis to include CLPlus hybrids from a broader range of genetic backgrounds.

Key words: *Ahasl1*; Clearfield; *CLHA-Plus*; ImiSun; *Helianthus annuus*; oil yield

INTRODUCTION

The Clearfield® production system in sunflowers, which refers to the use of regionally tailored imidazolinone herbicides together with imidazolinone-tolerant hybrid varieties, has been available commercially in North America, South America and in the EU (including Turkey) since 2003 (Tan et al, 2005). Imidazolinone-tolerant ImiSun varieties used in the Clearfield production system are derived from USDA sunflower maintainer and fertility restorer germplasm that inherited their imidazolinone tolerance gene (*Ahasl1-1*) from an imidazolinone tolerant wild sunflower line (Al-Khatib et. Al., 1998) (Miller and Al-Khatib,, 2002). Mapping studies conducted on different ImiSun hybrids have shown that most carry a 40 cM genomic region originating from the wild parental source surrounding the *Ahasl*1 gene (Sala and Weston, 2010). Since this genomic region includes mapped quantitative trait loci (QTLs) for grain oil content and seed weight, it was hypothesized that the wild parental sunflower sequences around the *Ahasl*1 locus in ImiSun sunflowers caused an agronomic drag for yield, thousand kernel weight and oil content (Trucillo et al, 2010). In 2010, a novel imidazolinone tolerance gene, *CLHA-Plus* or *Ahasl1-3*, was commercialized under the brand Clearfield Plus. This novel tolerance was created, via seed mutagenesis, in an elite sunflower inbred line and therefore did not contain linked wild sunflower genomic regions surrounding the *Ahasl*1 gene (Sala et al., 2008a; Sala et al., 2008b).

To better understand how Clearfield Plus (CLPlus) hybrids perform in terms of grain oil content and yield, a set of isogenic conventional, ImiSun , and CLPlus hybrids were developed. These hybrids are in the process of being tested over a number of years under different environmental conditions in South America, North America and in the EU. The preliminary results from these ongoing studies are presented in this publication.

MATERIALS AND METHODS

<u>Conventional versus CLPlus</u>

Three sets of conventional (P2, P3 and P4) and CLPlus (CL++P2, CL++P3, CL++P4) isohybrids (homozygous for the *Ahasl1-3* or *CLHA-Plus* gene) were produced by backcrossing and marker assisted selection. These hybrids were field tested in Argentina for two years (2007-2008 and 2008-2009) at various locations (6 trials over 2 years) in conventionally treated randomized full plot field trials with 3 repetitions. P3 and CL++P3 isohybrids were also trialed in the USA in 2010 and 2011 and in the EU in 2011 in randomized field trials (Randomized Complete Block Design, RCBD) with 3 to 4 repetitions over a range of environments typical for sunflowers grown in each respective region. A combination of conventional herbicides and hand weeding were employed for weed management.

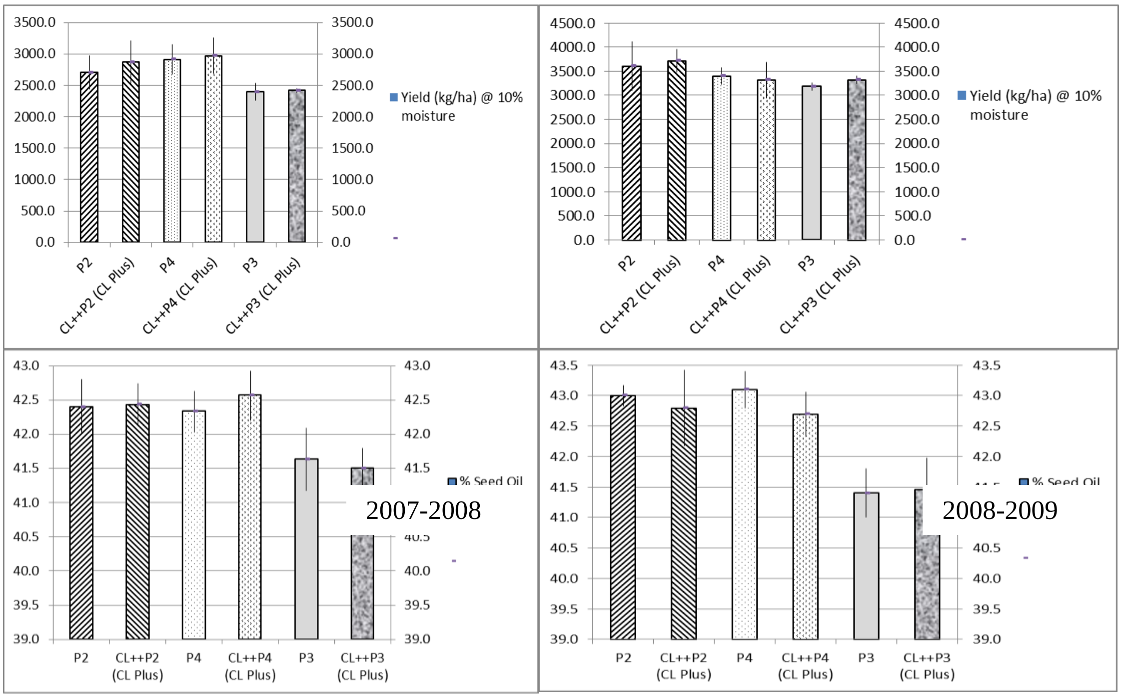
<u>Conventional versus ImiSun versus CLPlus</u>

One set of conventional (P3), ImiSun (CLP3) and CLPlus (CL++P3; homozygous for *Ahasl1-3* or *CLHA-Plus*) isogenic hybrids was also developed via backcrossing coupled with marker assisted selection. A near isogenic CLPlus hybrid (CL+P3*; heterozygous *Ahasl1-1*/*Ahasl1-3*) was also produced by crossing an ImiSun (*Ahasl1-1*) parent with a CLPlus (*Ahasl1-3*) parent, where one parent was common to P3, CLP3 and CL++P3. These 4 entries, P3, CLP3, CL+P3* and CL++P3, were tested along with other hybrid entries and local checks in both untreated (conventional herbicides plus hand weeding) and imidazolinone-treated field trials. Imidazolinone treated field trials were organized in a split plot design with 3 – 4 repetitions and at least 2 different herbicide treatment rates. In the EU (France and Hungary) Pulsar40 (Imazamox 40 g/l + 400 g/l Tween 20) was used at the 50 g ai/ha rate (1x) and at the 100 g ai/ha rate (2x). In the USA, Beyond (Imazamox 120 g/l LC) plus 0.25% non-ionic surfactant was used at the 35 g ai/ha rate (1x) and at the 70 g ai/ha rate (2x). Herbicides were applied at the 2 – 6 leaf stage.

An isogenic ImiSun test hybrid (CLP3*, homozygous for *Ahasl1-1*), isogenic to the CLPlus hybrid CL+P3* (heterozygous *Ahasl1-1*/ *Ahasl1-3*), was also produced and field tested together with CLPlus (CL+P3*) over a two year period in 20 locations across diverse sunflower growing regions and environments in Argentina.

<u>General:</u>

Agronomic performance, including yield at 10% moisture, thousand kernel weight (TKW) and grain oil content were measured. Grain moisture and grain oil content were assessed by an independent central analytics laboratory using the AOAC method 960.39 and 948.22. Results were statistically analyzed using an ANOVA or using the Tukey's Studentized Test for the comparison of varieties versus different treatment rates.



RESULTS

<u>Conventional versus CLPlus</u>

Three genetically different sets of isohybrids (set = conventional isohybrid and CLPlus isohybrid (*Ahasl1-3* homozygous)) were field tested over 2 consecutive years in Argentina. P3 was isogenic to CL++P3, P2 was isogenic to CL++P2 and P4 was isogenic to CL++P4. The grain yield and the % grain

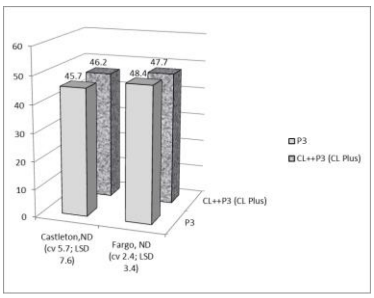
Figure 1: Mean Yield (kg/ha at 10% moisture) and % Grain Oil of Conventional and CLPlus Isohybrids tested at 3 Locations over 2 Consecutive Years in Argentina

oil were measured from each plot and means for each location were subjected to a statistical analysis.

In the USA, P3 and CL++P3 isohybrids were tested at two locations in 2010 (Castleton ND, Fargo ND). Data from these two sites are shown in Figure 2. Oil analysis data is still pending for the 2011 USA trials (4 locations) and therefore the 2011 data set will not be reviewed here.

In the EU, isogenic P3 and CL++P3 were tested at three locations (Aurade, FR, Gyor, HU and Hodmezovasarhely, HU) in 2011. A cross-site ANOVA analysis was conducted on all of the agronomic and seed composition parameters. The results from the P3 and CL++P3 isohybrid entries are illustrated in Figure 4 for the mean grain yield, the mean % grain oil (dry weight) and the mean thousand kernel weight.

No significant differences in either grain yield, thousand kernel weight or % grain oil were observed between conventional hybrids (P2, P3, P4) and hybrids with the CLHA-Plus trait (*Ahasl1-3* homozygous) (CL++P2, CL++P3, CL++P4) in any of the regions (Argentina, USA, France, Hungary) tested.



Yield (kg/ha @ 10% moisture) % Grain Oil (@ 4.3% moisture) Thousand Kernel Wgt (grams)

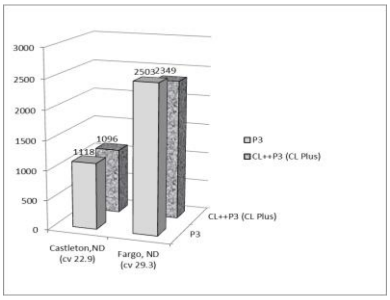
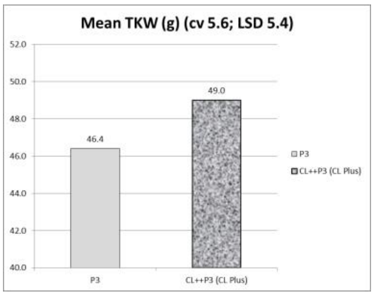


Figure 2: Yield, % Grain Oil and Thousand Kernel Weight (TKW) of a Conventional and CLPlus Isohybrid tested at 2 Locations (Untreated) in the USA in 2010



Conventional versus ImiSun versus CLPlus

In 2011, the agronomic performance of an ImiSun isohybrid (CLP3) was tested in RCBD full plot trials along with a conventional isohybrid (P3), a near-isogenic CLPlus hetero hybrid (CL+P3*) and a CLPlus (CL++P3) isohybrid in both the EU and in the USA. Isohybrids were evaluated along with grain composition in untreated trials (conventional herbicides plus hand weeding) and treated trials (treated with Clearfield herbicides). The grain composition results for the USA 2011 trials were not yet available.

The trend in the untreated trials was for higher yields, higher thousand kernel weights and higher % grain oil for the CLPlus isohybrid (CL++P3) in relation to the ImiSun isohybrid (CLP3) (Figure 3). The analyzed data, though, show that these differences were not significant. This was due to the higher level of variation (higher values for the least significant difference, LSD) observed in the untreated trials (Figure 3) versus trials that were treated with a Clearfield herbicide (Figure 4). Data from the trials that were treated with a 1x and 2x field rate of the locally registered Clearfield herbicide indicated a significant increase at both 1x and 2x rates in yield, thousand kernel weight and % oil for the CLPlus (CL++P3) isohybrid over the ImiSun (CLP3) isohybrid. In the treated trials, the CLPlus (CL++P3) had a 13% increase in yield over the CLP3 ImiSun isohybrid at the 1x Pulsar rate and an 18% increase in yield over the CLP3 isohybrid at the 2x Pulsar rate (Figure 4). For the % grain oil, the CL++P3 CLPlus isohybrid had at least a 2% significant increase in % grain oil over the CLP3 ImiSun isohybrid. There was no significant difference in the % grain oil between the treatment rates, 1x versus 2x. The only significant differences in % grain oil were between the varieties (Table 1). The thousand kernel weight, TKW, was also significantly higher in the CLPlus CL++P3 isohybrid versus the ImiSun CLP3 isohybrid in the treated trials (Figure 4). A significant increase in the TKW for the CLPlus (CL++P3) isohybrid was also observed at the 2x rate over the 1x rate, but the yield for the CL++P3 entry remained the same at 1x and 2x Pulsar40 treatment rates. The yield of the CLP3 (ImiSun) isohybrid was significantly lower at the 2x Pulsar40 treatment rate than at the 1x Pulsar40 rate (Figure 4).

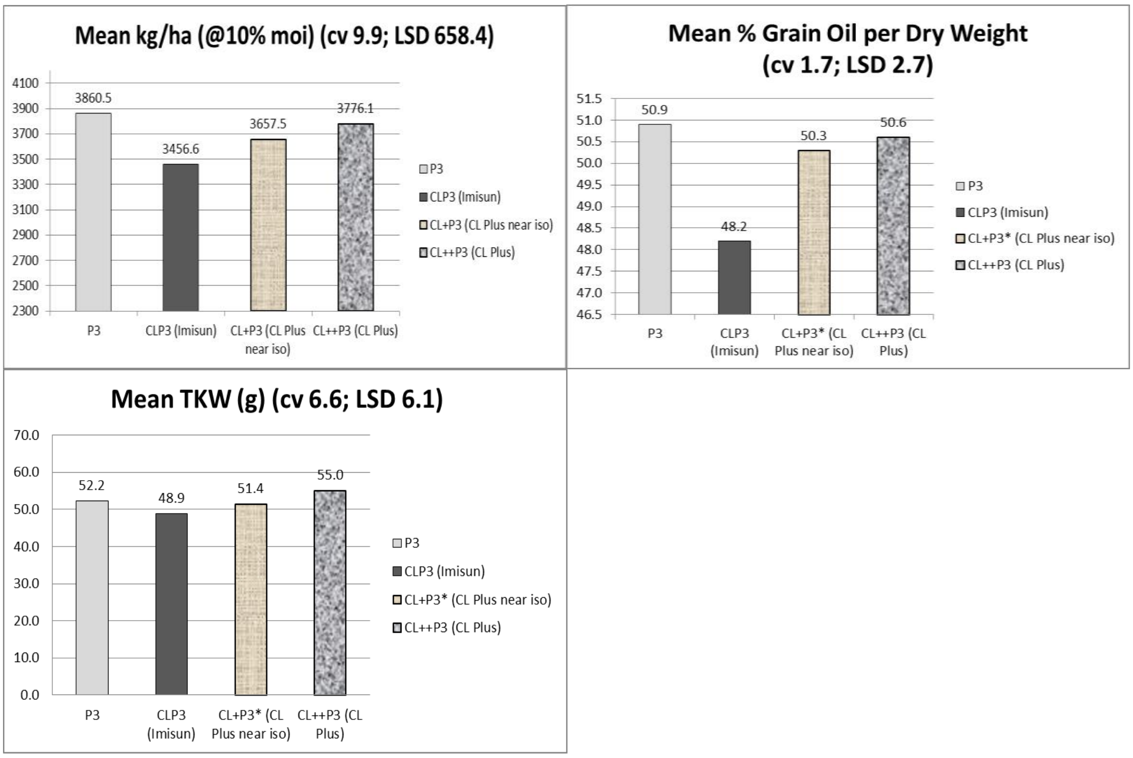


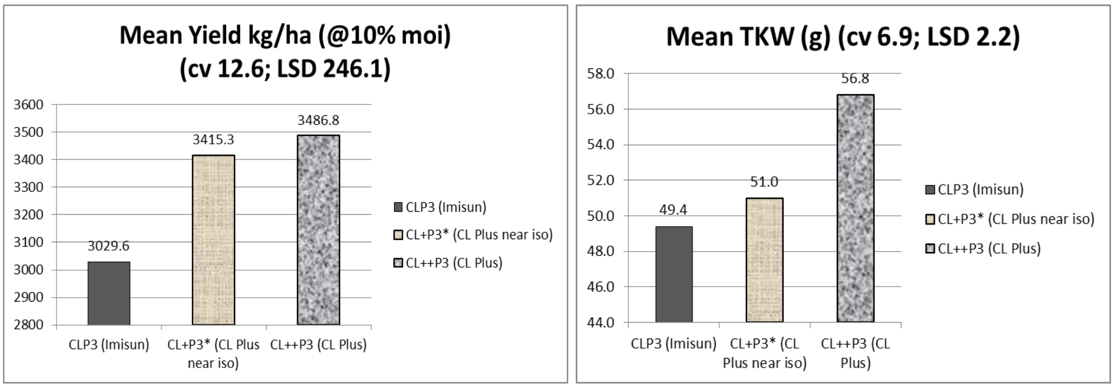
Figure 3: Yield (kg/ha), % Grain Oil and Thousand Kernel Weight (TKW) of a Conventional (P3) Isohybrid, a ImiSun (CLP3) Isohybrid, a CLPlus near-isogenic (CL+P3*) Hybrid and a CLPlus (CL++P3) Isohybrid from Untreated Field Trials; Mean of 3 EU Locations in 2011

Table1: % Grain Oil of an ImiSun (CLP3) Isohybrid, a CLPlus near-isogenic (CL+P3*) Hybrid and a CLPlus (CL++P3) Isohybrid when treated with 1x and 2x Pulsar40; Mean of 3 EU Locations in 2011

CL++P3	CL+P3*	CLP3	*Significance*	Herbicide Treatments
50.6	50.1	48.4	***A***	1x Pulsar (1.25 L/ha)
49.9	49.7	47.5	***A***	2x Pulsar (2.5 L/ha)
AB	*B*	*C*	***Significance***	***CV = 2.0 LSD =0.9***

CV = coefficient of variation; LSD = least significant difference

Treatment with 1 x Rate of Pulsar40:



Treatment with 2 x Rate of Pulsar40:

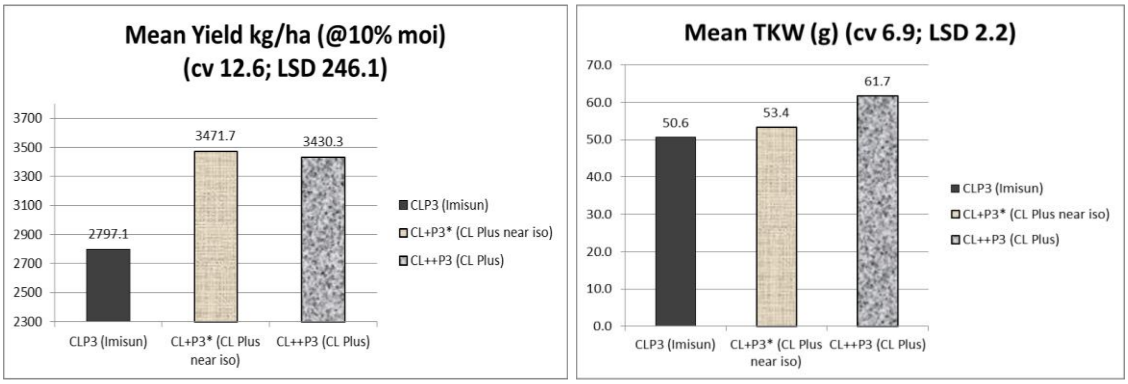


Figure 4: Yield (kg/ha) and Thousand Kernel Weight (TKW) of a ImiSun (CLP3) Isohybrid, a CLPlus near-isogenic (CL+P3*) Hybrid and CLPlus (CL++P3) Isohybrid when treated with 1x and 2x Pulsar40; Mean of 3 EU Locations in 2011

An ImiSun hybrid, CLP3*, isogenic to the CL+P3* CLPlus hetero hybrid (*Ahasl1-1* /*Ahasl1-3* heterozygous) was field tested in Argentina for 2 years at 20 locations. Two Clearfield ImiSun checks, Paraiso102CL (P102) and Paraiso 103CL (P103), were also included at each trial location. The harvested grain from all entries was analyzed for % grain oil. The environmental index for oil content was determined by averaging the oil contents of the two Clearfield ImiSun check varieties, P102 and P103, at each given location. The mean % grain oil for CLP3* and CL+P3* at each location was plotted on a graph versus the average of the two checks (Figure 5).

From this multilocation analysis (Figure 5), we observed that environments which support higher oil contents show lower differences between the CLPlus (CL+P3*) and the ImiSun (CLP3*) isohybrids than environments which support lower oil contents. For environments which generally produce lower oil contents, the CLPlus isohybrid produced on average 1% - 2% higher oil content than the CLP3* ImiSun isohybrid.

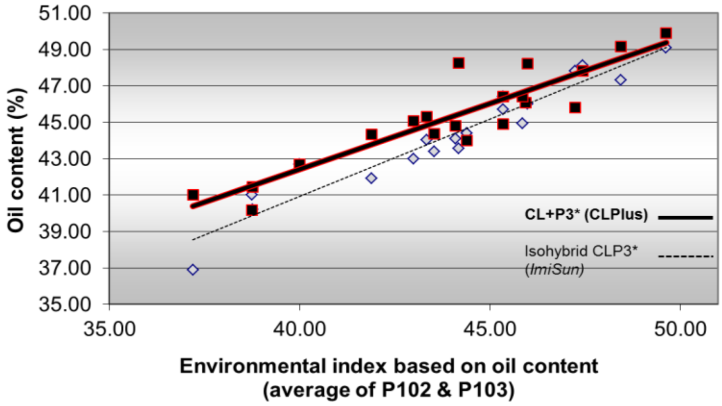


Figure 5: % Grain Oil Content of a CLPlus Hybrid (CL+P3*) in Relation to it's ImiSun Isohybrid (CLP3*) as a Function of the Environmental Index for Oil Content over 20 Locations and 2 Years in Argentina

DISCUSSION

Preliminary data gathered on a few sets of isogenic hybrids indicate that there is an increase in % grain oil, an increase in thousand kernel weight and an increase in yield of the CLPlus isohybrid (*CLHA-Plus* homozygous) over the ImiSun hybrid (*Ahasl1-1* homozygous), especially in environments which are more stressed. This data set is by far not complete and requires a number of additional years of field testing in different regions of the world. More importantly, additional genetic backgrounds from different sources need to be used to produce isogenic hybrids of conventional, ImiSun, and CLPlus hybrids to complement our current understanding of this novel trait. To date, conventional and CLPlus isogenic hybrids have been developed for 3 different germplasm backgrounds. All of these backgrounds showed no significant difference in agronomic parameters, including yield, and no significant differences in the thousand kernel weight of the grain and the % grain oil between conventional and CLPlus. This is in line with our hypothesis that the absence of wild sunflower sequences surrounding the *Ahasl*1 gene in CLPlus or Clearfield Plus hybrids (homozygous *Ahasl1-3*) results in no penalties on grain oil content and kernel size. Reduced grain oil and reduced kernel size are two QTLs which have been mapped to the wild sunflower genetic regions linked to the *Ahasl*1 gene in many ImiSun based hybrids (Trucillo et al. 2010).

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