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RESEARCH ARTICLE

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Genotype by environment interaction in sunflower (*Helianthus annus L.*) to optimize trial network efficiency

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Abstract

Modeling genotype by environment interaction (GEI) is one of the most challenging aspects of plant breeding programs. The use of efficient trial networks is an effective way to evaluate GEI to define selection strategies. Furthermore, the experimental design and the number of locations, replications, and years are crucial aspects of multi-environment trial (MET) network optimization. The objective of this study was to evaluate the efficiency and performance of a MET network of sunflower (*Helianthus annuus L.*). Specifically, we evaluated GEI in the network by delineating mega-environments, estimating genotypic stability and identifying relevant environmental covariates. Additionally, we optimized the network by comparing experimental design efficiencies. We used the National Evaluation Network of Sunflower Cultivars of Uruguay (NENSU) in a period of 20 years. MET plot yield and flowering time information was used to evaluate GEI. Additionally, meteorological information was studied for each sunflower physiological stage. An optimal network under these conditions should have three replications, two years of evaluation and at least three locations. The use of incomplete randomized block experimental design showed reasonable performance. Three mega-environments were defined, explained mainly by different management of sowing dates. Late sowings dates had the worst performance in grain yield and oil production, associated with higher temperatures before anthesis and fewer days allocated to grain filling. The optimization of MET networks through the analysis of the experimental design efficiency, the presence of GEI, and appropriate management strategies have a positive impact on the expression of yield potential and selection of superior cultivars.

Additional keywords: genotype by environment interaction; multi-environment trials; sunflower; network efficiency; yield stability.

Abbreviations used: CPD (critical percentage difference); GEI (genotype by environment interaction); GGE (genotype plus genotype by environment); LE (La Estanzuela); MET (multi-environment trial); NENSU (National evaluation network of sunflower cultivars of Uruguay); PLS (partial least squares); RCBD (randomized complete block design); YG (Young).

Authors' contributions: Designed the study, developed the methodology, performed the analysis, and wrote the manuscript: PGB and LG. Designed the experiments, collected the data and collaborated with the final drafting of the manuscript: MC, OP and DV.

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Introduction

Sunflower (*Helianthus annuus L.*) is one of the most important oil crops in the world (Fernandez-Martinez *et al.*, 2010). It is a relevant crop in agricultural rotations due to its short growing cycle, which provides more flexibility in planting dates and resistance to water stress periods (Zegada-Lizarazu & Monti, 2011). These attributes contribute to the sustainability of agricultural systems by increasing their functional diversity and breaking disease cycles (Hanson *et al.*, 2007). Even so, there is a gap between the yields obtained by farmers and those obtained in experimental stations using the best management practices (Fischer & Edmeades, 2010). On the other hand, the characterization of genotype by environment interaction (GEI) is necessary to understand the adaptation of cultivars and identification of superior cultivars (van Eeuwijk *et al.*, 1996; de León *et al.*, 2016; Lado *et al.*, 2016). The study of evaluation trials networks efficiency and the determination of relevant management variables that limit yield expression in sunflower are crucial to improve the selection efficiency of superior cultivars (de la Vega *et al.*, 2001).

Plant breeding programs require evaluation of new cultivars in experiments designed for a certain number of locations and years. This system has been defined as multi-environment trial (MET), where each environment refers to a particular combination of location and year (Smith *et al.*, 2001). The use of METs allows the analysis of performance of cultivars and the identification of genotypes with the best adaptation to a wide range of environments (Cooper & Byth, 1996). However, there are cultivars that have a specific adaptation to a certain environment, leading to changes in the ranking of means performance across environments, indicating the presence of GEI (Cooper & DeLacy, 1994). The MET's efficiency can be approached from two perspectives: 1) a global perspective associated to precise predictions of cultivar performance across a group of environments, and 2) a local perspective associated to precise predictions for defined regions or environments that require specifically adapted cultivars (Ceretta & van Eeuwijk, 2008).

An approach called Critical Percentage Difference (CPD) was proposed by Patterson *et al.* (1977) to indicate the efficiency of evaluation networks in terms of accuracy to detect differences among cultivars. This is from a global perspective using variance components of the GEI to estimate the network efficiency depending on the number of years, locations and replications *per* trial. Many studies have used this approach with positive results in terms of selection efficiency (Talbot, 1984; Ceretta & van Eeuwijk, 2008). The CPD is defined as the difference between a candidate cultivar and a check (expressed as a percentage of the general mean). To understand and diagnose the current utility of the National Evaluation Network of Sunflower Cultivars of Uruguay (NENSU), it is necessary to evaluate the efficiency of the network and study the variance components for determining the effects that have greater weight in the GEI.

The GEI can also be studied through multiplicative models like additive main-effects and multiplicative (AMMI) and genotype plus genotype by environment interaction (GGE) that combine the variance analysis with principal components analysis, and can be represented graphically by biplots (Gabriel, 1971; Yan & Kang, 2003). Yan *et al.* (2001) used the GGE biplots, where the genotype and GE interaction effects are two sources of relevant variability and are taken into account simultaneously at the time of selection of superior cultivars. The multiplicative models are useful when studying the GEI but do not allow the incorporation of environmental covariates. Another approach used to study the GEI has been the use of stability index, including Finlay-Wilkinson regression models proposed by Finlay & Wilkinson (1963). This index, that determines the stability of different cultivars through various evaluation environments, has shown successful results in several species, including sunflower (Sadras *et al.*, 2009).

Characteristics such as grain yield and oil content in sunflower are complex and are determined by genetic, environmental and genotype by environment interactions (Leon *et al.*, 2003). The number of grains per head, the weight per grain and grain oil content explains the determination of oil content per plant. Despite the fact that these components are strongly associated to genetic factors (Connor & Hall, 1997), environmental factors can also significantly affect these oil yield components (Bange *et al.*, 1997). Specifically, late sowing dates have been reported as the most influential factor in reducing the oil content and grain yield in sunflower (Beard & Geng, 1982; Bange *et al.*, 1997; Izquierdo *et al.*, 2009). Late sowing dates are related to higher temperatures during the growing season, which generate excessive growth of stems (Beard & Geng, 1982) and a reduction of time destined to flowering (Andrade, 1995). Exposure to higher temperatures and solar radiation negatively affect the grain filling phase (Andrade, 1995; Bange *et al.*, 1997; de la Vega *et al.*, 2001; de la Vega & Hall, 2002). In particular, the grain filling phase has been reported to be highly variable between years, locations and sowing dates due to the variations of temperature and solar radiation (Izquierdo *et al.*, 2009). How to design a good MET network to characterize and evaluate genotypic performance under strategic genotype by environment interaction is therefore challenging. The study of site-specific adaptation with a broader understanding of the performance limiting factors could contribute positively to sunflower competitiveness with other crops, making it more attractive for inclusion in agricultural rotations to improve long-term sustainability.

To evaluate the efficiency of the current NENSU and to study the factors that limit the expression of sunflower yield potential in Uruguay, information of the NENSU during the 1991 to 2009 period was analyzed. The objectives of this study were: (i) to quantify and analyze the GEI in sunflower, (ii) to discuss the reliability of the current NENSU, (iii) to evaluate stability of the superior cultivars to each environment and (iv) to identify environmental and management factors with higher incidence in determining yield potential of sunflower.

Material and methods

Field experiments

The NENSU has official records of 324 cultivars that were evaluated over a period of 20 years (1991-2010).

Each individual cultivar was evaluated between two to six years, but check cultivars were recurrently evaluated in a larger number of years. The NENSU is represented by two locations of evaluation, La Estanzuela (LE, latitude: 34°20'S, longitude: 57°41'W) and Young (YG, latitude: 32°42'S, longitude: 57°37'W) with two sowing dates in spring, early (1) and late (2).

The number of cultivars evaluated per year (diagonal) and the number of cultivars in common between years are shown in Table 1. Due to unbalanced data and the differences between the cultivars evaluated in the period 1991-1998 compared to the period 2002-2009, the results were analyzed separately for each period. Cultivars with at least three years of evaluation in the network were included in the analysis, resulting in 111 cultivars in the 1990's period and 11 cultivars in the 2000's period.

An alpha-lattice (resolvable incomplete blocks) experimental design with three replications was used, according to the protocol of the National Seed Council (INASE), Uruguay (www.inase.org.uy). The target population was 47,600 plants/ha located on plots of 7 m long with 2 rows separated by 0.7 m. Experiments were fertilized differently from year to year with non-limiting amounts of NPK. Also, pre and post emergent herbicides were routinely applied for weed control and insecticide when necessary.

Grain yield (kg/ha) corrected by moisture at 11% was recorded. The oil content (%) was determined by using a nuclear magnetic resonance spectrometry calibrated with a primary standard Soxhlet method (Jambunathan *et al.*, 1985). Oil yield (kg/ha) was calculated using the oil content (%) and yield. The number of days from plant emergence to anthesis (R5.5) and from R5.5 to maturity (R9) was obtained using phenological growth stages (Schneiter & Miller, 1981). Plant height (m) was evaluated at R9, from the base of the plant to the curvature of the stem. Vegetative cycle indicates the period in days between emergence and flowering time, while reproductive cycle covers the period from flowering time to harvest. An agronomic characterization of all environments is shown in Table S1 [suppl].

Climatic characterization of evaluation environments

The GRAS service of the National Institute of Agricultural Research of Uruguay (INIA) provided the meteorological information used for climatic characterization of the environments evaluated in the 2000's period. This information covers the period from 2003 to 2009, for both locations (LE and YG) and planting dates (1 and 2). The meteorological variables used were minimum, mean and maximum average temperatures and rainfall recorded daily. The phases evaluated were: pre-anthesis (from emergence to anthesis (R5.5)), anthesis (15 days ± R5.5) and post-anthesis (from the end of R5.5 to harvest). A singular

Table 1. Total number of shared cultivars for among years in the period 1991-2011 in the MET´s of sunflower (NENSU). The darker the shade of grey in the diagonal the larger the number of materials evaluated for that year. The darker the shade of grey for the off-diagonal, the larger the number of materials in common between the years.

	1991	1992	1993	1994	1995	1996	1997	1998	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011
1991	**82**																			
1992	54	**76**																		
1993	41	54	**82**																	
1994	32	42	59	**106**																
1995	24	32	44	71	**111**															
1996	21	29	39	53	79	**106**														
1997	9	15	19	28	42	51	**93**													
1998	5	9	16	25	34	39	50	**100**												
2000	0	1	0	1	1	1	3	11	**48**											
2001	0	1	0	1	1	1	1	3	11	**23**										
2002	0	0	0	1	1	1	1	1	2	7	**25**									
2003	0	1	0	1	2	2	2	4	5	4	7	**56**								
2004	0	1	0	1	2	2	3	4	2	2	2	28	**74**							
2005	0	0	0	0	1	1	1	2	1	1	1	4	12	**44**						
2006	0	0	0	0	1	1	1	2	0	0	1	1	2	17	**68**					
2007	0	0	0	0	0	0	0	1	1	0	0	1	1	5	11	**37**				
2008	0	0	0	0	0	0	0	1	2	1	1	2	2	1	1	9	**46**			
2009	0	0	0	0	1	1	0	1	0	0	0	2	2	1	3	4	17	**55**		
2010	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2	10	**25**	
2011	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	2	3	0	**12**

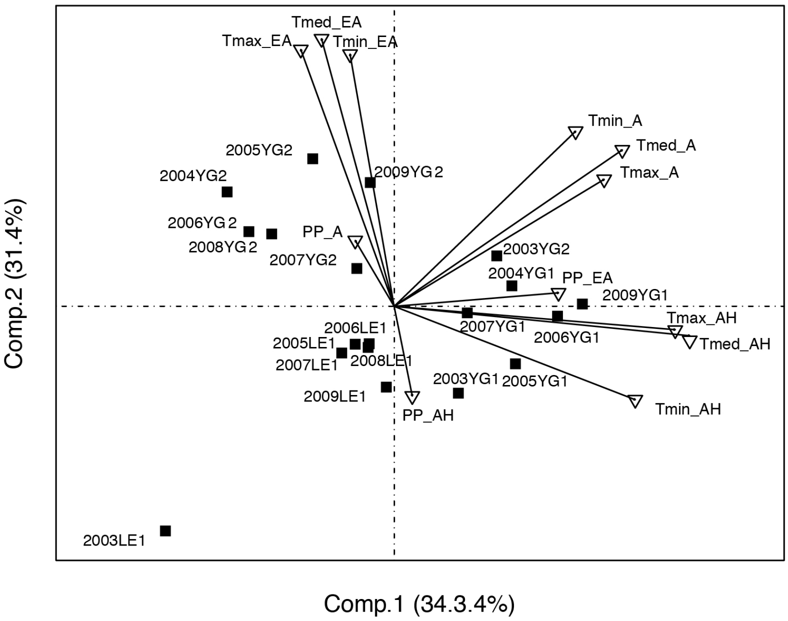

Figure 1. Biplot analysis for environmental characterization using the meteorological variables in pre-anthesis (EA), anthesis (A), and post-anthesis (AH) in all evaluation environments for the period 2003-2009.

value decomposition of the square Euclidean distance matrix of all environmental variables was used to create a biplot (Gabriel, 1971) (Fig. 1).

Experimental design efficiency

Spatial plot information (row and column position) for all replications, incomplete blocks and location in all 2000's period experiments were used to compare different experimental design efficiency through post-blocking. The three experimental designs most commonly used in cultivar evaluation were compared: randomized complete block design (RCBD), incomplete block design (IBD) and row-column design (RC). To determine the best fitting model, the AIC criterion was used. Comparisons were carried out for all environments in the 2000's period and implemented in PROC MIXED procedure of SAS (SAS Inst., 2011). The following models were used for each experimental design:

RCBD $\underline{Y_{ij}} = \mu + G_i + \beta_j + \underline{\varepsilon_{ij}}$

IBD $\underline{Y_{ijk}} = \mu + G_i + \beta_j + \underline{\gamma_{k(j)}} + \underline{\varepsilon_{ij}}$

RC $\underline{Y_{ijkl}} = \mu + G_i + \beta_j + \underline{\alpha_{k(j)}} + \underline{\delta_{l(j)}} + \underline{\varepsilon_{ijkl}}$

where μ is the overall mean, G_i is the effect of the i-th cultivar, β_j is the effect of the j-th complete block or replication, $\gamma_{k(j)}$ is the effect of the k-th incomplete block in j-th replication, $\alpha_{k(j)}$ is the effect of the k-th row in the j-th replication, $\delta_{l(j)}$ is the effect of the l-th column in the t-th replication and ε_{ij}, ε_{ijk} and ε_{ijkl} are the experimental errors for each model with $\gamma_{k(j)} \sim N(0,\sigma^2_S)$, $\alpha_{k(j)} \sim N(0,\sigma^2_R)$, $\delta_{l(j)} \sim N(0,\sigma^2_C)$ and ε_{ij}, ε_{ijk} and $\varepsilon_{ijkl} \sim N(0,\sigma^2_e)$.

Net efficiency through variance components

Yield variance components were estimated using a random effect model with information from each experiment for the 1990´s and 2000´s periods. The model used was:

$$\underline{Y_{ijklm}} = \underline{\mu} + \underline{Y_i} + \underline{L_j} + \underline{YL_{ij}} + \underline{G_k} + \underline{YG_{ik}} + \\ + \underline{LG_{jk}} + \underline{YLG_{ijk}} + \underline{\beta_{l(ij)}} + \underline{\gamma_{m(ijl)}} + \underline{\varepsilon_{ijklm}}$$

where μ is the overall mean, Y_i is the main effect of the i-th year, L_j is the main effect of the j-th environment, YL_{ij} is the year and location interaction effect, G_k is the effect of the k-th cultivar, YG_{ik} is the year and cultivar interaction effect, LG_{jk} is the location and cultivar interaction effect, YLG_{ijk} is the year, location and cultivar interaction effect, $\beta_{l(ij)}$ is the effect of the l-th complete block or replication within the ij-th environment, $\gamma_{m(ijl)}$ is the effect of the m-th incomplete block within the ij-th environment and l-th replication, and ε_{ijklm} is the residual error. All variance components were estimated by restricted maximum likelihood (REML), using PROC MIXED of SAS (SAS Inst., 2011).

Critical percentage difference (CPD)

The CPD for the NENSU was calculated by evaluating the effects of the number of years, locations and replications, where a lower value of CPD indicates greater efficiency to detect differences between means (Patterson *et al.*, 1977). The CPD was calculated as follows:

$$CPD(\%) = \frac{Z_{(\alpha)} 100 \sqrt{2V}}{\mu}$$

where $z_{(\alpha)}$ is the value to which the standard normal variable (Z) should be exceeded with α probability, μ is the yield overall mean and V is half of the variance of the difference between means of cultivars and was calculated as follows:

$$V = \frac{\sigma^2_{YG}}{n_Y} + \frac{\sigma^2_{LG}}{n_L} + \frac{\sigma^2_{YLG}}{n * n_Y} + \frac{\sigma^2_B}{n_B} + \frac{\sigma^2_I}{n_I} + \frac{\sigma^2_\varepsilon}{n_Y * n_L * n_B * n_I}$$

where n_Y, n_L, n_B and n_I, are the number of years, locations, incomplete blocks and replications, respectively.

Genotype by environment interaction analysis

The correlation between environments in years was studied through biplots (Gabriel, 1971; Yan & Kang, 2003) with the standardized mean of cultivars for each environment. This methodology is known as GGE biplot analysis and use the following multiplicative model:

$$Y_{ij} = \mu + \beta_j + \sum_{m=1}^{M} \lambda_m \gamma_{mi} \, \delta_{mj} + \rho_{ij}$$

where, Y_{ij} is the standardized mean yield of the i-th cultivar in the j-th environment, μ is the overall mean, β_j is the effect of the j-th environment, λ_m inertia or variance explained (eigenvalue) by the m-th axis, γ_{mi} and δ_{mj} are the projections of the cultivars and the environments in the m-th axis and ρ_{ij} is the residual. These analyses were performed to study the GEI in grain yield on all cultivars and the 11 environments selected in the 2000's period. Multiplicative models for these analyses were implemented using SAS (SAS Inst., 2011) software, reference to the codes developed by the CIMMYT's group for this analysis (Vargas & Crossa, 2000). Graphical representations of the GGE biplots were implemented through software R.

PLS regression

PLS regression method relates matrices X and Y through a multivariate linear model (Wold *et al.*, 2001), allowing to relate the responses of several cultivars with that of several environmental predictor variables. The response matrix of cultivars was performed with grain yields of the 11 cultivars described above and 11 evaluation environments periods from 2003 to 2006. Environmental predictor variables were grouped into two groups, one associated with crop phenological variables and another group depending on climatic variables during the growing cycle.

Finlay-Wilkinson regression analysis

Finlay-Wilkinson regressions were used to analyze stability of cultivars through different environments of evaluation (Finlay & Wilkinson, 1963). This technique involves the calculation and comparison of regression slopes for each cultivar, using the average yield of each environment in the adjustment of each regression line. Cultivars with a slope close to 1 and a high average yield indicate that cultivars are adapted to all evaluation environments. In contrast, slopes significantly lower or higher than 1 indicate that these cultivars show differential adaptation to particular environments. For this analysis, 11 cultivars and 11

Table 2. Agronomic characterization of 11 cultivars selected for the period 2003-2009

Cultivar	Grain yield (kg/ha)	Oil yield (kg/ha)	Oil content (%)	Height (m)	Emergence to anthesis (days)	Anthesis to harvest (days)
Agrobel 972	2298.7	1061.1	46.16	1.47	59.3	64.2
Dekasol 3810	2207.8	1054.7	47.77	1.40	61.3	66.2
Dekasol 4040	2412.2	1048.3	43.46	1.38	65.4	63.5
Dekasol 4050	2182.1	1000.7	45.86	1.52	66.7	64.6
INIA Butiá	1988.2	889.7	44.75	1.77	67.5	61.0
Macon RM	2345.1	1116.5	47.61	1.46	61.0	66.4
MG 52	2300.4	1072.4	46.62	1.69	65.2	63.3
NK 55 RM	2162.6	994.4	45.98	1.54	64.3	61.3
Pannar Pan 7031	2284.7	1064.0	46.57	1.62	65.4	65.4
Pannar Pan 7034	2097.7	946.7	45.14	1.61	65.4	61.3
Pannar Pan 7355	2579.2	1099.3	42.62	1.56	64.6	63.8

Table 3. Post-blocking model comparison assuming a randomized complete block design (RCBD), incomplete resoluble block design (IBD) and a row-column design (RC). The best adjustment was selected using the AIC criterion for each environment during the period 2003-2008. The best model for each environment is underlined.

Environment	Number of cultivars	Model		
		RCBD	IBD	RC
2003LE1	8	273.4	243.6	245.3
2003YG1	8	279.9	247.2	257.2
2003YG2	8	246.7	214.5	215.5
2004YG1	8	238.9	201.6	200.9
2004YG2	8	224.9	201.6	200.9
2005LE1	9	298.2	259.3	258.3
2005YG1	9	273.4	241.2	243.2
2005YG2	9	266.9	240.3	242.2
2006LE1	6	186.0	158.2	157.2
2006YG1	6	194.7	165.6	166.1
2006YG2	6	186.6	156.4	157.2
2007LE1	4	130.1	100.5	102.5
2007YG1	4	129.0	99.2	100.7
2007YG2	4	120.1	91.1	92.0
2008LE1	3	100.4	73.4	75.1
2008YG2	3	75.8	48.9	48.8

environments were used during the years 2003 to 2006. Analysis were implemented in GenStat (IBP Breeding View, 2015).

Results

Agronomic characteristics of evaluated cultivars

The grain yield and oil content yield means of the eleven cultivars evaluated in this period were 2260 and 1031 kg/ha, respectively (Table 2). ‘INIA Butia’ showed the lowest grain yield mean (1988 kg/ha) and ‘Pannar Pan 7355’ the highest (2579 kg/ha). Oil kernel concentrations showed less variability, with an average of 45.7%. ‘Pannar Pan 7355’ was the cultivar with lowest concentration of oil (42.6%), while ‘Dekasol 3810’ showed higher values with 47.8% (Table 2). The average plant height of the cultivars was 1.55 m, with differences of approximately 0.4 m between the extreme cultivars (Table 2). The durations of vegetative and reproductive cycles were less variable among cultivars, targeting an average of 60 days for each phase (Table 3).

Experimental design efficiency

The efficiency of the experimental designs used in MET's was evaluated for all environments during the 2000’s period (Table 3). The randomized complete block design was the worst model in any of the environments (Table 3). In most environments, the model analysis that showed better results in terms of model fit was the incomplete block design. However, in some situations, the use of row-column design was the best model based on AIC criterion.

MET network efficiency

Variance components

In the 1990's period the largest proportion of the variability was attributed to the location effect (29.0%), followed by the location by year interaction (16.9%) (Table 4). Although the estimated cultivar variance component was low (2.6%), it was greater than the cultivar by location and cultivar by year interactions. The effects of the GEI however, accounted for a small proportion of the total variance (10.1%). The analysis of the 2000's period shows a similar behavior of the 1990 period, where a higher number of cultivars were evaluated. In the 2000 period, the component with the highest proportion of variability was attributed to the year (29%), followed by the location by year interaction (18.2%) (Table 4). In this period, the proportion attributed to GEI was also low (8.7%).

Critical percentage difference

Increasing the number of locations from one to two decreased the CPD more than 3%, while incorporating a third location decreased the CPD 1.5% (Fig. 2a). On the

Table 4. Yield variance component estimation during the 1990's and 2000's periods in all the environments. Location is defined as a combination of location and sowing time.

Variance component	1990		2000	
	Yield (kg/ha)	Total (%)	Yield (kg/ha)	Total (%)
Location	264736	29.0	125187	15.9
Year	144719	15.9	227612	29.0
Cultivar	23648	2.6	16399	2.1
Location × Year	154341	16.9	143065	18.2
Cultivar × Location	13104	1.4	29889	3.8
Cultivar × Year	16853	1.8	1574	0.2
Cultivar × Location × Year	62493	6.9	37252	4.7
Replication	14666	1.6	36341	4.6
Incomplete block	33803	3.7	20493	2.6
Residual	183829	20.2	147722	18.8
Total	912192	100.0	785534	100.0

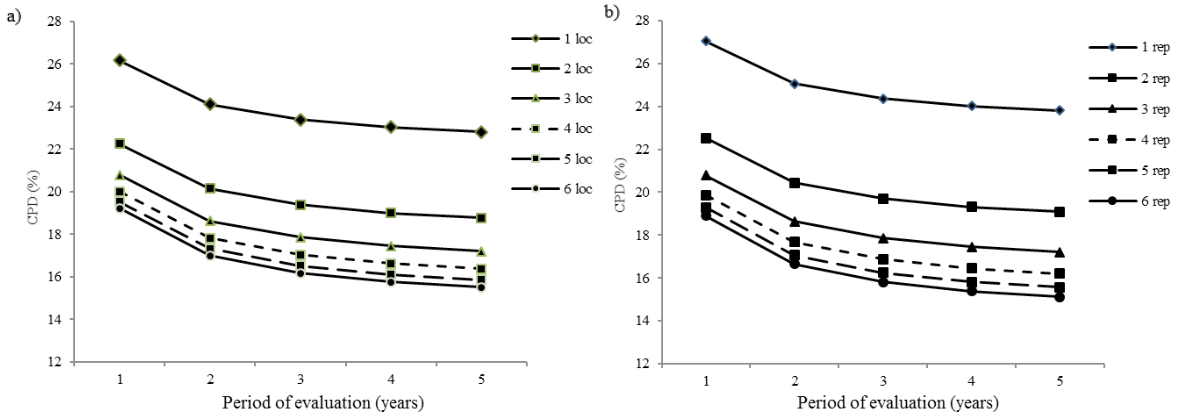

Figure 2. Effect of increasing the number of years of evaluation in terms of critical percentage difference (CPD) for different number (a) of replications per experiment in an evaluation network with three locations and (b) locations in an evaluation network with three replications.

other hand, increasing replication from one to two decreased the CPD more than 4%, while incorporating a third replication decrease the CPD 2% (Fig. 2b). Increasing from one to two years the CPD decreased 2% with no further decrease in CPD from third year of evaluation. Therefore, increasing the number of replications per trial has a larger impact than increasing the number of years (Fig. 2a *vs*. Fig. 2b).

Genotype by environment interaction

The first two principal components of the grain yield of the GGE biplot accounted 61.3% of the total variability for grain yield (Fig. 3). The ME1 included the 2005YG1, 2005YG1 and 2006LE1 environments. The winning cultivar in ME1 was 'NK 55 RM'. ME2 included all environments of 2003 and 2004, and 2006YG2. The winning cultivar in ME2 was 'Pannar Pan 7355'. The ME3 included the 2005LE1 and 2005YG2 environments and the winning cultivar was 'Dekasol 3810'. 'INIA Butia', 'Pannar Pan 7031' and 'Dekasol 4050' performed poorly in all environments.

PLS regression

Of the total variability, 80% was retained in the first two principal components for agronomic characteristics (Fig. 4a) and 71% was retained for meteorological variables (Fig. 4b). Plant height was positively correlated to number of days to flowering. Both variables were negatively associated to grain yield for all cultivars (Fig. 4). Oil yield and oil content were positively correlated with grain yield for all cultivars. All LE1 environments and 2003YG1 were associated with high yield performance and high oil yield and oil content (Fig. 4b). Furthermore, high temperatures during the whole growing cycle had a negative impact on grain yield. The YG locations on the second sowing date were the environments with highest temperature during the growing cycle.

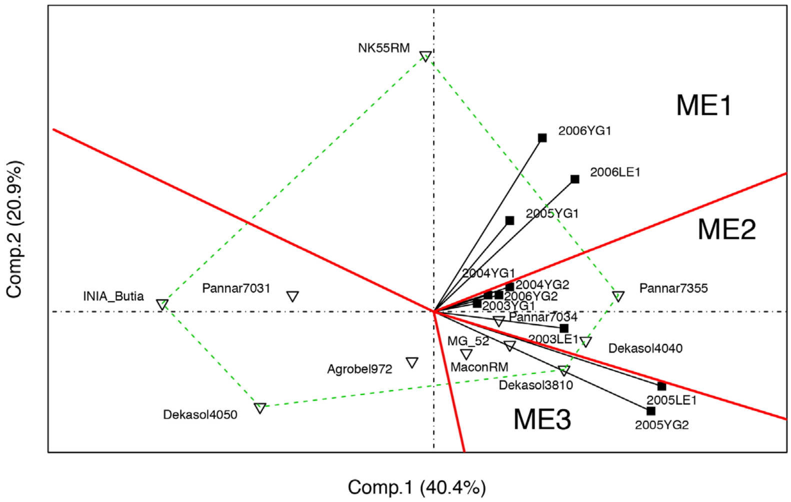

Figure 3. Genotypic main and genotype by environment interaction effects (GGE biplot) for grain yield through 11 environments of evaluation and 11 cultivars evaluated during the period 2003-2006. The environments are represented by squares and cultivars by triangles. The proportion of explained variance of each component (as a percentage of the total variability) is shown.

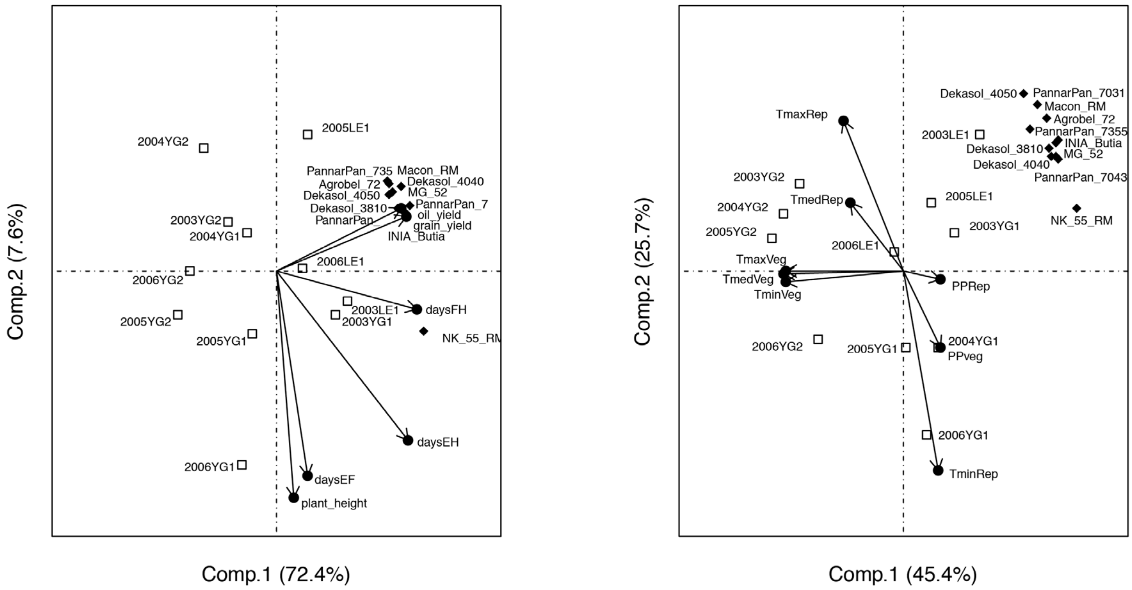

Figure 4. Biplot analysis for agronomic (a) and environmental variables (b) used in PLS regression analysis during the period 2003-2006 for all environments of evaluation. The proportion of explained variance of each component (as a percentage of the total variability) is shown.

Finlay Wilkinson regression analysis

Cultivars have shown differences in terms of grain yield stability (Fig. 5). Cultivars ‘Agrobel 972’ (s=1.0189) and ‘Dekasol 4050’ (s= 0.9882) were the most stable for type II stability. Cultivars with higher slopes as ‘Dekasol 4040’ or ‘Macon RM’ were able to exploit favorable conditions of the best environment, while the cultivars ‘INIA Butia’ and ‘NK 55 RM’ showed lower stability and poor average performance in all environments.

Discussion

MET network efficiency

The estimation of variance components is an efficient tool to quantify the relative magnitude of the genotype, genotype by environment interaction effects and to predict response to selection (Cooper & Delacy, 1994). The variance components proportion due to the GEI represented 10.1% and 8.7% for the 1990's

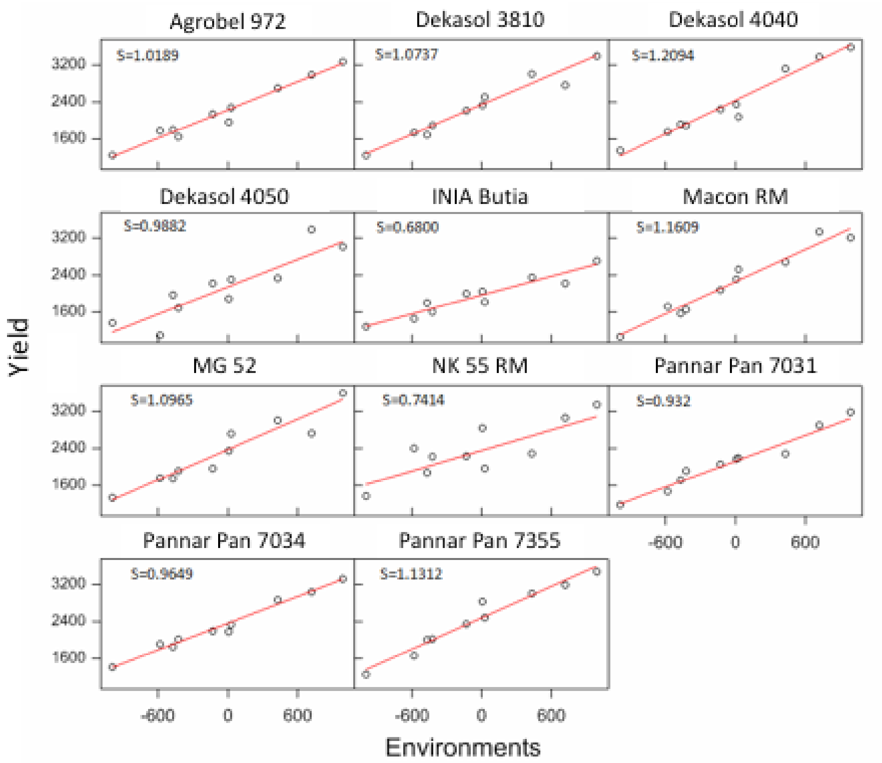

Figure 5. Finlay-Wilkinson regression for the 11 cultivars and 11 environments evaluated during the period 2003-2006. The Finlay-Wilkinson regression coefficient (slope) is indicated in the upper left corner of the graph for each cultivar.

and 2000's periods respectively (Table 4). In both situations, the magnitude of the GEI is larger than the cultivar effect, indicating the presence of a strong GEI in the NENSU. Similar results have been reported in sunflower (de la Vega *et al.*, 2007) as in other crops (*i.e.* rice, Cooper *et al.*, 1999; sorghum, Chapman *et al.*, 2000; corn, Chapman *et al.*, 1997; and barley, Ceretta & van Eeuwijk, 2008). In this study, the largest component was the cultivar by location by year effect with similar proportions in both periods; however, the greatest differences in both periods were found in the interaction location by cultivar (1.4% in 1990's *vs*. 3.8% in 2000's). The largest proportion of the total variability was explained by the location in 1990's (29.0%), while it was significantly lower (15.9%) in the 2000's period. Differences in the NENSU management during both periods could explain this difference; in the 1990s one location more was used in comparison with the 2000s period (YG2), and more cultivars were evaluated.

The evaluation of the NENSU efficiency in terms of the selection of superior cultivars using CPD indicates the relevance of the analysis of number of years, locations and replications to optimize MET networks. This result is consistent with other studies that have used the CPD to evaluate METs network efficiency (Cullis *et al.*, 1996; Ceretta & van Eeuwijk, 2008; Arief *et al.*, 2015). The factor that showed the strongest impact on the CPD indicator in our study was the number of locations, followed by the number of replications in each experiment (Fig. 2a,b). There is a gain in CPD by using up to three replications (*i.e.*, smaller CPD values), but no gain is obtained afterwards. Using more than one location is critical, and there is a gain in CPD by using a third location, but no gain after that. The use of a larger number of locations can be compensated with a reduction of the number of years of evaluation, showing that after the second year of evaluation no significant gains were obtained in terms of efficiency. Our results indicate that the best strategy to use when defining the NENSU is that it should have three replications per trial, at least three locations and two years of evaluation. If the conditions to expand the network are generated, efforts should be focused on increasing the number of locations and not the number of years or replications (Annicchiarico, 2002).

The RCBD had the worst model fit for all environments (Table 3). Generally, the RCBD is less efficient when compared with models that control the spatial variability (Patterson *et al.*, 1977; Yau, 1997; Qiao *et al.*, 2000). In the context of MET programs where it is necessary to evaluate a large number of treatments, the use of experimental designs such as alpha-designs and row-column design have proven to be efficient in estimating mean treatments effects (Williams *et al.*, 2002). Most scenarios presented a better model fit when they were analyzed under the experimental design used at the field level, the incomplete blocks design. However, better model fits were obtained using a RC design in certain environments. Piepho *et al.* (2006) showed good performance by the use of alpha design in the context of cultivar evaluation networks. In this sense, the use of IBD in current operation of the NENSU would be considered. It is relevant to obtain spatial information of all the plots evaluated in the experiments. The use of model analysis that incorporates spatial information through different adjustments could be beneficial, capturing the existing spatial variability (Qiao *et al.*, 2000; Piepho *et al.*, 2006).

GGE biplot analysis

Rose *et al.* (2008) compared the use of GGE biplot with nonparametric methods in a context of low number of cultivars and determined that the GGE was more efficient in the estimation of genotypic stability. The first two main components accumulated 61.3% of the total phenotypic variability. Specifically, three mega-environments were defined which grouped two environments in one of them, three in the second, and the remaining environments on the other. The 2005YG1, 2006LE1 and 2006YG1 environments, and the cultivar 'NK 55 RM' being the winning cultivar, defined the first mega-environment (ME1). The winning cultivar in the ME2 was 'Pannar Pan 7355', while in ME3 it was 'Dekasol 3810'. The mega-environments were defined by management practices such as sowing date and differences between years, mainly explained by meteorological conditions, rainfall and temperature, in flowering time. Our results coincide with other studies that analyzed the conformation of mega-environments in sunflower (de la Vega & Hall, 2002; Balalic *et al.*, 2012).

Agronomic and climatic factors

While the study of the GEI has great relevance in the process of genetic improvement, there is a growing interest in understanding more deeply which are the environmental and management factors affecting the determination of yield potential of different cultivars. In this sense, statistical tools have been developed such as Factorial Regression and PLS regression (van Eeuwijk *et al.*, 1996; Vargas *et al.*, 2001) in order to identify and quantify potential factors causing interactions (*i.e.* environmental covariates). The use of PLS regression, is helpful to identifying those agronomic management practices and climate information factors that have more relevance on the expression of sunflower yield performance.

In the current context of climate change, constant increases in global temperatures have been reported (Easterling *et al.*, 2000), which increases the frequency of periods of high temperatures in temperate and tropical climates. Additionally, more extreme events like storms with heavy rainfall in short periods have been reported. That is why summer crops such as sunflower could be more exposed to prolonged stress periods of heat stress that negatively affects the productive performance of cultivars (Rondanini *et al.*, 2006). Previous studies indicated that the exposure of sunflower head to temperatures above 34 °C for a period of seven consecutive days significantly reduced the grain quality and yield (Rondanini *et al.*, 2003). Our results are consistent with these authors indicating that high temperatures during the growth cycle have a negative impact on the potential yield expression, mainly the high temperatures until 15 days before anthesis (Fig. 4). In this sense, it is necessary to analyze the productive performance of different environments, where late sowing dates in Young (YG2) showed the lowest grain yields and oil content in all the years of evaluation (Table S1 [suppl]). Several authors show the negative effect of late sowing dates on the grain production (Andrade, 1995; Bange *et al.*, 1997). Yield losses are due to the reduced number of grains *per* square meter and lower oil content (%) (Andrade, 1995) explained by a reduction of the grain filling period (Hall *et al.*, 1985). While our results show that there are no significant differences in temperature between YG1 and YG2 during grain filling, the explanation of the differences in performance could be given by a shortening of both the pre-anthesis and post-anthesis phases in late sowing managements. Late sowing dates have a similar harvest moment to early sowing dates and this would explain the shorter cycles. The photoperiod is most likely the signal that cultivars receive to start the flowering and grain maturity (Craufurd & Wheeler, 2009). Therefore, regardless of sowing time, the cultivars will be harvested in relatively similar dates, making sowing date in sunflower relevant.

Another climatic factor analyzed in this work was the accumulated rainfall for different environments and cultivars during the vegetative and grain filling phases. Foucteau *et al.* (2001) studied the effect of environmental covariates in sunflower and concluded that the flowering time related to the water availability and the occurrence of high temperatures periods is crucial in determining the grain yield performance. They indicate that cultivars planted in later periods are more likely to flower in drought stress periods, penalizing the number of grains per head and grain filling cycle duration (Foucteau *et al.*, 2001). In a more recent study, Balalić *et al.* (2012)

identified that the level of rainfall and relative humidity at flowering time were the most important factors in the presence of GEI for a particular year of evaluation. On the other hand, for the remaining years the high levels of precipitation in the vegetative phase generated significant decreases in grain yield and oil concentration. Our results show that accumulated precipitations in both pre-anthesis and grain filling phases had no significant effect on yield determination.

Performance stability

The yield of a cultivar is explained by environmental factors, genetic factors and the presence of significant interactions between cultivar and environmental factors. One alternative to study this phenomenon is to evaluate the stability or phenotypic plasticity of the cultivars (Sadras *et al.*, 2009). The use of Finlay-Wilkinson regression (Finlay & Wilkinson, 1963) is one way to evaluate yield stability and group cultivars into stable, low or high response cultivars. 'INIA Butia' had the lowest yield response as expected because it has been used in NENSU as a check over the years. 'INIA Butia' had a low-level yield performance regardless of the environment. Cultivars such as 'Dekasol 4050', 'Pannar Pan 7034' and Agrobel 972' were more stable presenting an average yield across environments. These cultivars could be classified as global or with general adaptation. Finally, materials such as 'Dekasol 4040' and 'Macon RM' had high response to favorable environments and on average were cultivars with superior yield. These cultivars could be classified as of specific adaptation to certain conditions.

Our study was able to identify environmental and management factors that directly affect the NENSU in terms of network and experimental design efficiency. Early planting dates are more beneficial for sunflower performance in our conditions. No differences among locations were found in terms of ranking of cultivars for each sowing date. The adoption of any of these management practices could be contributing to the reduction of the gap existing between research trials and farmers yield performances, making it a more competitive and attractive crop.

The optimization of current MET network in the context of increments in the number of cultivars evaluated should focus on increasing the number of locations and/or sowing dates, using at least three replications and two years of evaluation. The use of incomplete block design shows acceptable performance in terms of efficiency. However, the use of spatial information of experimental units could contribute to improve network efficiency by the use of model analysis that includes such information in many situations. The three defined mega-environments were most affected by differences in sowing dates than by geographical differences. In this sense, the use of early sowing dates is preferred because they escape to periods of high temperature stress in relevant phases that determine the performance as the pre-anthesis period and increase the total growing cycle period. It is expected that the available methodological contributions like those applied in this study, be also increasingly applied on plant breeding programs and on the networks of cultivar evaluation to contribute positively in increasing the yield potential and decreasing the gap with the farmer yields.

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