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Identification of an ideal test environment for asparagus evaluation by GGE-biplot analysis

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Abstract
Asparagus is a perennial crop which remains in production for at least 10 years. Therefore, the appropriate election of cultivars is crucial for asparagus growers. The aim of this work was to determine which environment is most desirable for enhancing asparagus clonal hybrids selection. Thirty four asparagus clonal hybrids and two testers were planted in a complete randomized block design. Total yield was evaluated for each hybrid in four environments conformed by combinations between age of culture and type of production. Data were subjected to an ANOVA and broad sense heritability was calculated for each environment. GGE biplot methodology was also used. The second productive season (for blanched and green production) was the best test environment and the most powerful to discriminate genotypes. Selection in this productive season would reduce time and costs in asparagus cultivars evaluation.

Keywords: *Asparagus officinalis* L.; broad sense heritability; clonal hybrid; Multi-Environment Yield Trials; tester

Abbreviations: DF_Degree of freedom; E_Environment; G_Genotype; GE_Genotype by Environment; GEI_Genotype by Environment Interaction; MEYT_Multi-Environment Yield Trials; PC_Principal Components

Introduction

Asparagus (*Asparagus officinalis* L.) is a perennial crop which remains in production for at least 10 years. Every spring the young stems, known as spears, emerge through the ground. In asparagus commercial fields two typical types of managements are conducted: white or blanched asparagus when the stem grown under mounded soil, and green asparagus when the stem grown in raised beds. In general each country is devoted to one of the two types of production. Thus, green asparagus are commonly cultivated in the United States and Italy whereas white managment is generally used in the rest of Europe. Anyone cultivar can be used either for white asparagus or green asparagus (López Anido et al., 1999; Asprelli et al., 2005). Due to the perennial nature of this species and the unpractical cultivar replacement after the plantation has been established, an appropriate cultivar election becomes a crucial decision for the asparagus growers. An asparagus plantation must remain productive for several years to recover the initial investment and to obtain good returns. The hybrid 'UC-157 F_1' is the mostly used genotype for asparagus crops in Argentina (~ 90%). Often, the F_2 seed is harvested and sown by the growers to extend their plantations. The harvestable quality from F_2 seed is good. They obtain tight spears with a deep green color. However, this production is based on a unique variety and represents a risk from the sanitary point of view because a possible epiphyte could be quickly expanded to all production fields. The current world expansion of the asparagus production needs the development of hybrids adapted to new production areas as well as strategies of production. The asparagus biology allows the development of progenies by hybridization of selected pistillate and staminate plants. The selected parents are cloned by micropropagation and the obtained progeny is not strictly an F_1 hybrid because the parents are not lines. This type of hybrid denominated clonal hybrid is commercially well-accepted in asparagus. Development of new varieties highly productive, tolerant to the most frequent diseases and high marketable quality will be the challenge of breeders in the coming years (López Anido and Cointry, 2008). Although the evaluation period for potencial new varieties is long, several authors demonstrated that the performance of the crop during the first two years of production is highly associated to the long-term performance (Fallon and Nikoloff, 1986; Bussell et al., 1987). A suitable variety will be one that shows wide adaptation to different environments or interacts favorably with the environmental to maximize the yield. Therefore, different years, localities, types of production (blanched or geen asparagus) and age of cultivation could be considered as different environments. In this context, the interpretation of the Genotype by Environment Interaction (GEI) is decisive to define breeding strategies. GEI have been studied to determine the cultivars stability (Lin and Binns, 1988; Kang, 1993; Yan, 2001) and to group similar environments (Gauch and Zobel, 1997; Atlin et al., 2000; Trethowan et al., 2003; Yang et al., 2005).

Table 1. Analysis of variance for yield data from 36 asparagus genotypes tested across 4 environments

Source	DF	Sum of Square	Mean Square	% variability explained
E	3	1144656093	381552031***	61.75
G	35	261693703	7476963***	14.12
GE	105	356588827	3396084***	19.24
Error	216	90747865	420129	

However, relatively few researchers have studied GEI to determine the desirability of test environments. The GGE biplot methodology (Yan et al., 2000; Yan, 2001, 2002; Yan and Kang, 2003; Yan and Tinker, 2006) consists of a set of biplot interpretation methods, whereby important questions regarding genotype and test-environment evaluation can be visually addressed. Even more, plant breeders and other agronomists have found GGE biplots useful in test environment evaluation (Yan and Rajcan, 2002; Blanche and Myers, 2006; Thomason and Phillips, 2006). The aim of this work was to determine which environment of those typically concerned by asparagus breeders is most useful for genotype selection during asparagus breeding programmes.

Material and methods

Thirty four experimental clonal hybrids were obtained from the Argenteüil cultivar, an OP (*Open-pollinated*) variety obtained by mass selection. One year old crowns of these clonal hybrids were planted in 2004, together with two testers, UC-157 F_1 and Argenteüil (T1 and T2 respectively), in the Experimental Field of Rosario University, Argentina (33º1´S; 60º53´W). The climate of the region is typically Mediterranean, with mean yearly precipitation about 1,200 mm and average yearly temperature of 13.1 °C, with a minimum of 3.0 °C in July and a maximum of 28.6 °C in January. Soil reaction (pH) is neutral (6.6 - 7.3), which is suitable for asparagus. Genotypes were randomized within six replications and 20 plants per plot. The planting distances were 2.1 m between rows and 0.4 m between plants within the row. The planting depth was 20 cm. Three replications were manteined for green asparagus production, while the other three replications were used for blanched asparagus by ridging 30 cm high, with a ridging machine. The irrigation schedule was dictated by the availability of water. The area was irrigated for two o three hours every other day. This proved to be quite sufficient in this location. Fertilizing was adjusted to soil analyses. In general no more than 150 kg ha^{-1} of urea were spread between lines prior the spring sprouting. No herbicides were applied during the course of the experiment; weeding was done as deemed necessary. Total yield (in grams per plot) was evaluated for each hybrid for white and green production. Data were collected during the years 2005 and 2006 (first and second productive seasons). Productive season and type of production were combined as 'environments' for the analysis, thus each productive season - type of production combination represents a different environment as follow: E1: first productive season and blanched production. E2: second productive season and blanched production. E3: first productive season and green production. E4: second productive season and green production. Only spears with diameter higher than 12 mm for blanched asparagus and 10 mm for green asparagus were considered for yield records. The data were collected during 40 days, considering as the first day when the first spear appears in each plant. Harvests were carried out three times a week

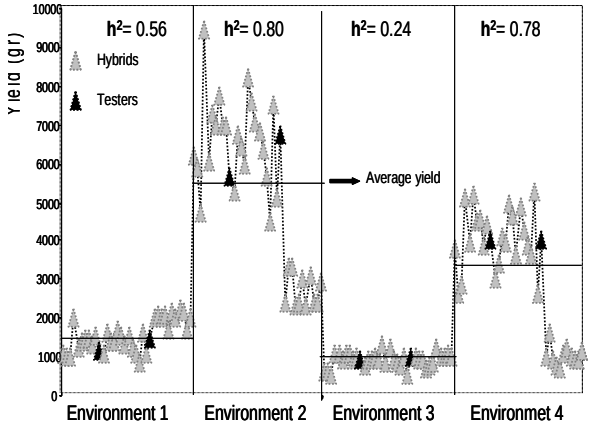

Fig 1. Mean values for yield of 34 asparagus clonal hybrids in the four tested environments and broad sense heritability in each environment.

and the spears were trimmed to a length of 15 cm before weighing. Combined analysis of variance (ANOVA) was used to determine the effects of genotype (G), environment (E) and genotype by environment interaction using the SAS software (SAS Institute, 1999). The genotypes, environments and replications were considered as random effects and in each environment broad sense heritability based on mean values were calculated from variance components following Toker (2004) and Cakmakcı et al. (2006). The GGE biplot methodology, which is composed of two concepts, the biplot concept (Gabriel, 1971) and GGE concept (Yan et al., 2000) was also used to identify the ideal test environment. The GGE biplot shows the first two principal components (PC1 and PC2, also referred as primary and secondary effects, respectively) derived from subjecting environment-centered yield data (the yield variation due to GGE) to singular value decomposition (Yan et al., 2000).

Results and discussion

The analysis of variance showed that asparagus total yield was significantly affected by environments (E) and genotypes (G), which explained 61.75 and 14.12% of the sum of squares, respectively (Table 1). Genotype x environment interaction (GEI) significantly explained 19.24% of the total variation. It is very common for Multi-Environment Yield Trials (MEYTs) data to embody a mixture of crossover and non-crossover types of GEI. Gauch and Zobel (1997) reported that E accounts for about 80% of the total variation, while G and GE each account for about 10% in normal MEYTs. Similar results were found in this study since the E effect was about three times higher than G and GE effects. On the other hand, the differential rankings of genotypes observed across test environments revealed a plausible existence of crossover GEI. The heritability value for yield per se is one of the most important parameters to select high yielding genotypes adaptable to target environments.

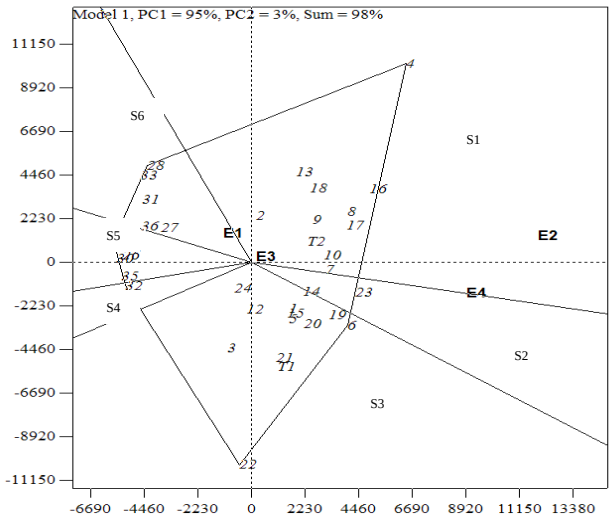

Fig 2. Polygon views of the GGE-biplot showing the mega-environments and their respective highest yielding cultivars. PC, T, E and S mean principal component, tester, environments and sectors, respectively

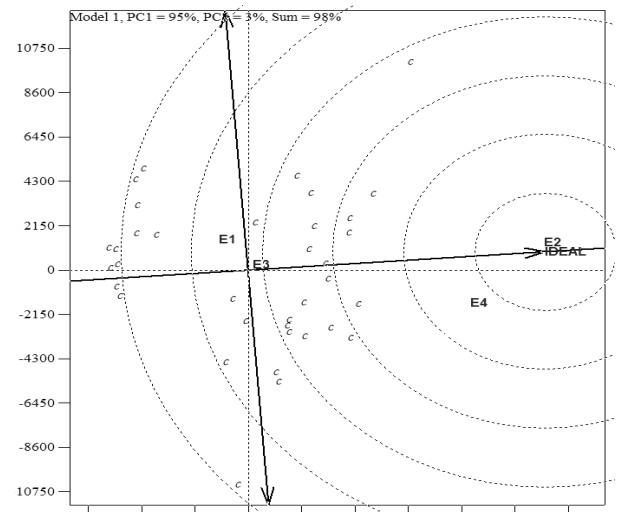

Fig 3. GGE-biplot based on environment-focused scaling for comparison the environments with the ideal environment. PC and E mean principal components and environments, respectively

Broad sense heritability values ranged between 0.24 (E3) and 0.80 (E2) (Figure 1). The heritability values reflect the variation detected among the genotypes, which will be greater when increase the environment discriminating ability. In this study, the highest heritability values were obtained for E2 (0.80) and E4 (0.78), second productive season for both blanched and green production, respectively. These values suggest that the genetic component of variance was more influenced by the plant age than by the non-genetic component. Cointry et al. (2000) and Gatti et al. (2000) demonstrated that the yield increases along years or age of cultivation, when equal harvest period in each year is considered. However, Cointry et al. (1996) established that the spears weight and diameter (principal yield components) become stabilized in the second year of harvest, expressing at this moment its maximun potential. This may explain why the first productive year was less effective and failed to discriminate among cultivars.

A high discriminating environment maximizes the observed genotypic variation among genotypes for a given trait. The efficiency and accuracy of cultivar selection for a given trait is greatly enhanced in high discriminating environments compared with non discriminating ones. The discriminating ability of an environment is comprised of a variety of factors, but the presence of GEI complicates the identification of an ideal test environment. When crossover GEI is present, it is necessary to reveal the nature of this interaction. GGE model analysis turns out very usefull to analyze this kind of interactions partitioning them into their Principal Components (PC). According to this analysis, ideal cultivars are those that should have large PC1 scores (high mean yield) and small (absolute) PC2 scores (high stability). Also, ideal test environments should have large PC1 scores (more power to discriminate genotypes in terms of the genotypic main effect) and small (absolute) PC2 scores (more representative of the overall environments) (Yan et al., 2000; Yan and Rajcan, 2002). The partitioning of GEI through GGE model analysis showed that the first two Principal Components (PC) were significant factors that explained 98% of the G and GE sums of squares. The polygon is a succinct summary of the GEI pattern of a MEYT data set (Figure 2). This polygon is formed by connecting the markers of the genotypes that are further away from the biplot origin in a way that all other genotypes are contained in the polygon. Six rays (blunt lines) in Figure 2 divide the biplot into six sectors (S1 to S6) and the environments fall into two of them. Three environments, E2, E3 and E4, fell into sector 1 (S1) and the vertex genotype for this sector was genotype 4. A single environment, E1 fell into sector 6 (S6). The vertex genotype for this sector was the genotype 28. These sectors (S1 and S6) were identified as two mega-environments.The polygon allows establishing associations between cultivars and environments; in this case, cultivar 4 show the highest yield in E2, E3 and E4, whereas genotype 28, shows better adaptation to E1. The length of an environmental vector (in pointed lines) is proportional to the standard deviation of cultivars in that particular environment. This length is an estimation of discriminating power of the environment if the experimental errors of the test environments are comparable (Yan et al., 2007). Test environments with longer vectors (see E2 in Figure 2) are more discriminating of the genotypes. If a test environment marker is close to the biplot origin (see E3 and E1 in Figure 2) means that all genotypes performed similarly and therefore it provided little or no information about the genotype differences.

Although the ideal environment is unlikely, its estimation can be used as reference for genotype selection in the MEYTs. GGE Biplot generates a biplot (Figure 3) of an ideal environment, which is highly discriminating and representative of every environment in the dataset. An environment is more desirable if it is located closer to the ideal environment. Thus, localizing the ideal environment at the center, concentric circles were drawn to help visualize the distance between each environment and the ideal environment. Figure 3 indicated that E2, which fell into the center of concentric circles, was the ideal test environment for discriminating genotypes. E4 can also be considered a favorable environment as far as its discriminating ability, whereas E1 and E3 were less effective because they failed to

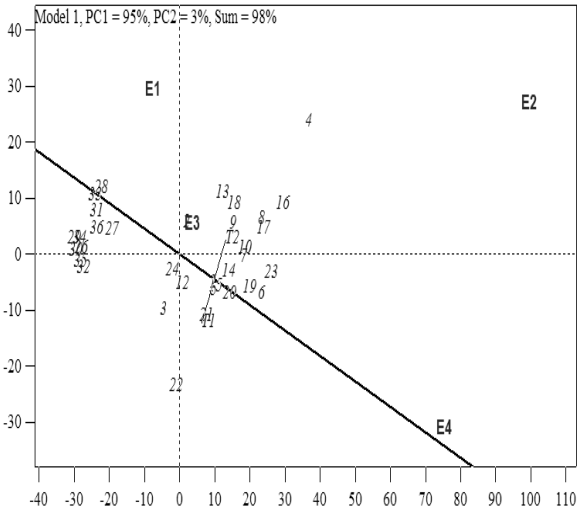

Fig 4. GGE-biplot grouping the environments into those where T2 outyields T1 (above the thick line) and where T1 outyields T2 (below the thick line)

discriminate among cultivars. In summary, the most discriminant environments are E4 and E2, second productive season for both blanched and green production respectively. Although, it is hard to determine the ideal cultivars since anyone presented high values of PC1, the cultivars 23, 17, 10, and 7 showed a good relation between yield and stability. Another important factor in the genotypes selection by MEYTs is the use of suitable testers. The performance of the two testers (T1 and T2) when considering only the G and GE interactions was also compared by the GGE biplot (Figure 4). A line that connected the markers of T1 and T2 was drawn. Also, another line (a thick line) that was perpendicular to the first line and that passed through the origin was drawn. The thick line separated the GGE coordinates into two groups, with each cultivar yielding better than the other within its respective side of the broken line. Thus, T2 would yield better than T1 at three environments (E1, E2 and E3), while T1 would yield better than T2 at only one environment (E4). The superiority of T2 in most of the environments may be because T2 is an open pollinated population (Argenteuil) and it would have greater homeostasis to support possible environmental changes. On the other hand, T1 is a clonal hybrid (UC-157F1) with less homeostasis than T2. The correct tester selection is as important as the selection of the test environments when the objective is to evaluate the performance of new genotypes. Plant breeding aims to improve crop production either within a given mega-environment or in a wide range of growing conditions. These two approaches have important implications on breeding methodologies and strategies (Ceccarelli, 1989). The knowledge of GEI can help to reduce the cost of extensive genotype evaluation by eliminating unnecessary testing sites and by fine-tuning breeding programs. Identification of an ideal test environment based on discriminating ability and representativeness implies that selections made at that site would have the highest probability of choosing superior genotypes that perform well in all environments in the growing region. Thus, major benefits to breeders would include the increased efficiency of selecting in discriminating environments and the discontinued use of poorly discriminating environments. In this way, cultivar development can be achieved most efficiently taking account the limited resources available to breeders.

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