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Biomass Yield Stability in Alfalfa

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In addition to biomass production, alfalfa (*Medicago sativa*) cultivars also need to express yield stability across diverse environments. The objective of this experiment was to analyze the nature of biomass yield stability in ten commercial alfalfa cultivars by evaluating performance of individual genotypes. Biomass yield was measured in each of five environments across two years, and the yield stability computed for the overall cultivar mean performance and the mean performance of each of the genotypes comprising the cultivars using the genotype x environment variance statistic of Shukla and the superiority statistic of Lin and Binns'. The GxE variance of the cultivars was not correlated with the mean GxE variance of the genotypes comprising the cultivar. A strong positive correlation was observed between the superiority value of the cultivar as a whole and the mean superiority value of its genotypes. Alfalfa cultivars can be stable, as measured by the GxE variance, without being composed of stable genotypes. However, cultivars identified as superior only result if the individual genotypes are also superior. The top 10% of individual genotypes selected based on GxE variance do not include any genotypes with high yield. However, truncation based on the superiority statistic selected seven of the ten top yielding genotypes. It seems that for an applied breeding program selection based on the superiority statistic would have a greater chance of improving yield and yield stability concurrently.

INDEX DESCRIPTORS: Alfalfa, biomass, GxE, stability, yield.

Selection of genotypes in breeding programs and making cultivar recommendations to farmers is complicated by genotype-by-environment interaction (GxE). The problem lies in the fact that genotypes or cultivars that are superior across all locations are rare. How to integrate GxE and performance to make selections has been the source of considerable research, but despite its importance, the optimal method of using GxE information is unclear (Allard and Bradshaw 1964; Bernardo 2002). Stability analysis is the general method used to assess performance across a set of environments, but several methods exist, complicating use in breeding or evaluation experiments.

Stability has several connotations, ranging from homeostasis, in which the performance of a genotype or cultivar is the same in every environment, to assessments of a cultivar's performance relative to the mean or the best entry's performance across environments (Lin et al. 1986; Becker and León 1988; Lin and Binns 1991; Bernardo 2002). Each stability statistic has corresponding strengths and weaknesses for their usefulness to applied breeding programs, and interpretation of their values needs to be made with a knowledge of these limitations. Two stability parameters, Shukla's (1972) (σ_i^2) statistic and Lin and Binns (1988) superiority statistic (P_i) represent two markedly different statistics and have been the focus of previous studies (Helland and Holland 2001). Shukla's σ_i^2 indicates how closely the performance of a genotype of interest parallels the mean performance of all genotypes evaluated, providing an unbiased estimate of genotype x environment interaction. However, a genotype whose performance parallels the mean genotypic performance does not necessarily imply that it is more desirable than others; some indication of performance is also needed. Lin and Binn's P_i describes the similarity between the performance of a genotype of interest and the best genotype in each environment; stable genotypes will have a performance close to the maximum in each environment. The incorporation of the magnitude of the phenotypic performance into the stability formula may be more useful in an applied breeding situation.

Individuals or populations have differential abilities to buffer their phenotypes from environmentally imposed perturbations (Allard and Bradshaw 1964). Individual buffering is genotype specific and is associated with heterozygosity, including residual heterozygosity present in ostensibly pure lines. Population buffering is due to the interactions among genotypes within the population and is associated with heterogeneity. Mixing seeds of two or more cultivars prior to planting has been repeatedly evaluated as a means of increasing stability and performance, but mixtures are not always more stable than their individual component parts (Clay and Allard 1969; Helland and Holland 2001). Both individual and population buffering appear to be important in the development of stable cultivars with superior performance in both inbreeding and outbreeding species (Jones 1958; Allard 1961; Allard and Bradshaw 1964; Helland and Holland 2001).

Synthetic cultivars are populations of plants that each has a unique genotype. Thus, the phenotypic stability of a synthetic cultivar could be partitioned into components for individual and population buffering. Synthetic cultivars are developed by intercrossing selected clones or inbred lines followed by two to four generations of random mating to increase seed for sale (Fehr

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Table 1. Number of parental clones, mean forage yields, genotype x environment interaction variance (σ_i^2) and superiority (P_i) values (mean values correspond to average values of individual genotypes), genetic variance (σ_G^2), and average Spearman rank values (r_{rank}) of ten alfalfa cultivars evaluated at five Iowa environments in 2001 and 2002.

Cultivar	No. Parental Clones	Mean Forage Yield	σ_i^2	Mean σ_i^2	P_i	Mean P_i	σ_G^2	r_{rank}
		g plant^{-1}						
5454	12	149	85	2251	635	15815	1295	0.51
Affinity+Z	91	164	393	2198	511	14777	1036	0.36
DK140	18	143	74	1913	992	16991	516	0.19
Enhancer	22	138	323	1681	1255	19751	1215	0.53
Innovator+Z	112	152	272	1323	732	15712	895	0.37
Jade	8	150	125	2051	606	15181	482	0.30
Stampede	228	138	212	2264	1237	18605	1810	0.60
Vernal	11	163	672	2685	254	12717	756	0.39
WetLand	156	141	239	3055	1206	18139	487	0.17
WL324	99	158	484	3354	340	14073	689	0.20
Mean		150	291	2178	777	16176	918	0.36
LSD (5 %)		14	59	263	106	1809	155	0.24

1987). In alfalfa, the difficulty of producing inbred lines and the prevailing seed production practices essentially dictate that most cultivars are synthetics (Hill 1987). Most current alfalfa cultivars are broad-based synthetics derived from more than 25 parents that incorporate multiple pest resistances, avoid high levels of inbreeding during the seed increase, and maintain high levels of heterogeneity (Hill 1987). The importance of heterogeneity to alfalfa cultivar stability could be assessed by studying the relationship between stability statistics and parental number.

We hypothesized that (i) alfalfa synthetic cultivars with individual genotypes that expressed high stability for forage biomass production would be more stable and (ii) cultivars developed from more parents would have greater stability for forage biomass production than cultivars with fewer parents. The objective of this experiment was to test these two hypotheses using clonal ramets of genotypes derived from 10 commercial alfalfa cultivars grown in five environments for two years. As a consequence of evaluating these genotypes, we also evaluated the suitability of various stability statistics for selecting high yielding and stable genotypes.

METHODS

Plant Materials

Ten commercial alfalfa cultivars were evaluated in this experiment: 'Affinity +Z' and 'Innovator +Z' (ABI Alfalfa, Lenexa, KS); 'Enhancer,' 'Jade,' 'Stampede,' and 'WetLand' (Dairyland Seed Co., West Bend, WI); 'DK140' (Dekalb Seed, Dekalb, IL); '5454' (Pioneer Hi-Bred Intl., Des Moines, IA); 'Vernal' (Univ. Wisconsin, Madison, WI); and WL324 (W-L Research, Madison, WI). The cultivars were derived from different numbers of parent plants (Table 1). During the winter and spring 2000, 10 randomly selected genotypes from each cultivar, for a total of 100 genotypes, were cloned by stem cuttings in the greenhouse.

Experimental Design

In August 2000, clones were transplanted at three Iowa field locations: the Agronomy and Agricultural Engineering Research Farm, Ames, IA in a Nicollet loam soil (fine-loamy, mixed, superactive, mesic Aquic Hapludolls); the Northeast Research Farm, Nashua, IA in a Readlyn loam soil (fine-loamy, mixed, mesic Aquic Hapludolls); and the Western Research Farm, Castana, IA in a Monona silt loam (fine-silty, mixed, superactive, mesic Typic Hapludolls). At each location, the experimental design was an α-lattice, consisting of two replications each with 10 incomplete blocks comprised of 10 plots. Three clones of each genotype were placed in each plot. Plants were spaced 30 cm within plots, with 60 cm between plots within a row, and 90 cm between rows. The Ames location included two other environments. The second environment was planted as described except that it was overseeded with orchardgrass. In the third environment, plants were spaced 15 cm within rows and 30 cm between rows. The three Ames environments will be labeled Ames-1, -2, and -3, respectively. Data were collected in two years, to produce a total of 10 environments.

Data Collection

In October 2000, all plants were clipped 7.5 cm above ground level and the forage discarded. Plots were harvested for forage biomass determination in June, August, and September 2001 and 2002. At each harvest, all plants were hand harvested at 7.5 cm above ground level, and the forage from each plot was placed into paper bags and dried with forced air for five days at 60°C. Dry forage was weighed to determine the dry matter per plot. The number of plants per plot was recorded at each harvest, and the forage biomass yield for each plot was adjusted by the number of plants and recorded as g plant^{-1}. Total yearly forage yields for each plot were determined by summing the biomass yield from each harvest during each year.

Data Analysis

For each environment, least squared means (lsmeans) of forage yield were calculated for each cultivar and individual genotype. The cultivar yields were calculated as the average yield of the 10 individual genotypes. Lsmeans were used to calculate the superiority statistic (P_i) of Lin and Binns (1988) and the genotype-by-environment interaction variance statistic (σ_i^2) of

Shukla (1972). The P_i statistic is calculated as follows:

$$P_i = \frac{\sum_{j=1}^{n} (X_{ij} - M_j)^2}{2n},$$

where X_{ij} is the yield of the ith genotype (or the ith cultivar) in the jth environment, M_j is the yield of the highest yielding genotype (or cultivar) in the jth environment, and n is the total number of environments included in the experiment. Shukla's σ_i^2 is calculated as follows:

$$\sigma_i^2 = \left[\frac{p}{(p-2)(q-1)} \sum_{j=1}^{q} (X_{ij} - \bar{X}_{i\cdot} - \bar{X}_{\cdot j} + \bar{X}_{\cdot})^2\right] - \frac{\sum_{i=1}^{p}\sum_{j=1}^{q} (X_{ij} - \bar{X}_{i\cdot} - \bar{X}_{\cdot j} + \bar{X}_{\cdot})^2}{(p-1)(p-2)(q-1)},$$

where p equals the number of genotypes (or cultivars) and q equals the number of environments, X_{ij} is the yield of the ith genotype (or the ith cultivar) in the jth environment. Both statistics were calculated on an individual genotype and on a cultivar basis. The values of the statistics calculated on an individual genotype basis were averaged across genotypes to calculate mean σ_i^2 and P_i values for each cultivar. The jackknife procedure (Weir, 1996) was used to determine the variance associated with these values.

Two other statistics also were computed to assess stability. First, the genetic variance (σ_G^2) in forage yield among clones within a cultivar across locations and years was estimated using a random statistical model. Second, the stability of genotype performance within cultivars was estimated as follows. Genotypes within cultivars were ranked from high to low based on forage yield lsmeans in each environment, and Spearman rank correlations were computed between the ranks in all pairwise environmental combinations. The mean of all the pairwise correlations for each cultivar was designated r_{rank}; the standard error of the mean was calculated as described by Steel and Torrie (1980).

Simple correlations between the number of parental clones, mean forage yield, and the stability statistics were calculated using overall cultivar means. Statistical analyses were run using the MIXED, GLM, and CORR procedures of SAS (SAS Institute, 2003). Statistical significance is at the 5% level of probability unless otherwise noted.

RESULTS AND DISCUSSION

Forage Yield

The overall mean forage biomass yield differed among the 10 cultivars (Table 1) and was highly variable on a cultivar and on an environment basis. Averaged across environments, yields ranged from 138 g plant^{-1} (Enhancer and Stampede) to 164 g plant^{-1} (Affinity +Z) (Table 1). The range was greater on an individual genotype basis, from 59 g plant^{-1} (a Stampede genotype) to 205 g plant^{-1} (a 5454 genotype) (data not shown). Environments ranged in yield from 80 g plant^{-1} at Ames-3 in 2001 to 267 g plant^{-1} for Ames-2 in 2002. Cultivar rankings varied throughout the experiment and extensive crossover GxE was observed. In all but one environment, Affinity +Z had higher yield and DK140 and Stampede had lower yields than the environmental mean.

Most individual genotypes outperformed the mean in some environments and underperformed in others (data not shown). Three genotypes (one each from Affinity +Z, Enhancer, and WL324) produced more biomass than the mean in each environment. Conversely, ten genotypes (two from Affinity +Z, three from Enhancer, one from Innovator +Z, three from Stampede and one from WL324) yielded less than the mean in each environment.

Stability

All stability measures differed among cultivars (Table 1). For Shukla's GxE variance and Lin and Binn's superiority statistic, cultivar stability was assessed based on average performance across genotypes (σ_i^2 and P_i) and on average stability of genotypes (mean σ_i^2 and mean P_i). The mean values reflect the stability of the genotypes comprising a cultivar. Relating the mean values to the overall cultivar values provides an indication of whether overall cultivar stability is a property of genotypic or population buffering.

The cultivars that performed similarly to the mean performance of all entries had low σ_i^2 values; they were 5454, DK140, Jade, Stampede and WetLand and were the most stable according to this measure (Table 1). Vernal had the highest σ_i^2 (σ_i^2=672) value and was the least stable based on this measure. There was no correlation between the σ_i^2 value and the mean σ_i^2 values (Table 2). Cultivars that showed low σ_i^2 values (more stable) did not have correspondingly low mean σ_i^2 values. These results suggest that the Shukla stability of individual genotypes has no bearing on the stability of the cultivar, at least under the conditions of this experiment.

In contrast, the P_i and mean P_i statistics were very congruent; cultivars that had high P_i values also contained genotypes with high P_i values (Tables 1 and 2). WetLand, stable based on σ_i^2 (σ_i^2=239), was unstable based on P_i (P_i=1206). Vernal, which had low stability based on σ_i^2 (σ_i^2=672) was among the most stable based on P_i (P_i=254). Thus, cultivars that are superior do not necessarily parallel the mean performance across environments. The presence of consistently low yielding genotypes likely contributed to the high P_i values of Enhancer and Stampede, although Affinity +Z also had poor yielding genotypes and had among the lowest P_i.

We also used an estimate of the genetic variation (σ_G^2) and the nonparametric r_{rank} values of each cultivar to assess phenotypic stability. We reasoned (i) that higher σ_G^2 may increase a cultivar's ability to buffer itself against diverse environmental conditions and (ii) that cultivars with individual genotypes whose performance was positively correlated across environments would be more stable. Neither of these measures was correlated with the four previously discussed stability statistics (Table 2), suggesting that they measure different aspects of stability. Stampede had the highest σ_G^2 (σ_G^2=1810) of any cultivar, and it was among the most stable based on σ_i^2 (σ_i^2=212). However, it was among the most unstable based on its P_i value (P_i=1237). Jade, DK140, and WetLand had among the lowest σ_G^2 for yield, yet all were among the more stable cultivars—and similar to Stampede—based on low values of σ_i^2. Thus, cultivars with lower σ_G^2 are not necessarily less stable or lower yielding than those with high variance (Table 2). Differentiation among cultivars based on r_{rank} was not strong, and no relationship with yield or with any stability measure was noted (Tables 1 and 2). Thus, the performance ranking of genotypes within cultivars across environments has little effect on cultivar stability. σ_G^2 and r_{rank} were highly positively correlated (r=0.91); cultivars with higher

Table 2. Correlations—based on overall cultivar means—among the number of parental clones used for each cultivar, forage yield, overall and individual genotype mean values of the genotype x environment interaction variance (σ_i^2) and superiority (P_i) statistics, genetic variance (σ_G^2), and average Spearman rank values of individual gentoypes (r_{rank}).

	Forage Yield	σ_i^2	Mean σ_i^2	P_i	Mean P_i	σ_G^2	r_{rank}
No. Parental Clones	−0.27	−0.003	0.22	0.42	0.35	0.41	0.11
Forage Yield		0.64*	0.26	−0.94***	−0.93***	−0.27	−0.23
σ_i^2			0.42	−0.54	−0.53	−0.07	−0.03
Mean σ_i^2				−0.30	−0.32	−0.25	−0.42
P_i					0.98***	0.32	0.23
Mean P_i						0.38	0.31
σ_G^2							0.91***
r_{rank}							

*- significant at the 5 % level
***- significant at the 0.1 % level

σ_G^2 tended to include genotypes whose ranks were more consistent across environments. The importance of this result is unclear, and should be verified with further studies to ensure that it is not an artifact if this experiment's data.

Correlations

The number of parental clones used in the creation of the cultivars was not correlated with mean forage yield or with any stability statistic (Tables 1 and 2). This is not necessarily surprising because we have no way of knowing the actual amount of genetic diversity represented by the parental genotypes. More parents derived from the same population may be less diverse than parents derived from different populations. However, this result suggests that synthetics with many parents are not more diverse than those with fewer parents.

Forage yield was positively correlated with σ_i^2 and negatively correlated with P_i and mean P_i. Higher yields were associated with more unstable cultivars based on σ_i^2 (larger values), but were associated with stable cultivars based on P_i (smaller values). The lack of correlation between σ_i^2 and mean σ_i^2, but the presence of a positive correlation between P_i and mean P_i indicates that individual genotypes in a synthetic have a greater influence on P_i than on σ_i^2. These same trends were seen in the analysis of individual genotype forage means and stability measures (data not shown). Among the individual genotypes, P_i and σ_i^2 were weakly and negatively correlated, but the small correlation coefficient (−0.22) is of limited biological significance (data not shown).

Table 3. Mean forage yield, genotype x environment interaction variance (σ_i^2), and superiority parameter (P_i) values for the selected highest and lowest 10% of genotypes, based on selection for each criterion.

Selection Criteria	Forage Yield	σ_i^2	P_i
	g plant^{-1}		
Top 10% Yield	196	2532	8565
Bottom 10% Yield	93	1629	28709
Lowest 10% σ_i^2	152	504	14670
Highest 10% σ_i^2	167	7019	12320
Lowest 10% P_i	193	4122	8201
Highest 10% P_i	94	2177	28885

The importance of individual buffering (Allard and Bradshaw 1964) on cultivar yield stability is difficult to assess. The lack of a correlation between σ_i^2 and mean σ_i^2 indicates that stable alfalfa cultivars, as defined by σ_i^2, do not need to consist of stable genotypes. However, the strong positive correlation between P_i and mean P_i indicates that superior alfalfa cultivars are composed of superior genotypes, which may not have stability based on σ_i^2.

Selection for yield and stability

The lack of agreement between the statistics evaluated in this study reflects the different parameters that each defines. Agreement does not exist among plant breeders for the best measure of stability (Becker and Léon 1988), so individual breeders will need to make that decision for themselves based on their needs.

We considered what effect selecting genotypes based on yield or the two stability statistics would have (Table 3). Depending on which stability measure was used for selection, the mean values of the selected individuals for forage yield, σ_i^2, and P_i vary significantly and for each criterion different sets of genotypes would be selected. Although σ_i^2 is likely a good measure of stability, highly stable genotypes tended to yield poorly (Table 3), suggesting that it is not adequate for selection, as has been noted previously (Kang 1993). Based on the genotypes included in this study, selection of the most stable 10% based on σ_i^2 would identify none of the genotypes also among the 10% highest yielding genotypes. Three of the highest yielding genotypes are also among the 10% with highest values of σ_i^2.

For applied breeding programs, where emphasis is usually placed on high phenotypic performance, P_i would be a better indicator of phenotypic stability. Seven of the 10 highest yielding genotypes were also identified as highly stable and nine of the 10 lowest yielding genotypes were identified as unstable based on P_i. Selection on P_i would result in the selection of some of the higher yielding genotypes and could have the effect of concurrently improving the phenotypic performance.

Conclusions

This study has several limitations. First, we assessed cultivar stability based on individual genotypes, cloned with stem cuttings, and planted in spaced plant conditions. Cultivar stability based on swards may be different. However, this planting arrangement is similar to that used in breeding

programs that evaluate single plants or clonal rows. For individual genotypes, these results may have applicability. Second, no populations were synthesized from the selected genotypes. An unanswered question is whether selection for stability, in concert with yield, would result in cultivars improved for both traits. To our knowledge, no alfalfa breeding program has ever included stability in its traits under selection. Cultivars developed from genotypes selected for both yield and stability—particularly based on the superiority statistic—may have superior characteristics to those selected solely for yield.

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