

# GENOTYPE X ENVIRONMENT INTERACTION AND STABILITY PARAMETERS FOR ESTABLISHMENT IN ANNUAL MEDICAGO SPP.

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## INTRODUCTION

Annual *Medicago* species originated in the Mediterranean region (5) but encountered in most bioclimatic stages (9). They, along with subterranean clover (*Trifolium subterraneum* L.), are widely used in Australia (5, 10). Selected Australian cultivars are being introduced for extensive ley-pasture use in North Africa and the Middle East (1, 3). Research has been initiated in Southern Europe to investigate the potential use of medics under rang conditions. El Tomi (7) found that planting date and seeding rate are important factors in the establishment of alfalfa (*M. sativa* L.). Bakhitri (1) indicated that optimal medic seeding rates were 10 kg ha<sup>-1</sup> in North Africa. In Morocco, farmers adopting the ley-farming program are advised seed medics at a rate of 20 kg ha<sup>-1</sup> for all

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varieties. Cocks (3) demonstrated that dry matter production increased with increased plant density. Seeding rate as high as 50 kg/ha-1 gave the maximum dry matter production in *M. rigidula* L. The significance of genotype x environment interactions (GXE) lies in their impact on reliability of estimates for variance components in breeding programs (4) and technology transfer. Eberhart and Russell (6) used linear regression model to evaluate the performance and stability of cultivars across environments. Regression coefficient (b) and residuals ( $s^2_d$ ) were used as criteria for responsiveness and stability to environmental changes, respectively. By definition a stable variety would have  $b = 1$  and  $s^2_d = 0$

The objectives of this study were : 1) to evaluate the effects of genotype, environment, and GXE interactions on annual medic emergence and forage production, 2) to estimate stability parameters for emergence, and 3) to assess the relationship between emergence and cultivar performance.

## MATERIALS AND METHODS

This study was conducted in Oklahoma, USA, from 1983 to 1985 and in Morocco, during 1985-86 growing season. Oklahoma has a continental climate with severe, cold winters and year around precipitations concentrated in late spring. Morocco has a Mediterranean climate with mild temperatures and precipitations between October and May with a peak in January. three locations, differing ecologically in soils and rainfall regimes were used in each macroclimate (Table 1); Sites in Oklahoma were Stillwater, Haskell, and Woodward. Stillwater was replaced by Perkins in 1984/85 growing season. During the first year, the Haskell site was divided in to two subsites differing in pH; one was not limed and had a pH of 4.8 and the other was limed to bring pH to 6.8. Lime was also added in the second year at Hskell because the effect of pH was not significant in the previous year. Locations in Morocco were Sidi el Aydi, Jemaa Shaem, and Tessaout; all in semi-arid areas. Two planting dates : fall (late October) and spring (late March) were evaluated in Oklahoma. Only fall plantings (early November) were used in Morocco since water is a limiting factor to plant growth in spring and summer. Combination of sites, years and planting seasons resulted in 17 test environments.

Treatments included fourteen genotypes, representing six annual medic species, one alfalfa (*M. sativa* L.) , and three clovers (Table 2). They were sown in a randomized complete block design with four replications (three in Perkins). Seeds of each accession were inoculated with proper *Rhizobium* sp. and

Table I. Ecological characteristics of the test environments \*.

Location	Soil Type	Annual precipitation	Temperature	
			Min.	Max.
Stillwater, Payne, co.	Fine, silty, mixed Thermic Cumalic Haplustolls	-- mm -- 860	----- OC ---- - 8.9	36.7
Haskell Muskogee, co.	Fine, mixed, Thermic Mollic Albaqualfs	1025	- 6.7	33.9
Perkins, Payne, co.	Fine-loamy, mixed Thermic Udic Argiustolls	839	- 10.0	35.6
Woodward, Woodward, co.	Sandy, mixed, Thermic Psammentic Haplustalfs	575	- 11.0	38.0
Sidi el Aydi, Morocco	Calcixerollic chromoxerert	408	4.4	33.6
Jemaa Shaem, Morocco	Typic chromoxerert	297	7.0	33.5
Tessaout, Morocco	Vertic xerofluent	257	4.3	39.8

\* Average over 65 and 33 years for Oklahoma and Moroccan sites, respectively. Precipitations include snow. Temperatures min. and max. are monthly means for January and August, respectively.

Table II : Emergence percentage of genotypes at 12 environments.

Genotype		Environment*											
Species	Cultivar	Sf3	Af3	Ss4	As4	Ms4	Pf4	Mf4	Wf4	Ms5	Yf5	Jf5	Tf5
		..... % .....											
M. littoralis	Harbinger	88	59	41	14	9	62	45	63	34	37	96	33
M. polymorpha	Circle Valley	87	47	83	37	19	65	49	68	46	53	69	47
M. rugosa	Paragosa	60	40	66	29	11	41	35	63	18	40	16	41
M. sativa	OK - 61	63	38	67	17	12	72	85	69	19	87	87	42
M. scutellata	Robinson	nd+	nd	48	nd	31	53	40	61	42	29	22	28
M. tornata	Tornafield	28	33	22	13	12	37	35	40	20	42	50	47
M. truncatula	Borong	55	49	32	20	7	61	55	47	15	82	63	38
M. truncatula	Cyprus	51	48	42	15	11	66	67	49	32	37	41	41
M. truncatula	Jemalong	37	44	23	15	6	56	29	55	19	36	52	30
M. truncatula	Paraggio	67	52	56	28	11	56	62	71	43	58	76	45
T. incarnatum		37	31	18	9	7	34	51	50	16	24	29	39
T. subterraneum	Mount Barker	78	38	51	45	31	64	46	65	26	45	56	28
T. vesiculosum	Yuchi	91	48	42	24	8	57	63	72	27	34	66	33
LSD (P = 0.05)		11	17	11	10	6	17	18	ns++	22	25	16	ns

\* Sf3, Af3, Ss4, As4, Ms4, Pf4, Mf4, Wf4, Ms5, Yf5, Jf5, and Tf5 are, respectively, Stillwater and Haskell (no lime) 1983 fall, Stillwater, Haskell (no lime), and Haskell (lime)1984 spring, Perkins, Haskell, and Woodward 1984 fall, Haskell 1985 spring, sidi el Aydi, Jemaa Shaem, and Tessaout 1985 fall plantings.

+ not determined

++ not significant

hand-sown at the rate of 100 scarified, viable seeds  $m^{-1}$  in single rows 2 m long and 1 m apart. In addition to commercial inoculum, nitrogen was applied at sowing at the rate of 20 kg  $ha^{-1}$ . Phosphorus, potassium, and lime were applied as needed according to soil test recommendations for alfalfa. All plots were hand-weeded and conducted under non-irrigated, clean-tilled conditions except in Tessaout where two irrigations by flooding were applied to allow germination and emergence. Seedlings were counted 30 days after planting. Emergence percentage was calculated on the basis of 200 seeds per plot. Forage was harvested at each location when half of the accessions were at the flowering stage. Samples for dry matter determination were taken and dried to a constant weight at 70°C. Some accessions were harvested twice in Morocco and hence, total forage yield is reported. Experiments were terminated when all medics died.

Gabriel (8) demonstrated that regular analysis of variance may be applied to proportions. Thus, responses within each experiment were analyzed using standard analyses of variance, and mean comparisons were evaluated with the protected least significant difference (LSD) at  $p = 0.05$ . Wind in the spring at Woodward (Oklahoma) and water from irrigation at Tessaout (Morocco) created heterogeneity by displacing soil and seeds. Woodward spring plantings has poor emergence and were not included in the analysis. Three other environments; Woodward, the limed site at Haskell in fall 1983, and Perkins spring 1985, were also rejected from the combined analyses due to the lack of homogeneity of variances which was performed using Bartlett's test. Combined analyses for emergence were made on 12 environments (Table 2). Data from all genotypes, except *M. scutellata* (L.) Mill. (not evaluated in certain environments) were used. The combined analyses were performed by using a "mixed" linear model, random environments and fixed plants accessions. The average of four single combined analyses was used to construct the analysis of variance table according to the Jackknife method (11). Each analysis was based on three replications; i.e., the first combined analysis had replications 1, 2 and 3, while the second had replications 2, 3 and 4, and so on. Stability analyses of genotypes for emergence were studied by linear regression method as outlined by Eberhart and Russell (6). An environment index (I) was calculated by taking the mean of all cultivars grown at the  $i^{th}$  environment and subtracting from this the general mean of all genotypes over all environments. The linear regression of I on the mean of the parameter measured was obtained for each genotype. Forage yield was also regressed on emergence percentage.

## RESULTS AND DISCUSSION

### Emergence

Differences in emergence among genotypes were significant in all environments, except at Woodward with spring plantings and at Tessaout where irrigation was applied. Emergence percentages for each genotype in each environment are exhibited in Table 2. Environment, genotype, and GXE effects were highly significant ( $P < 0.01$ ) (Table 3). Higher emergence percentages were obtained from fall plantings than from spring plantings in Eklahoma. This concurs with results of El Tomi (7) obtained on alfalfa. No major differences were detected in emergence from one year to another in Oklahoma. Fall plantings in Oklahoma were not different from those in Morocco with regard to the number of emerged plants. Certain cultivars had either high or low emergence in most environments. Species with consistently high emergence were *M. polymorpha* L. Circle Valley, *M. littoralis* Rhode Harbinger, and *M. sativa* OK-61. Genotypes ranking low were *M. rugosa* Desr. Paraponto, *M. tornata* (L.) Mill. Tornafeld, and *T. incarnatum* L.

Highly significant GXE may partly be attributed to the extreme contrasts both in environments and genotypes as was shown by Comstock and Moll (4). The large contribution of GXE in *Medicago* spp. and *Trifolium* spp. seedling emergence variance strongly demonstrated that recommendations for uniform seeding rates across locations as presented by Bakhtri (1) and by the extension service in Morocco should be reconsidered. Cocks (3) found that dry matter increased with higher plant density and a seeding rate of 50 kg ha<sup>-1</sup> produced the highest forage yield in *M. rigidula* L., *M. noeana* L., and *M. truncatula*, but there were seeding rate x species interactions. Furthermore, large differences in seed size exist among medic species. *M. scutellata* L. (Mill) seed is five-fold bigger than *M. polymorpha* and 10 times bigger than *M. minima* L. Seeding rates should be adjusted for specific seed weight, emergence percentage, and prevailing environmental conditions when optimal stand density is desired. Also, the large contribution of environmental component to total variance confirmed that improvement in emergence may be realistically achieved with good seedbed preparation, adequate soil moisture, pest and disease control, and good quality seed.

Regression coefficients (b) of cultivar emergence on environmental index differed significantly ( $P < 0.05$ ) from zero in the stability analysis (Tableau 3). A genotype with  $b = 1$  represents, by definition, average responsiveness to

**Table III.** Mean squares (MS), regression coefficients (b), and residuals (sd) about the regression line for emergence.

Source	df	MS	b	sd
Environment (Env.)	11	2574.0***		
Env. (linear)	1	28066.9***		
Replication in Env.	24	115.1		
Genotype (Gen.)	12	1362.3***		
Gen. x Env.	132	121.7**		
Gen. X Env. (linear)	12	212.9***		
Pooled deviations	120	114.6		
Harbinger	10		1.29**	8.00*
Circle Valley	10		0.95	5.15
Paragosa	10		0.70***	6.24
Paraponto	10		0.60***	10.80***
OK-61	10		1.59***	15.70**
Tornafield	10		0.60***	0.00
Borong	10		1.12*	0.00
Cyprus	10		0.84	6.33
Jemalong	10		0.80**	0.00
Paraggio	10		0.97	0.00
Crimson clover	10		0.60***	0.00
Mount Barker	10		0.89	1.24
Yuchi	10		1.32***	4.93
Pooled error	288	65.8		

\*, \*\*, \*\*\* Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

environment changes. Mean square ( $s_d^2$ ) for deviation from regression measures how well the predicted response agrees with observed response and it includes GXE. Cultivars with  $b$  not different ( $P > 0.05$ ) from 1 were Circle Valley, Cyprus, Paraggio, and Mount Barker. Their  $s_d$  were statistically equal to zero. According to Eberhart and Russell (6), such cultivars had average responsiveness to environmental changes and were stable for emergence. Circle Valley cultivar had high emergence percentage in most environments. Borung and Yuchi had  $b > 1$  and  $s_d$  not different from zero. Borung was medium and Yuchi had above average emergence and both were relatively stable in the field. Harbinger and OK-61 exhibited a  $b > 1$  and a  $s_d$  different from zero, but OK-61 was unstable. Under field conditions these two genotypes emerged well. Paragosa, Tornafeld, Jemalong, and crimson clover (*T. incarnatum* L.) possessed a  $b < 1$  and  $s_d$  not different from zero. Paragosa and Jemalong had in fact medium emergence rates in many environments, while Tornafeld and crimson clover emerged poorly. Paraponto was the least desirable cultivar with regard to emergence because it was below average both on field and in the analysis ( $b < 1$  and  $s_d > 0$ ).

Results obtained in stability analyses confirmed the information obtained from individual experiment analyses for cultivars which had  $b < 1$ . It was difficult, however, to group cultivars with a  $b > 1$ . Deviations observed between individual and stability analyses may have originated from the use of linear regression. Nguyen et al. (12) demonstrated the limitations in using linear models for perennial grass forage yields for it is based on mean values. Taliafero et al. (13) indicated that cultivars of alfalfa with  $b > 1$  had either higher or lower forage yield than average response.

## Forage Yield

Winter kill precluded harvests of medics from fall plantings in Oklahoma except in Stillwater (Table 4) where snow cover preserved *M. polymorpha* in 1983-84. Waterlogging at Haskell 1984 and poor emergence along with drought at Woodward during tow years resulted in negligible forage yield from spring-sown experiments. Differences among genotypes were significant in the remaining six environments (Table 4) from which two sets of data resulted from the variance homogeneity test : one consistend of Stillwater in 1984, and Haskell and Perkins in 1985. The second was composed of Morroccan environments. Effects associated with environment, genotype, and GxE interactions were significant in both sets. The highest production was obtained from Borung, Paraggio, and Robinson at Sidi El Aydi, Jemaa Shaem, and



Tessaout, respectively. Relatively high yields were also obtained from Circle Valley and Tornafield in Morocco. *M. sativa* was not different from medium to low producing medic genotypes. *M. rugosa* Paraponto and *Trifolium* spp. had low forage production.

Most of the GXE resulted from crossover interactions. Noncrossover interaction, caused by varying performance of some genotypes among environments, also existed. A third potential source of GXE may have arisen

Table IV : Dry matter yield (g m<sup>-2</sup>) of genotypes in seven environments.

Genotype		Environment						
Species	Cultivar	Sf3	Ss4	Ps5	Ms5	Yf5	Jf5	Tf5
<i>M. littoralis</i>	Harbinger	0	58	34	16	118	184	112
<i>M. polymorpha</i>	Circle Valley	21	109	40	52	50	282	251
<i>M. polymorpha</i>	Paragosa	0	53	15	1	57	213	188
<i>M. rugosa</i>	Paraponto	0	24	18	1	52	74	116
<i>M. sativa</i>	OK-61	98	40	8	86	22	99	40
<i>M. scutellata</i>	Robinson	nd+	104	67	112	124	268	606
<i>M. tornata</i>	Tornafield	0	36	27	16	218	157	75
<i>M. truncatula</i>	Borong	0	41	23	56	285	233	205
<i>M. truncatula</i>	Cyprus	0	62	36	42	113	136	128
<i>M. truncatula</i>	jemalong	0	41	20	15	73	169	103
<i>M. truncatula</i>	paraggio	0	65	57	74	151	329	223
<i>T. incarnatum</i>		35	20	10	7	39	145	71
<i>T. Subterraneum</i>	Mount Barker	48	21	3	28	1	50	66
<i>T. Vesiculosum</i>	Yuchi	81	22	1	20	1	94	53
LSD (P=0.50)		17	14	33	37	80	72	71

\* Sf3, Ss4, Ps5 Ms5, Yf5, Jf5 and Tf5 are, respectively, Stillwater 1983 fall and 1984 spring, Perkins and Haskell 1985 spring, Sidi El Aydi, Jemma Shaem, and Tessaout 1985 fall plantings.

+ not determined

from the use of wide spectra of species (genotypes) and environments (4). Variation between cultivars of the same species was observed and confirmed the presence of genetic variation in annual medics reported by Crawford (5). Generally, yield of annual *Medicago* spp. was higher in Morocco than in Oklahoma. Most growth in Morocco occurred during a cooler, shorter photoperiod months (December through February) compared to April and May growth in Oklahoma. Also, plants took 4 months to flower in Morocco as compared to 2 months in Oklahoma. Flowering stage at this latter environment was hastened with long photoperiod and high temperatures according to Clarkson and Russell (2). Apparently, plants do not have enough time to accumulate biomass prior to reproductive growth phase in the US Southern Great Plains.

**Table V. Linear regression (b) and simple correlation coefficients (r) between forage yield and emergence Percentage in each environment and medic species.**

	b	r
<b>environment</b>		
Stillwater spring 1984	0.84***	0.69
Perkins spring 1985	1.67***	0.78
Haskell Spring 1985	1.87*	0.45
Jemaa Shaem fall 1985	0.88	0.23
Sidi El Aydi fall 1985	1.29	0.32
Tessaout fall 1985	2.88	0.03
<b>Species</b>		
<i>M. littoralis</i>	1.51*	0.72
<i>M. polymorpha</i>	2.00	0.39
<i>M. rugosa</i>	1.85	0.49
<i>M. scutellata</i>	0.31	0.10
<i>M. tornata</i>	4.61**	0.75
<i>M. truncatula</i>	3.01***	0.66

\*, \*\*, \*\*\* significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

## Relationships between Emergence and Forage Yield

Linear regression of forage yield on emergence percentage was significant ( $P < 0.05$ ) when plants were spring-seeded in Oklahoma (Table 5). Emergence percentage might be used as a predictor for forage production but with a varying magnitude depending on environmental conditions. Cocks (3) reported that forage production increased with seeding rate in Syria. Duration of vegetative growth was short and thus, interplant competition was minor in Oklahoma. This situation may occur in pastures when plants are either grazed early in the season or when animals continuously graze medic pasture, particularly with a high stocking rate. The linear model, however, was not significant in Morocco. Fall sown plants in Morocco had enough time prior to harvesting to express their potential of production, to compete for light and nutrients, and to compensate any deficiency caused by low plant density. Changes in emergence percentage induced a significant ( $P < 0.001$ ) variation in forage yield for *M. truncatula* and to a lesser degree for *M. tornata* and *M. littoralis* (Table 5). Very low association between the two agronomic parameters existed in *M. polymorpha*, *M. rugosa*, and *M. scutellata*. Cultivars of these species were earlier maturing and had fewer stems than those of *M. truncatula* which may explain the differences observed among species.

The study showed that seedling emergence and dry matter production of medics were subjected to G X E interaction. Seeding rates of medics should be conceived according to cultivar characteristics and management. Most annual medics tested produced higher forage yield than alfalfa and clovers under rainfed conditions when planted in spring in Oklahoma or in fall in Morocco. Finally, in situations of short growing season or under grazing an increase in plant population would result in a better stand and consequently, substantial increase in forage yield.

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## ABSTRACT

Genotype x environment interaction (G X E) and stability parameters were evaluated in annual *Medicago* spp. For seedling emergence and forage yield. The study was conducted under a continental climate of the southern Great Plains in the USA and a mediterranean climate in Morocco between 1983 and 1986 in cleantilled field conditions. Combination of sites, years and planting seasons (fall vs. spring) resulted in 12 test environments. Plant materials included ten annual *Medicago* spp. accessions, one alfalfa (*M. sativa* L.), and three clovers (*Trifolium* spp.) .Differences among genotypes in seedling emergence and seed size, and the existence of G X E affecting emergence, indicate that similar seeding rate for all annual *Medicago* spp. would result in different stand densities and subsequent forage yield. Improvement of this establishment parameter through genetic manipulation would require efforts and time. The best forage producing genotypes were *M. truncatula* cv. Borung at Sidi El Aydi, *M. truncatula* cv. Paraggio at Jemaâ Shaem, and *M. scutellata* cv. Robinson at Tessaout. Forage yield appeared to be dependant on seedling emergence, particularly when the growth cycle is short (late seeding, grazing situations, and use of early maturing genotypes).

## RESUME

L'interaction Génotype x milieu (G X M) et les paramètres de stabilité ont été évalués pour la levée et la production fourragère des luzernes (*Medicago*) annuelles. L'étude a été conduite sous deux macroclimats, continental aux Etats Unis d'Amérique et méditerranéen au Maroc entre 1983 et 1986. Dix génotypes de ces luzernes annuelles (medics), un cultivar de luzerne pérenne (*M. sativa* L.), et trois trèfles (*Trifolium* spp.) ont été testés dans 12 environnements. Les différences apparues entre génotypes pour la levée et le poids spécifique des semences, et la présence de l'interaction GxM recommandent que la dose de semis des variétés de luzernes annuelles soit une caractéristique génotypique et doit être raisonnée en fonction des conditions du milieu. Les variétés ayant produit les plus hauts rendements en fourrage ont été *M. truncatula* cv. Borung à Sidi El Aydi, *M. truncatula* cv. Paraggio à Jemaâ Shaem, et *M. scutellata* cv. Robinson à Tessaout. La production fourragère a été positivement liée à la réussite de l'installtion surtout dans les conditions où le cycle de végétation et d'exploitation est court.

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**KEY WORDS** : Medics, clovers, emergence, seeding rate, seeding date, mediterranean area.

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