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ADAPTABILITY AND PHENOTYPIC STABILITY OF LETTUCE CULTIVARS IN A SEMIARID REGION[1]

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ABSTRACT – Studies on lettuce genotypes growing in different environments and planting seasons are important for this crop because its productive potential is greatly affected by environmental conditions. The mixed model methodology (REML/BLUP) have been used to evaluate the effects of the genotype-environment interaction on crops. Thus, the objective of the present work was to estimate parameters of adaptability and the phenotypic stability in lettuce cultivars grown in a semiarid region of Brazil, using a mixed model. Twelve lettuce cultivars from the Americana (Angelina, Amélia, and Tainá), Crespa (Scarlet, Vera, Isabela, and Vanda), Mimosa (Mila, Mimosa, and Lavínia), and Lisa (Elisa and Regiane) groups were evaluated. The experiments were conducted in a randomized complete block design, with four replications. The genetic parameters were estimated by the REML/BLUP method; and the simultaneous selection of productivity, stability, and adaptability of the genotypes was based on harmonic means of relative performances of predicted genotypic values (HMRPPGV). The Amélia, Angelina, and Tainá cultivars stood out for yield. The Regiane and Elisa cultivars presented significant responses in yield and number of leaves and are the most suitable for growing in the environmental conditions of Mossoró, RN, Brazil. The Crespa and Mimosa groups presented no stability and adaptability to the environmental conditions in which the experiments were conducted. More genotypes of all lettuce groups should be evaluated, and breeding programs should be developed to obtain genotypes with higher productivity, quality, and resistance to heat for semiarid regions.

Keywords: *Lactuca sativa* L. Mixed models. Productivity. Plant breeding.

ADAPTABILIDADE E ESTABILIDADE FENOTÍPICA DE CULTIVARES DE ALFACE EM REGIÃO DE CLIMA SEMIÁRIDO

RESUMO - O estudo de genótipos, em diferentes ambientes ou épocas de cultivo, tem grande importância para a cultura da alface, cuja expressão do potencial produtivo é influenciada pelas condições ambientais. Deste modo, a aplicação da metodologia de modelos mistos (REML/BLUP) tem sido utilizada para avaliar os efeitos da interação genótipo x ambiente. Assim, o objetivo do presente trabalho foi estimar os parâmetros de adaptabilidade e estabilidade fenotípica que estão relacionados a produção de cultivares de alface em região de clima semiárido utilizando modelo misto. Avaliaram-se doze cultivares de alface dos grupos Americana (Angelina, Amélia, Tainá), Crespa (Scarlet, Vera, Isabela, Vanda), Mimosa (Mila, Mimosa, Lavínia) e Lisa (Elisa e Regiane). O delineamento utilizado foi em blocos casualizados completos, com quatro repetições. Os parâmetros genéticos foram estimados pela metodologia REML/BLUP e a seleção simultânea da produtividade, estabilidade e adaptabilidade dos genótipos, baseou-se no método da média harmônica da performance relativa dos valores genotípicos preditos (MHPRVG). As cultivares Amélia, Angelina e Tainá sobressaíram-se para a produtividade. As cultivares Regiane e Elisa foram responsivas para o número de folhas e produtividade, sendo as mais indicadas para o cultivo nas condições de Mossoró. Os grupos Crespa e Mimosa não foram adaptados e estáveis nas condições ambientais em que foram conduzidos os ensaios. Recomenda-se que mais genótipos de todos os grupos de alface sejam testados e que, sobretudo, programas, para obter genótipos com maior resistência ao calor, produtivos e com boa qualidade, sejam desenvolvidos em regiões semiáridas.

Palavras-chave: *Lactuca sativa* L. Modelos mistos. Produtividade. Melhoramento.

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) is widely cultivated throughout Brazil in practically all seasons of the year. This is possible due to some intrinsic characteristics of this species, such as short cycle, possibility of successive crops, and safe marketing, which makes it one of the preferred crops by vegetable producers (FILGUEIRA, 2008).

Despite widely grown in Brazil, lettuce yield is low in the Northeast region of the country when compared to mild climate regions, mainly due to lack of research and development of cultivars that are adapted to the region. Thus, evaluating cultivars under different environmental and climatic conditions is important to identify the most promising genotypes.

However, the genotype-environment interaction is commonly found when evaluating cultivars in different environmental conditions. This is explained by the different response of genotypes to different environments (YAN et al., 2007). Genotype -environment interaction affects phenotypic manifestation and may hinder plant breeding and the recommendation of cultivars from the selection process (GUIMARÃES et al., 2016).

Few studies on genotype-environment interaction in lettuce are found (GUALBERTO; OLIVEIRA; GUIMARÃES, 2009; QUEIROZ et al., 2014). Despite their importance, the sole analysis of the genotype-environment interaction does not provide information about the response of a genotype in different environmental conditions. Adaptability and stability analyses are necessary to assist the recommendation of the most adequate genotypes for a given region.

Several methods for the study of adaptability and production stability of genotypes were proposed (NUNES et al., 2011b), such as AMMI (Additive Main effects and Multiplicative Interaction) and GGE Biplot (Genotype main effects and Genotype-Environment interaction), which explain the main effects of the genotype-environment interaction (SILVA et al., 2011). Mixed models have been used in studies of several subjects related to plant breeding, including genotype- environment interaction.

Analysis through the mixed model methodology can include the REML/BLUP (Restricted Maximum Likelihood/Best Linear Unbiased Prediction) to estimate variance components required by the model, making better non-biased linear predictions to obtain the genotypic value (RESENDE, 2007).

An alternative is to include the harmonic means of relative performances of predicted genotypic values (HMRPPGV) in the mixed models, as reported by Resende (2004). This method has been used in studies of different crops, but there are no reports of its use with lettuce. The HMRPPGV method shows the genotype adaptability, stability, and productivity, simultaneously, in a single measure on the scale of the assessed character. The model adjusts the effects of environments, and blocks within environments in the vector of fixed effects, encompassing all degrees of freedom available in the sources of variation referring to environments and to blocks within environments. Thus, data from all environments are used simultaneously for the predicted genotypic values obtained for a given genotype in each environment. Thus, the random effects (genotypes and genotype-environment interaction) are predicted with greater precision, and the interaction noises are eliminated by the BLUP (RESENDE, 2007).

In this context, the objective of the present work was to estimate parameters of adaptability, and the phenotypic stability in lettuce cultivars grown in a semiarid region of Brazil, using a mixed model.

MATERIAL AND METHODS

Six experiments were carried out in Mossoró, Rio Grande do Norte (RN), Brazil (5º11'S, 37º20'W, and altitude of 18 m). The region has a BSh, semiarid, dry, very hot climate, according to the Köppen classification. The soil of the experimental area was classified as abrupt eutrophic Red-Yellow Argissolo of loamy sand texture.

A randomized complete block design was used in all experiments, with four replications. Twelve lettuce cultivars from the Americana (Angelina, Amélia, and Tainá), Crespa (Scarlet, Vera, Isabela, and Vanda), Mimosa (Mila, Mimosa, and Lavínia), and Lisa (Elisa and Regiane) groups were evaluated.

The field experiments were conducted in between February/2012 and September/2013, during different seasons—summer (02/2012), autumn (05/2012), summer (12/2012), autumn (03/2013), winter (06/2013), and spring (09/2013).

Seedlings were produced in 200-cell expanded polystyrene trays and transplanted when they had four to six leaves.

The plots consisted of beds (0.20 m height, 1.20 m width, and 1.85 m length) with five plant rows, with spacing of 0.20 m between rows and 0.20 m between plants. The evaluation area of each plot (0.84 m^2) consisted of the three central rows, excluding the plants in the ends of the rows.

Soil samples were collected from the 0-20 cm layer for chemical analysis. Planting fertilization was carried out with 40 kg ha^{-1} of N, 60 kg ha^{-1} of P_2O_5, and 30 kg ha^{-1} of K_2O (urea, simple superphosphate, and potassium chloride, respectively) and an organic fertilizer (23 Mg ha^{-1}) consisting of bovine and chicken manures.

Topdressing fertilization consisted of urea (40 kg ha^{-1} of N) was carried out at 15 days after transplanting (DAT). Two foliar fertilization were performed at 10 and 20 DAT, with applications of Rizammina 420 (Biolchim, São Paulo, Brazil)—13% N, 8% P_2O_5, 21% K_2O, 2% Mg, 5.5% S, 0.03% B, 0.05% Cu, 0.2% Fe, and 0.1% Zn.

The plants were harvested when they presented a commercial pattern—at maximum vegetative development and with no signs of flowering. Their roots and external leaves were separated from the aerial part.

Four plants of the evaluation area were sampled at harvesting to determine plant diameter and height, number of leaves per plant, and shoot dry weight, which was determined by drying the samples in a forced air circulation oven at 65 °C until constant weight. The average plant weight was determined; and plant yield (Mg ha^{-1}) was based on the plant fresh weight of the evaluation area.

The 54 statistical model of the Selegen-Reml/ Blup software (RESENDE, 2007) was used to evaluate the adaptability and stability of the studied lettuce cultivars. This model is represented by the equation $y = Xb + Zg + Wc + e$, wherein *y, b, g, c,* and *e* are, respectively, the means of the blocks through the environments (fixed vectors), genotype effects (random), effects of the genotype-environment interaction (random), and random errors; and *X, Z,* and *W* are the incidence matrices for *b, g,* and *c*, respectively. The distributions and structures of means (E) and variances (Var) were:

$$E\begin{bmatrix} y \\ g \\ c \\ e \end{bmatrix} = \begin{bmatrix} Xb \\ 0 \\ 0 \\ 0 \end{bmatrix}; \; Var\begin{bmatrix} g \\ c \\ e \end{bmatrix} = \begin{bmatrix} I\sigma_g^2 & 0 & 0 \\ 0 & I\sigma_c^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

The model was adjusted from the mixed model equations as:

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + I\lambda_1 & Z'W \\ W'X & W'Z & W'W + I\lambda_2 \end{bmatrix} \times \begin{bmatrix} \hat{b} \\ \hat{g} \\ \hat{c} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix},$$

with $\lambda_1 = \frac{\sigma_e^2}{\sigma_g^2} = \frac{1-h_g^2-c^2}{h_g^2}$, wherein $h_g^2 = \frac{\sigma_g^2}{\sigma_g^2+\sigma_c^2+\sigma_e^2}$ is the individual heritability in the broad sense in the block; $c^2 = \frac{\sigma_c^2}{\sigma_g^2+\sigma_c^2+\sigma_e^2}$ is the coefficient of determination of the effects of the genotype-environment interaction; σ_g^2 is the genotype variance among the lettuce genotypes; σ_c^2 is the variance of the genotype-environment interaction; σ_e^2 is the residual variance between plots; $r_{gloc} = \frac{\sigma_c^2}{\sigma_g^2+\sigma_c^2} = \frac{h_g^2}{h_g^2+c^2}$ is the genotypic correlation of the genotypes through the environments.

The interactive estimators of variance components, by REML via the EM algorithm, are:

$$\hat{\sigma}_e^2 = \frac{[y'y-\hat{b}'X'y-\hat{g}'Z'y-\hat{c}W'y]}{[N-r(x)]}; \; \hat{\sigma}_g^2 = \frac{[\hat{g}'\hat{g}+\hat{\sigma}_e^2 tr\, C^{22}]}{q}; \; \hat{\sigma}_c^2 = \frac{[\hat{c}'c+\hat{\sigma}_e^2 tr\, C^{33}]}{s};$$

Wherein C^2 and C^3 are from

$$C^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} \\ C_{21} & C_{22} & C_{23} \\ C_{31} & C_{32} & C_{33} \end{bmatrix}^{-1} = \begin{bmatrix} C^{11} & C^{12} & C^{13} \\ C^{21} & C^{22} & C^{23} \\ C^{31} & C^{32} & C^{33} \end{bmatrix},$$

and *C* is the matrix of the coefficients of the mixed model equations; *tr* is the matrix trace operator; *r(x)* is the rank of the matrix *X*; *N* is the total number of data, *q* is the number of genotypes, and *s* is the number of genotype-environment combinations.

This model was used to obtain the eBLUP or REML/BLUP predictors of genotypic values free from interaction, which were obtained by $\hat{\mu} + \hat{g}_i$, wherein $\hat{\mu}$ is the mean of all environments and $\hat{g}_i$ is the genotypic effect free from the genotype-environment interaction. The genotypic values (Vg) are predict for each environment *j* by $\hat{\mu}_j + \hat{g}_i + (\hat{g}e)_{ij}$, wherein $\hat{\mu}_j$ is the mean of the environment *j*, $\hat{g}_i$ is the genotypic effect of the genotype *I* in the environment *j*, and $(\hat{g}e)_{ij}$ is the effect of the genotype -environment interaction that corresponds to the genotype *i*.

The prediction of genotypic values by capitalizing the average interaction (gem) in the different environments is given by $\hat{\mu}_j + \hat{g}_i + (\hat{g}e)_m$,

which is calculated by: $\hat{\mu} + \frac{\left(\hat{\sigma}_g^2 + \frac{\hat{\sigma}_c^2}{n}\right)}{\hat{\sigma}_g^2}\hat{g}_i$, wherein $\hat{\mu}$ is the overall mean of all environments; *n* is the number of environments, and $\hat{g}_i$ is the genotypic effect of the genotype *i*.

The simultaneous selection for productivity, stability, and adaptability of the evaluated lettuce genotypes was based on the harmonic means of relative performances of predicted genotypic values (HMRPPGV), according to the equation $HMRPPGV_i = \frac{n}{\sum_{j=1}^{n}\frac{1}{Vg_{ij}}}$ wherein *n* is the number of sites where the genotype *i* was evaluated, and Vg_{ij} is the genotypic value of the genotype *i* in the environment *j*, which is expressed as the ratio of the mean of that environment.

RESULTS AND DISCUSSION

The differences among genotypes are, in general, reduced at the final stages of breeding programs, thus, high accuracy is necessary to detect these differences. Coefficient of variation (CV) is the most used measure to compare the experimental precision. The estimates of the present study were within the range found in other studies evaluating lettuce cultivars (RODRIGUES et al., 2008; GUALBERTO; OLIVEIRA; GUIMARÃES, 2009; QUEIROZ et al., 2014; ZUFFO et al., 2016).

Selective accuracy has been used to evaluate experimental precision when using mixed models. In addition to the coefficient of variation, this parameter includes information on the number of replicates and the coefficient of genotypic variation. According to the classification presented by Resende and Duarte (2007), the accuracy found in the present study for yield and number of leaves was very high (≥0.86), indicating high precision (Table 1). High accuracies indicate high correlation between predicted and true genotypic values. The present work reports the first estimates of accuracy for experiments with lettuce grown in semiarid conditions and can be useful to design experiments with lettuce in the agricultural region encompassed by the municipalities Mossoró and Assu, RN, Brazil.

Table 1. Deviance, likelihood ratio test (LRT), and estimates of components of variances via REML (Var), and genetic parameters of yield and number of leaves of lettuce cultivars growing in six different environmental conditions.

Effect	Yield (Mg ha^{-1})			Number of leaves		
	Deviance	LRT	Var	Deviance	LRT	Var
Complete model	974.53			1057.31		
Genotype (G)	985.34^{+}	10.81**	4.16	1132.72^{+}	75.41**	33.39
G×E interaction	1037.15^{+}	62.62**	6.86	1083.35^{+}	26.04**	4.78
Residue			7.59			10.88
Phenotypic			18.61			49.05
h^2_{mg}			0.74			0.96
Ac_g			0.86			0.98
c^2			0.37			0.10
$\hat{r}_{gloc}$			0.38			0.88
CV_g			17.63			29.91
CV_e			23.82			17.08
Mean			11.57			19.32

E = environment; LRT (χ^2) = tabulated Chi-square of 3.84 and 6.63 for the significance levels of 5% and 1%, respectively.; + Deviance of the model adjusted without the respective effect; Values in parenthesis are the likelihood ratio (LRT); **and* = significant at 1% and 5%, respectively, by the Chi-square test with 1 degree of freedom, ^{ns} = not significant.

A significant effect (p<0.05) was found for the yield and number of leaves in the evaluated lettuce cultivars (Table 1), indicating presence of heterogeneity among the evaluated genotypes. Considering that the evaluated cultivars are from distinct lettuce groups, differences between them were expected. Moreover, the estimates of heritability corroborate the variability among the cultivars. The average heritability found in the present work was estimated when using the averages of the blocks as a selection criterion (RESENDE, 2002). The yield and number of leaves found were high. Heritability is the ratio between genetic variance and phenotypic variance; the greater the heritability, the greater the reliability of the selection of cultivars based on predicted genotypic values.

Genotype-environment (G×E) interaction was found for these two variables (yield and number of leaves) in all evaluated hybrid groups (Table 1). Presence of interaction indicates different responses of hybrids to different environment conditions (TORRES et al., 2015). G×E interaction is essential in experiments evaluating cultivars and can influence the genotype selection process. The pragmatic consequence is the difficulty in the selection process of promising genotypes. G×E interaction in lettuce has been found in some studies (GUALBERTO; OLIVEIRA; GUIMARÃES, 2009; LUZ et al., 2009; VIANA et al., 2013; QUEIROZ et al., 2014; BLIND; SILVA FILHO, 2015).

In addition to detecting G×E interaction, estimating its magnitude on the phenotypic manifestation is important, since significant G×E interaction denotes the need for evaluation of genotypes in different environments to surely select the best ones. The c^2 component shows how much of the phenotypic variance is explained by the G×E interaction. The G×E interaction had more effect on lettuce yield because it explained 37% of the phenotypic variance, whereas it explained only 10% of the variation of number of leaves (Table 1). The significance of the G×E interaction found in the present work was higher than those found in other studies (GUALBERTO; OLIVEIRA; GUIMARÃES, 2009; QUEIROZ et al., 2014); in addition, all the experiments of the present work were conducted in open field, whereas these other studies usually were conducted in greenhouse and field conditions (QUEIROZ et al., 2014) or in hydroponic system (GUALBERTO; OLIVEIRA; GUIMARÃES, 2009).

G×E interaction is explained by two components; the first (simple part) represents the magnitude of the variability among genotypes, and the second (complex part) depends on the genetic correlation among genotypes in the environments (CRUZ; CASTOLDI, 1991). The average genotypic correlation of performances of the cultivars in the environments (r_{loc}) shows how constant is the ordering of the cultivars and, therefore, measures the complex interaction. Corroborating with estimates of the c^2 component, the estimate of r_{loc} was low for yield (0.38) and high for number of leaves (0.88) (Table 1), indicating a predominance of the complex component of G×E interaction for yield and predominance of the simple component for number of leaves. Gualberto, Oliveira and Guimarães (2009) evaluated lettuce cultivars in a hydroponic system under protected environment and found no predominance of the complex component in yield and number of leaves.

The growing of a cultivar in different environments is important in researches. The identification of productive materials with greater stability and adaptability in the evaluated group can attenuate the G×E interaction. The HMRPPGV-BLUP technique developed by Resende (2004, 2007) allows the incorporation of stability, adaptability, and the mean of the character of interest in a single statistic from the genotypic data.

The harmonic mean of genotypic values (HMGV) allows the selection based on the stability and productivity. According to these criteria, the Angelina, Amélia, and Tainá lettuce cultivars (Americana group) stood out for yield, followed by Elisa (Lisa group). Scarlet and Vanda (Crespa group) had the lowest HMGV estimates (Table 2). Elisa and Regiane (Lisa group) stood out for number of leaves, followed by Mila and Lavínia cultivars (Mimosa group).

Table 2. Stability (HMGV) and adaptability (RPGV) of genotypic values for yield and number of leaves of lettuce cultivars grown in six different environmental conditions.

Hybrids	Yield (Mg ha^{-1})			Number of leaves		
	HMGV	RPGV	RPGV*MG	HMGV	RPGV	RPGV*MG
Vera	7.85	0.78	9.01	13.26	0.69	13.36
Vanda	8.52	0.84	9.67	14.87	0.78	14.98
Isabela	8.89	0.88	10.20	17.08	0.90	17.31
Scarlet	6.97	0.69	8.01	18.39	0.96	18.53
Mimosa	7.51	0.87	10.08	18.21	0.95	18.35
Mila	10.37	1.06	12.21	19.32	1.01	19.48
Lavínia	8.85	0.96	11.08	19.37	1.02	19.79
Angelina	13.22	1.31	15.15	17.21	0.92	17.81
Amélia	13.23	1.33	15.39	15.57	0.81	15.70
Tainá	11.33	1.13	13.12	14.34	0.75	14.51
Elisa	11.13	1.08	12.49	30.54	1.60	30.87
Regiane	10.88	1.07	12.38	30.90	1.61	31.11

Adaptability is the positive response of a genotype to environmental improvements (MARIOTTI et al., 1976). This characteristic is evaluated using relative performance of the genotypic values (RPGV), which capitalizes the response capacity of each lineage to environment improvements. The lettuce cultivars that stood out for yield were Angelina, Amelia, and Tainá (American group), followed by Elisa and Regiane (Lisa group), and Mila (Mimosa group) (Table 2). The number of leaves had similar results to those found through HMGV.

The harmonic means of relative performance of genotypic values (HMRPPGV) is based on genotypic values predicted via mixed models, which groups stability, adaptability, and productivity in a single statistic evaluation, facilitating the selection of superior genotypes (RESENDE, 2007). The HMRPPGV*MG provides the genotypic values of each genotype penalized by the instability and capitalized by the adaptability in the unit of measure of the character. The Amélia, Angelina, and Tainá cultivars (Americana group) stood out for yield; they had, on average, 1.31, 1.28, and 1.11-fold the average of the environment where they were cultivated. However, these cultivars were not responsive to number of leaves, since their HMRPPGV were lower than the unit (Table 3).

Table 3. Stability and adaptability of harmonic means of relative performance of genotypic values (HMRPPGV) for yield and number of leaves of lettuce cultivars grown in six different environmental conditions.

Hybrids	Yield (Mg ha^{-1})		Number of leaves	
	HMRPPGV	HMRPPGV*VG	HMRPPGV	HMRPPGV*VG
Vera	0.76	8.75	0.69	13.32
Vanda	0.82	9.49	0.77	14.95
Isabela	0.87	10.10	0.89	17.24
Scarlet	0.69	7.98	0.96	18.52
Mimosa	0.79	9.17	0.95	18.30
Mila	1.02	11.81	1.00	19.41
Lavínia	0.90	10.37	1.01	19.56
Angelina	1.28	14.76	0.89	17.26
Amélia	1.31	15.15	0.81	15.67
Tainá	1.11	12.80	0.75	14.46
Elisa	1.04	12.08	1.59	30.73
Regiane	1.06	12.20	1.61	31.10

According to the HMRPPGV criterion, the Regiane and Elisa cultivars (Lisa group) were very responsive in number of leaves, with 1.61 and 1.59-fold the average of the environment in which they were grown, respectively, and they responded to a lesser degree to yield, with 1.06 and 1.04-fold the average of the environment in which they were grown, respectively. Thus, these cultivars were the most adapted and stable in the tested group; therefore, they are the most suitable cultivars for the environmental conditions of Mossoró, RN, Brazil.

Considering both evaluated variables, the lettuce cultivars of the Crespa and Mimosa groups presented no stability and no adaptation to the environmental conditions of the experiment. This low performance occurred because the cultivars evaluated in the present study were not selected for high temperature conditions. The Mossoró region has high temperatures throughout the year, with average of 31.24 °C and maximum above 40 °C (NUNES et al., 2011a). The results showed the difficulties in identifying cultivars adapted to high temperatures, denoting that the development of lettuce cultivars to semiarid regions is a great challenge, since this plant species is originally suitable for mild climate with temperatures between 15 and 20 ºC.

CONCLUSIONS

The Amélia, Angelina, and Tainá lettuce cultivars had the best results for yield.

The Regiane and Elisa cultivars had positive responses for yield and number of leaves and are the most recommended for growing on the environmental conditions of Mossoró, RN, Brazil.

The Crespa and Mimosa groups showed no stability and no adaptability to the environmental conditions of the experiments.

More genotypes of all lettuce groups should be evaluated, and breeding programs should be developed to obtain more productive genotypes with good quality and high resistance to heat for growing in semiarid regions.

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