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RESEARCH ARTICLE

ANALYSIS OF MEANS (ANOM) – A STATISTICAL METHOD FOR BETTER VISUALIZATION OF RESULTS OF MULTI-ENVIRONMENT TRIAL DATA OF VARIETIES

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Abstract

Multi-Environment trials (MET) involve testing of new entries or genotypes along with the check entries in several locations/ environments and plant breeders select new entries on the basis of high mean across environments and high stability. Significant G x E interaction as indicated in a pooled analysis of variance (ANOVA) can help in estimating stability variances of individual entries based on which superior entries can be recommended by combining mean and stability variance as a criterion (Shukla, 1972;Eskridge, 1990; Kang 1993). More advanced methods involving biplot analysis of G x E data have been recently followed by many breeders in advanced stages of selection, though they require careful interpretation. As an alternative to these statistical methods, this article explains the utility of a technique known as Analysis of Means (ANOM in short) in analyzing the results of multi-environment breeding trials. The method is very similar to ANOVA in concept but more useful in visualization of the main effects and the interaction effects by depiction of the results through control charts with decision lines. The results can be easily understood and interpreted even by non-statisticians. The method can be used as a first step in initial stages of selection in identifying superior entries and can further supplement the results of procedures dealing with stability analysis in Advanced Variety Trials.

Key words: ANOM, ANOVA, Control charts, Decision lines, Safety-first index, MET, Shukla's Stability Variance.

Introduction

Analysis of multi-environmental trials (MET) of crops for evaluation of cultivars is an important aspect of research in plant breeding. The technique of analysis of groups of experiments proposed by Yates and Cochran (1938) led to extensive research on the methodology of recommending the best of cultivars using stability of cultivar performance as an important criterion. A plethora of stability parameters has been propounded by various authors with different objectives and it is not easy to judge which method is best for a given situation. Lin et al. (1986) provided a broad based review of these methodologies as a guideline for selecting a good stability parameter from among the several measures. More recently biplot analysis of genotype x environment data (Yan and Kang 2002; Gauch 2006; Gauch et al. 2008) has gained popularity among plant breeders. Nevertheless, plant breeders need a better visualization of the immediate data on hand arising out of a MET before venturing into any one of the stability analysis methods.This article examines the potential usage of a procedure known as Analysis of Means (ANOM) in analyzing data from MET for better visualization and interpretation of the results. ANOM can be used as a first step

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before a more exhaustive analysis is taken up for evaluation of cultivar performance using a stability parameter.

ANOM is a graphical decision tool for comparing a set of multiple group means with respect to their overall average and can be used as an alternative to an analysis of variance (ANOVA). In an ANOVA, a significant F-test just indicates that the means are statistically different, but it does not reveal where the differences are coming from, unless one goes for further tests for comparison of means either pair-wise or simultaneously. By contrast, the ANOM provides a "confidence interval type of approach" that allows the investigator to determine which, if any, of the entries or treatments has a mean significantly different from the overall average of all the entry means combined. Instead of looking at the lower and upper limits of a confidence interval of each entry or treatment, one will be studying which of the entry means are not contained in an interval formed between a *lower decision line* and an *upper decision line*. Any individual entry mean not contained in this interval is deemed significantly higher than the overall average of all entries if it lies above the upper decision line (Fig.1). Similarly, any entry mean that falls below the lower decision line is declared significantly lower than the overall average. The term "Analysis of means" was introduced by Ellis Ott (1967) and was suggested as a tool for statistical quality control. It became popular during the early 1980s, when it was applied to experimental data in manufacturing. Nelson (1982, 1993) provided exact critical values for ANOM tests. Nelson *et al*. (2005) provide details on the concepts and applications of ANOM in experimentation.

In circumstances in which one might use ANOVA to analyze fixed main effects, ANOM is equally appropriate and generally produces a more useful result. While ANOM can be used to study main effects and interactions as well, its main advantages occur when it is used to study main effects. When studying main effects, ANOM is more advantageous than ANOVA: (1) if any of the treatments are statistically different, ANOM indicates exactly which ones are different; and (2) ANOM can be presented in a graphical form, which allows one to easily evaluate both the statistical and the practical significance of the differences. Typically while testing the main effects of varieties / genotypes in plant breeding trials, ANOM can produce results that are easier to visualize and interpret in comparison to results produced by pair-wise comparisons or multiple range tests where overlapping of groups of variety means often leads to difficulties in interpretation of the results. An advantageous feature of ANOM is that the decision chart is easy for non-statisticians to understand and ANOM makes assessing practical significance easy.

With continuous data, the ANOM procedure is based on two assumptions:

(i) The data are at least approximately normally distributed.

(ii) The different treatments have the same variance.

Under the assumption of same variance for the levels of a factor (say, varieties), we need to only check if the factor has an effect on the means. That is, one would test

$H_0 : \mu_1 = \mu_2 = \cdots = \mu_I$ versus the alternative hypothesis that at least one of the μ_i is different.

Using the ANOM to test this hypothesis not only answers the question of whether there are any differences among the factor levels but, when there are differences, it also indicates how the treatment levels differ. The idea of the Analysis of Means is that if H_0 is true, the I factor levels all have the same population mean. Therefore, all the $\boldsymbol{y}_{i\cdot}$ (mean for factor level $\boldsymbol{i}$) should be close to the overall mean $\boldsymbol{y}_{\cdot\cdot}$. So we will reject H_0 if any one of the $\boldsymbol{y}_{i\cdot}$ is too far away from $\boldsymbol{y}_{\cdot\cdot}$.

The results of ANOM are presented through control charts, wherein a central horizontal axis is constructed corresponding to overall average of all the treatments; then vertical bars are constructed for each treatment mean; and the upper and lower decision lines are drawn on either side of the central axis across the vertical bars representing the treatment means (see Fig.1). Any treatment for which the mean value falls outside these decision lines is inferred to be significantly superior (if the vertical bar is above the central axis and upper decision line) or inferior (if the vertical bar is below the central axis and lower decision line). Treatments, whose mean values lie between the upper and lower decision lines are inferred to be on par with the overall mean.

In the case of single factor ANOM, say when we want to compare only one factor i.e Entries or genotypes, the upper and lower decision lines are computed as follows:

$$UDL = \overline{Y..} + h(\alpha; I, \gamma)\sqrt{MSe * \frac{I-1}{N}}$$

$$LDL = \overline{Y..} - h(\alpha; I, \gamma)\sqrt{MSe * \frac{I-1}{N}}$$

The critical values ***h(α; I, ν)*** termed as *Nelson's h statistic* (Nelson, 1982) depend on

α = the level of significance desired, say 0.05
I = the number of means being compared,
N = total number of observations
ν = the degrees of freedom for MS*e*, the Mean Square Error.

They are derived based on the joint distribution of the $|y_{i\cdot} - y_{\cdot\cdot}|$, which is an equi-correlated multivariate *t*- distribution with correlations $\rho = -1/(I - 1)$ (Nelson (1982, 1993). Tables of critical values of Nelson's statistic are available readily in Nelson *et al.,* (2005). The table of critical values can be accessed also through the following web resources in 2 parts:

(a) http://www.pmean.com/07/AnomTable05Part1.html

(b) http://www.pmean.com/07/AnomTable05Part2.html

ANOM is included as a standard option in some statistical software (including SAS® and MINITAB®).

In the present investigation where our interest is on two factor ANOM (the first factor being the entries or genotypes and the second factor being environments (combination of test centers and crop-type, i.e 1st plant, 2nd plant and ratoon), the ANOM charts can be separately constructed for entries, environments and their interaction. In case of limited levels of both the factors (say, up to 5 x 5 combinations) the upper and lower decision lines can be computed using the formulas and tables (Nelson *et al*. 2005):

$$UDL = 0 + g(\alpha;(I,J), \gamma)\sqrt{MSe * \frac{2(J-1)}{Jn}}$$

$$UDL = 0 - g(\alpha;(I,J), \gamma)\sqrt{MSe * \frac{2(J-1)}{Jn}}$$

For larger levels of factor A and Factor B, the formulae are given in Appendix I. We may compute the critical values of Student's-t statistic for a given P-value and degree of freedom using any freely available on-line software.

Material and methods

A large amount of data emanating from multi-environment varietal trials conducted during the last 13 years (2000-01 to 2012-13) in sugarcane under the All India Coordinated Research Project has been considered for this investigation. The trials were conducted in four agro-climatic zones in India growing sugarcane, viz., Peninsular zone, East Coast Zone, North West and North Central & Eastern Zone. The number of locations in each zone varies, from about 13 locations in Peninsular Zone to about 5-6 locations in other zones. The experiments were conducted in Randomized Complete Block designs in these locations with a minimum of three replications. In the case of Initial Varietal Trials (IVT), the data pertains to single year, whereas in the case of Advanced Varietal Trials (AVT) the data pertains to three trials, viz., I plant crop during the 1st year followed by a 2nd crop and a ratoon crop of the 1st year. In this analysis, given ***n*** locations, we have considered the data as pertaining to 3 x ***n*** environments for the AVT experiments. In these cases, 2 factor ANOM was

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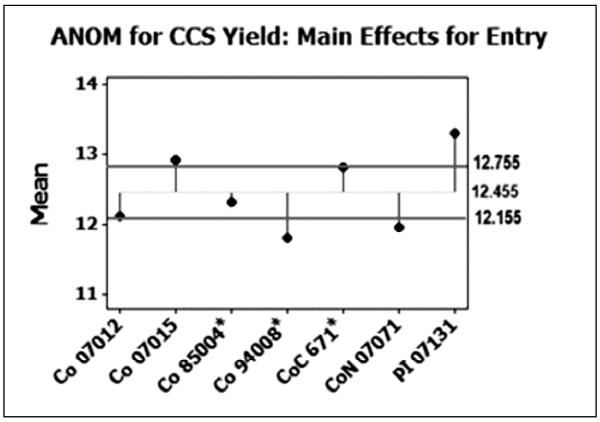

Fig.1 A typical output of ANOM bringing out the statistical significance of a single factor (Entries)

performed to bring out the main effects (Entries and Locations) as well as the interaction effect (Nelson 1988) which helped in visualizing which of the entries had positive interaction at a given test site and which of the entries had a negative interaction. The data were analyzed using Minitab Statistical software that has a module for ANOM analysis. The data were also subjected to stability analysis by evaluating Shukla's stability variance (Shukla 1972), Kang's mean-stability ranking (Kang 1993) and safety-first index of Eskridge (Eskridge 1990).

Results and discussion

Fig.1 displays a typical output of the main effect (entries) results from a MET in sugarcane under the All India Coordinated Research Project. The data pertains to Advanced Variety Trial (AVT - 1st plant crop of 2011-12; 2nd Plant crop of 2012-13 and ratoon crop of 2012-13) pooled from individual trials conducted in Randomized Complete Block designs across 13 locations with three replications in each location. Of course, a 2-factor ANOM would be more appropriate if the G x E interaction is significant (see Fig.2) in clearly bringing out the significance of main effects as well as the interaction effects. From Fig.1 it is very easy to visualize the results and interpret that the CCS (commercial cane sugar) yield of Co 07015 and PI 07031 (test entries), CoC 671 (a check variety) are significantly higher than the overall mean across locations, whereas the performance of test entries Co07012 and CoN 07071is significantly lower than the overall mean apart from the check entry Co 94008. Other entries performed on par with the overall mean and hence did not differ significantly among themselves.

Fig. 2 presents a typical output from data of AICRP 2008-10 AVT Early, North Central Zone (pooled

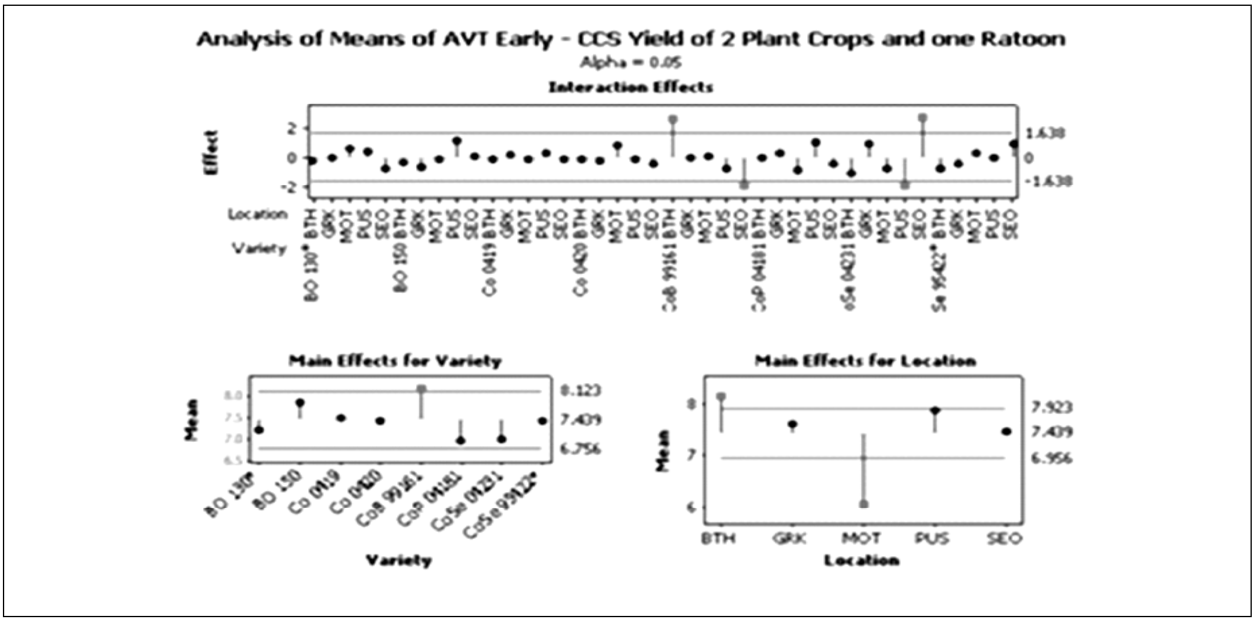

Fig.2 A typical output of ANOM for main effects (entries and locations) and their interaction

from 1st plant crop of 2008-09, 2nd Plant crop of 2009-10 and ratoon of 2009-10). Mean CCS Yield for test entry CoB 99161 was significantly higher than the overall mean across five locations and two plant crops and one ratoon. The mean CCS yield at Bethuadhari was significantly higher than the overall mean. CCS yield at Motipur, was significantly lower than the overall mean across two plant crops and oneratoon. The Interaction was positive for CoB-99161 at Bethuadhari but negative at Seorahi, whereas for CoSe 04231 it was positive at Seorahi but negative at Pusa.

In order to compare the evaluation of entries using stability measures and ANOM procedure, pooled data from AICRP varietal trials were subjected to Kang's method of mean and stability rankings (Kang 1993), Safety-first index ranking of Eskridge (1990) and ANOM procedure. The pooled error mean squares were estimated from the pooled ANOVA and Shukla's stability variance (Shukla 1972) was used in Kang's method and Safety-first index method. Table 1 provides a sample result for CCS yield (t/ha) and cane yield (t/ha) for the AICRP trials during 2011-13. The results suggest that, in general, whenever the rankings as per Kang's method and Safety-first index were in the top order (1 to 3) for a genotype, one can check if ANOM also indicated a positive significant effect for the genotype over the overall mean. In Table1, though CoC 671 had a rank of 1 on safety-first index for cane yield, the results were negative from the point of view of Kang's method and ANOM. In such cases (setting aside check entries like the one above), the breeder may opt out in recommending such entries.

Performance of locations

While analyzing the results of METs, plant breeders in general are more concerned with one main effect, namely entries (genotypes) and the interaction of entries x environments, to identify entries that are better adapted to a given location or environment. However, not much attention is paid to the 2nd main

Table1. Evaluation of genotypes as per stability measures and ANOM (AICRP 2011-13, Peninsular, AVT Early pooled across 2 plant crops and 1 ratoon in 13 locations)

Entry / genotype	CCS Yield				Cane Yield			
	CCS Yield (t/ha)	Kang's method (Select or not)	ANOM Sig 5% level	Safety 1st Index Rank	Cane Yield (t/ha)	Kang's method (Select or not)	ANOM Sig 5% level	Safety 1st Index Rank
Co 07012	12.10	No	NS	6	94.12	No	NS	6
Co 07015	12.92	Yes	+VE	2	96.51	Yes	NS	4
CoN 07071	11.96	No	-VE	7	91.69	No	-VE	7
PI 07131	13.29	Yes	+VE	3	101.42	Yes	+VE	3
CoC 671*	12.81	Yes	+VE	1	93.64	No	NS	1
Co 94008*	11.80	No	-VE	4	94.19	Yes	+VE	2
Co 85004*	12.30	No	NS	5	92.25	No	NS	5
Mean	12.45				94.83			

CCS Yield: Upper Critical Limit for ANOM at 5% level = 12.755
Lower Critical Limit for ANOM at 5% level = 12.155

Cane Yield: Upper Critical Limit for ANOM at 5% level = 96.97
Lower Critical Limit for ANOM at 5% level = 92.69
NS: Not significantly different from overall mean

Entries marked with '*' indicate check varieties

Table 2. Performance of test centers in Peninsular Zone during 2000-2011 in relation to the overall mean in each trial

	CCS Yield				Cane Yield		
Location	% trials above overall mean	% trials on par with overall mean	% trials below overall mean	Location	% trials above overall mean	% trials on par with overall mean	% trials below overall mean
AKL	11.76	23.53	64.71	AKL	18.75	25.00	56.25
BSM	40.00	26.67	33.33	BSM	18.75	50.00	31.25
CBE	45.16	38.71	16.13	CBE	43.75	43.75	12.50
KLP	12.12	54.55	33.33	KLP	3.13	34.38	62.50
MND	25.00	67.86	7.14	MND	24.14	65.52	10.34
NAV	27.27	57.58	15.15	NAV	41.18	41.18	17.65
PAD	40.63	53.13	6.25	PAD	17.65	79.41	2.94
PRV	18.18	63.64	18.18	PRV	17.65	64.71	17.65
PUG	47.37	52.63	0.00	PUG	60.00	30.00	10.00
PUN	83.33	16.67	0.00	PUN	56.25	34.38	9.38
RUD	5.26	78.95	15.79	RUD	5.26	52.63	42.11
SMW	6.25	31.25	62.50	SMW	3.13	34.38	62.50
SNK	34.38	31.25	34.38	SNK	36.36	51.52	12.12
TVL	3.03	63.64	33.33	TVL	2.94	85.29	11.76

AKL: Akola; BSM: Basmathnagar; CEB: Coimbatore; KLP: Kolhapur; MND: Mandya; NAV: Navsari; PAD: Padegaon; PRV: Pravaranagar; PUG: Pugalur; PUN: Pune; RUD: Rudrur; SMW: Sameerwadi; SNK: Sankeshwar; TVL: Tiruvalla

effect, i.e. the locations (environments). One important aspect is that ANOM can bring out the performance of the locations also in the same chart. From Fig.2, one can easily visualize that the mean performance of the entries in Bethuadhari is significantly higher than the overall mean across all locations, whereas, the mean performance of the entries at Motipur is significantly lower than the overall mean. To emphasize the importance of location/ environment effects, the ANOM was carried out using long term data for Peninsular Zone under the AICRP on Sugarcane. The ANOM for all individual trials (IVT- Early and Midlate; AVT-Early and Midlate with 2 plant crops and one ratoon, totaling around 88 individual trials) was performed for the period 2000-01 to 2010-11 and the results are presented in Table 2.

It is clearly seen from the ANOM results for CCS yield that two centers namely, Akola (AKL) and Sameerwadi (SMW) had performed significantly below the overall mean in more than 60% of the trials during the 11 year period under study. Similarly, four other centers had performed below-par in about 30% of the trials. The real reason for this may be due to prevalence of high stress factors in these centers and hence an objective assessment of the performance of entries in these centers should be taken up. In the case of Pune center, it had performed significantly better than the overall mean in more than 80% of the trials indicating a highly favorable environment in this center. Four more centers had a significantly higher CCS yield than the overall mean in about 40% of the trials. The trend was more or less similar for cane yield. Hence the performance of entries in the stress locations should be properly weighted while evaluating them in initial variety trials.

During recent times many reports have been published advocating advanced graphical methods like AMMI Biplots or GGE Biplots in selecting stable entries with high mean acrossenvironments. While ranking of entries on the basis of high mean and stability by AMMI or GGE Biplots is visibly easier, it should be noted that it does not bring out the statistical significance of the difference among top-ranked entries that are close to each other. Apart from that, interpretation of interaction effects also requires a good judgment on the part of the breeder.In comparison to the more advanced techniques, ANOM is far simpler in visualizing the results of MET even for non-statisticians and can be very useful in supplementing the results of other stability oriented methods.

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Appendix-I

Upper and lower decision limits for main effects

The calculation of the upper and lower decision limits varies based on the number of levels in the factor and the number of observations at each level. The formulas below show the upper and lower decision limits for factor A. To calculate the decision limits for factor B, replace terms specific to factor A with equivalent terms for factor B.

$UDL_A = y_{...} + h_\alpha * Sqrt[MSE * (a - 1) / (a * n_1)]$

$LDL_A = y_{...} - h_\alpha * Sqrt[MSE * (a - 1) / (a * n_1)]$

where h_α = absolute value (t(α/2; abn - ab), MSE = mean square error (from an ANOVA with terms A, B, and AB), a = number of factor levels in factor A, and n_1 = number of observations at each level of the factor. Tables of critical values are provided in Nelson *et al*. (2005) and also in the web pages cited in the text.

Upper and lower decision limits for interaction effects

These Indicate whether the interaction is significant. Points that lie outside the upper decision limit (UDL) or lower decision limit (LDL) indicates that the interaction is statistically significant. Listed below are the general formulas for the upper and lower decision limits for the interaction of factors A and B. The terms are defined differently based on the number of levels and observations in each factor.

$UDL_{AB} = h_\alpha * Sqrt[MSE * (q/(a * b * n)]$

$LDL_{AB} = h_\alpha * Sqrt[MSE * (q/(a * b * n)]$

where h_α = absolute value (t(α2, dfe), *a* = number of levels in factor A, *b* = number of levels in factor B, *n* = number of observations for each interaction between factors, *q* = degrees of freedom for interaction effects= *(a - 1)(b - 1)* and *dfe* = degrees of freedom for error = *abn - ab.*

Factors A and B have more than two levels

$\alpha2 = [1-(1- \alpha)**(1/(a * b)] / 2$

where *a* = number of levels in factor A and *b* = number of levels in factor B and '**' indicates 'to the power of' (e.g. $x^{0.05}$). (The above formulas adopted from MINITAB help documentation).