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CHI-SQUARE TEST

Document prepared by an expert from Australia

CHI-SQUARE TEST

In DUS trials, many of the characteristics are observed by measurements such as plant height, leaf length, leaf width, flower diameter etc. These are continuous variables and are expected to follow normal distribution with μ mean and σ^2 variance. These can be in general, statistically analyzed using 'Student t criterion' or F test. However, in some cases, distinctness may be established by classifying individual varieties into broad groups and demonstrating statistically different grouping patterns for different varieties. Such examples include counts based on the flower color groups - red, pink or white etc. and the disease/pest/nematode infection classes. Data based on counts of individuals in a sample/population belonging to each of several classes require a different kind of statistical analysis. A commonly used method called *Chi-square* (χ^2) for analyzing such enumeration data.

To use the Chi-square analysis for plant breeder rights' (PBR) purposes, we should consider how we are going to arrive at certain conclusions about distinctness and stability by formulating certain hypotheses using the classification data.

The standard formula for chi-square used in such analysis is:

$$\chi^2 = \Sigma \frac{(\text{Observed value of a class - Expected value of a class })^2}{\text{Expected value}}$$

This factor by definition is *the sum of squares of independent, normally distributed variables with zero mean and unit variance.* Hence, Chi-square distribution is a continuous distribution based upon an underlying normal distribution.

Note: The following precautions are to be considered before using chi-square test.

(1) Selection of the hypothesis to be tested should be based on previously known facts or principles

(2) Given the hypothesis, you should be able to assign expected values for each class correctly. Avoid using chi-square if the smallest expected class is less than five. By increasing the sample size the size of the smallest expected value can be made larger. Alternatively, if some classes have a size less than five, pool those classes to bring the size of the pooled class to five or more than five.

(3) The number of degrees freedom to look up the chi-square table is not always obvious. *Degrees of freedom is defined as the number of classes that are independent to be assigned an arbitrary value.* For example, if we have two classes the degrees of freedom is 2-1 = 1. *Hence, in testing any hypothesis, degrees of freedom for chi-square is one less than the number of classes.*

(4) Avoid using two class situations which follow more like binomial distribution. If you encounter such situations, calculate expected values using formulae based on binomial distribution. Always use Yates Correction for determining chi-square with only one degree of freedom.

Let us examine the following data on the disease scoring of two generations of a lucerne candidate variety and its four comparator varieties. The disease scored was Coletotrichum crown rot in lucerne. The scoring was on 1-5 scale, note 1 being resistant and note 5 being susceptible.

Table: Number of plants counted in different classes in each variety after 7-10 days of inoculation

Class/Score	**Candidate Generation 1**	**Candidate Generation 2**	**Comparator 1**	**Comparator 2**	**Comparator 3**	**Comparator 4**
1	34	32	12	6	1	7
2	4	3	7	6	5	10
3	1	3	9	5	5	5
4	1	2	7	9	8	7
5	6	4	9	19	9	15
Total	**46**	**44**	**44**	**45**	**28**	**44**

It can be seen from the table that the two generations of the candidate variety have more number of plants in the resistant category than the comparators. However, to statistically test the significance of these differences, we need to formulate two hypotheses:

(1) Whether the comparator varieties differ significantly or not from the generation 1 of the candidate in the distribution of scores i.e. by testing the null hypothesis. The null hypothesis in this case is all the varieties show similar reaction to the Coletotrichum crown rot. This can be done by testing the **"distinctness χ^2"**.

(2) If the two generations of the candidate differ from one another in the distribution of scores. This can be approached by testing another null hypothesis that the two generations behave similarly to the inoculation of Colectrichum crown rot. This can be done by testing **"stability χ^2".**

The generation 1 of the candidate variety is considered as a reference variety for PBR comparisons. Hence, the distribution of scores in different classes observed for this reference variety is considered to be expected distribution. The expected values of classes 2, 3 and 4 for generation 1 of the candidate are less than 5 and it would be appropriate to pool all the values in those classes to form a new intermediary pooled class for all the varieties under consideration.

Now the observed data is reduced to:

Class/Score	**Candidate Generation 1**	**Candidate Generation 2**	**Comparator 1**	**Comparator 2**	**Comparator 3**	**Comparator 4**
1	*34*	32	12	6	1	7
2	*6*	8	23	20	18	22
3	*6*	4	9	19	9	15
Total	***46***	**44**	**44**	**45**	**28**	**44**

The distribution of expected values for different varieties are as using the distribution of the scores for the reference variety (0.74 (34/46) for class 1, 0.13 (6/46) for class 2 and 3 respectively) is as follows:

Class/Score	**Candidate Generation 1**	**Candidate Generation 2**	**Comparator 1**	**Comparator 2**	**Comparator 3**	**Comparator 4**
1	*34*	32.52	32.52	33.26	20.70	32.52
2	*6*	5.74	5.74	5.87	3.65	5.74
3	*6*	5.74	5.74	5.87	3.65	5.74
Total	***46***	**44**	**44**	**45**	**28**	**44**

The total χ^2 for the whole set of data is as follows:
χ^2 = $(34 - 34)^2/34 + \ldots (32-32.52)^2/32.52 + \ldots (12 - 32.52)^2 /32.52 + \ldots (6 - 33.27)^2/33.27 + (1 - 20.70)^2/20.70 + \ldots (7-32.52)^2/32.52 + \ldots (15-5.74)^2/5.74$
= 317.87

At v(n-1) degrees of freedom i.e., 6(2) = 12 df the table χ^2 value is 26.22 at P = 0.01. The calculated value is more than table value and hence there are significant differences among varieties for Coletotrichum crown rot (CCR). Hence, the null hypothesis that there are no significant differences in reaction to CCR among the varieties is rejected.

For calculating the "distinctness χ^2" for comparator 1

$\chi^2 = (12 - 32.52)^2/32.52 + (23 - 5.74)^2/5.74 + (9 - 5.74)^2/5.74$
= 35.1 + 12.95 + 1.18
= 49.23

The degrees of freedom for looking up the χ^2 table is one less than the number of classes i.e., 3 - 1 =2.

At P = 0.01, for 2 df, the tabular value is 9.21. The calculated distinctness χ^2 is more than table χ^2 value. Therefore, we reject the null hypothesis that the comparator variety 1 has similar reaction to the disease as that of the first generation of the candidate variety.
Similarly the calculated "distinctness χ^2" for comparator-2, comparator-3 and comparator-4 are 142.92, 402.53 and 110.79, respectively, which are all greater than the table χ^2 value of 9.21 at 2 df.

Hence, all the comparator varieties are significantly different from the generation 1 of the candidate variety in reaction to Coletotrichum crown rot.

Similarly, for calculating the **"stability χ^2"** the observed and expected values of generation 2 of the candidate variety are to be used.

Thus, "Stability χ^2" **is**

χ^2 = $(32 - 32.52)^2/32.52 + (8 - 5.74)^2/5.74 + (4 - 5.74)^2/5.74$
= 0.01 + 0.64 + 0.76
= 1.41

This should be tested again at 2 df and it turns out to be non-significant. Hence, the null hypothesis is accepted and it is concluded that the two generations of the candidate show similar reaction to Coletotrichum crown rot.

Thus, χ^2 analysis is a useful analytical tool to analyze such categorical data for PBR.

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