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REDUCING THE NUMBER OF PLANTS PER VARIETY IN THE ASSESSMENT OF DISTINCTNESS OF A SEGREGATING CHARACTERISTIC

Document prepared by experts from France

INTRODUCTION

1. Generally, qualitative characteristics have stronger genetic heritability than quantitative characteristics and therefore are more consistent from year to year. So these kind of characteristics are very good for describing and distinguishing varieties.

2. In homogeneous species, these characteristics are widely used for distinction and are often grouping characteristics. In allogamous species the qualitative characteristics could be also very useful for distinction, but the situation is different because there are several states of expression in a given variety (see a list of examples in annex 1).

3. In this case, to establish distinctness in pair-wise comparisons, we have to compare the distribution of two varieties and check if the difference is significant in 2 successive years and in the same direction. In doing this, we take into account the effect of the year.

4. These segregated characteristics should be analysed with specific statistical tests like Chi-square and Fischer exact tests. Even so, it is necessary to explore the possibility of using other tests, as agreed by the TWC at its twenty-third session.

5. That is the case of the characteristic of flower colour in Lucerne. In the DUS trials in France more than 45,000 plants are visually assessed each year and this characteristic proves to be very efficient: more than 60% of varieties of the reference collections are distinguished with this criterion.

6. This paper deals with the possibility of reducing the number of plants used in the DUS trial for Lucerne varieties. This has to be done without reducing too much efficiency of the characteristic for distinctness assessment. The final objective is to reduce the cost for the trial.

MATERIALS AND METHODS

7. The data used for this investigation has come from the French Lucerne DUS trials from 2001 to 2005. The “herbage DUS” team of GEVES La Minière (78) near Paris made the measurements.

8. There are specific trials for assessing the characteristic flower colour. This trial is composed of 240 plants per variety. The number of varieties of the reference collection varies from 109 to 182 depending on the year, so about 30,000 to 50,000 plants are observed per year. The flower colour of each plant is visually observed and the state of expression recorded as one of the colours presented in Table 1. For the purpose of the statistical analysis, these 9 notes are transformed into 4 classes. This characteristic is described in the UPOV Test Guidelines for Lucerne (TG/6/5).

Table 1: Notes and states of expression used to describe the flower colours of Lucerne varieties.

Level of expressions	Note	Classe
White	1	1
Yellow	2	1
Light Violet	3	2
Light variegated	5	4
Violet	6	2
Dark violet	7	3
Very dark violet	8	3
Dark variegated	9	4

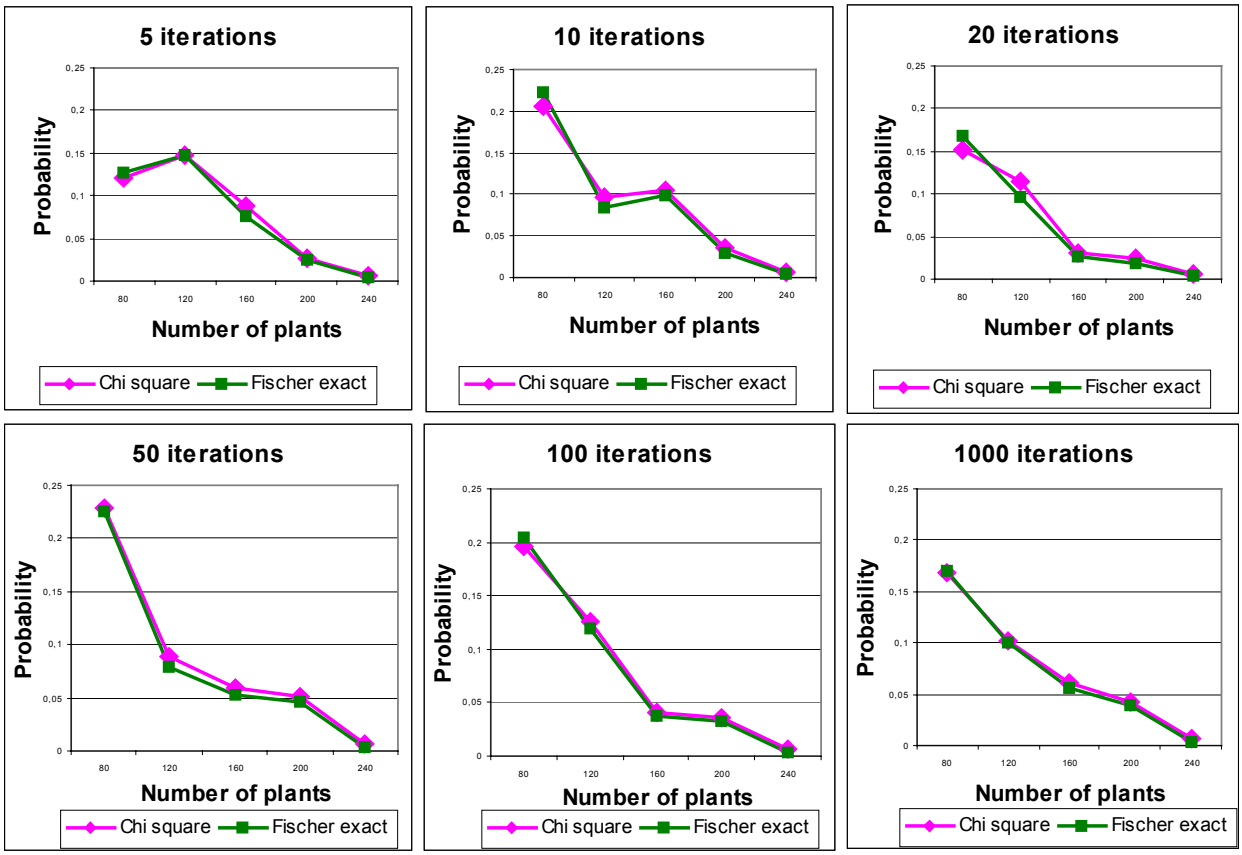
9. The chi-square test is performed with the classes of a given year at the significance level of 1 %. A variety is declared distinct from another one if they are different for at least two years. The difference must be in the same direction.

Statistical methods for testing the reduction of the number of plants assessed.

10. A statistical study was done by the GEVES team in Montpellier. 8 varieties were compared to the whole reference collection with the Chi-square test and the Fisher test based on 240 plants per variety, then based on subsets of the complete data set. This was done to determine if either test allowed a decrease in the number of plants assessed without loosing distinction power. The subsets were obtained by random sampling among the complete data set.

11. The number of random sampling to be performed was determined previously by performing 5, 10, 20, 50, 100 and 1000 iterations of the random sampling of 200, 160, 120 and 80 plants and averaging the obtained probabilities for the Chi-square and Fisher tests (bootstrap procedure). The results showed that 100 iterations are almost similar to 1000, so the study was done with 100 iterations of the random sampling.

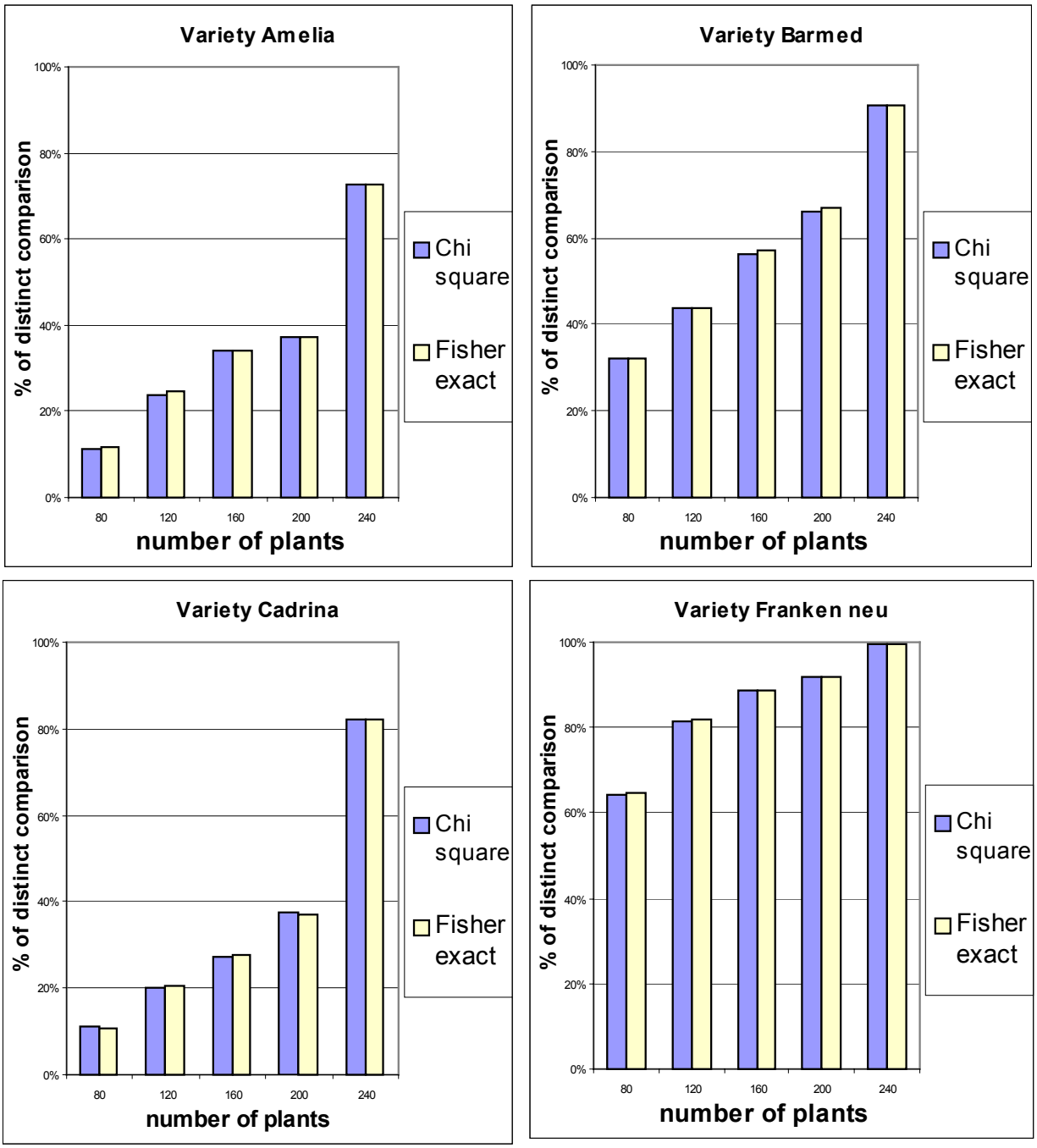
Graph 1: Number of iterations and probabilities of distinctness.



12. The Chi-square test and the Fisher exact test were performed to compare 8 varieties to the whole reference collection based on 240 plants observed, then on 200, 160, 120 and 80 plants observed by the bootstrap procedure previously described.

13. The graphs show the percentage of distinct comparisons with both tests for 8 varieties. (see Graph 2 and Annex 2)

Graph 2: Percentage of distinct comparison with the 2 tests for 4 varieties.



RESULTS AND DISCUSSION

14. There is a significant difference between the percentages of distinction obtained with 240 plants and those obtained with 200. This is very clear when the distinction is intermediate, as with the varieties Amelia, Cadrina and Sonate (see Graph 2 and Annex II). When the percentage of distinction is close to 100 %, like with the variety Franken neu, the decrease in distinction is lower, when the number of plants reduces.

15. This result is not so clear, at the statistical level of 5 %. In that case the decrease is more progressive.

16. In this study it seems very difficult to decrease the number of plants observed in the field each year. The number of distinction with the chi-square and with the Fisher tests gives an equivalent result in term of distinctness.

17. Perhaps an approach based on an ANOVA on the frequency of plants will be more efficient (COY D or an other statistical model). When doing that, we have to keep in mind that the random pooling effect will increase the experimental error, especially with a low number of plants.

18. Another solution could be to compare observations from different growing cycles with the GAIA method. It seems possible to do so, because the characteristic colour of flowers has strong genetic heritability and therefore it is very stable across years.

ANNEX I

Annex I: List of qualitative characteristics and descriptions of how they can be used to assess Distinctness and Uniformity

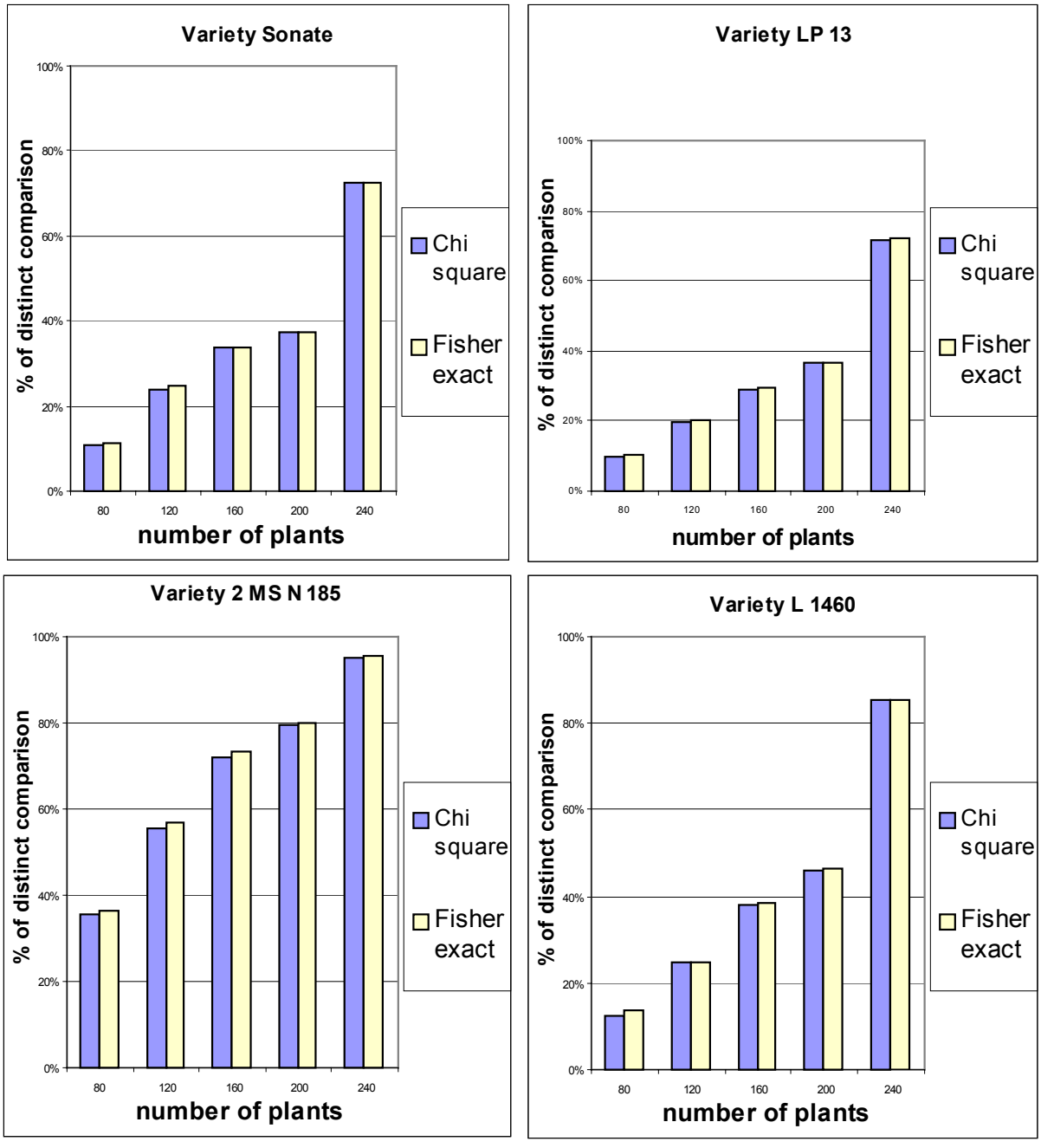
Name of characteristic	Distinctness			Uniformity		
	States for assess-ment	Description (states of expression)	Type of scale	Unit of assess-ment	Description (states of expression)	Type of scale
Sex of plant	1 2 3 4	dioecious female dioecious male monoecious unisexual monoecious hermaphrodite	nominally scaled qualitative data [1]	True-type Off-type	Number of plants belonging to the variety. Number of off-types	nominally scaled qualitative data
Ploidy	2 4 6	diploid tetraploid hexaploid	nominally scaled qualitative data [3]	True-type Off-type	Number of plants belonging to the variety. Number of off-types	nominally scaled qualitative data
Flower colour for Lucerne varieties	1 2 3 4	white or yellow violet very dark violet variegated	combination of ordinal and nominal scaled qualitative data [2]		It is not possible to assess uniformity.	
Colour of hypocotyls for beetroots varieties	1 2 3 4	green white pink orange	combination of ordinal and nominal scaled qualitative data [3]		It is not possible to assess uniformity.	
Tendency to form inflores-cences in year of sowing	1 9	absent present	nominal scaled qualitative data [4]		It is not possible to assess uniformity.	
Resistance to *Xanthomonas translucens (campestris) pv graminis* for Rye-grass varieties	1 9	dead plant living plant	nominal scaled qualitative data. [5]		It is not possible to assess uniformity.	

[Annex II follows]

[1] Distinctness occurs when varieties express different states of expression.
[2] Distinctness can be assessed by applying the Chi square test.
[3] Distinctness can be assessed by applying the Chi square test.
[4] Distinctness can be assessed by applying the chi-square test or the COYD criterion on percentage.

ANNEX II

Annex II: Percentage of distinct comparison with the 2 tests for 4 varieties.



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