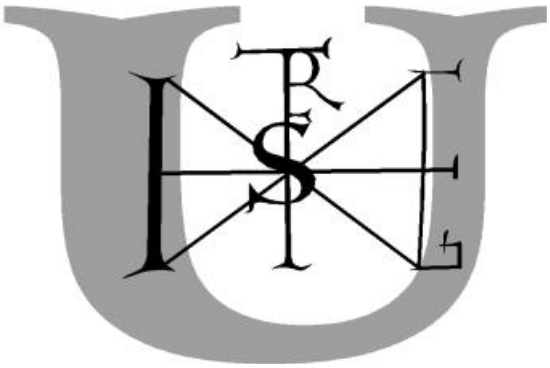

SZENT ISTVÁN UNIVERSITY

IMPROVEMENT OF DUS TEST METHODOLOGY OF PLANT VARIETIES

DOCTORAL (PHD) THESIS

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1. BACKGROUND AND OBJECTIVES

Appearance of new hybrids and resistant varieties from the second half of the 20th century resulted in the appreciation of genetic information in plant varieties. Spreading of modern varieties with high biological input led to the established of plant breeders' right system.

The system is based on the UPOV (International Union for the Protection of New Varieties of Plants) Agreement signed in 1963 in order to provide efficient legal protection for genetic know-how as intellectual property. The triple condition of the so called DUS test had been elaborated by the countries signing the Agreement. Testing of *Distinctness*, *Uniformity* and *Stability* ensures the definite identification and distinction of the variety concerned.

DUS test is a plant variety comparing small plot test described in methodology guidelines. Candidate varieties are compared to varieties of common knowledge so, that appropriate state of expression value of qualitative and quantitative trait has to be selected. The result of DUS test is a code series expressing the variety description in form of a numerical vector.

Variety description matrices compiled of variety descriptions are suitable for further detailed statistical analysis. Columns of the matrix refer to the morphological traits, and rows refer to the varieties. Matrices may have internal relation concerning intervarietal similarity that can be revealed by statistical methods. Beyond similarity the variety description matrix is suitable for assessment of morphological distance serving the basis of distinctness. Morphological similarity is mostly revealed by cluster analysis based on pairvise calculation of distances. New approaches were elaborated in my study concerning the assessment of the histograms of distribution frequencies of pair distances.

Detailed study of intervarietal similarity effectively contributes to a better selection of most similar varieties to compare the candidates during DUS test design. Joint analysis of similarity and distinctness, and detailed assessment of distinctness enables an efficient DUS test result evaluation. The following objectives were designated in order to improve variety testing methodology by the use of variety description matrix:

- Elaboration of new method for DUS test design and efficient test guideline application, and development by assessing relation between DUS traits and distribution of state of expression values within each trait.
- Study the possible further development of random number control matrix by changing its value range, data type (nominal, ordinal) and distribution (normal, uniform).

- Establishment of random number variety description models on the basis of similarity groups and their detailed evaluation of model histograms in order to reveal intervarietal similarity.
- Comparing histograms of random number models to dendrograms of cluster analysis and the evaluation of real DUS variety description matrices according to the models.
- Assessment of molecular and morphological similarity of white grape varieties, and comparison of variety pairs and similarity groups.
- Analysis of distinctness by setting the threshold value with respect to similarity.

2. MATERIALS AND METHODS

2.1. Test material

Model plants in the study were winter wheat, barley and white grape varieties. DUS test (observation and assessment of qualitative and quantitative traits) as well as collection of plant material for molecular analysis was carried out at the Variety Test Stations of National Food Chain Safety Office. Microsatellite marker allele sizes were determined by the generally proposed 9 grapevine SSR primers described by Halász et al. (2005).

2.2. Variety description matrices and data

DUS variety description matrix compiling state of expression values of morphological traits, microsatellite allele size matrix, different forms of random number control matrices, and many derived (calculated) matrices (i.e. morphological dissimilarity) were used in the study. For correlation analysis and for the evaluation of distribution of state of expression values 588 annual DUS descriptions of 350 winter wheat varieties were used. For barley morphological distance distribution evaluation 44 varieties and 28 DUS traits were used. Morphological and molecular similarity assessment of 39 DUS traits of 38 white grape varieties was carried out. Study of distinctness was based on variety description matrix of 61 winter wheat varieties and 20 DUS traits. Random number control matrix was compiled of 1-9 random numbers in uniform distribution. Improvement of random number control matrix meant different intervals, data types and distributions. One or more reduced intervals of random number matrices were defined in order to model similarity groups. Morphological dissimilarity

distribution histograms were characterized by mode (x), deviation (s), highest distance frequency and interval.

Morphological distance (dissimilarity) was calculated by pairwise comparison of varieties. Morphological distance meant the sum of state of expression value differences at all traits. Calculated morphological distances and similarities were summarized in morphological distance and similarity matrices. Molecular similarity was calculated from binary matrix derived from allele size matrix.

2.3. Applied statistical methods

Frequency distribution histograms of variety pairs were evaluated by descriptive statistical analysis. Middle value parameters (average, and mode), deviation parameters (deviation, range, maximum and minimum values) and distortion parameters (skewness and curtosis) were applied. Correlation analysis and cluster analysis as multivariate statistical tools were applied to assess DUS traits and molecular and morphological similarity.

Pearson correlation coefficient and Spearman rank correlation coefficient were applied in correlation analysis. In cluster analysis agglomerative hierarchical clustering method was applied to random number matrices and divisive method to molecular similarity. Morphological distance was calculated by city block (Manhattan) algorithm and Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm to grouping according to Sokal és Michener (1958). Jaccards' similarity index was applied for calculating molecular similarity.

Calculation of distinctness was also based on the differences in state of expression values but the difference was related to a $d_{threshold}$ value defined for the trait concerned. Variety pairs were considered distinct, if the difference in one trait exceeded the appropriate $d_{threshold}$ value.

Statistical analysis and graphical layout was carried out by SPSS 11.0 (Software Package for Social Sciences) and MS Excel 2003 programs. Random numbers were generated by RND function of MS Excel.

2.4. Forms of visual display

Frequency distribution of state of expression values and variety pair distances were displayed in histogram form. The x axis of the DUS trait histograms referred to the state of expression values, the y axis meant the proportion of state of expression values. In the case of random number matrices x axis referred to distances obtained in pairwise comparison, and y axis

meant the sum of equal distances. Deviation from normal distribution of the histogram was characterized by skewness and curtosis.

Output of cluster analysis was visualized in form of dendrograms. The dendrogram showed the relation of connecting varieties and the distance value where the link was established.

3. RESULTS

3.1. Correlation between DUS traits

Information on the correlation between DUS traits was important point when traits are selected for the test. Extracting the most significant correlations on 99% significancy level, it can be seen that traits related to glaucosity had correlation between 0,32-0,81.

The correlation between C*ulm: glaucosity of neck* and *Flag leaf: glaucosity of sheath* traits was the highest (0,81), and between *Ear: glaucosity* and *Culm: glaucosity of neck* traits 0,72 correlation was observed also very high (Table 1).

Table 1. *Pearson correlation of winter wheat glaucosity traits*

Pearson correlation	*Flag leaf: glaucosity of sheath*	*Flag leaf: glaucosity of blade*	*Ear: glaucosity*	*Culm: glaucosity of neck*
Flag leaf: glaucosity of sheath	-			
Flag leaf: glaucosity of blade	0,61	-		
Ear: glaucosity	0,59	0,32	-	
Culm: glaucosity of neck	0,81	0,51	0,72	-

It was proved that effective correlation (0,4) occured between *Flag leaf: glaucosity of blade* and *Flag leaf: glaucosity of sheath* if the effect of *Culm: glaucosity of neck* trait was removed (Table 2).

Table 2. *Partial correlation of winter wheat waxiness traits*

Partial correlation	*Flag leaf: glaucosity of sheath*	*Flag leaf: glaucosity of blade*	*Ear: glaucosity*
Flag leaf: glaucosity of sheath	-		
Flag leaf: glaucosity of blade	0,40	-	
Ear: glaucosity	0,01	0,08	-

3.2. Evaluation of distribution frequency of state of expression values

Distribution frequencies of state of expression values were evaluated in traits of winter wheat final DUS variety descriptions. Distribution frequencies of qualitative and quantitative traits was displayed by histograms. Varieties were found to be grouped easily according to the distribution of qualitative traits. Distribution of state of expression values of wheat varieties followed near uniform, normal and atypical patterns. Near uniform distribution was found at *Flag leaf: glaucosity of blade* trait, near normal distribution at *Time of ear emergence* trait. The frequency of one state of expression value was significantly higher in case of atypical distributions. The descriptive parameters of such patterns were skewness and curtosis.

High curtosis value was observed at *Straw: pith cross section* trait (Figure 1), high skewness value was found at *Coleoptile: anthocyanin coluration* trait. Such histograms unambiguously indicate the decreased usefulness of the trait.

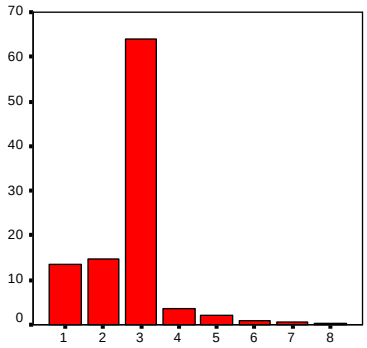

Figure 1. *Histogram of state of expression value distributions of winter wheat Straw: pith cross section trait*

3.3. Improvement of random number control matrix

The improvement of the uniform distribution 50x30 element 1-9 random number control matrix meant the change of state of expression intervals, distribution (uniform, normal) and data type (nominal ordinal). Three typical interval version model matrices were studied. Evaluating histograms of variety description martix models with minimum interval (1-2 in histogram A), maximum interval (1-9 histogram B) and an intermediate interval (1-4 histogram C) (Figure 2), it was found that morphological diversity can be statistically characterized by distance range, the most frequent distance percentage defining the peak size

(Ymax) and x axis position of mode. These parameters describe the internal diversity of the matrix data set (Table 3).

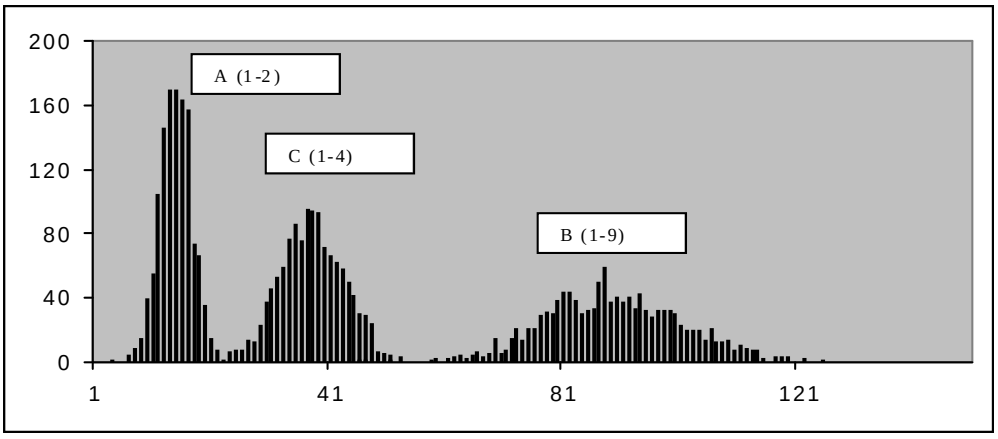

Figure 2. *Histograms A, B és C matrix distance frequency distributions*

Table 3. *Statistical parameters of 3 random number control matrix model (A, B és C) histograms*

	Matrix A (1-2)	**Matrix B (1-9)**	**Matrix C (1-4)**
interval min.	4	46	24
interval max.	19	125	53
Mode (x)	15	88	37
Y_{max}	169	59	95

The model matrix with normal distribution in all traits resulted in a narrow peak distribution pattern closer to the origo, because of the decreased proportion of extra small and large distances. Modelling nominal (qualitative type) traits morphological distance can be 0 or 1 since dissimilarity between two traits either exists or not. Histogram of such model matrix proved to be very narrow with mode value of x=24.

A special case of random number matrix was elaborated where randomization was achieved by the use of original DUS data. The intervarietal similarity was eliminated by random mixing of state of expression values within each trait (column). Such column mixed DUS matrix saved its original data set and trait distribution so that randomization was solved. Comparing the x axis positions (mode) of the random number matrix, the column mixed matrix and the original DUS data matrix, it was found, that histograms of the original DUS and column mixed matrices nearly coincided (x_A=64 and x_B= 62). Mode of the 1-9 random number matrix was closer to the origo x_C=28 (Table 4). Comparing column mixed and real DUS matrix histograms the effective similar variety pairs can be easier determined.

Table 4. *Statistical parameters of DUS variety description, column mixed and 1-9 random number matrices*

paraméter	A	B	C
	DUS variety description matrix	Column mixed matrix	1-9 random number matrix
Mode (x)	64,00	62,00	28,00
Deviation (s)	6,98	5,54	6,09
Minimum	44,00	46,00	11,00
Maximum	94,00	84,00	52,00

3.4. Modelling similar groups by random number matrices

Distribution frequency of variety pairs can show diverse pattern in function of similarity groups hidden in the description matrix. Interpretation of the histograms tends to be difficult as complexity of similarity relation in matrices increases. Some basic versions of similarity groups served as model for the definition of reference histograms. Models form ed the following similarity groups:

- one similar group with 10 and 40 elements,
- two similar groups with different overlaps,
- three non overlapping groups.

In the one similar group models the two plotted histogram peaks properly reflected distance frequencies and numbers. In case of the 3 overlaping models the random number matrix was divided into two equal parts. The models followed an increasing data range overlap. The ranges of state of expression values were 1-5 and 5-9, 1-6 and 4-9, 1-7 and 3-9. The increased overlap resulted in a closer peaks on the histograms. The histogram with widest overlap showed that peaks of high frequency distances of within group and between group similarities already fused.

In case of the matrix with 3 not overlapping similarity groups 3x10 rows (varieties) consisted of random numbers in 1-3, 4-6 and 7-9 ranges. The histogram included a smaller peak on the left and right side and a large one in the middle combined with two smaller peaks on its sides (Figure 3). The left peak had x axis mode position of x=25 indicating the internal similarity frequencies within the 3 groups. The smaller peak on the right side at x=180 refers to the greater distances of similarity groups ranging 1-3 and 7-9.

The large peak refers to the distance distribution of the remaining 20 1-9 random number rows. The left and right small peaks in the middle includes distances between groups with

range 1-3 and 4-6, and range 4-6 and 7-9, respectively. The right peak refers to the large distances of groups of 1-3 and 7-9.

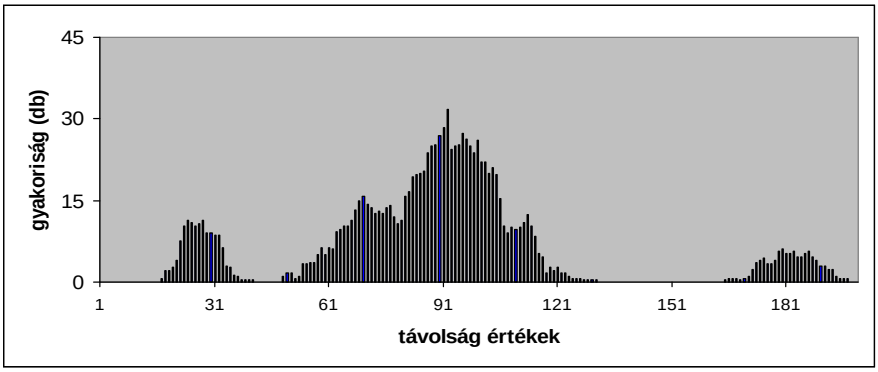

Figure 3. *Histogram of random number matrix consisting of three different 10 element similarity groups*

3.5. Comparing random number matrix histograms to dendrograms of cluster analysis

Cluster analysis of random number control matrices was carried out and the dendrograms indicated that matrices had homogenious data set since cluster cutting point could be not identified. In case of similarity group models dendrograms revealed clearly visible clusters. Similarity groups were clearly visible on the three similar group models as well (Figure 4). The analogy between the histograms and dendrograms were detected and the tendencies were recognised.

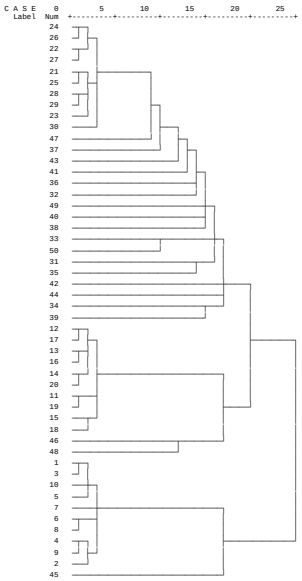

Figure 4. *Histogram of random number matrix consisting of three different 10 element similarity groups*

3.6. The new way of variety pair plotting

Result of cluster analysis is mostly displayed in form of dendrograms. Variety pair distances are overwritten during cluster construction, and this fact makes it difficult to reveal actual variety-variety links on dendrogram. A new alternative plotting method was elaborated in order to solve this problem. The calculation saves the original distances since variety pairs related to each distance level are displayed. The principle of the plotting that pairs do not form bigger groups, but they are paralelly displayed side by side on the appropriate distance level. (Figure 5). Variety pairs positioned on different distance levels can be introduced individually by this plotting method.

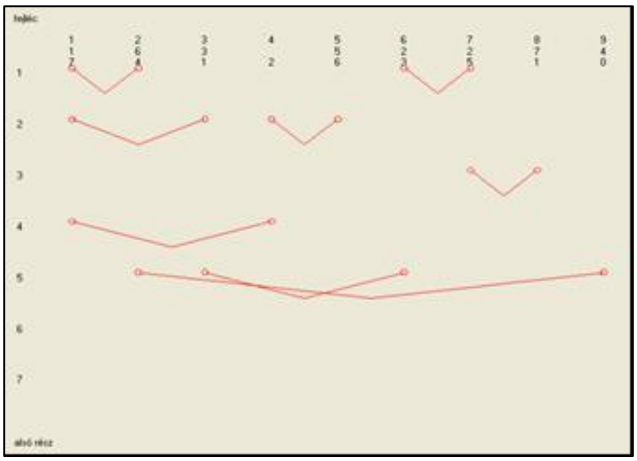

Figure 5. *Layout of variety pair plotting by example of 9 varieties and 8 pairs*

The advantage of new variety pair plotting is that it makes univocal the similarity relation of variety pairs. The display becomes too complex if many pairs are plotted together therefore, it is completed properly cluster analysis when applied for smaller similarity groups.

3.7. Comparison of barley DUS variety description mátrix histograms

The applicability of model histograms was studied on barley varieties. Spring and winter barley varieties are different concerning their morphological traits but compared within the same DUS test. Histogram obtained after pairvise comparison of 27 winter and 17 spring barley varieties showed double peak pattern. The histogram had a larger and a smaller peak at x=59 és x=76 mode values. The peaks referred to a possibly smaller and a larger similarity group in the matrix (Figure 6). Comparing separately winter and spring barley varieties it the two histograms indicated that winter barley varieties had peak at x=55 mode, and spring barley varieties at x=76 mode. It was justified that the large peak on left side on Figure 6 was

created by the winter barley varieties while the smaller one on the right side by the spring varieties.

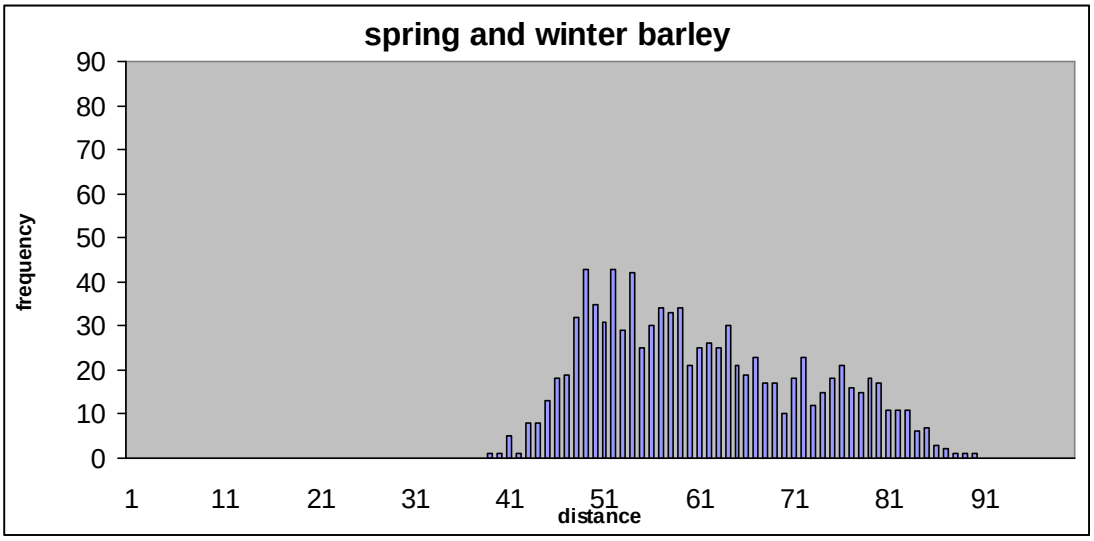

Figure 6. *Distribution pattern of morphological distance frequencies of 44 barley varieties*

3.8. Comparing morphological and molecular similarity of white grape varieties

Development of PCR technology opened new ways to improvement of variety testing methodology. DNA analysis is currently used for pedigree relation detection, to separate varieties, or to justify identity. Geographical origin and pedigree was considered during assessment of molecular and morphological similarity.

Arranging molecular similarity values of variety pairs in descending order some pairs had no common SSR alleles. High molecular similarity was observed at ‘Rózsakő’/‘Zeusz’ (72.2%) ’Pátria’/‘Tramini’, ‘Irsai Olivér’/‘Csabagyöngye’, and ’Generosa’/ ‘Tramini’ (50-60%) pairs.

High morphological similarity was found at ’Pátria’/’Kabar’ (89,4%) and ‘Trilla’/‘Csillám’ (87,0%) pairs without having any parent progeny relation. The most similar and dissimilar variety pairs were classified into 4 groups according to their molecular and morphological similarity. In group 1 and 4 the similarities and dissimilarities coincided, in group 2 and 3 they were opposites. These variety pairs confirmed that molecular and morphological similarity can be significantly different. The obvious reason of this difference is that genes expressing morphological tratis and SSR marker genes can inherit independently.

The average similarity index was introduced for describing similarity of each variety. It was calculated as the average of similarity values of n-1 pair combinations of the variety

concerned. High similarity index expressed that the variety is similar to many others. Molecular and morphological average similarity indices were ranked. Assessing the ranked index differences varieties with molecular or morphological similarity dominance was determined.

Divisive cluster analysis was carried out on molecular and morphological similarity values of the pairs setting cluster numbers 4 to 12. Clusters were compiled by larger (6-13 elements), smaller (2-4 elements) groups of varietries and individuals. Coincidence was found to be partial between molecular and morphological similar groups.

3.9. Application of variety description matrix in assessment of DUS distinctness

DUS distinctness is decided by the relation of calculated variety pair distance and the preset distinctness threshold value. Distinctness is to be calculated for each trait during DUS test. If the difference between two state of expression values at a particular trait exceeds the appropriate threshold value than the variety pair is considered to be distinct.

Distinctness was studied on 61 of winter wheat varieties (1830 pairs). The impact of similarity three threshold levels - $d_{threshold}=4$, $d_{threshold}=3$ and $d_{threshold}=2$ - was analysed to intervarietal distinctness. Distinct variety pairs had DI=1 designated, non distinct pairs had DI=0 respectively. The most stringent condition for distinction was $d_{threshold}=4$, where 30 % (188 pairs) of the 610 most similar pairs was non-distinct.

Comparing non-distinct pairs occuring in the most similar, in the medium similar and in the least similar groups, it was found that non-distinct pairs occured more frequently among the most similar pairs and the trend is non linear. In the case of $d_{threshold}=3$ the number of non-distinct pairs in the most similar first third group was 62 pairs and at $d_{threshold}=2$ only 4 pairs. Distinct variety pairs among the most similar ones are proposed to check in variety testing. Application of homogeneous (univalent) threshold vector served reference for the calibration of the level and proportion of distinctness.

DUS traits can be individually evaluated by the use of heterogeneous threshold vector. For such evaluation of $d_{threshold}$ two winter wheat variety groups - Martonvásár (Mv) varieties and Szeged (Gk) varieties - were selected. First step-by-step cumulative transition of the $D_{threshold} = 3 \rightarrow D_{threshold}=4$ vector was studied on the 20 traits. My results showed that Mv varieties reached 144 non distinct pairs from 42 and Gk varieties 92 from 14 respectively (Figure 7).

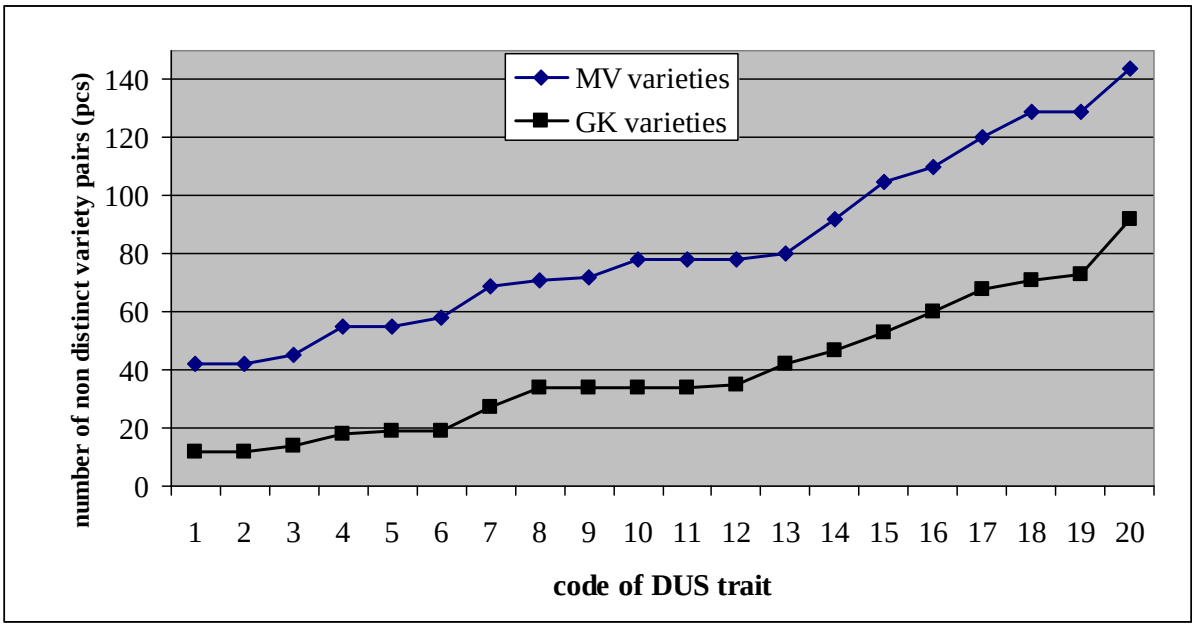

Figure 7. *Proportion of non distinct variety pairs by the application $D_{threshold}$=3-4 cumulative transition at Mv and Gk varieties*

In the next evaluation $d_{threshold}$=3 was replaced by $d_{threshold}$=2 (decrease) and $d_{threshold}$=4 (increase) in each trait. The change in the number of non-distinct pairs indicated how the traits reacted to the threshold modification. Increase of the threshold resulted in the increase of the number of non-distinct pairs. The individual increase of $d_{threshold}$ resulted in the increase of non-distinct pairs at 9 Mv and 11 Gk traits and the decrease of the threshold resulted in the decrease of non distinct pairs at 19 Mv and 20 Gk traits. It was concluded that decrease of threshold value had greater impact on the change of non-distinct pair numbers. The 20 studied DUS traits were classified concerning their sensibility to threshold value modification. The *Coleoptile: anthocyanin coloration, Plant: growth habit, Flag leaf: glaucosity of blade* and *Ear: density* traits proved stable. The difference between the two variety groups was at *Culm: glaucosity of neck, Straw: pith in cross section, Apical rachis segment: hairiness of convex surface, Lower glume: shoulder shape* és a *Lower glume: beak shape* traits. The 20 traits of the two variety groups were classified in the following 5 groups according to their sensitivity to threshold value change. The meaning of the letters in Table 5 is the following:

A) Not sensitive
B) Sensitive to threshold value decrease only
C) More sensitive to decrease
D) More sensitive to increase
E) Sensitive to changes of both.

It was underlined that selecting the proper state of expression value is important where the change of threshold value have significant influence on distinctness.

Table 5. *Sensitivity cathegories of 20 DUS traits of Gk and Mv winter wheat varieties to the individual change of distinctness threshold value*

DUS trait	GK 1-10	MV 1-10	DUS trait	GK 11-20	MV 11-20
1. Coleoptile: anthocyanin coloration	B	B	*11. Ear: density*	B	A
2. Plant: growth habit	B	B	*12. Ear: length*	C	B
3. Plant: frequency of plants with recurved flag leaves	C	C	*13. Awns or scurs at tip of ear: length*	E	B
4. Time of ear emergence	C	C	*14. Apical rachis segment: hairiness of convex surface*	C	D
5. Flag leaf: glaucosity of sheath	B	B	*15. Lower glume: shoulder width*	C	C
6. Flag leaf: glaucosity of blade	B	B	*16. Lower glume: shoulder: shape*	C	B
7. Ear: glaucosity	D	C	*17. Lower glume: beak length*	E	C
8. Culm: glaucosity of neck	D	B	*18. Lower glume: beak shape*	B	C
9. Plant: length	B	B	*19. Lower glume: extent of internal hair*	B	B
10. Straw: pith in cross section	B	C	*20. Grain: coloration with phenol*	E	C

3.10. New scientific results

1. I have proved that relation between DUS traits can be revealed by correlation analysis of the columns of variety description matrix, and this statistical method is suitable for detection of highly correlated DUS traits.

2. By the analysis of frequency distribution of state of expression values I have justified that distribution pattern of the state of expression values within a trait determines the practical applicability of the trait concerned. The evaluation of trait distributions and contributes to the effective improvement of DUS test guideleines.

3. I proved that modification of trait intervals, distributions and data types of random number control matrices determine the frequency distributions of variety pair distance histograms and statistical parameters of such histograms serve as numerical references for the assessment of DUS variety description matrices.

4. I have elaborated the column mixed version of random number control matrix which contribute to the detailed detection of actually similar variety pairs.

5. I have justified that there is complying relation between the histogram pattern of random number matrix containing similar groups, and the matrix dendrogram obtained in cluster

analysis and the elaborated variety pair plotting scheeme provides additional information to the result of cluster analysis on pair similarities

6. Difference between molecular and morphological similarity using white grape varieties was determined, and the similarity positions of the varieties were described by the new mean similarity index.

7. In my study I have justified that non-distinct variety pairs occure among the most similar pairs and modification of the distinctness threshold value at each DUS trait significantly influences the number of non-distinct pairs. DUS traits of winter wheat were cathegorized according to their sensitivity to value change.

4. CONCLUSIONS AND PROPOSALS

Statistical analysis of DUS traits and impact analysis of morphological distance to distinctness are not part of variety testing methodology described in test gudelines or protocols. Several new methods were proposed in the study in order to improve the efficiency of DUS test design and result evaluation and test guideline and for the better acquintance of intervarietal similarity and proper definition of distinctness.

Determination of morphological traits and setting their proper state bof expression values are important questions during the compilement and periodical revision process of DUS test guideliens. Assessment of winter wheat variety description matrices proved that correlation among DUS traits in matrix columns can be revealed by correlation analysis assuming sufficient number of varieties available. Multiple correlation of traites was filtered by calculation of partial correlation. Collective application of highly correlated traits in DUS test is proposed for consideration.

The distribution of state of expression values within the traits is also provide important information therefore it was also evaluated. Many winter wheat quantitative traits had wide range of state of expression values in the variety description matrix. They followed nearly uniform, normal or atypical (overwhelming frequency in a state) distribution patterns. My results showed that drifting of state of expression values traits especially in case of the atypical distribution reduces the efficient application of such trait in the decision on distinctness. (Veress (1999) and Huw et al. (2003))

Varieties comprising the rows of variety description matrices are suitable for analysis of intervarietal relation as well. Dendrogram as common output of cluster analysis provides general overview on similarity of varieties. However, in the DUS test it is necessary to get information on the similarity of variety pairs. The elaborated variety pair plotting method means that pairs graphically displayed on each similarity level. The new application contributes to the detailed evaluation of intervarietal similarity of smaller groups obtained in cluster analysis.

The interval, the distribution and the data type of the random number matrix determine the shape and x axis position of the compiled histogram. It was concluded according to my results that the more the DUS data histogram reaches the shape and position of the 1-9 random number control matrix the higher is the morphological diversity.

Random number control matrix can be used for compiling a calibration series that make possible to evaluate numerically any DUS data histogram on the basis of its mode, height and interval.

The histograms of random number control matrix and DUS variety description matrix did not overlap. The new column mixing randomization method enabled the histograms to have full overlap. Random number models of different similarity groups showed properly the increased distance frequencies within and between groups in forms of histogram peaks. The shape of the model histograms contributed to more efficient interpretation of a complex histogram.

Evaluating molecular and morphological similarity of white grape varieties my results confirmed that the two kind of similarity may coincide but there was no correlation between them confirming the relevant literature (Lopez et al. 2008). Nevertheless both similarities is worthwhile to calculate despite the different genetical background if parental relation is important. Morphological trait based DUS test does not take pedigree into account while molecular similarity can reflect it more precisely.

My results confirmed in evaluating distinctness that threshold values should be defined individually (Garzó et al. (1997) and carefully at all species. Modification of the threshold value by trait may result significant change in the number of non distinct variety pairs thus, it is proposed to carry out an analysis as part of DUS methodology to reveal the changing dynamics of distinctness. It is further suggested that the elaborated sensitivity categories to be considered during definition or refinement of per trait threshold values.

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5. PUBLICATIONS

Publications in English

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