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DIAGNOSTICS OF SUBTROPICAL PLANTS FUNCTIONAL STATE BY CLUSTER ANALYSIS

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ABSTRACT

The article presents an application example of statistical methods for data analysis on diagnosis of the adaptive capacity of subtropical plants varieties. We depicted selection indicators and basic physiological parameters that were defined as diagnostic. We used evaluation on a set of parameters of water regime, there are: determination of water deficit of the leaves, determining the fractional composition of water and detection parameters of the concentration of cell sap (CCS) (for tea culture flushes). These settings are characterized by high liability and high responsiveness to the effects of many abiotic factors that determined the particular care in the selection of plant material for analysis and consideration of the impact on sustainability. On the basis of the experimental data calculated the coefficients of pair correlation between climatic factors and used physiological indicators. The result was a selection of physiological and biochemical indicators proposed to assess the adaptability and included in the basis of methodical recommendations on diagnostics of the functional state of the studied cultures. Analysis of complex studies involving a large number of indicators is quite difficult, especially does not allow to quickly identify the similarity of new varieties for their adaptive responses to adverse factors, and, therefore, to set general requirements to conditions of cultivation. Use of cluster analysis suggests that in the analysis of only quantitative data; define a set of variables used to assess varieties (and the more sampling, the more accurate the clustering will happen), be sure to ascertain the measure of similarity (or difference) between objects. It is shown that the identification of diagnostic features, which are subjected to statistical processing, impact the accuracy of the varieties classification. Selection in result of the mono-clusters analysis (variety tea Kolhida; hazelnut Lombardsky red; variety kiwi Monty and Hydrangea forma rosea) shown as a helpful tool to detect drastically different varieties.

Keywords: subtropical crops; cluster analysis; diagnostics; adaptability; water regime; pigments; enzymes activity

INTRODUCTION

Recently in Sochi, the importation of tropical and subtropical plants has increased, in the fruit and ornamental areas. These crops are deservedly popular among the local residents, especially given the existence of the municipal program “Development within territories of municipal formation the city-resort Sochi» **(Resolution of the Sochi city administration, 2014)**. At the same time, in light of recent political events and the imposition of sanctions, Russia is actively carrying out activities on import substitution, which implies the saturation of the farmer’s market local adapted varieties, process of restoring tea plantations is in full swing **(Resolution of the Russian Federation Government, 2012; Decree of the Russian Federation President, 2014)**. The subtropics of Krasnodar region are in undoubted interest and as well as a year-round family resort direction, therefore, the issues of urban green space in settlements are required as a must. In this regard, of paramount importance in the development of horticulture in Russia is the development of a scientifically-based selection of crops and use of better varieties and garden forms that meet modern requirements and the most adapted to the conditions of damp subtropics.

However, often in the cultivation of new varieties, we have to face failure, because of the classical approach to plant care carried out by analogy with well known established varieties. At the same time, it is known that the choice of crop conditions is individual and depends on the varietal characteristics. All this leads to the need for a thorough, comprehensive research not only by each culture but by each class. Research is often lengthy; require large analysis, which in connection with the use of phenological observations and the large number of morphological, anatomical, physiological indicators, sometimes is difficult. Thus, before researchers there is a question about the search for more rapid methods of assessment introduced new material.

In this regard, in the laboratory of biotechnology, plants physiology and biochemistry in recent years conducted a comprehensive study of adaptive reactions of various subtropical and tropical crops (tea, kiwi, hazelnut, gidrangea, weigela, etc.) aimed at the search of diagnostic criteria for their evaluation.

For a number of crops already established diagnostic indicators, and established scales for assessment of drought resistance varieties and crops such as tea, kiwi, gidrangeya, and weigela **(Belous, 2009; Malyarovskaya and Belous, 2012; Klemeshova and Belous, 2013; Malyarovskaya and Belous, 2015)**. However, attracting new assortment leads to more specific research in respect of obtaining a large number of indicators. As is known, methods of multivariate analysis are effective quantitative tools for the study of fundamental processes described by a large number of characteristics. Cluster analysis most clearly reflects the characteristics of a multivariate analysis in classification and its use allows you to quickly organize the extensive material available in the laboratory. In this case, combining objects into clusters so that way, similar classes maximally got into one class, and objects from different classes would maximally differ from each other.

MATERIAL AND METHODOLOGY

As objects of researches were made by different varieties of the following plants: tea (*Camellia sinensis* L); filbert (*Corylus pontica* C. Koch); kiwi (*Actinidia deliciosa*); gidrangeya large-leaved (*Hydrangea macrophylla* (Thunb.) Ser.); weigela (*Weigela* X *Wagnera*). The accounting of plants resistance to adverse climate conditions was determined visually (in balls) by Technique of the State grades researches **(Technique, 1968)** and methods of laboratory and field assessment of water regime parameters: water deficiency (for a drought resistance assessment) **(Pochinok, 1976)**; the concentration of cell sup (CCS) (for heat tolerance testing) **(Filippov, 1975)**; express diagnostics of change parameters of leaf blade (for a drought resistance assessment) **(Goncharova, 2005)**; activity of enzyme catalase - gasometrical method **(Gunar, 1972)**; thickness of leaf was determined by field turgor meter, coefficient of heat resistant - method express diagnosing **(Kushnirenko, et al., 1986)**.

When processing the data and evaluating the results of the research used the statistical software package STATGRAPHICS Centurion XV standard mathematical software package MS Excel XP. To construct the dendrograms and partition the varieties into homogeneous the adaptability of the group used clustering k-average, or «nearest neighbor».

RESULTS AND DISCUSSION

The establishment of the indicator organ that can serve as a reliable diagnostic authority in the evaluation of the adaptability of cultures was the paramount importance. In the result of conducted research we have established that when long diagnosis culture of the kiwi should consider the existence of tiers in plants (to take away the leaves from the middle tier) and the location of leaves in relation to inflorescences and fruits; for the diagnosis of plant resistance gidrangea and weigela gives a more accurate physiologically formed the third, starting from the terminal of a leaf bud; while the research on tea should be selected physiologically Mature leaves that are after the so-called «fishy».

The following moment was the selection of diagnostic indicators to assess the sustainability of the studied cultures. In the order of the country's scientific institutions developed different methods of diagnosis of plant resistance, recommending for practical use a variety of techniques for evaluation of resistance to extreme factors **(Tsukanov, 2007; Goncharova, 2011)**. Their analysis showed that the entire diversity of ways to diagnose plant resistance lies with a small number of general principles of evaluation based on views on the adaptation mechanisms of plants to stresses **(Tsukanova, 2007; Goncharova, 2011)**.

As a rule, when determining the resistance of the varieties we used two or three well-known varieties (grown in the area), clearly differing from each other in terms of resistance to a specific type of stress: highly resistant, moderately resistant and unstable. However, variety, highly resistant to extreme factors, but not with great productivity potential (realized only in optimal conditions) and gives the highest absolute yield. Most often, the introduction of such varieties in production is recognized as inappropriate; however, it retains its value for breeding as a genetic source of high resistance to stress, which does not preclude his selection as recommended.

We also took into account the fact that the sustainability of any plant organism changes in ontogenesis: it is low at a young age, then gradually increases. From this overall biological patterns should be that a comparative assessment of crops for resistance to stress factors is possible only on the basis of the same age.

Since the main disadvantage of our subtropical zone is the uneven distribution of rainfall, with recurrent drought periods and high temperatures, often accompanied by hair dryers, the main focus of the research was done on plant resistance to drought. In particular, we used evaluation on a set of parameters of water regime. These settings are characterized by high liability and high responsiveness to the effects of many abiotic factors that determined the particular care in the selection of plant material for analysis and consideration of the impact on sustainability. Methods of using identified indicators that are closely related to water status of crops, are: determination of water deficit of the leaves, determining the fractional composition of water and detection parameters of the concentration of cell sap (CCS) (for tea culture flushes).

To confirm the validity of the studies conducted statistical processing of experimental data summarizing and averaging of results and involvement analysis of variance, according to the methodical instructions on conducting field experiments specific to perennial crops.

As a result, on the basis of the experimental data calculated the coefficients of pair correlation between climatic factors and used physiological indicators. The result was a selection of physiological and biochemical indicators proposed to assess the adaptability and included in the basis of methodical recommendations on diagnostics of the functional state of the studied cultures **(Ryndin at all 2014)**. The tea culture is established that a significant relationship exists between enzyme activity – temperature and activity of the enzyme – solar insulation; the culture of hazelnuts dependence between temperature and physiological indicators of above average or even high, the strong correlation observed between temperature and amount of carotenoids ($r = 0.98$), the temperature rise causes a decrease in the synthesis of chlorophylls ($r = -0.73$) and indicators related to water regime: the water content ($r = -0.81$), water-holding capacity ($r = -0.83$) and the amount of free water ($r = -0.78$); revealed close correlations between the indicators of water status weigela (water deficit, the concentration of cell sap (CCS) and the activity of the enzyme catalase **(Belous, 2008; Belous, Ryndin and Pritula, 2009; Klemeshova and Belous**

2011; Kozhevnikova, 2014; Malyarovskaya and Belous 2015).

However, as already mentioned, the analysis of complex studies involving a large number of indicators is quite difficult, especially does not allow to quickly identify the similarity of new varieties for their adaptive responses to adverse factors, and, therefore, to set general requirements to conditions of cultivation. In this case, for the separation of the studied cultivars on the most similar in agility classes (clusters), it is desirable to connect the cluster analysis, which is the most effective way to solve this problem.

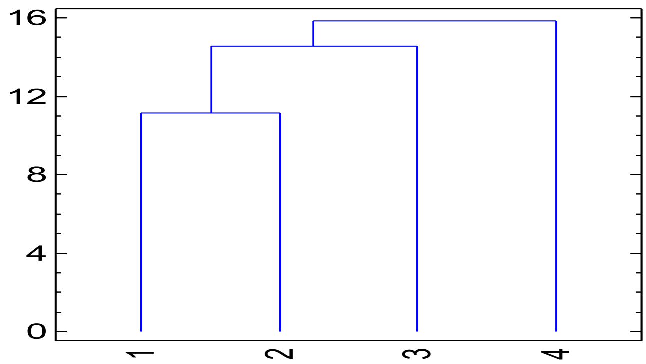
As known, cluster analysis is a multivariate statistical procedure that performs the data collection that contains information about the sample objects, and then marshalling them into homogeneous groups **(Gorsky and Orlov, 2002; Bessokirny, 2003; Savvina, 2013)**. Thus, the objective of cluster analysis is that the newly introduced varieties appear to refer to one of the already defined classes.

Use of cluster analysis suggests that in the analysis of only quantitative data; define a set of variables used to assess varieties (and the more sampling, the more accurate the clustering will happen), be sure to ascertain the measure of similarity (or difference) between objects. In addition, it is necessary that the sample should be homogeneous (not to contain «emission») and the distribution of indicators should be close to normal **(Acopov at all 2013; Sidorenko, 2001)**.

Cluster analysis of the tea plants allowed determining similar to the adaptive potential of varieties. In close clusters are of local plant populations, varieties, and Kimyn and Karatum (Figure 1). Moreover, the more similar the sustainability of local plant populations (sufficiently adapted to growing conditions) and grade Karatum (anthocyanin pigment flushes which is an indirect confirmation of its stability). At the same time the cultivar Kolkhida is located in a separate cluster, as a highly variable grade. High temperature instantly results in drying of young flushes, which are unsuitable for the collection and production of the drink.

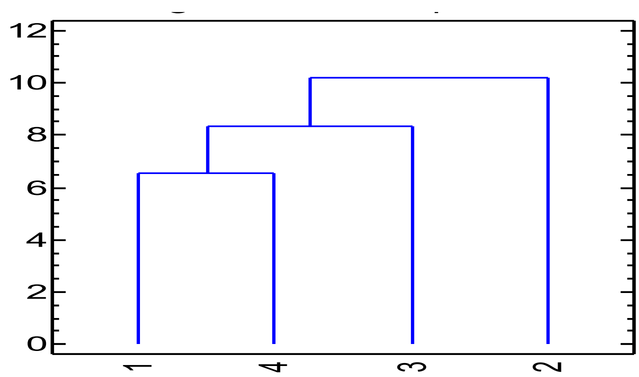
According to the results of the cluster analysis were divided into classes that demonstrate their adaptive capacity, these varieties of hazelnut, as the Cherkessky -2, Lombardsky red, President and Futkurami (Figure 2).

As can be seen from the dendrogram 2 varieties Cherkessky -2, President and Futkurami form a group similar in terms of resistance to stress factors, while variety Lombardsky red according to the degree of adaptability differs from the others. At the same time, considering the combined group of varieties within this cluster are the President that differ in their adaptive potential of varieties Cherkessky -2 and Futkurami.



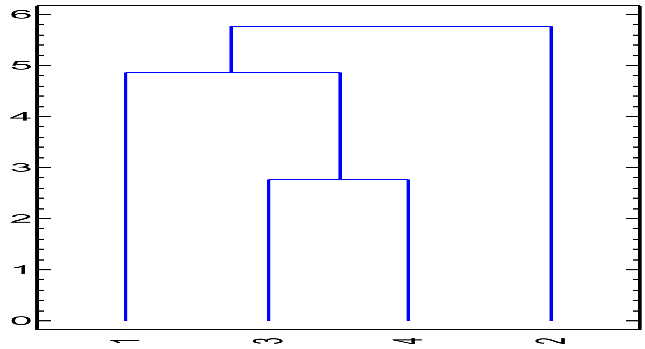
1 – Karatum, 2 – local plants, 3 – Kimyn and 4 – Kolhida

Figure 1 Dendrogramm of tea plants.



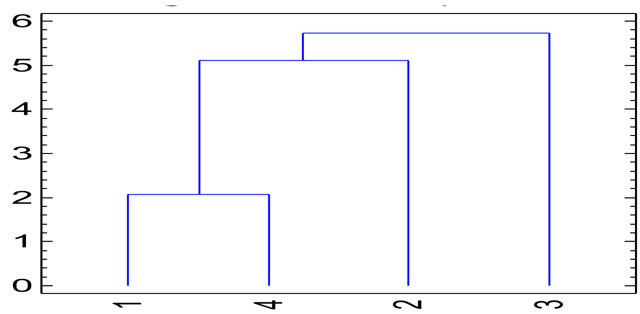
1 – Cherkessky-2, 2 – Lombardsky red, 3 – President and 4 – Futkurami

Figure 2 Dendrogramm of varieties filbert.



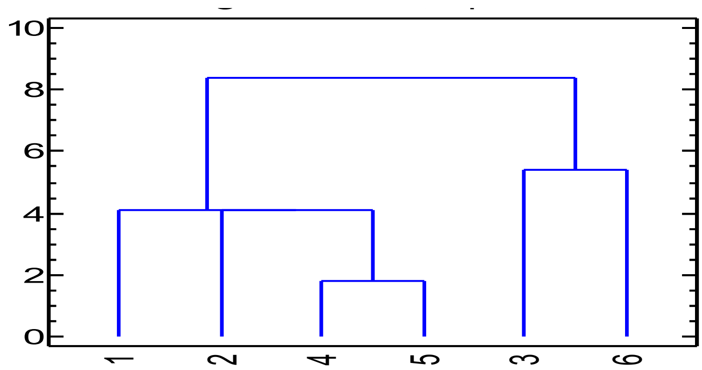
1 – Hayward, 2 – Monty, 3 – Allisson and 4 – Bruno

Figure 3 Dendrogramm of varieties kiwi.



1 – Sester Teresa, 2 – Bichon, 3 – f. rosea and 4 – Draps Wonder

Figure 4 Dendrogramm of varieties gidrangeya.



1 - Eva Ratke, 2 - Gustav Male, 3 – Avgusta, 4 – Arleqin, 5 - Mon Blanc, 6 - Variegata

Figure 5 Dendrogramm of varieties weigela.

As in previous stat analysis using the method of «Nearest neighbor» we managed to get the explanation for the greater stability of the Monti varieties compared to other varieties of kiwi (Figure 3). As can be seen from the dendrogram 3, this variety occupies a position in a separate cluster, which causes the difference of metabolic reactions, including the action of stress factors.

Similarly, the distribution of the studied varieties hydrangea large groups, characterized by similar adaptive potential (Figure 4). As can be seen from the dendrogram 17 variety Draps Wonder, be determined by us on the basic physiological indicators as the most responsive, reasonably is a separate cluster, variety Sester Teresa close to it in terms of resistance to hydrothermal factors, occupying a total cluster.

While form rosea is unstable, rapidly losing its decorative qualities when exposed to high temperatures and lack of water availability (Figure 4). It is not surprising, since form rosea genotypic and phenotypic different from other varieties.

According to the results of the cluster analysis all the studied varieties weigela were divided into the following groups that demonstrate their adaptive capacity (Figure 5).

As can be seen from the dendrogram 5 varieties of Gustav Male, and Arleqin form a cluster, characterized by high resistance to stress factors, while the varieties Avgusta and Variegata according to the degree of adaptability differ greatly, being unstable and leaving a separate group. At the same time, variety Eva Ratke as melatonin, stands in its phenotypic characteristics closer to the group Gustav Male and Arleqin.

CONCLUSION

Thus, it is possible to establish the fact that the cluster analysis reliably and sufficiently illustrates the analyzed material, which gives the opportunity to use it in classification purposes. Moreover, this method is confirmed by the findings on sustainability of cultures that we have done on the results of physiological and biochemical tests. In the end, we propose a two-stage analysis to select the most informative features and classification of the studied cultivars, where the first phase involves a correlation analysis, the second step - cluster analysis. In this case, the value of the correlation coefficient affects the accuracy of further classification. The resulting dendrogram can be used for inter cluster distance quickly assess the differences in functional state of the species and its place in the classification of resistance to stress factors. In addition, the selection in the result of the analysis of mono clusters (as in the case of dendrograms: 1 - variety tea Kolkhida, 2 – hazelnut Lombardsky red; 3 – variety kiwi Monty and 4 – hydrangea forma rosea is a good tool to detect drastically different varieties.

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