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# Radiobiological Method of Studying Sustainability of Grafted Grape Georgian Varieties towards Extreme Factors

**M.E. Gogebashvili, N.I. Ivanishvili, S.A. Kiparoidze, A.E. Kontselidze**

Laboratory of Radiation Safety Problems of Ivane Beritashvili Center for Experimental Biomedicine, 14, Gotua Str., Tbilisi, 0160, Georgia

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## A B S T R A C T

Prognosis of the grape endemic varieties sustainability represents an important scientific-practical task for Georgia. This is conditioned by the circumstances that more than 500 endemic varieties of grape grow in different climatic zones of Georgia. Especially noteworthy is problem of sustainability towards high temperatures of endemic grape grafting with regard to expected global-climatic processes. The present work deals with the radiobiological method of studying sustainability of grafted grape endemic varieties based on several radiobiological and radiological methods. In particular, using the primary fluorescence of grape tissues by the high quality accuracy can be determined structural-functional condition of the grafted grape accretion zone. In addition, by examining the xylem transport of graft, it's possible to test stability of grape in condition of optimum and extreme temperature regime. Overall, conducted research allows complex diagnostics of accretion quality of plant transplants and their sustainability level prediction towards unfavorable environmental factors.

***Keywords:*** Georgian grape, Radioisotopic method, Grafting sustainability, High temperature, Radiological studies, Antigenic structure.

*Corresponding author: Mikheil Gogebashvili; E-mail address; gogebashvili@gmail.com

## Introduction

The central task of live nature protection represents to maintain biological diversity. Based on the data of International - World Wildlife Fund, biological diversity is defined as the diversity of all forms of life on earth. These include: millions species of plant, animal and microorganism and their gene sets in complex ecosystems, which form the living nature [1-3]. At the same time, in relation to agricultural plants, the problem involves not only the protection issues of genetically unique organisms, but also the mission of creating favorable conditions for their real use in agricultural production. This applies to many rare, endemic varieties and species. In this aspect very important is the circumstance, that cultivation of perennial plants requires a number of practical tasks to be solved, in particular, to obtain high quality grafting material.

In many countries of the world the development of viticulture as a field depends on the supply of farmland by quality seedling materials [4-7]. Apart from this different problems arises to Georgian winegrowers caused by the large number of endemic varieties of Georgian grape and their agrobiological peculiarities [8,9].

Therefore, the agenda is not only the issue of providing farmland healthy seedling material, but also the necessity of survival and distribution of endemic varieties. In biological view, grafted plant is a unique organism which combines two genetically different individuals. If we consider that each grafting component for it' s partner is different and often carry "foreign" genetic information during grafting period, then the diversity of reactions which hinder as the accretion process of plant transplants as well as their viability will be clear [10,11,12]. It is clear that the specific of the biological incompatibility mechanism of the tissues is determined by the taxonomic location of the organism in the hierarchy. If we consider this issue in the area of stock and grafting tissues incompatibility, there appear a number of different processes: Anatomical incompatibility of the stock and grafting structures, different dynamics of callusogenesis, antigenic structures incompatibility of the plant transplants and many other physiological-biochemical processes, so biological incompatibility of the tissues is based a combination of events on various structural-functional levels.

Today is successfully implemented different settings by affinity of stock and grafting through many forms of stock. Nevertheless, in most cases, certain types of grapes demote ability to overcome environmental adverse conditions, which is due to the imperfect conductive system restored by differentiation of callus tissue between stock and grafting. In such situation, during optimal conditions for a long time fully retains normally parameters characteristic of its viability, but in case of extreme conditions of environment (eg. extreme temperature), due to the demote ability of conductive system restoration develops not only the negative changes of the physiological-biochemical parameters of the plant, but also partial necrotization of the grafting zone of tissues [13,14,15,16]. Therefore, there is necessity for effective methodologies to be developed, able to assess the accretion degree of the graft by considering their further use in plantation development. At the same time it is necessary to take into account the structural-functional condition of the plants not only for constitution of accretion quality, but also to predict their viability for a long time. The present work presents the methods of determining qualitative indicators of grafting that are based on the results of radiological studies.

## Oobjectives and methods

Quality indicators of grafting are basically defined by two parameters - functional condition of tissues in the accretion zone and the post transplantation restore level of conductive system. The functional condition assessment of the tissues was based on the primary fluorescence of the tissues. For induction of primary fluorescence of tissues as a source ultraviolet radiation (Ultraviolet Mercury-quartz lamp of high pressure (250 Watt)) was used. Induction factor of lighting was a blue part of the spectrum, which was obtained by combination of violet and blue filters of different width. The “locking” yellow filter provided a visibility of reflected wave, excited by ultraviolet irradiation. Observation on microtome slices of accretion zone of grafting was carried out with a standard light microscope.

To study the process of intergrowth of grape grafting and the quality of the resulting seedlings, we carried out various experiments using the radioisotope method. Studies were carried out at various stages of the formation of experimental grafting. The study of the regeneration process of the conducting system during the stratification of graftings was carried out in two ways. The first method consisted in analyzing the “flow” of the radioactive label from the stock to the graft part during the stratification on an isotopic solution. The second is when the cuttings are saturated with an isotopic solution and the stratification on the water. After grafting they were placed on an open stratification into a climatic chamber with moistened air at a temperature of 28-30°C. The basal ends of the grafting were kept in the water at all times with a radioindicator. In experiments, sodium phosphate orthophosphate was used. The radioactivity of the solution used in the work was 2.5 millicuries per liter. After stratification, grafting was divided into parts and the total content of labels in different parts of the grafting was determined.

## Results and discussion

Primary fluorescence of living tissue, due to its potential capabilities, has advantages compared to other research methods. Such capabilities can be attributed to the observation of living cells and tissues, without fixation and processing with specific reagents. A practical solution to this approach is appropriate that unlike other plants, grape is characterized by a good ability of primary fluorescence. 15-20 micron thicknesses slices were obtained on microtome for analysis of primary fluorescence of grape tissue. During the ultraviolet irradiation of the slice, various tissues gave a different fluorescence; In particular: The fluorescence of the xylem cells was dark green when the cells of the walls were fluorescent by pale yellow color. Different picture of the primary fluorescence was obtained in case of irradiation already formed healing zone of graftings. The preliminary examination of the function of the grafting components was carried out by the intensity criterion of callus formation. Endemic varieties of grape characteristic to different zones of Georgia were used in the work. As the first picture shows, most of them are characterized by the specifications of callusogenesis (Fig.1).

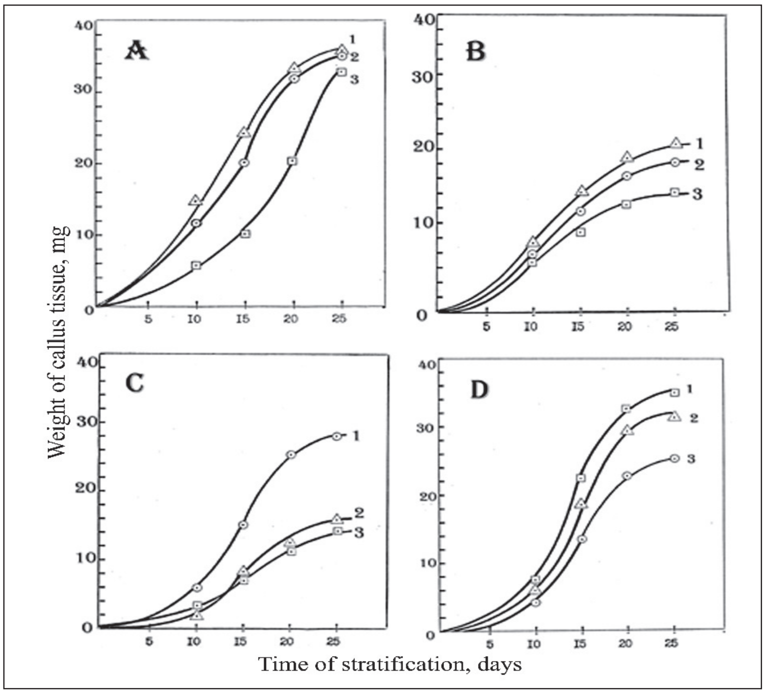


**Fig. 1**. *SCallusogenesis dynamics of Georgian grape varieties*

*A-Kakhetian varieties: 1- kharistvala black, 2-Kisi, 3-Vardisperi;*

*B- Kartli varieties: 1-Asuretuli, 2-Gorula, 3-Ananura;*

*C- Racha-Lechkhumi varieties: 1-Alexandrouli, 2-Green from Oni, 3- Usaxelouri;*

*D-Megrelian varieties: 1-Paneshi pocxos, 2-Ojaleshi, 3- Zerdagi*

Therefore, the cause of the detection of accretion hidden defects during the grafting can be differences in the dynamics of the callusogenesis of stock and graftings.

After that, grafts were adopted by the so-called omega and indirect copulation method. The results showed that a healthy, normal functioning lignified tissue causes a sharp, yellow fluorescent light. Non viable areas, lesions, necrotic areas and other anatomical and functional defects of accretion are less fluorescence. The full fluorescence of tissue was observed in the accretion zone of grape grafting, which indicating the complete restoration of the damaged tissues and all tissues were functioning normally in the given grafting.In second case we got another picture, when on healing area was observed a dark zones, indicating the presence of necrosis (Fig.2).

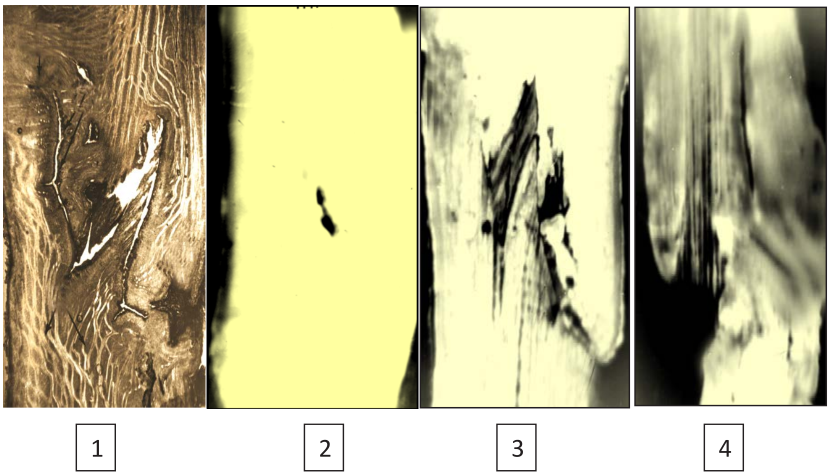


**Fig. 2**. *Structural-functional condition of tissue of grape grafting accretion zone*
*1- Histological picture of grafting accretion zone (light microscope); 2- First ultraviolet light of fully recovered tissues of grafting; 3-Average structural-functional defects of grafting accretion zones (ultraviolet microscopy); 4-Strong structural-functional defects of grafting accretion zones (ultraviolet microscopy).*

The sharp fluorescent light of graftings in graft indicates the viability of these tissues, which means, that conductive system was fully recovered between the grafting components. During the analysis, there were detect non-fluorescent dark areas, where the wide necrotic zone was fully isolated, which led the reduction of nutrients in grafting area. Such graftings, as a rule, are characterized by low ability of growth-development and are unfit for cultivating plantation. In many cases the grafts were found, in which, at first glance, the grafting components were perfectly combined and the healing zone was flourished fluorescent, but in the grafting area of the graft, there was a weak fluorescence and the sharp expressed border between the stock and grafting. This picture indicates a low level of vitality which is the result of weak differentiation between the grafting components. It is noteworthy that the fluorescence analysis method can be used during early stage-stratification of grafting formation. In this case, we can discuss about normal course of accretion processes by the level of tracheid strand creation. Ultraviolet irradiation with blue-ultraviolet spectrum causes yellow flashes when non-differentiated callus mass is not fluorescent. If stratification is going on light, due to the formed chlorophyll, callus acquires red, but tracheid strand yellow color. In order to check adequacy of the treated method we had used labeled atoms method (saturated solution by $^{32}$Pisotope). Isotope migration was observed by radioautography.

The use of radiation methods for various types of tissue incompatibility basically reduces to the analysis of primary contacts caused by the processes of recognition at the cellular level of transplants. At the heart of the proposed method is the analysis of transport of radioactive phosphorus from a stock saturated with radioactive solution into an unsaturated grafting. Thus, the parameters of the investigated processes involve both the time characteristics of the primary contact and the beginning of the functioning of the joint conductive system that formed as a result of cytodifferentiation of the accretion callus tissues. In order to characterize the dynamics of redistribution of radioactive phosphorus in a stock-graft combination of grafting under study, we analyzed the isotope label localization zones using the radioautography method. The radioautoradiographs shown in Fig.1 testify that the pattern of localization of the isotope label at various times of the stratification period varies significantly. So, on the radioautogram of the grape grafting indicates, that 5 days after the onset of stratification, main zone of radioactive label localization is at stock (Fig.3-1). Non-luminescence zone of the grafting may indicate that active processes, associated with the formation of callus cells are taking place below the accretion zone, but the accretion of callus and the restoration of the conduction system has not yet occurred.

A somewhat different picture of the localization of radiophosphorus in different areas of grafting was observed 10 days after the beginning of the stratification (Fig. 3-2).

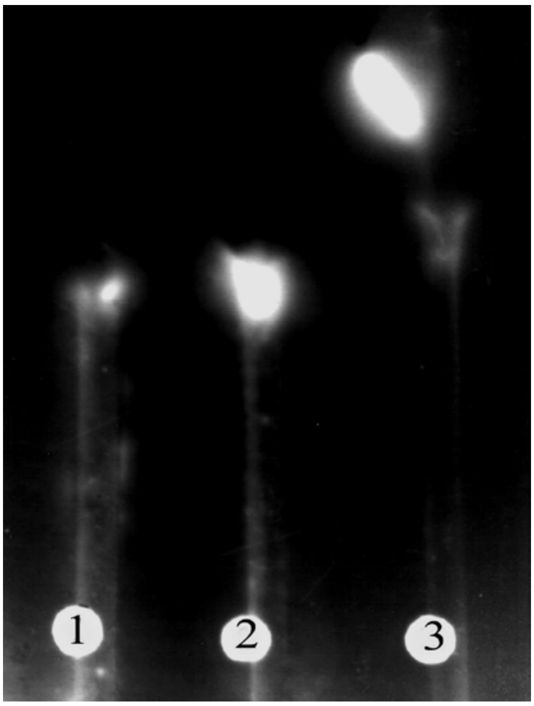


**Fig. 3**. *Localization of radioactive phosphorus in various zones of the grafted plant*
*1-on the 5th day of the stratification period;*
*2-on the 10th day; 3-on the 15th day.*

The similar to 5th day of stratification, there is no fluorescence of grafting zone; This is an indication that the entry of a radioactive label into this part of the grafting has not yet begun because of a dense physiological barrier in the form of undifferentiated callus. At the same time, it should be noted that the luminescence caused by the action of the radioisotope on the photographic plate has become more intense. In interpreting these data, it can be concluded that a conglutination of wound callus of the grafted components occurred and a solution with a radioisotope label entered the basal part of the graft adjacent to the accretion zone.

An even more significant change in the pattern of radiophosphorus localization in grape grafting was observed on the 15th day of the stratification period (Fig. 3-3). Here the main zone of localization was in the developing bud of grafting. At the same time, the radioactivity of the accretion zone itself decreased markedly, which was manifested in a decrease of the luminescence in accretion zone. The appearance of a significant amount of radiophosphorus in the grafting part of the graft is an indication that the conductive system is restored and the active xylem transport between the stock and the graft part is started.

The analysis of the change in the distribution of radiophosphorus label in grafting at various stages of stratification showed the importance of changes in the dynamics of accumulation of radiophosphorus in the accretion zone when the structural integrity and functional activity of the conducting system are restored.

In recent years, regarding to the risks of global climate changes, the issue of sustainability of endemic species towards the high temperature regime has become especially actual. In addition, if the research direction in relation to various agricultural plants can be achieved through the selection of genetic material, depending on the need to maintain genetic diversity while working with endemic varieties, it is impossible to use the methods of selection. In this case, for sustainability analysis of grape plant grafting towards high temperature, the leading factor is grafting adaptation level with the xylem transport intensity, which is provided by stock part of the transplant. In this aspect, the recovery rate of accretion zone tissues provide active xylem transport, which is the criterion of sustainability towards the high temperature regime of the grafted plant. In order to study the restoration level of transplants accretion zone, the method of labeled atoms was used by us. In particular, the xylem transport intensity was studied during optimum and extreme temperatures. As radioautography showed, the last place of xylem transport is the leaf surface tissue, which indicates the radioactive label localization (Picture 4–A). Naturally, in case of temperature regime increases, transpiration intensity allows to prevent the leaves surface overheating, which in total ensures the sustainability of the grafted plant. For the study of the importance of tissue restoration levels, experiments were done on such grafts which were characterized by different structural-functional conditions of the accretion zone tissue. As the graph shows (Fig. 4-B), plant transplant different by restoration level indicators of accretion zone tissues were characterized by different intensity of xylem transport. Difference in transition intensity of radioactive label in grafting area of grafts, in case of high and relatively low recovery levels indicators of tissues in the accretion zone was 29,5%. It's no less important how the same characteristic is changing in relation to extreme temperature regime. It is possible that the temperature factor is the criterion of sustainability of the tissue with respect to different levels of tissue restoration. As shown in 4B-3 and 4B-4 graphs, in condition of extreme temperature regime, in both versions are stated a high rate of radioactive label overflow in the accretion zone; However, quantitatively among the variants are stated- Increase by 14% within a good accretion ability variants, but 6.8% in grafting of low-accretion ability.

Based on the obtained results was shown, by means of structural-functional analysis of grafting components It's possible to monitor cytodifferentiation process of callus tissue, which, in turn, helps to assess the active transport of conductive tissues, and the latter is a precondition to increase the sustainability of plant towards high temperature. It should be noted, that the approach offered by us is noteworthy when the researcher has a goal of maintaining the biodiversity of the endemic varieties with respect to global warming. If sustainability of non-endemic perennial agricultural plants towards high temperature is resolved through the selection of new drought resistant varieties, this methodological approach is not acceptable regarding to endemic varieties, because of phenomenon of genetic purity should be considered.

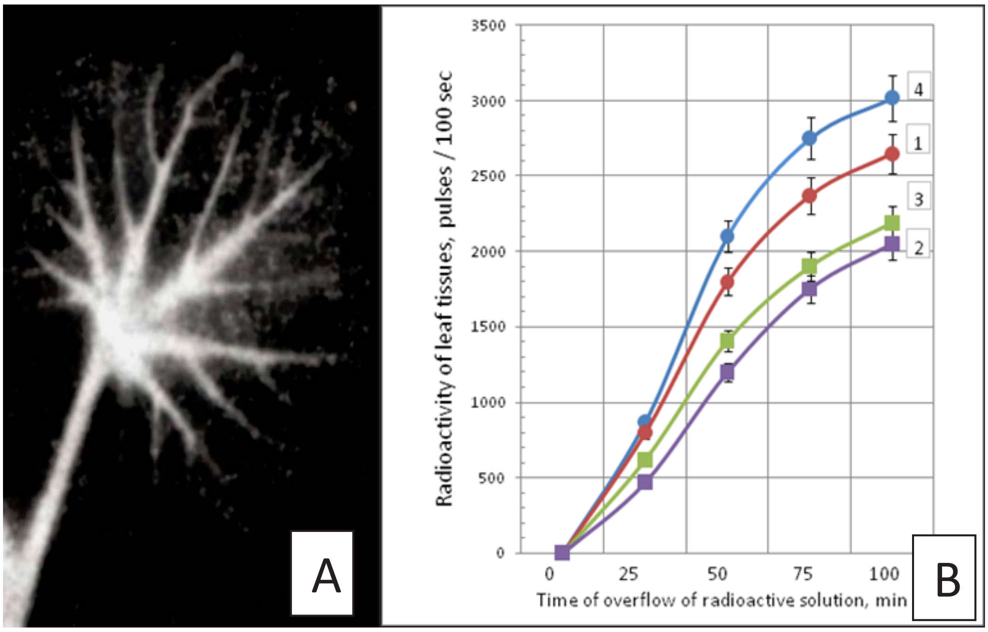


**Fig. 4**. *Radioisotope ($^{32}P$)solution transportin stock-grafting system of Grafted grape A-Grape leaf Radioautograph; B-Dynamics of radiophosphorus movement in the grafting, 1- Intensity of radiophosphorus transport ($25^0C$) in stock-grafting system of high quality grafting, 4- Same, in condition of extreme (40-$45^0C$) temperature; 2-Intensity of radiophosphorus transport ($25^0C$) in stock-grafting system of incomplete accretion grafting,3- Same, in condition of extreme (40-$45^0C$) temperature.*

## Conclusion

Obtained results received on the basis of radioisotopic method, were adequate to the primary fluorescence picture, which indicates high efficiency of the treated method. It is noteworthy, that proposed approach is especially important when are dealing with grafting components having various affinity. Conclusion can be made based on the performed work, that the method of structural-functional analysis of grafting, based on the first fluorescence of the tissues, can be used as an efficient method of early diagnostic of grafting quality. The use of this method will enable us to enrich the arsenal of the methods to verify the large party of grafting for the purpose of planting the grape.

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