Fruit Growing Research, Vol. XXXIX, 2023 DOI 10.33045/fgr.v39.2023.09
https://publications.icdp.ro/index.php

REZULTATE PRIVIND CONCENTRAŢIA, PURITATEA, INTEGRITATEA ADN-ULUI ȘI OPTIMIZAREA REACȚIILOR MULTIPLEX SCAR-SSR PENTRU UNELE SOIURI ROMÂNEȘTI DE MĂR

RESULTS REGARDING CONCENTRATION, PURITY, INTEGRITY OF DNA AND OPTIMIZATION OF SCAR-SSR MULTIPLEX REACTIONS FOR SOME ROMANIAN APPLE VARIETIES

Iancu Adina Floricica[1,2], Militaru Mădălina[2], Cosmulescu Sina[3]
[1]Doctoral School of Plant and Animal Resources Engineering, Faculty of Horticulture, University of Craiova, 13 A. I. Cuza Street, 200585 Craiova, Romania
[2]Research Institute for Fruit Growing Pitești, Romania
[3]Department of Horticulture & Food Science, Faculty of Horticulture, University of Craiova, 13 A. I. Cuza Street, 200585 Craiova, Romania

Abstract

The study was performed to optimize the method of DNA extraction with CTAB, considering reducing working time (non-repeatability of the extraction stage) by obtaining an optimal quantitative concentration to make dilutions and avoid contamination, which would adversely affect the correct and clearly visible amplifications of areas of interest. On the other hand, the multiplexing of SCAR and SSR techniques aimed at reducing reagents consumption and working time. The 48 samples were represented by young (from the top of annual branches) and mature apple leaves harvested in different periods (June and July) from the same year. The leaves come from the apple collections located in two Romanian research units (Research Institute for Fruit Growing Pitesti, Argeș and Research Station for Fruit Growing Voinești, Dâmbovița). DNA was quantified for 48 samples and extracted from young leaves harvested in June and mature leaves, harvested in July. The readings made with the help of the Nanodrop 2000 spectrophotometer showed high concentration values for young leaves and lower concentrations for leaves harvested in July. Evaluation of the DNA concentration for the 48 samples allowed the quantitative/qualitative evaluation and a correlation of the amount of DNA with the stage of development of the leaf. Optimization of SCAR-SSR multiplex reactions for the same DNA concentration (50 ng / µl), led to optimal amplifications for the fragments associated with genes of interest in the SCAR technique, as well as for those amplified by the SSR technique.

Cuvinte cheie: măr, PCR multiplex, *Rvi2, Rvi4, Rvi5, Rvi6, Rvi8*, tehnica SCAR, tehnica SSR, gene de rezistenţă la rapăn
Key words: apple, multiplex PCR, *Rvi2, Rvi4, Rvi5, Rvi6, Rvi8*, SCAR technique, SSR technique, apple scab resistance genes

1. Introduction

DNA was first isolated in 1869 by a Swiss physician, Friedrich Miescher (Dahm R., 2004), who, in an attempt to determine the chemical composition of cells, obtained a substance soluble in a basic medium and insoluble in an acidic medium, which it called nuclein, which was later recognized as nucleic acid. Over time, DNA isolation protocols have been optimized, both depending on the nature of the biological material and to increase the purity and concentration of DNA, respectively to optimize working time in the laboratory (Tan and Yiap, 2009; Buckingham et al., 2007). The inorganic method is often used for isolating DNA for the apple species (Ansari and Khan, 2012; Bosetto et al., 2017; Collins et al., 2021). The CTAB extraction method, introduced by Doyle et al. (1991), was very useful for isolating plant DNA, which contains large amounts of polysaccharides (Tan and Yiap, 2009; Dairawan et al., 2020). Yan et al. (2018) optimized the protocol for DNA isolation using the CTAB extraction method by increasing the concentration of β-mercaptoethanol and PVP to inhibit polyphenol oxidation. For protein denaturation, it is recommended a serum protease, proteinase K (Dieki et al., 2022), which was first discovered by Ebeling et al. (1974), in the fungus *Tritirachium album*. Methods for selective binding of DNA in high salt conditions, known as „solid phase isolation", have been developed, making DNA extraction faster and more efficient, a technique which is based on the high affinity of the negatively charged DNA backbone towards the positively charged silica particles (Buckingham et al., 2007; Tan et al., 2009; Dairawan & Shett, 2020). Plants have specific characteristics like the rigid polysaccharide cell wall, antioxidants, chemical heterogeneity of secondary metabolites and necessitate special attention and skill during the isolation procedure for ADN (Varma et al., 2007). Most proteins absorb light at 280 nm through the

aromatic tryptophan and tyrosine residues. Phenol absorbs ultraviolet light at 270 nm to 275 nm, but and at 230 nm. Some proteins can absorb light at 260 nm, and some peptide bonds which absorb at 228 nm, are often a more constant indicator of the presence of protein in a sample (Buckingham et al., 2007; O'Neill et al., 2011). The main contaminant as a protein is histone which is closely bound to DNA to form chromatin (Bryce et al., 1998). Knowledge of the concentration and purity of DNA is of major importance in obtaining correct results when conducting studies in the field of molecular analysis.

Some techniques (RFLP, AFLP) require large amounts of DNA and high quality (300 ng/µl), for other techniques (SCAR, SSR, ISSR, CAPS) an AND concentration in the range [10-100] ng/µl is sufficient, and other techniques (RAPD) have low reproducibility conducting to different results, even in the same laboratory, if the PCR reaction is repeated but with the different purity and concentrations of DNA. The purpose of the study is to obtain genetic material (DNA) of high concentration and purity and to verify the integrity of the DNA, respectively the insulation efficiency of nucleic acids using the extraction kit „ Isolate II Plant DNA kit" for the apple species increasing the amount of plant material from 100 mg (recommended by kit) at 130 mg and the volume of Lysis buffer from 400 µl (recommended by kit) to 500 µl.

2. Material and methods

Plant Materials

The plant material was represented by young leaves, harvested in June and mature leaves, harvested in July. The young leaves from June were harvested from the top of annual branches, and mature leaves, from July, were harvested from the entire length of the branch. 40 apple varieties were introduced in this study from the collections located at Research Institute for Fruit Growing (RIFG) Pitesti and Research Station for Fruit Growing (RSFG) Voinesti: 'Alex', 'Aura', 'Auriu de Cluj', 'Bistriţean', 'Ciprian', 'Colmar', 'Colonade', 'Dany', 'Delicios de Voineşti', 'Doina', 'Estival', 'Generos', 'Iris', 'Irisem', 'Ionaprim', 'Luca', 'Nicol', 'Precoce de Ardeal', 'Productiv de Cluj', 'Rebra', 'Redix', 'Remus', 'Romus 3', 'Romus 4', 'Romus 5', 'Rustic', 'Salva', 'Starkprim', 'Voinicel', 'Inedit', 'Dacian', 'Voinea', 'Valery', 'Real', 'Discoprim', 'Cezar', 'Frumos de Voineşti', 'Pomona', 'Revidar', 'Remar'.

Reagents and Chemicals

The DNA was extracted using Bioline's Isolate II Plant DNA kit. In our study, DNA extraction was performed according to the working mode recommended by the kit protocol. The lysis buffer was optimized to a pH of 8 and contains a mixture consisting of: 2% CTAB, 1% polyvinylpyrrolidone, 100 mM Tris-HCl, 1.4 M NaCl and 20 mM EDTA. RNA was removed from the AND sample by adding ribonuclease A to the lysis, „RNase A" from Bioline. The DNA binding buffer on silicon columns contains: 24-36% guanidine hydrochloride and 35-55% ethanol. For the purification of nucleic acids, the protocol recommends one step of washing the silicon columns with a buffer, called PAW1 (guanidine hydrochloride 36-50% and isopropanol 20-50%) and two stages of washing with 96-100% ethyl alcohol. The buffer used for DNA elution is optimized to a pH of 8.0 and contains: 10 mM Tris-Cl, and 1 mM EDTA. For nucleic acid migration, a 1% agarose gel was prepared in 1X TBE buffer and subsequently stained with "RedSafe Nucleic Acid Staining". The viscosity of the DNA sample and its sinking on the bottom of the well was achieved by adding in 2 µl per sample of a loading buffer (glycerol 80%: 47 ml; bromophenol blue: 125 mg; xylene cyanol: 125 mg; TRIS 1M, PH 7.4: 500 µl; NaCl 5M: 100 µl; EDTA 0.5 M: 100 µl; SDS 10%: 500 µl). The amplification of multiplex reactions was performed with the help of a PCR analyzer FastGene, using the 2x PCRBIO Taq Mix Red kit from Biosystems.

Genomic DNA Isolation from Apple leaves

In specialized works, it is known that, before extraction, the plant material must be stored in liquid nitrogen or 96% ethanol (Bressan et al., 2014). The leaves were stored at -70^{0}C before extraction and crushed with an electric press. Immediately after the shredding, the plant tissue was weighed, 130 mg and homogenized in 500 µl the lysis buffer. The stages of DNA isolation were followed, as described by the kit protocol, which is a combination of the inorganic method and the method of DNA binding on silicon columns and takes place in five main stages.

Homogenization and cell lysis

The plant material, the leaves, were ground, weighed and collected in sterile Eppendorf tubes free of DN-ase and RN-ase. Over 130 mg of plant material 500 µl of lysing buffer was added. The addition of lysing buffer immediately after grinding is intended to protect the DNA from the cellular contents released by cellular and nuclear membrane disruption.

RNA digestion

After vortexing the lysed plant material, the ribonuclease "RNase A" was added for RNA removal, followed by further vortexing for homogenization and incubation at 65^{0}C for 10 minutes.

Filtration of fresh lysate

Filtration of the lysate as a column was achieved by transferring the lysate from Eppendorf tubes into tubes fitted with filters (2 ml) that prevent filtration of the plant material, followed by centrifugation at 11,000 revolutions for 2 minutes and collection of the clear liquid (supernatant) by removing the filter used. If the pellet is visible the clear liquid is transferred, without disturbing the pellet, into a new 1.5 ml tube. If, after the centrifugation step, the plant material does not look sufficiently compact and liquid is still visible in the pellet, they will be repeated the centrifugation step not before homogenizing the material with a pipette tip.

Adjusting and binding DNA

This was done by adding DNA binding buffer, and PB Buffer (guanidine hydrochloride and ethanol) over the supernatant and homogenizing followed by transferring the mixture into tubes fitted with silica membranes. The step was completed after washing the silica membranes with 2-propanol and guanidine hydrochloride in the first step, followed by two-step washing with 96-100% ethanol and drying the silica membranes by centrifugation at 11000 rpm for 1 min after the first two washing steps, respectively drying by centrifugation at 11000 rpm for 2 min after the last washing step.

DNA elution

DNA was recovered by adding 50 µl of elution buffer (Elution Buffer PG) in two steps, followed by centrifugation at 11000 rpm for 1 minute.

DNA Quantification and assessment of nucleic acid integrity

ADN concentration and purity were analyzed using the NanoDrop 2000 spectrophotometer, and integrity assessment and qualitative concentration estimation by migrating DNA particles into the electrophoretic system (Dilhari et al., 2017) with the help of horizontal electrophoresis. DNA concentration was calculated using the Lambert-Beer law from spectrophotometric absorption analysis at 260 nm (O'Neill et al., 2011; Lucena-Aguilar et al., 2016). The level of DNA purity was determined by the A260/A280, respective A260/A230 absorbance. The concentration of agarose gel was 1%, because the best results for verifying the integrity and concentration of DNA were obtained in agarose gel at 1% (Cseke et al., 2004; Buckingham et al., 2007; Abdel-Latif and Osman, 2017). The volume of a migrated sample was optimized to 8 µl, being represented by 6 µl DNA and 2 µl loading buffer. The sample migration time was 1 hour and 30 seconds at a voltage of 70 volts. The gels were visualized and photographed under UV light with the help of a high-quality Uvitec Cambridge Essential imaging system.

Molecular multiplex PCR analysis

The optimization of amplification reactions using the multiplexing of two different pairs of primers, one of SCAR type, and the other SSR type was made to reduce the working time in the stages preceding the cloning step, the cutting of the amplified fragments of interest from the gel being a laborious method. Seven molecular markers of resistance genes to scab *Venturia inaequalis* were introduced in the study, four being of SCAR type and three of SSR type, and for the study of powdery mildew resistance only an SSR-type marker (Table 2). PCR multiplex mixture was performed in a reaction volume of 15 µl, including the following components in final concentration: 12 µl 2x PCRBIO Taq Mix Red, for each of the primer F and R: 0.1 µl (0.6 µM/µl in the final reaction volume), 2 µl DNA (50 ng /µl) and 0.6 µl ultrapure water for all multiplex reactions. Amplifications were performed under the following conditions: for AL07 with CH03d01 (initial denaturation step at 95°C for 2 min., followed by 35 cycles of 1 min. at 94°C, 1 min. at 58°C, 2 min. at 72°C and final extension 10 min. at 72°C); for AD13 with CH05e03 (initial denaturation step at 95°C for 2.45 min., followed by 40 cycles of 55 sec. at 94°C, 55 sec. at 58°C, 1.39 min. at 72°C, and a final extension of 10 min. at 72°C); for OPL19 with CH02B10 and OPB12 with CH02d12 (initial denaturation step at 94°C for 3 min., followed by 40 cycles of 1 min.. at 94°C, 1 min. at 58°C, 2 min. at 72°C, and a final extension of 10 min. at 72°C).

Analysis of PCR Amplification Products

Evaluation of PCR products was performed using a horizontal electrophoresis system after migration of samples through agarose gel (3% concentration) in 1X TB buffer. The gel was colored with Midori Green Advance and read with a high-quality Uvitec Cambridge Essential imaging system using UVITec1D analysis software. The duration of sample migration was 4 hours, at a voltage of 50 volts.

3. Results and discussions

DNA was quantified (Fig. 3 and Fig. 4) for the twenty-eight varieties from the apple collection of the Research Institute for Fruit Growing Pitesti, whose young leaves were harvested in June and for the fourteen varieties in the apple collection from Research Station for Fruit Growing Voinesti, whose leaves were harvested in July (Fig. 5 and Fig. 6). The number of samples was higher than the number of varieties because for some varieties the extraction was duplicated, as can be seen in Table 1. The results related to the concentration values were obtained by reading the absorbances with the help of the spectrophotometer falling under the range of values [20.46-355.02] ng/µl (Fig. 1). The lowest values in the

range [20.46-79.36] ng/µl belong to the 14 varieties whose leaves were harvested in July. For 18 apple varieties from *ex situ* collection from RIFG Pitesti, the concentration was higher than 100 ng/µl and less than 200 ng / µl ('Aura', 'Dany', 'Productiv de Cluj', 'Luca', 'Irisem-P1', 'Salva-P1', 'Salva-P2', 'Ionaprim', 'Iris-P1', 'Auriu de Cluj', 'Generos-P1', 'Generos-P2', 'Colonade-P1', 'Nicol-P2', 'Delicios de Voinești-P1', 'Delicios de Voinești-P2', 'Redix' and 'Doina'); greater than 200 ng /µl and less than 300 ng/µl for 11 varieties ('Estival', 'Rebra' ,'Bistriţean', 'Romus 3', 'Romus 5', 'Rustic', 'Precoce de Ardeal', 'Starkprim', 'Colmar-P1', 'Remus', and 'Alex'); greater than 300 ng/µl in one of the samples for the variety 'Colonade' (in the other sample being 187.54 ng/µl) and 'Colmar' in one of the samples (in the other sample being 238.53) and a concentration of less than 100 ng/µl f or 'Ciprian' (81.97 ng/µl) 'Nicol' (59,13 ng/µl) and 'Romus 4' (83.59 ng/µl). An analysis of the concentration expressed as a percentage, over the range of values, shows that at 37.5% of the total number of samples the concentration values are in the range [100-200] ng / µl, 35.41% are samples with a concentration of less than 100 ng/µl, 22.91% of the samples have a concentration in the range [200-300] ng/µl and at 4.16%, the concentration exceeded 300 ng/µl (Fig. 2).

For the 28 apple varieties from *ex situ* collection from RIFG Pitesti, the ratio of absorbents 260/280 nm indicates a DNA with high purity, the report values being in the range [1.8-1.9], except for two samples ('Salva-P2' and 'Starkprim') at which this ratio was slightly less than 1.8 (1.78 and 1.79), respectively, but sufficient to consider the samples pure, the kit's recommendations for this ratio values being in the range [1.6-1.9].

For 14 varieties from the apple collection located at RSFG Voinesti, the values of this ratio were lower compared to the others and in the range [1.63 / 1.92], only for two samples ('Pomona' and 'Inedit') the values being below 1.7 (1.63; 1.69) (Table 1). The absorbance ratio 260/230 nm did not show low values (below 1.8), so it can't talk about the presence of copurified contaminants (reagents used in the extraction stage) for none of the samples, and the values of the 230/260 nm ratio were below 0.5, thus excluding protein or phenol contamination (Fig. 3 and Fig. 5). Comparing the results on the gel for the leaf samples, harvested from the two different locations, in June and July differences can be seen between the bands of the migrated DNA. DNA bands are wider for samples corresponding to leaves harvested in June (Fig. 4 and Fig. 6), but in both cases, the integrity of genomic DNA is seen as a single, well-defined band. Optimization of the extraction protocol by increasing the concentration of PVP when adding a volume of 500 µl lysis buffer over 130 mg plant material led to a reduction in polyphenolic contamination and to obtaining very good values for 260/280 absorption ratio.

Starting from the initial concentration, DNA dilutions were performed for each variety, optimizing the DNA concentration at 25 ng/µl.

To highlight the 5 genes of interest regarding scab resistance (*Rvi2, Rvi4, Rvi5, Rvi6, Rvi8*), four SCAR markers and three SSR markers were introduced in this study. Only one SSR marker was introduced for the study of powdery mildew resistance (Table 2). Molecular screening performed using the SCAR technique resulted in the identification of 7 varieties with tetragenic resistance, 12 varieties with trigenic resistance, 2 varieties with digenic resistance, 14 varieties with monogenic resistance and 5 varieties in which the presence of any gene *Rvi2, Rvi4, Rvi5, Rvi6, Rvi8* (Table 3) was not observed.

The optimization of multiplex reactions made in a mixture with two primers, one SCAR type and the other SSR type, had as an objective of reducing working time and reagents consumption, the results being useful in the cutting stage of the amplified fragments from the agarose gel to clone and sequence the fragments of interest and which will be performed in a subsequent study (Fig. 7, Fig. 8, Fig. 9, Fig. 10).

4. Conclusions

Analyzing the results of this study, we can say that there is a correlation between the values of the AND concentration and the stage of development of the leaf because in the case of varieties from *ex situ* apple collection located at Research Institute for Fruit Growing Pitesti for the young leaf harvested from the top of annual branches, in June the concentration values were higher compared to those associated with the mature leaf samples harvested from the entire length of the branch in July from the collection of Research Station for Fruit Growing Voinesti. In the stages of the extraction process, more than likely in the stage of separating the supernatant from the vegetal material, DNA can be lost, because between the concentration values for the same sample (identical plant material), considerable differences of 98.47 ng/µl were observed in the case of 'Colmar' cv., 167.48 ng/µl for 'Colonade' cv., 21.37 ng / µl for 'Generos' cv., 71.38 for 'Salva' cv. and 128.53 ng/µl for 'Nicol' cv.. Low values of concentration associated with mature leaf samples, harvested in July from apple varieties located at Research Station for Fruit Growing Voinești, show that this harvest period is not recommended for a quantitative DNA extraction to achieve a greater number of dilutions. For the apple species, there was no need to optimize the lysis buffer with β-mercaptoethanol involved both in preventing the oxidation of polyphenols and in the degradation of

proteins and nucleases, especially in the degradation of RN-ases. The DNA concentration was sufficient to perform the amplification reactions, obtaining values higher than those estimated by the extraction kit manufacturer.

Optimizing the initial DNA concentration at 50 ng / ml to perform the SCAR-SSR multiplex reaction, allowed clear amplifications for both molecular techniques.

References

1. Abdel-Latif A., Osman G., 2017. Comparison of three genomic DNA extraction methods to obtain high DNA quality from maize. Plant Methods, 13:1.
2. Ansari I.A. and Khan M.S., 2012. An efficient protocol for isolation of high quality genomic DNA from seeds of apple cultivars (*Malus* × *domestica*) for Random Amplified Polymorphic DNA (RAPD) analysis. Pharmaceutical Crops. 3: 78-83.
3. Bosetto L., Brancher T. L., Carlesso C., Kvitschal M. V., Crestani Hawerroth M., 2017. The best tissue to obtain DNA from apple tree using CTAB extraction protocol, II International Symposium on Science and Biotechnology. Entrepreneurship and innovation. https://periodicos.unoesc.edu.br/ISSB/article/view/16158
4. Bressan E. A., Rossi, M. L. Gerald, L. T., Figueira A., 2014. Extraction of high quality DNA from ethanol-preserved tropical plant tissues. BMC Research Notes, 268.
5. Bryce C. F.A., Pacini D., 1998. The structure and function of nucleic acids. Bolton: Biochemical Society.
6. Buckingham L., Flaws M.L., 2007. Nucleic acid amplification in Molecular Diagnostics: Fundamentals, Methods & Clinical Applications, Philadelphia, PA: F.A. Davis Company.
7. Boudichevskaia A., Flachowsky H., Peil A., Fischer C., Dunemann F., 2006. Development of a multiallelic SCAR marker for the scab resistance gene *Vr1/Vh4/Vx* from R12740-7A apple and its utility for molecular breeding. Tree Genetics & Genomes, 2: 186–195.
8. Bus V.G.M., Rikkerink E.H.A., van de Weg W.E., Rusholme R.L., Gardiner S.E., Bassett H.C.M., 2005a. The *Vh2* and *Vh4* scab resistance genes in two differential hosts derived from Russian apple R12740-7A map to the same linkage group of apple. Molecular Breeding, 15(1): 103-116.
9. Bus V.G.M., Laurens F.N.D, van de Weg W.E, Rusholme RL, Rikkerink E.H.A, 2005b. The *Vh8* locus of a new gene-for-gene interaction between *Venturia inaequalis* and the wild apple *Malus sieversii* is closely linked to the *Vh2* locus in *Malus pumila* R12740–7A. New Phytol. 166: 1035–49.
10. Bus, V.G.M., Bassett H.C.M., Bowatte D., Chagne D., Ranatunga, C.A., Ulluwishewa, D.; Wiedow, C.; Gardiner, S.E., 2010. Genome mapping of an apple scab, a powdery mildew and a woolly apple aphid resistance gene from open-pollinated Mildew Immune Selection. Tree Genet. Genomes, 6: 477–487.
11. Cheng F.S., Weeden N.F., Brown S.K., Aldwinckle H.S., Gardiner S.E., Bus V.G., 1998. Development of a DNA marker for *Vm*, a gene conferring resistance to apple scab. Genome 41:208–214.
12. Collins T., Kennedy G., Sarah W., Kenneth M., Geoffrey M., Henry R., Evans N., Joshua N., 2021. Comparative evaluation of two DNA isolation protocols for PCR detection in processed fruit juices. IOSR Journal of Biotechnology and Biochemistry, 7(2): 1–12. https://doi.org/10.9790/264X-0702010112
13. Cseke L.J., Kaufman P.B., Podila G.K., Tsai C.J., 2004. Handbook of Molecular and Cellular Methods in Biology and Medicine, CRC Press, Boca Raton, Fla, USA, 2nd edition.
14. Dairawan M., Shett P.J., 2020. The evolution of DNA extraction methods. Am. J. Biomed. Sci. Res., 8: 39–46
15. Dieki R., Nsi Emvo E., Akue J.P., 2022. Comparison of six methods for Loa Ioa genomic DNA extraction. PLOS ONE 17: 3.
16. Dilhari A., Sampath A., Gunasekara C., Fernando N., Weerasekara D., Sissons C., McBain A., Weerasekera M., 2017. Evaluation of the impact of six different DNA extraction methods for the representation of the microbial community associated with human chronic wound infections using a gel-based DNA profiling method. AMB Expr. 7: 179.
17. Doyle J.,1991. DNA Protocols for Plants. Molecular Techniques in Taxonomy, 57: 283-293.
18. Ebeling W., Hennrich N., Klockow M., Metz H., Orth H. D., Lang H., 1974. Proteinase K from *Tritirachium album* Limber. European Journal of Biochemistry, 47,1: 91–97.
19. Hemmat M., Weeden N.F., Manganaris A.G., Lawson D.M., 1994. Molecular marker linkage map for apple, 85: 4-11.
20. Hemmat M., Brown S.K., Weeden N.F., 2002. Tagging and mapping scab resistance genes from R12740–7A apple. J. Am. Soc. Hortic. Sci. 127: 365–370.
21. Höfer M., Flachowsky H., Schröpfer S., Peil A., 2021. Evaluation of scab and mildew resistance in the Gene Bank collection of apples in Dresden-Pillnitz. Plants, 10 (6): 1227

22. Gardiner S.E., Murdoch J., Meech S., Bus V., Rusholme R., Rikkerink E., Bassett H., Cook M., Gleave A., Crowhurst R., Ross G., Warrington I., 2003. Candidate resistance genes from an EST database prove a rich source of markers for major genes conferring resistance to important apple pests and diseases. Acta Horticulturae, 622: 141 - 151.
23. Gianfranceschi L., Seglias N., Tarchini R., Komjanc M. and Gessler C. 1998. Simple sequence repeats for the genetic analysis of apple. Theor. Appl. Genet. 96: 1069-1076.
24. Lucena-Aguilar G., Sánchez-López A.M., Barberán-Aceituno C., Carrillo-Ávila J.A., López-Guerrero J.A., and Aguilar-Quesada R., 2016. DNA source selection for downstream applications based on DNA quality indicators analysis. Biopreservation and Biobanking 14,4: 264-270.
25. Dahm R., 2004. Friedrich Miescher and the discovery of DNA. Genetics, Spemannstr. 35/III, D-72076 Tu¨bingen, Germany.
26. O'Neill M., McPartlin J., Arthure K., Riedel S., McMillan N., 2011. Comparison of the TLDA with the Nanodrop and the reference Qubit system. Journal of Physics: Conference series 307.
27. Tan S.C., Yiap B.C., 2009. DNA, RNA, and Protein Extraction: The Past and the Present. Journal of Biomedicine and Biotechnology, 2009, Article ID: 574398.
28. Tartarini S., Gianfranceschi L., Sansavini S. and Gessler C., 1999. Development of reliable PCR markers for the selection of the *Vf* gene conferring scab resistance in apple. Plant Breed. 118(2): 183–186.
29. Varma A., Padh H., Shrivastava N., 2007. Plant genomic DNA isolation: an art or a science. Biotechnology Journal, 2: 386-392.
30. Yan J., Ma C., Bo C., Fan X., Li Z., Yang Y., et al., 2018. A modified CTAB method for genomic DNA extraction from apple fruit. Mol. Plant Breed, 15: 3610–5. doi: 10.5376/mpb.2018.09.0005.
https://www.science.smith.edu/cmbs/wp-content/uploads/sites/36/2015/09/How-to-Use-Guide-for-the-Nanodrop-Spectrophotometer.pdf

Tables and Figures

Table 1. Concentration and ratio values at 260 and 280 nm for 40 apple varieties

No.	Provenience/Cultivar	DNA concentration (ng/µl)	260/280 ratio	Cultivar cod
Research Institute for Fruit Growing Pitesti				
1	Estival	298.06	1.89	2
2	Rebra	227.92	1.87	3
3	Bistriţean	211.63	1.88	4
4	Aura	105.01	1.87	5
5	Romus 3	273.05	1.89	6
6	Dany	100,.83	1.9	7
7	Romus 5	217.72	1.86	9
8	Productiv de Cluj	198.02	1.85	10
9	Luca	175.58	1.89	11
10	Ciprian	81.97	1.87	12
11	Irisem-P1	151.96	1.89	13-1
12	Salva-P1	193.6	1.87	14-1
13	Salva-P2	122.22	1.78	14-2
14	Ionaprim	179.86	1.89	15
15	Rustic	272.82	1.87	16
16	Precoce de Ardeal	273.96	1.82	17
17	Iris-P1	174.89	1.87	18-1
18	Starkprim	202.76	1.79	19
19	Auriu de Cluj	159.2	1.89	20
20	Generos-P1	169.27	1.90	21-1
21	Generos-P2	147.9	1.89	21-2
22	Colonade-P1	187.54	1.87	23-1
23	Colonade-P2	355.02	1.83	23-2
24	Nicol-P1	59.13	1.91	30-1
25	Nicol-P2	187.66	1.88	30-2
26	Colmar-P1	238.53	1.87	31-1
27	Colmar-P2	337.74	1.85	31-2
28	Delicios de Voineşti-P1	132.75	1.84	44-1
29	Delicios de Voineşti-P2	198.95	1.84	44-2
30	Romus 4	63.59	1.92	47
31	Remus	214.86	1.97	53
32	Redix	153.6	1.90	54
33	Alex	215.76	1.87	55
34	Doina	103.61	1.85	56
Research station for Fruit Growing Voinesti				
35	Irisem-P2	43.94	1.82	13-2
36	Voinicel	79.36	1.79	59
37	Iris-P2	20.46	1.83	18-2
38	Inedit	63.19	1.69	60
39	Dacian	37.22	1.87	61
40	Voinea	31.23	1.92	62
41	Valery	23.16	1.87	63
42	Real	36.84	1.75	64
43	Discoprim	31.79	1.76	65
44	Cezar	41.9	1.85	66
45	Frumos de Voineşti	49.29	1.74	67
46	Pomona	44.17	1.63	68
47	Revidar	78.65	1.73	70
48	Remar	46.44	1.75	71

Table 2. Primers used for amplification of scab and powdery mildew resistance genes

Gene	Name/type marker	Primer sequence (5'→3')	Fragment size (bp)	References
Rvi6 (Vf)	AL07 SCAR	F:TGGAAGAGAGATCCAGAAAGTG R: CATCCCTCCACAAATGCC	570; 823	Khajuria et al., 2014 Tartarini et al., 1999
Rvi4 (Vr1,Vh4, Vx)	AD13 SCAR	F: GGTTCCTCTGTAAAGCTAG R: GGTTCCTCTGCCCAACAA	950; 1200	Boudichevskaia et al., 006
Rvi2 (Vh2) *Rvi8 (Vh8)*	OPL19 SCAR	F:ACCTGCACTACAATCTTCACTAATC R:GACTCGTTTCCACTGAGGATATTTG	433; 1200	Bus et al., 2005a
Rvi5 (Vm)	OPB12 STS	F: CCTTGACGCAGCTT R: CCTTGACGCATCTACG	687	Cheng et al., 1998
Rvi2 (Vh2)	CH02b10	F:CAAGGAAATCATCAAAGATTCAAG R:CAAGTGGCTTCGGATAGTTG	122	Gianfranceschi et al., 1998 Hemmat et al., 1994 Hemmat et al., 2002
Rvi2 (Vh2)	CH05e03	F:CGAATATTTTCACTCTGACTGGG R:CAAGTTGTTGTACTGCTCCGAC	165 163	Bus et al., 2005 Höfer et al. 2021
Rvi8 (Vh8)	Ch03d01	F: CGCACCACAAATCCAACTC R: AGAGTCAGAAGCACAGCCTC	124	Bus et al., 2005b
Plm	CH02d12	F: AACCAGATTTGCTTGCCATC R: GCTGGTGGTAAACGT GGTG	205	Gardiner et. al., 2003 Bus et. al, 2010

Table 3. The molecular screening of apple cultivars obtained from Romanian breeding program

Cultivar	Genes / molecular markers					Genetic profile
	Rvi2	***Rvi4***	***Rvi5***	***Rvi6***	***Rvi8***	
	OPL19	**AD13**	**OPB12**	**AL07**	**OPL19**	
Estival	+	+	-	-	+	*Rvi2+Rvi8+Rvi4*
Rebra	-	-	-	+	-	*Rvi6*
Bistriţean	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Aura	-	-	-	+	-	*Rvi6*
Romus 3	+	+	-	+	+	*Rvi2+Rvi6+Rvi8+Rvi4*
Dany	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Romus 5	+	-	-	+	+	*Rvi2+Rvi6+Rvi8*
Productiv de Cluj	-	-	-	-	-	
Luca	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Ciprian	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Irisem	-	+	+	-	-	*Rvi5*
Salva	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Ionaprim	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Rustic	-	-	-	+	-	*Rvi6*
Precoce de Ardeal	-	-	-	-	-	
Iris	-	+	-	+	-	*Rvi4+Rvi6*
Starkprim	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Auriu de Cluj	-	-	-	-	-	
Generos	-	-	+	-	-	*Rvi5*
Colonade	-	-	-	+	-	*Rvi6*
Nicol	-	-	+	-	-	*Rvi5*
Colmar	-	-	-	+	-	*Rvi6*
Delicios de Voineşti	+	-	-	-	+	*Rvi2+Rvi8*
Romus 4	+	+	-	+	+	*Rvi2+Rvi8+Rvi6+Rvi4*
Remus	-	-	-	-	-	
Redix	+	+	-	+	+	*Rvi2+Rvi8+Rvi6+Rvi4*
Alex	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Doina	-	-	-	+	-	*Rvi6*
Voinicel	+	+	-	+	+	*Rvi2+Rvi8+Rvi6+Rvi4*
Inedit	-	-	-	+	-	*Rvi6*
Dacian	-	-	-	+	-	*Rvi6*
Voinea	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Valery	-	-	-	+	-	*Rvi6*
Real	-	-	-	+	-	*Rvi6*
Discoprim	+	+	-	+	+	*Rvi2+Rvi8+Rvi6+Rvi4*
Cezar	+	+	-	+	+	*Rvi2+Rvi8+Rvi6+Rvi4*
Frumos de Voineşti	-	-	-	-	-	
Pomona	+	-	-	+	+	*Rvi2+Rvi8+Rvi6*
Revidar	-	-	-	+	-	*Rvi6*
Remar	+	+	-	+	+	*Rvi2+Rvi8+Rvi6+Rvi4*

DOI 10.33045/fgr.v39.2023.09

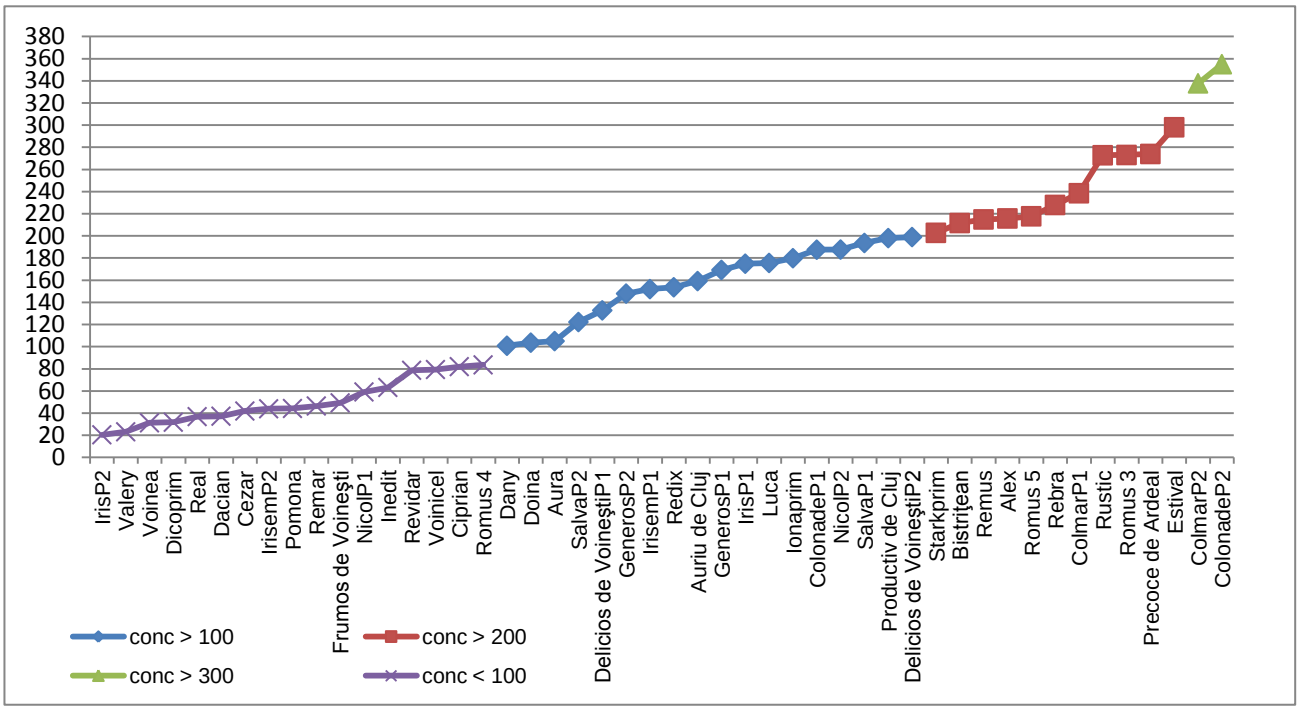

Fig. 1. DNA concentration values in the range [20.46-355.02]

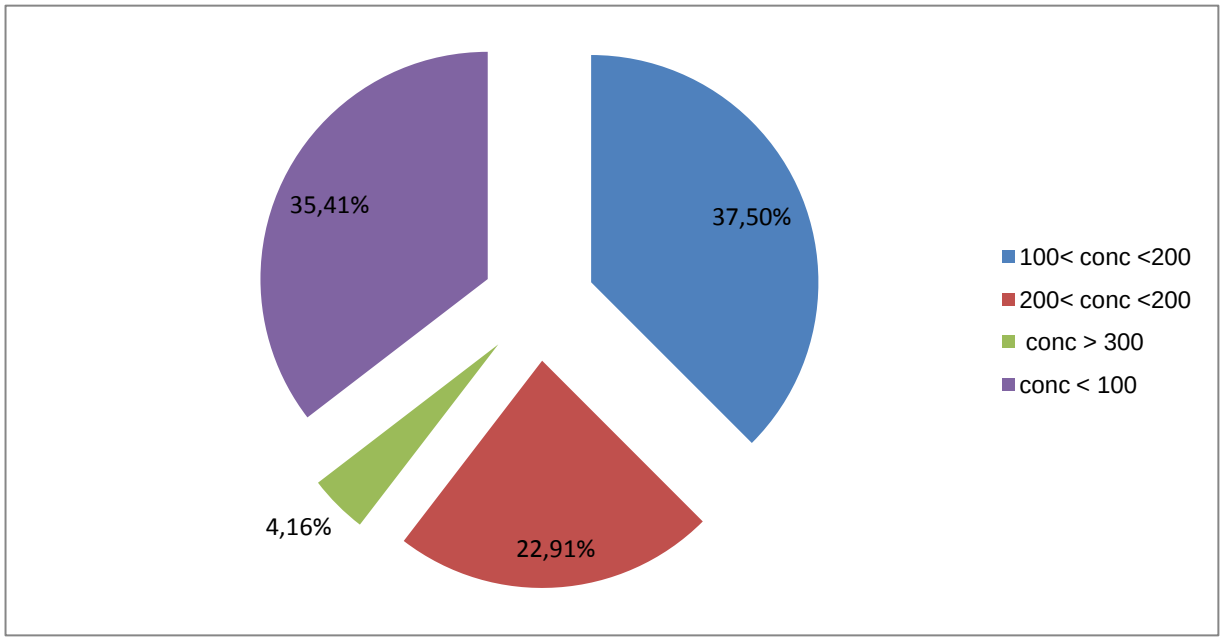

Fig. 2. The values of the percentage concentration of DNA expressed over the range of values for 40 apple varieties

Plots | Report

Test type: Nucleic Acid 7/13/2023 10:32 AM Exit

Report Name mar gDNA 13 iulie 2023 Report Full Mode Ignore

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
2	Default	7/13/2023	9:37 AM	298.06	5.961	3.147	1.89	4.25	50.00	230	1.402	0.155
3	Default	7/13/2023	9:39 AM	227.92	4.558	2.434	1.87	5.71	50.00	230	0.798	0.383
4	Default	7/13/2023	9:40 AM	211.63	4.233	2.254	1.88	6.78	50.00	230	0.624	0.219
5	Default	7/13/2023	9:41 AM	105.01	2.100	1.124	1.87	4.06	50.00	230	0.516	0.183
6	Default	7/13/2023	9:42 AM	273.05	5.461	2.894	1.89	4.87	50.00	230	1.121	0.096
7	Default	7/13/2023	9:43 AM	100.83	2.017	1.064	1.90	5.83	50.00	230	0.346	0.093
9	Default	7/13/2023	9:45 AM	217.72	4.354	2.345	1.86	5.48	50.00	230	0.795	0.267
10	Default	7/13/2023	9:46 AM	198.02	3.960	2.146	1.85	4.46	50.00	230	0.888	0.507
11	Default	7/13/2023	9:47 AM	175.58	3.512	1.862	1.89	3.96	50.00	230	0.885	0.160
12	Default	7/13/2023	9:48 AM	81.97	1.639	0.877	1.87	3.87	50.00	230	0.424	0.096
13-1	Default	7/13/2023	9:50 AM	131.96	2.639	1.398	1.89	3.49	50.00	230	0.756	0.054
13-2	Default	7/13/2023	9:51 AM	43.94	0.879	0.484	1.82	1.74	50.00	230	0.506	0.205
14-1	Default	7/13/2023	9:52 AM	193.60	3.872	2.068	1.87	7.14	50.00	230	0.542	0.165
14-2	Default	7/13/2023	9:53 AM	122.22	2.444	1.371	1.78	5.51	50.00	230	0.444	0.951
15	Default	7/13/2023	9:53 AM	179.86	3.597	1.906	1.89	8.00	50.00	230	0.450	0.183
16	Default	7/13/2023	9:54 AM	272.82	5.456	2.921	1.87	4.83	50.00	230	1.131	0.166
17	Default	7/13/2023	9:55 AM	273.96	5.479	3.014	1.82	3.22	50.00	230	1.701	0.941
18-1	Default	7/13/2023	9:56 AM	174.89	3.498	1.875	1.87	5.40	50.00	230	0.648	0.089
18-2	Default	7/13/2023	9:57 AM	20.46	0.409	0.224	1.83	2.43	50.00	230	0.168	0.385
19	Default	7/13/2023	9:59 AM	202.76	4.055	2.260	1.79	3.40	50.00	230	1.193	1.162
20	Default	7/13/2023	10:00 AM	159.20	3.184	1.685	1.89	4.24	50.00	230	0.750	0.255
21-1	Default	7/13/2023	10:01 AM	169.27	3.385	1.779	1.90	3.60	50.00	230	0.939	0.151
21-2	Default	7/13/2023	10:02 AM	147.90	2.958	1.562	1.89	3.01	50.00	230	0.981	0.873
23-1	Default	7/13/2023	10:03 AM	187.54	3.751	2.005	1.87	7.46	50.00	230	0.503	0.093
23-2	Default	7/13/2023	10:04 AM	355.02	7.100	3.882	1.83	3.20	50.00	230	2.220	0.591

Fig. 3. Results of the concentration and purity of DNA samples read with the Nanodrop 2000 spectrophotometer

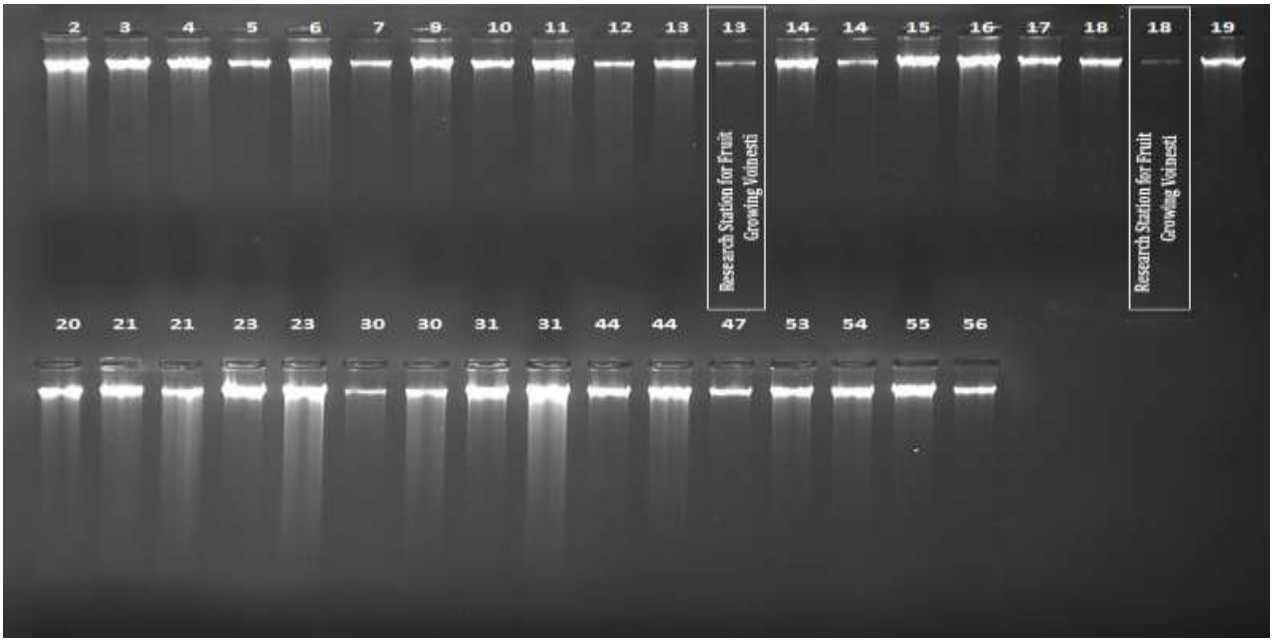

Fig. 4. Electrophoretic profile samples from Research Institute for Fruit Growing Pitesti, harvested in June

2. 'Estival', 3. 'Rebra', 4. 'Bistriţean', 5.'Aura'. 6.'Romus 3', 7. 'Dany', 9. 'Romus 5', 10.'Productiv de Cluj', 11.'Luca', 12.'Ciprian', 13.'Irisem', 14.'Salva', 14.'Salva', 15.'Ionaprim', 16.'Rustic', 17.'Precoce de Ardeal', 18.'Iris', 19.'Starkprim', 20.'Auriu de Cluj', 21.'Generos', 21.'Generos', 23.'Colonade', 23.'Colonade', 30.'Nicol', 30.'Nicol', 31.'Colmar', 31.'Colmar', 44.'Delicios de Voineşti', 44.'Delicios de Voineşti', 47.'Romus 4', 53.'Remus', 54.'Redix', 55.'Alex', 56.'Doina'

 DOI 10.33045/fgr.v39.2023.09

Plots | Report

Test type: Nucleic Acid | 7/13/2023 10:33 AM | Exit

Report Name: mar gDNA 13 iulie 2023 | Report Full Mode: Ignore

Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
23-2	Default	7/13/2023	10:04 AM	355.02	7.100	3.882	1.83	3.20	50.00	230	2.220	0.591
30-1	Default	7/13/2023	10:05 AM	59.13	1.183	0.620	1.91	2.25	50.00	230	0.526	0.293
30-2	Default	7/13/2023	10:06 AM	187.66	3.753	1.991	1.88	8.04	50.00	230	0.467	0.146
31-1	Default	7/13/2023	10:07 AM	238.53	4.771	2.547	1.87	5.68	50.00	230	0.841	0.085
31-2	Default	7/13/2023	10:09 AM	337.74	6.755	3.653	1.85	3.71	50.00	230	1.821	0.130
44-1	Default	7/13/2023	10:10 AM	132.75	2.655	1.446	1.84	3.24	50.00	230	0.817	0.107
44-2	Default	7/13/2023	10:11 AM	198.95	3.979	2.162	1.84	4.49	50.00	230	0.885	0.598
47	Default	7/13/2023	10:12 AM	83.59	1.672	0.870	1.92	3.82	50.00	230	0.437	0.080
53	Default	7/13/2023	10:13 AM	214.86	4.297	2.301	1.87	6.43	50.00	230	0.669	0.111
54	Default	7/13/2023	10:14 AM	153.60	3.072	1.615	1.90	4.57	50.00	230	0.672	0.084
55	Default	7/13/2023	10:15 AM	215.78	4.316	2.306	1.87	6.14	50.00	230	0.703	0.082
56	Default	7/13/2023	10:16 AM	103.61	2.072	1.121	1.85	3.80	50.00	230	0.545	0.331
59	Default	7/13/2023	10:17 AM	79.36	1.587	0.885	1.79	2.04	50.00	230	0.777	0.228
60	Default	7/13/2023	10:18 AM	63.19	1.264	0.747	1.69	1.94	50.00	230	0.651	0.664
61	Default	7/13/2023	10:19 AM	37.22	0.744	0.398	1.87	2.37	50.00	230	0.313	0.279
62	Default	7/13/2023	10:20 AM	31.23	0.625	0.325	1.92	2.23	50.00	230	0.280	0.237
63	Default	7/13/2023	10:21 AM	23.16	0.463	0.248	1.87	2.08	50.00	230	0.222	0.214
64	Default	7/13/2023	10:22 AM	36.84	0.737	0.420	1.75	1.78	50.00	230	0.414	0.690
65	Default	7/13/2023	10:23 AM	31.79	0.636	0.362	1.76	2.67	50.00	230	0.238	0.166
66	Default	7/13/2023	10:24 AM	41.90	0.838	0.453	1.85	2.00	50.00	230	0.418	0.359
67	Default	7/13/2023	10:25 AM	49.29	0.986	0.567	1.74	1.91	50.00	230	0.516	0.638
68	Default	7/13/2023	10:27 AM	44.17	0.883	0.543	1.63	1.91	50.00	230	0.462	0.604
70	Default	7/13/2023	10:28 AM	78.65	1.573	0.911	1.73	2.34	50.00	230	0.672	0.873
71	Default	7/13/2023	10:29 AM	46.44	0.929	0.532	1.75	2.01	50.00	230	0.462	0.556
water	Default	7/13/2023	10:29 AM	-0.01	-0.000	0.007	-0.02	-0.03	50.00	230	0.006	0.021

Fig. 5. Results of the concentration and purity of DNA samples read with the Nanodrop 2000 spectrophotometer

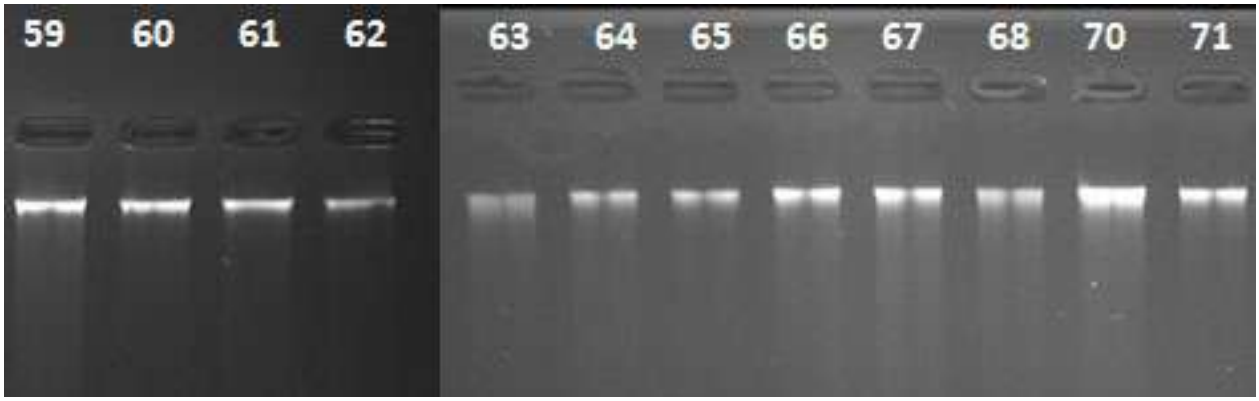

Fig. 6. Electrophoretic profile samples from Research Station for Fruit Growing Voinești harvested in July

59.'Voinicel', 60.'Inedit', 61.'Dacian', 62.'Voinea', 63.'Valery', 64.'Real', 65.'Discoprim', 66.'Cezar', 67.'Frumos de Voineşti', 68.'Pomona', 70.'Revidar', 71.'Remar'

 DOI 10.33045/fgr.v39.2023.09

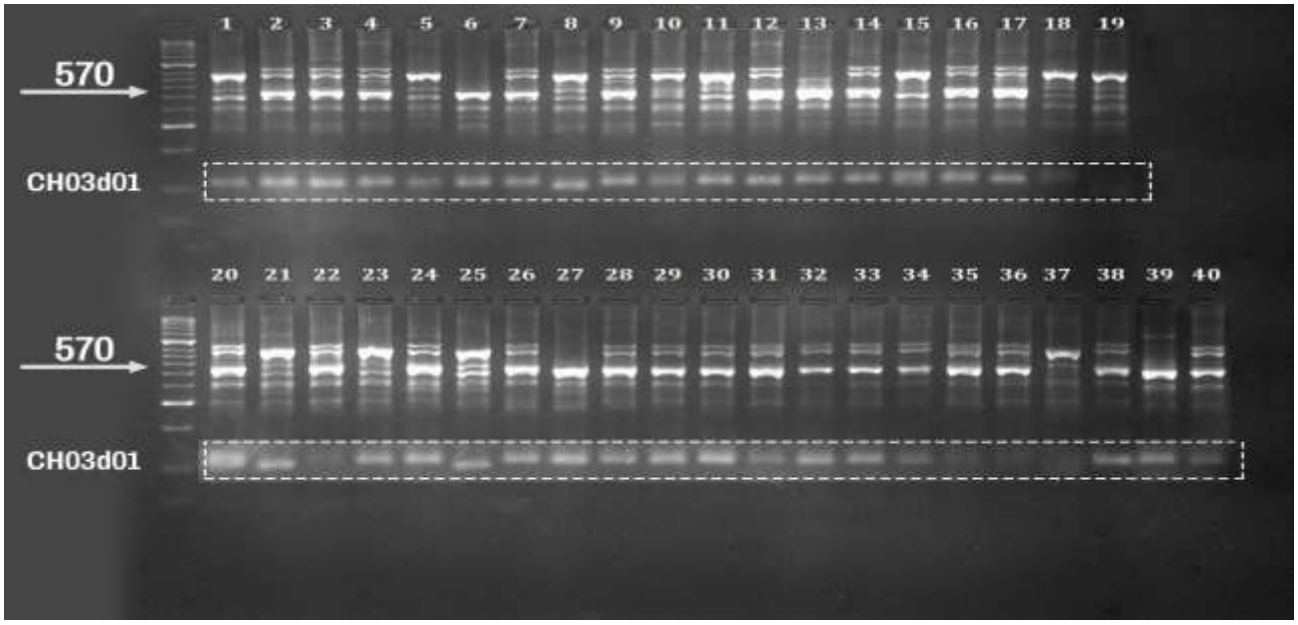

Fig. 7. Multiplex reaction. The electrophoretic profile obtained with AL07 and CH03d01 markers
1. 'Estival', 2. 'Rebra', 3. 'Bistriţean', 4. 'Aura', 5. 'Romus 3', 6. 'Dany', 7. 'Romus 5', 8. 'Productiv de Cluj', 9. 'Luca', 10. 'Ciprian', 11. 'Irisem', 12. 'Slava', 13. 'Ionaprim', 14. 'Rustic', 15. 'Precoce de Ardeal', 16. 'Iris', 17. 'Starkprim', 18. 'Auriu de Cluj', 19. 'Generos', 20. 'Colonade', 21. 'Nicol', 22. 'Colmar', 23. 'Delicios de Voineşti', 24. 'Romus 4', 25. 'Remus', 26. 'Redix', 27. 'Alex', 28. 'Doina', 29. 'Voinicel', 30. 'Inedit', 31.'Dacian', 32. 'Voinea', 33. 'Valery', 34. 'Real', 35. 'Discoprim', 36. 'Cezar', 37. 'Frumos de Voineşti', 38. 'Pomona', 39. 'Revidar', 40. 'Remar'

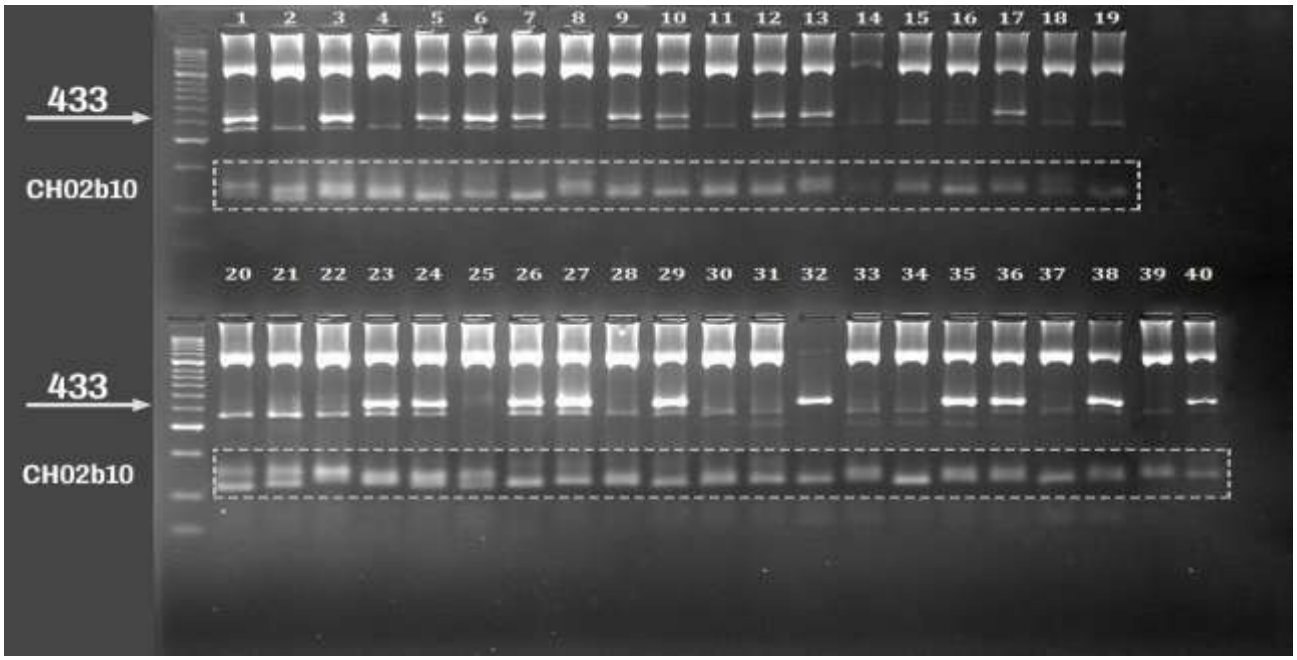

Fig. 8. Multiplex reaction. The electrophoretic profile obtained with OPL19 and CH02B10 markers
1. 'Estival', 2. 'Rebra', 3. 'Bistriţean', 4. 'Aura', 5. 'Romus 3', 6. 'Dany', 7. 'Romus 5', 8. 'Productiv de Cluj', 9. 'Luca', 10. 'Ciprian', 11. 'Irisem', 12. 'Slava', 13. 'Ionaprim', 14. 'Rustic', 15. 'Precoce de Ardeal', 16. 'Iris', 17. 'Starkprim', 18. 'Auriu de Cluj', 19. 'Generos', 20. 'Colonade', 21.'Nicol', 22. 'Colmar', 23. 'Delicios de Voineşti', 24. 'Romus 4', 25. 'Remus', 26. Redix'', 27. 'Alex', 28. 'Doina', 29. 'Voinicel', 30. 'Inedit', 31. 'Dacian', 32. 'Voinea', 33. 'Valery', 34. 'Real', 35. 'Discoprim', 36. 'Cezar', 37. 'Frumos de Voineşti', 38. 'Pomona', 39. 'Revidar', 40. 'Remar'

 DOI 10.33045/fgr.v39.2023.09

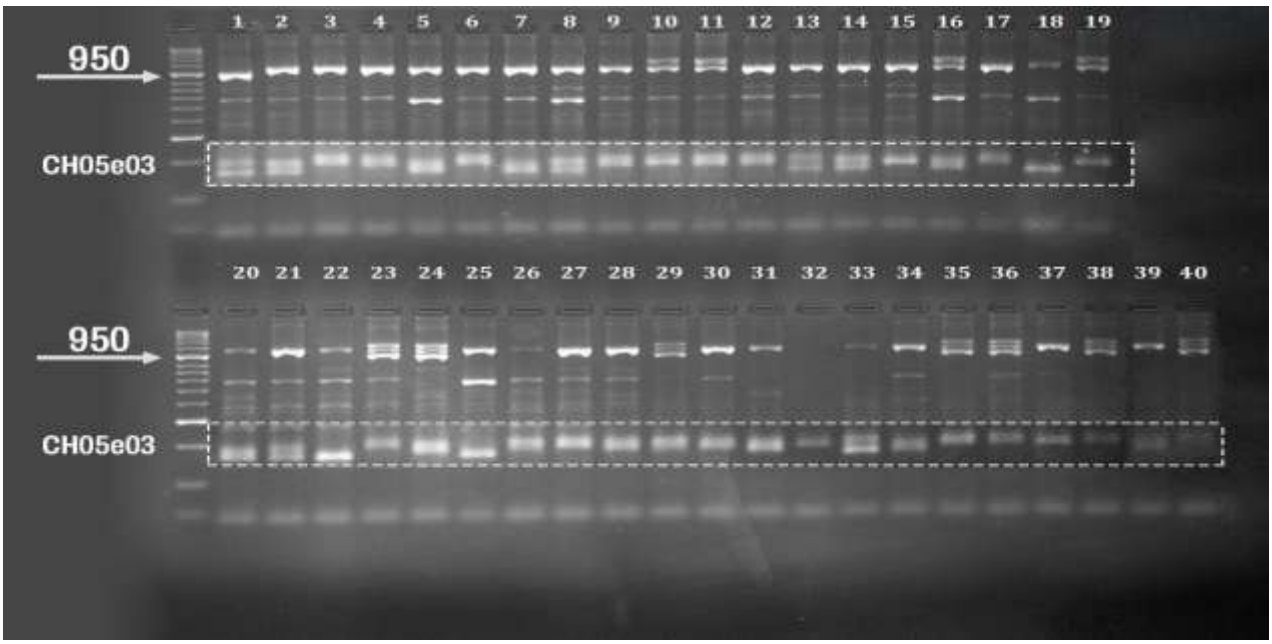

Fig. 9. Multiplex reaction. The electrophoretic profile obtained with AD13 and CH05e03 markers
1. 'Estival', 2. 'Rebra', 3. 'Bistriţean', 4. 'Aura', 5. 'Romus 3', 6. 'Dany', 7. 'Romus 5', 8. 'Productiv de Cluj', 9. 'Luca', 10. 'Ciprian', 11. 'Irisem', 12. 'Slava', 13. 'Ionaprim', 14. 'Rustic', 15. 'Precoce de Ardeal', 16. 'Iris', 17. 'Starkprim', 18. 'Auriu de Cluj', 19. 'Generos', 20. 'Colonade', 21.'Nicol', 22. 'Colmar', 23. 'Delicios de Voineşti', 24. 'Romus 4', 25. 'Remus', 26. Redix'', 27. 'Alex', 28. 'Doina', 29. 'Voinicel', 30. 'Inedit', 31. 'Dacian', 32. 'Voinea', 33. 'Valery', 34. 'Real', 35. 'Discoprim', 36. 'Cezar', 37. 'Frumos de Voineşti', 38. 'Pomona', 39. 'Revidar', 40. 'Remar'.

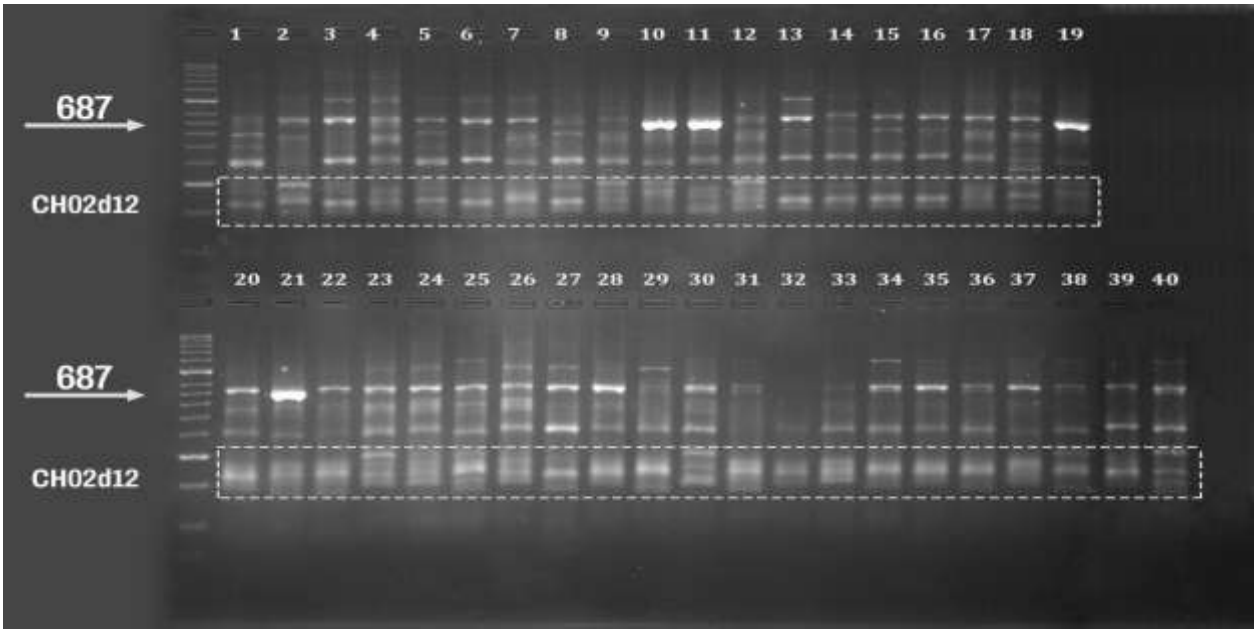

Fig. 10. Multiplex reaction. The electrophoretic profile obtained with OPB12 and CH02d12 markers
1. 'Estival', 2. 'Rebra', 3. 'Bistriţean', 4. 'Aura', 5. 'Romus 3', 6. 'Dany', 7. 'Romus 5', 8. 'Productiv de Cluj', 9. 'Luca', 10. 'Ciprian', 11. 'Irisem', 12. 'Slava', 13. 'Ionaprim', 14. 'Rustic', 15. 'Precoce de Ardeal', 16. 'Iris', 17. 'Starkprim', 18. 'Auriu de Cluj', 19. 'Generos', 20. 'Colonade', 21.'Nicol', 22. 'Colmar', 23. 'Delicios de Voineşti', 24. 'Romus 4', 25. 'Remus', 26. Redix'', 27. 'Alex', 28. 'Doina', 29. 'Voinicel', 30. 'Inedit', 31. 'Dacian', 32. 'Voinea', 33. 'Valery', 34. 'Real', 35. 'Discoprim', 36. 'Cezar', 37. 'Frumos de Voineşti', 38. 'Pomona', 39. 'Revidar', 40. 'Remar'.