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EFFECT OF DNA EXTRACTION METHOD IN THE *ROSA CANINA* L. IDENTIFICATION UNDER DIFFERENT PROCESSING TEMPERATURE

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ABSTRACT
Rosa canina, L. is widely used for medicinal purposes as well as in food industry where it is a valuable source, bioactive compounds and natural colorants. Actually, no specific method is recommended as suitable one for DNA extraction from rose hips. The aim of the study was to compare three commercial and three non-commercial methods to extract total genomic DNA from rose hips hyphanthium. Four methods are based on the precipitation in principle and two methods are based on resin-binding. Extracted DNA was proved for the effectivity in following PCR. In total, six different DNA isolations was performed for differently heat processes rose hips - fresh hyphanthium, 2-weeks frozen hyphanthium, dried hyphanthium (50 °C) and boiled hyphanthium (100 °C). The amplification parameters of 500 bp chloroplast gene amplicon were evaluated. Obtained amounts of extracted DNA was very variable not only for every individual method used but for individual treatment of samples, too. In general, non-commercial method provided higher amount of extracted DNA, but the A260/280 ratio was lower. When regarding the processing treatment of the samples, high differences were found among the samples untreated by heat and those that were dried or boiled for three of the used extraction methods. All the samples were positive for amplification, but different amounts of amplified product were obtained. The comparison of data for concentrations of extracted DNA and concentrations of amplified product showed large differences when regarding the achieved purity of DNA in extraction.

Keywords: dog rose; DNA extraction; PCR effectivity

INTRODUCTION

Dog rose – *Rosa canina* L. is a shrub that is a widespread across a whole Europe where for more than 2,000 years it is used in traditional herbal medicinal purposes in many different ways **(Winther et al., 2016)**. The pseudo fruit of dog rose is called a rose hip and comprises from aggregate achenes enclosed in hypanthium **(De Cock et al., 2008)**. Actually, many of the natural rose hip content is explained about the manner of medical active action. Vitamin C is crucial for the physiological functions in human body and is important in cardiovascular and atherosclerosis prevention **(Halliwell, 2006)**. Carotenoids as a significant part of rose hips secondary metabolites are presented by beta-caroten that act as a precursor of vitamin A as well as a lycopen that is used in food production **(Hornero-Méndez and Mínquez-Mosquera, 2000; Tozzi et al., 2008)**. Beside the above mentioned, flavonoids, fatty acids and antioxidant properties are reported for rose hips as well as anti-inflammatory agents **(Winther et al., 2016)**. Regarding this rich chemical composition, dog rose is used widely in food industry where rose hips are a source of bioactive compounds and color **(Stănilă et al., 2015)**.

Raw rose hips are not used to be eaten (or very rare) without previous processing due to the hairs inside the pseudo fruit. Here, the problems arise not only because of destroying of water soluble chemical. Once the DNA is needed to be extracted from heat processed foods or food stuffs, different aspects must be considered. The processing is connected to the mechanical stress, high temperatures, rapid changes of pH or different enzymatic reactions that all has the potential to change the primary structure of DNA due to the hydrolysis, oxidation or deamination. These all results are significant in degradation or removal of DNA from the sample **(Kharazmi et al., 2003)**. External factors that affect the extraction efficiency are the presence of chemicals in the sample and physicochemical changes during processing. Both of them result to binding of DNA to insoluble form **(Gryson, 2010)**. Furthermore, in processed foodstuffs and food, the efficiency of the DNA extraction method affects the results of molecular analysis strongly, too. Food processes such as thermal treatment lead to a decrease of DNA fragment length and change DNA extraction efficiencies **(Gryson, 2010)**. In all these cases, an inappropriate extraction method leads to the impossibility

A B C

Figure 1 Biological material used in the study. A-2 – weeks frozen hyphanthium; B – dried hyphanthium; C – boiled hyphanthium.

to isolate only clean DNA present in the sample without PCR inhibitors **(Cankar et al., 2006)**.

Actually, no data was found by authors that refer to the method of DNA extraction from rose hips hyphanthium. Different molecular studies od *Rosa canina*, L. are reported, but the extraction was performed always from fresh, frozen or silica dried plant material, leaflets, callus or rhizoids **(Jűrgens et al., 2007; De Cock et al., 2008; Kaul et al., 2009; Kedong et al., 2011; Ritz et al., 2017)**.

Similarly, no specific information was found about the *Rosa canina* L. PCR based authentication in foodstuffs. Here, different sequence data exist in the public databases that can be used for the purpose of specific molecular identification of this specie. Actually, DNA or RNA sequences are known for cytokinin dehydrogenase 5 mRNA, RcSERK1 gene, HK1 gene, rpoB, rpoC1 or matK1 genes.

In this study, different DNA extraction method was proved for the effectivity in following PCR. In total, six different DNA isolations were performed for differently heat processes rose hips and the amplification parameters of amplification of chloroplast gene were evaluated.

MATERIAL AND METHODOLOGY

Biological material of *Rosa canina* L. was collected in autumn 2016 in the locality of town Skalica. The rose hips were transported in the laboratory and devided into seeds and red fleshy floral cups – hyphanthium.

The first part of fresh hyphanthium was immediately processed by DNA extraction (Table 1, Figure 1), the second was dried at 50 °C and the third part was boiled at 100 °C. The last part of the hyphanthium was stored by – 20 °C for two weeks and was processed by DNA extraction after this storage period.

DNA extraction methods

In total, six different methods were tested to extract a total genomic DNA from rose hips (Table 2). Three of them are non-commercial and three of them are commercial extraction kits. For all the extraction methods, a 100 µg of rose hips were used.

A different strategies were a part of the tested method – based on lyses and purification with cetyltrimethylammonium bromide; using a dodecyl sulfate as detergent or commercial kits with membrane system of cleaning the lysate **(Rogers and Bendich, 1985; Dellaporta et al., 1983)**. All the extractions were performed in biological triplicates. Nanodrop Nanophotometer™ was used for quantity and quality setting of the extracted DNA.

PCR reactions

The PCRs were performed by using the primer pair that amplifies the region of plant chloroplast as referred in **Thion et al. (2002)**. A fragment of a length of 500 bp was the obtained product of amplification. Dream Tag DNA maser mix (2x) (Thermo Scientific) was used for the PCR reactions that were performed in 20µL.

Thermal cycling profile was as follows: 95°C 5 min; (95 °C 40 sec; 55 °C 30 sec; 72 °C 40 sec) 45 x with final extension 72 °C 5 min. All the amplifications were performed in technical triplicates in a Bio-Rad C1000™ Thermocycler.

Analyses of PCR products

The amplified product was screen for the right amplification without non specific products on 1% agarose gel electrophoresis in 1xTBE buffer stained by GelRed™. The screening analyses were performed from the first of

Table 1 Codes of samples under processing variants.

Code in sample	Processing method
1	Fresh hyphanthium
2	2-weeks frozen hyphanthium
3	Dried hyphanthium (50 °C)
4	Boiled hyphanthium (100 °C)

Table 2 Methods of DNA isolation performed in the study

Sample code	Extraction method/kit	Principle of the method
A	Rogers and Bendich, 1994	precipitation
B	Dellaporta et al., 1983	precipitation
C	Padmalatha and Prasad, 2006	precipitation
D	Power Plant Pro DNA Isolation Kit, www.mobio.com	precipitation
E	Illustra DNA Extraction Kit Phytopure, www.gelifesciences.com	resin-binding
F	NucleoSpin® Food, www.mn-net.com	resin-binding

technical triplicates of PCR. The second of the technical triplicates of PCR was used for specific amplicons cleaning. This was performed with Agencourt AMPure XP solid-phase paramagnetic bead purification system (Beckman Coulter) following the manufacturer´s instructions. Pure amplicons were dissolved in water and measured for the quantity and quality by Nanodrop Nanophotometer™.

RESULTS AND DISCUSSION

In the study, differently processed rose hips hyphanthia were subjected to six DNA extraction methods to find the most appropriate for PCR product amplification. Dog roses possess the high levels of different secondary metabolites what in combination with heat processing decreases the quality of extracted DNA. All the extraction methods that are used for plant tissues consists from carbohydrates and enzymes that ensure lysis of cell wall **(Manen et al., 2005)**, because polysaccharides, polyphenols and other organic compounds very often pose problems in plant DNA isolation process **(Cota-Sánchez et al., 2006)**. That is why mature plant tissues are not the most suitable for DNA extraction **(Dabo et al., 1993; Zhang et al., 2000)** and juvenile leaves are most often chosen in molecular analyses **(Jűrgens et al., 2007; De Cock et al., 2008; Kaul et al., 2009; Kedong et al., 2011; Ritz et al., 2017)**. Until now, to knowledge of authors, no specific extraction protocol was published for the total genomic DNA extraction from rose hips hyphanthium as well as in connection to the *Rosa canina* L. authentication in food.

When **Rogers and Bendich (1994)** and **Padmalatha and Prasad (2006)** extraction protocols were used for rose hips, the high level of contamination (Figure 2) and viscose pellet formation was observed through the

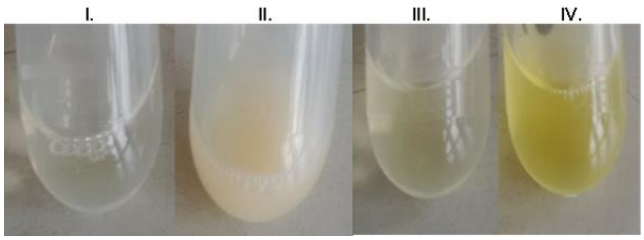

Figure 2 Differences of extraction lysates after the first incubation step when different extraction protocols used. I. – NucleoSpin® Food; II. – Padmalatha and Prasad; III. – Dellaporta; IV. – Rogers and Bendich.

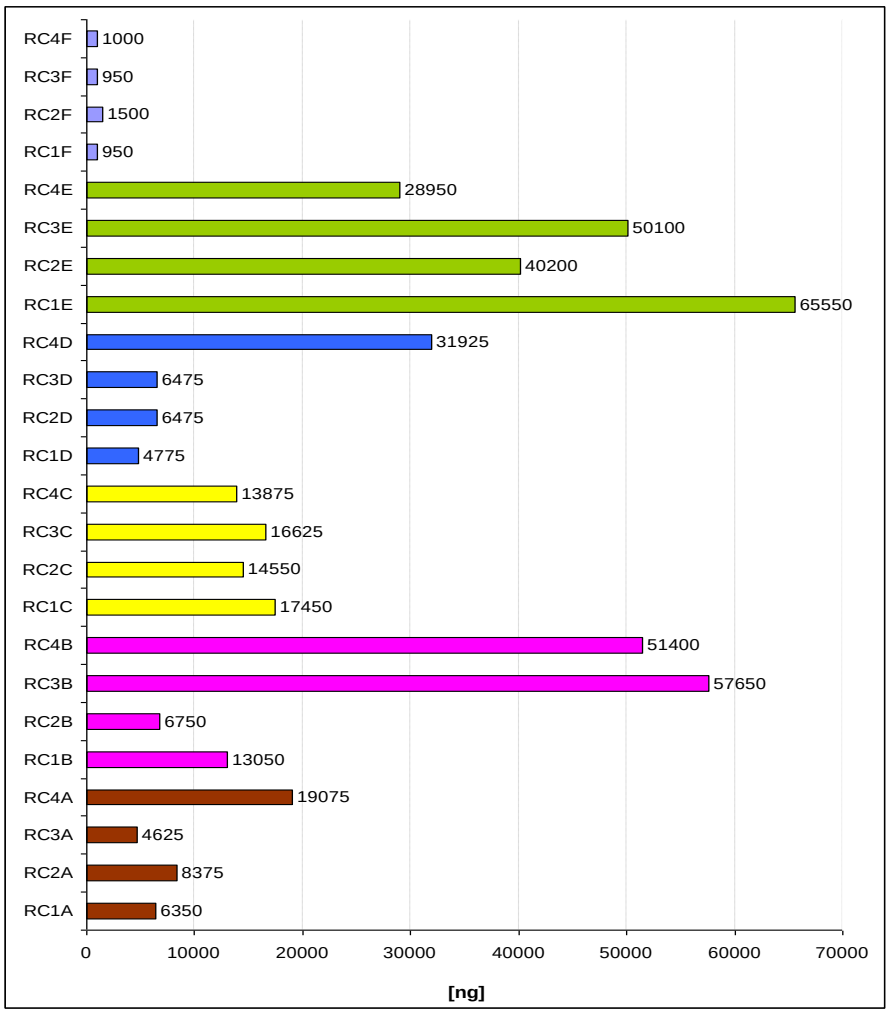

Figure 3 Total genomic DNA concentration in samples obtained by extraction methods.

Table 3 Average A260/A280 ratios in triplicates of extracted DNA from rose hips under tested extraction methods and processing variants.

Sample	A260/A280 ratios	Sample	A260/A280 ratios
RC1A	1.65	RC1D	1.8
RC2A	1.55	RC2D	1.8
RC3A	1.6	RC3D	1.75
RC4A	1.7	RC4D	1.8
RC1B	1.4	RC1E	1.7
RC2B	1.4	RC2E	1.8
RC3B	1.4	RC3E	1.8
RC4B	1.45	RC4E	1.75
RC1C	1.65	RC1F	1.75
RC2C	1.7	RC2F	1.8
RC3C	1.7	RC3F	1.75
RC4C	1.6	RC4F	1.7

extraction. In the case of **Dellaporta et al. (1983)** lysate protocol was comparable to the commercial kits that use membrane microtubes from the first steps of extraction process.

Total amounts of extracted DNA was very variable not only for every individual method used but for individual treatment of samples, too (Figure 3).

Only in the case of NucleoSpin® Food extraction kit and **Padmalatha and Prasad (2005)** extraction protocol, total amount of extracted DNA was comparable for the different treatments of rose hips hyphanthium with the results of the GeneJET™ Plant Genomic DNA Purification Mini Kit where the amount ranged from 10 – 200 ng.μL^{-1} and no contamination was detected, so both of the protocol gives the DNA suitable for PCR analyses.

When comparing all tested methods, the highest average concentration of DNA was obtained by the Illustra DNA Extraction Kit Phytopure – 46 200 ng.μL^{-1}, followed by extraction method according to **Dellaporta et al. (1983)** – 32 212 ng.μl^{-1}. When regarding the processing treatment of the samples, high differences were found among the samples untreated by heat and those that were dried or boiled for **Dellaporta et al. (1983)** protocol. **Rogers and Bendich (1994)** protocol and Power Plant Pro DNA Isolation Kit give the higher concentration of extracted DNA for the fresh rose hips and all the other treatments were comparable within themselves. The lowest amount of isolated DNA was obtained by NucleoSpin® Food extraction kit. The average values of DNA purity was in range of 1.45 – 1.8 (Table 3), but the commercial extraction kits provide extracted DNA that was less contaminated by potential PCR inhibitors.

All the samples were diluted for 100 ng.μL^{-1} and in subsequent PCR, the analyses of the effectivity of extracted DNA in enzymatic amplification was proved. The chloroplast gene target **Thion et al. (2002)** was chosen to be amplified from 50 ng of. All the samples were positive for amplification, but different amounts of amplified product were obtained (figures 4 and 5). Here, a correlation (*p*-value 0.011; correlations at the 95.0% confidence level) can be seen between the amount of extracted DNA and the amount of amplified PCR products. The strongest amplification was obtained for samples where the DNA was extracted by NucleoSpin® Food extraction kit. This kit is specifically designed for the purposes of DNA extraction from food samples, that are

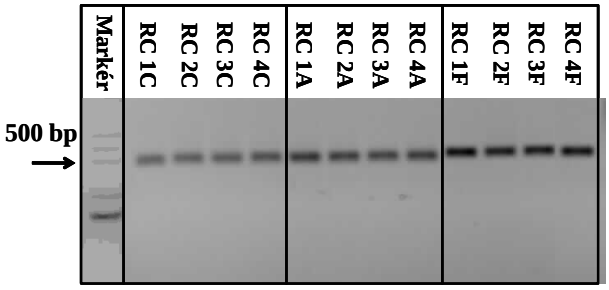

Figure 4 Amplification of target chloroplast sequence for samples extracted by methods of Padmalatha and Prasad, Rogers and Bendich and NucleoSpin® Food extraction kit.

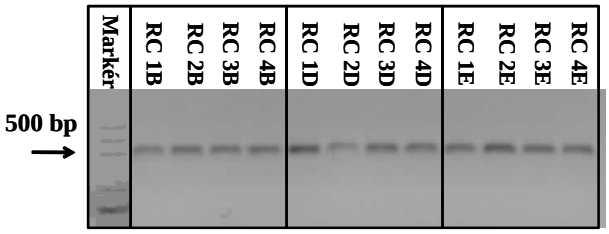

Figure 5 Amplification of target chloroplast sequence for samples extracted by method of Dellaporta et al. and Power Plant Pro DNA Isolation Kit and Illustra DNA Extraction Kit Phytopure.

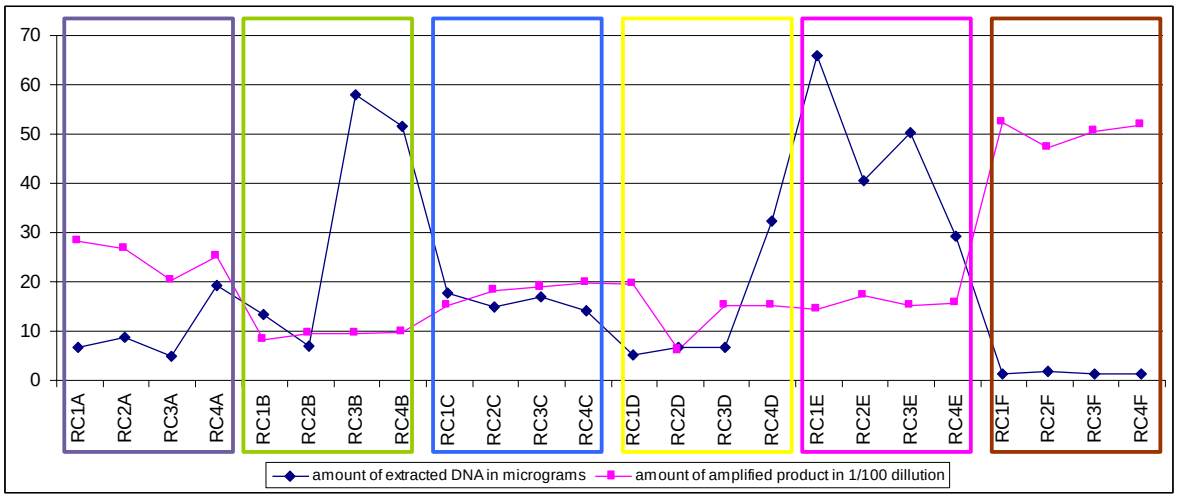

Figure 6 Comparison of tendencies between amount of extracted DNA (blue line) and amount of amplified PCR product (purple line). Different colour boxes corresponds to colours in Figure 2 and differs the extraction methods used in the study.

Table 4 Average amounts of amplified PCR products when 50 ng . μL^{-1} DNA used as starting point.

Sample	µg of PCR product	Sample	µg of PCR product
RC1A	2.76	RC1D	1.97
RC2A	2.65	RC2D	0.65
RC3A	2.10	RC3D	1.58
RC4A	2.65	RC4D	1.53
RC1B	0.81	RC1E	1.36
RC2B	0.86	RC2E	1.65
RC3B	0.94	RC3E	1.53
RC4B	0.98	RC4E	1.47
RC1C	1.60	RC1F	5.25
RC2C	1.89	RC2F	4.83
RC3C	1.85	RC3F	5.17
RC4C	1.98	RC4F	5.23

very heterogeneous and contain many different compounds that often lead to suboptimal extraction. NucleoSpin® Food guarantees good recovery for small genomic DNA fragments that are shorter than 1 kbp and is recommended for samples where a very low DNA content is present.

A specific situation was found for the amount of extracted DNA and the amount of amplified product for the results of Illustra DNA Extraction Kit Phytopure. Here, the highest concentration of extracted DNA was obtained, but the amout of amplified product ranged from 1.36 to 1,53 $\mu g.\mu L^{-1}$ (Table 4).

The comparison of data for concentrations of extracted DNA and concentrations of amplified product (figure 6) show large differences when regarding the achieved purity of DNA in extraction (Table 3).

When comparing the commercial and non-commercial methods used in the study, no preference can be done for this kind of dividing of them. Residual PCR contaminants were present mostly in samples extracted by non-commercial methods, but the amount of amplified product was not affect by this (*p*-value 0.0692 at the 95.0% confidence level). The phenol based extraction methods are reported to possess the disadvantage that phenol contaminants inhibit and reduce the efficiency of PCR **(Hiesinger et al., 2001)**. Similar results and decreased amount of amplified PCR products as obtained in this study was reported by **Drábková et al. (2002)**. According to A260/A280 ratio and the repeatability of the extractions, all commercial methods used in the study yielded relatively pure DNA. On the other side, for commercial kits, the largest differences were obtained, when the kit with the highest amount of extracted DNA provided only the average amounts of amplicons and the kit with the lowest amount of extracted DNA provided the highest amounts of generated amplicons. This may be a result of the presence of inhibitors in extracted DNA that originated from the sample **(Volk et al., 2014)**. In the case of non-commercial kits, the reasons with the lower amounts of amplicons achieved in PCR could be connected to EDTA and isopropanol that was used for DNA extraction and those remain as traces in the extracted DNA **(Bar et al., 2012; Hedman and Rådström, 2013)**. The functionality of extracted DNA in PCR is the most important evaluation factor as this determines the suitability of an extraction method. Molecular analyses based on the precisely extracted DNA are a part of many different types of DNA based analyses today **(Gálová et al., 2015; Petrovičová et al., 2015; Balážová et al., 2016)** For plant tissues, determination of the most suitable method is always a crucial step where a success of the analyses starts. **Huaqiang et al. (2013)** reported the comparison of 6 DNA extraction methods for *Vigna unguiculata* L – Rogers and Bendich, Dellaporta, Doyle, Saghai-Maroof, Aljanabi and E.Z.N.A. commercial kit. The highest yields were obtained

for Aljanabi and Dellaporta extraction methods and the highest purity was obtained by Doyle method and E.N.Z.A kit. **Doosty et al. (2012)** compared 4 extraction method in the isolation of total genomic DNA of *Satureja khuyistanica* – **Dellaporta (1983)**; **Doyle and Doyle, (1990)**; **Murry and Thompson (1980)** and **Kang and Yang (2004)**. A very low quality and quantity was reported in extracted DNA for Dellaporta protocol and the authors referred it to the SDS buffer used in the method that interfered with the secondary metabolits. **Abu-Romman, (2011)** reported the comparison of 4 plant DNA extraction protocols – **Bokszczanin and Prazybyla (2006)**; **Doyle and Doyle (1987)**; **Krizman et al. (2006)** and **Sarwat et al. (2006)** for *Salvia officinalis* L. In this study again – non-commercial CTAB method by Doyle and Doyle provided the lowest DNA yield with insufficient quality.

CONCLUSION

Molecular DNA based methods are routinely applied in many fields of analyses connected to the plant tissues or food today. The precise results are fully dependent on the successful extraction of DNA with an appropriate quantity and quality. In this study, six DNA extraction methods were compared and analysed for the effectivity of PCR amplification. Four types of differently processed rose hips were used as a biological material. The yield of extracted DNA was in range of 0.9 up to 65 µg x μL^{-1}. The purity of extracted DNA is higher for commercial kits that were used. Functionality of the extracted DNA was proved in the PCR analyses and the amounts of amplified products were measured. Based on the results, the most suitable DNA extraction method for *Rosa canina* L. hyphanthium was proved NucleoSpin® Food extraction kit for all the differently processed rose hips.

REFERENCES

Abu-Romnan, S. 2011. Comparison of methods for isolating high quality DNA from sage (*Salvia officinalis*). *Journal of Medicinal Plants Research*, vol. 5, no. 6, p. 938-941.

Bar, T., Kubista, M., Tichopad, A. 2012. Validation of kinetics similarity in qPCR. *Nucleic Acids Research*, vol. 40, p. 1395-1406. https://doi.org/10.1093/nar/gkr778

Balážová, Ž., Petrovičová, L., Gálová, Z., Vivodík, M. 2016. Molecular characterisation of rye cultivars. *Potravinarstvo*, vol. 10, no. 1, p. 54-58. https://doi.org/10.5219/522

Bokszczanin, K., Prazybyla, A. A. 2006. Copper (II) acetate improves the quality of pear (*Pyrus*) DNA during extraction. *Plant Molecular Biology Reporter Journal*, vol. 24, p. 249a-249d. https://doi.org/10.1007/BF02914065

Cankar, K., Stebih, D., Dreo, T., Zel, J., Gruden, K. 2006. Critical points of DNA quantification by real-time PCR – effects of DNA extraction method and sample matrix on quantification of genetically modified organisms. *BMC Biotech*, vol. 6, p. 37. https://doi.org/10.1186/1472-6750-6-37

Cota-Sánchez, H. J., Remarchuk, K., Ubayasena, K. 2006. Ready-to-Use DNA Extracted with a CTAB Method Adapted for Herbarium Specimens and Mucilaginous Plant Tissue. *Plant Molecular Biology Reporter*, vol. 24, no. 2, p.161-167. https://doi.org/10.1007/BF02914055

Dabo, S. M., Mitchell, E. D. Jr, Melcher, U. 1993. A method for the isolation of nuclear DNA from cotton (*Gossypium*) leaves. *Analytical Biochemistry*, vol. 210, no. 1, p. 34-38. https://doi.org/10.1006/abio.1993.1146 PMid:8098189

Dellaporta, S. L., Wood, J., Hicks, J. B. 1983. A Plant DNA Minipreparation: Version II. *Plant Molecular Biology Reporter*, vol. 1, no. 4, p. 19-21. https://doi.org/10.1007/BF02712670

De Cock, K., Mijnsbrugge, K. V., Breyne, P., Van Bockstaele, E., Van Slycken, J. 2008. Morphological and AFLP-based differentiation within the taxonomical complex section *Canine* (subgenus *Rosa*). *Annals of Botany*, vol. 102, no. 5, p. 685-697. https://doi.org/10.1093/aob/mcn151 PMid:18723861

Doosty, B., Drikvand, R., Salahvarzi, E., Amiri, H., Hadian, J. 2012. Comparative Analysis and Optimization of Different DNA Extraction Protocols in *Satureja khuzistanica*. *International Journal of Biology*, vol. 4, no. 4, p. 112-116. https://doi.org/10.5539/ijb.v4n4p111

Doyle, J. J., Doyle, J. L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, vol. 19, no. 1, p.11-15.

Drábková, L., Kirschner, J., Vlček, E. 2002. Comparison of seven DNA extraction and amplification protocols in historical herbarium specimens of *Juncaceae*. *Plant Molecular Biology Reporter*, vol. 20, no. 2, 161-175. https://doi.org/10.1007/BF02799431

Gálová, Z., Vivodík, M., Balážová, Ž., Kuťka-Hlozáková, T. 2015. Identification and differentiation of *Ricinus communis* L. using SSR markers. *Potravinarstvo*, vol. 9, no. 1, p. 556-561. https://dx.doi.org/10.5219/516

Gryson, N. 2010. Effect of food processing on plant DNA degradation and PCR-based GMO analysis: a review. *Analytical and Bioanalytical chemistry*, vol. 396, no.6, p. 2003-20022. https://doi.org/10.1007/s00216-009-3343-2

Halliwell, B. 2006 Polyphenols: antioxidant treats for healthy living or covert toxins? *Journal Science Food Agriculture*, vol. 86, no. 13, p. 1992-1995. https://doi.org/10.1002/jsfa.2612

Hedman, J., Rådström, P. 2013. Overcoming inhibition in real-time diagnostic PCR. *Methods in Molecular Biology*, vol. 943, p. 17-48. https://doi.org/10.1007/978-1-60327-353-4_2

Hiesinger, M., Löffert, D., Ritt, CH., Oelműller, U. 2001. The effects of phenol on nucleic acid preparation and downstream applications. *Qiagen News*, vol. 5, p. 23-26.

Huaqiang, T., Haitao, H., Mamman, T., Jianyao, M., Huanxiu, L. 2013. Comparative Analysis of Six DNA Extraction Methods in Cowpea (*Vigna unguiculata* L.Walp). *Journal of Agricultural Science*, vol. 5, no. 7, p. 82-90. https://doi.org/10.5539/jas.v5n7p82

Hornero-Méndez, D., Mínguez-Mosquera, M. I. 2000. Carotenoid pigments in *Rosa mosqueta* hips, an alternative carotenoid source for foods. *Journal of Agricultural Chemistry*, vol. 48, no. 3, p. 825-828. https://doi.org/10.1021/jf991136n

Jűrgens, A. H., Seitz, B., Kowarik, I. 2007. Genetic differentiation of *Rosa canina* (L.) at regional and continental scales. *Plant Systematic and Evolution*, vol. 269, no. 1, p.39-53. https://doi.org/10.1007/s00606-007-0569-3

Kang, T. J., Yang, M. S. 2004. Rapid and reliable extraction of genomic DNA from various wild-type and transgenic plants. *BMC Biotechnology*, vol. 4, p. 20. https://doi.org/10.1186/1472-6750-4-20

Kaul, K., Dhyani, D., Sharma, R. K. 2009. Evaluation of DNA Extraction Methods for RAPD, SSR and AFLP Analyses of Wild Rose Species. *Floriculture and Ornamental Biotechnology*. vol. 3, no. 1, p. 25-30.

Kedong, X., Qinglin, L., Huifang, Y., Li, Z., Lili, D., Fengluan, L., Ling, Bi., Nan, M., Liangjun, Z. 2011. Isolation and molecular characterization of RcSERK1: A Rosa canina gene transcriptionally induced during initiation of protocorm-like bodies. *African Journal of Biotechnology*, vol. 10, p. 4011-4017.

Kharazmi, M., Bauer, T., Hammes, W. P., Hertel, C. 2003. Effect of food processing on the fate of DNA with regard to degradation and transformation capability in *Bacillus subtilis*. *Systematic and Applied Microbiology*, vol. 26, no 4, p. 495-501. https://doi.org/10.1078/072320203770865774 PMid:14666975

Krizman, M., Jakse, J., Baricevic, D., Javornik, B., Prosek, M. 2006. Robust CTAB-activated charcoal protocol for plant DNA extraction. *Acta Agriculturae Slovenica*, vol. 87, no. 2, p. 427-433.

Manen, J. F., Sinitsyna, O., Aeschbach, L., Markov, A. V., Sinitsyn, A. 2005. A Fully Automatable Enzymatic Method for DNA Extraction from Plant Tissue. *BMC Plant Biology*, vol. 5, no. 23, p. 1-9. https://doi.org/10.1186/1471-2229-5-23

Murry, M. G., Thompson, W. F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research*, vol. 8, no. 19, 4321-4325. https://doi.org/10.1093/nar/8.19.4321

Padmalatha, K., Prasad, M. N. V. 2006. Optimization of DNA isolationand PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. *African Journal Biotechnology*, vol. 5, no.3, p. 230-234.

Petrovičová, L., Gálová, Z., Balážová, Ž., Vivodík, M., Wójcik-Jagla, M., Rapacz, M. 2015. Assessment of RAPD polymorphism in rye (*Secale cereale* L.) genotypes. *Journal of Microbiology, Biotechnology and Food Sciences*, vol. 4, no. 2, p. 94-97. https://doi.org/10.15414/jmbfs.2015.4.special2.94-97

Ritz, Ch. M., Wissemann, V. 2011. Microsatellite analyses of artificial and spontaneous dogrose hybrids reveal the hybridogenic origin of *Rosa micrantha* by the contribution of unreduced gametes. *Journal of Heredity*, vol. 102, no. 2, p. 217-227. https://doi.org/10.1093/jhered/esq124

Sarwat, M., Negi, M. S., Lakshmikumaran, M., Tyagi, A. K., Das, S., Srivastava, P. S. 2006. A standardized protocol for the genomic DNA isolation from *Terminalia arjuna* for genetic diversity analysis. *Electronic Journal of Biotechnology*, vol. 9, no. 1, p. 86-91. https://doi.org/10.2225/vol9-issue1-fulltext-3

Stănilă, A., Diaconeasa, Z., Roman, I., Sima, N., Măniuţiu, D., Roman, A., Sima, R. 2015. Extraction and Characterization of Phenolic Compounds from Rose Hip (*Rosa canina* L.) Using Liquid Chromatography Coupled with Electrospray Ionization – Mass Spectrometry. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 43, no. 2, p. 349-354.

Rogers, S. O., Bendich, A. J. 1994. Extraction of total cellular DNA from plants, algae and fungi. In: Gelvin, S. B. et al. *Plant Molecular Biology Manual D1*. 2nd ed. Belgium : Kluwer Academic Publishers, pp. 1-8. ISBN 0-7923-2868-2. https://doi.org/10.1007/978-94-011-0511-8_12

Tozzi, R., Mulinacci, N., Storlikken, K., Pasquali, I., Vincieri, F. F., Bettini, R. 2008. Supercritical extraction of carotenoids from *Rosa canina* L. hips and their formulation with beta-cyclodextrin. *AAPS PharmSciTech*, vol. 9, no. 2, p.693-700. https://doi.org/10.1208/s12249-008-9100-6 PMid:18528762

Volk, H., Piskernik, S., Kurinčič, M., Klančnik, A., Toplak, N., Jeršek, B. 2014. Evaluation of different methods for DNA extraction from milk. *Journal of Food and Nutrition Research*, vol. 2, p. 1-10.

Winther, K., Vinther Hansen, A. S., Campbell-Tofte, J. 2016. Bioactive ingredients of rose hips (*Rosa canina*, L) with special reference to antioxidative and anti-inflamatory properties: in vitro studies. *Botanics: Targets and Therapy*, vol. 6, p. 11-23. https://doi.org/10.2147/BTAT.S91385

Zhang, J., Stewart, J. M. 2000. Economic and rapid method for extracting cotton genomic DNA. *Journal of Cotton Science*, vol. 4, p. 193-201.

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