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EFFECTS OF STORAGE TEMPERATURE ON THE QUALITY AND QUANTITY OF DNA EXTRACTED FROM MAIZE LEAVES

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Abstract: This study was carried out to evaluate the effect of temperature during storage of maize leaves and extracted DNA on its quality and quantity in order to be efficiently amplified using PCR. Leaves were collected from the four-week-old plants and divided into three groups of 20 samples. The first group of leaves was processed immediately, while the other two were stored at -20°C or -80°C for 30 days. The DNA extracted from the fresh leaves was divided into three portions with the first being amplified immediately and the other two were stored at -20°C or -80°C for 30 days. The DNA quality and quantity were examined using a biospectrometer, after which the samples were diluted for the PCR assay. The quality of all DNA samples was at an acceptable level with their average $OD_{260/280}$ ratio in the range from 1.85 to 1.87. The concentration of the DNA extracted immediately from fresh leaf tissue was not statistically different from the stored samples. Both the quality and quantity of DNA in all samples were sufficient for successful PCR amplification with two *opaque2*-specific molecular markers. Phi057 amplified a ~170bp fragment in QPM and ~160bp in non-QPM, while umc1066 amplified a ~150bp fragment in QPM and ~160-170bp in non-QPM. Our results suggest that appropriate storage conditions do not affect the DNA quality and quantity. This could be useful in marker-assisted selection of target genes, when a large number of samples must be processed prior to pollination, allowing breeders to discard plants lacking the desired alleles and reduce the size of the breeding population.

Key words: DNA quality, DNA quantity, extraction, maize, storage temperature.

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Introduction

Marker-assisted selection (MAS) has become an efficient method for crop improvement programs due to its precise and accelerated approach (Vivekananda et al., 2018). According to Eathington et al. (2007), MAS methodologies have increased the mean performance of progeny as compared to the conventional breeding methodologies. As a method used worldwide to rapidly incorporate valuable traits into new cultivars, it is used in breeding to select progenies with the desired genes. Once the DNA has been extracted from the plants, genetic markers are used to tag and track the genetic variation in the DNA samples, which can be identified by polymerase chain reaction (PCR) and electrophoresis (Begna, 2020).

The extraction of DNA with good quality and high yield is a limiting factor in the genetic analysis of plants (Abdel-Latif and Osman, 2017). The quality and quantity of the DNA extracted from cereals are often affected by the presence of polysaccharides, proteins, and DNA polymerase inhibitors, rendering the sample non-amplifiable (Sarwat et al., 2006). Although many DNA extraction protocols have been developed for plants (Dellaporta et al., 1983; Saghai-Maroof et al., 1984; Doyle and Doyle, 1987), some of them include time-consuming steps, laborious works, or the use of liquid nitrogen, which can be hard to procure. Furthermore, some DNA isolation methods from plant tissues produce either small amounts or DNA of inconsistent quality (Abdel-Latif and Osman, 2017). On the other hand, there are methods of DNA extraction that can be performed within a short period of time in a laboratory with basic facilities, especially suitable for foreground selection in the MAS program (Dorokhov and Klocke, 1997; Vivekananda et al., 2018). The quality and quantity of the extracted DNA can be assessed spectrophotometrically. The A_{260}/A_{280} absorption ratio provides an estimation of DNA purity by considering contaminants that absorb UV light, such as proteins (Vahdani et al., 2024). Besides the acceptable DNA purity, the sufficient quantity of DNA for successful PCR amplification must also be considered.

Researchers and scientists from the Maize Research Institute Zemun Polje have integrated conventional and molecular breeding programs aimed at converting standard maize inbred lines to quality protein maize (QPM) genotypes for growing in temperate regions (Kostadinovic et al., 2016; Ignjatovic Micic et al., 2020). Marker-assisted breeding involves a large number of individual plants that need to be analyzed prior to pollination. Therefore, in addition to a rapid and reliable DNA extraction protocol, adequate storage conditions are important due to its large-scale requirements. The aim of our study was to evaluate the effect of storage temperature on the quality and quantity of DNA extracted from maize leaves for use in marker-assisted selection to improve maize quality.

Material and Methods

Leaves were collected from the four-week-old plants that are parental lines in the marker-assisted conversion of maize inbred lines to quality protein maize (QPM) adapted to temperate climate. The collected maize leaves were divided into three groups of 20 samples. The first group was processed immediately, while the other two were stored at -20°C or -80°C for 30 days. The genomic DNA was extracted following the modified protocol of Dorokhov and Klocke (1997). The DNA extracted from fresh leaves was divided into three portions with the first being processed immediately and the other two stored for 30 days at -20°C or -80°C. The DNA quantity and quality were examined using a biospectrometer (BioSpetrometer kinetic, Eppendorf, Germany). The DNA concentration was measured directly in µg/mL, while the DNA quality was measured as DNA/RNA ratio ($A_{260/280}$). The t-test (Microsoft Excel) was used to compare the mean values among the treatments at the probability level $P<0.05$.

All samples were diluted to a working concentration of 20 ng/µl for the application in polymerase chain reaction (PCR). Two SSR markers specific for the *opaque2* gene (phi057 and umc1066) were employed in the PCR assay to evaluate the efficiency of DNA amplification (Table1). The polymerase chain reaction was carried out in a reaction volume of 20 µl containing the following: DreamTaq™ Green PCR Master Mix (Thermo Scientific, USA), 0.25 µM primers and 20 ng of DNA. Amplifications were performed in the Biometra TProfessional Standard 96 thermocycler (Biometra, Germany) with the following program: an initial denaturation at 94ºC /2min, followed by 40 cycles each of denaturation at 94ºC/1min, annealing at 60ºC/2min and extension at 72ºC/2min, with final extension at 72ºC/10min. The amplified fragments were resolved by electrophoresis on 8% polyacrylamide gel (Mini Protean Tetra-Cell, Bio-Rad, USA).

Table 1. The set of *opaque2* gene-specific markers used for PCR.

Primer		Sequence (5'-3')	Fragment size
phi057	F	CTCATCAGTGCCGTCGTCCAT	160–170 bp
	R	CAGTCGCAAGAAACCGTTGCC	
umc1066	F	ATGGAGCACGTCATCTCAATGG	150–160 bp
	R	AGCAGCAGCAACGTCTATGACACT	

After staining with ethidium bromide, they were visualized under UV transilluminator and documented in the gel documentation system BioDocAnalyze (Biometra, Germany). The approximate size range of the amplification products for each SSR locus was determined based on the positions of the bands relative to the 50 bp molecular weight ladder.

Results and Discussion

Extraction and purification of DNA represent one of the basic steps in molecular biology and therefore the preparation of high-quality DNA from various sources, such as fresh and frozen tissue, is the most important first step (Samoo et al., 2017). Although fresh tissue is the best source for high molecular weight DNA extraction, in some cases fresh tissue cannot be obtained or only previously collected and stored samples are available (Alrokayan, 2000). Furthermore, some methods and large-scale experiments require DNA extraction of a large number of samples in a short period of time, so the storage conditions are of great importance.

In this research, the concentration and purity of DNA extracts from fresh maize leaves were compared to extracts and leaves stored at -20°C or -80°C for 30 days. The quality and purity of the DNA is presented as $A_{260/280}$ ratio. A high A_{260}/A_{280} value indicates RNA contamination, while a low A_{260}/A_{280} ratio indicates DNA contamination with proteins (Meyer, 2003). With an average $OD_{260/280}$ ratio in the range from 1.85 to 1.87, the quality of all DNA samples was at an acceptable level with values between 1.8 and 2.0 (Gryson, 2010). No significant differences were found for A_{260}/A_{280} values between the DNA extracted from fresh leaf tissue immediately after sampling and stored samples. Similarly, there were no significant differences between the concentration of the DNA extracted from fresh leaf tissue immediately after sampling and the samples that were stored. However, concentration of DNA was numerically the highest in the samples extracted from the leaves stored at -20°C. The concentration and purity of DNA extracted from maize leaves are presented in Table 2.

Table 2. The concentration and purity of DNA extracted from maize leaves.

	Fresh	Fresh/-20°C	Fresh/-80°C	-20°C	-80°C
DNA concentration (μg/mL)	523.84^{ns}	536.28^{ns}	538.64^{ns}	544.57^{ns}	534.08^{ns}
DNA purity ($A_{260/280}$)	1.87^{ns}	1.87^{ns}	1.85^{ns}	1.85^{ns}	1.87^{ns}

Both the quantity and quality of DNA in all samples were sufficient for successful PCR amplification with two *opaque2*-specific molecular markers. The phi057 amplified a ~170bp fragment in QPM and ~160bp in non-QPM, while umc1066 amplified a ~150bp fragment in QPM and ~160-170bp in non-QPM. The amplification with the SSR marker umc1066 is given in Figure 1. The dominant homozygotes (lanes 1, 3 and 12) were clearly distinguished from the recessive homozygotes (lanes 7, 8 and 10) and the heterozygous individuals (lanes 2, 4, 5, 6, 9 and 11).

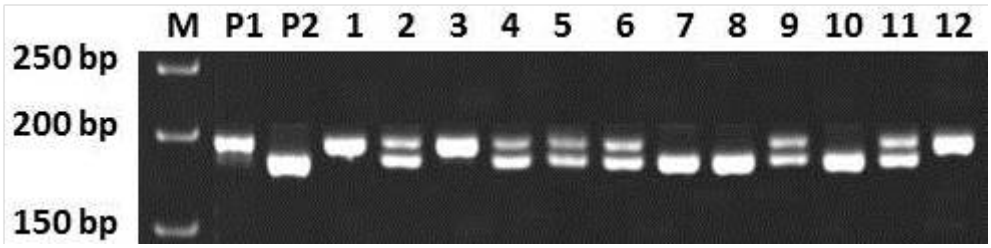

Figure 1. Amplification with the *opaque2*-specific marker umc1066. M: 50 bp DNA ladder, P1: standard line, P2: QPM line, 1–12: analyzed individual plants.

Based on these results, it can be concluded that the DNA was intact and the banding patterns showed no obvious difference or any form of DNA degradation. This is an indication that the extracted DNA from all samples is of good quality and suitable for PCR analysis (Adetumbi et al., 2013). This could be useful in marker-assisted selection of target genes, when a large number of samples must be processed prior to pollination, allowing breeders to discard plants without alleles of interest and reduce the size of the breeding population. The time elapsed between sampling and DNA extraction depends principally on the experiment and storage conditions. If tissue preservation conditions and sampling are appropriate, the storage time will not be a factor at least for short storage periods (Samoo et al., 2017).

Conclusion

This study provides useful information for marker-assisted breeding, where a large number of plants need to be processed and therefore a large number of DNA extracts need to be handled. Our results indicate that adequate storage of leaves/extracted DNA does not have a negative effect on DNA yield in terms of quality, quantity, and integrity, at least for short storage periods. The samples extracted from stored leaves as well as the stored extracts, exhibited not only a sufficient DNA amount, but also ensured their purity for PCR applications.

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UTICAJ TEMPERATURE SKLADIŠTENJA NA KVALITET I KOLIČINU DNK IZOLOVANE IZ LISTA KUKURUZA

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R e z i m e

Cilj ovog istraživanja je bio ispitivanje efekta temperature skladištenja listova kukuruza i iz njih izolovane DNK na njen kvalitet i količinu radi efikasnog umnožavanja pomoću lančane reakcije polimeraze. Listovi su uzeti sa biljaka starih četiri nedelje koji su podeljeni u tri grupe od po 20 uzoraka. Prva grupa listova je analizirana odmah, dok su druge dve čuvane 30 dana na -20°C i -80°C. DNK izolovana iz svežih listova je podeljena u tri grupe, od kojih je prva analizirana odmah, a druge dve su skladištene 30 dana na -20°C i -80°C. Kvalitet i količina DNK određene su na biospektrofotometru, nakon čega su uzorci razblaženi za lančanu reakciju polimeraze. Kvalitet svih uzoraka DNK bio je na zadovoljavajućem nivou sa prosečnim vrednostima odnosa $OD_{260/280}$ od 1,85 do 1,87. Koncentracija DNK koja je izolovana odmah iz svežih listova nije se statistički razlikovala od uzoraka koji su čuvani 30 dana. I kvalitet i količina DNK u svim uzorcima bili su dovoljni za uspešno umnožavanje pomoću lančane reakcije polimeraze sa dva molekularna markera specifična za gen *opaque2*. Pomoću markera phi057 umnožen je fragment od ~170bp kod linije visokog kvaliteta proteina (VKP) i ~160bp kod linije koja nije VKP, dok je pomoću markera umc1066 dobijen fragment od ~150bp kod VKP i ~160-170bp kod ne-VKP. Rezultati su potvrdili da odgovarajući uslovi čuvanja ne utiču ni na kvalitet ni na količinu DNK. Ovo može biti korisno u selekciji pomoću molekularnih markera kada veliki broj uzoraka mora biti obrađen pre polinacije, što omogućuje selekcionerima da odbace biljke bez željenih alela i smanjuje obim populacije koju treba testirati.

Ključne reči: kvalitet DNK, količina DNK, ekstrakcija, temperatura skladištenja.

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