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Comparison of RNA extraction methods for the detection of BNYVV rhizomania virus from roots of sugar beet

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ABSTRACT

Rhizomania is one of serious threat to sugar beet production in Morocco and in several parts of the world. This disease led to a statistically significant decrease in the quality and yield of sugar beet plantations. Therefore, this study aimed at comparing the efficacy of six commonly used RNA extraction methods for the detection, recovery of RNA of beet necrotic yellow vein virus (BNYVV) and removal of amplification inhibitors by reverse transcription-polymerase chain reaction (RT-PCR). The efficiency of these extraction methods was then compared to that of a commercial isolation kit with high content of phenolic compounds. The results showed that the extraction with the lithium chloride technique, the commercial kit, and direct and membrane spotting crude extract methods were found effective in yielding a higher purity and a higher concentration of RNA when compared to the other tested methods. Extraction with the lithium chloride technique and the Qiagen kit (RNeasy Plant Mini Kit) allowed the most intense band, whereas the CTAB method has generated the least intense band. Furthermore, the silica capture extraction method did not yield any RNA after extraction and electrophoresis. Consequently, it was concluded that, of these six methods, the lithium chloride technique and the Qiagen kit are the most appropriate for the extraction of viral RNA from sugar beet samples prior to RT-PCR for detecting BNYVV.

1. Introduction

Rhizomania, which causes both abnormal rootlet proliferation and sugar yield loss, is the most devastating disease of many sugar beet (*Beta vulgaris* L.) growing areas including Europe, Asia, America, and Morocco [1]. This disease is commonly caused by the beet necrotic yellow vein virus (BNYVV) [2]. The disease was first reported in Italy during the growing season of 1950 [3] and has since spread to most of sugar beet growing areas around the world, including Morocco [4], causing serious losses of sugar yield and crop quality.

Detection of the virus in sugar beet roots is relatively simple and is usually based on the use of antisera and/or monoclonal antibodies in an enzyme-linked immunosorbent assay (ELISA). In the past decades, several suitable antibodies were produced [5,6] and detection kits are now available in the market from commercial companies.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), which is based on the replication of specific nucleic acids, was a very sensitive method commonly used for routine detection of viruses and viroids. The accuracy and reliability of RT-PCR bioassay was mainly linked to the

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quality and quantity of total nucleic acids, which are used in PCR [7]. Subsequently, RT-PCR is a highly sensitive detection method, and usually requires pure highly purified nucleic acids.

Likewise, phenols, polyphenols, and polysaccharides in plant materials were usually found to affect the sensitivity of PCR and might lead, in some cases, to false negative results [8,9]. These substances existing in plant tissues prevent reverse transcriptase, notably in RNA extraction phase and lowered, therefore, the RT-PCR's reliability [7].

Although numerous studies were carried out on RNA extraction methods, a standard method could not be found for all plants or viruses [10]. Furthermore, nucleic acid extraction protocols were unable to eliminate the phenolic compounds and polysaccharides that reduced the PCR's effectiveness [11]. In 2004, Cieślińska [9] performed a study about effective RNA extraction methods on strawberries and found the lithium chloride method was the most effective of all tested methods. MacKenzie et al. [12] underlined that high amounts of nucleic acids can be obtained with the silica capture method. Therefore, the main objective of this study was to evaluate and compare the effectiveness of six RNA extraction methods, including a commercial RNA extraction kit in obtaining a high-quality RNA for beet necrotic yellow vein virus detection.

2. Material and methods

Sugar beet samples were collected from the Tadla plain during the growing seasons 2014–2016. The collected samples were stored at −20 °C until use for RNA extraction. The extraction methods used for comparison purposes are the CTAB method [13], the silica capture method [14], Hughes and Galau's lithium chloride method [15], the direct and membrane spotting crude extract methods [16], and a commercial plant RNA extraction kit (RNeasy Plant Mini Kit Qiagen).

2.1. Silica capture method (SC)

The silica capture protocol was applied as previously described by Boom et al. [14]. Briefly, 100 mg of each root sample were used and grinded in sterile sample plastic bags with grinding buffer (Table 1). After homogenization, each tube was centrifuged at 13,000 g for 10 minutes. The supernatant of the flow-through was then transferred to a new microcentrifuge tubes and stored at −20 °C until use. Twenty microliters of sodium dodecyl sulfate (10%) were added to each tube and then incubated at 55 °C for 15 min. Hundred µl of acetate potassium (3 M) were added to each tube, and the tubes were placed on ice. All tubes are centrifuged at 13,000 *g* for 5 min, and the supernatant of the flow-through was transferred to a new microcentrifuge tube previously stored at −20 °C. These tubes were amended with 700 µL of 6 M NaI and 10 µL of a suspension of autoclaved silica powder, then incubated at room temperature for 10 min under gentle stirring. After centrifugation at 5000 *g* for 1 min, the supernatant was discarded, and the pellet was washed twice with a washing buffer. The obtained pellets were dried and resuspended in 400 µL of sterile distilled water, gently vortexed, and incubated for 5 min at 50 °C. After centrifugation for 2 min at 13,000 *g*, the supernatant from each tube was collected and stored in sterile tubes at −20 °C until use for PCR amplification.

Table 1
Chemicals used in the silica capture method.

Buffer	Chemical
Grinding buffer	137 mM NaCl 1.5 mM KH_2PO_4 3 mM KCl 8 mM Na_2HPO_4, pH 7.2 0.05% Tween 20 20 mM sodium diethyldithiocarbamate 2% PVP 25
NaI	NaI 6 M 1.87% Na_2SO_3
Washing buffer	20 mM Tris, pH 7.5 1 mM EDTA 100 mM NaCl 50% ethanol
Silica	6 g/50 mL

2.2. Lithium chloride method (LC)

The lithium chloride extraction method was performed following the protocol developed by Hughes and Galau's [15]. One hundred milligrams of root sample were weighted and placed in a tube containing 0.5% 2-mercaptoethanol and 1 mL of an extraction buffer (Table 2). Each sample was grinded, homogenized using a mortar and pestle, and 500-µL aliquots of the extract were transferred into 1.5 mL microcentrifuge tubes. The tubes were then incubated at 65 °C for no more than 15 min. These tubes were supplemented with 500 µL of 5 M potassium acetate (pH6.5) each, and kept on ice for 10 min. The tubes were centrifuged at 14,000 rpm for 15 min, and 600 µL of supernatant were transferred into new sterilized microcentrifuge tubes. Afterwards, 600-µL aliquots of isopropanol were added to each tube, and the tubes were then incubated at −20 °C overnight. The mixture, which has become pellets, was centrifuged at 14,000 rpm for 15 min and washed with 70% ethanol. The tubes were stored at −20 °C until use.

2.3. CTAB extraction method

The CTAB extraction method was used following the protocol of Chang et al. [13], with slight modifications. One hundred mg of the root sample were added to 1 mL of the extraction buffer (Table 3), and grinded to a fine powder in

Table 2
Chemicals used in the lithium chloride method.

Buffer	Chemical
Extraction buffer	200 mM Tris-HCl (pH 8.5) 1.5% sodium dodecysulphate 300 mM lithium chloride 10 mM EDTA 1% sodium deoxycholate 0.5% 2-β-mercaptoethanol 5 M potassium acetate (pH 6.5) Isopropanol Ethanol

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