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Development of methodologies for virus detection in soybean and wheat seeds

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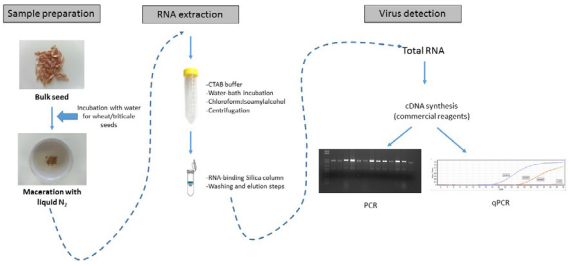
GRAPHICAL ABSTRACT



ABSTRACT

Seeds that contain large amounts of oil, starch, fibers and phenols are the most difficult tissues for RNA extraction. Currently, there are some reports of virus detection in seeds using commercial kits for RNA extraction. However, individual seeds were used, which may not be always suitable for analyses that deal with large amounts of seeds. Sangha [1] described a simple, quick and efficient protocol for RNA extraction and downstream applications in a group of seeds of jatropha (*Jatropha curcas*), mustard (*Brassica* sp.) and rice (*Oryza sativa*). We tested this protocol for soybean (*Glycine max*), maize (*Zea mays*), wheat (*Triticum aestivum*) and triticale (×*Triticosecale*) seeds and further reverse transcription PCR (RT-PCR)/quantitative real-time PCR (qPCR) in order to have a faster and more practical method for virus detection from seeds than the traditional scheme of seed planting and subsequent Elisa/RT-PCR from leaves. The essential points in the method are:

- Some modifications in the protocol [1] were done in order to increase performance: Wheat and triticale seeds are incubated with water prior to maceration. An amount of 1.2 g of dry soybean seeds is used to maceration.

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- RT-PCR is used for detection of *Wheat streak mosaic virus* from wheat seeds and RT-qPCR for detection of *Soybean mosaic virus* from soybean seeds.
- The method may be tested for other viruses, however, pre-validation will be needed.

ARTICLE INFO
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Methods detail

The protocol described for RNA extraction of immature seeds of jatropha (*Jatropha curcas*), mustard (*Brassica* sp.) and rice (*Oryza sativa*) seeds is a simple, quick and efficient protocol for RNA extraction and downstream applications [1]. To use it for virus detection in soybean (*Glycine max*) and wheat (*Triticum aestivum*) seeds, some improvements are needed to be done in the maceration step. Two viruses of quarantine importance, *Soybean mosaic virus* (SMV), family *Potyviridae*, genus *Potyvirus* [2] and *Wheat streak mosaic virus* (WSMV), family *Potyviridae*, genus *Tritimovirus* [3,4] were chosen to check if the protocol is suitable for application in their identification in seeds. The molecular tools: RT-PCR test for WSMV detection [3] and the RT-PCR for SMV detection [5] adapted to RT-qPCR were compared to other techniques and selected to perform virus detection with the RNA obtained from seeds.

Sample preparation

Soybean plants were mechanically inoculated with an isolate of SMV (named 165.09 GenBank: KC331990) intercepted at Plant Quarantine Laboratory of Embrapa Genetic Resources and Biotechnology. Wheat plants of cv. Guabiju were also mechanically inoculated with WSMV (isolate 915 GenBank: KC152463.1 from Passo Fundo, Brazil). Plants were cultivated in greenhouses to obtain seeds. Seeds were harvested and stored at 4 °C until the maceration procedure began. In addition, seeds of soybean (several accessions), wheat cv. BRS Guabiju and BRS Guamirim, triticale cv. BRS Saturno and BRS Ulisses and maize hybrid HS201 from healthy plants were collected for RNA extraction procedure. Prior to maceration, the wheat and triticale seeds were incubated with water in order to soften the tissues.

Maceration

(1) Put an amount of 0.5 g of wheat/triticale seeds into a mortar and subsequently cover the seeds with about 20 mL of distilled water. Cover the mortar with a paper towel and wait at least 12 h at room temperature to start the maceration in order to allow the seeds to absorb water.
(2) Pulverize 1.2 g of dry soybean seeds, 0.5 g of dry maize seeds and the previously incubated wheat and triticale seeds in liquid nitrogen. Put the seed powder into 50 mL pre-chilled polypropylene (Falcon) tube.

RNA extraction

(3) In a fume hood, add 5 mL of pre-heated (65 °C) total RNA extraction buffer {2% (w/v) CTAB, 2% (w/v) polyvinylpyrrolidone (PVP-40), 100 mM Tris HCl (pH 8.0), 25 mM EDTA, 2 M NaCl, 0.1% spermidine and 2% β-mercaptoethanol} to the frozen samples and keep them in a water bath (65 °C) for 30 min, vortexing every 5 min.