Supporting Information

Rapid identification of quarantine invasive *Solanum elaeagnifolium* by real-time, isothermal recombinase polymerase amplification assay

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Plant DNA extraction

Total DNA from plant was extracted using plant DNA extraction kit with magnetic beads (Tiangen Biotech, Beijing, China). A total of 40 mg dry specimen, or 100 mg seeds, or 100 mg fresh plant leaves were finally ground to powder in liquid nitrogen with a mortar and a pestle. The powder was transferred into 1.5-mL tube and subjected to extraction of genomic DNA according to the manufacturer's instructions. Briefly, the powder were vortexed mixed with a mixture of 400 μL lysate buffer (GPM) and 5 μL RNase A (10 mg/mL) and incubated for 10 min at 70°C. Lysate was centrifuged for 4 min at 20,000×*g*. Three hundred microliters of the supernatant was transferred to a fresh 1.5 mL tube, followed by adding 300 μL GHB, 300 μL isopropanol and 15 μL magnetic beads suspension, and vortex mixed for 5 min. Tubes were placed on the magnetic frame for 1 min, and the solution was removed. The magnetic beads were add with 500 μL RD solution to wash the residual proteins. The magnetic beads were washed with 75% ethanol two times, then were put to dry at 37°C. 50-100μL autoclaved distilled water was added and incubated for 3 min at 65°C to elute the DNA.

The first primer screening for the RPA assay

We firstly screened a characteristic conserved voucher Bohs 3204 UT granule-bound starch synthase (GBSSI) gene region of *S. elaeagnifolium* (AY996412.1) using basic TwistAmp™ basic kit (Cambridge, UK). Four forward and three reverse primers (Table S1) were designed to amplify the *S. elaeagnifolium* genomic DNA template. Primers were synthesized by Sangon Biotech

(Shanghai, China).

RPA reaction was performed in a 50 μL volume according to the manufactures protocol, respectively. Besides the rehydration buffer, 480 nM primers and 14 mM magnesium acetate were contained for the basic RPA reaction. To select the optimized primers, all reagents except for the primers and magnesium acetate were prepared in a master mix; for the assay of sample, all reagents except for the template and magnesium acetate were prepared in a master mix. The master mix was distributed into each 0.2 ml reaction tube containing a dried enzyme pellet. After the addition of the primers or template, the solution was vortexed briefly and spun down, subsequently magnesium acetate was pipetted into the tube lids, the lids were closed carefully, and magnesium acetate was centrifuged into the rehydration buffer using a minispin centrifuge. The sample was vortexed briefly and spun down. The tubes were immediately placed in an incubator at 39°C for 20-60 min.

The amplicon in the reaction tube was purified using Beckman Agencourt AMPure XP beads. Briefly, add 80 μL beads buffer into the 50 μL reaction tube, mix and stand for 8 min in room temperature. Then the tube was separated with a magnet, and the supernatant was removed. Wash the beads with 200 μL of 70% ethanol for twice. When washing, turn the tube on the magnet shelf for 30 s, then remove the solution with pipette, dry the beads at 37°C. Subsequently, add 30 μL of DiH_2O into the tube, vortex 3 s, stand for 5 min, then put the tube on the magnet shelf for 30 s, then transfer the solution to a clean tube. Ten microliter of amplicon were detected by 1% agarose gel electrophoresis.

The gel electrophoresis photo of the RPA amplicons of *S. elaeagnifolium* showed that the

combination of Elae-BF1/BR1, Elae-BF2/BR1, Elae-BF3/BR1 and Elae-BF4/BR1 can produce amplicates (Fig. S1). The further amplification resulted from primer BF2/BR1 and BF3/BR1showed that BF2/BR1 amplified only *S. elaeagnifolium*, but not *S. rostratum, S. carolinense* , or *S. torvum* (Fig. S2).

Table S1. Sequence of primers for the construction of basic-RPA assays.

Name	Sequences 5'-3'	Position in GBSSI gene sequence (AY996412.1)
Elae-BF1	AGCTTGTTCTGTCAAGTAAGTTACTAGCTGTATGG	388
Elae-BF2	ACTAGCTGTATGGTTGTCTTGACTTAATGTGGC	410
Elae-BF3	ATTCAGCTTGTTCTGTCAAGTAAGTTACTAGCTG	383
Elae-BF4	CAAGTAAGTTACTAGCTGTATGGTTGTCTTG	400
Elae-BR1	ATTGCCAAGGAGATTTCTGAAACTGGGATGT	597
Elae-BR2	CTTCTCCTGCAAAATAAAGATGATTACCTACAAGG	642
Elae-BR3	CAATGAAGAGAACATCTTCTCCTGCAAAATAAAG	657

The second primer and probe screening for the RPA assay

Then three forward primers (exo-F), four reverse primers (exo-R), and probe (Elae-P) (Fig. S3) were designed based on the sequence of the specific amplicons of *S. elaeagnifolium*, in order to select the combination producing the highest analytical sensitivity for the *S. elaeagnifolium.* Primers and the probes were synthesized by Sangon Biotech (Shanghai, China).

The primers screening for the real-time PCR assay

The primers were designed across the whole GBSSI gene sequence (AY996412.1), and were listed in Table S2. To investigate whether the amplification curves were resulted by specific amplification or non-specific amplification, the melting curves of the four primers for S. *elaeagnifolium* (Fig. S4, A) showed that primer 3 produced the best single peak.

Table S2. Sequence of primers for the construction of real-time PCR assays

Primer No.	Name	Sequences 5'-3'	Position in GBSSI gene sequence (AY996412.1)
1	**E-rtPCR-F1**	**CTCATAACCGCAGATGATACAA**	34
	E-rtPCR-R1	**TGCCAATAAAGTAGGGATCACAT**	97
2	**E-rtPCR-F2**	**TAAATTTCACATTGCCTCCAGGTC**	807
	E-rtPCR-R2	**TGAGAAGAGGGAAGTCAGAGAAAGT**	894
3	**E-rtPCR-F3**	**CCTACCAAGGCCGATTTAC**	853
	E-rtPCR-R3	**GATCTGATTTGAAGCATTAGATTTC**	966
4	**E-rtPCR-F4**	**GAAATCTAATGCTTCAAATCAGATC**	942
	E-rtPCR-R4	**TTAGCAGATGCTGAGGTCAGAA**	1298

The validation of time-dependent S. elaeagnifolium RPA assays

The real-time fluorescence intensity showed that the fluorescence signal went up at about 6 min, and to a stage at about 20 min. To check if the fluorescence signal is related to amplification degree, we used basic RPA kit to amplify the genomic DNA template and adopted agarose gel electrophoresis to check the amount of amplicons (Fig.S5). The results showed that 10 minutes amplification could produce enough amplicons to be measured by gel electrophoresis, and 20 minutes longer amplification didn't increase the amplicon obviously.

Supplementary Figure legends

Fig. S1. Gel electrophoresis photo of the RPA amplicons of *S. elaeagnifolium.* M, 100 bp DNA Marker; For *S. elaeagnifolium,* lane 11, 12, 13, 21, 22, 23, 31, 32, 33, 41, 42, 43 means the combination of primers Elae-BF1/BR1, BF1/BR2, BF1/BR3, BF2/BR1, BF2/BR2, BF3/BR3, BF4/BR1, BF4/BR2, BF4/BR3, respectively. The sequences of the primers were listed in Table S1.

Fig. S2. The gel electrophoresis of basic RPA amplicons of different DNA template with BF2/BR1 (lane 1-4) or BF3/BR1 (lane 5-8) primers. Lane 1,5: *S. rostratum;* Lane 2,6: *S. carolinense*; Lane 3,7: *S. torvum*; Lane 4,8: *S. elaeagnifolium*, respectively. M: 100 bp DNA Marker.

Fig. S3. Real-time RPA primers and probes sequence aligned with the S. elaeagnifolium amplicon; Elae-P: fluorescence probe for real-time RPA.

Fig. S4. A. Fluorescent amplification curves of *S. elaeagnifolium* (E), *S. rostratum* (R), *S. torvum* (T), *S. nigrum* L (N) and water (W) using different primers listed (A. pirmer 1; B, primer 2; C, primer 3; D. pirmer 4) in Table S2. E: *S. elaeagnifolium*; R: *S. rostratum*; C: *S.carolinense*; T: *S. torvum*; N: *S. nigrum* L; W: water.

Fig. S5. **A.** Melting curves of real-time PCR assay of S. *elaeagnifolium* genomic DNA using primer 1 (curve 1), primer 2 (curve 2), primer 3 (curve 3) and primer 4 (curve 4). **B.** Melting curves of real-time PCR assay of S. *elaeagnifolium* (E), *S. rostratum* (R), *S. torvum* (T), *S. nigrum* L (N) and water (W).

Fig. S6. The amplicon produced from different RPA amplification time. Lane 1, 10 min; Lane 2, 20 min; Lane 3, 30 min; Lane 4, 40 min; Lane 5, 60 min; Lane 6, 90 min; M, 100 bp DNA Marker.

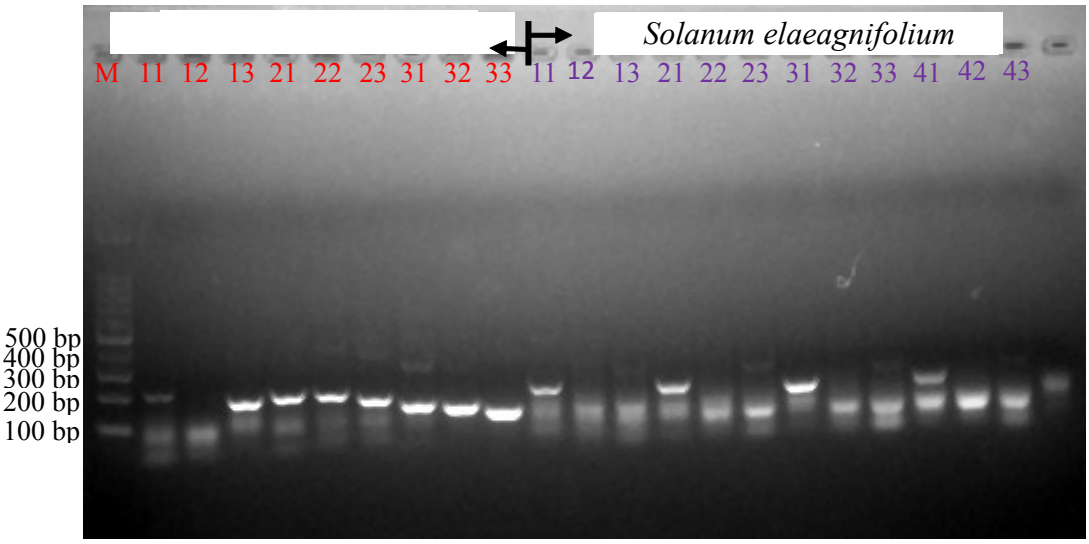


Fig. S1. 1.0% agarose gel electrophoresis with 1×TAE buffer to separate the RPA amplicons of *S. elaeagnifolium*. M, 100 bp DNA Marker; For *S. elaeagnifolium,* lane 11, 12, 13, 21, 22, 23, 31, 32, 33, 41, 42, 43 means the combination of primers Elae-BF1/BR1, BF1/BR2, BF1/BR3, BF2/BR1, BF2/BR2, BF3/BR3, BF4/BR1, BF4/BR2, BF4/BR3, respectively. The sequences of the primers were listed in Table S1.

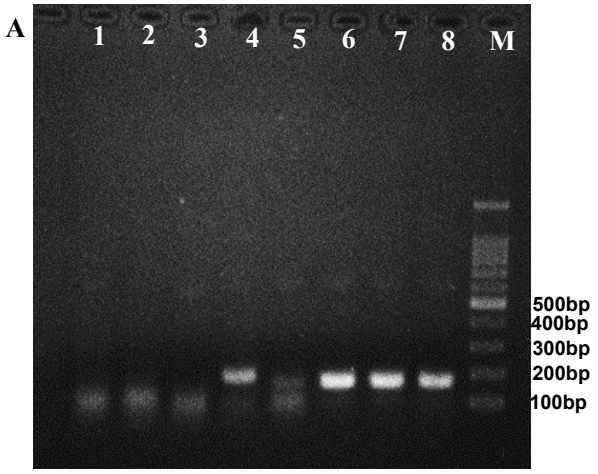
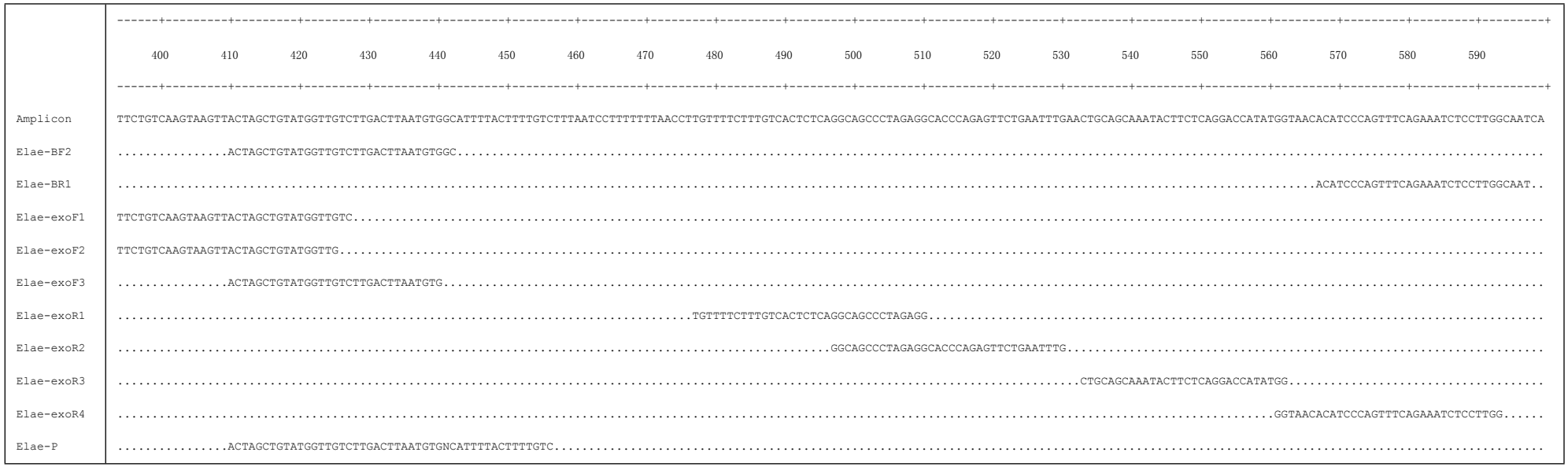
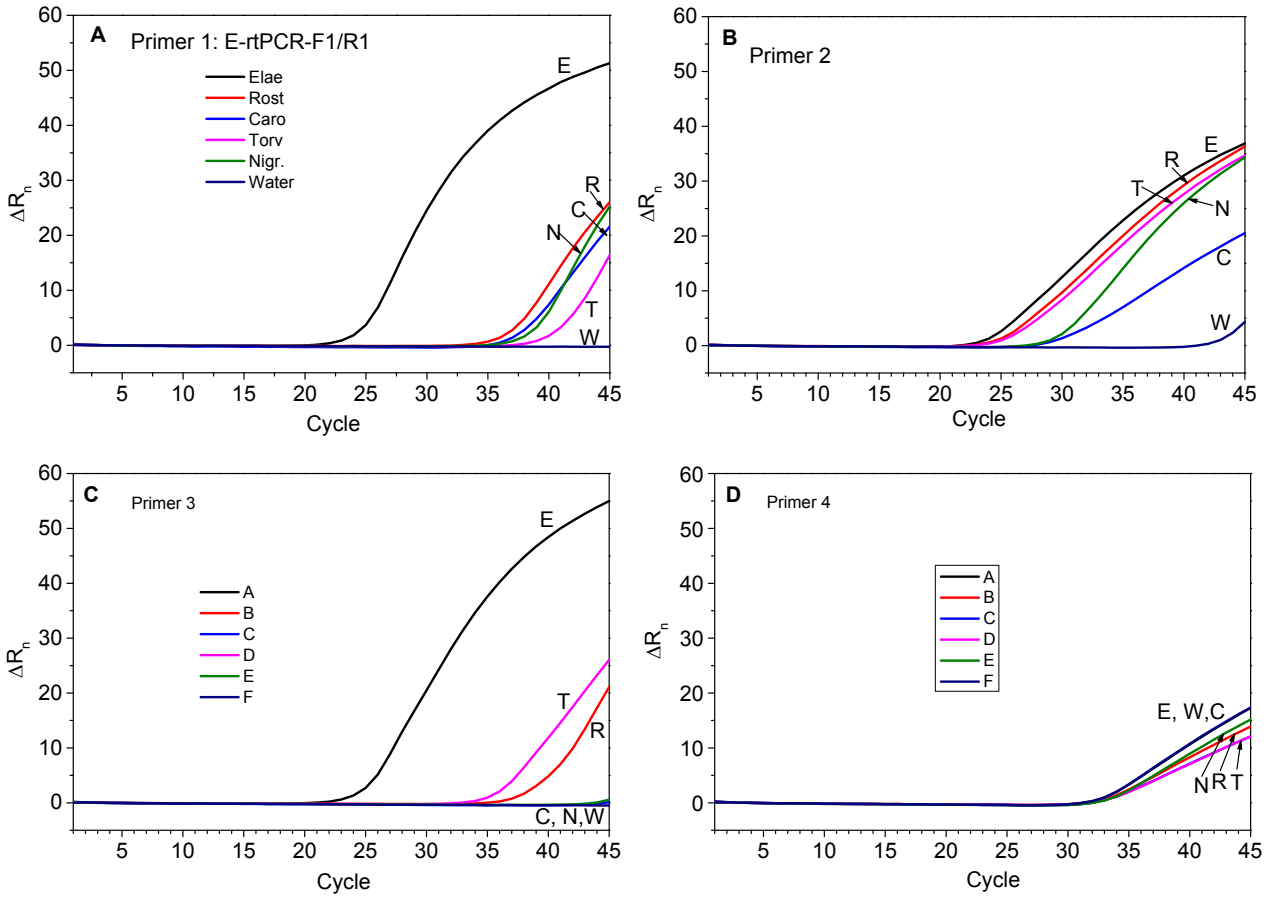


Fig. S2. 1.0% agarose gel electrophoresis with 1×TAE buffer to separate of basic RPA amplicons of different DNA template with BF2/BR1 (lane 1-4) or BF3/BR1 (lane 5-8) primers. Lane 1,5: *S. rostratum;* Lane 2,6: *S. carolinense*; Lane 3,7: *S. torvum*; Lane 4,8: *S. elaeagnifolium*, respectively. M: 100 bp DNA Marker.



105 **Fig. S3.** Real-time RPA primers and probes sequence aligned with the *S. elaeagnifolium* amplicon; Elae-P: fluorescence probe for real-time RPA



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108 **Fig. S4.** Fluorescent amplification curves of *S. elaeagnifolium* (E), *S. rostratum* (R), *S. torvum* (T), *S. nigrum* L

109 (N) and water (W) using different primers listed (A. pirmer 1; B, primer 2; C, primer 3; D. pirmer 4) in Table S2.

110 E: *S. elaeagnifolium*; R: *S. rostratum*; C: *S.carolinense*; T: *S. torvum*; N: *S. nigrum* L; W: water.

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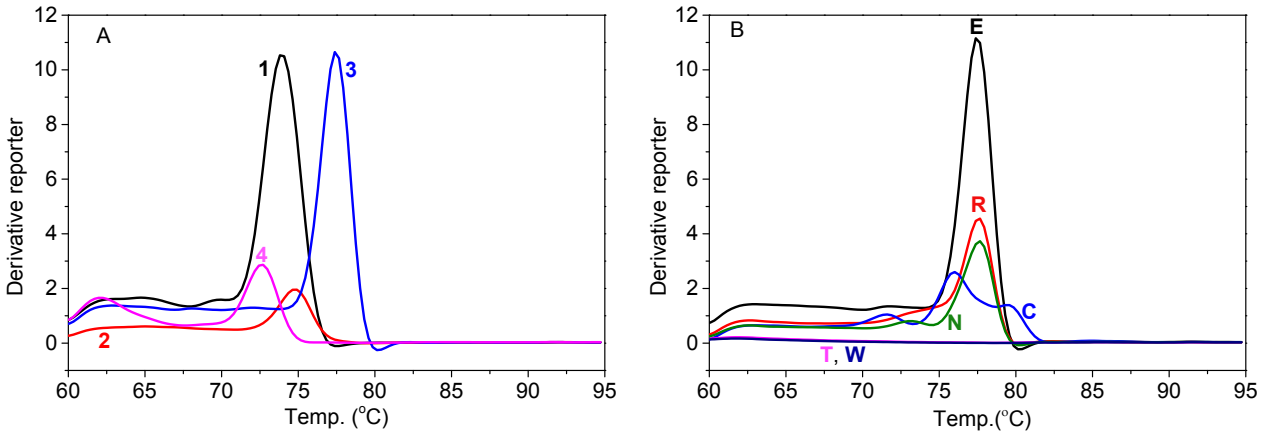

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113 **Fig. S5. A.** Melting curves of real-time PCR assay of S. *elaeagnifolium* genomic DNA using primer 1 (curve 1),

114 primer 2 (curve 2), primer 3 (curve 3) and primer 4 (curve 4). **B.** Melting curves of real-time PCR assay of S.

115 *elaeagnifolium* (E), *S. rostratum* (R), *S. torvum* (T), *S. nigrum* L (N) and water (W).

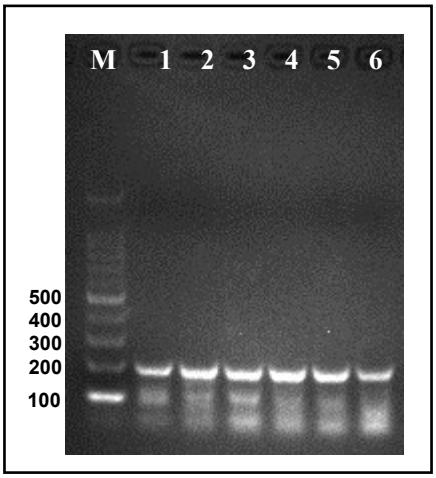

Fig. S6. 1.0 % agarose gel electrophoresis with 1×TAE buffer to separate the amplicon produced from different RPA amplification time. Lane 1, 10 min; Lane 2, 20 min; Lane 3, 30 min; Lane 4, 40 min; Lane 5, 60 min; Lane 6, 90 min; M, 100 bp DNA Marker.