

1 **Supplemental Data**

2 **Supplemental Figure 1. Physical interaction between SHW1 and COP1 as shown by**
3 **in vitro binding assay. A,** GST-HY5, GST-SHW1 and GST were individually incubated
4 with Ni-NTA bound COP1-His protein (2 µg) in equimolar ratio. Supernatant and pellets
5 were fractioned by 10% SDS-PAGE, blotted and probed with anti-GST antibodies. Lane
6 1 shows the interaction of COP1-His with GST-HY5 (positive control). Signal in
7 supernatant serves as loading control. An autoradiograph of a typical in vitro binding
8 experiment has been shown. **B,** Quantification of proteins retained by COP1-His, as
9 described in A. The experiment was repeated for three times with similar results. Error
10 bars indicate standard error of the mean of three independent experiments.

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12 **Supplemental Figure 2. ABA mediated responsiveness of *shw1 hy5* double mutants.**
13 **A,** Germination of the wild type, *shw1*, *hy5*, and *shw1 hy5* at 1 µM concentration of ABA
14 **B,** Quantification of seed germination after 3 day in WL. Results presented are obtained
15 from three biological replicates. Error bars indicate SD. The *t* tests show the significant
16 differences (*P < 0.05).

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18 **Supplemental Figure 3. Physical interaction between SHW1 and HY5 as shown by**
19 **in vitro binding assay. A,** GST-HYH, GST-SHW1 and GST were individually incubated
20 with Ni-NTA bound HY5-His protein (2 µg) in equimolar ratio. Supernatant and pellets
21 were fractioned by 10% SDS-PAGE, blotted and probed with anti-GST antibodies. Lane
22 1 shows the interaction of HY5-His with GST-HYH (positive control). Signal in
23 supernatant serves as loading control. An autoradiograph of a typical in vitro binding
24 experiment has been shown. **B,** Quantification of proteins retained by HY5-His, as
25 describe in A. The experiment was repeated for three times with similar results. Error
26 bars indicate standard error of the mean of three independent experiments.

27
28 **Supplemental Figure 4. Transcript levels of *HY5*.** **A,** The transcript levels of *HY5* in
29 wild type (Col), *shw1*, *cop1-6*, *shw1 cop1-6* mutants in 5-day-old dark grown seedlings.
30 A representative result is shown. M indicates the DNA molecular size marker. **B,**
31 Quantification of the data in A. The experiment was repeated for three times. The error

32 bars indicate standard deviations of three technical repeats (one of the three biological
33 replicates).

34

35 **Supplemental Figure 5. SHW1 Enhances the Ubiquitylation Activity of COP1 but**
36 **not it Self ubiquitinated by COP1.** Recombinant COP1-His, GST-SHW1, and GST-
37 HY5 fusion proteins were purified from E.coli (BL21DE3) and used for in vitro
38 Ubiquitination assays. Ubiquitination assays were performed using UBE1 (E1), UbcH5b
39 (E2), and His-tagged ubiquitin (His6-Ub). Recombinant COP1-His was pre incubated
40 with 20 mM ZnCl₂. Immunoblot against ubiquitin antibodies shows that HY5, however
41 not SHW1, is a substrate of COP1 for ubiquitination. Lower panel shows the equal
42 loading of GST-HY5 and GST-SHW1 probed with anti-GST antibodies.

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44 **Supplemental Figure 6. The Expressed proteins in Yeast Cells.** The expressed
45 proteins used in two-hybrid assays are shown.

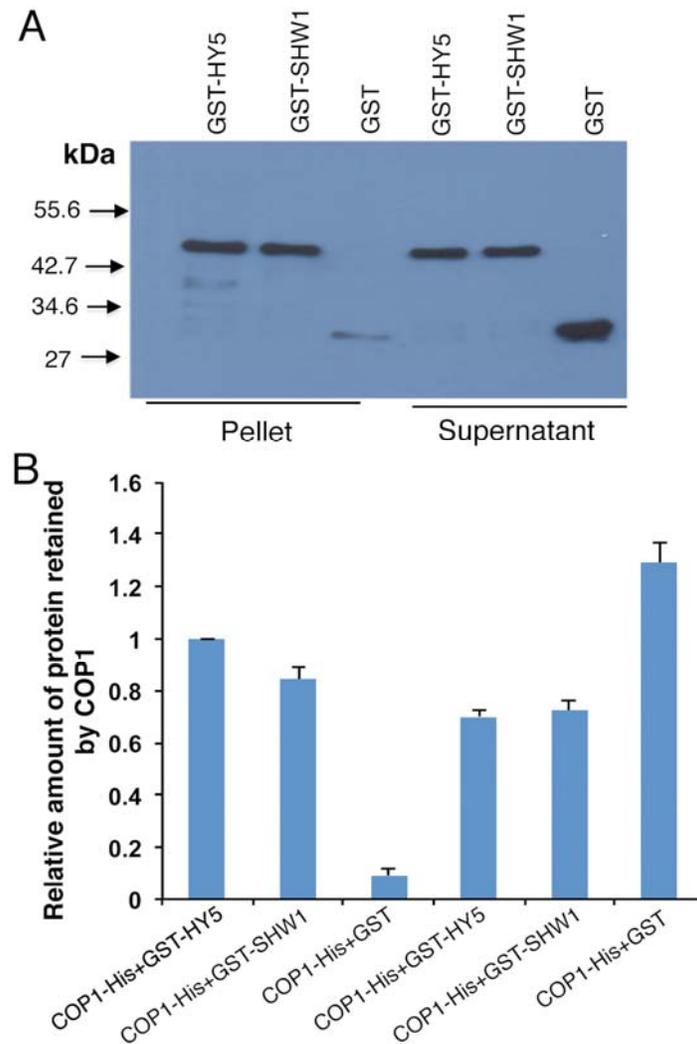
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47 **Supplemental Table 1. Primers used in this study.** The primers used in this study are
48 summarized.

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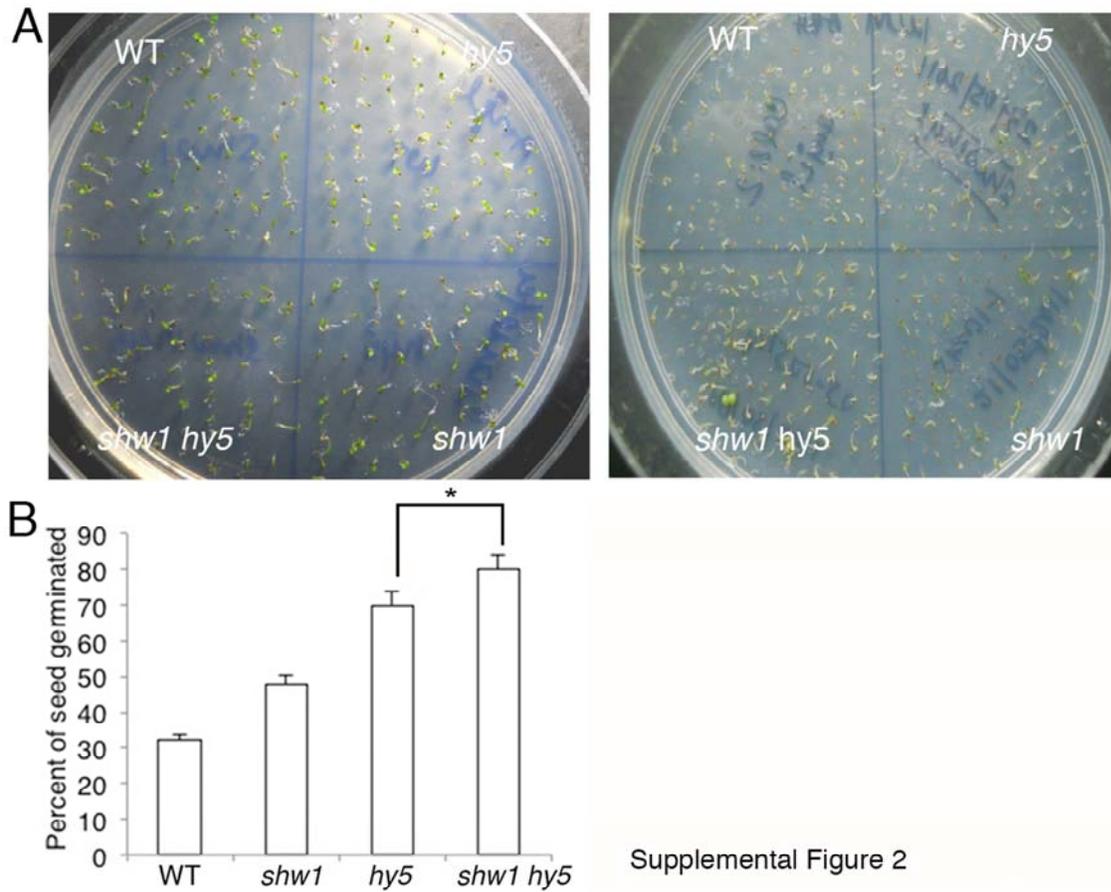
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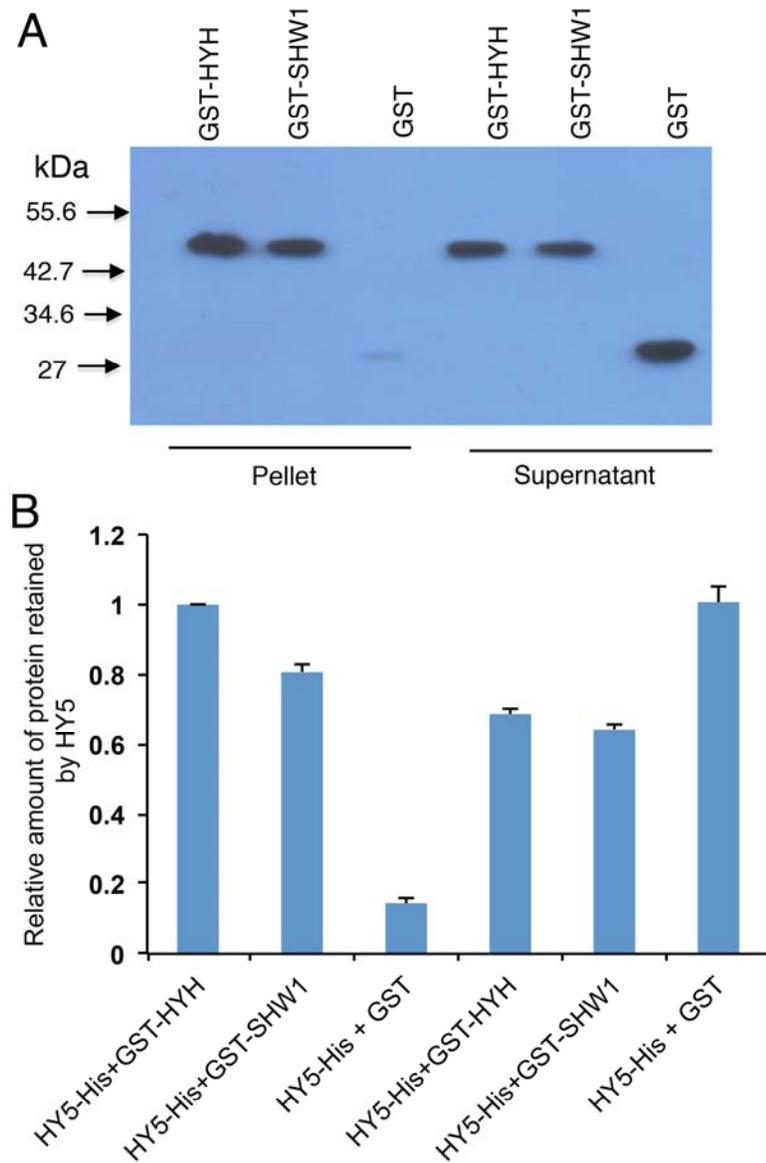


Supplemental Figure 1

Supplemental Figure 1. Physical interaction between SHW1 and COP1 as shown by in vitro binding assay. **A**, GST-HY5, GST-SHW1 and GST were individually incubated with Ni-NTA bound COP1-His protein (2 μ g) in equimolar ratio. Supernatant and pellets were fractionated by 10% SDS-PAGE, blotted and probed with anti-GST antibodies. Lane 1 shows the interaction of COP1-His with GST-HY5 (positive control). Signal in supernatant serves as loading control. An autoradiograph of a typical in vitro binding experiment has been shown. **B**, Quantification of proteins retained by COP1-His, as described in A. The experiment was repeated for three times with similar results. Error bars indicate standard error of the mean of three independent experiments.

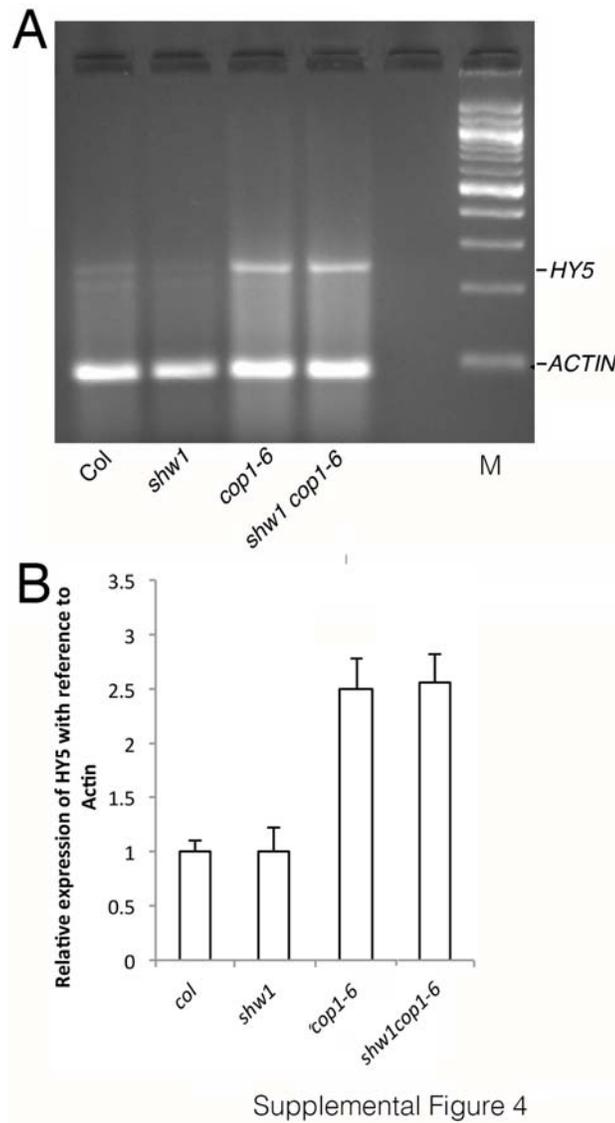


Supplemental Figure 2. ABA mediated responsiveness of *shw1 hy5* double mutants. **A**, Germination of the wild type, *shw1*, *hy5*, and *shw1 hy5* at 1 μ M concentration of ABA **B**, Quantification of seed germination after 3 day in WL. Results presented are obtained from three biological replicates. Error bars indicate SD. The *t* tests show the significant differences (* $P < 0.05$).



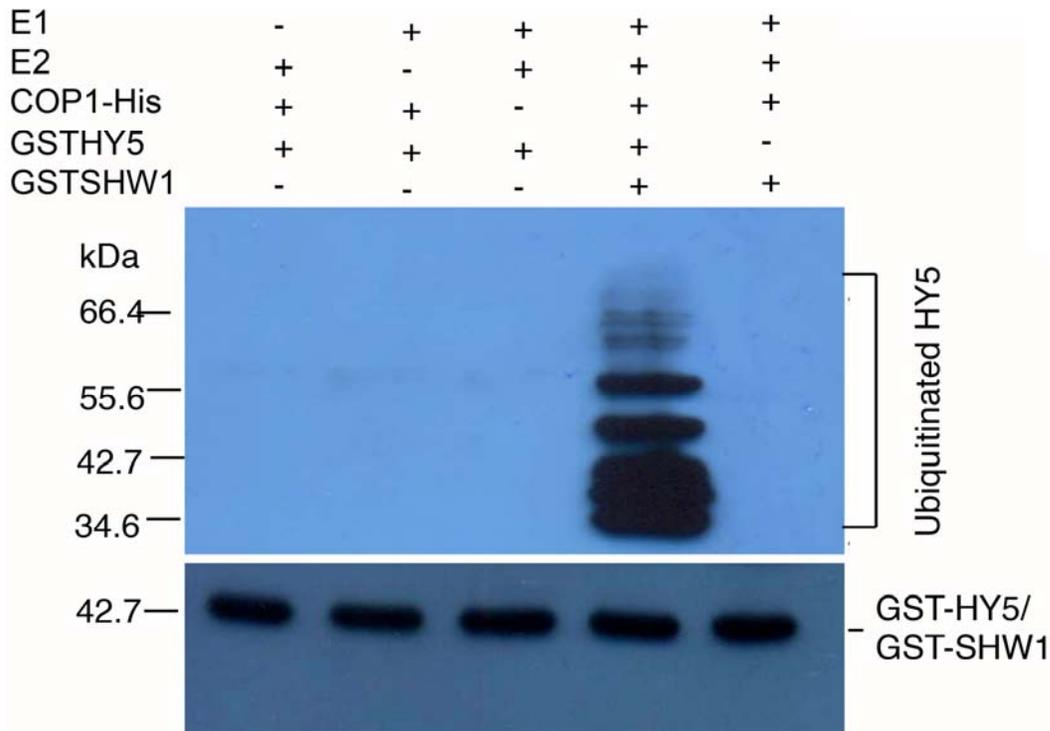
Supplemental Figure 3

Supplemental Figure 3. Physical interaction between SHW1 and HY5 as shown by in vitro binding assay. **A**, GST-HYH, GST-SHW1 and GST were individually incubated with Ni-NTA bound HY5-His protein (2 μ g) in equimolar ratio. Supernatant and pellets were fractionated by 10% SDS-PAGE, blotted and probed with anti-GST antibodies. Lane 1 shows the interaction of HY5-His with GST-HYH (positive control). Signal in supernatant serves as loading control. An autoradiograph of a typical in vitro binding experiment has been shown. **B**, Quantification of proteins retained by HY5-His, as describe in A. The experiment was repeated for three times with similar results. Error bars indicate standard error of the mean of three independent experiments.



Supplemental Figure 4. Transcript levels of *HY5*. **A**, The transcript levels of *HY5* in wild type (Col), *shw1*, *cop1-6*, *shw1 cop1-6* mutants in 5-day-old dark grown seedlings. **A** representative result is shown. **B**, Quantification of the data in **A**. The experiment was repeated for three times. The error bars indicate standard deviations of three technical repeats (one of the three biological replicates).

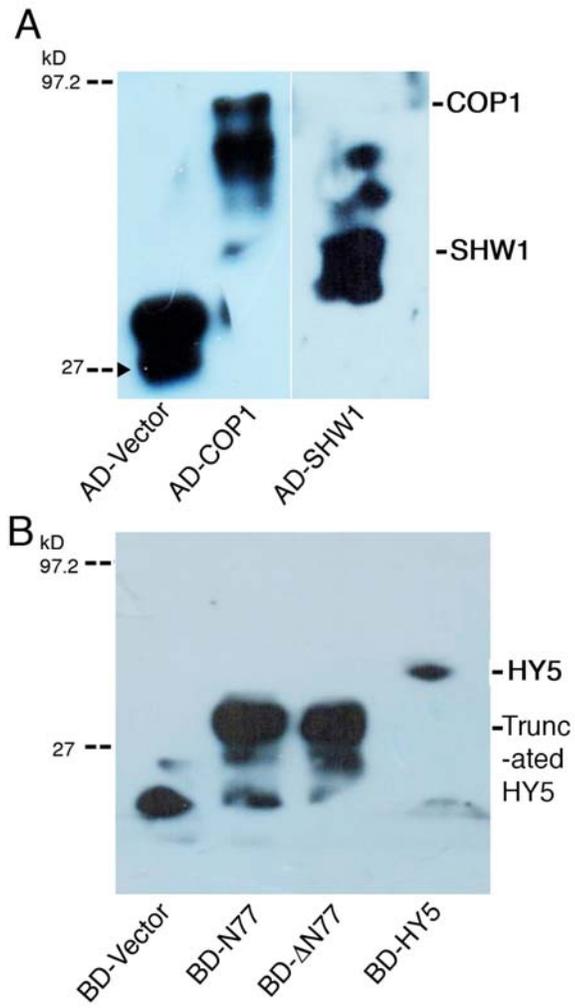
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Supplemental Figure 5

Supplemental Figure 5. SHW1 enhances the ubiquitylation Activity of COP1 but not it self ubiquitinated by COP1. Recombinant COP1-His, GST-SHW1, and GST-HY5 fusion proteins were purified from E.coli (BL21DE3) and used for in vitro Ubiquitination assays. Ubiquitination assays were performed using UBE1 (E1), UbcH5b (E2), and His-tagged ubiquitin (His6-Ub). Recombinant COP1-His was pre incubated with 20 mM ZnCl₂. Immunoblot against ubiquitin antibodies shows that HY5, however not SHW1, is a substrate of COP1 for ubiquitination. Lower panel shows the equal loading of GST-HY5 and GST-SHW1 probed with anti-GST antibodies.

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Supplemental Figure 6

Supplemental Figure 6. The Expressed proteins in Yeast Cells. The expressed proteins used in two-hybrid assays are shown.

63 **Supplementary Table 1. Primers used in this study.**

64 **List of primers used in in-vitro pull down assays**

65 SHW1-FL: FP (BamHI): CGGGATCCATGGCCGCAGCTACAACAAC
66 SHW1-FL: RP (EcoRI):GGAATTCTTAATTTACCGGGTTTGGTC
67 HY5-FL: FP (NdeI): GGAATTCCATATGCAGGAACAAGCGACTAG CTCTTT
68 HY5-FL: RP (ClaI): CCATCGATTCAAAGGCTTGCATCAGCATTAG
69 COP1-FL: FP (EcoRI):GCGAATTCATGGAAGAGATTTTCGACGGATC
70 COP1-FL: RP (PstI): CGGGATCCTCACGCAGCGAGTACCAGAAC

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72 **List of primers used in yeast two hybrid assays**

73 SHW1-FL FP (EcoRI): GGAATTCATGGCCCGCAGCTACAACAAC
74 SHW1-FL RP (BamHI): CGGGATCCTTAATTTACCGGGTTTGGTC
75 HY5-FL FP (EcoRI): GGAATTCATGCAGGAACAAGCGACTAGC
76 HY5-FL RP (BamHI): CGGGATCCTCAAAGGCTTGCATCAGC
77 HY5-ΔN77 FP (EcoRI): GGAATTCAGGAAGCGAGGGAGGACACCG
78 HY5- ΔN77 RP (BamHI): CGGGATCCTCAAAGGCTTGCATCAGC
79 HY5- N77 FP (EcoRI): GGAATTCATGCAGGAACAAGCGACTAGC
80 HY5 N77 RP (BamHI): CGGGATCCTTGACTTTCTCCGACAGTCGC
81 COP1-FL FP (EcoRI): GGAATTCATGGAAGAGATTTTCGACGG
82 COP1-FL RP (PstI): AACTGCAGAGCTCGGTATAAATCTATTC
83 COP1-Zn+CC FP (EcoRI): GGAATTCATGGAAGAGATTTTCGACGG
84 COP1-Zn+CC RP (PstI): AACTGCAGAGCTCGGTATAAATCTATTC
85 COP1-WD-40 FP (EcoRI): GGAATTCGCCACTGCTGGTGTCTTAG
86 COP1-WD-40 RP (BamHI): CGGGATCCCGCAGCGAGTACCAGAAC

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88 **List of primers used in BiFC assays**

89 SHW1-FL: FP (BamHI) CGGGATCCATGGCCGCAGCTACAACAACC
90 SHW1-FL: RP (XhoI) CCGCTCGAGTTAATTTACCGGGTTTGGTCC
91 COP1- FL: FP (AscI) GCGCGCCATGGAAGAGATTTTCGACGGATC
92 COP1-FL: RP (XhoI) CCGCTCGAGCGCAGCGAGTACCAGAACTTTG

93

94 **List of primers used in RT-PCR**

95 HY5-FP: CTGAAGAGGTTGTTGAGGAACAG

96 HY5-RP: AGAAGAAGAAGGAGATCAAAGGC

97 ACTIN2-FP: AAAGGCTTAAAAAGCTGGGG

98 ACTIN2-RP: GGGACTAAAACGCAAACGA

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100 **List of primers used in Genomic PCR**

101 LBP : GCGTGGACCGCTTGCTGCACCT

102 *SHW1* specific primers LP11: TGCAAAAGACACCTGCAAATCA and

103 RP11: ATGCAAAGAAC CGAGAGGTCG

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