

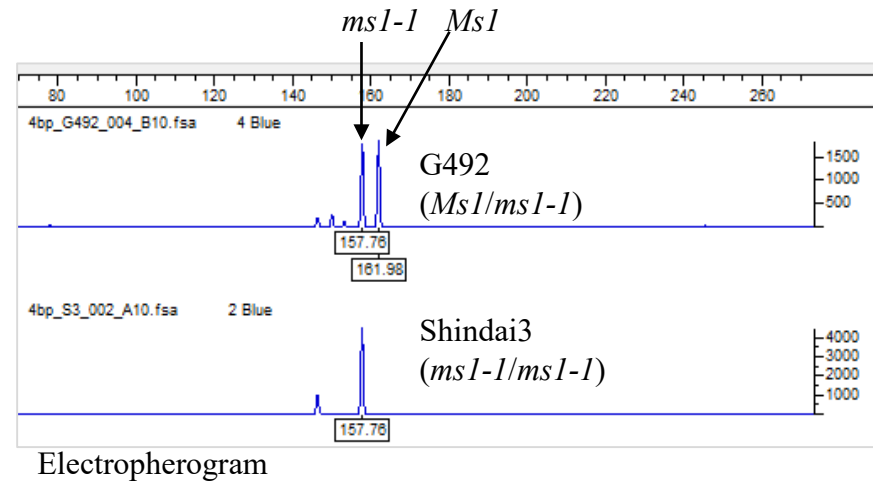
(a) ALP_ms1-1

Amplified fragment length polymorphism (ALP) marker to detect *ms1-1* allele in *MALE STERILITY 1 (MS1)* in sugi.

Reaction	Volume (μL)
2 \times Multiplex (QIAGEN)	3
2 μM CJt020762_F145	1
2 μM CJt020762_R294_A	0.5
2 μM Tail_A	0.5
dH ₂ O	4
DNA (10—50 ng)	1
Total (μL)	10

Temperature ($^{\circ}\text{C}$)		Cycles
95	15 min	1
94	15 sec	
63	45 sec	38
72	15 sec	
60	30 min	1
4	∞	1

Primer ID	Primer sequence
CJt020762_F145	CCATGCCTTTCTTACATGACGAG
CJt020762_R294_A	GCCTCCCTCGCGCCATTGATTAATGGGAAAGCCCAGAA
Tail A	[FAM]-GCCTCCCTCGCGCCA



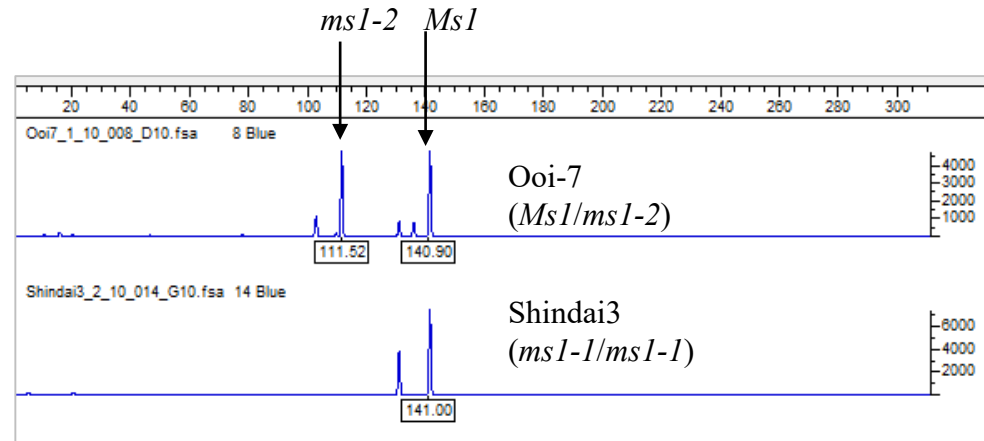
(b) ALP_ms1-2

Amplified length polymorphism (ALP) marker to detect *msl-2* allele in *MALE STERILITY 1* (*MSI*) in sugi.

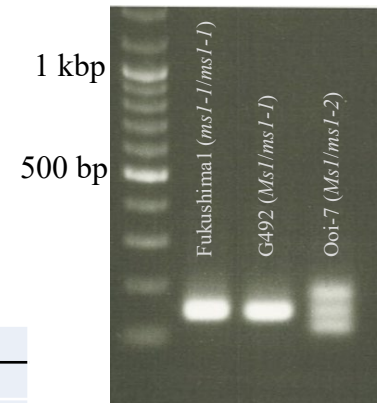
Reaction	Volume (μL)
2 \times Multiplex (QIAGEN)	3
2 μM CJt020762_F1O7.F_A	1
2 μM CJt020762_F1O7.R	0.5
2 μM Tail_A	0.5
dH ₂ O	4
DNA (10–50 ng)	1
Total (μL)	10

Temperature ($^{\circ}\text{C}$)		Cycles
95	15 min	1
94	15 sec	
63	45 sec	38
72	15 sec	
60	30 min	1
4	∞	1

Primer ID	Primer sequence
CJt020762_F1O7.F_A	GCCTCCCTCGCGCCACCTCCGGTGTATCAAACCTTCAA
CJt020762_F1O7.R	ATTCGCCCTTTCCAAATGTTAGC
Tail A	[FAM]-GCCTCCCTCGCGCCA



Electropherogram



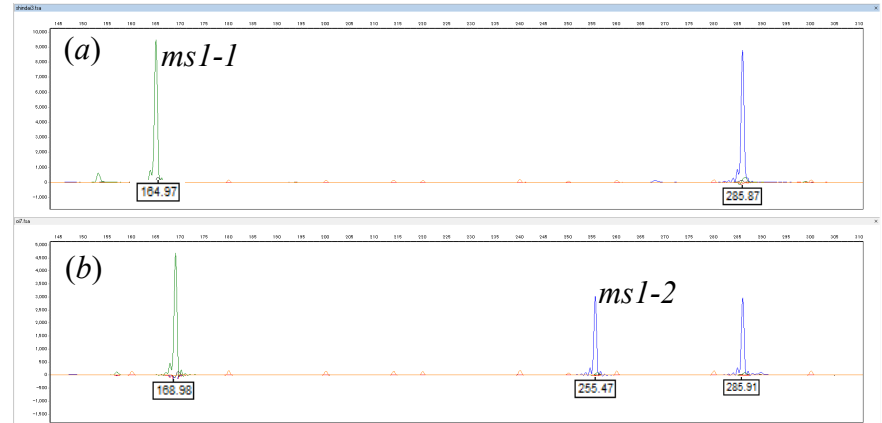
Gel image (2% agarose)

(c) ALP_ms1

Amplified length polymorphism marker (ALP) for *MALE STERILITY 1* (*MS1*) in sugi. Two mutant alleles (*ms1-1* and *ms1-2*) and rest of the wild type alleles (*Ms1*) can be detected simultaneously by multiplex PCR.

Reaction	Volume (mL)
KAPA2G Fast DNA Polymerase (5 U/ μ L)	0.1
5 \times KAPA2G Buffer A [§]	2.0
25 mM MgCl ₂	0.2
dNTP Mix (10 mM each)	0.2
5 μ M CJt020762_ms1-1_F	0.4
5 μ M CJt020762_ms1-1_R	0.4
5 μ M CJt020762_ms1-2_F	0.2
5 μ M CJt020762_ms1-2_R	0.2
dH ₂ O	5.3
DNA (5 ng/ μ L)	1.0
Total (mL)	10

[§] 1 \times KAPA2G Buffer A contains 1.5 mM MgCl₂



Electropherogram for (a) Shindai3 (*ms1-1/ms1-1*) and (b) Ooi-7 (*Ms1/ms1-2*)

Temperature (°C)		Cycles
95	3 min	1
95	15 sec	
60	15 sec	35
72	1 sec	
72	1 min	1
4	∞	1

Primer ID	Primer sequence
CJt020762_ms1-1_F	GCTATAGACTGCACCGACCC
CJt020762_ms1-1_R	AGCCCAGAAAATCGTCCCTG
CJt020762_ms1-2_F	GAATCCACCGCCACGACTAT
CJt020762_ms1-2_R	TGAACTCTGTTTCCATGGCA

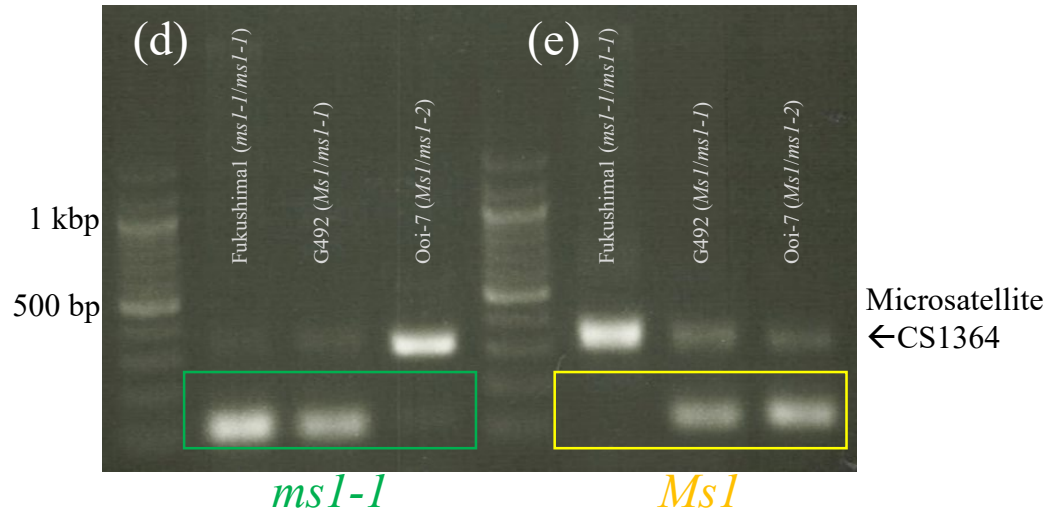
(d) ASP_ms1-1 and (e) ASP_ms1-1_wt

Allele specific PCR reaction to detect *ms1-1* (d) and *Ms1* (e) allele in *MALE STERILITY 1 (MSI)* in sugi.

Reaction	
2 × Multiplex (QIAGEN)	3
2 μM CJt020762_ASP ^s	1
2 μM CJt020762common.r	1
2 μM CS1364_F	1
2 μM CS1364_R	1
dH ₂ O	2
DNA	1
Total (μL)	10

^s Primer ID for ASP targeting (d) *ms1-1* and (e) *Ms1* is CJt020762_mt-224F_C.f and CJt020762_WT-227F_A.f, respectively.

Temperature (°C)		Cycles
95	15 min	1
94	15 sec	
56	45 sec	38
72	15 sec	
4	∞	1



Gel image (2% agarose)

Amplification of CS1364 is suppressed if that of ASP surpasses.

Primer ID	Primer sequence
CJt020762_mt-224F_C.f	CTGTGACTCACTGGCCACAGTAAC
CJt020762_WT-227F_A.f	GTGACTCACTGGCCACAGTCATAAA
CJt020762common.r	AGAGAGTGATGGTTTGATTAATGGGAAA
CS1364_F	TGATTATGGTTCGGTGGTCTT
CS1364_R	GTGATGTGGTGTTATCTTGT

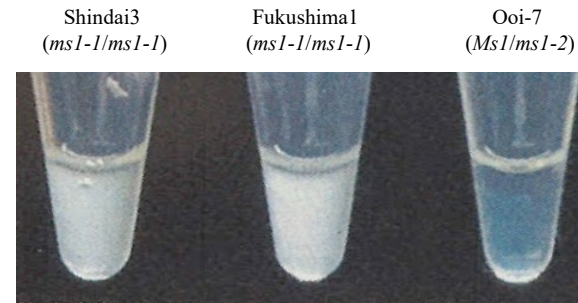
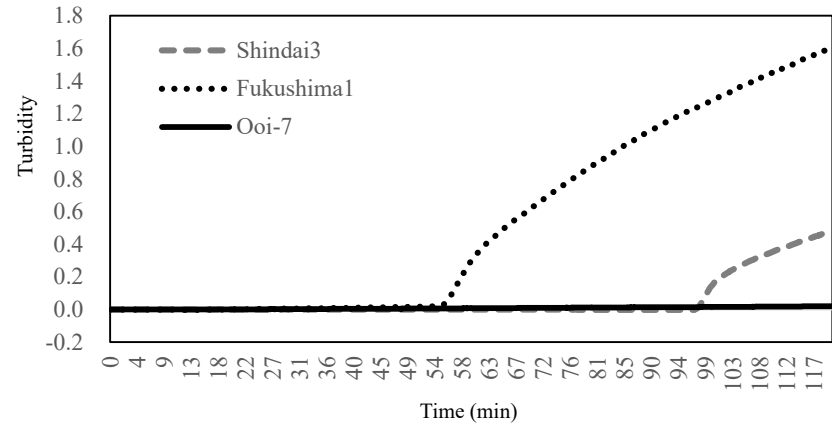
(f) LAMP_ms1-1

LAMP reaction to detect *ms1-1* allele in *MALE STERILITY 1* (*MS1*) in sugi.

Reaction	
2 x RM	12.5
40 μ M FIP (4D-1_FIP)	1
40 μ M BIP (4D-1_BIP-3C)	1
5 μ M F3 (4D-1_F3)	1
5 μ M B3 (4D-1_B3)	1
<i>Bst</i> DNA polymerase	1
DNA	2
dH ₂ O	5.5
Total (μ L)	25

Temperature (°C)	
63	120 min
80	5 min

Primer ID	Primer sequence
4D-1_FIP	ACGATGGGGTCGGTGCAGTCTAGCGGTAGCAGAAAGTGTGAT
4D-1_BIP-3C	ACGAGATCAGCCAAGCTTTCCAACCTGCGTGGGTCTG
4D-1_F3	GCGGCAATTGTGAGAGCATT
4D-1_B3	GAAAATCGTCCCTGGAGACG



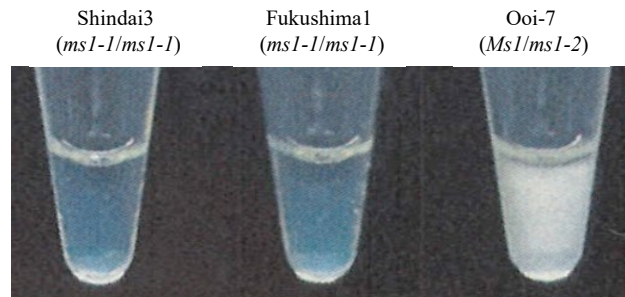
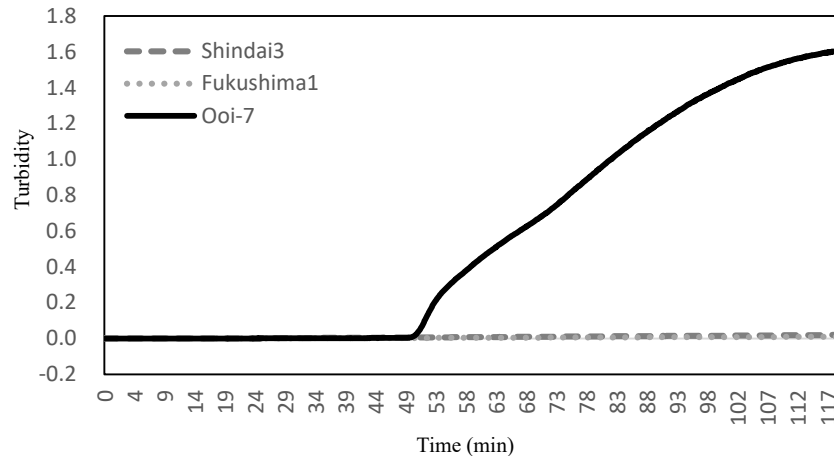
White precipitate was generated after 120 min incubation for assay with *ms1-1*.

(g) LAMP_ms1-1_wt

LAMP marker to detect wild type allele (*MsI*) without 4-bp deletion.

Reaction	
2xRM	12.5
40 μ M FIP (MS1-190727_FIP-2A)	1
40 μ M BIP (MS1-190727_BIP)	1
5 μ M F3 (MS1-190727_F3)	1
5 μ M B3 (MS1-190727_B3)	1
<i>Bst</i> DNA polymerase	1
DNA	2
dH ₂ O	5.5
Total (μ L)	25

Temperature (°C)	
63	120 min
80	5 min



White precipitate was generated after 120 min incubation for assay with *MsI*

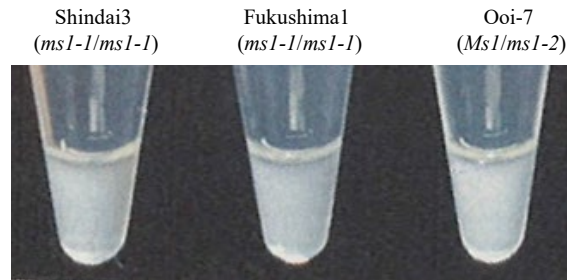
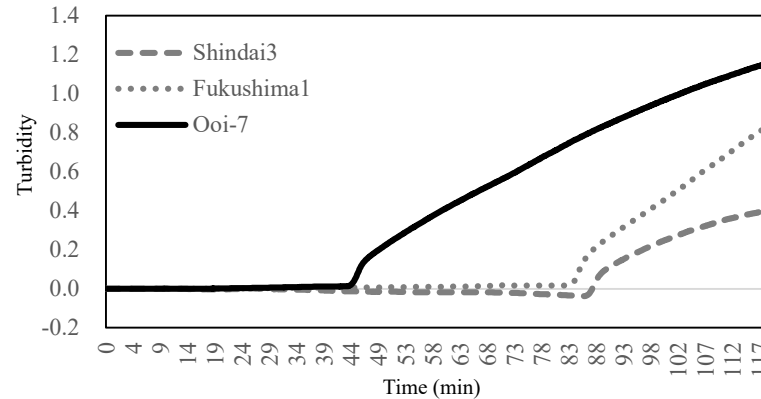
Primer ID	Primer sequence
MS1-190727_FIP-2A	TATTGATGACTGTGGCCAGTGAAGATCAGCCAAGCTTTCCAT
MS1-190727_BIP	GGTGCTTGTGCGAGCTCGTCGGAGCGAGAGAGTGATGGTT
MS1-190727_F3	ATCGTGAGCCTCTCACCA
MS1-190727_B3	GGTCAAGTTCACGCGGATA

(h) LAMP_ms1-2

LAMP reaction to detect *ms1-2* allele in *MALE STERILITY 1 (MS1)* in sugi.

Reaction	
2xRM	12.5
40 μM FIP (30D-1-FIP-3T)	1
40 μM BIP (30D-1-BIP)	1
5 μM F3 (30D-1_F3)	1
5 μM B3 (30D-1_B3)	1
<i>Bst</i> DNA polymerase	1
DNA	2
dH ₂ O	5.5
Total (μL)	25

Temperature (°C)	
65	120 min
80	5 min



White precipitate was generated after 120 min incubation for all assays. Inclusion of positive control and stopping the reaction at 60 min. is necessary.

Primer ID	Primer sequence
30D-1-FIP-3T	TGTAGTGATCCCCAAAATAGCCGTTAGCATTGCTGGAGCC
30D-1-BIP	TGAAGTTTGATACACCGGAGGTTTCTGCTAAATATAACTTTGAACTCTG
30D-1-F3	TGATTCGCCCTTTCCAAAT
30D-1-B3	AGACAACAGCAATTCATTCA