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1 *Article*

2 Gnotobiotic evaluation of *Dalbergia sissoo* genotypes 3 for resistance against *Fusarium solani* via dual culture 4 set up

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15 **Abstract:** *Dalbergia sissoo* (shisham), an important timber yielding multipurpose tree species of the
16 Indian subcontinent, has been afflicted with large scale mortality due to wilt in natural forests and
17 plantations, causing huge economic losses. *Fusarium solani* f. sp. *dalbergiae* (Fsd) has been identified
18 as one of the causal organisms for wilt disease in *D. sissoo*. Present study comprises *in vitro*
19 screening of ten selected genotypes of *D. sissoo* against two strains of *Fsd* in a dual culture set up
20 under axenic condition. Callus and plantlets of ten genotypes of host plant were multiplied *in vitro*
21 and were inoculated with conidial suspension of two strains of *Fsd* at three concentrations; 1×10^1,
22 1×10^3, and 1×10^5 conidia/ml. Gnotobiotic evaluation of dual culture set up shows variations among
23 *D. sissoo* genotypes in their response towards *in vitro Fsd* infection; and two genotypes (14 and 66)
24 exhibited resistance against the pathogen strains. Callus of genotypes 14 and 66 significantly
25 restricted the fungal mycelium growth whereas callus of remaining eight genotypes were
26 completely infested by *Fsd* mycelium within 9 days. Similarly, plantlets of genotype 14 and 66, had
27 lesser disease severity and remained green, and had fewer necrotic lesions in the roots whereas
28 plantlets of remaining eight genotypes died within 15 days.

29 **Keywords:** Shisham mortality, *Fusarium solani* f. sp. *dalbergiae*, *in vitro* screening, genetic variation,
30 *Fusarium* wilt, plant-microbe interaction, gnotobiota
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32 1. Introduction

33 *Dalbergia sissoo* Roxb. ex DC., commonly known as shisham, is a valuable timber species in
34 Fabaceae family, native to Afghanistan, Bangladesh, Bhutan, India, Iraq, Iran, Myanmar, Nepal and
35 Pakistan and introduced into Africa, Australia, China and the USA [1,2]. It is a pioneer tree of
36 primary succession in natural riverine forest of rivers Indus, the Ganges, Yamuna and Brahmaputra
37 with their tributaries and also an important multipurpose tree growing outside forest which yields
38 beautiful dark brown wood for furniture and panels, additionally used for strong poles, quality
39 fodder, fuel wood and folk medicine [3]. The species is already a suitable tree for wheat-based
40 agroforestry system as it is deciduous and fixes nitrogen [4,5].
41 Widespread mortality in *D. sissoo* has been reported from many parts of India [6-13] as well as
42 from Bangladesh [14], Nepal [15] and Pakistan [16]. An estimate by Shukla (2003) suggested that
43 damage to 400,000 mature trees of *D. sissoo* in India could have incurred a loss equivalent to US$ 200
44 million [17]. *Fusarium solani* (Mart.) Sacc. f. sp. *dalbergiae* Bakshi and Singh (hereafter referred as *Fsd*)
45 has been reported as one of the primary pathogenic fungi causing mortality in *D. sissoo* due to

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46 vascular wilt which is prevalent in the Gangetic and other riverine plains of India, Pakistan, Nepal
47 and Bangladesh [7]. Other fungal pathogen *viz*. *Fusarium oxysporum*, *Oxyporus latemarginatus* [18],
48 *Ganoderma lucidum* (Curtis) P. Karst [19], *Cercospora sissoo* [20], *Colletogloeum sissoo*, *Glomerella*
49 *cingulata* and *Septothyrella dalbergiae* [21], *Mycosphaerella dalbergiae* [22], *Phyllachora dalbergiae*
50 (=*Phyllachora viventis*; [23]), *Phyllactinia dalbergiae* [24], *Maravalia achroa* and *Uredo sissoo* [25],
51 *Ceratocystis manginecans* M. Van Wyk, Al Adawi & M. J. Wingf [26] and *Lasiodiplodia theobromae* (Pat.)
52 Griffon and Maubl. [27] have also been reported.

53 Tree improvement programmes in India has largely been focused on productivity
54 improvement of timber and to a lesser extent on disease resistance [28]. Rotation and maturation age
55 of important agro-forestry tree species *D. sissoo* 20 and 50 years, respectively, have also added to the
56 problem of screening for disease resistance in field despite widely reported infections and hence
57 necessitate to find quicker and robust system to develop disease resistance genotypes of *D. sissoo*.
58 Screening and selection of disease resistant clones by *in vitro* co-cultivation of desired cloned
59 genotypes with the fungal pathogen or toxin is an important crop improvement tools and is being
60 utilized gradually by different researchers [29-33]. *In vitro* growth condition is not only highly
61 reproducible but also minimize influence of abiotic and biotic factors and ensures homogenous
62 interaction between *in vitro* cultures of desirable host genotypes and inoculums or toxins of specific
63 pathogen and thus evincing a robust system to study plant microbe interaction. Callus of genotypes
64 of *D. sissoo* along with *Fsd* can be grown gnotobiotically under *in vitro* conditions in dual culture
65 setup and functional effects of host and microbe interaction can be analyzed. This further help to
66 understand mechanistic effect of host *D. sissoo* genotypes against *Fsd* [34]. Thus, *in vitro* screening in
67 dual culture setup facilitates estimation of resistance of desirable host genotypes [35]. Therefore, it
68 can be used as an indirect selection method for disease resistant genotypes of tree species [36]. Hence,
69 an approach of *in vitro* screening of *D. sissoo* genotypes against virulent strains of *Fsd* was
70 undertaken in a dual-culture setup with an aim to study the variability and selection of resistant *D.*
71 *sissoo* genotypes.

72 **2. Materials and Methods**

73 *2.1. Plant material*

74 Phenotypically superior trees of *D. sissoo* have been selected from various parts of northern
75 India, cloned through branch cuttings are being maintained in vegetative multiplication garden of
76 the Forest Research Institute (FRI), Dehra Dun, Uttarakhand, India. Ten genotypes which were part
77 of long-term field trials for improved productivity were selected for this study (Table 1). Genotype
78 14, a commercial cultivar of *D. sissoo* resistant to shisham die-back was also chosen for the study [37].

79 *2.2. Fungal Material*

80 *F. solani* f. sp. *dalbergiae* strains (Isolate no. 1145 and 1149) were obtained from the National Type
81 Culture Collection (NTCC) maintained at the Forest Protection Division, FRI and were cultured in
82 Petri dishes (9 cm) on potato dextrose agar (PDA) medium at 25 ± 2 °C.

83 *2.3. In vitro establishment of aseptic cultures of D. sissoo*

84 2.3.1. Explant sterilization

85 Healthy and uninfected nodal segments of the selected genotypes of *D. sissoo* were used for
86 aseptic culture establishment. Explants were washed in running tap water followed by soaking in a
87 0.5 % solution of antiseptic Cetrilak® (Cetrimide 5% w/v, India) and then in an aqueous solution of
88 0.1 % Bavistin® (Carbendazim WP; 50 % w/v, India). Surface sterilization of explants was carried out
89 in a laminar air flow with 0.1% Mercuric chloride as described by Panwar (2014) and Kunwar et al.
90 (2018) [38,39].

91 **Table 1.** Source location of selected genotypes of *D. sissoo* in the study

Sl. No	Genotype number	Source Location			
		Locality	District	State	Country
1.	10	Sabalgarh, Pathri	Haridwar	Uttarakhand	India
2.	14	Sabalgarh, Pathri	Haridwar	Uttarakhand	India
3.	19	Shah Mansurpur	Saharanpur	Uttar Pradesh	India
4.	24	CollectorbuckGanj	Bareilly	Uttar Pradesh	India
5.	41	Hasanpur, Tulsipur	Gonda	Uttar Pradesh	India
6.	66	Chhachhrauli Range	Yamuna Nagar	Haryana	India
7.	201	Hasanpur, Tulsipur	Gonda	Uttar Pradesh	India
8.	204	Tulsipur	Gonda	Uttar Pradesh	India
9.	232	Birpur-Bhambar	Gonda	Uttar Pradesh	India
10.	237	Bankatwa	Gonda	Uttar Pradesh	India

92 2.3.2. Axillary bud proliferation and *in vitro* rooting

93 Nodal segments of branch containing single axillary bud from each accession were collected,
94 sterilized and inoculated. For bud induction and multiplication, best responded treatments;
95 Murashige and Skoog (MS) medium [40] comprising 4.44 µM 6-Benzylaminopurine (BAP) + 2.69 µM
96 1-Naphthaleneacetic acid (NAA) and MS medium comprising 4.44 µM BAP + 1.34 µM NAA, were
97 used, respectively [38]. Cultures were maintained on standardized multiplication medium at 5
98 weeks interval and repeated further in subsequent sub-culturing. Micro shoots of size > 2.5 cm were
99 excised and transferred for root induction in half strength MS medium supplemented with
100 Indole-3-butyric acid (IBA) at 4.92 µM concentrations [38]. Culture medium pH was adjusted to 5.8
101 and autoclaved for 15 min at 121 °C and 1.0 x 10^5 Pa. Incubation temperature of culture room was 25
102 ± 2 °C and 55 ± 5 % relative humidity under a 16/8 hr (light/dark) photoperiod with light supplied by
103 cool-white fluorescent tubes (Philips, India) at an intensity of 35 µmoles/m^2/s.

104 2.3.3. Callus induction

105 Nodal explants of *D. sissoo* were collected from each genotype and sterilized as mentioned
106 earlier, further inoculated on MS medium supplemented with BAP alone or in combination with
107 2,4-Dichlorophenoxyacetic acid (2,4-D) for callus induction. The callus was maintained on MS
108 medium supplemented with 4.44 µM BAP and 2.69 µM NAA in culture condition as mentioned
109 above for further *in vitro* screening [38].

110 *2.4. In vitro screening and selection of tolerant D. sissoo genotypes against Fsd*

111 2.4.1. Fungal inoculum preparation

112 A mycelial disc (4 mm dia) from growing margins of the *Fsd* culture was transferred to an
113 Erlenmeyer flask (250 ml) containing 100 ml Carboxy Methyl Cellulose (CMC) medium [41] for
114 sporulation. The culture was incubated for 15 days at 25 ± 2 °C and then viewed in a
115 haemocytometer slide for conidial count. Consequential conidial suspension was diluted to the
116 desired concentration (1× 10^1, 1× 10^3, and 1× 10^5 conidia/ml) in the appropriate inoculation medium.

117 2.4.2. Screening of *D. sissoo* callus against *Fsd*

118 Callus of each genotype of *D. sissoo* were inoculated on standardized multiplication medium.
119 On growing callus 5µl droplet of conidial suspensions of *Fsd* (1145 and 1149) at three concentrations
120 (1×10^1, 1×10^3 and 1×10^5 conidia/ml) were inoculated atop the center of the callus and incubated at

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25 °C ± 2 as described before. After inoculation of callus tissue with conidial suspensions of *Fsd*, the extent of infection was assessed by measuring the diameter of fungal growth on the tissue as well as condition of callus i.e., either dead or alive. Fungal growth (cm) was measured by taking average of diameters at both X-X′ and Y-Y′axes of spread. Means of fungal radial diameter were analyzed using parametric test.

2.4.3. Screening of *D. sissoo* plantlets against *Fsd*

In vitro rooted plantlets of each genotype of *D. sissoo*, were inoculated as described before with *Fsd* (1145 and 1149) conidia (1× 10^5 conidia/ml) in 0.1% water agar supplemented with MS salts, and incubated in culture condition as described before. The extent of infection was assessed at regular intervals throughout a 15-day period of incubation using following disease scores; 0 = healthy plant; l = main root tip necrotic; 2 = whole root system infected; 3 = stem infected and appearance of wilt symptoms; 4 = whole plant wilted; 5 = plant dead. Infection extent of each genotype was scored from 0 to 5 on end of 5th, 7th, 9th, 11th, 13th and 15th days. On each observed day, means of infection extent score of genotypes were compared using non parametric test.

2.5. Experimental design and Statistical analyses

The experiments were laid in completely randomized design (CRD) with five replicates for each treatment and data were analyzed using analysis of variance (ANOVA) in Genstats 5 edition 3.2 for PC/Windows NT (Copyright 1995, LAWES Agricultural Trust (Rothamsted Experimental Station) and means were compared with least significance difference (LSD). Extent of plantlet infection score of 10 genotypes on 5th, 7th, 9th, 11th, 13th and 15th day against *Fsd* isolates 1145 and 1149 were non normal and analyzed using Kruskal-Wallis (KW) test in SPSS statistics-23. Results with significant difference were compared using rank corresponding to mean of infection extent of plantlet of genotype on each observed day. The genotype having rank with low numerical value were resistant whereas genotype having rank with high numerical were susceptible against the inoculated isolate of *Fsd*.

3. Results

3.1. In vitro response of callus of ten genotypes of D. sissoo against infection to Fsd

Results of the experiment suggest that treatments of both *Fsd* isolates (1145 and 1149), *D. sissoo* genotypes as well as interaction between them had significantly affected the extent of fungal infection on callus. High concentration (1×10^5 conidia/ml) of both isolates resulted in maximum spread of fungus 3.12 cm and 3.14 respectively, on callus after 9 days whereas low concentration (1×10^1 conidia/ml) of both isolates resulted in minimum spread of fungus on callus 1.04 cm and 1.28 cm, respectively. Also, the extent of fungal infection on callus caused due to each treatment differed significantly. Effect of *D. sissoo* genotypes on growth of *Fsd* mycelium on callus was significant; fungal spread of both *Fsd* isolates 1145 and 1149 was least on callus of genotype 14 (0.9 cm and 1.16 cm, respectively) whereas genotypes 232 and 41 could not restrain the fungal spread of isolates 1145 and 1149, respectively, and thus, resulted in maximum spread of 2.9 cm and 2.89 cm, respectively. Fungal growth on callus of genotype 66 for *Fsd* isolates 1145 and 1149 was 1.00 cm and 1.23 cm, respectively, which is at par with genotype 14 (Table 2, Figure 1a-d). Among the genotypes, callus of genotypes 14 and 66 show resistance to fungal growth of both *Fsd* isolates.

Observations on interaction between host genotypes and conidial concentrations of *Fsd* isolates (1145 and 1149) revealed that maximum spread of fungus was 5.02 cm and 4.68 cm, respectively, on callus of genotype 232 at 1×10^5 conidia/ml concentration whereas minimum spread of fungal growth 0.77 cm and 1.06 cm, respectively, was observed on callus of genotype 14 inoculated with 1×10^1 conidia/ml concentration. An increase in *Fsd* mycelium spread was observed in callus of genotype 232 from 1.42 cm to 5.02 cm upon increasing conidial concentration from 1×10^1 conidia/ml to 1×10^5

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167 conidia/ml and similarly from 1.53 cm to 4.68 cm for isolate 1149. On the other hand, genotype 14
168 apparently restricted fungal growth of isolate 1145 on callus as no significant difference in fungal
169 spread was observed when conidial concentration was increased from 1×10^1 conidia/ml to 1×10^5
170 conidia/ml. However, for isolate 1149 an increase in conidial concentration from 1×10^1 conidia/ml to
171 1×10^5 conidia/ml significantly affected the fungal spread on callus of genotype 14. It was interesting
172 to note that the fungal spread on callus of genotype 14 was non-significant when concentration was
173 increased from 1×10^1 conidia/ml to 1×10^3 conidia/ml as well as from 1×10^3 conidia/ml to 1×10^5
174 conidia/ml. Genotype 66, though not as promising as genotype 14, showed some resistance as fungal
175 spread on callus was non-significant when conidial concentration of both isolates was increased
176 from 1×10^1 conidia/ml to 1×10^3 conidia/ml, however fungal spread on callus differed significantly
177 when conidial concentration of both isolates was increased from 1×10^3 conidia/ml to 1×10^5
178 conidia/ml. In other genotypes spread on callus differed significantly when conidial concentration
179 of both isolates was increased from 1×10^1 conidia/ml to 1×10^3 conidia/ml as well as from 1×10^3
180 conidia/ml to 1×10^5 conidia/ml (Table 2, Figure 1a-d).

181 **Table 2.** Effect of conidial concentration of *Fsd* (isolate 1145 and 1149) and genotypes of *D. sissoo* on callus

Genotypes	**Spore Concentration**							
	1×10^1	**1×10^3**	**1×10^5**	**Mean (cm)**	**1×10^1**	**1×10^3**	**1×10^5**	**Mean (cm)**
	Fungal diameter for isolate 1145 (cm)				**Fungal diameter for isolate 1149 (cm)**			
14	0.8	0.8	1.1	0.9	1.1	1.1	1.3	1.2
66	0.9	0.9	1.3	1	1.1	1.1	1.5	1.2
19	0.9	1.3	2.4	1.5	1.2	1.6	2.6	1.8
41	1.0	1.9	3.0	2.9	1.2	2.0	3.0	2.1
24	1.0	1.8	3.4	2.1	1.3	2.0	3.6	2.3
10	1.1	2.2	3.9	2.4	1.4	2.3	3.8	2.5
201	0.9	1.1	2.2	1.4	1.2	1.5	2.6	1.7
204	1.2	2.1	4.2	2.5	1.4	2.3	4.0	2.6
232	1.4	2.4	5.0	2.9	1.5	2.5	4.7	2.9
237	1.3	2.3	4.78	2.8	1.5	2.4	4.5	2.8
Mean (cm)	1.04	1.68	3.12		1.28	1.88	3.14	

Variable	**LSD at 5%**	**Variable**	**LSD at 5%**
Genotypes	0.20	Genotypes	0.13
Treatment	0.29	Treatment	0.17
Genotypes × Treatment	0.34	Genotypes × Treatment	0.22

182 *3.2. In vitro response of plantlets of ten genotypes of D. sissoo against infection of Fsd*

183 Results of the experiment suggest that genotype 14 ranked first on all observed days except on
184 day seven, where it was a joint second against isolate 1149. The corresponding mean infection score
185 of genotype 14 on day five was 0.4 against both isolates which increased to 2.4 and 2.6 against
186 isolates 1145 and 1149, respectively, on 15th day. Genotype 66 ranked second consistently on all
187 observed days against both isolates and its corresponding mean infection score was 0.6 on 5th day
188 which grew to 2.8 on 15th day against both isolates. Genotype 19 managed rank 3.5 and 3.0 against

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189 1145 and 1149, respectively on 5th day, 3.0 against both isolates on 9th day, 11th day and 13th day but
190 rank 6.5 on 15th day. The corresponding mean infection score was 0.8 on day 5th which steeply
191 reached to 5.0, which meant completely dead plantlets, on 15th day, which suggests that slight
192 resistance was shown by genotype during initial days of treatment but was lost by the 15th day.
193 Similar results were obtained for genotype 41. Genotypes 10, 24, 201, 204, 232 and 237 on 5th day had
194 infection score more than one and their relative ranks were 6, 8.5, 6.0, 10, 6 and 8.5, respectively,
195 against isolates 1145 whereas against isolate 1149 their ranks were 5, 8, 8, 8, 8 and 8, respectively.
196 Five genotypes 24, 201, 204, 232 and 237 scored more than three against isolate 1149 on 7th day and
197 their corresponding ranks were 8, 8, 8, 8 and 10. Though, against isolate 1145 genotypes 10, 24, 201,
198 204, 232 and 237 had scores of 1.8, 2.0, 1.0, 1.8, 1.0 and 2.4 and corresponding ranks were 7.5, 9, 4, 7.5,
199 4 and 10. On 11th day genotypes 24, 232 and 237 had the highest infection scores of 5 and
200 corresponding ranks were 9 against isolate 1149 whereas same genotypes had infection scores of 4, 4
201 and 4.2 with corresponding ranks 8.5, 8.5 and 10 against isolates 1145. Genotypes 10, 24, 201, 204, 232
202 and 237 had infection score 5 on 13th and 15th day against both isolates (Table 3, Figure 1e-i).

203 **4. Discussion**

204 Vascular wilt, blight, bakanae disease etc. caused by *Fusarium* species have been reported for
205 widespread plant mortality [41-43]. Selection of disease resistant plant varieties through *in vitro*
206 screening has been utilized in improvement of crops; against *Fusarium* spp, *viz*. strawberry [29],
207 wheat [44, 45], date palm [46], alfalfa plants [47], passion fruit [48] and *Musa* spp [32].
208 This study is a first report of *in vitro* screening and selection of resistant *D. sissoo* genotypes
209 against *Fsd* vascular wilt. Results highlight that *in vitro* raised callus as well as plantlets of *D. sissoo*
210 genotypes 14 and 66 exhibited improved resistance against both virulent strains of *Fsd* (1145 and
211 1149) as compared to other eight genotypes. Genotype 14, which had been tested in the field and
212 showed better survival against shisham mortality than other genotypes was taken as a positive
213 control for the study and interestingly this exhibited similar disease resistance capabilities *in vitro* as
214 well.
215 Resistant characteristics of genotypes were apparent on the 9th day of *in vitro* infection on callus
216 of *D. sissoo* with *Fsd*. Fungal diameter growth increased by more than 350% in genotype 232 and 237
217 whereas only by 50% in genotype 14 on increasing conidial concentration from 1×10^1 to 1×10^5
218 conidial/ml (Table 2). Among ten genotypes, it can be said that callus of genotype 14 has resisted the
219 infection by fungal mycelium, followed by genotype 66. But in genotype 66, the resistance
220 diminished gradually with increase in conidial concentration to 1×10^5 conidial/ml. Similar studies of
221 callus-fungal interactions for disease resistance selection have been reported in woody species like;
222 *Pinus eschinata* and *P. virginiana* [49], *Acacia pulchella*, *Eucalyptus calophylla*, *E. marginata* [30], *Prunus*
223 *persica* [50], *Pinus ellottii* [51], *Citrus sinensis* and *C. limon* [52], *Fagus sylvatica* [53], *Pinus nigra* and *P.*
224 *sylvestris* [54] and *Malus domestica* [55].
225 *In vitro* cloned plantlets of *D. sissoo* genotypes infected under *in vitro* condition with conidial
226 suspension of *Fsd* showed results similar to callus. After infection, it was observed that, fungal
227 mycelium grew rapidly and a cottony mass of mycelium could be seen around rhizosphere of
228 plantlets, which may be due to humid conditions of the culture vessels providing favorable
229 environment for mycelial growth [56]. Nonetheless, plantlets of genotype 14 and 66 had a
230 significantly lesser disease severity index as compared to the other genotypes and, also remained
231 green, healthy and had fewer necrotic lesions in the roots whereas plantlets of remaining eight
232 genotypes died slowly during the course of the study. Similar findings have been reported for other
233 plantlet-microbe interactions [32, 48, 50, 57-61].
234 *In vitro* screening of clonal host genotypes against specific strains of pathogen in a dual culture
235 set up gives an opportunity to estimate the resistance or susceptibility of clones. Plantlets with
236 complete root and shoot system or callus (mostly representing the unorganized cellular growth)
237 both of the same genotype showed similar trend. Thus, the results obtained after screening of *in*
238 *vitro* plantlets and callus signify that either can be used to screen disease tolerant genotypes of *D.*
239 *sissoo*. Pathogenesis-related (PR) proteins have often been reported in plant systems for

Table 3. Disease score of plantlets of ten genotypes of *D. sissoo* inoculated with *Fsd* (isolate 1145 and 1149). On each observation day mean and rank of each genotypes was compared. Mean reflects disease severity and rank reflects ability to resist *Fsd* infection.

	5th day				**7th day**				**9th day**				**11th day**				**13th day**				**15th day**			
Isolate no.	**1145**		**1149**		**1145**		**1149**		**1145**		**1149**		**1145**		**1149**		**1145**		**1149**		**1145**		**1149**	
Genotype no.	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
10	1	6	1.8	5	1.8	7.5	2.6	5	2.8	5.5	3.8	6	3.8	6.0	4.8	6	5.0	7.5	5	7	5.0	6.5	5	6.5
14	0.4	1	0.4	1	0.6	1.0	1	2	1.0	1.0	1.6	1	1.6	1.0	1.8	1	2.2	1.0	1.8	1	2.4	1.0	2.6	1.0
19	0.8	3.5	0.8	3	1.2	6.0	2	4	1.6	3.0	2.6	3	2.8	3.0	3.8	3	3.6	3.0	4.8	3	5.0	6.5	5	6.5
24	1.2	8.5	2	8	2.0	9.0	3	8	3.0	7.5	3	5	4.0	8.5	5	9	5.0	7.5	5	7	5.0	6.5	5	6.5
41	0.8	3.5	1	4	1.0	4.0	2	4	1.8	4.0	2.8	4	3.0	4.0	4	4	3.8	4.0	5	7	5.0	6.5	5	6.5
66	0.6	2	0.6	2	0.8	2.0	1	2	1.2	2.0	2	2	1.8	2.0	2.4	2	2.6	2.0	2.6	2	2.8	2.0	2.8	2.0
201	1.0	6	2	8	1.0	4.0	3	8	2.8	5.5	4	8	3.8	6.0	4.8	6	5.0	7.5	5	7	5.0	6.5	5	6.5
204	1.4	10	2	8	1.8	7.5	3	8	3.0	7.5	4	8	3.8	6.0	4.8	6	5.0	7.5	5	7	5.0	6.5	5	6.5
232	1.0	6	2	8	1.0	4.0	3	8	3.2	9.5	4	8	4.0	8.5	5	9	5.0	7.5	5	7	5.0	6.5	5	6.5
237	1.2	8.5	2	8	2.4	10.0	3.2	10	3.2	9.5	4.2	10	4.2	10.0	5	9	5.0	7.5	5	7	5.0	6.5	5	6.5

**Disease Scores* (0-5)*

0= Healthy plant, 1= Main root tip necrotic, 2= Whole root system infected, 3= Stem infected and appearance of wilt symptoms, 4= Whole plant wilted, 5= Plant dead

administering biotic stress tolerance [62] especially against fungal pathogens [63]. PR proteins reportedly enhanced tolerance to somatic embryos of *Vitis vinifera* against *Elsinoe ampelina* pathogen [64, 65] hence, antifungal activity observed during *in vitro* screening and selection of *D. sissoo* genotypes (Figure. 1a, f-g) might reflects probable role of PR proteins.

5. Conclusions

In vitro screening of candidate genotypes of *D. sissoo* against *Fsd* in gnotobiotically reduces time, resources and space otherwise required and a large number of *D. sissoo* genotypes could be screened in limited time thus assisting in the process of selection of disease resistant genotypes. The study thus concludes that callus of two genotypes of *D. sissoo* (14 and 66) showed resistance against *Fsd in vitro* infection whereas remaining eight genotypes were susceptible. Similar trend was observed after *in vitro* screening of plantlets of *D. sissoo* genotypes, further establishing that genotypes 14 and 66 exhibited resistance to *Fsd* infection *in vitro*.

(a)

(b)

(c)

(d)

(e)

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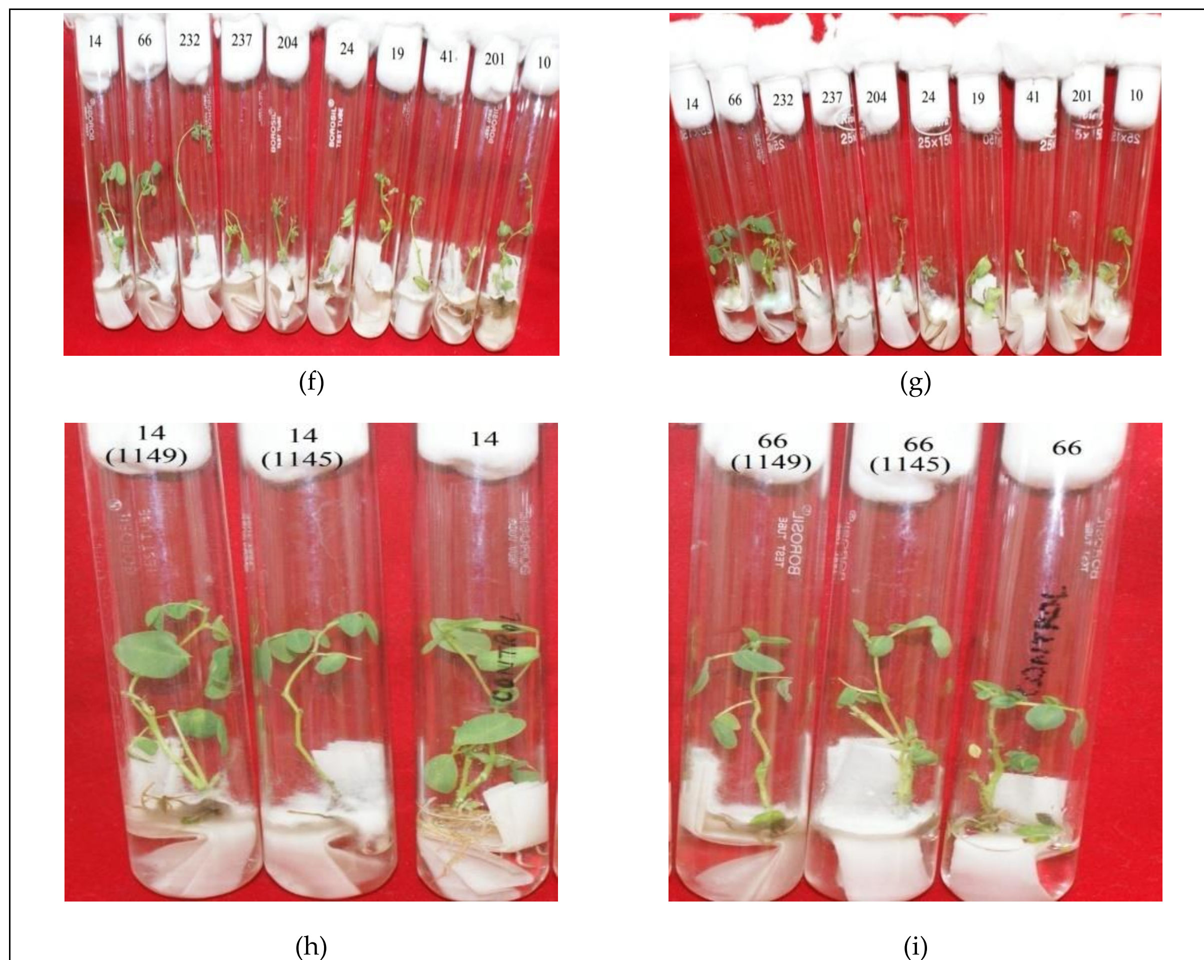

Figure 1. (a) Effect of *Fsd* isolate 1145 on *in vitro* raised callus of *D. sissoo* genotypes, from left to right genotype 19, 237, 204, 14, 66, 41, 232, 10, 24, and 20; **(b)** Callus of genotype 14 inoculated with *Fsd* (1149) after 30 days of infection; **(c)** Callus of genotype 66 inoculated with *Fsd* (1145) after 30 days of infection; **(d)** Control for a; **(e)** Control for f and g; **(f)** Effect of *Fsd* isolate 1145 on *in vitro* raised plantlets of ten genotypes of *D. sissoo*; **(g)** Effect of *Fsd* isolate 1149 on *in vitro* raised plantlets of ten genotypes of *D. sissoo*; **(h)** Plantlets of genotype 14 infected with both isolates of *Fsd* and uninfected control (third test tube from left) after three weeks of infection; **(i)** Plantlets of genotype 66 infected with both isolates of *Fsd* and uninfected control (third test tube from left) after three weeks of infection.

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