Production of interspecific hybrids between *Sesamum alatum* Thonn and *Sesamum indicum* L. through ovule culture and screening for phyllody disease resistance

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Abstract

Sesame (*Sesamum indicum* L.) is an important oilseed crop grown in the tropical and subtropical regions of the world. Despite the nutritional value and cultural importance, the biotechnological research on sesame is very limited. In this study, we have optimized a simple and efficient protocol for producing an interspecific hybrid between *Sesamum alatum* and *S. indicum* through ovule culture. In the cross *S. alatum* × *S. indicum*, capsule retention without embryo abortion was extended up to 7 days after pollination by spraying the growth regulator mixture containing 289 µM gibberellic acid (GA₃), 80.6 µM α-naphthalene acetic acid (NAA) and 23.3 µM kinetin. Direct organogenesis was successfully achieved when the ovules, excised from 7-day-old capsules, were cultured on MS medium containing 8.8 µM benzylaminopurine (BAP), 2.8 µM indole acetic acid (IAA) and 1712.3 µM glutamine. The regenerants produced roots on half strength MS medium supplemented with 0.27 µM NAA. Phenotypically, the *S. alatum* × *S. indicum* hybrid plants were intermediate to those of parents for majority of the traits. Cytological studies revealed normal meiosis in the hybrid without any chromosomal abnormalities. Peroxidase and esterase isozymes were demonstrated to be useful in the identification of hybrid plants. Screening against phyllody disease under greenhouse conditions revealed that the hybrids were moderately resistant.

Keywords: Interspecific hybridization; Ovule culture; Phyllody disease; Post-zygotic barrier; *Sesamum alatum*; *Sesamum indicum*

1. Introduction

Sesame (*Sesamum indicum* L.) is a member of the order Tubiflorae, family Pedaliaceae. It is one of the most ancient crops and an important oilseed crop grown in tropical and subtropical areas on 6.5 million hectares worldwide, producing more than 3 million tonnes of seed (FAOSTAT, 2005). India, Sudan, Myanmar and China are the major contributors with 68% of the world production. Sesame seed is highly nutritious (50% oil and 25% protein) which is traditionally used for direct consumption and as a source of oil of excellent quality because of the presence of natural antioxidants such as sesamin and sesamol (Pastorello et al., 2001). Sesame oil also contains high level of unsaturated fatty acids, which has a reducing effect on the plasma cholesterol (Banerjee and Kole, 2006). The seed is also used for confections and for decorating bread and cakes as an extra-rich source of protein in many developing countries. Due to promising beneficial attributes on human health and increasing demand for vegetable oil worldwide, research activities have recently been accelerated with great interest in this crop.

Despite the economic importance for food, oil and medicine, the yield potential of sesame is not spectacular due to its cultivation in submarginal lands and non-availability of superior high yielding varieties lacking inbuilt resistance to biotic and abiotic stresses. Among various biotic stresses, phyllody is a highly destructive disease of sesame and is caused by a pleomorphic mycoplasma-like organism (phytoplasma) transmitted by leaf hopper (Vasudeva and Sahambi, 1955). The
affected plants become stunted and the floral parts are transformed into green leaf-like structures followed by abundant vegetative growth resulting in a yield loss up to 33.9% or even 100% during severe incidence (Abraham et al., 1977; Sarwar and Haq, 2006). Control of this disease by killing the hoppers through foliar application of insecticides has been effective only to some extent. Therefore, the development of cultivars with durable resistance to phyllody forms an integral component of sesame breeding programs. Since the cultivated germplasm are mostly susceptible, exploitation of wild relatives as sources of resistance genes could be a viable and sustainable approach. The resistance to phyllody disease was reported in the wild species S. indicum through artificial screening (Srinivasulu and Narayanaswamy, 1992; Singh et al., 2007).

However, the transfer of this trait from wild to the cultivated varieties was largely unsuccessful due to operation of high degree of crossability barriers (Kedhamath, 1961). Though Ramalingam et al. (1992) was later able to successfully produce an interspecific hybrid between S. alatum and S. indicum, the crossability was very low (0.04%) and only one crossed seed germinated. Subsequent studies have shown that the interspecific hybridization has led to post-zygotic barriers in the cross S. alatum × S. indicum, as clearly demonstrated by an early abortion of young embryos (Rajeswari and Ramaswamy, 2004a). Among different in vitro techniques employed, ovule culture has been proved to be an effective method in circumventing the post-zygotic barriers in a number of interspecific crosses involving oilseed species such as Arachis (Liang et al., 1990; Zhuang et al., 1999), Brassica (Inomata, 1990), Brassica × Orychophragmus (Jiaming et al., 1998) and Helianthus (Dahlhoff et al., 1992). But, the major hurdles in adopting in vitro regeneration systems in sesame are that the species is highly recalcitrant for tissue culture manipulations and the protocols are generally genotype-dependent. This is clearly reflected by the fact that only two reports are available presently on the recovery of interspecific hybrids of sesame through ovule culture. Qu et al. (1994) was first to demonstrate the embryo rescue and plant regeneration of interspecific hybrids between S. indicum and a wild species S. schizinixinum. Recently, Dasharat et al. (2007) employed both ovary and ovule culture to recover the plantlets from aborting embryos of interspecific hybrids between S. indicum and two wild species S. radiatum and S. occidentale. Besides these successful reports, however no consistent attempts have so far been made to obtain the interspecific hybrids between S. alatum and S. indicum using ovule culture technique. In this study, we report a simple and efficient in vitro regeneration system for efficient recovery of viable plantlets from the ovules of S. alatum × S. indicum hybrid. In addition, for the first time, the recovered plants were tested to confirm the hybrid nature by morphological, cytological and molecular means and screened for their reaction to phyllody disease.

2. Materials and methods

2.1. Plant materials and hybridization

Four S. indicum cultivars (SVPR 1, CO 1, TMV 3 and TMV 6) and the wild species S. alatum were used in this study. Plants were grown in the greenhouse of Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India and pollinated in both direct and reciprocal directions. To effect hybridization, four epipetalous stamens were emasculated in the previous day evening and covered with a butter paper bag. The anthers were collected for crossing by next day morning before 7:30 a.m. and the pollens were dusted on the stigmatic surface. After 7 days of pollination, both self pollinated and crossed capsules were collected.

2.2. In vitro culture of hybrid embryos and plant regeneration

Since sesame embryos are very smaller in size, ovules of 7-day-old at heart shaped stage were selected for in vitro culture. The capsules were first surface sterilized with 0.1% (w/v) HgCl₂ for 3 min, washed thoroughly three times with sterile distilled water and were disinfected under aseptic conditions using a dissection microscope. To optimize the plant regeneration system, ovules were cultured onto Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with different combinations and concentrations of BAP (4.4, 8.8 and 13.3 μM), IAA (2.8 μM) and glutamine (1027.4, 1369.9 and 1712.3 μM). The pH of the medium was adjusted to 5.8 by 0.4 N NaOH before autoclaving at 121 °C for 20 min. Culture tubes were maintained with a controlled temperature of 25±2 °C under dark for 4 days and then incubated at 25±2 °C with light intensity of approximately 3000 lux for 16 h. To induce rooting from the regenerated plant, half strength MS basal media containing different levels of NAA (0.05, 0.16, 0.27, 0.38 and 0.54 μM) were tested. After 70 days of culture, hybrid plantlets were transferred to plastic micropots filled with sterilized mixture of vermiculite and sand (1:1) and incubated in the culture room for one week before being transferred to mist chamber. After 4 weeks, the hardened plants were transferred to earthen pots containing sterilized garden soil and grown under greenhouse conditions.

2.3. Cytological and pollen fertility studies

The young developing flower buds of both parents and hybrids were fixed in ethyl alcohol:acetic acid (3:1 v/v) mixture at 11:30 a.m. Mcuagenous secretions were removed by treating the flower buds in 1 N HCl for 3 min, washed thoroughly three times with sterile distilled water and were disinfected under aseptic conditions using a dissection microscope. To optimize the plant regeneration system, ovules were cultured onto Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with different combinations and concentrations of BAP (4.4, 8.8 and 13.3 μM), IAA (2.8 μM) and glutamine (1027.4, 1369.9 and 1712.3 μM). The pH of the medium was adjusted to 5.8 by 0.4 N NaOH before autoclaving at 121 °C for 20 min. Culture tubes were maintained with a controlled temperature of 25±2 °C under dark for 4 days and then incubated at 25±2 °C with light intensity of approximately 3000 lux for 16 h. To induce rooting from the regenerated plant, half strength MS basal media containing different levels of NAA (0.05, 0.16, 0.27, 0.38 and 0.54 μM) were tested. After 70 days of culture, hybrid plantlets were transferred to plastic micropots filled with sterilized mixture of vermiculite and sand (1:1) and incubated in the culture room for one week before being transferred to mist chamber. After 4 weeks, the hardened plants were transferred to earthen pots containing sterilized garden soil and grown under greenhouse conditions.

The young developing flower buds of both parents and hybrids were fixed in ethyl alcohol:acetic acid (3:1 v/v) mixture at 11:30 a.m. Mcuagenous secretions were removed by treating the flower buds in 1 N HCl for 3–5 min. Flower buds were then washed thoroughly with water, stored in ethyl alcohol:acetic acid (3:1 v/v) fresh fixative for 24 h and finally preserved in 70% (v/v) ethyl alcohol for further use. Smears of pollen mother cells (PMCs) were prepared by squashing and staining the pollen grains with 1% (w/v) acetocarmine on a microscopic slide. The slides were warmed on a hot plate and observed for meiotic stages using a Meiopha research microscope. Photomicrographs were taken using a Pentax Spotmatic camera. For pollen fertility estimation, matured anthers of parents and hybrids were collected, squashed on a microscopic slide with acetocarmine:glycerol (1:1 v/v) mixture and observed under a light microscope. The swollen and well stained pollens were counted as fertile, while the shrunken and unstained pollen as sterile.
2.4. Isozyme analysis

Leaf tissues (500 mg) of *S. indicum* (cv. TMV 3), *S. alatum* and the interspecific hybrids were homogenized in 1 ml of ice-cold extraction buffer (0.1 M phosphate buffer) in a prechilled pestle and mortar. The homogenate was centrifuged at 12,000 rpm for 25 min at 4 °C and the supernatant was used for isozyme analysis. Electrophoresis was carried out on a 15% polyacrylamide gel in a Hoefer mini gel. The gels were maintained at 4 °C with refrigerated circulating water bath. A constant current of 30 mA was applied throughout the run. Staining for peroxidase and esterase isozymes was done according to Vallejos (1993).

2.5. Artificial screening for phyllody disease

The parents and the hybrids were screened for their reaction against phyllody disease under greenhouse conditions (Sertkaya, 1999). After 30 days, the disease incidence was measured as disease intensity percentage by counting the number of plants infected. Based on the intensity or infection percentage, the disease reaction was evaluated on 0–6 scale as field immune (0), highly resistant (1), resistant (2), moderately resistant (3), moderately susceptible (4), susceptible (5) and highly susceptible (6) as described by Sarwar et al. (2007).

2.6. Enzyme quantitative estimation

Leaf samples of parents and hybrids were assayed for three different enzymes phenylalanine ammonia-lyase (PAL), peroxidase, polyphenol oxidase (PPO) and phenolics using a Beckman spectrophotometer and the data were recorded as five replicates for each enzyme. Leaf samples from disease infected and healthy uninfected *S. indicum* cv. TMV 3 plants served as positive and negative controls, respectively. The enzyme assay procedures as described by Dickerson et al. (1984) for PAL, Langcake and Wicking (1975) for peroxidase, Mayer et al. (1965) for PPO and Zieslin and Ben-Zaken (1993) for phenolics were followed.

2.7. Statistical analyses

Unless indicated otherwise, all the experiments were replicated thrice and statistically analyzed in a factorial randomized block design (Gomez and Gomez, 1992). The data on ovule germination and mean enzyme activities were subjected to analysis of variance (ANOVA) and significant differences among genotypes or species were determined by Duncan’s Multiple Range Test (DMRT) using statistical software Statgraphics Centurian version XV.

3. Results and discussion

3.1. Crossability and hybrid survival

In this study, direct crosses involving four cultivated varieties of *S. indicum* SVPR 1, CO 1, TMV 3 and TMV 6 as ovule parents and the wild species *S. alatum* as pollen parent failed to set seeds. This was due to existence of pre-zygotic barriers especially the inhibition of pollen tube growth in the mid-stylar region preventing the pollen tubes to reach the ovule (Rajeswari and Ramaswamy, 2004a). In the reciprocal crosses using *S. alatum* as female parent, capsule formation was observed confirming successful fertilization. However, the capsules dropped prematurely as the embryo was found to degenerate after 48 h of cross pollination. This showed that the post-zygotic barriers were strongly prevalent in the *S. alatum × S. indicum* cross. In order to extend the capsule retention for 7 days after pollination for the isolation of ovules, a mixture of growth regulators containing 289 µM GA₃, 80.6 µM NAA and 23.3 µM kinetin was applied to the flowers immediately after cross pollination and to the developing capsules (Rajeswari and Ramaswamy, 2004b). For *in vitro* culture experiments, ovules of 7-day-old were isolated from the capsules and subsequently germinated on a suitable media to recover the regenerants (Fig. 1a).

3.2. Regeneration of hybrid plants through ovule culture

The results on the effect of BAP (4.4, 8.8 and 13.3 µM) and glutamine (1027.4, 1369.9 and 1712.3 µM) in the presence of 2.8 µM IAA for direct plantlet regeneration from the ovules of parents and hybrid were presented in Tables 1 and 2. Among the different hormonal combinations tried, successful regeneration of the plantlets was observed after 25 days of culture of both selfed and crossed ovules on MS medium supplemented with 8.8 µM BAP, 2.8 µM IAA and 1712.3 µM glutamine (Table 1; Fig. 1b). For unsuitable combinations, the ovules showed varied response in the form of improper growth patterns like enlarging, greening and browning. This suggested that the phytohormone regime is critical for the successful germination of ovule into a whole plant irrespective of parental species or combination used. The germination frequencies in the crosses *S. alatum × S. indicum* cv. TMV 3 and *S. alatum × S. indicum* cv. TMV 6 were recorded as 12.0%, 10.0%, 9.7% and 9.3%, respectively in the best responding medium containing glutamine (Table 2). This clearly indicated the positive influence of glutamine in the induction of ovule germination apart from BAP and IAA. Also, this view was further supported by our results that the ovule germination occurred when higher level of glutamine was added in the medium. It has been proposed that glutamine may serve as a suitable nitrogen source in supporting the active protein synthesis associated with embryo germination being a central intermediate in the synthesis of other amino acids (Miflin and Lea, 1976). In Black spruce, a two-fold increase in the number of embryos was noticed when glutamine was used as the only nitrogen source or as a supplement to inorganic nitrogen (Khelifi and Tremblay, 1995).

To induce rooting from well developed shoots, we tested different concentrations of NAA (0.05, 0.16, 0.27, 0.38 and 0.54 µM). Normal root proliferation was achieved upon culture of shooted plantlets on half strength MS medium.
containing 0.27 µM NAA (Fig. 1c). The induction of rhizogenesis could be attributed due to the action of NAA, which is well known for its root promoting characteristics (Smith et al., 1986). Taskin and Turgut (1997) also reported that the plants regenerated from cotyledonary explants of sesame rooted profusely in the medium without growth regulators or with NAA only. After proper shoot and root growth in vitro, the plantlets were transferred to greenhouse and grown until maturity.

3.3. Morphological, cytological, and molecular characterization of the interspecific hybrid

The *S. alatum* × *S. indicum* hybrid was found to be intermediate between their parents for most of the morphological characteristics and resembled *S. indicum* cv. TMV 3 for stem and leaf traits. Flowers were white coloured with purple tinch on the lip of the corolla (Fig. 1d–f). Hybrid seeds were rough with wingless dull brown colour. Hybrid plants were
mostly fertile as they recorded pollen fertility of 75.5%. Meiotic behaviour was normal as that of the parents and revealed regular bivalent (13II) formation at diakinesis and metaphase I stages (Fig. 1g). No typical chromosomal abnormalities, usually associated with interspecific crosses, were detected in the hybrid. This may be reasoned as intergenomic homology between the chromosomes of the two parental species having the same chromosome status ($2n = 26$).

Isozyme polymorphism is one of the most practical and direct applications for confirmation of hybridity. Isozymes generally exhibit Mendelian inheritance, co-dominant expression, complete penetrance and absence of pleiotropic and epistatic interactions. Such features facilitated several researchers to utilize isozymes as molecular markers to confirm the authenticity of interspecific hybrids in *Vigna* spp (Gomathinayagam et al., 1998), *Allium* spp (Hou et al., 2001), *Arachis* spp (Mallikarjuna, 2002) and *Helianthus* spp (Prabakaran and Sujatha, 2004). In this study, we employed two different isozymes peroxidases and esterases to prove the hybridity of F1 plants of sesame developed through ovule culture. The results showed that both isozymes could clearly differentiate the hybrids from their parents as they expressed distinct banding patterns. For peroxidase, the interspecific hybrids possessed bands of both parents, indicating the presence of genomes of both parents in the hybrids (Fig. 2a). In case of esterase, the hybrids resembled their paternal parent in the isozyme profile which revealed that the genetic introgression had taken place (Fig. 2b). This phenomenon may be explained that different functions of individual isozymes can be expected because each isozyme catalyses specific reactions reflecting in their end products. Parani et al. (1997) reported that the inheritance of the esterase locus est D and peroxidase loci prx A and prx L were useful in the identification of *S. alatum* × *S. indicum* hybrid. Nanthakumar et al. (2000) also confirmed by genetic diversity analysis that the varieties of *S. indicum* stayed away from their wild counterpart *S. alatum*. Thus, the potential of isozymes for the identification of interspecific hybrids has been well demonstrated in this study.

### Table 1

<table>
<thead>
<tr>
<th>Growth regulator (µM)</th>
<th>Parents</th>
<th>Crosses</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAP</td>
<td>Glutamine</td>
<td>S. alatum</td>
</tr>
<tr>
<td>4.4</td>
<td>1027.4</td>
<td>NR</td>
</tr>
<tr>
<td>4.4</td>
<td>1369.9</td>
<td>E</td>
</tr>
<tr>
<td>4.4</td>
<td>1712.3</td>
<td>E</td>
</tr>
<tr>
<td>8.8</td>
<td>1027.4</td>
<td>G</td>
</tr>
<tr>
<td>8.8</td>
<td>1369.9</td>
<td>G</td>
</tr>
<tr>
<td>8.8</td>
<td>1712.3</td>
<td>G</td>
</tr>
<tr>
<td>13.3</td>
<td>1027.4</td>
<td>E</td>
</tr>
<tr>
<td>13.3</td>
<td>1369.9</td>
<td>G</td>
</tr>
<tr>
<td>13.3</td>
<td>1712.3</td>
<td>B</td>
</tr>
</tbody>
</table>

* B: Browning; E: Enlarging; G: Greening; GR: Germination; NR: No response.

** The medium used was MS+8.8 µM BAP+2.8 µM IAA+1712.3 µM glutamine.

** Means followed by the same letters within a column are not significantly different at a 5% probability level by DMRT.

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**Table 2**

<table>
<thead>
<tr>
<th>Parents/crosses</th>
<th>No. of ovules inoculated</th>
<th>No. of ovules germinated</th>
<th>% of ovule germination **</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. alatum</em></td>
<td>200</td>
<td>16</td>
<td>8.0*</td>
</tr>
<tr>
<td><em>S. indicum</em> cv. SVPR 1</td>
<td>200</td>
<td>26</td>
<td>13.0*</td>
</tr>
<tr>
<td><em>S. indicum</em> cv. CO 1</td>
<td>165</td>
<td>20</td>
<td>12.1b</td>
</tr>
<tr>
<td><em>S. indicum</em> cv. TMV 3</td>
<td>170</td>
<td>20</td>
<td>11.8b</td>
</tr>
<tr>
<td><em>S. indicum</em> cv. TMV 6</td>
<td>210</td>
<td>17</td>
<td>8.1*</td>
</tr>
<tr>
<td><em>S. alatum</em> × <em>S. indicum</em> cv. SVPR 1</td>
<td>150</td>
<td>18</td>
<td>12.0b</td>
</tr>
<tr>
<td><em>S. alatum</em> × <em>S. indicum</em> cv. CO 1</td>
<td>140</td>
<td>14</td>
<td>10.0º</td>
</tr>
<tr>
<td><em>S. alatum</em> × <em>S. indicum</em> cv. TMV 3</td>
<td>155</td>
<td>15</td>
<td>9.7cd</td>
</tr>
<tr>
<td><em>S. alatum</em> × <em>S. indicum</em> cv. TMV 6</td>
<td>140</td>
<td>13</td>
<td>9.3º</td>
</tr>
</tbody>
</table>

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Fig. 2. Molecular identification of interspecific hybrids of sesame with isozyme markers. Banding patterns of peroxidase (a) and esterase (b) from leaf tissues of *S. alatum* (lane 1), *S. indicum* (lane 2) and *S. alatum* × *S. indicum* hybrids (lanes 3 and 4). Arrows indicate specific bands distinguishing the hybrid plants.
3.4. Screening of parents and the hybrids for phyllody disease

As one of the prime goals of the present study, the parents *S. alatum* and *S. indicum* and the hybrids were evaluated for their response against phyllody disease under controlled conditions. The results revealed that the disease incidence was less than 5% or even negligible in the resistant parent *S. alatum* and was within a range of 60–70% in the susceptible parent *S. indicum*. These findings are in accordance with the previous reports by Srivinasulu and Narayanawamy (1992) and Singh et al. (2007). The interspecific hybrids were found to be moderately resistant as they exhibited an infection percentage ranging from 10 to 20% with a disease scale of 3 (data not shown).

Disease and pest resistance in plants is multifaceted, involving structural and chemical components that are produced in response to pathogen infection. A survey of literature indicated that increased activity of many plant metabolizing enzymes like chitinase, peroxidase, glucanase, polyphenol oxidase, phenylalanine ammonia-lyase, as a result of pathogen attack, is usually implicated in the disease resistance of many crops (Stahmann et al., 1966; Gawande and Sharma, 2003; YongLan and Zhong, 2003; Katoch et al., 2004). In this study, we chose to analyze the activities of the enzymes phenylalanine ammonia-lyase (PAL), peroxidase, polyphenol oxidase (PPO) and phenolics in the phyllody resistant parent (*S. alatum*) and susceptible parent (*S. indicum*) to establish the relationship between the enzyme activity and disease resistance. We used infected and healthy plants of *S. indicum* as positive and negative control, respectively in the assay in order to ensure that variation for enzyme activity actually exists between them. Furthermore, to avoid the effects of experimental variation on the results, all the plants were grown under identical conditions and the plant samples were collected and assayed at the same time.

ANOVA revealed a significant effect of different enzyme activities studied (Table 3). The results indicated enhanced activity for *S. alatum* irrespective of enzymes analyzed. As expected, the enzyme activities differed significantly between healthy and infected plant samples of the susceptible parent *S. indicum* and the healthy plants showed more enzyme activity than the infected plants (Table 3). Based on these results, we have characterized the hybrids for the level of resistance to phyllody disease and found that the activity was intermediate between resistant and susceptible parent for all the three enzymes assayed. The phenolics content in the hybrids was also intermediate to those of parents (Table 3). However, further studies with more number of hybrid combinations should be carried out to conclusively establish a direct relationship between the increased level of enzyme activity and disease resistance.

Importantly, the above quantitative measurement of enzyme reaction in parents and hybrids also corresponded well with the disease response obtained through artificial screening studies. PAL is a key enzyme of the phenyl propanoid pathway and is associated with the production of some antifungal phytoalexins, lignins and specific compounds that have been proven to significantly contribute disease resistance in plants (Vidhyasekaran, 1998). Increase in peroxidase activity had been correlated with disease resistance in a number of crop species including barley, cucurbits, cotton and rice (Young et al., 1995; Dalisay and Kuc, 1995).

In conclusion, the present study has demonstrated the successful recovery of interspecific hybrids between *S. alatum* and *S. indicum* by employing an optimized embryo rescue protocol. The phytohormone regime and age of the ovule were the crucial factors for the germination of ovules into complete plantlets. The authenticity of the interspecific hybrids was confirmed through detailed morphological, cytological and isozyme studies. Disease screening and enzyme activity assays revealed moderate level of resistance in the hybrid for phyllody disease. Thus, the present study extends the scope for producing interspecific hybrids, which is limited by post-zygotic barriers, possessing economically important characters transferred from wild species of sesame through ovule culture techniques.

**Acknowledgements**

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<table>
<thead>
<tr>
<th>Table 3</th>
<th>Enzyme activity and phenolic content in leaf samples of parents and hybrid of sesame.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species</strong></td>
<td><strong>Phenylalanine ammonia-lyase (nmol/min/g)</strong></td>
</tr>
<tr>
<td><em>S. alatum</em></td>
<td>0.839&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. indicum</em> cv. TMV 3 (Healthy)</td>
<td>0.526&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. indicum</em> cv. TMV 3 (Phytophthora infected)</td>
<td>0.469&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>S. alatum</em> × <em>S. indicum</em> hybrid</td>
<td>0.621&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE</td>
<td>0.09</td>
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</table>

**ANOVA summary table**

<table>
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<th>Sources of variation</th>
<th>df</th>
<th>MS</th>
<th>F</th>
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<tbody>
<tr>
<td>Treatments</td>
<td>3</td>
<td>1,279,542.588</td>
<td>162.93 *</td>
</tr>
<tr>
<td>Error</td>
<td>12</td>
<td>7853.052</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>1,287,395.64</td>
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</tr>
</tbody>
</table>

SE = Standard error. Each value represents the mean of five replicates (n = 5). Means followed by the same letters are not significantly different at a 5% probability level by DMRT.

* * Significant at 1% level.
References


Mallikarjuna, N., 2002. Gene introgression from<br>


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