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Research Article

Induced Vivipary in *Sesamum indicum* L. By Seed Borne Infection of *Phytophthora parasitica* var. *sesame*

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ABSTRACT

The seed samples of white seeded sesame collected from Jaipur, Rajasthan carried oospore and chlamydospores of *Phytophthora parasitica* var. *sesame*. Such seeds on sowing in earthen pots produced infected plants with characteristic symptoms of blight disease. Plants nearing maturity induced morphological abnormalities in green pods. The pods while green and immature get splitted lengthwise due to emergence of few seedlings from the pods. The pathogen induced emergence of the radicle, hypocotyls and cotyledons through the seed coat within the pod. Such viviparous condition occurred in 25-48.8% pods and 27.08-36.12% seeds. The viviparous pods were characterized by internal browning of pedicel, septum and placenta. The seeds carried white cottony growth of *P. parasitica* var. *sesame*. Such viviparous condition was not visible in pods with normal looking seeds. Vivipary in our case might be due to fungal stimulation. Similar work can be carried out in this and other plant species to further strengthen the proposed correlation between vivipary and fungal infection. Presence of pathogen in different parts of pods and seedlings were established by incubation and cleared preparation. In immature developing pods hyphae were observed in tissues of pericarp, placenta, locules and ovules.

Key Words: Induced Vivipary, *Sesamum indicum* L. Seed Borne, *Phytophthora parasitica* var. *sesame*

INTRODUCTION

Vivipary is a kind of vegetative reproduction in some plants, including grasses (Stebbins, 1950) and can also be induced artificially by altering phytohormones balance in plant not known to show vivipary in nature. During present investigation, besides causing blight symptoms, *Phytophthora parasitica* var. *sesame* has been found to induce vivipary in seeds of sesame contained in green pods. In grasses the plantlets produced by viviparous species are viable and have many advantages over seeds for reproduction under certain etiological conditions (Langham, 2008). But in the present study of the crop plant (sesame) it is an unusual phenomenon, increasing rapid seed infection and poor seed quality. *Phytopthora parasitica* var. *sesame* causing blight of sesame is predominantly soil-borne pathogen which over-winters through chalamydospores of the pathogen as seed contaminants (Dubey and Singh, 1999).

MATERIALS AND METHODS

The seeds of white sesame sample (accession number 17 and 118) of Churu district, carrying 10,000-22,000 chalamydospore and oospore (per gram seed) of *Phytophthora parasitica* var. *sesame*, were sown in earthen pots of 12 cm diameter (10 seeds/pot) and thinned to make 4-5 seedlings/pot. The pots were kept under natural field condition during September to December. The pots were watered at regular interval and observations were taken on seed germination and various phytopathological parameters. Morphological observations were made by naked eye and under steriobinocular microscope (SMZ-10, 10X-40X). For recovery of pathogen the affected seed and seedlings were incubated on potato dextrose agar and corn meal agar media. To confirm the presence of pathogen in plants showing vivipary in which seedling emerges out from partially dehisced capsules, various parts of pod, seed and seedlings were cleared by boiling in 10% KOH for 5-20 min. (time depending on the softening of the tissue), washed thoroughly in warm water and again boiled in lactophenol + cotton blue (5:2 v/v), for 3-5 min. the stained softened plant part was kept on slide under coverslip in a drop of polyvinyl alcohol and pressed gently to spread the tissues uniformly. The slides were dried by keeping in an oven at 60^{O}C for 48h and observed under bright field microscope.

RESULTS AND DISCUSSION

The plants raised in earthen pots were critically observed for morphological and histological alterations. The infected plants showing vivipary showed length wise splitting of green and immature pods and 1-4 seedling per pod emerged, their cotyledon protruded out of the pod after rupturing their seed coat (Fig. 1). Observation recorded on number of plants studied, number of pod/plant, incidence of vivipary and number of seed/pod are summarized in Table 1. The viviparous pod/plant and

viviparous seed/pod varied from 25- 48.88% and 27.08-34.34%. In such pods the number of seed/pod was as low as compared to healthy pods (38.2- 50.8%). The viviparous pods carried white cottony growth with sporangia of *Phytopthora parasitica* var. *sesame*.

Such pods when split open longitudinally, showed internal browning of pedicel, septum, placenta and seeds. The seeds were either normal looking or shriveled and closely adhered due to hyphal growth.

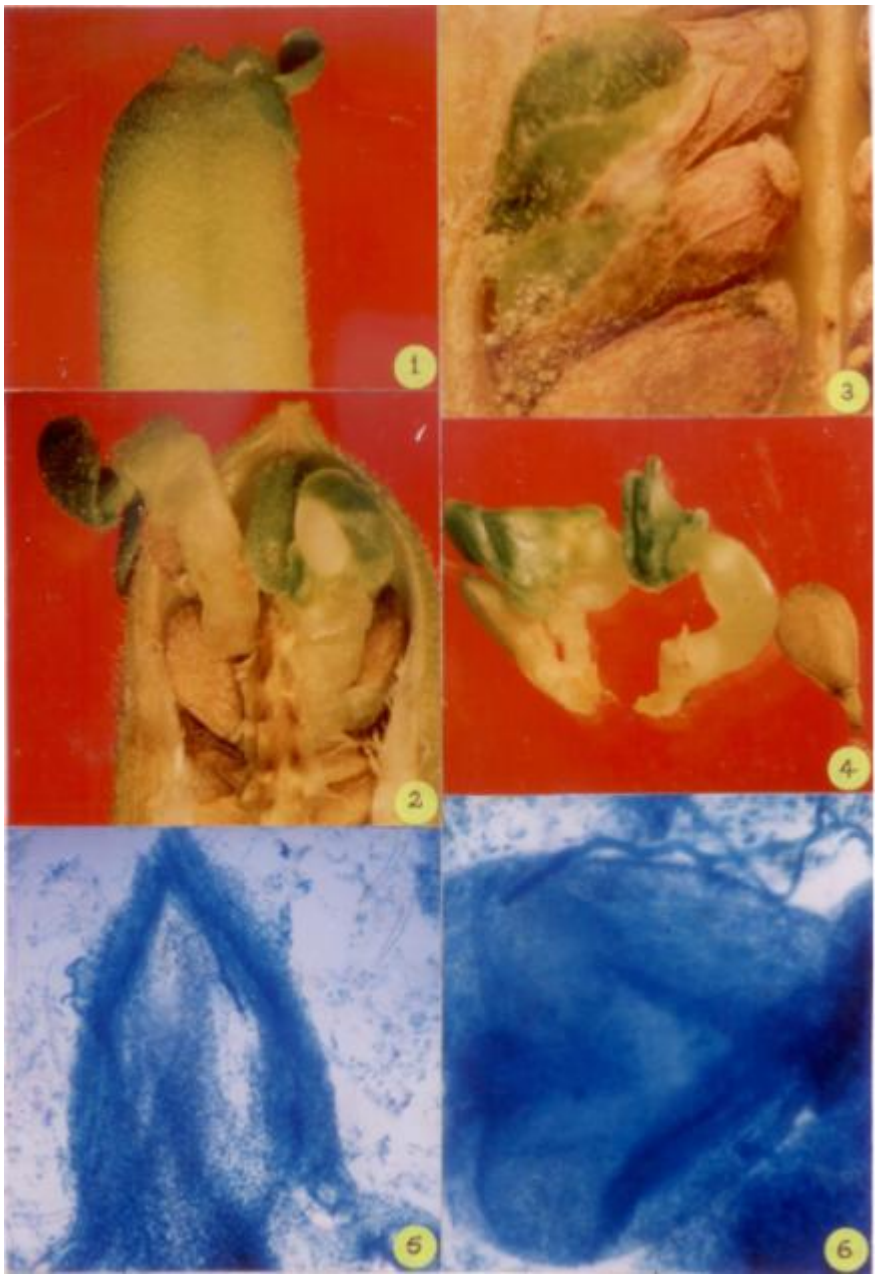

Figure 1. Infection of *Phytophthora parasitica* var. *sesame* inducing vivipary in sesame. 1 A young pod of sesame showing emergence of cotyledonds X35; **2** Splitted pod showing ruptured seed coat and emergence of green cotyledons, while seeds are attached to placenta X 62; **3** Splitted pod with germinated seeds. Note seedlings with green cotyledons and swollen hypocotyl X35; **4** Different categories of viviparous seedlings showing radicle emergence with browning of tip (right), green cotyledon with swollenstunted hypocotyl and rudimentary radicle X27; **5** Cleared tissue of young fruit showing hyphae in the fruit wall (pericarp) and wall of the carpel X35; and **6** An immature ovule with hyphae in ovuliferous tissues X700.

Table 1. Occurrence of vivipary in sesame plants induced by infection of *Phytophthora parasitica* var. *sesame*

No. of plant/pot	No. of plant showing vivipary	No. of pods per plant	Average percent of normal pod/plant	Average percent of vivipary pod/plant	Average seed/pod	Average and percent seed/pod showing morphological changes
4	2	45	23	22(48.88)	46.0	15.8(34.34)
3	2	51	36	15(29.41)	49.0	16.6(33.87)
4	3	62	35	27(43.54)	47.6	15.4(32.35)
2	2	42	30	12(28.57)	38.2	13.8(36.12)
1	1	21	12	9(42.85)	44.8	14.6(32.58)
3	3	57	38	19(33.33)	48.0	13.0(27.08)
4	3	63	38	25(39.69)	50.8	16.0(31.49)
2	1	17	12	5(29.41)	44.4	14.0(31.53)
3	2	56	41	15(26.78)	39.0	13.0(33.33)
4	2	68	51	17(25)	44.0	14.0(31.81)

Note: *In the present investigation viviparous condition occurred in 25-48.8% pods and 27.08-36.12% seeds due to the infection of Phytophthora parasitica var. sesami that leads to browning of various region of pod. The presence of pathogen was confirmed by cleared whole mount preparation of the tissue showing hyphal ramification.*

The pods showed partial to complete vivipary. Partial vivipary was seen in seed of the lower part of capsule in which only seed coat was ruptured from one side due to enlargement of the cotyledons (Fig. 2). In case of complete vivipary emergence of both radicle and hypocotyls through the seed coat were observed (Fig. 3). The nongerminated seeds either were normal looking or showed abnormalities like ruptured seed coat, necrotic spot and white hyphal growth. Among the germinated viviparous seedlings three categories could be made (i) seed with only radicle emergence (ii) seed with cotyledon emergence (iii) and both radicle and cotyledon emergence.

The radicle was thin showing brown to black necrotic areas. Hypocotyls in most of the seedling were stunted, swollen and fleshy. Cotyledons were green with brown to black lesions and partially opened. Softening of cotyledonary tissues was usually observed in such seedlings. The seedlings did not survive when transferred to soil and the seeds obtained from the viviparous pods had poor germination.

Different components of the viviparous pod, seed and seedlings were cleared to study the presence of pathogen. Characteristic branched, coenocytic, non-septate, thin to broad mycelium with granular cytoplasm Dubey and Singh 1999, Dastur, 1913, chlamydospores and oospores of *Phytopthora parasitica* var. *sesame* were frequently observed in the tissues of pedicel, pericarp, septum and placenta. Immature developing pods revealed hyphae in tissues of pericarp, placenta, and locules (Fig. 5) and also in ovuliferous tissues (Fig. 6). In seed the mycelium and chlamydospores were observed in seed coat and endosperm tissues. In seedlings the pathogen ramified in all the parts, the radicle, hypocotyls and cotyledons. Fungal structures were commonly associated with their vascular elements. The hyphae frequently spread along the middle lamellae and intercellular spaces. Intracellular hyphae were also seen in parenchymatous tissues and vascular elements of radicle and hypocotyl. No haustoria could be seen. Chlamydospore formation was more frequent than oospore. The former was spherical, thick smooth walled, yellow in colour and develops from vegetative hyphae terminally or intercalary. Whereas the oospores were having outer thick and rough and inner thin wall containing homogeneous mass of granular protoplasm with a few nuclei and oil globules.

Vivipary, a form of vegetative reproduction where bulbils and plantlets are formed within the inflorescence and replace the flowers, is found in plants of many families such as *Allium*, *Festuca*, *Poa*, *Polygonum*, *Saxifraga* (Stebbins, 1950) it can also be artificially be induced by altering the phytohormone balance in plants.

Plantlets or bulbils produced by viviparous species are viable and may have considerable advantages over seeds for reproduction under certain etiological conditions. According to Sampson and Western (1959), the *Poa bulbosa* L., a viviparous grass, when infected by the fungus *Epichloe typhina* (Pers.) Tul also shows infection in its bulbils. During present investigation the sesame plants raised in earthen pots from naturally infected seeds

with *P. parasitica* were allowed to grow up to maturity for disease transmission and carry over pathogen. Besides causing blight symptoms, the pathogen *Phytophthora parasitica* var. *sesame* was found to be associated with vivipary in immature seeds of sesame contained in green pods of plants raised from naturally infected seeds. It is an unusual phenomenon which besides increasing the seed infection also rendered poor quality seeds.

In infected plants the pods while green got splitted due to emergence of seedlings. The presence of white cottony growth of *P. parasitica* on viviparous pods and internal browning of pedicel, septum, placenta and seeds were suggestive of the possible pathogen inducing vivipary in sesame. Such viviparous condition was not visible in plants with normal looking (uninfected) seeds. In addition to these, the seeds of such pods were either normal looking or shriveled and carried hyphal growth. The pathogen also induced abnormalities in non germinated seeds of viviparous pods. But the phenomena of vivipary and pseudovivipary have been reported in 21 genera from subfamilies of Poaceae, such as Pooideae, Panicoideae and Chloridoideae. A previously overlooked description of pseudovivipary in *Digitaria angolensis* is confirmed and constitutes the first record of pseudovivipary in the genus *Digitaria*. This species is illustrated for the first time and the proliferation phenomenon in the spikelets is described and documented (Vega *et al.*, 2006).

The host and parasite interaction results in abnormal seedling emergence which lacked vigor and further survival. According to Farnsworth (2000) the differences in the production and function of plant hormones are implicated in the occurrence of recalcitrance and vivipary in plant families. Plant hormones are key regulators of seed physiology and simultaneously coordinate responses of the seedling and mature plant to their environment. Infection of *Phytophthora parasitica* var. *sesame* induced vivipary was somehow positively correlated by cleared wholemount preparation of different parts of viviparous pod, seed and seedlings which showed presence of characteristic, mycelium, chlamydospores and oospores of *P. parasitica*. The vivipary in sesame by *Phytophthora parasitica* var. *sesame* is highly disadvantageous since the pathogens infects the plant as well as pods and also reduce the seed quality by early maturation and adversely affect the yield (seed/pod). Also the rupturing of the seed coat and the emergence of seedlings provide suitable means for the spread of the pathogen which further contaminate the seed of the same pod as well as other pods and plants. In plant tissue culture fungal cell extract is being used to stimulate production of secondary metabolites. Using a bioelicitor the secondary metabolite production can be increased.

As per the study conducted by Devi and Srinivasan (2011) gymnemic acids have become a valuable drug in diabetes treatment due to their potent antidiabetic activity. These compounds are extracted commercially from *Gymnema sylvestre*. Since the intact plant contains low concentrations of active compound, therefore *Aspergillus niger* cell extract was used as an elicitor to stimulate the production of secondary metabolite and 9 fold increase of gymnemic acid yield was obtained. Vivipary in our case might be due to fungal stimulation. Similar work can be carried out in this and other plant species to further strengthen the proposed correlation between vivipary and fungal infection.

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