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COMPARATIVE DEVELOPMENTAL STUDIES OF TWO FUNGI FORMING VESICULAR ARBUSCULAR ASSOCIATION WITH GINGER AND BLACK PEPPER[1]

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ABSTRACT

Two VA mycorrhizal fungi viz: *Glomus versiforme,* an endophyte associated with black pepper, and *G. multicaule,* associated with ginger were inoculated in sorghum and in their respective native hosts to study and compare development in these hosts. In case of *G. versiforme* inoculated in black pepper and sorghum, hyphae, arbuscules and vesicles were produced on both hosts. However, there was delay in the appearance of vesicles in the case of sorghum. Developmental studies of *G. multicaule* in sorghum and ginger showed that the time of appearance of arbuscules and vesicles were similar in both hosts. However, the unusual appearance of vesicles in the stelar region was confined to ginger only.

INTRODUCTION

Comparative developmental studies of VA mycorrhizae colonizing ginger, black pepper and a convenient test host viz. sorghum was undertaken. Black pepper, (*Piper nigrum* L.) the spice crop and host of *Glomus versiforme* Danels and Tappe (Berch and Fortin, 1983) does not produce as many roots as *Sorghum bicolor,* L. the test host, within a short time. Raising pepper cuttings of uniform growth in sufficient numbers is more labour intensive, time consuming and expensive when compared to that of raising sorghum. In the case of ginger, the host for *G. multicaule* Gerd. and Bakshi (Gerdemann and Bakshi, 1976), the occurrence of rhizomes, the delayed germination, slower rate of root production are all to be taken into account while accepting it as a test system. Moreover, the rate of biomass production which is an indicator of growth, is slower in pepper and ginger in comparison to sorghum which forms a convenient crop for studying VAM fungi. It is necessary to have an idea of the similarities and differences this system has, with that where the native host is employed. With this point in view, studies on the developmental cycle of the VA endophytes in these different systems were taken up.

MATERIALS AND METHODS

Soil from the root zone of ginger and black pepper from the experimental fields in CPCRI, Kasaragod was collected from the top 25 cm. depth. VA mycorrhizal spores were collected using wet sieving and decanting method (Gerdemann and Nicolson 1963). The most frequented species namely *Glomus versiforme* from pepper and *G. multicaule* (Gerdemann and Bakshi, 1976) from ginger were isolated to be used for further multiplication by funnel technique (Menge and Timmer, 1982) and later in pots using sorghum.

Soil fumigated with methyl bromide was treated with 5 per cent formalin at the rate of 1 litre/10kg soil. After 72h this was uncovered and the soil spread thinly on tarpaulin to make it air-dry. After the lapse of one week's time this soil was used for the study. Thirty centimeter pots were filled with 10kg sterilized soil and were used for pot culture studies with sorghum, pepper and ginger.

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Developmental studies of G. versiforme in sorghum

Five hundred g of soil (from sorghum root zone) containing 100 chlamydospores of *G.versiforme* was added to each pot (30 cm) containing fumigated soil. Each pot was seeded with 100 seeds of sorghum. Root samples were taken on the second, fourth, 6th, 8th, 17th, 24th, 39th, 46th and 53rd days after inoculation for microscopic study.

Developmental studies of G. versiforme in black pepper

Hanging runner vines of black pepper *(Piper nigrum* L., Karimunda variety) were kept coiled around stakes at the base of the plant in December-January. Two to four noded cuttings were made and their bases were dipped in Seradix-B (May and Baker). These were planted in sterilized (by autoclaving) potting mixture in polythene bags of size 20 x 15 cm. They were watered and kept under shade to promote rooting. When sufficiently rooted, these were transferred to 30 cm pots containing treated potting mixture. The pots were irrigated with Hoagland's solution (25%) initially for the first two weeks on alternate days. Subsequently, regular fertilizers were added @ 3 g urea, 1.25g superphosphate and 0.5 g muriate of potash/ pot.

Inoculation of black pepper was done by adding 500g of soil which contained 100 chlamydospores of the VAM fungus. Root samples were taken on second, fourth, 6th, 8th, 10th, 11th, 13th, 14th, 21st, 28th, 35th, 49th, 56th, 70th, 77th and 84th days after inoculation. The samples were processed, stained and examined according to Phillips and Hayman, 1970).

Development of G. multicaule in sorghum

To every pot containing 10 kg fumigated soil, 200 chlamydospores (500g soil inoculum) of *G. multicaule* was added. This was seeded with 100 grains of sorghum and irrigated with Hoagland's solution (25%) once a week.

Root samples were taken on the second, fourth, sixth 8th, 24th, 31st, 46th and 53rd days after inoculation to study the development of *G. multicaule* in sorghum.

Development of G. multicaule in ginger

Seed rhizomes of ginger (*Zingiber officinale* Rosc.) variety Maran, treated with Dithane M-45 (Mancozeb) 0.3% and Quinalphos (Ekalux) 0.005% stored in sand-lined pits of $1\frac{1}{2}$ ft. depth, were taken out, washed thoroughly to remove adhering pesticides, and were drained and dried. Hundred g bits of this material possessing 2 or 3 sound 'eyes' were planted in each pot containing 10 kg sterilized soil. These were kept mulched with dry leaves till sprouts appeared. After sprouting, these were watered with 25% Hoagland's solution on alternate days for the first two weeks. Later, since rainy season had set in, irrigation was stopped and fertilizers were added @ 3g urea, 1.25 g superphosphate and 0.5 g muriate of potash/pot.

Cultures of *G. multicaule* which were raised on sorghum were inoculated @ 200 spores/pot while planting the seed bits. Root samples were taken on the 31st, 39th, 53rd, 67th and 74th days after VAM inoculation. The root samples taken from sorghum as well as ginger were processed, stained and examined as per Phillips and Hayman (1970).

Observations

Incidence was indicated by the positive presence of the fungi in the roots.

Extent of infection (infection grading) was calculated based on the length of the root traversed by the VAM fungus (Giovanetti and Mosse, 1980).

RESULTS AND DISCUSSION

Development of G. versiforme in sorghum

Hyphae and appresorium-like structures were seen only on the 6th day after inoculation. Subsequently, arbuscules were

observed on the 17th day and vesicles on the 39th day respectively.

The percentage incidence rose upto 100 by the 17th day and maintained this status fairly well throughout. Though the infection grading was very low on the 6th day, it gradually increased and reached the highest peak of 72.5% on the 24th day after which it slowly declined to a level of 55% on the 46th day. From this time onwards, empty vesicles were also seen along with fertile ones (Fig. 1).

Development of G. versiforme in black pepper

Infection appeared in the roots of pepper on the 6th day after inoculation. Arbuscules and vesicles were observed on the 11th day after inoculation (Fig. 2 & 3). Maximum number of vesicles were observed on the 35th day, after which it decreased reaching zero level by the 84th day. Infection grading registered two peaks at 66% and 54% on the 13th and 49th days respectively as in the case of percentage incidence (100% on both days).

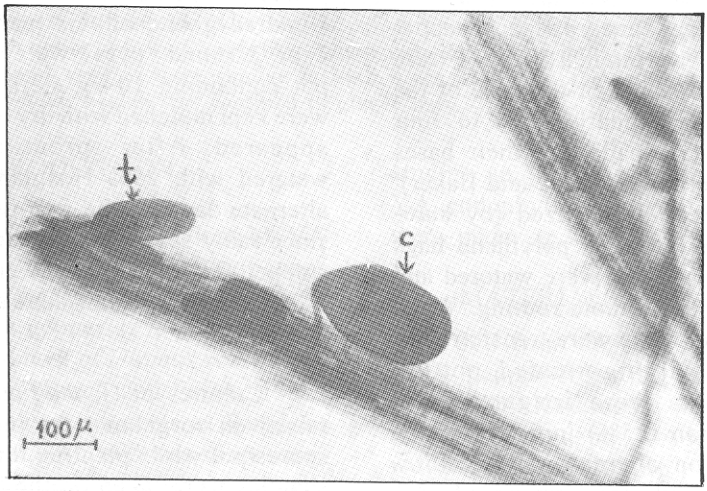

Fig. 1. Development of *G.versiforme* in sorghum-empty (C) & fertile (t) vesicles.

Table I. *G. versiforme* ***in sorghum: Development of the VAM endophyte***

Days after inoculation of *G. versiforme*	% incidence*	Infection grading (%)	Remarks
2	0	0	–
4	0	0	–
6	20	2.5	Hyphae and appresorium-like structure
8	40	4	Hyphae and appresorium-like structures
17	100	69	Arbuscules
24	100	72.5	Arbuscules
39	80	34	Arbuscules
46	100	55	Vesicle/spore formation
53	100	55	Vesicle and spores light brown coloured, empty vesicles.

* % incidence = No. of root pieces showing VAM / Total no. of roots examined × 100

Table II. *G. versiforme* ***in black pepper: Development of the VAM endophyte***

Days after inoculation of *G. versiforme*	% incidence	Infection grading (%)	Remarks
2	0	0	–
4	0	0	–
6	20	2	Hypha seen
8	40	18	Hypha seen
10	40	6	Hypha seen
11	90	24.5	Arbuscules and vesicles (initials) Vesicles growing in size
13	100	66	Vesicle number increasing
14	90	28	Vesicle number increasing
21	90	11.5	Vesicle number increasing
28	80	10	Vesicle number increasing
35	70	34	Maximum number of vesicles
49	100	54	Vesicle number increasing
56	80	21	Vesicle number decreasing
70	50	19	Vesicle number decreasing
77	80	24	Vesicle not found
84	20	6	Vesicle not found

Development of *G. multicaule* ***in sorghum***

VAM fungal hyphae were seen in the fixed root samples on the sixth day after inoculation. There was only very low percentage of incidence (10%), while from the 8th day onwards appresorium-like structures could be seen. Vesicles could not be observed eventhough distinct spores could be seen from the 46th day onwards. The data are presented in Table III.

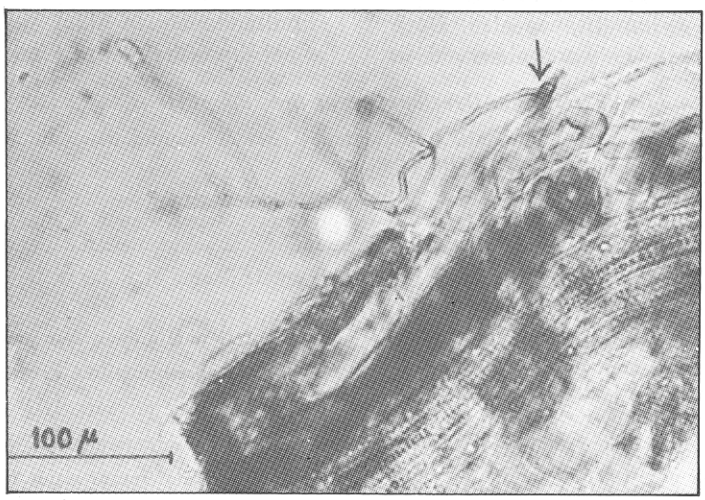

Fig. 2. Development of *G.versiforme* in black pepper-entry point and arbuscules.

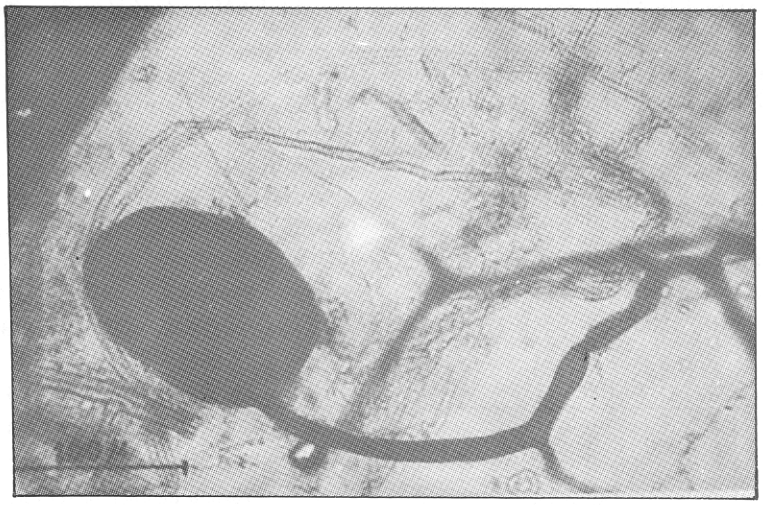

Fig. 3. ***G.versiforme*** **in black pepper–development of vesicles**

Development of *G. multicaule* ***in ginger.***

Root samples of inoculated ginger were taken only from the 31st day onwards, since root production was observed only on the 24th day after sowing. Though only hyphal growth was observed on the 31st day, arbuscules and vesicles were seen along with hyphae on the 39th day (Table IV). Vesicles of two types, rectangular and oval were observed (Fig. 4). An interesting feature noticed was the growth of the hyphae through the xylem vessels. Though arbuscules were visible only in the cortex, vesicles were visible inside the xylem tissues also. Both large and small vesicles were observed in the cortex on the 53rd day after inoculation. Vesicles were seen in the xylem too. On the 67th day, many vesicles appeared to be empty and took up only very light stain. On the 74th day the number of empty vesicles were found to increase in proportion.

In both hosts, namely sorghum and black pepper, initials of *G. versiforme* infection were seen on the sixth day. Formation of arbuscules were distinctly earlier in black pepper when compared to that in sorghum. Though vesicles were seen simultaneously in black pepper, in the case of sorghum they were seen only on the 53rd

Table III. *G. multicaulae* ***in sorghum-Development of the endophyte***

Days after inoculation	% incidence	Infection grading %	Remarks
2	0	0	–
4	0	0	–
6	10	10	Hyphal growth seen inside the root
8	10	2	Appresorium-like structure present
24	100	26	Hyphae present
31	100	41	Hyphae present
46	100	28	Vesicles/spores inside the root
53	88.8	2.33	Vesicles/spores inside the root

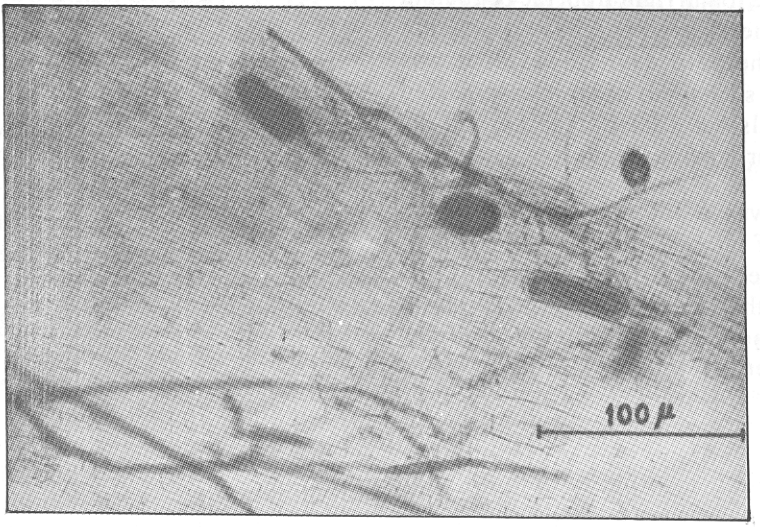

Fig. 4. Development of *G.versiforme* in ginger-rectangular and oval vesicles

day. Even though the structures produced were similar, there was dissimilarity in the periodicity of their occurrence.

Initial stages of penetration and mycelial development of *G. multicaule* was first observed on the sixth day after sowing (as well as inoculation) in sorghum and on the thirty-first day after sowing (as well as inoculation) in ginger. This time lag in the case of ginger is due to the delayed start of root formation (twenty fourth day after sowing).

However, arbuscules were produced after an interval of six days in both hosts ie., 12th day after root initiation in sorghum and 15th day in ginger. Vesicle production was simultaneous in the case of ginger, whereas in the case of sorghum it was delayed upto the 21st day. An interesting feature noticed was the presence of vesicles even in the xylem vessels of ginger. Taber and Trappe (1982) also noticed such an unusual phenomenon in ginger infected with *Glomus* sp. Lysis of vesicles was seen on the 53rd day after sowing in sorghum and on the 43rd day after root initiation in ginger. In ginger, two types of vesicles, namely rectangular

Table IV. *G. multicaulae* ***in ginger: Development of the endophyte***

Days after inoculation	% incidence	Infection grading %	Remarks
31	20	2	Hyphal growth
39	60	19	Hyphae growing through xylem vessels. Arbuscules observed in cortical cells. Vesicles (Oval and rectangular) seen. Vesicles visible inside xylem also.
53	77.7	31.1	Large and small vesicles observed in cortex. Vesicles seen in xylem
67	40	72	Poorly stained empty vesicles noticed
74	30	9	Empty vesicles increase in number

and oval, were found right from the beginning whereas in the case of sorghum it was not noticed till the 53rd day. However, it was observed that such types of vesicles appeared in sorghum also at a later stage i.e., on the 89th day after sowing.

There was a lot of similarity in the production of internal structures by *G. multicaule* in the two different hosts. At the same time, the exceptional production of vesicles in the stellar region was confined only to ginger.

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