**Table 1** *Mean per cent VAM colonization and spores on roots, and phosphorus content, dry weight of shoot and yield of gamma ray-induced micromutants of greengram*

| Micromutant/ parent | External root colonization | | | Internal root colonization | | | Average number of spores/vesicles per cm of root | | | P content at anthesis (%) | Dry wt of shoot at maturity (g) | Yield per plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30* | 45* | 60* | 30 | 45 | 60 | 30 | 45 | 60 | | | |
| LGG 403 | 30.75 | 63.50 | 90.50 | 42.50 | 67.00 | 93.75 | 41.25 | 125.75 | 459.50 | 0.45 | 24.51 | 9.63 |
| LGG 405 | 19.75 | 51.25 | 67.50 | 30.00 | 50.00 | 73.75 | 20.25 | 70.25 | 215.75 | 0.42 | 19.45 | 8.45 |
| LGG 407 | 25.00 | 38.25 | 65.00 | 23.75 | 47.50 | 68.25 | 13.25 | 39.50 | 115.50 | 0.39 | 21.03 | 8.58 |
| LGG 410 | 23.75 | 63.25 | 83.75 | 38.50 | 63.25 | 83.00 | 25.50 | 87.75 | 228.00 | 0.44 | 25.70 | 10.18 |
| LGG 127 ($P_1$) | 12.50 | 22.50 | 47.50 | 14.75 | 30.00 | 54.25 | 8.25 | 15.25 | 65.00 | 0.34 | 16.42 | 6.20 |
| ML 26–10–3 ($P_2$) | 15.75 | 36.75 | 50.00 | 20.50 | 45.00 | 61.50 | 16.00 | 23.75 | 81.00 | 0.36 | 19.00 | 6.85 |
| SE of mean | 1.11 | 1.40 | 2.00 | 1.84 | 1.43 | 1.05 | 1.41 | 1.63 | 2.73 | 0.004 | 0.78 | 0.16 |
| CD at 5% | 2.35 | 2.99 | 4.26 | 3.92 | 3.05 | 2.23 | 3.00 | 3.47 | 5.82 | 0.01 | 1.66 | 0.34 |

*Days after sowing.

Seeds were sown in a randomized block design with four replicates in the rainy season (August 1986). Roots were sampled at 15-day intervals up to 60 days (harvest). The roots of each plant were dug out carefully, washed gently, and cut to one cm segments. These were cleared and stained by the method of Phillips and Hayman[4] and 20 segments were examined for each replication. External and internal colonization by VAM fungi and presence of vesicles/spores were recorded. Phosphorus content of the plant was analysed at the time of flowering by the standard method[5]. The dry weight of the plant was recorded at the time of harvest. All the observations were made on five randomly selected plants of each replication and the pooled data were subjected to ANOVA test.

There is significant variation in the mycorrhizal colonization among the six genotypes of greengram by the VAM fungi indigenous to the botanical garden soil. The mean mycorrhizal colonization was highest in LGG 403 (90%), followed by LGG 410, LGG 405, LGG 407, ML–26–10–3 and LGG 127 in decreasing order. The progeny showed more colonization than either of the parents (table 1).

There was no VAM colonization in the first sampling (15 days after sowing) but after 30 days all the features of colonization such as entry points, external hyphae with vesicles, internal mycelium with arbuscules and vesicles were observed. The amount of external mycelium and the number of vesicles/spores were also greater in 403 than in others. Most of the spores observed belong to the species of *Glomus* while a cluster of crenulate spores were also noticed in the root bits of LGG 410.

The variation in mycorrhizal colonization between the genotypes was clearly evident in all the samplings. Analysis of variance showed that phosphorus content of the six genotypes differed significantly. Dry weight of shoot and yield per plant were also higher in the micromutants than in the parents. There was a correlation between mycorrhizal colonization, phosphorus uptake and yield (table 1).

Thus in addition to the fact that mycorrhizal efficiency is largely under the influence of soil characters, other factors such as host plant species[2] or the genotype of the host[3] are also likely to influence VAM colonization.

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## ON THE PHYSIOLOGY OF FERN GAMETOPHYTE PRECEDING SEX ORGAN INITIATION

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THE gametophyte of ferns passes through several distinct morphological stages before the reproductive

phase, which is morphologically distinguished by the formation of sex organs, the antheridia usually appearing first. Ten to fifteen days prior to the formation of antheridia, growth of the cordate prothallus (gametophyte) almost ceases, signalling the achievement of vegetative maturity. The switch from vegetative to reproductive phase in higher plants has been correlated with a change in the nitrogen metabolism of the organ concerned[1]. In the present study an attempt has been made to understand the influence of the chemical form of inorganic nitrogen on the switch from vegetative to reproductive phase in the gametophyte of *Cyclosorus parasiticus*, a tropical homosporous fern, collected from the foothills of the eastern Himalayas. Assimilation of $NO_3^-$, the usual form of combined nitrogen available in nature, was also studied by assaying nitrate reductase (NR) at different developmental stages of the gametophyte growing on $NO_3^-$ medium. Collection of spores, their preservation, sterilization and sowing, growing of gametophytes, *in vivo* NR assay, etc. were carried out as described earlier[2].

Spores were germinated and grown on nitrogen-free, $NO_3^-$, $NH_4^+$ and ($NO_3^-$+$NH_4^+$) media to determine the optimum concentration of each of the nitrogen sources for the best growth of the gametophyte. In the next step, mature cordate prothalli about 15 days prior to formation of antheridia, growing on medium containing one form of nitrogen, were transferred to a medium containing the same or another form of nitrogen. After about a week the prothalli were again transferred to medium containing the same or different form of nitrogen. The number of days to formation of antheridia in the final medium was noted. NR activity at different developmental stages of the gametophyte was determined in gametophytes grown on $NO_3^-$ medium.

It is evident from the results in table 1 that the gametophyte of *C. parasiticus* is capable of assimilating $NO_3^-$ and $NH_4^+$ nitrogen at the same time; the prothalli grew much better on ($NO_3^-$+$NH_4^+$) media than on media containing either of the two N sources. This has been reported in higher plants[3,4] as well as in some cryptogams[5]. Antheridiogenesis was also accelerated in gametophytes growing on ($NO_3^-$+$NH_4^+$) medium. Since the threshold vegetative growth required for antheridiogenesis seems to be much less than the growth seen in ($NO_3^-$+$NH_4^+$) medium, as is shown by gametophytes grown on $NO_3^-$ medium, it is possible that the form of combined nitrogen present in the medium has some role in bringing about the observed acceleration of antheridiogenesis. The results of the transfer experiment (table 2) show that, irrespective of the form of inorganic nitrogen received by the prothallus up to maturity, acceleration of antheridiogenesis could be brought about if the gametophytes obtained the combination of $NO_3^-$ and $NH_4^+$ during the period between vegetative maturity and morphogenetic expression of the reproductive phase. This period is 10–15 days. Further experiments showed that the

**Table 1** *Vegetative growth and antheridiogenesis in Cyclosorus parasiticus gametophytes grown in media containing different nitrogen sources*

| | | | Size of prothallus (24 days old) | | | Days to sex organ initiation | |
|---|---|---|---|---|---|---|---|
| | N source | Optimum conc. (mg/ml) | Length (μm) | Breadth (μm) | Area ($\mu m^2 \times 10^4$) | Anth. | Arch. |
| $NO_3^-$(a) | $KNO_3$ + $Ca(NO_3)_2$ | 0.12 + 1.44 | 345.92 | 364 | 12.5 | 37 | 41 |
| $NO_3^-$(b) | $KNO_3^-$ | 2.88 | 370.00 | 508 | 18.8 | 37 | 41 |
| $NH_4^+$ | $NH_4Cl$ | 1.875 | 382.00 | 739 | 28.8 | 35 | 40 |
| $NO_3^-$(a) +$NH_4^+$ | $KNO_3$ + $Ca(NO_3)_2$ + $NH_4Cl$ | 0.12 + 1.44 + 1.875 | 727.00 | 1055 | 76.7 | 30 | 35 |
| None | — | — | 160.00 | 180 | 2.8 | — | — |

$NO_3^-$(a) is the usual nitrate present in standard A. F. Dyer's medium; $NO_3^-$(b) is the nitrate supplementation to A. F. Dyer's medium without the usual nitrogen source. In this case $Ca(NO_3)_2$ was replaced by $CaCl_2$.

**Table 2** *Effect of changing nitrogen source during growth of gametophyte of Cyclosorus parasiticus on antheridiogenesis*

| Initial N source | Intermediate N source (6 days duration) | Final N source | Days to sex organ initiation in final medium |
|---|---|---|---|
| | $NO_3^-+NH_4^+$ | $NO_3^-+NH_4^+$ | 3 |
| | $NO_3^-$ | $NO_3^-+NH_4^+$ | 3 |
| | $NH_4^+$ | $NO_3^-+NH_4^+$ | 6 |
| $NO_3^-+NH_4^+$ (optimum) | $NO_3^-$ | $NO_3^-$ | 8 |
| | $NH_4^+$ | $NH_4^+$ | 6 |
| | $NO_3^-$ | $NO_3^-$ | 8 |
| $NO_3^-$ (A.F.D. conc.) | $NH_4^+$ | $NO_3^-$ | 7 |
| | $NH_4^+$ | $NH_4^+$ | 6 |
| | $NO_3^-+NH_4^+$ | $NO_3^-+NH_4^+$ | 2 |
| | $NH_4^+$ | $NH_4^+$ | 6 |
| $NH_4^+$ (optimum) | $NO_3^-$ | $NO_3^-$ | 7 |
| | $NO_3^-$ | $NH_4^+$ | 6 |

combination of nitrogen compounds should be available throughout this period to bring about the acceleration of antheridiogenesis. Any interruption of supply of the combination during this period, substituting $NO_3^-$ or $NH_4^+$ alone for the combination, results in failure to accelerate antheridiogenesis. Interestingly this period coincides with the period in which there is a decline in $NO_3^-$ assimilation by the gametophyte during growth on $NO_3^-$ medium. This is shown in table 3. The gametophytes failed to form sex organs in nitrogen-free medium in spite of attaining the cordate stage. This could be because of the small size of the prothalli, never more than half of the normal ones growing on $NO_3^-$.

The observations indicate that the *C. parasiticus* gametophyte perhaps establishes within itself a subtle $NO_3^-/NH_4^+$ ratio during or prior to the switch from vegetative to reproductive phase, as a result of which the vegetative prothallus starts forming reproductive organs. Normally, in presence of $NO_3^-$ this is brought about by a decrease in NR activity. An external supply of $NH_4^+$ along with $NO_3^-$ increases the chances of establishment of the desired ratio (intracellularly), either by inhibition of NR[6] or by reduction of $NO_3^-$-induced NR synthesis[7]. This type of in-built mechanism to delay or hasten the advent of reproductive phase by 4–5 days may be of significance, because fern prothalli show abundant intergametophytic crossing *in vitro*[8,9]. It may be useful in nature also, where the prothalli grow in separate groups in the crevices of rocks in different microenvironments, awaiting rain for fertilization.

**Table 3** *In vivo nitrate reductase activity of Cyclosorus parasiticus gametophyte at different stages of growth*

| Age (days) | NR activity ($A_{543}$ per 100 mg fresh wt per h) |
|---|---|
| 14 (cordate) | 0.205 |
| 21 (cordate) | 0.120 |
| 24 (cordate) | 0.120 |
| 28 (cordate, antheridial stage) | 0.093 |
| 35 (cordate, hermaphrodite) | 0.077 |
| 50 (sporophyte) | 0.041 |

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