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REGENERATION OF DOWNY MILDEW RESISTANT PLANTS FROM INFECTED TISSUES OF PEARL MILLET (*PENNISETUM AMERICANUM*) CULTURED *IN VITRO*.

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DOWNY mildew of pearl millet caused by *Sclerospora graminicola* (Sacc.) Schroet.—an obligate pathogen, receives much importance in pearl millet cultivation. Much emphasis has been placed on the study of the biology and control of the disease[1], and work on the development of resistant lines to downy mildew through breeding techniques is being continued at the Downy Mildew Research Laboratory in the University of Mysore, India. Selection of resistant cell lines by exposing the host cells to toxins extracted from the concerned pathogens appears to be the most recent and advantageous technique[2-5]. Such selected cultures and even mutated cell lines well screened for resistance frustrate researchers by the lack of regenerability[3]. Exposing the plant cells directly to the pathogen attack, instead of exposing to toxin extract, and further screening for resistant cells has been a new and present approach. An experiment[14] conducted with the explants from mycoplasma-diseased brinjal plant indicated the possibility of obtaining disease-symptomless regenerants through tissue culture, but whether the regenerants were actually disease-free or carried latent infection was not confirmed. So far no attempt has been made to understand the nature of regenerants from obligate fungal pathogen infected tissue. The results obtained were novel and seem to indicate an important breakthrough in the field of plant science.

A genotype of pearl millet susceptible to downy mildew disease (HB_3) was used in the experiment. Diseased immature inflorescence explants (with phyllody—a symptom of disease) served as the source material. Inoculation and incubation were done as explained earlier[6]. The nutrient medium for the growth of the callus as well as the pathogen was standardized earlier[7]. Callus cells were screened regularly to ensure the presence of downy mildew mycelia. For this, a smear preparation with propionohematoxylin was used (1% hematoxylin in a mixture of 4.5 : 5.5 propionic acid : ethanol). Screening for disease resistance was done by sowing the seeds collected from regenerated plants in a sick plot between the rows of diseased plants (the latter 30 days old), because soil borne inoculum has been considered as the most important source of disease initiation[8-10], and also because root, coleoptile and leaves act as excellent sites for infection by zoospores[7] (also results obtained in our laboratory by Subramanya, 1982). The results were compared with control plants raised similarly from seeds collected from diseased plant groups from which the explants for *in vitro* culture were taken. Screening experiment was conducted in three different seasons, *i.e.* February-April, May-July and August-October of 1981.

Callus formation of soft coherent type and also of hard opaque nodular type was observed at least twenty days after inoculation. Younger the explant more was the formation of opaque nodular callus. The latter, upon transfer to IAA (3–5 ppm) supplemented MS medium[11] very rarely differentiated into shoots. However, when the mycelial mat was observed at the surface of the callus, such cultures produced only roots upon transfer to MS + IAA medium. Although some mycelial strands ramified from the callus on to the medium, the pathogen never grew on the medium alone. All the differentiating cultures did not succeed in completing the process. Hence some of the shoots, soon after initiation, degenerated. Some shoots, soon after the onset of differentiation, grew rapidly and produced roots on the same medium. The plantlets thus formed could be reared to maturity in pots. Only four plants could be obtained from infected callus tissue over a period of eighteen months. When dry, the seeds were harvested from the regenerated plants.

In the screening experiment conducted under field conditions in downy mildew sick plots, none of the plants in all the three seasons expressed the disease symptoms but the control plants did.

As indicated before, by exposing the callus tissues to pathogen extracts and the subsequent screening for viable and active tissue, disease resistance has been evaluated[2,4,5]. The mechanism of obtaining resistant cultures in such experiments could be based on the simple fact that the cells which are already endowed with resistance get selected during the treatment and hence remain viable. In the present experiment the pathogen present in the callus cultures itself serves as pathotoxin. As the plants so obtained could be reared to maturity and were always resistant, one can infer that in a mass of callus, certain cell groups or patches of tissues retain their ability to resist the disease which could be comparable to the absence of virus infection in the shoot apices of some species[12]. When once such cells were provided with factors which support them to morphogenise and develop into whole plants they naturally regenerate into resistant plants. The mechanism could be similar to that involved in the experiments conducted with pathotoxin supplementation and further aquisition of resistant cell lines. The present method has an advantage over earlier ones, because, in the latter, due to repeated subcultures, although resistant cell lines get selected, the cultures may fail to regenerate into whole plants[3,13]. The achievement in the present experiment is a step ahead of the earlier ones as it evades many complicated steps like induction of mutant cell lines, exposures to stress conditions like freezing and toxins separately and further selection. Extracts from pathogens, although generally toxic, may lack some of the active principles which the live pathogen in direct contact with the host tissue may have. This probable disadvantage has been circumvented in the present investigation. The method applies only to obligate parasite systems.

The plants produced in the present experiment were scanty, which in itself proves that an extremely limited population of cells retain the so-called 'resistant factor' in a susceptible genotype but when such cells dominate (the regenerated plants in the present experiment) resistance results. Under *in vitro* conditions, there may also be synthesis of phytoalexins (host-induced antimicrobial agents which bring about resistance) which may play a role in contributing to the resistant cell population. This is only a speculation and needs further extensive investigation.

While the present report provides a new insight into the problem of obtaining disease resistant lines, it could also probably herald a significant breakthrough in the field of crop science, especially since a number of laborious and time consuming steps have been eliminated. Further investigations in other species and genotypes are certainly warranted.

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