CALLUS INDUCTION AND GROWTH IN TWO VARIETIES OF *CYMBOPOGON NARDUS* (L.) RENDLE (CEYLON CITRONELLA)

IN this note, the effect of different auxins on callus induction and sub-culture in two varieties of the Ceylon Citronella *Cymbopogon nardus* (L.) Rendle, is communicated. This report is first of its kind in *Cymbopogon*; the two varieties studied are *C. nardus* var. *nardus* ($2n = 40$) a cultivar and *C. nardus* var. *confertiflorus* (Steud) Stapf ex Bor ($2n = 20$) a wild form[1,2].

Seeds, entire seedlings, root and shoot segments of the seedlings and also, of the tillering plants were used as the source material for callus induction. The material was surface sterilized with 0·1% $HgCl_2$ for five minutes, rinsed several times with distilled water and transferred to the culture media. Murashige and Skoog[3] (MS) medium supplemented with different auxins, individually and in combination with kinetin (KN), was used. The pH of the medium was 5·8 and gelled with 1% w/v Difco bacto-agar. All the cultures were maintained at 23° ± 2° C and 60–70% R.H. under alternating conditions of light and darkness.

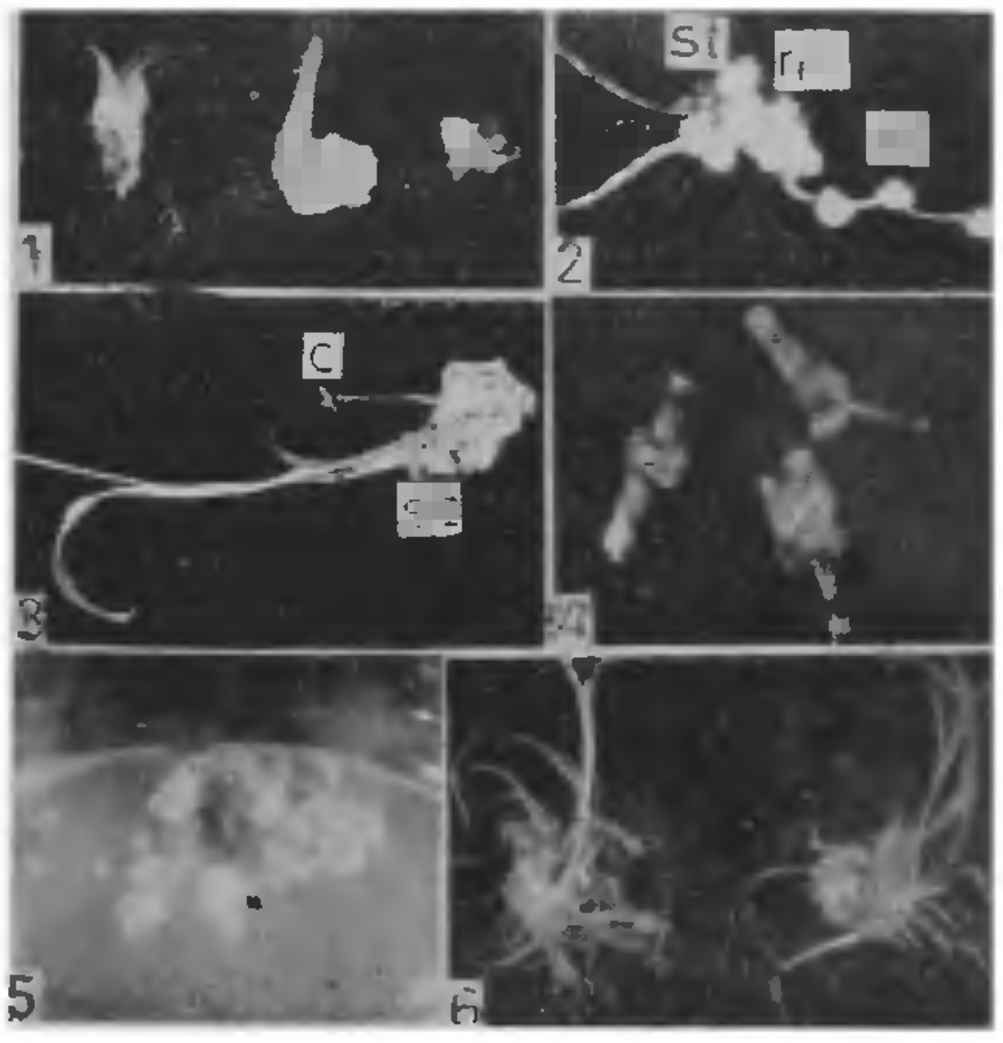
For the sub-culture studies calli were first initiated from the seeds on MS medium with 5 mg/l of 2,4–D and 0·4 mg/l of KN. Calli weighing 15–20 mg were incubated in the media containing different concentrations of the auxin with or without KN. The average fresh weight increase for a period of 30 days was recorded.

Callus was induced from the seeds, entire seedlings, shoot sections of the seedlings and the nodal regions of the stem, at 1–15 mg/l of 2,4–D. Callus was also induced, along with the roots, from the cultured seeds at 5 mg/l NAA. IAA (5 & 50 mg/l) failed to induce the callus even with added KN (0·4 mg/l). Root explants failed to respond to either of the auxins. KN alone could not induce the callus; however, it enhanced callus induction in combination with 2,4–D.

Callus was induced from the mesocotyl region of the seeds (Fig 1); while, in the seedlings, callus was initiated at the stem tip, mesocotyl and on the root (Fig. 2) and subsequently callusing extended to the entire seedling axis. The growth of the callus from the stem tip was 2–3 times faster than that from the mesocotyl or the root. The shoot sections of the seedlings produced callus only at the apical meristem (Fig. 3). The stem bits of the tillering plants produced only callus at the nodal regions (Fig. 4).

The callus thus formed has been sub-cultured in the medium containing 2,4–D, after isolation from the primary explants (Fig. 5). These calli are grown for 12–14 months and even after sub-culturing 8–10 times, the calli have maintained a steady rate of growth. IAA and NAA failed to support good callus growth in sub-culture. 2,4–D was found to be essential for the sub-culture of the callus without redifferentiation of the shoots and the roots. In both the varieties of the Citronella grass, the maximum proliferation of the callus occurred at 1 mg/l of 2,4–D with 0·4 mg/l of KN. Higher concentrations of 2,4–D, however, reduced the callus growth.

The callus, when transferred to auxin-free medium, differentiated many shoot and root primordia that developed into leafy shoots and roots respectively (Fig. 6); many roots also differentiated from the bases of the shoots.



FIGS. 1–6. Figs. 1–4. Callus induction from the seeds (Fig. 1), seedling (Fig. 2), shoot explant (Fig. 3), and the stem bits (Fig. 4) of the Ceylon Citronella on the MS + 2,4–D. Fig. 5. Isolated callus in the sub-culture on the MS + 2,4–D. Fig. 6. Plantlets regenerated from the callus tissue on the auxin-free medium. (*st*—stem tip, *ml*—mesocotyl, *rt*—root, *c*—caryopsis remnant. Note—Figs. 1, 3 and 4 of *C. nardus* var. *confertiflorus*. Figs. 2, 5 and 6 of *C. nardus* var. *nardus*.)

Studies conducted on the two varieties of *C. nardus* differing in ploidy, clearly establish that as in the other grasses[4–10], 2,4–D is the most effective auxin for both the callus induction and the sub-culture, in this grass also. In both the varieties of Ceylon Citronella, IAA failed to induce callus, suggesting the presence of a stronger IAA-Oxidase system in this non-cereal, *i.e.*, aromatic grass. Induction of the callus, only from the roots of the intact seedlings indicates the need for endogenous factors. *C. nardus* behaves similar to the cereal grasses[8] in the regeneration of the entire plant from the callus culture, in the absence of auxins or at low levels of IAA or NAA. The success obtained

in culturing the callus from the seeds and the explants, and the regeneration of the whole plant, on the specific medium is quite useful in the rapid clonal multiplication of this important grass.

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REACTIONS OF *AZOSPIRILLUM* TO CERTAIN DYES AND THEIR USEFULNESS IN ENUMERATION OF THE ORGANISM

REACTIONS of bacteria to various dyes have been studied by several workers and their differential responses made use of, in formulating useful selective media[1-4]. In the context of the potential use of *Azospirillum brasilense* as a bacterial fertilizer[5-7], it became necessary to evolve methods for its quantitative enumeration in inoculated cultures and carrier materials for effective quality control. With this aim, the reactions of *Azospirillum* to certain acidic and basic dyes were studied and the results are discussed here.

Solid sodium malate medium of Okon *et al*[8] containing ammonium chloride was made use of in the present study with slight modifications. The phosphate buffer portion of the medium was made in half of the total volume (not one-tenth as suggested by the authors) required and also contained enough agar for solidification of the total volume (2%). Minerals were dissolved in the remaining half, autoclaved separately and mixed with the agar at the time of plating. Required concentrations of the respective dyes (Table I) (stock solution in 50% alcohol suitably diluted with distilled water) were sterilized separately and added to the medium prior to plating. The pH of the medium was maintained between 6·5 to 6·8. Incubation temperature was 35° C.

The usual pour plate method, involving the addition of serially diluted suspension of the culture to molten and cooled agar, resulted in the formation of innumerable minute colonies of *Azospirillum*, partially embedded in the medium due perhaps to its microaerophilic tendencies. On the other hand, spreading the dilution evenly on the solidified agar led to the formation of well-defined surface colonies of uniform size and shape. Hence, surface plating of 0·1 ml of the required serial dilution was adopted.

Majority of the strains of *Azospirillum brasilense* being alkali producers[9], within 24 hr, the acidic dyes bromothymol blue (BTB) and bromo cresol purple (BCP) reacted to the rise in pH by a noticeable colour change into blue or purple respectively, initially formed as a halo surrounding the individual colony which later coalesced and spread through the entire plate. On the contrary, basic dyes, brilliant green (BG) and congo red (CR) got themselves decolorised around bacterial growth (Table I), but more slowly.

The colonies were thin, dry, slightly convex and rugose with a granular wavy surface and undulate margins often giving a somewhat fried egg appearance. The central, slightly raised portion of the colony was the first to absorb the colour of the acidic dyes.

The few acid forming strains, which could easily be identified by the colony characteristics, did not change the colour of the medium but absorbed the acidic colour of the dyes and stood out as golden yellow colonies. Common contaminants did not respond with such a quickly perceptible colour reaction and dye absorption, and could easily be discounted by their morphological characters as well.

Of the four dyes tested congo red was slow to react to the growth of the organism. In decreasing order of suitability, the four dyes could be rated as BTB > BCP > BG > CR.

Admittedly, the response of bacterial growth to dyes contained in a medium due to changes in the pH of a substrate is a non-specific reaction. Nevertheless, in