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SOMATIC VARIATION OF *PLUMBAGO ZEYLANICA* IN VITRO

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Abstract: *Plumbago zeylanica* is the most important medicinal plant of the genus Plumbago with manifold uses in folk/tribal medicine. Protocol for cultural conditions was established using nodal explants. Genetic uniformity of tissue-culture derived plants (TCD) was tested in a small field experiment using four characters. Low degree of developmental instability was noted in floral symmetry and lobe number of corolla, but no major variation. One unusually vigorous and apparently stress-tolerant plant in TCD population suggested possibility of using in vitro organogenesis for rapid regeneration and also for obtaining growth/physiological variant.

Key words: Somatic Variation; *In Vitro*; *Plumbago zeylanica*

INTRODUCTION

Plumbago zeylanica is the most important medicinal plant of the genus Plumbago with manifold uses in folk/tribal and traditional medicine. Since it is an important root-drug plant, it is likely to be endangered in no time unless early measures are taken to counteract the risk due to overexploitation. Development of a rapid method of micro propagation should be a step in this direction and would help to meet the possible demand of the drug in future. There remain many gaps in our understanding of differentiation and organogenesis in callus cultures, etc. Such basic studies are likely to contribute to our manipulation capabilities in cell/tissue cultures and thus ultimately help realizing full potential of tissue culture biotechnology of medicinal plants in general and selected important medicinal plants in particular.

Figure: Variation in the number of petals.

The micro propagation of *P. zeylanica* was done with MS medium which showed a satisfactory multiplication rate of one to about 50 within a short period of about three months. A cultural condition for accelerated organogenesis was also established with the aid of hormones BA or Kn along with MS medium. Genetic uniformity of tissue-culture derived plants (TCD) was tested in a small field experiment using four characters.

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MATERIALS AND METHODS

For the purpose of micro propagation and organogenesis young stems were taken as explants. The culture media consisted of Murashige and Skoog's basal medium containing basal salts and organic components along with 20g/l sucrose and 8g/l agar. The MS medium was further supplemented with different concentration of BA or Kn for shoot multiplication and shoot bud differentiation; 2,4-D for callusing in stem internode and IAA,NAA,IBA for rooting of shoot. After rooted plantlets obtained through micro propagation and organogenesis, the plants were hardened for a week and exposed to field conditions. One experimental plot of tissue culture derived plants was maintained for testing of genetic uniformity.

RESULTS

Variation in tissue culture derived (TCD) plants: The plantlets were first grown in pots (earthen) containing garden soil and compost (1:1) and kept in the greenhouse for 3-4 weeks before transferring to the field. Subsequently, they grew quite vigorously under field conditions.

One plant was rather was purposely maintained throughout (for a period of more than three years) in a small polythene container (25 cm tall and 10 cm in diameter) without being transferred even to a larger earthen pot. It was interesting to note that even under such stressed conditions, this plant grew vigorously compared to any other tissue culture derived (TCD) plant or normally propagated (NP) plants. Further, this plant appeared to be exceptional in flowering earlier and long before any other plant in the TCD and NP populations. Moreover, this plant bore more flowering branches, secondary per flowering branch and larger number of flowers per ultimate inflorescence. It is suspected from the known conditions to which it was exposed, that this plant may be stress (drought) tolerant.

This part of the investigation was considered necessary in view of the well-known fact that micro propagation through callus culture gives rise to variants i.e. show soma clonal variation. It is only through meristem culture that such variation can be reduced or eliminated and genetically uniform population obtained. However, genetic uniformity would depend on the size of the meristem 'dome' involved in the culture, the minimum being the most useful for this purpose. But often as in the case of nodal explants, non-meristematic tissue might contribute to regeneration to some extent. Hence, it was deemed necessary to ascertain the degree of somaclonal variation, if any, in the micro propagated (TCD) plants.

A small population of micro propagated/TCD plants grown in an experimental plot along with another small population of seed propagated (SdP) plants were compared for possible variation. Unusual growth and flowering behavior of a single TCD plant grown in a small polythene container has been mentioned. The field grown TCD plants did not show any gross morphological differences from the SdP plants, nor from the mother plant (MP), the source of explants. However, a critical search for non-apparent and micro-morphological characters revealed some variation in respect of four characters viz. (a) two of the corolla i.e. petal (corolla lobe) number and symmetry/asymmetry of petals (Figs.) and (b) two of the young bracts i.e. presence or absence of pigment of glandular hairs.

The first two characters were considered more important as they were indicators of developmental stability (homeostasis) and hence each plant of the two different populations (TCD and SdP) was examined at regular intervals during the flowering period besides observations at one time on the bract characters. These data are presented in the form of a pictorialized scatter plot i.e. metroglyph diagram. Referring to Fig., it is evident that the TCD plants show greater developmental instability of the corolla than the SdP plants, whereas, the petal is normally 5, more plants in the TCD population showed deviation i.e. 5, all being of equal size i.e. with a symmetrical corolla. As compared with SdP a greater number of plants in the TCD population showed corolla asymmetry, one or more being clearly smaller than other petals. For each petal character the typical normal condition was scored zero and the atypical/abnormal condition was scored as one.

Similarly, in the case of young bracts, the absence of pigment or gland was scored zero and the presence was given the score one. In Fig., the bract character has been indicated as rays. However unlike petals, in respect of bract characters. It reveals little difference between the TCD and SdP populations, though some TCD plants show difference from the mother plant.

DISCUSSION

A small but significant part of this study is concerned with ascertaining variation in the TCD plants compared with normally (clonal or seed) propagated plants. Although it is often tacitly assumed that meristem culture would not give rise to genetic variation, yet it should not be taken for granted, when a protocol development for rapid regeneration/propagation is the goal. However, this assumption is seldom subjected to appropriate field testing (Narayanswamy, 1994).

Since clonal propagation would be ordinarily expected to be genetically uniform, seed propagated plants were taken for comparison with TCD plants. Excepting one plant, maintained for a period of three years under rather stress condition, other plants did not show any evident change in morphological growth or vigor. However, this particular plant was highly vigorous even under pot bound (in small polythene container) and stress condition. This would suggest possible origin of vigorous and stress tolerant plants through tissue culture. Some other plants in the TCD population showed developmental instability in corolla lobe number (4 or 6 compared with normal 5) and corolla asymmetry in higher frequency than in seed population. Hence, certain degree of (genetically controlled) instability cannot be ruled out in the TCD plants.

In fact, a purposeful and critical study of in vivo clonal propagation in a cereal, *Eleusine coracana* did reveal (contrary to expectation) polygenic variation in quantitative traits, which could be detected only through appropriate field experiments (Behera, 1968; Sinha and Behera, unpublished). Extrapolating to the in vitro culture, the possible presence of polygenic/minor genetic variations cannot be entirely ruled out, in the absence of any critical study. Hence the need arises for such a study attempted here.

Such variation may be desirable or undesirable depending on the purpose of TC and also the magnitude of variation. It is certainly undesirable if high degree of genetic uniformity is required. In the present case of micro propagation in *Plumbago zeylanica,* absence of macro-morphological variation and only a certain degree of developmental instability (which is of a quantitative nature and not qualitative) may be taken to mean that the protocol in the investigation can be adopted for rapid micro propagation. The exceptional plant would suggest that useful growth/bigger variants

may result through such micro propagation, which is rather desirable.

REFERENCES

1. Behra RK, A study of variation in clonal propagation in Ragi (*Eleusine coracana* Gaertn.) (1968) Unpublished M.Sc. (Ag) thesis OUAT.

2. Mohan Jain S, Brar DS, Ahluwalia BS Somaclonal variation and induced mutations in crop improvement. Springer Publication, (1998).

3. Murashige T and Skoog F A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, (1962) 15: 473 – 497.

4. Narayanswamy S, Plant Cell and Tissue Culture. Tata McGraw-Hill. Publishing Company Limited, New Delhi. (1994).

5. Patnaik J, Sahoo S, Debata B K, Somaclonal variation in cell suspension culture derived regenerants of *Cymbopogon martinii* (Roxb.) Wats. Plant breeding. (1999), 118(4): 351 – 354.

6. Swaminathan MS, Biotechnology and agricultural betterment in the developing countries. In Natesh, S. *et al.,* ed. Biotechnology in Agriculture. (1987), Pp 1-11.

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