SUMMARY

Plants are used as one of the major food materials of the animals including human being. The members of the family Rosaceae are very important as most of them are economically very useful. The genus Duchesnea indica is important as its fruit is very similar to the economically important strawberry, Fragaria sp. The extract of this plant has been used by Chinese in their traditional medicines. In this context the genus D. indica has to be explored to evaluate the cytogenetical aspects and the related phytochemical effects. Among the different species of the strawberry Duchesnea is one which is less explored and thus the study on this plant is important.

Since this plant is sterile, the induction of variability is very difficult in natural conditions. Assessment of genetic variability is the basic aspect of any breeding programme. An alternative method to produce new forms of the plant is selecting a potential variant from tissue culture method. Plant tissue culture has the potential to induce genetic variability in Duchesnea genotype through somaclonal variants, somatic hybrids or transegenic plants. The exploitation of tissue culture technique for the production of plants with superior quality is attempted here to produce a novel variety of D. indica. Comparison of the in vitro derived plants with the parent was carried out to find out the performance of the micropropagated one over the parent. In the present study comparative figures in different parameters like cytogenetical, phytochemical, biochemical activity etc. were tested to find out the efficiency of the micropropagated plants.
Micropropagation

An efficient tissue culture method was developed by this study for the production of a variant plant which has some superiority over the parent plant. Murashige and Skoog basal medium with different hormonal combinations of auxins and cytokinins were tried in this venture to produce the variant. Though different explants tried, satisfactory result was given by nodal explant.

Among the different combinations of hormones used with MS medium, multiple shoots were induced with a combination of 2 mg/l IAA and 2 mg/l NAA. For the induction of multiple shoot regeneration, nodal explants were inoculated in the medium with combination of auxins and cytokinins. Multiple shoot were noticed after 3 – 4 weeks in 90 – 100% cultures. The frequency of shoot induction and percentage of initiation was higher in the medium containing 2 mg/l IAA and 2 mg/l NAA. When 2, 4-D (2 mg/l) was used the nodal explant turned to callus. The combinations of 2, 4-D (0.2 mg/l), IAA and NAA (0.5 ml/ each), had developed shoots and roots. When 2, 4-D (0.2 mg/l) was used with IAA (0.5 mg/l), profuse callusing was observed. When this calli were subcultured in the medium supplemented with the hormones 2 mg/l IAA and 2 mg/l NAA, they developed into multiple shoots along with profuse rooting. Since no morphological variation was noticed among the regenerated plants, further analysis at cytological, molecular and phytochemical levels was conducted to search for the possible potential variants.

Cytological analysis

To examine the karyomorphological changes in the chromosome complement of the tissue culture derived plants, mitotic studies were carried out on actively growing root tip meristem of in vitro plants and also on callus cells. Then it was compared with the in vivo plant. The ploidy level of all the cells studied, i.e., in vivo, in vitro and callus were found to be invariably dodecaploid (2n = 7x = 84). Chromosome morphology of the regenerated
plants showed slight variation. Some of the chromosomes are not the exact replica of the parent plant and exhibited structural changes, like variation in total chromosome length, average chromosome length, centromeric positions, disparity index and total forma percentage. The chromosomes ranged in size from 1.5323 μm to 0.3838 μm in the parent plant, from 1.5323 μm to 0.4402 μm in the variant and from 1.6191 μm to 0.3838 μm in the callus. The total chromosome length of the cells of the parent plant was noticed as 34.566 μm and that of the variant was 35.7675 μm. The total chromosome length of the callus was 33.3151 μm. The disparity indices observed in the chromosome complement of parent, variant and callus were 61.6755 and 55.3662 and 59.9394 respectively. The total forma percentages of the parent, variant and callus were estimated as 40.6758, 42.0408 and 41.5358.

The karyotype formula of the parent and the in vitro plant was A_{12} B_{60} C_{12}. The karyotype formula of the callus was found to be slightly different (A_{12} B_{58} C_{14}).

**Random Amplified Polymorphic DNA (RAPD) Analysis**

To obtain more information on the genetic variability between the plants, RAPD analysis was carried out. For this DNA was extracted from the fresh young leaves of the parent plant and two in vitro regenerated plants, viz., T1 and T2. Amplification of DNA was carried out with 27 primers out of which only 10 responded successfully. The number of bands resolved per primer ranged from a minimum of 5 to a maximum of 28. The size of amplification product also differed and ranged from approximately 500 bp to over 10000 bp in the 10000 bp ladder. RAPD fingerprints of the variant, T1 differed from that of the donor plant with all the 10 primers whereas the variant, T2 differed with only eight primers, viz,

OPA 01, OPA 03, OPA 09, OPB 13, OPC 03, OPC 06, OPD 01 and OPD 03.
Altogether 20 bands were absent and 14 additional bands were present in T1 when compared with parent during RAPD analysis with 10 primers. However only 18 bands were absent and 8 new bands were present in T2 when compared with the parent. The reproducibility of these genomic DNA bands was consistent in successive repetitions.

Noticeable genetic polymorphism was observed in one tissue cultured plant (T1) as some parental bands are found to be missing in it. Some additional bands were also found in it with some primers. Since the T1 plant has shown more differences than the parent plant, it was considered as a variant and further investigations were carried out on this variant plant.

**Essential oil analysis**

The essential oil of both *Duchesnea indica* (Andr.) Focke and its tissue culture derived variant (T1) was subjected to GC - MS analysis. Both the plants yielded similar quantity of essential oil (0.1%). The analysis of the essential oil samples revealed a range of variation in their constituents. The major components identified from the essential oils of *in vivo* and *in vitro* plants were similar (carvacryl acetate, valencene, nona hexa contanoic acid, aristalone, dihydro aromadendrene, 2-hexa-decan-1-ol, eicosane), even though, there is marked difference in the percentage of occurrence. Considerable increase in the quantity of carvacryl acetate (30.5% and 31.2%) and valencene (7.6 % and 7.7%) and a decrease in percentage in the quantity of α- humulene (0.8% and 0.5%) was observed in the *in vitro* (T1) plant. The major component (carvacryl acetate) was observed in an enhanced quantity in the *in vitro* plant supports the better performance of the essential oil biosynthesis in the *in vitro* plants.

Dissimilarity was evident in the essential oil quality with respect to composition of oil components. Although 25 components were observed in the parent plant, the *in vitro* plant showed only 21 components.
**Total carbohydrate analysis**

The estimation of the total carbohydrate in both the parent plant and the variant (T1) also showed differences in quantity. The total carbohydrate present in the *in vivo* plant is 3% and that of *in vitro* (T1) plant is 3.2 % as per the present study. This also shows the superiority of the culture derived plant (T1) to the parent plant.

In most cases, study of somaclonal variations has been limited to phenotypic variation and has been associated with changes in the chromosome structure and function. The somaclonal variation in yield and quality characters will have importance in studies of crop improvement. The present study shows there is a significant change in the essential oil content and composition and the total carbohydrate content. The biosynthesis of secondary metabolites is controlled by genetic factors. Since there is a connection between the development and differentiation processes and the metabolism in plants, the growth regulators and the stress induced by the artificial environment might have influenced the metabolic pathways. The phytochemical differences observed in the present investigation may be due to the differences caused in the genetic make up of the *in vitro* plant of *Duchesnea indica*. 