INTRODUCTION

Endosperm is a product of double fertilization in angiosperms and develops through the fusion of three haploid nuclei to form endosperm in walnut (1). It functions as a storage organ and provides nourishment during the development of embryo and its germination to form seedling. Since endosperm formation results from the fusion of two female nuclei and a male nucleus, the resulting hybrid vigour may be utilized to raise vigorous triploid plants by the use of endosperm cultures. This would avoid time consuming less efficient conventional techniques through sexual hybridization, provided a dependable system of morphogenesis is evolved. To date, successful establishment of callus culture has been induced in 29 taxa of angiosperms, of which 8 semi-parasitic and 13 autotrophic taxa have been known to differentiate embryoids/plantlets (2). The woody taxa in these are represented by apple, Citrus, Jatropha, peach, Putranjiva and sandalwood. Two different views on the role of embryo in the endosperm culture have emerged. According to one view (3,4), there is necessity of 'embryo factor/s' for callus proliferation and/or organogenesis. It has now been suggested that gibberellin can replace the 'embryo factor/s'. There are others (5,6) who suggest that presence of embryo is not an absolute necessity. Recently, however, Wang and Chang (7) observed that although endosperm callus could be induced without embryo the callus differentiation into embryoids and plantlets required gibberellic acid (embryo factor). Our interest to resolve this problem grew out of our observations on the immature and mature endosperm cultures of walnut being raised in this laboratory since 1979. This report is a preliminary account of detailed work to be published later.

MATERIALS AND METHODS

The materials consisted of young immature and mature nuts of a thin-shell variety of walnut (Juglans regia L.). The developing nuts were examined at weekly intervals by dissection starting with the onset of stigma withering of pollinated pistils. From May to last week of June, the endosperm was constituted of free nuclear liquid stage and the young embryo at late heart stage. In the first week of July, the endosperm turned into cellular mass and the embryo was fully developed with a small hypocotyl and cotyledons. By this stage the oils were relatively scarce and perisperm easy to separate. Thereafter the endosperm became oily, perisperm turned papyraceous, brownish and difficult to separate from the endosperm. The young fruits were flame sterilized by dipping in alcohol, the nut was broken and endosperm scooped out of the perisperm. It was inoculated in two ways i) with embryo (EE) ii) without embryo (EWE). The culture medium consisted of modified MS (8) containing 3% sucrose and various concentrations of auxins (IAA, IBA, NAA, NOA, 2,4-D), cytokinins (K, BAP), GA, and complex growth substances casein hydrolysate (CH), yeast extract (YE), malt extract (ME) singly and in combinations. The pH of the medium was adjusted to 5.7 before adding agar (1%) and autoclaving. All manipulations were conducted in sterile conditions in laminar flow chamber. The cultures were maintained at 25°C in total dark, and 25±3°C in 16h light (3500 lux)/8h dark. The cultures of mature endosperms of pecan nut (Carya illinoiensis cv. Mahan (Wangh.) K. Koch) were also tried.
RESULTS AND DISCUSSION

There were some striking differences in growth response of the two types of explants, while EWE showed two modes of differentiation i.e. direct regeneration of roots without intervening callus, and indirectly through differentiation of callus, the EE explants showed rhizogenesis from proliferating callus at excised ends of endosperm. However, in contrast to root- ing which was observed in 100 percent of both types of cultures in varying degrees in different media tried, the formation of shoot-bud like structures was observed only in EWE cultures (Fig. 3). This observation was further confirmed with mature endosperm explants without embryo. In this case the subcultured callus obtained on MS+CH(500mg/l)+2,4-D+K(1 mg/l) when transferred to basal MS differentiated roots and shoot-bud like structures thus indicating that embryo association with endosperm in culture may delay/inhibit morphogenesis- an observation also noted in Taxillus ventitum (9). The endosperm cultures of walnut show that different concentrations of growth substances are needed to evoke an optimal response with respect to morphogenesis in two types of explants. Furthermore the immature explants are found to be much more responsive than mature ones in which accumulation of brownish exudates poses severe problems. Also total dark is ideal for the purpose. The cytological study of the root-tips and callus colls revealed triploid nature 3n=48 as expected. The mature endosperm explants in pecan nut could similarly be induced to rhizogenesis.

CONCLUSION

The studies demonstrate that callus induction and/or differentiation of roots and shoot-bud like structures do not require embryo factor (or GA), at least in walnut and rhizogenesis in pecan nut as is also the case in apple (5) and Vitis (6). The independent system of endosperm culture offers an advantage that it eliminates the chances of mixoploid calli during callusing simultaneously of endosperm and embryo.

REFERENCES
first shoot-tips of M 9 and M M 106 since July 1979 have been
induced and observed to consistently continue to maintain en-
tire viability potential. In somatic embryos do not grow beyond
certain stages (Cheema, unpublished). Hutchinson (1971) also
reported the induction of callus from apple rootstock Crab C
and M M 9 but failed to get any organogenesis. Sporadic low fre-
quency differentiation of shoots from triploid endosperm cal-
lus of apple has been reported by Wu et al. (1977). And though en-
tryogenesis from the apple cv. Golden Delicious was accom-
plished by Eichholtz et al. (1979) plant development from the
embryos was not reported. Fett and Xue (1981) reported the in-
duction of haploid plantlets by anther culture of apple cv.
Delicious.

However, a dependable system of inducing organization of
shoots in somatic interzones and/or its callus in apple has re-
cently been reported by us (Cheema and Sharma, 1981).

This paper reports the dependable methods established and
repeatedly used in our lab for: i) shoot multiplication and
elongation; ii) induction of adventitious shoots from isolated
internodal segments and internodes and its callus from develop-
ing shoot-tip; iii) rooting of shoots and iv) establishment of
plantlets transferred to soil in pots.

Material and methods

The plant material of EMLA-25 (the virus free indexed clo-
line) was obtained from East Malling Research Station, England
by kind courtesy of Dr. C. J. James as shoot cultures in. Jones
et al. (1977) medium. Two media viz. Jones et al. (1977) and
Murashige and Skoog (1962) were used. The latter consisted of
Y2 macro AND micro inorganic salts and following supple-
ments (mg/l): myo-inositol-100; thiamine HCl - 0.1; pyridoxine
HCl - 0.5; nicotinamide acid - 0.5; glycine - 2.0; sucrose-30000.

The medium was further supplemented with casein hydrolysate
(CR 150, phoroglucinol PG)162, activated charcoal (A; 15 g,
25 x 150 mm test tube), zeatin (0.3 mg/l), 6-benzyladenine (BA),
6-furfurylamino-purine (K; 0.2-0.5) - naphtylacetic acid (NAA) 1.0
as required for the experiment. The rooting medium consisted of
1/2 strength macro and micro-elements of MS and organic
supplements of Linsmaier ans Skoog (1965) medium (mg/l): myo-
inositol-100, thiamine HCl - 0.1; sucrose-20000. The pH of
the medium was adjusted to 6.8 with 1N HCl or 1N NaOH before
adding agar (40000) and autoclaving. The medium was sterilized at
1.5 kg/cm² pressure for 15-20 minutes. 25 ml medium was
dispensed in 25 x 150 mm conning test tubes and plugged with
nonsterile cotton wrapped in muslin.

Its test propagules were selected for uniform size and
single shoots were plantets in cultures. The rate of shoot mul-
tiplication was determined by counting the number of shoots
in each shoot-propagule unit and the medium shoot length of 2 cm.
Test treatment consisted of over ten replications and the response
of a minimum of 3 cultures on the same medium were
taken into consideration.

Cultural conditions

All the cultures were kept in a temperature controlled
room maintained at 25 ± 2°C. The source of light consisted of
two four feet white fluorescent tubes (40W and an inconces-
scent lamp (25W, 100W). The intensity of light at the level of
cultures was 1000-1500 lux. The light regime consisted of 16h
light/8h darkness. The subculture of cultures by transfer onto fresh
media was generally performed after 4 weeks unless re-
quired otherwise and mentioned.

Propagating subculture

After 3 weeks on the rooting medium the plantlets were
transferred from the propagation temperature of 25 ± 3°C to a
low temperature condition at 6 ± 10°C, for another 6-8 weeks on
the same medium.

Potting

Rooted plantlets with shoots ca 2.5 cm and with dormant
bud like structure (OBLS) characteristics were carefully re-
moved from agar and gently washed of all adherent material. The plant-
lets were treated in the following manner i) dip or irri-
igation with starter solution; ii) dip and irrigation with starter solution
and potted in unsterilized soil mixture consisted of leaf could;
soil sand (5:1:2) in 200 ml beaker with broken base to cre-
ate efficient drainage facility and covered with petri dish.

The starter solution consisted of 200 mg/l FeSO₄; 15 mg/l of
ammonium nitrate, potassium dihydrogen orthophosphate 2; supply (2.2
mg/l of MS (1962) and IBA (5mg/l).

RESULTS

Each of the explants from stock culture was tested for
latent infection by raising the concentration of CK to 2000
mg/l in the same medium and incubaing the cultures for 4 weeks.
This procedure showed that infected cultures produce profuse contaminant growth within a