J. Himalayan Ecol. Sustain. Dev. Vol. 1 (2006)

Exploiting the Potential of Organ Culture in *Juglans Regia* L.

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ABSTRACT

To trigger the morphogenetic response, various explants of *J.regia* were cultured on MS basal medium supplemented with different concentrations and combinations of plant growth regulators. MS basal medium with full strength favored the morphogenetic response in shorter period of time as compared to half strength which delayed the results. Multiple shoot formation and elongation was achieved from explants like embryo, *in vitro* raised shoot tips and nodal segments. The cotyledons were found to possess high rhizogenic potential whereas callus formation of different colors and textures was observed in case of leaf, petiole, stem, root as well as from the explants of the cotyledons. This indicates that all explants of *J.regia* are highly responsive to dedifferentiation but are comparatively recalcitrant to redifferentiation.

Keywords: Organ culture, dedifferentiation, redifferentiation, multiple shoot regeneration.

Abbrevations: MS-Murashige and Skoog; BAP-6 benzylamino-purine; IAA-indole acetic acid; IBA-indole butyric acid;

INTRODUCTION

The walnut, genus *Juglans* belongs to order Juglandales and family Juglandaceae. It is tree of great utility and almost every part of it is highly valued. Almost all parts of walnut are utilized in one way or the other but the fruit and timber have been put to maximum use by man.

Walnut is an important fruit crop of J&K state and its germplasm in J&K having a high bearing potential with better nuts and kernel quality needs special attention for propagation. Amongst the major problems identified as bottlenecks to successful walnut cultivation in the state of J&K is the lack of adequate propagation techniques, (c. f. Rathore, 1991). Presently very little attention is being paid in the valley for the production of these important fruit trees which forces us to resort to some unconventional method of propagation. Micropropagation a proven means of producing millions of identical plants with genetic uniformity has come of years. Well tested protocols are now available in case of apple, citrus, and grapes (Masoodi,2002).Efforts to standardize techniques for micropropagation of walnut have proved disappointing (Masoodi, 2002). So this study was undertaken with an aim to work out the possibility of developing an approach for in vitro propagation of walnut.

MATERIAL AND METHODS

Fresh material of various organs i.e,embryo, shoot apices, nodal segments, cotyledon, root, leaf, petiole and stem of *Juglans regia* L.from field grown plants were collected and prepared for culture. Mature seeds collected at the end of the growing season were used for excising embryo and cotyledons for culture after properly separating them from the seed shell. Various fresh explants and kernels were washed

separately with lab. detergent(Cedpol) containing few drops of Tween-20(wetting agent)under running tap water for 15-20 minutes till no traces of detergent were left. Surface sterilization was carried out with 5% NaOCl varying from 15-25 min for various explants. Finally these were rinsed 3-4 times with autoclaved double distilled water. Explants obtained from *invitro* raised seedlings need no sterilization process as these were growing aseptically.

Sterilized explants were inoculated separately on MS (1962) medium (both full and half strength) supplemented with different concentrations and combinations of phytohormones. pH of the medium was adjusted to 5.6 and 0.8% agar was used as gelling agent. The cultures were maintained at 23+2C with 55-65%RH and exposed to 16hr photoperiod provided by cool fluorescent tubes (3000 lux).

RESULTS

Embryo Culture

The varying responses of cultured embryos to various phytohormonal regimes were observed. The embryos when cultured on full strength MS basal medium resulted in growth and development of single shoot and a thick root from the root pole. The hypocotyl resulted in swelling and subsequent callus formation. However MS(x1/2) medium supplemented with NAA (1mg/l) resulted in root development only and the shoot growth got arrested. The same based medium supplemented with cytokinin alone i.e. BAP (5mg/l) resulted in shoot formation only and friable callus got developed at the root pole.

Best results were obtained when combination of IAA and BAP was used. Stimulation and development of (6-8) multiple shoots on both sides of embryonal axes were observed after 10 weeks of culture on MS (x1/2) medium augumented with IAA (2mg/l) + BAP (2mg/l). Friable callus formation was seen at root pole. Use of full strength MS medium supplemented with same hormonal concentration revealed same results but only after 8 weeks of culture period. Decrease in the concentration of IAA and BAP to (1mg/l) each in the medium resulted in vitrification of shoots. However, usage of higher concentrations of IAA and BAP i.e. more than 2mg/l hampered shoot growth.

Culture of Shoot Apices and Nodal Segments

Shoot apices and nodal segments from *in vitro* raised seedling and from mature field grown plants were trimmed and cultured on various hormonal concentrations .In case of field grown plants both the explants showed regeneration of shoots on MS medium supplemented with BAP (1mg/l) as well as on MS +BAP (2mg/l) + IAA (0.2mg/l) whereas a lesser number of shoots got initiated on MS medium supplemented with BAP (4mg/l) +IBA (0.1mg/l)

Shoot apices and nodal segments from the seedling showed very good response i.e. 18 multiple shoots were recovered after culturing on medium augmented with IAA (2mg/l) + BAP (2mg/l).Multiplication continued for months together provided the material was transferred to fresh medium after every 4-6 weeks or else the proliferation would stop because of the deficiency of the nutrients in the medium.

Multiple shoots obtained after multiplication phase from nodal segments and shoot apices as well as the embryonal shoots were sub cultured for elongation on different hormonal combinations. The best medium observed for the elongation of shoots was found to be the same as was for stimulation of shoot buds i.e. MS (x1/2) + IAA (2mg/l)+ BAP(2mg/l).

Different trials for rooting were made by culturing these *in vitro* raised elongated shoots on different rooting media. Rooting of shoots obtained was successful to a moderate percentage only. Initiation of rooting became possible only at higher concentration of IBA added to MS medium. No response was observed below 2mg/l

auxin concentration. After 6 weeks 2 small white root initials were observed on MS +IBA (3mg/l). Increase in the concentration of IBA above 3mg/l in the medium resulted in well developed small thick roots at cut end of shoots after 6 weeks.

Cotyledons Culture

Cotyledon explants from mature seeds were isolated and inoculated on MS medium enriched with various growth regulators. On MS basal medium these explants showed direct root initial development without any callus formation but the percentage was too low and the process was too slow. Presence of BAP (1mg/l) in the medium favored the development of friable callus all over the surface of explant. Orangish white callus developed on MS + BAP (5mg/l) whereas callus with juicy appearance was formed on the medium supplemented with IBA (0.5mg/l) +BAP (5mg/l).

Juglans cotyledons have shown high rooting potential directly from the surface of explants. Multiple adventitious roots were initiated under the influence of different auxin concentrations like IAA (2mg/l) and NAA (6mg/l).

Root Culture

Root segments used for raising primary cultures were obtained from regenerated roots of cotyledon as well as from *in vitro* raised seedling from cultured embryo. Callus formation was observed after 6 weeks of culture period of root explants on MS(x1/2) basal medium. The explants showed swelling followed by friable callus formation on MS medium supplemented with NAA (1mg/l). Similar response was observed on medium supplemented with IAA (0.001mg/l) and BAP (1mg/l). However callus cultures were nondifferentiating.

Leaf,Petiole and Stem Culture

Leaf segments from field as well as from *in vitro* raised seedlings were grown in presence of various hormonal concentrations. Nodular callus formation from leaf explant was observed on medium supplemented with IAA (2mg/l); whereas NAA (2mg/l) added medium favoured compact nature of callus but was appearing spiny. Combination of IAA (2mg/l) and BAP (2mg/l) resulted in friable callus differentiation. Leaf expansion without any callus formation was observed on medium supplied with BAP (5mg/l).

Petiole explants also showed the same response of friable callus formation on MS medium supplemented with only IAA (6mg/l) and IAA(2mg/l)+ BAP(2mg/l).Stem segments developed creamish white friable callus on cut ends on BAP (5mg/l) supplemented medium.

DISCUSSION

The investigations carried out suggest that different explants of walnut possess different degrees of morphogenetic potential for multiple shoot formation, elongation, callus formation and root regeneration which these express under different phytohormonal concentrations and combinations. Use of MS medium both half and full strength was followed for almost all the types of experimental studies primarily because of the higher and quicker response on this medium. A number of earlier workers namely Caruso (1983); Meynier (1984); Pijut (1993); Tang *et al.* 2000 have also found MS medium quite responsive for walnut culture.

Morphogenetic response of walnut explants was studied by Jay-Allemand (1982); Sommers *et al.* (1982); Rodriguez *et al.* 1985 and Fernandez *et al.* (2000) who have reported multiple shoot-bud stimulation and proliferation from embryonal axes of different species of *Juglans* on various media supplemented with BAP. However, in present experimental work only single shoot formation was observed on medium supplemented with different concentrations of BAP.Contrary to this present investigation revealed multiple shoot bud stimulation on medium supplemented with a combination of auxin and cytokinin which is exactly in accordance with that of Caruso (1983)

and Pope *et al.* (1990). Rooting of elongated shoots was reported on medium supplemented with low concentration of auxin by Meynier (1984); Berros *et al.* (1993) and Helie Schdolt *et al.* (1986). Current studies have drawn contradictory conclusion wherein no rooting was observed when the concentration of auxin in the medium was low.

Cummins and Ashby (1969) and Rodriguez (1982) reported callus formation from cotyledon explants on medium supplemented with combination of auxin and cytokinin. Present findings are confirmed by the earlier observations. Direct rooting without intervening callus occurred in these cotyledon explants on MS medium without growth regulators However, profuse rooting was achieved on medium supplemented with auxins IBA (6 mg/l) on both half and full salt strength concentration.These results are in conformity with those of Rodriguez (1982); Driver and Kuniyuki (1984) and Sallanon *et al.* (1997) who reported rooting from cotyledons on IBA (40 µM). All these findings reveal that *Juglans* cotyledons in addition to callus formation possess a high rooting potential also.

Development of callus from stem segments of *Juglans* was observed with cytokinin alone showing that medium without auxin is also capable of callus induction. On contrary Cummins and Ashby (1969) reported callus formation under the combined influence of auxin and cytokinin. Studies on other explants of walnut viz (leaf blade, petiole, leaf vein and internodes) by Liu and Hans (1986) have revealed callus inducing potentials even without growth regulators which could not be confirm the present studies wherein only friable callus formation was observed from leaf/petiole explants on medium with auxin/cytokinin combination.

Present investigations reveal that apart from phytohormonol influences, salt strength of MS medium also influence the morphogenetic response of the explants. Dedifferentiation of all the explants is easily exploited as compared to there redifferentiation potential to which these are comparatively recalcitrant. Best regeneration capacity was found in embryos followed by shoot apices and nodal segments. These observations reveal that after refinement of the protocol the technique can be used for propagating elite cultivars of *Juglans regia* for economic benefits.

ACKNOWLEDGEMENTS

The author wish to thank Director, C.O.R.D for providing laboratory facilities to carry out these investigations. The findings are a part of M.Phil work of the first author.

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