Pterocarpus marsupium Roxb. is grown in deciduous and evergreen forests of central, western and southern regions of India. It is found mostly in the states of Gujarat, Madhya Pradesh, Bihar and Orissa. The role of P. marsupium as anti-diabetic has been very well established. It is one of the valuable multipurpose forest trees that yield excellent timber for the international market.

In order to develop an efficient in vitro regeneration protocol of Pterocarpus marsupium were conducted on the following aspects:

**AXILLARY SHOOT PROLIFERATION**

In the present study, nodal segments from the 10 year old tree grown at nursery of AFRI, Jodhpur were used as explants. These nodal segments were pre-disinfected with 0.2% bavistin and 0.1% streptomycin for 10 min, followed by disinfection with 0.1% Hgcl$_2$ for 7 min, which gave maximum aseptic explants.

Seasonal variation was observed for morphogenetic response of nodal segments. It was found that maximum bud break response was obtained, when nodal segments were collected during month of March to May period of the year.

Effect of cytokinins on axillary bud proliferation was investigated. Nodal explants were cultured on MS medium supplemented with BAP or Kn or TDZ or Zeatin. Kn at 13.95µM concentration was found best for axillary bud proliferation than other cytokinins, on which 64.44% explants responded.

Effect of various additives was also studied for in vitro shoot proliferation. MS medium supplemented with 13.95µM Kn, with additives (568 µM Ascorbic acid, 260µM Citric acid, 605µM Ammonium sulphate and 217µM Adenine sulphate) was found to be optimal for bud break.

Out of various basal media (MS, B$_5$ and WPM) tried, MS medium was found to be effective for axillary bud break in P. marsupium.
**IN VITRO SHOOT MULTIPLICATION**

*In vitro* proliferated shoots were excised from mother plant and subjected to different physio-chemical manipulations to increase their number as well as their long-term maintenance.

Different cytokinins (BAP, Kn or TDZ) were tried to select the cytokinin supporting optimal *in vitro* shoot multiplication. Of these different cytokinins tested, no cytokinin alone was found optimal for shoot multiplication. Cytokinin with auxin enhanced *in vitro* shoot multiplication. Therefore, cytokinin-auxin interaction was also studied for *in vitro* shoot multiplication. MS medium supplemented with 9.30 µM Kn + 0.54 µM NAA was found to be optimal for *in vitro* shoot multiplication.

After selection of best cytokinin, trails were conducted with different mineral salt formulations (MS, B₅ and WPM) to optimize shoot multiplication. Of these mineral salt formulations, MS medium was found superior to other for shoot multiplication.

Various additives (Ascorbic acid, Citric acid, Ammonium sulphate and Adenine sulphate) were used for *in vitro* shoot multiplication. MS medium supplemented with 9.30 µM Kn along with 0.54 µM NAA with additives (568 µM Ascorbic acid, 260µM Citric acid, 605µM Ammonium sulphate and 217µM Adenine sulphate) was found to be optimal for *in vitro* shoot multiplication.

Studies were conducted to find out the effect of liquid (without agar) and semisolid media on shoot multiplication. Results exhibited that, semi-solid medium supported shoot multiplication better than semisolid medium.

It was observed that subculture period of four weeks gave highest number of multiplied shoot and an average of 3 fold *in vitro* shoot multiplication was obtained on 4 weeks subculture frequency, which was significantly higher than other subculture duration.

Studies were further conducted to optimize carbon source for shoot multiplication. For this purpose sucrose, glucose and fructose were used. Results
showed that sucrose to be more efficient for in vitro shoot multiplication. Different concentrations of sucrose (1% - 6%) were tested to study its effect on in vitro shoot multiplication. Of these concentration, sucrose at 3% in the multiplication medium, elicited optimal response.

Effect of pH of the medium was studied for in vitro shoot multiplication; six different (3.8-7.8) levels were tried. Of all the levels, the optimal pH of the medium was 5.8 for in vitro shoot multiplication.

To see the effect of myo- inositol on in vitro shoot multiplication, four concentrations (50,100,150,200 mg/l) of myo- inositol were incorporation in MS medium supplemented with 9.30 µM Kn + 0.54 µM NAA + additives. The best shoot multiplication was obtained at 100 mg/l myo- inositol supplemented with 9.30 µM Kn + 0.54 µM NAA + additives.

**IN VITRO ROOTING**

Induction of roots on in vitro raised shoots remains a critical step for in vitro propagation. In the present study, IBA was found effective as compared to other auxin (NAA or IAA) in inducing roots. Amongst all the auxins tried for in vitro rooting, IBA supplemented MS medium produced maximum number of roots as compared to NAA. On medium supplemented with 4.92µM IBA, 42.22% rooting with 2-3 roots per shoot was observed.

In order to increase the percentage of rooting a two step procedure was also applied where a short-term treatment of various concentrations of auxins IBA or NAA (100, 200, 300 µM) was given and then subsequently treated shoots were transferred on hormone free medium. In the first step, in vitro shoots were transferred on medium supplemented with high concentration of auxin for seven days. As a results root primordia developed from the base of the shoot. The IBA supplemented medium responded positively and showed the development of promordial tips. In the second step, these treated in vitro shoots with developed root primordial were transferred on basal medium (MS medium without auxins) for further elongation of root primordial into roots. When the in vitro shoots were kept
in the medium with high concentration of auxin for more than seven days in the first step, the developed root primordial become thick that lead to stunted growth of the roots. It was observed that two-step treatment with NAA did not show development of root primordial.

In order to increase the percentage of rooting a two step procedure was adapted where a short-term treatment of various concentrations of auxins IBA or NAA (100, 200, 300 µM) was given and then subsequently treated shoots were transferred on hormone free medium. In the first step, in vitro shoots were transferred on medium supplemented with high concentration of auxin for seven days. As a results root primordia developed from the base of the shoot. The IBA supplemented medium responded positively and showed the development of promordial tips. In the second step, these treated in vitro shoots with developed root primordial were transferred on basal medium (MS medium without auxins) for further elongation of root primordial into roots. When the in vitro shoots were kept in the medium with high concentration of auxin (200 µM) for more than seven days in the first step, the developed root primordial become thick that lead to stunted growth of the roots. It was observed that two-step treatment with NAA did not show development of root primordial. The maximum percentage (53.33%) of rooting response was observed only in those in vitro shoots which were given two-step treatment in medium supplemented with IBA. Best rooting percentage was observed in 200 µM IBA supplemented MS medium.

Various concentrations of auxins (IBA, NAA and IAA) were used for pulse treatment for ex vitro root induction. None of the shoots rooted in any of the treatment tested.

Hardening was carried out in two steps, primary hardening and secondary hardening. The in vitro rooted plantlets were first washed with water so as to remove adhered agar/medium and then transferred to autoclave culture bottles containing soilrite. These plantlets were nurtured with half strength MS medium twice a week and were kept for four weeks in culture room. Secondary hardening was carried out by transferring the plantlets to mist chamber at relative humidity of
80-85% and temperature at 32 ± 2°C. The caps of the bottles were opened and the bottles were kept for 3-4 days.

After mist chamber stage plants became hardened and were shifted to open shade house condition for acclimatization of outer environment. Hardened plants were transferred to polybags containing sand: soil: FYM in 1:1:1 proportion. After mist chamber stage the plants became hardened and were shifted to open shade house conditions. During this phase the shoots elongated, leaves turned greener and expanded.

A total of 22 plants were ready to shift in shade house. After one month of transplanting all the plants in polybags kept in shade house, only 14 plants were survived. The height of the plants was recorded after 3 months. The height of plants reached up to the average height of 17.57 cm in 9 months under shade house conditions.

**IN VITRO CALLUS CULTURE**

In the present study, callus culture was raised for *Pterocarpus marsupium* for obtaining somatic embryogenesis. *In vitro* raised leaves and cotyledonary explants that were from seeds which was *in vitro* germinated on MS medium were cultured on auxin supplemented MS medium. Results illustrated that leaves obtained from *in vitro* raised shoots were not responsive in any treatment and only the cotyledons showed callus induction. Callus induction was maximum 62.22% at 9.04µM 2,4-D.

To assess the effect of auxin –cytokinin interaction on *in vitro* callus induction, 2,4-D (4.52 µM and 9.04 µM) and Kn (0.93 µM and 2.32 µM) were tried. It was again observed that *in vitro* raised leaves were not responsive for callus induction.

To find the effect of basal medium on *in vitro* callus induction, MS, B5 and White’s media supplemented with 2, 4 –D were tried. Among the three basal media MS medium was found to be best for *in vitro* callus induction. Maximum *in vitro*
callus induction (62.22%) was obtained on MS medium supplemented with 9.04µM 2,4-D. However, at no stage somatic embryogenesis could be induced in the callus.

CONCLUSION

The present investigation concluded that *Pterocarpus marsupium* could be successfully micopropagated by the pathway of axillary shoot proliferation. The objective set in the research investigation was completed by developing in vitro propagation technique for *Pterocarpus marsupium* Roxb.

Axillary buds collected from 10 year old tree of *Pterocarpus marsupium* - induced axillary shoots were multiplied, rooted and plants were produced.

A limited success has been achieved in callus culture of *Pterocarpus marsupium*. However studies on callus culture can further be utilized for organogenesis, genetic manipulation and molecular studies of *Pterocarpus marsupium*.

The findings of the present investigation proved to be noteworthy study for micropropagation of *Pterocarpus marsupium* and conservation of this economically important multipurpose tree. Further it would facilitate its use for future tree improvement programme.