SUMMARY
AND
CONCLUSION
SUMMARY

Explants from mature male tree-

Nodal sector

1. In *Ceratonia siliqua* L. nodal sectors collected in summer (of all age) responded by callus initiation at the basal cut end of NS and cut end of petiole by first to third week, on all the media used. Callus continued to grow upto ninth week on all media without morphogenesis.

2. NS of all age responded by axillary bud release by fourth to sixth week, on all the media used. By ninth week, the released buds elongated into shoots of variable length ranging from 0.7 to 2.5 cm on most of the media used.

3. NS (6 to 7 weeks old), on MSB + 0.10 mg/l BA + 0.10 mg/l Kn and MSB + 0.15 mg/l BA + 0.15 mg/l Kn, showed maximum percentage of axillary bud release.

4. NS (1 to 2 week old) showed maximum percentage of axillary bud release and elongation on MSB with 0.5 mg/l Kn.

5. NS (3 to 4 weeks old) showed maximum percentage of axillary bud release and elongation, on MSB + 0.5 mg/l BA + 0.5 mg/l Kn. Release and elongation of axillary bud was not observed on MSB + 1.0 mg/l BA + 0.5 mg/l Kn and MSB + 1.0 mg/l BA + 1.0 mg/l Kn.

6. NS (5 to 6 weeks old) showed maximum percentage of axillary bud release and elongation on MSB + 0.1 mg/l BA + 1.0 mg/l Kn.

7. On MSB + 0.5 mg/l BA + 0.5 mg/l Kn, NS (6 to 7 weeks) showed maximum percentage of axillary bud release whereas NS (1 to 2 weeks) showed maximum percentage of axillary bud elongation.

The in vitro response of NS seems to be directly related to the endogenous phenolics.

DNA, RNA and protein values of shoots with callus at the base (NS culture-incubation period 9 weeks for all) on the different nutrient media increased with increase in the conc. of BA and Kn in the medium.
Increase in DNA is associated with corresponding increase in RNA and proteins.

8. NS collected in winter responded by callus initiation at the base of NS and cut end of petiole by second to fourth week on all media used. Callus turned brown on MSB + 0.5 mg/l BA + 1.0 mg/l Kn and continued to grow up to ninth week on remaining media without morphogenesis.

9. Axillary bud was released by fourth to fifth week, on all media used. The released buds elongated into shoots of varying length from 0.8 to 2.0 cm.

10. NS showed maximum percentage of axillary bud release and elongation on MSB with 0.5 mg/l BA and 0.5 mg/l Kn.

11. Responses of NS in summer and winter showed that NS (5-6 weeks old) responded best in terms of percentage of axillary bud release and elongation on MSB + 0.5 mg/l BA + 0.5 mg/l Kn in winter.

12. NS (5-6 weeks old) collected in summer responded by more percentage of release and elongation of axillary bud on MSB + 0.1 mg/l BA + 0.1 mg/l Kn as compared to winter.

Shoot apex

1. In Ceratonia siliqua L., shoot apex from mature tree collected in summer responded by callus initiation at the basal cut end by third to fifth week, on all media except MSB, MSB with 0.1 mg/l Kn and MSB with 0.5 mg/l Kn. Callus grew on all media and shoot buds initiated from callus on MSB + 1.0 mg/l BA.

2. SA (2 weeks old) responded by release of apical bud by fifth to sixth week on all media, except MSB, MSB with 0.1 mg/l Kn and MSB with 0.5 mg/l Kn. All the released buds elongated into a shoot on all media with shoot length ranging from 0.7 to 2.23 cm.

3. SA (2 weeks old) showed maximum percentage of apical bud release on MSB + 0.1 mg/l BA + 0.5 mg/l Kn.
4. SA (5 to 6 weeks old) responded by release of apical bud by seventh week only on MSB + 1.0 mg/l BA + 0.1 mg/l Kn and MSB + 1.0 mg/l BA + 0.5 mg/l Kn. All the released buds elongated into a shoot of varying length from 0.7 to 1.5 cm.

On MSB + 1.0 mg/l BA + 0.5 mg/l Kn percentage of apical bud release and elongation was more.

5. SA collected in Winter, showed callus initiation at the basal cut end, by third to fourth week on all the media except MSB with 0.5 mg/l Kn. Callus turned brown on MSB with 0.1 mg/l Kn, 1.0 mg/l Kn and MSB with 0.1 mg/l BA + 1.0 mg/l Kn and grew on remaining media. Indirect organogenesis (shoot bud induction and development) was observed on MSB + 1.0 mg/l BA.

6. SA (5-6 weeks old) showed maximum percentage of apical bud release and elongation on MSB + 0.5 mg/l BA.

7. SA (7 weeks old) showed maximum percentage of apical bud release and elongation on MSB + 1.0 mg/l BA + 0.5 mg/l Kn.

8. From the SA (5-6 weeks old) collected in Summer and Winter release and elongation of apical bud was observed on MSB + 0.5 mg/l BA + 0.5 mg/l Kn only in Winter.

Composition of medium and response had an effect on amounts of DNA, RNA and proteins from SA culture.

Leaf explants

1. In Ceratonia siliqua L. leaf explants from mature tree collected in three seasons responded by callus initiation and growth on all the media except MSB.

2. LP collected in summer responded by callus initiation at cut ends and on surface by second to fourth week. Maximum percentage of callus initiation was observed on MSB + 0.5 mg/l NAA + 0.5 mg/l 2,4-D.

3. LP collected in rainy season responded by callus initiation at cut ends and on surface by second to fourth week. Maximum percentage of callus initiation was observed on MSB + 1.5 mg/l 2,4-D.
4) LP collected in winter responded by callus initiation at cut ends and on surface by fourth week. Maximum percentage of callus initiation was observed on MSB + 10% CM + 0.5 mg/l 2,4-D.

As response is callus formation on all the media increase or decrease in the values of DNA, RNA and proteins seems to be due to change in the composition of nutrient media.

On subculture callus either continued to grow or turned brown without morphogenesis on modified media.

Explants from mature female tree-

Nodal sector

Nodal sectors collected in summer responded by either callus initiation at the basal cut end or turned brown on media with BA, Kn and combinations of BA and Kn. Axillary bud release was not observed on any medium.

Leaf explants

Leaf explants collected in summer showed no signs of growth and turned brown on media with 2,4-D, CM and 2,4-D, CM and BA.

Response of explants from mature male and female tree are different in terms of callus formation and axillary bud release on media with same composition.

Explants from in vitro shoot

Nodal sector

1. On medium MSB + 0.5 mg/l BA + 0.5 mg/l Kn ns developed into a shoot with callus at the base up to sixth subculture. Indirect organogenesis (shoot bud induction from callus) was observed at every subculture, except at fourth subculture on same medium.

2. ns (6 and 8 weeks old) responded by callus initiation by first or second week, on all the media used except MSB. Shoot buds developed from callus on MSB + 1.0 mg/l BA, MSB + 2.0 mg/l BA + 2.0 mg/l Kn, MSB + 0.5 mg/l BA + 0.5 mg/l IAA and MSB + 1.0 mg/l BA + 0.5 mg/l IAA.
3. ns (8 weeks old) responded by release of axillary bud by first to second week on all the media. The released bud elongated into a shoot of variable length (0.7 - 2.5 cm) by fifth week on all media. Maximum percentage of axillary bud release and elongation was observed on medium with 1.0 mg/l BA.

4. ns (6 weeks old) responded by release of axillary bud by second week on all media except MSB, MSB with Kn and NAA and MSB with 1.0 mg/l Kn + 0.5 mg/l IAA. The released bud elongated into a shoot of variable length (0.5-1.5 cm) on all media by fifth week. Maximum percentage of axillary bud release was observed on medium with 1.0 mg/l BA + 0.5 mg/l IAA.

Leaf explant

Leaf explant from in vitro shoot responded by callus initiation at cut ends and on surface by second week. Callus grew and roots initiated from callus on subculture. Callus initiation was 100% on media with 1.0, 2.0 mg/l 2,4-D with 1.0, 2.0 mg/l BA, from 4 week old Ip. Maximum callus initiation was observed on medium with 15% CM + 1.5 mg/l NAA + 0.5 mg/l Kn, from 8 week old Is.

Seed Explants

Cotyledon pieces

1. In *Ceratonia siliqua* L., cotyledon pieces (irrespective of size) responded by callus initiation at cut ends and on surface by first to second week on all the media used except MSB5, MSB5 + 5.0 mg/l 2,4-D with 0.25 mg/l Kn or 0.25 mg/l 2-ip.

2. Cotyledon explants responded by direct and indirect embryogenesis on MSB + 1.0 mg/l NAA + 2.0 mg/l BA. On remaining media callus grew and on subculture of callus to different media roots initiated and developed on MSB and MSB with BA.
Embryo axis

Embryo axis responded by elongation into an axis with shoot and root ends, on both the media used. Shoot buds initiated and developed from callus at the shoot base on MSB + 0.5 mg/l BA + 0.5 mg/l Kn.

Seedling explants

Hypocotyl

In *Ceratonia siliqua* L., hypocotyl responded by callus initiation by first or second week on the four media used. Maximum percentage of callus initiation was observed on MSB + 10% CM + 0.5 mg/l 2,4-D. Callus grew on all the media without morphogenesis.

Cotyledonary leaves

Cotyledonary leaves responded by callus initiation and growth in 100% cultures on MSB + 100 mg/l CH + 1.0 mg/l 2,4-D + 0.5 mg/l BA. On subculture of the callus to media without 2,4-D and CH, it continued to grow without morphogenesis.

Seedling apex

Seedling apex elongated into a shoot with callus at the base on MSB + 0.5 mg/l BA + 0.5 mg/l Kn in 100% cultures. Shoot buds were induced from callus in 11.11% cultures.
CONCLUSION
1. Response of mature male tree explants is dependent on the season of collection of explant, age of explant and composition of nutrient media.
2. Indirect caulogenesis was obtained from shoot apex of mature tree.
3. Mature tree leaf responded by callus initiation and development.
4. Indirect caulogenesis was obtained from nodal sectors of in vitro shoots.
5. Direct and indirect embryogenesis was obtained from cotyledons.
6. Nucleic acid and protein values vary with age of explant, composition of nutrient media and specific response in terms of callus formation, organogenesis or embryogenesis.