The papaya, *Carica papaya* is one of the most widely grown fruit plant in tropics and subtropics and this plant is conventionally propagated through seeds. Also there are reports of asexual propagation of papaya using techniques like grafting, cutting etc. though of limited success. The major problem of propagation through seeds is that elite nature of the plants can not be maintained in a population because plants have open pollination system. Also, rate of seed germination is poor. Attempts were taken to overcome these problems by using techniques of *in vitro* culture of shoot buds of elite varieties. Problem was that different varieties had different cultural need; and the suitability of a culture medium published for a variety could not be used for another variety. We, for example, were not successful in getting shoot buds established following the works previously reported. So, we took an attempt to standardize the technique of clonal propagation *in vitro* of Honey Dew variety of papaya. This variety is widely cultivated in eastern and northern India due to its good quality pulp and flavour. So, the major objective of the work presented in this thesis was to standardize the technique of *in vitro* cloning of *C. papaya* var. Honey Dew. In addition, some other basic works related to *in vitro* culture of papaya shoots for cloning, or of using the callus of papaya for production of papain in culture were also done. The complete work has been divided into four chapters.
In chapter-I, the results of investigations on collection of shoot buds, treatments for controlling contamination, establishment of shoot buds in culture, multiplication, rooting and finally transplantation of plantlets to field condition have been included. In chapter-II, work on callus induction and regeneration of shoot from callus, total phenol content of callus during different stages of growth and the activity of proteolytic enzymes of leaves of in vitro grown plantlets and mother plant and of callus were included. In chapter-III, results of studies of gel electrophoretic pattern of isozymes of five enzymes were included. In chapter-IV, chlorophyll content in leaves of cloned plantlets, subjected to different treatments with cytokinins and sucrose was studied. Also, phenol content of leaves and multiplication rate and vitrification of shoots treated in different concentrations of sucrose was investigated.

Apical buds, both from fruit bearing plants and saplings and axillary buds only from fruit bearing plants were used as explants. A major constraint for the establishment of shoot buds of papaya in culture was the high susceptibility of papaya explants to bacterial contaminations. So, it was necessary to obtain contamination free explants for use in subsequent experiments. It was observed that it was necessary to spray the plants with Gentamycin (1000 mg/l) for 7 days before removing the explants. Subsequently, the shoot buds were treated with the same antibiotic (500 mg/l) in laboratory. As a result, only 43% of explants from fruit bearing plants and 69% of those from saplings could be made contamination free and those explants retained their
regeneration capacity. MS was selected for future work because efficiency of this medium in comparison to other media tested was good. Maximum number of buds remained green without any other morphological changes in this medium. Ultimately, MS was further enriched with vitamins of SH, 800 mg/l casein hydrolysate, 500 mg/l malt extract, 50 mg/l adenine sulphate and 100 mg/l inositol for inducing the establishment of explants in culture. This modified MS was designated as M-MS. 1 mg/l GA$_3$ along with 2 mg/l kinetin in M-MS supported the establishment of maximum number of buds in culture. Treatment with GA$_3$ and kinetin was necessary to shorten the establishment period to 6-10 days. The establishment period reported earlier was more. Different experiments with established shoot buds to induce multiplication were made and it was observed that a combination of 1 mg/l NAA and 3 mg/l kinetin in medium was suitable. Treatment in this medium produced a film of callus at cut end of established buds and provided a conditioning effect for multiplication of those in subsequent stages of culture in a different medium. Maximum multiplication was observed in a medium with 0.1 mg/l NAA and 0.5 mg/l BAP. So, for an effective multiplication a sequential treatment was necessary. The maximum rate of multiplication induced was 14 shoots/15 days. Most of the previous reports also showed that NAA and BAP in combination was suitable for shoot multiplication of papaya in vitro. Percentage of responding buds from saplings were always more in number than those from mature fruit bearing plants. A steady state of multiplication was being maintained for last 24 months in the laboratory and this period was much
more than most of the other works reported earlier. Rooting efficiency of
shoots obtained from buds of saplings or from fruit bearing plants were
similar. Half strength of M-MS along with 2 mg/l IBA in agar-gelled
medium was found to be suitable for induction of rooting. It was also
observed that when shoots were pretreated for 5 days in half strength of
liquid M-MS with 2 mg/l IBA before culturing those shoots for 15 days in
hormone-free liquid M-MS, shoots elongated and also formed healthy roots
within 20 days of culture. Survival of transplanted plantlets in field
was always higher when rooting was induced in liquid rooting medium.
Rainy season was most suitable for transplantation of plantlets from
laboratory to the soil. In other seasons most of the plantlets were
dessicated when exposed to outside environment.

Half strength of M-MS supplemented with 2 mg/l IBA and 0.5 mg/l
BAP was found to be best medium for inducing callus from the roots of in
vitro cloned plantlets and juvenile roots were more suitable explant for
obtaining callus. But, the problem was that callus growth could not be
maintained beyond 20-25 days in culture. Work on more callusing for an
extended period of culture can be taken up in future. It was possible to
regenerate shoots from callus in full strength of M-MS with 2 mg/l IBA
and 1 mg/l kinetin from callus derived from the roots. Callus production
from some other explants of papaya was poor. Growth value of non-
differentiating callus was significantly higher than that of the differentiating
callus. Anatomical studies revealed that root initials and shoot bud
initials were formed from the meristematic cells occurring in the
periphery of callus. Investigations of total phenol content during different stages of growth of callus revealed that phenol accumulation in callus increased with age of callus and it was maximum during stationary phase of growth. Studies of the proteolytic enzymes indicated that the younger callus contained low proteolytic activity than that in older calli. Similarly, leaves of in vitro grown plantlets contained lower amount of proteolytic activity than that in leaves of field grown fruit bearing plants. Observation of the presence of proteolytic activity in callus was interesting. Potentiality of callus to produce the enzymes can be used in a future programme of work.

Investigations on isozyme pattern of esterase, peroxidase, acid phosphatase, LAP and GOT were done following conventional techniques of disc gel electrophoresis using materials like (1) mother plant, (2) in vitro cloned plantlets and (3) plantlets transferred to soil from culture. It was observed that basic banding pattern of all those isozymes in different materials investigated was similar excepting minor differences. The differences might be due to differences in maturity of material; and indicated that apparently characters of the cloned plantlets were similar to those of the mother plant and no change was introduced due to long process of culture.

Chlorophyll content in leaves was maximum at 0.5 mg/l BAP in medium. Maximum rate of shoot multiplication was also obtained in medium with 0.5 mg/l BAP. Similarly, chlorophyll content and maximum
multiplication rate were observed in medium with 30 gm/l sucrose. Gradual reduction of sucrose from medium caused decline in chlorophyll content in leaves. Thus, sucrose starvation from medium did not stimulate chlorophyll synthesis in tissue or photoautotrophism of the plantlets. Percentage of vitrified shoots increased with decreasing sucrose concentration in medium. Maximum shoots vitrified at 0 and 10 gm/l sucrose in medium. Very few shoots were vitrified at 20 gm/l sucrose in medium and no shoots vitrified at 30 gm/l and 40 gm/l sucrose in medium. Phenol content in leaves increased with increasing concentration of sucrose in medium.