Chapter 6

SUMMARY
The present studies carried out on four indigenous cultivars of almond \textit{[Prunus dulcis (Mill.) Batsch.]} namely \textit{Parbat}, \textit{Shalimar}, \textit{Waris} and \textit{Mukhdoom}, displayed a very good potential of in vitro regeneration from almost all types of explants except leaf discs. Marked differences were observed in in vitro nature of explants from juvenile and mature trees in all the cultivars under reference.

Maximum sterilization of explants taken from field grown trees e.g. shoot tips and seeds was achieved by using a solution containing HgCl$_2$ (0.2g) and NaOCl (1ml) per 100ml of DDW for 20-25 minutes. In case of forced shoots 7\% solution of Ca(OCl)$_2$ was used for 15 minutes and for embryos a solution containing HgCl$_2$ (0.05\%) plus NaOCl (1ml) per 100ml of DDW proved to be most effective. All the sterilizing solutions also contained Tween-20 (0.05ml). The pres-sterilized explants (shoot tips and nodal segments) established well on modified MS (1962) medium with BAP (1.5, 2.0 and 2.5\mu M) either alone or in combination with IBA (0.5 and 1.0\mu M). Amongst all the four cultivars used, cv. \textit{Parbat} showed the maximum rate of in vitro establishment on BAP (2.5\mu M) and IBA (0.5\mu M) combination, followed by cv. \textit{Shalimar} on BAP (1.5\mu M) and IBA (0.5\mu M) combination and cv. \textit{Mukhdoom} and cv. \textit{Waris} on BAP (2.0\mu M) only. Further, explants from nursery grown plantlets showed healthy percentage of in vitro establishment as compared to explants from cultivated mature (10 years old) trees.
Among three different growth media tested viz., Modified MS (1962), QL (1977) and AP (1986) for shoot proliferation in all the 4 almond cultivars under study. Modified MS (1962) medium in fortification with BAP (4.5μM) proved best for cv. Parbat and Waris followed by QL (1977) media with BAP (3μM) for cv. Shalimar and AP (1986)/QL(1977) medium with BAP (4.5μM) for cv. Mukhdoom. The elongation of microshoots in all the cases was achieved on their respective shoot proliferating basal media either in presence or in absence of BAP (0.5μM).

Normal in vitro seed germination of all the four cultivars was achieved by culturing the peeled off (without testa) kernels on MS (½) basal medium within 4 weeks. Addition of PGR’s to MS basal medium decreased the germination percentage. Further, a chilling period of 40 days at 4°C was necessary for normal in vitro seed germination without which seedlings showed shoot tip necrosis.

The juvenile explants like internodal segments, hypocotyl segment and root segments from in vitro born seedlings were cultured for in vitro regeneration of shoots. Direct shoot regeneration from juvenile internodal segments (of all the four cultivars) was achieved on MS (½) medium with either NAA (10μM) or a combination of NAA (2.5 and 5μM) and BAP/Kn (5.0μM) or NAA (2.5) and BAP/Kn (7.5μM). While shoot regeneration from internodal segments of mature trees was observed on MS (½) medium with NAA (2.5 and 5μM) and BAP/Kn (5 and 7.5μM). Similarly the hypocotyl segments from in vitro germinated seedlings differentiated into multiple shoots when cultured on MS (½) medium with BAP (2.5, 5.0 and 7.5μM) and NAA/IBA/2,4-D (5 and 10μM). The in vitro born root segments produced callus of different nature (friable and nodular) on MS (½) medium with or without glutamine (0.5g/l) and either BAP (2.5μM) in combination with NAA/IBA(5μM) or BAP (5μM) in combination with NAA/IBA/2,4-D (10.0μM) in all cultivars. Only 10% of indirect shoot
regeneration was recorded from root segments of cv. Parbat upon subculturing on MS (1/2) medium with BAP (5\mu M) and IBA (10\mu M).

Cotyledons of both immature and mature almond from all the four cultivars were cultured under in vitro condition for shoot and root regeneration. Immature cotyledon showed maximum (60.00% for cv. Waris) and fast shoot and root regeneration response on MS (1/2) medium with BAP and IBA / NAA combination. Shoot regeneration was observed on BAP (2.5, 5, 7.5, 10 and 20\mu M) in combination with IBA (2.5, 5, 7.5 and 10\mu M) or BAP (5, 10 and 20\mu M) in combination with NAA (5 \mu M) in cv. Shalimar. In other cultivars shoot regeneration with varied percentage was also achieved on such phytohoromonal combination. Similarly root regeneration in all the four cultivars with different percentage was also observed on MS (1/2) medium supplemented with various growth adjuvants like IBA (10\mu M) or BAP (20\mu M) and IBA (10\mu M) or NAA (5 and 10\mu M). Contrary to this the cotyledons from mature almonds showed very low regeneration potential. Only 10-20% shoot regeneration was recorded on MS (1/2) medium with various growth adjuvants as BAP (5 and 7.5\mu M) and IBA (2.5\mu M) or BAP (7.5\mu M) and IBA (5\mu M) or BAP (2.5\mu M) and IBA (10\mu M) or NAA (10\mu M) or NAA (2.5 and 10\mu M) and Kn (1\mu M) or NAA (2.5\mu M) and Kn (5 and 10\mu M).

Leaf discs from in vitro sprouted shoots of all the 4 cultivars of almond produced either nodular or friable callus on MS (1/2) medium fortified with BAP (3.5, 4.5, 7.5, 10 and 20\mu M) in combination with NAA/IBA (0.5, 1.5, 2.5, 5.0 and 10\mu M). All these calli failed to differentiate into either shoots or roots or both, after subculturing either on same callus proliferating media or on MS (1/2) medium with BAP (0.5\mu M) or on MS (1/2) basal medium even after 4 week.

Embryos, both mature and immature of almond cultivars under study were cultured on MS (1/2) medium with BAP, IBA and GA3 combinations. Multiple shoots were produced directly from 40 day chilled (4°C) mature
embryonal axes in all cultivars with maximum percentage (66.66%) and average shoot number (8) in cv. *Mukhdoom*. In other cultivars under reference the regeneration percentage was comparatively low and average shoot number ranged from 6-7 on MS (1/2) medium with BAP (2.5μM) and IBA (1.0μM). Addition of GA$_3$ (0.5μM) to this medium decreased the regeneration percentage. Immature embryos of all the cultivars from 90-120 days after pollination (DAP) were also cultured on MS (1/2) medium with BAP (2.5, 5.0, 7.5 and 10μM) and IBA (2.5 and 5.0μM).

Embryos produced nodular callus first on BAP (7.5 and 10μM) and IBA (2.5 and 5.0μM) combination. The nodules later on differentiated into shoots on the same callus proliferating medium without subculturing after 8 weeks with varying percentage and shoot number on different combinations. Maximum shoot regeneration percentage with 10-12 adventitious shoot number in all the cultivars was achieved on BAP (7.5μM) and IBA (2.5μM) combination followed by BAP 10.0μM and IBA (2.5μM) combination. Lower combinations of BAP (2.5 and 5.0μM) and IBA (2.5μM) produced non-regenerative callus in all the cultivars.

Rooting trials in all the four cultivars were tried by employing three different methods viz. long dip, short dip and quick dip. The 2-4 cm long in vitro born shoots either from shoot tip proliferation or from cotyledon regeneration or from embryo regeneration were cultured for induction of in vitro rooting. Both long dip and short dip methods proved successful for all the cultivars under reference. In long dip method 60% rooting percentage was achieved by covering the lower portion of culture tubes with black paper on MS (1/2) medium enriched with IBA (2.5μM) for all the cultivars except cv. *Waris*. Here both root initiation as well as elongation were achieved on same medium after 4 weeks, while 80% of root induction in all the 4 cultivars was recorded on IBA (3.0μM) fortified MS (1/2) medium followed by root elongation on MS (1/2) basal medium after 4 weeks. Rooting percentage got decreased in cv. *Parbat*, while it was completely
absent in other cultivars with IBA (5.0μM). Rooting was completely absent in all the four cultivars on different concentrations of NAA (2.0, 2.5, 3.0, 5.0 and 10.0μM) after 4 weeks.

In short dip method only cv. *Parbat* and cv. *Shalimar* produced 6-8 white roots with 90 and 70 rooting percentage under dark incubation in water agar with IBA (0.2g/l or 0.1mM) for 18hrs, followed by subculturing on MS (½) basal medium after 4 weeks. The remaining two cultivars cv. *Waris* and cv. *Mukhdoom* totally failed in rooting response.

In quick dip method of rooting in vitro born shoots from none of the 4 cultivars produced roots in which their basal ends were dipped in IBA/NAA (1.0g/l or 5.0mM) solution for 5, 7 and 10min followed by subculturing on MS (½) basal medium for 4 weeks.

In general cv. *Parbat* and cv. *Shalimar* showed the highest rooting percentage, followed by cv. *Muckhdoom* under different treatments as mentioned above. Cultivar *Waris* proved to be recalcitrant for in vitro rooting as compared to other cultivars under study. Finally cv. *Parbat* and cv. *Shalimar* showed the best rate of acclimatization on peat moss: vermiculite (1:1) mixture after 3 weeks, followed by cv. *Mukhdoom* and cv. *Waris*. Among all the four cultivars the chronological order of plantlet survival from high to low was found to be as cv. *Parbat*, cv. *Shalimar*, cv. *Mukhdoom* and cv. *Waris*. 