IN VITRO APPROACHES TO THE ESTABLISHMENT AND MULTIPLICATION OF TEAK (*TECTONA GRANDIS* L.)

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By

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TO MY

LOVING PARENTS &

MY SINCERE TEACHERS

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CERTIFICATE

This is to certify that the research work entitled **"*In vitro* approaches to the establishment and multiplication of teak (*Tectona grandis* L.)"** described in this thesis by **Mr. Muhammad Akram** is an original work of the author and has been carried out under my direct supervision. I have personally gone through all the data, results materials reported in the manuscript and certify their correctness and authenticity. I further certify that the material in this thesis has not been used in part or full in a manuscript already submitted or in the process of submission of partial or complete fulfillment of the award of any other degree from an institution. I also certify that the thesis has been prepared under my supervision according to the prescribed format and I endorse its evaluation for the award of Ph.D degree through the official procedures of the University of the Punjab, Lahore.

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ABBREVIATIONS/UNIT ABBREVIATIONS

µM	Micro Molar
µm	Micrometer
µmol $m^{-1}s^{-1}$	Micromole per meter per second (Light intensity)
2, 4-D	2, 4-Dichlorophenoxyacetic acid
2iP	Isopentenyl adenine
8-HQC	8-hydroxyquiniline citrate
ABA	Abscissic acid
ABT	1-aminobenzotriazole
Ads	Adenine sulphate
BA	N^6-Benzyleadenine
Cm	Centimeter
cm^2	Square centimeter
cm^3	Cube Centimeter
CMF-PT	Changa Manga Forest Plus Trees
CMF-TS	Changa Manga Forest Teak Stand
CPPU	N_1-(2-chloro-4-pyridyl)-N_3-phenylurea
CrO_3	Chromic acid
df	Degree of Freedom
DKW	Driver and Kuniyuki (1984) medium
DMRT	Duncan's Multiple Range Test
DW	Dry weight
EDTA	Ethylene diamine tetraacetate
FW	Fresh weight
g	Gram
g^{-L}	Gram per liter
GA_3	Gibberellic acid
h	Hour
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulphuric acid
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IAAsp	Indol-3-acetyl-aspartic acid

IBA	Indole-3-butyric acid
IPA	N^6- (2-isopentyl) adenine
Kin	Kinetin
KNO_3	Potassium Nitrate
L.	Linneus
LSD	Least Significant Difference
M	Molar
mg	milligram
min	Minute
ml	Milliliter
mM	Milli molar
mm	Millimeter
MS	Murashige and Skoog (1962) basal medium
MW	Molecular weight
NAA	Naphthaleneacetic acid
NaOCl	Sodium hypochlorite
NaOH	Sodium Hydroxide
Ø	Diameter
PGRs	Plant Growth Regulators
pH	Hydrogen ion concentration
ppm	parts per million
PUBG	Punjab University Botanical Garden
PVP	Polyvinyl polypyrolidone
RH	Relative humidity
SE	Standard Error
SWS	Softwood shoots
TDZ	Thidiazuron (*N*-phenyl-*N'*-1,2,3-thidiazol-5-ylurea)
UV	Ultra violet light
V/V	Volume per volume
W/V	Weight per volume
WPM	Woody plant medium
$\bar{x}$	Mean
$\bar{\bar{x}}$	Grand mean
Zt	trans-Zeatin

SUMMARY

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Propagation and multiplication of teak, *Tectona grandis* L., was investigated by using the conventional methods of seed germination and rooting of cuttings, and *in vitro* techniques of callus induction and plant regeneration. Teak fruits were collected from different provenances and graded on the basis of their size. Most of the fruits were of 6-8 mmØ. A total mean number of fruits of 2560 ± 89.5, 2870 ± 71.1 and 3356 ± 105.5 per kilogram were collected from CMF-TS, CMF-PT and PUBG, respectively. The large-sized fruits of mean diameter of ≥17mmØ were heavier ($\bar{x}$=0.58 ± 0.02 g) than the smaller ones (12-16mm, 9-11mm or 6-8mmØ). Most of the fruits were one-seeded as analyzed by X-rays radiography collected from either fruit size grade. After fresh water scarification of seeds, the mean seed germination under nursery conditions was 44.3 percent. The rate of *in vitro* seed germination was 100 % on MS medium supplemented with 0.22 µM N^6-benzyladenine (BA). After 40 days of culture, the *in vitro* germinated seedlings had 4.4 ± 1.2 cm long shoots and 4.7 ± 1.3 cm long roots. On MS medium with 0.8 µM thidiazuron an average of 27.4 ± 4.8 shoot buds were formed on intact seedlings, which further developed into shoots. Mature shoot cuttings were also used for developing a simple and reproducible method for clonal propagation of teak. For this purpose, mature shoot cuttings were treated with IBA or NAA or in combination. After 60 days of planting, the best rooting (80 %) was obtained with a combination of 14.76 + 14.76 mM (IBA + NAA) as compared with 4.92 IBA or 9.84 mM NAA. Softwood shoots were forced from latent epicormic buds on various media under various environmental conditions. It was found that continuous light (35µmm^{-1}s^{-1}) at 25 ± 2 °C enhanced softwood shoot production ($\bar{x}$=2.7 ± 1.3) as compared with glasshouse ($\bar{x}$=2.1 ± 1.0) or natural environment ($\bar{x}$=1.8 ± 0.9). Potting medium had a significant role in

softwood shoot production; the highest ($\bar{x}$=3.4 ± 0.4) shoots were formed on coco peat as compared with sand or sawdust during autumn season after 38 days of initial potting. The forcing solution of 8-hydroxyquinoline citrate, 2 % sucrose, 10 µM TDZ in combination with 2 µM each of IBA and gibberellic acid was effective in shoot forcing from dormant shoot tips with 56.6 % success. Softwood shoots were used for further studies on rooting and axillary shoot proliferation. Softwood cuttings treated with IBA + NAA (3000 + 3000 µM) had the highest rooting (89.3 %) with an average of 5.5 ± 0.05 roots. Addition of glutamine and PVP were equally effective with 60 % establishment of shoot apices on MS medium containing 10 µM BA and 5 µM NAA and produced on average 43 ± 3.6 shoots. In the present study, as compared with other auxins (2, 4-D, Dicamba, Picloram or NAA), thidiazuron (0.1 or 1 µM) was found to be highly effective for callus induction, the highest (100 %) callus induction was obtained after 35 days from shoot-tip explants taken from forced-softwood shoots. Calluses were creamy, nodular and embryogenic with high proliferation. After 63 days of the culture, 100 % of the calli underwent somatic embryogenesis with an average 36.4 ± 6.6 globular somatic embryos on MS medium supplemented with 8 µM TDZ, 2.2 µM BA and 5mM ascorbic acid. The rate of shoot regeneration was 100 % with an average of 16.4 ± 2.6 after culture up to 120 days on the same medium. Shoots were elongated and rooted (70 %) on half-strength MS medium supplemented with 8 µM IBA and 8 µM NAA, and were acclimatized successfully under field conditions. In another experiment, highest rate of callus induction (100 %) was achieved from cotyledon as compared with that derived from node, internodes or leaf explants of *in vitro* germinated 50 day old seedlings. For callus induction MS medium was better than Woody Plant Medium (WPM). Thidiazuron (1 or 4 µM) gave highest callus induction response (93.3 ± 8.2 %) as compared with woody plant medium (WPM) for callus induction. Thidiazuron (1 or 4 µM) induced

highest callus induction response of 100 %. Thidiazuron was better than the other phytohormones, 2, 4-D, Dicamba, NAA and Picloram. A high frequency of shoot regeneration (100 %) was obtained on medium supplemented with a combination of 8 μM Thidiazuron and 0.5 μM IBA; also the highest number of shoots ($\bar{x}$=42.3 ± 4.2) was obtained on the same medium after 120 days of culture. Shoots were elongated by transferring them on half-strength MS basal medium supplemented with IBA and NAA. Best rooting ($\bar{x}$=75.4 %) was obtained on medium containing 8 ft M IBA and 8 ft NAA after 35 days of culture; a mean number of 1.6 ± 0.7 and mean root length of 3.2 ± 0.1 cm. The rooted shoots were transferred to plastic pots or trays filled with mixer of sand : soil : peat moss of 2 : 1 : 1 and kept in a growth room at 25 ± 2 °C for a month and then shifted to a glasshouse at 28 ± 5 °C for an another 28 days. The plants in baby glass jars were covered with polythene sheet to maintain high relative humidity, and after 28 days these were transfered to soil in large plastic pots (25 x 30 mm). After 4 months of growth, these were planted in field plot in botanical garden. The results showed that *in vitro* plant regeneration can be used to complement the conventional propagation of teak from seed and rooted cuttings.