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Abstract

Teak (Tectona grandis L.) is amongst the precious tropical trees produces luxurious wood items and metabolites of great medicinal value. However, teak growth is hampered due to high dormancy and poor viability of the seeds under the QRUPDO ¿HOG FRQGLWLRQV +HUH ZH LQYHVWLJDWHG WKH HIIHFW RI YDULRXV F\ In vitro and ex vitro conditions of seedlings. Completely randomized design was used and data were analyzed to ascertain whether or not the tested cytokinins can improve seed germination and growth of the teak plants. Seeds were cultured on MS medium supplemented with various concentrations (0.08, 0.22, 0.35, 0.80, 2.20 or 3.50 μM) of adenine sulphate (ADS), N6-benzyleadenine (BA), kinetin (KIN), thidiazuron (TDZ), and zeatin (ZEA) under In vitro conditions for 40 days. Highest (100%) germination was obtained at 0.22 μM BA with 4.41 cm shoot and 4.67 cm root lengths as well as 75.32% highest seedling's survival was observed in glasshouse. TDZ inhibited seedling's growth and induced hyperhydricity (16.36–35.36%). Improved growth of seedlings in both conditions may be linked with enhanced production of chlorophyll (Chl) both In vitro (1.86 Chl a, 1.30 Chl b mg/g FW) and in acclimatized seedlings (1.91 Chl a, 1.70 Chl b mg/g FW) at 0.22 μM BA. The same concentration of BA also produced highest soluble proteins in In vitro (7.52 mg/g FW) and DFFOLPDWL]HG VHHGOLQJV PJ J): 8QGHU VSHFL¿F WLVVXH FXOWXUH FRQG growth of teak seedlings can be improved with BA.

Keywords: Chlorophyll; Hyperhydricity; In vitro seedlings; N^6-benzyleadenine; Teak

Introduction

Teak (Tectona grandis L.) is a well-known timber producing deciduous tree belonging to family verbenaceae. It grows well in tropical parts of Myanmar, India, Laos and ailand and is considered among top ve tropical hardwood species in terms of area established worldwide [1]. It plays an important role in the timber industry due to its luxury applications. For example, it is used for making doors, window pans, plywood, furniture as well as water boats and ships. It provides highly demanding wood that makes it most expensive in the timber industry of the world. Teakwood is extremely resistant against termite and fungal attack due to the presence of various repellents, for example, naphthoquinones is reported to have antifungal activity [2]. Moreover, teakwood also contains lapachol [3] which has recently been demonstrated as an anticancer agent [4]. Endogenous plant hormones play fundamental role in the growth and development throughout the life cycle of plants. e level of such hormones decreases with the age of plant tissues [5]. e quiescent zone of seeds is a target site of phytohormones for germination and plant growth [6]. In vitro seed germination depends upon the physiology and viability of seeds [7]. Our previous experience on teak (Tectona grandis L.) showed 10-15% germination under eld conditions which further decreases with the passage of time during storage. is may be due to the lack of some important growth factors in the seeds; exogenous cytokinins may substitute the lost features of seeds for enhanced germination when cultured In vitro [6]. Cytokinins in uence various physiological processes essential for In vitro germination, growth and development of plant [6,8]. Adenine derivative cytokinin N^6-Benzyladenine (BA) promotes the growth of In vitro shoot as well as improved seed germination in di erent plants such as Citrus reticulata [9] and Lotus corniculatus [10] by enhancing protein and photosynthetic pigments [11].

Hyperhydricity is a water logging condition in plants [12] caused by various factors including gelling agent and nitrogenous compounds. Various cytokinins and their concentrations are also responsible to induce this disorder [13,8]. Under such conditions, developing shoots lose chlorophyll and soluble protein contents which may eventually prevent the plant growth [14]. Prevalence of hyperhydricity is more frequent when phenylurea derivative TDZ was used in the medium [13].

Renewed interest in teak wood around the world in the recent years has received attention of researchers for its multiplication using diverse means to meet the requirements of the timber industry. However, limited number of seed production and recalcitrant nature of mature tissues are major bottlenecks for its rapid multiplication. Plant tissue culture o ers a solution by direct manipulation of seeds or vegetative tissues under In vitro conditions. e use of cytokinins for both In vitro seed germination enhancement and changes in growth is an interesting research aspect [7]. ere is no previous information vis-à-vis e ect of cytokinins on seed germination and seedling growth of teak in In vitro developing and acclimatized seedlings as well as transplantation in glasshouse. e aim of the present study was therefore to elucidate the e ect of cytokinins on In vitro seed germination and growth of teak seedlings under both In vitro and ex vitro glasshouse conditions.

Materials and Methods

Plant material

Mature dried drupes were collected from Changa Manga Forest, Kasur District, Punjab, Pakistan (31°05 cN 73°58 cE). Locular seeds were extracted out by breaking down both the outer exocarp as well as the inner hard bony endocarp by carefully tapping with a suitable iron

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hammer. Extracted seeds from various trees from the above location were pooled and used in germination experiments.

Culture conditions

Fleshy locular seeds were surface sterilized with 6% (v/v) solution of Sodium hypochlorite (NaOCl) containing 0.1% Tween-20 for 10 min followed by at least five rinsing with autoclaved distilled water under aseptic condition. The pH of all culture media was adjusted to 5.8 solidified with 0.8% agar (Agar Technical, Oxoid, UK) and autoclaved at 121 °C at 104 kPa for 15 min. All cultures were incubated in growth room under 16 h photoperiod (35 ± 5 µmol $m^{-1}s^{-1}$) fitted with white fluorescent tube lights (Philips, 40W, Pakistan) at 25 ± 2 °C with 44 % relative humidity of growth room.

In vitro seed germination and growth of seedlings: Healthy seeds were inoculated in culture vessels (25 x 150 mm, Pyrex, Germany) containing 10 ml agar-solidified MS [15] medium with 3% sucrose supplemented with Sigma-Aldrich grade adenine sulphate (ADS), N^6-benzyleadenine (BA), kinetin (KIN), zeatin (ZEA), and thidiazuron (TDZ) at various levels (0.08,0.22,0.35,0.8,2.2 or 3.5µM). Medium without cytokinins was considered as control. The data for percent seed germination were recorded after 40 days of initial culture. Shoot as well as root length (cm) was also recorded on the same day of harvesting.

Hyperhydricity

The hyperhydricity was calculated according to Kadota and Niimi (2003) by using the following formula:

$$\text{Hyperhydricity \%} = \frac{\text{Number of hyperhydric seedlings}}{\text{Number of normal seedlings}} \times 100$$

The percentage of hyperhydricity (vitreous, chlorophyll deficient water logging shoots) was recorded on the same day (40) of harvesting. Green shoot with normal growth was considered as morphologically normal seedlings.

Acclimatization and survival of seedlings

Seedlings were immersed in 1% (v/v) fungicide (Dithane M-45, Dow AgroSciences, USA) for 30 sec and planted in poly-cups filled with peat moss + sand + soil (1:1:1) in glasshouse at natural day/night low light conditions (67 µmol $m^{-1}s^{-1}$) at 27 ± 2 °C with 60 ± 5% relative humidity. The survival percentage of seedlings was calculated after 40 days of transfer in the glasshouse by the following formula.

$$\text{Survival in glasshouse (\%)} = \frac{\text{Survived seedlings}}{\text{Total seedlings planted}} \times 100$$

Biochemical analysis (determination of soluble proteins)

A sample of 0.1 g leaves from 40-day old In vitro and acclimatized seedlings grown on different concentrations of each cytokinin were collected for extraction and determination of total soluble proteins (TSP) following the method of Premkumar et al. [16]. Leaf tissues were crushed in pestle and mortar in liquid nitrogen to a fine powder and homogenized in extraction buffer and centrifuged at 13,000 rpm for 15 minutes. The supernatant was separated and TSP were determined spectrophotometrically (HITACHI U-1100) at 750 nm. After the extraction, quantitative analysis of TSP was performed according to the method of Bradford [17].

Physiological analysis (determination of chlorophyll contents)

For determination of the chlorophyll a (Chl a) and chlorophyll b (Chl b), plant material as described above was collected and ground in pestle and mortar to a fine pulp in 80% acetone and centrifuged (Sorvall® RC-5B) at 5,000 rpm for 15 min. The supernatant was collected and concentration of Chl contents was determined with spectrophotometer on the basis of mg/g fresh weight (FW) of tissue according to Premkumar et al. [16].

Experimental design and data analysis

Completely randomized design was used of 5 cytokinins with 7
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4). Generally, survival percentage of acclimatized seedlings was low with high concentrations of cytokinins. Seedlings grown on BA were healthy and vigorous with large leaves and stout stems irrespective of seedlings on TDZ hardly survived or very low survival frequency (5.21 to 10.22%) was obtained (Figure 2c).

Chlorophyll and TSP contents

Generally, the amount of photosynthetic pigments was signi cantly improved by increasing the concentration of each cytokinin In vitro (Figure 5) as well as in glasshouse growing plants (Figure 6). Both Chl a (1.86 mg/g FW) and Chl b (1.30 mg/g FW) were highest at 0.22 µM BA from In vitro seedlings as compared to control (Chl a 0.86, Chl b 0.27 mg/g FW). Seedlings on TDZ and ZEA had least amount of photosynthetic pigments. Similarly, acclimated seedlings for 40 days in the glasshouse also had more chlorophyll pre-cultured on all cytokinins especially BA and ADS (Figure 4). Highest amount of Chl a (1.91 mg/g FW) and Chl b (1.70 mg/g FW) were recorded from plantlets previously grown at 0.22 µM BA as compared to control (Chl a 0.91 and Chl b 0.40 mg/g FW). However, TDZ and ZEA were least e ective produced chlorophyll contents in the acclimatized seedlings.

e amount of TSP was highest in acclimatized as compared to In vitro seedlings (Figure 6). Generally, amount of TSP was signi cantly improved by increasing the concentration of all cytokinins. In case of In vitro seedlings, BA at 0.22 µM produced highest TSP contents (7.52 mg/g FW) as compared to other cytokinins and control (2.35 mg/g FW) followed by 4.12 mg/g FW TSP with ADS (0.35 µM). TSP contents in acclimatized plantlets were also highest at 0.22 µM BA (8.88 mg/g FW) followed by 5.63 mg/g FW at 0.35 µM ADS. iourea derivative TDZ and ZEA at 3.50 µM were least e ective for production of TSP, i.e., 2.88 and 3.75 mg/g FW, respectively than other cytokinins, albeit higher than control (2.75 mg/g FW).

Discussion

In vitro seed germination is an important aspect for axenic seedling production under the in uence of various plant growth regulators (PGRs) for subsequent micropropagation and organogenesis of woody and non-woody plants. e present investigation clearly showed that germination of teak seeds was signi cantly improved with di erent cytokinins under In vitro conditions. Our previous report demonstrated the likelihood of teak seed germination on MS basal medium [18]. In the present study, we achieved improved rate of germination with cytokinins.

Signi cant relationship exists between endogenous hormones and their action on the targeted developmental seed germination loci. Exogenous application of cytokinins plays signi cant role by stimulating speci c metabolic activity for enhanced germination and seedling growth [6,19]. In the present study, BA was most e ective as compared to ADS, ZEA, KIN, and TDZ for In vitro seed germination. Existing information vis-à-vis the use of cytokinins added in tissue culture medium for In vitro seed germination of trees is scanty. However, Bhattacharya and Khuspe [20] obtained 70% In vitro seed germination with BA in Carica papaya. Stewart and Kane [21] reported 47.2% In vitroseed germination in Habenaria macroceratitis with KIN (1 µM). Dutra et al. [22] and Samuel et al. [23] also demonstrated the similar