IN VITRO PROPAGATION OF *PARKIA BIGLOBOSA* (JACQ.) BENTH USING COTYLEDONARY NODES

*A. O. AKINYELE and I. N. ABDULLAHI
Department of Forest Resources Management,
University of Ibadan, Nigeria.

Email: akinyelejo@yahoo.co.uk

INTRODUCTION

• *Parkia biglobosa Jacq Benth* popularly called African Locust Bean is a multipurpose tree belonging to the family Leguminaseae.

• The tree is widely distributed on the sandy loam soils of Sudan and Guinea savannah areas of Nigeria.

• It is known to spread across the semi arid zone of sub-saharan Africa from Senegal to Sudan.

• *Parkia* tree is threatened and has been listed among the trees for *in situ* conservation.

OBJECTIVES

The aims of this study are to:

- Propagate Parkia *in vitro* using explants for multiple shoot induction from locally preserved seeds.

- Determine the plant hormone and concentration that induces more shoot proliferation

- Observe the type and age of explant that induces rapid shoot multiplication of this woody species.

PLATE 1: Mature tree, pods and seeds of *P. biglobosa*

METHODOLOGY

Study Location

The study was carried out in the Tissue Culture Laboratory of National Centre for Genetic Research and Biotechnolgy, (NACGRAB), Moor Plantation, Ibadan, Nigeria.

Seed Source

- *Parkia biglobosa* seeds were preserved in woven sack stored in earthen pots in a cool dry area.
- Batch of the seeds stored for 3 years was collected from Lafiagi, Kwara State, Nigeria.
- The seeds were identified as those of *Parkia biglobosa* (Jacq.) R.Br. Ex G.Don at the Herbarium of National Centre for Genetic Research and Biotechnolgy, (NACGRAB), Moor Plantation, Ibadan.

Seed Scarification and Disinfection

•Seeds of *Parkia biglobosa* were washed thoroughly using detergent under running tap water to remove dirt particles from the seed coat.

•Three hundred (300) seeds were thoroughly rinsed and soaked in concentrated H_2SO_4 for 30 minutes and rinsed in four changes of distilled water to remove the traces of acid.

•Scarified seeds were surface sterilized by soaking in 70% ethanol for 5 minutes after which the chemical was decanted.

• The seeds were disinfected in 15% solution NaOCl, (Jik) with two drops of teepol detergent as a wetting agent for 5 min, followed by 4 successive rinsing and left in the fourth rinse to soften.

• Only seeds that settled at the sterilization jar bottom were used to ensure viability.

Explant preparation

• Treated *Parkia* seeds were placed in a Petri-dish for 24 hours. The seeds had 100% germination.

•3000ml Woody Plant Medium (WPM) with pH 5.7 was mixed with 18.0gL^{-1} of agar.

• The media was dispensed into 200 sterilized test tubes at a rate of 15 ml/test tube and allowed to solidify.

•Two hundred (200) germinated *Parkia* seeds were introduced in pure Woody Plant Medium (WPM) at one seed per test tube.

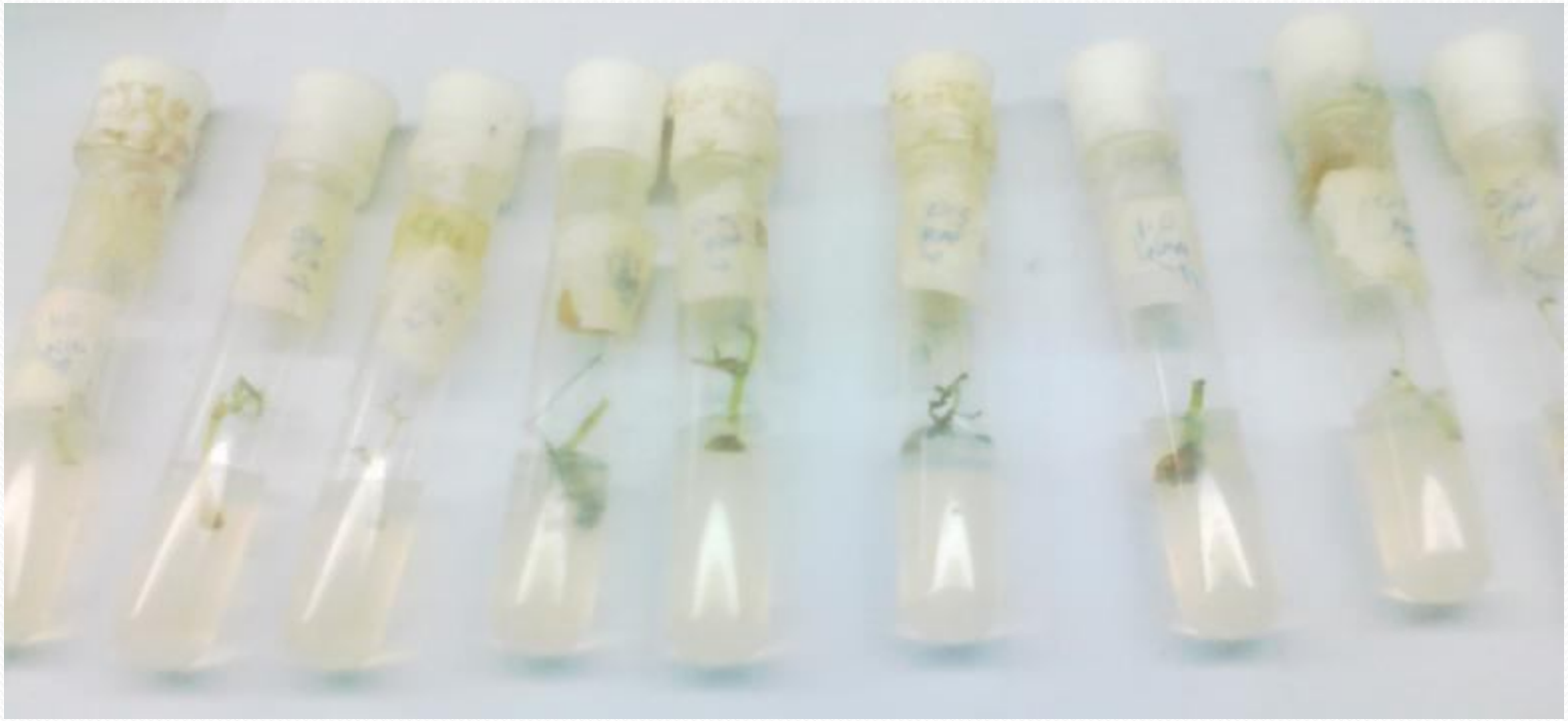
Plant Growth Media

- 8 and 10 weeks old cotyledonary nodes (the axillary and the apical) of two length types (1 and 2 cm) were introduced into modified WPM.
- WPM was modified with 3% sucrose and
    a) 6-benzylaminopurine (BAP)
    b) Kinetin (KIN) at 0, 0.5, 0.75, 1.0mgl^{-1} were used.
    c) 1-naphthaleneacetic acid (NAA)
- Concentration of each hormone was 0.5, 0.75 and 1.0mgl^{-1}.
- Four replicates of explants were cultured with a single explant per culture tube for the apical and the axillary nodes.
- The tubes were stored at room temperature of 27±1^{o}C under a photoperiod of 16 h day and an incidental light of 101.4 µmoles.$m^{-2}s^{-1}$.
- Measurement was taken at weekly interval for four weeks.

Data Collection and Analysis

- The experimental design is a 2x2x3x4 factorial experiment in a Complete Randomized Design (CRD).
- The factors are
  - The plant parts - 2
  - Age of the explants- 2
  - Hormones - 3
  - Concentrations of the Hormones - 4
- The number and length of the shoots, and the number of nodes of the cultured explants from the treatments were measured and subjected to statistical analysis (ANOVA).
- Significant means were separated using Least Significance Difference (LSD).

PLATE 2: Eight-week old Axillary and apical nodes 2 weeks after inoculation.



RESULTS

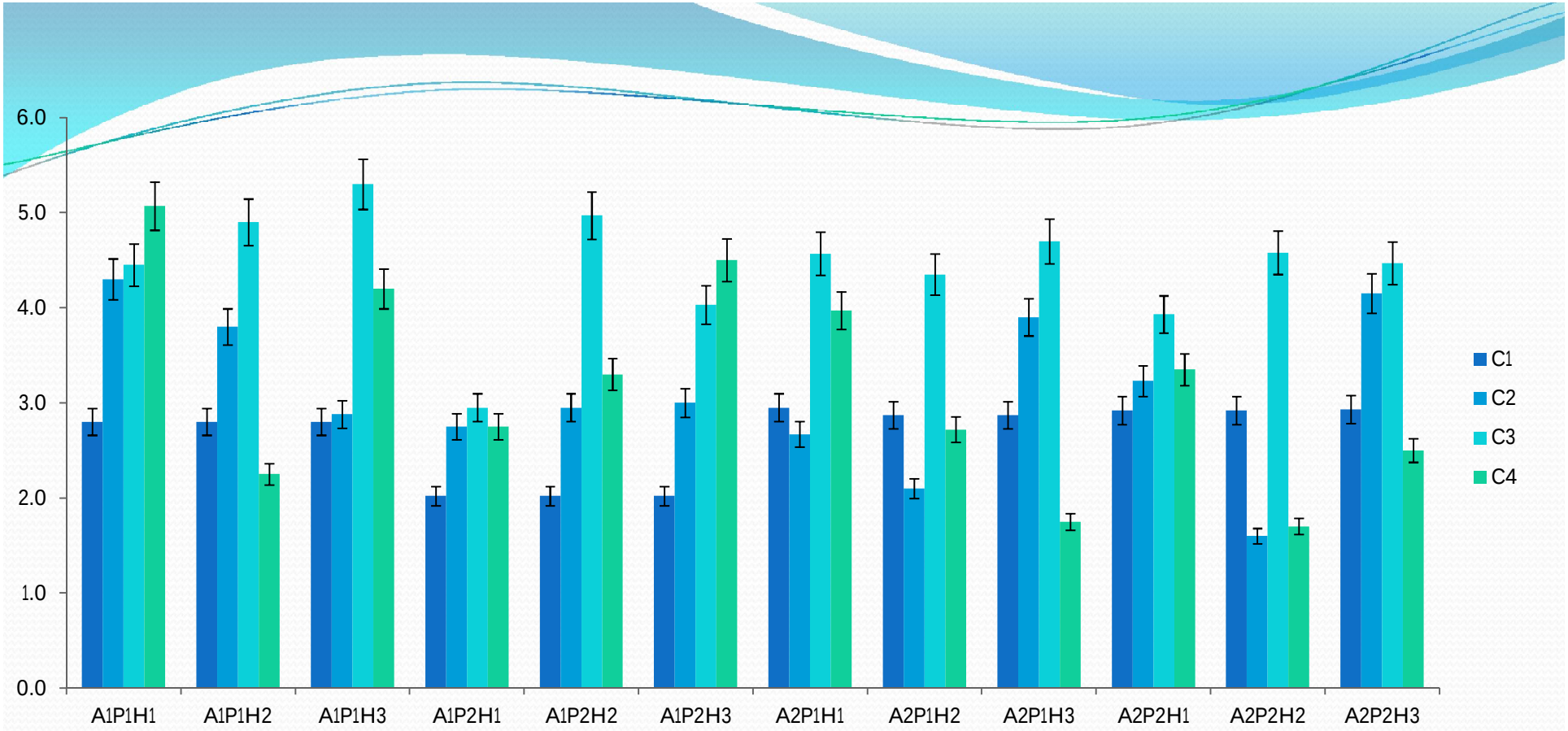
Effects on shoot length

- Shoot initiation was affected by age (8 and 10 weeks old explants), plant parts (axillary and apical nodes), hormones (NAA, KIN and BAP) and concentrations of the hormones (0.0, 0.5, 0.75, and 1.0 all in mg/L).

- Hormones and plant parts affected the growth of shoots from the nodal explants of *P. biglobosa*.

- Although many of the explants produced shoots, the shoot length varies according to treatment combination and affected its length in different ways.

- The results indicated that the concentration of the hormones had a significant effect on the shoot length (Figure 1).



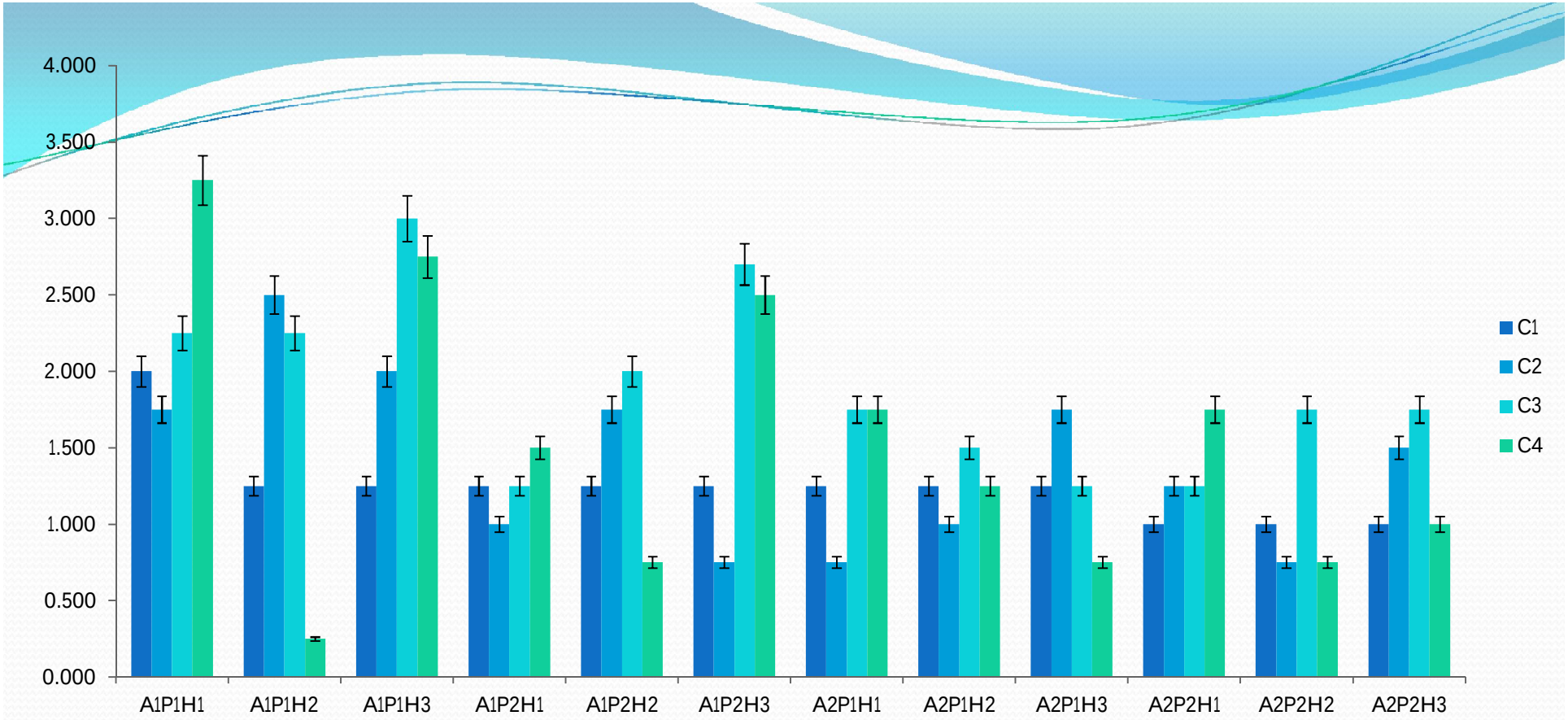
A1= 8 weeks old explants, A2= 10 weeks old explants, P1= Axillary nodes, P2= Apical nodes,
H1= NAA, H2= KIN, H3= BAP, C1= 0.00mg/l, C2= 0.50mg/l, C3= 0.75mg/l, C4= 1.00mg/l

Fig. 1: Effect of treatments on Shoot length.

PLATE 3: Eight weeks old explants at different concentrations of the hormone.

Effects on number of shoots

- Significant effect was observed in the hormone-concentration interaction and the age-hormone-concentration interaction (P=0.01) on number of shoots.
- Concentration of hormone at 0.75mg/l produced more shoots in all the treatments.
- BAP hormone across all the treatments induces more shoot proliferation except at 10 weeks old apical nodes where it tends to decrease.
- The elongation and multiplication of shoots increases as the concentration increases up to 0.75mg/l in most treatments with BAP treatments recording more shoots (Figure 2).

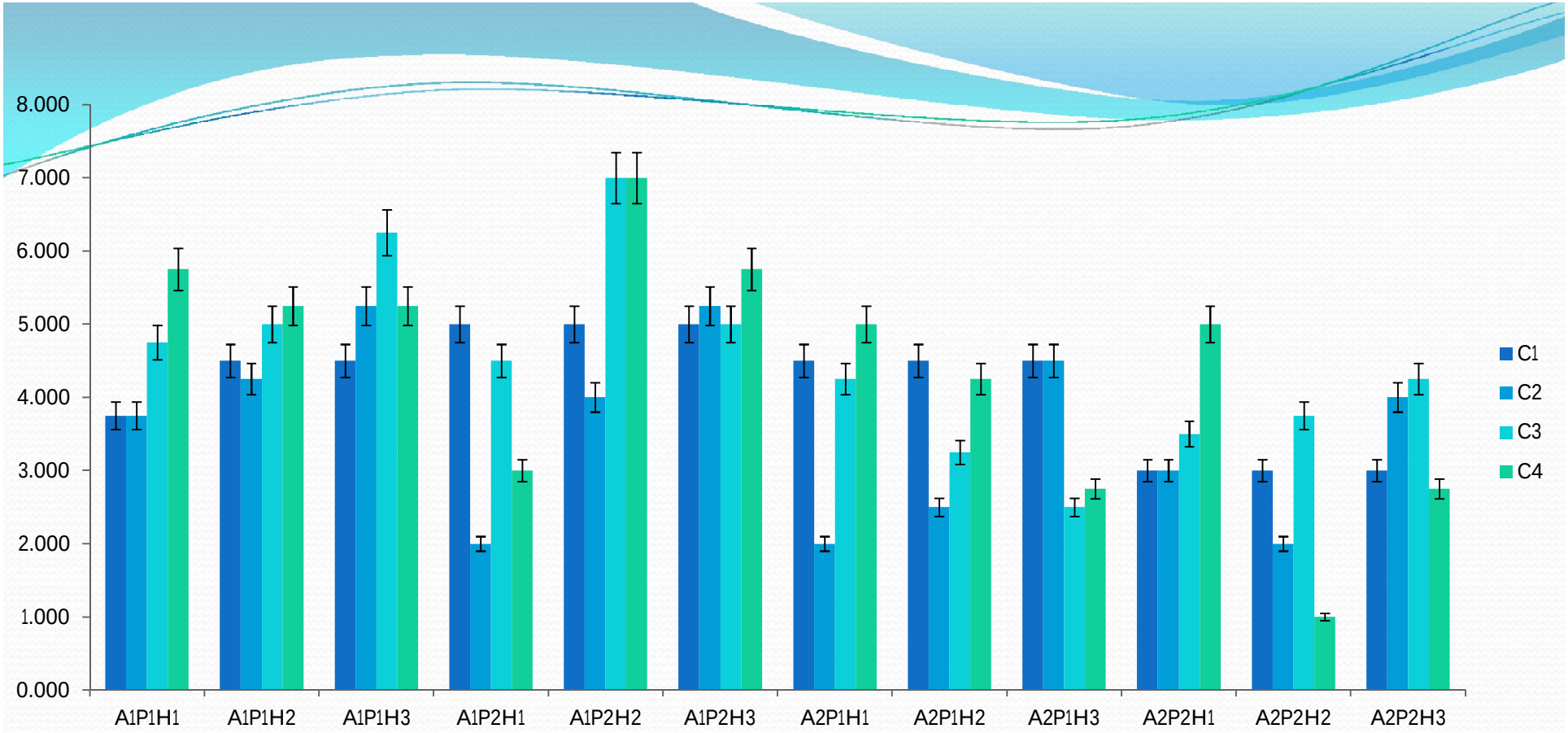


A1= 8 weeks old explants, A2= 10 weeks old explants, P1= Axillary nodes, P2= Apical nodes, H1= NAA, H2= KIN, H3= BAP, C1= 0.00mg/l, C2= 0.50mg/l, C3= 0.75mg/l, C4= 1.00mg/l. Bar indicates standard error

Fig. 2: Effect of treatments on Number of Shoots

Effects on number of nodes

- The number of nodes were significant (P=0.05) at the age and the age-hormone interaction.
- Mean of 7.00 was recorded in 8 weeks apical nodes in 0.75mg/l and 1.00mg/l KIN respectively) and 1.00 in 10 weeks apical nodes in 1.00mg/l KIN) (Fig. 3).
- It was observed that 0.75mg/l and 1.00 mg/l of all the treatment had increase in number of nodes with the exception of 10 weeks old axillary node in 0.75mg/l BAP and apical nodes in 1.00mg/l of KIN and BAP respectively



A1= 8 weeks old explants, A2= 10 weeks old explants, P1= Axillary nodes, P2= Apical nodes, H1= NAA hormone, H2= KIN hormone, H3= BAP hormone, C1= 0.00mg/l, C2= 0.50mg/l, C3= 0.75mg/l, C4= 1.00mg/l. Bar indicates standard error.

Fig. 3: Effect of treatments on Number of nodes

CONCLUSION

- Juvenile nodal cuttings from inoculated seeds would be effective in carrying out rapid clonal propagation *Parkia biglobosa*.
- The nutrient medium and the hormone combinations at different concentrations can be used for multiple shoot induction.
- There can be increase in the number of plantlets produced using in vitro propagation.

THANK YOU FOR LISTENING