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Research Paper

Vegetative Propagation of the Medicinal Plant *Picralima Nitida* (Stapf)

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Abstract: *Several individual factors which also interact in their effects are important in the rooting of juvenile leafy stem cuttings. Cuttings were set in three blocks of a split-split plot experimental design. Each main block contains three rooting media while three different hormones and control were tested at the sub-plot levels. Also, four leaf areas and three hormone concentrations were investigated. Results showed that effect of hormone type (HT) and concentration (HC), leaf area of cuttings (LA) and sowing media (SM) as well as interaction of HT x LA, HT x LA x SM were significant (P≤0.05)on the number, height and diameter of sprouts. The effect of HT, LA, SM and interaction of HT x SM were significant on number and length of roots of sprouted cuttings. Sowing media significantly affected callus formation in P. nitida. Leaf area, SM and interaction effects of HC x HT, HC x SM, HT x LA, HC x HT x LA, HC x HT x SM were significant on the number of leaves of sprouts. Cuttings sown in mixture of sand/sawdust had the highest mean values for sprout diameter (0.97cm); number of sprouts (0.50); number of leaves of sprout (0.65), root length (5.82cm) and height of sprout (0.63cm) while the highest mean number of roots (4.77) and number of callused cuttings (0.44) were recorded in cuttings sown in sand. Cuttings treated with IBA had the highest mean values of 0.94cm, 0.55cm, 0.43, and 5.99cm for diameter of sprout, sprout height, number of sprouts and length of root respectively. Cuttings which had 0.1 mg/L of IBA gave the highest mean values of 0.79cm, 0.47cm, and 0.40 for diameter, height and number of sprouts respectively. Overall, cuttings with 50cm^2 leaf treated with 0.1 mg/L of IBA and sown in mixture of sand/sawdust gave the best performance and thus recommended for vegetative propagation of P. nitida.*

Keywords: *P.nitida*, Hormone, Sowing Media, Leafy Stem Cutting.

Introduction

Deforestation is a major environmental issue of global concern which attendant consequences remain a formidable threat to the rich flora and fauna biodiversity of the tropical rainforest. Natural and man-made forests have economic, social and environmental benefits and they play important roles in the economic development of any society (Okonkwo *et al.,* 2002). Forests with its common property status in Nigeria (Gbadamosi, 2002) provide the stop gap for the poor rural people who depend on its produce for food, medicine and shelter. Mass propagation therefore offers a long term panacea to massive exploitation of useful forest species that are often harvested to the point of endangering and/or extinction.

Clonal propagation of plus trees remain a veritable means of harnessing and strengthening the genetic potentials of useful forest tree species. The long history of selection and improvement of many agricultural crops contrasts with that of forest tree species where the shift from exploitation to domestication is much more recent. Vegetative propagation shortens the reproductive cycle of a tree; that is particularly important when the flowers, fruits or seeds are the desired product of use or commerce (Gbadamosi, 2008) like *Picralimanitida*, a forest tree species which seed is a major item of commerce in local markets in Nigeria and this greatly limits its regeneration in the wild. Tchoundjeu *et al.* (2006) opined that the development of cost-effective vegetative propagation protocol has become a *sine qua non* for the domestication of choice trees.

Surendran *et al*. (2003) submitted that vegetative propagation is an indispensable component of most tree improvement programmes, ensuring quick genetic gains through mass multiplication of selected genotypes and establishment of clonal seed orchards. It is also useful in producing true to type plants with shorter juvenile period leading to early productivity (Raju and Rao, 2006).

Vegetative propagation using cuttings in propagators have shown that the percentage rooting is highly influenced by rooting media, the amount of auxins and the surface area of the leaf (Tchoundjeu *et al.*, 2002). Auxins play a major role in cell elongation, bud formation and root initiation (Went and Thiamann, 1937; Akinyele, 2010); and production of other hormones (Kumari *et al*., 2010).Although, auxins have been reported to increase the number of roots per cutting in different plant species (Aminah*et al*., 1995); very high dosage could result in death of the cutting (de Klerk *et al*., 1997).

Therefore, this study was conducted to determine the optimum auxin concentration, leaf surface area and appropriate sowing media for the clonal propagation of *P. nitida*. This is critical for the mass propagation and subsequent successful integration of the species to agro-ecosystem.

Materials and Methods

Experimental Plants

Mature fruits of *P.nitida* collected from Benin City (Lat. 6^0 23^1N; Long. 5^0 13^1E), Nigeria were allowed to ferment for four weeks in the nursery of the National Centre for Genetic Resources and Biotechnology, Moor Plantation, Ibadan (lat. 7^0 24^1N; Long. 3^0 49^1E); Nigeria under ambient temperature ranges of 28 and 32ºC and relative humidity of between 65 and 76% to enable decomposition thus, easy extraction of seeds. One thousand de-coated seeds were sown in germinating trays containing washed and sterilized river sand and watered daily in the morning using a fine meshed watering can. At the two leaf stage, juvenile seedlings were transplanted into black polythene bags measuring 16×14×12cm filled with forest top soil and watered daily to saturation. After three weeks of acclimatization under the shade, seedlings inside polythene bags were transferred to the open nursery at the Centre. No nutrient or fertilizer was supplied for the plant growth.

Preparation of Cuttings

After four months of growth, seedlings were sprayed with water early on collection day and cuttings were harvested for vegetative propagation. Seedlings were cut at the base and kept inside water in a bucket. Cuttings of 3 to 4cm length were then excised; each cutting consisted of a single node with the full internode underneath and a full leaf (Mpeck and Atangana, 2007). The non-mist propagator were constructed following the modification by Leakey *et al.* (1990) and as described by Mpeck and Atangana (2007). Assessment of rooting success and other parameters such as callused cuttings, number of sprouts, number of roots, root length, height and diameter and number of leaves of sprouts were done 16weeks after planting.

Experimental Design

A total of 1296 cuttings were excised and set in three blocks of a split-split plot experimental design. Each main block contain three rooting media (cured sawdust, river sand and a mixture of sand and sawdust at 1:1); while three different hormones (Indole-3-butyric acid (IBA), Indole-3-acetic acid (IAA), 1-Napthalene acetic acid (NAA) and control (Alcohol) were tested at the sub-plot level. At the sub-sub plot level, four leaf areas (20, 30, 40 and 50cm^2); at the sub-sub-sub plot, three hormone concentrations of 0.1, 0.15 and 0.2 mg/L were investigated. At each level, treatments were assigned at random to experimental units comprising three rooting media × four hormones × four leaf areas × hormone concentration × nine cuttings. Leaf area was determined using a set of templates cut from graph paper by trimming the leaf to the correct size. Hormones were applied to the cutting base as a 10µl droplets containing 50µgb per cutting of IBA, IAA or NAA dissolved in ethanol at 95% concentration. The control treatment was10µl of ethanol only.

Statistical Analysis

Data collected were subjected to statistical analysis for ANOVA; and mean separation done by Duncan' multiple range test (Duncan, 1995) using the SAS 9.1 (SAS 1999).

Results

Sprout Diameter

The effect of hormone concentration (HC), type (HT), leaf area of cutting (LA) and sowing media (SM) as well as interactions of HC x LA; HT x LA; LA xSM; HC x HT xLA; HC x HT x SM; and HT x LA x SM were significant ($P \leq 0.05$) on the stem diameter of sprouts (Table 1). Mean diameter of sprout of IBA treated cutting had the highest value (0.94cm) which is significantly different from NAA treated cutting (0.63cm) and control (0.37cm). The highest mean diameter of sprout among different leaf area treatment was obtained in 50cm^2 leaf area cuttings (1.05cm) which is significantly different from 30cm^2 leaf cuttings (0.64cm) and 20cm^2 leaf cuttings (0.29cm). Mean diameter of sprouts of 0.97cm among cuttings sown in sand/saw dust is significantly higher than 0.64cm for sand and 0.27cm for sawdust. Mean diameter of sprouts was significantly different from sand (0.64cm) and saw dust (0.27cm). Mean diameter of sprouts of cuttings treated with 0.15mg/L of hormone had the lowest value of 0.43cm which is significantly different from 0.2mg/L treated cuttings (0.68cm) and 0.1mg/L (0.79cm) (Table 2).

Height of Sprout

The effects of HT, HC, LA and SM as well as interactions of HT × LA; HC × HT × LA; and HT × LA × SM were significant ($P \leq 0.05$) on the height of sprouts (Table 3). Mean height of sprout of cutting under control treatment had the lowest value of 0.18cm which was significantly ($P \leq 0.05$) different from NAA (0.37cm) and IBA (0.55cm). Cuttings with 50cm^2 leaf area had the highest mean height of

sprout value of 0.70cm which is significantly (P≤0.05) different from 40cm^2 leaf area cutting (0.31cm) and 20cm^2 cuttings (0.91cm) which had the lowest value. Highest mean height of sprout of 0.63cm was obtained in S/SD sowing medium which is significantly (P≤0.05) different from those in sand which had (0.29cm) and saw dust (0.19cm). Cuttings that had 0.15mg/L of hormone had the lowest mean height of sprout value of 0.22cm which is significantly (P≤0.05) different from those of 0.2mg/L cuttings (0.43cm) and 0.1mg/L cuttings (0.47cm) (Table 2).

Number of Sprouts per Cutting

Hormone type, HC, LA, SM as well as the interactions of HC × LA; HT × LA; LA × SM; HC × HT × LA and HT × LA × SM significantly affected the number of sprouts per cutting (Table 4). Mean number of sprouts differed significantly due to hormone type; IBA treated cuttings had the highest mean number of sprout (0.43); NAA had 0.34 while the lowest value of 0.19 was obtained under the control. Leaf area of cutting affected significantly the mean number of sprout; cutting with 50cm^{2}leafhad the highest value of 0.45, cuttings with 30cm^2 leaf had 0.35 while the lowest value of 0.15 was obtained in cutting with 20cm^2 leaf area. Mean number of sprouts varied significantly due to sowing media; mixture of sand and saw dust had the highest value of 0.50 while the lowest was obtained in cuttings sown in saw dust (0.15). Concentration of hormone significantly (P≤0.05) affected the mean number of sprouts. Cuttings that received 0.1mg/L had the highest mean value of 0.40, followed by 0.2mg/L while cuttings which received 0.15mg/L had 0.24 (Table 2).

Number of Leaves of Sprouts

The effects of LA, SM as well as interactions of HC × HT; HC × SM; HT × LA; HC × HT × LA; HC × HT × SM; and HT × LA × SM were significant (P≤0.05) on the number of leaves produced by the sprouted cuttings (Table 5). Mean number of leaves of sprouts of 50cm^{2}leaf area cuttings was highest with a value of 0.58 and was significantly different from 40cm^2 leaf area cuttings (0.34) and 30cm^2 cuttings which had 0.23. Mean number of leaves of sprouts in mixture of sand/saw dust was significantly different with the highest value of 0.65 from those in sand (0.27) and saw dust (0.15) (Table 2).

Root Length of Sprouted Cuttings

The effect of HT, LA of cuttings and SM were significant on the root length of cuttings. Also, the interaction effects of HC x SM, HT x LA; HT x SM; HC × HT × SM; HT × LA × SM were significant on root length (Table 6). Mean root length of sprouted cuttings treated with IBA and NAA had mean root length of 5.99cm and 5.69cm respectively were significantly different from cuttings under control (4.99) and IAA treated cuttings (4.32). Mean root length of cuttings with 20cm^2 leaf area (4.38) was significantly different from those of 30, 40 and 50cm^2 leaf areas with values of 5.11cm, 5.58cm and 5.92cm respectively. Mean root length of sprouted cutting in saw dust (4.45) was significantly different from cuttings in sand (5.47cm) and mixture of sand/saw dust (5.82cm) (Table 2).

Number of Roots of Sprouted Cuttings

Hormone type, LA and SM had significant (P ≤ 0.05) effects on the number of roots in sprouted cuttings. The effects of interactions of HT × SM; LA × SM as well as HC × HT × LA × SM were significant on number of roots of cuttings (Table 7). Cuttings treated with IAA had the lowest mean number of roots (3.57) which is significantly different (P ≤ 0.05) from control (4.31), IBA (4.54) and NAA (4.77) treated cuttings. 20cm^2 leaf area cutting had the lowest mean number of roots of 3.56 which is significantly different from 30cm^2 leaf area (4.37), 40cm^2 leaf area (4.44) and 50cm^2 leaf area (4.81). Lowest mean number of roots of 3.65 obtained in cuttings sown in sawdust was significantly different from those in sand/saw dust (4.47) and sand (4.77) (Table2).

Callused cuttings

The effect of SM was significant ($P \leq 0.05$) on the number of callused cuttings (Table 8). The highest mean number of callused cuttings (0.44) was obtained in cutting sown in sand, this was significantly different from values obtained in saw dust (0.13) and sand/saw dust (0.23) (Table 2).

Number of Rooted Cuttings

The effects of HT and HC, LA and SM as well as their interactions were not significant on the number of rooted cuttings (Table 9).

Discussion

Development of vegetative propagation protocol for choice species is grist for the mill of domestication of such species, thus, successfully rooting of *P.nitida* is a step forward in the integration of the species into agro ecosystem. Results of this study indicate that number of root and callused cuttings in *P.nitida* were strongly influenced by sowing media with sand giving the best performance. This is in agreement with the findings of Atangana *et al.*, (2006), for *Allanblackia floribunda*, Gbadamosi and Oni, (2005) for *Enantiachlorantha*; and Nyansi (2004) for *G.kola.* However, root length of cuttings was best in moisture retaining mixture of sand/sawdust; this could be due to the better aeration and high retention ability of sand/sawdust media, this agreed with the findings of Tchiogio and Duguma (1998) who reported that stem cuttings of *Calliandracalothyrsus* rooted better in a 1:1 mixture of fine sand and rotted sawdust. On the contrary, Avana *et al*. (1999) proposed that saw dust was the best substrate for rooting cuttings (84% of rooting).

As corollary, vegetative parameters in *P. nitida* cuttings such as number, heights and diameter of sprout as well as number of leaves of sprout gave the best performance in mixture of sand/sawdust. Auxins stimulate rooting in most tree species. Hormone types strongly affected root length in the species with IBA producing the best performance. This is supported by the submission of Mukta and Sreevalli (2009) who recommended IBA as the best agent for rooting of *Pogamiapinnata.* Majeed *et al.* (2009) also recorded the highest rooting rate (50%) for *Aesculusindica* cuttings treated with IBA at 2000ppm. Baul *et al.* (2008) also observed a similar trend in the vegetative propagation of *Stereospermumsuaveolens* with cuttings treated with 0.2% IBA producing the longest root. On the other hand, NAA gave the best performance for number of roots per cutting as hormone type affected the rooting in *P.nitida.* Application of NAA hormone tended to increase the rate of root growth as submitted by Mialoundama *et al.* (2002).

Concentration of hormone strongly affects vegetative characters of sprouts in *P.nitida,* number, diameter and height of sprouts was best in 0.1g/L hormone. This agreed with the findings of several authors that higher doses of auxins led to a supra-optimal level which usually had inhibitory effects on roots (Puri and Verma, 1995; Gbadamosi and Oni, 2005).

Leaf area of cutting is another strong endogenous factor which affects rooting in this study. *P.nitida* cuttings with $50cm^2$ leaf area gave the optimum results for number and length of roots; number, diameter and height of sprouts as well as number of leaves of sprouts. This agreed with the findings of Tchoundjeu *et al.* (2004) for *Pausinystaliajohimbe.* Leakey *et al.* (1982) reported a leaf area of 25-$50cm^2$ for *G.kola*. However, Mpeck and Atangana, (2007) reported a lower rate of rooting at $50cm^2$ within the range of leaf areas tested for *Baillonellatoxisperma.* Since $50cm^2$ was the largest leaf area tested in *P.nitida,* further studies using larger sizes in combination with other factors may further enhance rooting in the species.

The significant effect of interactions of hormone type and leaf area of cuttings on various rooting parameters indicates that these factors could be studied further. In same vein, non-significance of all factors on number of rooted cuttings may require further studies.

Conclusion and Recommendation

Conclusively, *P.nitida* stem cutting which had 50cm^2 leaf area treated with 0.1g/L IBA and inserted in sand were found to root and callused optimally while vegetative characteristics of rooted cuttings were optimum in mixture of sand and sawdust. The successful rooting of *P.nitida* is an important step forward in the integration of the species into agro ecosystem; the combination of factors seemed to favour optimum rooting of the species. Also, the rooted cuttings sprouted well within 16 weeks and ready for seedlings establishment. Therefore, it is recommended that for optimum vegetative propagation of *P.nitida* cuttings with leaf area of 50cm^2 treated with 0.1g/L IBA be inserted in mixture of sand/saw dust for a period of 16weeks.

Figures and Tables:

Control 40cm^2

0.15g IAA 50cm^2

0.20g IBA 50cm^2

0.20g NAA 50cm^2

Fig. 1: Rooting of leafy single node cuttings of *P. nitida* in different sowing media

Table 1: Analysis of variance for diameter of sprouts in cuttings of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Effect	df	MS	F	P-level
Hormone concentration(HC)	2	5.01	8.05*	0.000
Hormone type (HT)	3	5.93	9.51*	0.000
Leaf area of cutting (LA)	3	10.51	16.87*	0.000
Sowing media (SM)	2	18.22	29.25*	0.000
HC × HT	6	1.20	1.93	0.075
HC × LA	6	1.35	2.16*	0.047
HC × SM	4	1.32	2.12	0.079
HT × LA	9	3.66	5.88*	0.000
HT × SM	6	0.51	0.83	0.551
LA × SM	6	1.75	2.81*	0.011
HC × HT × LA	18	1.11	1.78*	0.027
HC × HT × SM	12	1.23	1.98*	0.026
HC × LA × SM	12	0.61	0.98	0.471
HT × LA × SM	18	3.79	6.09	0.000
HC × HT × LA × SM	36	0.69	1.11	0.314

*Significantly different at P≤0.05

Table 2: Mean of metrical traits of cutting of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Parameter	Sprout diameter	Sprout height	Number of sprout	Number of leaves	Length of root	Number of roots	Callused Cuttings
Sowing Medium							
Sawdust	0.27[a]	0.19[a]	0.15[a]	0.15[a]	4.45[a]	3.65[a]	0.13[a]
Sand	0.64[b]	0.29[a]	0.29[b]	0.27[a]	5.47[b]	4.77[b]	0.44[b]
SD/SWD	0.97[c]	0.63[b]	0.50[c]	0.65[b]	5.82[b]	4.47[b]	0.23[a]
Hormone Conc.							

A	0.79[b]	0.47[b]	0.40[c]	-	-	-	-
B	0.43[a]	0.22[a]	0.24[a]	-	-	-	-
C	0.68[a]	0.43[b]	0.31[b]	-	-	-	-
Hormone Type							
Control	0.37[a]	0.18[a]	0.19[a]	-	4.99[a]	4.31[b]	-
IAA	0.60[b]	0.39[b]	0.29[ab]	-	4.32[a]	3.57[a]	-
NAA	0.63[b]	0.37[b]	0.34[bc]	-	5.69[b]	4.77[b]	-
IBA	0.94[c]	0.55[b]	0.43[c]	-	5.99[b]	4.54[b]	-
Leaf Area							
20	0.29[a]	0.19[a]	0.15	0.27[a]	4.38[a]	3.56[a]	-
30	0.64[b]	0.28[a]	0.35	0.23[a]	5.58[b]	4.37[b]	-
40	0.56[b]	0.31[a]	0.30	0.34[a]	5.11[bc]	4.44[b]	-
50	1.05[c]	0.70[b]	0.45	0.58[b]	5.92[c]	4.81[b]	-

abc: Values in the same column with similar letters are not significantly different from each other ($P \leq 0.05$)

Table 3: Analysis of variance for height of sprouts in cuttings of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Effect	df	MS	F	P-level
Hormone concentration(HC)	2	2.48	5.01*	0.007
Hormone type (HT)	3	2.39	4.82*	0.002
Leaf area of cutting (LA)	3	5.49	11.09*	0.000
Sowing media (SM)	2	7.59	15.34*	0.000
HC × HT	6	0.99	2.00	0.065
HC × LA	6	0.52	1.04	0.540
HC × SM	4	0.54	1.09	0.359
HT × LA	9	1.49	3.02*	0.002
HT × SM	6	0.82	1.66	0.132
LA × SM	6	0.10	0.20	0.976

HC × HT × LA	18	1.64	3.30*	0.000
HC × HT × SM	12	0.74	1.49	0.128
HC × LA × SM	12	0.33	0.66	0.789
HT × LA × SM	18	1.62	3.28*	0.000
HC × HT × LA × SM	36	0.65	1.32	0.112

*Significantly different at P≤0.05

Table 4: Analysis of variance for number of sprouts in cuttings of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Effect	**df**	**MS**	**F**	**P-level**
Hormone concentration(HC)	2	0.92	7.39*	0.001
Hormone type (HT)	3	1.02	8.17*	0.000
Leaf area of cutting (LA)	3	1.76	14.04*	0.000
Sowing media (SM)	2	4.56	36.50*	0.000
HC × HT	6	0.23	1.83	0.092
HC × LA	6	0.32	2.52*	0.021
HC × SM	4	0.19	1.56	0.186
HT × LA	9	0.28	2.27*	0.018
HT × SM	6	0.14	1.09	0.367
LA × SM	6	0.32	2.60*	0.018
HC × HT × LA	18	0.30	2.43*	0.001
HC × HT × SM	12	0.19	1.48	0.130
HC × LA × SM	12	0.13	1.02	0.426
HT × LA × SM	18	0.72	5.74*	0.000
HC × HT × LA × SM	36	0.17	1.33	0.107

*Significantly different at P≤0.05

Table 5: Analysis of variance for number of leaves of sprouts in cuttings of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Effect	df	MS	F	P-level
Hormone concentration(HC)	2	1.71	2.80	0.057
Hormone type (HT)	3	1.32	2.23	0.085
Leaf area of cutting (LA)	3	2.70	4.58*	0.004
Sowing media (SM)	2	10.04	17.02*	0.000
HC × HT	6	1.55	2.62*	0.017
HC × LA	6	1.23	2.08	0.055
HC × SM	4	1.86	3.15*	0.015
HT × LA	9	2.86	4.80*	0.000
HT × SM	6	0.23	0.39	0.882
LA × SM	6	0.89	1.51	0.174
HC × HT × LA	18	1.75	2.96*	0.000
HC × HT × SM	12	1.46	2.48*	0.004
HC × LA × SM	12	0.56	0.94	0.503
HT × LA × SM	18	2.93	4.96*	0.000
HC × HT × LA × SM	36	0.58	0.98	0.508

*Significantly different at $P \leq 0.05$

Table 6: Analysis of variance for root length in sprouted cuttings of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Effect	df	MS	F	P-Level
Hormone concentration(HC)	2	10.49	2.57	0.078
Hormone type (HT)	3	60.54	14.85*	0.000
Leaf area of cutting (LA)	3	47.94	11.76*	0.000
Sowing media (SM)	2	72.64	17.82*	0.000
HC × HT	6	3.72	0.91	0.486
HC × LA	6	7.72	1.89	0.082

HC × SM	4	15.84	3.89*	0.004
HT × LA	9	11.43	2.81*	0.004
HT × SM	6	50.57	12.41*	0.000
LA × SM	6	5.47	1.34	0.238
HC × HT × LA	18	6.43	1.58	0.065
HC × HT × SM	12	16.07	3.94*	0.000
HC × LA × SM	12	5.48	1.34	0.192
HT × LA × SM	18	13.99	3.43*	0.000
HC × HT × LA × SM	36	3.82	0.94	0.576

*Significantly different at P≤0.05

Table 7: Analysis of variance for number of roots in sprouted cuttings of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Effect	**df**	**MS**	**F**	**P-Level**
Hormone concentration(HC)	2	3.85	0.96	0.384
Hormone type (HT)	3	28.90	7.21*	0.000
Leaf area of cutting (LA)	3	29.83	7.44*	0.000
Sowing media (SM)	2	48.91	12.20*	0.000
HC × HT	6	5.74	1.43	0.202
HC × LA	6	2.52	0.63	0.708
HC × SM	4	3.44	0.86	0.489
HT × LA	9	7.19	1.79	0.069
HT × SM	6	28.45	7.10*	0.000
LA × SM	6	20.70	5.16*	0.000
HC × HT × LA	18	6.32	1.58	0.065
HC × HT × SM	12	4.86	1.21	0.274
HC × LA × SM	12	4.25	1.06	0.394
HT × LA × SM	18	6.00	1.50	0.090

HC × HT × LA × SM	36	6.27	1.56*	0.025

*Significantly different at P≤0.05

Table 8: Analysis of variance for number of callused cuttings of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Effect	df	MS	F	P-level
Hormone concentration(HC)	2	0.99	1.73	0.18
Hormone type (HT)	3	0.56	0.99	0.40
Leaf area of cutting (LA)	3	0.40	0.71	0.55
Sowing media (SM)	2	3.82	6.68*	0.00
HC × HT	6	0.36	0.64	0.69
HC × LA	6	0.14	0.24	0.96
HC × SM	4	0.16	0.27	0.90
HT × LA	9	0.44	0.76	0.65
HT × SM	6	0.11	0.18	0.98
LA × SM	6	0.24	0.42	0.86
HC × HT × LA	18	0.48	0.83	0.66
HC × HT × SM	12	0.13	0.23	0.99
HC × LA × SM	12	0.16	0.28	0.99
HT × LA × SM	18	0.53	0.93	0.54
HC × HT × LA × SM	36	0.22	0.39	0.99

*Significantly different at P≤0.05

Table 9: Analysis of variance for number of rooted cuttings of *P. nitida* under different hormone types, concentration, leaf area and sowing media

Effect	df	MS	F	P-level
Hormone concentration(HC)	2	0.79	0.09	0.908
Hormone type (HT)	3	6.25	0.75	0.521
Leaf area of cutting (LA)	3	5.02	0.61	0.612

Sowing media (SM)	2	4.60	0.56	0.575
HC × HT	6	0.17	0.02	0.999
HC × LA	6	0.74	0.09	0.997
HC × SM	4	1.27	0.15	0.962
HT × LA	9	0.54	0.65	0.999
HT × SM	6	6.08	0.73	0.623
LA × SM	6	0.53	0.06	0.998
HC × HT × LA	18	0.71	0.09	1.000
HC × HT × SM	12	0.85	0.10	0.999
HC × LA × SM	12	0.16	0.19	1.000
HT × LA × SM	18	1.19	0.14	0.999
HC × HT × LA × SM	36	0.48	0.06	1.000

*Significantly different at $P \leq 0.05$

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