Micro-propagation of *Spathiphyllum wallisii* Regel by Tissue Culture Technique

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ABSTRACT

The purpose of this research is to develop a protocol for micro propagation on *Spathiphyllum wallisii* Regel. This study was carried out during the years 2020 and 2021. In the sterilization phase using 5% clorox for 15 min recorded very good results for survival and free contamination %. In establishment, full salt strength MS medium + 30 g/l sucrose increased shootlet formation. Chlorophyll a content increased on full salt strength MS medium with 20 g/l sucrose while augmented with 40 g/l increased chlorophyll b. On the other hand, ¼ salt strength MS with 30 g/l sucrose increased carotenoids. In multiplication, adding 1.5 mg/l BAP resulted in the largest number of shootlets, while using 0.5 mg/l BAP recorded the largest number of leaves. Adding 0.5 mg/l Kin increased the contents of chlorophyll a and b while carotenoids increased with BAP at 1.5 mg/l. For rooting, culture on ¼ strength medium without or with activated charcoal gave the highest root numbers. There were no significant differences in root length. At acclimatization, studying the effect of media substrate (peat moss, peat moss + perlite or peat moss + sand) recorded good results without significant effect on the length, leaf number, root length and root number of plantlet.

Key words: *In vitro* culture, peace lily, cytokinin types, MS medium, medium salt strength.

INTRODUCTION

Spathiphyllum wallisii Regel (Peace Lily) is a popular flowering indoor house plant that belongs to the Araceae family native to tropical regions of the Americas and South east Asia. It is a very profitable for its large leaves and attractive inflorescence consisting of a brilliant white spathe and spadix (Dewir et al., 2006) which add to popular and mercantilism skylight as a florescence foliage plant (Henny et al., 2004). *Spathiphyllum* plant is one of the most common imported and exported ornamental plants. Its monetary value has increased greatly over the last two contracts and there is a great possibility for sustainable growth in both local and international markets (Rout et al., 2006).

Plant tissue culture is considered to be simple and fast method, which enable mass production of true to- type plant material (Singh, 2003). The optimal growth of tissues perhaps differs for various plants according to nutritional needs. Variegated segments of tissues plants perhaps have various needing for specific growth (Sulaiman et al., 2020). Surface sterilization is the first step in the preparation at of a hygienic in vitro explants taken from the field that are greatly exposed to contamination of microbial (Sameer and Nabeel, 2016). The quantity and quality of plant growth regulators (PGRs) play a significant role for the ability to produce specific in-vitro propagation (Youssef et al., 2021). Cytokinins are commonly used to build up composition of buds and differentiation of shoots in micro propagation. BAP (benzyl aminopurine) was more potent of cytokinin for shoot proliferation of explant Asiatic of lily (Taha et al., 2018) and *Nephrolepis exaltata* (Nofal et al., 2022). The effects of activated charcoal perhaps due to do a darkened environment; adsorption of growth regulators adsorption of undesirable/ inhibitory elements; and latest organic composites, or their lease of growth enhancing substances (Panand Van Staden, 1998). Vegetative propagation of *Spathiphyllum* is slow and hard, and seed

production is abnormal in temperate countries (Hennen and Hotchkiss 1995). Plant tissue culture industries mainly propagate this plant on a large scale by micro propagation. A lot of research revealed the overcoming of micro propagation of *Spathiphyllum* by axillary bud culture (Malagon et al., 2001and Dewir et al., 2006). The aim of this study was to find a micro propagation protocol for *S. wallisii*.

MATERIALS AND METHODS

This research was carried out in Tissue Culture and Germplasm Conservation Research Laboratory, Horticulture Research Institute, Agricultural Research Center, in 2020 and 2021.

Source and sterilization explant:-

The nodal explant was immersed for 1 min in 70% solution of ethanol under an aseptic condition in the laminar air-flow cabinet then immersed in (5% v/v) clorox or (0.1% w/v) mercuric chloride (MC) solution at (5, 10, 15, 20, 25 or 30 min) supplemented a few drops of tween-20 then rinsed three times by sterile distilled water. The sterilized explants were cultured on MS medium without growth regulator (control). After three weeks survival% and free contamination% explants were calculated.

Culture medium and conditions:-

Murashige and Skoog, (1962) (basal MS medium) contained sucrose and 7 g/l agar. It was adjusted pH to 5.7±0.1, poured into the jars (200ml) and autoclaved for 20 min at 121°C with 1.2 kg/cm^2 pressure. The explants culture in 200 ml jars glass containers containing 25 ml medium. The cultures were incubated at 24±1° C under fluorescent tube light illumination of 2000-2500 lux at 16/8h (daylight/dark).

Culture establishment:-

The explants were cultured on different MS salt strengths (full, ¾ or ½) with sucrose concentrations at (20, 30 or 40 g/l) without plant growth regulators. In this experiment fifteen explants in three replicates were cultured for one month. After that, the shootlet number per explant, leaf numbers per shootlet, chlorophyll a, b and carotenoids (mg/100g f.w.) were recorded.

Multiplication stage:-

The shootlet produced from the previous experiment culture were cultured on full salt strength MS medium supplemented with 30 g/l sucrose (the best treatment from the above experiment) supplemented with different types of cytokinins Benzyl aminopurin (BAP),6-Furfuryl-aminopurine (Kinetin or Kin)or 6-y-y-dimmethylallyl aminopurin (2ip) each at (0.0, 0.5, 1.0 or 1.5 mg/l). The cultured explants were incubated for one month. The shootlets were subculture three times, after that shootlet number/explant and leaf number/shootlet and chlorophyll a, b and carotenoids (mg/100g fw) were determined.

Pigments determination:-

For determination of pigments, was done according to Saric et al. (1967) using ethanol for extraction.

Rooting stage:-

Shootlets resulted from multiplication treatments were cultured on different MS medium salt strength (full, ½ or ¾) without or with 1 g/l activated charcoal. Shootlets cultured were incubated one month. After that, root number and length were then examined.

Acclimatization stage:-

The rooted shootlet resulting from rooting stage were transferred to plastic pots containing peat moss, peat moss + sand (1:1 v:v) or peat moss + perlite (1:1 v:v). The pots irrigated and then covered with transparent polyethylene bags. The plantlets were kept in acclimatization house for four weeks then transplanting out-of- door then the plantlet length, leaf number, root length and root number were evaluated.

Statistical analysis:-

Factorial (two factors) design of experiments for start, multiplication and rooting while one factor was used in acclimatization experiment. The experimental design was a Complete Randomized Design (CRD). L.S.D. (Least Significant Differences) at $p \leq 0.05$ were used to comparison the means of the treatments according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Sterilization stage:-

In Table (1), the data indicate that there were significant effects on explants due to the treatments of clorox and mercuric chloride (MC) for various times and their interaction. For sterilization, no significant differences between the two sterilizing agents (clorox and MC) on survival percentage, while the free contamination % was significantly increased by using clorox (64.83%) compared to MC which gave (46.30%). For different immersion times, the survival % was significantly decreased with increasing the time of sterilization while free contamination% was significantly increased by decreasing the time of sterilization. The interaction between the two sterilizing agents and the period of sterilization indicated that the highest % of survival and lowest % of free contamination were recorded when the explants immersed in MC 0.1% for 5 min which gave (100.0 and 22.22%). On the other hand, the best percentage of survival and free contamination was recorded when explants immersed in clorox 5% for 15 min which gave (77.80 and 55.58%).

In this respect**,** Sayed and Gabr (2007) recorded that immerged in 15% clorox for 10 min gave the best results for contamination free explant% and survival% on *Deutzia scarba*. Moreover, Pawan et al., (2016) on *Spathiphyllum floribundum* L., found that the best result was obtained when the explants treated with 20 % clorox with few drops of tween-20. Using clorox at concentrations of 30 or 35 % for 25 min recorded high survival% of *Phytolacca andioicaex* explants (El-Afry et al., 2017).

Table (1). Effect of different types and times of sterilization agents for survival % and free contamination % on *S. wallisii*.

Time (min.)	Survival (%)			Free contamination (%)		
	Clorox 5%	MC 0.1%	Mean B	Clorox 5%	MC 0.1%	Mean B
5	77.80	100.0	88.90	44.45	22.22	33.34
10	77.80	66.70	72.25	55.58	33.33	44.45
15	77.80	66.70	72.25	77.80	55.58	66.69
20	44.45	55.58	50.01	44.45	33.33	38.89
25	44.45	33.33	38.89	77.80	44.45	61.13
30	11.11	33.33	22.20	88.90	88.90	88.80
Mean A	55.57	59.27		64.83	46.30	
LSD $_{0.05}$		A= NS B = 17.60 A×B = 24.89			A= 4.019 B = 6.961 A×B = 9.844	

LSD $_{0.05}$ = Least Significant Different at 0.05 level of probability, MC: Mercuric Chloride, NS: Non Significant

Establishment stage:-

1. **Effect of salt strength of MS medium and concentrations of sucrose for shootlet establishment on *S. wallisii*:**

Data in Table (2), and Fig. (1), showed significant differences between MS salt strength and sucrose concentration and their interaction.

For shootlet number, culture explants on full MS medium recorded the largest shootlet number (3.479 shootlets/explant) and gradually decreased on ¾ or ½ MS medium to 2.924 or 1.706 shootlets/explant, respectively. Growing explant on MS medium with 30 g/l sucrose produced the largest number of shootlets (3.220 shootlets/explant) compared to 20 or 40 g/l sucrose (2.407 or 2.482 shootlets/explant, respectively). In interaction between MS salt strength and concentration of sucrose, culture explants on full MS supplemented with 30 g/l sucrose recorded the largest shootlet number (4.66 shootlets/

explant) compared to ½ MS with all concentrations of sucrose which gave the lowest shootlets/explant.

For leaf number, the explants culture on ½ MS formed the highest leaf number (3.961 leaves/shootlet) compared to full or ¾ MS strength medium. Supplementation of MS medium with 40 g/l sucrose increased leaf number to (3.616 leaves/shootlet). In interaction, culture explant on ½ salt strength MS supplemented 30 g/l sucrose increased the formed of leaves (4.15 leaves/shootlet) whereas culture explant on full MS with 20 or 30 g/l sucrose reduced it (3.11 or 3.10 leaf/shootlet, respectively).

This result was in agreement with Dewir et al. (2006) who found that full salt strength MS medium with 30 g l/l sucrose was favorable for shoot tip culture of *Spathiphyllum cannifolium*. Furthermore, Chinthala and Ciddi (2014) on *Toddali asiatica* found full salt strength MS medium fortifies 30 g/l sucrose was found to be optimal for growth and biomass accumulation.

Table (2). Effect of salt strength MS medium and sucrose concentrations on *S. wallisii*

	Shootlet number/explant				Leaf number/shootlet			
	Full MS	**¾MS**	**½MS**	**Mean B**	**Full MS**	**¾MS**	**½MS**	**Mean B**
20 g/l sucrose	2.33	3.11	1.78	2.407	3.11	3.28	3.73	3.377
30 g/l sucrose	4.66	3.33	1.67	3.220	3.10	3.30	4.15	3.518
40 g/l sucrose	3.44	2.33	1.67	2.482	3.32	3.53	4.00	3.616
Mean A	3.479	2.924	1.706		3.179	3.700	3.961	
LSD $_{0.05}$		A= 0.4834 B = 0.4834 A×B = 0.8373				A= 0.2096 B =0.2096 A×B = 0.3631		

LSD $_{0.05}$ = Least Significant Different at 0.05 level of probability, MS: Murashige and Skoog

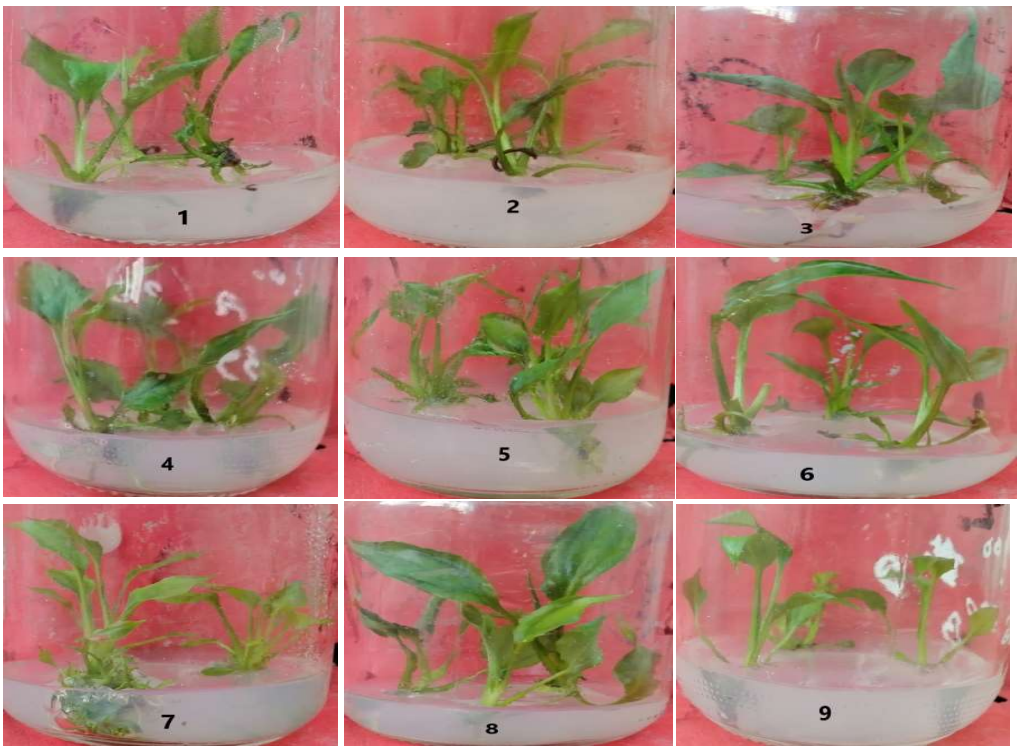

Fig. (1). Effect of salt strength of MS medium and sucrose concentrations 1- full MS+20g Suc., 2- ¾MS+20g Suc., 3- ½ MS+ 20g Suc., 4- full MS+30g Suc., 5- ¾ MS+30g Suc., 6- ½ MS+ 30g Suc., 7- full MS+40g Suc., 8- ¾ MS+40g Suc., 9- ½ MS+ 40g Suc.

2. Effect of MS salt strength medium and concentrations of sucrose for pigments on *S. wallisii*:

The data recorded in Table (3), indicate a significant effect of salt strength of MS medium and sucrose concentrations on chlorophyll a, b and carotenoids.

In regard to chlorophyll a, culturing the explants on full salt strength MS medium, regardless the level of sucrose recorded the highest content of chlorophyll a (11.18 mg/100g f.w.) whereas ¾ or ½ MS medium gave (9.693 or 9.147 mg/100g f.w., respectively). For sucrose concentrations, the medium augmented with 20 g/l sucrose recorded the highest chlorophyll a (11.23 mg/100g f.w.). The interaction revealed that, culture explants on full MS with 20 g/l sucrose recorded the highest content of chlorophyll a (15.25 mg/100g f.w.), while full salt strength MS medium fortify 40 g/l sucrose or ½ MS salt strength medium with 20 g/l sucrose reduced chlorophyll a to the minimum value (5.63 and 5.20 mg/100g fw, respectively).

Regarding chlorophyll b, for MS salt strength, the explants culture on full MS medium recorded the highest chlorophyll b (9.57 mg/100g f.w.). For concentrations of sucrose, supplemented medium with 40 g/l sucrose recorded the highest chlorophyll b (8.474 mg/100g f.w.). For interaction, culture explants on full MS adding with 40 g/l sucrose recorded the highest chlorophyll b content (13.54 mg/100g f.w.) in compared to ½ MS medium supplemented 20 g/l sucrose recorded (1.873 mg/100g fw).

The carotenoids content, culture explants on ¾ or ½ MS medium was the highest content (4.795or 5.326 mg/100g f.w., respectively). For concentrations of sucrose, supplemented medium 30 g/l sucrose recorded the highest carotenoids content (5.594 mg/100g f.w.). The interaction between salt strength MS medium and concentrations of sucrose showed that explants culture on ½ MS medium + 30 g/l sucrose recorded the highest content (6.813 mg/100g f.w.), while culture explants on full MS medium augmented with 40 g/l sucrose reduced the carotenoids content to the lowest one (1.98 mg/100f.w.).

In this respect, Tessa et al., (2004). Gao et al., (2000) revealed that adding high sucrose concentration increased the production of pigments on *Carthamus tinctorius.*

Table (3). Effect of salt strength MS medium and sucrose concentrations for pigments on *S. wallisii*.

	Full MS	¾ MS	½ MS	Mean B
Chlorophyll a (mg/100g f.w.)				
20 g/l sucrose	15.25	13.24	5.20	11.23
30 g/l sucrose	12.65	8.09	10.27	10.34
40 g/l sucrose	5.63	7.75	11.97	8.45
Mean A	11.180	9.693	9.147	
LSD5%	**A= 0.666**	**B= 0.666**	**A×B = 1.155**	
Chlorophyll b (mg/100g f.w.)				
20 g/l sucrose	7.88	8.77	1.87	6.174
30 g/l sucrose	7.28	5.31	3.33	5.310
40 g/l sucrose	13.54	5.35	7.35	8.474
Mean A	9.570	6.477	4.184	
LSD5%	**A= 0.5086**	**B= 0.5086**	**A×B = 0.8809**	
Carotenoids (mg/100g f.w.)				
20 g/l sucrose	3.60	4.667	4.13	4.136
30 g/l sucrose	5.18	4.789	6.81	5.594
40 g/l sucrose	1.98	4.919	5.04	3.978
Mean A	3.587	4.795	5.326	
LSD5%	**A= 0.6733**	**B= 0.6733**	**A×B = 1.166**	

LSD $_{0.05}$ = Least Significant Different at 0.05 level of probability, MS: Murashige and Skoog

Multiplication stage:-

1. Effect of cytokinins of different types and concentrations for shooting behaviour on *S. wallisii*:-

The illustrated data in Table (4), and Fig. (2), indicate a significant effect on micro-propagation of explant (shootlet number, shootlet length and leaf number) obtained from the effect of cytockinins treatments.

For shootlet number, in the type of cytokinins, the medium supplemented with 2iP recorded the highest shootlet number (4.90 shootlet/explant) compared with BAP or Kin which reduced to (3.542 or 3.283). For concentrations of cytokinins, culture explant on medium containing 1.5 mg/l recorded the highest number of shootlets (6.022 shootlets/explant) compared to control (free hormone) which gave the lowest shootlet number (1.7 shootlets/ explant). For interaction, adding 1.5 mg/l 2iP produced the highest shootlet number (7.40 shootlets/explant) meanwhile the control gave the minimum value (1.70 shootlet/ explant).

With regard to leaf number, adding BAP to culture medium increased the leaf formation per shootlet (3.358 leaves/ shootlet) whereas the addition either Kin or 2ip reduced it. For the concentration of cytokinins, adding 0.5 mg/l gave the largest number of leaves (3.378 leaves/shootlet) meanwhile 1.5 mg/l gave the lowest number (2.889 leaves/shootlet). In interaction, leaf number was increased to maximum value by adding 0.5 mg/l BAP compared 1.0 mg/l 2iP which reduced to minimum value (2.73 leaves/shootlet).

Cytokinins are known to activate cell division, stimulate RNA synthesis, cell elongation and protein synthesis and enzyme activity (Kshanika and Niranjini, 1995). In this respect, Dewiret al. (2006) found that shoot buds of *Spathiphyllum cannifolium* induced on 2iP-supplemented media had a larger leaves. Sayd et al. (2010) revealed that for shooting stage adding 1.5 mg/l 2iP produced the highest shootlet number/ explant on *Gardenia jasminoides*.

Table (4). Effect of different type and concentrations of cytokinin on *S. wallisii*.

	Shootlet number/explant				Leaf number/shootlet			
	BAP	**Kin**	**2iP**	**Mean B**	**BAP**	**Kin**	**2iP**	**Mean B**
0.0 (cont.)	1.70	1.70	1.70	1.700	3.30	3.30	3.30	3.300
0.5 mg/l	2.60	3.53	5.00	3.711	4.03	3.13	2.97	3.378
1.0 mg/l	3.30	3.80	5.50	4.200	3.40	3.53	2.73	3.222
1.5 mg/l	6.57	4.10	7.40	6.022	2.70	2.97	3.00	2.889
Mean A	3.542	3.283	4.900		3.358	3.158	3.000	
LSD%5	A= 0.6364 B = 0.7349 A×B =1.273				A= 0.3069 B =0.3538 A×B = 0.6129			

LSD $_{0.05}$ = Least Significant Different at 0.05 level of probability, BAP: Benzyl aminopurine, Kin: 6-Furfuryl-aminopurine, 2iP: 6-y-y-dimmethylallyl aminopurin

Fig. (2): Effect of different types and concentrations of cytokinins (mg/l): 1-Control, 2- 0.5 BA, 3- 1.0 BAP, 4- 1.5 BA, 5- 0.5 kin, 6- 1.0 kin, 7- 1.5 kin, 8- 0.5 2iP, 9-1.0 2iP and 10- 1.5 2iP.

2. Effect of types and concentrations of cytokinins for pigments on *S. wallisii*

Data in Table (5), indicate a significant effect of cytokinines type on chlorophyll a, b and carotenoids.

With regard to the effect of cytokinin types on chlorophyll a, the explants culture on MS medium with Kin recorded the highest chlorophyll a (14.48 mg/100g f.w.) meanwhile MS medium containing 2ip or BAP recorded (13.93 or 11.91 mg/100 f.w., respectively). For concentrations of cytokinine, medium contain 0.0, 0.5, 1.0 mg/l recorded the highest chlorophyll a (13.73, 14.42, 14.25 mg/100g f.w., respectively). For interaction between type and concentrations of cytokinin, culture explants on MS fortify 0.5 mg/l Kin or 2ip recorded the highest chlorophyll a (17.21, 17.22 mg/100g f.w., respectively).

Concerning chlorophyll b**,** the explants culture on MS medium enriched with Kin or 2ip recorded the highest chlorophyll b (7.901or 8.066 mg/100g f.w., respectively). For concentrations of cytokinin, adding 0.5 mg/l to medium recorded the highest content (9.31 mg/100g f.w.). The interaction indicated the culture explants on medium augmented with 0.5 mg/l Kin or 2ip exhibited the highest content (11.62 or 11.64 mg/100g f.w.).

With regard to carotenoids content, regard to the type of cytokinins, the explants culture on MS medium with BAP had the highest content of carotenoids (4.974 mg/100g f.w.). For concentrations of cytokinins, medium supplemented with 1.5 g/l showed the highest carotenoids (5.482 mg/100g f.w.). For interaction between types and concentrations of cytokinine, culture explants on medium with 1.5 mg/l BAP recorded the highest carotenoids content (6.053 mg/100g f.w.).

The relationship between cytokinins application and chlorophyll content was studied by Kapchina- Toteva and Stoyanova (2003) who found that chlorophyll a and b contents in of *Gypsophila paniculata* shoots decreased when adding cytokinins in culture medium. Nofal et al. (2022) on *Nepherolipis exltata* revealed that, the highest content of total chlorophylls was recorded by using Kin at 1.0 mg/l, while the lowest value was obtained when BAP at 1.5 mg/l.

Table (5). Effect of different types and concentrations of cytokinin for pigments on *S. wallisii*

	BAP	Kin	2iP	Mean B
Chlorophyll a (mg/100g f.w.)				
0.0(cont.)	13.37	13.73	13.72	13.73
0.5 mg/l	8.82	17.21	17.22	14.42
1.0 mg/l	14.51	14.14	15.01	14.25
1.5 mg/l	10.56	13.85	9.68	11.36
Mean A	11.91	14.48	13.93	
$LSD_{5\%}$	**A= 0.5260**	**B= 0.7429**	**A×B = 1.052**	
Chlorophyll b (mg/100g f.w.)				
0.0(cont.)	8.43	8.43	8.43	8.43
0.5 mg/l	4.67	11.62	11.64	9.31
1.0 mg/l	6.99	5.59	7.52	6.70
1.5 mg/l	4.09	5.96	4.68	4.91
Mean A	6.045	7.901	8.066	
$LSD_{5\%}$	**A= 0.552**	**B= 0.6373**	**A×B = 1.104**	
Carotenoids (mg/100g f.w.)				
0.0(cont.)	4.29	4.29	4.28	4.285
0.5 mg/l	5.02	2.69	2.52	3.409
1.0 mg/l	4.54	5.27	4.15	4.651
1.5 mg/l	6.05	4.87	5.52	5.482^{s}
Mean A	4.974	4.279	4.117^{B}	
$LSD_{5\%}$	**A= 0.6757**	**B=0.7803**	**A×B = 1.351**	

$LSD_{0.05}$ = Least Significant Different at 0.05 level of probability; BAP: benzyl aminopurine; Kin: 6-Furfuryl-aminopurine, 2iP: 6-y-y-dimmethylallyl aminopurin.

-Rooting stage: Effect of MS medium salt strength and activated charcoal for rooting behavior on *S. wallisii*

The data presented in Table (6), and Fig. (3), indicate that significant differences were obtained in the number of root /shoot and root length (cm) from different MS salt strength medium and activated charcoal treatments.

For activated charcoal, no significance effect was noticed in medium supplemented without or with activated charcoal on root number and length. For salt strength of MS medium, ¼ salt strength recorded the largest root number and longest roots (7.667 roots /shootlet and 4 cm length, respectively). While culture on full or ½ MS medium recorded the lowest root number (6.0 or 5.0 roots/shootlet, respectively) but root length was reduce to 2.3 cm when cultured on full MS. For interaction, culture on ¼ MS without or with activated charcoal gave the largest root number (7.33 and 8.00 roots/ shootlet, respectively). No significant differences in root length were recorded.

In term of rooting, concentration of macro-and micro elements of culture medium without or with activated charcoal remarkably effect on rooting rate, root length and root number of rooted shootlets was observed by Sakr et al. (1999) on Yucca elephantips. In this regard, Máximo et al. (2020) showed that increasing concentrations of activated charcoal in culture medium increased the percentage of formed roots in shoots of *Handroanthus impetiginous*. Woldeyes et al. (2021) obtained the largest root number and longest root length at ½ MS strength on Okra (*Abelmoschus esculentus*).

Table (6). Effect of salt strength of MS medium and activated charcoal for rooting behaviour on *S. wallisii*

	Root number/shoot			Root length (cm)		
	Without AC	**With AC**	**Mean B**	**Without AC**	**With AC**	**Mean B**
Full MS	7.00	5.00	6.000	2.27	2.33	2.300
½MS	3.66	6.33	5.000	2.33	4.00	3.167
¼ MS	7.33	8.00	7.667	3.67	4.33	4.000
Mean A	6.000	6.444		2.756	3.556	
$LSD_{0.05}$	A= NS B = 1.442 A×B = 2.039			A= NS B = 1.482 A×B = NS		

LSD $_{0.05}$ = Least Significant Different at 0.05 level of probability, MS: Murashige and Skoog, AC: Activated charcoal, NS: Non Significant

Fig. (3). Effect of salt strength of MS-Medium and activated charcoal for rooting behaviour (1-Full MS without AC, 2-Full MS with AC, 3- ½ MS without AC, 4- ½ MS with AC, 5- ¼ MS without AC, 6- ¼ MS with AC).

- **Acclimatize stage: Effect of media substrate on acclimatization behavior of *S. wallisii***

The illustrated data in Table (7), and Fig. (4) indicate the influence of media substrate (peat moss, peat moss+ perlit or peat moss + sand) on acclimatization of plantlet, which recorded 100% survival with no significant effect on plantlet length, leaf number, root length and root number.

In this respect, acclimatization of *S. wallisii* plantlet on medium containing (peat: sand 3:1 v/v) produced the best result by Abou Dahab et al., (2000). Mohamad et al. (2021) showed that peat moss: sand (1:1, v/v) or peat moss: sand (1:2, v/v) soil mixtures during acclimatization stage on *Paulownia* species achieved good result.

Table (7). Effect of media substrate on acclimatization behavior of *S. wallisii*

	Plantlet length	**Leaf number**	**Root length**	**Root number**
Peat moss	7.90	4.40	3.40	4.60
Peat moss+ perlit	6.40	4.60	2.30	4.60
Peat moss + sand	6.40	5.80	2.30	4.20
$LSD_{0.05}$	NS	NS	NS	NS

LSD $_{0.05}$ = Least Significant Different at 0.05 level of probability, NS: Non Significant

Fig. (4). Effect of media substrate on acclimatization behavior (1- Peat moss, 2- peat moss + perlit, 3- Peat moss + sand).

Conclusion:-

The current study magnificently recognized the optimal requirements for the *in vitro* propagation of *S. wallisii*. For a protocol of micro-propagation we can sterilization by use 5% clorox for 15 min and establishment by full salt strength MS medium fortify 30g/l sucrose. In multiplication adding 1.5 g/l 2ip to MS medium after that rooting with culture on ¼ MS without or with activated charcoal finally acclimatization on any media substrate (peat moss, peat moss+ perlit or peat moss + sand).

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الاكثار الدقيق للاسباثيفيلم ويلسياي بواسطة تكنيك زراعة الانسجة

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الغرض من هذا البحث عمل بروتوكول للاكثار الدقيق للاسباسيفيلم ويلسياى خلال الاعوام 2020-2021. فى مرحلة التعقيم استخدام الكلوروكس بتركيز 5% لمدة 15 دقيقه سجل نتيجه جيده جدا بالنسبه للنسبه المئويه للبقاء حيه وخلو الاجزاء النباتيه من التلوث. وفى المرحله التأسيسيه، كانت بيئة املاح مورشيجى وسكوج كاملة الاملاح و المزوده 30 جرام/لتر سكروز مؤثره فى زيادة عدد الفريعات. الكلوروفيل أ زاد فى بيئة مورشيجى وسكوج كاملة الاملاح و المزوده بـــ 20 جرام/لتر بينما زيادة السكروز الى 40 جرام/لتر ادى الى زيادة كلوروفيل ب. على العكس من ذلك ادى استخدام ربع تركيز املاح مورشيجى وسكوج الى زيادة محتوى الكاروتين. وفى مرحلة التضاعف، وجد ان اضافة 1.5ملجم/لتر بنزيل امينو بيورين انتج اكبر عدد من الفريعات بينما استخدام 0.5 ملجم/لتر بنزيل امينو بيورين انتج اكبر عدد من الاوراق. اضافة 1.5 ملجم/لتر كينتين زاد من الكلوروفيل أ ، ب بينما الكاروتين زاد باضافة بالبنزيل ادنين 1.5 ملجم/لتر. فى مرحلة التجذير، وجد ان الزراعه على ربع تركيز املاح بيئة مورشيجى وسكوج بدون او باضافة الفحم النشط ادى الى زيادة عدد الجذور. بينما طول الجذور لم يكن هناك فروق معنويه. بالنسبه للاقلمه، دراسه تأثير البيئات المختلف (بيت موس، بيت موس + برليت او بيتموس + رمل) سجلت نتائج جيده دون فروق معنويه على طول النبته، عدد الاوراق، طول الجذور، عدد الجذور.