Micropropagation of Sweet Cherry Dwarf Rootstock (PHL-C)

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ABSTRACT

PHL-C is one of dwarf sweet cherry rootstocks which is a hybrid between P*runus*.avium L. × *Prunus.cerasus* L. and is recommended for high density plantation of the commercial orchards. In this study the effect of different media types and plant growth regulators for proliferation and rooting of PHL-C rootstock were investigated in Khorasan Razavi Agriculture and Natural Resources Research and Education Center. For proliferation, three culture media (MS, DKW, WPM) and four concentrations of BAP (0.0, 1.0, 1.5, 2.0 mgL^{-1}) were experimented. Also, effects of six culture media (solid and double phase of MS, DKW, WPM) supplemented with four concentrations of IBA (0, 1, 1.5 and 2 mgL^{-1}) on rooting were investigated. Results showed that, the highest shoot multiplication rate was observed in MS + 1 BAP mgL^{-1}, while the highest quality (strong and vigorous Explants, with no signs of vitrification, necrosis, yellow apex and leaves) of plantlet was observed in DKW medium. Moreover, MS media supplemented with either 1.5 or 2 mgL^{-1} IBA proved to be superior to other treatments in terms of root number and length.

Keywords: Double phase medium; micropropagation; Proliferation; Rooting.

INTRODUCTION

Iran is one of the largest producers of sweet cherry in world after turkey and United States (FAO, 2012). The conventional rootstocks like Mazzard (*Prunus avium* L.) and Mahlab (*Prunus. Mahlab* L.) are used as rootstock for sweet cherry in Iran. Due to the basic role in growth rate and resistance to disease; selection of rootstock plays an important role in the management of orchard. Since 1963, experiments on the dwarf cherry trees rootstocks were conducted in the Research and Breeding Institute of Pomology Holovousy Ltd. In Czech Republic. Among the plants grown from the seeds of cherry trees, three clones the numbers 6, 84 and 224 were selected from six clones in numbers 6, 4, 5, 84, 103 and 224. There were named PHL-A, PHL-B and PHL-C rootstocks, respectively (Erbenova et al, 2001). PHL series are dwarf rootstock resulted from a cross between *P. avium* L. and *P.cerasus* L. PHL-C rootstock is compatible with a variety of cherries and might result in 80% growth reduction in trees compared to PHL-A and PHL-B as they reduce growth by 70% and 50%(Erbenova et al, 2001). It is tolerant to waterlogging, calcareous soils, pseudomonas syringae, and agrobacterium and recommended to be used for high density cultivation (Sulusoglu and Cavusoglu, 2013). Direct rooting of sweet cherry rootstocks is difficult which can be solved by using in vitro propagation.

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Moghaddam *et al* (2013) reported that, MS[1] medium containing 1.2 mgL^{-1} BAP was best for proliferation while 1/2 MS contains 1 mgL^{-1} IBA was suitable for rooting of Gisela 6. Mahdavian *et al* (2011) reported full rooting rate was obtained in DKW medium without growth regulator in PHL-A rootstock. In proliferation stage, shoot multiplication rate of (5.37 microshoots/ explant) was achieved in hormone free MS medium. In a study about the effect of different culture media and growth regulators on proliferation and rooting of St. Lucie rootstock 64, Mahdavian *et al* (2011) reported that DKW medium without hormone was the best medium for Rooting of shoots, with up to 100 percent efficiency. Erbenova *et al* (2001) reported that 1.5 mgL^{-1} BAP in MS medium was the optimum concentration for multiplication. Hussain *et al* (2003) in micropropagation of plum reported that MS medium containing 0.5 mgL^{-1} BA+ 0.2 mgL^{-1} NAA was the best treatment for proliferation phase, and 1/2 MS medium supplemented with 1 mgL^{-1} IBA showed to be the best medium for rooting stage. We studied micropropagation of PHL-C dwarfing rootstock. The objective of this work was to investigate the effects of different culture media and growth regulators on shoot proliferation and rooting of a dwarfing cherry rootstock.

2. Materials and Methods

2.1 Shoot proliferation

The axillary bud were taken from one years old tree of PHL-C rootstock maintained in the experimental garden of Khorasan Razavi Natural Resource and Agricultural Research Center. Nodal segments, 1-1.5 cm in length, without leaves were then excised from the shoots.

The explants were washed by water and dishwashing liquid to removed surface contamination. Then they were divided to some parts containing one bud and were pre sterilized by immersion in 70% ethanol for 60 sec followed by a rinse with sterile distilled water. These pre-sterilized explants were then exposed to 0.1% and 0.2% mercuric chloride for 1 and 2 minutes. After being sterilized by ethanol and mercuric chloride, explants were washed 3 times with sterile distilled water and then culture in medium. Three media (MS, DKW and WPM[2]) were used in presence of BAP and IBA plant growth regulators (Table 1). In proliferation stage, different concentrations of BAP (0.0, 1.0, 1.5 and 2.0 mgL^{-1}) were added to the mentioned media. After three subcultures, number and length of shoots were measured. Number of shoots after three subculture was compared with number of shoots at first for proliferation rate. After three subcultures(21 days between each subculture), number and length of shoots were measured. Number of shoots after three subculture intervals was compared with number of shoots at first for proliferation rate.

2.2 Rooting

To determine the best rooting medium, after removal of lower leaves, suitable shoots from elongation phase transferred to solid and double-phase medium(Liquid medium and perlite). containing IBA at four levels (0, 1, 1.5 and 2 mgL^{-1}). All media were supplemented with 6.7 gL^{-1} agar and 30 gL^{-1} sucrose. In all media, pH was adjusted to 5.7 before autoclaving. The cultures were grown under 16-h photoperiod and 23±1º. After 6 weeks, number and length of root, rooting percent, leaf number and stem length were measured.

[1] Murashige and Skoog

[2] Woody plant medium

2.3 Acclimation

For acclimation, rooted shoots washed in tap water for traces of agar and planted in pots filled with cocopeat and perlite and put in moist chamber in greenhouse with 70% humidityAll media were supplemented with 6.7 gL^{-1} agar and 30 gL^{-1} sucrose. In all media, pH was adjusted to 5.7 before autoclaving. The cultures were grown under 16-h photoperiod and 23±1º

2.4 Experimental design

A factorial experiment was laid out in a completely randomized design with three replications and each consisted of three explants that repeated 3 times. Collected data were analyzed statistically by jmp8 software and significant differences among treatment means were compared by Tukey test at $P < 0.05$.

3. Result

3.1 Proliferation phase:

Infection rate for initial deployment explants about 4% for the next subculture were less than 1% .To determine the best medium, different medium and growth regulators for proliferation were used. At the end of the fourth week, the number and height of explants were recorded. There was significant difference ($P< 0.05$) in multiplication rate between media and BAP concentrations. Results showed that the highest proliferation rate, 6.20 micro-shoots, was observed in MS medium enriched with 1 mgL^{-1} BAP (Figure 1). Positive effect of BAP on proliferation of Gisela 6 and Gf rootstock was reported by daneshvar hoseini et al (2010) and nazary moghaddam and yadollahi(2011). Moreover, shoot branching was always reported to be controlled hormonally mainly by cytokinin (Dobránszki, and Silva, 2010), by way of initiation and activity of auxiliary meristems which results in shoot formation, but influence of cytokinin can be differed based on the kind of culture, variety of plant and age of explants (Thorpe *et al*, 2008). Medium without growth regulator had the lowest number of shoot. There were significant differences ($P< 0.01$) in shoot length between media and different concentrations of BAP. The longest shoots were observed in the DKW medium. The best plantlet in terms of quality was observed in DKW medium and least at concentration of 2 mgL^{-1} BAP, which may be due rosette growth and large number of branches (results are not shown). The function of the BAP is breaking the apical dominance and stimulate growth of new shoots. The longest stem and leaf growth in proliferation stage observed in control treatment which can be explained in this way that all plantlets rooted in proliferation phase before being transferred to rooting phase. Optimal BAP concentration in proliferation stage was 1 mgL^{-1} (Figure 3-A). The results in our study were similar to those reported by Ruzic and Vajovic(2013) and Movsiuw(2011) who showed that MS medium with 1 mgL^{-1} of BAP had highest number of shoot in Myrobelan rootstock multiplication. So, it can be concluded that higher concentrations of BAP reduced shoot number, therefore, lower concentrations are recommended to reduce the adverse effects of high BAP concentrations.

3.2 rooting

Rooting percentage was 100% in most treatments. The lowest percentage of rooting was observed in control without IBA. Regarding root number, there was significant difference ($P< 0.05$) between media and IBA concentrations. The highest root number was observed in MS medium containing 1.5 mgL^{-1} IBA with an average of 14/62 roots (Figure 2 and 3-B). Reults reported by Hossain et al (2003) indicated that, highest root number is obtained by using of 1.5 mgL^{-1} IBA. Plantlets grown in MS medium with 2 mgL^{-1} IBA had the longest roots with average length of 4.75 cm. The minimum root length, on average 1.67 cm, was observed in MS double-phase

medium containing 2 mgL^{-1} IBA (Table 2). Maximum shoot elongation was observed in MS+ 1 mgL^{-1} IBA with the average of 4.64 cm. DKW medium without hormone resulted in minimum elongation data of the shoot . The ability of plant tissues to form adventitious roots depends on the interaction of many exogenous and endogenous factors such as hormone, elements and type of culture medium (Frankel and Hess, 1973) and roots formation in vitro can be induced by exogenous auxins such as IBA, NAA and IAA. Another related study showed that, IBA had a better effect on rooting compared to IAA. In our study, the aim was to study the effect of different concentrations of IBA on rooting phase. IBA at different concentrations improved rooting but the best results were obtained at the concentration of 1.5 mgL^{-1}. This hormone is more stable and less sensitive to reducing auxin enzymes (Riov, 1993). Different media have different effects on rooting stage. In this study, root number and length in MS medium were higher than other media. Putting roots in the dark increased rooting. Root formation in double -phase media was obtained earlier than other media types which can be explained by low concentration of agar, which provides adequate contact between plant tissue and medium (suthar *et al*., 2010). In our study, survival percentage of plantlets, 100% in most treatments, was higher than previous many other studies (Mahdavian et al., 2011; Daneshvar Hossini et al., 2010; and Sulusoglu and Cavusoglu, 2013) who conducted in vitro rooting experiments on prunus rootstocks.

3.3 Acclimatization

Plantlets with better quality were more successful in acclimatization. For survival rate, there was no difference between plantlets rooted in liquid media and those rooted in agar-gelled media. Roots from double-phase medium were not injured during transplantation. The lowest survival rate was 71% in double-phase WPM medium. In general, survival percentage in most treatments was 100%. Acclimatization was affected by rooting treatment and Leaf development. Plantlets with longer roots were more successful than plantlets with short roots. Plantlets from liquid media produced more lateral roots and root hair compared to agar-gelled media, therefore they showed good performance in acclimatization phase (Figure 3-C) but there was no significant difference between agar and double-phased media.**5. Conclusion**

The results of this study indicate that MS medium with BAP at concentration of 1 mgL^{-1} had about the highest number of shoots in proliferation phase with 6.20 micro shoot . In rooting stage, MS medium with 1.5 mgL^{-1} IBA had average 14.62 roots. application of the double-phase media, composed of liquid medium with perlite, instead of commonly used agar-gelled medium gave better support for the root formation and resulted in better compatibility.

4. Acknowledgment

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Table1. Media used for shoot proliferation and rooting

Composition	MS	WPM	DKW
NH4NO3	1650	400	1416
KNO3	1900	-	-
CA(NO3)2.4H2O	-	556	1367.41
CaCL2.2H2O	440	96	149
K2SO4	-	990	1559
MgSO4.7H2O	370	370	361.38
KH2PO4	170	170	265
MnSO4.4H2O	22.3	370	-
MgSo4.H2O	-	-	33.5
Na2MoO4.2H2O	0.25	0.25	0.39
ZnSO4.7H2O	8.6	8.6	-
Zn(NO3)2.6H2O	-	-	17
KI	0.83	-	-
H3BO3	6.2	6.2	4.8
CuSO4.5H2O	0.025	0.25	0.25
CoCL.6H2O	0.025	-	-
FeSO4.7H2O	27.8	27.8	33.8
Na2EDTA.2H2O	37.3	37.3	45.4
Myo-inositol	100	100	100
Thiamino-HCL	0.1	1	2
Nicotinic acid	0.5	0.5	1
Pyridoxine-HCL	0.5	0.5	-
Glicine	2	2	2

Table 2. Comparison of various culture media' effects on growth rooted plantlets

Medium	IBA (mgL^{-1})	Rooting	Leaf number	Shoot length(cm)	Root length (cm)	Survival rate
	0.0	65.5 ±0.5	7.8 c-f	2.41 d-g	3.43 bc	85±0.2
	1.0	99± 0.5	10.53 a-d	4.64 a	3.40 bcd	97±0.2
MS	**1.5**	99± 0.5	10.12 a-d	3.62 a-e	4.37 ab	99±0.2
	2.0	100± 0.5	11.75 ab	3.37 a-e	4.75 a	100 ±0.2
	0.0	76 ± 0.5	8.08 b-f	3.25 b-f	2.29 e-i	89±0.2
WPM	**1.0**	99 ± 0.5	8.76 a-f	3.66 a-d	2.5 c-i	100 ±0.2
	1.5	93± 0.5	11.81 ab	3.55 a-e	3.45 bc	91±0.2
	2.0	93± 0.5	12.16 a	3.91 abc	3.24 cde	85±0.2
	0.0	92 ± 0.5	7.68 c-f	1.8 g	2.61 c-i	100 ±0.2
DKW	**1.0**	99 ± 0.5	9.22 a-f	2.65 c-g	2.94 c-h	100±0.2

Medium	IBA (mgL^{-1})	Rooting	Leaf number	Shoot length(cm)	Root length (cm)	Survival rate
	1.5	100 ± 0.5	11.28 abc	3.34 b-e	2.78 c-h	100 ±0.2
	2.0	66 ± 0.5	8.36 a-f	2.87 b-g	3.07 c-f	100±0.2
	0.0	75± 0.5	6.02 f	2 fg	2.37 d-i	100 ±0.2
	1.0	75 ± 0.5	8.45 a-f	2.68 c-g	2.62 c-i	99 ±0.2
Double phase MS	**1.5**	66 ± 0.5	6.26 ef	2.37 efg	1.9 hi	100±0.2
	2	75± 0.5	7.35 def	2.37 efg	1.67 i	100±0.2
	0.0	92 ± 0.5	8.75 a-f	2.41 d-g	3.18 cde	81±0.2
	1.0	51± 0.5	8.66 a-f	2.62 d-g	3.28 cde	71 ±0.2
Double phase WPM	**1.5**	80 ± 0.5	9.10 a-f	4 ab	2.79 c-h	73±0.2
	2.0	58± 0.5	7.75 c-f	3.32 b-e	3.07 c-f	100±0.2
	0.0	92± 0.5	8.5 a-f	3.3 b-e	2.12 f-i	81 ±0.2
	1.0	100± 0.5	10.1 a-e	3.97 ab	3 c-g	81±0.2
Double phase DKW	**1.5**	92 ± 0.5	8.62 a-f	3.22 b-f	1.97 ghi	80 ±0.2
	2.0	92± 0.5	9.75 a-f	3.25 b-f	2.42 c-i	100±0.2

Values in the same column with different lower-case letters are significantly different at $P<0.01$.

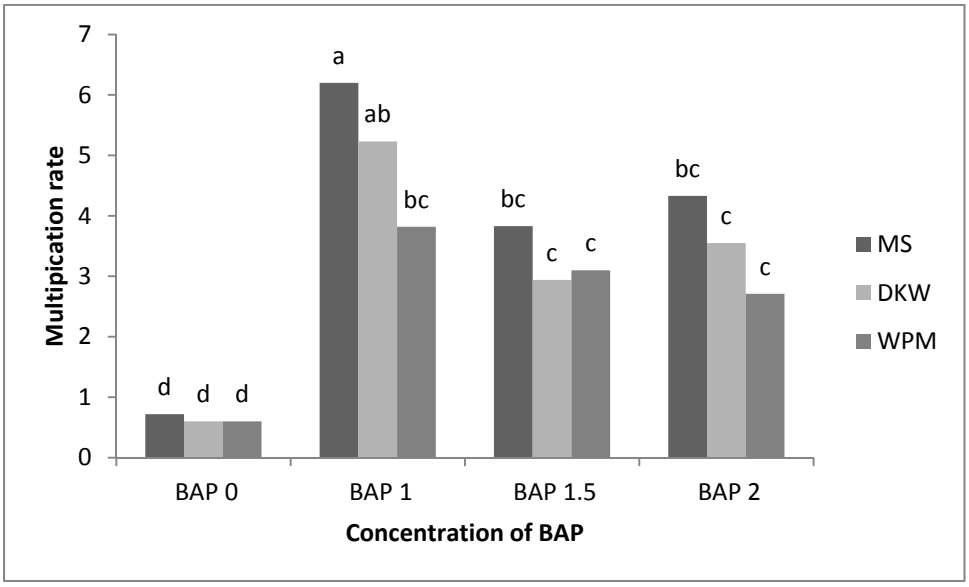

Fig 1. Effect of BAP on shoot proliferation of PHL-C rootstock

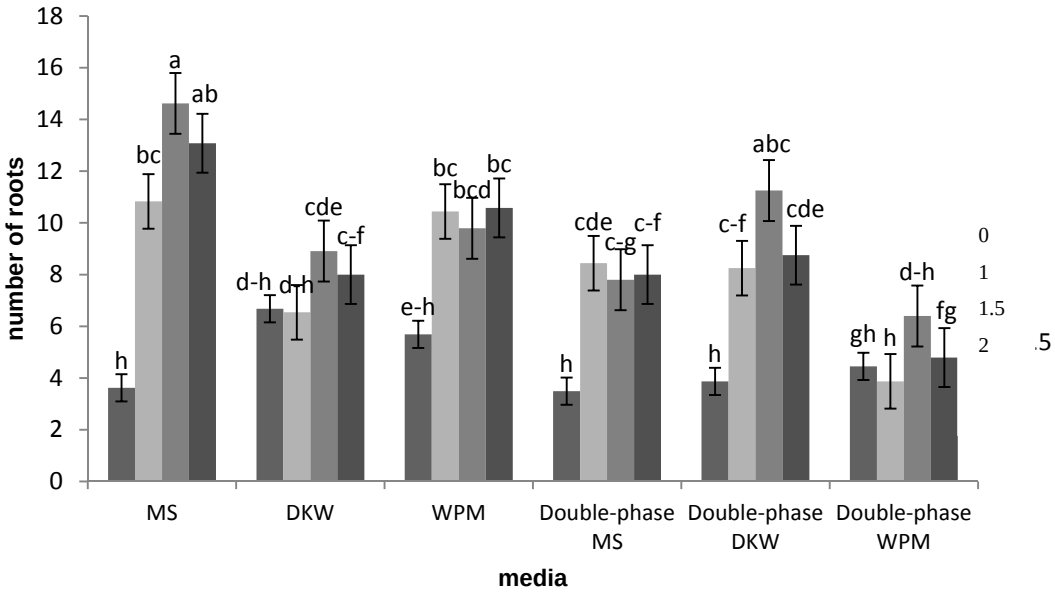

Fig 2. Effect of IBA on number of roots in PHL-C rootstock

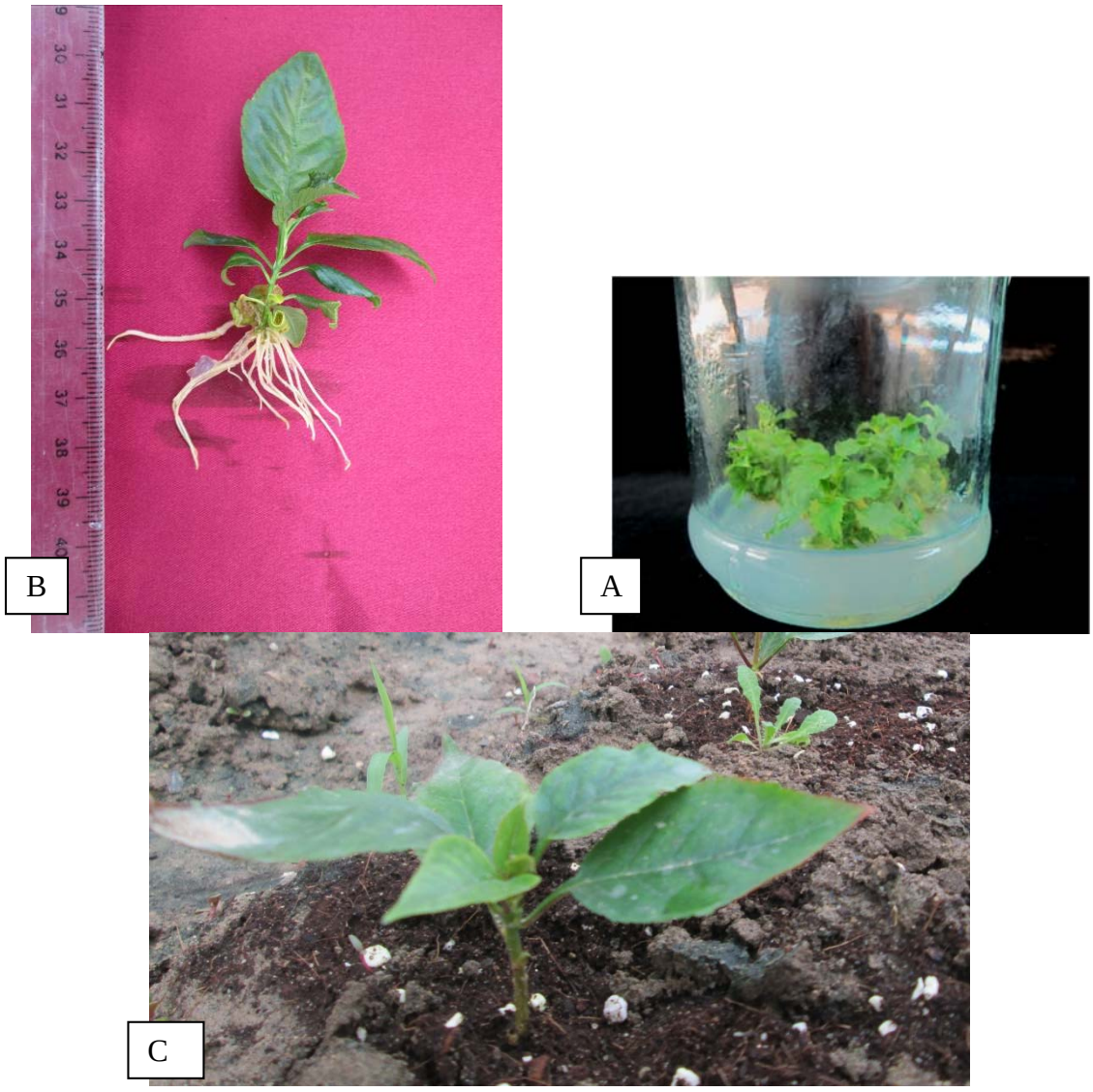

Fig. 3. A: Shoot proliferation in MS basal medium supplemented with 1.0 mgL^{-1}BAP four weeks after culture multiplication. B: Roots from regenerated shoots on MS basal medium supplemented with 1.5 mgL^{-1} IBA C: Plantlets in acclimatization stage

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إكثار الأصول المقاومة المقزمة من الكرز الحلو (PHL-C)

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ملخص

بين هو واحد من الأصول المقاومة المقزمة للكرز الحلو والذي يعد هجين ما .*runus cerasus* L(PHL-C) و *Prunus avium* L. وينصح به لزراعة عالية الكثافة من البساتين التجارية. وفي هذه الدراسة ، تم اختبار تأثير أنواع مختلفة من وسائط ومنظمات النمو النباتية لانتشار وتجذير هذا الأصل المقاوم في مركز خراسان رضوي للبحوث الزراعية والموارد الطبيعية. لدراسة الانتشار، قد جربت ثلاث وسائط نمو (MS,DKW, WPM)مختلفة BAP وأربعة تراكيز مختلفة من (0.0, 1.0, 1.5, 2.0 mg/L). وقد تمت أيضاً دراسة آثار استخدام ستة وسائط نمو مختلفة (Solid and double phase of MS, DKW, WMP) على عملية التجذير. IBA (0, 1, 1.5 and 2.0 mg/L) مع أربعة تراكيز من وقد أظهرت النتائج أن أعلى معدل لتكاثر النمو الخضري قد لوحظ عند استخدام ، (MS+ 1BAP mg/L) في حين أن أعلى مستوى من الجودة من حيث انتاج نباتات قوية ونشطة مع عدم وجود علامات نخر وقمة وأوراق صفراء (DKW. قد لوحظ عند استخدام وسط(علاوة على ذلك ، فقد أثبتت الدراسة تفوق وسط (mg/L) 1.5 أو 2مزوداً ب (MS) على غيره من المعاملات من حيث عدد وطول الجذور.

الكلمات الدالة: وسط النمو المزدوج، الاكثار الدقيق، الانتشار، التجذير.

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