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Effect of various concentrations of indole butyric acid and plantation techniques on rooting performance of grapes germplasm

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Abstract

Climate is changing universally, however it is severely affecting the arid areas of the World in particular. Highlands of Balochistan, Pakistan are also under this threat where underground water has gone down to a drastic level due low rain falls and water mining. To overcome these issues there is a need of alternative approach i.e. Cultivation of high value low delta crops like grapes, olives, pistachio and pomegranate instead of low value high delta crops apple, apricot, cherries and peaches etc. For speeding-up the cultivation of high value low delta crops access to their true to type, disease free nursey plants are very important, which have been addressed in this experiment by developing nursery plants of grapes germplasm, under hydroponics system with semi controlled conditions and supplementation of IBA in the media. The aim of this research was to standardize the protocol for rooting of grapes in hydroponics and to find the optimized concentration of IBA for rooting of grape. Two local germplasm i.e. Haita and Sahibi and two exotic genotypes i.e. Autumn Royal and Red globe of grapes were planted in soil, static hydroponics and continuous hydroponics systems. Shoot cuttings were treated with different concentrations of Indole Butyric Acid (IBA). The grapes genotypes treated with 4000 mg.l-1of IBA had more number of roots and greater root length in both static and continuous hydroponics after one month compared with those which were treated with low concentrations of IBA. Furthermore, cuttings produced their roots in short span of time i.e. one month in hydroponics system compared with those kept in soil.

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Introduction

Balochistan, the largest province of Pakistan covers 44% of the total land of the country. The peculiar climatic conditions of the province, particularly in highlands are favorable for growing variety of high quality fruits at large scale. The nature has presented this region with special climatic environment for the production of good assortment and nature of fruits products. Because of this beauty the region is known as fruit basket of Pakistan. In Balochistan fruits are cultivated on an area of 0.221 million hectors with a yield of 0.9 million tons per annum (Dawnnews 2011).On national level, 90% of grapes cherry almonds and 60% of peach pomegranates apricot, 34% of apple and 70% of dates are produced in this region. Despite all its stated potential, drought has been a major limiting factor in the region hampering fruit production both qualitatively and quantitatively. The prevailing drought since 2000 and onward has not only badly affected the yield of agriculture crops but also lowered down the water table in the area (Wasim 2011). Likewise, lowering of ground water table owing to excessive and indiscriminate water mining emanating from the government policy of subsidized electricity tariff to the farming community has further aggravated the situation (ICARDA 2010).

To overcome these issues there is a need for alternative approaches. In addition to the revised government policy of shrugging off the subsidy on electricity, cultivation of high value low delta crops like grape, olive, pistachio and pomegranate instead of apple, apricot, cherry and peach can ameliorate the situation to a great extent. Remedy on the similar lines has also been proposed earlier emphasizing low delta crops and modern techniques of irrigation to tackle the water scarcity challenge in the province (Faiz.M.Kakar 2012).

Land races of grapes are under cultivation in the highlands of the province from centuries. With the passage of time, exotic varieties had been added to the cropping system. Red Globe and Autumn Royal have been imported in the recent past from USA.

Fruits of these varieties have longer shelf life due to thicker fruit skin, which helps them to with stand against heavy rains at ripening stage and maintain their quality even after long transportation intervals. Exotic varieties with short span of time have proven themselves as best juicy fruit producing ones along with showing better tolerance against biotic and abiotic stresses On top of all these advantages yield and market value of fruits harvested from these varieties are almost double then local germplasm. (Personal Comm: Malik Naseer Shahwani, Chairman Farmer's Association, Balochistan).

The overall requirement of water for Grapes is 40% less than apple and 30% less than apricot (Faiz.M.Kakar 2012). Grapes require under 60% of water than apple in the age of 1year in like manner it step by step required less water when it gets developed i.e. it requires 30% less water than apple at 7years years old. In first year Apple requires 17 times watering system, 19 times in year 3 and in year 7 around 22 times in overwhelmed condition and these watering systems are around 40% more than grapes (Faiz.M.Kakar 2012).

Hydroponics is the strategy for developing plants in a dirt free medium. The nutrient rich solution contains all the necessary elements which feeds the plant for its growth and development Fruits species like grapes (Albuquerque and Dechen 2000), seed potatoes (Corrêa *et al.* 2009), fruits like strawberries and melon (Andriolo *et al.* 2009), pineapple (Macêdo *et al.* 2003) can also be propagated.

Viewing the advantageous position of these exotic varieties and scarcity of water in Balochistan, rapid multiplication of these varieties as a potential alternate to low value high delta crops is always advisable. One potential economical and technology based technique for producing grapes nursery plants on large scale is the use of hydroponics systems for nursery development, with supplementation of rooting hormone i.e. indole butyric acid in the nutrient media under controlled temperatures.

The fragmented published data on growing rooted grape plants under hydroponics system with supplementing rooting hormones demands for designing and conducting, such a study on economically important crops like grapes. The work, at the same time, will provide an understanding for the use of low water consuming and less labor intensive technology for the production of rooted nursery plants of high value crops. We will use and test the hydroponics system for development of rooted nursery plants in short span of time and to compare the rooting performance of local germplasm with exotic ones under hydroponics and soil for the development of rooted nursery plants of grapes on large scale.

Materials and methods

Experiment was designed to use hardwood cuttings of four grapes genotypes, out of which two local indigenous namely HAITA and SAHIBI and two exotic AUTUMN ROYAL and RED GLOBE. These cuttings were collected from Agriculture Research Institute Sariab Quetta for testing their rooting performance under three planting techniques viz. Soil, Static hydroponics and Continuous hydroponics systems.

Under soil polythene bags of 15x20 cm^2 size were filled with 850 to 900gm of soil in equal combination of sand and surface soil. About 20 to 30cm long shoot cuttings were prepared with 1 cm distance to the buds and angled for increasing the surface area of lower part of cutting for maximum contact with the soil and rooting hormone i.e. Indole Butyric Acid. For rooting hormone treatments, approximately 2.5cm from the bottom of the shoot cuttings were dipped in the rooting solution of various concentrations i.e. 10mM and 20mM for 5 seconds.

For static hydroponics system plastic tubs with 10litres water holding capacity were used for placing shoot cuttings. Thermo-pole sheets with foam were used for covering the media and supporting the shoot cuttings. For aeration of media aquarium pumps were used along with transparent rubber tubing. Two different concentrations of IBA were used i.e. 10mM and 20mM.

Experiment was conducted under the controlled conditions with 25 to 30°C temperature and 14 h day light throughout the course of experiment. 200µl house hold bleach was added in the solution to make it chlorinated for avoiding contaminations. In the beginning shoot cuttings were kept under distilled water for 1 week. The Hoagland's nutrients solution of $1/4^{th}$ concentration was also supplied along with IBA after 1 week. The media solution was changed twice in a week for avoiding contaminations and depletion of nutrients and hormones

Continuous hydroponics system was made with the help of 5cm wide PVC pipes and iron stands. Two PVC pipes of 40cm each and one pipe of 80cm were cut and joined by elbow and binding solution. Small holes were made on the upper side of these pipes for plantation of shoot cuttings. This system was kept above the level of plastic tub with the help of iron stands connected with the plastic tubs of 15 litre water holding capacity. Media solution which was kept in the tubs were made to flow easily and continuously in the system by pumping it with small water pumps kept submerged in the solution media from tubs to system and flowing back in the tubs due to the elevated position of system. Plastic tubs were filled with 12L of Hoagland's nutrients solution of $1/4^{th}$ concentration supplemented with different IBA treatments i.e. 10mM and 20mM. Physical conditions were kept same as in static hydroponics. For every phase of experiment there were two IBA concentrations used with following details.

For Soil

Control: No IBA.

T_1: 10mM ($2g.l^{-1}$) IBA. Shoot cuttings were dipped for 5 seconds in IBA solution and then planted.

T_2: 20mM ($4g.l^{-1}$) IBA. Shoot cuttings were dipped for 5 seconds in IBA solution and then planted.

For Hydroponics

Control: No IBA.

T_1: IBA with the concentration of 10mM ($2g.l^{-1}$) was supplied along with the nutrients solution. The volume of the IBA for static hydroponics was 15ml and the volume of IBA for continuous hydroponics was 60ml.

T_2: IBA with the concentration of 20mM (4g.l^{-1}) was supplied along with the nutrients solution. The volume of the IBA for static hydroponics was 15ml and the volume of IBA for continuous hydroponics was 60ml.

Shoot cuttings were taken out from soil and both hydroponics systems after one month with intense care. The rooted cuttings were washed with distilled water and were dried on towel paper. The rooting data was recorded and compared for conventional and non-conventional methods under each IBA concentrations. For statistical analysis software SPSS was used for calculating the significance of results.

Results

Soil

After one month time when shoot cuttings were taken out from soil there was no root development in all four genotypes under all IBA concentrations.

Static hydroponics

Number of roots

All the treatments showed significant mean difference with each other for number of roots. The mean difference of control with T_1 and T_2 was 11.1667* and 18.333* respectively, while the mean difference of T_1 with T_2 was 7.1667* (Table 1). With respect to genotypes in their respective treatments, Autumn Royal with Red Globe didn't show any significant mean difference while other genotypes show significant mean difference with each other (Table 2).

Taking each genotype individually the highest number of roots of genotype Haita was found underT_2 which was 16.33. The number of roots in three replicates of Haita was 5 which was lowest in all other genotypes under T_1 while in control there was no rooting at all (Fig. 1A). The number of roots in three replicates of Red Globe under static hydroponics was also highest in T_2. The number of roots of Red Globe under T_2 was 26.66. Similarly the number of roots of Red Globe in T_1 was 21 and in control it was found 7. Under all IBA treatments the numbers of roots in Red Globe were greatest compared to all other genotypes (Fig. 1A). Autumn Royal gave rise highest number of roots in static hydroponics under T_2. The number of roots by Autumn Royal in T_2 was 26.66. Similarly in static hydroponics system the number of roots of Autumn Royal in T_1 and control were 19.33 and 6.33 respectively. Under T_1 and control the number of roots were lesser than Red Globe but greater than other indigenous genotypes (Fig. 1A).

The results of number of roots of Sahib showed same pattern as other genotypes i.e. the highest number of roots of Sahibi was found in in T_2under static hydroponics system followed by T_1 and control. The average number of roots in three replicates of Sahib under T_2, T_1 and control was 23, 19 and 6.33 respectively. Under T_1 and T_2 the number of roots was lesser than Autumn Royal and Red Globe but greater than Haita while under control the result was similar to Autumn Royal but lesser than Red Globe (Fig. 1A).

Table 1. Post hoc analysis of no. of roots in static hydroponics with respect to treatments.

Multiple Comparisons

Dependent Variable: number of roots LSD

(I) Indole Butyric Acid	(J) Indole Butyric Acid	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment one control	Treatment two 2g/l	-11.1667*	.41388	.000	-12.0209	-10.3125
	Treatment three 4g/l	-18.3333*	.41388	.000	-19.1875	-17.4791
Treatment two 2g/l	Treatment one control	11.1667*	.41388	.000	10.3125	12.0209
	Treatment three 4g/l	-7.1667*	.41388	.000	-8.0209	-6.3125
Treatment three 4g/l	Treatment one control	18.3333*	.41388	.000	17.4791	19.1875
	Treatment two 2g/l	7.1667*	.41388	.000	6.3125	8.0209

Based on observed means.
The error term is Mean Square (Error) = 1.028.
*The mean difference is significant at the 0.05 level.

Table 2. Post hoc analysis of no. of roots in static hydroponics with respect to genotypes.

Multiple Comparisons

Dependent Variable: Number Of Roots Static LSD

(I) Grapes Genotypes	(J) Grapes Genotypes	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Haita	Red Globe	-11.1111*	.47791	.000	-12.0975	-10.1248
	Autumn Royal	-10.3333*	.47791	.000	-11.3197	-9.3470
	Sahibi	-9.1111*	.47791	.000	-10.0975	-8.1248
Red Globe	Haita	11.1111*	.47791	.000	10.1248	12.0975
	Autumn Royal	.7778	.47791	.117	-.2086	1.7641
	Sahibi	2.0000*	.47791	.000	1.0136	2.9864
Autumn Royal	Haita	10.3333*	.47791	.000	9.3470	11.3197
	Red Globe	-.7778	.47791	.117	-1.7641	.2086
	Sahibi	1.2222*	.47791	.017	.2359	2.2086
Sahibi	Haita	9.1111*	.47791	.000	8.1248	10.0975
	Red Globe	-2.0000*	.47791	.000	-2.9864	-1.0136
	Autumn Royal	-1.2222*	.47791	.017	-2.2086	-.2359

Based on observed means. The error term is Mean Square (Error) = 1.028.
*The mean difference is significant at the 0.05 level.

Root length (cm)

The root length of static hydroponics under all IBA treatments showed significant difference with each other. Comparison of control with T_1 and T_2 showed significant mean difference with the value of 1.9750* and 2.658* respectively, while mean difference of T_1 and T_2 was 0.6833* (Table 3).

Genotypes Red Globe and Sahib didn't show any significant mean difference with each other under T_1 and T_2. Whiles hoot cuttings obtained from genotype Autumn Royal did not show any difference when kept under all IBA treatments including control (Table 4).

The root length comparison of Sahib and Autumn Royal with Red Globe also didn't show significant mean difference. The maximum root length in three replicates of Haita under all treatments was greater in T_2 followed by T_1 and control. The root length in T_2 of static hydroponics system was 6cm greater than Sahibi and Red Globe but smaller than Autumn Royal. Similarly the root length of genotype Haita kept under T_1 in static hydroponics was 4.5cm which was smaller than all other grapes genotypes while Haitha did not bear root under control (Fig. 1B).

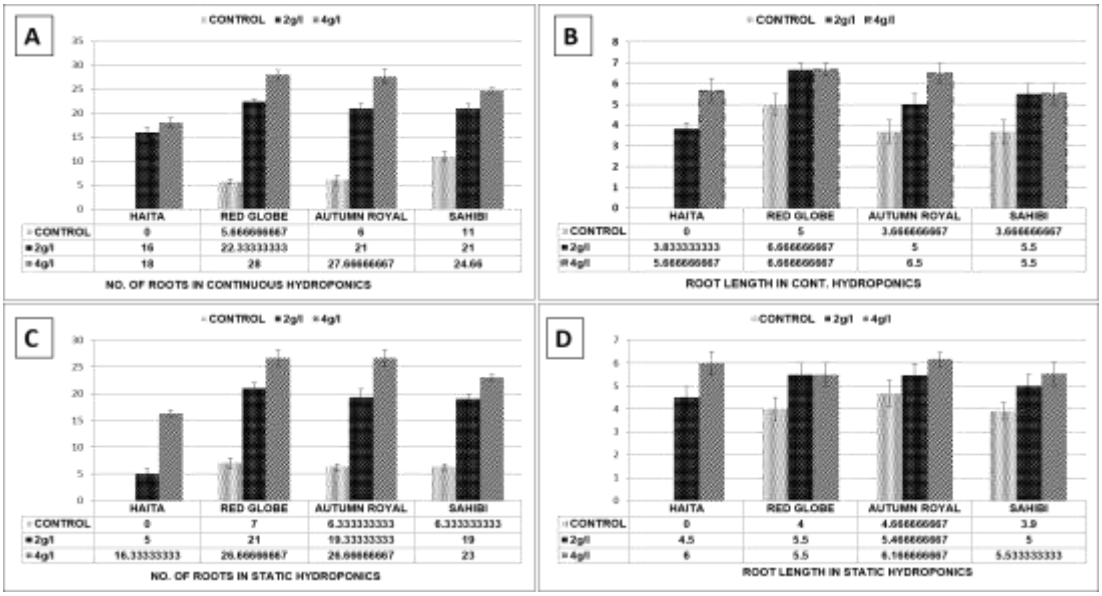

Fig. 1. Shows the number of roots and root length in both static and continuous hydroponics.
[A] Number of roots in continuous hydroponics. [B] Root length in continuous hydroponics. [C] Number of roots in static hydroponics. [D] Root length in static hydroponics. All has 4 grapes genotypes with 2 treatments and a control. The P value is the significant value if it is less than 0.05.

The maximum root length of Red Globe was found in T_2under static hydroponics which was 5.5cm; It was smaller than all other grapes genotypes.

The maximum root length of Red Globe in T_1 was also 5.5cm which was greater among all other grapes genotypes and the maximum root length of Red Globe under control of static hydroponics was 4cm in all three replicates which was greater than Sahibi but lesser than Autumn Royal (Fig. 1D). The results of maximum root length of Autumn Royal in static hydroponics were as followed. The average maximum root length in three replicates of control, T_1 and T_2 was 4.6cm, 5.4cm and 6.1cm respectively.

Under T_2 the maximum root length was greater among all genotypes but under T_1 the maximum root length was greater than Haita and Sahibi but smaller than Red Globe (Fig. 1D). The maximum root length of genotype Sahibi were found greatest in T2 compared to other treatments. The maximum root length of Sahibi in three replicates of T_1 and T_2 was 5.5cm and 5cm respectively. The maximum root length of Sahib under control IBA was 3.9 cm, it was smaller than all other grapes genotypes. Under T_1 the root length of Sahibi was greater than Haita and in T_2it was smaller than Haita and Autumn Royal as well but greater than Red Globe (Fig. 1D). After one month there was no rooting in soil in all the genotypes and treatments (Fig. 2).

Table 3. Post hoc analysis of root length in static hydroponics with respect to treatments.

Multiple Comparisons

Dependent Variable: Maximum Root Length LSD

(I) Indole Butyric Acid	(J) Indole Butyric Acid	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment one control	Treatment two 2g/l	-1.9750*	.18832	.000	-2.3637	-1.5863
	Treatment three 4g/l	-2.6583*	.18832	.000	-3.0470	-2.2697
Treatment two 2g/l	Treatment one control	1.9750*	.18832	.000	1.5863	2.3637
	Treatment three 4g/l	-.6833*	.18832	.001	-1.0720	-.2947
Treatment three 4g/l	Treatment one control	2.6583*	.18832	.000	2.2697	3.0470
	Treatment two 2g/l	.6833*	.18832	.001	.2947	1.0720

Based on observed means. The error term is Mean Square (Error) = .213.
*The mean difference is significant at the 0.05 level.

Table 4. Post hoc analysis of root length in static hydroponics with respect to genotypes.

Multiple Comparisons

Dependent Variable: Maximum root length Static LSD

(I) Grapes Genotypes	(J) Grapes Genotypes	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Haita	Red Globe	-1.5000*	.21745	.000	-1.9488	-1.0512
	Autumn Royal	-1.9333*	.21745	.000	-2.3821	-1.4845
	Sahibi	-1.3111*	.21745	.000	-1.7599	-.8623
Red Globe	Haita	1.5000*	.21745	.000	1.0512	1.9488
	Autumn Royal	-.4333	.21745	.058	-.8821	.0155
	Sahibi	.1889	.21745	.394	-.2599	.6377
Autumn Royal	Haita	1.9333*	.21745	.000	1.4845	2.3821
	Red Globe	.4333	.21745	.058	-.0155	.8821
	Sahibi	.6222*	.21745	.009	.1734	1.0710
Sahibi	Haita	1.3111*	.21745	.000	.8623	1.7599
	Red Globe	-.1889	.21745	.394	-.6377	.2599
	Autumn Royal	-.6222*	.21745	.009	-1.0710	-.1734

Based on observed means. The error term is Mean Square (Error) = .213.
*The mean difference is significant at the 0.05 level.

Continuous hydroponics

Number of roots

The number of roots in all genotypes and treatments under continuous hydroponics system was significantly greater in T_2containing 4mM IBA. The comparison of number of roots of control with T_1 and T_2 showed significant mean differences with the value of -14.4176* and -18.9167*. All genotypes showed significant mean difference when kept under different IBA treatments, however non-significant differences between each other on each treatment (Table 5 & 6). Taking each variety individually the number of roots of Haita was greatest in T_2 which was 18. The number of roots in three replicates of Haita under T_1 was 16 while there was no rooting in control. In continuous hydroponics the number of roots of Haita under all treatments was lower as compare to other genotypes (Fig. 2A).

Table 5. Post hoc analysis of number of roots in continuous hydroponics with respect to treatments.

Multiple Comparisons

Dependent Variable: Number of Roots LSD

(I) Indole Butyric Acid	(J) Indole Butyric Acid	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment one control	Treatment two 2g/l	-14.4167*	.37884	.000	-15.1986	-13.6348
	Treatment three 4g/l	-18.9167*	.37884	.000	-19.6986	-18.1348
Treatment two 2g/l	Treatment one control	14.4167*	.37884	.000	13.6348	15.1986
	Treatment three 4g/l	-4.5000*	.37884	.000	-5.2819	-3.7181
Treatment three 4g/l	Treatment one control	18.9167*	.37884	.000	18.1348	19.6986
	Treatment two 2g/l	4.5000*	.37884	.000	3.7181	5.2819

Based on observed means. The error term is Mean Square (Error) = .861.

*The mean difference is significant at the 0.05 level.

Table 6. Post hoc analysis of no. of roots in continuous hydroponics with respect to genotypes.

Multiple Comparisons

Dependent Variable: no of roots continuous LSD

(I) Grapes Genotypes	(J) Grapes Genotypes	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Haita	Red Globe	-7.3333*	.43744	.000	-8.2362	-6.4305
	Autumn Royal	-6.8889*	.43744	.000	-7.7917	-5.9860
	Sahibi	-7.5556*	.43744	.000	-8.4584	-6.6527
Red Globe	Haita	7.3333*	.43744	.000	6.4305	8.2362
	Autumn Royal	.4444	.43744	.320	-.4584	1.3473
	Sahibi	-.2222	.43744	.616	-1.1251	.6806
Autumn Royal	Haita	6.8889*	.43744	.000	5.9860	7.7917
	Red Globe	-.4444	.43744	.320	-1.3473	.4584
	Sahibi	-.6667	.43744	.141	-1.5695	.2362
Sahibi	Haita	7.5556*	.43744	.000	6.6527	8.4584
	Red Globe	.2222	.43744	.616	-.6806	1.1251
	Autumn Royal	.6667	.43744	.141	-.2362	1.5695

Based on observed means. The error term is Mean Square (Error) = .861.
*The mean difference is significant at the 0.05 level.

The number of roots in three replicates of Red Globe in continuous hydroponics was greatest in T_2. The number of roots of Red Globe under T_2 was 28 which were greater than all other grapes genotypes. Similarly the number of roots of Red Globe in T_1 was 22.3 that were also greater than all other genotypes of grapes and the number of roots of Red Globe in control was 5.6, lower than Autumn Royal and Sahibi (Fig. 2B).

The number of roots of Autumn Royal in T_2 was 27.66 which were greater than Haita and Sahibi. Similarly in continuous hydroponics system the number of roots of Autumn Royal under T_1 and control was 21 and 6 respectively (Fig. 2C).

The results of number of roots of Sahibi were same as other genotypes i.e. the greatest number of roots of Sahibi was observed under T_2 followed by T_1 and control.

The number of roots in three replicates of Sahibi in T_2, T_1 and control was 24.66, 21 and 11 respectively. The number of roots was lower than Autumn Royal and Red Globe under T_2while the numbers of roots were equal to Autumn Royal but lesser than Red Globe under T_1 (Fig. 2D).

Root length (cm)

The root length of all genotypes and treatments of continuous hydroponics was significantly greater in T_2 which contain 20mM IBA.

The comparison of control with T_1 and T_2 showed the significant mean difference with the value of -2.1667* and -3.00* while the comparison of T_1 and T_2 showed significant mean difference with the value of -0.8333* (Table 7).

With respect to genotypes, the comparison of Autumn Royal with Sahibi didn't show any significant mean difference while the comparison of other genotypes showed significant mean difference with each other (Table 8).

Table 7. Post hoc analysis of root length in continuous hydroponics with respect to treatments.

Multiple Comparisons

Dependent Variable: Maximum Root Length LSD

(I) Indole Butyric Acid	(J) Indole Butyric Acid	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Treatment one control	Treatment two 2g/l	-2.1667*	.18634	.000	-2.5513	-1.7821
	Treatment three 4g/l	-3.0000*	.18634	.000	-3.3846	-2.6154
Treatment two 2g/l	Treatment one control	2.1667*	.18634	.000	1.7821	2.5513
	Treatment three 4g/l	-.8333*	.18634	.000	-1.2179	-.4487
Treatment three 4g/l	Treatment one control	3.0000*	.18634	.000	2.6154	3.3846
	Treatment two 2g/l	.8333*	.18634	.000	.4487	1.2179

Based on observed means. The error term is Mean Square (Error) = .208.
*The mean difference is significant at the 0.05 level.

Table 8. Post hoc analysis of root length in continuous hydroponics with respect to genotypes.

Multiple Comparisons

Dependent Variable: Maximum root length Continuous LSD

(I) Grapes Genotypes	(J) Grapes Genotypes	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Haita	Red Globe	-2.9444*	.21517	.000	-3.3885	-2.5004
	Autumn Royal	-1.8889*	.21517	.000	-2.3330	-1.4448
	Sahibi	-1.7222*	.21517	.000	-2.1663	-1.2781
Red Globe	Haita	2.9444*	.21517	.000	2.5004	3.3885
	Autumn Royal	1.0556*	.21517	.000	.6115	1.4996
	Sahibi	1.2222*	.21517	.000	.7781	1.6663
Autumn Royal	Haita	1.8889*	.21517	.000	1.4448	2.3330
	Red Globe	-1.0556*	.21517	.000	-1.4996	-.6115
	Sahibi	.1667	.21517	.446	-.2774	.6107
Sahibi	Haita	1.7222*	.21517	.000	1.2781	2.1663
	Red Globe	-1.2222*	.21517	.000	-1.6663	-.7781
	Autumn Royal	-.1667	.21517	.446	-.6107	.2774

Based on observed means. The error term is Mean Square(Error) = .208.
*The mean difference is significant at the 0.05 level.

The maximum root length in three replicates of Haita under all treatments was greater in T_2 followed by T_1 and control. The maximum root length of Haita under T_2 was 5.6cm.

Similarly the root length of Haita in continuous hydroponics under T_1 was 3.83cm while control had no rooting. The maximum root length of Haita was lower than all other grapes genotypes under all the treatments (Fig. 1B).

The maximum root length in T_2 of Red Globe under continuous hydroponics was 6.6cm. The maximum root length of Red Globe in T_1 was also 6.6cm and the maximum root length of Red Globe in control of continuous hydroponics was 5cm in all three replicates. Under all the treatments the maximum root length of Red Globe was greater than all other grapes genotypes (Fig. 1B). The results of maximum root length of Autumn Royal in continuous hydroponics were as followed. The average maximum root length in three replicates under control, T_1 and T_2 was 3.6cm, 5cm and 6.5cm respectively.

The maximum root length was lower than Red Globe and at par with Sahib under control while the root length was greater than Haita but lesser than Red Globe and Sahib under T_1 (Fig. 1B).

The maximum root length of Sahibi in all three treatments of continuous hydroponics system was same in T_1 and T_2 which was 5.5 cm each. The root length of Sahibi was lower than all other grapes genotypes under T_2 and the maximum root length of Sahibi was greater than Haita and Autumn Royal under T_1. The maximum root length of Sahibi was 3.66cm under control which was same to Autumn Royal but lower than Red Globe (Fig. 1B).

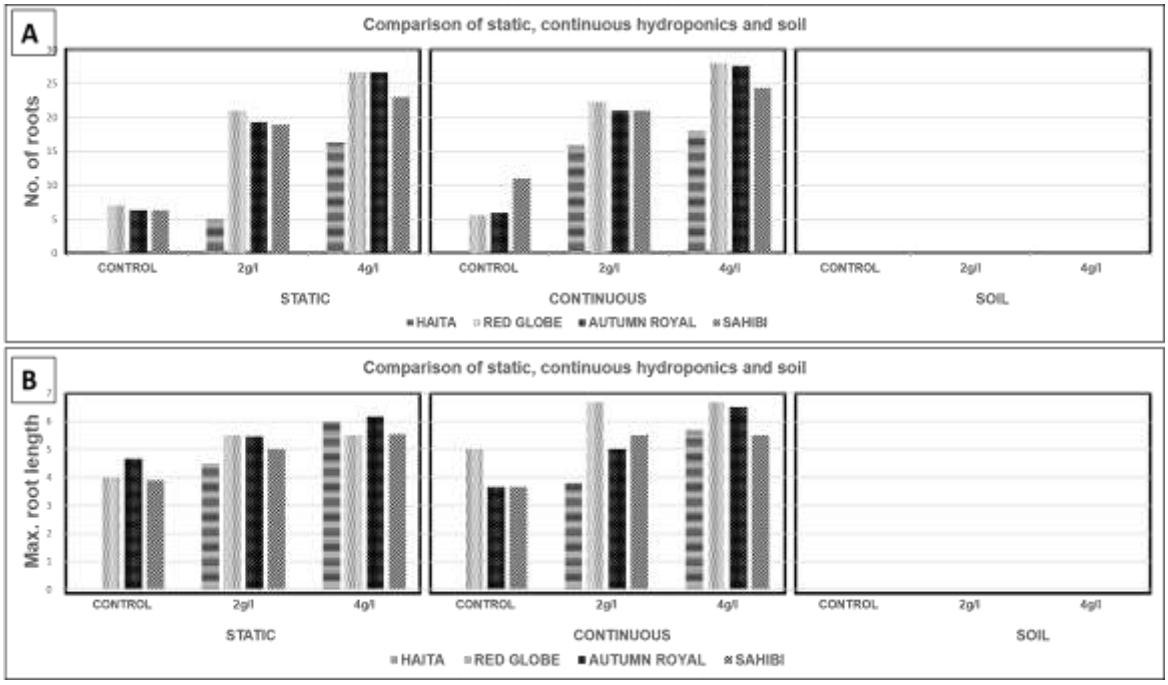

Fig. 2, Comparison of number of roots and root length in both static and continuous hydroponics with soil after one month.

[A] Comparison of number of roots in soil and both continuous and static hydroponics after one month. Soil has no rooting in both the treatments and control. [B] Comparison of root length in soil and both hydroponics systems after one month.

Discussion

We were not able to found any published material from Pakistan about standardizing the protocols for rooting of grapes in hydroponics, therefore this study was novel. After facing lots of physical and operational problems limited success was achieved. Initially there were severe problem due to not having any literature about controlling contamination, formulating optimal IBA concentrations, deciding about type of nutrients solution to be used for purpose and avoiding decomposition due to continuous dip of cuttings in nutrients solution. Due to contamination a sticky growth called slime occurred on the plants cuttings which blocked the way of nutrients for the cuttings.

The part of shoot cuttings that were under the nutrient solution was developing slime on it and the part of shoot cuttings that was above the solution was showing slight contamination of fungal growth. To avoid these contaminations tissue culture sterilization method was used i.e. 10% NaOCl with 70% Ethanol. Shoot cuttings were soaked in it for 10min (Yen and & Hain) and then planted both in hydroponics and soil. Furthermore to control the contamination in nutrient media slightly chlorinated water was used with a concentration of 3ppm. This concentration is in accordance with (Deniel *et al.,* 2009).

The amount of IBA for hydroponics was also not known. Initially IBA concentration was kept lower i.e. 200 and 400 µM(Nissen and Sutter 1990) but due to not obtaining good results it was increased to 0, 2 and 4mM which are in accordance with (Galavi *et al.,* 2013). As for as supplementation of IBA in hydroponics system is concerned it was continuously supplied in the nutrient medium.

The growth occurred on $1/4^{th}$ strength Hoagland's media, while according to literature (Albuquerque and Dechen 2000) studied the absorption of macronutrients by rootstocks of grapevine cultivars in hydroponics. These reports were showing use of mixture of solutions for macronutrients (Furlani 1995) and micronutrients (Hoagland and Arnon 1950). The results of continuous hydroponics were better than static hydroponics because in static hydroponics there were more chances of contamination. After one month there were more rooting in treatment two i.e. $4g.l^{-1}$ of IBA among all genotypes in continuous hydroponics. The maximum root length was also greater in treatment two i.e. 4mM. Genotype Red Globe and Sahib showed better root length in T_1 i.e. 2mM was also equal to treatment two. Very same type of results was obtained in static hydroponics experiments. On the basis of these results it could be concluded that the advance technique of hydroponics along with supplementation of rooting hormone can be used as an efficient system to produce rooted nursery plants of grapes in significantly short span of time compared with conventional methods. Furthermore, these finding also showed importance of IBA for development of roots in grapes which is an accordance with the findings of (de Klerk *et al.,* 1999).

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