Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/1382
DOI URL: http://dx.doi.org/10.21474/IJAR01/1382
ISSN NO. 2320-5407

RESEARCH ARTICLE

MULTIPLICATION OF NEMAGUARD AND MARIANA ROOTSTOCKS: INFLUENCE OF CYTOKININS AND ADENINE SULFATE AND THEIR CONCENTRATIONS.

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Manuscript Info

Manuscript History

Received: 18 June 2016
Final Accepted: 16 July 2016
Published: August 2016

Key words:-
Nemaguard and Mariana rootstocks, multiplication, cytokinin, AS.

Abstract

This study was conducted during the period 2015 to 2016 at plant tissue culture laboratory, Department of Horticulture, College of Agriculture, University of Baghdad.

In this research, the effect of cytokinin (6-Benzyladenin (BA) and Kinetin (Kin)), Adenine Sulfate (AS) and their concentrations on shoot multiplication were evaluated. Young nodal segments with a single bud from 5-8 years old of peach and plum rootstocks (Nemaguard and Mariana) were used as explants. The results can be summarized as follows:

For Nemaguard and Mariana shoot induction, MS (Murashige and Skoog, 1962) medium supplemented with (BA) $2mgl^{-1}$ and $0.2mgl^{-1}$ GA_3 (Gibbrelic Acid) and $0.4mgl^{-1}$ (GA_3), resulted in high shoot regeneration percentage (100%). For shoot multiplication, $6mgl^{-1}$ (Kin) in combination with 50 mgl^{-1} (AS) increased the mean of shoots number up to 13.62, 15.62 shoot per explant for Nemaguard and Mariana respectively. From the tested combination $6mgl^{-1}$ (Kin) with 50 mgl^{-1} (AS) was best performing by resulting in 4.20 cm mean length of shoots for Nemaguard rootstock.

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Introduction:-

Plum (*Prunrs cerasifera* Ehrh) is belonging to the Prunoideae subfamily of the Rosaceae family, which involves all of the stone fruits such as: apricot, peach and cherry. Plums have the most taxonomically diverse of the stone fruits and are more compatible to the diverse climatic and soil condition, as well as they are having the resistance to drought, crown rot and root knot nematodes (Griesbach, 2007). Nemaguard (Prunuspersica L. Batsch × PrunusdavidianaCarriere) and Mariana (*Prunus mariana*) are important rootstocks of plum (Revees *et al*., 1983).

Conventional methods of propagation do not produce true to type from seed, and also do not ensure disease free plants (Holtz *et al*., 1995), conversely, *in vitro* techniques have overcome these problems and have the potential to supply the good multiplication rates of uniform genotypes (Hammatt and Grant, 1993). Also Estifanos (2014) observed the potentiality of *in vitro* regeneration of plum variety, Myrobalan, and showed it is an efficient and reproducible protocol for genotype regeneration.

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Different factors of woody plants micropropagation have been studied as the influence of different combinations of growth regulators (Pruski *et al*., 2000; Andreu and pilar, 2005), the application of AS (Vicas, 2011; Al Dabbagh and Salman, 2000; Dodds and Roberts, 1985) and the impact of various cytokinins (Farag *et al*., 2012; Mansvelt *et al*., 2006).

Additional additives (such as AS) used in culture medium have proved their efficiency and beneficially at the plant species that have a difficult reproduction *in vitro* by stimulating the growth and development for number of plant tissues (Kulcarni *et al*., 2007).

Materials and methods:-

Micropropagation study:-

Preparation of explants:-

Five to eight years old stock plants of Nemaguard and Mariana rootstocks were taken from fruit cultivation station/ Iraqi ministry of agriculture (Al Hawija, at the north of Iraq). Actively growing shoots of the donor plants were cut off, then the leaves were removed and cut into nodal parts with approximate size of 4-5 cm to be disinfected and cultured. Explants were washed under running tap water 5 times, surface sterilization was carried out under complete aseptic conditions at the Laminar air flow cabinet. These explants were sterilized with 70% ethanol-alcohol for a minute and rinsed them three times with sterile distilled water, followed by sterilization with (10% v/v) commercial Clorox solution with a drop of Tween-20 for 20 minutes with continues shaking and rinsed. Finally, the nodal parts were washed 3 times with sterile distilled water to remove the bleach off the explants and trimmed to 2 cm long (V-shape) to increase the surface area of absorption.

The basic nutrient medium:-

Nodal explants were planted vertically on solid basal medium of MS supplemented with vitamins ($100mgl^{-1}$ myo-inositol and $30\ gl^{-1}$ sucrose. BA, Kin, GA_3, and AS were used independently or in combination at different concentrations. The value of pH was adjusted to 5.7±0.1 with few drops of 0.1N either HCl or NaOH prior to agar addition and autoclaving. The media were dispensed into 40 ml jars and autoclaved for 20 minutes at 121 C° and $1.2kg/cm^2$ pressure, then left at room temperature (22±2C°) to cool.

Culture growth conditions:-

Tissue culture jars were placed in an incubation room at 25±1C° under 16 hours photoperiod of 1000 lux supplied with cool white fluorescent lamps.

Influence of different growth regulators on shoot induction:-

The shoot induction development from the stem node segments was attempted with MS medium supplemented with different concentrations of BA (0.0, 1.0, 2.0, 3.0 mgl^{-1}) in combination with GA_3 (0.0, 0.1, 0.2, 0.3 mgl^{-1}) in the presence of 0.3 mgl^{-1} NAA.

Percentage of explants forming axillary shoots% and mean length of these shoots (cm) were recorded after 6 weeks of culture.

Multiplication stage:-

Shoots were placed in MS hormone-free medium for 4 weeks in order to improve shoot elongation. 0.7-0.8 cm – long shoots from previous culture were subjected to be multiplied on MS medium containing different concentration of Kin (0.0, 2.0, 4.0, 6.0 mgl^{-1}) in combination with AS (0.0, 25.0, 50.0, 75 mgl^{-1}) in the presence of 0.1 mgl^{-1} GA_3. For further multiplication, the shoots were subcultured 2 times on the best medium. Mean number and length (cm) of axillary shoots/explant were recorded after 6 weeks of subculture.

Statistically analysis:-

This experiment was carried out based on Completely Randomized Design (CRD), each treatment was done in 10 replications. SPSS 16 software was used for statistically analysis of the data, and differences among means of treatments were compared by using (LSD) Least Significance Design (Steel *et al*., 1997).

Results and Discussion:-

Influence of growth regulators on shoot induction:-

Influence of growth regulators on shoot induction(%):-

The basic goal of the establishment stage is to get a large percentage of free pathogens explants (Murashige, 1974). There are many factors that affect the success of establishment stage involved:

- The choice of plant material
- Elimination of contamination
- Culture conditions

(Hartman and Kester, 1983)

In this experiment, various concentrations of BA as cytokinin and GA_3 in the presence of NAA as an auxin were added to MS medium for shoot induction of the studied rootstocks; Nemaguard and Mariana. Table 1 showed the effect of different concentrations of BA and GA_3 with 0.3mgl^{-1} NAA on the shoot induction (%) of both rootstocks stem node sections. The interaction between genotype, BA and GA_3 and concentration was highly significant for shoot induction. For Nemaguard, the highest percentage (100%) was observed on media supplemented with 2mgl^{-1} BA and 0.1mgl^{-1} GA_3. For Marina, the optimum percentage of shoot induction (100%) was on media with 1mgl^{-1} BA and 0.3mgl^{-1} GA_3.

It´s well known that BA promotes cell division and cell expansion in plant tissue culture, These results agreed with those gained by Magalhaes *et al*. (2012) who reported that addition of GA3 to the culture medium was superior in increasing proliferation of Japanese plum (*Prunus salicina* Lindl.) cv. América shoots.

Table 1:- Effect of genotype, kind and concentration of BA and GA_3 on shoot induction (%) of Mariana and Nemaguard rootstocks cultured on MS media supplemented with 0.3mgl^{-1} NAA.

Genotype X BA conc.	GA_3 conc. mgl^{-1}				BA conc. mgl^{-1}	Genotype
	0.30	0.40	0.20	0.00		
0.25	0.40	0.30	0.30	0.00	0.00	Mariana
0.78	1.00	1.00	0.60	0.50	1.00	
0.72	1.00	1.00	0.60	0.30	2.00	
0.73	1.00	1.00	0.50	0.40	3.00	
0.16	0.30	0.20	0.17	0.00	0.00	Nemaguard
0.50	0.60	0.60	0.60	0.20	1.00	
0.88	1.00	1.00	1.00	0.50	2.00	
0.85	1.00	1.00	1.00	0.40	3.00	
Genotype						
0.62	0.85	0.82	0.50	0.30	Mariana	Genotype X GA_3 conc.
0.60	0.73	0.70	0.69	0.27	Nemaguard	
Mean of BA conc.						
0.21	0.36	0.26	0.23	0.00	0.00	BA conc. X GA_3 conc.
0.64	0.80	0.79	0.60	0.35	1.00	
0.80	1.00	1.00	0.80	0.40	2.00	
0.79	1.00	1.00	0.75	0.40	3.00	
	0.79	0.76	0.60	0.28	Mean of GA_3 conc.	
BA X GA_3=0.23 Genotype X GA_3 = 0.16 Genotype XGA_3 X BA= 0.33				Genotype = 0.08 GA_3 =30.11, BA= 0.11 Genotype X BA=0.16		L.S.D 0.05

Influence of growth regulators on induced shoot length (cm):-

Table 2 showed the effect of different concentrations of BA and GA_3 with 0.3mgl^{-1} NAA on the induced shoot length (cm)of both rootstocks stem node sections. It is important to note the interaction of BA, GA_3 and hormonal concentrations, highest average (1.95 cm) was obtained from the treatment (3mgl^{-1} BA+ 0.6 mgl^{-1} GA_3). Highly shoot length of Nemaguard rootstock was observed between the interaction of genotype, growth regulator type and hormonal concentrations producing maximum shoot length (2.22 cm) in treatment containing (3mgl^{-1} BA+ 0.6 mgl^{-1} GA_3)which proved to be the best treatment.Peirik (1987) reported that the cytokinin promoted division of cell by activating the synthesis of DNA; promoting the growth of an axillary buds and inducing shoot formation.

These results were agreed to this obtained by Yepes and Aldwinckle (1994) who found that the balance between GA_3 and auxin affected shoot elongation positively.

Table 2:- Effect of genotype, kind and concentration of BA and GA_3 in presence of 0.3mgl^{-1} NAA on induced shoot length (cm) of Mariana and Nemaguard explants.

Genotype X BA conc.	GA_3 conc. mgl^{-1}				BA conc. mgl^{-1}	Genotype
	0.60	0.40	0.20	0.00		
0.21	0.41	0.26	0.17	0.00	0.00	Mariana
0.94	1.62	0.97	0.57	0.58	1.00	
1.15	1.66	1.73	0.84	0.36	2.00	
1.18	1.69	1.80	0.76	0.47	3.00	
0.09	0.19	0.15	0.01	0.00	0.00	Nemaguard
0.80	1.03	1.02	0.90	0.25	1.00	
1.35	1.67	1.65	1.41	0.66	2.00	
1.56	2.22	2.02	1.44	0.53	3.00	
Genotype						
0.87	1.34	1.19	0.58	0.35	Mariana	Genotype X GA_3 conc.
0.95	1.28	1.21	0.96	0.34	Nemaguard	
Mean of BA conc.						
0.16	0.31	0.21	0.11	0.00	0.00	BA conc. X GA_3 conc.
0.87	1.32	0.99	0.73	0.42	1.00	
1.25	1.67	1.69	1.12	0.51	2.00	
1.37	1.95	1.91	1.10	0.50	3.00	
	1.31	1.20	0.77	0.35	Mean of GA_3 conc.	
BA X GA_3=0.31 Genotype X GA_3 = 0.22 Genotype XGA_3 X BA= 0.45				Genotype = 0.11 GA_3 =0.16, BA= 0.16 Genotype X BA=0.22		L.S.D 0.05

multiplication stage: Influence of growth regulators and AS on Mean number and length of axillary shoots/explant:-

following the successful growth of plant tissue culture, the establishment stage is followed by multiplication, which is defined as a rapid increase of organs which is achieved by enhancing axillary shoot initiation, through repeated this process, hundreds or thousands of plants may be produced from a single explant sample (Smith and Murashige, 1970; Murashige, 1974).

In this experiment, lateral shoots produced from the previous stage were excised and cut into 2 bud segments. Tables 3 & 4 and fig.1&2 showed the effect of different concentration of Kin in combination with various concentrations of AS in the presence of 0.1 mgl^{-1} GA_3 on the mean number and length of lateral buds for both Nemaguard and Mariana rootstocks.

Increasing Kin concentration up to 6 mgl^{-1} in combination with AS led to an enlargement in mean number and length of lateral buds, data in table 3 and 4 revealed that the mean number and length of Nemaguard, Mariana axillary shoots increased significantly as Kin concentration increased until reached the highest value of 13.62, 15.62 shoot/explant and 4.20, 0.78 cm respectively at treatment of 6 mgl^{-1} Kin+50mgl^{-1} AS.

The effect of adenine sulfate in known in the tissue cultures in many plant species and types of vegetal tissues, affect that is superior in combination with a balanced dose of cytokinin and auxin **(**Yepes and Aldwinckle**,** 1994**).**

Table 3:- Effect of genotype, kind and concentration of Kin and AS in the presence of 0.1 mgl^{-1} GA_3 on mean number of shoots (shoot per explant) of Nemaguard, Mariana axillary shoots.

Genotype X Kin conc.	AS conc. mgl^{-1}				Kin conc. mgl^{-1}	Genotype
	75	50	25	0		
2.09	3.62	2.38	2.38	0.00	0.00	Mariana
5.06	5.38	7.62	4.62	2.62	2.00	
7.81	6.25	11.62	8.00	5.38	4.00	
9.50	11.38	15.62	7.62	3.38	6.00	
1.19	1.63	1.77	1.34	0.00	0.00	Nemaguard
4.28	3.50	7.62	3.38	2.62	2.00	
7.37	7.15	12.11	7.11	3.11	4.00	
8.37	9.40	13.62	7.25	3.25	6.00	
Genotype						
5.30	6.66	9.31	5.66	2.84	Mariana	Genotype X AS conc.
6.12	5.41	8.78	4.77	2.24	Nemaguard	
Mean of Kin conc.						
1.64	2.63	2.08	1.86	0.00	0.00	Kin conc. X AS conc.
4.67	4.44	7.62	4.00	2.62	2.00	
7.59	6.70	11.86	7.55	4.24	4.00	
8.94	10.38	14.62	7.43	3.31	6.00	
	6.04	9.05	5.21	2.55	Mean of AS conc.	
Kin X AS=0.32 Genotype X AS = 0.23 Genotype XAS X Kin= 0.46				Genotype = 0.12 Kin =0.16, AS= 0.16 Genotype X Kin=0.23		L.S.D 0.05

Table 4:- Effect of genotype, kind and concentration of Kin and AS in the presence of 0.1 mgl^{-1} GA_3 on mean length of shoots (cm) of Nemaguard, Mariana axillary shoots.

Genotype X Kin conc.	AS conc. mgl^{-1}				Kin conc. mgl^{-1}	Genotype
	75.00	50.00	25.00	0.00		
0.54	0.86	0.74	0.69	0.00	0.00	Mariana
0.68	0.44	0.58	0.71	0.66	2.00	
0.70	0.40	0.69	0.53	0.60	4.00	
0.57	0.59	0.78	0.80	0.70	6.00	
1.55	0.62	0.68	0.59	0.00	0.00	Nemaguard
1.56	0.77	0.97	0.93	1.15	2.00	
1.80	1.66	1.38	0.99	1.13	4.00	
1.54	3.62	4.20	3.72	3.91	6.00	
Genotype						
0.62	0.72	0.55	0.65	0.57	Mariana	Genotype X AS conc.
1.61	3.86	1.16	0.96	0.47	Nemaguard	
Mean of Kin conc.						
0.52	0.74	0.71	0.64	0.00	0.00	Kin conc. X AS conc.
0.80	0.60	0.78	0.82	1.00	2.00	
0.86	0.78	1.03	0.76	0.87	4.00	
2.29	2.10	2.50	2.26	2.30	6.00	
	1.06	1.25	1.12	1.05	Mean of AS conc.	
Kin X AS=0.56 Genotype X AS = 0.39 Genotype XAS X Kin= 0.79				Genotype = 0.20 Kin =0.28, AS= 0.28 Genotype X Kin=0.39		L.S.D 0.05

Figure 1:- Proliferation of Mariana axillary shoots on MS medium containing 6 mgl^{-1} Kin+50mgl^{-1} AS+0.1 mgl^{-1} GA_3

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