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Full Length Research Paper

The effect of plant growth regulators, cultivars and substrate combination on production of virus free potato minitubers

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Nowadays large amounts of potato seed in the world is produced by *in vitro* virus free minitubers. Therefore, evaluation of commercial varieties in production of virus free potato minitubers is critical. In this study virus free plantlets of 4 potato cultivars of Agria, Marfona, Sante and Burren were achieved with meristem culture method. The meristem-derived plantlets which had optimum growth were transferred to the MS-medium without any growth regulators to stimulate the same vigor of the plantlets. Then DAS-ELISA test was conducted and the effects of different level of 2 growth regulators either singly or in combination were evaluated on single node culture of potato. In all cultivars the best and most economical medium for single node culture was MS medium without growth regulators. Afterwards virus free plantlets were selected and transferred to the green house. The effects of substrate combination including 4 planting bed in minituber production were evaluated, after 90 days, numbers and diameters of minitubers for each cultivar were counted and recorded. Marfona had the greatest number of minitubers and Agria had the lowest. There are no any significant differences in minituber diameter between the cultivars. A mixture of peat moss and sand in the ratio of 1:1 was proved to be the best for minituber production.

Key words: Potato, plant growth regulators, single node culture, substrate combination, minituber.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is the fourth ranked world crop which had 20 million ha planting area in the world in 2005 and produced nearly 325 million tons annually (FAO, 2007). It is the most widely cultivated food crop after wheat, rice and maize (Anonymous, 2000). It originates from the western hemisphere and the Andes mountain range in southern America (Woolf, 1986).

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Abbreviations: MS, Murashige and skoog; **BA**, butyric acid; **IBA**, indol butyric acid; **NAA**, naphthalene acetic acid; **GA3**, gibberlic acid; **BAP**, (6-benzyl-amino purine; **KIN**, 6-furfuryl amino purine; **DAS-ELISA**, double antibody sandwich ELISA; **CRD**, completely randomized design.

Planted area in Iran is 189670 ha which produces 25763 kg/ha (Anonymous, 2005). In the common environment conditions, potato is infected with 25 viruses and 1 viruid (Salazar, 1996). Potato production is being seriously hampered due to certain viruses, fungus and bacterial diseases. Researchers showed that some viruses can decrease the yield by 40% singly and in combination with other viruses, the loss is 90% (Siddiqui et al., 1996). In Iran many of the used tubers have virus infection and a virus free field is rarely found. The meristem in the virus infection plants have a minimum concentration of virus or are virus free and the best control method for potato viruses is production of healthy plants from meristem culture (Espinoza et al., 1992).

The numerous and different tissue culture methods were done in potato as one of the first nutrition productions (Wersuhn and Dathe, 1998). These methods

Figure 1. Meristem grown in liquid medium.

allow rapid multiplication of potato clones (Ahloowalia, 1994). At first White (1943) reported the production of virus free plantlets with tissue culture method. He showed that mosaic virus concentration in the old region of tomatoes root is less than the younger region (White, 1943). Potato virus free clones with meristem culture-methods were conducted by researchers such as Ebadi et al. (2007) and Nagib et al. (2003). Producing mini-tubers from *in vitro* plantlets allows a faster multiplication rate in seed tuber production programs and reduces the number of required field generations (Imma and Mingo-Castel, 2006). The mini-tubers must have a minimum of phytopathogenic infections and must be true-to-type. The difficulty lies in the fact that tubers naturally tend to accumulate and transmit viral, bacteriological and fungal diseases to the next generation, which weakens the plant production potential progressively (Balali et al., 2008). Therefore production of virus free plantlets and minitubers are important. Ebadi et al. (2007) cultured meristem-derived plantlets in MS (Murashige and Skoog) medium containing 0.5 mgl^{-1} GA3 + 0.4 mgl^{-1} NAA and produced regenerated plants with single node culture method (Ebadi et al., 2007). Modarres Sanavy and Jami Moeini (2003) studied the effect of cultivars, NAA (Naphthalene acetic acid), BAP (6-Benzyl-amino purine) growth regulators and different soil combinations in production of minituber of Agria and Marfona in the plantlets that were achieved with meristem culture. In their study to multiply plantlets that were achieved in meristem culture technique, they cultured single node in the MS solid media containing NAA and BAP. They showed that the best media for potato single node culture is MS media without any growth regulators and the Marfona produced the largest number of minituber and the best mixture for growing plantlets and minituber production is peat moss/ sand with 4 to 1 ratio (Modarres Sanavy and Jami Moeini, 2003). Shah Zaman et al (2001) showed that the highest stem length and the largest single nodes were achieved in MS medium containing 0.5 mgl^{-1} NAA and highest root length in MS containing 1 mgl^{-1} NAA (Shah Zaman et al., 2001). Bostan and Demirel (2004) indicated that the best medium for potato single node culture is MS medium without any growth regulators (Bostan and Demirel, 2004).

Vanaei et al. (2008) studied the effects of 2 commercial cultivars of Marfona and Agria that were achieved by meristem culture, substrate combination including 3 planting beds and 2 pot sizes on number and total weight of minitubers. Results showed that the Marfona produced the largest number of minitubers and mixture of turb/perlite in the ratio of 1:1 had the highest value for total weight and number of minitubers (Vanaei et al., 2008). Balali et al. (2008) studied the effect of genotype, planting date and pot size in production of minituber in the plantlets that originated from virus free sprouts and a genotype of the same cultivar (Marfona) originated from apical meristem. They used sterilized soil (2 parts sandy loam, one part decayed compost). The results showed that the number of mini-tubers per plant was higher for genotypes originated from meristem culture than genotypes obtained from sprouts (Balali et al., 2008).

MATERIALS AND METHODS

Due to the importance of production of virus free plants and minitubers and necessity of production of minituber in countries, the growth regulators effects and different cultivars in single node culture and different planting beds on the production of potato minitubers were evaluated in this study. Four commercial cultivars of Burren, Agria, Marfona and Sante were cultured in the Potato, Onion and Irrigated Legumes Department of Seed and Plant Improvement Institute's green house. After 1 month the pots were moved to laboratory for meristem isolation. Lateral buds were used for meristem isolation under complete aseptic conditions in the laboratory. Isolated meristems were placed on filter paper bridge in liquid medium (Figure 1). Modified MS medium with GA3 (Gibberlic acid) and KIN (6-Furfuryl amino purine) were applied at this stage. Later the test tubes were put in the growth chamber of 25°C temperature, 16 h light (3000 - 4000 Lux) and 8 h darkness period. Meristem derived plantlets which had optimum growth were transferred to MS medium without any growth regulators to stimulate the same vigor of the plantlets. After optimum growth some leaves from each plantlet were separated under laminar flow and a double antibody sandwich ELISA (DAS-ELISA) test was done. To distinguish the best media for potato single node culture, virus free plantlets were cut and cultured in test tubes containing MS solid media consisting of IBA (Indole butyric acid) and NAA in 3 levels of 0, 0.5 and 1 mgl^{-1} singly and in combinations. The experimental design was factorial on basis of completely randomized design (CRD) with 9 replications. The test tubes were then put in a growth chamber of 25°C, 16 h light and 8 h darkness period.

After 3 to 4 weeks the plantlets were evaluated for single node number, length and number of root and stem length and the best media was evaluated. Then the viruses free plantlets were moved to greenhouse and the effects of 4 planting beds were studied on

Figure 2. Transferred virus free plantlets after seven days.

minituber production in the greenhouse (Figure 2). Planting beds were prepared using soil/sand/perlite (S/S/P) (1:1:1), peat moss/ sand (P/S) (1:1), soil/hull rice (S/H) (1:2) and turb/hull rice/perlite (T/H/P) (1:1:1) with 5 replications. One half of pots loaded with planting beds with above mentioned substrate and then irrigated. The next day the best plants were selected and transferred in pots and covered with transparent glasses to prevent the stress. The pots were irrigated day to day. After 1 week, depending on the plants growth, the glasses were taken off gradually, until they were removed completely. After 90 days plants were removed and the number and diameter of minitubers and the best planting bed were evaluated.

RESULTS AND DISCUSSION

ANOVA results of stem and root length, root number and single node number in all cultivars showed that different hormonal combination had significant effects on studied characters in Burren (Table 1). In Agria different combination of IBA in root length did not have any significant effects, but another treatment had significant effects on other characters. In Marfona different densities of IBA in root number and single node number did not have any significant effects, but other treatment had a significant effect on other characters. Different hormonal treatments had a significant effect on studied characters in Sante (Table 2).

In spite of different plants growth regulators treatments had different effects on the evaluated characters, the study of means comparisons of stem length, single node number and root length and number in single node stage showed that in Burren the best media for single node culture was MS media without any growth regulators or MS media containing low amount of IBA. Therefore, the best and economically media for single node culture was MS media without any growth regulators (Table 3).

The study of means comparisons of stem length, single node number and root length and number in single node stage showed that in Agria (Figure 3), in spite of different plants growth regulators treatments had different effects on the evaluated characters. Differences between MS without any growth regulators and 2 other treatments were insignificant and number of root that achieved in MS0 was suitable, efficient and economical. Therefore MS should be introduced without any growth regulators for Agria (Table 4).

The study of means comparisons of stem length, single node number, root length and root number in single node stage showed that in Marfona, in spite of different plants growth regulators treatments had different effects on the evaluated characters. Differences between MS without any growth regulators and 2 other treatments were insignificant and root number and single node number that were achieved in MS0 was suitable efficiency and economic. Therefore MS should be introduced without any growth regulators for Marfona (Table 5).

Duncan's mean comparisons of stem length, single node number, root length and root number in single node stage of Sante showed that MS without any growth regulators and MS media containing low amount of IBA are suitable media for single node culture. Root number and single node number achieved in MS0 was suitable, efficiency and economic. Therefore MS should be introduced with-

Table 1. Analysis of variation on the stem length, number and length of roots and number of single node in single node stage in all varieties.

Burren	DF	SS	MS	F	Probability
Stem length					
IBA	2	3137.876	1568.938	18.17	0.0001**
NAA	2	17946.395	8973.197	103.90	0.0001**
IBA*NAA	4	5578.345	1394.586	16.15	0.0001**
Error	72	6218.000	86.361		
Root number					
IBA	2	114.246	57.123	73.74	0.0001**
NAA	2	1446.246	723.123	933.43	0.0001**
IBA*NAA	4	237.975	59.493	76.80	0.0001**
Error	72	55.777	0.774		
Root length					
IBA	2	6857.407	3428.703	45.70	0.0001**
NAA	2	48466.666	24233.333	323.00	0.0001**
IBA*NAA	4	3348.148	837.037	11.16	0.0001**
Error	72	5401.777	75.024		
Single node number					
IBA	2	21.629	10.814	7.63	0.0001**
NAA	2	151.185	75.592	53.36	0.0001**
IBA*NAA	4	47.407	11.851	8.37	0.0001**
Error	72	102.000	1.416		
Agria	**DF**	**SS**	**MS**	**F**	**Probability**
Stem length					
IBA	2	1282.098	641.049	3.05	0.0534 n.s
NAA	2	24980.246	12490.123	59.49	0.0001**
IBA*NAA	4	3793.827	948.456	4.52	0.0026**
Error	72	15116.666	209.953		
Root number					
IBA	2	169.555	84.777	87.48	0.0001**
NAA	2	873.185	436.592	450.50	0.0001**
IBA*NAA	4	151.703	37.925	39.13	0.0001**
Error	72	69.777	0.969		
Root length					
IBA	2	3697.876	1848.938	43.93	0.0001**
NAA	2	28921.209	14460.604	343.59	0.0001**
IBA*NAA	4	9489.086	2372.271	56.37	0.0001**
Error	72	3030.222	42.086		
Single node number					
IBA	2	62.395	31.197	16.57	0.0001**
NAA	2	188.913	94.456	50.17	0.0001**
IBA*NAA	4	24.419	6.104	3.24	0.0167*
Error	72	135.555	1.882		
Marfona	**DF**	**SS**	**MS**	**F**	**Probability**
Stem length					
IBA	2	5893.654	2946.827	22.54	0.0001**
NAA	2	20237.358	10118.679	77.41	0.0001**
IBA*NAA	4	3880.938	970.234	7.42	0.0001**
Error	72	9411.333	130.712		

Table 1. continues…

Root number					
IBA	2	1.950	0.975	0.38	0.6850 n.s
NAA	2	2833.358	1416.679	552.35	0.0001**
IBA*NAA	4	96.345	24.086	9.39	0.0001**
Error	72	184.666	2.564		
Root length					
IBA	2	2267.283	1133.641	16.27	0.0001**
NAA	2	85276.543	42638.271	611.95	0.0001**
IBA*NAA	4	1356.790	339.197	4.87	0.0016**
Error	72	5016.666	69.675		
Single node number					
IBA	2	3.283	1.641	0.92	0.4030 n.s
NAA	2	97.061	48.530	27.20	0.0001**
IBA*NAA	4	49.975	12.493	7.00	0.0001**
Error	72	128.444	1.783		
Sante	**DF**	**SS**	**MS**	**F**	**Probability**
Stem length					
IBA	2	3137.506	1568.753	6.18	0.0033**
NAA	2	13779.358	6889.679	27.15	0.0001**
IBA*NAA	4	9346.493	2336.623	9.21	0.0001**
Error	72	18269.111	253.737		
Root number					
IBA	2	130.296	65.148	26.48	0.0001**
NAA	2	807.629	403.814	164.16	0.0001**
IBA*NAA	4	1188.962	297.240	120.84	0.0001**
Error	72	177.111	2.459		
Root length					
IBA	2	11713.185	5856.592	344.01	0.0001**
NAA	2	16448.962	8224.481	483.09	0.0001**
IBA*NAA	4	62081.629	15520.407	911.64	0.0001**
Error	72	1225.777	17.024		
Single node number					
IBA	2	112.024	56.012	32.01	0.0001**
NAA	2	16.765	8.382	4.79	0.0111*
IBA*NAA	4	37.679	9.419	5.38	0.0008**
Error	72	126.000	1.750		

**Significant at 1% probability level; * Significant at 5% probability level; ns: Not significant.

out any growth regulators for Sante (Table 2).

The considered the results revealed that NAA did not have any effects on single nodes number and stems length and the best media for these characters were MS media without any growth regulators or MS containing IBA and NAA had effects on characters related to root only when it combined with IBA. In Agria and Sante, using NAA single caused growth reduction. Also, increasing in NAA concentrations caused decrease in roots number of all cultivars. Results showed that different cultivars had a different response to the same medium. These results had some differences with those of Shah Zaman (2001), but confirmed Bostan and Demirel (2004) and Modarres Sanavy and Jami Moeini results (2003).

ANOVA results revealed that there were no significant differences between cultivars in minituber diameter (Table 6). Studying means of minituber per plant showed that Marfona produced the greatest number of minituber and Agria produced the lowest (Figure 4). The results confirmed the work of Sanavy and Moeini (2003), Vanaei et al. (2008) and Balali et al. (2008). Therefore the best genotype for commercial production of minituber is Marfona.

In greenhouse, planting beds showed different effects on minituber production (Figure 5). There were significant

Table 2. Means comparison of stem length, length and number of root and single node number in different hormonal treatments at single node stage of Sante.

Means of single node number	Means of roots length (mm)	Means of roots number	Means of stems length (mm)	Treatment
7.777 ab	91.111 a	13.444 a	86.667 ab	MS0
8.555 a	4.444 c	4.000 b	98.333 a	0.5 IBA
6.555 cd	5.889 c	5.333 b	72.778 bc	1 IBA
4.222 e	0.000 d	0.000 d	46.333 d	0.5 NAA
7.888 ab	71.111 b	12.888 a	82.222 bc	0.5 IBA+0.5 NAA
7.000 bc	2.111 cd	2.333 c	75.000 bc	1 IBA+0.5 NAA
4.888 e	0.000 d	0.000 d	48.889 d	1 NAA
5.333 de	0.000 d	0.000 d	44.444 d	0.5 IBA+1 NAA
6.888 bc	0.000 d	0.000 d	68.889 c	1 IBA + 1 NAA

Means in each column, followed by at least one letter in common one not significantly different at 5% probability level-using Duncan's multiple range test.

Table 3. Means comparison of stem length, length and number of root and single node number in different hormonal treatments at single node stage of Burren.

Means of single node number	Means of roots length (mm)	Means of roots number	Means of stems length (mm)	Treatment
6.444 a	78.333 a	9.222 b	74.444 a	MS0
6.333 a	50.556 b	10.888 a	47.778 b	0.5 IBA
7.111 a	37.778 c	11.111 a	70.556 a	1 IBA
3.777 b	3.333 e	2.222 d	36.667 c	0.5 NAA
4.222 b	0.000 e	0.000 e	40.556 bc	0.5 IBA+0.5 NAA
2.000 c	0.000 e	0.000 e	15.000 e	1 IBA+0.5 NAA
6.222 a	20.000 d	7.111 c	44.444 bc	1 NAA
3.666 b	0.000 e	0.000 e	35.000 c	0.5 IBA+1 NAA
2.000 c	0.000 e	0.000 e	25.778 d	1 IBA + 1 NAA

Means in each column, followed by at least one letter in common one not significantly different at 5% probability level-using Duncan's multiple range test.

Figure 3. Agria virus free plantlets after subculture in Ms0 medium.

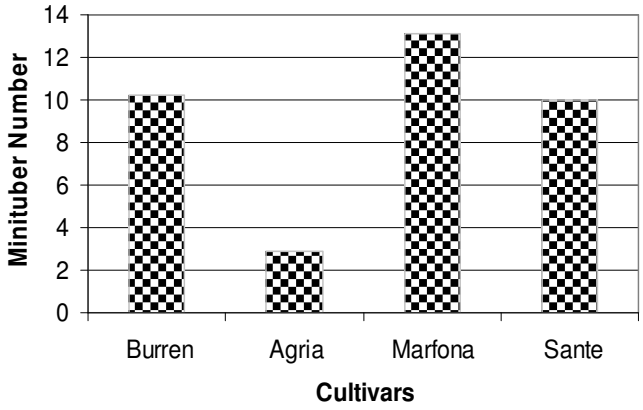

Figure 4. Means of minituber number per plant in commercial potato cultivars.

differences between peat moss/sand (1:1) and other planting beds. The numbers of minituber in treated plants

Table 4. Means comparison of stem length, length and number of root and single node number in different hormonal treatments at single node stage of Agria.

Means of single node number	Means of roots length (mm)	Means of roots number	Means of stems length (mm)	Treatment
7.888 b	47.778 b	8.111 b	73.333 b	MS0
7.444 b	43.333 b	10.666 a	89.444 a	0.5 IBA
9.222 a	46.111 b	7.333 c	81.111 ab	1 IBA
4.333 cd	10.889 c	5.888 d	45.556 cd	0.5 NAA
6.888 b	60.000 a	9.000 b	71.667 b	0.5 IBA+0.5 NAA
7.666 b	16.111 c	2.666 e	56.667 c	1 IBA+0.5 NAA
3.888 d	0.000 d	0.000 f	45.556 cd	1 NAA
3.888 d	0.000 d	0.000 f	32.222 d	0.5 IBA+1 NAA
5.555 c	0.000 d	0.000 f	37.222 d	1 IBA + 1 NAA

Means in each column, followed by at least one letter in common one not significantly different at 5% probability level-using Duncan's multiple range test.

Table 5. Means comparison of stem length, length and number of root and single node number in different hormonal treatments at single node stage of Marfona.

Means of single node number	Means of roots length (mm)	Means of roots number	Means of stems length (mm)	Treatment
6.222 b	83.333 a	11.222 b	85.556 a	MS0
5.333 bc	69.444 b	13.333 a	52.778 bc	0.5 IBA
7.555 a	60.556 c	14.444 a	62.778 b	1 IBA
5.000 bcd	0.000 e	0.000 d	44.444 cd	0.5 NAA
5.000 bcd	0.000 e	0.000 d	26.000 fg	0.5 IBA+0.5 NAA
3.444 ef	0.000 e	0.000 d	31.667 ef	1 IBA+0.5 NAA
3.888 def	14.444 d	2.888 c	40.000 de	1 NAA
4.666 cde	0.000 e	0.000 d	41.111 de	0.5 IBA+1 NAA
2.777 f	0.000 e	0.000 d	17.889 g	1 IBA + 1 NAA

Means in each column, followed by at least one letter in common one not significantly different at 5% probability level-using Duncan's multiple range test.

Table 6. Analyze of variation of minituber per plant for commercial potato cultivars.

Parameter	DF	SS	MS	F	Probability
Minituber number					
Cultivar	3	456.625	152.208	39.01	0.0001**
Error	28	109.250	3.901		
Minituber diameter					
Cultivar	3	77.593	25.864	0.30	0.8255 n.s
Error	28	2418.136	86.362		

** Significant at 1% probability level; ns: not significant.

with peat moss/sand (1:1) were more than other plants (Figure 6). Vanaei et al. (2008) and Balali et al. (2008) observed differences due to differences in cultivars, environmental conditions and studied substrate combina-

Figure 5. Minituber obtained from Sante cultivar.

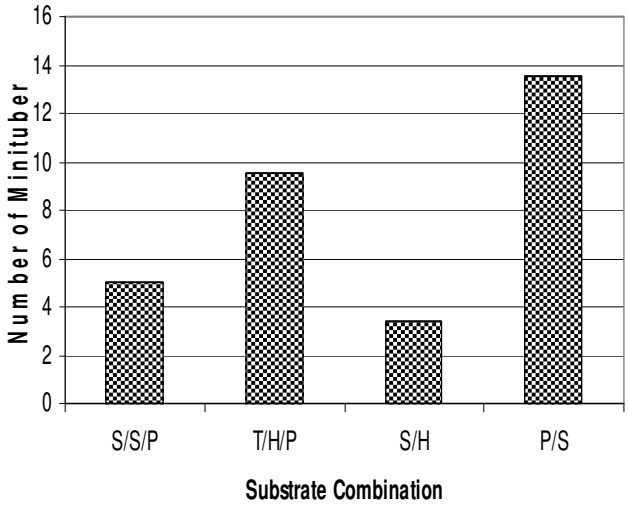

Figure 6. Means of minituber number per plant beds in commercial potato cultivars. The substrates are Soil/Sand/Perlite (S/S/P), Turb/Hull rice/Perlite (T/H/P), Soil/Hull rice (S/H) and Peat moss/Sand (P/S).

tion. Our results confirmed that of Sanavy and Moeini (2003), although there were some differences in substrate combination ratio.

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