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UNIFORMITY OF POTATO MINITUBERS DERIVED FROM MERISTEM CULTURES OF NUCLEAR SEED STOCKS

Gülsüm ÖZTÜRK* Zihin YILDIRIM

Ege University, Faculty of Agriculture, Department of Field Crops, Turkey
**Corresponding author: gulsum.ozturk@ege.edu.tr*

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ABSTRACT

The uniformity of minitubers of meristem plantlets obtained from the meristems of nuclear seed stocks in the laboratory were studied in the greenhouse during 2008 and 2009. Tuber number per plant (TNP), tuber yield per plant (TYP) and single tuber weight (STW) were recorded on the 10 randomly selected plants in two consecutive years. Based on the statistical analysis there was significant variation for genotype in terms of TNP and TYP. Year and genotype x year components of variation were not significant as expected. STW did not have any significant variation for genotype, year and their interaction. Granola had higher means such as 3.3 in 2008 and 3.7 in 2009 than Hermes for TNP (2008: 2.6 and 2009: 3.0). Within Granola and Hermes the means of TNP, TYP and STW of 2008 and 2009 were not different from each other. In conclusion, the uniformity of minitubers within cultivars during the propagation of 2 years indicated that nuclear seed stocks could be used in minituber production in the greenhouse for more than one year.

Key Words: Potato (*Solanum tuberosum* L.), meristem culture, nodal cuttings, seed stock, minituber, greenhouse growing

INTRODUCTION

In the seed potato programs, basic seed stocks are obtained through meristem cuttings from the sprouts of healthy potato tubers. The aim of such programs are to supply healthy potato seed tubers coming from minitubers obtained by propagating of healthy meristem plants obtained from nuclear seed stocks. Therefore growing the first generation seed tubers and effective utilization seed stocks are important (Rosenberg et al., 2007). The qualities of the plantlets as well as their health are important (Struik, 2007). Since viruses can cause great damage (Ražukas, 2002), at the beginning of preparation of seed stocks; selected potato tubers are tested for pathogens by using the Elisa test technique.

The minitubers are produced in the greenhouse by using the meristem plantlets obtained from meristems of basic seed stocks micro-propagated by node culture (Regan et al., 1995; Ahloowalia, 1999; Nielsen et al., 2007; Pruski, 2007).

There has been a controversy about the maintenance of basic seed stock in the laboratory and the duration of their usage in the production of minitubers in a seed potato program. The healthy and genetic purity of the basic seed stock should be kept for a suitable time (Struik, 2007; Rosenberg et al., 2010). To prevent deterioration meristem plants are transferred to subcultures in certain time periods (Rosenberg et al., 2010).

On the other hand some researchers have accepted the meristem plantlets as a starting material for clonal selection. Thus the minitubers originated from the meristem plantlets were tested for genetic variation under field condition (Wright, 1983; Rosenberg et al., 2007; Rosenberg et al., 2010). Until now a satisfactory genetic variation has not been reported.

Another aspect of discussion is the intervals of time in renewing basic seed stocks. On this subject several suggestions have been made in different programs although some people proposed to renew stocks every year by taking meristem tip cuts (Nielsen et al., 2007). Some others suggested to maintain the seed stocks in the laboratory for certain time period (Wright, 1983; Rosenberg et al., 2007). A widely accepted hypothesis is the usage of seed stocks for long time.

Therefore, the purpose of this study was to observe the uniformity of minitubers obtained from meristem plantlets of the basic seed sources of two genotypes Granola and Hermes grown in the greenhouse for two consecutive years, 2008 and 2009.

MATERIALS AND METHODS

The study was conducted in the Tissue Culture Laboratories and in the greenhouse of the Department of Field Crops, Faculty of Agriculture, of the Aegean University located in Bornova-Izmir, Turkey during the 2008 and 2009 growing years.

Development of Nuclear Seed Stocks

The nuclear seed stocks of two cultivars, Hermes and Granola, were used as starting material. The tubers of these cultivars were kept at room temperature until sprouting.

Meristem cuts from the tip of the sprouts were taken in the tissue culture laboratory. The sprouts were cultivated on the MS medium (Murashige & Skoog, 1962) modified as following MS + 0.1 mg/l GA_3 + 0.1 mg/l IAA + 0.1 mg/l BAP (Ozturk and Yildirim, 2010). About 30-40 meristem explants from each cultivar were taken in the laboratory in March 2007. The meristems of seed stocks were grown in the growth chamber at 23±1 for 16 h day length. The meristem plantlets were kept in the separate test tubes as nuclear seed stocks. The meristem plantlets of these seed stocks were used in the 2008 and 2009 for minituber production. The seed stocks were maintained by sub-culturing at 60 day intervals. The modified MS nutrient media used in the study are given in Table 1.

Table 1. The modified MS medium used in the study

Culture	IAA (mg/l^{-1})	IBA (mg/l^{-1})	BAP (mg/l^{-1})	GA_3 (mg/l^{-1})	Sucrose g^{-1}	Agar g^{-1}	Reference
Meristem Culture	0.1	-	0.1	0.1	30	6	Ozturk and Yildirim, 2010
Micropropagation Culture		2			20	6	Yildirim, 1995

Growing Meristem Plantlets in the Greenhouse

The meristem plantlets, about 3-4 cm in length, were taken into subculture. Then the cuttings of these cultivars were tested for viruses by using the Elisa test. The plantlets found healthy were micro-propagated by nodal cuttings to get sufficient numbers. Single nodal cuttings were transferred into the medium supplemented with auxin for rooting (Yildirim, 1995). The meristem samples taken from each genotype were micro-propagated by using the plantlet cuttings done in the fall of 2007.

After acclimatization plantlets grown in the test tubes were transferred to the plastic cups containing 2:1:1 sand: turf: fertilizer in September, 2007, for each genotype. At maturity, the minitubers were harvested by hand in February 2008.

The same procedures were followed in the fall of 2008 and the meristems taken from seed stocks of two genotypes were micro-propagated first and the plantlets developed were transferred to the plastic cups in September, 2008. The minitubers were harvested in February, 2009.

In two years, before the harvest 10 plants from each genotypes were sampled randomly and tuber number (TNP), tuber yield (TYP) and single tuber weight (STW) were recorded.

Statistical Analyses

The data obtained for minitubers were analyzed by the standard technique of Analyses of Variance (ANOVA) in Completely Randomized Plot Design with 10 replications. The means of the TNP, TYP and STW compared by using the LSD test as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

The results of the analysis of variance for minituber characteristics and the means of tuber number per plant (TNP), tuber yield per plant (TYP) and single tuber weight (STW) pertinent to genotypes grown for two years are shown in Table 2.

Table 2. Means of genotypes for tuber number per plant (TNP), tuber yield per plant (TYP) and single tuber weight (STW) of the minitubers grown in the greenhouse in 2008 and 2009.

Genotype	Tuber number per plant (TNP)			Tuber yield per plant (TYP) (g)			Single tuber weight (STW) (g)		
	Year			Year			Year		
	2008	2009	Mean	2008	2009	Mean	2008	2009	Mean
Granola	**3.3a**	**3.7a**	**3.5a**	**15.0a**	**14.8a**	**14.9a**	4.5a	4.0a	4.3a
Hermes	**2.6b**	**3.0b**	2.8b	**10.5b**	**11.0b**	**10.8b**	4.0a	3.9a	4.0a
Mean	3.0a	3.4a		12.7a	12.9a		4.3a	4.0a	
LSD (% 5)	0.66	0.66	0.47	3.19	3.19	2.25	0.63	0.63	0.44
F_{value}	$F_{genotypes}$: 8.832**			$F_{genotypes}$: 13.580**			$F_{genotypes}$: 1.729ns		
	F_{year} : 2.020ns			F_{year} : 0.026ns			F_{year} : 1.729ns		
	F_{gxy} : 0.007ns			F_{gxy} : 0.129ns			F_{gxy} : 0.526ns		

*: significant at the 0.05 probability level
**: significant at the 0.01 probability level
ns: non-significant

It could be seen in Table 2 that the variation among the genotypes for TNP and TYP are significant at the $p \leq 0.01$ significance level. Therefore the means of two genotypes Granola and Hermes for TNP and TYP were significantly different. The variation for STW had non-significant F value. The insignificant variation between two years (2008 and 2009) as well as genotype x year interaction component indicated that the TNP, TYP and STW of minitubers had similar means in 2008 and 2009.

Granola had higher means for TNP than Hermes in 2008 (3.3 vs 2.6) and in 2009 (3.7 vs 3.0). Granola had higher means for TYP than Hermes in 2008 (15.0 g vs 10.5 g) and in 2009 (14.8 g vs 11.0 g). Two genotypes had similar STW in 2008 and 2009.

The random sampling of the plantlets in two consecutive years resulted in similar means for TNP, TYP and STW of the minitubers. This finding supported the hypothesis that minitubers obtained from the micro-propagation of meristem originated from a seed stock had a genetic uniformity and purity. These results are based on data obtained for 2 years of growing plantlets originated from nuclear seed stocks in the greenhouse. This result is in good agreement with Wright (1983) who stated that within in a cultivar all clones had similar TNP for several years. Rosenberg et al (2010) also reported non-significant variation among the meristem clones within the cultivar Reet.

The uniformity of minitubers for TNP, TYP and STW for two genotypes in the two years could also be seen in Figures 1 and 2.

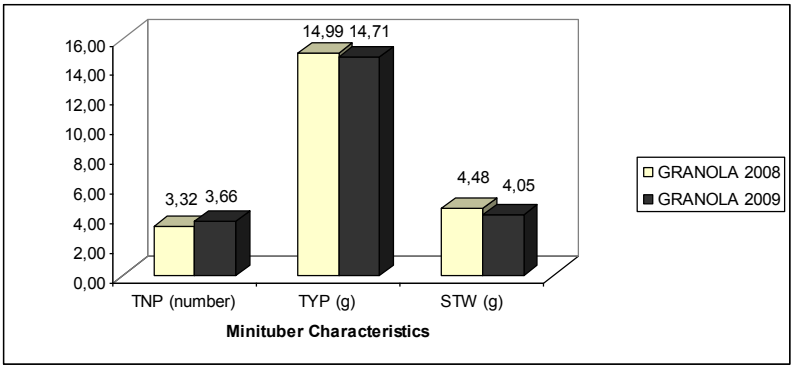

Figure 1. Histograms of tuber number per plant (TNP), tuber yield per plant (TYP) and single tuber weight (STW) of the minitubers of Granola grown in 2008 and 2009.

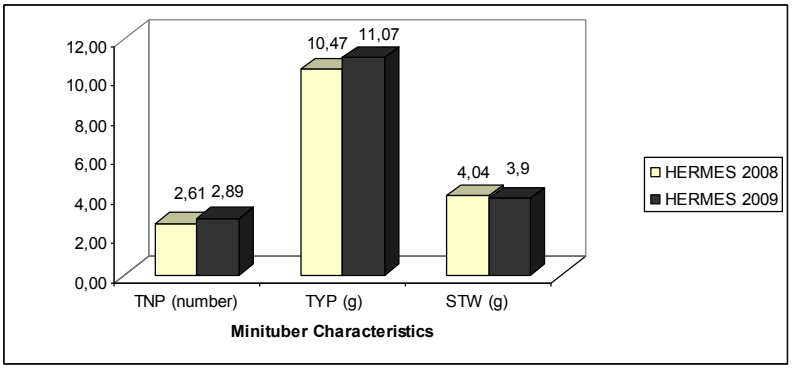

Figure 2. Histograms of tuber number per plant (TNP), tuber yield per plant (TYP) and single tuber weight (STW) of the minitubers of Hermes grown in 2008 and 2009.

Total number of plantlets grown, total number of minitubers and total yield of minitubers obtained in the study and the means for TNP, TYP and STW for genotypes Granola and Hermes are shown in Table 3 and Table 4.

It could be seen in Table 3 that for Granola had similar means for TNP, TYP and STW based on a total of 3851 plantlets grown in 2008 and 2009 (TNP: 3.0 vs 3.2); (TYP: 13.2 g vs 13.5 g and STW: 4.5 g vs 4.2 g. These means could be accepted as more reliable than the means based on samplings given in Table 2. Thus could be inferred that minituber characteristics of Granola, were not different in two consecutive years of 2008 and 2009.

The similar results could also be observed for Hermes in terms of minituber characteristics (Table 4). Minituber characteristics such as TNP, TYP and STW for Hermes were not different for two years based on the means obtained from observations done on 2206 meristem plantlets. Means of TNP, TYP and STW were 2.8 vs 2.8; 11.2 g vs 10.4 g and 4 g vs 3.8 g in 2008 and 2009 respectively.

The uniformity of minitubers obtained from meristem plantlets were reported by Rosenberg et al., (2007 and 2010) although they were tested under field conditions.

Table 3. Minituber characteristics based on the plantlets grown for Granola in the greenhouse in 2008 and 2009

Tuber Characteristics	Means		
	2008	2009	Over two years
Tuber Number Per Plant	3.0	3.2	3.1
Tuber Yield Per Plant (g)	13.2	13.5	13.4
Single Tuber Weight (g)	4.5	4.2	4.3
Totals			
Number of Plantlets Grown	1950	1901	3851
Number of Minitubers	5801	6158	11959
Yield of Minitubers (kg)	25.8	25.7	51.6

Table 4. Minituber characteristics based on the plantlets grown for Hermes in the greenhouse in 2008 and 2009

Tuber Characteristics	Means		
	2008	2009	Over two years
Tuber Number Per Plant	2.8	2.8	2.8
Tuber Yield Per Plant (g)	11.2	10.4	10.7
Single Tuber Weight (g)	4.0	3.8	3.9
Totals			
Number of Plantlets Grown	819	1387	2206
Number of Minitubers	2289	3823	6112
Yield of Minitubers (kg)	9.2	14.5	23.7

In this study the uniformity of minitubers obtained from meristem plantlets in the greenhouse indicated the possibility of using potato nuclear seed stocks for longer periods. Rosenberg et al (2010) concluded that starting material in good quality can be obtained and also be preserved long terms as seed stocks. Nielsen et al (2007) proposed keeping several meristems as seed stocks instead of using only one single clone. Thus the genetic purity of the seed stock could be preserved.

CONCLUSION

The uniformity of minitubers produced in the greenhouse was found to be consistent over two years so the usage of nuclear seed stocks more than one year could be proposed in the minituber production.

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