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Studies on Microtuberization of Five Potato Genotypes

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MATERIALS of *in vitro* culture are the first step in most seed potato programs worldwide. Microtubers are considerable tool for implementation of a national seed potato production system in countries lacking isolated high cool latitude and vector free areas for seed potato production. The current study investigates microtuber production ability of 5 genotypes imported from CIP (International Potato Center, Peru) comparing with Diamant variety. Also, studied the impact microtuberization agent (80 g/l sucrose, 5 mg/l benzyladenine or 500 mg/l Chlorocholine chloride) added to MS medium on *in vitro* microtuberization. Significant differences recorded between genotypes in microtuber weight and number. Unica and Costanera yielded the highest microtubers weight, number and average microtuber weight. The best media for microtuberization was MS supplied with 80 g sucrose. As a conclusion it is recommended to use elevated sucrose for microtubers production of Unica, Costanera, Yayla Kizi and Meva genotypes.

Keywords: Potato, Microtuberization, CIP, Genotypes.

Introduction

Potato is one of the most important vegetable crops in Egypt. Potato has a crucial part of the exported crops. However, the cost of seeds concern about more than halve the cultivation costs especially in summer season which cultured with imported seeds. Potato propagated by vegetative methods which transmit diseases and pests from generation to another causing the degeneration of seed quality (Struik and Wiersema, 2012). Around the world the means of *in vitro* culture and micropropagation is the milestone in potato seed production programmes (Ranalli, 1997, Allhloowalia, 1999, Struik & Wiersema, 2012 and Kawakami et al., 2015). Minituber production in greenhouses is the intermediate step between *in vitro* multiplication and the normal seed in field. Use of *in vitro* plantlets produced from nodal cutting is the popular way for production of minituber in greenhouses (Struik, 2007). Microtubers are more vigorous, easy to store and easier to handle comparing with *in vitro* plantlets (Seabrook & Coleman, 1988 and McCown& Joyce, 1991). Recently, many researches compared the performance of microtubers with traditional potato seed tubers and minitubers (Struik & Lommen, 1999, Wróbel, 2015 and Srivastava, 2015). Furthermore, microtubers is used in potato seed production in many countries they improved self-sufficiency, reduce number of field generation and reduce disease frequency in the final product (Donnelly et al., 2003). Microtubers are produced in vitro in a wide range of different growing systems with varying environment, media constituents, and storage intervals. Many of the interactions between growth parameters *in vitro* and subsequent productivity appear to be genotype specific (Donnelly et al., 2003).

The current study investigates the microtuberization of five CIP genotypes *i.e*., Costanera, Meva, Sissay, Unica and Yayla Kizi with different characteristics (Table 1). Costanera and Unica genotype has advantage of adaptability to high temperature which could be an advantage for early planting in august in autumn cultivation under Egyptian conditions. Furthermore, all the tested cultivars have high content of tuber dry matter which of great importance for processing industry. Moreover, the five genotypes are moderately resistance (Costanera, Sissay, Unica and Yayla Kizi) or resistance (Meva) to late blight.

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Materials and Methods

The current study was conducted in Vegetable Crops Research Departments Tissue Culture Laboratory in Dokii (2015 / 2016). *In vitro* micropropagated explants of five genotypes i.e., Costanera, Meva, Sissay, Unica and Yayla Kizi (Table 1) imported from international Potato Center (CIP), Peru in addition to Diamant variety plantlets were the source of single node cuttings explants used in the study. The used media for multiplication was MS (Murashige and Skoog, 1962) salts and vitamins media (Cassion Laboratories Inc. USA) supplemented with 10 mg/l Adenine sulfate, 5 mg/l calcium pantothenate, 30 g/l sucrose and 7 g/l agar. The pH was adjusted to 5.7 before autoclaving at 1.45 Kg/cm^2 for 20 min. Microtuberization behavior of the imported genotypes comparing with Diamant was tested using nodal cutting sunder the effect of various additives (microtuberization agents) i.e. sucrose, benzyladenine (BA), Chlorocholine chloride (CCC) or sucrose + BA + CCC to MS nutrient media. Cultures were incubated under 16 hour light at 23°C. The experiment statistical design was factorial analysis in complete randomized design. Each treatmenten closed 4 replicates each one contained 5 jars. 5 nodal cuttings were cultured per jar (500 g jar containing 50 ml media). Data of microtuberization ratio, microtuber number and weight were collected after 8 weeks. Data were submitted to ANOVA and treatment means were compared using multiple comparison tests at 5 % level of probability using Statistix 10 software.

TABLE 1. Characteristics of 5 CIP genotypes used in the study*

Name	Origin	Late blight resistance	Growing period (days)	Shape	Dry matter (%)	Adaptation
Costanera	Peru	Moderately Resistance	90	Oblong	23%	Tropical- subtropical to temperate
Meva	Madagascar	Resistance	90	Oblong	23%	Cool, warm and temperate
Sissay	Ethiopia	Moderately Resistance	90	Ovoid	19%	Cool tropical
Unica	Peru	Moderately Resistance	90-120	Oblong	20%	Tropical and sub-tropical to temperate
Yayla Kizi	Turkey	Moderately Resistance	90-120	Oblong	23%	Cool tropics

* CIP catalogue of potato varieties (2014)

Results and Discussion

The obtained results (Fig. 1, Fig.2-a, b, and Table 2) showed a significant differences between genotypes in microtuberization. Unica and Costanera gave the highest microtuberization ratio and microtuber weight per jar. In the second place came Diamant and Yayla Kizi. However, the lowest microtuberization ratio and average microtuber weight produced by Meva and Sissay. The different microtuberization agents caused significant differences in microtuberization ratio and microtuber weight per container (Fig 3-a andb). The media containing sucrose without growth regulators produced the uppermost microtuberization ratio and microtuber weight per jar followed by the media contained sucrose plus BA and CCC media. On the other hand, the lowest microtuber weight per jar resulted when media contained CCC or BA alone. Data presented in Table 2 show significant effects for the interaction between varieties and microtuberization agent.

According to average tuber number per jar the genotypes differed significantly (Fig 2-c). Unica yielded the highest average tuber number per jar followed by Costanera. Another time Sissay recorded the lowest average microtuber number per jar. Moreover, the effect of microtuberization agent on average tuber number was significant (Fig. 3-c). The treatments which contained sucrose or CCC alone or with BA gave higher tuber number per jar than BA alone. The interaction between the two studied factors was significant with the highest average tuber number per jar in Unica on media containing CCC alone, sucrose alone or sucrose plus CCC and BA as well as Costanera on media containing CCC alone as microtuberization agent (Table 2).

Significant differences were recorded between the different genotypes in average microtuber weight (Fig.2.d). Costanera produced the heaviest microtubers followed by Unica. The lowest average microtuber weight was obtained in Meva. Concerning the microtuberization agent sucrose alone resulted in the highest values of average

microtuber weight followed by treatments contained sucrose, CCC and CCC together in the same media (Fig. 3-d). On the other hand, BA or CCC as sole microtuberization agent recorded the lowest values of average microtuber weight. The interaction between genotypes and Microtuberization agent on average microtuber weight was significant (Table 2). The maximum average tuber weight was obtained in Unica on media containing sucrose alone.

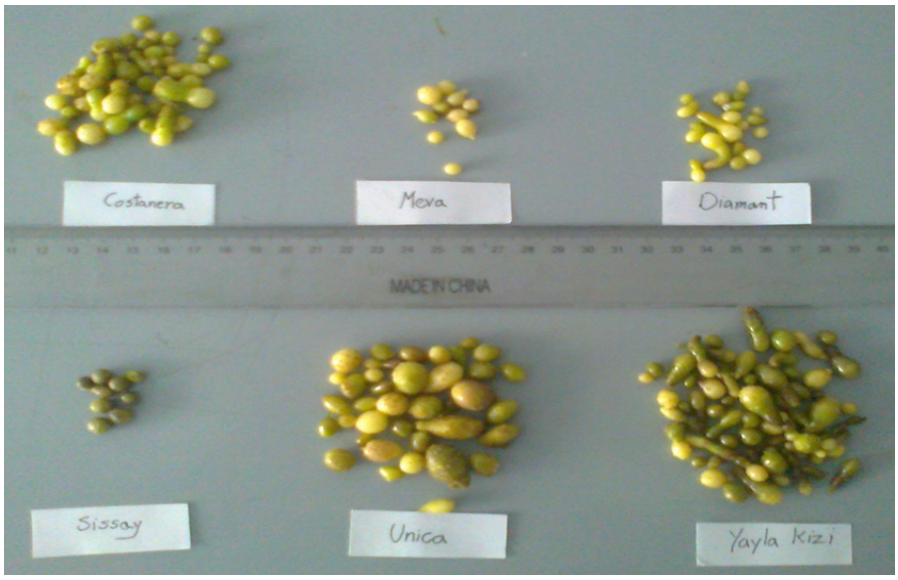

Fig. 1. Microtubers of 5 CIP genotypes comparing with Diamant formed after 8 weeks from culture *in vitro* on media containing sucrose only as microtuberization agent.

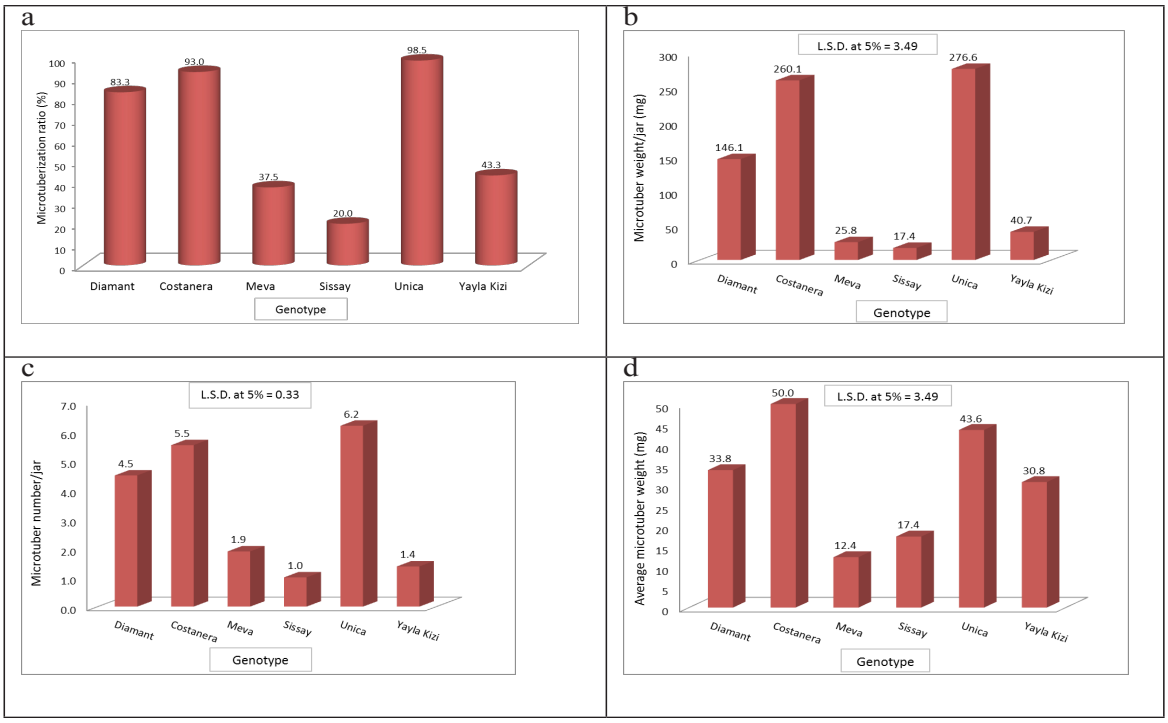

Fig. 2. Main effects of genotypes (a,b,c,d) on microtuberization ratio, microtuber weight per jar, number and average micotuber weight after 8 weeks from culture.

TABLE 2. Effect of interaction between genotype and microtuberization agent on microtuber average weight jar, number and microtuber weight after 8 weeks from culture *in vitro*.

Genotype	Microtuberization agent	Microtuber weight/ jar (mg)	Microtuber number/ jar	Average microtuber weight (mg)
Diamant	Sucrose	275.8	4.7	61.8
	BA*	60.8	3.0	23.0
	CCC*	118.3	4.2	28.8
	Sucrose+BA+ CCC	129.5	6.1	21.5
Costanera	Sucrose	306.0	5.7	57.0
	BA	200.5	4.0	53.0
	CCC	228.3	6.7	34.8
	Sucrose+BA+ CCC	305.5	5.8	55.3
Meva	Sucrose	58.8	3.4	18.3
	BA	13.8	1.4	10.0
	CCC	14.3	1.4	9.8
	Sucrose+BA+ CCC	16.3	1.4	11.5
Sissay	Sucrose	22.3	1.0	22.3
	BA	12.8	1.0	12.8
	CCC	15.8	1.0	15.8
	Sucrose+BA+ CCC	19.0	1.0	19.0
Unica	Sucrose	575.3	6.4	89.5
	BA	104.0	4.8	21.8
	CCC	199.8	7.1	28.3
	Sucrose+BA+ CCC	227.5	6.5	35.0
Yayla Kizi	Sucrose	64.3	1.4	50.5
	BA	27.5	1.3	21.0
	CCC	29.3	1.4	21.5
	Sucrose+BA+ CCC	41.8	1.4	30.3
LSD (*p* 0.05)		41.59	0.67	6.97

*BA: Benzyladenine , CCC: Chlorocholine chloride.

The differences between studied genotypes related to its genetic makeup since they are different in origin and adaptability. The obtained data are in accordance with Gopal and Minocha (1997) who found that significant genotypic differences for different characters in microtuber and normal seed tubers. The obtained effects of high sucrose concentrations on microtuberization are in line with those obtained by Garner and Blake (1989) and EL-Sharabasy et al. (2012). Also Wang and Hu (1982) recommended the addition of 80% sucrose to media contain BA to enhance microtuberization. In this respect, Khuri and Moorby (1995) suggested that sucrose play major role in microtuberization, hence high sucrose trigger tuber initiation while high osmolality insure starch deposition. Furthermore, Xu *et al*. (1998) stated that sucrose regulates tuber formation by changing gibberellin level. According to BA and CCC effects on the reduction of microtuber weight obtained results are agreed with those obtained by Harvey et al. (1991) who stated that CCC caused reduction in microtuber fresh weight. Furthermore, Leclerc *et al*. (1994) stated that CCC and BA reduced fresh weight and number of microtubers. Although, other literatures indicate that CCC reinforce BA effect leading to earlier microtuberization (Hussey & Stacey, 1984 and Protacio & Flores, 1992) and larger microtubers (Protacio and Flores, 1992). This could be attributed to the difference in light treatment since we used 16h light/ 8h dark while Hussey and Stacey (1984) indicated that BA effect

was more pronounce in short (8h) than long day (24h). On the other side, El-Sawy and El-Sherif (2014) indicated that microtuberization was better on media with 16 µM kinetin 6% sucrose than which contain 8% sucrose and other growth regulators combinations. Also, El-Sawy and Girgis (2015) obtained the highest microtubers number and weight on media which contain 2 mg/l BA, 2 mg/l NAA, and 100 mg/l CCC.

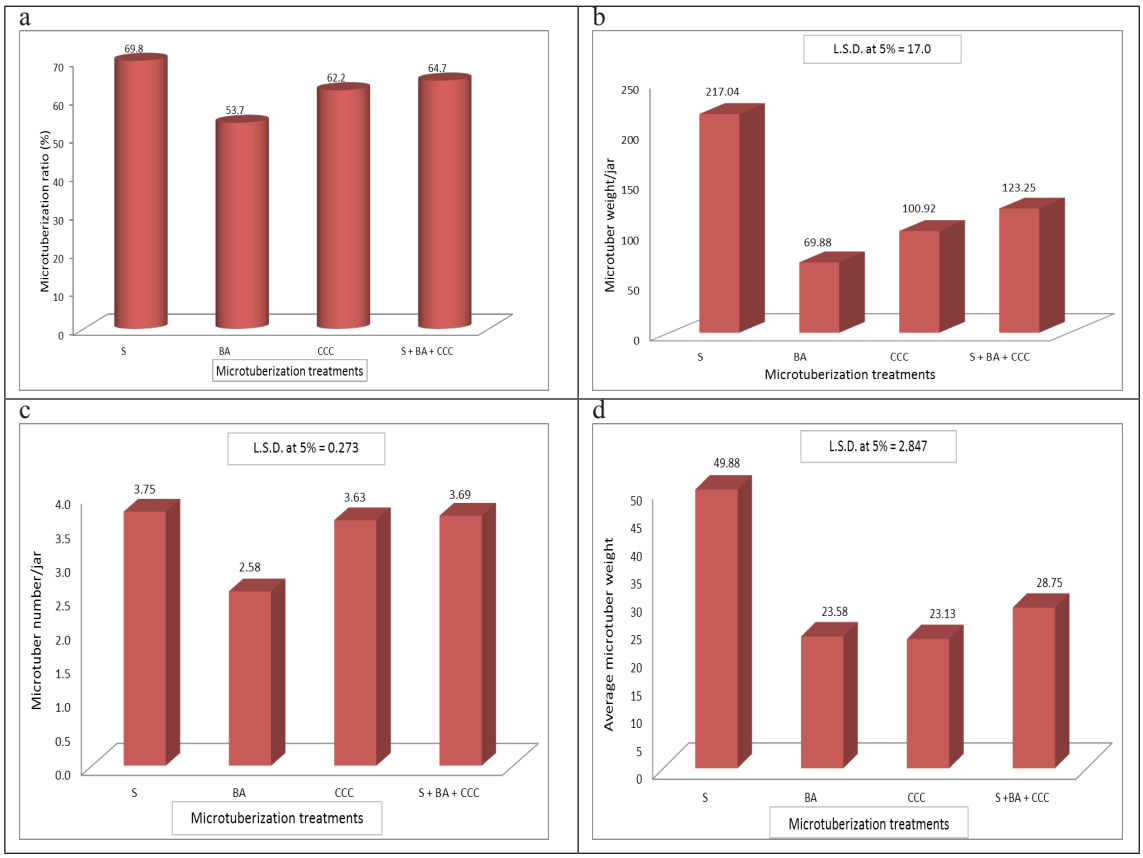

Fig.3. Main effects of microtuberization agent (a,b,c,d) on microtuberization ratio, microtuber weight per jar, number and average micotuber weight after 8 weeks from culture.

The difference in the microtuberization rate and weight could be resulted from the difference in genotypes used, the growth conditions (light and temperature) or the physiological age and type explant. Concerning the interaction between genotypes and microtuberization agent in the same line based on the significant interactions between twenty two potato genotype and different *in vitro* cultural condition Gopal et al. (1998) stated that it is important to develop genotype specific protocols for optimum microtuberization. In conclusion, CIP genotypes Unica, Costanera, Yayla Kizi and Meva could be micropropagated successfully by microtuber production on MS media with 80% sucrose without growth regulators. The obtained microtubers could be a reliable material for a national pre-basic potato seed program.

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دراسات على تكوين الدرينات لخمس تراكيب وراثية للبطاطس

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تمثل المواد الناتجة من مزارع الأنسجة الخطوة الأولى لبرامج إنتاج عالميا. تعتبر الدرينات أداة واعدة لعمل برنامج وطنى لنظام إنتاج تقاوى البطاطس فى الدول التى تفتقر للمناطق المعزولة ذات الإرتفاعات الباردة الخالية من نواقل الأمرض . تتناول الدراسة الحالية إنتاج الدرينات لخمس طرز وراثية مستوردة من المركز الدولى للبطاطس ببيرو مقارنة بالصنف دايمونت. دُرس تأثير إضافة عوامل تكوين الدرينات (80 جرام سكروز أو 5 ملليجرام بنزيل أدينين أو 500 ملليجرام كلوروكولين كلوريد) إلى بيئة موراشيج وسكوج. سُجلت إختلافات معنوية بين الطرز الوراثية فى وزن وعدد الدرينات. أعطى الصنف يونيكا وكوستانيرا أعلى وزن وعدد ومتوسط وزن درينات. أفضل وسط غذائى لتكوين الدرينات هى التى أحتوت على 80 جرام سكروز. يوصى بإستخدام تركيزات السكر المرتفعة لإنتاج درينات أصناف يونيكا وكوستانيرا ويلاكيزى وميفا.

كلمات مفتاحية : بطاطس ، تكوين الدرينات ، تراكيب وراثية ، المركز الدولى للبطاطس.