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EFFECT OF PH AND PHOSPHORUS CONCENTRATION ON POTATO MICROTUBERIZATION

M. M. Khalil
Potato and Vegetative Propagated Vegetables Research Department, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

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ABSTRACT: ***An in vitro study was conducted to investigate the effect of phosphorus concentration and pH on microtuberization of two potato varieties. Diamant variety produced higher microtuber number while Spunta gave heavier microtubers. Increasing phosphorus concentration to 2 mM gave the best results of microtuber number and average microtuber weight per jar. Moreover, lowering pH for 24 hr. produced higher microtuber number and average microtuber weight per jar. The results demonstrated that increasing phosphorus concentration and pH temporary lowering could improve microtubers number and weight magnifying the possibility of microtuber utilization in seed potato programs.***

Key words: ***Potato, Microtuberization, Phosphorus, pH.***

INTRODUCTION

Potato is the fourth major world food crop produced after maize, wheat and rice (FAO, 2019). In most developing countries the main problem facing the spread of potato cultivation is the lack of high quality seed tubers. The use of tissue culture techniques could improve self-sufficiency of seed potato, reduce number of field generation and reduce disease frequency in the final product. *In vitro* produced potato tubers (Microtubers) are formed in a wide range of different growing systems with varying environment, media constituents, and storage intervals (Donnelly *et al*., 2003). Microtubers are used in seed potato program for minituber production (Kawakami *et al*., 2015). Some studies used microtubers for direct seeding in open field (Pruski *et al*., 2003; Kawakami, J. and Iwama, K., 2012; Wróbel, 2015). Also, Microtubers are an alternative model for biochemical and physiological studies (Coleman *et al*., 2001). Furthermore, *in vitro* microtuberization was used for comparisons between potato genotypes salinity tolerance (Morpurgo, 1991; Naik and Wildholm, 1993; Zhang and Donnelly, 1997 and Khalil, 2009), heat stress (Gopal and Minoch, 1997) and frost resistance (Martinez *et al*., 1996).

Phosphorus studies on potato concentrated mainly on yield quantity. Although, some literatures indicated that phosphorus play a role in increasing potato tuber number in open field (Freeman *et al.* 1998; Jenkins and Ali 2000; Rosen *et al*. 2014) and in solution cultures (Rolot and Seutin 1999; Rolot *et al*., 2002). Another factor was manipulated by researcher is the acidity of the nutrient medium; Wan *et al*. (1994) stated that reducing pH in solution culture increased potato tuber numbers. Recently Teng *et al.* (2019) mentioned that acid pretreatment can efficiently promote microtuber formation and growth, which can be used for industrial production of potato seeds. On the other hand, Oraby *et al*., (2015) indicated that reducing pH reduced growth and tuber number. The optimum pH for tuberization need more studies specially in relation to

phosphorus availability which could take different trend than in soils because of the rapid shift in pH. The current *in vitro* study investigates the relationship of phosphorus and temporary acidity on two potato varieties microtuber production to be used in pre-basic seed production program.

MATERIALS AND METHODS

The plant material for the study were plantlets multiplied *in vitro* on MS media (Murashige and Skoog, 1962) salts and vitamins supplemented with 30 g/l sucrose solidified with 7 g/l agar with adjusted pH to 5.7 prior autoclaving at 1.05 Kg/cm^2 and 121°C for 20 min. For the study of the effect of experiment treatments stem segments containing three nodes were cultured on microtuberization medium. The microtuberization liquid medium consisted of MS salts and vitamins with 80 g/l sucrose. The culture vessels were 400 g glass jars containing 50 ml liquid medium. Only one gram of Cotton pieces was used as supporting material in each jar. The pH was adjusted according to the treatments prior autoclaving. Cultures were incubated at 25±2°C in darkness for 60 days. The treatments of the study contained three factors i.e., two varieties, three phosphorus concentrations and three pH levels. The two varieties were Diamant and Spunta, three phosphorus concentrations were the stander concentration recommended in MS medium 1.25 mM as well as 2 mM and 2.5 mM; potassium phosphate was used as the source of phosphorus and the increase in potassium was adjusted by reducing potassium nitrate concentration and the reduction in nitrate was corrected by ammonium nitrate. The pH levels used in this experiment to study its effect on microtuber formation were: 1) initial culture for 24 hours on pH 3.5 then transferring to fresh medium with pH 5.7; 2) initial culture for 24 hours on pH 4.0 then transferring to fresh medium pH 5.7 or 3.5) culture on constant pH (5.7). Each treatment was replicated three times each replicate contained five jars with three stem cuttings in each jar. Data of microtuber number per explant, microtuber weight, and weight of the largest microtuber per jar, microtubers weight per jar and microtuberization ratio (percent of the number of tuberized explants divided by the total number of explants) were determined after 60 days. The experiment contained 18 (2x3x3) treatments laid out in a factorial (2x3x3) in completely randomized design. The experiment was repeated twice its results were analogous hence data presented for the last one. Statistix 10 software was used for data analysis; separation between means was performed by the L.S.D at 5 % level (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The main effects of the varieties, phosphorus concentrations and pH levels on microtuber number were illustrated in Fig. (1-a). Diamant cv. produced higher average microtuber number and tuberization ratio than Spunta cv. (Fig. 1-d). Increasing the phosphorus concentration from stander concentration in MS media (1.25 mM) to 2 mM produced the highest microtuber number (Fig 1-b) followed by 2.5 mM, however the stander phosphorus concentration in MS medium produced the lowest average microtuber number and tuberization ratio (Fig. 1-e).

According to pH effect (Fig. 1-c, f), lowering pH to 3.5 before transferring to stander pH (5.7) resulted in the highest average number of microtuber and tuberization ratio followed by initial pH 4.0 comparing with constant pH 5.5.

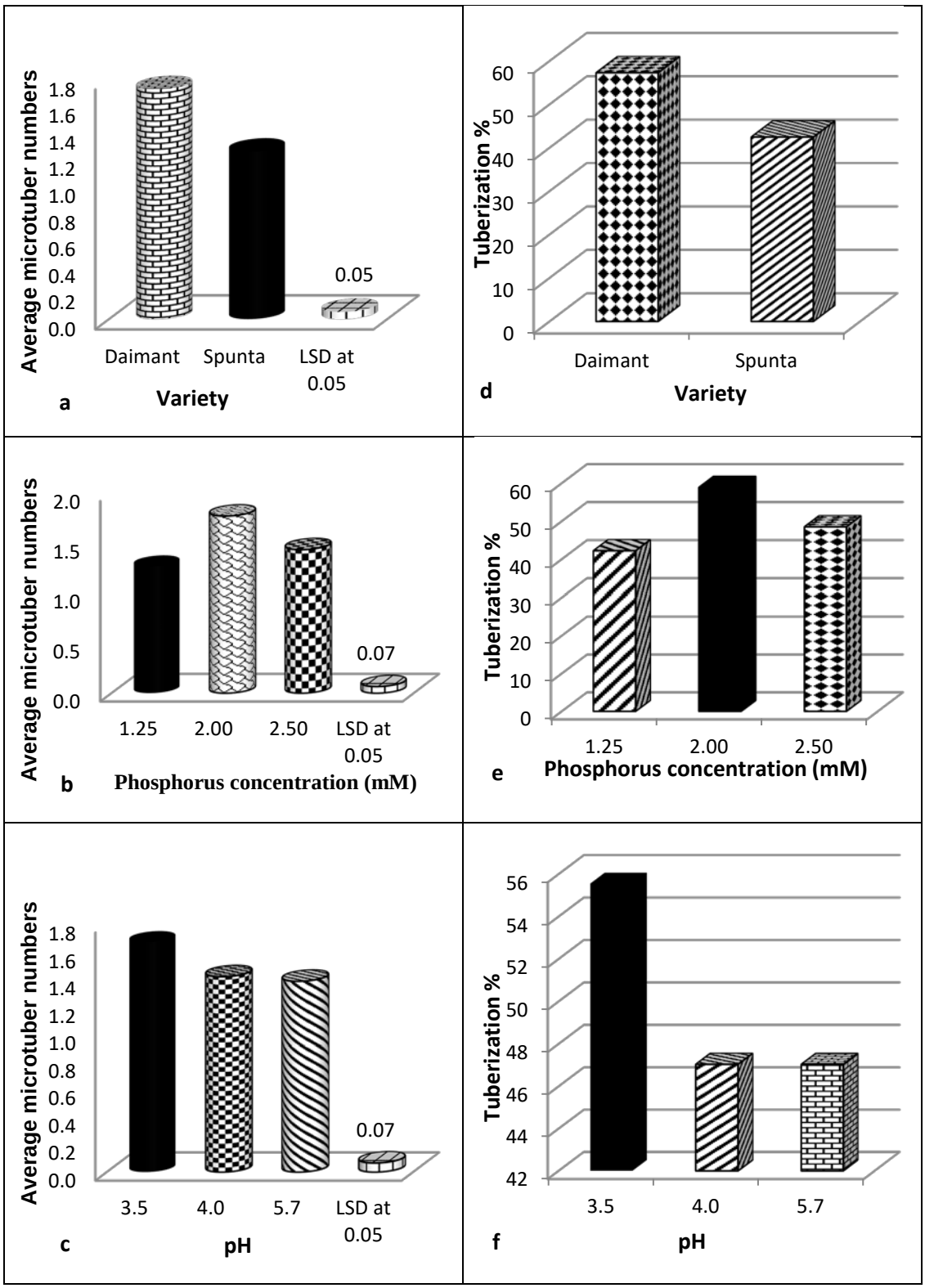

Fig.1: Main effects of varieties, phosphourus concentration and initial pH on microtuber number per explant (a., b. and c. respectively) and on tuberization ratio (d., e. and f. respectively).

The interaction between varieties and phosphorus concentration showed that the significant highest average of microtuber number produced when Diamant variety combined with 2 mM Phosphorus (Table 1). Also, the interaction between varieties and pH was significant thus the highest average of microtuber number produced by Diamant cv. on initial pH 3.5. Furthermore, the interaction between phosphorus and pH was significant with the highest average microtuber number in 2 mM P either with pH 5.7 or initially on pH 3.5 before transferring to pH 5.7. Data presented in Table 1. show the significant effect of interaction between the three tested factors on average tuber number; as Diamant variety gave the largest average microtubers number when initially cultured on pH 3.5 with 2 mM phosphorus.

Regarding to the average of individual microtuber weight, Spunta cv. formed heavier microtubers than Diamant (Fig. 2-a, 3, 4). Furthermore, stander phosphorus concentration 1.25 produced higher average of microtuber weight (Fig. 2-b). Moreover, lowering initial pH to 4 or 3.5 increased the average of microtuber weight than culture on constant pH 5.7 (Fig. 2-c). Concerning the interaction between varieties and phosphorus concentration, Spunta cv. cultured on 1.25 or 2 mM gave the highest average of microtuber weight comparing with other combinations between the two varieties and phosphorus concentrations. Also, Spunta cultured on initial pH 3.5 gave the highest average of microtuber weight in comparison with other combinations between the two varieties and pH levels. Regarding the interaction between phosphorus and pH level; the highest microtuber weight was observed when P concentration at 2 mM combined with pH 4 and P concentration at 1.25 mM combined with pH 3.5 (238) or 4 (239). Furthermore, the triple interaction between varieties, phosphorus and pH was significant producing the highest average microtuber weight by Spunta explants cultured on stander MS phosphorus concentration (1.25mM) with initial pH 3.5 before transfer to pH 5.7 while the lowest one produced in Diamant in 1.25 mM phosphorus with constant pH 5.7 (Table 1).

Regarding the main effect of varieties, phosphorus concentration and pH treatments on average microtuber weight per jar was illustrated in Fig. 2; Diamant cv. produced higher microtuber weight per container than Spunta cv. This could be attributed to the increase in Diamant microtubers number which compensates the higher Spunta individual microtuber weight. Two mM phosphorus concentration gave higher average weight per jar. Furthermore, the highest average microtuber weight per container was obtained by initial 3.5 followed by 4 while constant pH 5.7 gave the lowermost values. Concerning, the interactions between varieties and P concentration; Diamant on 2 or 1.25 mM and Spunta on 2 mM produced were significantly superior to other treatments. Furthermore, the interaction between varieties and pH was significant leading to the highest microtuber weight per jar for Diamant on pH 3.5 or 4. Regarding the interaction between phosphorus and pH 1.5 mM or 2 mM phosphorus with pH 3.5 and 2 mM with any pH treatment surplus other treatments. The interaction between the three tested factors was significant raising the heaviest microtuber weight per container by Diamant when the medium contained 1.25 mM P was cultured on media adjusted to 3.5 initial pH without significant difference between Diamant on the medium containing 1mM phosphorus with initial pH 4 (Fig. 3).

Table 1: Effect of variety, phosphorus and pH interactions on microtuber average number per explant, average weight and average weight of largest microtuber per jar.

Variety	Phosphorus concentration (mM)	pH	Average microtuber Number	Average microtuber weight (mg)	Average largest microtuber weight (mg)
Daimant	1.25	3.5	2.0	214	399
	1.25	4.0	1.5	269	415
	1.25	5.7	1.1	108	124
	2.00	3.5	2.7	134	198
	2.00	4.0	2.0	192	414
	2.00	5.7	1.6	152	398
	2.50	3.5	1.8	136	436
	2.50	4.0	1.4	146	310
	2.50	5.7	1.4	192	323
Spunta	1.25	3.5	1.0	261	443
	1.25	4.0	1.0	208	295
	1.25	5.7	1.0	233	319
	2.00	3.5	1.1	292	475
	2.00	4.0	1.0	266	345
	2.00	5.7	2.2	165	348
	2.50	3.5	1.4	210	282
	2.50	4.0	1.6	186	308
	2.50	5.7	1.0	237	419
LSD at 0.05			0.16	17.1	123
Daimant	1.25		1.5	197	313
	2.00		2.1	159	337
	2.50		1.5	158	356
Spunta	1.25		1.0	234	353
	2.00		1.4	241	389
	2.50		1.3	211	337
LSD at 0.05			0.09	9.9	ns
Daimant		3.5	2.2	161	344
		4.0	1.6	202	380
		5.7	1.4	151	282
Spunta		3.5	1.2	254	400
		4.0	1.2	220	316
		5.7	1.4	212	362
LSD at 0.05			0.09	9.9	71
	1.25	3.5	1.5	238	421
	1.25	4.0	1.3	239	355
	1.25	5.7	1.1	171	222
	2.00	3.5	1.9	213	336
	2.00	4.0	1.5	229	380
	2.00	5.7	1.9	159	373
	2.50	3.5	1.6	173	359
	2.50	4.0	1.5	166	309
	2.50	5.7	1.2	215	371
LSD at 0.05			0.11	12.1	87

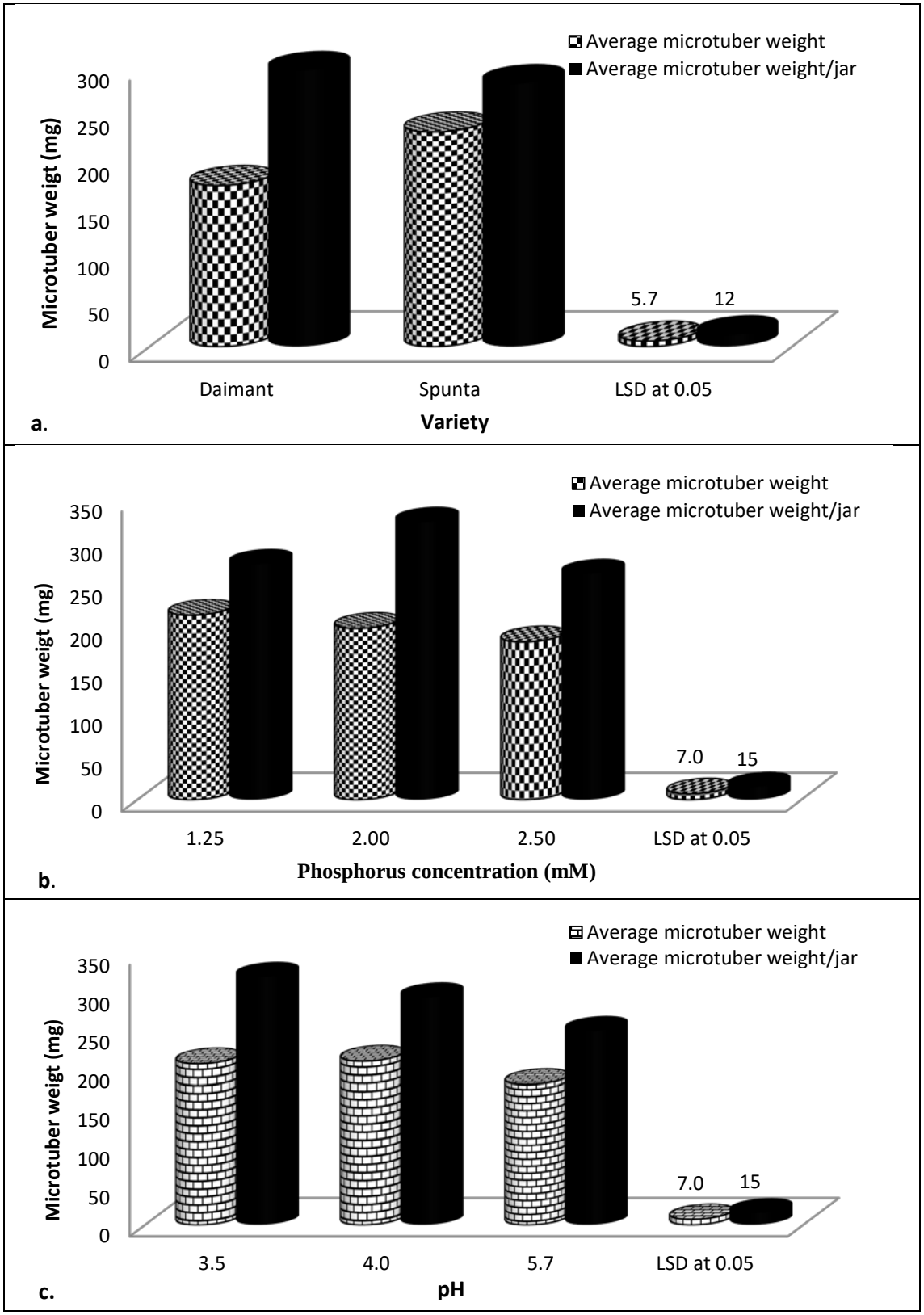

Fig. 2: Main effects of a. varieties, b. phosphorus concentration and c. initial pH on microtuber weight.

Fig. 3: Diamant (D) and Spunta (S) microtubers.

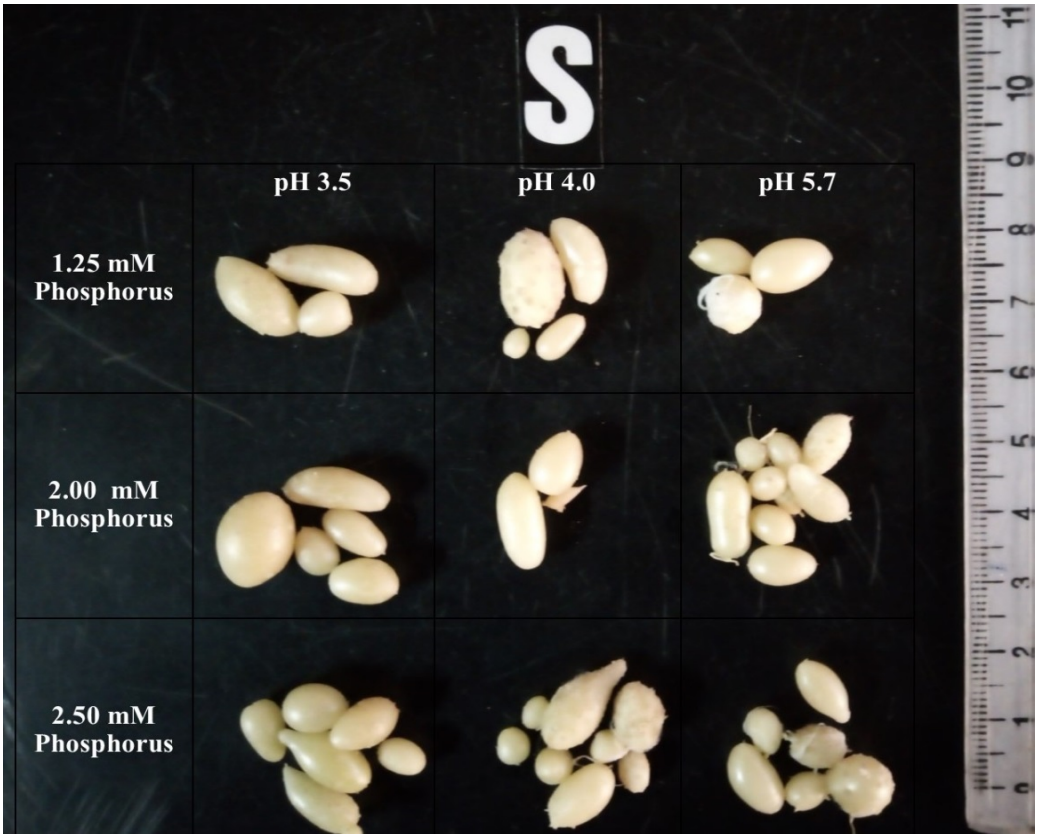

Fig. 4: Spunta microtuber yield on different combinations of phosphorus concentration and initial pH (per one jar).

The main effects of the three tested factors didn't significantly affect the weight of the largest microtuber per treatment. However, the interaction between the three tested factors effect on the largest microtuber weight was significant (Table 1). In this respect, 7 treatment combinations produced the highest average weight for the largest microtuber i.e., for Spunta 1.25 or 2 mM phosphorus with initial pH 3.5 or 2.5 mM on constant pH 5.7; for Diamant were 1.25 mM phosphorus with pH 3.5 or 4, 2 mM phosphorus with pH 4 or 5.7 and 2.5 mM phosphorus with pH 5.7.

The obtained results could be discussed as potato tuberization is a distinctive developmental process controlled by many factors and influenced by the physiological status as a result for that stress could play a major role in driving the plants to tuberization in spite of shoot and root growth. The difference between the two varieties in microtubers number and weight can be related to its genotypic differences (Gopal and Minocha, 1997; Khalil *et al.,* 2017). Regarding the beneficial effects of raising phosphorus concentration to 2 mM, it could be attributed to increasing tuber number of small tubers versus reduced number of large tubers in open field experiments (Benepal, 1967; Sharma and Arora, 1987; Freeman *et al*., 1998; Jenkins and Ali, 2000; Allison *et al.* 2001; Kumar *et al*. 2007; Rosen *et al*. 2014; Rosen and Bierman, 2008. Although, some reports claimed that P did not affect the total tuber number (Sharma and Arora, 1987; Allison *et al*., 2001; Mohr and Tomasiewicz, 2011). However, phosphorus is a major player in synthesis and composition of starch (Nielsen *et al.*, 1994; Houghland, 1960). Furthermore, Addition of P had a positive effect in controlling the severity and progression of Mn toxicity symptoms; this effect was pH-dependent and genotype-specific with its effect more prominent at pH 4.0 than 5.8 (Sarkar *et al*., 2004). Concerning the obtained results of lowering pH to 3.5 before transferring to stander pH (5.7) in the same line Teng *et al.* (2019) mentioned that acid pretreatment resulted in earlier microtuberization, maximum microtuber number per plantlet, fresh and dry weight. Also, Wan *et al*. (1994) reported that reducing pH to 3.5 in solution culture resulted in formation of more tubers per plant. Also, Cao and Tibbitts (1994) reported higher plant dry weight obtained in solution culture containing nitrate with pH 5. This effect could be attributed to the inhibition of assimilate partitioning to shoots and roots by lower pH in favor of tuber initiation However, Oraby *et al*., (2015) reported that reducing pH reduced growth and tuber number. The effects of phosphorus concentration and pH could be related either to availability of nutrients or stress effects favoring tuberization.

From the present study it could be concluded that microtubers number and weight can be improved by manipulation of nutrient media phosphorus concentration and temporary acidity treatment which can lower the cost and enhance the use of microtubers in seed potato programs.

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تأثير درجة الحموضة وتركيز الفوسفور على تكوين درينات البطاطس

محمد مصطفى على خليل

قسم بحوث البطاطس والخضر خضرية التكاثر، معهد بحوث البساتين، مركز البحوث الزراعية، الجيزة، مصر.

الملخص العربى

أجريت دراسة معملية لبحث تأثير تركيز الفوسفور ودرجة الحموضة على تكوين الدرينات لصنفي بطاطس. أنتج الصنف دايامونت عدد أكبر من الدرينات بينما أعطى الصنف سبونتا درينات أكبر وزنا. أدى رفع تركيز الفوسفور ل ٢ مللي مول لأفضل النتائج لعدد الدرينات ومتوسط وزن الدرينات للبرطمان. أيضا، أدى خفض درجة الحموضة لمدة ٢٤ ساعة لإنتاج أعلى عدد من الدرينات ومتوسط وزن الدرينة للبرطمان. تبين النتائج إمكانية تحسين أعداد وأوزان الدرينات بزيادة تركيز الفوسفور والخفض المؤقت لدرجة الحموضة مما يعظم من إمكانية استخدامها في برامج انتاج تقاوي البطاطس.

كلمات مفتاحية: بطاطس، تكوين الدرينات، فوسفور، درجة الحموضة.

أسماء السادة المحكمين

أ.د/ محمد السيد أحمد كلية الزراعة – جامعة طنطا

أ.د/ نبيل محمد ملـش كلية الزراعة – جامعة المنوفية