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Full Length Research Paper

Effects of plant growth regulators and photoperiod on *in vitro* microtuberization of potato (*Solanum tuberosum* L.)

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In vitro **microtuber production of potato (*Solanum tuberosum* L.) cvs. Sante and Savalan were studied on solid Murashige and Skoog (MS) basal medium applying different plant growth regulators 2,4-dichlorophenoxyacetic acid and benzylamino purine (2,4-D and BAP) and photoperiods. Cultures were exposed to 16, 8 and 16 h+utter darkness photoperiodic regimes. The experimental design, complete randomized with three replications was applied. The results indicate that the effect of cultivar, hormone and photoperiod significantly had influence on whole traits. Sante cultivar had higher productivity than Savalan for whole measured traits. The results of mean comparison for photoperiod show that highest productivity for whole traits is gained by this treatment 16 h photoperiod+ utter darkness (P_3). In this experiment by using the combination of 2,4-D (2.26 µM) and BAP (22.19 µM), microtubers number, diameter and weight was increased. In this study, the highest number for microtuber in Sante cultivar with hormone 2,4-D and photoperiod P_3 is 9.47 which this high number for Savalan cultivar is gained by using the combination of two plant growth regulators and same photoperiod.**

Key words: Tissue culture, potato, microtuber, photoperiod, hormone.

INTRODUCTION

The potato (*Solanum tuberosum* L.) is a vegetable crop of major economic importance world wide. It is the fourth most cultivated food crop after wheat, rice and maize (Jones et al., 1994). As such, potato growers produce about 325 million tons of potato annually. Potato tuberization is characterized by anatomical modifications, hormone and physiological changes. The use of *in vitro* growth of plants for production of microtuber has the advantage of higher control of the different factors that might affect the tuber formation compared to plants grown in soil (Veramendi et al., 1999). Furthermore, by using microtubers it is possible to maintain genebank accessions in a much smaller space, and to remove virus-infection in asexually propagated species (Zobayed et al., 2001). Potato (*S. tuberosum* L.) microtubers offer several advantages over *in vitro* propagated plants, since they can be stored and transplanted directly into the field without an acclimatization stage. Also handling and shipping are easier, thus facilitating commercialization and international exchange of germplasm (Jimenez-Gonzales, 2005).

Growth regulators and photoperiod influence potato tuberization (Hussey and Stacey, 1984; Villafranca et al., 1998; Silva et al., 2001). Plant hormones have been studied for decades, but the interactions that take place between them are still being discovered (Ross and O'Neill, 2001). Hormones play a crucial role in the control of potato tuberization (Vreugdenhil and Struik, 1989), and the effect of exogenous plant growth regulators are commercially significant for the inducing of potato tuberization (Zhang et al., 2005). For inducing tuberization *in vitro*, much attention has so far been focused on the use

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Abbreviations: BAP, 6-benzylaminopurine; **IAA,** indole-3-acetic acid; **2,4-D,** 2,4-dichlorophenoxyacetic acid; N6-(2-isopentenyl)adenine

Table 1. Analysis of variance for potato microtuber production.

(S.O.V)	d.f	Mean square (MS)			
		Total number of axillary shoots per Erlenmeyer	Total number of microtuber per Erlenmeyer	Microtuber diameter (mm)	Microtuber weight (mg)
Treatment	23	427.537 **	22.789 **	20.892 **	45538.1 **
Cultivar (a)	1	132.573 **	113.628 **	82.133 **	202738.02 **
Hormone (b)	3	850.186 **	25.77 **	63.387 **	149039.67 **
Photoperiod (c)	2	230.539 **	12.624 **	5.907 **	22526.202 **
a×b	3	596.213 **	14.626 **	8.826 **	18509.185 **
a×c	2	576.08 **	61.869 **	44.527 **	82370.119 **
b×c	6	531.494 **	11.381 **	1.977 *	7591.523 **
a×b×c	6	93.23 **	12.009 **	11.502 **	14441.778 **
Error	48	9.402	0.932	0.754	1994.366

* and **: Significant at p≤ 0.05 and 0.01, respectively

of cytokinins such as BAP (Lentini and Earle, 1991), N6-(2-isopentenyl) adenine (Levy et al., 1993), kinetin (Pelacho and Mingo-Castel, 1991), and zeatin (Koda and Okazawa, 1983). In spite of the fact that the auxins indole-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at lower concen-trations induce tuberization (Mangat et al., 1984), surprisingly little attention has been given to these hormones.

Cytokinins play an important role in creating the sink during plant development, and through regulating the expression of a gene involved in the partition of assimilates towards the stolons as observed in potato (Prat, 2004).

Light-Microtuberization efficiency increased when micropropagated source plants were grown under long days (16/8 h d/n) compared with short days (8/16 h d/n), followed by microtuber induction under short days or continuous darkness (Seabrook et al., 1993). For example, decreased daylight from long to short days promoted earlier (Garner and Blake, 1989) or more numerous (Wang and Hu, 1982) microtubers and increased microtuber size (Seabrook et al., 1993). Microtuberization response varied with the relative maturities of the cultivars tested and appeared to be partly controlled by photoperiod (Lentini and Earle, 1991; Seabrook et al., 1993). Gopal (1996) reported a faster rate of microtuberization and an early senescence of plantlets cultured under continuous darkness.

The objective of this research was therefore to investigate the role of 6-benzylaminopurine (BAP), 2,4-dichloro-phenoxyacetic acid (2,4-D) and photoperiod on production of microtuber in two potato cultivars grown *in vitro.*

MATERIALS AND METHODS

In this experiment, plantlets of potato cvs. *Sante* and *Savalan* by age 4 weeks produced in the laboratory were used in *in vitro* condition. Plantlets were put out from the medium in *in vitro* condition, then separated into segments about 1 cm in length each containing an axillary bud.

In this research, four hormonic treatments and three photo-periods were studied. Plant growth regulator (PGR) treatments included were H0=control, H1=2.26 µM 2,4-D, H2= 12.19 µM BAP, H3= 2.26 µM 2,4-D + 12.19 µM BAP and photoperiods include 16 h (P1), 8 h (P2), 16 h to produce first microtuber and then place in utter darkness until microtubers harvest (P3).

Before transferring plantlets into Erlenmeyers flask, trays containing medium were autoclaved by PH adjusted to 5.7 prior to autoclaving at 121°C under 100 Kpa for 15 min.

To enforce hormonic treatments, single-node segments of each cultivar were separated into four equal groups. Each group is put inside a 250 ml Erlenmeyers flask which contain one hormonic treatment.. Medium used in this trial is based on MS (Murashige and Skoog, 1962) to which 50 g l^{-1} sucrose and 7 g l^{-1} agar was added.

To perform photoperiod, culture trays of each hormonic treatment separated into three categories were separately inserted into growth compartments with the above mentioned three photo-periods. At eight weeks post-culture, the number of sprouts (internode) on plantlets inside each Erlenmeyer were measured. Four weeks after this measurement, plantlets were pull out from Erlenmeyers completely and micronodes (microtuber) on them were counted and their diameter and weight was measured. The experimental design was factorial on the basis of complete randomized design (CRD) with three replications per treatment, and each experimental unit was five flask containing five single-node segments each. Data were analyzed using SAS version 6.0 program [SAS (Statistical Analysis System) 1990]. The effect of each treatment was quantified and mean values were compared using Ducans Multiple Range Test (p ≤ 0.05).

RESULTS AND DISCUSSION

Variance analysis results showed that there was a significant difference between used cultivars, hormones and photoperiod. This difference for all traits is 1% (Table 1). Mean comparison showed that *Sante* cultivar is superior for most traits than *Savalan* (Table 2).

Table 2. Main effects of various factor on number of axillary shoots and microtuber production.

Treatment		Total number of axillary shoots per Erlenmeyer	Total number of microtuber per Erlenmeyer	Microtuber diameter (mm)	Microtuber weight (mg)
Cultivar	Sante	32.19 b	4.65 a	5.34 a	195.57 a
	Savalan	34.91 a	2.14 b	3.2 b	89.44 b
PGR treatments	Control	34.68 b	1.95 c	2.03 c	45.97 d
	2.26 µM 2,4-D	42.73 a	3.72 b	4.28 b	146.51 b
	12.19 µM BAP	29.6 c	3.1 b	4.16 b	113.86 c
	2.26 µM 2,4-D + 12.19 µM BAP	27.19 d	4.82 a	6.62 a	263.69 a
Photoperiod	16 h (p1)	35.67 a	3.22 b	3.81 b	169.56 a
	8 h (p2)	29.99 b	2.78 b	4.21 b	109.24 b
	16 h+utter darkness (p3)	34.99 a	4.19 a	4.8 a	148.72 a

Means within the same column and treatment followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$)

This cultivar is superior in terms of microtubers number, diameter and weight respectively 117.3, 66.9 and 118.7 percent than *Savalan* cultivar. These results coincide with the findings of Jones et al. (1989) who noticed a marked genotype effect on the induction of microtubers *in vitro*. Difference in response to culture *in vitro* condition because of genotype factor in potato has been reported ago (Leclerc et al., 1994; Ziv and Shemesh, 1996; Anjum and Villiers, 1997). In research by Leclerc et al. (1994) on microtuber production potential, three potato cultivars called *Kennebec*, *Russet Burbank* and *Superior*, a significant effect of genotype on studied traits is reported. In this research, the mean weight for microtubers varies from 358 mg for *Superior* cultivar to 629 mg for *Russet Burbank.* Notwithstanding *Sante* superiority for most studied traits than *Savalan* cultivar, number of axillary shoots per Erlenmeyer for *Savalan* is 34.91 while in *Sante* cultivar this amount reaches to 32.19 (Table 2). This result shows that various cultivars' potential for various traits differs, so that one cultivar might present superiority for some traits in comparison to other cultivars, while this cultivar presents inferiority for other traits.

Different responses by potato cultivars to various traits *in vitro* condition has been reported by many researchers (Leklerck et al., 1994; Gopal et al., 1997; Ochotorena et al., 1999). In addition to genotype effect, other factores including hormone and photoperiod would effect on microtuber production in potato. In this trial, usage of plant growth regulators BAP and 2,4-D in medium led to a significant increase in number, diameter and weight of microtuber (Table 2). Gained values for number and diameter of produced microtubers in control treatment is 1.95 and 2.03 respectively while these values in hormone treatment by using hormone 2,4-D is 3.72 and 4.28 respectively. This superiority in treatment using hormone BAP than Control treatment is evident in a way that this treatment for mentioned traits is 59.5 and 104.9 respectively. For these traits (number, diameter mean) between two treatments BAP and 2,4-D no significant difference was seen, although between these two treatments for mean weight trait, there was a significant difference and in using 2,4-D shows its superiority at 146.51mg than treatment BAP at 113.86. Also, using these plant growth regulators lead to increase in microtuber mean weight from 45.97mm in Control to 146.51mm and 113.86 mm in treatments with BAP and 2,4-D respectively. Also the results show that using both plant growth regulators BAP and 2,4-D concurrently in medium would influence more effectively on related traits to microtuber productivity in comparison to using each hormone separately. In this study, treatment by two plant growth regulators combination (BAP+2,4-D) in comparison to treatment by hormone BAP alone for microtuber number and mean diameter is 1.55 and 1.59 respectively. Superiority of this treatment (two plant growth regulators combination) in comparison to treatment by hormone 2,4-D for mentioned traits is 1.29 and 1.55 respectively. Anjum and Villiers (1997) study on the effects of three PGR treatments on microtuber number and mean weight of four potato genotypes. They reported that a medium containing both BAP and 2,4-D proved best for *in vitro* tuberization in *S. tuberosum* cvs. Desiree and Marls Piper and medium lacking growth regulators for *S. commersonii*.

Other traits studied in this research include the total number of axillary shoots which by various hormonic treatments, different responses were seen (Table 2). The results show that largest axillary shoots number (42.73) on cultured plantlets is seen inside those mediums containing hormone 2,4-D. For this trait, most less response is seen by those plantlets which replaced in medium containing both plant growth regulators BAP and 2,4-D, although this treatment had most optimum

Table 2. Main effects of various factor on number of axillary shoots and microtuber production.

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Cultivar	Sante	32.19 b	4.65 a	5.34 a	195.57 a
	Savalan	34.91 a	2.14 b	3.2 b	89.44 b
PGR treatments	Control	34.68 b	1.95 c	2.03 c	45.97 d
	2.26 µM 2,4-D	42.73 a	3.72 b	4.28 b	146.51 b
	12.19 µM BAP	29.6 c	3.1 b	4.16 b	113.86 c
	2.26 µM 2,4-D + 12.19 µM BAP	27.19 d	4.82 a	6.62 a	263.69 a
Photoperiod	16 h (p1)	35.67 a	3.22 b	3.81 b	169.56 a
	8 h (p2)	29.99 b	2.78 b	4.21 b	109.24 b
	16 h+utter darkness (p3)	34.99 a	4.19 a	4.8 a	148.72 a

Means within the same column and treatment followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$)

response to microtuber number and its mean weight and diameter. Same reports contain this fact that various treatments induce different responses. In a research by Zhang et al. (2005) on the effect of three plant growth regulators IAA, GA3 and BAP, they report that to produce high plantlets, using auxins like IAA is efficient and by adding hormone GA_3 to the medium its effect is doubled, while BAP hormone notably reduces the effect. The results of photoperiod effect on studied traits are shown in Table 2. As shown in this table, for studied traits, there is a significant difference among photoperiod treatments. For number of axillary shoots, those treatments which photoperiod spent more time in light exposure reacted more efficiently. In this experiment, there was no significant difference between treatments P1 (16 h photoperiod) and P_3 (16 h photoperiod +utter darkness) which both spent same time against light exposure until microtuber production and gained values are 35.67 and 34.99 axillary shoots per Erlenmeyer flask respectively. This value for photoperiod P_2 (8 h photoperiod) notably reduces into 29.99 sprouts. For microtuber number and diameter traits, treatment by P_3 reacts more efficiently. In this treatment, plantlets before microtuber production expose to 16 h photoperiod, then were transferred to permanent dark place. This treatment for microtuber number trait per Erlenmeyer (containing 5 plantlets) in comparison to treatments P1 and P2 shows 23.2 and 33.7% superiority respectively. This superiority to above mentioned treatments (P_1 and P_2) for mean diameter is respectively 20.6 and 12.3%. The results for this experiment show that the highest value for microtubers mean weight is gained 169.56 mg from treatment P_1. Although treatment P3 produced microtubers by mean weight 148.72 mg which is not too far from treatment P_1 (Table 2). Seabrook et al. (1993) report that decreased daylight from long to short days increased microtuber size.

In Table 3, the interaction effects for cultivar × hormone × photoperiod is shown. As shown in the table, due to significant difference for cultivar × hormone × photoperiod interaction effects, different responses was seen among treatments. In Sante cultivar largest axillary shoots number gained by hormonic treatment 2,4-D and 16 h photoperiod had significant difference against other treatments, while in Savalan cultivar for this trait there is no significant differences among hormonic and photoperiod treatments. For axillary shoots number, Sante cultivar in photoperiod P_1 and PGR treatment H_1 (2.26 µM 2,4-D) shows optimum response by 63.73. Concerning this trait, highest productivity for Savalan cultivar is gained by photoperiod P_2 and PGR treatment H_2 (12.19 µM BAP) (47.27). As shown in Table 3, highest value for microtuber number and weight traits in Sante cultivar is 8.02 and 405.9 mg respectively while Savalan cultivar shows 6.85 and 241.2 mg for these traits. For traits related to microtuber productivity, Sante cultivar shows more proper response than Savalan. In this cultivar (Sante) for diameter and microtuber weight in PGR, photoperiod treatments decrease from 405.9 to 33.5 mg. In Sante cultivar for microtuber diameter in PGR, photoperiod treatments decrease from 8.02 to 1.22 mm. Results show that the highest number for microtuber in Sante cultivar with hormone 2,4-D and photoperiod P3 is 9.47. It can be concluded that a medium containing both 2.26 µM 2,4-D + 12.19 µM BAP (H3) and photoperiod 16 h to produce first microtuber and then they place in utter darkness until microtubers harvest (P3) proved best for *in vitro* tuberization in *S. tuberosum* cvs. Savalan.

Table 3. Effects of PGR treatments and photoperiod on microtuber production in two potato cultivars.

Cultivar	PGR treatment	Photoperiod	Total number of axillary shoots per Erlenmeyer	Total number of microtuber per Erlenmeyer	Microtuber diameter (mm)	Microtuber weight (mg)
Sante	H0	p1	43.2 bc	7.27 b	4.19 f	107.8 fghi
		P2	18.72 i	2.63 fg	4.71 def	117.2 efgh
		P3	40.47 cd	1.03 gh	1.89 g	33.5 hijk
	H1	p1	63.73 a	7.13 b	7.47 ab	405.9 a
		P2	22.67 i	1.4 gh	3.63 f	78.81 ghijk
		P3	61 a	9.47 a	6.88 abc	249.8 bc
	H2	p1	21.07 i	4.2 def	5.72 cde	190.2 cdef
		P2	28 gh	0.87 h	1.22 gh	33.61 hijk
		P3	19.88 i	5 cde	6.2 bcd	189.1 cdef
	H3	p1	22.6 hi	5.67 bcd	8.02 a	431.5 a
		P2	22.67 hi	4.73 cde	6.67 abc	197.5 cde
		P3	22.33 hi	6.47 bc	7.48 ab	312 b
Savalan	H0	p1	38.53 cde	0 h	0 h	0 k
		P2	27.72 gh	0 h	0 h	0 k
		P3	39.47 cde	0.8 h	1.36 gh	17.42 jk
	H1	p1	37.2 de	0 h	0 h	0 k
		P2	34.67 ef	3.2 ef	4.16 f	86.73 ghij
		P3	37.13 de	1.13 gh	3.55 f	57.74 hijk
	H2	p1	30.60 fg	0.73 h	1.1 gh	30.57 ijk
		P2	47.27 b	4.53 de	6.56 abc	150.7 defg
		P3	30.8 fg	3.27 ef	4.15 ef	88.97 ghij
	H3	p1	28.4 g	0.8 h	3.99 f	190.5 cdef
		P2	38.25 cde	4.85 cde	6.71 abc	209.4 cd
		P3	28.87 g	6.4 bc	6.85 abc	241.2 bc

H0=control , H1=2.26 µM 2,4-D, H2= 12.19 µM BAP, H3= 2.26 µM 2,4-D + 12.19 µM BAP. P1= 16 h photoperiod, p2 = 8 h photoperiod, p3= 16 h photoperiod+ utter darkness. Means within the same column and treatment followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$).

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