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Micro tuber Induction of two Potato (Solanum tuberosum L.) Varieties namely, Almera and Diamant

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Abstract

Two potato varieties namely, Almera and Diamant were induced to form micro tubers under two in vitro culture conditions (darkness and light). Murashige and Skoog (MS) medium verified with varied concentrations of thiadizuron (TDZ), benzylaminopurine BAP and Sucrose, were evaluated for micro tuber induction using node segment explant from the in vitro culture micro plant. Highest micro tubers number (6.0±0.5 micro tuber/jar) obtained by Almera on MS medium verified with sucrose 8% only under dark, whereas higher micro tuber number obtained by Diamant cultivar is (3.0±0.0 micro tuber/jar) on MS medium verified with sucrose 8% only at dark too. In case of micro tuber weight, maximum tuber weight (1250.3±13.0 mg/tuber) obtained by Almera on MS medium supported with 60 g/l sucrose without hormone in dark. Higher micro tuber weight obtained by Diamant cultivar was (420.9±1.3) mg/tuber obtained on MS medium supported with 60 g/l sucrose in dark, followed by (248.6±25.5) mg/tuber produced on MS medium verified with 60.0 g/l sucrose plus 5.0 mg/l TDZ under light, at 30g/l sucrose no micro tuber observed. Highest micro tuber number is related to high sucrose concentration than the level of growth hormones in the medium. It was also observed that twenty four hour dark was best for tuber initiation.

Keywords: Micro tuber, sucrose, in vitro , Thidiazuron, Benzylaminopurine.

Introduction

The potato (*Solanum tuberosum* L) belongs to the family *Solanaceae*, is a crop of worldwide importance. It supplies at least 12 essential vitamins, minerals, proteins, carbohydrates and iron[1,2] . Micro tubers are minute tubers produced under *in vitro* conditions. Generally each plantlet can obtained one micro tuber with a weight of 0.2-0.7 g and 3-10 mm diameter[3]. They are usually produced on media supplemented with growth regulators, like cytokinins. Several workers have focused on the application of exogenous growth regulators for stimulating *in vitro* tuberization[4,5]. Micro propagation of virus-free potato plantlets is an important method of potato in vitro multiplication[6]. Under certain culture conditions, axillary buds of micro plants can be induced to produce aerial micro tubers[7].

Microtuberizations is a method used to increase specific pathogen tested materials in addition to single node cuttings[8]. Micro tuber can provide different recourse for *Agrobacterium*-mediated gene transformation[9]. Microtuberizations of potato has been one of the successful methods of increasing potato at *in vitro* conditions[10]. Micro tubers can be preserve for a long time and thus these could be an ideal propagation material[11]. Many research was done on microtuberization[12-16]. Very little data is handy about the influence of thiadizuron (TDZ) in micro tuber induction on the potato. Thus, Therefore, the present research was carry out to induce in vitro microtumer from two potato cultivars grown in vitro, to assess the effect of 6-benzylaminopurine (BAP), thiadizuron (TDZ) and sucrose on *in vitro* micro tuber induction of potato (*Solanum tuberosum* L.) plant under two *in vitro* culture conditions (darkness and light).

Material and Methods

Explant preparation and micro tubers production: Nodal segments excision from *In vitro* micro plant, six week old of cultivars Almera and Diamant were used for micro tuber induction. The plantlets were cut in to 1.0-2 cm long segments, each with about two node (2 axillary buds), incubated on Murashige and Skoog MS medium[17.] MS media with 6% and 8% sucrose without hormone or supplemented with thiadizuron (TDZ) and benzylaminopurine (BAP) each alone at two concentration (5.0mg/l and 8.0 mg/l) and the two sucrose concentration. The pH of the medium was disciplined to 5.8±0.02 before adding agar and the autoclaving. 25 ml of media was dispensed to culture jar (9x5cm), the culture were incubated at incubator room, half of treatment incubated at 25±2°C room temperature and 16 hour light, 8 hour dark photoperiod, the other half incubated at 25±2°C and 24 hour dark for two month, the progress of tuberization was monitored for micro tuber induction daily , final data recorded after three month is micro tuber number and average weight.

Statistical analysis: Data on micro tubers number and weight were statistically analyzed using analysis of variance

(ANOVA) and clarified as mean ± standard error, means were discriminated using Duncan's Multiple Range Test (DMRT) Duncan[18].

Results and Discussion

Micro tubers Induction: In the present study protocols for the production of micro tubers were established. The micro tubers were produced using BAP, TDZ and sucrose under two *in vitro* culture conditions (darkness and light).

The results of these experiment which were conducted to detect the influence of varied concentrations of sucrose single or in blended with BAP and TDZ, under two *in vitro* culture conditions regimes, Dark (24 h dark) and light (16 light/8h dark) on microtuberizations of two potato cultivars namely Almera and Diamant as explain in table-1.

Over all treatment, MS medium with only 30 g/l sucrose did not initiate any tuber after 12 weeks in culture except with BA at 8.0 mg/l in dark.

Loftiest number of micro tubers/bottle is (6.0±0.5) obtained by Almera on MS medium fortified with 80 g/l sucrose without hormone, under dark condition in a short period of time for micro tuber initiation (46 day), followed by (3.0±0.0) in the same cultivar induced on MS medium verified with BAP at 8.0 mg/l +60 g/l sucrose under light condition (16 h), see (figure-1).

Table-1
Effect of different concentrations of Sucrose, BAP and TDZ on MS medium on micro tuberization of two potato cultivars under two *in vitro* culture conditions (darkness and light)

Treatments	Days to tuber initiation		Number of micro tubers/ jar		Average fresh weight (mg)	
cultivars	Almera	Diamant	Almera	Diamant	Almera	Diamant
Dark						
S 30	00	00	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
S 60	57	66	2.0±0.0^{d}	1.0±0.0^{e}	1250.3±13.0^{a}	420.9±1.3^{a}
S 80	46	58	6.0±0.5^{a}	3.0±0.0^{a}	498.5±6.3^{b}	40.1±1.4^{fghi}
BA8+S30	66	46	1.3±0.1d^{e}	1.0±0.9^{e}	50.5±1.5^{fg}	21.1±0.3^{hi}
BA8+S60	66	46	1.0±0.0^{e}	1.0±0.0^{e}	51.8±1.2^{fg}	23.5±0.7^{hi}
BA8+S80	64	58	1.0±0.0^{e}	1.0±0.0^{e}	8.0±0.5^{g}	9.9±0.3^{hi}
BA5+S 30	00	00	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
BA5+S 60	62	33	2.5±0.1^{c}	1.9±0.2^{c}	139.9±31.1^{de}	49.9±7.4^{fgh}
BA5+S 80	49	28	2.0±0.0^{d}	1.3±0.1^{de}	36.5±0.7^{fg}	101.6±40.0^{de}
TD8+S30	00	00	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
TD8+S60	65	72	1.0±0.0^{e}	1.0±0.0^{e}	34.7±1.1^{fg}	119.3±12.9^{d}
TD8+S 80	65	29	1.0±0.0^{e}	1.5±0.1^{d}	102.9±7.5^{ef}	33.4±3.1^{ghi}
TD5+S30	00	00	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
TD5+S 60	70	72	1.0±0.0^{e}	1.3±0.1^{de}	10.1±0.5^{g}	43.3±8.7^{fghi}
TD5+S 80	00	58	0.0±0.0^{f}	1.5±0.2^{d}	0.0±0.0^{g}	28.3±4.8^{hi}
light						
S 30	00	00	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
S 60	70	33	2.5±0.1^{c}	2.5±0.1^{b}	328.5±47.5^{c}	205.7±45.0^{c}
S 80	60	46	2.0±0.0^{d}	2.5±0.4^{b}	505.3±1.7^{b}	80.8±19.4^{def}
BA8+S30	00	00	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
BA8+S60	58	62	3.0±0.0^{b}	1.0±0.0^{e}	200.2±1.8^{d}	23.2±0.7^{hi}
BA8+S80	70	36	1.0±0.0^{e}	2.0±0.0^{c}	11.1±0.8^{g}	22.5±2.6^{hi}
BA5+S 30	00	00	0.0±0.0^{f}	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
BA5+S 60	33	46	1.3±0.1^{e}	2.0±0.2^{c}	66.0±10.3^{efg}	41.3±8.7^{fghi}
BA5+S 80	52	46	2.3±0.3^{cd}	2.5±0.1^{b}	287.4±122.8^{c}	16.8±1.9^{hi}
TD8+S30	00	00	0.0±0.0f	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
TD8+S60	46	58	1.0±0.0^{e}	1.0±0.0^{e}	9.8±0.3^{g}	73.6±12.0^{def}
TD8+S 80	54	36	2.0±0.3^{d}	2.1±0.2^{c}	24.2±1.6^{fg}	21.1±2.2^{hi}
TD5+S30	00	00	0.0±0.0f	0.0±0.0^{f}	0.0±0.0^{g}	0.0±0.0^{i}
TD5+S 60	66	36	1.0±0.0e	1.4±0.1^{de}	21.1±1.2^{fg}	248.6±25.5^{b}
TD5+S 80	66	39	2.5±0.4c	1.5±0.1^{d}	318.1 ±26.4^{c}	101.4±1.5^{de}

Means followed by the same letter(s) are not different significantly according to Duncan's Multiple Range Test (P=0.05), Duncan[18].

Whereas higher micro tuber numbers produced by Diamant cultivar is (3.0±0.0) obtained on MS medium fortified with 80 g/l sucrose without hormone at dark condition. On the other hand the maximum micro tuber weight, was given by Almera cultivar on MS medium involving 60 g/l sucrose without hormone (1250.3±13.0 mg/tuber) under dark conditions, followed by 505.3±1.7mg/tuber with 80 g/l sucrose under light and 498.5±6.37mg/tuber with 80 g/l sucrose, without hormone under dark. The higher micro tuber weight produced by Diamant cultivar is 420.9±1.3 on MS medium containing 60 g/l sucrose without hormone under dark, followed by 248.6±25.5b produced on MS medium verified with 60 g/l sucrose and 5.0 mg/l TDZ under light, while most treatments of BAP and TDZ at 8.0 mg/l + 80g/l sucrose under dark and light produced the least tuber weight. Higher micro tuber number was more related to sucrose concentration and photoperiod than to the level of growth hormones in the medium .The finding is agree with Fatima et al.[19] and Imani et al.[14]. The addition of BAP, TDZ and high sucrose concentration to the MS medium influenced the induction of micro tubers than MS medium at basal components in agreement with Piao et al.[20].

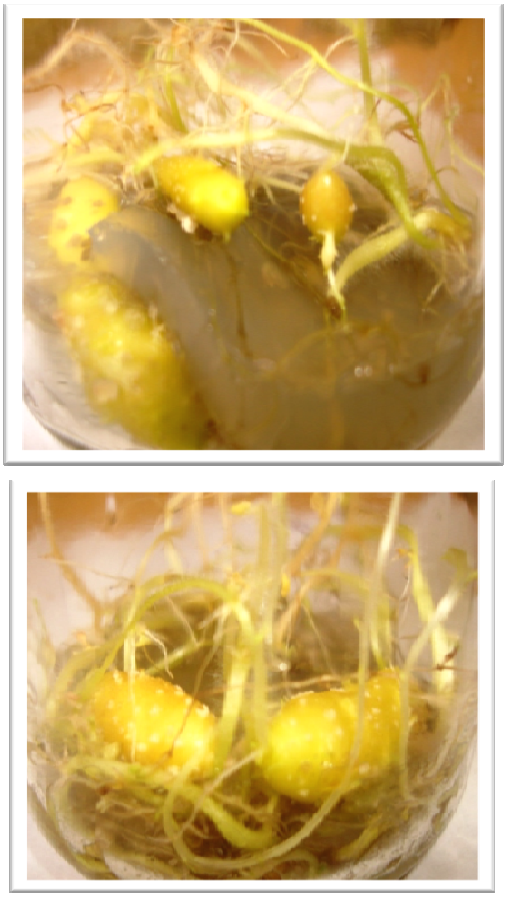

Figure-1
***In vitro* micro tuber formation from potato plant cultivar Almera on MS medium without growth regulators, supplemented with sucrose 80 g/l at left and 60 g/l at right**

The effect of sucrose on microtuberizations: The highest numbers of tubers (6.0±0.5[a] and 3.0±0.0[a]) were obtained from cultivar Amera and Diamant respectively on MS medium containing 80 g/l of sucrose. It was observed that 30 g/l sucrose produced shoots without micro tubers; indicating that high sucrose concentration increased osmotic pressure, creating a stress which shifted the plantlets to maturity and tuber genesis. This may be the stimulus for producing utmost tuber digit with both cultivars at 80 g/l sucrose. This result concise with the finding of Hussain et al.[14].

Higher micro tuber weight of cv Almera was induced with 60 g/l sucrose under dark followed by 80 g/l sucrose under dark and light. For cv Diamant higher micro tuber weight was obtained by 60 g/l sucrose under dark. This result in harmony with the finding of Fufa and Diro[16].

The effect of BAP and TDZ on Microtuberizations: When MS medium supplemented with sucrose at 60 and 80 g/l plus growth regulators (BAP, TDZ) microtuberizations occurred but at significantly less values than sucrose alone at 60 or 80g/l. This outcome is harmonize with the finding of Rosell et al.[11] and Hussain et al.[14]. They recommended the use of sucrose alone at high concentration for microtuberizations as a cheap component for microtuberizations as compared to plant growth regulators like BAP and TDZ.

The effect of Dark and Light on Microtuberizations: All culture showed tuber formation (except with sucrose 30g/l) under dark and light) conditions,as shown at (figure-2, 3 and 4). However maximum tuber number and weight were obtained under dark condition. This outcome is harmonize with Simko et al.[21] and Aslam, and Iqbal[22] . They reported microtuberizations efficiency was increased by being confronted with short day's or complete dusk.

Conclusion

A protocol for micro tuber induction of potato varieties 'Almera' and 'Diamant' from in vitro single node explant has been developed. The result indicated that micro tuber induction of potato was highly dependent on sucrose concentration, dark, growth regulators and genotype interaction.

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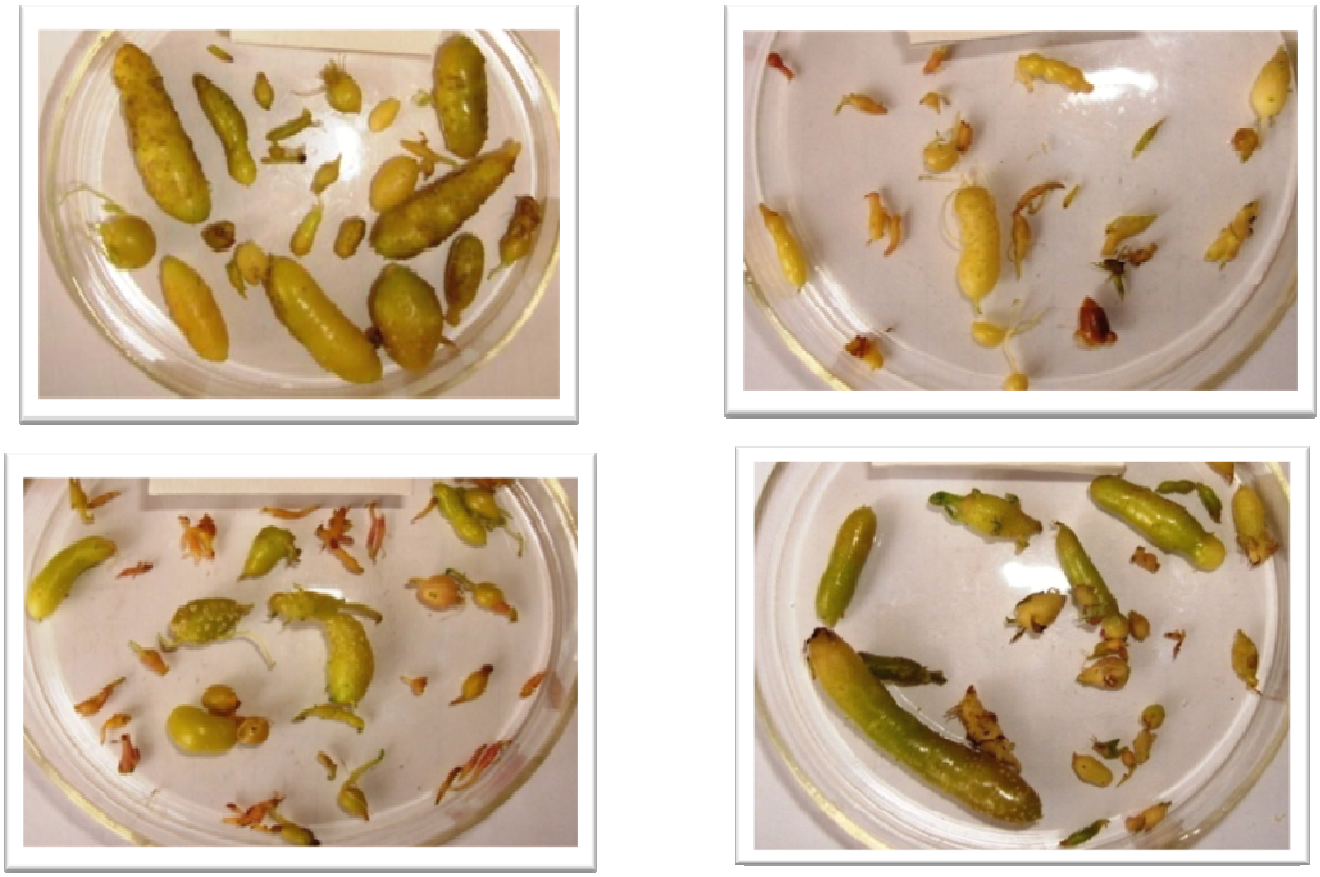

Figure-2
Micro tuber obtained after harvesting , the left (upper and bottom) under dark condition, the right (upper and bottom) under light condition from cvs Almera and Diamant respectively

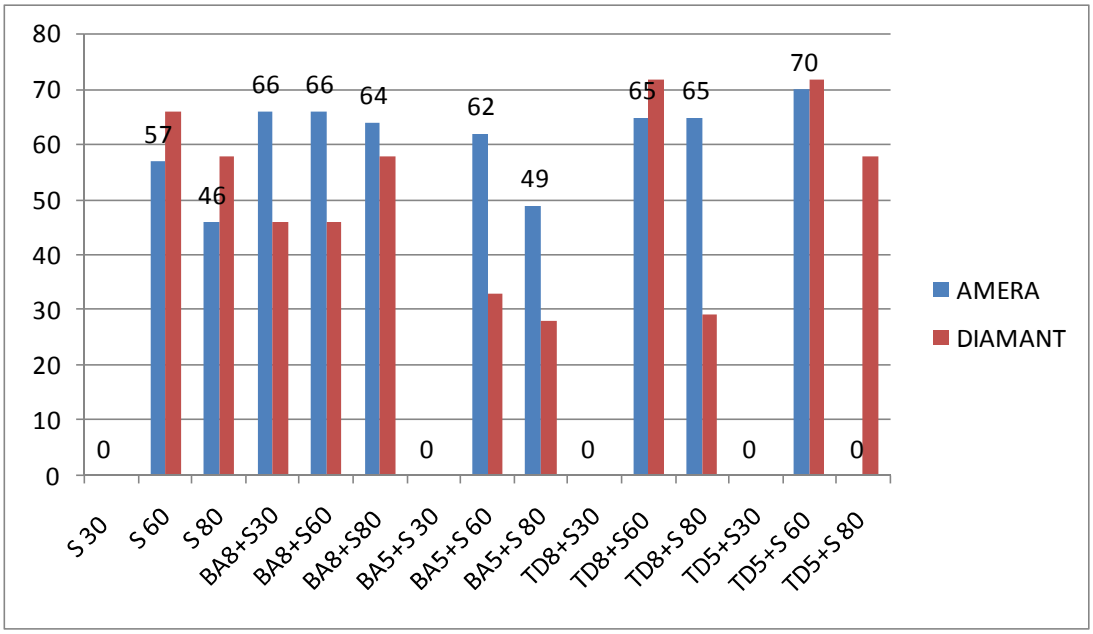

Figure-3
Effect of different concentrations of Sucrose, BAP and TDZ on days to tuberization at dark

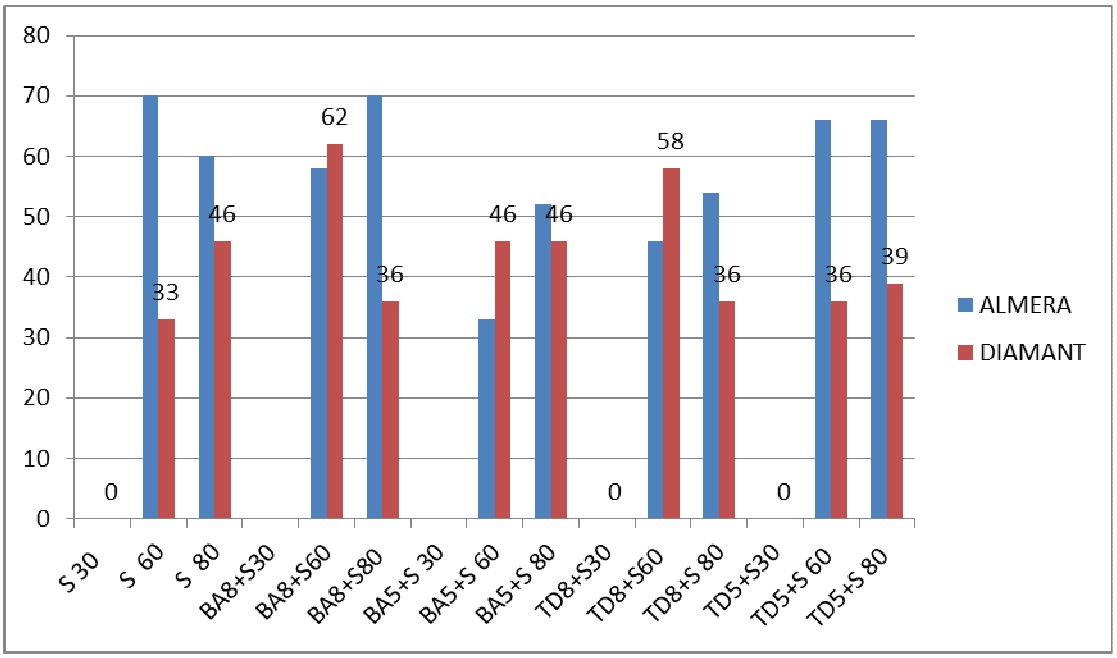

Figure-4
Effect of different concentrations of Sucrose, BAP and TDZ on days to tuberization at light

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