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In Vitro Microtuberization of Potato (*Solanum tuberosum* L.) Cultivar Kuroda-- A New Variety in Pakistan

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ABSTRACT

In vitro microtubers were obtained in potato (*Solanum tuberosum* L.) cultivar Kuroda, a new variety of potato introduced in Pakistan. The cotton based liquid MS medium supplemented with 8% sucrose and 0.75 mg L^{-1} BAP, The pH 5.7 gave better results when using node as a source of explant. Since microtubers can be induced economically at low concentration of BAP, it is recommended that this cultivar may be adopted for commercial cultivation in Pakistan.

Key Words: Potato; Tissue culture; Culture medium; Micropropagation; Microtuberization
Abbreviations: MS: Murashige and Skoog (1962) basal medium, BAP: 6 -Benzyl amino purine, Kin: Kinetin (6 -furfurylaminopurine), NAA: Naphthalene acetic acid, 2, 4 -D: 2, 4 –Dichlorophenoxyacetic acid, min: Minutes, mmt: million metric ton, mg: milligram, L: Litre.

INTRODUCTION

The potato is the fourth most important crop after wheat, rice and maize (Jones, 1973; Dowling, 1995). Potato growers produce 325 million tons of potato annually the world over (WorldBook, 2000). Pakistan's economy is agriculture-based. At the time of independence (in 1947) the area under potato crop was 3 thousand hectares, which increased to 40.5 thousand hectares during 1990 - 2000, with the total production of about 1.8 million metric tons (mmt), out of which 0.28 mmt is used as seed and remaining is consumed (MINFAL, 2000). With the population of 150 million, this accounts to 11 kg per capita per annum. During the last ten years, the yield per hectare has increased by 60% due to improved seed and better crop management practices. Tubers are the most common source of planting material in potato reproduction. Approximately 15% of the total area under potato cultivation around the world is used for the production of seed tubers (FAO, 2000). However, with the conventional method of vegetative propagation, potatoes are often prone to pathogens such as fungi, bacteria and viruses; thereby resulting in poor quality and yield. Consequently, much attention has been focused on the *in vitro* production of virusfree potatoes (Djurdjina *et al*., 1997).

Plant tissue culture is the only technique that can eliminate approximately 100% viruses in seed production programs and microtuber is one of the strategies in this perspective. Because of their small size and weight, microtubers have tremendous advantages in terms of storage, transportation and mechanization. They can be directly sown into the soil and can be produced in bulk in any season. They have the similar morphological and biochemical characteristics to field produced tubers. Therefore, mass production of potato microtuber is likely to revolutionize the world potato production. A number of research groups all over the world are trying to bring about this revolution (Sakha e*t al.,* 2004; Gopal *et al.,* 2004; Zhijun *et al.*, 2005).

The objectives of present study were to produce virus free microtuber *in vitro* from shoot apex and nodal segments, and to optimize the culture condition (pH, liquid, solid medium & liquid medium with cotton bases) for increasing the number of microtuber and selection of appropriate medium for optimum growth (based on number, culture time & fresh weight) of microtuber of a newly introduced promising potato (*Solanum tuberosum* L.) cultivar, Kuroda.

MATERIALS AND METHODS

Healthy and disease-free explants of potato (*Solanum tuberosum* L.) cv. Kuroda were obtained from Seed Centre, Punjab University, Lahore. Potato tubers were washed several times with detergent followed by several times rinsing with distilled water, dried and placed in dark room for one month till sprouting started. One-week old sprouts were dipped in 20% NaOCl solution for 15 - 20 min, given several washings with autoclaved distilled water, before incubating on the MS basal medium for culturing. After 4 weeks, the buds sprouted into full plantlet having 7 - 8 nodes. Single node cuttings were excised and inoculated on MS basal medium for further *in vitro* multiplication. The process was continued until complete plantlets were obtained in sufficient numbers. Four-weeks old plantlets were excised into shoot apices and nodal cuttings, which were used as explants. One apical explant was inoculated on medium in each test tube and 2 - 3 multinodal explants were inoculated in jars.10 test tubes and 10 jars were prepared in such a way. Seven concentrations (3, 4, 5, 6, 7, 8 & 9%) of sucrose in MS medium and different concentrations of two

cytokinins i.e. BAP and kinetin both at 0.25, 0.50, 0.75 & 1.0 mg L^{-1} were used for microtuberization. Moreover, sucrose (3%) in MS medium in combination with above formulations of cytokinins and different concentrations of two auxins i.e. 2, 4 -D and NAA at 0.25, 0.5 mg L^{-1} were used for shoot formation. So, for each concentration 10 replicates were prepared. After 10 weeks of incubation, the microtubers were harvested in pre-weighed and sterilized polypropylene bags aseptically and their fresh weight was recorded.

RESULTS AND DISCUSSION

In vitro microtuber production is very beneficial to propagate and store valuable potato stock and may be adaptable for automated commercial propagation and large-scale mechanized field planting (McCown & Joyce, 1991), in addition to economizing time, space and money (Venter & Steyn, 1997). However, an adequate supply of explants is a prerequisite. In this study shoot formation, from internodal explant on full strength as well as half strength MS medium with or without plant growth regulators was also carried out. The multiple shoot regeneration was optimized and shoots derived from internodal explants were used for tuberization.

***In vitro* shoot formation.** Maximum (96.6%) shoot formation was obtained in full strength MS medium supplemented with 1.0 mg L^{-1} BAP. Kinetin also faired well (93.3% success) at 0.25 mg L^{-1}, while formulations containing NAA, i.e. 33.3% and 26.6% at 0.25 and 0.5 mg L^{-1} performed poorly (Table I). Different concentrations of 2, 4 -D (0.25 & 0.5 mg L^{-1}) in MS medium failed to induce shoot production instead the explant was covered with hyaline and friable callus (Plate 1). It was observed that with the increase of BAP concentration in MS medium the rate of shoot formation was increased. These results are in conformity to the earlier findings (Haque, 1996; Kotkas & Peter, 1998; Al-Momani *et al.,* 2000; Shibli *et al.,* 2001; Rida *et al.,* 2001).

***In vitro* microtuberization on MS medium supplemented with sucrose without growth regulators.** MS medium supplemented with 8% sucrose produced microtubers in a minimum period (29 - 30 days), and 96.6% cultures showed microtuberization with 5 - 6 microtubers per culture. Average fresh weight of microtuber was high (0.115 g). The tubers were not formed on MS medium supplemented with 3 and 4% sucrose (Table II; Plate 2). This showed that 8% sucrose in MS medium was the best culture medium for *in vitro* healthy microtuber formation. Lawrence and Barker (1963) reported the *in vitro* tuberization from leaf-less and etiolated potato sprout sections in the presence of high sucrose concentration. Dobranszki & Mendis (1993) found that after culturing of shoots for 4 -weeks under long days, tuberization was induced with pouring of 8% sucrose in the culture medium. In line with the present findings various other studies report that high level of sucrose was beneficial in producing larger micotubers (Khuri & Moorby, 1995; Khuri, 1996; Yu *et al.,* 2000; Shibli *et al.,* 2001; Rida *et al.,* 2001; Gopal *et al.,* 2004).

***In vitro* microtuberization on MS medium supplemented with sucrose and growth regulators.** The MS medium supplemented with 8% sucrose and 0.75 mg L^{-1} BAP exhibited maximum microtuberization (6.33 explant^{-1}) within comparatively short period of inoculation (29 days), along with maximum average fresh weight (0.442 g) of microtuber (Plate3 & 4), whilst the kinetin at 0.25 mg L^{-1} showed poorest response (Table III). Seabrook *et al.* (1993), Gopal *et al.* (1998), Pelacho *et al.* (1994), Haque (1996), Sadder (1996), Vinterhalter *et al.* (1997), Amma and Maity (1998) and Rodrigues-otubo *et al.* (1999) have also obtained maximum number of microtubers in different cultivars in media supplemented with cytokinins, although at high concentrations (4 - 5 mg L^{-1}). In this study the optimal concentration of BAP was fairly low (0.75 mg L^{-1}), which is attributable to the genotypic differences. This makes cv. Kuroda a better candidate for *in vitro* manipulations.

Effect of different types of explants on *in vitro* microtuberization. Nodal explant produced sufficient microtubers (4 - 5 explant^{-1}) in 29 - 30 days of inoculation with high average fresh weight on MS medium with 8% sucrose. Contrarily, shoot apex produced 4 microtubers per explant in 34 days, and mean fresh weight was also significantly lower (Table IV). Yiem *et al.* (1990) and Myeong *et al.* (1990) studied the influence of several factors affecting *in vitro* tuberization of shoot nodes in potato cv. Dejima, More microtubers were produced on the nodes taken from middle and basal part of shoots than on those from upper parts. Leclerc *et al.* (1994) observed that layered shoots produced microtubers rapidly and in higher numbers, compared with nodal cuttings. Escalante and Langill (1998) found that nodal location influenced tuber development, as basal explants produced significantly more and larger tubers, as well as longer rhizomes, than did apical explants. While sub-apical segments produced more and larger tubers than did apical segments, there were no significant differences between medial and basal nodal segments with respect to tuber number or tuber/rhizome size. The present study on cv. Kuroda is also in accordance with these published reports. Nodal explant produced better tubers in all respects in nodal cuttings or layered shoots protocols.

Effect of physical nature of media on microtuberization. Maximum number of microtubers (4 - 5 explant^{-1}) was obtained within 29 - 30 days of inoculation in liquid with cotton base MS medium supplemented with 8% sucrose, while their number was low even in prolonged time on the same formulation of agar solidified MS medium (Table V). Although many studies recommend solidified media for microtuberization of potato (Myeong *et al.,* 1990; Kiji *et al.,* 1997; Pelacho *et al.,* 1999), reports favouring liquid medium are also available. Khomyak (1998) and Jimenez (1999), reported that use of liquid medium instead of solid medium increased microtuber yield of potato. We recommend cotton based liquid medium for cultivar Kuroda.

Table I. Effect of MS medium with and without different growth regulators on shoot formation

Media	Composition	No. of explants cultured	No. of explants showing shoot formation	Days for shoot formation	Rate of shoot formation (%)
MS	Half strength	10	8.66±0.16	11.33±0.43	86.66±1.63
	Full strength	10	9.00±0.14	6.66±0.22	90.00±1.41
MS+	MS + 0.25	10	8.66±0.16	6.66±0.08	86.66±1.63
BAP (mg L^{-1})	MS + 0.5	10	9.00±0.14	7.00±0.14	90.00±1.41
	MS + 0.75	10	9.00±0.14	7.00±0.14	90.00±1.41
	MS+1.0	10	9.66±0.08	6.66±0.22	96.66±0.82
MS + Kinetin	MS + 0.25	10	9.33±0.08	7.00±0.00	93.33±0.82
(mg L^{-1})	MS + 0.5	10	9.00±0.14	7.00±0.14	90.00±1.41
	MS + 0.75	10	8.66±0.08	8.33±0.29	86.66±0.82
	MS+1.0	10	8.66±0.08	9.00±0.16	86.66±0.82
MS + BAP(mg L^{-1}) +	MS + 0.25+ 0.25	10	8.00±0.14	7.00±0.14	80.00±1.41
Kinetin (mg L^{-1})	MS + 0.5+ 0.25	10	7.33±0.08	8.33±0.08	73.33±0.82
MS + NAA	MS + 0.25	10	3.33±0.08	15.00±0.14	33.30±0.82
(mg L^{-1})	MS + 0.5	10	2.66±0.08	15.00±0.28	26.60±0.82
MS + 2,4-D	MS + 0.25	10	NDA	NDA	NDA
(mg L L^{-1})	MS + 0.5	10	NDA	NDA	NDA

NDA = No data available.

Table II. Effect of different concentrations of sucrose on microtuber formation

Media	Sucrose concentration	No.of testtubes inoculated	Days to microtuber formation	No. of cultures showing microtuber formation	No. of microtubers per culture	Average fresh weight of microtuber (g.)
MS+Sucrose	3%	10	0	0	0	0
MS+ Sucrose	4%	10	0	0	0	0
MS+ Sucrose	5%	10	60.66±0.29	5.33±0.16	1.66±0.08	0.019±0.00022
MS+ Sucrose	6%	10	53.00±0.14	6.66±0.22	2.00±0.14	0.056±0.00036
MS+ Sucrose	7%	10	36.66±0.22	9.00±0.14	3.00±0.14	0.075±0.00057
MS+ Sucrose	8%	10	29.00±0.08	9.66±0.08	5.00±0.14	0.115±0.0017
MS+Sucrose	9%	10	34.66±0.36	8.33±0.08	2.66±0.22	0.066±0.0014

Table III. Effect of different concentrations of BAP, Kin. and BAP + Kin. on microtuberization at 8% sucrose in MS medium

Media	Concentration (mg L^{-1})	No. of explants inoculated	Days for microtuber formation	No. of microtuber per explant	Average fresh weight of microtuber(g.)
MS +	0.25	10	31.00±0.14	5±0.14	0.175±0.00050
BAP	0.5	10	29.33±0.16	5.66±0.08	0.204±0.0012
	0.75	10	29.00±0.14	6.33±0.08	0.442±0.0066
	1.0	10	30.33±0.22	5.66±0.08	0.245±0.0046
MS +	0.25	10	31.33±0.22	3.00±0.14	0.123±0.00067
kinetin	0.5	10	31.33±0.16	3.33±0.08	0.132±0.00051
	0.75	10	30.00±0.14	3.33±0.16	0.144±0.00043
	1.0	10	31.33±0.22	3.33±0.08	0.112±0.0048
MS +BAP +kinetin	0.25+0.25	10	30.00±0.14	2.66±0.08	0.245±0.0025
	0.5+0.25	10	29.00±0.14	3.33±0.08	0.294±0.0016

Table IV. Effect of different types of explant on microtuberization

Explant	Media composition	No. of explant cultured	No. of microtuber formed per explant	Days to microtuberization	Average fresh weight of microtuber (g.)
Shoot apex	8%	10	4±0.14	34.33±0.16	0.067±0.00088
Node	8%	10	4.66±0.08	29.33±0.22	0.115±0.0022

Table V. Effect of physical nature of media on microtuberization

Physical nature of media	Media composition	No. of explant cultured	Days to microtuberization	No. of microtuber formed per Explant
Solid	8%	10	38.33±0.08	2.66±0.22
Liquid	8%	10	37.00±0.14	3.33±0.08
Liquid with cotton base	8%	10	29.33±0.22	5.0±0.14

CONCLUSIONS

The investigations on potato cv. Kuroda revealed that it was easy to manipulate for *in vitro* tuberization and production of seed as compared to previously introduced cultivars in Pakistan. Since, this cultivar has good yield and palatability, we recommend this cultivar for mass propagation in Pakistan.

Plate 1. Hyaline and friable callus formation on shoot apex of potato cv. Kuroda in MS medium Supplemented with 0.25 mg L^{-1} 2, 4-D.
Plate 2. Microtuberization in potato cv. Kuroda from nodal explant in MS medium supplemented with 8% sucrose.Tubers with high mean fresh weight.
Plate 3. *In vitro* tuberization from nodal explant in potato cv. Kuroda on MS medium supplemented with 0.75 mg L^{-1} BAP and 8% sucrose.
Plate 4. Microtubers of potato cv. Kuroda harvested from MS medium supplemented with 0.75 mg L^{-1} BAP and 8% sucrose from nodal explant

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