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Comparison of Kinetin and 6-benzyladenine (BA) on *in vitro* Microtuberization of Potato Under Short Days Conditions

Serkan URANBEY[(1)]

Abstract: The influence of different concentrations of BA (6-benzyladenine) and Kinetin on microtuberization was compared. *In vitro* single node stem segments of potato were cultured on solid MS media supplemented with different concentrations of BA and Kinetin in dark at 22±1°C. No tuberization occurred 2.5 and 5.0 µM BA. Plantlets cultured on media containing 15 µM Kinetin exhibited the greatest microtuberization response. Moreover, considerable increases in yield of microtubers per plantlet were achieved on media containing 15 µM Kinetin. It was observed that high Kinetin concentrations promoted massive microtubers compared to BA at the same concentrations.

Key words: Potato, microtuberization, Kinetin ve 6-benzyladenine (BA)

Kinetin ve 6-benzyladenine (BA) Sitokininlerinin Kısa Gün Koşulları Altında Patateste *in vitro* Mikro Yumru Üretimi Bakımından Karşılaştırılması

Özet: Bu çalışmada, faklı konsantrasyonlardaki Kinetin ve 6-benzyladenine (BA) sitokininlerinin patateste *in vitro* mikro yumru üretimi üzerine etkileri karşılaştırılmıştır. Çalışmada, *In vitro*'da elde edilen boğum gövde parçaları, karanlık koşullarda ve 22±1°C'de farklı konsantrasyonlarda BA ve Kinetin içeren MS besin ortamında kültüre alınmıştır. 2.5 and 5.0 µM BA içeren MS besin ortamında mikro yumru oluşumu gözlenmezken, 15 µM Kinetin içeren besin ortamı, mikro yumru oluşumu bakımından en iyi tepkiyi vermiştir. Ayrıca bitki başına mikro yumru verimi bakımından 15 µM Kinetin içeren besin ortamında önemli derecede artışlar saptanmıştır. Aynı konsantrasyonlardaki Kinetin'in BA'ne göre daha yoğun olarak mikro yumru oluşumunu teşvik ettiği görülmüştür.

Anahtar kelimeler: Patates- mikro yumru, Kinetin ve 6-benzyladenine (BA)

Introduction

In vitro micropropagation in potato is generally used to bulk up new cultivars and breeding lines, for germplasm storage, transport and production of minitubers which are easy to store, transfer and distribute. *In vitro* propagated plantlets produce microtubers when incubated under suitable conditions (Estrada *et al.,* 1986). Microtubers are very important in terms of selection and transport of germplasm. In addition to contributing to selection and evaluation of germplasm (Gopal and Minocha, 1997; 1998), spotato microtubers have provided a valuable addition to the collection of tools for studies of genetic transformation (Choi *et al.,* 1997; Sandhu *et al.,* 1998; Choi *et al.,* 1999; Inui *et al.,* 1999). The successfull development of transgenic potato plants has been achieved primarily by the use of disarmed strains of A*grobacterium tumefaciens.* This transformation was followed by regeneration of plants from on *vitro* grown microtubers (Ishida *et al.,* 1989). Recent studies also demonstrate the potential use of microtubers as seed tubers. Microtuber is an alternative end product of micropropagation, produced by allowing *in vitro* plantlets to grow under tuber inducing conditions. *In vivo* and *in vitro* tuberization of potato is influenced by genetic, physiological and environmental factors including photoperiod, temperature, irradiance, mineral elements and hormone concentrations. This suggests that culture conditions must be optimized for optimal *in vitro* microtuberization. Because growth regulators considerably affect microtuber formation, many studies have been done on microtuberization of potato and focused on the use of growth regulators (Wang and Hu 1982; Estrada *et al.,* 1986; Vecchio *et al.*, 1994). Therefore, this study was initiated to assess the effects of concentrations of BA and Kinetin, in addition, to evaluate comparison of between BA and Kinetin on microtuberization of potato.

[(1)] Central Research Institute for Field Crops, ANKARA

Materials and Methods

Six week old axenic plantlets were subcultured aseptically using single nodal segments. Single node stem segments of *Solanum tuberosum* L. Rosetta cv. were isolated from *in vitro* plantlets cultured on medium on half-strength basal MS medium (Murashige and Skoog, 1962) containing 6% sucrose and 2 g/l gelrite different concentrations (2.5, 5.0, 10.0 20.0 and 25.0 µM) of BA and Kinetin. Cultures were maintained in dark at 22±1°C, for six weeks. Growth regulators such as Kinetin and BA were filter-sterilized using a Milipore filter (0.22 µm, pore size) and added to hot autoclaved medium before dispensing it into culture tubes. Six weeks later, cultures were evaluated for microtuberization. Microtubers were then harvested, weighted and scored to determine the number of microtubers per plantlet, average weight of microtubers and microtubers yield per plantlet. The experiment was conducted using randomized block design with four replications. Each replication consisted of 10 nodal segments per Magenta GA-7 vessels. Significances were determined by analysis of variance (ANOVA) and the differences between the means were compared by Duncan's multiple range test using MSTAT-C computer programme (Michigan State University).

Results and Discussion

Efficiency of microtuberization was dependent on Kinetin and BA concentration. Cytokinins gave different responses in terms of microtuberization. Some of the BA and all of Kinetin concentrations induced the formation of microtubers. Microtubers started to form after 20-26 days. No tuberization occurred at 2.5 and 5.0 µM BA. Tuberization began almost at the same time on media containing BA and Kinetin after 28-35 days culture initation. Analysis of variance showed that mean squares due to BA and Kinetin concentrations were highly significant ($P<0.01$) for number of microtuber per plantlet and microtuber yield per plantlet (Table 1).

The media supplemented with high concentrations of Kinetin (15.0 and 20 µM) produced the maximum numbers of microtubers per plantlet. The highest microtuber yield was also obtained MS medium containing 15 µM Kinetin. Among Kinetin concentrations, 2.5 µM was the least effective. Kinetin at concentrations greater than 10 µM resulted in induction of microtuberization.

A comparison of number of microtuber obtained under different concentrations of BA indicated that 15 µM BA was the most effective and 2.5 and 5.0 µM BA were the least effective. The results related to the effect of various BA and Kinetin on numbers of microtubers were similar to that for microtuber yield. As the numbers of microtubers increased, microtuber yield increased too. 15 µM Kinetin gave the highest microtuber yield per plantlet (421.00 mg). At the same time in terms of microtuber yield per plantlet among BA treatments, 15 µM was the most effective (226.66 mg) and 2.5 and 5.0 µM were the least effective (0.00 mg). Comparison of microtuber yield between BA and Kinetin indicated that the use of Kinetin led to much more microtuber formation.

Table 1. Effect of various concentrations of BA and Kinetin on number of microtuber and on microtuber yield

Growth regulator (µM)		Number of microtuber (per plantlet)	Microtuber yield (mg/plantlet)
BA	Kinetin		
2.5		0.00 f*	0.00 h*
5.0		0.00 f	0.00 h
10.0		0.80 d	98.33 g
15.0		1.20 c	226.66 e
20.0		0.63 de	136.66 f
25.0		0.67 de	143.00 f
	2.5	0.40 ef	89.66 g
	5.0	1.37 bc	336.66 c
	10.0	1.13 c	281.66 d
	15.0	2.43 a	421.00 a
	20.0	2.27 a	370.00 b
	25.0	1.63 b	249.30 e

* Values within a column followed by different letters are significantly different at the 0.01 probability level using Duncan's multiple range test.

Efficiency of tuberization in potato was fluctuated according to BA and Kinetin concentration on the medium in the study. This variation may be due to the degree of cell sensitivity towards growth regulators, which depends on origin of explants and endogenous levels of growth regulators. The promotion of microtuberization on cultured shoots by cytokinins has been demonstrated by many workers (Palmer and Smith, 1969; Wang and Hu, 1982; Hussey and Stacey, 1984; Estrada *et al.*, 1986; Ortiz-Montiel and Lozoya-Saldana, 1987). High sucrose concentration, kinetin and growth retardants are required for tuberization as an inductive medium (Lawrence and Barker, 1963; Hus-sey and Stacey, 1984: Estrada *et al.*, 1986; Chandra *et al.*, 1988; Peri *et al.*, 1991; Alchanatis *et al.*, 1994). Our findings with regard to effect of Kinetin on microtuberization agree with previous studies (Palmer and Smith, 1970; Wang and Hu 1982; Chandra *et al.* 1988; Alchanatis *et al.* 1994).

It was concluded that more improvements in microtuberization of potato could be induced by use of kinetin as an inductive cytokinin. To achieve satisfactory microtuberization in potato Kinetin as a cytokinins source should be used.

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