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# The Effect of Silver Nitrate (Ethylene inhibitor) on *in vitro* Shoot Development in Potato (*Solanum tuberosum* L.)

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**Abstract:** Ethylene produced by tissue, callus and plantlets in closed vessels may lead to abnormal plantlet growth and branching *in vitro*. Silver nitrate ($AgNO_3$) is known as an ethylene inhibitor. Therefore, the objective of this study was to determine the effect of $AgNO_3$ on potato plantlets with measuring several characters. The results showed that MS basal medium supplemented with $AgNO_3$ resulted in an inhibitory effect on ethylene gas produced by potato plantlets. The response of the cultivars used showed genotypic dependence to different $AgNO_3$ concentrations. In general, the best results for all cultivars were obtained from 5 or 10 μM $AgNO_3$. On the other hand, the higher $AgNO_3$ concentrations (25 and 50 μM) can be also used for cultivar Nicola and Desiree that initially had branching and abnormal plantlet growth.

**Key words:** *Solanum tuberosum*, tissue culture, silver nitrate

## INTRODUCTION

In tissue culture, closed vessels are used with the purpose of avoiding contamination. However, sometimes this may cause abnormal plant growth due to gas accumulation such as ethylene in tissue culture vessels. Ethylene, as a plant growth regulator, produced by tissue, callus and plantlets is known to influence *in vitro* morphogenesis[1]. Excessive ethylene gas accumulation in a closed vessel may inhibit plant growth and constrain establishment of appropriate plantlets to use for repropagation. To overcome this problem, various chemicals such as silver nitrate ($AgNO_3$), 2,5-norhornadiene or anti cobalt chloride ($CoCl_2$) have been used in media. Among these chemicals, silver nitrate ($AgNO_3$) has been widely and in most cases successfully used one. Silver nitrate was also used in order to reduce the occurrence of hyperhydricity in tissue culture of sunflower[2]. Moreover, Kotsias and Roussos[3] pointed out that adding 3 mg $l^{-1}$ silver nitrate to media enhanced shoot elongation in lemon. Similarly, Hyde and Phillips[4] also used silver nitrate for bud enlargement in Chile pepper *in vitro*. Therefore, the objective of this study was to examine effect of media containing various concentrations of silver nitrate ($AgNO_3$) on four potato cultivars in terms of reducing the hyperhydricity or abnormal plantlet growth.

## MATERIALS AND METHODS

The potato cultivars, Maris Bard, Desiree, Nicola and Russet Burbank were obtained as plantlets from the collection in the Department of Agricultural Botany at Reading University, UK. Stock shoot cultures were maintained by culturing single node stem sections on Murashige and Skoog[7] MS basal medium without growth regulators used. Explants (1 cm long stem cuttings) were taken from 4 weeks old *in vitro* plantlets. In the light of the previous experiments, cultivar Nicola and Desiree were chosen because they show significant rate of abnormal growth *in vitro* culture. This problem does not occur in the other two cultivars, Maris Bard and Russet Burbank. In the experiment as in stock shoot cultures, 150x20 mm glass test tubes (borosilicate) containing a single node were used. Growth room was illuminated with cool white fluorescent tubes at 23±1°C with a 16 h photoperiod and a photosynthetic photon flux density (PPHD) of 95 $\mu$ mol $m^{-2}$ $s^{-1}$.

The basal medium MS was supplemented with four different concentrations of $AgNO_3$ (5, 10, 25 and 50 μM). Each treatment including the control without $AgNO_3$ comprised ten culture tubes and each tube was inoculated with one node. The experiment was of a factorial randomised block design. After four weeks, several characters were observed and scored. These include shoot length (SL) (mm), number of leaves (LNO), number of roots (RNO), fresh shoot weight (SFW)(g), dry shoot weight (SDW) (g), number of usable nodes (NNO), root score (RSC) and number of branches (NBR). For NNO, stem section, 10±2 mm long with a central node which can be used for sub-culturing *in vitro*. Root score was based on a 1 to 9 scale where 1 was no root, 9 very well-established root system. The statistical analysis was carried out by using the SAS computer package[5].

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## RESULTS AND DISCUSSION

The data from four cultivars grown in media supplemented with four different $AgNO_3$ concentrations were analysed in terms of the effect of cultivars, silver nitrate and their interaction on a range of growth parameters (Table 1). From the table, the effect of silver nitrate, cultivar and their interaction on all measured characters were significant. The significant silver nitrate*cultivar interactions indicate that the cultivars respond differentially to the $AgNO_3$ concentrations.

As seen in Table 2 and Table 3, response cultivar Nicola and Desiree to $AgNO_3$ was different from Maris Bard and Russet Burbank. Mohiuddin *et al.*[6] also found that there were differences among cucumber cultivars in terms of genotypic response to different $AgNO_3$ concentrations (10, 20 and 30 μM). In Russet Burbank and Maris Bard, there were generally decreases in all measured characters, except root score of Russet Burbank, by application of $AgNO_3$ into MS medium. This decrease was more obvious at higher $AgNO_3$ concentrations. $AgNO_3$ reduced the number of roots per plantlet whereas it increased the length of the roots. Therefore, root score values seemed better than those of number of roots or root score did not changed much with $AgNO_3$ application.

*In vitro,* plantlets tend to form branches because of damage of ethylene gas on tip of the plantlets or causing stress. This reduces number of usable nodes in a potato plantlet. However, less branching and high number of usable nodes is required in sub-culturing of potato *in vitro*. To solve this problem, the results showed that application of silver nitrate decreased branching in potato genotypes. Consequently, number of usable nodes in Desiree and Nicola increased with increasing $AgNO_3$ levels. In addition, from the visual observation, shoot colour became darker green and more vigour with increasing $AgNO_3$ concentrations.

Table 1: Analysis of variance for determination of effect of silver nitrate on potato cultivars *in vitro*

| | Source | | | | |
|---|---|---|---|---|---|
| Parameters | $AgNO_3$ | Cultivar | $AgNO_3$*Cultivar | Rep | Error |
| df | 4 | 3 | 12 | 9 | 171 |
| SL | 13289.23*** | 7517.57*** | 781.82*** | 67.44 | 178.41 |
| LNO | 60.77*** | 23.39*** | 6.37** | 0.14 | 2.32 |
| RNO | 24.10*** | 44.87*** | 15.54*** | 1.27 | 2.46 |
| NNO | 44.73*** | 36.66*** | 18.28*** | 1.12 | 2.35 |
| SFW | 8790.67*** | 136482.79*** | 5136.07*** | 453.61 | 1548.62 |
| SDW | 34.42** | 489.44*** | 27.51*** | 4.03 | 8.19 |
| NBR | 3.63** | 3.07*** | 0.75** | 0.19 | 0.28 |
| RSC | 8.68* | 79.01*** | 9.21*** | 1.19 | 2.59 |

*, **, ***, Significant at 0.05, 0.01, 0.001 level, respectively

Table 2: Effect of silver nitrate on measured characters in potato varieties *in vitro*

| | $AgNO_3$ Concentrations (μM) | | | | | |
|---|---|---|---|---|---|---|
| Cultivar | 0 | 5 | 10 | 25 | 50 | Mean |
| | Shoot length (mm) ($LSD_{0.05}$ : $AgNO_3$ = 5.90, Variety = 5.27) | | | | | |
| M. Bard | 87.20 | 81.80 | 48.80 | 36.80 | 43.20 | 59.56a* |
| Desiree | 79.00 | 83.20 | 61.00 | 28.80 | 37.60 | 57.92a |
| Nicola | 62.60 | 43.20 | 38.80 | 34.20 | 36.40 | 43.04b |
| R.Burbank | 54.40 | 50.00 | 27.60 | 24.20 | 13.80 | 34.00c |
| Mean | 70.80a | 64.55b | 44.05c | 31.00d | 32.75d | |
| | Number of leaves ($LSD_{0.05}$ : $AgNO_3$ = 0.67, Variety = 0.60) | | | | | |
| M. Bard | 10.40 | 9.20 | 7.20 | 6.40 | 6.60 | 7.96a |
| Desiree | 9.80 | 9.60 | 7.80 | 5.80 | 7.00 | 8.00a |
| Nicola | 8.80 | 8.60 | 9.80 | 7.80 | 7.80 | 8.56a |
| R.Burbank | 8.40 | 7.80 | 6.60 | 6.00 | 5.80 | 6.92b |
| Mean | 9.35a | 8.80a | 7.85b | 6.50c | 6.80c | |
| | Number of roots ($LSD_{0.05}$ : $AgNO_3$ = 0.69, Variety = 0.62) | | | | | |
| M. Bard | 8.00 | 7.60 | 5.80 | 3.40 | 4.40 | 5.84a |
| Desiree | 5.20 | 4.60 | 6.20 | 2.60 | 4.80 | 4.68b |
| Nicola | 3.40 | 3.20 | 4.20 | 4.60 | 4.00 | 3.88c |
| R.Burbank | 6.80 | 7.00 | 5.00 | 5.40 | 4.80 | 5.80a |
| Mean | 5.85a | 5.60a | 5.30a | 4.00b | 4.50b | |
| | Number of usable nodes ($LSD_{0.05}$ : $AgNO_3$ = 0.68, Variety = 0.61) | | | | | |
| M. Bard | 6.40 | 7.00 | 4.60 | 3.40 | 3.60 | 5.00a |
| Desiree | 3.40 | 6.60 | 4.80 | 3.00 | 3.00 | 4.16b |
| Nicola | 1.40 | 3.20 | 3.80 | 2.60 | 4.00 | 3.00c |
| R.Burbank | 5.80 | 5.00 | 3.20 | 3.00 | 0.80 | 3.56 bc |
| Mean | 4.25b | 5.45a | 4.10b | 3.00c | 2.85c | |

* Means with the same letter are not significantly different at p=0.05

Table 3: Effect of silver nitrate on measured characters in potato varieties *in vitro*

| Cultivar | $AgNO_3$ Concentrations (µM) 0 | 5 | 10 | 25 | 50 | Mean |
|---|---|---|---|---|---|---|
| | Shoot fresh weight ($LSD_{0.05}$ : $AgNO_3$ = 0.017, Variety = 5.27) | | | | | |
| M. Bard | 0.15 | 0.15 | 0.13 | 0.13 | 0.12 | 0.14a* |
| Desiree | 0.17 | 0.18 | 0.19 | 0.19 | 0.09 | 0.15a |
| Nicola | 0.05 | 0.04 | 0.07 | 0.07 | 0.06 | 0.06b |
| R.Burbank | 0.06 | 0.08 | 0.06 | 0.06 | 0.05 | 0.06b |
| Mean | 0.10ab | 0.11a | 0.11a | 0.08c | 0.09bc | |
| | Shoot dry weight ($LSD_{0.05}$ : $AgNO_3$ = 0.001, Variety = 0.001) | | | | | |
| M. Bard | 0.013 | 0.012 | 0.011 | 0.010 | 0.011 | 0.011a |
| Desiree | 0.009 | 0.012 | 0.012 | 0.007 | 0.011 | 0.010a |
| Nicola | 0.004 | 0.004 | 0.006 | 0.005 | 0.006 | 0.005b |
| R.Burbank | 0.007 | 0.007 | 0.007 | 0.006 | 0.004 | 0.006b |
| Mean | 0.008a | 0.009a | 0.009a | 0.007b | 0.008ab | |
| | Number of branches ($LSD_{0.05}$ : $AgNO_3$ = 0.23, Variety = 0.21) | | | | | |
| M. Bard | 0.40 | 0.00 | 0.00 | 0.00 | 0.00 | 0.08bc |
| Desiree | 1.20 | 0.00 | 0.00 | 0.00 | 0.00 | 0.24b |
| Nicola | 1.40 | 0.40 | 0.40 | 0.60 | 0.00 | 0.56a |
| R.Burbank | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00c |
| Mean | 0.75a | 0.10b | 0.010b | 0.15b | 0.00ab | |
| | Root score ($LSD_{0.05}$ : $AgNO_3$ = 0.71, Variety = 0.64) | | | | | |
| M. Bard | 8.20 | 8.60 | 7.80 | 6.60 | 7.80 | 7.80a |
| Desiree | 6.20 | 8.60 | 8.20 | 5.40 | 7.00 | 7.08b |
| Nicola | 5.00 | 5.00 | 5.00 | 5.40 | 4.60 | 5.00d |
| R.Burbank | 4.60 | 6.20 | 5.80 | 7.40 | 5.00 | 5.80c |
| Mean | 6.00b | 7.10a | 6.70ab | 6.20b | 6.10b | |

* Means with the same letter are not significantly different at p=0.05

$Ag^{2+}$ ions in silver nitrate ($AgNO_3$) inhibits ethylene action, consequently reduces the receptor capacity to bind ethylene[8]. Bais *et al.*[9] reported that addition of 40 µM $AgNO_3$ into medium increases shoot proliferation and *in vitro* flowering. Moreover, Chi *et al.*[10] and Gerats *et al.*[11] also obtained the similar results for Brassica and Petunia genotypes, respectively. Our results for potato genotypes were also in line with the results of these previous studies.

As a conclusion, $AgNO_3$ could be used in the cultivars or genotypes that have a proliferation problem *in vitro* due to branching, lack of usable nodes or hyperhydric shoot growth. Depending upon genotype, in general, 5 or 10 mM $AgNO_3$ seem to be the optimum concentrations in potato for increasing number of usable nodes and decreasing hyperhydric or abnormal shoots. However, one should consider that the higher $AgNO_3$ concentrations shorten shoot length, thus number of usable nodes decreases. Since there was a genotypic response to $AgNO_3$, it would be better to determine the appropriate $AgNO_3$ concentrations for each species or genotypes.

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