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Effect of Silver Nitrate During *Ex vitro* Acclimatization of Micropropagated Ginger Cultivars

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Abstract

Silver nitrate ($AgNO_3$) was used under *in vitro* conditions to study the response of ginger cultivars 'Nadia' and 'Baishey' under *ex vitro*. Micropropagated plants treated with $AgNO_3$ showed significant difference ($p \leq 0.05$) compared to those plantlets without $AgNO_3$ and control type in almost all the different quantitative traits analyzed. Significant difference in number of finger per plant and minirhizome yield indicated the repercussion of $AgNO_3$ during acclimatization.

Keywords: acclimatization, microrhizome, minirhizome, silver nitrate, yield

Introduction

Ginger (*Zingiber officinale* Rosc.) is an important spice crop used in various medicinal and culinary preparations. It produces pungent, aromatic rhizomes that are primarily grown in tropical areas of Asia and has been used in medicine since ancient times for conditions including colds, fevers, digestive problems, and as an appetite stimulant. Further, ginger has been evaluated for treatment of motion sickness (Grontved *et al.,* 1988; Mowrey and Clayson, 1982; Stewart *et al.,* 1991; Wood *et al.,* 1988), nausea, and vomiting (Borrelli *et al.,* 2005; Chaiyakunapruk *et al.,* 2006). There is scanty report on the *in vitro* production of diseases free ginger clones and the performance of tissue culture-derived plants under field conditions (Sharma *et al.,* 1997). Since, the silver ion is a potent inhibitor of ethylene action and its incorporation into tissue culture media has produced beneficial effects on growth and multiplication (Beyer, 1976). It promotes regeneration in *Brassica campestris* (Palmer, 1992) and *Helianthus annuus* (Chraibi *et al.,* 1991) and improved somatic embryogenesis in *Hevea brasiliensis* (Auboiron *et al.,* 1990), *Solanum tuberosum* (Tiainen, 1992) and *Hordeum vulgare* (Evans and Batty, 1994). To our knowledge, there is no detailed report on the effect of silver nitrate ($AgNO_3$) of micropropagated ginger plant of cultivars 'Nadia' and 'Baishey' under *ex vitro*. In the present paper, we report on the potential of *in vitro* microrhizome treated with $AgNO_3$, resulted in highly efficient morphological escalation and development of minirhizomes.

The purpose of the present investigation was to evaluate the comparative field performance and the high-frequency establishment of micropropagated ginger cultivars 'Nadia' and 'Baishey' and to observe whether $AgNO_3$ has an effect on microrhizome morphology and development. Interaction of $AgNO_3$ and development of microrhizome with propagation method under *ex vitro* condition have not been documented with ginger cultivars 'Nadia' and 'Baishey'.

Materials and methods

In vitro derived microshoots ('Nadia' and 'Baishey') obtained from aseptic grown shoots were inoculated on microrhizome inducing liquid Murashige and Skoog's (MS) medium supplemented with and without silver nitrate (11 μM), 8 % sucrose, 1 mg/L α-napthalene acetic acid (NAA), and 2 mg/L 6-benzyl-amino-purine (BAP). Plantlets with well developed shoots along with microrhizome were acclimatized in plastic pot containing soil in 70% shade house (Saveer Biotech Ltd., New Delhi, India) condition maintained at ca. 90% relative humidity and 16 h photoperiod. The experiments for comparative performance were carried out in shade house of Bioresource Park of IBSD (Institute of Bioresources and Sustainable Development) at Harourou, Imphal. Plant propagules of each type ('Nadia' and 'Baishey' plantlets treated with and without $AgNO_3$) were planted in plots, size 8 x 40 m, with 2 replications containing 112 plants in a 2 x 56 arrangement per replication spacing at 0.3 x 0.3 m. Morphological characters like number of leaves, plant height, average no. of finger/plant and total fresh weight of minirhizome/plant. Statistical analyses of the non-parametric data (frequencies) were carried out by the test for homogeneity of proportions and significant treatment differences selected by a nonparametric statistical Post Hoc Multiple Comparisons Test. Discrete data were subjected to analysis of variance (ANOVA) using 17.00 SPSS (SPSS Inc., Chicago, IL, USA)

followed by the least significant difference (LSD) test at $p \leq 0.05$ to compare means.

Results and discussions

In vitro derived plantlet of ginger cultivars 'Nadia' and 'Baishey' with well developed microrhizome and root were successfully transferred to plastic pots containing unsterilized potting mixture under the shade house condition. 92-95% survival rates (92% in 'Baishey' and 95% in 'Nadia') were documented in the plants treated with $AgNO_3$ and 80-83% survival rates (80% in 'Baishey' and 83% in 'Nadia') in those plants without $AgNO_3$ treatment. The plants were acclimatized for 5 weeks before being transferred directly in field condition (bed prepared under shade house). The control was treated in a larger plastic pot. The plantlets for both cultivars were well established even in unsterilized soil, which was in support with the finding of Sharma and Singh (1997) in *Zingiber officinale* Rosc. The performance of micropropagated ginger treated with and without $AgNO_3$ for four quantitative traits under field conditions for minirhizome production is given in Tab. 1. The preliminary experiments, designed to investigate the role of ethylene in rhizome development and analyses of variance for the combined effect of $AgNO_3$ with propagation method, were significant ($p \leq 0.05$) for 3 qualitative traits for both cultivars (Tab. 1). The dependency between presence and absence of $AgNO_3$ and propagation method for number of leaves was, however, not evident as the interaction between these two factors was insignificant (Tab. 1) for the investigated ginger cultivars.

The increase in the size of minirhizome production as well as the no. of fingers was dependent on propagation type. Field propagated plants had more vigour in growth and showed significant result in no. of finger per plant, producing also twice the minirhizome yield compared to the control type. Large differences between the minirhizome yields of the two different cultivars of ginger were reliant in $AgNO_3$ treatment during *in vitro* culture. The morphological differences of the rhizome, observed in the present study, can at least partly be attributed to the presence of $AgNO_3$, BAP and propagation system. Although the effect of $AgNO_3$ on ginger propagation has not been studied previously, Dikash *et al.* (2012) found similar results in turmeric *in vitro*. According to the previous study of Kavyashree (2009), BAP incorporation in the medium shows significant response in the shoot production of ginger. However, the obtained results from our study were not only compatible with this finding, but also clearly indicate that the use of $AgNO_3$, a potent inhibitor of ethylene action, with BAP had a synergistic effect on plant height, average number of finger/plant and total fresh weight of minirhizome/plant of ginger cultivars 'Nadia' and 'Baishey' during *ex vitro*. This result suggested that ethylene could play a negative regulatory role in micropropagation of ginger and $AgNO_3$ seemed to be effective in counteracting this regulation. As shoot regeneration capacity of the explant and stimulation of ethylene biosynthesis might vary, depending on the growth used regulators (Kumar *et al.,* 1998), the medium containing BAP can possibly release higher amount of ethylene, which is in agreement with the findings of Ozen-Tokatli *et al.* (2005).

As a result, the inhibitory effect of silver nitrate on ethylene action could be more significant on ginger cultured on BAP containing media, where higher minirhizome frequencies were more often obtained than on $AgNO_3$ free media under field condition. Ag^{2+} ions interact with the ethylene binding sites located in cell membranes (Yang and Hoffman, 1984) and blocks ethylene binding *in vivo* (Goren *et al.,* 1984); usage of $AgNO_3$ can possibly counteract this ethylene-caused recalcitrance. However, production of higher finger number with larger minirhizome may be a direct result of residual $AgNO_3$ with the microrhizome from the *in vitro* treatment used to increase microrhizome production. Although an effect of AgNO3 on minirhizome response during acclimatization has not been studied previously, Dikash *et al.* (2012) and Chithra *et al.* (2005), show increased microrhizome production under *in vitro*

Tab. 1. Field performance of micropropagated ginger cultivars 'Nadia' and 'Baishey' for four quantitative traits

Traits	'Nadia'			'Baishey'		
	With $AgNO_3$	Without $AgNO_3$	Control	With $AgNO_3$	Without $AgNO_3$	Control
Number of leaves**	11.10 ± 0.10^{a}	10.30 ± 0.15^{a}	9.40 ± 0.26^{a}	12.20 ± 0.20^{a}	11.60 ± 0.30^{a}	11.10 ± 0.10^{a}
Plant height (cm) *	22.30 ± 0.15^{d}	17.30 ± 0.21^{c}	14.20 ± 0.13^{b}	21.30 ± 0.15^{d}	15.30 ± 0.15^{b}	15.40 ± 0.16^{b}
Number of finger per plant*	48.33 ± 0.18^{g}	31.209 ± 0.13^{f}	27.20 ± 0.13^{e}	48.65 ± 0.15^{g}	29.97 ± 0.18^{e}	28.20 ± 0.13^{e}
Average weight of finger per plant (g) *	20.50 ± 0.16^{j}	17.90 ± 0.15^{i}	13.30 ± 0.15^{h}	23.10 ± 0.10^{j}	16.30 ± 0.15^{i}	14.20 ± 0.20^{h}

Values consist means of 112 replicates for each treatment
*Significant at $p \leq 0.05$, **Non-significant
Means followed by the same letters are not significantly different at p=0.05

conditions. In conclusion, this study shows that BAP and $AgNO_3$, and their interaction, most prominently influenced minirhizome development of ginger cultivars 'Nadia' and 'Baishey'. The hypothesis was that increased minirhizome production on $AgNO_3$ rich medium accelerates the minirhizome characteristics that provide tissue culture plants ($AgNO_3$) with desirable morphogenesis and growth habits. This observation emphasises the necessity of a highly productive regeneration method using $AgNO_3$ in order to raise the frequency of micropropagated plants to increase the response of minirhizome development under *ex vitro*. The addition of the ethylene inhibitor $AgNO_3$, to the culture medium, represented an important practical step for further experiments. Additional field work is needed to better comprehend the function of $AgNO_3$.

Conclusions

In conclusion, the present study demonstrates the scope of selecting improved ginger clones with higher rhizome yield by addition of silver nitrate during *in vitro* condition. Field evaluation of ginger cultivars 'Nadia' and 'Baishey' treated with silver nitrate shows superior growth. This protocol for *in vitro* microrhizome formation and its role in improvement of rhizome yield of micropropagated plants can be utilized by commercial growers for production of disease-free ginger in large scale.

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