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SYNERGISTIC EFFECTS OF SULFHYDRYL COMPOUNDS ON CYTOKININ-INDUCED POTATO MICROTUBERIZATION *IN VITRO*

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ABSTRACT The synergistic effects of two sulfhydryl compounds, *viz.,* L-cysteine and 2-mercaptoethanol each at 0, 2.0, 4.0, 6.0, 8.0 and 10.0 mM during cytokinin-induced microtuberization were studied in two potato (*Solanum tuberosum* L. subsp. *tuberosum*) genotypes. The sulfhydryl compounds were tested on induction medium based on Murashige and Skoog's basal medium supplemented with 10 mgl^{-1} N^6-benzyladenine (BA) and 80 gl^{-1} sucrose There were significant genotype x sulfhydryl compound interactions for microtuber initiation and development Except for cysteine which showed a positive influence on microtuber initiation only in early maturing genotype Kufri Ashoka, sulfhydryl compounds in general significantly affected microtuber development. Cysteine at ≥ 4.0 mM inhibited microtuber development Mercaptoethanol at 2.0 mM increased microtuber fresh mass and microtuber yield. However, increasing concentrations of mercaptoethanol in the medium significantly declined microtuber fresh mass and yield Thus 2.0 mM mercaptoethanol can be used in the induction medium to improve potato microtuber production and utilization efficiency during cytokinin-induced tuberization *in vitro*.

INTRODUCTION

In potato (*Solanum tuberosum* L.) microtuberization is an established technique for the production of virus-free planting material (10, 16). Over the past two decades, a wide range of physical (5, 26, 28) and nutritional (2, 14, 18) factors have been reported for their varying degrees of effectiveness for potato microtuber production *in vitro* and its successful integration in seed production programmes (11, 17, 21) Of these, cytokinin-induced microtuberization has been found to be most effective for large-scale microtuber production in potato (9, 26). In spite of phenomenal success of this technology in recent years, its effective utilization is severely limited due to inherent tendency of potato microplants to produce small sized tubers *in vitro* (10). Small microtubers are particularly inconvenient for post–harvest handling and/or storage (8), dormancy release (4) and subsequent field establishment (11, 17). Therefore, in recent years much attention has been directed towards increasing the size of potato microtubers

Sucrose synthase (UDPG-D-fructose-2-aα-D-glucosyl transferase, EC 2.4.1.13) is one of the major sucrolytic enzymes present in excessive amounts in potato plants (1) It catalyzes a reversible reaction yielding UDP-glucose and fructose (from sucrose), the precursors of sucrose-starch transformation. The catalytic property of sulfhydryl (nucleophilic) groups in enzyme molecules is well known. It has been shown that sulfhydryl compounds such as cysteine and mercaptoethanol markedly activate the sucrolytic property of sucrose synthase (15) Since sucrose synthase plays a major role in determining sink strength during tuberization in potato (29), it was of interest to study the effects of sulfhydryl compounds on potato microtuber production *in vitro*. As to the best of our knowledge, there appears to be no information about the efficacy of sulfhydryl compounds on potato tuberization *vis-à-vis* microtuberization. We, therefore, decided to examine as to how two sulfhydryl compounds,

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viz., cysteine and mercaptoethanol affect potato microtuberization *in vitro* when exogenously supplemented in the microtuber induction medium.

MATERIALS AND METHODS

Two tetraploid (2n=4*x*=48) potato (*Solanum tuberosum* L. subsp. *tuberosum*) genotypes belonging to different maturity groups, *viz.*, Kufri Ashoka (early maturing) and Kufri Chipsona-1 (medium maturing) were used in the present experiment. These were selected for their contrasting response to *in vitro* microtuber production (9). Disease-free microplants of these genotypes were maintained and multiplied through shoot cuttings following the method described earlier (20). To reduce the carry-over effects of growth hormones on subsequent microtuberization, the axillary shoot cuttings were pre-conditioned in hormone-free semisolid (8 gl^{-1} agar) medium for 21 d (18)

For microtuber induction, pre-conditioned double node cuttings (DNCs) were used. The induction medium was based on MS (7) medium supplemented with 10 mgl^{-1} N^6-benzyladenine, 80 gl^{-1} sucrose and different concentrations (0, 2 0, 4 0, 6 0, 8 0 and 10.0 mM) of L-cysteine or 2-mercaptoethanol (Sigma, Missouri). Three DNCs were cultured per tube (25 × 150 mm) containing 13 ml of induction medium, and the tubes were closed using polypropylene closures (Kasablanka, Mumbai). Both the sulfhydryl compounds were added by filter sterilization (Millipore, USA) after autoclaving the medium at 121 °C for 20 min. The microtuber induction cultures were incubated in the dark at 20 °C (8).

After 60 d of incubation, observations were recorded on the number of microtubers per tube, average microtuber fresh mass (mg) and microtuber yield (g) per tube The experiment was conducted in a factorial (2 × 2 × 6) completely randomized design with 15 replicate culture tubes in each treatment. Before statistical analyses, the data on number of microtubers per tube were transformed into square roots' ($\sqrt{x+0\ 5}$), and the three-way analyses of variance (ANOVAs) were computed using the standard procedure (22). Means were separated by Least Significant Difference (LSD) or Student-Newman-Keul's tests.

RESULTS AND DISCUSSION

The analyses of variance **(Table 1)** showed that sulfhydryl compounds had significant ($P \leq 0\ 01$-0.05) main effects on induction and development of potato microtubers *in vitro*. Variation due to genotypic differences was also significant ($P \leq 0\ 01$) ' Significant ($P \leq 0\ 05$) genotype × sulfhydryl compound interaction for microtuber number indicated that the effect of sulfhydryl compound for this character was not consistent over the two genotypes tested. Also there were strong sulfhydryl compound × concentration interactions, suggesting that the effect of sulfhydryl compound on microtuberization was not uniform over the concentration used in the experiment. However, there were no significant ($P \leq 0.05$) genotype × sulfhydryl compound × concentration interactions

Microtuber initiation: The effects of sulfhydryl compounds on microtuber initiation as recorded by the number of microtubers developed per culture tube (i.e. 3 DNCs) are shown in **Table 2** In cv. Kufri Ashoka, cysteine was effective in significantly ($P \leq 0\ 05$) increasing the microtuber number at 6 0 mM level, and at concentrations beyond this level, no further significant ($P \leq 0\ 05$) increment in microtuber number occurred. In comparison, cysteine did not have any significant effect on microtuber initiation in cv. Kufri Chipsona-1. The effect of mercaptoethanol on microtuber initiation was uniform in both the genotypes Addition of mercaptoethanol to induction medium did not result in any significant

Table 1. Analyses of variance for microtuber growth parameters in sulfhydryl compounds experiment (* = $P \leq 0.05$ and ** = $P \leq 0.01$)

Source	*d f*	*MSS*		
		Number of microtubers	*Average microtuber fresh mass (mg)*	*Microtuber yield (g)*
Genotype	1	0.97**	269013.61**	0.75**
Sulfhydryl compound	1	0.34*	88915.25**	0.39**
Genotype × sulfhydryl compound	1	0.37*	407.44	0.14
Concentration	5	0.23**	117005.36**	0.99**
Genotype × concentration	5	0.03	10910.79*	0.06
Sulfhydryl compound × concentration	5	0.45**	21721.97**	0.35**
Genotype × sulfhydryl compound × concentration	5	0.05	3863.53	0.03
Error	336	0.07	4846.27	0.04

Table 2. Effects of sulfhydryl compounds on microtuber number during cytokinin-induced microtuberization in potato. Data in parenthesis represent square root transformed values ($LSD_{0.05}$ = 0.13). Average effect means with common letter are not significantly different at $P \leq 0.05$, according to Student-Newman-Keul's test

Concentration (mM)	*Cysteine*			*Mercaptoethanol*		
	Kufri Ashoka	*Kufri Chipsona-1*	*Average effect*[a]	*Kufri Ashoka*	*Kufri Chipsona-1*	*Average effect*[a]
2.0	2.9 (1.84)[b]	3.1 (1.89)	3.0 (1.86) *a*	2.8 (1.80)	3.4 (1.96)	3.1 (1.88) *a*
4.0	2.5 (1.73)	3.0 (1.87)	2.8 (1.80) *a*	2.9 (1.82)	3.6 (2.01)	3.3 (1.92) *a*
6.0	3.1 (1.88)	2.9 (1.83)	3.0 (1.85) *a*	2.7 (1.76)	3.3 (1.93)	3.0 (1.84) *a*
8.0	3.1 (1.87)	3.1 (1.90)	3.2 (1.89) *a*	2.5 (1.71)	2.9 (1.82)	2.7 (1.76) *a*
10.0	3.4 (1.95)	3.1 (1.87)	3.2 (1.91) *a*	1.7 (1.43)	2.4 (1.64)	2.0 (1.53) *b*
Control	2.4 (1.67)	2.9 (1.83)	2.6 (1.75) *a*	2.3 (1.67)	2.9 (1.83)	2.6 (1.75) *a*

increase in microtuber number; rather mercaptoethanol at > 8.0 mM concentration was found to decrease microtuber number significantly. The average effects of sulfhydryl compounds over the two genotypes showed that cysteine did not have any effect on microtuber initiation, while mercaptoethanol did inhibit microtuber initiation when supplemented in the induction medium at a concentration of 10.0 mM **(Table 2)**.

Microtuber development: The effects of sulfhydryl compounds on microtuber fresh mass are shown in **Figure 1**. Cysteine significantly ($P \leq 0.05$) decreased the microtuber fresh mass at 4.0 and 6.0 mM concentrations in cvs Kufri Chipsona-1 and Kufri Ashoka, respectively. However, no further significant decline in microtuber fresh mass occurred with increasing concentrations of cysteine in both the genotypes. In cv. Kufri Ashoka, a significant ($P \leq 0.05$) improvement in microtuber fresh mass occurred when the induction medium was supplemented with 2.0 mM mercaptoethanol. In contrast, mercaptoethanol was not effective in improving the microtuber fresh mass in cv. Kufri Chipsona-1. However, mercaptoethanol at higher concentrations (8.0 - 10.0 mM) was inhibitory to microtuber development in both the genotypes. The average effects of sulfhydryl compounds on microtuber development showed that there was no significant ($P \leq 0.05$) improvement in microtuber fresh mass when the medium contained cysteine **(Figure 1)**, rather cysteine at ≥ 4.0 mM inhibited microtuber development as indicated by a reduction in average microtuber fresh mass. Microtuber fresh mass was significantly improved at 2.0 mM mercaptoethanol and thereafter, declined gradually with increasing concentrations **(Figure 1)**.

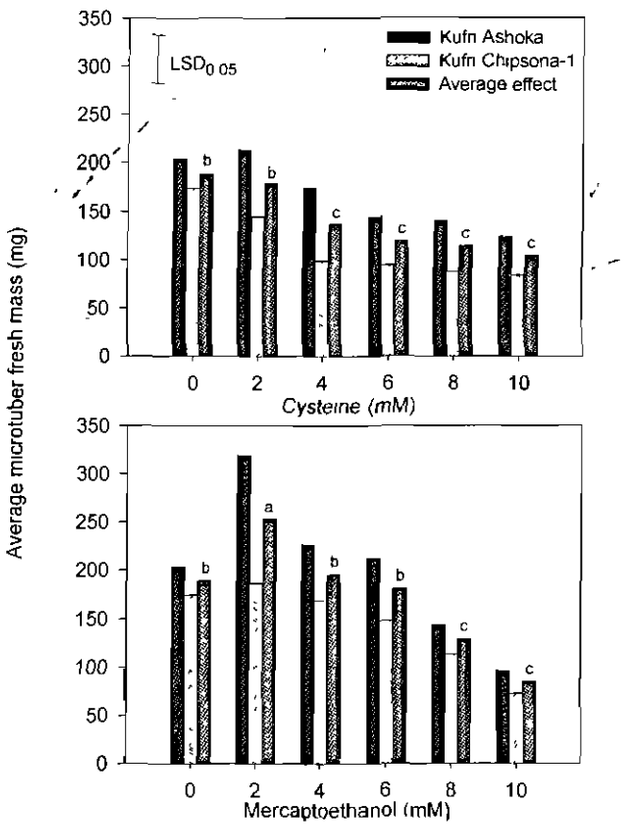

Fig 1 Effects of sulfhydryl compounds on average microtuber fresh mass (mg) during cytokinin-induced microtuberization in potato Average effect means with common letter are not significantly different at P ≤ 0 05, according to Student-Newman-Keul's test

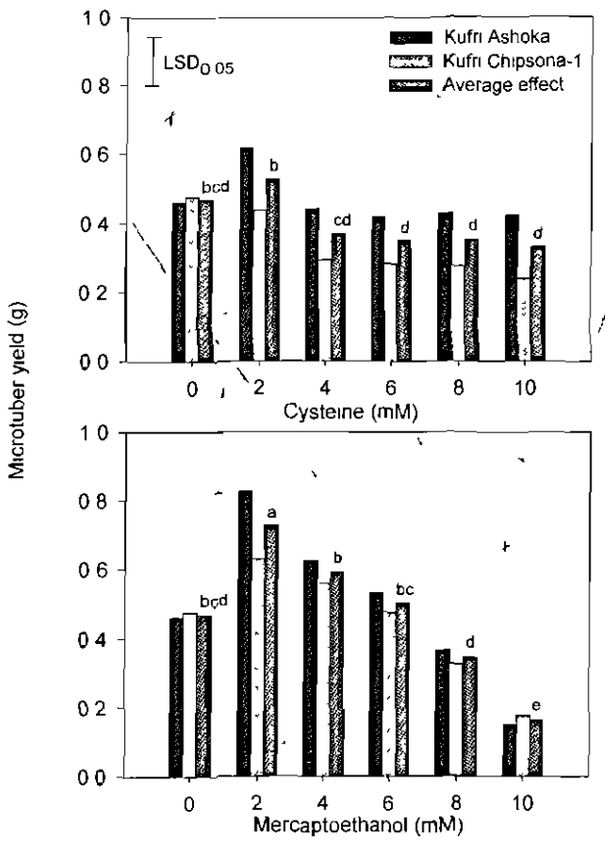

Fig 2 Effects of sulfhydryl compounds on microtuber yield (g) during cytokinin-induced microtuberization in potato Average effect means with common letter are not significantly different at P ≤ 0 05, according to Student-Newman-Keul's test

Microtuber yield: The effects of sulfhydryl compounds on microtuber yield are shown in **Figure 2**. In cv. Kufri Ashoka, a significant improvement in microtuber yield occurred at 2 0 mM cysteine, however, it did not have any further effect with increasing concentrations In contrast, cysteine decreased the microtuber yield in cv Kufri Chipsona-1 at ≥ 4.0 mM Mercaptoethanol significantly ($P \leq 0.05$) improved the microtuber yield at 2 0 mM level in both the genotypes However, a significant decline in microtuber yield was observed in both the genotypes with increasing concentrations of mercaptoethanol in the induction medium The average effects of sulfhydryl compounds showed that cysteine did not have any promoting effect on microtuber yield, and mercaptoethanol was effective in increasing the microtuber yield at 2.0 mM. Averaged over the genotypes, mercaptoethanol was found to decrease microtuber yield with increasing concentrations **(Figure 2)**

The present study clearly established the synergistic effects of sulfhydryl compounds on cytokinin-induced microtuberization process in potato. However, this synergistic effect was more conspicuous for microtuber development than their initiation. Microtuber initiation in potato is essentially hormonal

(13, 25) There are numerous evidences in potato that upon tuber formation, the activity of sucrose synthase increases (1) resulting in a shift from apoplastic unloading in stolons to symplastic unloading in tubers (12, 23, 24) In view of this and considering the fact that sulfhydryl compounds are involved in activating the enzyme sucrose synthase (15), it is likely that their effects are confined to microtuber development rather than microtuber initiation as reported in the present study. Between the two sulfhydryl compounds tested in the present investigation, cysteine did show some beneficial effect on microtuber initiation at higher concentrations in early bulking (maturing) genotype Kufri Ashoka, which is known to microtuberize earlier *in vitro* (19) This beneficial effect is difficult to explain, but it may perhaps be due to some positive interactions between cysteine and endogenous hormonal balance of the genotypes. In numerous studies on potato microtuber induction, such sort of favourable interactions between the exogenously applied growth additives and endogenous growth regulators have been reported (6). Furthermore, this beneficial effect of cysteine on microtuber initiation may perhaps explain as to why it did have a negative effect on microtuber development (microtuber fresh mass) at higher concentrations in both the genotypes. It is well established that there always exists an inverse relationship between microtuber number and microtuber fresh mass during *in vitro* tuberization in potato (18, 27).

Mercaptoethanol exhibited a positive influence on microtuber development (microtuber fresh mass) at 2.0 mM concentration. This is in agreement with its mode of activating effect on cleavage activity of potato sucrose synthase at this level (15) Although the sucrose cleavage activity has been shown to be markedly activated by mercaptoethanol at concentrations ≥ 6 0 mM (15), it was found to exert a detrimental effect on potato microtuber fresh mass when supplemented in the induction medium at concentrations > 6.0 mM as recorded in the present study Kim *et al.* (3) reported an increase in sucrose synthase activity up to the attainment of 1 0 g microtuber fresh mass during microtuberization in potato. However, in the absence of any report on potato sucrose synthase activity during microtuberization process in the presence of sulfhydryl compounds, this detrimental effect of mercaptoethanol on microtuber development at higher concentrations is difficult to explain A possible explanation may be that it somehow at higher concentrations interferes adversely in metabolic process(es) to inhibit the sucrose-starch transformation, which is essentially associated with tuber development. The superiority of mercaptoethanol to cysteine in microtuber development as reported in the present study can further be substantiated by greater activating effect of the former as compared to the latter on sucrose synthase activity (15).

The results further showed that addition of 2.0 mM mercaptoethanol to induction medium resulted in about two-fold increase in microtuber yield This microtuber yield improvement (affected by mercaptoethanol) was more conspicuous in early maturing (bulking) genotype than that in medium maturing one The greater effectiveness of mercaptoethanol in increasing microtuber yield can be attributed to the increase in microtuber fresh mass It has been shown earlier that average microtuber fresh mass has maximum direct effect on microtuber yield during cytokinin-induced microtuberization in potato (11) Thus it can be concluded that induction of potato microtubers on 2.0 mM mercaptoethanol-supplemented medium during cytokinin-induced *in vitro* tuberization would affect significant improvement in

microtuber fresh mass *vis-á-vis* microtuber size and yield. Microtubers with greater fresh mass are more amenable to effective storage and utilization in seed potato production programmes.

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