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Effects of spermidine, spermine and cyclohexylamine on mitotic activity of 2X, 4X and 6X wheats

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Abstract

The effects of spermidine (Spd), spermine (Spm) and cyclohexylamine (CHA) on seed germination, root elongation, mitotic index and chromosomal behavior were studied in diploid (*Triticum monococcum L.*), tetraploid (*T. durum* Desf.) and hexaploid (*T. aestivum* L.) wheats. The polyamines (PA) inhibited seed the germination in three species. CHA inhibited seed germination in diploid and tetraploid species, but it showed a stimulation in hexaploid seeds.

Spd, Spm and CHA reduced root elongation in three species. Spd and Spm decreased mitotic index in diploids and tetraploids. In hexaploids, Spd slightly increased the division frequency whereas Spm decreased it. The root tips of the three species treated with CHA showed maximum inhibition in mitotic index. Some mitotic abnormalities like unoriented metaphase, anaphase bridges, unequal distribution, laggards, multipolar spindle fibers were observed in the control groups of diploid, tetraploid and hexaploid. Spd, Spm and CHA affected the percentages of aberrations. Our results suggested that the reason of the inhibition in root elongation was due to the reduction in the mitotic index.

Key words: Spermidine, spermine, cyclohexylamine, mitotic index, chromosomal abnormalities

Spermidin, spermin ve sikloheksilaminin 2X, 4X ve 6X buğdaylarda mitotik aktiviteye etkileri

Özet

Bu çalışmada, spermidin (Spd), spermin (Spm) ve sikloheksilamin'nin (CHA), diploid (*Triticum monococcum* L.), tetraploid (*T. durum* Desf.) ve heksaploid (*T. aestivum* L.) buğdaylarda tohum çimlenmesi, kök uzaması, mitotik indeks ve kromozomların davranışına etkileri incelendi. Poliaminler üç türde de tohum çimlenmesini inhibe etti. CHA diploidlerde ve tetraploidlerde tohum çimlenmesini inhibe ederken, heksaploidlerde çimlenmeyi teşvik etti.

Spd, Spm ve CHA üç türün kök uzamasını inhibe etti. Spd ve Spm diploidlerde ve tetraploidlerde mitotik indekste inhibisyona neden olurken, heksaploidlerde Spd artışa, Spm ise düşüşe neden oldu. CHA üç türün kök uçlarında da mitoz bölünme frekansını düşürdü. Her üç türün kontrol grubunda da mitotik anormallikler gözlendi. Bu anormallikler bozuk metafaz tablası, anafazda kromozom köprüleri, eşit olmayan dağılım, geç kalan kromozomlar, çok kutuplu iğ ipliği şeklinde özetlenebilir. Spd, Spm ve CHA üç türde de anormallik yüzdesini etkiledi. Sonuçlar, kök uzamasındaki inhibisyonun bu maddelerin mitotik indeksi düşürmelerinden kaynaklandığını gösterdi.

Anahtar sözcükler: Spermidin, spermin, sikloheksilamin, mitotik indeks, kromozomal anormallikler

Introduction

Polyamines, (PA) putrescine (Put), spermidine (Spd) and spermine (Spm) are found ubiquitous in higher plants. The exact function of these polycations is unknown, but many studies indicate their involvement in various events such as cell division, DNA and protein synthesis, growth and differentiation, senescence inhibition, fruit ripening (Slocum and Flores., 1991; Martin-Tanguy, 2001).

PAs were found as powerful inhibitors of seed germination in *Arabidopsis* (Mirza and Bagni, 1991). On the other hand cyclohexlyamine (CHA), an inhibitor of spermidine synthase, accelerated radicle emergence in *Cicer arietinum* seeds (Gallardo et al., 1992).

Although high level of PAs is generally related to cell division some inhibitory effects of exogenously applied PA have been reported (Gatta et al., 1992; De Agazio et al., 1992). The inhibition of root growth observed during the Spd treatment of maize seedlings is due to reduction of both mitotic index and cell elongation (De Agazio et al., 1995).

PAs can bind to DNA and associate with chromosomes (Hougaard, 1992). Direct binding of PAs to DNA and their ability to modulate DNA-protein interactions appear to be important in mitotic activity and chromosomal behavior. Recently, Ünal et al. (2002) observed mitotic abnormalities in barley seedlings with Put treatment however little is known about the effect of PAs and their biosynthetic inhibitors on chromosome behavior.

Presence of ploidy series is well known since long years in wheat and the basic number is seven in this genus. It is also known diploid wheat species having 2n=2X=14, tetraploid 2n=4X=28, and hexaploid species 2n=6X=42 chromosomes. Plants at different ploidy level giving different responses are also well documented (Kerby and Kuspira, 1988).

The aim of this research is to investigate the effect of PAs on seed germination, root elongation, mitotic index and chromosome behavior and also to reveal the response of plants at different ploidy levels to exogenous PAs.

Material and methods

The seeds of diploid *Triticum monococcum* L. cv. TUR O2343, tetraploid *Triticum durum* Desf. cv. *Ankara 09/96* and hexaploid *Triticum aestivum* L. *cv. Kutluk-94* were used as experimental materials. The seeds were surface sterilized with 1% sodium hypochloride for 5 min and washed with tap water. They were soaked in water (control) or in experimental solutions (1 mM Spd, 1 mM Spm, 10 mM CHA) overnight. These concentrations were chosen, based on previously reported favorable results (De Agazio et al., 1992). After that, the seeds were placed to petri dishes containing filter paper moistened with distilled water or experimental solution. 30 seeds were used to estimate germination percentages and each experiment has 5 replications. 2 days after sowing, root tips were cut 0.5 cm and hydrolyzed with 1 N HCl at 60^0 C for 10 min. Then they were transferred in to basic fuchsin for 1.5-2 h in dark. Squash preparations were made in 2% aseto-orsein. Cytological analysis included mitotic index and scoring of aberrant cells. Ten well spread slides were chosen and more than 5000 cells were scored for each treatment. Mitotic index was calculated as the percent ratio of dividing cells and total number of cells observed.

Results

In control, the seeds of diploid (2X) and hexaploid (6X) species started to germinate at 12^{th} hours after sowing but the germination started at 18^{th} hours in tetraploids (4X). No difference was established in Spd and Spm treated seeds in respect to the starting time of germination. Seed germination was started at 12^{th} hours in 2X, 4X and 6X seeds treated with CHA. Spd and Spm inhibited seed germination at three species (Table 1). The seeds of T. *monococcum* were the most sensitive to PAs.

Table 1: The effect of spermidine, spermine and cyclohexylamine on seed germination in diploid, tetraploid and hexaploid wheats. Control: Distilled water, Spd: Spermidine (1mM), Spm: Spermine (1mM), CHA: Cyclohexylamine (10mM).

	Seed Germination (%)		
Treatment	*T. monococcum* (2X)	*T. durum* (4X)	*T. aestivum* (6X)
Control	100.0	100.0	100.0
Spd	75.1	80.6	85.0
Spm	18.8	60.4	72.7
CHA	89.1	98.5	107.2

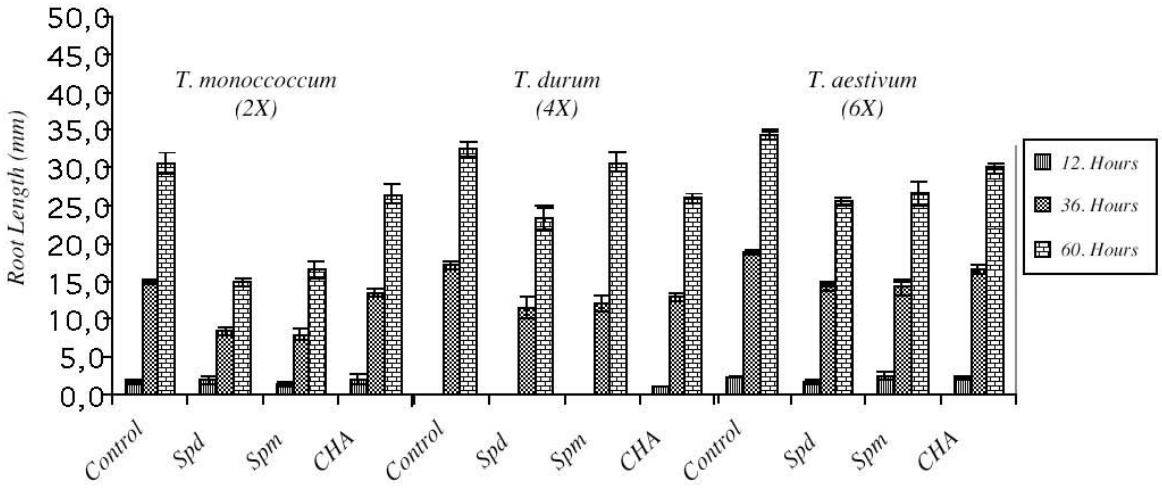

Figure 1: Effect of spermidine, spermine and cyclohexylamine on the primary root growth of 2X, 4X and 6X wheats. Control: Distilled water, Spd: Spermidine (1mM), Spm: Spermine (1mM), CHA: Cyclohexylamine (10mM).

Table 2: Mitotic index and frequency of chromosome aberration in the root tips of 2X, 4X and 6X wheats treated by Spd, Spm and CHA. Control: Distilled water, Spd: Spermidine (1mM), Spm: Spermine (1mM), CHA: Cyclohexylamine (10mM).

Species	Treatment	Total cell scored	Mitotic index (%)	Abnormal metaphase	Abnormal anaphase	Total aberration frequency (%)
T. monococcum	Control	4608	18.6±0.009	8/114*	11/84**	9.6
(2X)	Spd	4604	13.4±0.009	31/104	10/52	26.3
	Spm	2951	12.3±0.003	6/52	4/49	9.9
	CHA	2988	9.1±0.006	3/36	14/28	26.6
T. durum	Control	5563	19.2±0.006	21/142	22/75	19.8
(4X)	Spd	2045	18.8±0.006	7/40	9/38	20.5
	Spm	1634	17.8±0.009	8/35	3/23	19.0
	CHA	6851	10.6±0.003	40/124	34/79	36.5
T. aestivum	Control	8722	18.5±0.006	35/173	40/116	26.0
(6X)	Spd	6709	18.8±0.006	24/131	26/136	18.7
	Spm	5784	17.8±0.009	15/115	21/121	15.3
	CHA	5295	7.1±0.003	5/38	10/24	24.2

** Ratio of cells at abnormal metaphase to total metaphase*
*** Ratio of cells at abnormal anaphase to total anaphase*

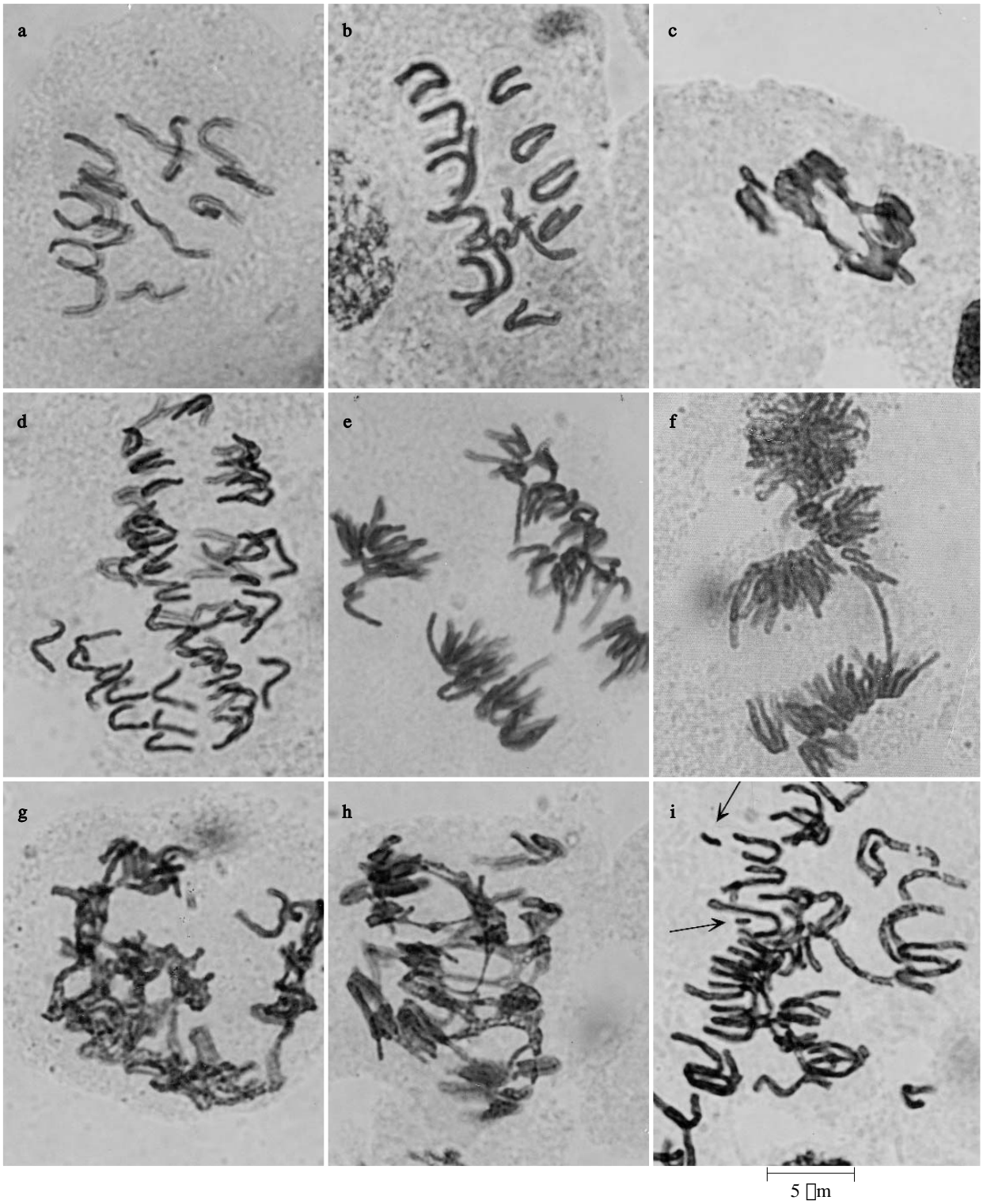

Figure 2: Mitotic abnormalities in the root tip cells treated with Spd, Spm and CHA of diploid tetraploid and hexaploid wheats. a,b: Abnormal metaphase in diploids; c: Abnormal anaphase in diploids; d-f: Abnormal anaphase in tetraploids; g-h: Stickness of chromosomas in hexaploids; i: Abnormal metaphase in hexaploids. Arrow points B chromosomes.

Any morphological change was not observed by Spd, Spm and CHA treatments. Primary root lengths were measured after Spd, Spm and CHA inhibited seed germination up to 60 h. Root elongation in diploid, tetraploid and hexaploid wheats (Fig 1). Root lengths were reduced by 51.6% and 46.1% with Spd and Spm treatments respectively in diploids after 60 hours. Spd and Spm applications also caused by 28.6% and 5.5% decrements in tetraploids and by 25.7% and 22.4% in hexaploids respectively.

Mitotic index was summarized in Table 2. Mitotic index reflects frequency of cell divisions and it is an important parameter to determine the rate of root growth. Mitotic index was almost the same in the control groups of wheats at different ploidy levels and it was determined to be 18.6% in *T.monococcum*, 19.2% and 18.5% in *T.aestivum* (Table 2). Spd decreased the division frequency from 18.6 to 13.4 in the root tips of diploid wheat but no significant difference was observed in mitotic index at Spd treated root tips of tetraploid and hexaploid wheats. Spm decreased the division frequency from 18.6 to 12.3 in the root tips of diploid wheat. This value was also reduced to 17.8 in tetraploids and to 17.6 in hexaploids. CHA, a biosynthetic inhibitor of Spd and Spm, showed a drastic reduction in the mitotic index of the three species studied; to 9.1 in diploids, to 10.6 in tetraploids and to 7.1 in hexaploids. The results show that the root tip meristems of diploid wheats are more sensitive to PAs and to their inhibitor in respect to division frequency.

Mitotic abnormalities like unoriented metaphase, anaphase or telophase bridges, unequal anaphase separation, laggards, fragments, stickiness, multipolar spindle were observed in the control groups of three species. Spd, Spm and their biosynthetic inhibitor, CHA did not cause a considerable change on the shape of the cells and also in their cytoplasm but they affected the percentage of mitotic abnormality (Fig. 2) results were presented in the Table 2. The lowest percentage of chromosome aberration in the control existed in diploids, and the highest percentage in hexaploids. Although the percentage of aberration was highly increased in diploids by Spd and CHA, in tetraploids by CHA, whereas it was reduced in hexaploids by all treatments.

Discussion

PAs are activators of protein synthesis occurring in early phase of germination (Villanueva and Huang, 1993; Anguillesi et al., 1982). Spd and Spm inhibited seed germination in *Arabidopsis thaliana* but they had no effect on root growth whereas Put at low concentration stimulated root growth. This suggested that endogenous concentration of Put in the roots of *A. thaliana* could be growth limiting (De Agazio et al., 1995). In this paper, Spd and Spm inhibited seed germination in three wheat species. CHA also inhibited seed germination in 2X and 4X species but slightly increased in 6X species. Spd, Spm, inhibited Root growth and CHA in three species studied. Comparison of root length with mitotic index revealed close correlation for the investigated species.

Some inhibitory effect of exogenously applied PA in various cell functions has been described (Gatta et al., 1992; De Agazio et al., 1995). De Agazio et al. (1992) reported that Spd pretreatment induced 50% inhibition of root extension in intact maize seedlings after 24 h. They concluded the inhibition of root growth of maize seedlings is a complex phenomenon due to reduction of both the mitotic index and cell elongation accompanied by stiffening and lignification of cell wall (De Agazio et al., 1995). Although much evidence supports the involvement of PA in various growth and developmental processes in higher plants it is not completely clear whether PA per se, compounds made from PA, or their catabolites are responsible for some of the observed effects. Therefore, the possibility exists that its degradation products mediate the inhibitory effects of Spd in the inhibition of maize root growth (de Agazio et al., 1995).

Bharti and Rajam (1995) investigated the effect of DFMO on growth, PA levels and chromosome behavior in *Triticum aestivum* L cv. Agra. They sprayed 5 days old wheat seedlings with DFMO (0.1, 1.5 and 10 mM) and they calculated the mitotic index. They established no significant effect on seedling height, root length, number of roots in seedlings treated with DFMO at any concentration tested (0.1-5 mM). 10 mM DFMO treatment resulted in a marked decrease in plant height, root length and number of roots. They observed some mitotic alterations like unoriented chromosomes at metaphase and anaphase upon treatment with 10 mM DFMO, however they established no significant difference in mitotic index.

These researchers suggested that DFMO could be safely used as a protecting agent against the fungal diseases without affecting the host plant.

In our previous study it was established an inhibition in mitotic index by applying 10 mM DFMO in three wheat species differing from the results of Bharti and Rajam (1995).

Sauve et al. (1999) determined that PAs play some role in chromosome condensation in human breast cancer cells (MCF-7) and they also found more Spd content in interphase cells than in mitotic cells. These findings are agreed with the studies that PAs are almost entirely cytoplasmic during interphase but associate with chromosomes in mitosis (Hougaard et al., 1987; Hougaard, 1992). The close relation between PAs and chromosomes elucidates the chromosomal abnormality observed in PAs treated root tip cells of wheat.

In conclusion, The PAs, Spd and Spm and their biosynthetic inhibitor, CHA caused a reduction in root growth resulted from the reduction of mitotic index and mitotic irregularities in the root tips of diploid, tetraploid and hexaploid wheats and diploid species is more sensitive to Spd and Spm and CHA than the others.

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