*Korean J. Environ. Agric.*
*Vol. 14, No. 1, April 1995*

# 식물체 내에서 Strontium의 대사 : 밀(*Triticum aestivum* L.)의 발아과정 중 Polyamine 생합성에 미치는 Strontium의 영향

김태완 · 게오르그 하인리히

# Strontium Metabolism in Higher Plants: Effect of Strontium on the Polyamine Biosynthesis during Germination of Wheat(*Triticum aestivum* L.)

Tae-Wan Kim and Georg Heinrich

## Abstract

Wheat (*Triticum aestivum* L.) seeds were used to study a possible relationship between strontium and polyamines (PAs) in the coleoptile, root, and endosperm during germination. When $Sr^{2+}$ (0.001 mM + 10 mM) was applied to the incubation medium with 10 μM $GA_3$, great increases in putrescine (Put) were observed in root and spermidine (Spd) in the coleoptile, depending on the concentration. In germinating seeds, putrescine accumulation was induced even at a low concentration (0.01 mM Sr), whereas spermidine accumulation was stimulated considerably at a high concentration (10 mM Sr). The putrescine levels, on a gram fresh weight (g-fr-wt) basis, in the roots which were growth-inhibited by 1 and 10 mM $Sr^{2+}$ were 22.4 and 15.3 fold higher respectively than at the same concentrations of $Ca^{2+}$. The accumulation of total polyamine (TA), in particular Put and Spd, induced by Sr seemed to be an important physiological response not only on a g fr wt basis but also on an RNA basis. In contrast, the levels of agmatine (Agm) and cadaverine (Cad) were notably enhanced by 10 mM $Ca^{2+}$ in the coleoptile and root. Cadaverine was detected only in $Ca^{2+}$-treated seedlings. However, $Ca^{2+}$-treatment in the range of 0.001 mM to 1.0 mM resulted in reduction of TA content.

The distinction of accumulated polyamines and the change in diamine (DA) / TA and tri- and tertiary (tPA) / TA ratios were likely to be a physiological difference between $Sr^{2+}$ and $Ca^{2+}$ during germination in wheat.

Key words: Strontium-polyamine-*Triticum aestivum*-germination-Calcium-RNA

오스트리아 그라쯔대학 식물생리연구소 (Institut für Pflanzenphysiologie, Karl-Franzens Universität Graz, Schubertstrasse 51, A-8010 Graz, Austria

## Introduction

The diamines putrescine and cadaverine and the polyamines spermidine and spermine (Spm) are widely distributed in bacteria, animals and plants[3,23,24,28]. Plant amines play an important role in plant growth, correlated with the action of plant regulators, *i.e.* IAA, GA, cytokinine, ethylene and ABA. Although the study of metabolic changes on polyamines associated with cell growth and division has been well-characterized in higher plant systems under various conditions, it is still unclear how PAs can physiologically act on the Ca-modulated cellular reaction. It has been demonstrated that PAs appear to substitute at least in part for the physiological responses under Ca-regulation such as protoplast stabilization, pollen germination, preservation of chlorophyll during senescence, phosphorylation of several membrane proteins and so on. Under a wide range of stress conditions such as potassium deficiency, low pH, cadmium treatment, osmotic stress, chilling injury and ozone stress[17,26,29,32,34,35], PA accumulation is also induced. Especially, the level of Put seems to increase in response to many of the plant stress conditions tested. The induction of Put has been thought to be a protective response against stress. It has been found that the induction of PA synthesis does have a connection with RNA synthesis, activation of RNA polymerase[7] and stabilization of DNA and RNA during replication[14,19,20].

Although Sr closely resembles Ca in a wide range of chemical characteristics[8,16], Sr cannot be substituted for Ca in all physiological processes. To investigate what their physiological differences are is of major interest.

This is the first study to report changes in polyamine levels during the germination of wheat seed in the presence of strontium. The effect of Sr on polyamine accumulation and the possible relationship between the change in polyamine levels and the nucleic acid synthesis have been observed.

## Materials and Methods

**Chemicals-** Put- and Spd-free base were purchased from Sigma, and Cad-dihydrochloride and Agm-sulfate from Serva. Spm tetrahydrochloride (Sigma, molecular biology grade) was a gift from Dr. H. W. Pfeifhofer, Univ. Graz. Diphenylamine and benzoylchloride were obtained from Merck.

**Germination-** Wheat seeds (*Triticum aestivum* L. gift from the Botanical Garden, Univ. Graz) were sterilized in 1% sodium hypochlorite for 10 min and then after washing several times with sterile re-distilled water, immediately transferred into media (30 μM chloramphenicol, 10 μM $GA_3$, 1 mM acetate buffer, pH 5.0) containing a appropriated $SrCl_2$ or $CaCl_2$ under aseptic condition at 22°C. Twenty washed seeds were incubated in the dark for 4 days in autoclaved petridishes containing 7 ml of each media, placing embryos on the upper side. Coleoptiles, roots and endosperms were dissected with an aseptic razor blade (if necessary, done under a light microscope, AO instrument Co. Mod 570).

**Diamine and polyamine isolation, and HPLC analysis-** Benzoylation of polyamine standards and plant tissue extracts were carried out according to earlier methods with slight modifications[5,18]. Briefly, tissues were soaked in 5% $HClO_4$ (v/v) at a ratio of 50 mg fr wt/ml overnight for endosperms and ground at 4°C. Coleoptiles and roots were used directly after soaking for 6 hr.

After standing for 1 hr on an ice bath, samples were pellets through centrifugation (48,000 × g) for 20 min. Supernatants and pellets were used for benzoylation of polyamines and for isolation of DNA and RNA, respectively. $HClO_4$ extracts (500 μl) were added to 1 ml of 2 N NaOH in centrifuge tubes and incubated for 20 min at room temperature. After adding of 20 μl benzoylchloride-HCl, vortexing for 20 sec, and standing for 30 min at room temperature, 2 ml saturated NaCl was added. The benzoylated amines were extracted in 2 ml ice-cold diethylether through vigorous shaking. After 1,500 x g centrifugation, 1 ml of the upper ether phase was collected, evaporated under the air stream of a hair dryer (not warm air), redissolved in 200 μl absolute methanol and then stored at −80°C until analysis (not over 1 week). The HPLC column was a reversed phase Spherisorb-ODS2 C18 (particle size 5.0 μm; 25 cm × 4.5 mm, Knauer). Elution (20 μl loading volume) was isocratically performed in 64/32 methanol/water (v/v) for 20 min.

Eluates from the column were detected at 254 nm. The retention times of Put, Cad, Spd, Agm and Spm were 4.2, 4.5, 6.3, 8.2, and 10.2 min, respectively. Detection limit was 1 nmol per g fr wt.

**Isolation of RNA and DNA-** Nucleic acids were extracted by earlier method[24] with the exception that the $HClO_4$ pellets were washed 3 times before precipitation in ethanol saturated with sodium acetate. If the ratio of absorbance at 260 nm : 280 nm was less than 1.75, the isolation procedure of RNA was repeated with the $HClO_4$ extracts (not diluted). Calf thymus DNA (Roth) was used as a standard.

All experiments were performed in duplicate and measured in triplicate. The mean values were expressed.

## Results

**The effect of Sr and Ca on the accumulation of polyamines-**

As shown in Fig. 1B and 1C, the Put level was considerably enhanced in roots grown in the medium containing high concentrations (1.0−10 mM) of Sr and in endosperms grown in the medium containing low concentrations (0.01−0.1 mM) of Sr. Although Put was not detected in untreated coleoptiles (Fig. 1A) and the levels of Put were highest at 0.01 mM Ca and Sr, it is difficult to evaluate a trend on the basis of g fr wt. In roots, which were markedly growth-retarded by 10 mM Sr treatment, the Put level was 11 fold higher than in the untreated control. In our experimental procedure, it was impossible to completely isolate the coleoptiles and the basipetal root cells from the embryonic tissue attached to the starchy endosperm. Taking that into account, the increase of Put in the endosperm in the range of 0.01 and 0.1 mM might be overestimated through a contamination by the residual part (particularly basipetal roots). However, a large (9-fold) increase in the endosperm is indisputable to interpret that Sr has affected the Put synthesis in the embryonic tissue or aleurone layer. As shown in Fig. 2C, the Spd levels per g fr wt in the endosperms were slightly decreased in the range of 0.001 to 1.0 mM of both Ca and Sr and increased 2.5 fold only in the presence of 10 mM Sr. If endosperms were contaminated, it might be expected that Spd would also be accumulated in the mitotic basipetal root, as observed by Mukhopadhyay *et al.*[15]. However, Spd was rather low in

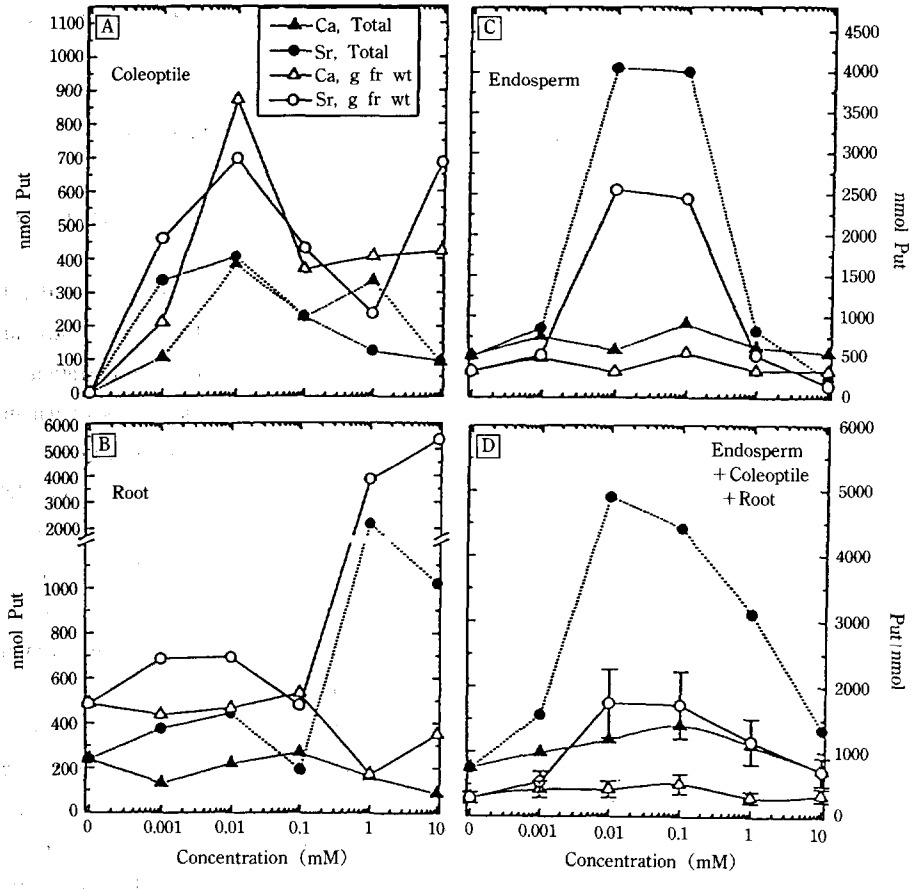


**Fig. 1. Changes in Put level in: coleoptile (A), root (B), endosperm (C), and the sum (D) of coleoptile, root and endosperm. Dotted lines represent the sum of each parts from 20 seedlings (Ca; triangle, Sr; circle symbol). Vertical bars of D represent standard deviation (n=6) on a g fr wt basis of the sum of A, B, and C.**

the range of Put increase. On the other hand, the Spd accumulation in the roots was induced in a wide range (0.1 to 10 mM) of Sr concentrations, but the increase on the nmol level was relatively minimal compared to the μmol level in coleoptile and endosperm. Therefore, the total Spd content in 20 seedlings increased only in the presence of 10 mM Sr. It is interesting that tht Put in the root and the Spd content in the coleoptile increased up to a level of μmol/g fr wt through a treatment of 10 mM Sr. At 10 mM Ca, Spd and Put syntheses were not induced, while Agm and Cad had notably accumulated in the coleoptile and roots (Fig. 3A, 3B, and Table 1). When wheat seeds were germinated in media containing Sr, Cad was not detected in all parts of the seedlings. In the coleoptiles of the untreated control, where Put was not detectable, the Cad level was 2.64 μmol/g fr wt (Table 1). These results may mean that the lack of Put and Spd causes the sy-

**Table 1. Effect of Ca on Cad levels (nmol Cad/g fr wt).**

| Conc of Ca (mM) | Coleoptile | Root | Endosperm | Total[a] |
|---|---|---|---|---|
| 0 | 2640± 319[b] | – | – | 528± 88 |
| 0.001 | –[c] | – | – | – |
| 0.01 | 348± 55 | – | 214± 39 | 199± 22 |
| 0.1 | 300± 57 | 396± 88 | – | 138± 29 |
| 1.0 | – | – | 5± 2 | 2.5± 1 |
| 10.0 | 1664± 265 | 2388± 334 | – | 452± 67 |

* The values are presented as the mean± SD of 2 independent experiments(n=6)

a : mean of nmol/g fr wt in the total of the coleoptile, root and endosperm,

b : standard deviation,

c : not detected.

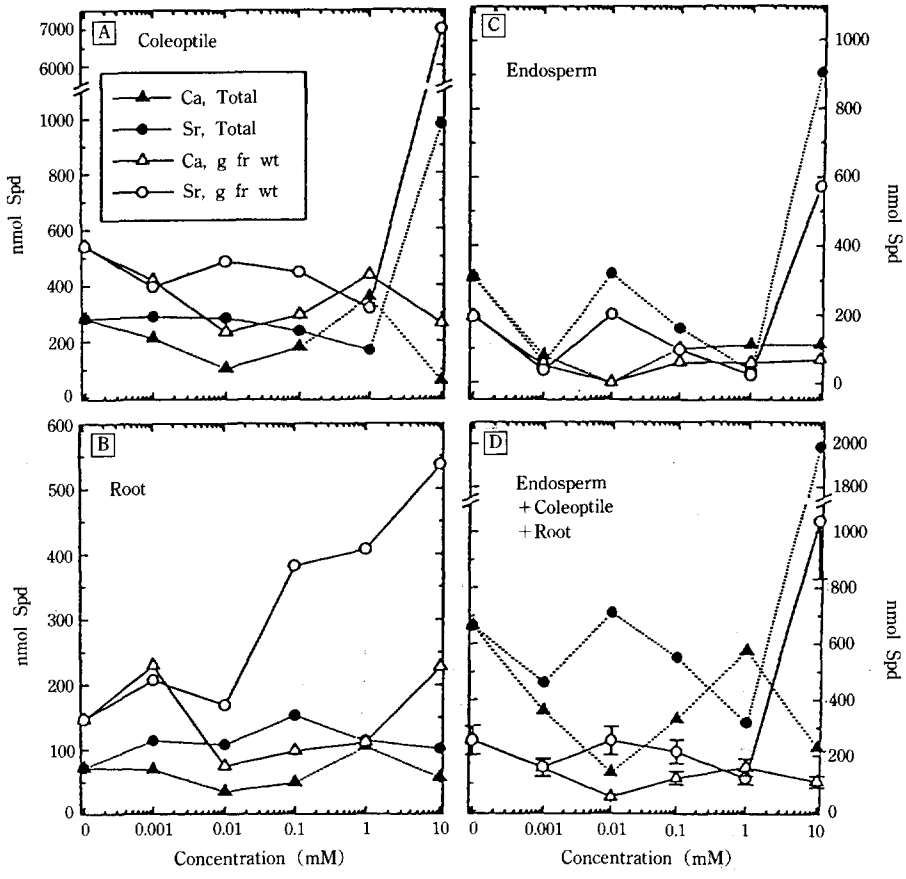


**Fig. 2. Changes in Spd level in: coleoptile (A), root (B), endosperm (C), and the sum (D) of coleoptile, root, and endosperm (see legend of Fig. 1).**

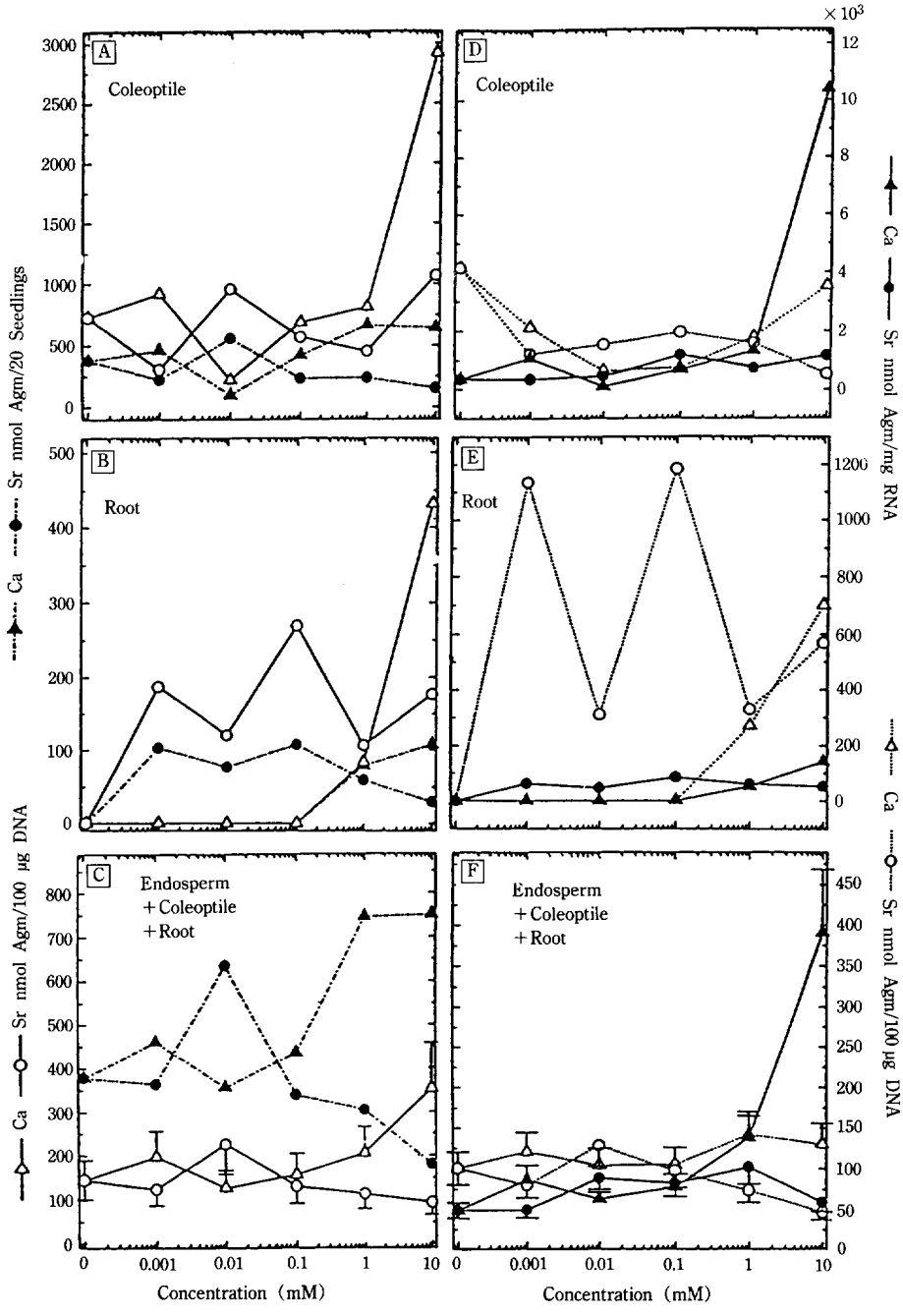


Fig. 3. Changes in Agm level in: coleoptile (A), root (B), and the sum (C) of coleoptile, root, and endosperm (see legend of Fig. 1). Agm levels in endosperms did not exceed 8 nmol except for 0.01 mM Ca (77.1 nmol). D, E, and F show nmol Agm/mg RNA (left axis) and Agm/100 μg DNA (right axis) in: coleoptile (D), root (E), and the sum (F) of coleoptile, root, and endosperm.

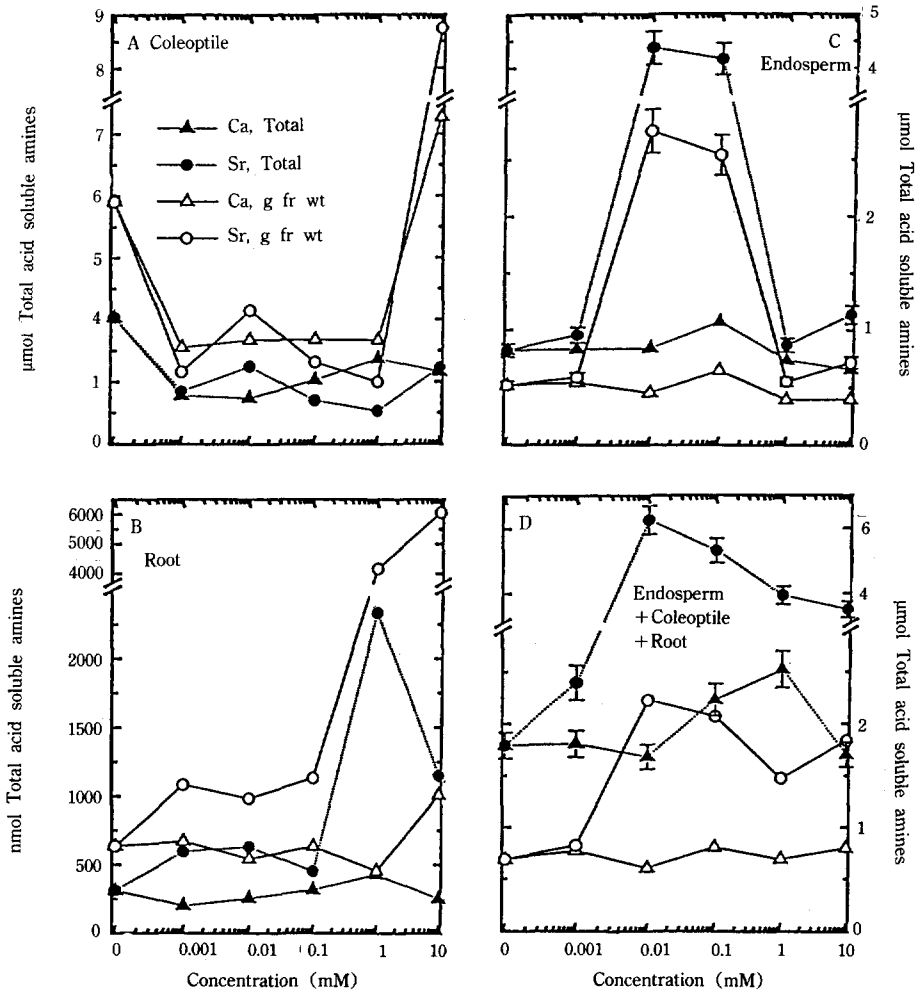


**Fig. 4. Changes in total amine content per g fr wt (solid line) and per 20 seedlings (dotted line) in coleoptile (A), root (B), endosperm (C), and the sum (D) of coleoptile, root, and endosperm.**

nthesis of other DA and PA, Cad and Agm.

Acid soluble Spm was not detected in this work with the exception of a small amount (<20−80 nmol/g fr wt) in seedlings incubated in 0.1 and 1.0 mM Ca (data not shown). It is presumed that most of it is present as a bound form.

As shown in Fig. 4, the level of TA on a basis of g fr wt was increased by Sr. In particular, the TA levels has notably increased in the root. In the range of 0.001 to 0.1 mM, TA levels induced by Sr were about 2 fold higher than those induced by Ca, and at the 1.0 and 10 mM Sr concentrations, 6.5 and 9.5 fold higher than in untreated control (Fig. 4B). However, TA content in the coleoptiles was about 2 fold decreased in the range of 0.001 to 1.0 mM of Ca or Sr and increa-

sed only in 10 mM. Both Ca and Sr do not have a harmful effect at concentrations between 0.001 and 1.0 mM and 0.001 to 0.1 mM, respectively. As shown in Fig. 4D, the TA level was increased even at the low Sr concentration of 0.01 mM. It can be concluded that Sr does induce an accumulation of polyamines on a basis of g fr wt. It may paradoxically have to be assumed that plants are not in need of amine synthesis during germination in the presence of Ca, suggesting that Ca can be substituted, at least in part, for the physiological roles of DAs and PAs. It is also interesting that DA (Put + Cad) and PA (Agm + Spd + Spm) are accumulated in different parts, *i.e.* roots and coleoptiles, respectively (Fig. 5A and 5B). Fig. 5C shows that the increase of TA in the endosperm (0.01 and 0.1 mM Sr) results from DA (especially Put) accumulation (see also Fig. 1C and 4C).

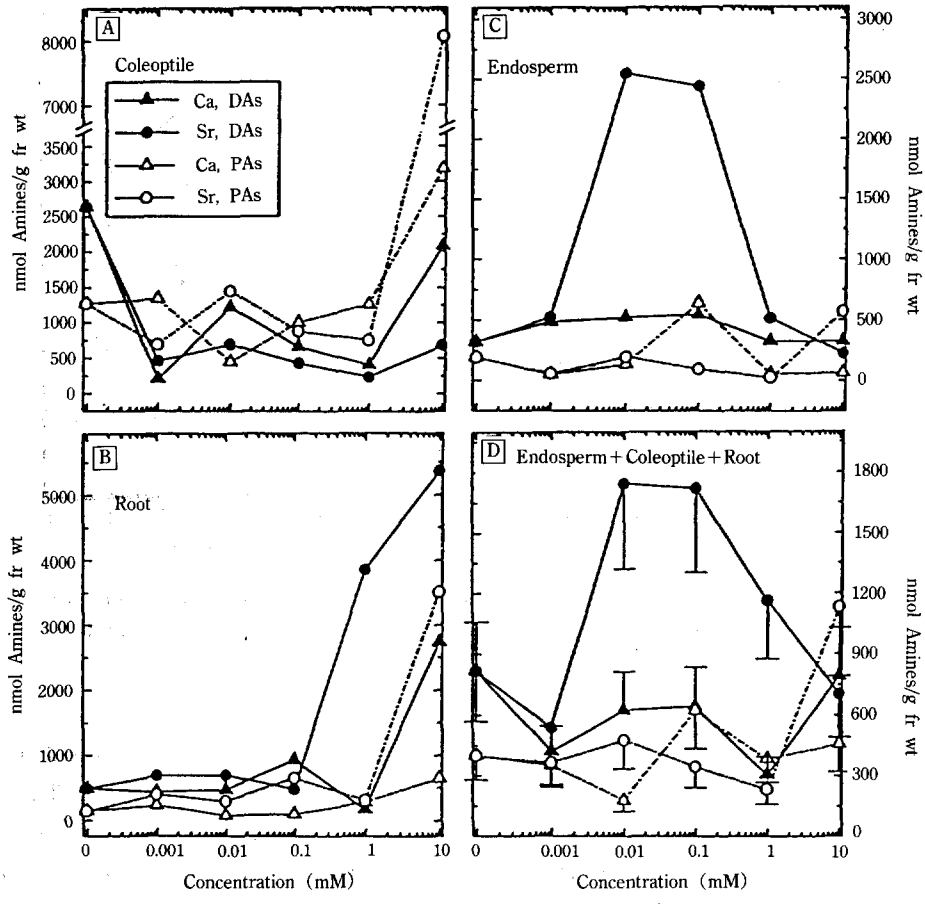


**Fig. 5. Effect of Ca (triangle) and Sr (circle) on diamine (Cad + Put) and polyamine (Agm + Spd + Spm) content in coleoptile (A), root (B), endosperm (C), and the sum (D) of coleoptile, root, and endosperm. Solid line and striked line express diamines and polyamines, respectively.**

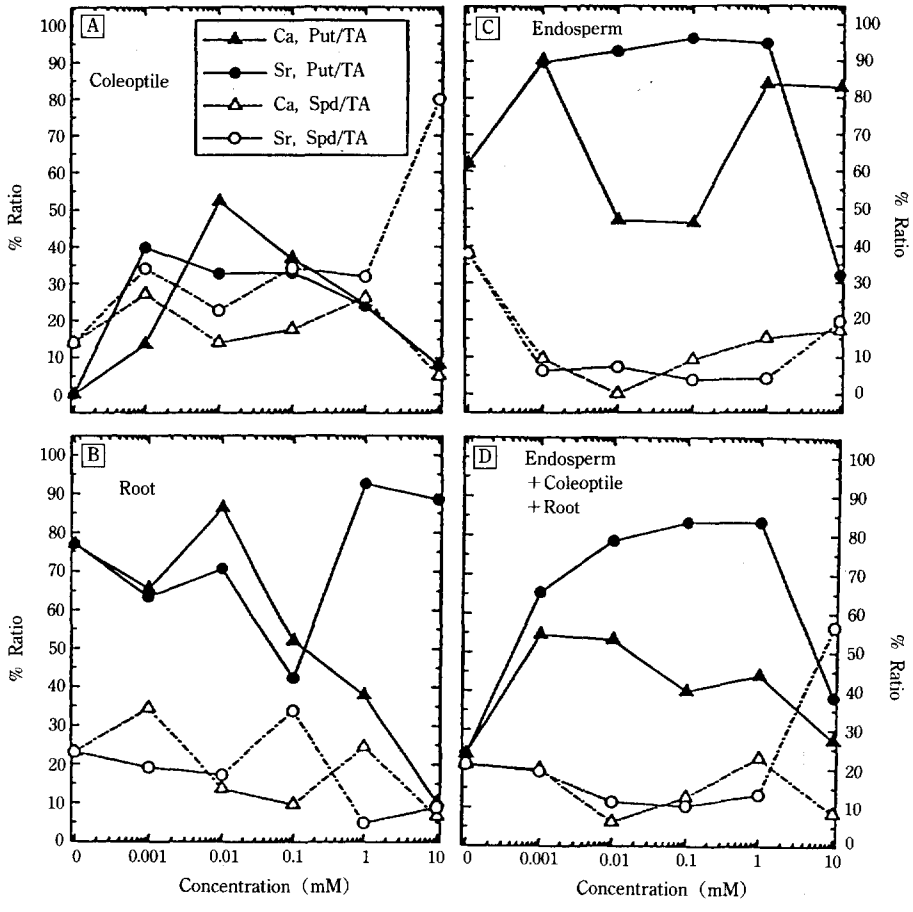


**Fig. 6. Changes in ratio of Put/TA (solid line) and Spd/TA (striked line).**

**Ratios of Put and Spd to TA-**

Fig. 6A shows the changes in the ratio of Put and Spd to TA in the coleoptiles. The ratios of Put/TA decreased at least in the range of 0.01 mM to 10 mM both of Ca and Sr. However, although the Put/TA ratios in Sr treated coleoptiles were higher than in Ca treated ones, the changes in the ratio of Spd to TA were not distinguishable in the range of 0.001 mM to 1.0 mM, whereas the Spd/TA ratio was equal to 80 percent in the presence of 10 mM Sr.

As shown in Fig. 7B, when germinated at a concentration of 1.0 and 10 mM Sr, the ratios of Put to TA were dramatically increased in the roots. In the presence of Ca, the ratios decreased slightly with concentrations in the range of 0.01 mM to 10 mM as seen in the coleoptiles (see also Fig. 6A). Taking into account that Sr is stress inducible in 1 mM and 10 mM as previously indicated, the increase of the Put/TA ratio may be closely correlated with growth retardation through stress. It was observed that the growth of the coleoptile and the first leaf in the presence of 10 mM Sr recovered somewhat with incubation

time (5 to 10 days after germination), whereas the root growth never recovered (data not shown). Therefore, we would like to assume that the recovering effect may be caused by a difference between the ratio of Put to TA in the root and the coleoptile. Although Put accumulation is not induced by Ca in the coleoptile and the root, what is of interest is that the ratios of Put to TA show a considerable decline at concentrations of 0.1 mM and 1.0 mM Ca in the range in which Agm increased (Fig. 4).

In endosperms, Put/TA ratios were almost constant at a level of 90 percent in Sr concentrations of 0.001 mM to 1.0 mM (Fig. 6C). However, the ratio was notably reduced at 10 mM Sr. It was also observed that the ratio of Spd to TA was highest (about 38 percent) in the untreated control. At 0.01 mM and 0.1 mM Ca, the ratios of Put to TA were 47 and 46 percent, respectively. At the same concentrations of Sr, the levels of Put/g fr wt are interestingly highest.

As shown in Fig. 6D, when Put/TA ratios were expressed as the sum of all parts (on a g fr wt basis), they increased markedly in the range of 0.001 to 1.0 mM of Sr. At 10 mM Sr, however, Spd/TA ratios were higher than Put/TA. This could have resulted from the conversion of Put to Spd or the delayed mitotic activity through growth retardation in the presence of high Sr concentrations (see discussion).

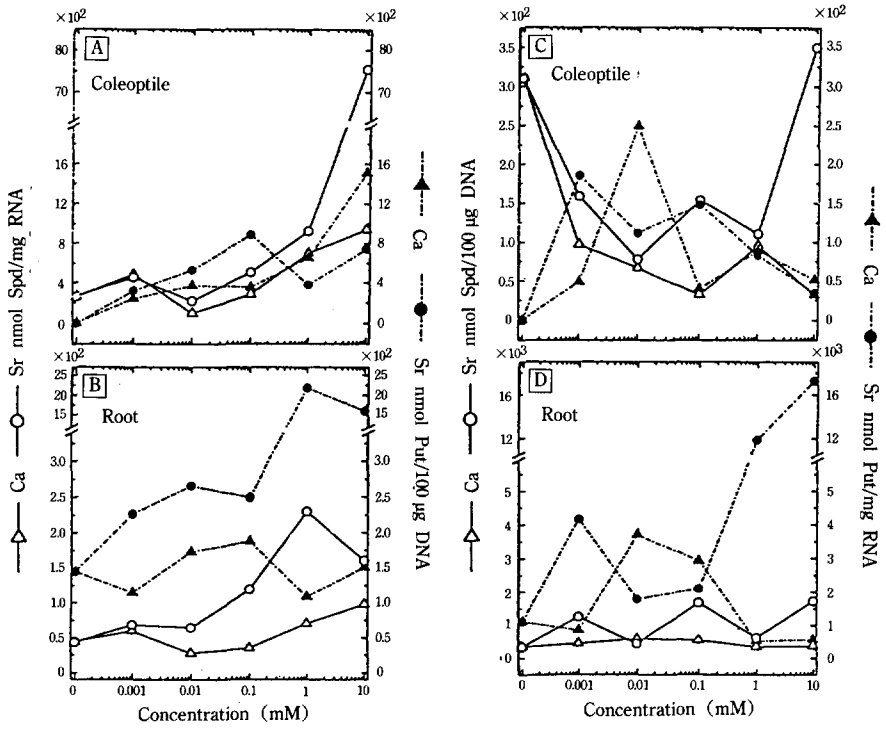


**Fig. 7. Changes in Spd (left axis) and Put (right axis) in coleoptile (A, C) and root (B, D) on the basis of RNA (A, B) and DNA (C, D).**

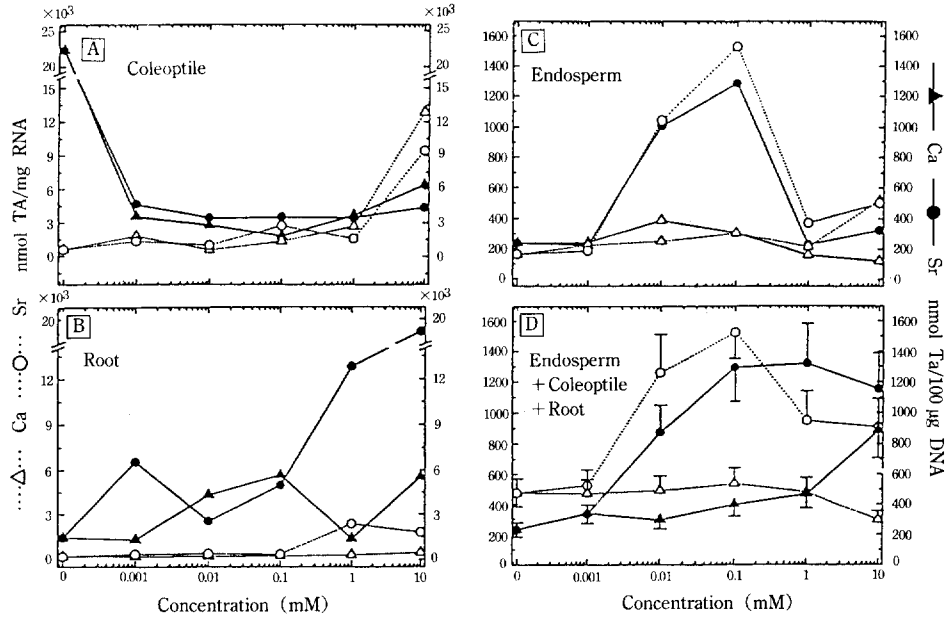


**Fig. 8. Changes in total amine level in coleoptile (A), root (B), endosperm (C) and the sum (D) of coleoptile, root, and endosperm on the basis of RNA (dotted line) and DNA (solid line).**

**Relationship between polyamine synthesis and nucleic acids-**

On a nucleic acid (DNA and RNA) basis, TA accumulation was remarkably induced by Sr (Fig. 8D). In coleotiles, RNA synthesis increased notably at 0.01 mM Sr and Ca (data not shown). Therefore, on a RNA basis, the increase in Put and Agm represents an easement curve in low concentrations (Fig. 3D and 8A). This increase may be evidence of a relationship between Put (or Agm) and RNA synthesis. Exogenous 10 μM Ca is near the intracellular Ca concentration. In this study, Put- and Spd contents seem to be more closely correlated with RNA than DNA (Fig. 7A and 7B). In coleoptiles, the Spd level (7.5 μmol) on a basis of RNA at 10 mM Sr was 29 and 8 fold higher than in the untreated control and at 10 mM Ca, respectively, while the Put level (1.5 μmol) at 10 mM Ca was 2 fold higher than at 10 mM Sr, resulting from a low RNA content per g fr wt in the presence of 10 mM Ca (data not shown). On a basis of RNA, the accumulation of Agm induced by 10 mM Ca (9 fold) was also more profound than on a g fr wt basis (3 fold) in coleoptile (Fig. 3D). Fig. 7B shows the increase of Spd and Put levels in the root such as expressed in g fr wt (Fig. 1B and 2B). In particular, the levels of Put/mg RNA at 1 and 10 mM Sr were 20 and 11 fold higher than at same concentrations of Ca, respectively. In the root, however, it was so inconsistent that a trend of Spd on a DNA basis could not be predicted unlike for Put (Fig. 7C). Put and Spd accumulation in the root induced by Sr seemed to be an important physiological response not only on the basis of g fr wt but also on the basis of RNA.

The changes in DNA did not correlate well to changes in the content of Agm (Fig. 3). In the coleoptiles, TA levels in terms of DNA did not exceed the levels in the untreated control at all concentrations (Fig. 8A), whereas TA on a basis of RNA increased at 10 mM Ca and Sr. In the roots, an increase in TA on a RNA basis did not occur through Ca, and the change in TA through Sr was not also remarkable (1−1.5 fold) in the range between 0.001 mM and 0.1 mM. In fact, Ca or Sr concentrations between 0.001 and 0.1 mM were not inhibitory to coleoptile and root growth. But TA content at 1.0 and 10 mM Sr was 12.5 and 9.6 fold higher than in the untreated control, respectively. Fig. 8D shows that the TA accumulation per 20 seedlings (the sum of all parts; coleoptile, root, and endosperm) on a RNA basis was induced at 0.01 mM Sr and maximized at 0.1 mM Sr. Increases in TA at 0.01 and 0.1 mM were due to the increases in the endosperms as occurred on a DNA basis (Fig. 8C and 8D). In conclusion, it is suggested that polyamine accumulation through Sr is also intimately related with RNA biosynthesis (in particular between Put and RNA in roots and Spd and RNA in coleoptiles).

## Discussion

Although there was much evidence of a physiological similarity between Sr and Ca[8,12,16,21], it is still not clear in which processes Sr plays a different role than Ca. Sugar beet, a calcicole plant, can survive over 20 weeks in a Hoagland's solution containing 10 mM Sr instead of Ca, showing markedly gowth-retardation and inhibition of reproductive growth (unpublished data). At least some intracellular reactions related with calmodulin, to which Sr can also bind, were almost similar to Ca in some higher plants (data in preparation) . Acid-induced wall loosening may also be another possible explanation related to the inhibition or retardation of cell elongation in roots. Namely, Sr may be more effective in binding to cell walls than Ca[16,21], resulting in a decrease in $H^+$-induced wall loosening according to the auxin regulated acid growth theory. But the longitudinal growth of the roots did not recover strongly even in the presence of 10 μM IAA instead of $GA_3$. Hence, the inhibition of root elongation can not be explained only with the acid growth theory. Actually, the growth recovery of the roots was rather more remarkable in the same IAA medium with 50 μM ruthenium red, suggesting that the inhibitory effect of Sr may occur in mitochondrial processes after an ATP-dependent transport via the plasma membrane[9]. In addition, the fact that DAs and PAs can be protonated at the physiological pH[36] suggests that the increase of polyamines may result in scavenging protons which loosen the cell wall.

When germinated in the range of 0.001 mM to 1.0 mM Ca or Sr, the levels of DAs and PAs were decreased in coleoptile where the intracellular concentration of Ca or Sr could be in a relatively better condition to maintain the optimal concentration than in the roots. Since roots are surrounded by exogenous media, even if it is difficult to estimate whether concentrations of Ca or Sr are in the optimal range or not, Ca or Sr may have a more direct effect than in coleoptiles. Therefore, polyamine synthesis may be, as a physiological protective response, not strongly needed in the coleoptiles. It should be mentioned that the previous reports on the substitutive effects of Ca on PAs were mainly carried out in a range of 1.0

to 10 mM Ca. This range is too high to evaluate an endogenous *in vivo* intracellular reaction.

It is also well known that ethylene biosynthesis is inhibited by polyamine, whereas the flux of SAM into polyamines is enhanced when ethylene biosynthesis rates are inhibited[27]. Moreover, Fuhrer *et al.*[6] reported that Ca (1 mM) competitively reduced the PA-mediated inhibition of the (1-amino cyclopropane-1-carboxylic acid) ACC conversion. We would like to postulate that if ethylene synthesis is not inhibited (slightly activated) by the treatment of high concentrations of Ca or Sr, SAM, as a methyldonor in Spd synthesis, may be able to continuously metabolize to ethylene at least in the roots as earlier observed by Lao and Yang[11]. Accordingly, the lack of Spd accumulation in the coleoptile and root induced by a high concentration of Ca (10 mM) may result from a decrease in the conversion of SAM to Spd. However, the accumulated intermediate, induced by 10 mM Ca, is interestingly not Put but Agm. Although this assumption may partially explain the roles of Sr and Ca in the ethylene and Spd synthesis, the Put accumulation at high concentrations (1 to 10 mM) of Sr is on the μmol level not only on a g fr wt basis but also on a mg RNA basis. On the other hand, Spd accumulation through Sr is already induced at a concentration of 0.1 mM, although the level is under a μmol. This amount of Sr is not stress-inducing concentration. This (Put accumulation and Spd induction at a low concentration) may be a difference between Ca and Sr. Again, Ca and Sr may have an effect on the increase (μmol) of the Agm and Put levels, respectively. The conversion of Agm to Put has been indicated as a fast reaction[13]. Therefore, although Sr may substitute Ca in the methionine cycle (methionine → SAM → ACC → $C_2H_4$) as shown in a previous observation[11], it may have to assume that Sr facilitates a more rapid conversion of Agm to Put than one of Put to Spd, at least in a growth stage during germination in a high concentration of Sr.

Also, as reported by Berlin and Forche[1] and Kyriakidis[10], if an increased ornithine decarboxylase activity (ODC, EC 4.1.1.17) and arginine decarboxylase (ADC, EC 4.1.1.19) are correlated with cell division and cell elongation, respectively, the accumulation of Agm in the roots and coleoptiles germinated in a medium containing 10 mM Ca may be associated with cell elongation or with a low conversion of Agm to Put via the putrescine synthase complex. By contrast, in the same parts germinated in the presence of 1.0 to 10 mM Sr, the increase of DA, Put, and Spd, may be a delayed increase in cell division activity. The biosynthesis of DA or PA is often greatest where cell division is most active[15]. It has been observed that a high ratio of Put/TA can be harmful in plant systems[22,23]. However, Cho[2] reported that the growth-enhancing effect in lettuce hypocotyls could be produced by Agm and Cad, as well as arginine and ornithine in the presence of 0.1 and 1.0 μM $GA_3$, and recovered only through Put combined with acrain (1,4-diguanidinobutane) which is an Agm iminohydrolase inhibitor, *i.e.* in the conversion of Agm to *N*-carbamyl-putrescine. Even though both Ca and Sr at 10 mM are stress inducing concentrations, ratios among polyamines were different. In particular, the ratio of Put/TA is obviously increased through Sr in the roots. The ratio of Spd/TA also increased considerably in the coleoptiles. However, to determine a correlation between the ratio changes and the Ca treatment was difficult. In which processes this difference occurs between Ca and Sr remains to be

clarified in future works.

Cad was detected in considerable amounts in the coleoptiles, as reported by Felix and Harr[4], whether it was Ca treated or not. In the present work, Cad accumulation in the roots and coleoptiles was eminently induced by the 10 mM Ca treatment. It is of interest that a Sr treatment does not induce Cad synthesis. These results may mean that the lack of Put and Spd caused by a Ca treatment demands physiologically that other DAs or PAs are synthesized.

For whatever reason, the inhibition of cell growth results eventually in a decrease in DNA, RNA, and protein synthesis. In the roots and endosperms, total RNA content per 20 seedlings was obviously reduced with Sr concentrations (data not shown). On a g fr wt basis, however, the RNA level is highest at 10 mM Sr, suggesting that cell elongation or expansion were markedly inhibited. The increase of Put and Spd in the roots may be closely related with the RNA synthesis as shown in the present work. It has been observed that the replication of DNA, with DNA polymerase, and the transcription to RNA, with RNA polymerase, are mediated by Mg and Mn[3,28]. The effect of similar metal ions, such as Be and Sr in high concentrations, on the pyrophosphate cleavage in the above replication reaction would be inhibitory[31,33]. Therefore, PA synthesis at high concentrations of Sr is putatively a protective response for nucleic acid stabilization. In view of the high concentration of both divalent cations (Ca and Sr) and PAs in plant cells, it may not be possible to decide what influenced the nucleic acids stabilization and synthesis more effectively. Additionally, it had been found that the Spd concentration increased 15-fold in the first 2 hr after imbibition of the wheat seeds[30]. The Spd level, even in the presence of Ca and Sr (10 mM), also increases before nucleic acid synthesis (unpublished data). Therefore, albeit it is clear that PAs are required for the stabilization of DNA or RNA, it is very difficult to explain which specific nuclear process is directly affected by the PAs in the presence of Sr. To investigate whether the induction of nucleic acid synthesis in an extremely high concentration (10 mM) of Sr indicates simply a delayed start of cell division through the retardation effect of Sr or not remains to next works.

## 요 약

소맥 종자의 발아 과정중 strontium의 생리적 작용을 규명하기 위해 초엽, 종근 및 배유에서의 free amine의 함량변화가 측정되었다. $GA_3$를 포함한 배양액에 strontium 농도의 증가는 종근에서의 putrescine과 초엽에서의 spermidine의 축적을 현저히 유발하였다. 10 μM 정도의 저농도에서도 strontium에 의한 putrescine 증가는 뚜렷한 반면, spermidine 증가는 10 mM의 고농도 처리에서 현저했다.

종근 생장을 억제하는 1mM 내지 10mM의 strontium 농도에서, g 생체중당 putrescine 수준은 동일농도 calcium 처리에서 보다 각각 22.4배와 15.3배 높았다. 특히 putrescine과 spermidine 증가에 의한 총 free amine 수준의 증가는 생체중 대비 뿐만이 아닌 RNA 함량 대비에서도 중요한 생리적 반응으로 보여졌다. Strontium과 대조적으로 고농도 calcium (10 mM) 처리는 초엽과 종근에서의 agmatine과 cadaverine 함량의 증가를 유발했다. Cadaverine은 calcium을 처리했을 경우에만 검출되었다. 그러나 1 μM에서 1 mM 수준의 calcium 처리는 총 free amine 함량의 감소를 가져왔다.

Strontium과 calcium 존재하에서의 발아과정중 축적되는 amine 종류의 차이와 총 free amine 중 diaminine 비율과 총 free amine 중 polyamine 비

율의 변화는 strontium과 calcium 간의 생리적 대사 반응의 차이중 하나로 판단되었다.

## Acknowledgments

We thank Dr. H. W. Pfeifhofer for his excellent technical advice and spermine gift, and Ms. L. Forsberg for her English proof-reading. This work was chosen as a project for a Ph. D. student (T. W. Kim) by the Universität Graz (Universitätverbund 1993) and supported in part by "Fonds zur Förderung der wissenschaftlichen Forschung" (FWF, Bioproject 8968) from the Austrian Ministry.

## References

1. Berlin, J. and Forche, E. (1981). DL-α-Difluoromethylornithine causes enlargement of cultured tobacco cells. Z. Pflanzenphysiol. **101** : 277−282.
2. Cho, S.-C. (1983). Enhancement by putrescine of gibberellin-induced elongation in hypocotyls of lettuce seedlings. Plant Cell Physiol, **24** : 305−308.
3. Evans, P. T. and Malmberg, R. L. (1989). Do polyamines have roles in plant development ? Annu. Rev. Plant Physiol. Plant Mol. Biol. **40** : 235−269.
4. Felix, H. and Harr, J. (1987). Association of polyamines to different parts of various plant species. Physiol. Plant. **71** : 245−250.
5. Flores, H. E. and Galston, A. W. (1982). Analysis of polyamines in higher plants by high performance liquid chromatography. Plant Physiol. **69** : 701−706.
6. Fuhrer, J., Kaur-Sawhney, R., Shih, L.-M. and Galston, A. W. (1982). Effects of exogenous 1,3-diaminopropane and spermidine on senescence of oat leaves. II. Inhibition of ethylene biosynthesis and possible mode of action. Plant Physiol. **70** : 1597−1600.
7. Guilfoyl, T. J. and Hanson, J. B. (1973). Increased activity of chromatin-bound ribonucleic acid polymerase from soybean hypocotyl with spermidine and high ionic strength. Plant Physiol. **51** : 1022−1025.
8. Hutchin, M. E. and Vaughan, B. E. (1968). Relation between simultaneous Ca and Sr transport rates in isolated segments of vetch, barley and pine roots. Plant physiol. **43** : 1913−1918.
9. Kim, T. W. and Heinrich, G. (1994). Calmodulin stimulation f $Sr^{2+}/nH^{+}$ antiport in vacuoles of red beet (*Beta vulgaris* L.) storage tissue. Biol. Plant. **36** : S−1525 (1994).
10. Kyriakidis, D. A. (1983). Effect of plant growth hormones and polyamines on ornithine decarbonylase activity during the germination of barley seeds. Physiol. Plant. **57** : 499−504.
11. Lao, O. L. and Yang, S. F. (1974). Stimulation of ethylene production in the mung bean hypocotyls by cupric ion, calcium ion, and kinetin. Plant Physiol. **57** : 88−92.
12. Lenihan, J. M. A., Loutit, J. F., and Martin, J. F. (1967). Strontium Metabolism, eds. Academic Press, New York.
13. Lin, P. P. C. (1984). Polyamine metabolism and its relation to response of the aleurone layers of barley seeds to gibberellic acid. Plant Physiol. **74** : 975−983.
14. Liquori, A. M., Constantino, L., Grescenzi, V., Elia, A., Giglio, E., Puliti, R., De Santis Savino, M. and Vitagliano, V. (1967). Complex between DNA and polyamines: a molecular model. J. Mol. Biol. **24** : 113−122.

15. Mukhopadhyay, A. and Choudhuri, M. M. Sen, K. and Ghosh, B. (1983). Changes in polyamines and related enzymes with loss of viability in rice seeds. Phytochemistry **22** : 1547-1551.
16. Myttenaere, C. (1964). (F) Effect of the strontium-calcium ratio on the localization of strontium and calcium in *Pisum sativum*. Physiol. Plant. **17** : 814-827.
17. Reddy, G. N., Arteca, R. N., Y.-R. Dai, Flores, H. E., Negm, F. B. and Pell, E. J. (1993). Changes in ethylene and polyamines in relation to mRNA levels of the large and small subunits of ribulose bisphosphate carboxylase /oxygenase in ozone-stressed potato foliage. Plant Cell Environ. **16** : 819-826.
18. Redmond, J. W. and Tseng, A. (1979). High pressure liquid chromatographic determination of putrescine, cadaverine, spermidine and spermine. J Chromatogr. **170** : 479-481.
19. Sasaki, I., Dufour, M. N. and Gaudemer, A. (1982). Interaction between nucleic acids and metal complexes. 1. Synthesis of polyamines and polyamines derived from uracil. J. Chim **6** : 341-344.
20. Seyfried, C. E. and Morris, D. R. (1979). Relationship between inhibition of polyamine biosynthesis and DNA replication in inactivated lymphocytes. Cancer Res. **39** : 4861-4867.
21. Simon, W., Morf, W. E. and Meier, P. C.,. (1973). Specificity for alkali and alkaline earth cations of synthetic and natural organic complexing agents in membrane. Structure and Bonding **16** : 113-160.
22. Slocum, R. D., Kaur-Sawhney, R., and Galston, A. W. (1984). The physiology and biochemistry of polyamines in plants. Arch. Bioch. Biophys. **235** : 283-303.
23. Smith, T. A. (1985). Polyamines. Annu. Rev. Plant Physiol. **36** : 117-143.
24. Smith, T. A. and Best, G. R. (1977). Polyamines in barley seedlings. Phytochemistry **16** : 841-843.
25. Smith, T. A., Best, G. R., Abbott, A. J. and Clements, E. D. (1978). Polyamines in Paul's Scarlet rose suspension cultures. Planta **144** : 63-68.
26. Stroinski, A. and Szczotka, Z. (1989). Effect of cadmium and *Phytophthora infestans* on polyamine levels in potato leves. Physiol. Plant. **77** : 244-246.
27. Suttle, J. C. (1981). Effect of polyamines on ethylene production. Phytochemistry **20** : 1477-1488.
28. Tabor, C. W. and Tabor, H. (1984). Polyamines. Ann. Rev. biochem. **53** : 749-790.
29. Tiburicio, A. F., Masdeu, M. A., Dumortier, F. M. and Galston, A. W. (1986). Polyamine metabolism and osmotic stress. I. Relation to protoplast viability. Physiol. Plant. **82** : 369-374.
30. Villanueva, V. R., R. G. Adlakha and A. M. Cantera-Soler (1978). Changes in polyamine concentration during seed germination. Phytochemistry **17** : 1245-1249.
31. Wacker, W. E. C. and Vallee, B. L. (1959). Nucleic acids and metals I, Cr, Mn, Ni, Fe and other metals in ribonucleic acid from diverse biological sources. J. Biol. Chem. **234** : 3257-3262.
32. Wang, S. Y. (1987). Changes of polyamines and ethylene in cucumber seedlings in response to chilling stress. Physiol. Plant. **69** : 253-257.
33. witschi, H. P. and Aldridge, W. N. (1968).

Uptake, distribution and binding of beryllium to organelles of the rat liver. Biochem. J. **106** : 811－817.

34. Young, N. D. and Galston, A. W. (1983). Putrescine and acid stress. Induction of arginine decarboxylase activity and putrescine accumulation by low pH. Plant Physiol. **71** : 767－771.
35. Young, N. D. and Galston, A. W. (1984). Physiological control of arginine decarboxylase activity in K-deficient oat shoots. Plant Physiol. **76** : 331－335.
36. Young, N. H. and Kauss, H. (1983). Release of calcium from suspension-cultured *Glycine max* cells by chitosan, other polycations and polyamines in relation to effects on membrane permeability. Plant Physiol. **73** : 698－702.