Phytochemistry, Vol. 22, No. 7, pp. 1547–1551, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

CHANGES IN POLYAMINES AND RELATED ENZYMES WITH LOSS OF VIABILITY IN RICE SEEDS

A. MUKHOPADHYAY, M. M. CHOUDHURI, K. SEN and B. GHOSH

Botany Department, Bose Institute, Calcutta 700009, India

(*Revised received* 12 *August* 1982)

Key Word Index—*Oryza sativa*; Gramineae; rice; spermine; spermidine; putrescine; arginine; arginine decarboxylase; polyamine oxidase.

Abstract—Putrescine, spermidine and spermine of high vigour, low vigour and non-viable (classes 1, 2 and 3 respectively) seeds of *Oryza sativa* increased with loss of viability. The largest concentration of spermine was found in non-viable embryos. Spermine was absent in the husks of all the three categories of seeds. Arginine decarboxylase was greatest in high vigoured seeds and its activity gradually declined with loss of viability. However, diamine oxidase and polyamine oxidase activities gradually increased with the loss of viability of the seeds while DNA, RNA and protein contents decreased. The total content of polyamines increased on kinetin treatment but declined on ABA treatment. DNA, RNA and protein followed the same trend as polyamines. The polyamine contents increased by *ca* 3- and 4-fold, respectively, in high vigoured and low vigoured seeds on 10^{-4} M kinetin treatment. The activity of ADC followed the same change as that of the polyamines in both cases, but the reverse was observed for the activities of diamine and polyamine oxidases.

INTRODUCTION

Ageing, a universal problem in seed storage, is also of considerable importance in rice seeds. This commences just after the formation of the embryo. Although the exact mechanism relating to ageing is not known it has been associated with the degradation of enzymes, chromosomal damage and loss of membrane integrity [1, 2].

The polyamines, spermidine and spermine, and the diamine, putrescine are widely distributed in animals, bacteria and higher plants. Although their exact function is not yet clear, they are believed to play an important role in a number of biochemical processes [3, 4]. Some reports suggest a close correlation between polyamine biosynthesis and changes in the rate of DNA, RNA and protein synthesis [5–10]. Since the importance of polyamines in cellular growth and differentiation is well recognized, studies on polyamines and their related enzymes during seed life is of considerable interest. Moreover, polyamines have also been reported to inhibit senescence [11]. Although there are several reports regarding the changes in polyamine levels in rapidly growing animals, plants and bacterial systems, changes in the levels of the polyamines with age are not well documented, particularly in plant systems [12]. In view of this, we have studied the polyamine contents and the activities of arginine decarboxylase and of polyamine and diamine oxidases with the aim to establish a correlation, if any, between the polyamine contents and their related enzymes with the loss of viability of the rice seeds.

RESULTS AND DISCUSSION

A distinct role for polyamines in cell proliferation and differentiation had been established in various microorganisms, animals, and higher plants [13]. Surprisingly, in the non-growing rice seeds (class 3) polyamine levels were the highest, and compared to high vigoured ones (classes 1 and 2) more than *ca* five-fold increment in total polyamine contents occurred (Table 1). Although all the polyamines increased with loss of viability of the seeds, the increase in spermine content was more marked. The distribution pattern in the different parts of the seeds showed that the embryo was the richest source of polyamines while the husks contained the least. No spermine was detected in the husks of rice seeds.

The specific activity of arginine decarboxylase, an important enzyme of polyamine biosynthesis, being highest in class 1 seeds gradually decreased with ageing (Table 2). This was analogous to the behaviour of ornithine decarboxylase, a key enzyme for putrescine biosynthesis in animals, which was reported to decline with ageing of human fibroblasts [14]. Highest ADC activity was noted in the embryo of class 1 seed.

Activities of polyamine/diamine oxidase which oxidatively degraded polyamines/diamines, on the other hand, increased gradually with loss of viability of the seeds (Table 2). Highest activities were obtained for embryos of class 3 where *ca* two-fold rise was noted compared to that of class 1 seeds. This type of age-related increment in oxidase activities was found to be quite similar to that of animal systems in which diamine oxidase activity increased with ageing [15].

Since there is a good correlation between seed life and nucleic acids and protein contents, these were estimated in the seeds of different ages, to establish a possible correlation between polyamines and nucleic acids during the ageing process. Table 3 clearly indicated a gradual decrease in nucleic acids and protein content with the age of rice seeds. Embryos of class 1 seeds contained the highest amount of DNA, RNA and protein. As the contents of nucleic acids and protein gradually decreased with ageing,

Table 1. The contents of polyamines in rice seeds with loss of viability

Status of seeds	Total polyamine content in seeds (nmol/g of fr. wt)	Parts of seeds	Polyamine content (nmol/g of fr. wt)		
			Putrescine	Spermidine	Spermine
Full germination with high vigour (100% germination, class 1)	950	Husk	17.8	18.8	nd
		Endosperm	19	10	21
		Embryo	476	112	275
Partial germination with low vigour (25–30% germination, class 2)	2510	Husk	102	66	nd
		Endosperm	64	41	17
		Embryo	744	281	1197
Non-germination (0% germination, class 3)	5420	Husk	111	99	nd
		Endosperm	79	49	258
		Embryo	836	759	3225

The procedures are described in the Experimental. Results are expressed as a mean value from three experiments.
nd, Not detected under the experimental conditions.

Table 2. The specific activities of certain biosynthetic and degradative enzymes in rice seeds with loss of viability

Status of seeds	Parts of seeds	Sp. act. of arginine decarboxylase (ADC) (pkat/mg protein)	Sp. act. of polyamine oxidase (units*)	
			Putrescine	Spermidine
Full germination with high vigour (100% germination, class 1)	Endosperm	0.09	3	44
	Embryo	0.18	8	43
Partial germination with low vigour (25–30% germination, class 2)	Endosperm	0.08	5	43
	Embryo	0.03	15	80
Non-germination (0% germination, class 3)	Endosperm	0.002	6	48
	Embryo	0.003	11	70

Arginine decarboxylase was assayed using [^{14}C]arginine and shaking in a Dubnoff metabolic shaker at 40° for 1 hr. The methods for estimating polyamine oxidase activity are described in the Experimental. Activities are expressed as a mean value from two experiments.
*1 unit = 0.0001 A/mg of protein·min.

Table 3. Protein and nucleic acids contents of rice seeds with loss of viability

Status of seeds	Parts of seeds	Protein content (mg/g of fr. wt)	Nucleic acids content (mg/g of fr. wt)	
			RNA	DNA
Full germination with high vigour (100% germination, class 1)	Husk	0.2	0.04	nd
	Endosperm	2.3	0.3	0.1
	Embryo	8.3	2.5	0.7
Partial germination with low vigour (25–30% germination, class 2)	Husk	0.2	0.04	nd
	Endosperm	1.6	0.2	0.1
	Embryo	6.4	2.1	0.3
Non-germination (0% germination, class 3)	Husk	0.2	0.04	nd
	Endosperm	0.9	0.2	0.05
	Embryo	5	1.1	0.2

nd, Not detected under the experimental conditions.

in contrast to that of polyamines, a negative correlation was indicated in the ageing seeds with a low level of metabolism, while in the case of actively synthesizing tissues a positive correlation was reported [16, 17].

Prompted by our earlier observation [18] that the content of cytokinin gradually diminished with ageing while abscisic acid (ABA) increased, we have studied the effects of these substances on polyamine metabolism in the embryo of rice seeds after 24 hr imbibition with the above hormones. From Table 4 it was clear that there was

Table 4. The polyamine content in rice embryos after 24 hr treatment with growth regulators

Status of seeds	Concn of substances added	Total polyamine content (nmol/g fr. wt)	Polyamine content (nmol/g of fr. wt) Putrescine	Spermidine	Spermine
Full germination with high vigour (100% germination, class 1)	Control (distilled H_2O)	2550	1110	940	500
	10^{-4} M ABA	1180	440	230	500
	10^{-6} M ABA	1870	670	710	500
	10^{-8} M ABA	1900	670	240	1000
	10^{-4} M Kinetin	7560	1070	4890	1600
	10^{-6} M Kinetin	3120	590	1000	1520
	10^{-8} M Kinetin	2380	960	500	920
Partial germination with low vigour (25–30% germination, class 2)	Control (distilled H_2O)	1570	560	240	760
	10^{-4} M ABA	920	390	100	440
	10^{-6} M ABA	1210	540	260	410
	10^{-8} M ABA	1390	730	260	410
	10^{-4} M Kinetin	6160	1980	770	2420
	10^{-6} M Kinetin	2920	560	1540	820
	10^{-8} M Kinetin	2150	740	590	820

Results are mean values of two duplicate experiments.

a dose-dependent increase or decrease in the polyamine contents on kinetin or ABA treatments, respectively. Highest contents of polyamines were found on 10^{-4} M kinetin treatments while with 10^{-4} M ABA treatment the content decreased markedly. Kinetin at 10^{-4} M caused 3- and 4-fold rises in polyamine contents in class 1 and 2 embryos, respectively, and the spermidine content was almost seven-times greater than that of the control in class 2 embryos. On the other hand, with 10^{-4} M ABA treatment the polyamine content decreased to about half that of control in both class 1 and 2 embryos and a marked decrease was noted in putrescine and spermidine contents. An increase in polyamine with cytokinin treatment has been reported by Galston and Kaur-Sawhney [19].

Both the biosynthetic and oxidative enzymes were regulated by these two phytohormones (Table 5). Activity of ADC was increased by 10^{-6} M kinetin and decreased by 10^{-4} M ABA treatment. On the other hand, the polyamine oxidase showed the reverse picture, i.e. increased by ABA and decreased by kinetin treatment. The dose-dependent response of ADC by kinetin and ABA treatment was also reported by Suresh *et al.* [20] and an

Table 5. Changes in the specific activities of ADC and polyamine oxidase in embryos of rice seeds after 24 hr treatment with growth regulators

Status of seeds	Concn of substances added	Sp. act. of arginine decarboxylase (ADC) (pkat/mg protein)	Sp. act. of polyamine oxidase (units*) Putrescine	Spermidine
Full germination with high vigour (100% germination, class 1)	Control (distilled H_2O)	0.21	12	66
	10^{-4} M ABA	0.12	13	70
	10^{-6} M ABA	0.19	14	77
	10^{-8} M ABA	0.20	16	113.3
	10^{-4} M Kinetin	0.24	10.6	62
	10^{-6} M Kinetin	0.34	6.2	43
	10^{-8} M Kinetin	0.23	6.0	38
Partial germination with low vigour (25–30% germination, class 2)	Control (distilled H_2O)	0.09	4	35
	10^{-4} M ABA	0.03	9	45
	10^{-6} M ABA	0.04	13	123
	10^{-8} M ABA	0.04	7	68
	10^{-4} M Kinetin	0.14	3	27
	10^{-6} M Kinetin	0.20	3.5	27
	10^{-8} M Kinetin	0.1	0.6	5

*1 unit = 0.0001 A/mg of protein · min.

Table 6. Changes in protein and nucleic acid content after treatment of rice embryos with growth regulators

Status of seeds	Concn of substances added	Protein content (mg/g of fr. wt)	Nucleic acid content (mg/g of fr. wt) RNA	DNA
Full germination with high vigour (100% germination, class 1)	Control (distilled H_2O)	11.4	3	1.4
	10^{-4} M ABA	8.6	1.3	0.5
	10^{-6} M ABA	9.3	1.8	0.8
	10^{-8} M ABA	10.4	2.3	1.1
	10^{-4} M Kinetin	13.7	3.8	1.8
	10^{-6} M Kinetin	12.3	3.3	1.7
	10^{-8} M Kinetin	11.4	3	1.4
Partial germination with low vigour (25–30% germination, class 2)	Control (distilled H_2O)	10.7	1.4	0.6
	10^{-4} M ABA	7.4	0.7	0.4
	10^{-6} M ABA	8	1.1	0.5
	10^{-8} M ABA	9.6	1.3	0.6
	10^{-4} M Kinetin	12.4	2.0	1.0
	10^{-6} M Kinetin	11.6	1.7	0.9
	10^{-8} M Kinetin	10	1.4	0.6

analogous situation was observed in the case of *Lathyrus sativus* where ADC activity was increased by IAA and GA [21]. Although Srivastava *et al.* [22] reported that the activity of polyamine oxidase was decreased *in vivo* by IAA while kinetin has no effect, in our *in vitro* system we noted a prominent decrease in polyamine oxidase activity both by kinetin and IAA (the results of IAA are not shown).

DNA, RNA and protein content followed the same pattern as that of polyamines with ABA and kinetin (Table 6).

EXPERIMENTAL

Winter variety of rice (*Oryza sativa* L. cv Rupsail) seeds of different status; class 1, full germination with high vigour; class 2, 25–30% germination with low vigour; and class 3, non-germination used as exptal materials in our study, were collected from the Institute's own field station at Shyamnagar, West Bengal.

Chemicals, putrescine, spermidine, spermine, kinetin, abscisic acid, BSA and the enzyme, peroxidase, were purchased from Sigma. Si gel used for TLC was obtained from Merck. L-[U-^{14}C]Arginine (sp. act. 228 mCi/mmol) was from Bhaba Atomic Research Centre. All chemicals used for this expt were commercial products of the highest purity grade available.

Treatment of seeds. After removal of the husks, the seeds were surface sterilized with 0.1% $HgCl_2$ and were imbibed for 5 hr in double distilled H_2O at 30°. The different parts, viz. embryo, endosperm and husk, were extracted for determining polyamines and the activities of the enzymes. To study the effect of plant growth regulators, the embryos from class 1 and 2 seeds were treated with kinetin and ABA for 24 hr at 30°.

Determination of amines. Polyamines extracted with 5% TCA were dansylated and separated by Si gel TLC by the procedure of ref. [23] using EtOAc–cyclohexane (2:3) as solvent. After removal from the solvent mixture, the plates were sprayed immediately with $(C_2H_4OH)_3N$–*iso*-PrOH (1:4). The spots were marked under UV-light with reference to the standard used. The dansylated polyamines were eluted with Me_2CO and the fluorescence intensities were measured in a spectrofluorometer with the excitation and emission wavelengths at 360 and 506 nm, respectively.

The activity of arginine decarboxylase (arginine carboxylase, EC 4.1.1.19) in the tissue extract was estimated by measuring the release of $^{14}CO_2$ from L-[U-^{14}C]arginine under the conditions reported in ref. [24].

Polyamine oxidase was assayed by the method of ref. [25]. The enzyme was extracted with 0.1 M KPi buffer, pH 7.6, filtered through muslin, centrifuged at 10 000 *g* for 20 min and the supernatant obtained was used for enzyme assay. All processes were done at 0–4° unless otherwise stated. The assay mixture consisted of 0.1 M KPi buffer (pH 7.6), peroxidase (1 mg/ml), hydroquinone (1 mg/ml) and crude enzyme in a total vol. of 3.9 ml. After 2 min, 0.1 ml of 10 mM putrescine, spermidine or spermine was added and incubated at 30° for 1 hr. The reading was taken at 470 nm in a colorimeter against the corresponding blank in which 0.1 ml of 0.1 M KPi buffer (pH 7.6) was added instead of the substrate.

DNA, RNA and protein. These were estimated by the diphenylamine reaction [26], orcinol reaction [27] and Lowry's method [28], respectively.

Acknowledgements—Financial support from West Bengal Government and INSA is gratefully acknowledged.

REFERENCES

1. Sircar, S. M. (1970) *J. Indian Bot. Soc.* **XLIX**, 1.
2. Abdul Baki, A. A. and Anderson, J. D. (1972) *Seed Biology* (Kozlowski, T. T., ed.) Vol. II, pp. 283. Academic Press, New York.
3. Tabor, C. W. and Tabor, H. (1976) *Annu. Rev. Biochem.* **45**, 285.
4. Cohen, S. S. (1971) *Introduction to the Polyamines*, Prentice-Hall, Englewood Cliffs.
5. Bachrach, U. (1973) *Functions of Naturally Occurring Polyamines*. Academic Press, New York.

6. Caldarera, C. M. and Moruzzi, G. (1970) *Ann. N. Y. Acad. Sci.* **171**, 709.
7. Chin, J. F. and Sung, S. C. (1972) *Biochim. Biophys. Acta* **281**, 535.
8. Villanueva, V. R., Adlakha, R. C. and Cantera-Soler, A. M. (1978) *Phytochemistry* **17**, 1245.
9. Hunter, A. R., Farrell, J. F., Jackson, J. R. and Hunt, T. (1977) *Eur. J. Biochem.* **75**, 149.
10. Guilfoyle, T. J. and Hanson, J. B. (1973) *Plant Physiol.* **51**, 1022.
11. Altman, A., Kaur-Sawhney, R. and Galston, A. W. (1977) *Plant Physiol.* **60**, 570.
12. Anguillesi, M. C., Bagni, N. and Floris, C. (1974) *G. Bot. Ital.* **108**, 305.
13. Tabor, H. and Tabor, C. W. (1964) *Pharmacol. Rev.* **16**, 245.
14. Duffy, P. E. and Kremzner, L. T. (1977) *Exp. Cell Res.* **108**, 435.
15. Buffoni, F. (1966) *Pharmacol. Rev.* **18**, 1163.
16. Stevens, L. (1970) *Biol. Rev.* **45**, 1.
17. Bagni, N. (1970) *New Phytol.* **69**, 159.
18. Bose, S., Ghosh, B. and Sircar, S. M. (1977) *Ind. J. Agric. Sci.* **47**, 634.
19. Galston, A. W. and Kaur-Sawhney, R. (1981) *What's New Plant Physiol.* **11**, 5.
20. Suresh, M. R., Ramakrishna, S. and Adiga, P. R. (1978) *Phytochemistry* **17**, 57.
21. Ramakrishna, S. Ph.D. thesis, IIS, Bangalore, India.
22. Srivastava, S. K., Prakash, V. and Naik, B. I. (1977) *Phytochemistry* **16**, 185.
23. Seiler, N. (1971) *J. Chromatogr.* **63**, 97.
24. Sen, K., Choudhuri, M. M. and Ghosh, B. (1981) *Phytochemistry* **20**, 631.
25. Kenten, R. W. and Mann, P. J. G. (1952) *Biochem J.* **50**, 361.
26. Burton, K. (1968) *Methods Enzymol.* **12B**, 163.
27. Aswell, G. (1955) *Methods Enzymol.* **3**, 87.
28. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.