Phytochemistry, Vol. 22, No. 12, pp. 2681–2686, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

EFFECT OF AMINES AND GUANIDINES ON PEROXIDASE FROM MAIZE SCUTELLUM

S. K. SRIVASTAVA* and P. RAJBABU

Biochemistry Department, M.S. University of Baroda, Baroda 390002, India

(*Revised received* 23 *April* 1983)

Key Word Index—*Zea mays*; Gramineae; maize scutellum; peroxidase; putrescine; spermidine; spermine; guanidino-acetic acid; dodine; guazatine; cysteine; sulfhydryl reagents; activation; inhibition; membranes.

Abstract—The membrane-bound peroxidase activity of excised maize scutellum is inhibited by putrescine, spermidine and spermine and activated by guanidino-acetic acid, guanidino-butyric acid, guazatine and dodine as a result of their binding to the membranes. The inhibition of polyamines is reversed by guanidino compounds but the activation by guanidines is not reversed by polyamines. Other guanidino compounds like arginine, agmatine, creatine and creatinine have no effect by themselves but they reverse the effect of polyamines, except in the case of creatinine which does not have a free guanidino group. Peroxidase present in the soluble fraction or the ionically bound peroxidase from particulate fractions solubilized by Ca^{2+} is not affected by polyamines or guanidines. The sulfhydryl reagents iodoacetate and *p*-chloromercuri-benzoate (*p*-CMB) activate peroxidase activity and compete for the polyamine binding site. The effect of dodine is potentiated by sulfhydryl reagents.

INTRODUCTION

Polyamines, which occur ubiquitously in all living organisms, are believed to be involved in growth processes through their interaction with nucleic acids [1, 2]. In plants, however, they are also involved in the control of several stress-related processes such as senescence [3–8], wounding [9] and temperature and salt stress [10]. The protective effect of polyamines in these processes may be associated with the stabilization of membrane structure. This has been demonstrated, for instance, in the stabilization of protoplasts from oat leaf mesophyll cells [11], thylakoid membranes of barley chloroplast [12] and reduction of betacyanin efflux from beet root discs [9, 13–16]. Guanidino compounds, which are structurally related to polyamines, are known to destabilize the membranes [16–18], and to interfere with the mitochondrial metabolism [19] and transport of ions [20, 21]. Recent studies have shown that the destabilization produced by guanidines could be reversed by polyamines [16, 17].

Peroxidase, which is widely distributed in plants [22], has been implicated in growth and development [23, 24], hormonal balance [25], fruit ripening [26, 27], senescence [28–30] as well as in membrane integrity [31]. Peroxidase has also been used as a parameter for various physiological conditions such as stress by salt [32–34], water [35, 36] and temperature [37], senescence [28–30], wounding [38], air pollution [39], infection by pathogens [40–42] and γ-irradiation [43]. Since polyamines appear to protect plants against some of these conditions, it was of interest to investigate if peroxidase activity is affected by amines and guanidines. The results of these studies in excised maize scutellum are reported in the present paper.

*To whom correspondence should be addressed.

RESULTS AND DISCUSSION

When scutella were excised from seedlings germinated for different periods and assayed for peroxidase in slices on the day of excision (0 day incubation) (Table 1), the enzyme activity increased with germination up to 8 days and then remained constant. If the excised scutella were kept for incubation for different periods, the peroxidase activity increased continuously until it reached a maximum level (*ca* 200 units/g tissue). The development of enzyme was more rapid in the scutella excised and incubated rather than in the freshly excised scutella from

Table 1. Development of peroxidase activity in excised maize scutellum

Scutellum excised from seeds germinated for (days)	Peroxidase activity (units/g tissue) in excised scutellum incubated for (days)					
	0	1	2	3	4	5
1	5	17	77	92	145	156
2	25	92	135	150	160	172
3	61	97	137	163	184	190
4	77	116	140	173	186	196
5	112	143	163	176	185	195
6	131	151	173	182	197	201
7	140	162	175	196	201	202
8	148	162	182	196	202	201
9	151	173	198	201	202	200
10	152	173	190	200	202	201

Scutella were excised from seedlings germinated for different days, starting from the day of transfer to Petri dishes, and incubated for up to 5 days. The peroxidase activity was assayed in the slices.

the seeds germinated for the same period. Increased oxygen availability during incubation of the excised scutella may account for the early development of peroxidase activity.

Scutella from seedlings germinated for 4 days were used for most of the studies reported below. Peroxidase activity in these scutella was 77 units/g tissue when assayed in slices. However, when assayed in the homogenate, the total activity was *ca* 300 units/g tissue showing that only 25% of the total activity had been assayed in slices. Later studies (Table 5) showed that the membrane fraction (40 000 g) had 30% of the total activity present in the homogenate suggesting that only membrane-bound peroxidase was assayed in the slices.

Preincubation of scutellum slices from seedlings germinated for 4 days with amines and guanidines showed (Table 2) that among the diamines the inhibition of peroxidase activity increased with chain length up to diaminobutane (putrescine), which gave 80% inhibition, and then decreased with further increase in diamine chain length. The polyamines spermidine and spermine gave maximum inhibition among all the amine compounds. Out of the guanidino compounds tested, arginine, agmatine, creatine and creatinine had no effect, but guanidino-acetic acid (GAA), guanidino-butyric acid (GBA) and the two guanidino-fungicides dodine and guazatine [44, 45] showed 40–45% activation. In a similar experiment with scutella excised from 8-day germinated seedlings, none of the compounds had any effect. These results indicated that with increase in the period of germination from 4 to 8 days, which may result in disintegration and loss of membrane integrity, the effect of amines and guanidines was abolished. The compounds thus appear to affect peroxidase activity by altering the membrane structure. This was further evident when a homogenate was used instead of slices. These compounds had very little effect in the homogenate due to the breakdown of membrane structure during homogenization (data not given). The compounds showing an effect on enzyme activity were then tested at different concentrations with slices or homogenates. The results showed (Table 3) that in slices the inhibition of peroxidase by putrescine, spermidine and spermine increased with concentration and activity was completely inhibited with 3 mM spermidine and spermine. With GAA and dodine, however, the activity increased up to 2–3 mM and then decreased to the control level with increasing concentrations. With homogenate the inhibition by putrescine, spermidine and spermine and the activation by GAA and dodine was considerably reduced. The guanidino compounds even showed some inhibition at higher concentrations.

The question whether polyamines and guanidines compete for a common binding site on the membrane to produce their effect on peroxidase activity was studied by preincubating the slices with one compound followed by the other after washing, and the activity was compared with a control group treated with water for the same period. When the preincubation was carried out with water first and followed by different compounds, putrescine, spermidine and spermine showed inhibition but GAA and dodine showed activation, whereas arginine, agmatine, creatine and creatinine had no significant effect (Table 4). However, if the slices were first incubated with

Table 2. Effect of amines and guanidines on peroxidase activity in maize scutellum slices

Compound	Peroxidase activity in scutellum slices (% of control) from seedlings germinated for (days)	
	4	8
1,2-Diaminoethane 2HCl	88	98
1,3-Diaminopropane 2HCl	66	100
1,4-Diaminobutane 2HCl (putrescine)	21	98
1,5-Diaminopentane 2HCl (cadaverine)	63	100
1,6-Diaminohexane 2HCl	70	98
1,8-Diaminooctane 2HCl	86	98
1,10-Diaminodecane 2HCl	88	98
1,8-Diamino-4-azaoctane 3HCl (spermidine)	18	97
1,12-Diamino-4,9-diazadodecane 4HCl (spermine)	16	96
2-Amino-5-guanidino-*n*-valeric acid HCl (arginine)	109	102
1-Guanidino-4-aminobutane H_2SO_4 (agmatine)	104	101
N-Guanyl *N*-methyl glycine (creatine)	90	100
2-Imino-*N*-methyl hydantoin (creatinine)	101	100
N-Amidino-glycine (guanidino-acetic acid)	138	104
4-Guanidino-ethyl acetic acid (guanidino-butyric acid)	138	103
Guanidino-*n*-dodecane HOAc (dodine)	140	105
1,17-Diguanidino-9-azaheptadecane 3HOAc (guazatine)	145	105

Scutellum slices from 4- or 8-day germinated maize seedlings were incubated with the compounds (2 mM) for 1 hr. After incubation, the slices were washed with water and used for enzyme assay. Control activity on the fourth and eighth days was 75 and 150 units/g tissue, respectively.

Table 3. Effect of concentration of polyamines and guanidines on peroxidase activity in maize scutellum slices and homogenate

Concentration (mM)	Peroxidase activity (% of control) with				
	Putrescine	Spermidine	Spermine	GAA*	Dodine
			Slices		
0.5	83	43	58	114	113
1	67	25	29	125	136
2	34	18	16	138	140
3	24	7	3	121	146
4	15	2	2	97	128
5	15	2	2	94	107
			Homogenate		
0.5	99	94	91	117	113
1	96	85	89	119	123
2	93	78	84	120	121
3	85	73	78	97	86
4	80	69	74	86	80
5	71	62	62	84	73

Scutella from 4-day germinated seedlings were used. Slices and homogenates were preincubated at 37° with the compounds at different concentrations for 1 hr. Control activity in the slices and homogenate was 75 and 305 units/g tissue, respectively.

*GAA, Guanidino-acetic acid.

Table 4. Interaction between polyamines and guanidines for peroxidase activity in maize scutellum slices

Treatment* 1st / 2nd	Peroxidase activity (%)									
	Water	Putrescine	Spermidine	Spermine	Arginine	Agmatine	Creatine	Creatinine	GAA†	Dodine
Water	100									
Putrescine	36				123	123	110	37	145	164
Spermidine	21				120	114	111	21	142	156
Spermine	20				110	102	100	19	140	150
Arginine	119	120	115	122						
Agmatine	112	115	112	109						
Creatine	108	112	108	107						
Creatinine	102	40	24	21						
GAA	147	140	139	135						
Dodine	163	153	140	153						

*Scutellum slices from 4-day germinated seedlings were preincubated (first treatment) for 1 hr in polyamines and guanidines (2 mM) at 37°. After washing in water, the polyamine-treated slices were again incubated in guanidines, and guanidine-treated slices were incubated in amines (second treatment) for 1 hr. The slices after the second incubation were washed in water and used for enzyme assay. A control group incubated in water for the same period was included and its activity (76 units/g tissue) was taken as 100%.

†GAA, Guanidino-acetic acid.

putrescine, spermidine and spermine and then with the guanidino compounds, the inhibitory effect of the polyamines was completely abolished by all the guanidino compounds except creatinine. GAA and dodine then activated to the same extent as without the amines' pre-treatment. Further, if the pre-treatment was carried out first with the guanidino compounds and then with polyamines, the polyamines could not reverse the effect of the guanidino compounds. These results suggested that the binding of polyamines was reversed by compounds having free guanidino groups, but the guanidine binding was not reversed by polyamines. It is interesting to note that although arginine, agmatine and creatine had no significant effect by themselves, they prevented the binding of polyamines to the membrane. Creatinine, which has no free guanidino group and had no effect by itself, was unable to prevent the polyamine binding and consequently the inhibitory effect.

Peroxidase has been reported to be present in soluble as well as in membrane fractions [38, 40, 46]. The membrane-bound enzyme may be bound ionically or covalently and the ionically-bound enzyme could be solubilized by Ca^{2+} [46]. In order to investigate whether polyamines and guanidines affect the membrane-bound

or the soluble enzyme, the subcellular fractions were prepared from scutella of seedlings germinated for 4 days.

The fractions 1000 *g*, 10 000 *g*, 40 000 *g*, 105 000 *g* and the soluble fraction had 11, 25, 30, 1 and 33% activity, respectively. These fractions were then incubated with polyamines and guanidines (Table 5). None of the compounds had any effect on the enzyme activity present in the soluble fraction. The polyamines inhibited all the particulate fractions to varying degrees, the maximum effect (*ca* 70%) being in the 40 000 *g* fraction. GAA and dodine activated the 1000 *g*, 10 000 *g* and 40 000 *g* fractions but had no effect on the 105 000 *g* fraction. In another experiment the particulate fractions 10 000 *g* and 40 000 *g* which had 25 and 30% of the total activity were treated with calcium chloride, Triton X-100 and deoxycholate to solubilize the membrane-bound enzyme. After treatment with these compounds the fractions were centrifuged to separate the soluble and residual fractions which were then incubated with spermine or dodine and assayed for enzyme activity. The results (Table 6) showed that Ca^{2+} could solubilize *ca* 80% of the enzyme from both the 10 000 *g* and 40 000 *g* fractions whereas Triton and deoxycholate did not solubilize the enzyme. The enzyme which remained in the residual fractions was inhibited by spermine to *ca* 50 and 65% in the 10 000 *g* and 40 000 *g* fractions, respectively. The activation of the residual enzyme by dodine was *ca* 45 and 30% in the 10 000 *g* and 40 000 *g* fractions, respectively. The enzyme solubilized by Ca^{2+} was not affected by spermine or dodine, indicating that polyamines and guanidines modulate peroxidase activity only when it is bound to the membranes. The effects produced by these compounds are not due to their direct binding to the peroxidase enzyme but are a result of alteration in membrane structure. The differential response obtained with the subcellular fractions may be associated with the differences in the composition of their membranes.

Table 5. Effect of polyamines and guanidines on peroxidase activity in subcellular fractions of maize scutellum

Compound	Peroxidase activity (% of control) in fractions				
	1000 *g*	10 000 *g*	40 000 *g*	105 000 *g*	Soluble
Putrescine	75	60	44	84	100
Spermidine	67	54	39	84	100
Spermine	61	52	30	81	100
Arginine	99	108	102	103	100
Agmatine	101	99	103	108	100
Creatine	104	101	100	95	99
Creatinine	102	102	99	97	101
GAA*	120	128	122	105	100
Dodine	123	151	147	103	102

Subcellular fractions from scutella of 4-day germinated seedlings were prepared. These fractions had 11, 25, 30, 1 and 33% activity of the homogenate (306 units/g tissue). The fractions were incubated with polyamines and guanidines (1 mM) for 1 hr at 37° before the addition of other assay components for the enzyme activity test.

*GAA, Guanidino-acetic acid.

Sulfhydryl groups appear to be important for membrane function. For instance, the non-permeant sulfhydryl reagent *p*-chloromercuri-benzene sulfonate inhibited membrane transport in beet leaf discs [47]. They also affect permeability of red blood cells [48]. Interaction between sulfhydryl groups and polyamines has been demonstrated in beet root discs [15]. The sulfhydryl reagent *p*-chloromercuri-benzoate (*p*-CMB) enhanced betacyanin efflux in beet root discs. The stabilizing effect of polyamines in beet root discs was abolished but the toxic effect of guanidines was potentiated by *p*-CMB [16]. Since membrane-bound peroxidase of maize scutellum is affected by polyamines and guanidines, and peroxidase has also been reported to be inhibited by cysteine [49], it was of interest to study the interactions between polyamines and guanidines with cysteine and sulfhydryl reagents. When the slices were incubated with iodoacetate and *p*-CMB, they activated peroxidase by 37 and 77%.

Table 6. Effect of spermine and dodine on peroxidase activity in the 10 000 *g* and 40 000 *g* fractions treated with calcium chloride, Triton X-100 and sodium deoxycholate

Fraction	Treatment*	Peroxidase activity (units/g tissue) in Residue			Supernatant		
		Control	+ Spermine	+ Dodine	Control	+ Spermine	+ Dodine
10 000 *g*	—	73	39	105	4	4	5
	$CaCl_2$	13	9	17	60	57	58
	Triton X-100	73	38	108	4	4	4
	Deoxycholate	67	36	99	9	9	9
40 000 *g*	—	86	30	113	4	5	5
	$CaCl_2$	12	4	14	78	77	81
	Triton X-100	85	29	109	4	4	5
	Deoxycholate	78	27	101	9	8	9

*The 10 000 *g* and 40 000 *g* fractions from scutella of 4-day germinated seedlings were treated with calcium chloride (0.8 M), Triton X-100 (1%) and sodium deoxycholate (1%) in the grinding medium for 1 hr at 4° and then centrifuged at 10 000 *g* and 40 000 *g*, respectively, to separate the supernatant and residue fractions. These fractions were then incubated for 1 hr at 37° with spermine or dodine (1 mM) before the addition of other assay components for the enzyme activity test.

Table 7. Interaction between iodoacetate, *p*-chloromercuri-benzoate (*p*-CMB), cysteine, spermine and dodine for peroxidase activity in maize scutellum slices

Treatment 1st / 2nd	Peroxidase activity (%)					
	Water	Iodoacetate	*p*-CMB	Cysteine	Spermine	Dodine
Water	100					
Iodoacetate	137				122	153
p-CMB	177				134	194
Cysteine	68	79	77		29	81
Spermine	23	22	38	18		
Dodine	138	170	190	81		

Scutellum slices from 4-day germinated seedlings were preincubated (first treatment) for 1 hr in iodoacetate (0.5 mM), *p*-CMB (0.5 mM), cysteine (0.5 mM), spermine (2 mM) and dodine (2 mM) at 37°. After washing in water, the iodoacetate- and *p*-CMB-treated slices were incubated in cysteine, spermine and dodine, while the cysteine-treated slices were treated with spermine and dodine. Similarly, the spermine- and dodine-treated slices were then incubated with iodoacetate, *p*-CMB and cysteine (second treatment) for 1 hr. The slices after the second incubation were washed with water and used for enzyme assay. A control group incubated in water for the same period was included and its activity (73 units/g tissue) was taken as 100%.

respectively, whereas cysteine inhibited the enzyme by 32% (Table 7). Incubation of slices first with iodoacetate or *p*-CMB and then with cysteine after washing completely prevented the increase by sulfhydryl reagents and showed inhibition to the same extent as with cysteine alone. Similar results were obtained with spermine. However, incubation of the slices with dodine after the sulfhydryl reagents gave an additive effect. The inhibitory effect of spermine was not evident if the slices were treated with it first and then with sulfhydryl reagents, but showed 22 and 34% activation, respectively. Incubation of slices with dodine first followed by sulfhydryl reagents showed more activation compared to sulfhydryl reagents and dodine alone. *p*-CMB and iodoacetate had no effect on the enzyme present in the soluble fraction, whereas cysteine inhibited it by 62%. Also the presence of *p*-CMB or iodoacetate along with cysteine did not change the inhibition pattern of cysteine (data not given).

Polyamines are known to affect several membrane-bound enzymes such as ATPases [50–53], acetylcholine esterase [54, 55] and glucose-6-phosphatase [56]. It is suggested that they may bind directly to the regulatory sites of the enzyme as in the case of acetylcholine esterase [55] or to the negatively charged phospholipids of the membrane, thus neutralizing the charge which could lead to a change in the membrane-bound enzyme. Membrane permeability changes appear to result from their binding to membrane lipids [9, 13, 16]. Sulfhydryl groups of membrane proteins also play a role in membrane function [15, 47, 48]. The present studies suggest that polyamines, guanidines and sulfhydryl reagents modulate membrane-bound peroxidase due to their binding to the membrane, and not to the enzyme itself since the soluble and the ionically-bound enzyme solubilized by Ca^{2+} was not affected by these compounds. Polyamine binding was reversed by guanidines and sulfhydryl reagents whereas guanidine binding was not reversed by polyamines but was potentiated by sulfhydryl reagents, indicating that there must be some common binding sites on the membrane between polyamines and guanidines and between polyamines and sulfhydryl reagents. However, the non-reversibility of guanidine binding by polyamines or sulfhydryl reagents and the additive response between guanidines and sulfhydryl reagents would indicate the presence of additional sites for guanidines. The inhibition produced by cysteine may be associated with its metal-binding property.

EXPERIMENTAL

Plant material. Maize seeds (*Zea mays* L. var. Ganga-2) were obtained from the National Seed Corporation, Baroda. The seeds were soaked in running tap H_2O for 24 hr and then kept in Petri dishes on 2 layers of moist filter papers and allowed to germinate in the dark at 22°. The scutella were excised from the seeds and after removal of the embryo, they were cut transversely into thin slices with a razor blade. The slices were washed in cold H_2O until the wash H_2O remained clear and then were blotted on filter paper. In the case where homogenate rather than slices was used, a 1% homogenate was prepared with 0.95% KCl, pH 7, using a chilled pestle and mortar. In expts where scutella were incubated for a further period after excision, they were kept in Petri dishes with 2 layers of moist filter papers for specified periods at 22° before preparation of slices for enzyme assay. The time when the seeds were kept for soaking was considered as zero time.

For treatment with amines and guanidines, the scutellum slices from 4- or 8-day germinated seedlings were incubated for 1 hr at 37° in specified concns of these compounds. After incubation the slices were washed thoroughly with H_2O and assayed for enzyme activity. Incubation of slices in H_2O for over a 4 hr period did not change peroxidase activity present in freshly prepared slices. In the case of homogenates, the compounds were added to the homogenate and incubated at 37° for 1 hr before the other assay components were added. For expts where amines, guanidines and sulfhydryl reagents were used to investigate their interaction, the scutellum slices were incubated at 37° with either amines, guanidines or sulfhydryl reagents first, followed by the other compounds after washing with H_2O.

Isolation of subcellular fractions. Scutella (30 g) from 4-day germinated seedlings were homogenized with 120 ml 0.25 M

sucrose in 25 mM Tris–maleate buffer, pH 7.2. The homogenate was strained through 4 layers of cheesecloth and centrifuged at 1000 *g* for 10 min, 10000 *g* for 15 min, 40000 *g* for 30 min and 105000 *g* for 60 min. Pellets obtained from these fractions, representing crude nuclear, mitochondrial, membrane and microsomal fractions, were suspended in the grinding medium. The fractions without any further purification were used for enzyme assay with or without preincubation with amines and guanidines for 1 hr as described above for slices.

Enzyme assay. Peroxidase activity was assayed essentially according to the method of ref. [57]. The assay system consisted of 2.5 ml 50 mM acetate buffer, pH 5.5; 0.1 ml 0.25% *o*-dianisidine; 0.1 ml 3% H_2O_2 and enzyme (0.1 ml homogenate or 40 mg slices) in a vol. of 4 ml. After incubation for 30 sec at room temp., the reaction was terminated with 0.5 ml 5 N H_2SO_4 and the *A* of the coloured complex was measured at 400 nm. Enzyme unit is defined as the amount of enzyme giving 1 *A* unit change per min under the assay conditions.

Chemicals. The homologous series of diamines, spermidine, spermine, guanidino-acetic acid and guanidino-butyric acid were purchased from Sigma. Guazatine was donated by KenoGard AB, Sweden and dodine (Melprex Tech) was donated by Cyanamid of Great Britain, London. Other chemicals of analytical grade were purchased locally.

Acknowledgements—We are grateful to KenoGard AB, Sweden and Cyanamid of Great Britain, London for the donation of chemicals as cited in the text; to Dr. B. I. Naik for help and discussion during the course of investigations; and to the CSIR, New Delhi, for the provision of a fellowship to P. R.

REFERENCES

1. Cohen, S. S. (1971) *Introduction to Polyamines*. Prentice-Hall, New Jersey.
2. Bachrach, U. (1973) *Function of Naturally Occurring Polyamines*. Academic Press, New York.
3. Cohen, A. S., Popovic, R. B. and Zalik, S. (1979) *Plant Physiol.* **64**, 717.
4. Kaur-Sawhney, R. and Galston, A. W. (1979) *Plant Cell Environ.* **2**, 189.
5. Altman, A. (1980) *Isr. J. Botany* **28**, 55.
6. Altman, A. and Bachrach, U. (1981) in *Advances in Polyamine Research* (Caldarera, C. M., Zappin, V. and Bachrach, U., eds.). Vol. 3, p. 365. Raven Press, New York.
7. Srivastava, S. K., Raj, A. D. S. and Naik, B. I. (1981) *Indian J. Exp. Biol.* **19**, 437.
8. Altman, A. (1982) *Physiol. Plant.* **54**, 189.
9. Altman, A. (1982) *Physiol. Plant.* **54**, 194.
10. Nezgovorova, L. A. and Borisova, N. N. (1967) *Fiziol. Rast.* **14**, 644.
11. Galston, A. W., Kaur-Sawhney, R., Altman, A. and Flores, H. (1980) in *Advances in Protoplast Research, Proceedings of the 5th International Protoplast Symposium* (Ferenczy, L. and Farkas, G. L., eds.), p. 485. Pergamon Press, Oxford.
12. Popovic, R. B., Kyle, D. J., Cohen, A. S. and Zalik, S. (1979) *Plant Physiol.* **64**, 721.
13. Naik, B. I. and Srivastava, S. K. (1978) *Phytochemistry* **17**, 1885.
14. Naik, B. I., Sharma, V. and Srivastava, S. K. (1980) *Phytochemistry* **19**, 1321.
15. Naik, B. I. and Srivastava, S. K. (1981) *Indian J. Exp. Biol.* **19**, 479.
16. Srivastava, S. K. and Smith, T. A. (1982) *Phytochemistry* **21**, 997.
17. Srivastava, S. K. and Smith, T. A. (1982) *Ann. Botany* **50**, 265.
18. Gomez-Lepe, B., Lee-Stadelmann, O. Y., Palta, J. P. and Stadelmann, E. J. (1979) *Plant Physiol.* **64**, 131.
19. Papa, S. M., Gomez-Puyou, M. T. and Gomez-Puyou, A. (1975) *Eur. J. Biochem.* **55**, 1.
20. Gomez-Puyou, A., Sandoval, F., Lotina, B. and Gomez-Puyou, M. T. (1973) *Biochem. Biophys. Res. Commun.* **52**, 74.
21. Gomez-Lepe, B. and Hodges, T. K. (1978) *Plant Physiol.* **61**, 865.
22. Saunders, B. C., Holmes-Stedle, A. G. and Stark, B. P. (1964) *Peroxidase: The Properties and Uses of a Versatile Enzyme and Some Related Catalysts*. Butterworths, London.
23. Trinh, T. H., Gasper, T. H., Van, T. T. and Marcotte, J. L. (1981) *Physiol. Plant.* **53**, 153.
24. Van Huystee, R. B. and Cairns, W. L. (1982) *Phytochemistry* **21**, 1843.
25. Schneider, G. (1970) *Annu. Rev. Plant Physiol.* **21**, 499.
26. Mattoo, A. K. and Modi, V. V. (1969) *Plant Physiol.* **44**, 308.
27. Ranadive, A. S. and Haard, N. F. (1972) *J. Food. Sci.* **37**, 381.
28. Parish, R. W. (1968) *Planta* **82**, 1.
29. Kar, M. and Mishra, D. (1976) *Plant Physiol.* **57**, 315.
30. Parida, R. K., Kar, M. and Mishra, D. (1981) *Biol. Plant.* **23**, 249.
31. Dilley, D. (1970) *The Biochemistry of Fruits and Their Products*, p. 195. Academic Press, New York.
32. Stevens, H. C., Calvan, M., Siegel, B. Z. and Siegel, S. M. (1978) *Phytochemistry* **17**, 1521.
33. Sheorane, I. S. and Garg, P. (1979) *Physiol. Plant.* **46**, 147.
34. Gettys, K. L., Hancock, J. F. and Covalieri, A. J. (1980) *Bot. Gaz.* **141**, 453.
35. Mali, P. C., Nanda, B. B., Bhattacharya, D. P. and Lodh, S. B. (1980) *Plant Biochem. J.* **7**, 126.
36. Mukherjee, S. P. and Chaudhari, M. A. (1981) *Physiol. Plant.* **52**, 37.
37. Hofferek, H. (1981) *Biochem. Physiol. Pflanz.* **176**, 170.
38. Bireeka, H. and Miller, A. (1974) *Plant Physiol.* **53**, 569.
39. Castillo, F. J. and Greppin, H. (1980) *C. R. Seances. Soc. Phys. Hist. Nat. Geneve* **15**, 57.
40. Gollios, T., Pugin, A., Peresse, M. and Dufouchet, J. (1980) *Phytopathol. Z.* **99**, 215.
41. Kaul, J. L. and Munjal, R. L. (1980) *Indian J. Phytopathol.* **33**, 530.
42. Neetria, G. B. (1981) *Phytopathol. Z.* **101**, 43.
43. Thomas, P. and Henry, D. (1979) *Phytochemistry* **18**, 917.
44. Ascher, K. R. S., Nemny, N. E., Wysoki, M. and Gurtelzak, L. (1978) *Pestic. Sci.* **9**, 566.
45. Higgins, R. A. and Pedigo, L. P. (1979) *J. Econ. Entomol.* **72**, 680.
46. Haard, N. F. (1973) *Phytochemistry* **12**, 555.
47. Giaquinta, R. (1976) *Plant Physiol.* **57**, 872.
48. Knauf, P. A. and Rothstein, A. (1971) *J. Gen. Physiol.* **58**, 190.
49. Lotti, G. and Galoppini, C. (1961) *Agrochemica* **6**, 21.
50. Peter, H. W., Wolf, H. U. and Seiler, N. (1973) *Hoppe-Seyler's Z. Physiol. Chem.* **354**, 1146.
51. Heinrich-Hirsch, B., Ahlers, J. and Peter, H. W. (1977) *Enzyme* **22**, 235.
52. Tashima, J., Hasegawa, M. and Mizunuma, H. (1978) *Biochem. Biophys. Res. Commun.* **82**, 13.
53. Peter, H. W., Pinheiro, M. R. and Lima, M. S. (1981) *Can. J. Biochem.* **59**, 60.
54. Kossorotow, A., Wolf, H. U. and Seiler, N. (1974) *Biochem. J.* **144**, 21.
55. Peter, H. W., Gies, A., Neumeier, M., Schadler, R. and Wegener, I. (1979) *Gen. Pharmacol.* **10**, 133.
56. Nordlie, R. C., Johnson, W. T., Cornatzer, W. E., Jr. and Twedell, G. W. (1979) *Biochim. Biophys. Acta* **585**, 12.
57. Shanon, L. M., Kay, E. and Lew, J. Y. (1966) *J. Biol. Chem.* **241**, 2166.