J. Phytol. Res. **15** (2) : 191-195, 2002

EFFECT OF FREE RADICAL QUENCHERS AND PARAQUAT ON LIPOXYGENASE, PROTEASE ACTIVITY AND TOTAL SOLUBLE SUGARS ACCUMULATION DURING *IN VITRO* MAIZE (*ZEA MAYS L.*) KERNEL DEVELOPMENT

M.K. MAHATMA*, G.C NANAWATI, V. SHARMA and H.R. MEHTA
Department of Biochemistry, Rajasthan College of Agriculture, Udaipur, Rajasthan Agricultural University, Bikaner - 334001, India.
*Plant Biotechnology Centre, Agricultural Research Station, Beechwal, Rajasthan Agricultural University, Bikaner (Rajasthan), India.

Studies of free radical quenchers and paraquat on lipoxygenase, protease activity and total soluble sugar accumulation during *in vitro* maize (*Zea mays* L Ganga-2) kernel development showed that 1mM of Na-thiosulfate improved intrinsic free radical caused impaired kernel growth rate from 4.50 mg to 5.06 mg (12.6%) kernel^{-1} day^{-1} and prevent effectively browning and necroses of cob tissue. 0.5 mM paraquat base reactive oxygen species system had about 50% reduce kernel growth, 27.7% lower kernel ethanol soluble sugars, 133.3% and 54.4% higher lipoxygenase and protease activities than kernels from control. The intrinsic reactive oxygen species may be a possible constrain for low productivity of maize grown under Indian tropical conditions.

Keywords : Free radicals; Lipoxygenase; Protease; *Zea mays* L.

Introduction

Free radicals have been implicated in a number of biological phenomena ranging from stress induced ageing to natural cause of senescence[1]. The biochemical milieu favourable for assimilate transport seems to be impaired by stresses viz. edaphic, drought, higher temperature, ageing related senescence etc.

The free radicals may protonate with trace amount of oxygen in the presence of iron and copper in the cell system and be converted into more reactive species perhydroxy radicals (HO_2), hydroxyl radicals (OH^-) etc. Free radicals react more selectively with target at critical cell location and cause membrane damage due to reaction with protein and lipid components of the membrane.

The existance of lipoxygenase (Lox) throughout phloem loading pathway suggests that the aberrations in assimilate transport seem to occur by hydroperoxide radicals, a product of Lox reaction. Thus damage to membrane fluidity in phloem loading cells, symplastic degree of fusion/ connection between sieve element may be limiting the phloem loading of sucrose for potential yield of maize. The study of intrinsic free radical impaired grain development in intact maize plant is complicated by interaction of grain with the mother plant. These problems can be overcomed by isolation of each limiting factor through *in vitro* kernel culture technique. In recent past many researchers[2-4] have made attempts in this direction by means of *in vitro* kernel culture techniques. In the light of above consideration we studied the influence of exogenously supplied free radical quenchers and paraquat on lipoxygenase and protease activity during *in vitro* kernel development of maize and assess the efficacy of these enzymes on soluble sugar accumulation.

Materials and Method

Hand pollinated ears were collected into polythene bags from field grown maize (Vr-Ganga-2) plants at 7 days after pollination (DAP) for explant preparation in various experiments. Cob pieces were taken from the middle of the ear so that the kernels were of relatively same size. The ear middle part was sliced horizontally into triple rows without disturbing structural integrity. The white central pith removed, and cut into 6 kernels bearing cob piece segment. The constant cob piece weight by 6 times the

total of 6 kernels (i.e. about 2.470±0.1 g) was taken for each treatment. So kernel cob ratio by weight is 1x6. Cob pieces were immediately sterilized by 0.5% sodium hypochloride for 2 minutes and rinsed 3 times with sterile distilled water and excess water was blotted by sterilized filter paper. Two of these explants cob piece were placed aseptically in a 250 ml erlenmeyer flasks containing 50 ml of basal medium. 4.3 g/l MS medium was supplemented with 440 mg calcium chloride, 500 mg/l activated charcoal, 30 g/l sucrose, and 8.0 g/l agar. The basal medium was further supplemented with different chemical as per the requirement of the various experiments. The pH of the medium was adjusted to 5.8 before autoclaving. Standard conditions were followed for autoclaving and inoculation. All the the cob pieces were positioned upright as in the ear position. The flasks were incubatd at room temperature (28±4°C) in the dark for 8 days.

Kernel fresh and dry weight : Intact kernel were excised by pressing forcep below the glume andd weighed . The fresh and dry weight of the kernel before and after 8 days culture was recorded. After seperating, kernels were dried at 60°C till constant weight. Kernel water content was calculated by substracting the dry weight from the fresh weight. The kernel growth rate i.e. mg/ kernel/day was calculated by dividing total number of days in culture.

Soluble sugars, protease and lipoxygenase were estimated with UV/vis spectrophotometer by the methods described earlier[6-8].

Statistical analysis : "F" test was applied to evaluate the significance of data.

Results and Discussion

Effect of Free Radical Quenchers : 1 mM of each free radical quenchers viz., thiourea, n-propyl gallate, Na-benzoate, Na-thiosulfate and salicylic acid were added separately to MS basal medium. Kernel dry weight was 9.7% and 12.5% greater with Sodium-benzoate and Na-thiosulfate than that observed for control basal medium. These two chemicals found to hasten the kernel mass more faster to parallel upsurge in growth rates than observed with other chemicals (Table 1). Thus it appears that reducing agents sodium thiosulfate[10] and sodium benzoate may lower the leakage of electrolytes and contents of malondialdehyde (MDA) and H_2O_2 as reported for maize callus culture under osmotic stress and paraquate (o.5 and 5mM/litre) treatments[11]. Kernel dry weight was higher with n-propylgallate and salicylic acid to the extent of 5% is in agreement with reported observation that maize infused salicylic acid produce 9% more kernel dry matter compared to control[12].

Effect of Paraquat : The effect of paraquat at four different concentrations on developing kernels during 8 days in culture are presented (Table 2). Kernels cultured on 0.5 mM and 1.0 mM paraquat compared with 30 g/l of sucrose had resulted in lowering of kernel dry matter by 42-57%, fresh weight by 30-43% and kernel water content by 20-30%. Paraquat at the level above 2.5 mM completely inhibited kernel growth and development. These results indicate the phytotoxic effects of paraquat leading to disruption of membrane integrity[13-15] probably via free radical generation[16].

These results are in agreement with the hypothesis that free radicals and lipid peroxidation are major contributor to paraquat toxicity[17,18].

Effect of lipoxygenase and protease activities on kernel sugar content : Role of lipoxygenase (LOX) and protease activities on the accumulation of ethanol soluble sugars by the kernels was investigated. Free radical quenchers (1 mM each) and paraquat (0.5mM) were added separately in the medium and the changes in the enzyme activities and ethanol soluble sugars were determined (Table 3).

Table 1. Effect of free radical quenchers on growth of *in vitro* culture maize kernels.

Treatments	Weight gain (mg kernel^{-1})		Water content (mg kernel^{-1})	Growth rate (mg kernel^{-1}day^{-1})		Water uptake (mg kernel^{-1} day^{-1})
	FB	DB		FB	DB	
Thiourea	81.66	37.00	44.66	10.20	4.62	5.58
n-Propyl gallate	83.33	38.00	45.33	10.41	4.74	5.66
Sodium benzoate	85.00	39.50	45.50	10.62	4.93	5.69
Sodium thiosulfate	86.94	40.50	46.44	10.86	5.06	5.80
Salicylic acid	83.66	38.20	45.46	10.45	4.77	5.68
Control	79.44	36.00	43.44	9.93	4.50	5.43
SEm ±	1.398	0.441	1.416	0.162	0.102	0.170
C.D. at 5%	4.30	1.359	NS	0.482	0.304	NS

- Kernels were harvested after 8 days in M.S. medium (10% M.S. salt + 30% sucrose). FB = fresh weight basis, DB = dry weight basis.
- All chemicals were added @ 1.0 mM.
- The mean of initial kernel fresh/dry mass and water content at 7 DAP was 68.76±1.18 mg/10.42±0.28 mg and 58.34±1.05 mg respectively.
- The data are mean of three replications.

Table 2. Effect of exposure to paraquat on growth of *in vitro* culture maize kernels.

Paraquat (mM)	Weight gain (mg kernel^{-1})		Water content (mg kernel^{-1})	Growth rate (mg kernel^{-1}day^{-1})		Water uptake (mg kernel^{-1} day^{-1})
	FB	DB		FB	DB	
0.5	55.33	20.80	34.53	6.91	2.6	4.32
1.0	45.05	15.20	29.85	5.63	1.90	3.73
2.5	10.61	1.36	9.25	1.33	0.173	1.156
5.0	10.39	1.35	9.04	1.30	0.170	1.130
Control	79.44	36.00	43.44	9.93	4.50	5.43
SEm ±	0.898	0.257	0.743	0.11	0.036	0.092
C.D. at 5%	2.831	0.811	2.343	0.35	0.115	0.290

- The Kernels were cultured on 10% M.S. salt medium for 8 days. FB = fresh weight basis, DB = dry weight basis.
- The mean of initial kernel fresh/dry weight and water content at 7 DAP was 68.69±1.35 mg/10.41±0.30 mg and 58.28±1.47 mg respectively.
- The data are mean of three replication; each kernel weight is mean of 6 kernels cob piece^{-1}.

Table 3. Effect of free radical quenchers and paraquat on lipoxygenase, protease activity and soluble sugars during *in vitro* culture maize kernel.

Chemical	Enzyme Activity		Ethanol Soluble Sugars
	LOX	Protease	
	Units min^{-1} g^{-1} fresh wt.	Units min^{-1} g^{-1} fresh wt.	(% dry weight)
Thiourea (1mM)	50	4.81	17.50
n-Propyl gallate (1mM)	45	4.71	17.50
Sodium benzoate (1mM)	55	5.85	16.25
Sodium thiosulfate (1mM)	60	5.93	16.00
Salicylic acid (1mM)	25	5.49	17.25
Paraquat (0.5mM)	105	7.86	13.00
Control	45	5.09	18.00
SEm ±	1.345	0.198	0.948
C.D. at 5%	4.08	0.603	2.875

The kernels were cultured on 10% M.S. salt medium with 30% sucrose for 8 days.

Kernel cultured on 0.5 mM paraquat had 133.3 and 54.4% higher lipoxygenase and protease activity, which lead to 27.7% lower levels of ethanol soluble sugars in the kernel than control.

This indicates involvement of paraquat base reactive oxygen species in the membrane lipid peroxidation and protein degradation. In contrast moderate increase in the level of LOX (22 to 33%) and protease (14.9 to 16.5%) by Na-benzoate and Na-thiosulfate did not significantly affect kernel ethano soluble sugar compared to control. Furthermore, significantly more sugars from thiourea, n-propylgallate and salicylic acid than paraquat or even decreased in lipoxygenase activity by 44.4% with salicylic acid compared to control. This result was in fair agreement with reported results[19] that the elimination of LOX resulted in a decrease in the level of protease inhibitors in soyabean seeds. These results indicated that suboptimal level of LOX and protease activity in the developing kernel tissues may be necessary but higher levels of the enzymes would contribute to inhibition of sugar utilization. The kernel intrinsic reactive oxygen species may be constrain for low productivity of maize grown under Indian tropical condition.

Acknowledgement

The authors are thankful to the Head, Department of Biochemistry, RAU, Bikaner for providing the necessary facilities to execute these studies.

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