Australian Journal of Basic and Applied Sciences, 3(2): 1104-1112, 2009
ISSN 1991-8178

The Role of Epibrassinolide in Improving Performance of *Cucurbita Pepo* Seeds Germination

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Abstract: In a trial for increasing performance of *Cucurbita pepo* (*C .pepo*) seeds (increased number of uniform, rapidly germinated seeds) via the priming technique a relatively high concentration of epibrassinolide (EB) was required to act as a priming agent. Using this concentration only 18% of the primed *C .pepo* seeds showed visible protruded radicles. In case of hydroprimed seeds, 28% of the primed seeds showed protruded radicles. Upon germination of dry, hydroprimed and EB-primed *C. pepo* seeds, the percentage of germination reached 78, 80 and 99 % respectively. The activity level of catalase, peroxidase and superoxide dismutase revealed a relatively low activity level in the dry seeds compared with that detected in hydroprimed and EB-primed seeds with the highest activity being obtained in EB-primed seeds .Through this way EB priming could reverse the detrimental effects of seed deterioration that occur during seed storage and counteract the effect of lipid peroxidation prevailed during seed germination. RAPD-PCR results showed that unique DNA fragment with different sizes were characteristic for EB-primed seeds. EB via enhancing the activity level of the above free radical scavenging enzymes had reduced DNA damage and / or induced the appearance of new DNA in the EB-primed seeds.

Key words: epibrassinolide, germination, priming, DNA, catalase, peroxidase and superoxide dismutase

INTRODUCTION

Rapid, uniform and complete emergence of seeds upon their germination is desirable attributes. However, optimum seed performance is seldom achieved. This lack of uniform performance may be attributed to differences in genetics, seed maturity, seed size, period and conditions of storage ….etc. (McDonald, 2000). Thus, a seed lot represents an amalgam of individuals, each with its own unique ability to germinate. But this lack of uniform performance is undesirable in agriculture, which demands that all seeds germinate rapidly and uniformly for optimum stand establishment. To minimize this problem growers tend to plant more seeds to achieve the desired stand.

The question here: why not all planted seeds succeed to germinate? The answer is that during seed storage, cellular damage occurs to some of them depending on the above mentioned factors. This cellular change led to seed deterioration. As seeds deteriorate a cascade of disorganization ensues ultimately leading to complete loss of cell function. The current model of seed deterioration accepts lipid peroxidation as a central cause of cellular degeneration through free radical assault on important cellular molecules and structures as mitochondrial dysfunction, enzyme inactivation and membrane perturbation. Most, if not all, of these effects undoubtley expected to decrease or led to loss of seed potential to germinate.

One of the modern advances in seed technology, aimed to increase seed performance to germinate, is called seed priming. Seed priming is defined as the uptake of H_2O to initiate the early events of germination but not sufficient to permit radicle protrusion.

In the present work, hydropriming of *C.pepo* seeds was carried out in absence and presence of 24-epibrassinolide in a trial to evaluate its efficiency in enhancing hydropriming technique.

MATERIALS AND METHODS

A- Materials:

The plant material used in the present investigation was the seeds of squash (*Cucurbita pepo* L. cv. Eskandarany). The seeds were obtained from the ministry of agriculture Giza, Arab Republic of Egypt.

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The chemical used in the present work was 24-epibrassinolide (EB) which was obtained from Sigma Chemical Company, St. Louis, Mo, USA. 24-epibrassinolide is an analogue of brassinolide which has potential use as a synthetic form of brassinolide.

B-Methods:

PCR-RAPD Analysis:

DNA was extracted according to the method of Junghans and Metzlaff (1991). Eleven mer primers from operon kit a (operon tech. INC, USA) were used for amplification of the extracted DNA .The sequences and GC ratios of these primers are shown in table (1).

A standard PCR reaction was performed in a total volume of 50 uL containing 2 mM $MgC1_2$, 200 uM each of dATP, dCTP, dGTP and dTTP, 50 pmol of each, 2.5 units of Tag polymerase and the DNA template. The reaction mixture was overlaid with a drop of mineral oil. The optimal condition for PCR reaction was as follows: denaturation at 94 °C for 6 min , 40 cycles of 94 °C for 1 min at 36 °C for 2 min and at 72 °C for 3 min and a final extension step at 72 °C for 1 min. The amplified products were assessed using 1.2% agarose electrophoresis in 1 X TAE (tris –acetate- EDTA), five ul DNA. Molecular size marker was also loaded as a standard. The gel was stained with ethidium bromide (Maniatis *et al.,* 1982).

Data analysis: the similarity coefficient between the three treatments was calculated based on pairwise base comparison between them for each particular primer using the formula:

$$S = \frac{2Nab}{Na + Nb} X100$$

where Na and Nb are the number of bands in individuals a and b. Nab is the number of shared (common) bands (Lynch, 1990)

Table I: The nucleotide sequences and GC ratios of 11 decamer primers used in RAPD-PCR analysis.

Primer code	Sequences 5¯- 3¯	% GC
OPA-03	AGTCAGCCAC	60
OPB-03	CATCCCCCTG	70
OPB-04	GGACTGGAGT	60
OPB-05	TGCGCCCTTC	70
OPB-09	TGGGGGACTC	70
OPB-11	GTAGACCCGT	60
OPO-14	AGCATGGCTC	60
OPC-04	CCGCATCTAC	60
OPC-19	GTTGCCAGCC	70
OPC-08	TGGACCGGTG	70
OPC-06	GAACGGACTG	60

Extraction and Assaying Activity of Certain Enzymes:

Enzyme Extraction:

The method adopted in enzyme extraction was that described by Mukheriee and Choudhuri (1983). A fresh tissue (250 mg) were frozen in liquid nitrogen and finely grounded by pestle in a chilled mortar. The frozen powder was added to 10 ml of 100 mM phosphate buffer (KH_2PO_4 / K_2HPO_4) (pH 6.8). The homogenates were centrifuged at 20000 xg for 20 minutes. The supernatant was made up to a known volume with the same buffer and used as enzyme preparation for assaying the activity of certain enzymes.

a) Superoxide Dismutase (SOD.EC.1.12.1.1) Assay:

SOD activity was measured according to the method of Dhindsa *et al* (1981). Three ml of reaction mixture contained 13mM methionine, 0.025 mM p–nitro blue tetrazolium chloride (NBT), 0.1 mM EDTA, 50mM Phosphate buffer (pH 7.8), 50 mM sodium bicarbonate and 0.5ml enzyme extract. Reaction was started by adding 0.002mM riboflavin and placing the tubes below two 15W fluorescent lamps for 15min. The reaction was stopped by switching off light and covering the tubes with black cloth. The tubes without enzyme developed maximal colours. A nonirradiated complete reaction mixture served as blank. The absorbance was measured at 560nm using Spekol Spectrocolourimeter VEB Carl Zeiss. The enzyme activity was calculated as change in absorbance g^{-1} F.wt. min^{-1}**.**

b) Peroxidase (POX, EC 1.11.1.7) Assay:

POX activity was assayed using the method of Bergmeyer (1974). The reaction mixture contained 5.8ml of 50mM phosphate buffer pH 7.0, 0.2 ml of the enzyme extract and 2.0ml of 20mM H_2O_2 after addition of 2.0ml of 20mM pyrogallol. The rate of increase in absorbance as pyrogallol is oxidized into purpurogallin was determined spectrophotometrically by Spekol Spectrocolourimeter VEB Carl Zeiss within 60 seconds at 470 nm and 25°C. The blank sample was made by using buffer instead of enzyme extract. The enzyme activity was expressed as change in absorbance g F.wt. min^{-1}.

c) Catalase (CAT, EC 1.11.106) Assay:

CAT activity was assayed according to the method of Chen *et al*. (2000). The reaction mixture with final volume of 10ml, containing 40 µl enzyme extract, was added to 9.96ml of H_2O_2 contained in phosphate buffer, pH 7.0 (0.16 ml of 30% H_2O_2 to 100 ml of 50 mM phosphate buffer). CAT activity was determined by measuring the rate of change of H_2O_2 absorbance in 60 second using a Spekol Spectrocolourimeter VEB Carl Zeiss at 250nm. The blank sample was made by using buffer instead of enzyme extract. The enzyme activity was calculated as change in absorbance g F.wt. min^{-1}.

Time Course Experiment:

In this work, a homologous lot of *C.pepo* seeds was selected to subject to hydropriming in presence of serial dilutions of epibrassinolide (EB) namely 10, 5.0, 2.5, 1.25 and 0.63 µM. To achieve the purpose, the seeds were arranged in five sets of Petri-dishes, each set comprises five Petri-dishes. Filter papers of the 1st, 2nd, 3rd, 4th and 5th sets were moisted with the dilution series of EB depicted above, respectively. The experiment was conducted at room temperature (25°C ± 2°C) for 5 days. At the end of this period, seeds were thoroughly rinsed with water, left to dry at room temperature on filter paper for at least two hours.

To exclude the variance expected to occur as a result of difference in water content between dry seeds and EB- primed ones, a third lot of seeds were arranged in Petri-dishes misted with water and left for the same period employed in EB-primed seeds, referred to as hydroprimed seeds.

RESULTS AND DISCUSSION

Effect of EB on the Number of Seeds with Visible Protruded Radicle:

The number of seeds with visible protruded radicle was recorded in each concentration used and the data were represented in table (2).

As the applied concentrations 5.0, 2.5, 1.25 and 0.63 µM visibly induced radicle protrusion with the highest figure being obtained on applying the lowest concentration (0.63 µM), this range of concentrations were eliminated and only the concentration 10 µM, which sharply reduced radicle protrusion (18.77%) was selected to be used in experiments designed to detect the action mechanism of EB as a priming agent.

The results represented in table (2) show that the number of seeds with visible protruded radicle was gradually decreased with each increase of the applied concentration of EB. Thus, whereas it reached 100% on applying the lowest concentration (0.63 µM) it was reduced to 90, 88.5, 35.5 and 18.77 % in case of applying the concentrations 1.25, 2.5, 5.0 and 10 µM of EB, respectively.

Table 2: Number of seeds with visible protruded radicle. Each value is a mean of ten variables ± SE.

Treatments, EB (µM)	% of seeds with visible protruded radicle
0.63	100 ± 1.154
1.25	90 ± 0.577
2.5	88.5 ± 0.577
5.0	35.5 ± 1.154
10	18.77± 0.011

Effect of EB as a Priming Agent on the % of Germination of C.pepo Seeds:

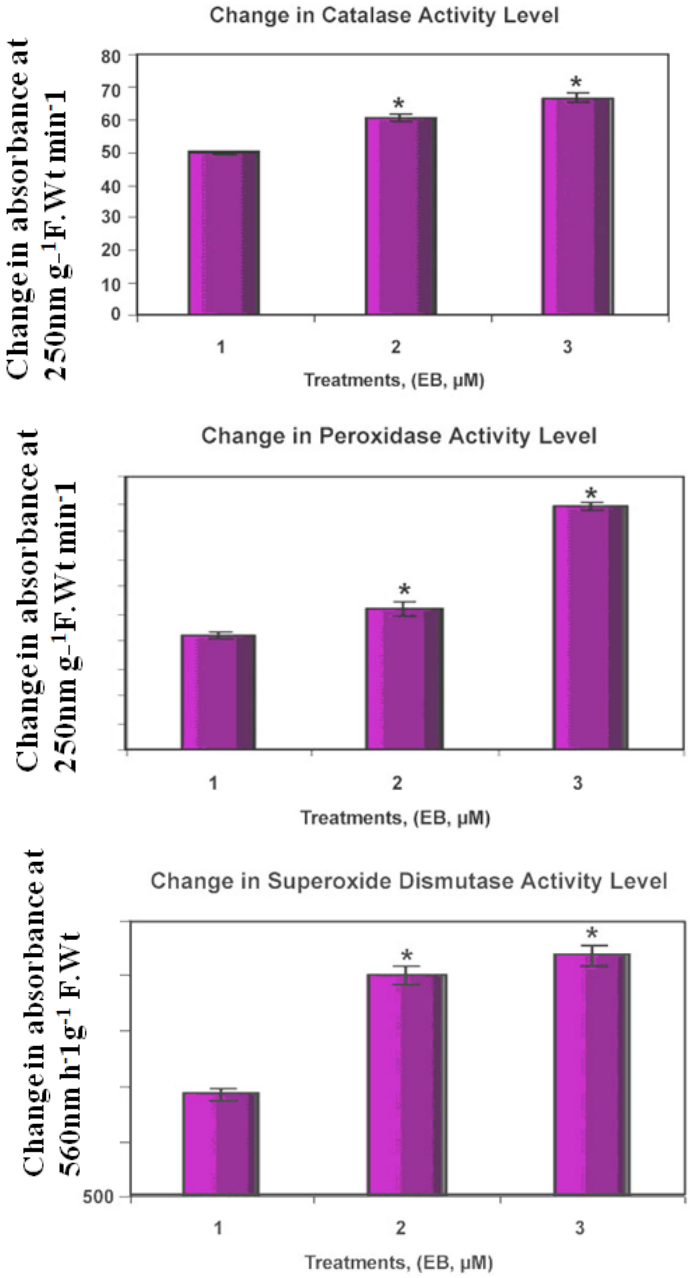
The dry, hydroprimed and EB-primed seeds were germinated in Petri-dishes in incubator (25±2.0°C) for five days and the percentage of germination in each was calculated and the results are represented in table (3). The results shown in table (3) indicate that whereas the % of germination of dry and hydroprimed seeds reached 78 and 80%, respectively it increased to 99% in EB-primed seeds.

Effect of EB as a Priming Agent on the Activity Level of Catalase, Peroxidase and Superoxide Dismutase: The activity levels of catalase, peroxidase and superoxide dismutase were assayed in dry, hydroprimed and EB-primed seeds and the results obtained are represented graphically in figure (1). The results of the enzymes assayed were statistically analysed using the least significant difference at 5% level.

Table 3: Percentage of germination. Each value is a mean of ten variables ± SE.

Sample	% of Germination
Dry seeds	78 ± 1.15
Hydroprimed seeds	80 ± 1.73(NS)
EB-primed seeds	99 ± 0.58(+HS)
L.S.D at 5% level	4.31
L.S.D at 1% level	6.54

It is evident from figure (1) that the activity level of catalase, peroxidase and superoxide dismutase was relatively low in dry seeds if compared with that detected in hydroprimed and EB-primed seeds, with the highest activity being obtained in EB-primed seeds.



1- Dry Seeds 2- Hydroprimed Seeds 3- EB-primed Seeds

Fig. 1: Effect of Epibrassinolide (EB) as a priming agent on the activity level of catalase, peroxidase and superoxide dismutase. Each value is a mean of three replicates ±.SE * Refers to a significant change.

RAPD analysis has been performed in the present study to evaluate the variability in the genomes of squash seeds challenged with EB treatment, thereby to detect the molecular changes associated with priming of squash seeds in the presence of EB and to elucidate the mechanism of epibrassinolide at the gene level. Eleven-mer primers (sequences are shown in the methods, table 1) were used to screen the genomic DNA. Ten primers (OPA-03, OPB-04, OPB-05, OPB-09, OPB-11, OPB-14, OPC-04, OPC-19, OPC-08, and OPC-06) could efficiently align genomic DNA of squash seeds; however the primer OPB-03 gave poor reproducibility (Tables 4, 5 & Figure 2). Table (4) shows molecular sizes and number of DNA fragments that represent positive or negative markers for dry, hydroprimed and EB-primed squash seeds.

Table 4: PCR-RAPD Pattern of Genomic DNA of Dry, Hydroprimed and EB-Primed *C.pepo* Seeds Using Eleven-Mer Random Primers

Primers	Samples No.	No. Of band												
		1	2	3	4	5	6	7	8	9	10	11	12	Total No. Of Bands
OPA-03	1	+	+	+	-	+	+	+	+					7
	2	+	+	+	-	+	+	+	+					7
	3	+	+	+	+	+	+	+	+					8
	Molecular size (bp)	2000	1500	1100	900	700	600	400	300					
OPB-03	1	+	-	-	-	+	+	+	-	+	-	-	-	5
	2	+	+	-	+	-	-	-	+	-	+	+	+	7
	3	+	-	+	-	+	-	-	-	+	-	+	+	6
	Molecular size (bp)	1200	900	850	750	700	650	550	500	450	400	350	300	
OPB-04	1	+	+	+	+	-	+	+	+	+	+	+		10
	2	+	+	-	-	+	+	+	-	+	+	+		8
	3	+	+	-	+	+	+	+	-	+	+	+		9
	Molecular size (bp)	1700	1550	1200	1000	900	700	650	600	500	300	250		
OPB-05	1	+	+	+	+	+	-	+	+					7
	2	+	+	+	+	+	-	+	+					7
	3	+	+	+	+	+	+	+	+					8
	Molecular size (bp)	1300	1250	1000	900	800	700	400	380					
OPB-09	1	+	+	+	+	+	-	+	+	-				7
	2	+	+	+	+	+	+	+	+	-				8
	3	+	+	+	+	+	-	+	-	+				7
	Molecular size (bp)	1500	1250	1200	900	800	650	500	400	350				
OPB-011	1	+	+	+	+	+								
	2	+	+	+	+	+								
	3	+	+	+	+	+								
	Molecular size (bp)	900	700	600	400	250								
OPO-14	1	+	+	+	+	+	+	+						7
	2	+	+	+	+	+	+	+						7
	3	+	+	+	+	+	-	+						6
	Molecular size (bp)	1400	1300	1000	800	650	400	250						
OPC-04	1	-	+	+	+	+	+	+						6
	2	+	+	+	+	+	+	+						7
	3	+	+	+	+	+	+	+						7
	Molecular size (bp)	1400	1350	1000	900	700	500	400						
OPC-019	1	+	+	+	-	+	+	+	+	+	+	+		10
	2	+	+	+	-	+	+	+	+	+	+	+		10
	3	+	+	+	+	+	+	+	+	+	+	+		11
	Molecular size (bp)	1500	1400	1300	1100	1000	950	850	800	700	600	550		
OPC-08	1	+	+	+	-	+	+	+	-	-	+			7
	2	+	+	+	-	+	+	+	+	-	+			8
	3	+	+	+	+	+	+	+	-	+	+			9
	Molecular size (bp)	1200	1000	950	900	700	600	500	450	400	250			
OPC-06	1	+	+	+	+	+	+	+	+	+	+	+	+	12
	2	-	-	-	+	+	+	+	+	+	+	+	+	9
	3	-	-	-	+	+	+	+	+	+	+	+	+	9
	Molecular size (bp)	2500	2000	1200	1150	750	700	600	550	450	400	300	250	

+ Amplification Fragment
- Non Amplification Fragment
Sample No. 1 Dry seeds
2 Hydroprimed seeds
3 EB – Primed seeds

Table 5: PCR-RAPD patterns of genomic DNA (with 11-mer random primers) of *C. pepo* seeds, dry seeds (A), hydroprimed seeds (B) and EB-primed seeds (C), distriibution of monomorphic, polymorphic bands and similarity Coefficients

Primer codes	RAPD fragments			Monomorphic Fragments				Polymorphic fragments					Similarity coefficients		
	A	B	C	Total	A/B	A/C	B/C	A,B/C	A/B	A/C	B/C	A,B/C	A/B	A/C	B/C
OPA-03	7	7	8	22	7	7	7	7	-	1	1	1	100	93.33	93.33
OPB-03	5	8	6	18	1	3	4	1	7	5	6	11	15.4	54.5	57.14
OPB-04	10	8	9	27	7	8	8	7	4	3	1	4	77.8	84.21	94.12
OPB-05	7	7	8	22	7	7	7	7	-	1	1	1	100	93.33	93.33
OPB-09	7	9	7	23	7	6	6	6	2	2	2	3	87.5	85.7	75.0
OPB-11	5	5	5	15	5	5	5	5	-	-	-	-	100	100	100
OPO-14	7	7	6	20	7	6	6	6	-	1	1	1	100	92.3	92.3
OPC-04	6	7	7	20	6	6	7	6	1	1	-	1	92.3	92.3	100
OPC-19	10	10	11	31	10	10	10	10	-	1	1	1	100	95.2	95.2
OPC-08	7	8	9	24	7	7	7	7	1	2	3	3	93.33	87.5	82.4
OPC-06	12	9	9	30	9	9	9	9	3	3	-	3	85.1	85.1	100
Total	83	85	85	253	93	74	78	85	18	20	16	29	-	-	-

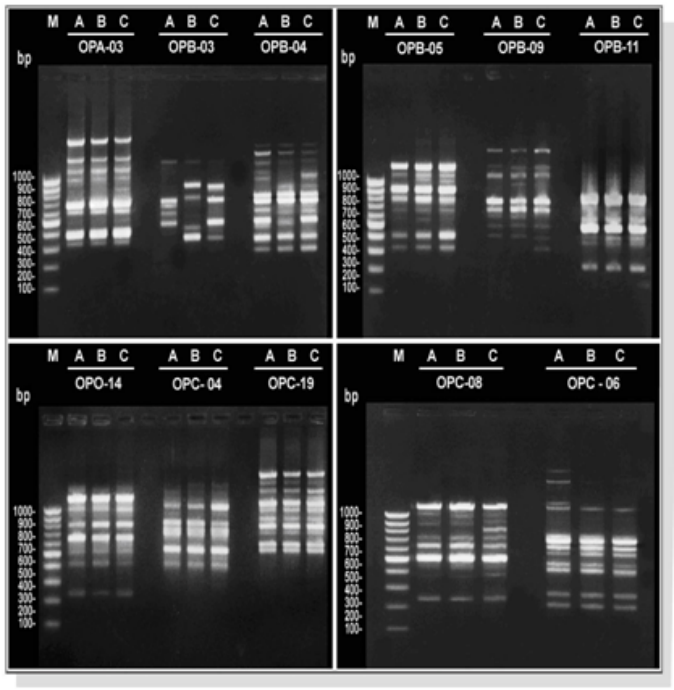


Fig. 2: DNA polymorphism using randomly amplified polymorphic DNA-RAPD for dry (A), hydroprimed (B) and EB-primed

All amplifications were found to be reproducible when repeated at different times under the same amplification conditions. The primers used in this investigation generated 253 fragments distributed as 83 fragments in dry squash seeds, 85 fragments detected in both hydroprimed and EB- primed squash seeds (Table 5). EB-priming and hydropriming stimulated the production of new RAPD-fragments while other fragments disappeared compared with those of dry seeds (Tables 4, 5& Figure 2). Primer OPC-19 produced the highest number of fragments, up to 31 fragments, while primer OPB-11 showed the lowest number of RAPD fragments where it reached around 15 fragments. Different primers also have different performances for evaluation of genetic polymorphism. The extensive polymorphism detected among the dry, hydroprimed and EB-primed squash seeds measure the degree of changes occurs in DNA sequences. The pattern generated by using primer OPB-03 clarify the responsive changes occurred in DNA fragments in response to EB-treatment, compared with both dry and hydroprimed seeds (Tables 4 & 5). This primer showed that, the similarity coefficient was comparable in squash seeds subjected to EB-priming (C) and hydroprimed seeds (B) (around 55% similarity coefficient). On the other hand, it was completely different in dry seeds (A) (around 15.4 % similarity coefficients). Furthermore, the pattern of RAPD fragments generated by using primers OPA-03, OPB-03, OPB-05, OPC-08 and OPB-09, OPC-19 explain the responsive changes occur in DNA fragments in response to EB treatment as compared with those of hydroprimed and dry squash seeds (Figure 2).

Primer OPB-03 produced fragments with similarity coefficient ranged from 15.4, 54.5, and 57.14 in dry, hydroprimed and EB-primed seeds, respectively. On the other hand, the primers OPB-04 and OPC-06 gave 77.8 and 85.1 % similarity coefficient in dry seeds, 84.21 and 85.1 % in hydroprimed seeds and 94.12 & 100 % similarity coefficients in EB-primed seeds. On the other hand, the primer OPB-11 (Tables 4, 5 & Figure 2) showed the specific pattern of squash seeds.

Discussion:

The results represented by table (2) refer to the validity of a relatively high concentration (10 µM) of EB in priming technique where radicle protrusion was highly reduced where it appeared in only 18.77% of the tested seeds. Meanwhile the other applied concentrations enhanced appreciably radicle protrusion (37.5, 87.5, 90 &100%) in response to 5.0, 2.5, 1.25 and 0.63 µM, respectively. EB is reported to enhance water inflow (Takeuchi *et al*. 1995). Thus, the obvious decrease in EB efficiency in priming of *C.pepo* seeds encountered on applying relatively low concentrations could be explained on the basis of increased water content of the EB-treated seeds and which bypass the threshold of active metabolic changes which induce radicle protrusion.

To realize the mode of action of the highest applied concentration of EB (10 µM) in enhancing seed priming, analyses were made in the dry, hydroprimed and EB- primed seeds. The results represented by table (2) show that whereas the percentage of germination of dry and hydroprimed seeds was 78 and 80 % respectively, it increased to 99% in EB-primed seeds.

One of the main causes of decreased percentage of seed germination is their deterioration during storage. The most frequently cited case of seed deterioration is lipid peroxidation (McDonald, 1999), with the result of the release of free radicals. Once the free radicals are accumulated, they create profound damage to membranes, change protein structure of seeds (Dell´Aquila, 1994, Jeng & Sung, 1994, Kalpana & Madhava Rao, 1997a). Free radicals are also suspected of assault on chromosomal DNA (Larson, 1997), leading to genetic mutation and loss of DNA (Cruz-Garcia *et al*. 1995, Kalpana & Madhava Rao 1997b). Many of these mutations delay the onset of mitosis necessary for cell division and germination (Begnami & Cortelazzo, 1996). Thus, one of the main features of seed deterioration is the predominance of elements of oxidative stress. Moreover, during seed germination a high production of toxic O_2 species can be expected in view of the high O_2 consumption and respiratory activity following imbibition of water (Bewley & Black 1994). So, to clarify the participation of EB in seed priming, its effect on the main enzymes having a crucial role in alleviating oxidative stress, mainly catalase, peroxidase and superoxide dismutase were assayed in the present work. The results represented by figure (1) refer to a relatively low activity level of these enzymes in dry seeds when compared with that detected upon water imbibition of hydroprimed and EB-primed seeds, with the highest activity being obtained in EB-primed seeds. In this connection, it must be remembered that upon imbibition of water, the cascade of cellular damage caused by autoxidation is furthered by free-radical damage. Thus, in addition to the cellular damage established during storage, the damage associated with water imbibition leads to unalterable detrimental physiological consequences which led to a nongerminable seeds (McDonald, 1999), resulting in reduced percentage of germination, lower seed performance. However, if germination medium was enriched with antioxidants or the seeds were pre-primed with the treatments which could counteract the hazards of oxidative stress and /or reverse the detrimental effects occur during seed dessication, high seed performance could be achieved. The results obtained in the present work clearly show that the lowered percentage of seed germination (≈78%) of *C.pepo* seeds is mainly attributed to membrane competency problems associated with lipid peroxidation and which is expected on the basis of a relatively low activity level of the free radical scavenging enzymes detected in the dry seeds (catalase, peroxidase and superoxide dismutase). On the other hand, obvious increase in their activity level was observed in hydroprimed seeds, however it was not quite enough to remove a lot of the free radicals and this was expressed in a relatively low % of germination if compared with EB-primed seeds. In response to seed priming in EB solution (10 µM) significant increase in the activity level of these enzymes was detected, if compared with the dry and hydroprimed seeds.

Thus, EB via increasing the activity level of these lipid-peroxidation-detoxifying enzymes could reverse the detrimental effects of seed deterioration and counteract the effect of lipid peroxidation prevailed with seed germination.

In the present experiment, RAPD analysis has been performed to evaluate the variability in *C.pepo* genomes challenged with EB-treatment, thereby to detect the molecular changes associated with priming *C.pepo* seeds in the presence of EB. In the present work, eleven-mer primers were used. The results obtained refer to that ten primers of them (A3, B4, B5, B9, B11, B14, C4, C19, C8 & C6) could efficiently align genomic DNA of *C.pepo* seeds, while primer B3 gave poor reproducibility. On using different primers, unique DNA fragments with different sizes were characteristic for EB-primed seeds. Thus, EB-primed seeds exhibited the

DNA fragments: 900bp with primer A3, 850 bp with primer B3, 700 bp with primer B5, 350bp with primer B9, 1100 bp with primer C19, 900 and 400 bp with primer C8. All the above fragments were not detected in dry or hydroprimed *C.pepo* seeds.

Thus, it is evident that the different used primers have different performances for evaluation of genetic polymorphism. The extensive polymorphism detected among dry, hydroprimed and EB-primed *C.pepo* seeds measure the degree of change occurs in DNA sequences.

The results of RAPD-PCR indicated the existence of differences in RAPD fragments. The quantitative polymorphism obtained might be due to the changes of some regions of the nucleotide sequences aligned by arbitrary primers as a result of the promotive effects of EB or due to the enhancement of annealing efficiency between primers and DNA templates by activating the recognition of sequences and / or activation of Tag polymerase activity by the steroidal hormones.

In this regard, a general consensus refer to that degradation of DNA belong to the main reasons of seed deterioration (Clark & James 1991). This lead to impaired transcription causing the incomplete or faulty synthesis of enzymes essential for the earliest stages of germination (Sung and Chang, 1993). Moreover, it was observed that *in vivo* protein synthesis began 12 hrs after imbibition of deteriorated legume seeds and suggested that this was due to degradation of pre-existing "long-lived" mRNA that is unable to be rapidly and properly transcribed (Gidrol *et al*. 1988).

Also, the free radicals associated with water imbibition are suspected of assault on chromosomal DNA (Larson, 1997). Thus, EB via enhancing the activity level of free radical scavenging enzymes could reduce the incidence of DNA damage, explaining the appearance of new DNA in EB-primed seeds. In this connection, numerous studies have demonstrated that priming is associated with an increase in protein synthesis (Bray *et al*. 1989, Dell´Aquila & Spada, 1992) as well as in nucleic acid synthesis and repair (Bray *et al.* 1989, Bray 1995).

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