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Effect of Sodium-Dikegulac on Maintenace of Viability of a Few Crop Seeds Under Adverse Storage Conditions

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Abstract

In short-term accelerated ageing (98.2% relative humidity, RH) experiment for 21 days, leaching of sugar and amino acids from the seeds of gram (*Cicer arietinum*), soybean (*Glycine max*), sunflower (*Helianthus annuus*) and safflower (*Carthamus tinctorius*) cultivars steadily increased with the progress of ageing duration. Such deleterious changes were associated with proportional reduction in the levels of protein, RNA, insoluble carbohydrate and the activity of total dehydrogenase enzyme in the seed kernels along with reduction in percentage TTC-stained seeds. Pretreatment of the gram, soybean, sunflower and safflower seeds with sodium-dikegulac (Na-DK, 2, 3 : 4-6-di-O-iso-propylidene-α-L-xylo-2-hexalofuranosate) significantly arrested the leakage of soluble substances and checked the declining of the levels of some vital cellular components such as carbohydrate, protein, RNA and total dehydrogenase enzyme and also of the percent TTC-stained seeds. Accelerated ageing-induced damage in overall cellular metabolism and its substantial alleviation by Na-DK were conclusively proved from the accelerated ageing experiments.

Deterioration of seeds is a natural catabolic process which terminates their life-span resulting in loss of viability. This process may be accelerated by some pathogenic attack and/or by adverse environmental conditions. This inevitable detrimental processes leading to deterioration of seeds is a matter of concern to the seed physiologists and crop growers all over the world. The problem of retention of seed viror in Darjeeling and surrounding areas of India is more acute because of extremely high RH which is conducive to the growth of microorganisms resulting in epeditious deteriopration of seeds. As most crop seeds require storage for either one or several planting seasons, agriculturists and horticulturists of this region are often handicapped by non-availability of standard vigor seeds for healthy seedling production. Keeping in mind this problem of seed storing in the hilly region of Darjeeling, an attempt was made in this investigation to prolong the storage life of a few crop seeds having viability problems. All experiments of this investigation were performed at accelerated ageing conditions just to expedite experimental processes. In fact, accelerated ageing treatment provides a powerful manipulative tool which makes it possible to study the process of seed deterioration over a short period (1) and this mimics the natural ageing process (2, 3).

Although efficacy of several classes of chemicals, namely, hormones, retardants, redox chemicals, phenols, vitamins and some salts on maintenance of seed health under storage has been reported (4, 5), Na-DK is a recent addition to this group of chemicals (6) and its effect on seed viability on a wide range of seeds should be established. Being stimulated from the literature on the regulatory action of Na-

DK on delaying of plant senescence (7, 8), the present investigators tested the possibility of this chemical on the regulation of seed senescence of a varied group of crops under storage environment.

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Methods

Plant Materials. Experiments of the present investigation were carried out with certified seeds of gram (*Cicer arietinum* L. cv B-108), soybean (*Glycine max* L. cv. DS-73—16), sunflower (*Helianthus annuus* L. cv. Morden) and safflower (*Carthamus tinctorius* L. cv JLA-900), procured from the Government Oil Seeds and Pulses Research Station, Berhampore, West Bengal, India.

Seed Pretreatment. After surface sterilization (0.1% $HgCl_2$ for 90 seconds) all the seed varieties were separately presoaked in the aqueous solutions of 100, 2000 and 4000 μg/ml sodium dikegulac (2, 3:4-6-di O-isopropylidene-α-L-xylo-2-hexalofuranosate) or distilled water for 6 hours and dried back to original weight of seeds with the moisture contents ranging from 6.45 to 6.98%. At 48-hour intervals such soaking drying treatments were repeated thrice to make the total duration of pretreatment to 18 hours. This mode of pretreatment enabled maximum penetration of the chemical while avoiding the commencement of germination. The pretreated seed lots (200 g each) were put into separate cloth bags and thus stored in a desiccator in which an environment of 98.2% RH was preimposed by keeping 250 ml 5.96% H_2SO_4 (vol/vol) within it. This experimental setup was kept at 19±1 C allowing the seeds to experience forced ageing treatment and H_2SO_4 was changed periodically to restore the desired RH within the desiccator throughout the experimental period. Starting from 0-day, analyses were made at 7-day intervals upto 21 days after imposition of accelerated ageing condition, and then the experiment was terminated.

Biochemical Analyses. Soluble carbohydrate level from the seed leachates were analyzed after immersing 1 g seed sample of gram, soybean, sunflower and safflower cultivars in 20 ml deionized distilled water for 16 hours. This was determined following the method of McCready et al. (9) with anthrone reagent using glucose as reference standard. Samples for determining free amino acids were taken from the same seed leachates and amino acid level was quantified as per the method of Manglik (10). Test tubes containing 1 ml seed leachate and 3 ml of 0.1% ninhydrin solution (in 80% ethanol) were kept in a water bath for 30 minutes with glass marbles at the top of the test tubes. When the reaction mixture turned to violet color, the test tubes were taken out, cooled and the volume was made upto 4 ml with 80% ethanol. The absorbance of the solution was measured at 580 nm in a spectrophotometer. Quantitative estimation was made by comparing the OD values of the standard curve prepared from glycine.

Samples for protein were taken from seed kernels of each cultivar. Extraction of protein was made following the method of Kar and Mishra (11), and estimation was done using folinphenol reagent according to the method of Lowry et al. (12). Extraction of RNA was made from seed kernels following the method described by Cherry (13) and quantitative estimation was done as per the method described by Markham (14) modified by Choudhuri and Chatterjee (15).

Extraction of soluble and insoluble carbo-

Table 1. Effect of accelerated ageing and seed pretreatment with Na-DK on leaching of soluble carbohydrate (mg/g per 20 ml) from seeds of a few cultivars. Seeds were presoaked with 0, 1,000, 2,000 and 4,000 μg/ml Na-DK for 18 hours. The pretreated seed lots were then subjected to accelerated ageing treatment at 98.2% in a desiccator. Data were recorded from seed leachate after immersing 1 g seed sample of each cultivar in 20 ml distilled water for 16 hours.

Cultivar	Pretreatment	(μg/ml)	Days after accelerated ageing 0	7	14	21
Gram	Na-DK	0	2.88	4.45	8.98	9.12
		1000	2.95	4.06	6.20	7.45
		2000	2.95	3.92	5.95	7.22
		4000	3.14	4.18	5.88	7.04
	LSD	(P=0.05)	0.14	0.32	0.70	0.72
Soybean		0	7.08	13.62	22.30	24.50
		1000	7.18	12.15	17.25	21.42
		2000	7.39	11.28	16.55	19.80
		4000	7.56	12.30	16.08	19.62
	LSD	(P=0.05)	0.45	1.05	1.95	2.01
Sunflower		0	2.37	4.80	7.38	8.55
		1000	2.42	3.95	5.05	6.24
		2000	2.45	3.80	4.80	5.90
		4000	2.56	4.10	4.78	5.88
	LSD	(P=0.05)	0.15	0.37	0.58	0.65
Safflower		0	2.18	4.56	6.69	7.25
		1000	2.24	3.58	5.18	5.25
		2000	2.25	3.40	4.88	4.86
		4000	2.40	3.35	4.72	4.80
	LSD	(P=0.05)	0.12	0.30	0.60	0.55

hydrates was made from 100 mg kernels of each seed type with 5 ml 80% boiling ethanol in a mortar. After thorough homogenization, this was centrifuged at 6,000 g for 10 minutes. The supernatant was taken in a test tube. This extraction was repeated thrice, and from the pooled supernatant (10 ml) soluble carbohydrate was analyzed. For the analysis of insoluble carbohydrate, the residue after centrifugation of the sample was digested with 5 ml 25% H_2SO_4 (vol/vol) at 80 C in a water bath for 30 minutes. The extracted material was taken as source of insoluble carbohydrate. Estimation of both the fractions of carbohydrate was done following the method of McCready et al. (9).

To determine TTC stainability, four groups of 40 dehusked seeds of each sample were allowed to imbibe 1% TTC (2, 3, 5-triphenyl tetrazolium chloride) solution (wt/vol) in separate beakers and kept them 8 hour in dark condition. Subsequently, percentage TTC-stained (red colored) seeds was calculated from the total stained seeds of each cultivar. This method was adopted after Halder (16). The activity of total dehydrogenases of intact seeds was analyzed by the reaction of TTC according to the method of Rudrapal and Basu (17).

Statistical Analysis. All the data were statistically analyzed at the treatment and replication levels, and least significant difference (LSD) were calculated at 95% confidence limits (18).

Table 2. Effect of accelerated ageing and seed pretreatment with Na-DK on leaching of free amino acids (mg/g per 20 ml) from seeds of a few cultivars. Data were recorded from seed leachate at 7-day intervals upto 3 weeks of accelerated ageing. NS, Not significant.

Cultivar		Pretreatment (μg/ml)	Days after accelerated ageing 0	7	14	21
Gram	Na-DK	0	1.43	2.71	5.69	10.05
		1000	1.47	2.45	4.12	8.18
		2000	1.52	2.05	3.96	7.32
		4000	1.55	2.20	3.90	7.09
	LSD	(P=0.05)	NS	0.16	0.42	1.01
Soybean		0	2.68	5.02	10.85	25.00
		1000	2.78	4.15	8.52	20.80
		2000	2.86	3.80	7.45	17.98
		4000	2.95	3.98	7.28	17.05
	LSD	(P=0.05)	0.16	0.38	0.88	1.70
Sunflower		0	0.95	1.70	3.67	9.53
		1000	1.04	1.50	3.02	7.05
		2000	1.10	1.39	2.80	6.67
		4000	1.08	1.48	2.73	6.60
	LSD	(P=0.05)	0.09	0.12	0.30	0.80
Safflower		0	0.30	0.58	1.23	3.12
		1000	0.35	0.48	1.00	2.40
		2000	0.40	0.46	0.95	2.15
		4000	0.40	0.45	0.91	2.06
	LSD	(P=0.05)	0.05	0.04	0.09	0.19

Results

Table 1 shows that leaching of soluble carbohydrates occurred from all seed lots and the magnitude of leaching showed positive correlation with the duration of accelerated ageing. Data further reveal that the 0-day after ageing Na-DK at its highest concentration significantly increased leaching but during subsequent analyses the pretreating chemical, regardless of its concentrations, checked higher leakage of sugars in all the seed species.

Accumulation of amino acids in seed leachates went on increasing in water-soaked seed samples of all the crop seeds with the advancement of ageing duration. The same trend was found in the chemical-pretreated seed samples, but here the rate of increase was considerably slowed down at all the concentrations. However, the chemical initially increased the amino acid level at the 0-day after ageing treatment (Table 2).

Table 3 shows that unlike the changes of leaky products, protein content of seed kernels gradually declined keeping pace with the days of accelerated ageing both in water-soaked and Na-DK pretreated seed samples of all the four cultivars. Out of the four sampling periods (0, 7, 14 and 21 days after seed ageing), initially the chemical reduced the protein level over control values; at the second sampling period the adverse effects were erased and during two subsequent sampling periods, the chemical partially averted the adverse effects as evidenced by higher protein level in Na-DK presoaked seeds.

Overall changing pattern of RNA level (Table 4) was identical with that of protein. However, the changes noted at 0-day after acc-

Table 3. Effect of accelerated ageing and seed pretreatment with Na-DK on protein (mg/g wet weight) contents of the seeds of a few cultivars. Data were recorded from seed kerne's at 7-day intervals upto 3 weeks of accelerated ageing. NS, Not significant.

Cultivar		Pretreatment (μg/ml)	Days after accelerated ageing 0	7	14	21
	Na-DK	0	138.7	99.0	89.4	81.5
		1000	132.6	105.7	97.8	88.7
Gram		2000	126.8	105.0	101.6	91.9
		4000	124.9	100.9	98.7	93.5
	LSD	(P=0.05)	NS	NS	8.60	7.06
		0	190.8	135.5	120.6	105.8
		1000	182.8	143.4	133.8	118.8
Soybean		2000	170.6	139.8	136.0	125.7
		4000	167.5	132.6	128.5	126.0
	LSD	(P=0.05)	17.58	NS	10.08	10.12
		0	88.5	72.9	61.5	53.1
		1000	84.7	76.7	70.5	61.8
Sunflower		2000	78.8	74.0	72.0	64.3
		4000	78.0	75.2	69.3	66.0
	LSD	(P=0.05)	7.80	NS	6.07	4.88
		0	83.30	64.6	52.3	49.5
		1000	78.5	71.5	62.8	57.0
Safflower		2000	76.7	68.8	65.0	61.5
		4000	76.2	68.6	65.6	63.0
	LSD	(P=0.05)	NS	NS	5.85	5.88

elerated ageing were found insignificant in all the seed samples. Na-DK, regardless of its concentrations, substantially alleviated the gradual fall of RNA level in all the seed samples.

Table 5 reveals that with the progress of age ing, soluble carbohydrate level in the kernels of four crop seeds increased upto 14 days after accelerated ageing which is followed by a decrease after 21 days. In chemical pretreated seeds the same trend was apparent except that it occurred at a much slower pace. Unlike the changes of protein and RNA levels, Na-DK initially increased the level of internal soluble carbohydrates over control values.

So far the overall changes of insoluble carbohydrate level is concerned, a clear reverse picture was noted against the changes of soluble carbohydrate level. All the concentration, of Na-DK were found almost equally efficient in averting the loss of insoluble carbohydrate content to a considerable extent (Table 6).

Initial percentage of TTC-stained seeds of gram, soybean, sunflower was found 100, 90, 80 and 85 respectively. This started declining with the progress of ageing, and at the final observation period, percent TTC-stained seeds in water-soaked samples was reduced to 40, 40, 50 and 36 in gram, soybean, sunflower and safflower cultivars respectively. This reduction was substantially arrested with Na-DK (Table 7).

Table 8 shows that activity of total dehydrogenase enzyme gradually declined with the advancement of accelerated ageing days both in control and in chemical pretreated seed samples regardless of cultivars. But the speed of fall

Table 4. Effect of accelerated ageing and seed pretreatment with Na-DK on RNA (μg/g wet weight) contents of the seeds of a few cultivars. Data were recorded from seed kernels at 7-day intervals upto 3 weeks of accelerated ageing. NS, Not significant.

Cultivar		Pretreatment (μg/ml)	Days after accelerated ageing 0	7	14	21
	Na-DK	0	995.4	843.5	626.0	562.4
		1000	962.8	890.0	765.8	672.8
Gram		2000	950.5	900.5	798.0	695.6
		4000	943.8	885.4	780.8	692.0
	LSD	(P=0 05)	NS	NS	68.08	54.98
		0	658.8	549.8	409.2	379.2
		1000	641.7	597.5	501.0	462.7
Soybean		2000	630.5	505.2	526.6	490.5
		4000	621.6	588.6	518.0	478.7
	LSD	(P=0.05)	NS	52.06	49.18	40.08
		0	935.8	781.8	594.9	522.7
		1000	902.7	835.6	682.7	608.2
Sunflower		2000	887.6	847.0	728.2	647.4
		4000	875.5	840.8	707.5	625.6
	LSD	(P=0.05)	NS	60.28	62.50	54.72
		0	833.5	699.8	541.2	465.6
		1000	810.0	737.0	619.5	548.8
Safflower		2000	786.8	749.9	638.9	580.5
		4000	773.9	735.5	628.0	559.2
	LSD	(P=0.05)	NS	NS	49.90	48.88

of the enzyme activity was found to occur slowly in latter cases. The changes noted at the initial observation period were statistically insignificant in all the crop seeds.

Discussion

Maintenance of vigor and viability of seeds is an important problem in agriculture and horticulture. The two environmental factors, namely, temperature and humidity have profound influence on seed health under storage. In recent years some physical and chemical manipulative techniques have been developed by seed physiologists to get rid of such climatic and biotic hazards which are conducive to earlier deterioration of stored seeds (19—21).

The present study shows that high RH treatment stimulated the ageing processes of gram, soybean, sunflower and safflower seeds at accelerated ageing condition. Data revealed that the leakage of soluble carbohydrates (Table 1) and amino acids (Table 2) from the forced aged seeds progressively increased with the ageing duration, and this increase of leaky substances was considerably checked by seed pretreatment with Na-DK. The results are indicative of the fact that accelerated ageing caused to damage membrane which consequently resulted in higher leakage of soluble substances, and Na-DK alleviated this deleterious effect to a considerable extent. There are reports that at accelerated ageing condition, along with fall of seed germination, phospholipid and phosphatidyl choline—the important components of membrane also decline (22, 23) leading to loss of membrane integrity. This loss is reflected in enhanced leaching of organic and inorganic metabolites from seeds into the imbibing medium (16, 24, 25). Na-DK-induced changes of

Table 5. Effect of accelerated ageing and seed pretreatment with Na-DK on soluble carbohydrate (mg/g wet weight) contents of the seeds of a few cultivars. Data were recorded from seed kernels at 7-day intervals upto 3 weeks of accelerated ageing. NS, Not significant.

Cultivar		Pretreatment (μg/ml)	Days after accelerated ageing 0	7	14	21
	Na-DK	0	22.5	26.8	40.8	36.6
		1000	25.0	26.2	35.0	28.5
Gram		2000	27.0	27.8	32.8	27.6
		4000	26.8	27.5	29.9	27.0
	LSD	(P=0.05)	2.18	NS	3.28	2.08
		0	35.0	40.6	58.7	51.4
		1000	37.7	39.0	47.7	44.5
Soybean		2000	40.5	42.1	45.6	42.7
		4000	40.7	42.5	45.0	41.9
	LSD	(P=0.05)	3.25	NS	4.32	3.70
		0	24.8	29.4	43.8	38.5
		1000	26.2	27.5	38.9	33.9
Sunflower		2000	29.0	29.8	32.8	33.0
		4000	29.5	30.6	31.7	31.2
	LSD	(P=0.05)	2.03	2.70	3.08	2.54
		0	21.5	24.3	38.7	35.5
		1000	25.6	27.0	33.0	31.6
Safflower		2000	27.3	28.7	31.5	29.0
		4000	27.9	28.9	30.2	28.4
	LSD	(P=0.05)	1.98	2.66	2.98	2.66

membrane permeability have also been reported (26, 27). The supporting references, thus, indicate that accelerated ageing triggered seed leaching by acting on seed membrane and Na-

Table 6. Effect of accelerated ageing and seed pretreatment with Na-DK on insoluble carbohydrate (mg/g wet weight) contents of the seeds of a few cultivars. Data were recorded from seed kernels at 7-day interval upto 3 weeks of accelerated ageing. NS, Not significant.

Cultivar		Pretreatment (μg/ml)	Days after accelerated ageing 0	7	14	21
	Na-DK	0	25.9	23.8	18.9	15.8
		1000	23.0	22.4	21.0	17.6
Gram		2000	20.9	20.0	19.2	18.8
		4000	20.4	19.8	19.0	18.0
	LSD	(P=0.05)	2.08	2.20	1.87	1.60
		0	150.6	139.0	114.4	100.0
		1000	143.7	138.5	125.9	117.9
Soybean		2000	130.9	128.0	123.0	119.5
		4000	128.0	126.9	123.9	121.0
	LSD	(P=0.05)	12.18	12.15	8.52	9.08
		0	28.7	26.5	18.0	16.0
		1000	20.5	18.9	18.5	18.0
Sunflower		2000	20.0	19.0	18.4	17.8
		40 0	19.4	19.3	19.0	18.4
	LSD	(P=0.05)	1.98	2.05	NS	1.40
		0	24.4	22.0	16.5	12.5
		1000	22.5	21.7	18.9	15.2
Safflower		2000	21.0	20.1	19.0	17.5
		4000	17.2	16.9	16.2	15.8
	LSD	(P=0.05)	2.01	1.90	1.50	1.49

Table 7. Effect of accelerated ageing and seed pretreatment with Na-DK on percentage TTC-stained seeds of a few cultivars, Data were recorded at 7-day intervals upto 3 weeks of accelerated ageing. NC, Not calculated ; NS, Not significant.

Cultivar		Pretreatment (μg/ml)	Days after accelerated ageing 0	7	14	21
Gram	Na-DK	0	100	100	80	40
		1000	100	100	88	60
		2000	100	100	95	62
		4000	100	100	95	62
	LSD	(P=0.05)	NC	NC		
Soybean		0	90	85	60	40
		1000	90	88	75	56
		2000	90	90	80	58
		4000	90	87	80	55
	LSD	(P=0.05)	NC	NS	5.95	4.44
Sunflower		0	80	70	58	50
		1000	80	78	75	59
		2000	80	75	72	65
		4000	80	78	74	65
	LSD	(P=0.05)	NC	6.98	5.88	6.08
Safflower		0	85	75	40	36
		1000	85	80	66	62
		2000	65	80	70	65
		4000	85	78	70	60
	LSD	(P=0.05)	NC	NS	5.54	4.48

DK rendered the seeds tolerant against rapid storage deterioration by retaining the integrity of seed membrane.

The level of protein (Table 3), RNA (Table 4) and insoluble carbohydrate (Table 6) gradually decreased in control samples with ageing duration and this decreasing trend was considerably slowed down by Na-DK. The chemical also arrested the rise of internal soluble carbohydrate level in seed kernels (Table 5). Results, therefore, point out that although deterioration is a common phenomenon both in treated and control samples of the crop seeds, the catabolic processes within the treated seeds remained somewhat subdued, thereby rendering them tolerant against unfavorable storage environment. Available reports show that during seed ageing loss of some vital cellular components occur (24, 25). Increase of soluble substances such as sugars and amino acids (28, 29) and decrease of nucleic acids (30) during the process of seed deterioration has also been decumented. The present result is thus corroborated from the reported observations.

That the pretreating chemical is efficient in substantial alleviation of the damaging effect of accelerated ageing can be supported from the analyses of total dehydrogenase activity of seed kernels and from percent TTC-stained seeds. Data showed that both dehydrogenase activity and percent TTC-stained seeds gradually declined with seed ageing and Na-DK adverted the adverse effects. Dehydrogenase activity is generally used as a reliable index for the evaluation of seed viability (31). There are also reports that as seeds age, they lose vigor which is evaluated by counting percentage TTC-stained seeds and/or by observing the pattern of TTC-staining. Data, thus, point

Table 8. Effect of accelerated ageing and seed pretreatment with Na-DK on total dehydrogenase (expressed as Δ OD/g wet weight/ml) activity of the seeds of a few cultivars. Data were recorded at 7-day intervals upto 3 weeks of accelerated ageing. NS, Not significant.

Cultivar	Pretreatment	(μg/ml)	Days after accelerated ageing 0	7	14	21
Gram	Na-DK	0	0.72	0.65	0.58	0.40
		1000	0.69	0.68	0.64	0.55
		2000	0.68	0.69	0.66	0.58
		4000	0.70	0.65	0.63	0.54
	LSD	(P=0.05)	NS	NS	0.059	0.045
Soybean		0	0.66	0.58	0.42	0.30
		1000	0.67	0.64	0.15	0.42
		2000	0.64	0.64	0.53	0.46
		4000	0.64	0.60	0.51	0.41
	LSD	(P=0.05)	NS	0.057	0.044	0.036
Sunflower		0	0.54	0.48	0.40	0.32
		1000	0.56	0.52	0.47	0.36
		2000	0.56	0.53	0.48	0.41
		4000	0.54	0.49	0.48	0.39
	LSD	(P=0.05)	NS	0.040	0.039	0.033
Safflower		0	0.55	0.50	0.34	0.27
		1000	0.55	0.52	0.45	0.39
		2000	0.53	0.55	0.48	0.39
		4000	0.54	0.53	0.45	0.38
	LSD	(P=0.05)	NS	0.045	0.035	0.033

out that in spite of experiencing accelerated ageing treatment, the chemical-pretreated seeds got hardened and retained higher vigor than the control ones, and such hardening is effected at the metabolic level.

From these results, Na-DK appears to be a promising chemical manipulative agent for restoration of seed viability. Now, it is a challenge to the modern researchers working in this field to devise newer strategies for better exploitation of Na-DK working on seeds of a wide range of crops.

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