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Effect of Different Priming Methods on Seed Quality,Biochemical Changes and Storability of China Aster (*Callistephus Chinensis* L. Nees)

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Abstract

A laboratory experiment, "Effect of different priming methods on seed quality,biochemical changes and storability of china aster (Callistephus chinensis L. Nees)" was conducted at College of Horticulture, Dr.Y.S.R. Horticulture University, Rajendranagar, Hyderabad during the year 2011-12. This experiment had two factors. The experiment consisted of two different ages of the seed viz., one year old seed (S_1) and Half year old seed (S_2) and four treatments viz., Hydro priming (T_1), Osmo priming(T_2), Halo priming(T_3), Unprimed as control (T_4) as another factor, which was replicated four times in completely randomized design with factorial concept. Seed samples were primed and kept in polyethythelene bag and stored for six months at ambient condition. The results emanated from the experiment revealed that, amongest the two different ages of the seed, six months old seed recorded maximum germination percentage, field emergence, speed of germination, seedling length, seedling dry weight and seedling vigor index and least was observed in one year old seed. Amongst the priming treatments, priming of seed with KNO_3 @ 0.5% resulted in best performance of the seed regarding all the physiological and biochemical parameters followed by hydro priming. Unprimed seed (control) failed to exert any significant influence on the quality parameters. Storage of the treated seeds up to six months resulted in a gradual decrease in performance of the seed. However, storage of the six months old KNO_3 treated seed up to six months was found to be good compared to one year old seed regarding all the quality parameters.

Keywords: China aster; Seed; Priming

Introduction

China aster (Callistephus chinensis L. Nees) an important commercial flowering annual, belongs to the family Asteraceae and is native to China. It ranks next to chrysanthemum and marigold. Commercially grown in many parts of the world in open conditions as cut flower, loose flower, bedding plant, herbaceous boarder and potted plant. The wide spectrum of flower colour ranges available made China aster the most important popular flower crop all over the world. In India china aster is widely grown in Andhra Pradesh, Karnataka, Tamil Nadu, Maharashtra and West Bengal. Adopts well to varying soil and climatic conditions, flowers comparatively last longer, thus it can be used for various purposes like keeping in vase, flower decoration, preparation of bouquets and garlands etc. In addition, it is grown in landscape gardens to provide mass aesthetic effect.

With the development of flower industry in India, the area under flower crops is increasing year after year and requirement of high quality flower seeds has become the basic need of the growers for production of flowers to cater the market demand. Although the demand for flower seeds fluctuates very often and there may be a large surplus of seeds which need to be stored for the subsequent 2-3 sowing seasons with good quality and viability.

Seed being a living entity, deterioration in its quality occurs with the advancement in ageing which is natural, inevitable, irreversible and continuous process. During ageing, unsaturated fatty acid components of lipid membranes i.e. phospholipids are converted into free radicals and cytotoxic aldehydes by the reaction with atmospheric oxygen and lipoxygenase activity. The resulting chemical changes bring about alterations in the permeability properties of bio-membranes making them leaky, results in loss of viability. Seed priming has presented surprise results for flower crops like Pansy, marigold, gladiolus and china aster many other crop seeds like field crops, vegetables and grasses [1]. Information on the effectiveness of using different priming methods on aged flower seeds especially the china aster is scanty.

Physiological parameters

Germination percent

At random hundred undamaged China aster seeds per replication were selected. The selected seeds were planted between two layers of wetted germination paper towels. The paper towels were rolled and placed in walk- in germinator in upright position at 25 ± 1°C temperature and 70 ± 2% RH for 14 days (ISTA, 1985). Germination counts were taken on 7th and 14th day. The number of seeds germinated were counted and expressed as germination percentage.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds put for germination}} \times 100$$

Seed infestation

At random hundred undamaged seeds per replication were selected, and then the seeds were planted between two layers of wetted germination paper towels. The paper towels were rolled and placed in walk- in germinator in upright position at 25 ± 1°C temperature and

70 ± 2% RH for 14 days (ISTA, 1985). The infested seeds i.e., fungal infected seed count was recorded and expressed in percentage.

Biochemical Parameters

Electrical conductivity of leachates

The test is based on the leakage of solutes from the cell membrane of all the seeds into deionized distilled water. The amount of electrolyte leakage was assessed by measuring the electrical conductivity of the seed soaked water with a conductivity meter.

Fifty undamaged seeds in four replications were taken, and the seeds were soaked in 25 ml of distilled water at 25 ± 1°C for 24 hrs. Then the electrical conductivity of seed leachate was measured by using digital EC meter at ambient conditions and the average mean of EC was calculated by using the formula illustrated as follows and expressed in dSm-1.

Formula: E.C. (dSm-1) = Value × Range × Cell constant /10 × number of soaked seeds.

Where, Range 1K = 1000

10K = 10,000

Cell constant = 1.0

Tetrazolium test

Fifty seeds were taken from each priming treatment and soaked in water for 24 hours at 25°C temperature. Then these seeds were taken out from the water and seed coat was removed with the help of sharp blades and needle. After removing the seed coat, the seeds were immersed in 50 ml of 1% tetrazolium salt (2,3,5-triphenyl tetrazolium chloride) solution for 3 hours at 25°C in dark for staining. The seeds were washed with water, uniformly pink stained seeds were considered as viable.

Dehydrogenase activity

The viable China aster seeds were taken into a beaker, and then 6 ml methoxy ethane was added and allowed to shake till all the pink color of seed was converted to red due to the dehydrogenation of TZ salt solution (Figure 1).

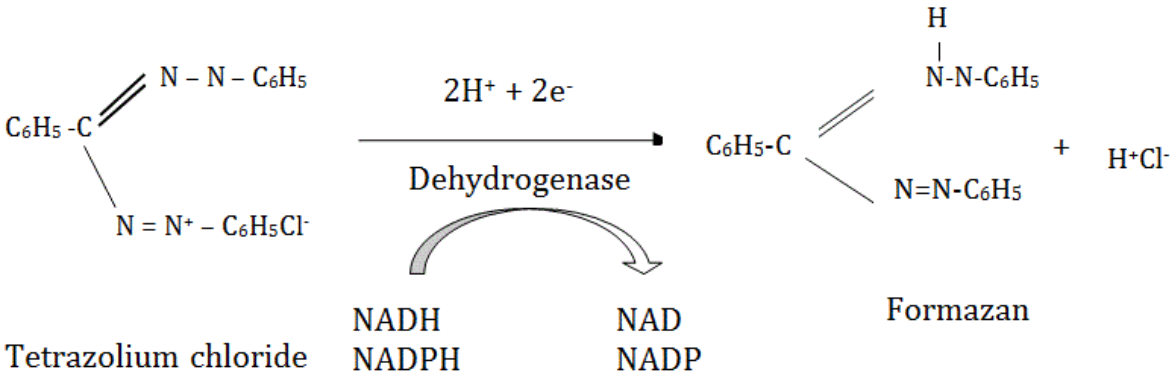

Figure 1: Absorbancy of the supernatant was measured using calorimeter at 470 nm.

Lipid peroxidation

This involves measuring the amount of a products lipid peroxidation, by the colorimetric method [2]. The dry seeds were ground to powder form after removing the seed coat with the help of sharp knife. Hundred milli grams (0.100 g) of seed powder was soaked in 2 ml distilled water for 24 hrs at 25°C. After 24 hrs the water was decanted and 2 ml solution of 0.5% TBA prepared in 20% TCA was added to the water soaked seed powder in test tube in four replications. The mixture was boiled for 20 minutes at 95°C, was cooled in ice box and then centrifuged at 16,000 rpm for 15 minutes. The orange red colored supernatant was siphoned out and its absorbance was read at 532 nm and the value of non-specific absorption at 600 nm was subtracted from this lipid peroxidation value, expressed as the absorbance at 532 nm/ gm fresh wt. of the sample(after subtracting the absorbance at 600 nm).

Reagents used

20% TCA (Trichloro– acetic acid): Dissolve 20 g TCA salt in 100 ml distilled water.

0.5% TBA (Thiobarbituric acid) in 20% TCA: Dissolve 500 mg (0.5 g) TBA salt in 100 ml of 20% TCA solution.

The lipid peroxidation model of seed deterioration (Figure 2).

Results and Discussion

Germination percentage (%)

Six months old seed (S_2) recorded significantly maximum germination (64.37 per cent) and minimum (47.00 per cent) in first and six months of storage respectively, was illustrated in Table 1. In one year old seed (S_1) also similar trend of decreasing germination per cent was noticed, but the percentage of germination initially and at subsequent months of storage was drastically ceased, reduction in germination percentage is attributed to cytoplasmic or physiological changes in sub cellular system (membrane, mitochondria, protein synthesis, ribosomes and DNA) and enzyme machinery during storage with preceding age of the seed resulting in slow germinaton rate of embryo, which intended to continue its ontogenic effect on the developing seedling.

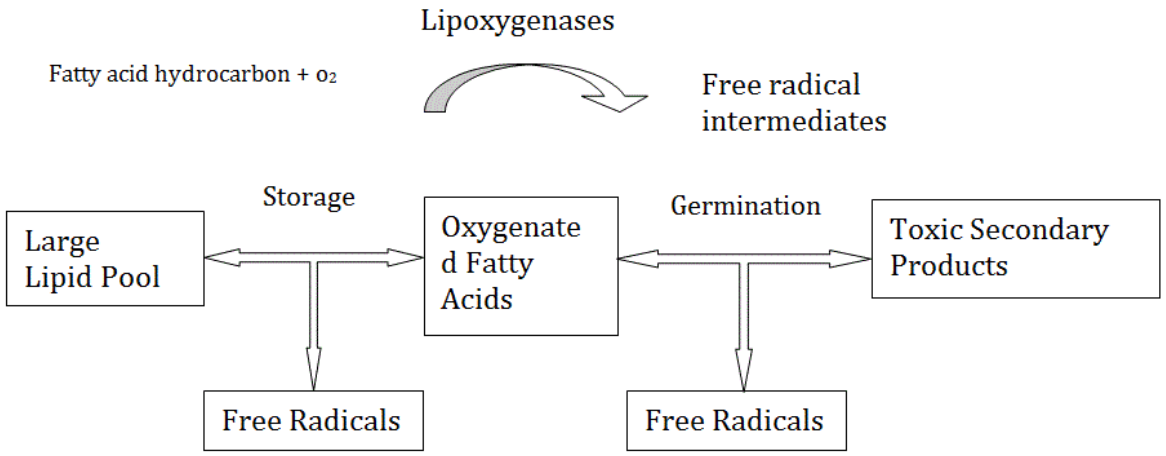

Figure 2: Hypothetical model of the damage mechanism of lipid peroxidation in seeds

Treatments	Storage months					
	S_1	17.87	18.5	16.37	14.56	12.87
Age of the seed (S)						
S_1	17.87	18.5	16.37	14.56	12.87	11.43
S_2	64.37	60.81	58.62	56.56	52.19	47
SEm ±	0.82	0.44	0.29	0.64	0.68	0.46
CD (0.05)	2.38	1.83	0.86	1.86	2	1.34
Priming treatments (T)						
T_1	41.5	42.87	40.62	37.37	33.5	31.5
T_2	44.5	39.62	36.5	35.37	31.37	28.37
T_3	44.25	44.5	42.87	40.87	38.12	34
T_4	34.25	31.62	30	28.62	27.12	23
SEm ±	0.8	0.63	0.42	0.9	0.97	0.65
CD (0.05)	2.33	1.83	1.22	2.63	2.83	1.9
Interactions (S×T)						
S_1T_1	16	20.75	18.5	14.5	12.5	12.25
S_1T_2	21.5	18.75	16	14	11.75	10
S_1T_3	19.5	22.5	20.5	19.75	17.25	15.25
S_1T_4	14.5	12	10.5	10	10	8.25
S_2T_1	67	65	62.75	60.25	54.5	50.75
S_2T_2	67.5	60.5	57	56.75	51	46.75
S_2T_3	69	66.5	65.25	62	59	52.75
S_2T_4	54	51.25	49.25	47.25	44.25	37.75

SEm ±	1.6	0.88	0.59	1.27	1.37	0.92
CD (0.05)	4.67	2.585	1.72	3.72	4	2.68

Table 1: Effect of seed priming on germination percentage of china aster seed (per cent). S_1: One year old seed, S_2: six months old seed, T_1: Seed primed with distilled water, T_2: Seed primed with polyethylene glycol (PEG), T_3: Seed primed with potassium nitrate (KNO_3), T_4: Unprimed half year old seed (Control)

Among the priming methods, seeds primed with KNO_3 (T_3) performed well and followed by hydro priming (T_1). The possible effect of KNO_3 might be due to its role in influencing the turgidity of membranes, lead to activation of enzymes involved in protein synthesis and carbohydrate metabolism. Besides, the nutrient potassium has a positive effect on keeping quality.

Subsequently, a decline in per cent germination was observed in all the priming treatments with advance in storage period, which may be attributed to the phenomenon of ageing and due to depletion of food reserves, decline in synthetic activity.

Seed infection/ infestation

As the age of the seed increases, seed infection was severed. In both one year (S_1) and six months old seed (S_2) there was an increase in the seed infestation by prolonging the period of storage. Among the priming treatments KNO_3 primed seed recorded less seed infection, but the severity of infestation increased from the first and last months of storage (48.75 to 66.00 per cent respectively), it might be due to loss of membrane integrity, lipid peroxidation and invasion of fungi, leaching of seed exudates can stimulate microbial activity, it contribute to increase of fungal proliferation. Similar trend was observed in one year old seed, but the severity of infection was high (Table 2).

Treatments	Storage months					
	October	November	December	January	February	March
Age of the seed (S)						
S_1	70.37	80.50	83.62	84.31	86.75	88.56
S_2	30.75	38.00	41.37	42.75	48.00	53.00
SEm ±	0.72	0.60	0.30	0.76	0.64	0.46
CD (0.05)	2.10	1.76	0.90	2.23	1.86	1.34
Priming treatments (T)						
T_1	50.75	54.00	59.37	61.37	66.25	68.50
T_2	53.62	59.62	63.50	62.25	68.62	71.62
T_3	48.75	54.50	57.12	59.12	61.62	66.00
T_4	65.12	68.87	70.00	71.37	72.62	77.00
SEm ±	1.01	0.85	0.42	1.08	0.90	0.65
CD (0.05)	2.96	2.49	1.22	3.15	2.63	1.89
Interactions (S×T)						
S_1T_1	75.75	77.75	81.50	85.50	87.00	87.75
S_1T_2	77.00	79.75	84.00	81.50	88.25	90.00
S_1T_3	74.25	75.50	79.50	80.25	82.25	84.75
S_1T_4	86.50	89.00	89.50	90.00	89.50	91.75
S_2T_1	25.75	30.25	37.25	37.25	46.25	49.25
S_2T_2	30.25	39.50	43.00	43.00	49.00	53.25
S_2T_3	23.25	33.50	34.75	38.00	41.00	47.25
S_2T_4	43.75	48.75	50.50	52.75	55.75	62.25

SEm ±	1.43	1.21	0.59	1.52	1.28	0.92
CD (0.05)	4.20	3.53	1.72	4.46	3.72	2.69

Table 2: Effect of priming treatments on seed infestation of China aster seed (per cent), S_1: One year old seed, S_2: six months old seed, T_1: Seed primed with distilled water, T_2: Seed primed with polyethylene glycol (PEG), T_3: Seed primed with potassium nitrate (KNO_3)

Electrical conductivity

Electrical conductivity of seed leachate has negative effect on seed quality. This parameter was studied for different seed priming treatments throughout the storage period. One year old seed recorded highest electrical conductivity, it was recorded lowest in the first month in half year old seed (Table 3).

At initial month of storage lowest electrical conductivity was recorded in KNO_3 primed seed and highest was recorded in unprimed seed. The differential EC values recorded among the seed treatments indicate the nature and extent of membrane protection offered may not be the same for all seed priming treatments, thus resulting in difference in EC values in cotton. Electrical conductivity of seed leachate increased with the increase in storage period due to the leakage of electrolytes from the seed and loss of membrane integrity due to ageing [3]. Generally, the electrical conductivity of seed leachate is inversely related to seed quality, higher the EC lower the seed quality and vice versa. In aged seeds or partly deteriorated seed, the EC will be higher owing to decrease in membrane integrity caused by detrimental changes occurring in seeds [4]. Seed deterioration is associated with enhanced permeability of seed membranes, which leads to higher leakage of electrolytes during imbibition [5,6], similar results by sung, 1996 in soybean.

Treatments	**Storage months**					
	October	November	December	January	February	March
Age of the seed (S)						
S1	1.48	1.58	1.59	1.74	1.75	1.82
S2	1.18	1.18	1.23	1.29	1.31	1.34
SEm ±	0.01	0.01	0.01	0.02	0.02	0.02
CD (0.05)	0.03	0.03	0.03	0.05	0.05	0.06
Priming treatments (T)						
T1	1.41	1.41	1.42	1.43	1.4	1.48
T2	1.23	1.24	1.42	1.51	1.54	1.6
T3	1.2	1.2	1.26	1.32	1.34	1.4
T4	1.47	1.47	1.58	1.79	1.83	1.9
SEm ±	0.01	0.01	0.01	0.03	0.02	0.03
CD (0.05)	0.04	0.04	0.04	0.08	0.07	0.08
Interactions (S×T)						
S1T1	1.57	1.58	1.59	1.56	1.48	1.6
S1T2	1.35	1.35	1.64	1.72	1.75	1.81
S1T3	1.31	1.31	1.36	1.47	1.5	1.56
S1 T4	1.67	1.67	1.85	2.2	2.25	2.32
S2T1	1.25	1.23	1.24	1.3	1.32	1.36
S2T2	1.11	1.12	1.2	1.29	1.33	1.38

S2T3	1.09	1.09	1.15	1.18	1.2	1.21

Table 3: Effect of priming treatments on electrical conductivity of china aster seed (dS m-1). S_1: One year old seed, S_2: six months old seed, T_1: Seed primed with distilled water, T_2: Seed primed with polyethylene glycol (PEG), T_3: Seed primed with potassium nitrate (KNO_3), T_4: Unprimed half year old seed (Control)

Dehydogenase activity

The dehydrogenase activity was high for aged seeds in initial months of storage, however with increase in storage period there was a decline in dehydrogenase activity. Greater dehydrogenase activity was reported in seeds subjected to KNO_3 (T_3) followed by hydro priming (T_1). Similar observations were made in mustard, soybean and maize. A sharpfall in dehydrogenase activity with ageing and found that direct correlation exists between germinability and dehydrogenase activity of seeds in sunflower reported by Dey and Basu [7]. Damage to membrane system could be repaired and protected against such changes by invigoration treatments particularly KNO_3 as indicated by low electrical conductivity of seed leachates, which presumably have extended the viability of seeds [8] (Table 4).

Treatments	**Storage months**					
	October	November	December	January	February	March
Age of the seed (S)						
S1	0.03	0.03	0.03	0.03	0.02	0.02
S2	0.2	0.2	0.19	0.19	0.11	0.1
SEm ±	0	0	0	0	0	0
CD (0.05)	0	0	0	0	0	0.01
Priming treatments (T)						
T1	0.13	0.14	0.13	0.13	0.13	0.12
T2	0.14	0.13	0.13	0.13	0.13	0.12
T3	0.14	0.14	0.14	0.13	0.13	0.13
T4	0.05	0.05	0.05	0.04	0.04	0.04
SEm ±	0	0	0	0	0	0
CD (0.05)	0	0	0	0	0.01	0.01
Interactions (S×T)						
S1T1	0.03	0.04	0.03	0.03	0.02	0.02
S1T2	0.03	0.04	0.03	0.03	0.03	0.02
S1T3	0.04	0.04	0.03	0.03	0.04	0.03
S1 T4	0.02	0.03	0.02	0.02	0.02	0.01
S2T1	0.24	0.24	0.23	0.23	0.23	0.22
S2T2	0.24	0.23	0.23	0.23	0.23	0.22
S2T3	0.24	0.24	0.24	0.24	0.23	0.23
S2T4	0.08	0.07	0.07	0.07	0.06	0.06
SEm ±	0	0	0	0	0	0

CD (0.05)	0	0.01	0.01	0	0.01	0.01

Table 4: Effect of priming treatments on dehydrogenase activity of China aster seed (OD value). S_1: One year old seed, S_2: six months old seed, T_1: Seed primed with distilled water, T_2: Seed primed with polyethylene glycol (PEG), T_3: Seed primed with potassium nitrate (KNO_3), T_4: Unprimed half year old seed (Control)

Lipid peroxidase activity

The lipid peroxidase activity was low with six months old seed after one month of storage (0.17) and subsequent months of storage the activity shoot up and finally it was 0.28 after six months of storage. In contrary, the lipid peroxidase activity was recorded higher values for one year old seed (S_2). The enhanced lipid peroxidation was indirectly supported by increased peroxide accumulation in connection with reduced germinability might explain the loss of vigor and viability as reported in cotton [9]. KNO_3 primed seed recorded less lipid peroxide activity value, which gradually increased from first to six months of storage ranging between 0.283 to 0.357 respectively, closely followed by hydro priming (T_1). Unprimed seed (T_4) showed a linear trend of increase in lipid peroxidation activity from first to six months of storage and the data ranged between 0.396 to 0.508. The level of lipid peroxidation decreased in seeds primed with KNO_3 might be malonaldehyde and its secondary by products, decrease the activity of enzymes and membrane potrubations leading to electrolyte leakage and exudation of simple sugars [10] (Table 5).

Treatments	**Storage months**					
	October	November	December	January	February	March
Age of the seed (S)						
S1	0.38	0.44	0.46	0.5	0.54	0.63
S2	0.17	0.18	0.19	0.2	0.2	0.28
SEm ±	0.01	0	0.01	0	0.01	0.04
CD (0.05)	0.03	0.01	0.02	0.01	0.01	0.13
Priming treatments (T)						
T1	0.27	0.25	0.26	0.28	0.31	0.38
T2	0.28	0.31	0.33	0.34	0.36	0.41
T3	0.28	0.25	0.27	0.29	0.3	0.36
T4	0.4	0.44	0.46	0.49	0.51	0.53
SEm ±	0.01	0.01	0.01	0.01	0.01	0.01
CD (0.05)	0.04	0.2	0.02	0.02	0.02	0.03
Interactions (S×T)						
S1T1	0.38	0.35	0.36	0.4	0.45	0.58
S1T2	0.4	0.46	0.48	0.5	0.55	0.64
S1T3	0.41	0.35	0.38	0.42	0.45	0.55
S1 T4	0.6	0.62	0.64	0.68	0.72	0.74
S2T1	0.16	0.16	0.16	0.17	0.17	0.18
S2T2	0.16	0.16	0.17	0.18	0.18	0.19
S2T3	0.16	0.15	0.16	0.16	0.16	0.17
S2T4	0.2	0.25	0.28	0.29	0.3	0.32
SEm ±	0.02	0.1	0.01	0.01	0.01	0.01

CD (0.05)	0.05	0.03	0.03	0.02	0.03	N.S

Table 5: Effect of priming treatments on lipid peroxidase activity of China aster seed. S_1: One year old seed, S_2: six months old seed, T_1: Seed primed with distilled water, T_2: Seed primed with polyethylene glycol (PEG), T_3: Seed primed with potassium nitrate (KNO_3), T_4: Unprimed half year old seed (Control)

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