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Effect of post priming storage duration on physiological and biochemical parameters of halo, hormonal and hydro primed cowpea seeds

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Abstract
The present investigation was undertaken to study the effect of storage duration on physiological and biochemical parameters of halo, hormonal and hydro primed cowpea seeds. The experiment was conducted in a completely randomised design with four replications. Eighteen months old cowpea seeds were primed with KCl (1%), KNO_3 (1%), GA3 (50 ppm), salicylic acid (50 ppm) and distilled water for 6 hours. Primed seeds were stored up to 45 days under normal room temperature. Experimental results revealed that at 45 days of storage duration, seeds primed with KCl recorded the highest germination count of 55.5% and the lowest electrical conductivity (1.0 dS/m); hydro primed seeds showed the highest seedling vigour index I (2374.60) & II (2532.95) along with the highest alpha amylase activity (0.271) and seeds primed with GA3 had the highest dehydrogenase activity (278.8 µg/ 20 ml/hr). Based on germination response index, vigour response index and biochemical response index it was suggested that priming of old cowpea seeds with distilled water and KCl could resist the seed quality deterioration that happened due to post priming storage.

Keywords: Cowpea, storage duration, halo priming, hormonal priming, hydro priming, alpha amylase and dehydrogenase activity

Introduction
Cowpea (*Vigna unguiculata* (L.) Walp.) is an important legume vegetable crop of India. It is also grown for its grain, vegetable and also for fodder purpose. Cowpea is adapted to warm weather and required less rainfall than other crops. Cowpea has also the ability to be intercropped with cereals such as millet and sorghum. Coupled with these attributes, its quick growth and rapid ground cover have made cowpea an essential component of sustainable subsistence agriculture in marginal lands and drier regions of the tropics, where rainfall is scanty and soils are sandy with little organic matter (Singh *et al.*, 1993) [31]. Being a drought tolerant and hot weather crop, cowpea is well-adapted to the semi- arid regions of the tropics where other food legumes do not perform well (Singh *et al*., 1993) [31].

In India, despite the fact that a large number of varieties/hybrids and agro-techniques have been developed, the productivity of cowpea has not still reached the desired level. Considering its versatile nature its production must be increased to benefit the farmer's community. Among several inputs used for increasing production, seed is the basic and key input for increasing yield. When adverse weather condition prevails or natural calamities like drought/flood occurs the farmers may not be able to collect fresh seeds and forced to use old seeds as planting material. Under such circumstances priming of old seeds gives better result as compared to unprimed seeds.

The primed seeds provide excellent seedling performance in the field by reversing some of the ageing inducing deteriorating events (Taylor *et al*., 1998) [33] and there by promote sustainable farming systems especially in marginal environments. The rate of germination and improvement of seedling stands were accelerated as a result of seed priming in vegetables (Corbineau *et al*., 2000) [12]. Variation in the results depended on temperature, priming duration, concentration of the priming chemical and the crop type. An important factor is to determine how long the benefits last during dry storage of seeds following priming (McDonalds, 2000) [22]. However, the general rule in this connection is that primed seeds should be considered vigorous but without prolonged storage periods. This rule was obvious with many plants such as sweet corn (Chang and Sung, 1998) [11] and pepper (Lanteri *et al*., 1997) [20]. But the literature available in this context for cowpea is very scanty.

Therefore in the present investigation an attempt has been made to study the effect of post priming storage duration on physiological and biochemical parameters of halo, hormonal and hydro primed cowpea seeds.

Materials and methods

Eighteen months old cowpea seeds of variety Utkal Manika were collected from AICRP on Vegetable Crops, OUAT, Bhubaneswar. The experiment was conducted in a completely randomised design with four replications in the department of Seed Science and Technology, OUAT, Bhubaneswar during the year 2017. The cowpea seeds were taken in speciemen tube. Prepared solution of KCl (1%), KNO_3 (1%), GA_3 (50 ppm) and salicylic acid (50 ppm) were poured in to the specimen tube such that all the seeds can equally be soaked. In case of hydropriming the seeds were soaked in distilled water. Seeds were soaked in solutions at ambient temperature for 6 hours. After 6 hours the soaked seeds were removed and rinsed with distilled water for three times and dried to regain original moisture content under shade. Before starting priming treatment the moisture content of fresh and old seeds were determined. The seeds treated with different priming agents were packed in separate cotton bags along with untreated seed (control) and stored up to 45 days under normal room temperature. Seed samples from the respective treatments were drawn at 0, 15, 30 and 45 days to take observations on physiological parameters like seed germination (%), shoot, root and seedling length (cm), seedling dry weight, seedling vigour index I & II and biochemical parameters like electrical conductivity (EC) of seed leachate, alpha amylase activity and dehydrogenase activity.

Electrical conductivity of seed leachate was estimated as follows. Four replications of 8 g of old cowpea seeds from each priming treatments along with control were taken in beakers and pre-washed thoroughly with distilled water to remove the adhering chemicals and then soaked in 40 ml of distilled water for 6 hrs at room temperature. After soaking, the seed steep water was decanted to obtain the seed leachate. The electrical conductivity of the seed leachate was measured in a digital conductivity meter and expressed as dS/m. Alpha amylase activity was determined following the procedure described by Black *et al.* (1996) [10]. Four replicates of old cowpea seeds from each priming treatments along with control were taken for the study. Alpha amylase activity was determined from the standard curve and expressed in terms of optical density (OD) value. Dehydrogenase activity was determined following the procedure described by Shenoy *et al.* (1990) [30]. Four replicates of old cowpea seeds from each priming treatments along with control were taken for the study. Dehydrogenase activity was estimated from the standard curve and expressed as µg/ 20 ml/hour.

The data were statistically analysed following SAS 9.3 version. The level of significance used in F test was P = 0.01. The data in percentage was transformed into angular transformation value and used for statistical analysis. Seed quality index (SQI) a new parameter is calculated to evaluate efficacy of priming treatments. Higher value of SQI indicated higher efficacy. SQI is obtained by adding germination response index, vigour response index and biochemical response index values. Evaluation of germination response index, vigour index and biochemical response index are described in result.

Results and Discussion

Analysis of variance indicated significant differences among the priming treatments in case of zero storage duration (Table 1). Hydro priming recorded the highest germination count (72.0%) followed by KCl (69.5%) as compared to control (53.0%). Among the treatments salicylic acid recorded the lowest germination count (56.5%). All the priming treatments produced higher germination than non primed seeds and the increase in germination count was 31.0%, 17.9%, 6.6%, 15.1% and 35.8% by KCl, KNO_3, salicylic acid, GA3and hydro priming respectively. At 15 days of post priming storage duration, all the priming treatments except KNO_3 achieved significantly higher germination count than control. Hydro priming recorded the highest germination count (66.5%) whereas salicylic acid recorded the lowest (48.50%). The reduction was more in salicylic acid (14.0%) followed by KNO_3 (11.0%), hydro priming (7.0%), KCl (5.0%), GA3 (5.0%) and the lowest was recorded in control (1.8%) as compared to zero storage duration. Coming to 30 days of post priming storage duration, it was observed that the halo priming agent KCl had the maximum germination count (63.5%) followed by hydro priming (62.0%). All the priming treatments scored significantly higher germination except salicylic acid as compared to non priming seeds. At 45 days of storage duration the germination count varied from 41.5% (salicylic acid) to 55.5% (KCl) and all the treatments recorded significantly higher germination percent as compared to non primed seeds. The percent increase in germination as recorded by KCl, KNO_3, salicylic acid, GA3 and hydro priming was 18.0%, 10.0%, -11.7%, 13.8% and 10.0% respectively as compared to dry seeds.

Table 1: Germination count of old seeds at different post priming storage duration

old seed				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1%)	69.50	64.00	63.50	55.50
KNO_3 (1%)	62.50	55.50	55.50	52.00
SALICYLIC ACID (50 ppm)	56.50	48.50	45.00	41.50
GA3(50 ppm)	61.00	58.00	59.00	53.50
WATER	72.00	66.50	62.00	52.00
CONTROL	53.00	52.00	49.50	47.00
CD 1%	5.93	5.61	4.52	4.21
CV%	4.67	4.80	3.99	4.12

Data presented in Table 2 indicated significant effect of priming treatments on root length during zero post-priming storage duration. The halo priming agent KCl recorded the maximum root length (21.63 cm) followed by KNO_3 (18.01 cm). All the treatments enhanced the root length except the hormonal priming agent GA3. At 15 days of post-priming storage period it was observed that the root length of all treatments decreased correspondingly as compared to zero post-priming storage periods. Root length of the treatments during this period varied from 14.25 cm (GA3) to 18.15 cm (KCl). All priming treatments were found to have positive effect as compared to non primed seeds except GA3. At 30 days of post-priming storage period, the hormonal priming agent salicylic acid recorded the highest root length of 16.18 cm followed by control (15.80 cm). The lowest root length was produced by GA3 (14.42 cm). The root length produced by the treatments during this period was definitely lower than zero days and 15 days. At 45 days of post-priming storage period, the hormonal priming agent salicylic acid recorded the highest root length of 13.86 cm followed by KNO_3 (13.64 cm). The hormonal priming agent GA3 and hydro priming had root length of 11.81 cm and 12.65 cm, thus showing negative effect in comparison to non primed seeds (13.08 cm).

Table 2: Root length (cm) in old seeds at different post priming storage duration

Old seed				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl(1%)	21.63	18.15	14.66	13.04
KNO_3(1%)	18.01	16.83	15.59	13.64
SALICYLIC ACID(50 ppm)	17.13	16.63	16.18	13.86
GA3(50 ppm)	14.70	14.25	14.42	11.81
WATER	16.02	15.60	15.26	12.65
CONTROL	15.32	15.14	15.80	13.08
CD 1%	1.54	1.67	1.41	1.32
CV%	4.41	5.11	4.53	5.00

The effects of halo, hormonal and hydro priming treatments on shoot length of old cowpea seeds are presented in Table 3. All the priming treatments exhibited significantly higher shoot length as compared to non primed seeds. Among the priming treatments, the halo priming agent KNO3 recorded the highest shoot length (28.32 cm) whereas hydro priming recorded the lowest shoot length (24.77 cm). At 15 days of post-priming storage period, very surprisingly the shoot length was found to increase in all treatments along with control except GA3 as compared to zero days. This may be due to favourable environmental effect. The highest shoot length was observed in KCl (30.71 cm) rather than KNO_3 (29.27 cm). KCl, KNO3, salicylic acid and hydro priming produced significantly higher shoot length as compared to non primed seeds. At 30 days of post-priming storage period, the shoot length was further found to increase as compared to 15 days. Here salicylic acid attained the maximum shoot length (33.67 cm) and the lowest was being recorded by GA3 (30.67 cm). There were no significant differences among priming treatment and control. At 45 days of post-priming storage period, all the treatments along with control showed decrease in shoot length as compared to 30 days. The shoot length was found to vary from 26.26 cm (control) to 33.04 cm (hydro priming). The hormonal and hydro priming treatments gave significantly higher shoot length as compared to dry seeds.

Table 3: Shoot length (cm) in old seeds at different post priming storage duration

Old seed				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl(1%)	26.07	30.71	32.40	27.53
KNO_3(1%)	28.32	29.27	33.40	26.74
SALICYLIC ACID(50 ppm)	27.31	28.45	33.56	30.20
GA3(50 ppm)	27.14	26.68	30.67	30.37
WATER	24.77	29.28	33.28	33.04
CONTROL	21.84	25.20	33.53	26.26
CD 1%	2.11	2.69	NS	2.96
CV%	4.00	4.68	4.69	5.01

Analysis of variance indicated significant differences among the treatments in case of 0, 15 and 45 days of post-priming storage period (Table 4). For halo priming agent KCl, the shoot: root length ratio gradually increased from 0 to 30 days at an increasing rate and from 30 to 45 days it increased at a decreasing rate. Similar trend was observed in case of KNO_3 and non primed seeds. But in case of hormonal priming agents (salicylic acid and GA3) and hydro priming agent shoot: root length ratio gradually increased from 0 to 45 days at an increasing rate.

Table 4: Shoot length: Root length ratio in old seeds at different post priming storage duration

Old seed				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl(1%)	1.21	1.69	2.22	2.12
KNO_3(1%)	1.57	1.74	2.15	1.96
SALICYLIC ACID (50 ppm)	1.60	1.71	2.09	2.18
GA3(50 ppm)	1.86	1.88	2.15	2.58
WATER	1.56	1.87	2.22	2.65
CONTROL	1.43	1.68	2.15	2.00
CD 1%	0.15	0.16	NS	0.20
CV%	4.90	4.58	3.80	4.41

At 0 day of post-priming storage period, seedling length ranged from 37.15 cm to 47.70 cm (Table 5). All priming treatments were found to produce higher seedling length as compared to non primed seeds. The highest seedling length was recorded by KCl (47.70 cm) followed by KNO_3 (46.33 cm) and these two halo priming agents were at par in their effect. Salicylic acid recorded 44.44 cm seedling length and GA3 recorded 41.83 cm seedling length and these two hormonal priming agents were also at par in their effect. At 15 days of post-priming storage period, significant differences were observed among the treatments. The two halo priming agents KCl and KNO_3 scored 1st (48.86 cm) and 2nd (46.10 cm) rank in their effect on seedling length. All the priming treatments exhibited higher seedling length as compared to non primed seeds (40.34 cm). At 30 days of post-priming storage period, the highest seedling length was recorded by salicylic acid (49.74 cm) followed by dry seeds (49.33 cm) and the lowest were reported in GA3 (45.09 cm). At 45 days of post-priming storage period, significant differences were observed among the treatments. All the priming treatments showed higher seedling length as compared to non primed seeds. Hydro priming recorded the highest seedling length (45.69 cm) followed by salicylic acid (44.05 cm). All primed seeds along with non primed seeds at 45 days produced decreased seedling length as compared to 30 days.

Table 5: Seedling length (cm) in old seeds at different post priming storage duration

Old seed				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl(1%)	47.70	48.86	47.06	40.56
KNO_3(1%)	46.33	46.10	48.99	40.38
SALICYLIC ACID(50 ppm)	44.44	45.08	49.74	44.05
GA3(50 ppm)	41.83	40.92	45.09	42.18
WATER	40.79	45.13	48.54	45.69
CONTROL	37.15	40.34	49.33	39.34
CD 1%	3.09	4.50	NS	3.49
CV%	3.52	4.99	4.60	4.09

Seedling dry weight of different priming treatments at 0, 15, 30 and 45 days is presented in Table 6. Significant differences were observed among the treatments for all the storage periods. At 0 days storage period, all priming treatments showed higher dry weight as compared to non primed seeds. Seedling dry weight ranged from 57.83 mg to 51.68 mg. The highest dry weight (57.83 mg) was observed in hydro priming treatment followed by salicylic acid (57.78 mg) and these two only showed significantly higher weight in comparison to non primed seeds. At 15 days of post-priming storage period, seedling dry weight was found to vary from 41.58 mg to 53.0 mg. Hydro priming recorded the highest weight (53.0 mg) followed by salicylic acid (44.85 mg) and KNO_3 (44.60 mg) and the lowest was being recorded by GA3 (41.58 mg) and

these three treatments produced significantly higher dry weight as compared to non primed seeds. All the treatments showed decreased seed weight as compared to zero day. At 30 days of post-priming storage period, hydro priming recorded the highest weight (50.83 mg) followed by salicylic acid (48.98 mg) and non primed seeds (48.43 mg). The lowest was being recorded in GA3 (41.80 mg). Halo priming treatments recorded lower dry weight than control. At 45 days of post-priming storage period, significant differences were observed among the treatments. Hydro priming recorded the highest weight (48.80 mg) followed by salicylic acid (44.20 mg) and non primed seeds (43.28 mg). Dry weight produced by non primed seedlings was found to be at par with KCl, KNO3, salicylic acid and GA3.

Table 6: Seedling dry weight (mg/plant) in old seeds at different post priming storage duration

Old seed				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl(1%)	55.48	44.60	40.15	40.30
KNO_3(1%)	52.18	44.85	45.15	42.50
Salicylic Acid(50 ppm)	57.58	43.48	48.98	44.20
GA3(50 ppm)	56.08	41.58	41.80	41.30
WATER	57.83	53.00	50.83	48.80
CONTROL	51.68	43.15	48.43	43.28
CD 1%	5.22	3.15	1.96	3.89
CV%	4.66	3.43	2.10	4.41

Significant differences were observed among the treatments for zero storage periods (Table 7). At zero day storage periods, all priming treatments recorded significantly higher seedling vigour index-I as compared to non primed seeds. SV-I values ranged from 1969.24 to 3313.49. The halo priming agent KCl recorded the highest SV-I value (3313.49) followed by hydro priming (2936.00) and KNO_3 (2894.61). The two hormonal priming treatments i.e. salicylic acid and GA3 were at par in their effect. SV-I value increased by 68.26%, 47.0%, 27.92%, 29.63% and 49.09% in case of KCl, KNO_3, Salicylic Acid, GA3 and hydro priming as compared to non primed seeds. At 15 days of post-priming storage period, significant differences were observed among the treatments. SV-I values ranged from 2095.37 to 3125.86 and all priming treatments recorded higher value tan non primed seeds. KCl recorded the highest value (3125.86) and at par with hydro priming (3000.26) but it was significantly different from other treatments. Seedling vigour index of all the treatments except hydro priming was reduced at 15 days as compared to zero days. Non-primed seeds showed higher SV-I value at 15 days than 0 day of storage period. At 30 days of post-priming storage period, hydro priming showed the highest SV-I value (3014.13) whereas Salicylic acid had the lowest value (2237.42). The hormonal priming agents showed lower effect as compared to halo priming agents. Significant difference was observed between KCl (2986.28) and KNO_3 (2716.78) as well as between salicylic acid (2237.42) and GA3 (2659.95). At 45 days of post-priming storage period, hydro priming recorded the highest value (2374.60) indicating its strong positive effect on SV-I parameter irrespective of the storage period. Significant differences were observed among the treatments. Salicylic acid exhibited the lowest value (1828.54) and found to be at par with non primed seeds (1848.93).

Table 7: Seedling vigour index-I (SV-I) in old seeds at different post priming storage duration

Old seed				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl(1%)	3313.49	3125.86	2986.28	2254.37
KNO_3(1%)	2894.61	2558.05	2716.78	2097.76
SALICYLIC ACID(50 ppm)	2519.54	2188.71	2237.42	1828.54
GA3(50 ppm)	2552.77	2369.85	2659.95	2256.61
WATER	2936.00	3000.26	3014.13	2374.60
CONTROL	1969.24	2095.57	2441.22	1848.93
CD 1%	263.80	216.85	189.33	206.96
CV%	4.81	4.17	3.48	4.82

Seedling vigour index- II of different priming treatments is presented in Table 8. SV-II values of the treatments varied from 2741.25 to 4161.95. Hydro priming exhibited the highest value (4161.95) followed by KCl (3856.00). The SV-II value of KNO3 (3262.55) was significantly lower than the SV-II value of KCl (3856.00). In case of hormonal priming treatments, GA3 scored higher value (3422.20) than salicylic acid (3252.70). All the priming treatments recorded high SV-II value than non primed seeds. After 15 days of storage, the SV-II values of different priming treatments though started to decline but showed positive effect as compared to non primed seeds. Hydro priming again attained the highest value (3525.20) followed by KCl (2853.00). The lowest SV-II value (2107.60) was reported in Salicylic acid. After 30 days of storage, almost all the priming treatments showed their positive impact on SV-II values in comparison to non primed seeds. Here also hydro priming and KCl scored 1st (3137.10) and 2nd (2547.95) rank. Primed seeds stored for 45 days recorded higher SV-II values than the non primed seeds. Among different treatments hydro priming secured the highest value (2532.95) followed by KCl (2237.15) and salicylic acid recorded the lowest value (1836.30). These results indicated that old cowpea seeds primed with KCl, KNO_3, GA3 and water consistently recorded higher germination count, SV-I and SV-II as compared to non primed seeds at 0, 15, 30 and 45 days of storage period.

Table 8: Seedling vigour index-II in old seeds at different post priming storage duration

Old seed				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1%)	3856.00	2853.00	2547.95	2237.15
KNO_3 (1%)	3262.55	2488.55	2477.70	2209.30
Salicylic Acid (50 ppm)	3252.70	2107.60	2206.15	1836.30
GA3(50 ppm)	3422.20	2412.75	2452.30	2201.90
Water	4161.95	3525.20	3137.10	2532.95
Control	2741.25	2242.05	2358.45	2033.75
CD 1%	342.66	232.61	178.16	187.86
CV%	4.88	4.39	3.46	4.24

The electrical conductivity of seed leachate (dS/m) is presented in Fig. 1. Old cowpea seeds treated with different

priming agents showed significant variation in their electrical conductivity without any storage period (0 day storage). Non primed seeds recorded the highest EC value (0.801 dS/m) and hydro primed seeds recorded the lowest EC value (0.597 dS/m) followed by KCl (0.617 dS/m). At 15 days of storage period, hydro priming and KCl had shown lower EC value as compared to other treatments. Similar trend was observed at 30 and 45 days. At 45 days of storage period, salicylic acid recorded the highest EC value whereas KCl had the lowest EC value. With the advancement of storage period, EC value was found to increase irrespective of the treatment type. High EC value indicated that seed quality deteriorated at a higher rate. The halo priming agent KCl and hydro priming consistently recorded low EC value at different days of storage and this indicated that these two priming treatments had positive effect in improving seed quality.

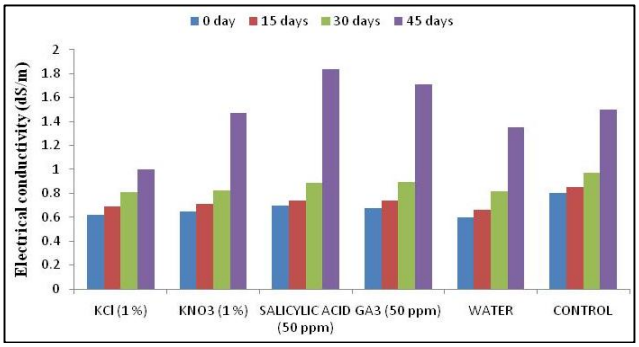

Fig 1: Electrical conductivity in primed cowpea seeds at different post priming storage duration

Alpha amylase enzyme activity as expressed in terms of optical density (OD) value at different post priming storage periods is depicted in Fig. 2. Significant differences were observed among the treatments for alpha amylase activity. From the figure it was observed that alpha amylase activity was more pronounced in seeds primed with KCl (0.446) followed by hydro priming (0.443) and lowest activity was reported in salicylic acid (0.119) during zero storage period. At 15 days of storage, the enzyme activity was found to decrease invariably for all the treatments as compared to zero storage periods. Alpha amylase activity varied from 0.086 to 0.390. All the treatments exhibited higher enzyme activity than non-primed seeds except hormonal priming treatments. Hydro priming and KCl were at par with each and significantly differed from rest of the treatments. The reduction rate of the enzymes in KCl, KNO_3, salicylic acid, GA3, deionised water and dry seeds were 12.5%, 27.2%, 27.7%, 29.4%, 13.7% & 23.6% respectively with the advancement of storage period from 0 to 15 days. At 30 days of storage period, the enzyme activity was the highest in case of hydro priming (0.336) and the lowest in salicylic acid (0.066). The rate of decrease in alpha amylase activity was 28.4%, 40.1%, 44.5%, 66.9%, 24.1%, and 44.4% in KCl, KNO_3, salicylic acid, GA3, distilled water and non primed seeds respectively as compared to zero storage periods. The highest decrease was observed in GA3 followed by salicylic acid and non primed seeds. At 45 days of storage period, the enzyme activity was the highest in case of hydro priming (0.271) and the lowest in salicylic acid (0.034). Alpha amylase activity ranged from 0.034 to 0.271. Significant differences were observed among the treatments. Salicylic acid and GA3 were at par with each other in influencing enzyme activity. The rate of decrease in alpha amylase activity was 43.0%, 62.4%, 71.4%, 78.8%, 38.8%, and 58.5% when old seeds treated with KCl, KNO_3, salicylic acid, GA3, distilled water and non primed seeds respectively as compared to zero storage periods.

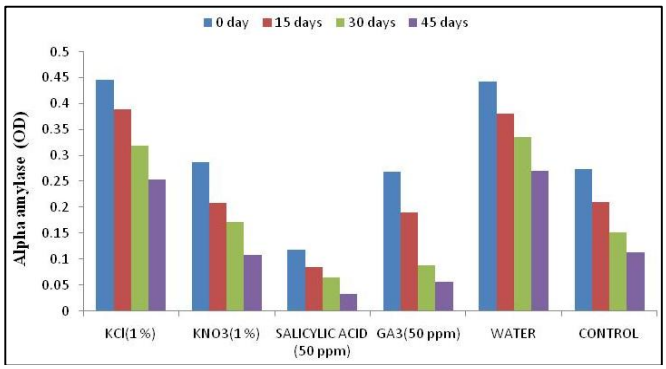

Fig 2: Alpha amylase activity in primed cowpea seeds at different post priming storage duration

Dehydrogenate activity of different priming treatments against post priming storage periods was reflected in Fig.3. Significant differences were observed among the treatments during zero storage periods. The highest dehydrogenase activity (383.4 µg/ 20 ml/hr) was observed in seeds treated with distilled water followed by salicylic acid and the lowest was recorded in seeds treated with KNO_3 (284.0 µg/ 20 ml/hr). Salicylic acid and GA3 were at par with each other in influencing dehydrogenase activity. With the advancement of storage period from 0 to 15 days, dehydrogenase activity was found to decrease for all priming treatments. Here seeds treated with GA3 showed the highest enzyme activity (333.5 µg/ 20 ml/hr) and the lowest was reported in non primed seeds (276.5 µg/20 ml/hr). Old seeds treated with hormonal priming agents executed high dehydrogenase activity as compared to halo priming agents KCl (299.5 µg/ 20 ml/hr) and KNO_3 (188.5 µg/ 20 ml/hr). The rate of decrease in dehydrogenase activity was 11.8%, 33.6%, 7.3%, 3.2%, 18.8% and 8.7% when old seeds treated with KCl, KNO_3, salicylic acid, GA3, distilled water and non primed seeds respectively as compared to zero storage periods. At 30 days of storage period, significant differences were observed among the treatments for dehydrogenase activity. There was a gradual decrease in dehydogenase activity for each category of primed seeds. Seeds treated with KNO_3 had the lowest dehydrogenase activity (147.0 µg/ 20 ml/hr). The rate of decrease in dehydrogenase activity was 23.7%, 48.2%, 16.6%, 12.5%, 29.5% and 24.9% when old seeds were treated with KCl, KNO_3, salicylic acid, GA3, distilled water and non primed seeds respectively as compared to zero storage periods. Increasing the storage period from 30 to 45 days showed further decrease in dehydogenase activity in primed seeds. There was a gradual decrease in dehydogenase activity for each priming treatment. The enzyme activity varied from 104.55 µg/ 20 ml/hr (KNO_3) to 278.8 µg/ 20 ml/hr (GA3). The rate of decrease in dehydrogenase activity was 38.4%, 63.2%, 28.9%, 19.1%, 44.6% and 35.7% when old seeds treated with KCl, KNO_3, salicylic acid, GA3, distilled water and non primed seeds respectively as compared to zero storage periods.

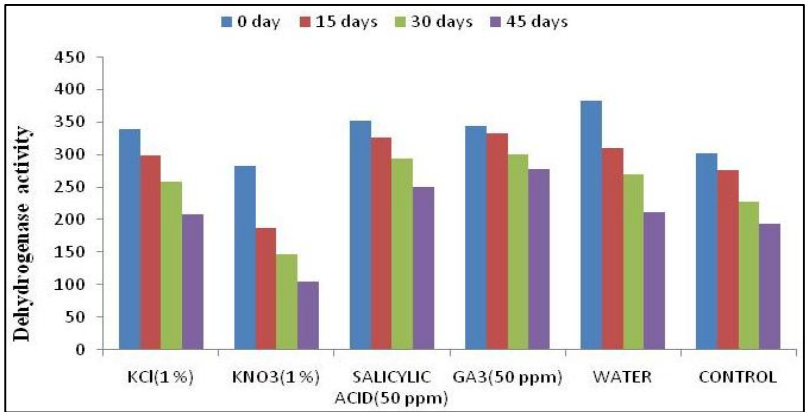

Fig 3: Dehydrogenase activity in primed cowpea seeds at different post priming storage duration

Comparing the effect of post priming storage duration on germination it was revealed that with the increase in storage period there is decrease in germination count (Fig. 4). The decrease rate was 7.9%, 8.6% and 20.0% at 15, 30 and 45 days of post priming storage duration when seeds were primed with KCl. For KNO_3 the decrease in germination percent was 11.2%, 11.2% and 16.8% at 15, 30 and 45 days; for salicylic acid the decrease was 14.2%, 20.4% and 26.5%; for GA3 the decrease was 4.9%, 3.3% and 12.3%; for hydro priming the decrease was 7.6%, 13.9% and 27.8% and for non primed seeds the decrease was 1.9%, 6.6% and 11.3%. It is very interesting to note that seeds primed with one of the hormonal priming agent salicylic acid showed the highest decrease rate whereas seeds primed with hormonal priming agent GA3 exhibited the lowest decrease rate.

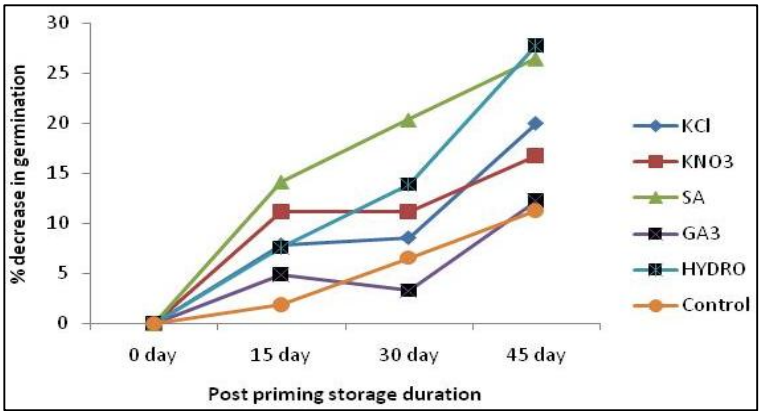

Fig 4: Reduction in germination rate of primed cowpea seeds at different post priming storage duration

Comparing the effect of post priming storage period on SV-I (Table 9), it was observed that the reduction rate in SV-I value increased with the increase in storage duration when the old cowpea seeds were treated with KCl (1%). In rest of the priming treatments the reduction rate was high at 15 days of storage duration than 30 days of storage duration and the maximum reduction was attained at 45 days of storage duration. This discrepancy may be due to effect of environmental factors on seedling growth. The average decrease in SV-I value was the highest (0.62) when seeds were primed with salicylic acid and lowest in hydro priming (0.06).

Table 9: Reduction in SV-I value of primed cowpea seeds at different post priming storage duration

Percent decrease in SV-I value					Av. decrease/day
Treatment	**0 day**	**15 days**	**30 days**	**45 days**	
KCl	0	5.7	9.9	31.9	0.47
KNO_3	0	11.6	6.14	27.5	0.53
Salicylic acid	0	13.1	11.2	27.4	0.62
GA3	0	7.2	- 4.2	11.6	0.20
Water	0	-2.2	-2.7	19.1	0.06
Control	0	-6.4	-24.0	6.1	-0.12

Comparing the reduction rate in SV-II values of the seeds treated with different priming agents (Table 10), it was observed that the reduction rate in SV-II value gradually increased with the increase in storage duration when the old cowpea seeds were treated with KCl, KNO_3 and distilled water. In case of salicylic acid and GA3 the reduction rate was high at 15 days of storage duration than 30 days of storage duration and the maximum reduction was 1.46) when seeds were primed with salicylic acid and lowest in hydro priming (0.90).

Table 10: Reduction in SV-II value of primed cowpea seeds at different post priming storage duration

Percent decrease in SV-II value					Av. decrease/day
Treatment	**0 day**	**15 days**	**30 days**	**45 days**	
KCl	0	26.0	33.9	42.0	1.26
KNO_3	0	23.7	24.1	32.3	1.03
Salicylic acid	0	35.2	32.2	43.5	1.46
GA3	0	29.5	28.3	35.7	1.23
Water	0	15.3	24.6	39.1	0.90
Control	0	18.2	14.0	25.8	0.75

Predicting the efficacy of priming treatments

The present study indicates that the priming agents taken for the study behave differently in influencing the physiological and biochemical parameters of primed seed. i.e Hence the efficacy of a priming treatment in improving seed quality parameters could not be judged on the basis of its effect on a single parameter. It would be better to consider all the parameters together in order to decide the efficiency of a priming treatment. In this investigation the efficacy of priming treatments in old seeds was judged by calculating germination response index (GRI), vigour response index (VRI) and biochemical response index (BRI). Finally these three indices are added to get seed quality index (SQI). The priming treatments giving significantly higher germination than control are coded as " 2" ; those are at par with control coded as " 1" and the treatments giving significantly lower germination than control coded as " 0" for each post priming storage duration (0, 15, 30 and 45 days). Then the coded values over post priming storage duration are added to get germination response index (GRI). Similar procedure is followed to evaluate vigour response index (VRI: coded value of SV-I + SV-II) and biochemical response index (BRI). The coded value of EC, alpha amylase activity and dehydrogenase activity are added to get BRI. The GRI, VRI and BRI values of different treatments are presented in Fig. 5. From Fig. 5, it is evident that hydro priming and KCl have high GRI, VRI and BRI values as compared to other treatments. The efficacy of salicylic acid as a priming agent is the poorest in improving seed quality parameters. The efficacy of GA3 is better than KNO_3. Hydro priming has the highest SQI value (25) followed by KCl (24) and GA3 (22). This result indicates that hydro priming is the best priming treatment followed by KCl and GA3.

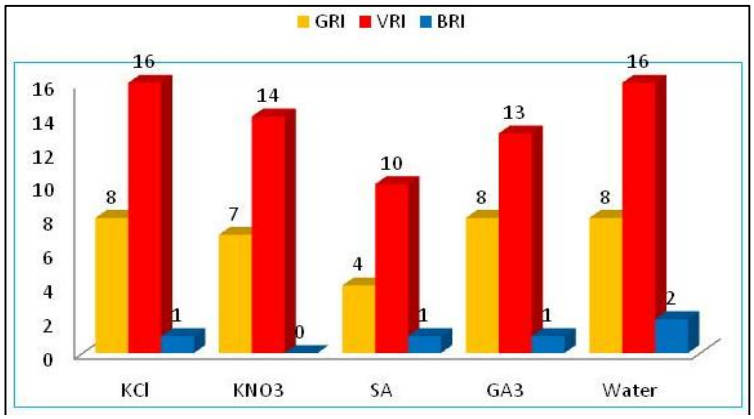

Fig 5: GRI, VRI and BRI values of different priming chemicals

Predicting appropriate post priming storage duration to retain seed germination

To predict appropriate storage duration of different priming treatments to retain seed germination three criteria are taken into consideration. The first one is average decrease in germination% per day; increase in germination% at 0 day as compared to non primed seeds and the last one is maintaining minimum 10% increase in germination over control after the completion of storage period. Prediction is essential to know that how many days the primed seeds can be stored with minimum loss in germination if unfavorable condition prevails at the time of sowing. In the present study it has been observed that the increase in germination count due to priming treatment of 18 months old cowpea seeds was 31.1%, 17.9%, 6.6%, 15.1% and 35.5% as compared to control in case of KCl, KNO_3, salicylic acid, GA3 and distilled water. The average decrease% per day was 0.42, 0.50, 0.67, 0.23 and 0.53 in case of KCl, KNO_3, salicylic acid, GA3 and deionised water. Maximum post priming storage period is obtained by dividing the permissible level of loss (%) with average germination decrease% per day. In Table 11 maximum post priming storage period of different treatments have been mentioned considering minimum 10% increase in germination over control after the completion of storage period. Old cow pea seeds primed with KCl, KNO_3, salicylic acid, GA3 and distilled water could be stored up to 50, 15, 0, 20 and 50 days respectively.

Table 11: Maximum post priming storage period of different priming treatments in old cowpea seeds

old seed							
Treatment	Increase in germination% over control	Permissible level of loss (%)	(% decrease in germination)			Av. decrease/day (%)	Maximum post priming storage period (days)
			15 days	30 days	45 days		
KCl (1%)	31.1	21.1	7.9	8.6	20.0	0.42	50
KNO_3 (1%)	17.9	7.9	11.2	11.2	16.8	0.50	15
Salicylic Acid (50 ppm)	6.6	0.0	14.2	20.4	26.5	0.67	0
GA3(50 ppm)	15.1	5.1	4.9	3.3	12.3	0.23	20
Water	35.8	25.8	7.6	13.9	27.8	0.53	50

The fate of primed seeds during storage is of great importance when farmers are not able to sow the primed seeds immediately. Therefore the objective of this study was to examine the deterioration in seed quality of primed seeds when subjected to storage under ambient environmental conditions. In the present study it was observed that old cowpea seeds primed with KCl were able to give higher germination percentage, seedling growth, seedling vigour, and seedling dry weight. Many other scientists also reported the same findings in many other crops Mohammadi, 2009; Armin

et al., 2010; Afzal *et al*., 2011; Mushtaq *et al*., 2012; Yadav *et al*., 2012; Kumar *et al*., 2013; Tiwari *et al*., 2014; Dutta *et al*., 2015) [23, 5, 1, 26, 36, 19, 14, 34, 13]. Hydro priming in the present study reflected its immense effect on physiological parameters and this finding was supported by others (Basra *et al*., 2002; Xiaoying *et al*., 2005; Neamatollahi *et al*., 2006; Filho and Kikuti, 2008; Moradi and Younesi, 2009; Azarnivand *et al*., 2010; Birendra and Shambhoo, 2011; Tiwari *et al*., 2014) [8, 35, 15, 25, 6, 9, 34].

Germination percentage of primed cowpea seeds in the present investigation was found to decrease as the post priming storage periods increase. This may be due to chromosomal aberrations (Akhtar *et al*. 1992) [3] or may be due to reduction in alpha amylase activity and carbohydrate content (Bailly, 2004) [7]. In the present study it was observed that EC value was increased with increase in storage period in both primed and non primed seeds. Similar finding was also reported by Kaewnaree *et al*. (2010). In the present investigation, the dehydrogenase and alpha amylase activity were found to decrease with the increase in storage period. These findings were supported by Lee and Kim (2000) [21], Jie *et al*. (2002) [17], EL-Arbay and Hegazi (2004) [14], Guzman and Aquino (2007) [16], Moosavi *et al*. (2009) [24], Amanpour-Balaneji and Sedghi (2011) [4], Oaikhena *et al*. (2013) [29] and Tabatabaei (2013) [32].

Conclusion

The results of the present investigation indicated that cowpea seeds primed with KCl (1%) and distilled water have better ability to resist seed quality deterioration under post priming storage duration.

References

1. Afzal I, Hussain B, Basra SMA, Ullah SH. Halopriming triggers higher germination potential and early seedling growth of tomato. Journal of Agricultural social Science. 2011; 7:105-108.
2. Ahammadi AK, Rahman MM, Ahmed M. Effect of osmopriming on the emergence of maize seedling. Journal of Agricultural Research. 2014; 39(3):427-435.
3. Akhter FN, Kabir G, Mannan MA, Shaheen NN. Ageing effect of wheat and barley seeds upon germination, mitotic index and chromosomal damage. J Islam Acad. Sci. 1992; 5:44-48.
4. Amanpour-Balaneji B, Sedghi M. Effect of Aging and Priming on Physiological and Biochemical Traits of Common Bean (*Phaseolus vulgaris* L.). Not Sci Biol. 2011; 4(2):95-100.
5. Armin M, Asghaipour M, Razavi-Omrani M. The effect of seed priming on germination and seedling growth of watermelon (*Citrullus lanatus*). Advanced Environmental Biology. 2010; 4:501-505.
6. Azarnivand H, Mohsen A, Enayati GH. Evaluation and determination of the best hydro and osmopriming treatments for germination properties of tall wheat grass (*Agropyron elongatum*). Journal of Water Management. 2010; 62:431-444.
7. Bailly C. Active oxygen species and antioxidants in seed biology. Seed Sci Res. 2004; 14:93-107.
8. Basra SMA, Zia MN, Mehmood T, Afzal I, Khaliq A. Comparison of different invigoration techniques in wheat (*Triticum aestivum* L.) seeds. Pakistan Journal Arid Agriculture. 2002; 5:11-16.
9. Birendra P, Shambhoo P. Standardization of seed hydro-priming time for rice *(Oryza sativa* L.). Journal of Hill Agriculture. 2011; 2:115-118.
10. Black M, Corbineau F, Grzesik M, Guy P, Come D. Carbohydrate metabolism in the developing and maturing wheat embryo in relation to its desiccation tolerance. Journal of Experimental Botany. 1996; 295:161-169.
11. Chang SM, Sung JM. Deteriorative changes in primed sweetcorn seeds during storage. Seed science & technology. 1998; 26:613-626.
12. Corbineau F, Ozbincol N, Vinel D, Come D. Improvement of tomato seed germination by osmo priming as related to energy metaboli, 2000.
13. Dutta SK, Singh AR. Effect of priming on germination and seedling vigour of bird's eye chilli seeds collected from eastern Himalayan region of India. The Bioscan. 2015; 10:279-284.
14. EL-Arbay MM, Hegazi AZ. Responses of tomato seeds to hydro and osmo priming and possible relations of some antioxidant enzymes and endogenous polyamine fractions, Egyptian journal of biology. 2004; 6:81-93.
15. Filho JM, Kikuti ALP. Hydropriming seed treatment and plant field performance. Hortic. Bras. 2008; 26:165-169.
16. Guzman P, Aquino AL. Longevity of hydro-primed rice seeds. Phillipines journal of crop science. 2007; 32:77-88.
17. Jie LL, Ong S, Dong MO, Fang L, Hua EW. Effect of PEG on germination and active oxygen metabolism in wild rye (*Leymus chinesis*) seed. Acta Prata culture Sinica. 2002; 11:59-64.
18. Kaewnaree P, Vichitphan S, Klanrit P, Siri B, Vichitphan K. Effect of accelerated aging process on seed quality and biochemical changes in sweet pepper (*Capsicum annuum* Linn.). Seeds Biotechnol. 2011; 10(2):175-182.
19. Kumar R, Singh R. Effect of Priming on emergence & vigour of bittergourd. Journal of Research. Punjab Agriculture University. 2013. 50:114-118.
20. Lanteri S, Belleti P, Marzach C, Nada E, Quaglitti L, Bino RJ. Priming induced replication activity in pepper (*capsicum annuum* L.) seeds. Effect on germination and storability. In: basic and applied aspects of seed biology RH Ellis (Edts). Kluwer Academic Publishers. The Netherlands. 1997, 451-460.
21. Lee SS, Kim JH. Total Sugars, alpha-amylase activity, and germination after priming of normal and aged rice seeds. Korean Journal of Crop Science. 2000; 45(2):108-111.
22. McDonald MB. Seed priming. In: Seed technology and its biological basis. M Black and JD Bewley (Eds.), Sheffield Academic Press, UK. 2000, 287-325.
23. Mohammadi GR, Amiri F. The effect of priming on seed performance of canola (*Brassica napus* L.) under drought stress. Am-Euras. J. Agric. & Environ. Sci. 2009; 9:202-207.
24. Moosavi A, Tavakkol-Afshari R, Sharif-Zadeh F, Aynehband A. Effect of seed priming on germination characteristics, polyphenoloxidase, and peroxidase activities of four amaranth cultivars. J. Food, Agr. Environ. 2009; 7:353-358.
25. Moradi A, Younesi O. Effects of osmo- and hydro-priming on seed parameters of grain sorghum (*Sorghum bicolor* L.). Aust. J. Basic Appl. Sci. 2009; 3:1696-1700.
26. Mushtaq S, Hafiz IH, Hasan SZU, Arif M, Shehzad AM, Rafique R *et al*. Evaluation of seed priming on

germination of *Gladiolus alatus*, African Journal of biotechnology. 2012; 11(52):11520-11523.
27. Nascimento WM, Souza de Aragao FA. Muskmelon seed priming in relation to seed vigor. Sci. Agric. 2004; 61:114-117.
28. Neamatollahi E, Bannayan M, Souhani DA, Ghanbari A. Hydropriming and osmopriming effects on cumin (*Cuminum cyminum* L.) seeds germination. World Academy of Science, Engineering and Technology. 2009; 57:526-529.
29. Oaikhena EE, Ajibade GA, Appah J, Bello M. Dehydrogenase enzyme activities in germinating Cowpea (*Vigna Unguiculata* (L) *Walp*). Journal of Biology, Agriculture and Health care. 2013; 3:32-36.
30. Shenoy VV, Dadlani M, Sheshu D. Association of laboratory assessed parameters with field emergence in rice - The non-ionic acid stress as a seed vigour test. Seed Research. 1990; 18:60-69.
31. Singh BB, Chambliss OL, Sharma B. Recent advances in cowpea breeding. Advances in cowpea research. Cooperation of the International Institute of Tropical Agriculture (IITA) and Japan International Research Center for Agricultural sciences (JIRCAS). IITA, Ibadan, Nigeria, 1993.
32. Tabatabaei SA. The effect of priming on germination and enzyme activity of sesame (*Sesamum indicum* L.) seeds after accelerated aging. Journal of Stress Physiology & Biochemistry. 2013; 9:132-138.
33. Taylor AG, Allen PS, Bennett MA, Bradford KJ, Burris JS, Misra MK. Seed enhancement. Seed Sci. Res. 1998; 8:245-256.
34. Tiwari TN, Dipti K, Singh RK, Prasad SR. Relative efficacy of seed priming with potassium nitrate and tap water in relation to germination, invigoration, growth, nitrate assimilation and yield of pigeonpea, Ann. Agric. Res. 2014; 35:164-170.
35. Xiaoying Z, Xiuqing L, Yong X. Germination barriers and hydropriming treatment of triploid watermelon seeds. Scientia Agricultura Sinica. 2005; 38:1238-1243.
36. Yadav VY, Kumari M, Ahmed Z. Seed priming mediated germination improvement and tolerance to subsequent exposure to cold and salt stress in capsicum. Res. J. Seed Sci. 2012; 4:125-136.