Response of cowpea (*Vigna unguiculata* L.) seeds to priming and post priming storage duration

A Thesis submitted to the
Orissa University of Agriculture and Technology
in Partial fulfilment of the Requirement for the degree of
Master of Sciences in Agriculture
(Seed science and technology)

By
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2017

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CERTIFICATE-I

This is to certify that the thesis entitled **"Response of cowpea (*Vigna unguiculata* L.) seeds to priming and post priming storage duration "** submitted in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (SEED SCIENCE AND TECHNOLOGY)** of the **ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY,** Bhubaneswar is a faithful record of *bona fide* and original research work carried out by **MANALI DASH** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

CHAIRMAN
ADVISORY COMMITTEE

CERTIFICATE-II

This is to certify that the thesis entitled **“Response of cowpea (*Vigna unguiculata* L.) seeds to priming and post priming storage durtion ”** submitted by **MANALI DASH** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN AGRICULTURE (SEED SCIENCE AND TECHNOLOGY)** has been approved by the students’ advisory committee and external examiner.

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ACKNOWLEDGMENT

"ananyas cintayato mam. ye janah paryupasate
teshamtesham nityabhiyuktanam. yoga-ksenam vahamy aham".

This couplet from the Bhagavath Gita (Chapter 9 ,sloka 55) states that for ones selfless worship to the lord, the almighty provides the soul with what it lacks and protects what one has.

Thus I am really thankful to the supreme personality of godhead for what I have received in my life because I believe his presence in my dedication. I am thankful to his greatness to bring me to this material world and caring and protecting me in every stage of life.

Many moments come in life where one falls short of words due to the overwhelming emotions (in both extremities of Life). Emotions can't always be conveyed so acknowledgment is a mere formality to pass it on as a token of thanks and abstract of remembrance.

It gives me immense joy to thank and mention the name of my guide Dr. Swarnalata Das, ASPO, BSP, NSP, OUAT, BBSR for her constant support, fabulous guidance, unending zeal and excellent advice during my course of research and the preparation of this thesis. In true sense she is a bona fide master. She has provided me the necessary materials and also helped me time to time. She was very friendly and co-operative in nature. Each moment spent with her was a good experience to me.

I am extremely grateful to Dr. S.K Swain ,Professor, HOD of Seed Science and Technology, College of Agriculture, OUAT, BBSR and a member of advisory committee for providing the required facilities for enabling successful completion of thesis work.

I am also thankful to Dr. Simanta Mohanty for his valuable advice, timely suggestion and inspiration. He used to guide me during this research work.

It is rightly said that "Gods cannot be everywhere, so he created Parents". My father Mr. Suryamani Dash and mother Mrs.Lalita Panda have very well nurturer me from childhood and have always helped me in taking the right decision in every step of my life. I really want to thank them from the core of my heart for their cumulative advice to keep my head strong during any type of academic and co-curricular problems. .

I remain a living memory to recollect the cooperation and help rendered to me by my family members. I would like to thank my eldest sister Mrs. Sushree Sangita Dash, scientist VSSC, ISRO (M. Tech, IIST) for her endless cooperation in completing the thesis and regular guidance regarding thesis writing and extraction of facts from necessary resources. I am really happy to mention the name of my two other elder sisters Mrs. Shubhra Subedita Dash and Mrs. Itishree Ranjita Dash for their care ,emotional support and loving advice. My younger brother Ambika Prasad Dash has really been a good companion during this entire period. He helped me a lot in finding resource from the internet, rearranging the documents and typing the research work.

Last but not the least I would like to thank my friends who have either set a benchmark for competition or helped me in any other way to complete the thesis. I really thank my best friend Babli for her loving attitude and always being at my side whenever I need her. I am equally thankful to Lucky and always wish her to be with me in future.

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ABSTRACT

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important legume vegetable crop of India. Its quick growth and rapid ground cover have made cowpea an essential component of sustainable subsistence agriculture in marginal lands. The present investigation was undertaken to study the effect of storage duration on primed cowpea seeds in addition to priming response of cowpea seeds to halo, hormonal and hydro priming. The experiment was conducted at the Department of Seed Science and Technology, college of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar. The experiment was conducted in a completely randomised design with four replications. Fresh seeds and 18 months old cowpea seeds were primed with potassium chloride (KCl, 1%), potassium nitrate (KNO_3, 1%), gibberellic acid (GA3, 50ppm), salicylic acid (50ppm) and deionised water for 6 hours. Primed seeds were stored upto 45 days under normal room temperature. Seed samples from the respective treatments were drawn at 15 days intervals and subjected to test the physiological and biochemical parameters. Observations were recorded on germination %, root length, shoot length, seedling length, shoot:root length ratio, seedling dry weight, SV-I, SV-II, electrical conductivity, alpha amylase and total dehydrogenase activity. In case of old cowpea seeds 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 % when seeds treated with KCl, KNO_3, Salicylic Acid, GA3and hydro priming respectively; the halo priming agent KCl recorded the highest SV-I value (3313.49); hydro priming exhibited the highest SV-II value (4161.95); non primed seeds recorded the highest EC value (0.801 dS/m) and hydro priming recorded the lowest value (0.597 dS/m); alpha amylase activity was more pronounced in seeds primed with KCl (0.446); the highest dehydrogenase activity was observed in seeds treated with deionised water (383.4 µg/ 20 ml/hr). In case of fresh seeds KNO_3 showed the highest germination count (89.5 %); seeds treated with KNO_3 produced the highest SV-I value (4416.60); seeds treated with deionised water produced the highest SV-II value (4645.80); salicylic acid recorded the highest EC value (0.651 dS/m); alpha amylase activity was more pronounced in seeds primed with KCl (0.462); the highest dehydrogenase activity was observed in seeds treated with KCl (590.0 µg/ 20 ml/hr).With the increase in post-priming storage duration all the physiological parameters started decline both in old and fresh primed and non primed seeds. But the primed seeds showed better quality parameters as compared to non primed seeds at different storage durations. 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). Hydro priming and KCl have high GRI, VRI and BRI values as compared to other treatments and salicylic acid has the lowest GRI, VRI and BRI values. 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. Old cow pea seeds primed with KCl and deionised water could be stored in ambient environment up to 50 days as per the prediction model. From the present investigation it may be concluded that hydro priming and the halo priming agent KCl (1 %) could improve the seed quality parameters of old cowpea seeds to a satisfactory level.

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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). 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).

Cowpea is an inexpensive source of vegetable protein. The protein content ranges from about 3 - 5% in green leaves, 4-5% in immature pods and 25-30 % in mature seeds. The amino acid profile reveals that lysine, leusine and phenylalanine content are relatively higher in cowpea (Bressani & Elias 1980). In India vegetable cowpea is grown over an area of 23,012 ha with production of 1, 33,587 tons of green pod and productivity of 5.8 t/ha. The leading states are UP, Bihar, Jharkhand, West Bengal, Odisha etc. Cowpea is called as vegetable meat due to high amount of protein in grain with better biological value on dry weight basis. It is a crop of sustainability and prosperity. Beside its use as vegetable, pulse and fodder it can also be used as green manure, nitrogen fixer, cover crop, leafy vegetable. Above all it is a drought tolerant and hardy crop and well adapted to relatively dry environment. Apart from this, cowpea forms excellent forage and it gives a heavy vegetative growth and covers the ground so well that it checks the soil erosion. As a leguminous crop, it fixes about 70-240 kg of nitrogen per ha per year. 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.

Since seed is a biological entity, deterioration beyond harvest is inevitable. The consequences of low quality seeds are poor germination, low and delayed emergence and weak growth leading to poor field stand and ultimately reflecting on reduced yield. Low productivity could be attributed broadly to use of poor quality seeds. At present to overcome this, several seed enhancement techniques are available for quality up gradation. Quality seed is the key for successful agriculture, which demands each and every seed should be readily germinable and produce a vigorous seedling ensuring high yield. The farmers are always very much interested in the best seed management practices which are safe, environmentally sound and scientifically proven technologies.

The primed seeds can be a technological tool to provide excellent seedling performance in the field (Leubner, 2000) by reversing some of the ageing inducing deteriorating events (Taylor *et al*., 1998) and there by promote sustainable farming systems especially in marginal environments. For resource poor farmers and indigenous people, who live and farm in marginal environmental, where rainfall is unpredictable and erratic, soil quality is poor and access to inputs is limited, the use of good quality seed can enhance the performance of the crops. The rate of germination and improvement of seedling stands were also accelerated as a result of seed priming in tomato (Corbineau *et al*., 2000). Variation in the results depend 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). 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) and pepper (Lanteri *et al*., 1997). But the literature available in this context is very scanty. Therefore in the present investigation an attempt has been made to study the response of cowpea seeds to priming and post priming storage duration with the following objectives.

- To study the effect of halo, hormonal and hydro priming on physiological and biochemical parameters of old cowpea seeds
- To study the effect of halo, hormonal and hydro priming on physiological and biochemical parameters of fresh cowpea seeds
- To study the effect of post-priming storage duration on physiological and biochemical parameters of old cowpea seeds
- To study the effect of post-priming storage duration on physiological and biochemical parameters of fresh cowpea seeds
- Evaluating the efficacy of priming treatments in order to identify the best one
- Predicting appropriate post priming storage duration to retain seed quality.

REVIEW OF LITERATURE

Seed enhancement through seed priming is an age old practice followed to improve rate of germination, seedling establishment, and faster growth rate. Numerous vegetable and ornamental crop species have been primed successfully. In order to maintain a superior product, seed companies have to maintain seed quality and longevity in the primed seed.

Now-a-days, various seed priming techniques have been developed, including hydropriming (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotica), thermo priming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices), and biopriming (hydration using biological compounds) (Ashraf, Foolad 2005). Each treatment has advantages and disadvantages and may have varying effects depending upon plant species, stage of plant development, concentration/dose of priming agent and storage period. In the present study the objective was focused on post priming storage duration. The literature is reviewed under following sections.

2.1 Effects of halo priming on physiological parameters

2.2 Effects of hormonal priming on physiological parameters

2.3 Effects of hydro priming on physiological parameters

2.4 Effects of priming on biochemical parameters

2.5 Effects of post priming storage duration on physiological and biochemical parameters

2.1 Effects of halopriming (KCl, KNO_3)

Nerson (1986) suggested that salt priming pre-treatment is an attempt to improve the germinability of watermelon seeds at sub-optimal temperatures. Priming in 2–3% solutions of KNO_3 for 1–5 days significantly increased the germination rate, and synchronization percentage. Enhancement of these effects was greatest at 10–11°C for the water melon variety “Persia 202” and at 15–16°C for ‘NoyYizre'el’.

Dehydration of seeds following treatments resulted in partial reversion of the positive effects of priming if incubation took place at the lower temperatures.

Trigo and Trigo (1999) observed that the priming in KNO_3 is efficient to improve germination of eggplant seeds.

Warley (1999) suggested that muskmelon seeds primed for 6 days in darkness at 25 °C in an aerated solution of KNO3 (0.35 M) affected both seed coat adherence and seedling development, and interaction between priming and orientation was significant for seed coat adherence. It indicates that seed priming can minimize seed coat adherence during emergence of muskmelon seeds.

Zheng *et al.* (2002) reported earlier and uniform emergence, improved seedlings fresh and dry weight in rice seeds treated with KCl.

Demir and Oztokat (2003) observed the effect of seed priming with KNO_3 on watermelon seeds and reported that salt priming is a useful technique for improving germination, seedling growth and uniformity of heterogeneously matured watermelon.

Yogananda *et al.* (2004) noticed that bell pepper seeds invigorated with KNO_3 (1%) recorded higher germination, root and shoot length seedling dry weight, rate of germination and seedling vigour index over control.

Farooq *et al.*(2005) studied the effect of priming on fresh seeds of four tomato cultivars and reported that KNO_3 (3%) had lower T_{50} and mean germination time; maximum germination percent, germination index, radicle length, plumule length; highest FEP, root length and shoot length as compared to control.

Iqbal *et al*. (2006) suggested that priming with KCl was helpful in removing the deleterious effects of salts.

Nascimento and Lima (2008) that priming of eggplant seeds in PEG decreasedgermination, while the use of KNO_3 provided the best result

Shim *et al.* (2008) reported that priming with KNO_3 solution for 48 to 72 hours in paspalum improved germination percentage, and uniformity. The increased duration of priming with KNO_3 was positively correlated with an improved germination percentage. The effect of increasing concentration was the most apparent at a constant temperature (30^0C) regime with the treatment of 0.2% KNO3 priming. Germination percentage was increased from 34.3% to 68.0% two weeks after imbibition (WAI) as the priming duration was increased from 24 to 72 hours. Therefore, priming with 0.2% or 0.5% solution of KNO3 for 72 hours is a recommended method that can be practically applied for increasing germination of paspalum under an alternating temperature ($25/35^0$C) condition.

Hassanpouraghdam *et al*. (2009) evaluated the effects of osmo-priming with KNO_3 on the germination traits and seedling growth of two *Brassica napus* L. cultivars under salinity conditions. Seeds of two spring rapeseed ('Heros' and 'Eagle') were primed with KNO_3 (-1.0 MPa) for 24 hrs at 30°C. Primed and un-primed seeds were cultured in medium grade perlite and placed in greenhouse for 40 days. Pots were daily irrigated with nutrient solutions containing different levels of salinity (0.2, 5, 10, 15 and 20 dSm^{-1}). Results showed that germination percentage of primed seeds was greater than that of un-primed seeds. Radicle length, seedling height and dry weight and leaf number of plants derived from primed seeds were higher compared with un-primed seeds.

According to Mohammadi and Amiri (2009) canola seeds primed with potassium nitrate showed the highest values for germination percentage (GP), germination rate (GR), seedling dry weight (SDW), height, LAI, the number of pods per plant, the number of seeds per pod, 1000-seed weight and yield as compared to control under both field and laboratory studies. Priming with GA3 caused an increase in germination percent of pot marigold and sweet fennel in various range of salinity, but in lower salinity levels percent of germination was higher than upper ones. The result of this experiment is consistent with the hypothesis that under undesirable conditions such as salinity stress, priming with GA3 can prepare a suitable metabolic reaction in seeds and can improve seed germination performance and seedling establishment (Sedghi*et al*., 2010a).

Tzortzakis (2009) suggested that priming with KNO_3 in endive and chicory may improve rapid and uniform seedling emergence and plant development in nurseries and/or in greenhouses, which is easily applicable by nursery worker with economic benefits.

Armin *et al*. (2010) evaluated the effect of priming media (PEG 6000 3%, KNO3 3% and none primed) on 3 watermelon cultivars (Niagara, Charleston Gray, and Crimson Sweet) and reported that priming increased watermelon emergence, emergence rate, and plumule length. No significant differences were found on plumule dry weight and radicle length. Priming with PEG negatively affected the rate and growth of emerged seedling. Among the assessed priming media, KNO_3 had the most effective impact on emergence and seedling growth. Compared with the non-primed seeds, seed priming with KNO_3 increased the germination, germination rate and plumule length by 17.87%, 18.65%, and 4.68%, respectively.

Mohammadi and Amiri (2010) obtained the best results in canola due to the priming treatments at the drought stress levels higher than -0.6 or -0.9 MPa. So that, at the -1.5 MPa of drought stress level, germination percentage, mean germination rate, radicle length, plumule length and seedling dry weight of canola were improved 128.62, 200, 223.08, 350 and 69.94, respectively by KNO_3 and 21.44, 71.43, 219.23, 100 and 41.18%, respectively by distilled water. However, seeds primed with KNO_3 showed better performance than those primed with distilled water.

Afzal *et al*. (2011) found that seed primed with varying concentration of KNO3 for 24h significantly improved final germination count, root and shoot lengths which was due to enhanced chlorophyll contents and better germination pattern.

Ghassemi-Golezani *et al*. (2011) conducted a laboratory experiment and found the highest germination rate and seedling dry weight when chick pea seeds primed with KNO_3. The highest grain and mucilage yields were produced by the plants from seeds primed with KNO_3.

Nawaz *et al*. (2011) indicated that halopriming with varying concentrations of KNO_3 improved germination potential and seedling establishment of tomato cultivars proved high emergence and seedling growth.

Mushtaq *et al*. (2012) suggested that seed priming was done in Gladiolus at different concentration of potassium nitrate (KNO_3). All the treatments had significant effect on germination percentage, germination test in growth room, time for 50% germination and mean germination time. Results showed that maximum invigoration was observed in seeds osmo-primed at lower concentrations of KNO_3 and minimum invigoration was observed at higher concentration of KNO_3. It was concluded that germination percentage can be increased by using lower concentrations of KNO_3.

Abdnadani and Ramezani (2012) showed that KNO_3 solutions increased the fresh and dry weight of roots in maize at 2% and 5% concentration primed for 12 h and 18 h. In addition it also increased the vigour index. Grain yield was significantly increased in many crops subjected to priming as compared to non-primed crops.

Reis *et al*. (2012) suggested that priming with KNO_3 improved germination, FEP, MET, GI and vigour in brinjal.

Yadav *et al*. (2012) treated diverse germplasm seeds of 15 okra genotypes with KNO_3 and recorded more than 65.33 % seed germination in two genotypes (IC282288, IC411698) over check (30.0%).

Kumar *et.al.* (2013) reported that bitter gourd seeds primed with 100 ppm KNO_3 for 24 hours gave better germination, field emergence, speed of emergence, seedling length and vigour index-I over the control .

Tiwari *et al*. (2014) studied the effect of potassium nitrate on seed quality parameters, growth and yield of pigeon pea (*Cajanus cajan* L.). Six varieties of pigeon pea viz: 'NDA 1', 'Bahar', 'LRG 30', 'UPAS 120', 'TS 30' and 'Pusa 2002-2' were primed with three concentrations (0.30, 0.40 and 0.50 %) of KNO_3 for 6 hours and one unprimed set was kept as control. Results based on two years data revealed that pigeon pea seeds primed with KNO_3 significantly enhanced the seed germination, seedling length, seedling dry weight, vigour index I and II and finally test weight and grain yield over unprimed control in all the varieties. Among KNO_3 concentrations, 0.30% showed significantly higher values in above characters over rest of KNO_3 concentrations. Deteriorations in seed quality parameters, growth and yield of pigeon pea varieties were noticed when the concentration of potassium nitrate exceeded beyond 0.30%. Pigeon pea varieties differed significantly in respect of all characters studied.

Dutta and Singh. (2015) while working in bird's eye chilli observed that 1% KNO3 recorded the highest germination percentage, root length, shoot length, SV-I, and FEP as compared to non primed control.

Faruk (2015) reported that priming the seeds of Lentil with KCl treatment improved the number of nodules, as well as root and shoot dry weight.

Faruk (2015) showed that lentil seeds primed with KNO_3 treatment improved the number of nodules, as well as root and shoot dry weight when compared to the control.

Yan (2015) soaked the seeds of Chinese cabbage at 20 °C for 8 hours in 200 mmol/l potassium nitrate (KNO_3). Both primed and unprimed seeds germinated under six levels of drought stress (0, − 1.0, − 2.0, − 3.0, − 4.0 and − 5.0 MPa osmotic potential) induced by PEG 6000. Results indicated that germination traits (germination percentage, potential and seedling vigour index) of Chinese cabbage decreased gradually with increasing drought intensity. Seed priming with KNO_3 increased germination traits at all levels of drought stress as compared to the unprimed seed. The results suggested that priming could serve as an appropriate treatment to increase the germination and early seedling growth of Chinese cabbage under drought stress conditions.

2.2 Effect of hormonal priming (salicylic acid, $C_7H_6O_3$; gibberellic acid, $C_{19}H_{22}O_6$)

Ashraf *et al*. (2002) found that GA3 treatment enhanced the vegetative growth of two wheat cultivars under but caused a slight reduction in their grain yield. GA3 treatment enhanced the deposition of Na+ and Cl- in both root and shoots of wheat plants under prevailing field conditions. It also caused a significant increase in photosynthetic activity in both lines at the vegetative stage of the crop.

Yogananda *et al.* (2004) noticed that bell pepper seeds invigorated with GA3 (200 ppm) recorded higher germination, root and shoot length seedling dry weight, rate of germination and seedling vigour index over control.

Afzal *et al.*, (2005) observed that hormone like salicylic acid has also proved for alleviating salinity stress in wheat.

Hussein *et al.* (2007) evaluated the effect of salinity and salicylic acid on growth of maize plants. The beneficial aspects of SA are that it could be used for the improvement of salt bearing capacity of many crops.

Tzortzakis (2009) suggested that priming with Gibberellin (GA3) treatments in endive and chicory may improve rapid and uniform seedling emergence and plant development in nurseries and/or in greenhouses, which is easily applicable by nursery workers with economic benefits.

Sedghi *et al*. (2010) reported that priming with GA_3 caused an increase in germination percent of pot marigold and sweet fennel in various range of salinity, but in lower salinity levels percent of germination was higher than upper ones. The result of this experiment is consistent with the hypothesis that under undesirable conditions such as salinity stress, priming with GA_3 can prepare a suitable metabolic reaction in seeds and can improve seed germination performance and seedling establishment.

Khan *et al.* (2011) reported that wheat seeds primed with GA_3 (20 ppm) recorded minimum mean germination and emergence time as compared to control.

Abnavi & Ghobadi (2012) evaluated the effect of hormonal priming on seed germination in wheat and showed that for hormonal priming, maximum shoot and root length, shoot and root dry weight and germination rate were observed at GA 50 ppm and 24 h treatmeant in Cross-Alborz and Sardari cultivars of wheat.

Anwar *et al*. (2013) assessed the role of salicylic acid in increasing germination of rice. Results of field experiment indicated that germination was maximum in seeds treated with 20 mg/L of salicylic acid for 40 hours.

Iqbal and Ashraf (2013) reported that the salt intolerant cultivar treated with GA_3 in wheat plants might have faced less stress compared with control. Thus, physiologically, GA_3-priming-induced increase in grain yield was attributed to the GA_3-priming-induced modulation of ions uptake and partitioning (within shoots and roots) and hormones homeostasis under saline conditions.

Kumar *et al.* (2013) reported that bitter gourd seeds primed with 100 ppm GA_3 for 24 hours gave better germination, field emergence, speed of emergence, seedling length and vigour index-I over the control.

Saeedipour (2013) reported that cowpea seeds treated with GA_3 shown better performance in overall seedling characters.

Das *et al*.(2014) reported that treatment of fresh bottle gourd seeds with GA_3 (500 ppm) for 24 hours gave significantly maximum germination, seedling vigour index-I and II and seedling dry weight than dry seeds.

Verma *et al*. (2014) studied the positive effect GA_3 on seed quality parameters of *Avena sativa*. The results indicated that priming as such improved the germination, seedling length, dry weight and vigour measured in terms of vigour index-I, II and speed of germination.

Faruk (2015) showed that priming of red lentil seeds with GA3 may be useful due to its positive effects on germination rate, germination percentage, yield component and grain yield of lentil. An experiment was conducted under laboratory and field conditions in order to evaluate the effect of GA3. GA3 treatment increased shoot length. In the pot experiments, GA3 treatment increased plant height and seedling emergence rate.

Pramanik *et al.* (2015) observed that sesame seeds primed with GA3 significantly improved plant height, aerial biomass production, chlorophyll content, relative leaf water content, number of primary branches/plant, number of capsules/ plant, number of seeds/ capsule, seed yield, stalk yield and harvest index were obtained. Crop receiving GA3 produced high seed yield (886 kg/ ha), stalk yield (1314 kg/ ha) asd compared to control.

2.3 Effects of hydro priming

Basra *et al*. (2002) reported that wheat seeds respond to different pre-sowing seed treatments and hydro priming for 48 h showed the maximum invigoration followed by hydro priming treatment for 24 hours.

Farooq *et al*. (2005) optimized hydro priming techniques (viz.12, 24, 36, 48 and 60 hour) for vigour enhancement in both rice types and they reported that maximum vigour enhancement was calculated in seeds hydro primed for 48 hour which was followed by 36 hours.

Xiaoying *et al*. (2005) observed that hydro priming can overcome the germination barriers related to seed coat of triploid watermelon seeds.

Joudi and Sharifzadeh (2006) observed that in lab experiment, hydropriming can improve germination percentage and rate, length of coleoptiles and root, dry weight of root and shoot as well as seed vigour index in barley.

Neamatollahi *et al.* (2006) reported that hydro priming increased seedling dry weight under saline condition.

Farooq *et al*. (2007b) stated that priming (hydro priming) resulted in improved germination speed, seedling fresh and dry weight, root and shoot length, seedling nitrogen and total sugar.

Filho and Kikuti (2008) suggested that hydropriming can promote speed of germination and speed of seedling emergence but have no effect on yield of cauliflower.

Ghassemi *et al*. (2008) reported that hydro priming resulted in higher seedling emergence in the field compared to seed priming with PEG 6000 in lentil.

Moradi and Younesi (2009) treated the seeds of sorghum by hydro-priming for different time (12, 24 and 36 hours) and they reported that hydro-priming improved the percentage and mean emergence time (MET) of seeds at sub- optimal temperature of 15°C. Seed treatment for 12 and 24 hours had a positive and statistically significant effect on percentage and speed of emergence.

Yu-jie *et al*.(2009) concluded that among the hydro priming treatments the highest speed of germination was observed for seeds hydrated in 500 μL of water per 1 g of seeds for 48 h at 15°C in case of chinese aster. This treatment accelerated seed germination at 10°C compared with the control.

Azarnivand *et al*. (2010) conducted experiment on hydro priming using five hydration time levels (6, 12, 18,24 and 30 hours) and they observed that the treatment for 12 hours had the highest effect compared to other levels in tall wheat grass.

Eniseh and Khourshid (2010) conducted a field experiment under drought stress and found that hydro priming increased LAI and net assimilation rate and proline, chlorophyll and carotenoid contents of aerial parts and protein and oil contents of the soyabean seeds under stress and non-stress conditions.

Ghassemi-Golezani *et al*. (2010b) obtained the highest chickpea yield with 16 hours of hydro priming and suggested that this priming duration is the best treatment for invigoration of chickpea seeds.

Mohammadi and Amiri (2010) obtained the best results due to the priming treatments at the drought stress levels higher than -0.6 or -0.9 MPa. So that, at the -1.5 MPa of drought stress level, germination percentage, mean germination rate, radicle length, plumule length and seedling dry weight were improved 21.44, 71.43, 219.23, 100 and 41.18%, respectively by distilled water.

Mohseney *et al*. (2010) evaluated the effect of seed priming with water and control on corn varieties (704 and 640 K.SC). They reported that varieties did not differ significantly but maximum speed of germination was recorded for hydro priming (water) and control (without pre-treatment) .

Amooaghaie (2011) observed that the seedlings primed with water showed more growth with respect to root and shoot length and germination percentage in comparison with seedlings obtained from non-primed seeds.

Birendra and Shambhoo (2011) reported that all the hydro priming treatments (8, 16, 24, 32, 40, 48, 56, 64 and 72 hours) resulted in enhancement of seed germination at both first and final count, and seedling vigour with respect to seedling length, fresh and dry weight of seedlings, relative growth index and mean daily emergence for rice except seed hydro priming for 64 and 72 hours.

Li *et.al*. (2011) conducted laboratory experiments on freshly harvested pyrethrum (*Tanacetum cinerariifolium*) seeds to investigate the effects of hydro priming on germination and it was concluded that hydro priming shortened the delay of MGT and improved the germination percentage.

Rouhi *et al*. (2011b) investigated the effect of hydro priming on the germination indexes and seed vigour of Tall fescue (*Festuca arundinacea*) and concluded that hydro priming had significant effect on the percent and speed of germination, length of radicle, coleoptile and seedling, as well as vigour indices in tall fescue.

Santos *et al*. (2011) suggested that priming with water for 24 hours was effective to promote the vigour of Moringa seeds.

Singh *et al*. (2011) observed that in laboratory and green house conditions hydro priming treatments of cowpea seeds were found to be superior over unprimed treatment regarding seed germination, emergence and growth at 3 weeks after sowing.

Tiwari *et al.* (2014) studied the effect of tap water on seed quality parameters, growth and yield of pigeon pea (*Cajanus cajan* L.). Six varieties of pigeon pea viz: 'NDA 1', 'Bahar', 'LRG 30', 'UPAS 120', 'TS 30' and 'Pusa 2002-2' were primed with tap water for 6 hours and one un primed set was kept as control. Results based on two years data revealed that pigeon pea seeds primed with tap water significantly enhanced the seed germination, seedling length, seedling dry weight, vigour index I and II and finally test weight and grain yield over un primed control in all the varieties.

2.4 Effects of priming on biochemical parameters

Ebukanson and Bassey (1992) states that in germinating seeds, stored food, e.g. starch, is hydrolysed to glucose for use in the growth. Reactivity of these dehydrogenases covered barely the first three days of cowpea seed germinations. The enzymes separately mediated the conversion of stored carbohydrates and lipids through the anaerobic respiratory oxidation process.

Jones *et al.*, (1999) observed that anaerobic respiration occurs during resting stages of seeds and the initial stages of seed germination. It was observed that for cowpea, the L-lactate pathway was the preferred pathway for anaerobic dehydrogenation, However the Alcohol pathway was also significant. This evidences that the enzymatic reaction was still substantially within the anaerobic phase. This was further sustained by the low reactivity of succinate dehydrogenase which is required mostly in the glyoxylate cycle of the aerobic respiratory phase. .

Lee and Kim (2000) suggested that activation of α- amylase is documented during priming. More the α-amylase activity higher will be the metabolic activity in seeds, which indicates the higher vigour of the seed.

Zheng *et al.* (2002) stated that in rice, seed primed with mixed-salt solution resulted in significant increase in activity of α-amylase, β-amylase, and root dehydrogenase, and moderate increase in the activity of shoot catalase under salt stress.

Jie *et al*. (2002) observed that in wild rye (*Leymus chinensis*) seed, priming with 30% PEG for 24 h resulted in increase in the activity of superoxide dismutase (SOD) and peroxidase (POD) and a rapid increase in the respiratory intensity, which were associated with an increase in germination vigour.

Farooq *et al*. (2005) reported that in tomato seeds primed with KNO_3 resulted in lower electrical conductivity of seed leachates as compared to non primed seeds.

Moosavi *et al*. (2009) studied effect of priming on biochemical parametes in amaranthus. They observed a strong correlation between lowering of the mechanical restraint and the activity of endo-beta-mannanase. Primed seeds significantly showed the increased emergence percentage, rate of emergence, root length and seed vigor in all amaranth cultivars. Trigin cultivar showed the best performance among cultivars. Total seed protein, POD and PPO were also increased significantly by seed priming. Almont and Plainsman cultivars exhibited high protein content and POD activity. PPO activity increased by seed priming comparing to controls for Amount, Plainsman and Mercado cultivars, but for Trigin cultivar, no increase was detected. The highest increase in PPO activity was observed in Mercado cultivar.

Amanpour-Balaneji and Sedghi (2011) studied the changes in physiological and biochemical characteristics of common bean under aging and priming treatments. Their results showed that osmo-priming had the ability to relatively ameliorate the aging effect and recover some of the seed aspects like germination rate, protein and phytin content for invigorate germination and seedling establishment.

Oaikhena *et al.* (2013) reported that dehydrogenases contributed to the catalyses of stored products in the anaerobic phase of seed germination in cowpea.

Tabatabaei (2013) showed that catalase and ascorbate peroxidase activity in sesame seeds decreased after ageing, but priming with GA3 increases enzyme activity in seeds after aging.

2.5 Effects of post-priming storage duration on physiological and biochemical parameters

EL-Arbay and Hegazi (2004) carried out experiments on priming of tomato seeds. They primed the seeds with pure water, PEG and K_2HPO_4 and after priming the seeds was sown at 0, 2 and 4 weeks following ambient air drying of the primed seeds. Germination percentage, seedling growth and enzyme activity showed the best results at zero dry storage.

Guzman and Aquino (2007) took two sets of non dormant naturally aged farmer bred (M12-22B5 and AG-5) and traditional (*Lantik*, *Kotong*) rice varieties and subjected those varieties to hydro priming by soaking for 10 hours and drying back to their original moisture content. Overall results showed that hydro priming has a favourable effect on germination specially deteriorated seeds with known low germination percentage. One month after hydro priming, the germination of M12-22B5 increased from 71 to 84 %, AG-5 from 69 to 78 %, and Lantik from 28 to 32 %. On the average different varieties reached 50 % of their initial germination after 2.4 to 3.2 months of storage for non primed seeds and 7.25 to 7.8 months for hydro primed seeds.

Abnavi and Ghobadi (2012) reported that maximum shoot and radicle length, shoot and radicle dry weight, rate of germination and germination percentage in cv. Cross Alborz was observed in 60 days after seed priming. In general, 60 days after seed priming was better than other storage duration after seed priming. These results showed that for both wheat cultivars germination test at 30, 45 and 60 days after seed priming had more shoot dry weight, more radicle dry weight, more shoot length, more radicle length, more speed of germination and more germination percentage than immediately after seed priming.

MATERIALS AND METHODS

The present investigation has been undertaken to study the effect of priming and post priming storage duration on seed quality parameters of fresh and old cowpea seeds. Priming is the process of controlled hydration of seeds to a level that allows pre-germination activities but does not permit primary root protrusion.The experiment was conducted at the Department of Seed Science and Technology, college of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar. The details of materials used and methods adopted are described in this chapter.

3.1 Experimental details

Seed material:

Fresh and 18 months old cowpea breeder seeds of variety Utkal Manika were collected from AICRP on Vegetable Crops to conduct the experiment

Experimental Design: Completely randomised design

Replications : 4

Priming treatments

Priming treatments used for the present investigation were mentioned below

Halo priming agent:

i) Potassium chloride (KCl, 1%)

ii) Potassium nitrate (KNO_3, 1%)

Hormonal priming agent:

iii) Gibberellic Acid (GA3, 50ppm)

iv) Salicylic Acid (50ppm)

Hydro priming:

v) Deionised water

Soaking period : 6 hours

Post priming storage period: 0, 15, 30 & 45 days

3.1.1 Seed priming procedure

Fresh seeds and 18 months old cowpea seeds were taken in specimen 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 hydro priming the seeds were soaked in deionised water. Seeds were soaked in solutions at ambient temperature for 6 hours. After priming for 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 seed 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 15 days intervals and subjected to test the proposed physiological and biochemical parameters.

3.2 Observations recorded

The 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 EC of seed leachate (dS/m), alpha amylase activity and dehydrogenase activity were recorded and documented under respective headings as explained below.

3.2.1 Seed germination (%)

Germination test was conducted on pure seed fraction using 100 seeds in four replicates following between papers (BP) methods at normal room temperature. Four replications of hundred seeds each were used for germination test. The numbers of normal seedlings were counted on 8th day (final count) of germination from all the replications.The average of four replications was expressed as germination percentage. The germination per cent was calculated based on the number of normal seedlings produced.

3.2.2 Root length (cm)

Ten normal seedlings were taken randomly from each replication for measuring the root length. The length between the collar region and the tip of the primary root was measured and mean value was recorded in cm.

3.2.3 Shoot length (cm)

The same ten normal seedlings used for root length measurement were also used for shoot length measurement. The length between the collar region and the tip of the shoot was measured and mean value was recorded in cm.

3.2.4 Seedling dry weight (mg/ seedlings)

After the germination test i.e., on 8th day, the same ten normal seedlings used for root length measurement from each replication were kept in a butter paper bag and dried for 24 hours in a hot air oven maintained at 80°C. The dried seedlings were removed and cooled under room temperature and then the dry weight of seedling was recorded using an electronic balance. The average weight of seedling was computed and expressed in milligram.

3.2.5 Seedling vigour index-I & II

SV-I = Seed germination (%) × seedling length (cm)

SV-II = Seed germination (%) × seedling dry weight (mg)

3.2.6 Electrical conductivity of seed leachates

Electrical conductivity of seed leachate was estimated as follows. Four replications of 8 g old and new 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.

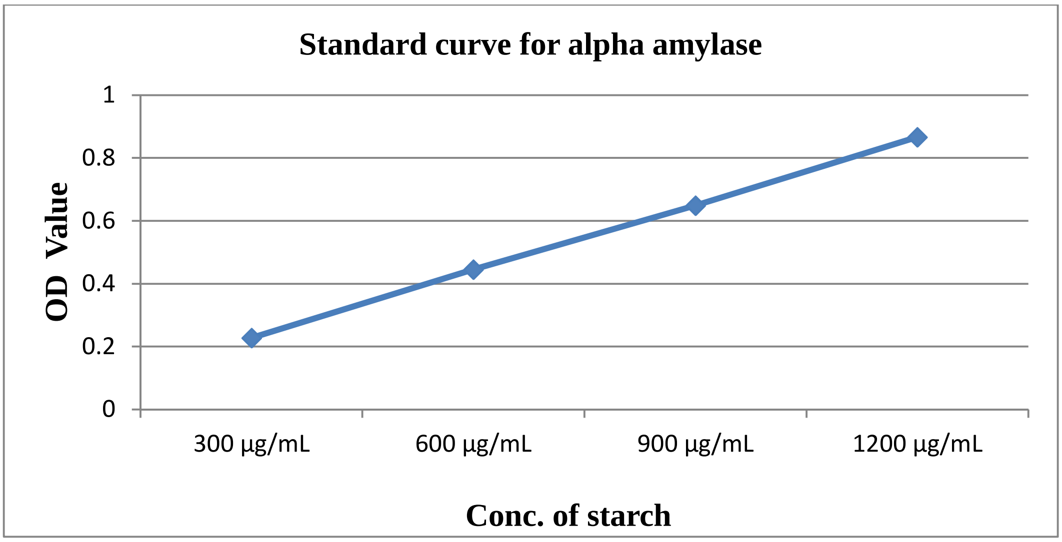
3.2.7 Alpha amylase activity

Alpha amylase activity was determined following the procedure described in appendix-ii. Four replicates of old and new cowpea seeds from each priming treatments along with control were taken for the study. Alpha amylase activity was expressed in terms of OD (optical density) value.

Procedure

- One gram of germinated cowpea seeds were taken from each treatment.
- Then seeds were macerated in a pestle and mortar separately by adding 10 ml of citrate buffer step wise slowly.

- Then we had transferred the content to the centrifuge tube and centrifugation was continued for 15 minutes at 2000 rpm.
- Then we had collected the supernatant for determination of α-amylase activity as µg starch digested per 10 minutes/g of seed.
- Before addition of reagents, all the test tubes should be kept at 37^0C.
- Also during reaction time the temperature should be maintained at 37^0 C.
- After development of colour, the OD was measured at 620 nm wave length of light Therefore, the α-amylase activity is calculated as µg starch digested /gram of seed /10 minutes or more by using the standard curve.



3.2.8 Dehydrogenase activity

Dehydrogenase activity was determined following the procedure described in appendix-iii. Four replicates of old and new cowpea seeds from each priming treatments along with control were taken for the study. Dehydrogenase activity was determined from the standard curve and expressed as µg/ 20 ml/hour.

Procedure

1. We had taken 1 gram of cowpea seeds of same size (i.e. well filled seeds) from each treatment of fresh and old seeds
2. Then the seeds were soaked for 12 hours
3. The seed coats were removed from the seeds.

4. Then we had kept the soaked seeds without the seed coat in 0.05 % tetrazolium solution (volume is 20 ml) for 4 hours.

5. Tetrazolium solution was decanted and the seeds were washed in water thoroughly.

6. We added 20 ml methanol (i.e the seeds were kept in methanol solution and we waited for the complete discolouration of the seeds).

7. Then we took the reading of the solution by the help of a spectrophotometer at 480 nm of light along with the blank methanol (as standard).

Preparation of standard curve

- We prepared the stock solution by taking 10 mg of formazan (TCF) in 100 cc methanol (conc. was 100 ppm).

- We took 1 ml stock solution and added 19 ml methanol so that the final volume was 20 ml (concentration was 5 ppm).

- From the stock solution we prepared 5 ppm, 10 ppm, 15 ppm and 20 ppm solution and made the final volume 20 ml by adding methanol as follows.

i) 1ml stock solution + 19 ml methanol = 5 ppm (vol 20ml)

ii) 2 ml stock solution + 18 ml methanol =10 ppm (vol 20 ml)

iii) 3 ml stock solution + 17 ml methanol = 15 ppm (vol 20 ml)

iv) 4 ml stock solutiom + 16 ml methanol = 20 ppm(vol 20 ml)

- We had measured the OD value and prepared the standard curve

Enzyme quantification

Let us consider the OD value of the solution is 2 and its concentration is 5 ppm (as per the standard curve) i.e the solution contains 5 mg formazan in 1000 cc solution.

1000 ml solution contains 5 mg formazan = 5×10^{-3} gram

1 ml solution will contain = (5×10^{-3} gram) ÷ 1000 = (5×10^{-6}) g of formazan

= 5×10^{-6} gram

= 5 µg

So 20 ml solution will contain 5 x 20 µg = 100 µg formazan

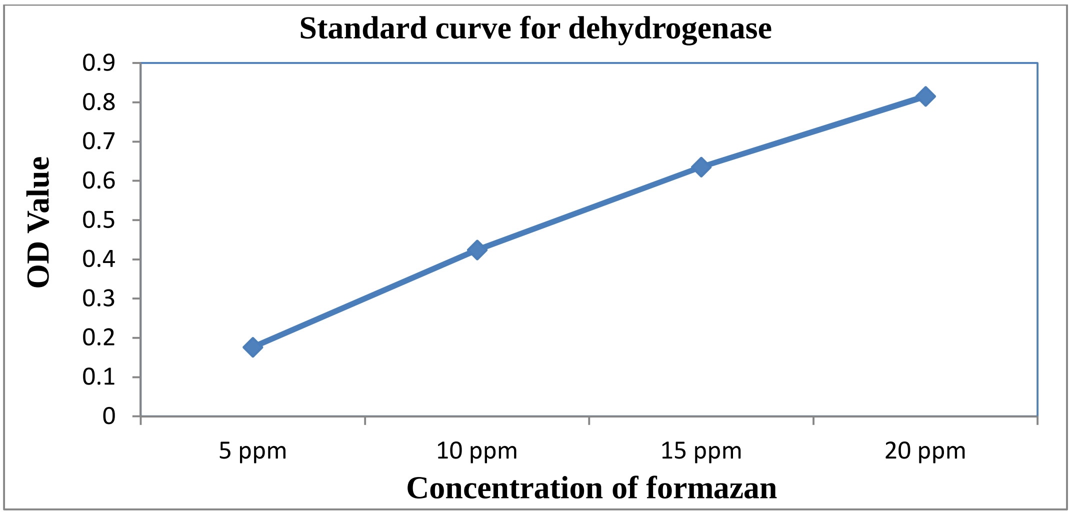
i.e. from 1 gram seeds we get 100 µg formazan.

Again we keep the seeds in tetrazolium for 4 hours.

Therefore quantity of formazan produced from 1 gm seeds in 1 hour will be

= 100µg ÷ 4 = 25µg

So, the quantity of formazan present is 25µg/20ml/hr.



3.3. Statistical analysis

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.

3.3.1 Estimation of seed quality index (SQI)

This parameter is used to evaluate efficacy of priming treatments. Higher value of SQI indicated higher efficacy. SQI is obtained by adding germination response index, vigour index and biochemical response index values. Evaluation of germination response index, vigour index and biochemical response index are described in result chapter.

RESULTS

The present investigation was aimed to study the effect of halo, hormonal and hydro priming in improving seed quality parameters of old cowpea seeds.The effect of post priming storage duration of different priming agents on fresh cowpea seeds was also investigated. The lab-test was carried out in a completely randomised design with four replications. Statistical analysis was done using SAS 9.3 version software. The result was presented under following sub-heads.

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

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

4.3 Comparing response of fresh and old cowpea seeds to post-priming storage duration

4.4 Correlation coefficient between physiological and biochemical parameters

4.5 Predicting the efficacy of priming treatments

4.6 Predicting appropriate post priming storage duration to retain seed quality

4.1 Effect of halo, hormonal and hydro priming and post priming storage duration on old cowpea seeds

4.1.1 Germination percentage

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, GA3 and hydro priming respectively.

After 15 days of post priming storage duration the germination count was found to decrease both in primed and non primed seeds. All the priming treatments except KNO_3 achieved significantly higher germination count than control. The highest germination percent was recorded by hydro priming (66.5 %) and the lowest by salicylic acid (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.

In case of 45 days of storage time 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.

Post priming storage duration study revealed that increased storage period would decrease the germination percent. The decrease in germination percent for the halo priming agent KCl was 7.9 %, 8.6 % and 20.0 % at 15, 30 and 45 days of post priming storage duration. 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 % . The decrease rate was high in case of hydro priming and low in KNO_3 and all the priming treatments had higher decrease rate as compared to non primed seeds with the increase in post priming storage duration.

Table 1 Germination percentage of old seeds

Old seeds				
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**

4.1.2 Root length

Data presented in Table 2 indicated significant effect of priming treatments on root length during zero post-priming storage periods. 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 day 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) of old seeds

Old seeds				
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**

4.1.3 Shoot length

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 KNO_3 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, KNO_3, 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) of old seeds

Old seeds				
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**

4.1.4 Shoot length: Root length ratio

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

Old seeds				
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**

4.1.5 Seedling length

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 was reported in GA3 (45.09 cm). No significant differences were observed among the treatments.

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) of old seeds

Old seeds				
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**

4.1.6 Seedling dry weight

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, KNO_3, salicylic acid and GA3.

Table 6: Seedling dry weight (mg/plant) of old seeds

Old seeds				
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**

4.1.7 Seedling vigour index-I (SV-I)

Significant differences were observed among the treatments for zero storage periods (Table 7). At 0 day storage period, 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, GA3and 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 day. 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) where as 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).

Comparing the effect of post priming storage period on SV-I, it was observed that there is a gradual decrease in SV-I value with the increase in storage period when the old cowpea seeds were treated with KCl. In rest of the treatments SV-I value started to decrease from 0 to 15 days then increase at 30 days and again decrease at 45 days. This discrepancy is may be due to effect of environment on germination and seedling growth.

Table 7 Seedling vigour index-I (SV-I) of old seeds

Old seeds				
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**

4.1.8 Seedling vigour index-II (SV-II)

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 KNO_3 (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.

Germination count, 0 day of storage photos taken on 5th day

Germination count, 15 days of storage (photos taken on 8th day)

Germination count, 30 days of storage (photos taken on 8th day)

Germination count, 45 days of storage (photos taken on 8th day)

Electrical conductivity test up to 45 days of storage

0 day

15 days

30 days

45 days

Conductivity Meter

α-amylase activity

0 day **15 days**

30 days **45 days**

Prepared Reagents **Spectrophotometer**

Dehydrogenase activity (15 days of storage)

New lots

Old lots

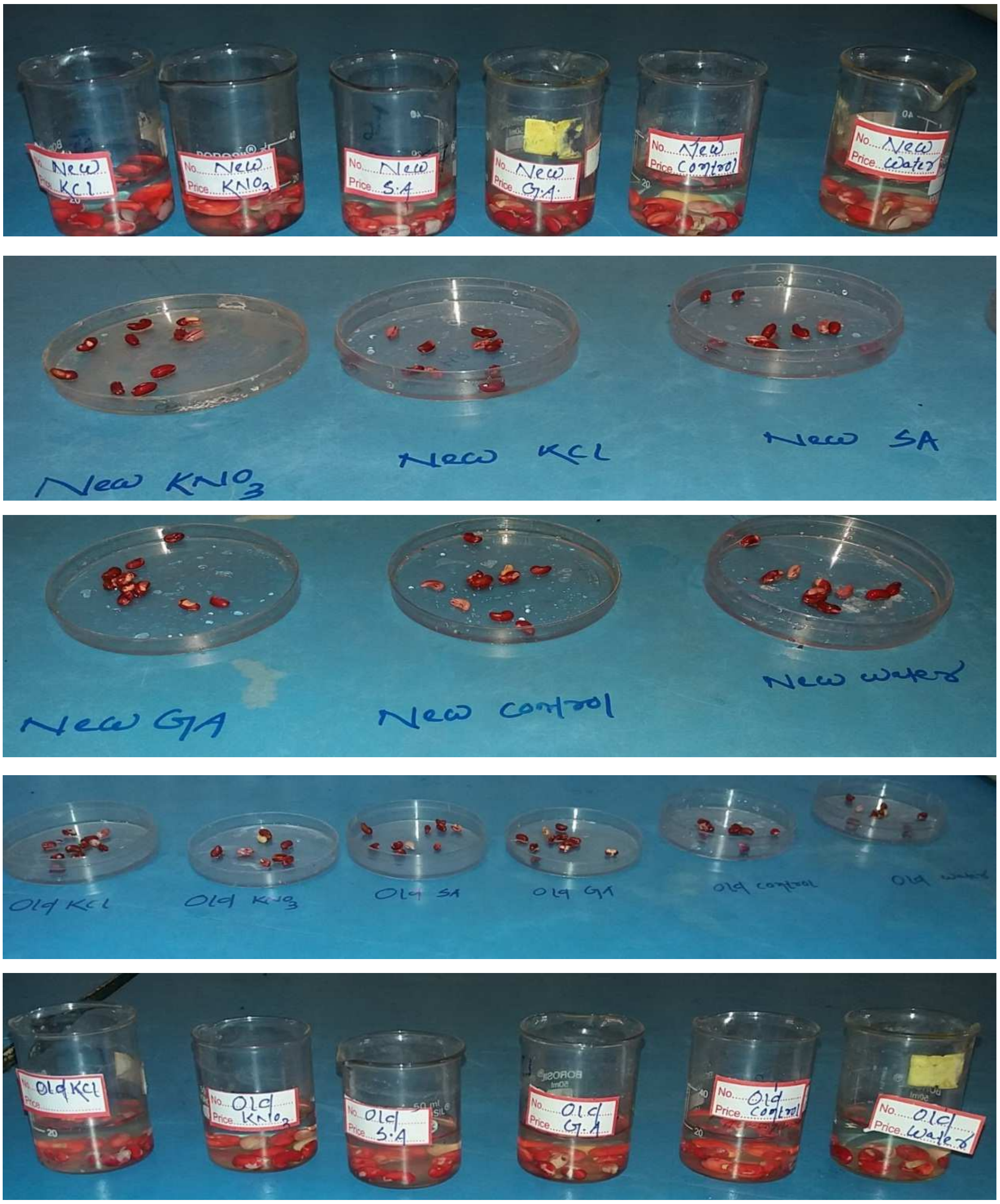
Colour development after 15 minutes

Colour development after 30

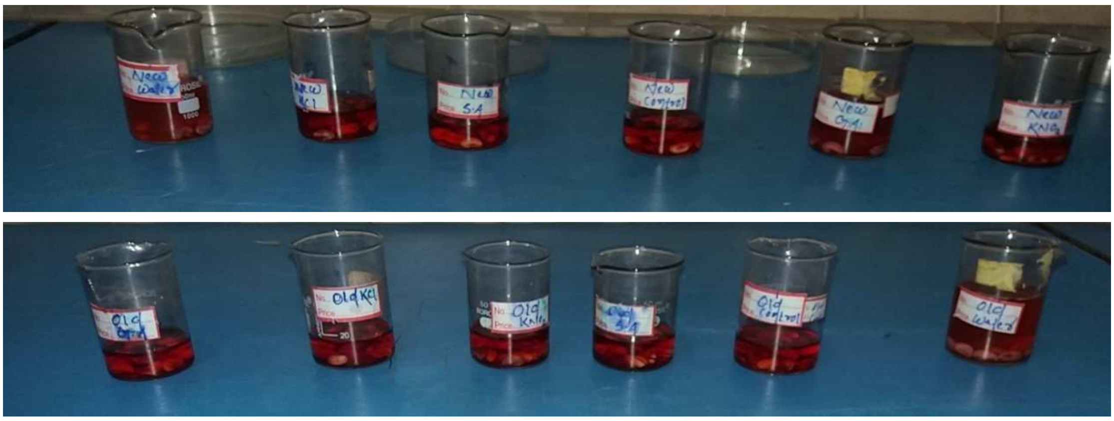
Colour development after one hours

Colour development after two hours

Dehydrogenase activity(30 days of storage)



Dehydrogenase activity, 45 days of storage



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, salicylic acid, 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 of old seeds

Old seeds				
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**

Biochemical parameters

4.1.9 Electrical conductivity of seed leachate (EC)

The data on electrical conductivity of seed leachate (dS/m) is presented in Fig. 4.1. 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 priming recorded the lowest 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 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.

4.1.10 Alpha amylase enzyme activity

Alpha amylase enzyme activity expressed in terms of optical density (OD) value for different post priming storage periods is depicted in Fig 4.2. 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, deionised water and non primed seeds respectively as compared to zero storage period. 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, deionised water and non primed seeds respectively as compared to zero storage periods.

4.1.11 Dehydrogenase enzyme activity

Dehydrogenate activity of different priming treatments against post priming storage periods was reflected in Fig.4.3. Significant differences were observed among the treatments during zero storage period. The highest dehydrogenase activity (383.4µg/ 20 ml/hr) was observed in seeds treated with deionised 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 had 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, deionised water and non primed seeds respectively as compared to zero storage period.

At 30 days of storage period, significant differences were observed among the treatments for dehydrogenase activity. There was a gradual decrease in dehydrogenase activity for each priming treatment. Seeds treated with KNO_3 had the lowest dehydrogenase activity (147.0µg/ 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 treated were with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

Increasing the storage period from 30 to 45 days showed further decrease in dehydogenase activity of priming treatments. There was a gradual decrease in dehydogenase activity for each priming treatments. The enzyme activity varied from 104.5.5 µg/ 20 ml/hr (KNO_3) to 278.8µg/ 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, deionised water and non primed seeds respectively as compared to zero storage period.

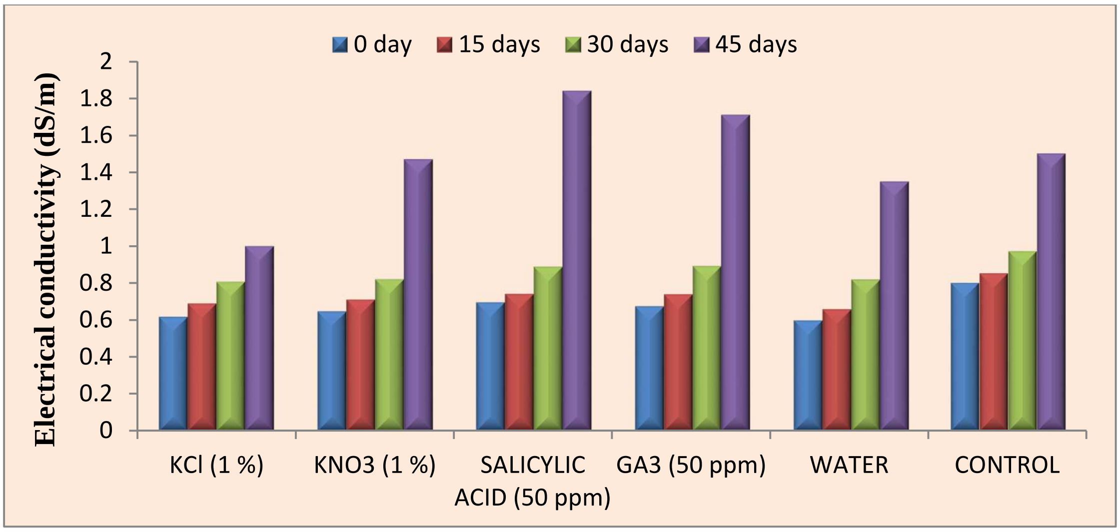


Fig.4.1 Electrical conductivity in old cowpea seeds treated with different priming agents

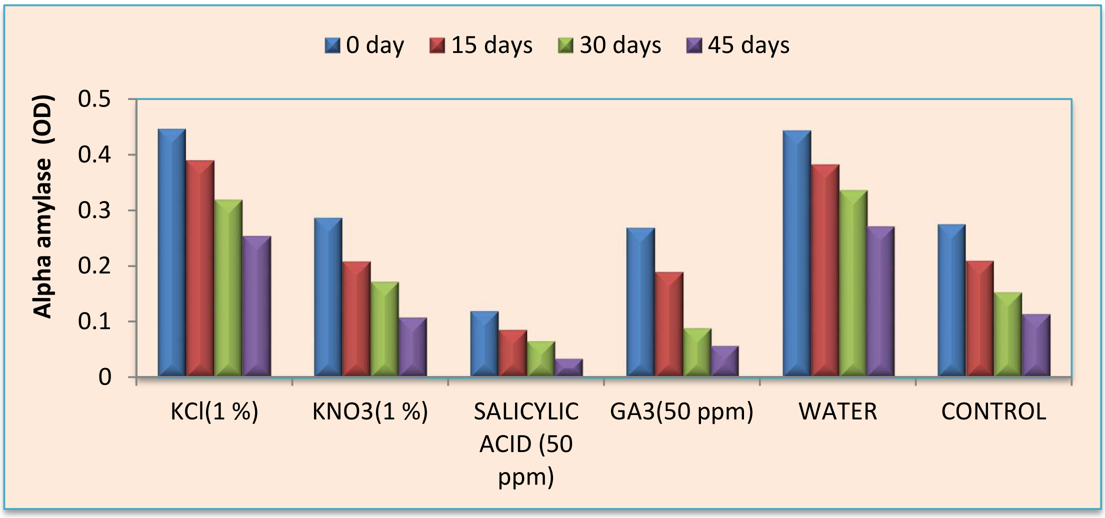


Fig.4.2 Alpha amylase activity in old cowpea seeds treated with different priming agents

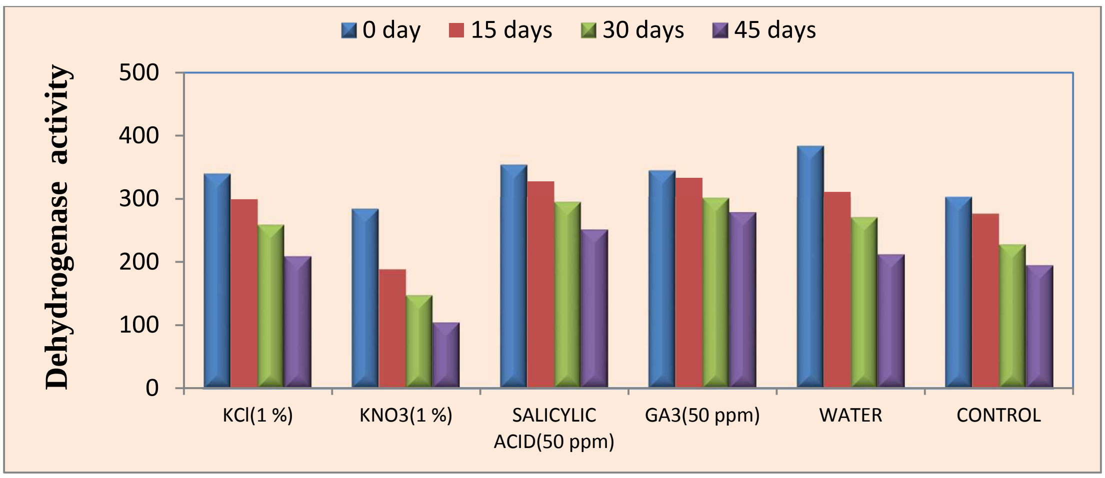


Fig.4.3 Dehydrogenase activity in old cowpea seeds treated with different priming agents

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

4.2.1 Germination percentage

Germination count of fresh cowpea seeds is presented in Table 9. Significant differences were observed among the treatments at zero storage period. Germination count varied from 83.0 to 89.5 %. Fresh seeds treated with KNO_3 showed the highest germination count (89.5 %) and it was at par with all other treatments and significantly different from non primed seeds.

With the increase of post priming storage duration from 0 to 15 days, it was observed that germination count of the corresponding treatments decreased. Germination count varied from 80.0 to 88.5 %. Fresh seeds treated with halo priming agents KCl (87.0 %) and KNO_3 (88.5 %) showed better performance than hydro priming (86.34 %) as well as from hormonal priming agents i.e. salicylic acid (81.5 %) and GA3 (82.5 %). Non primed seeds recorded the lowest count of 80.20%. The rate of decrease in germination count was 1.7 %, 1.1 %, 2.4 %, 6.8 %, 1.9% and 0.96 % when seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

At 30 days of post priming storage duration, significant differences were observed among the treatments. Seeds treated with KNO_3 had higher germination count (87.50 %) and the lowest was being recorded in salicylic acid (73.0 %).The rate of decrease in germination count was 7.3 %, 2.2 %, 12.6 %, 11.6 %, 6.6 % and 4.8 % when seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

During 45 days of post priming storage periods, germination count of corresponding treatments was further decreased. Seeds treated with KNO_3 had higher germination count (86.0 %) and the lowest was being recorded in GA3 (55.0 %). All the treatments except KNO_3 showed low germination count as compared to non primed seeds. The rate of decrease in germination count was 7.3 %, 2.2 %, 12.6 %, 11.6 %, 6.6 % and 4.8 % when seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

Table 9 Germination percentage of fresh cowpea seeds

Fresh seeds				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1 %)	88.50	87.00	82.00	77.00
KNO_3 (1 %)	89.50	88.50	87.50	86.00
SALICYLIC ACID(50 ppm)	83.50	81.50	73.00	60.50
GA3(50 ppm)	88.50	82.50	78.21	55.00
WATER	88.00	86.34	82.19	77.00
CONTROL	83.00	80.20	79.00	78.00
CD 1%	**6.29**	**7.81**	**7.98**	**7.03**
CV %	**3.56**	**4.55**	**4.89**	**4.78**

4.2.2 Root length

Experimental study on root length of the fresh seeds indicated gradual decrease in root length with the increase in post-priming storage period (Table 10) invariably for all the treatments. At zero day, seeds treated with KNO_3 resulted in higher root length (18.43 cm) followed by both hydro priming and KCl (16.71cm) and the lowest was reported in salicylic acid (14.85 cm).

At 15days of post priming storage duration , root length produced by different treatments varied from 14.57 cm to 17.23 cm. Seeds treated with KNO_3 resulted in higher root length (17.23 cm) and it was at par with KCl and hydro priming but significantly different from other treatments. Root length produced by salicylic acid (14.60 cm) and GA3 (14.57 cm) were significantly lower than non primed seeds.

At 30days of post priming storage duration, seeds treated with KNO_3 resulted in higher root length (16.16 cm) and it was at par with KCl (15.59 cm), hydro priming (15.26 cm) and control (15.38).

At 45 days of post priming storage duration, significant differences were observed among the priming treatments. Seeds treated with KCl recorded the highest root length (14.40 cm) followed by KNO_3 (14.42 cm) but these two were at par with non primed seeds.

Table 10   Root length (cm) of fresh cowpea seeds

Fresh seeds				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1 %)	16.71	16.38	15.59	14.40
KNO_3 (1 %)	18.43	17.23	16.16	14.12
SALICYLIC ACID(50 ppm)	14.85	14.60	14.11	12.96
GA3(50 ppm)	15.30	14.57	12.78	10.23
WATER	16.71	16.11	15.26	13.68
CONTROL	16.68	16.41	15.38	13.23
CD 1%	**1.29**	**1.21**	**1.28**	**1.62**
CV %	**3.85**	**3.75**	**4.23**	**4.57**

4.2.3 Shoot length

Shoot length of fresh cowpea seeds as influenced by different priming treatments is presented in Table 11. All priming treatments at zero days showed significant differences in their effect on shoot length. Shoot length was found to vary from 27.27 cm to 31.60 cm. Seeds treated with salicylic acid produced the highest shoot length (31.60 cm) followed by KNO_3 (30.93 cm) and hydro priming (30.53 cm). All the priming treatments recorded higher shoot length than non primed seeds.

At 15 days of post priming storage duration, significant differences were observed among the treatments. The shoot length produced by salicylic acid scored 1st rank (31.29 cm) and it was at par with KNO_3 (30.61 cm) and salicylic acid (30.53 cm). The lowest shoot length was recorded in non primed seeds (27.0 cm).

At 30 days of post priming storage duration, priming treatments were found to produce discrepancy in their effect on shoot length. Seeds treated with KCl recorded the highest shoot length (32.47 cm) among all the treatments and also exceeded the shoot length it produced at 0 and 15 days of storage periods. All treatments except hydro priming produced higher shoot length as compared to 15 days of storage periods.

At 45 days of post priming storage duration, significant differences were observed among the treatments. Seeds treated with KCl (31.31 cm), KNO_3 (31.85 cm), salicylic acid (30.49 cm) and hydro priming (30.47 cm) were at par with each other in their effect on shoot length.

Table 11 Shoot length (cm) of fresh cowpea seeds

Fresh seeds				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1 %)	27.80	27.39	32.47	31.31
KNO_3 (1 %)	30.91	30.61	31.41	31.85
SALICYLIC ACID(50 ppm)	31.60	31.29	31.10	30.49
GA3(50 ppm)	28.89	28.60	30.52	26.92
WATER	30.53	30.23	28.70	30.47
CONTROL	27.27	27.00	31.46	29.06
CD 1%	**2.63**	**1.59**	**2.43**	**2.71**
CV %	**4.38**	**2.67**	**3.86**	**4.45**

4.2.4 Shoot length: root length ratio

Shoot: root length ratio of fresh cowpea seeds is presented in Table 12. From this data table it was observed that all the treatments at different days of storage periods had higher shoot length than root length. At 0 day, significant differences were observed among the treatments in respect of shoot: root length ratio. Seeds treated with salicylic acid had the highest ratio (2.26) followed by GA3 (1.93).This indicated that these two hormonal priming had positive effect on shoot length as compared to root length. All treatments had higher ratio than non primed seeds.

At 15 days of post priming storage duration, seeds treated with salicylic acid and GA3 ranked 1st (2.18) and 2nd (2.01). Here, shoot: root ratio ranged from 1.67 to 2.18. Hydro priming (1.88) had more positive effect as compared to halo priming agents KCl (1.68) and KNO_3 (1.78).

With the increase of post priming storage period from 15 to 30 days, the ratio was found to increase at 30 days as compared to 15 days. The highest ratio was reported in seeds treated with GA3 (2.46) followed by salicylic acid (2.25). The effect of the halo priming agent KCl (2.09) was at par with non primed seeds (2.07).

At 45 days of post priming storage duration, the treatments showed significant ratio in respect of shoot: root length ratio. The highest ratio was reported in seeds treated with GA3 (2.64) followed by salicylic acid (2.36). Seeds treated with KCl showed the lowest ratio (2.17).

Table 12 Shoot: root length ratio of fresh cowpea seeds

Fresh seeds				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1 %)	1.67	1.68	2.09	2.17
KNO_3 (1 %)	1.68	1.78	1.95	2.27
SALICYLIC ACID(50 ppm)	2.26	2.18	2.25	2.36
GA3(50 ppm)	1.93	2.01	2.46	2.64
WATER	1.83	1.88	1.88	2.24
CONTROL	1.66	1.67	2.07	2.23
CD 1%	**0.18**	**0.19**	**0.21**	**0.22**
CV %	**4.82**	**4.94**	**4.76**	**4.65**

4.2.5 Seedling length

Seedling length of fresh cowpea seeds is presented in Table 13. Effect of priming treatments without storage (0 day) showed significant differences among the treatments. Seedling length was found to vary from 43.94 cm to 49.35cm. Seeds treated with KNO_3, deionised water and salicylic acid ranked 1st (49.35 cm), 2nd (47.24 cm) and 3rd (46.44 cm) respectively. Non primed seeds recorded the lowest seedling length (43.94cm).

At 15 days of post priming storage duration, the seedling length produced by different treatments started to decline. Seedling length was found to vary from 43.17 cm to 47.84 cm. Seeds treated with KNO_3, deionised water and salicylic acid ranked 1st (47.84 cm), 2nd (46.33 cm) and 3rd (46.44 cm) respectively.

At 30 days of post priming storage duration, significant differences were observed among the treatments. During this period seedling length produced by different treatments had increased as compared to 15 days. The highest seedling length was recorded in seeds treated with KCl (48.06 cm) followed by KNO_3 (47.56 cm). Non primed seeds recorded higher seedling length (46.83 cm) than the hormonal and hydro priming treatments.

With the advancement of post priming storage period from 30 to 45 days seedling length produced by the priming treatments was found to decrease at 45 days as compared to 30 days except in hydro priming. Seedling length varied from 37.15 cm to 45.98 cm.

Table 13 Seedling length (cm)of fresh cowpea seeds

Fresh seeds				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1 %)	44.50	43.77	48.06	45.70
KNO_3 (1 %)	49.35	47.84	47.56	45.98
SALICYLIC ACID(50 ppm)	46.44	45.89	45.21	43.45
GA3(50 ppm)	44.19	43.17	43.31	37.15
WATER	47.24	46.33	43.96	44.14
CONTROL	43.94	43.40	46.83	42.29
CD 1%	**3.88**	**3.83**	**3.51**	**3.78**
CV %	**4.15**	**4.18**	**3.77**	**4.31**

4.2.6 Seedling dry weight

Seedling dry weight of fresh cowpea seeds is presented in Table 14. At 0 day, it was observed that there were no significant differences among the treatments. Seedling dry weight was found to vary from 49.68 mg to 52.80 mg. Hydro priming recorded the highest weight (52.80 mg) followed by salicylic acid (50.98 mg), KCl (50.63 mg) and GA3 (50.18 mg).

By increasing the post priming storage duration from 0 to 15 days, significant differences were observed among the treatments. The dry weight was found to decrease after 15 days of storage. Non primed seeds recorded the highest weight (48.05 mg) and the lowest was being recorded by GA3 (41.08 mg).

Coming to 30 days of post priming storage period it was revealed that the dry weight increased in seeds treated with KCl, KNO_3, salicylic acid and GA3 at 30 days as compared to 15 days. Seeds treated with KCl showed the highest dry weight (48.03 mg) and the lowest was recorded in GA3 (41.65 mg).

At 45 days of post priming storage duration, seedling dry weight varied from 36.43 mg to 46.08 mg. Seeds treated with KNO_3 showed the highest dry weight (46.08 mg) and the lowest was recorded in GA3 (36.43 mg).

Table 14 Seedling dry weight (mg/plant) of fresh cowpea seeds

Fresh seeds				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1 %)	50.63	44.90	48.03	45.55
KNO_3 (1 %)	49.68	44.38	45.88	46.08
SALICYLIC ACID(50 ppm)	50.98	44.28	45.95	44.80
GA3(50 ppm)	50.18	41.08	41.65	36.43
WATER	52.80	45.73	42.48	42.83
CONTROL	49.78	48.05	47.35	45.10
CD 1%	**NS**	**3.58**	**4.20**	**3.61**
CV %	**2.96**	**3.93**	**4.56**	**4.08**

4.2.7 Seedling vigour index-I (SV-I)

Seedling vigour index-I of different priming treatments is given in Table 15. All the priming treatments without any post priming storage showed significant differences. The SV-I value varied from 3644.55 to 4416.60. Seeds treated with KNO_3 produced the highest SV-I value (4416.60) and the lowest was being recorded in non primed seeds (3644.55). All the priming treatments reflected their positive effect as compared to non primed seeds.

At 15 days of post priming storage duration, the SV-I value produced by different treatments started to decline. SV-I values were found to vary from 3474.80 to 4230.28. Seeds treated with KNO_3, deionised water ranked 1st (4230.28) and 2nd (3983.32) respectively. Halo priming treatments had found to have better impact on SV-I value as compared to hormonal priming treatments.

At 30 days of post priming storage duration, the priming treatments showed significant differences. Seeds treated with KNO_3 recorded the highest value (4148.47) followed by KCl (3992.04) and the lowest value was being recorded when seeds treated with salicylic acid (3322.06). Seeds treated with hormonal priming agents and deionised water had less value as compared to non primed seeds.

With the advancement of post priming storage period from 30 to 45 days, SV-I values produced by the priming treatments was found to decrease at 45 days as compared to 30 days except in hydro priming. SV-I value varied from 2043.16 to 3953.89. The decrease in SV-I values were 10.5 %, 10.5 %, 32.1% , 47.7 % , 18.8 % and 9.5 % for seeds treated with KCl, KNO_3, salicylic acid, GA3, hydro priming and in non priming condition respectively as compared to zero day.

Table 15 SV-I value of fresh cowpea seeds

Fresh seeds				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1 %)	3937.77	3808.55	3992.04	3522.76
KNO_3 (1 %)	4416.60	4230.28	4148.47	3953.89
SALICYLIC ACID(50 ppm)	3875.36	3739.51	3322.06	2630.60
GA3(50 ppm)	3908.80	3564.52	3447.41	2043.16
WATER	4155.67	3983.32	3599.36	3373.90
CONTROL	3644.55	3474.80	3693.29	3298.35
CD 1%	**252.80**	**280.37**	**336.52**	**271.17**
CV %	**3.11**	**3.63**	**4.47**	**4.25**

4.2.7 Seedling vigour index-II (SV-II)

Seedling vigour index-II of different priming treatments is given in Table 16. All the priming treatments without any post priming storage showed significant differences. The SV-II value varied from 4129.95 to 4645.80. Seeds treated with deionised water produced the highest SV-II value (4645.80) and the lowest was being recorded in non primed seeds (4129.95). All the priming treatments reflected their positive effect on SV-II value as compared to non primed seeds.

At 15 days of post priming storage duration, the SV-II value produced by different treatments started to decline. SV-II values were found to vary from 3389.98 to 3930.38. Seeds treated with deionised water and KNO_3 ranked 1st (3930.38) and 2nd (3928.25) respectively. Halo priming treatments had found to have better impact on SV-II value as compared to hormonal priming treatments.

At 30 days of post priming storage duration, the priming treatments showed significant differences. Seeds treated with KNO_3 recorded the highest value (4013.95) followed by KCl (3942.80) and the lowest value was being recorded when seeds treated with GA3 (3248.85). Seeds treated with hormonal priming agents and deionised water had less value as compared to non primed seeds.

With the advancement of post priming storage period from 30 to 45 days, SV-II values produced by the priming treatments was found to decrease at 45 days as compared to 30 days except in hydro priming. SV-II value varied from 2002.65 to

3965.05. The decrease in SV-II values were 21.6 %, 10.8 %, 36.3 % , 54.9 % , 29.1 % and 14.8 % for seeds treated with KCl, KNO_3, salicylic acid, GA3, hydro priming and in non priming condition respectively as compared to zero day.

Table 16 SV-II value of fresh cowpea seeds

Fresh seeds				
Treatment	**0 day**	**15 days**	**30 days**	**45 days**
KCl (1 %)	4480.95	3908.85	3942.80	3510.00
KNO_3 (1 %)	4445.00	3928.25	4013.95	3965.05
SALICYLIC ACID(50 ppm)	4253.50	3608.80	3352.85	2709.85
GA3(50 ppm)	4442.65	3389.98	3248.85	2002.65
WATER	4645.80	3930.38	3491.15	3296.13
CONTROL	4129.95	3846.65	3742.80	3517.00
CD 1%	**308.52**	**266.93**	**287.54**	**291.11**
CV %	**3.45**	**3.48**	**3.89**	**4.52**

Biochemical parameters

4.2.9 Electrical conductivity of seed leachate (EC)

The data on electrical conductivity of fresh cowpea seed leachate is presented in Fig.4.4. Fresh cowpea seeds treated with different priming agents showed significant variation in their electrical conductivity at zero storage period (0 day storage). EC value varied from 0.556 dS/m to 0.732 dS/m. Among the treatments seeds treated with salicylic acid recorded the highest EC value (0.651 dS/m) followed by GA3(0.0.638 dS/m). All the treatments had low EC value as compared to non-primed seeds (0.732 dS/m).

At 15 days of storage period, significant differences were observed among the treatments. EC value varied from 0.623 dS/m to 0.786 dS/m. Among the treatments seeds treated with salicylic acid recorded the highest EC value (0.651 dS/m) followed by salicylic acid (0.703dS/m). All the treatments had low EC value as compared to non primed seeds (0.786 dS/m).

At 30 days of storage period, salicylic acid recorded the highest EC value (0.797dS/m) followed by GA3 (0.789dS/m). With the advancement of storage period from 30 to 45 days, EC value was found to increase irrespective of the treatment type. Salicylic acid recorded the highest EC value (0.740 dS/m) whereas hydro priming had the lowest value (0.837dS/m).

High EC value indicated that seed quality is deteriorated at a higher rate and low EC value indicated low deterioration rate.

4.2.10 Alpha amylase enzyme activity

Alpha amylase enzyme activity was expressed in terms of optical density (OD) value. Significant differences were observed among the treatments for alpha amylase activity. From the fig 4.4 it was observed that alpha amylase activity was more pronounced in seeds primed with KCl (0.462) followed by hydro priming (0.408) and lowest activity was reported in salicylic acid (0.175) 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 period. Alpha amylase activity varied from 0.148 to 0.395. Seeds treated with KNO_3, salicylic acid and GA3 had low alpha amylase activity than non primed seeds. Hydro priming and KCl were at par with each other and significantly differed from rest of the treatments. The reduction rate of the enzymes in seeds primed with KCl, KNO_3, salicylic acid, GA3, deionised water and dry seeds were 14.5 %, 29.1 %, 15.4 %,21.7 %, 3.4 % &15.7 % 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 seeds treated with KCl (0.372) and the lowest in salicylic acid (0.106). The rate of decrease in alpha amylase activity was 19.5 %, 38.2%, 39.4 %, 30.9 %, 12.0 %, and 32.7 % in KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage periods. The highest decrease was observed in salicylic acid followed by KNO_3 and non primed seeds.

At 45 days of storage period, the enzyme activity was the highest in case of hydro priming (0.298) and the lowest in salicylic acid (0.087). 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 35.5 %, 59.5 %, 50.3 %, 63.4 %, 29.4 %, and 58.0 % when fresh seeds treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage periods.

4.2.11 Dehydrogenase enzyme activity in fresh seeds

Dehydrogenate activity of different priming treatments against post priming storage periods was reflected in Fig.4.6. Significant differences were observed among the treatments during zero storage period. The highest dehydrogenase activity was observed in seeds treated with KCl (590.0 µg/ 20 ml/hr) followed by KNO_3 (492.9 µg/ 20 ml/hr) and the lowest was recorded in non primed seeds.

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 KCl showed the highest enzyme activity (508.8 µg/ 20 ml/hr) and the lowest was reported in non primed seeds (336.6 µg/20ml/hr). Fresh seeds treated with halo priming agents had executed high dehydrogenase activity as compared to hormonal priming agents GA3 (351.5 µg/ 20 ml/hr) and salicylic acid (348.3µg/ 20 ml/hr).The rate of decrease in dehydrogenase activity was 13.8 %, 25.8 %, 6.2 %, 7.5 %, 14.5 % and 11.4 %in seeds treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

At 30 days of storage period, significant differences were observed among the treatments for dehydrogenase activity. There was a gradual decrease in dehydrogenase activity for each priming treatment. Seeds treated with KNO_3 had the lowest dehydrogenase activity (147.0µg/ 20 ml/hr). The rate of decrease in dehydrogenase activity was 21.41 %, 39.0 %, 14.2 %, 16.5 %, 18.9 % and 25.8 %when fresh seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

Increasing the storage period from 30 to 45 days showed further decrease in dehydrogenase activity of priming agents. There was a gradual decrease in dehydrogenase activity for each priming treatment. The enzyme activity varied from 104.5.5 µg/ 20 mL/hr (KNO_3) to 278.8µg/ 20 mL/hr (GA3). The rate of decrease in dehydrogenase activity was 55.1 %, 52.0 %, 28.2 %, 23.9 %, 42.3 % and 40.6 %when fresh seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

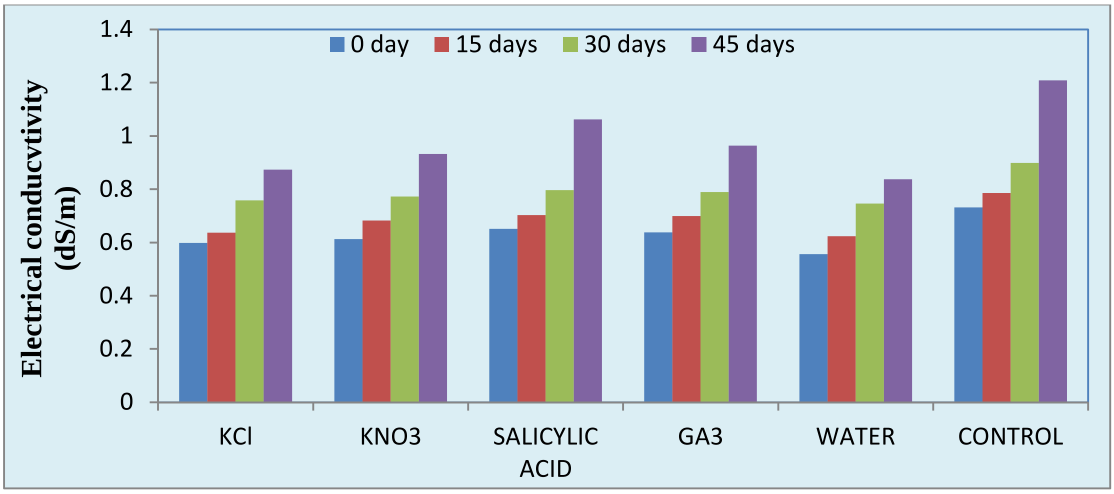


Fig.4.4 Electrical conductivity of fresh cowpea seeds treated with different priming agents

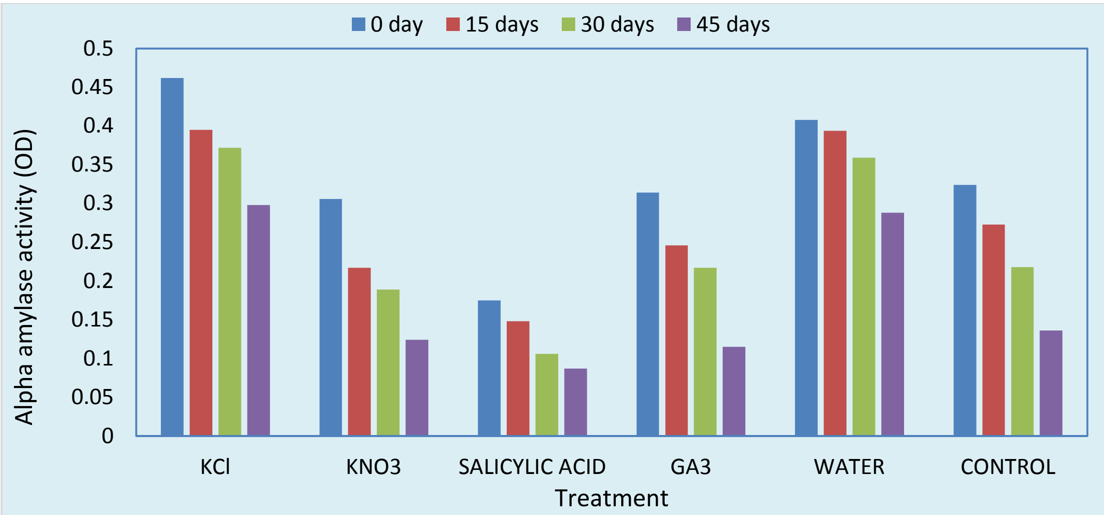


Fig.4.5: Alpha amylase activity of fresh cowpea seeds treated with different priming agents

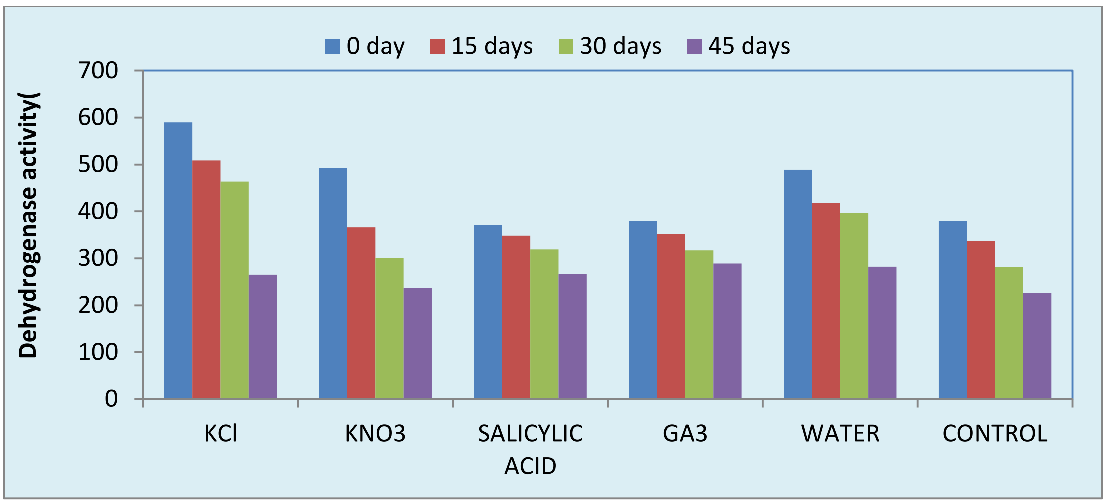


Fig.4.6: Dehydrogenase activity of fresh cowpea seeds treated with different priming agents

4.3 Comparing response of fresh and old cowpea seeds to priming and post-priming storage duration

4.3.1 Comparing response of fresh and old cowpea seeds to priming treatments

4.3.1.1 Germination response of fresh and old cowpea seeds

Old cowpea seeds treated with different priming agents showed a positive response in respect of germination count. All the treatments resulted in higher germination count as compared to non primed seeds. In old seeds hydro priming showed the maximum increase (35.8 %) in germination count and KCl showed second highest increase (31.1 %) as compared to non primed seeds. In fresh seeds KNO_3 showed the maximum increase (7.8 %) in germination count as compared to non primed seeds.

4.3.1.2 Seedling vigour response of fresh and old cowpea seeds

Seedling vigour index-I of the priming treatments was more as compared to non primed seeds. In case of old seeds, the maximum increase in SV-I value was recorded by KCl (68.3 %) followed by hydro priming. In case of fresh seeds, KNO_3 showed the highest positive effect (21.2 % increase) followed by hydro priming (14.0 % increase) over non primed seeds. In old seeds the highest increase in SV-II value (51.8 %) was achieved by hydro priming followed by KCl (40.7 %). Similar trend was observed in fresh seeds, where hydro priming recorded the highest increase of 12.5 %.

4.3.1.3 Response of fresh and old cowpea seeds to priming treatments in respect of bio chemical parameters

In both old and fresh cowpea seeds all priming treatments recorded lower EC value as compared to non-primed seeds. Amongst the priming treatments, hydro priming showed the lowest EC value and salicylic acid showed the highest value in both old and fresh seeds.

In old cowpea seeds, halo and hydro priming had increased alpha amylase activity whereas hormonal priming had decreased alpha amylase activity as compared to non-primed seeds. For old seeds, the increase in alpha amylase activity was 62.2 % and 61.1 % in KCl and deionised water respectively and – 56.7 % in salicylic acid. In fresh seeds, the increase in alpha amylase activity was the highest in KCl (29.9 %) followed by deionised water (25.9 %) and the lowest in salicylic acid (– 46.0 %).

In old cowpea seeds, halo and hydro priming had increased dehydrogenase activity whereas hormonal priming had decreased dehydrogenase activity as compared to non primed seeds. For old seeds, the increase in dehydrogenase activity was the highest in KCl (48.6 %) followed by KNO_3 and deionised water (33.3 %) and the lowest in GA3 (-5.1 %). In fresh seeds, the increase in dehydrogenase activity was the highest in KCl 55.3 % followed by KNO_3 (29.8 %) and the lowest in GA3 (– 2.2 %).

4.3.2 Comparing response of fresh and old cowpea seeds to post-priming storage duration

4.3.2.1 Germination response

Effect of post-priming storage duration on germination of fresh and oldcowpea seeds primed with KCl is depicted in Fig.4.7. From Fig.4.7 it was observed that with the increase in post priming storage duration, decrease in germination rate increases in both old and fresh seeds. From Fig.4.8 it was observed that the decrease rate in old cowpea seed was (11.2 %, 11.2 % and 16.8 %) higher as compared to fresh seeds (1.1 %, 2.2 % and 3.9 %) at 15, 30 and 45 days. Seeds primed with KNO_3under storage showed reduction in germination with the increase in storage period in both old and fresh seeds. The reduction rate was much higher in old seeds as compared to fresh seeds. In case of salicylic acid (Fig.4.9) the decrease rate was high in old seed than fresh seed at 15 days. But at 30 days the decrease rate or reduction in germination percent was high in fresh seed (12.6 %) though it was slightly less than old seed (20.4 %). At 45 days the reduction in germination per cent of fresh seed (27.5 %) exceeded the reduction rate of old seed (26.5 %). In case of GA3 (Fig.4.10) the result was quite interesting because the reduction in germination percent was higher in fresh seeds (6.8 %, 11.6 % and 37.9 %) as compared to old seeds (4.9 %, 3.3 % and 12.3 %) at 15, 30 and 45 days. In case of hydro priming (Fig.4.11) the reduction rate was higher in old seeds as compared to fresh seeds. Dry seeds and old seeds had higher rate of reduction than fresh seeds.

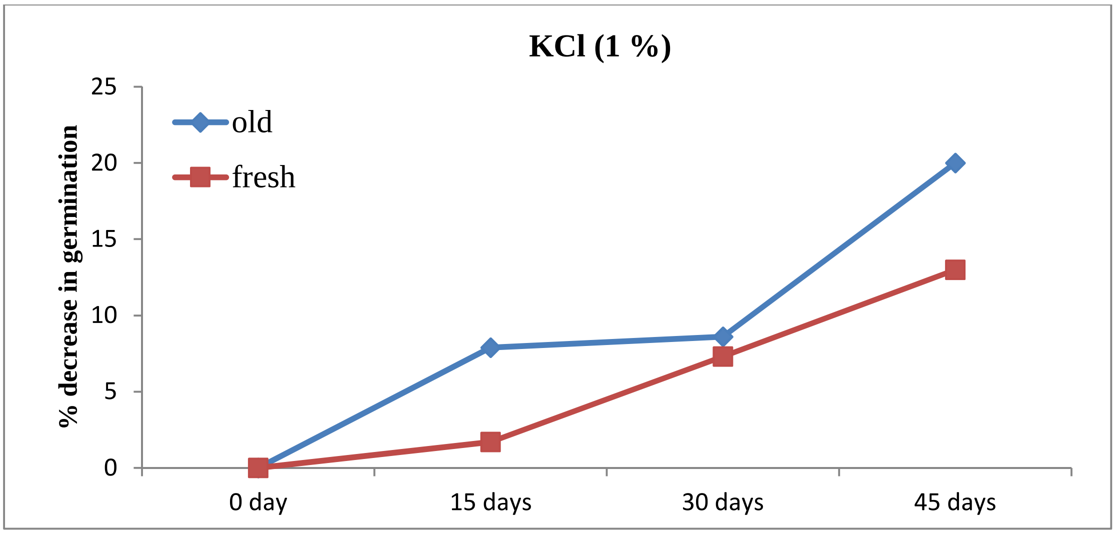


Fig.4.7 Effect of post-priming storage duration on germination of fresh and old cowpea seeds primed with KCl

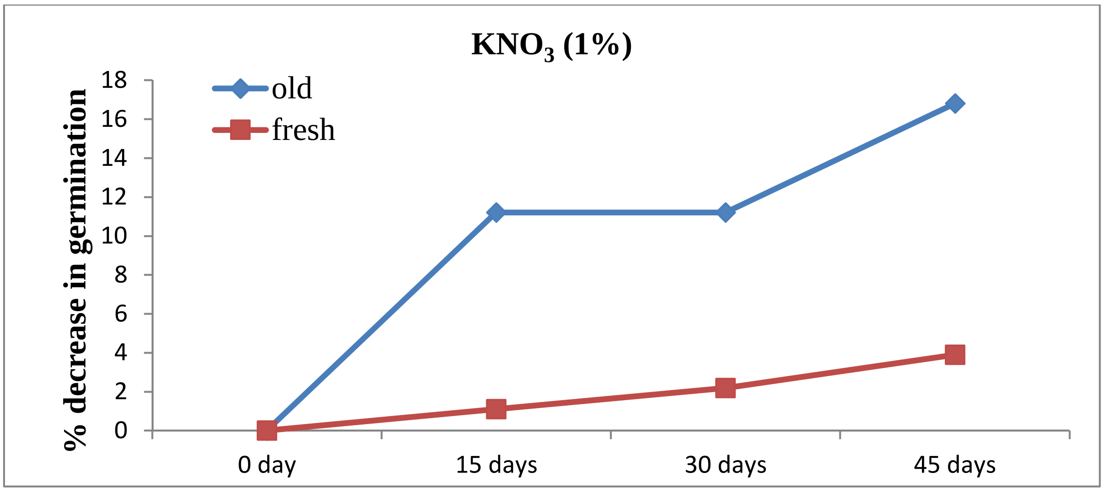


Fig.4.8 Effect of post-priming storage duration on germination of fresh and old cowpea seeds primed with KNO_3

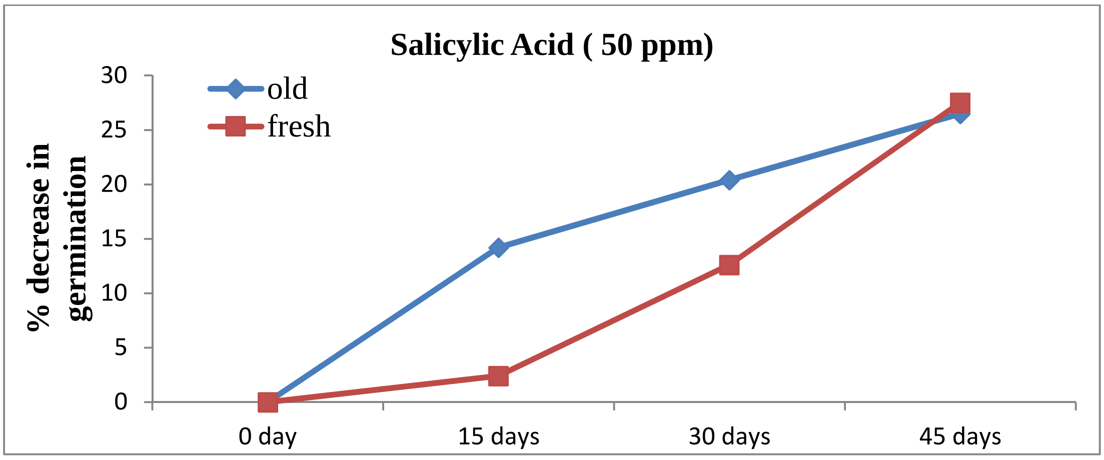


Fig.4.9 Effect of post-priming storage duration on germination of fresh and old cowpea seeds primed with salicylic acid

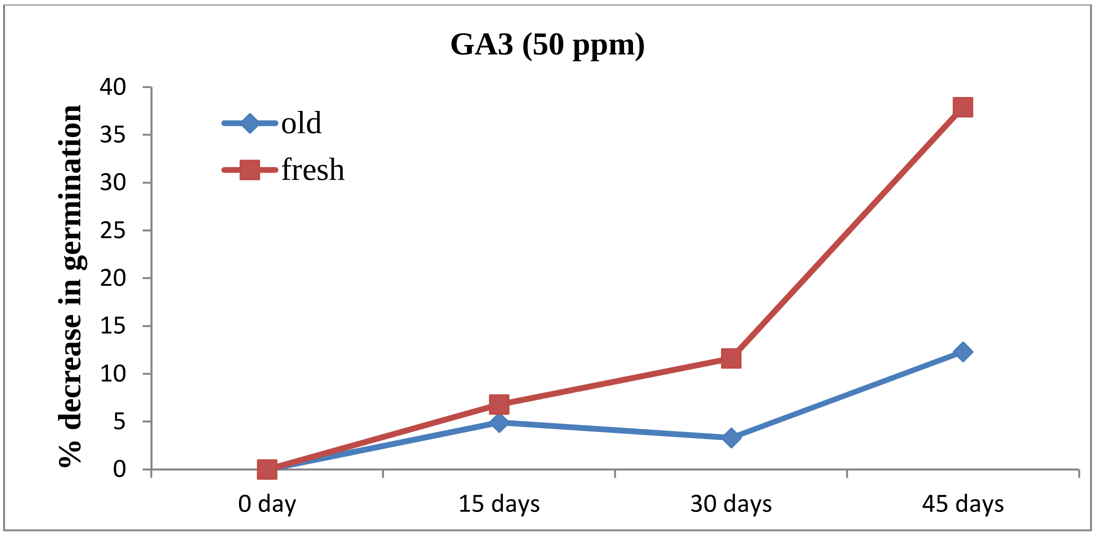


Fig.4.10 Effect of post-priming storage duration on germination of fresh and old cowpea seeds primed with GA3

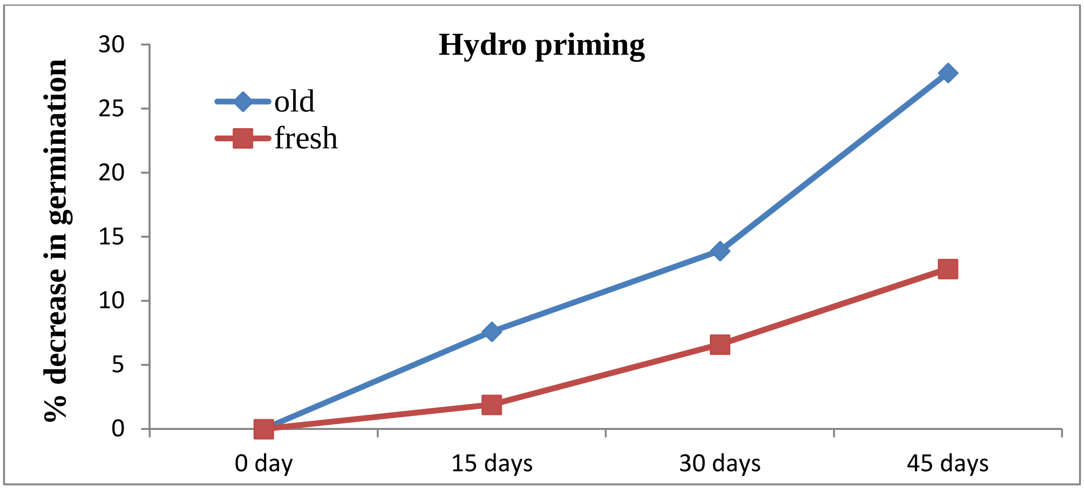


Fig.4.11 Effect of post-priming storage duration on germination of fresh and old cowpea seeds primed with deionised water

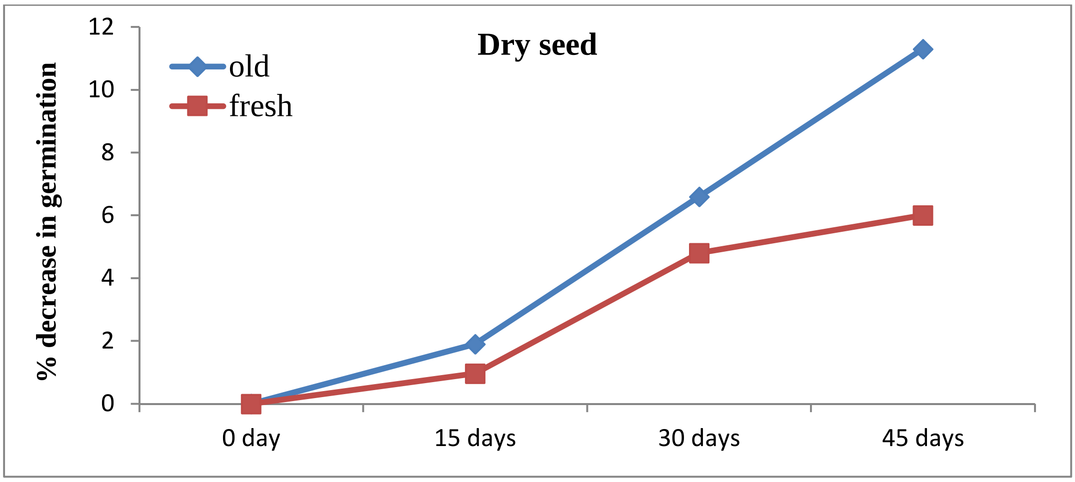


Fig.4.12 Effect of post-priming storage duration on germination of fresh and old non primed cowpea seeds

4.3.2.2 Seedling vigour response

In case of halo priming the rate of decrease in SV- I value at different post priming storage period was found to be higher in old seeds as compared to fresh seeds (Table 17). In case of salicylic acid (Table 17) the decrease rate was high in old seeds than fresh seeds at 15 days. But at 30 days the decrease rate or reduction rate in SV-I value was high in fresh seeds (11.2 %) though it was slightly less than old seed (14.3 %). At 45 days the reduction in SV-I value of fresh seeds (32.1 %) exceeded the reduction rate of old seeds (27.4 %). In case of GA3 (Table 17) the result was just reversed because the reduction in SV-I value was higher in fresh seeds (8.8 %, 11.8 % and 47.7 %) as compared to old seeds (7.2 %, -4.2 % and 11.6 %) at 15, 30 and 45 days. In case of hydro priming (Table 17) the reduction rate was higher in fresh seeds as compared to old seeds. Dry seeds and old seeds had lower rate of reduction than fresh seeds. The halo priming agents KCl and KNO_3 showed a higher rate of reduction in SV-II value with the increase of storage duration in old seeds as compared to fresh seeds (Table 18). The same trend was followed by hormonal and hydro priming along with dry seeds.

Table 17: Reduction in SV-I value of old and fresh cowpea seeds under post priming storage duration

Old seeds (% decrease) SV-I					Av. decrease/ day	Fresh seeds (% decrease)				Av. decrease/ day
Treatment	**0 day**	**15 days**	**30 days**	**45 days**		**0 day**	**15 days**	**30 days**	**45 days**	
KCl	0	5.7	9.9	31.9	0.47	0	3.4	-1.4	10.5	0.14
KNO_3	0	11.6	6.14	27.5	0.53	0	4.2	6.1	10.5	0.24
SA	0	13.1	11.2	27.4	0.62	0	3.5	14.3	32.1	0.47
GA3	0	7.2	- 4.2	11.6	0.20	0	8.8	11.8	47.7	0.68
Water	0	-2.2	-2.7	19.1	0.06	0	4.1	13.4	18.8	0.38
Control	0	-6.4	-24.0	6.1	-0.12	0	4.7	-1.3	9.5	0.16

Table 18 Reduction in SV-II value of old and fresh cowpea seeds under post priming storage duration

Old seeds (% decrease) SV-II					Av. decrease/ day	Fresh seeds (% decrease)				Av. decrease/ day
Treatment	**0 day**	**15 days**	**30 days**	**45 days**		**0 day**	**15 days**	**30 days**	**45 days**	
KCl	0	26.0	33.9	42.0	1.26	0	12.8	12.0	21.6	0.58
KNO_3	0	23.7	24.1	32.3	1.03	0	11.6	9.7	10.8	0.44
SA	0	35.2	32.2	43.5	1.46	0	15.2	21.2	36.3	0.84
GA3	0	29.5	28.3	35.7	1.23	0	23.7	26.9	54.9	1.23
Water	0	15.3	24.6	39.1	0.90	0	15.4	24.9	29.1	0.84
Control	0	18.2	14.0	25.8	0.75	0	6.9	9.4	14.8	0.47

4.3.2.3 Response in respect of biochemical parameters

Electrical conductivity measures the rate of deterioration in seeds kept for storage. With the increase in storage period the EC value was found to increase in both old and fresh seeds primed with KCl (Table 19) but the rate of increase was more in old seeds (11.7 %, 30.8 % & 62.1 % at 15, 30 & 45 days) as compared to fresh seeds (6.5 %, 26.8% & 46.0 %at 15, 30 & 45 days). In case of KNO_3, salicylic acid, GA3, deionised water and dry seeds the trend was same that of KCl.

Table 19 EC value of old and fresh cowpea seeds under post-priming storage duration

Old seeds (% increase in EC)					Fresh seeds (% increase in EC)			
Treatment	**0 day**	**15 days**	**30 days**	**45 days**	**0 day**	**15 days**	**30 days**	**45 days**
KCl	0	11.7	30.8	62.1	0	6.5	26.8	46.0
KNO_3	0	10.1	27.1	127.6	0	10.1	26.1	52.0
SA	0	6.6	27.9	164.7	0	8.0	22.4	63.1
GA3	0	9.8	28.2	154.1	0	9.6	23.7	96.4
Water	0	10.4	37.2	126.1	0	12.1	34.2	50.5
Control	0	6.4	21.3	140.9	0	7.4	22.7	65.0

By comparing alpha amylase activity in old and fresh seeds (Table 20) it was observed that with the increase in storage period alpha amylase activity decreased in both primed and non primed seeds. The rate of decrease was more in old seeds as compared to fresh seeds. In old seeds primed with KCl the rate of decrease was 12.6 %, 44.6 % & 43.0 % at 15, 30 and 45 days and the decrease rate in fresh seeds primed with KCl was14.5 %, 19.5 % & 35.5 %. In old non primed seeds the rate of decrease was 23.6 %, 44.4 % & 58.5 % at 15, 30 and 45 days and the decrease rate in non primed fresh seeds was 15.7 %, 32.7 % & 58.0 %.

Table 20 Alpha amylase activity of old and fresh cowpea seeds under post priming storage duration

Old seeds (% decrease)					Fresh seeds (% decrease)			
Treatment	**0 day**	**15 days**	**30 days**	**45 days**	**0 day**	**15 days**	**30 days**	**45 days**
KCl	0	12.6	44.6	43.0	0	14.5	19.5	35.5
KNO_3	0	27.2	40.1	62.4	0	29.1	38.2	59.5
SA	0	27.7	44.5	71.4	0	15.4	39.4	50.3
GA3	0	29.4	66.9	78.8	0	21.7	30.9	63.4
Water	0	13.8	24.2	38.8	0	3.43	12.0	29.4
Control	0	23.6	44.4	58.5	0	15.7	32.7	58.0

By comparing dehydrogenase activity in old and fresh seeds (Table 21) it was observed that with the increase in storage period dehydrogenase activity decreased in both primed and non primed seeds. The rate of decrease was more in old seeds as compared to fresh seeds. In old seeds primed with KNO_3 the rate of decrease was 33.6 %, 48.2 % & 63.2 % at 15, 30 and 45 days and the decrease rate in fresh seeds primed with KNO_3 was 25.8%, 39.0 % &52.0 %. In old non primed seeds the rate of decrease was 8.7 %, 24.9 % & 35.7 % at 15, 30 and 45 days and the decrease rate in non primed fresh seeds was 11.4 %, 25.8 % & 40.6 %.

Table 21 Dehydrogenase activity of old and fresh cowpea seeds under post priming storage

	Old seeds (% decrease)				Fresh seeds (% decrease)			
Treatment	**0 day**	**15 days**	**30 days**	**45 days**	**0 day**	**15 days**	**30 days**	**45 days**
KCl	0	11.8	23.7	38.4	0	13.8	21.4	55.1
KNO_3	0	33.6	48.2	63.2	0	25.8	39.0	52.0
SA	0	7.3	16.6	28.9	0	6.2	14.2	28.2
GA3	0	3.2	12.5	19.1	0	7.5	16.5	23.9
Water	0	18.8	29.5	44.6	0	14.5	18.9	42.3
Control	0	8.7	24.9	35.7	0	11.4	25.8	40.6

4.4 Correlation coefficient between physiological and biochemical parameters

The correlation coefficient between physiological and biochemical parameters in old seeds (Table 22) indicated that germination, SV-I & SV-II parameters had negative correlation with electrical conductivity in respect of all the priming treatments. Germination count showed positive correlation with alpha amylase and dehydrogenase activity in case of all the priming treatments. SV-I & SV-II showed positive correlation with alpha amylase and dehydrogenase activity in all priming treatments except KNO_3 that had negative correlation like non primed seeds. In fresh seeds physiological parameters like germination, SV-I & SV-II showed negative correlation with electrical conductivity and positive correlation with alpha amylase and dehydrogenase activity in case of all the priming treatments (Table 23).

Table 22 Correlation coefficient between physiological and biochemical parameters of old seeds

	Old seeds			
		Germination	**SV-I**	**SV-II**
KCl(1 %)	Electrical conductivity	-0.963	-0.978	-0.872
	Alpha amylase	0.946	0.923	0.936
	Dehydrogenase	0.963	0.943	0.936
KNO_3 (1 %)	Electrical conductivity	-0.762	-0.184	-0.699
	Alpha amylase	0.964	-0.170	0.945
	Dehydrogenase	0.975	-0.270	0.970
Salicylic Acid(50 ppm)	Electrical conductivity	-0.754	-0.893	-0.625
	Alpha amylase	0.980	0.951	0.894
	Dehydrogenase	0.946	0.928	0.819
GA3(50 ppm)	Electrical conductivity	-0.940	-0.683	-0.612
	Alpha amylase	0.776	0.269	0.862
	Dehydrogenase	0.832	0.352	0.759
Water	Electrical conductivity	-0.968	-0.931	-0.913
	Alpha amylase	0.989	0.728	0.999
	Dehydrogenase	0.978	0.685	0.999
Control	Electrical conductivity	-0.903	-0.515	-0.745
	Alpha amylase	0.963	-0.089	0.876
	Dehydrogenase	0.991	-0.022	0.813

Table 23 Correlation coefficient between physiological and biochemical parameters of fresh seeds

	fresh seeds			
		Germination	**SV-I**	**SV-II**
KCl(1 %)	Electrical conductivity	-0.999	-0.669	-0.864
	Alpha amylase	0.951	0.764	0.976
	Dehydrogenase	0.957	0.956	0.863
KNO3(1 %)	Electrical conductivity	-0.996	-0.979	-0.624
	Alpha amylase	0.974	0.994	0.831
	Dehydrogenase	0.973	0.937	0.499
Salicylic Acid(50 ppm)	Electrical conductivity	-0.991	-0.994	-0.940
	Alpha amylase	0.934	0.932	0.959
	Dehydrogenase	0.957	0.952	0.941
GA3(50 ppm)	Electrical conductivity	-0.984	-0.969	-0.963
	Alpha amylase	0.974	0.963	0.994
	Dehydrogenase	0.989	0.976	0.959
Water	Electrical conductivity	-0.986	-0.998	-0.948
	Alpha amylase	0.992	0.950	0.844
	Dehydrogenase	0.741	0.807	0.952
Control	Electrical conductivity	-0.826	-0.738	-0.911
	Alpha amylase	0.936	0.653	0.977
	Dehydrogenase	0.908	0.549	0.943

4.5 Predicting the efficacy of priming treatments

The present study indicates that the priming treatments taken for the study behaves differently in influencing the seed quality parameters. 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 was judged by calculating germination response index (GRI) and vigour response index (VRI) in old cowpea seeds. 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. In the Fig. 4.13 the GRI, VRI and BRI values of different treatments are presented. From the Fig. 4.13, it is evident that hydro priming and KCl have high efficiency as compared to other treatments. The efficacy of salicylic acid is the poorest in improving seed quality parameters. The efficacy of GA3 is better than KNO_3. Seed quality index parameter indicates that hydro priming is the best treatment followed by KCl and GA3 as the SQI values are 25, 24 and 22 respectively.

4.6 Predicting appropriate post priming storage duration to retain seed quality

To predict appropriate post priming storage duration of different priming treatments to retain seed quality, 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 % in case of KCl, KNO_3, salicylic acid, GA3 and deionised 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 24 maximum post priming storage period of different treatmentshave 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 deionised water could be stored up to 50, 15, 0, 20 and 50 days respectively.

Table 24 Predicting maximum post priming storage period of different priming treatments in old cowpea seeds

old seed						Av. decrease /day (%)	Maximum post priming storage period (days)
Treatment	Increase in germination % over control	Permissible level of loss (%)	(% decrease in germination)				
			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

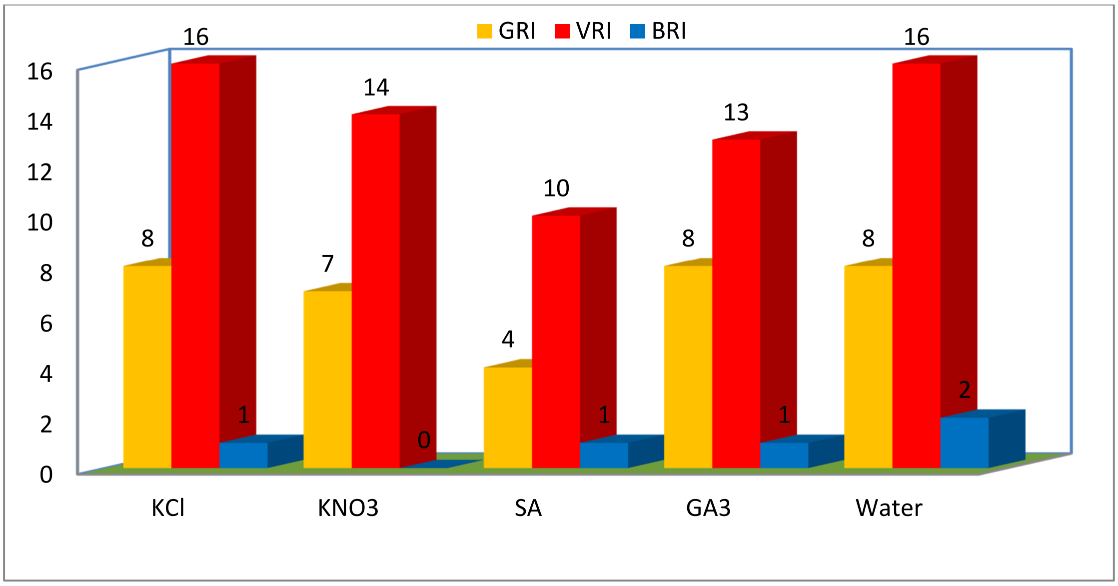


Fig.4.13 GRI, VRI and BRI values of different priming treatments in old cowpea seeds

DISCUSSION

Seed is the nucleus of life and is subjected to continuous ageing once it has reached maturity. This phenomenon results in an irreversible change in seed quality ultimately affecting viability. The quantitative deterioration during storage is mainly attributed to period of storage (Delouche and Baskin, 1973). Priming techniques help in improving seed quality that has occurred during storage. 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.

5.1 Storage of primed seeds and its effect on physiological parameters

Priming has definitely a positive effect on physiological parameters. In the present study it was observed that old and fresh seeds primed with KCl and KNO_3 were able to give higher germination percentage, seedling growth, seedling vigour, seedling dry weight. Many other scientists also reported the same findings (Zheng *et al.*,2002; Farooq *et al*.,2005; Nascimento and Lima,2008; 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). Old and fresh seeds primed with salicylic acid and GA3 were able to give higher germination percentage, seedling growth, seedling vigour, seedling dry weight. Many other scientists also reported the same findings (Ashraf *et al*., 2002; Yogananda *et al.*, 2004; Hussein *et al.*, 2007; Afzal *et al*., 2008; Tzortzakis,2009; Sedghi *et al*., 2010; Khan *et al.* ,2011; Ghobadi *et al*., 2012; Anwar *et al*.,2013; Das *et al*., 2014; Verma *et al*. , 2014; Pramanik *et al.*, 2015). Hydro priming in the present study reflected its immense effect on physiological parameters and this finding was supported by others (Basra *et al*., 2002; Farooq *et al*., 2005; Xiaoying *et al*., 2005; Neamatollahi *et al.*, 2006; Ahmadi *et al*., 2007; Filho and Kikuti , 2008; Moradi and Younesi , 2009; Azarnivand *et al*., 2010; Birendra and Shambhoo, 2011; Tiwari *et al.* , 2014; Mahmoudi *et al.*, 2015).

Germination percentage of old and fresh primed 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) or may be due to reduction in alpha amylase activity and carbohydrate content (Bailly, 2004) or denaturation of proteins (Nautiyal *et al*., 1985).

5.2 Storage of primed seeds and its effect on biochemical parameters

During storage, the seeds are bound to undergo ageing, an inevitable physiological phenomenon showing the pronounced effects on seed viability and seed vigour. It is widely accepted that loss in cellular membrane integrity is one of the chief causes for loss of viability and leads to increased leaching of seed constituents and ultimately death of the seed. The leachate exudates as measured by electrical conductivity are known to be associated with loss of viability and vigour in pea seeds as reported by Perry (1969). In aged seeds or partly deteriorated seed, the electrical conductivity will be higher owing to decrease in membrane integrity caused by detrimental changes occurring in seeds (Koostra and Harrington 1969). Electrical leakage is an indicator of relative permeability of plasma membrane and transition of liquid-crystal phase of membrane to solid-gel state maybe the first phenomenon that affect membrane transporters (Boonasiri *et al*., 2007). After this transition, phospholipid layer of membrane is destroyed by lipid peroxygenase and ROS (Torres and Androw, 2006) leading to de-construction of membrane integrity. 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 findings were reported by many other scientists. Aging of seeds can increase the electrical conductivity that is in accordance with Kaewnaree *et al*. (2010).

A seed is basically a kernel that encloses in itself a small embryonic plant covered by a hard seed coat and some stored food that upon receiving the appropriate climatic conditions, will promote growth of the embryo (Thomas *et al*., 2006). The stored foods in the plant seed include carbohydrates, proteins and lipids. The liberation of these foods are inhibited initially due to the impermeability to oxygen by the seed coat before they get soften and allow air influx. During germination, the stored food in the seed cotyledon is liberated initially by anaerobic respiration. Anaerobic respiration is made possible by the activity of enzymes such as dehydrogenases which catalyze catabolic chemical processes in anaerobic conditions (Turner and Turner, 1995).

Successful crop establishment requires rapid germination, high seed vigour and seedling vigour (Mackill and Redona, 1997). Regarding germination processes, key enzyme, α-amylase, is involved in the conversion of starch to simple sugars for growing seedlings (Sun and Henson, 1991). Amylase mainly carries out this process until the seedling becomes photo synthetically self sufficient. Germination ability of cereal seeds probably depend on the extent of α-amylase activity (Nandi *et al.*, 1995), while in seeds of reduced viability, low α-amylase activities have been reported (Livesley and Bray, 1991). Improved α-amylase activity increased the level of soluble sugars which triggered the faster and uniform emergence of seedlings (Lee and Kim, 2000: Farooq *et al.* 2006). The exact cause of seed deterioration is not fully elucidated yet. However, it is related to several physical, physiological and biochemical changes occurring in seed during storage (Chauhan *et al*., 1984).

Dehydrogenase is a respiratory enzyme whose activity proves that cell is alive. 2,3,5 Triphenyl Tetrazolium Chloride (TTC) is used in all seed test laboratories for viability test. In which the reduction of TTC (colourless) to Formazan (red) is the responsible chemical reaction. The tetrazolium test is widely recognized as an accurate means of estimating seed viability. Hydration of seed embryos increases the enzyme activity resulting in release of hydrogen ions which reduces TTC. Dehydrogenase catalyses transfer of H^+ atoms from a substrate to a hydrogen acceptor. The rate of reaction is measured according to the degree of reduction of the dye, usually methylene blue or TriphenylTetrazonium Chloride. Dehydrogenases belong to the class of enzymes known as oxidoreductases. The classification is based on donoracceptor reactions in which electron molecules are transferred from one molecule (the oxidant) to another (the reductant). Meyer and Anderson (1952) describe dehydrogenases as enzymes which accomplish intracellular oxidation and reduction by transfer of hydrogen from one kind of molecules to another. Most dehydrogenases use nicotinamide adenine dinucleotide (NAD+) or nicotinamide adenine dinucleotide phosphate (NADP+) while some make use of Riboflavin (Robert *et al*., 2009). Dehydrogenase enzymes include Alcohol dehydrogenase, Lactate dehydrogenase, Succinate dehydrogenase etc. Alcohol dehydrogenase (ADH) is one of two proteins in the ethanol fermentation pathway that is responsible for the reduction of acetylaldehyde, which is toxic to plant tissues, to ethanol, resulting in continuous regeneration of NAD+ in the cytoplasm (Chung and Ferl, 1999). Succinate dehyrogenase, a complex enzyme tightly bound to the inner mitochondrial membrane oxidizes succinate to fumarate (Devlin, 2011). Lactate dehydrogenase catalyses the reversible oxidation of lactate to pyruvate using NAD+ as a co-enzyme. In the present investigation the total dehydrogenase content of the primed seeds (old) was found to decrease with the increase of storage periods irrespective of priming treatments. All priming treatments except KNO_3 had higher dehydrogenase activity at 45 days of storage period as compared to non primed seeds and this indicated that priming has certainly beneficial effect.

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), Jie *et al*. (2002), EL-Arbay and Hegazi (2004), Guzman and Aquino (2007), Moosavi *et al*. (2009), Amanpour-Balaneji and Sedghi (2011), Oaikhena *et al.* (2013) and Tabatabaei (2013).

SUMMARY AND CONCLUSION

The present investigation was undertaken to study the effect of storage duration on primed cowpea seeds in addition to priming response of cowpea seeds to halo, hormonal and hydro priming. The experiment was conducted at the Department of Seed Science and Technology, college of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar. Fresh and old cowpea breeder seeds of variety Utkal Manika were collected from AICRP on Vegetable Crops, OUAT to conduct the experiment. The experiment was conducted in a completely randomised design with four replications. Fresh seeds and 18 months old cowpea seeds were primed with potassium chloride (KCl, 1%), potassium nitrate (KNO_3, 1%), gibberellic acid (GA3, 50ppm), salicylic acid (50ppm) and deionised water for 6 hours. Before starting priming treatment the moisture content of fresh and old seeds were determined. The seed 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 15 days intervals and subjected to test the proposed physiological and biochemical parameters. Observations were recorded on germination %, root length, shoot length, seedling length, shoot:root length ratio, seedling dry weight, electrical conductivity, alpha amylase and dehydrogenase activity. The results are presented below.

6.1 Effect of priming treatment on old cowpea seeds

6.1.1 Physiological parameters

- 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 % when seeds treated with KCl, KNO_3, Salicylic Acid, GA3and hydro priming respectively.
- 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.
- 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).

- Old seeds treated with GA3 showed the highest shoot: root length ratio (1.86) where as KCl showed the lowest shoot: root length ratio (1.21) and non primed seeds had the ratio 1.43.
- The highest seedling length was recorded by KCl (47.70 cm) followed by KNO_3 (46.33 cm).
- The highest dry weight (57.83 mg) was observed in hydro priming treatment followed by salicylic acid (57.78 mg).
- The halo priming agent KCl recorded the highest SV-I value (3313.49) followed by hydro priming (2936.00) and KNO_3 (2894.61).
- Hydro priming exhibited the highest SV-II value (4161.95) followed by KCl (3856.00).

6.1.2. Biochemical parameters

- Non primed seeds recorded the highest EC value (0.801 dS/m) and hydro priming recorded the lowest value (0.597 dS/m) followed by KCl (0.617 dS/m).
- 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)
- The highest dehydrogenase activity was observed in seeds treated with deionised water (383.4 µg/ 20 ml/hr) and the lowest was recorded in KNO3 (284.0 µg/ 20 ml/hr).

6.2 Effect of priming treatment on fresh cowpea seeds

6.2.1 Physiological parameters

- Fresh seeds treated with KNO_3 showed the highest germination count (89.5 %).
- Seeds treated with KNO_3, deionised water and salicylic acid ranked 1st (49.35 cm), 2nd (47.24 cm) and 3rd (46.44 cm) in respect of seedling length respectively.
- Hydro priming recorded the highest dry weight (52.80 mg) followed by salicylic acid (50.98 mg), KCl (50.63 mg) and GA3 (50.18 mg).
- Seeds treated with KNO_3 produced the highest SV-I value (4416.60) and the lowest was being recorded in non primed seeds (3644.55).

- Seeds treated with deionised water produced the highest SV-II value (4645.80) and the lowest was being recorded in non primed seeds.

6.2.2. Biochemical parameters

- Among the treatments, fresh seeds treated with salicylic acid recorded the highest EC value (0.651 dS/m) followed by GA3 (0. 0.638 dS/m). All the treatments had low EC value as compared to non primed seeds (0.732 dS/m).

- Alpha amylase activity was more pronounced in seeds primed with KCl (0.462) followed by hydro priming (0.408) and lowest activity was reported in salicylic acid (0.175).

- The highest dehydrogenase activity was observed in seeds treated with KCl (590.0 µg/ 20 ml/hr) followed by KNO_3 (492.9 µg/ 20 ml/hr) and the lowest was recorded in non primed seeds.

6.3 Effect of storage duration on primed old cowpea seeds

6.3.1 Physiological parameters

- At 15 days of post priming storage duration the germination count was found to decrease both in primed and non primed seeds. The highest germination percent was recorded by hydro priming (66.5 %) and the lowest by salicylic acid (48.50 %).

- At 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 %).

- In case of 45 days of storage time the germination count varied from 41.5 % (salicylic acid) to 55.5 % (KCl).

- At 15 days of post-priming storage period, 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.

- 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, hydro priming recorded the highest seedling length (45.69 cm) followed by salicylic acid (44.05 cm).
- At 15 days of post-priming storage period, hydro priming recorded the highest dry weight (53.0 mg) followed by salicylic acid (44.85 mg) and KNO_3 (44.60 mg).
- At 30 days of post-priming storage period, hydro priming recorded the highest dry weight (50.83 mg) followed by salicylic acid (48.98 mg) and non primed seeds (48.43 mg).
- At 45 days of post-priming storage period, hydro priming recorded the highest dry weight (48.80 mg) followed by salicylic acid (44.20 mg).
- 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.
- 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).
- 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.
- After 15 days of storage, hydro priming attained the highest SV-II 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. Hydro priming and KCl scored 1st (3137.10) and 2nd (2547.95) rank.
- At 45 days of post-priming storage period, hydro priming secured the highest SV-II value (2532.95) followed by KCl (2237.15) and salicylic acid recorded the lowest value (1836.30).

6.3.2. Biochemical parameters

- 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 15 days of storage, alpha amylase activity was found to decrease invariably in all the treatments as compared to zero storage periods. The reduction rate of the enzyme 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, alpha amylase 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, deionised water and non primed seeds respectively as compared to zero storage periods.
- At 45 days of storage period, 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, deionised water and non primed seeds respectively as compared to zero storage periods.
- With the advancement of storage period from 0 to 15 days, dehydrogenase activity was found to decrease for all priming treatments. 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, deionised water and non primed seeds respectively as compared to zero storage period.
- At 30 days of storage period, 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, deionised water and non primed seeds respectively as compared to zero storage period.
- At 45 days of storage period, 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, deionised water and non primed seeds respectively as compared to zero storage period.

6.4 Effect of storage duration on primed fresh cowpea seeds

6.4.1 Physiological parameters

- At 15 days of post priming storage duration, the rate of decrease in germination count was 1.7 %, 1.1 %, 2.4 %, 6.8 %, 1.9% and 0.96 % when seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

- At 30 days of post priming storage duration, the rate of decrease in germination count was 7.3 %, 2.2 %, 12.6 %, 11.6 %, 6.6 % and 4.8 % when seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.
- At 45 days of post priming storage duration, the rate of decrease in germination count was 7.3 %, 2.2 %, 12.6 %, 11.6 %, 6.6 % and 4.8 % when seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.
- At 15 days of post priming storage duration, seeds treated with KNO3, deionised water and salicylic acid ranked 1st (47.84 cm), 2nd (46.33 cm) and 3rd (46.44 cm) in respect of seedling length.
- At 30 days of post priming storage duration, the highest seedling length was recorded in seeds treated with KCl (48.06 cm) followed by KNO_3 (47.56 cm).
- At 45 days of post priming storage duration, seedling length was found to vary from 37.15 cm to 45.98 cm.
- By increasing the post priming storage duration from 0 to 15 days, the dry weight was found to decrease. Non primed seeds recorded the highest weight (48.05 mg) and the lowest was being recorded by GA3 (41.08 mg).
- Coming to 30 days of post priming storage period it was revealed that the dry weight increased in seeds treated with KCl, KNO_3, salicylic acid and GA3 as compared to 15 days.
- At 45 days of post priming storage duration, seedling dry weight varied from 36.43 mg to 46.08 mg. Seeds treated with KNO_3 showed the highest dry weight (46.08 mg) and the lowest was recorded in GA3 (36.43 mg).
- At 15 days of post priming storage duration, the SV-I value produced by different treatments started to decline. SV-I values were found to vary from 3474.80 to 4230.28.
- At 30 days of post priming storage duration, the priming treatments showed significant differences. Seeds treated with KNO_3 recorded the highest value (4148.47) followed by KCl (3992.04).

- With the advancement of post priming storage period from 30 to 45 days, SV-I values produced by the priming treatments was found to decrease as compared to 30 days except in hydro priming. The decrease in SV-I values were 10.5 %, 10.5 %, 32.1% , 47.7 % , 18.8 % and 9.5 % for seeds treated with KCl, KNO_3, Salicylic Acid, GA3, hydro priming and in non priming condition respectively as compared to zero day.
- At 15 days of post priming storage duration, the SV-II value produced by different treatments started to decline. Seeds treated with deionised water and KNO_3 ranked 1st (3930.38) and 2nd (3928.25) respectively.
- At 30 days of post priming storage duration, the priming treatments showed significant differences. Seeds treated with KNO_3 recorded the highest SV-II value (4013.95) followed by KCl (3942.80) and the lowest value were being recorded in GA3 (3248.85).
- With the advancement of post priming storage period from 30 to 45 days SV-II values produced by the priming treatments was found to decrease as compared to 30 days except in hydro priming. The decrease in SV-II values were 21.6 %, 10.8 %, 36.3 % , 54.9 % , 29.1 % and 14.8 % for seeds treated with KCl, KNO_3, Salicylic Acid, GA3, hydro priming and in non priming condition respectively as compared to zero day.

6.4.2. Biochemical parameters

- At 15 days of storage period, significant differences were observed among the treatments. Among the treatments seeds treated with salicylic acid recorded the highest EC value (0.651 dS/m) followed by salicylic acid (0.703 dS/m).
- At 30 days of storage period, salicylic acid recorded the highest EC value (0.797 dS/m) followed by GA3 (0.789 dS/m).
- With the advancement of storage period from 30 to 45 days, EC value was found to increase irrespective of the treatment type. Salicylic acid recorded the highest EC value (0.740 dS/m) whereas hydro priming had the lowest value (0.837 dS/m).
- At 15 days of storage, alpha amylase activity was found to decrease invariably for all the treatments as compared to zero storage periods. Seeds treated with KNO_3, salicylic acid and GA3 had low alpha amylase activity than non primed seeds.

- At 30 days of storage period, alpha amylase activity was the highest in seeds treated with KCl (0.372) and the lowest in salicylic acid (0.106). The rate of decrease in alpha amylase activity was 19.5 %, 38.2%, 39.4 %, 30.9 %, 12.0 %, and 32.7 % in KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage periods.
- At 45 days of storage period, alpha amylase activity was the highest in case of hydro priming (0.298) and the lowest in salicylic acid (0.087).
- With the advancement of storage period from 0 to 15 days, dehydrogenase activity was found to decrease for all priming treatments. Seeds treated with KCl showed the highest enzyme activity (508.8 µg/ 20 ml/hr) and the lowest were reported in non primed seeds (336.6 µg/20 ml/hr).
- At 30 days of storage period, there was a gradual decrease in dehydogenase activity for each priming treatment. Seeds treated with KNO_3 had the lowest dehydrogenase activity (147.0 µg/ 20 ml/hr).
- Increasing in the storage period from 30 to 45 days showed further decrease in dehydrogenase activity of priming agents. The rate of decrease in dehydrogenase activity was 55.1 %, 52.0 %, 28.2 %, 23.9 %, 42.3 % and 40.6 % when fresh seeds were treated with KCl, KNO_3, salicylic acid, GA3, deionised water and non primed seeds respectively as compared to zero storage period.

6.5 Correlation coefficient between physiological and biochemical parameters

- The correlation coefficient between physiological and biochemical parameters of all priming treatments in old seeds showed that germination count, SV-I & SV-II parameters had negative correlation with electrical conductivity.
- Germination count in old seeds showed positive correlation with alpha amylase and dehydrogenase activity in case of all the priming treatments.
- SV-I & SV-II parameters in old seeds showed positive correlation with alpha amylase and dehydrogenase activity for all priming treatments except KNO_3 that had negative correlation like non primed seeds.

- In fresh seeds physiological parameters like germination, SV-I & SV-II of all the priming treatments showed negative correlations with electrical conductivity and positive correlations with alpha amylase and dehydrogenase activity.

6.6 Predicting the efficacy of priming treatments

- 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)
- Hydro priming and KCl have high GRI, VRI and BRI values as compared to other treatments and salicylic acid has the lowest GRI, VRI and BRI values.
- 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.
- Old cow pea seeds primed with KCl, KNO_3, salicylic acid, GA3 and deionised water could be stored in ambient environment upto 50, 15, 0, 20 and 50 days respectively as per the prediction model described in result.

CONCLUSION

From the present investigation it may be concluded that hydro priming and the halo priming agent KCl (1 %) could improve the seed quality parameters of old cowpea seeds to a satisfactory level. Under unfavourable weather condition if the farmer is unable to sow the primed old seeds directly in the field then the primed cowpea seeds may be stored to a longer period by using these two priming treatments.

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APPENDICES

Appendix-I : Electrical conductivity test

When seeds are imbibed in water, a number of water soluble substances leach out of the seed. The extent of solute leaching is dependent on the kind of seed and its physiological state. Enhanced permeability of cellular membranes is one of the primary symptoms of seed ageing. Thus, seed lots low in vigour and viability leach out greater quantity of solutes, which include inorganic ions (electrolytes), water soluble sugars, aminoacids, organic acids etc. The degree of leaching can be detected among other methods by simple measurement of electrical conductance of seed leachate, which is directly propotional to the ionic concentration. High solute leakage, an indicator of membrane permeability is associated with low vigour and poor field emergence in most crops.

Procedure:

Old and new cowpea seeds (8 g) were taken in a beaker. Then soaked in 40ml distilled water for 6 hours at 20^{o}C-25^{o}C. The contents are stirred and the leachate is poured in another beaker to measure the electrical conductivity by help of a conductivity meter.

Appendix-ii : Determination of α- amylase activity

Theoritical aspects:

α-amylase is a hydrolytic enzyme which breaks down many polysaccharides, e.g. starch which is polymer of glucose units linked by α 1-4 linkages to yield the disaccharides(maltose) as the end product.

$(C_6H_{10}O_5)_{2n}$ +n H_2O--------→n$(C_{12}H_{22}O_{11})$
Starch maltose

Preparation of reagents:

1. Citrate buffer 0.025 M (pH-5.0):

(a) We had prepared 0.025 M citric acid solution :-weigh 1.313 g of citric acid (Mol.Wt= 210.14) and dissolved in 250 ml distilled water.

(b) Then we had prepared 0.025 M sodium citrate solution:- 1.838 g of trisodium citrate (Mol. Wt=294.10) + water=250 ml

For preparation of 400 ml citrate buffer, we had mixed 164 ml of (a) with 236 ml of (b) in a 500ml volume flask. if pH was less than 5,we had added sodium citrate solution for raising the pH to 5. If pH was above 5 then we had added citric acid solution to reduce the pH to 5.

2. Preparation of starch stock solution (0.2 % or 2000 µg/ml)

We had taken a few drops of citrate buffer in a 250 ml beaker .

Then we had put 200 mg starch in it so that the starch molecules are just soaked.

Then we added 100ml of cirtrate buffer and heating was continued till the solution was boiled for 1 minute.

Then we cooled the solution and made up the volume upto 100 ml with the citrate buffer and filtered through whatman No. 1

3. Preparation of starch standards (300, 600, 900,1200 µg /ml)

i) 1200 µg/ml:- 30 ml of starch stock solution + citrate buffer = 50 ml

ii) 900 µg/ml:- 22.5 ml of starch stock solution + citrate buffer = 50 ml

iii) 600 µg/ml:- 15 ml of starch stock solution + citrate buffer = 50 ml

iv) 300 µg/ml:- 7.5 ml of starch stock solution + citrate buffer = 50 ml

. 0.05 N HCl :- 0.42 ml conc. HCl + water = 100ml

4. Preparation of I_2KI Reagent:-

(a) KI (6%):- 300 mg KI + 5 ml 0.05 N HCl

(b) I_2 solution (0.6 %) :- 300mg I_2 + 50 ml of 0.05 N HCl mixed.

Then we had warmed it for dissolution and then filtered through Whatman no.1 filter paper.

Then we had mixed 1 ml of (a) with 50 ml of (b)+ 50 ml of water= 101 ml.

Procedure For Enzyme Extraction:

We had taken 5 numbers of germinated cowpea seeds of 12 different treatments. Then we had macerated the seeds in a pestle and mortar separately by adding 10 ml of citrate buffer step wise slowly.

Then we had transferred the content to the centrifuge tube and centrifugation was continued for 15 minutes at 2000 rpm or maximum speed of ordinary centrifuge at room temperature.

Then we had collected the supernatant for determination of α-amylase activity as µg starch digested per 10 minutes/gm of seed. If we would not have used the supernatant immediately then we would have preserved it in the defreeze.

	Test tube Sl. No.	**Starch standard solution (ml)**	**Citrate buffer solution (ml)**	**I_2KI solution (ml)**	**Water (ml)**	**O.D.**
For Standards	1		3	2	13	Blank
	2	2ml (300 µg/ml)	1	2	13	
	3	2ml (600 µg/ml)	1	2	13	
	4	2ml (900 µg/ml)	1	2	13	
	5	2ml (1200 µg/ml)	1	2	13	

	Test tube Sl. No.	**Enzyme extract(ml)**	**Citrate buffer (ml)**	**Starch solution of 1200 µg/ml (ml)**	**Keeping time for reaction (minutes)**	**I_2KT solution (ml)**	**Water (ml)**	**O.D.**
For samples	6	0.2	2.8	-	-	2	13	Blank
	7	0.2	0.8	2	5	2	13	
	8	0.2	0.8	2	10	2	13	
	9	0.2	0.8	2	20	2	13	
	10	0.2	0.8	2	30	2	13	

Before addition of reagents , all the test tubes should be kept at 37^0C.Also during reaction time the temperature should be maintained at 37^0 C. After development of colour, the O.D. was measured at 620 nm wave length of light .Therefore, the α-amylase activity is calculated as µg starch digested /gram of seed /10 minutes or more by using the standard curve.

Appendix –III: Estimation of total dehydrogenase activity

Principle

Dehydrogenase is respiratory enzyme whose activity proves that cell is alive. 2,3,5 Triphenyl Tetrazolium Chloride (TTC) is used in all seed test laboratories for viability test. In which the reduction of TTC (colourless) to Formazan (red) is the responsible chemical reaction. The tetrazolium test is widely recognized as an accurate means of estimating seed viability. Hydration of seed embryos increases the enzyme activity resulting in release of hydrogen ions which reduces TTC.

Dehydrogenase catalyses transfer of H^+atoms from a substrate to a hydrogen acceptor. The rate of reaction is measured according to the degree of reduction of the dye, usually methylene blue or Triphenyl Tetrazonium Chloride.

Reagents

Tetrazolium chloride (0.05%)

Methanol

Phosphate buffer (pH 7.00)

Procedure

1. We had taken 1 gram of cowpea seeds of same size (i.e. well filled seeds) from each treatment of fresh and old seeds
2. Then the seeds were soaked for 12 hours
3. The seed coats were removed from the seeds.
4. Then we had kept the soaked seeds without the seed coat in 0.05 % tetrazolium solution.(volume is 20 ml) for 4 hours.
5. Tetrazolium solution was decanted and the seeds were washed in water thoroughly.
6. We added 20 ml methanol (i.e the seeds were kept in methanol solution and we waited for the complete decolouration of the seeds).
7. Then we took the reading of the solution by the help of a spectrophotometer at 480 nm of light along with the blank methanol (as standard).

Preparation of standard curve

- We prepared the stock solution by taking 10 mg of formazan (TCF) in 100 cc methanol (conc. was 100 ppm).
- We took 1 ml stock solution and added 19 ml methanol so that the final volume was 20 ml (concentration was 5 ppm).
- From the stock solution we prepared 5 ppm ,10 ppm ,15 ppm and 20 ppm solution and made the final volume 20 ml by adding methanol.

i) 1ml stock solution + 19 ml methanol = 5 ppm (vol 20ml)
ii) 2 ml stock solution + 18 ml methanol =10 ppm (vol 20 ml)
iii) 3 ml stock solution + 17 ml methanol = 15 ppm (vol 20 ml)
iv) 4 ml stock solution + 16 ml methanol = 20 ppm(vol 20 ml)

- We had measured the O.D value and prepared the standard curve

ENZYME QUANTIFICATION

Let us consider the O.D value of the seed sample is 2.then its concentration will be 5 ppm (as per the standard curve) i.e the seed sample contains 5 mg formazan in 1000 cc solution.

1000 ml = 5 mg = 5×10^{-3} gram
1 ml = (5×10^{-3} gram) ÷ 1000 =(5×10^{-3})÷ 10^{3} gram
= $5 \times 10^{-3} \times 10^{-3}$ gram
= 5×10^{-6} gram
= 5 µg
So 20 ml solution will contain 5 x 20 µg = 100 µg formazan
i.e. from 1 gram seeds we get 100 µg formazan.
Again we keep the seeds in tetrazolium for 4 hours.
Therefore, quantity of formazan produced from 1 g seeds in 1 hour will be
= 100µg ÷ 4 = 25µg

100 mg + 100 ml methanol (1000 ppm stock solution)
↓
10 ml stock solution + 90 ml methanol (100 ppm stock solution)
↓
5 ml, 10 ml, 15 ml , and 20 ml + add methanol and make the final volume 100 ml to get 5 ppm , 10 ppm , 15 ppm , and 20 ppm solution

Appendix-iv : Maximum and minimum temperature, relative humidity during storage of primed seeds

Week		Date	Temperature (ºC)		RH (%)
			Maximum	Minimum	
6	February	5-11	33.3	17.2	94
7	February	12-18	33.6	17.4	92
8	February	19-25	35.1	22.4	92
9	Feb – March	26-4	35.7	20.6	94
10	March	5-11	33.2	22.8	93
11	March	12-18	33.1	21.6	92
12	March	19-25	35.9	22.7	89
13	Mar.- April	26-1	35.6	25.3	88
14	April	2-8	35.6	26.0	88
15	April	9-15	37.8	26.0	86
16	April	16-22	36.0	25.9	88