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Seed priming studies for vigour enhancement in onion CO onion (5)

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Abstract

Standardization of priming treatment for onion seeds, to improve the seed vigour under various stress conditions revealed that the seeds primed with 0.5 % $ZnSO_4$ for10 h (nutripriming) was found to enhance the speed of germination (9.9), germination (95 %), dry matter production (20.7 mg) and vigour index (1558) than the unprimed, hydroprimed and sand matrix primed seeds. The evaluation on the anatomical changes in the internal structure of nutriprimed seeds in onion through Scanning Electron Microscope (SEM) revealed that enhanced the cell division and cell elongation at the meristematic tissue of radicle as compared with unprimed seeds.

The evaluation on performance of primed seeds under moisture stress conditions, (water holding capacities of 20, 40, 60 and 80 %) revealed that the primed seeds performed better at 20, 40 and 60 % water holding capacities (WHC). The evaluation of primed seeds under different salt concentrations (0.25, 0.5, 0.75 and 1.0 %) to determine the salt tolerance of primed seeds revealed that, seeds primed with 0.5 % $ZnSO_4$ for 10 h could able to tolerate the NaCl salt stress up to 0.75 %. The performance of primed seeds under accelerated ageing condition was found superior over the unprimed seeds. The nutriprimed (0.5 % $ZnSO_4$for 10 h) and hormonal primed (0.2 % methionine for 8 h) seeds attained the 50 % reduction in germination on 9 and 10 days after accelerated ageing, respectively. Whereas the unprimed seeds attained 50 % reduction in germination within 7 days after accelerated ageing.

Keywords: Seed priming, moisture stress, salt stress, seed vigour, Onion

1. Introduction

Seed priming has been successful in improving seed vigour of many vegetable and agronomic crops, leading to rapid and uniform germination and seedling emergence. It can improve vigour especially under adverse conditions such as low/high temperatures, reduced water availability and salinity (McDonald, 2000) [34]. Poor crop stand establishment is one of the major abiotic constraints encountered by resource-poor farmers in marginal and sub-marginal areas particularly in developing and under developing countries. There are so many reasons like low quality seed, inadequate seedbed preparation, untimely sowing, poor sowing technique; abiotic stresses such as drought, high temperature, salinity and adverse soil properties (e.g. crusting) etc. Seed priming is one the means by which some of these constraints can be alleviate efficiently. It is a simple, low-cost, low-risk intervention that can be a useful technology for farmers and make a positive impact on farmers' livelihoods by increasing the rate of crop emergence, increasing rates of crop development, reducing crop duration and increasing production as well as productivity.

To improve the performance of onion seeds under varied stress conditions of moisture and salt stress, experiments were conducted to fond out the suitable priming treatments for invigourating onion seeds. In the present study, to improve vigour of onion seeds, different seed priming treatments like hydropriming, sand matrix priming, nutripriming (micronutrient) and methionine priming were attempted.

Materials and methods

Seeds of onion cv. CO (On) 5 with 8 % moisture content and 87% germination received from Horticultural College and Research Institute, Periyakulam, Tamilnadu, were used for priming experiments. The onion seeds were subjected to various priming treatments, under room temperature and dried back to the original moisture content to enhance the seed vigour status.

Seed invigouration methods

The different priming treatments given were, hydro priming, sand matrix priming, nutripriming and priming with methionine. They are as follows:

Hydro priming
Seeds were soaked in equal volume of water for 6 h and shade dried to original moisture content.

Sand matrix priming (SMP)
One kg of sand to which water was added @ 240 ml kg^{-1} of sand to create 80 % water holding capacities (WHC). The seeds of onion were placed in perforated plastic covers and burried in sand with 80 % WHC. Seed samples were retrieved after 24 h and shade dried to original moisture content.

Nutripriming
The nutrient solutions ($ZnSO_4$ were prepared in the concentrations *viz.*, 0.25, 0.5 and 1 % in which onion seeds were soaked for 6, 8, 10 and 12 h. After priming, the seeds were removed from the solutions and shade dried.

Hormonal priming
Seeds were primed with an aqueous solution of methionine. The methionine solutions were prepared in the concentrations *viz.*, 0.1, 0.2 and 0.3 % in which onion seeds were soaked for 6, 8, 10 and 12 h. After priming, the seeds were removed from the solutions and shade dry to bring back its original moisture content.

Histological changes in primed seeds
SEM FEI QUANTA 250 was used to study the anatomical changes of primed and unprimed onion seeds. The primed seeds and unprimed onion seeds radicle were sequentially dehydrated with ethanol (25, 50, 75, 95% for 20 minutes and 100 % for 30 minutes) to avoid contamination of bacterial cells adhered on the surface of radicle. The dehydrated seeds were observed under Scanning Electron Microscope (SEM).

Result and discussion
Effect of priming treatments on seed vigour improvement
In this study, there are four invigouration treatments used to improve the vigour of onion seeds *viz.*, hydropriming, sand matrix priming, nutripriming and hydropriming. Results revealed that among the priming treatments, nutripriming was found to be effective which recorded higher speed of germination, germination, seedling length and vigour index. Seed priming has been shown to enhance the speed of germination (Deering and Young, 2006) [21], reduce the time between sowing and emergence, improve seedling vigor (Harris, 1996) [26] and stand establishment.
Seeds may be treated with micronutrients either by soaking in nutrient solution of a specific concentration for a specific duration (seed priming) or by coating with micronutrients. Micronutrients are required in very small quantities (Abdel-Wahab, 2008) [1]. There are mainly three methods of micronutrient application in crops application to soil, foliar sprays, and seed treatment (Johnson *et al*., 2005) [30]. The application of micronutrients has been done recently for invigouration of seeds to improve the germination rate, hasten emergence time and improve the uniformity of seedling growth in some field crops (Basra *et al*., 2003) [12]. Sivritepe and Dourado (1995) [47] reported that priming is a physiological method that improves seed performance and provides faster and synchronized germination.
In micronutrient seed priming (nutripriming), micronutrients are used as osmotica (Imran *et al.*, 2004; Singh, 2007) [29, 46]. Primed seeds usually have better and more synchronized germination (Farooq *et al.*, 2009) [23] owing simply to less imbibition time (Brocklehurst and Dearman, 2008; McDonald, 2000) [17, 34] and build-up of germination-enhancing metabolites (Basra *et al.*, 2005; Farooq *et al.*, 2006) [11, 24].
Seed priming induce some of the metabolic changes necessary for germination. Primed seeds usually display increased germination rate, greater germination uniformity and total germination percentage (Basra *et al*., 2004) [10]. Increased germination rate and uniformity have been attained due to metabolic repair during imbibition (Bray *et al*., 1989) [15], a buildup of germination enhancing metabolites (Basra *et al*., 2005) [11]. In addition osmotic adjustment has been considered that one of the main merits of soaking treatment to increase germination and emergence rate. Thus, presowing hydration treatments improved seed germination by delivering various metabolites.
Seed priming with Zn can improve crop emergence, stand establishment and subsequent growth and yield. For example, priming *Echinacea purpurea* (L.) seed with 0.05 % $ZnSO_4$ solution increased germination and field emergence by 38 and 41 %, respectively (Babaeva *et al.*, 1999) [8]. Likewise, in common bean (*Phaseolus vulgaris* L.), seed priming with Zn significantly improved yield and related traits (Kaya *et al.*, 2007). In present investigation the speed of emergence was 11 % higher and seed germination by 12 % over the unprimed seeds due to 0.5 % $ZnSO_4$ priming than unprimed seeds (Table). The finding was supported by Ozturk *et al*. (2006) [40] who founded that Zn in newly-developed radicles and coleoptiles during seed germination was much higher(up to 200 mg kg^{-1}) thus highlighting the involvement of Zn in physiological processes during early seedling development, possibly in protein synthesis, cell elongation, membrane function and resistance to abiotic stresses (Cakmak, 2000) [18].

Table 1: Effect of seed primingtreatments on seed quality parameters in onion cv. CO (On) 5

Particulars	Speed of germination	Germination (%)	Shoot length (cm)	Root length (cm)	DMP (mg 10 Seedlings $^{-1}$)	Vigour index
T_1 – Control	8.3	85 (67.22)	8.2	5.5	17.2	1165
T_2 - Hydropriming (6 h)	8.6	88 (69.73)	8.3	7.0	18.2	1346
T_3 - 80 % SMP (24 h)	9.3	89 (70.63)	8.6	7.1	18.4	1397
T_4 - 0.5 % $ZnSO_4$ (10 h)	9.9	95 (77.08)	9.1	7.3	20.7	1558
T_5 - 0.2 % Methionine (8 h)	9.7	92 (73.57)	8.8	7.2	19.8	1472
Mean	9.2	90 (71.64)	8.6	6.8	18.9	1388
SEd	0.122	1.253	0.154	0.103	0.330	16.839
CD (P=0.05)	0.260**	2.671**	0.329**	0.218**	0.703**	35.892**

In the present study, it was observed that priming seeds with 0.5 % $ZnSO_4$for 10 h (nutripriming) resulted in a higher seedling vigour index (1558) compared to unprimed seeds (1165). Similarly, Parera and Cantliffe (1994) [41] founded that seed priming maintaining or enhancing seed vigour in onion. Seed priming induces the mobilization and solublization of

globulins and the synthesis of late embryogenesis abundant proteins (Capron *et al*., 2000 and Gamboa *et al*., 2006) [19, 25].
In the present study, the seedling length and dry matter production increased due to seed priming with 0.5 % $ZnSO_4$ for 10 h. The enhanced seedling length due to micronutrient priming was reported in many crops. Time to emergence shortened by priming with Fe, Zn and Mn, increased total biomass and enhanced the shoot length and root length of seedlings in plantain (Aylar *et al.*, 2014) [7].
Seed priming with 0.2 % methionine for 8 h (hormonal priming) was found to be the next best treatment followed by 0.5 % $ZnSO_4$ for 10 h. In the present investigation, the induction of seed germination in response to methionine may be due to changes in physiological as well as metabolic activities of seeds and also due to induction of hydrolytic enzymes responsible for endosperm degradation as indicated by Lokhande *et al.*, (2014) [33].
Plant hormone likes Gibberellin and Cytokinins regulate different developmental processes in plants. Cytokinins act early during shoot initiation and control meristem activity, while GA_3 are responsible for expansion and cell division in shoot elongation, flowering and seed germination. All phytohormones exert their regulatory role in close relation with each other. Additionally, exogenously applied growth regulators can alter the content of endogenous phytohormones (Pospisilova, 2003) [42].
Mirshekari (2014) [35] in wheat and Reza *et al*. (2014) [43] in sesame reported that seeds primed before sowing with Gibberellic acid and Kinetin recorded faster growth and development. In this view, present study on seed priming with 0.2 % methionine recorded higher speed of emergence (8.8) compared to unprimed seeds (8.2) (Table). Lokahande *et al*. (2014) [33] reported that in onion, increased biomass in terms of dry matter was evident in response to GA, methionine and cysteine pre-treatments. This treatment enhanced the carbon budget and productive ability of seedling, leading to development of healthy seedlings and more dry matter production. In the present study, increased biomass in terms of dry matter production (19.8 mg) and vigour index (1472) was evident in response to seed priming with 0.2 % methionine for 8 h.
Methionine is precursor of ethylene, Nascimento *et al.* (2004) [38] reported that plant responses to ethylene priming can be rather complex, it has been previously documented that seed priming with ACC (1-aminocyclopropane-1-carboxylic acid), an ethylene precursor, increased the germination rate of lettuce seeds. In present study also the ethylene precursor methionine enhanced the rate of germination, seedling length and vigour index.
In this present study, seed priming with 0.5 % $ZnSO_4$ and 0.2 % methionine seed invigouration treatments improved seed germination and viogur, increased shoot length and root length, seeds emerged more rapidly and also for other quality parameters *viz.*, germination and vigor index (Plate) compared to unprimed seeds.

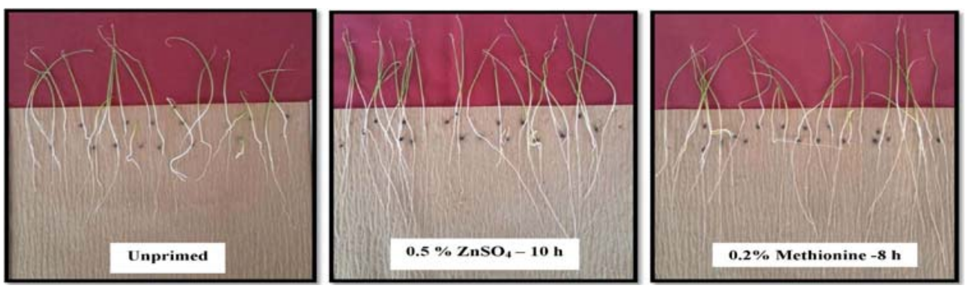
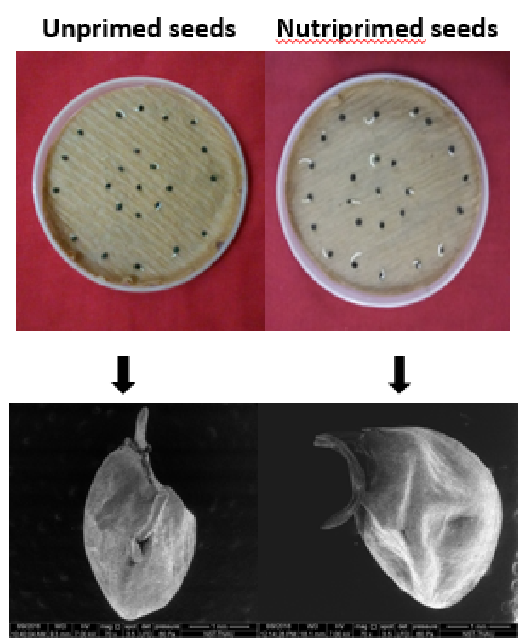


Plate 1: Effect of seed priming treatments on germination (%) in onion cv. CO (On) 5

In the present study, among the priming treatments 0.5 % $ZnSO_4$ for 10 h was recorded best over other treatments and control. The $ZnSO_4$ is one of the secondary nutrients and it involved in biosynthesis of amino acid (tryptophan). The tryptophan is a precursor of auxin. The second best treatment was 0.2 % methionine which is a precursor of ethylene. During seed germination ethylene cross talk with auxin induce cell division followed by cell elongation and also break seed dormancy.
In the present study, the observation on anatomical changes of 0.5 % $ZnSO_4$ primed and unprimed seeds were carried out with SEM (Scanning Electron Microscope). The results revealed that the primed seeds showed the early emergence of radicle and produced longer radicle as compared to unprimed seeds. This is because of rapid cell division and cell elongation at the tip of radicle as evidenced in the SEM picture (Plate 2). The rapid cell division might be due to triggered tryptophan biosynthesis in 0.5 % $ZnSO_4$ primed seeds. Tryptophan is a precursor of auxin, it is responsible for the rapid cell division followed by elongation of cells.



Radicle length of unprimed and primed seed

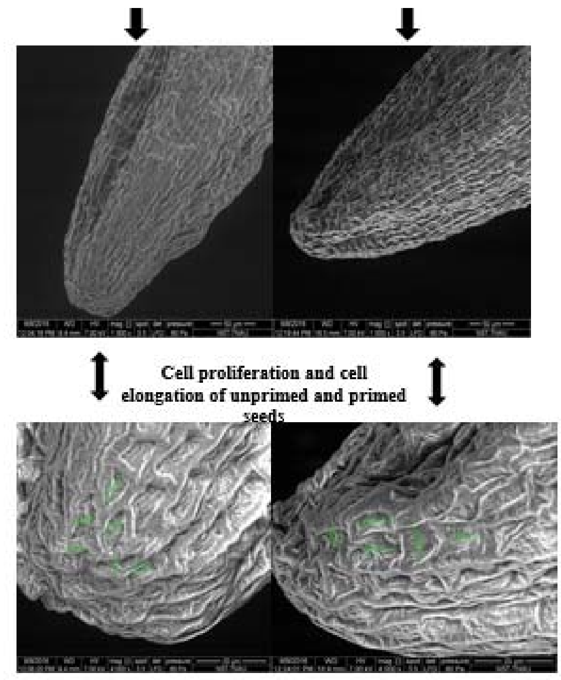


Plate 2: Anatomical changes of primed and unprimed seeds

Evaluation of primed seeds under stress conditions

Environmental pollution is a major global problem and plants are more and more subjected to a variety of abiotic stresses (Andrews *et al*., 2009; Cartes *et al*., 2010; Sarlikioti *et al*., 2010) [6, 20, 44]. Among these stresses, high concentrations of salt in the soil can result in severe detrimental effects, such as poor germination, seedling establishment and crop yield (Teklic *et al*., 2008; Bernstein *et al*., 2010; Soccio *et al*., 2010) [49, 14, 48]. This is mainly due to low soil water potential and an imbalance in the uptake of mineral nutrients and their accumulation within the plant (Munns and Tester, 2008) [37]. Afzal *et al.*, (2009) [2] reported that halo priming hastens and synchronizes seedling emergence and is capable of enhancing seedling tolerance to biotic and abiotic stresses including salinity stress.

Primed seeds tend to show better germination and growth even when imposed to stressful conditions and it has been suggested that the strategy activates a series of physiological processes that improve plant growth under stressful conditions, including the induction of antioxidant systems (Eisvand *et al*. 2010) [22]. Inorder to overcome these stresses encountered during seed germination in the field, several authors have recommended seed priming treatments such as hydropriming, halopriming, osmopriming, solid matric priming and hormonal priming. (Venkatasubramaniam and Umarani, 2007; Moosavi *et al.,* 2009; Nirmala and Umarani, 2014) [50, 36, 39].

The experiment carried out to study the performance of primed seeds of onion under different water holding capacities namely 20, 40, 60 and 80% revealed that the seeds primed with 0.5% $ZnSO_4$for 10 h enhanced the germination in all the water holding capacities followed by 0.2% methionine for 8 h. The percentages of germination increased due to ZnSO4 priming over unprimed seeds were39, 19, 14 and 28 % under 20, 40, 60 and 80% water holding capacities. This indicated that primed seeds could able to withstand extreme conditions like low moisture and high moisture. The high germination recorded at low moisture (20% WHC) stress condition could be attributed to the faster germination rate of primed seeds because of more metabolic and cell expansion activities.

Among the priming treatments 0.5% $ZnSO_4$ for 10 h recorded higher germination and vigour index followed by 0.2% methionine for 8h. (Fig). These also enhanced the seedling shoot length and dry matter production.

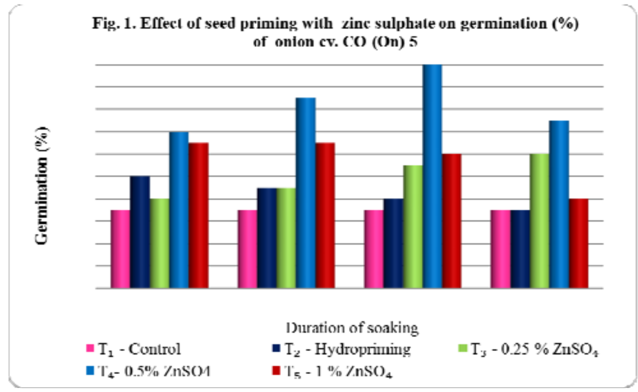


Fig 1: effect of seen priming with zinc sulphate on germination (%) of onion cv. CO (On) 5

In present study, that nutripriming with 0.5% $ZnSO_4$ for 10 h could tolerate the moisture levels upto 60% WHC all the quality parameters like speed of emergence, germination, seedling length and vigour index were reduced due to high moisture content of the soil.

Salinity is one of the major abiotic stresses that affect crop production in arid and semiarid areas. Seed germination and seedling growth are the stages most sensitive to salinity. Salt stress causes adverse physiological and biochemical changes in germinating seeds. The salinity delays or prevents the seed germination through various factors, such as a reduction in water availability, changes in the mobilization of stored reserves and affecting the structural organization of proteins. Various techniques can improve emergence and stand establishment under salt conditions. One of the most frequently utilized seed treatment is seed priming. It increases the antioxidant system activity and the repair of membranes. These changes promote seed vigour during germination and emergence under salinity stress (Ibrahim, 2016) [28].

Pre-treatment of seeds with different type of hormones and plant growth regulators is much effective in alleviating stress effects of salinity on the plants at different stages especially at early stage and it has been shown to improve crop germination under salt stress (Ashraf & Foolad, 2005; Ashraf *et al.*, 2008) [4, 5].

Halopriming increases the superoxide dismustase activity of plants under saline conditions. Bakht *et al*. (2011) [9] reported that, the increment of antioxidant enzymatic activity in various wheat cultivars due to halopriming was a key component in tolerance against salt stress. Afzal *et al*. (2008) [3] also observed that priming-induced salinity tolerance was linked with improved seedling vigor, metabolism of reserves, enhanced K^+, Ca^{2+} accumulation and reduced Na+ accumulation in wheat plants.

The present study aimed to delineate the effect of salt stress indicated that the seed priming with 0.5% $ZnSO_4$ for 10 h could able to withstand NaCl salt stress upto 0.75% concentration when compared to unprimed seeds. The percentage increase in germination over unprimed seeds accounted for 30, 34 and 20%at 0.25, 0.5 and 0.75% NaCl salt concentrations. Similarly the other physiological parameters *viz.*, seedling length, dry matter production and vigour index were high due to seed priming at different salt concentrations. The physiological parameters were high due to0.5% $ZnSO_4$ for 10 h followed by 0.2% methionine for 8 h.

From the results of this study, it is clear that the germination and seedling vigour of primed seeds reduced with increase in NaCl concentration. But, the detrimental effect was lower at lower concentration and no germination was recorded at 1.0% concentration. However, the performance of the primed seeds was better in all the NaCl concentrations (0.25 to 0.75%) compared to the nonprimed seeds. The results of this study are in accordance with the Shannon and Grieve (1999) [45] in vegetables and Kaymak *et al.* (2008) [32] in radish who reported that the salinity did not affect the germination of both primed and nonprimed seeds at low concentration, but higher levels of salinity reduce the germination percentage.

Hence this study to invigourate the onion seeds clearly revealed that soaking of seeds for 10 h with 0.5% $ZnSO_4$ (nutripriming) followed by 0.2% methionine (hormonal priming) for 8 h were highly suitable for invigouration the onion seeds for getting quality seedlings under various stress conditions for moist and salt stress. Since, priming induces metabolic activities of germination and the resulting sugars can be used for protein synthesis during germination which improves germination rate and uniform growth of the plant.

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