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Effect of priming treatments on seed quality enhancement in cucumber (*Cucumis sativus* L.) Seeds

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Abstract

Poor germination obstructs early and uniform seedling stand in cucumber. A laboratory experiment was conducted at the department of seed science and technology, College of Agriculture, University of Agricultural Sciences Raichur, during 2018-19 using Completely Randomized Design having 12 priming treatments with four replications to evaluate the possibility of improvement in emergence and early seedling growth in cucumber by pre-sowing treatments. Seeds of cucumber were soaked in different priming solution of for 24 h and evaluated for seed physiological and biochemical seed quality parameters. Results showed that seeds primed with KH_2PO_4 @ 0.001 M (T_8) registered significantly highest germination per cent, shoot length, root length, seedling dry weight, seedling vigour index I, seedling vigour index II and dehydrogenase enzyme activity (89.00%, 22.96 cm, 22.77 cm, 498.27 mg, 4070, 44347 and 0.494 respectively) and lowest (0.211 dSm^{-1}) electrical conductivity compared to other treatments and control (T_1) (76.50%, 11.14 cm, 10.22 cm, 400.32 mg, 1634, 30625 and 0.231 respectively). But significantly highest (0.695 dSm^{-1}) electrical conductivity was observed in control (T_1).

Keywords: Priming, germination, cucumber, Seed priming, Vigour index

Introduction

High-quality seeds play an important role in a successful crop production programme. Rapid germination and emergence are essential for successful crop establishment, for which seed priming could play an important role. Seed priming is a pre-sowing strategy for influencing seedling development by modulating pre-germination metabolic activity prior to the emergence of the radicle and generally enhances rapid, uniform emergence and plant development to achieve higher yields (Mc Donald, 2000) [11].

Delayed and reduced seedling emergence is a major setback to achieve a uniform and vigorous crop stand in early spring planted cucumber (*Cucumis sativus* L.) (Nerson and Govers, 1986) [14]. Moreover, erratic and non-uniform seedling emergence due to poor seed germination causes non uniform plant development, thereby extending cucurbit fruit maturation for early markets. Hence to improve the seedling establishment of old seeds need to go for seed quality enhancement techniques by priming with plant growth regulators, salt priming and other various chemical solutions. Seed priming technique is the one which will influence on seed quality in a short period. The seed priming will enhance the germination percentage, speed of germination and uniformity of germination, improves the resistance towards water and temperature stress, increase the yield. Several priming techniques have used widely including osmopriming, halopriming, hormone priming and other chemical solutions.

Growth regulators are most important in improving the growth of seed by increasing cell division and cell elongation. Mainly GA_3 and ethrel were used for seed quality enhancement in cucumber seeds as priming agent. GA_3 is one of the most important plant growth regulator application of GA_3 plays a key role in dormancy release and promotion of germination. GA_3 is widely used to break the dormancy of seeds of various plant species. Dormant seeds which require stratification, dry storage after ripening and light as a germination stimulator, are often treated with GA_3 .

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In addition to this various chemicals like water, KNO_3 , CaCl_2 , KH_2PO_4 , K_2HPO_4 , $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and chitosan are used as a priming agent. Several researchers have reported that priming with these chemicals will help in better germination per cent in both high and low vigour seeds. Besides germination, also increases seedling vigour index I and II due to seed priming. Priming with chemicals will appear beneficial as there was high per cent vigour.

KNO_3 is the most widely used chemical for promoting germination of many species. KNO_3 raises the ambient oxygen level by making less oxygen available for citric acid cycle (Bewley and Black, 1982) [4]. Chitosan seed priming increases germination and seedling growth in relation to physiological changes under low temperature stress.

Material and Methods

The seed material of cucumber variety Swarna Sheetal used for the present investigation "Effect of priming treatments on seed quality enhancement in cucumber seeds" which was obtained from National Seeds Corporation, Secunderabad, Hyderabad. The laboratory experiment was carried out at the department of Seed Science and Technology, College of agriculture Raichur, University of Agricultural Science, Raichur during the year 2018-19 and data was analyzed using Completely Randomized Design with 12 treatments and four replications. Cucumber seeds were primed with different priming treatments like organic, inorganic and growth regulators at different concentrations and evaluated for seed quality parameters. Cucumber seeds were primed with different solution with seed to solution ratio of 1:5 g.mL⁻¹ (weight/volume) for 24 h and then seeds were dried back to their original moisture content. The seeds were used to assess physiological and biochemical parameters by conducting standard germination test. The following were the different priming treatments imposed to enhance the seeds quality in cucumber as follows.

T₁: Control

T₂: Seed priming with old coconut water at 100%

T₃: Seed priming with GA₃ @ 100 ppm

T₄: Hydro priming

T₅: Seed priming with ethrel @ 100 ppm

T₆: Seed priming with KNO_3 @ 1%

T₇: Seed priming with CaCl_2 @ 0.001 M

T₈: Seed priming with KH_2PO_4 @ 0.001 M

T₉: Seed priming with K_2HPO_4 @ 0.001 M

T₁₀: Seed priming with $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ @ 0.2%

T₁₁: Seed priming with Chitosan @ 0.25%

T₁₂: Seed priming with Chitosan @ 0.5%

Seed germination (%)

The standard germination test was carried out by following between paper method as per ISTA procedure. Fifty seeds in eight replications were taken from each treatment and placed on germination paper uniformly. The roll towels were kept in germination chamber maintained at $25 \pm 2^\circ\text{C}$ temperature and 90 ± 5 percent relative humidity. Then the final count was taken on 8th day. The number of normal seedlings from each replication was counted and the mean germination was expressed in percentage (ISTA, 2013) [8].

$$\text{Seed germination (\%)} = \frac{\text{Number of normal seedlings}}{\text{Total no. of seeds}} \times 100$$

Seedling vigour index (SVI)

The seedling vigour index-I and II were determined by employing the formula given by (Abdul-Baki and Anderson, 1973) [1].

SVI-I = Germination (%) × Total seedling length (cm)

Whereas, SVI-II was calculated by using formula,

SVI-II = Germination (%) × Seedling dry weight (mg)

Electrical conductivity (dSm⁻¹)

For determining electrical conductivity of seed leachates five grams of seeds in four replications were soaked in acetone for half a minute and thoroughly washed in distilled water three times. Then, the seeds were soaked in 25 ml distilled water and kept in an incubator maintained at $25^\circ \text{C} \pm 1^\circ \text{C}$ for 12 h. The seed leachate was collected and the volume was made up to 25 ml by adding distilled water. The electrical conductivity of the seed leachate was measured in the digital conductivity bridge (ELICO) with a cell constant 1.0 and the mean values were expressed in deci simons per meter (dSm⁻¹) (Milosevic *et al.*, 2010) [12].

Dehydrogenase enzyme activity

Representative sample of 25 seeds were taken from each treatment and preconditioned by soaking in water for 18 h at room temperature. Seeds were taken randomly, remove the wings and cut through the midsection of distal end and expose the embryonic axis. Then the seeds were steeped in one per cent solution of 2, 3, 5 - triphenyl tetrazolium chloride (TZ) and kept in dark for 24 h for staining. Later on, the stained seeds were thoroughly washed with distilled water and soaked in 10 ml of methoxy ethanol (methyl cellosolve) solution overnight for destaining or extracting red colour. The intensity of red colour was measured using ELICO UV-VIS spectrophotometer using blue filter at 480 nm with methoxy ethanol as the blank. The OD value obtained was reported as the dehydrogenase enzyme activity (Kittcock and Law, 1968) [10].

Results and Discussion

It was observed that physiological seed quality parameters were significantly influenced by different priming treatments. Cucumber seeds primed with KH_2PO_4 at 0.001 M (T₈) registered highest (89.00%) seed germination and was on par with seeds primed with K_2HPO_4 at 0.001 M (T₉) and chitosan at 0.5 per cent (T₁₂) (88.3% and 87.8% respectively) followed by chitosan at 0.25 per cent (T₁₁) with 86.00 per cent. However the control (T₁) recorded lowest (76.50%) seed germination percentage. (Table 1). Seeds primed with KH_2PO_4 for 24 h produced higher germination in aged seeds of cucumber when compared with other treatments. From this study it is possible to presume that the enhanced germination with potassium dihydrogen phosphate due to ions absorption during priming as reported by Alvarado *et al.*, 1987 and Frett *et al.*, 1991 in tomato. Moreover, the potassium salts had been reported to raise the ambient oxygen level by making less oxygen available for the citric acid cycle (Bewley and Black, 1982) [4]. These results are in conformity with the findings of Sathish *et al.* (2011) [19] in maize hybrid and in cucumber by Pandey (2017) [16].

Significantly highest shoot and root length were observed in case of seeds primed with KH_2PO_4 @ 0.001 M (T₈) (22.96 cm and 22.77 cm respectively) followed by K_2HPO_4 @ 0.001 M (T₉) and lowest was recorded in control (T₁) (11.14 cm and 10.22 cm respectively) (Table 1). Phosphorus is one of the important macro nutrients essential for plant cell and tissue

culture. Phosphorus that found in meristematic and other fast growing tissue, is an essential element required in respiration and photosynthesis and its effect on plant maturation, root and shoot growth. The H_2PO_4^- and HPO_4^{2-} are two forms of phosphorus, the primary and secondary orthophosphate anions, were observed in plants by active process (George, *et al.*, 2008) [7]. Radicle and plumule growth was more in KH_2PO_4 primed seeds that can be due to availability of phosphorus that promotes root and shoot growth in crop plants. Jagadesh *et al.* (1994) also observed significant improvement in seedling size of tomato, capsicum and onion seeds when primed in KH_2PO_4 .

Significantly highest seedling dry weight, seedling vigour index I and II were recorded in seeds primed with KH_2PO_4 @ 0.001 M (T_8) (498.27 mg, 4070 and 44347 respectively) and lowest was observed in control (T_1) (400.32 mg, 1634 and 30625 respectively) (Table 2). In the present study, the increase in shoot length, root length and dry matter production due to priming mainly because of earlier start of emergence as evidenced by lesser days to 50 per cent germination and minimum days to maximum germination. This was in agreement with the earlier studies on maize by Murray (1990) [13] in sweet corn and Afzal *et al.* (2008) [2] in maize.

The increased germination per cent, root length and shoot length with KH_2PO_4 mainly due to increased phosphorous content both inside the seeds which can lead to better establishment of seedlings. Increase in germination and seedling length might have contributed for enhancement of seedling vigour index I. The results are in conformity with Chauhan *et al.* (2016) [5] in sorghum who reported that priming medium KH_2PO_4 produced the highest seedling vigour index. This finding also was similar with Umair *et al.* (2013) [22] who reported that osmo priming with KH_2PO_4 improved vigor index of mungbean (*Vigna radiata* L.) and concluded that early emergence in treated seeds may be due to the faster production of germination metabolites and better genetic repair.

Seedling vigour index II increased mainly due to higher germination, seedling length and dry matter. Priming with this potassium dihydrogen phosphate chemical able to repair the protein damage occurred during oxidative stress and initiates protein *de novo* syntheses. Thus cell will resume the normal metabolic activity *viz.*, mobilization of stored proteins, then the stored mRNA and restart of metabolism from stored

proteins metabolic transitions to support development. Proteomic evidence for this includes enzymes from energy production pathways in primed seeds: glycolysis [6-phosphofructokinase (PFK), phosphoglycerate kinase (PGK)], gluconeogenesis [PEP carboxykinase (PEPCK)], fermentation [alcohol dehydrogenase (ADH)], pyruvate dehydrogenase (PDH), tricarboxylic acid (TCA) cycle [succinate dehydrogenase, succinyl-CoA ligase, malate dehydrogenase (MDH)], glyoxylate cycle (isocitratelase) and the amino acid amino transferases will be recovered. Effective of functioning of metabolism pathways will circulate the energy required for seedling biomass accumulation in essential structures. The produced essential structures were become robust, normal and vigour seedlings thus there was increase in dry weight. The similar results were also recorded by Sowmya (2011) [20] in cucumber and Radha (2013) [17] in maize.

Biochemical parameter like electrical conductivity significantly highest (0.695 dSm^{-1}) was recorded in control (T_1) and lowest (0.211 dSm^{-1}) was observed in seeds primed with KH_2PO_4 @ 0.001 M (T_8) (Table 3). The electrical conductivity value is used as an index of loss of cell membrane integrity and it is negatively correlated with the seed quality attributes. The distinct reduction in electrical conductivity of seed leachate may be due to restoration of membrane integrity upon priming so that the leaching of electrolytes might be controlled in primed seeds (Sung and Chang, 1993) [21]. The similar results were also recorded by Sowmya (2011) [20] in cucumber and Radha (2013) [17] in maize.

Significantly highest (0.494 OD value) for dehydrogenase enzyme activity was recorded in primed with KH_2PO_4 @ 0.001 M (T_8) and lowest (0.231 OD value) was observed in control (T_1) (Table 3). Increased dehydrogenase enzyme activity might be an index of increased cellular biosynthetic activities like DNA and RNA synthesis that in turn indicate the higher protein and energy production necessary for germination and seedling emergence Osborne *et al.* (1980) [15]. Priming increased enzyme activity as well as counteracted the effects of lipid peroxidation. Saha *et al.* (1990) [18] showed that priming caused increased dehydrogenase activity in aged soybean seeds compared to unprimed seeds while decreased lipid peroxidation. The results are in line with Sowmya (2011) [20] in cucumber and Radha (2013) [17] in maize.

Table 1: Effect of seed priming on seed germination, length and root length in cucumber seeds

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)
T_1 - Control	76.5	11.14	10.22
T_2 - Old coconut water at 100%	83.3	17.15	16.21
T_3 - GA_3 at 100 ppm	84.3	18.40	18.28
T_4 - Hydropriming	83.0	16.97	16.14
T_5 - Ethrel at 100 ppm	83.8	17.23	16.92
T_6 - KNO_3 at 1%	84.5	19.41	19.26
T_7 - CaCl_2 at 0.001 M	85.0	19.43	19.27
T_8 - KH_2PO_4 at 0.001 M	89.0	22.96	22.77
T_9 - K_2HPO_4 at 0.001 M	88.3	21.72	21.48
T_{10} - $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ at 0.2%	85.5	20.59	20.51
T_{11} - Chitosan at 0.25%	86.0	20.89	20.59
T_{12} - Chitosan at 0.5%	87.8	20.96	20.79
MEAN	84.73	18.90	18.54
S.Em±	0.46	0.08	0.07
CD @ 1%	1.78	0.29	0.27

Table 2: Effect of seed priming on seedling vigour index I and II in cucumber seeds

Treatments	Seedling dry weight (mg)	Seedling vigour index I	Seedling vigour index II
T ₁ - Control	400.32	1634	30625
T ₂ - Old coconut water at 100%	470.73	2777	39188
T ₃ - GA ₃ at 100 ppm	484.96	3090	40854
T ₄ - Hydropriming	460.86	2748	38252
T ₅ - Ethrel at 100 ppm	473.53	2860	39660
T ₆ - KNO ₃ at 1%	486.78	3267	41131
T ₇ - CaCl ₂ at 0.001 M	488.36	3289	41511
T ₈ - KH ₂ PO ₄ at 0.001 M	498.27	4070	44347
T ₉ - K ₂ HPO ₄ at 0.001 M	496.72	3812	43835
T ₁₀ - Ca(NO ₃) ₂ .4H ₂ O at 0.2%	489.46	3514	41849
T ₁₁ - Chitosan at 0.25%	491.23	3567	42246
T ₁₂ - Chitosan at 0.5%	492.51	3663	43217
MEAN	477.81	3191	40560
S.Em±	1.20	16	235
CD @ 1%	4.62	63	902

Table 3: Effect of seed priming on electrical conductivity and dehydrogenase enzyme activity in cucumber seeds

Treatments	Electrical conductivity (dSm ⁻¹)	Dehydrogenase enzyme activity (OD value)
T ₁ - Control	0.695	0.231
T ₂ - Old coconut water at 100%	0.373	0.350
T ₃ - GA ₃ at 100 ppm	0.292	0.390
T ₄ - Hydropriming	0.429	0.241
T ₅ - Ethrel at 100 ppm	0.317	0.330
T ₆ - KNO ₃ at 1%	0.284	0.370
T ₇ - CaCl ₂ at 0.001 M	0.265	0.415
T ₈ - KH ₂ PO ₄ at 0.001 M	0.211	0.494
T ₉ - K ₂ HPO ₄ at 0.001 M	0.228	0.477
T ₁₀ - Ca(NO ₃) ₂ .4H ₂ O at 0.2%	0.261	0.350
T ₁₁ - Chitosan at 0.25%	0.247	0.430
T ₁₂ - Chitosan at 0.5%	0.240	0.441
MEAN	0.320	0.376
S.Em±	0.002	0.003
CD @ 1%	0.009	0.011

Conclusion

Seed priming with KH₂PO₄ at 0.001 M for 24 h was found to be best priming agent for obtaining highest physiological and biochemical parameters.

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