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Effect of Seed Priming on Biochemical Changes in Fresh and Aged Seeds of Cucumber

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Abstract

The present investigation was conducted at the laboratory of Seed Science and Technology, Indian Institute of Horticultural Research, Hessaraghatta, and Bangalore, India during March 2014 to September 2014. The objective of the study was to identify effect of seed priming on biochemical changes in cucumber seeds. This study showed that the enhancement in seed viability and vigor in primed seeds was due to low membrane injury coupled with high enzyme activities (dehydrogenase and amylase). The priming with KH_2PO_4 and K_2HPO_4 reduced the electrical conductivity (EC) significantly in seed. With seed priming with KH_2PO_4 10^{-3}M there was 44 and 45% lower EC values compared to unprimed. This priming with KH_2PO_4 10^{-3}M and $K_2HPO_4 10^{-3}$M reduced seed leachate values indicating priming initiates the process of repair by stabilizing membrane integrity. priming significantly increased dehydrogenase enzyme in low vigor seeds was noticed. These changes are to the tune of 16 and 21 per cent due to priming with $K_2HPO_4 10^{-3}$M and $KH_2PO_4 10^{-3}$M, respectively in cucumber. Similarly, higher amylase activity was noticed upon priming in low vigor seeds These are to the extent of 2.1, 1.94 folds higher due to priming with $K_2HPO_4 10^{-3}$M and $KH_2PO_4 10^{-3}$M, respectively, in cucumber and 1.4 & 1.4 folds higher due to priming with $K_2HPO_4 10^{-3}$M .Alteration in protein profiles in low vigor seeds compared to high vigor seeds was noticed in the crops. The disappearance of some low molecular weight proteins in low vigor seeds might be the reason for lesser performance of these seeds. Seed priming although not restored all the lost proteins, but induced synthesis of some other proteins which probably might helped in restoration of germination ability and vigor. Esterase Isozyme profiles also showed variation in their number and intensity in less vigor seeds.

Primed seeds further exhibited tolerance to abiotic stress condition (high temperature and water stress) as evident from higher germination compared to unprimed. Besides these studies, some advanced studies were initiated in the present investigation to identify changes in proteome profiles and isocitratelyase gene expression in high vigor and low vigor seeds during germination.

Keywords: seed ageing, seed priming, biochemical, enzymes

1. Introduction

Cucumber *(Cucumissativus L.*) is one of the most important cucurbitaceous vegetables grown throughout the world and ranks fourth after tomato, cabbage and onion. The crop has a wide range of utilities such as pickling, salad, cooked vegetable as an ingredient in Unani medicines and cosmetics. The world average productivity of cucumber is 16.53 t/ha but on the contrary it is only 6.67 t/ha (Anon, 2004) in India. The wide gap in productivity can be attributed to the lack of availability of quality seeds of high yielding varieties, promising hybrids and parthenocarpic varieties suited for glass or poly house cultivation. Seed quality is one of the key factors affecting the successful farming, but this seed trait inevitably declines during prolonged storage. Cucumber is a thermophilic crop which requires relatively high temperatures during its life cycle and generally seeds of cucurbits require relatively high temperature for successful germination and seedling emergence (Hegarty, 1973). Optimum seed germination and seedling growth in cucumber occur at 20-25^{0}C. Poor seed germination is a common phenomenon at sub optimal temperatures which causes a great concern for cucumber growers. According to McDonald (1999), primed seeds could shorten their steps of DNA and RNA synthesis. Consequently, seeds physiology tends to be closer to germination than the unprimed seeds. On the other hand, Higher rates of germination index (GI) might be due to the result of recovered development of genetic repair mechanisms during priming operations (Bradford,1986).Delayed and reduced germination and seedling emergence cause non- uniformity in stand establishment and tender seedling development susceptible to soil borne pathogens for long period. Cucumber plants are susceptible to chilling injury in fields of prolonged low temperatures below 55^{0}F (12.8^{0}C) which results in poor seedlings of splitting, water soaked lesions and decay. One of the major constraints in cucumber cultivation is the poor stand establishment at sub optimal temperature (<20^{0}C). Several priming treatments have been reported to enhance germination under stressful environmental conditions like low temperature, moisture stress and saline soil beds and even to improve poor quality seeds. The terms ‘seed priming’, ‘seed enhancement’ or ‘seed invigoration’ represents a series of treatments applied to a given seed lot in order to improve its germination, uniform emergence of seedlings and yield. Seed priming is a pre-sowing treatment that involves controlled hydration of seeds, sufficient to allow pre germinative metabolic events to take place place and to restrict radical protrusion through the seed coat (Heydecker et al., 1973). Seed priming enables a relatively prolonged Phase II, compared to the water absorption of un- primed seeds in a typical germination process, which presumably includes specific mechanism(s) that promotes rapid and uniform germination (Nascimento et al., 2001) The This technique has been used in some vegetables seeds including cucumber to augment the germination rate, total germination and seedling uniformity etc., mainly under

unfavorable environmental conditions. It is a useful technique to exploit seed potential in arid and desert ecosystem. Seed priming is a low cost technology in which controlled hydration of seeds followed by re-drying is done to break dormancy, improve germination and stand establishment (Afzal et al., 2009). Another benefit of seed priming is that damaged and poor quality seeds float when they are soaked during priming, making it easier for farmers to discard low quality seeds (Farooq and Basra, 2006). The knowledge gained on the repair mechanisms that take place upon various priming treatments has been used in many crops of seed industry. Hydro priming is nothing but soaking seeds in water for a precise time followed by re-drying. Besides, benefits of seed priming are used on pre-enlargement of embryo (Bradford et al., 1990) and extension speed of metabolites (Basra et al., 2005). Kazem et al. (2008) evaluated the effects of hydro priming, halo- priming (solutions of 1.5 % KNO3 and 15 mScm-1 NaCl) and osmopriming (PEG 6000 @ -0.8 MPa) on seedling vigour and field establishment of lentil. Analysis of variance of laboratory data showed hydro priming significantly improved the imbibition rate, germination rate, seedling vigour index, shoot, root and seedling dry weights and reduced electrical conductivity of seed leachates, compared to other seed treatments. Osmopriming involves soaking seeds in aerated water potential of different osmotica (Polyethylene glycol, mannitol, sorbitol, glycerol, etc.,) to control the amount of water imbibed by seeds. Chemo priming is soaking seeds in various inorganic salt solutions like KCl, KNO_3, $CaCl_2$, KH_2PO_4, etc.. the results for germination percentage confirmed the findings of Warley and Fernando (2004) who reported that priming improved the germination percentage and germination rate of un-aged and aged seeds of muskmelon.

2. Material and Methods

The present investigation on seed invigoration aspects of cucumber was conducted in the Section of Seed Science and Technology, Indian Institute of Horticultural Research, Hessaraghatta, and Bangalore-560089 March 2014 to September 2014. The details of the materials used and methods adopted for the conduct of various experiments on seed invigorationaredescribed hereunder:

Source of seeds: Freshly harvested and graded cucumber seeds of cv. Green long was obtained from the seed production unit of IIHR, Hessaraghatta, Bangalore

The investigation was carried to know the biochemical basis of seed enhancement due to seed invigoration.The different observations recorded are:

1.Electrical conductivity (dSm-1)

2.Total soluble seed protein (µg/ml)

3.Total soluble sugars ((µg/ml)4.

Total dehydrogenase activity (OD)

5. Amylase activity (µg starch hydrolyzed /ml/minute)

6.Catalase activity (OD / minute)

7.Protein profile- SDS- PAGE

8.Isozyme profile- NATIVE PAGE

3. Results and Discussion

3.1 Electrical Conductivity (Membrane Injury)

Dearman et al. (1986) reported that osmopriming of onion seeds with PEG-6000 at –1.5 MPa for 10 days at 150C significantly decreased the conductivity of seed leachate (98 to 28 µScm-1) over unprimed seeds. The effect of priming on electrical conductivity in fresh and aged seeds of cucumber is presented in figure 1. In case of fresh and aged seeds priming reduced the EC values significantly. Compared to unprimed and hydro primed, priming with KH2PO4 and K2HPO4 reduced the electrical conductivity significantly. Similarly in aged seeds also all priming treatment reduced the electrical conductivity. priming treatment applied to cucumber seeds after accelerated ageing, improved germination by inducing protein hydrolysis, increased dehydrogenase enzymatic activities and by decreasing electrolyte leakage (Habdas et al., 2000). When compared to hydro priming, priming with potassium salts reduced more significantly the electrical conductivity. Pallavi (2004) observed that osmopriming of cauliflower seeds with PEG-6000 at –1.0MPa for 72 hours significantly enhanced the germination (84 to 94 %), mean seedling dry weight (2.34 to 3.13 mg), SVI based on mean seedling dry weight (1009 to 1116) and decreased electrical conductivity of seed leachate (196 to 159 dSm-1). Electrical conductivity of seed leachate is considered as a simple and most reliable index of seed quality. In the present studies aged seeds showed 56.3 % higher electrical conductivity compared to unaged indicating there is damage to the membrane leading to loss of more solutes. Both high vigor and less viable seeds responded positively to the priming treatments but more so in priming with potassium salt. Due to priming with K2HPO4 there was 33 and 44% reduction in electrical conductivity when compared to control in high viable and less viable seeds, respectively.

3.2 Total Soluble Sugars and Total Soluble Protein

The effect of priming on total soluble sugars and total soluble protein in high viable and less viable seeds in cucumber is presented in fig 2 and 3. Both total soluble sugars and total soluble protein in seed leachate were found high in case of less viable seeds compared to high viable seeds indicating the loss of membrane integrity disturb the selective permeability of the solutes enhance the leakage of more amount of the solutes in the leakage. Bourgne et al. (2000) noticed significant increase in soluble protein content of individual primed seeds as compared to the untreated sugar beet seeds (42.1to 232.8 mg/seed)

Jungmoon and suksoon (2004) reported that osmopriming of sweet corn seeds with –1.2 MPa of PEG 8000 solution at 150C for 2 days resulted in increased -amylase activity, total soluble sugars and decreased electrolyte leakage in such seeds. There was 58 and 12 % higher total soluble sugars and total soluble protein, respectively, in less viable seeds compared to high viable seeds. Although, the priming reduced the total soluble sugars values but these values are almost on par with each other. In case of high viable seeds, however significant decrease in total soluble sugars was noticed due to hydro priming as well priming with KH_2PO_4 and K_2HPO_4. This reduction to the extent of 4.2, 8.95 and 10.6 respectively. Similar trend was noticed with respect to total soluble protein also.

3.3 Amylase Activity

Zuo et al. (1988a) reported that amylase activity during germination of primed peas and tomato seeds. The effect of seed priming on amylase activity in fresh and aged seeds of cucumber is presented in figure 1. Metabolic enzyme plays a key role in degrading seed storage reserve in to simpler and transportable form to supply and nourishment to the growing embryonic axis. Amylase is as such enzyme which breakdown starch in to the most common transportable form of sugar. In the present investigation, the less viable seeds showed 2.11 folds lower amylase activity compared to high viable seeds. Both high and less viable seeds responded to the priming treatments. Upon priming with water, KH_2PO_4 and K_2HPO_4, the percent increase in amylase activity in low vigor seeds was 1.5, 1.9 and 2.1 respectively. The high viability and vigor due to priming (table 1 and 2) might be activation of this enzyme during priming process. Suksoon and Jaehyeum (2000) noticed that osmopriming of rice seeds with PEG solution at –0.6 MPa for 4 days at 150C increased the -amylase activity and they also reported that amylase activity was positively correlated with the total soluble sugar content in such primed seeds.

3.4 Catalase Activity

Jeng and Sung (1994) found that free radicle scavenging enzymes such as superoxide dismutase, catalase, and peroxidase and glyoxysome enzymes such asisocitrate lyase and malate synthasewere increased by priming. The effect of seed priming on catalase activity in fresh and aged seeds of cucumber is presented in figure 2. Catalase activity is generally studied to understand an antioxidant scavenging capacity in the living tissue. These enzymes tend to fluctuate during production of super oxide radical or single oxide molecules. In the present investigation, the low vigor seeds showed higher catalase activity (38%) more indicating the requirement of anti-oxidation to counteract the oxidation that occurred during loss of vigor upon ageing. Although there was slight increase in less vigor seeds due to priming process but no clear trend was noticed. Afzal et al. (2009) reported that germination and seedling vigour can be enhanced by priming with Spermidine and Spermine in tomato cultivars through maintaining higher level of antioxidant enzymes like superoxide dismutase and catalase for eliminating excessive total peroxide.

3.5 Dehydrogenase Activity

Saha et al. (1990) reported that moisture equilibration soaking– drying treatment of soybean seeds increased the total dehydrogenase activity and amylase activity (0.48 to 0.53 and 33 to 35 µg starch hydrolyzed hour-1seed-1). The effect of priming on dehydrogenase activity in fresh and aged seeds of cucumber is presented in figure 3. All the living seeds those respire produce enzymes called dehydrogenase hence, dehydrogenase activity is considered as the positive biomarker for testing the seed viability status. In the present investigation less viable seeds show reduced dehydrogenase activity due to death of viable tissue. This was to the extent of 28.8% lower when compared to high viable seeds. In both high viable and less viable seeds the dehydrogenase activity was increased significantly due to priming. In case of high viable seeds the increase in dehydrogenase activity was to the extent of 48, 62 and 50 % respectively. Due to hydro priming, KH_2PO_4 and K_2HPO_4, this was to the tune of 111, 121

and 115% respectively. The increase dehydrogenase activity due to priming might be due to enzyme activity initiation during priming and also high cell cycle activity during priming process

3.6 Protein Profiles –Cucumber

Gurusinghe et al. (2002) used the seeds of tomato which were subjected to osmopriming with PEG and subsequently dried at 37 or 400C for 2 to 4 hours to study the banding pattern of total soluble seed proteins using SDS–PAGE. The effect of seed priming on protein profiles in fresh and aged seeds of cucumber is presented in figure 4. Variation in protein profiles were noticed between high vigor and less vigor seeds. High vigor seeds contain a total number of 6 bands against only 4 bands in low vigor seeds. In both high and low vigor seeds a common band was observed at Rm value 0.223 and 0.769. In less vigor seeds two additional protein bands were observed at Rm value 0.861 and 0.915. These additional low molecular weight bands might be due to breakdown of existing high molecular weight proteins or due to synthesis heat shock related proteins. Hameed et al. (2010) reported the expression of priming induced proteins (PIP) in wheat leaves, 36.7 kDa peptide, which was expressed by all priming treatments such as hydro priming, freezing (-200C), 100 mM CaCl2.2H20, 0.28 mM Ascorbate. In case of fresh and aged seeds, priming resulted in synthesis of lost protein and also synthesis of some new proteins. In low vigor seeds, hydro priming and showed five additional bands compared to unprimed. Whereas priming with potassium salts showed bands up to maximum of 12 numbers besides variation in the number, marked variation in the intensity of bands was also noticed due to priming in less vigor seeds a common band at Rm value 0.915 was noticed. This can be used as a marker for seed priming in cucumber.

3.7 Esterase Profile-Cucumber

The effect of seed priming on protein profiles in fresh and aged seeds of cucumber is presented in figure 14. Variation in protein profiles were noticed between high vigor and less vigor seeds. High vigor seeds contain a total number of 7 bands against only 2 bands in low vigor seeds. In both high and low vigor seeds a common band was observed at Rm value 0.245 and 0.505. In higher vigor seeds additional protein bands were observed at Rm value0.650 and 0.820. These additional bands might be due to breakdown of existing high molecular weight proteins or due to synthesis heat shock related proteins. In case of fresh and aged seeds, priming resulted in synthesis of lost protein and also synthesis of some new proteins. In low vigor seeds, K2HPO4 priming showed 1 additional bands compared to unprimed. Whereas priming with potassium salts showed bands up to maximum of 11 numbers besides variation in the number, marked variation in the intensity of bands was also noticed due to priming in less vigor seeds a common band at Rm value 0.820 was noticed. This can be used as marker for seed priming in cucumber.

4. Conclusion

Seed priming enhanced viability and vigor of low vigor seed and facilitated germination even under adverse conditions. Hence the technique of seed priming can be adopted as a simple

and cost effective technology for better crop stand establishment and higher productivity. The present studies have great practical utility particularly in the present scenario of global climate change and also in the context of safe seed storage and conservation (both formal and informal) in gene banks.

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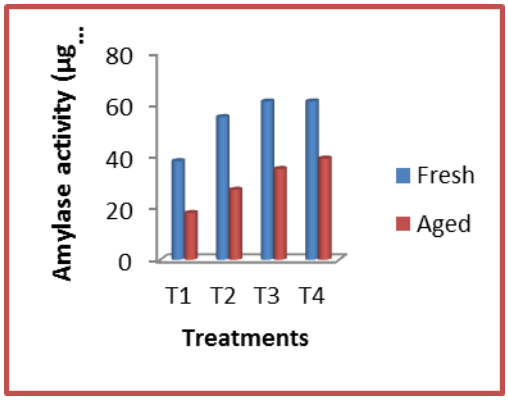
Table. 1 Effect of seed priming on electrical conductivity, soluble sugar and soluble protein in fresh and aged seeds of cucumber.

	EC (ds.m-1)		**Total Sugar (µg/ml)**		**Total Soluble Protein (µg/ml)**	
Treatment	Fresh	Aged	Fresh	Aged	Fresh	Aged
Control	225	400	197	313	152	181
Hydro Priming	200	315	190	300	150	170
KH_2PO_4	153	220	190	285	148	165
K_2HPO_4	150	223	190	280	151	165

Treatments: T1-Control, T2-Hydro priming,T3-KH_2PO_4@0.001M, T4- K_2HPO_4@0.001M



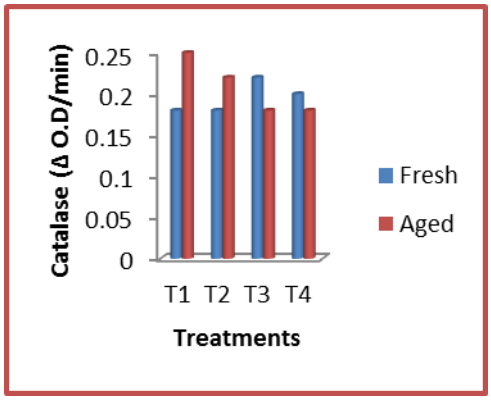


Fig.1 &2 Effect of seed priming on amylase and catalase activity in fresh and aged seeds of cucumber

Treatments: T1-Control, T2-Hydro priming,T3-KH2PO4@0.001M, T4- K2HPO4@0.001M

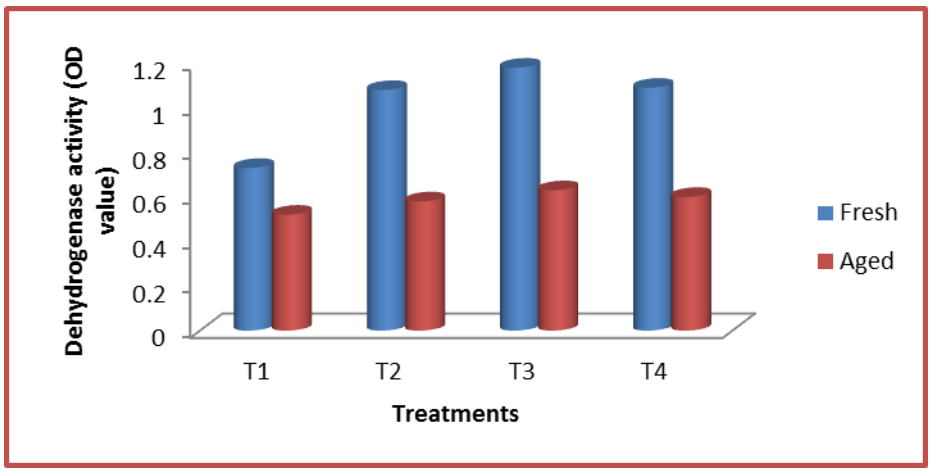
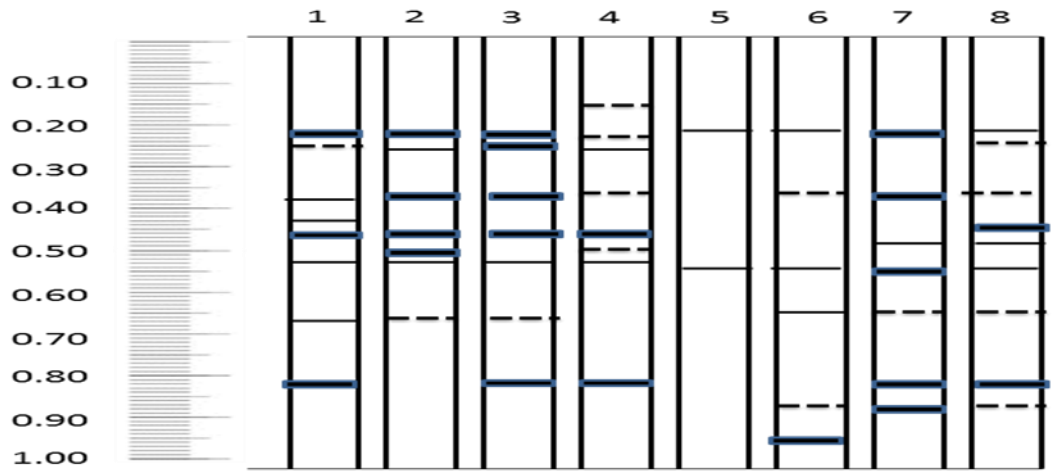


Fig. 3 Effect of seed priming on dehydrogenase activity in fresh and aged seeds of cucumber

Treatments: T1-Control, T2-Hydro priming,T3-KH_2PO_4@0.001M, T4 -K_2HPO_4@0.001M



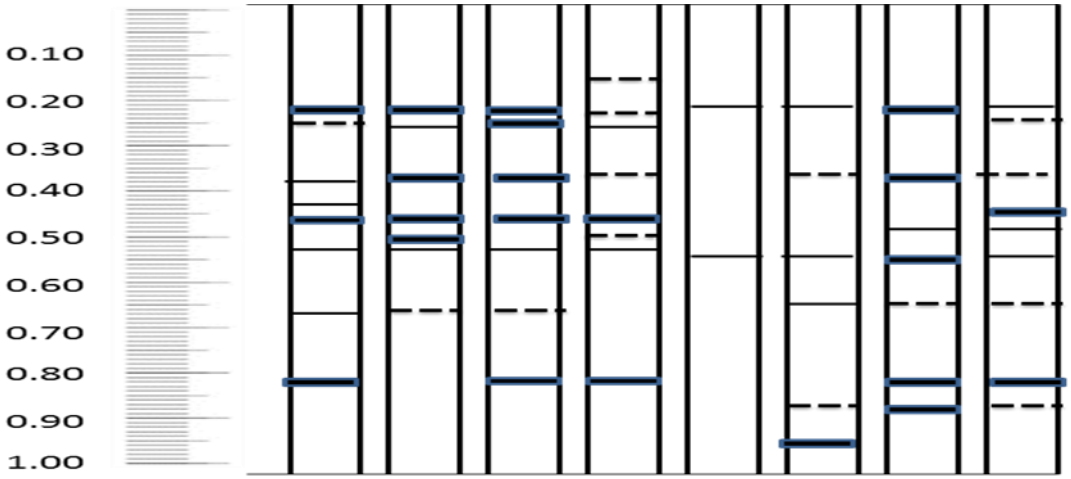
BAND NO.	RM VALUE	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1	0.223	+	-	+	+	+		+	+
2	0.323	+	+++	+	+	-		+	+
3	0.407	+	+	+	++	-	+++	+	+
4	0.423	+	++	++	+	-	+	+	+
5	0.600	-	+	+	+	-	+	+	+
6	0.615	++	+	+	+	-	+	+++	+++
7	0.730	-	-	+	+++	-	+	+	-
8	0.769	++	+++	+++	+	++	+++	+++	+
9	0.861	-	+	+	+	+	+	+	+
10	0.915	-	+	+	+	+	+++	+++	+++
11	0.938	-	+++	+++	+	-	+++	+++	+

Fig. 4 Protein profile as influenced by priming in fresh and aged seeds of cucumber

Vigor Levels: V1- Fresh seeds - high vigor (>90%); V2- low vigor (<50%);

Treatments: Fresh seeds: T1-Control; T2-Water; T3-KH_2PO_4@0.001M, T4-K_2HPO_4@0.001M

Aged seeds:T5-Control; T6-Water; T7-KH_2PO_4@0.001M, T8- K_2HPO_4@0.001M



BAND NO.	RM VALUE	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
1	0.166	-	-	+++	++	-	-	-	-
2	0.245	+++	+++	+++	++	+	+	+++	+
3	0.249	+	+	+	+	-			++
4	0.395	+	+++	+++	++	-	++	+++	++
5	0.456	+++	+++	+++	+++	-	-	+	+++
6	0.505	+	+++	+	++	+	+	+++	+
7	0.509	-	+		++	-	-		
8	0.650	+	++	+	+++	-	+	++	++
9	0.820	+++	-	+	-	-	-	+++	+++

10	**0.910**	-	-	-	-	-	++	+++	++
11	**0.950**	-	-	-	-	-	+++	-	-

Fig. 5 Esterase Isozyme profile as influenced by priming in fresh and aged seeds of cucumber
Vigor Levels: V1- Fresh seeds - high vigor (>90%); V2- low vigor (<50%);

Treatments: Fresh seeds: T1-Control; T2-Water; T3-KH2PO4@0.001M, T4-K2HPO4@0.001M

Aged seeds:T5-Control; T6-Water; T7-KH_2PO_4@0.001M, T8- K_2HPO_4@0.001

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