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Physiological and Biochemical Changes in Enhanced Seed and their Effects on the Field Emergence Characteristics of Chilli Seeds

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ABSTRACT : Rapid and uniform stand establishment is a prerequisite for good crop production, especially in high value vegetable crops. Seed enhancement treatments improve crop performance, the rate and uniformity of germination and provide early-stage protection for better crop establishment. An experiment was conducted on two chilli varieties; Kashi Anmol and Arka Lohit using various seed enhancement treatments; hydro (24 hr), halo (2% $NaCl_2$ for 24 hr), osmo (1.5 MPa PEG 6000 for 36 hr), solid matrix (Vermiculite for 24 hr) and magneto (50mT for 30 min) priming and then assessed for physiological, biochemical changes and their effect on field emergence characteristics. The results indicated that solid matrix priming followed by magnetopriming resulted in higher germination and vigour indices compared to the untreated control. Enhanced seeds exhibited lower electrical conductance and leachate sugars, and higher levels of respiratory enzymes regardless of vigour level. Subsequently, magnetopriming (67%) exhibited comparable efficacy in promoting field emergence with osmopriming (66%) and halopriming (66%) in Kashi Anmol. Emergence was slightly delayed in Arka Lohit compared to Kashi Anmol, but there was a significant improvement in emergence percentage with solid matrix priming (56%), followed by osmopriming (51%) and magnetopriming (50%) treatments. Overall, seed enhancement treatments improved germination, seedling vigour and improving field emergence under normal and adverse conditions in chilli seeds.

Keywords: Seed invigouration, magnetopriming, vigour index, dehydrogenase, electrical conductivity.

INTRODUCTION

Chilli (*Capsicum annuum* L.,) is an important spice crop of India. Chilli require a moist and warm season with an optimum temperature of 20-25°C for high seed germination and stand establishment in the field. Germination and emergence of chilli is slow and uneven due to insufficient moisture, low temperature, and pre-emergence damping off caused by *Pythium* spp under field conditions (Yadav *et al*., 2011). Seed enhancements, also known as seed invigoration, are beneficial post-harvest treatments applied to seeds to promote rapid and uniform germination and seedling growth. These treatments are particularly effective in mitigating stress effects and improving plant stand. The effectiveness of invigoration treatments depends on the level of stress experienced by the seeds (Taylor *et al*., 1998). Various approaches including primitive methods like hydration or invasive treatment used to manipulate seed vigour or physiological status (Yadav *et al*., 2018). It frequently entails the controls hydration of seed for a predetermined duration and temperature in a low water potential, which must be stopped before dessication tolerance is lost and then dehydrate to original moisture content. While, physical seed invigoration or non invasive methods is a non-destructive dry seed treatment through exposure to a magnetic field called magnetopriming that has been shown to increase the germination percentage, rate of germination, and seedling vigour of many agronomic and horticultural crops. However, the effectiveness of magnetopriming depends on genotype, exposure time and optimal dosage.

The invigoration treatment has a notable impact on enhancing seed vigour and germination and the effect can be attributed to completion of pre-germination process involved in DNA replication, increased RNA and protein synthesis, greater ATP availability (Ahmadi *et al*., 2007; Issam *et al*., 2012). Furthermore, enhancement treatment aids in repairing deteriorated seed parts, reducing metabolite leakage by enhancing membrane integrity and sugar content, controlling lipid peroxidation (Finch-savage and Bassel 2016). The increase in hydrolytic enzymes like aldolase, isocitratelyase and decreased alcohol dehydrogenase activity leads to improved reserve mobilization causes reduced time of imbibition required for onset of RNA and protein synthesis, poly ribosome formation, increased total RNA and total protein content were observed in hydro or osmopriming (Pandita *et al*., 2010).

The present study was undertaken to evaluate various seed enhancement treatments and to observe the physiological and biochemical changes, as well as their effects on the field emergence characteristics of chilli seeds.

MATERIALS AND METHODS

The experiment used high vigour (Kashi Anmol) and low vigour (Arka Lohit) chilli seeds procured from ICAR-Indian Institute of Vegetable Research, Varanasi (ICAR-IIVR) and ICAR-Indian Institute of Horticulture Science, Bengaluru (ICAR-IIHR) respectively. Both genotypes were subjected to various seed enhancement treatments like hydropriming (24hr), halopriming (2% NaCl for 24hr), osmopriming (1.5 MPa PEG 6000 for 36hr), solid matrix priming (vermiculite for 24hr) and magneto priming (50 mT for 30 min). Seeds of invasive treatments like hydro, halo and osmopriming were rinsed thoroughly while, solid matrix primed seeds were first separated from the matrix and then rinsed. The treated seeds were finally dried for 48 hr at room temperature. In non invasive magneto priming, the seeds were exposed to magnetic field at 50 mT for 30 min using "Resonance - 2K" generator. The untreated seeds were used as control.

Seeds of both treated and untreated control were further examined for germination, vigour index, membrane integrity test (Electrical conductivity and water soluable sugars), dehydrogenase enzyme test and then assessed for field emergence.

Standard germination and vigour index. Four replicates of fifty seeds was placed on top of moist filter paper in a Petriplate and incubated in a walk in germinator set at 20°C. First and final count was taken on 7th day and 14th day respectively, following ISTA (2011) guidelines. Germination percentage was calculated based on number of normal seedlings on the final day of the germination count. Ten normal seedlings were picked up randomly from each replication after the germination test on the final day. The seedling length (cm) was measured from seedling tip to end of root and dry weight (g) was recorded after drying seedlings for 17hr at 80°C. The seed vigour indices I and II were calculated by using the formula given by Abdul-Baki and Anderson (1973), where

Vigour Index I = Germination (%) × Total seedling length (cm)

Vigour Index II = Germination (%) × Seedling dry weight (mg)

Electrical conductivity of seed leachate. Fifty seeds from each treatment were weighed and soaked in 50 ml of distilled water taken in 100ml beaker. Each beaker was covered with aluminium foil and placed at 25°C ±2°C for 24hr. After incubation, the seeds were removed and the leachates were gently shaken and decanted. The conductance of seed leachate was measured at 25°C with the conductivity meter and expressed in $\mu Scm^{-1}g^{-1}$ seed.

Leakage of water soluble sugars. Seed leachates were used for determining the amount of sugar following the method described by Dubois *et al.* (1951). Seed leachate of 0.1ml was taken in a test tube with 5ml of 5% phenol solution. The final volume was made up by adding 5ml of concentrated H_2SO_4. The intensity of colour developed was observed at 490 nm in Spectrophotometer (Systronic, India).

Dehydrogenase activity. The dehydrogenase activity of the seeds was estimated as per the method described by Perl *et al.* (1978). Twenty five seeds were soaked in water for 24hr in three replicates for each treatment. The seeds were cut longitudinally and then subjected to 0.5 percent tetrazolium chloride solution, and incubated for 6 hr at 25ºC in dark. After incubation, the tetrazolium chloride solution was decanted and the embryo was thoroughly washed with distilled water and surface dried with blotter papers. The formazon was eluted by soaking the stained embryos in 5 ml of methyl cellosolve overnight and the colour intensity was measured at 480 nm using UV spectrophotometer with methyl cellosolve as blank. The total dehydrogenase activity was expressed in OD value.

(i) Field Emergence Percentage. The field emergence studies were conducted in order to elucidate the effect of enhancement treatments before undertaking seed production under field conditions. Seed enhancement treatments *i.e.* hydropriming (24 hr), halopriming (2% $NaCl_2$ for 24 hr), osmopriming (1.5 MPa PEG 6000 for 36 hr), solid matrix priming (Vermiculite for 24 hr) and magneto priming (50mT for 30 min) were sown in rows (600 seeds: 50 in each row; 2 row each treatment) under optimum (15 March 2020) and sub-optimum (10 Nov 2021) conditions. During sowing under sub-optimum and optimum conditions the temperature varied between 4.8 to 13.3^{o}C (Min temperature) and 16.1 to 28.5^{o}C (Max temperature) and 9.5 to 18.5^{o}C (Min temperature) and 21.0 to 31.8^{o}C (Max temperature) respectively (Fig. 1).

Number of seedlings emerged in two rows of each replication were counted in daily intervals until seedling establishment became stable and the emergence was expressed in percentage.

$$\text{Field emergence percentage} = \frac{\text{Number of Seedlings in two rows}}{\text{Total number of seeds sown}} \times 100$$

Statistical Analysis. The experiment data was laid in a completely and randomized block design respectively for lab and field emergence studies were statistically analysed using SPSS software (version 21.0). The data recorded as percentage were transformed to the respective angular (arcsine) values before subjecting them to statistical analysis. Various seed enhancement treatment means were separated by Duncan test at 5% probability level. Graphs were developed using Microsoft Excel.

RESULT AND DISCUSSION

Seed enhancement treatments under controlled conditions maintained at 20°C exhibited increased germination and vigour indices in both the chilli genotypes. Under controlled conditions, seeds of Kashi Anmol and Arka Lohit treated with solid matrix priming using vermiculite for 24 hr showed higher germination upto 87% and 63 % respectively followed by magnetopriming with 50mT for 30min reached 79 % and 61% respectively. While, the unprimed control seeds attain the lowest value of 65% in Kashi Anmol and 56% in Arka Lohit. The data pertaining to vigour index I conducted at 20°C showed that maximum vigour I (641,562) was recorded for solid matrix primed seeds which was stastically at par with magnetoprimed (545,545) in high and low vigour lot respectively (Table 1). The same trend was observed in vigour index II, where unprimed seeds recorded lowest in both the vigour lots. Solid matrix priming contributed to enhanced germination and vigour indices in both high and low vigour seed lots primarily through the mechanism of controlled or slow hydration facilitated by seeds (Jisha *et al*., 2013) and matric conditioning minimizing the aeration, enabling the repair mechanism to activate especially in low vigour seeds. On the other hand, magnetopriming is a dry treatment, there is no metabolic advancement towards complete germination but the stimulatory effect on germination and vigour index is due to changes in the structure of cell membrane and increases their permeability and ion transport ion channels, which finally alters the metabolic pathway (Vashisth and Nagarajan 2010).

Table 1: Effect of seed enhancement treatment on germination (%), vigour index I and vigour index II on chilli genotypes under controlled conditions.

Treatments	Kashi Anmol			Arka Lohit		
	Germination (%)	Vigour Index I	Vigour Index II	Germination (%)	Vigour Index I	Vigour Index II
T1- Unprimed	65^{c} (53.73)	355^{d}	1.2527^{d}	56^{c} (48.45)	409^{c}	1.4974^{d}
T2- Hydropriming (24hr)	77^{ab} (61.34)	485^{bc}	1.6317^{c}	57^{bc} (49.03)	478^{bc}	1.8347^{bc}
T3- Halopriming (NaCl2%, 24hr)	63^{c} (52.54)	389^{cd}	1.3112^{d}	58^{abc} (48.60)	461^{b}	1.7677^{c}
T4-Osmopriming (PEG -1.5MPa, 36hr)	72^{bc} (58.05)	441^{bcd}	1.6595^{c}	61^{ab} (51.36)	503^{ab}	1.9995^{abc}
T5-Solid Matrix Priming (Vermiculite, 24hr)	87^{a} (68.87)	641^{a}	2.6260^{a}	63^{a} (52.54)	562^{a}	2.2268^{a}
T6-Magnetopriming (50mT, 30min)	79^{ab} (62.73)	545^{ab}	2.1539^{b}	61^{ab} (51.36)	545^{a}	2.0537^{ab}
Mean	74 (59.34)	476	1.77	59 (50.19)	493	1.90
CD Value (0.05)	9.49	112.86	0.24	4.67	57.35	0.23

Means sharing same letter, for Kashi Anmol and Arka Lohit in a column do not differ significantly at $p \leq 0.05$ by Duncan test

Enhanced vigour and germination due to priming is an expression of increased membrane integrity (Ilyas *et al*., 2002), total soluble sugars (Nie *et al*., 2022), dehydrogenase (Thomas *et al*., 2013; Pandita *et al*., 2010), improved reserve mobilization, increased RNA and protein synthesis (Pandita *et al*., 2007, 2010), greater ATP availability and controlled of lipid peroxidation (Corbineau *et al*., 2000). In the study, EC of seed leachates ranged from 53.23 to 33.33 µS/cm/g and 77.37 to 59.27 µS/cm/g of seed during hydration treatments like hydro, halo, osmo and solid matrix priming of Kashi Anmol and Arka Lohit respectively. In Kashi Anmol seeds, the solute leakage values were 33.33 µS/cm/g and 37.10 µS/cm/g in solid matrix priming and osmopriming, respectively. Similarly, for Arka Lohit seeds, the EC was 59.27 µS/cm/g for solid matrix priming and 61.13 µS/cm/g for osmopriming which shows significant reduction in leakage of electrolytes than control seeds indicating that cell membrane undergoes rejuvenation and repair, leading to reduced solute leakage (Chiu *et al*., 1996) (Table 2). Low conductivity of seed leachate was also reported in egg plant and radish (Rudrapal and Naukamura 1988), onion (Choudhari and Basu 1988), okra (Sharma *et al*., 2014) and cabbage (Ziaf *et al*., 2015). Irrespective of treatments, enhanced seeds of Arka Lohit showed higher conductance as compared to Kashi Anmol, a low vigour genotype used in the study, the seed membrane integrity was poor in Arka Lohit as compared to Kashi Anmol which caused outward diffusion of ions during imbibition as reported by Simon and Mills (1983).

A significant reduction in water soluble sugars from primed seeds as compared to untreated seeds was observed in both Kashi Anmol and Arka Lohit seeds. Lower water soluble sugars was observed in solidmatrix priming (5.78 µ/ml) and osmopriming (6.54 µ/ml) treatments in Kashi Anmol. Similar trend was also observed in Arka Lohit where low soluble sugars were observed in osmopriming (10.27 µ/ml) followed by solid matrix primed seeds (10.94 µ/ml) (Table 2). Bewley (1997) reported that during the priming, the membranes returns to their normal or near-normal configuration in an orderly manner resulting in decreased release of sugars. Previous studies have also reported the influence of very low-frequency alternating magnetic field on ionic permeability of cell membrane (Bhardwaj *et al*., 2012).

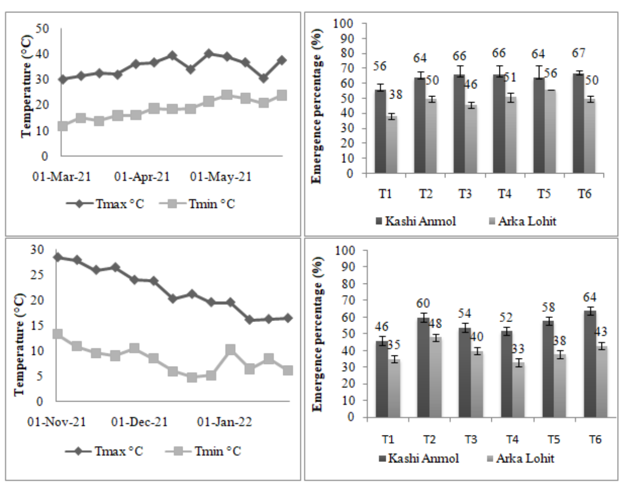
Dehydrogenase, an index of tissue respiration and metabolism was greater in treated seeds than untreated control seeds, irrespective of difference in their vigour. The maximum increase in dehydrogenase activity in primed and unprimed seeds ranged from 50-77% in Kashi Anmol and 33-101% in Arka Lohit. In Kashi Anmol, dehydrogenase activity was highest in solid matrix primed (Δ 0.6403) and osmoprimed seeds (Δ 0.6366) which were significantly higher over unprimed seeds (Δ 0.5772). Similarly, highest enzyme activity was observed in solid matrix (Δ 0.3449), osmoprimed (Δ 0.3242) followed by magnetoprimed (Δ 0.3127) seeds as compared to untreated control (Δ 0.1979) (Table 2). Similar reports of beneficial effect of priming on improved seedling vigour and elevated level of dehydrogenase activity is reported in carrot and tomato (Nagarajan *et al.,* 2003; Pandita *et al*., 2003) and okra (Sharma *et al.,* 2014).

Table 2: Effect of seed enhancement treatments on EC, water soluble sugar and dehydrogenase activity of chilli genotypes.

Treatments	Kashi Anmol			Arka Lohit		
	Electrical Conductivity (µS/cm/g of seed)	Water soluble sugars (µg/ml)	Dehydrogenase activity (OD Value)	Electrical Conductivity (µS/cm/g of seed)	Water soluble sugars (µg/ml)	Dehydrogenase activity (OD Value)
T1- Unprimed	71.43^{f}	12.30^{d}	0.5772^{c}	126.03^{d}	19.35^{e}	0.1979^{c}
T2- Hydropriming (24hr)	43.03^{c}	9.45^{c}	0.5023^{d}	77.37^{b}	13.48^{c}	0.3294^{ab}
T3- Halopriming (NaCl 2%, 24hr)	53.23^{d}	8.02^{bc}	0.4004^{e}	72.13^{b}	12.12^{b}	0.2902^{b}
T4-Osmopriming (PEG -1.5MPa, 36hr)	37.10^{b}	6.54^{ab}	0.6366^{a}	61.13^{a}	10.27^{a}	0.3242^{ab}
T5-Solid Matrix Priming (Vermiculite,24hr)	33.33^{a}	5.78^{a}	0.6403^{a}	59.27^{a}	10.94^{a}	0.3449^{a}
T6-Magnetopriming (50mT, 30min)	62.27^{e}	9.24^{c}	0.5604^{b}	110.83^{c}	16.42^{d}	0.3127^{ab}
Mean	50.07	8.55	0.5529	84.46	13.76	0.2999
CD (0.05)	3.53	1.68	0.01	8.19	0.76	0.04

Means sharing same letter, for Kashi Anmol and Arka Lohit in a column do not differ significantly at $p\leq0.05$ by Duncan test

Weather conditions have important bearing on field emergence and crop performance. In the study, Kashi Anmol and Arka Lohit exhibited low field emergence in the sub-optimum conditions due to the prevailing low temperature in the post sowing phase, which was below the optimum temperature required for seed germination in chilli (20-30°C as per ISTA, 2011). The prevailing sub-optimum conditions resulted in delayed and reduced emergence, particularly in low vigour genotype; Arka Lohit as compared to high vigour; Kashi Anmol. However, enhanced and early emergence was observed in magnetopriming, solid matrix and hydropriming treatments in both Kashi Anmol and Arka Lohit. On the other hand, under optimum conditions, the sowing was done on March 15, 2021, the weather conditions were optimum and favourable for early emergence and seedling growth which resulted in higher emergence in both the genotypes. Notably, unprimed control seeds of Kashi Anmol and Arka Lohit exhibited lower emergence as compared to enhanced seeds. Among the treatments; magnetopriming (67%) exhibited comparable efficacy in promoting emergence with osmopriming (66%) and halopriming (66%) in Kashi Anmol. Emergence was slightly delayed in Arka Lohit compared to Kashi Anmol, but there was a significant improvement in emergence percentage with solid matrix priming (56%), followed by osmopriming (51%) and magnetopriming (50%) treatments. The results of the study are in accordance with Heydecker and Coolbear (1977); Nayban *et al*. (2017) ; Chen *et al*. (2012) that seed enhancement treatments improved seed germination, rate and synchronization of field emergence and shortened exposure time of seed to adverse abiotic and biotic stresses.



[T_1- Unprimed; T_2- Hydropriming (24hr); T_3- Halopriming (NaCl 2%, 24hr); T_4-Osmopriming (PEG -1.5 MPa,36hr); T_5-Solid Matrix Priming (Vermiculite, 24hr); T_6-Magnetopriming (50mT, 30min)]

Fig. 1. Maximum and minimum temperature during optimum (Weekly weather data from March 2021 to May 2021) and sub-optimum condition (Weekly weather data from November 2021 to January 2022) and Emergence percentage in different enhancement treatments. First row represents optimum conditions and second row represents suboptimum conditions.

CONCLUSIONS

Seed enhancement treatments can positively impact the emergence performance of chilli seeds, especially for both high vigour (Kashi Anmol) and low vigour (Arka Lohit). Various seed enhancement treatments provide an opportunity to maximize the potential of seeds, leading to more uniform, vigorous stands, and ultimately, better crop establishment and productivity. Specifically, solid matrix priming and magneto priming had significant effect on field emergence both under suboptimum and optimum conditions. Changes in physiological (germination percentage, vigour index I, vigour index II, Electric conductivity and total soluble sugars) and biochemical parameters (dehydrogenase activity) in relation to enhancement in viability and vigour upon solid matrix priming were observed.

FUTURE SCOPE

Seed enhancement treatments are known to improve seed germination, emergence, early seedling growth, and overall plant growth, especially under stress conditions. These treatments effectively alleviate the impact of suboptimum temperatures during the sowing and transplanting of chilli plants in the off-season. They also provide sufficient isolation and reduce the incidence of diseases, thereby facilitating quality seed production.

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Conflict of Interest. None.

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