Physiological seedling vigour parameters of wheat as influenced by different seed invigoration techniques

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Abstract
The present investigation was carried out to study the response of physiological seedling vigour parameters of wheat as influenced by different seed invigoration techniques. Seeds of wheat variety UP 2565 were treated with electro-magnetic radiation (EMR) of 100 and 200 mT for one hour, hydroproming for 12 hours and plasma treatment for 6 minutes which were tested along the untreated seeds. The results revealed that all the seed invigoration treatments improved germination and seedling vigour parameters of wheat seed. The water absorption of plasma treated seeds increased by 42.2% and 23.9% after 6 and 12 hours of imbibitions period respectively over untreated seeds. The highest germination percentage was recorded in plasma treated seeds which was at par with electro-magnetic radiation (EMR) for 200 mT. The plasma treated seeds also emerged earlier than control and also took less time to emerge than rest of the treatment including untreated seeds.

Keywords: Electro-magnetic radiation, plasma, hydroproming, seed invigoration techniques

Introduction
Quality seed plays vital role for boosting agricultural production. Seed quality is influenced by many factors including its genetic makeup as well as environmental conditions prevailing during growing season. Post-harvest operations are also considered as imperative factors for maintaining seed quality in terms of viability and vigour of seed during storage. Declination in seed quality also termed as seed deterioration which is an irreversible and degenerative process. Seed deterioration is associated with various activities of seed occurring at cellular, metabolic and chemical level inside the seed. Deterioration causes reduced germination while those seeds that germinate produce weak seedlings. So, now a-days researchers are focusing on certain physical and physiological pre-sowing seed invigoration techniques which can improve seed viability and seedling vigour. In this context, one of the very traditional and oldest method of seed quality enhancement is hydroproming (soaking of seeds in water for a particular time and drying them back to their original moisture content before sowing) which perform magnificently under favourable as well as under adverse situations. It allows seeds to absorb sufficient water to initiate metabolic process but insufficient water to complete germination process. The major pre germination steps such as DNA and RNA synthesis are accomplished in the seed during the hydration consequently the seeds are physiologically close to germination and have fewer steps to complete germination process than unprimed seeds.

When hydropromised seeds were sown, they initiate germination rapidly in comparison to unprimed seeds. Hydro-priming is the most popular pre-sowing seed treatment among farmers but at large scale soaking and drying of seed is very tedious job. Therefore, physical seed invigoration methods offer great advantages to enhance seedling growth through manipulation of seed surface structural properties, increased DNA and RNA repair, quick breakdown of reserve food material and enhanced translocation of energy to the growing parts of seeds. One of the advance physical seed invigoration technique is application of electro-magnetic radiations which is also termed as magneto-priming. Electro- magnetic radiation described as eco-friendly and non-invasive approach to enhance the quality of seeds in terms
of germination and seedling growth parameters. Magnetic field changes the properties of cell membrane and cell division which brings alteration in functioning and metabolism of cell includes variation in protein biosynthesis, gene expressions and enzyme activities Atak et al. (2003) [1]. These changes inside the cell results in increment of physiological seedling vigour parameters. Another physical seed invigorating technique is plasma treatment, which is a modernized method and potentially improves seedling growth parameters. Plasma considered as substance having elevated energy with charged particle and reactive species which modify the outer surface as well as internal processes of seed after exposure. Plasma alters seed coat properties and makes it more permeable for water imbibition which improves physiological and biochemical properties of germinated seed. Plasma exposure resulted in etching of brown rice surface which allow water to be easily absorbed by rice kernel Chen et al. (2014) [8].

Every crop requires specific exposure intensity and duration of these physical methods. Some crops showed vast improvement even after the short duration of treatment with minute intensity while some perform outstandingly under higher doses of treatment. Proper standardization of exposure time and dose is important for better outcome. Therefore, the present investigation was carried out to study the influence of different seed invigoration techniques on seed germination and physiological seedling vigour parameters.

Materials and methods
For the present investigation, seeds of wheat variety UP2565 were procured from Breeder Seed Production Centre, GBPUA&T, Pantnagar in the year 2018. Immediately upon the receipt, the wheat seeds were packed in polylined cloth bag under ambient storage condition for further study.

Seeds invigoration techniques Electro-magnetic radiation
Seeds of wheat were treated with electro-magnetic radiation of 100 and 200 mT strength for 1 hour. The electro-magnetic radiation treatment was given in the Department of Physics, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology. A machine named ‘omega electromagnetic (input 0-4 Amp, output 7.5 kg gauss)’ was used for the treatment which have coil diameter of nine cm and spacing between the both north and south pole of five cm. A digital gauss meter model DHE-200 was used for measuring the frequency of magnetic field between the poles. Three hundred healthy seeds were taken in a transparent polythene packet at a time and put between the poles for the required duration. For creating magnetic field of 100 and 200 mT, current of 2.1, and 3.8 ampere along with voltage of 25.6 and 44.3 volt respectively were supplied. Gauss meter was used to measure the strength of magnetic field between the north and south poles. A unit of 10-4 gauss measure one tesla strength.

Hydropriming
For hydro priming, wheat seeds were soaked in normal tap water in the ratio of 1:2 (seed: water ratio) for 12 hours and then dried in shade up to seeds reached its original moisture content.

Plasma treatment
Plasma treatment of wheat seeds had done using air plasma at radio frequency of 13.56 MHz for 6 minutes with fixed 50 watts coupled power and 0.7 mbar pressure. This treatment was done at Institute of Plasma research, Ahmedabad.

Observation recorded
Germination test
A standard germination test was conducted in seed physiology laboratory, Department of Agronomy, to measure different germination and seedling growth parameters of wheat seeds. Seed germination was determined by following the method of ISTA (2004) [9]. Hundred seeds in each of three replications were placed on two layers of moist germination papers. Before placing seeds were treated with thiram @2.5g/kg of seed to control surface borne fungi. After that, seeds were covered with another sheet of moist germination paper. Then it was rolled and fastened with rubber band. The rolled towel paper samples were kept in an incubator maintained at 20 ±2 °C temperature for germination. The samples were evaluated after 8 days of incubation. The germination percentage was calculated on the basis of number of normal seedlings as per formula given below

\[
\text{Speed of germination} = \frac{n}{\text{di}}
\]

Where, \(n\) = number of newly germinated seeds on day \(di\), \(di\) = ith day after incubation

Mean germination time
For recording mean germination time taken by seeds during the germination test was computed by following formula:

\[
\text{Mean germination time} = \frac{n \times d_i}{\Sigma n}
\]

Where, \(n\) = no. of seeds newly germinated on day \(di\), \(di\) = ith day after incubation

Rate of water imbibition
Fifty seeds from each seed invigoration treatment in three replications were soaked in measured quantity of 100 ml tap water for 6 and 12 hour duration. After prescribed period the seeds were removed from water and volume of the water again measured and rate of water imbibition was calculated and expressed in percentage.

Results and Discussion
Seed Germination
The data on germination percentage of different seed invigoration techniques are depicted in Table 1. The highest seed germination was recorded in plasma treated seeds which was statistically at par with seeds treated with 200 mT strength of electromagnetic radiation for 1 hour. The increase in germination per cent was 4.34% and 3.58% in plasma and EMR 200 mT respectively over untreated seeds. Seed germination is an amphibolic process where both catabolic process in endosperm and anabolic process in embryonic axis take place simultaneously. Catabolic process is governed by hydrolytic enzyme activity of seed. Plasma treated seeds showed faster water uptake than rest of the treatment which leads to quick initiation in hydrolytic enzymes activity and responsible for rapid breakdown of stored food material. It resulted in higher germination in plasma treated seeds over untreated seeds. Meng et al. (2017) [14] also reported 26.7% increase in germination per cent of wheat seed treated with air plasma at

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atmospheric pressure and room temperature over untreated seeds. It may be due to the alteration in seed coat morphology, increased permeability and water uptake through seeds which promoted germination of seed. The results are also with strong agreement with Ling et al. (2014) \cite{11} in soybean. They reported that a brief exposure for 15 second with cold helium plasma improved germination percentage over untreated seeds. It may be because high energy ions used in plasma treatment causes erosion in seed surface and changed seed coat could increase the hydrophilic ability of seed which eventually improve the water uptake and germination of seed. Rochalska and Rywka (2005) \cite{13} treated wheat, maize and soybean seed with 16 or 50Hz homogenous magnetic field using a magnetic flux density of 5 mT for 2 hours. They reported that exposure of wheat seed with 50Hz magnetic strength for 2 hours increased germination per cent from 76 % to 91% in comparison to control seed and in soybean seed germination per cent increased from 77 % to 96%. Whereas magnetic treated seeds of maize did not show any increment on seed germination. Florez et al. (2018) \cite{7} reported that exposure of rice seeds to static magnetic field creates influx of Ca+2 ions through the plasma membrane & increase germination dynamics in seeds.

### Speed of germination

Plasma treatment enhanced speed of seed germination upto 24.2% which was significantly higher than rest of invigoration treatment including control. It might be due to the penetration of active species inside the seed through porous seed coat during treatment which is responsible to increase metabolic processes seeds (Sera et al. (2010) \cite{18}). This result agreed with the finding of Piza et al. (2019) in soybean where germination speed increased up to 10-23% in seeds treated with non -thermal plasma with oxygen and nitrogen gases for 2 and 3 hours. It may be due to the interaction of charged ions with hormones and enzymes inside the cell which stimulate quick germination over untreated seed. EMR 200 mT also showed 17.5% increment in speed of germination over untreated seeds. This result is in consistent with Florez et al. (2007) \cite{6} who treated maize seeds with magnetic strength of 125 mT and 250 mT for 24 hours. Maize seeds sprouted early and reduced total time taken for completion of germination process in comparison to untreated seeds. Mridha and Nagarajan (2014) \cite{15} treated chickpea seeds with magnetic flux of 50- 250 mT for 1 -3 hours. They reported 7-27% increment in speed of germination of treated seeds over untreated seeds, it may be due to the modification in internal energy status of seed due to magnetic radiation exposure which worked as a stimulatory factor in initiating germination.

| Parameters Seed invigoration techniques | Germination percentage (%) | Speed of germination (seedlings/day) | Mean germination time (days) |
|----------------------------------------|-----------------------------|-------------------------------------|----------------------------|
| Untreated                              | 92.0                        | 16.8                                | 3.04                       |
| EMR 100 mT                             | 92.7                        | 17.7                                | 2.94                       |
| EMR 200 mT                             | 95.3                        | 19.8                                | 2.67                       |
| Hydropriming                           | 93.3                        | 19.0                                | 2.71                       |
| Plasma treatment                       | 96.0                        | 20.9                                | 2.47                       |
| SEM±                                   | 0.9                         | 0.3                                 | 0.06                       |
| CD at 5%                               | 2.6                         | 0.9                                 | 0.20                       |

EMR: Electro-magnetic radiation; mT: mili tesla

Hydroprimed seeds also resulted in significantly higher germination speed (13.0%) than control. It might be due to quick commencement in enzymatic activities and breakdown of reserve food material. Similar results were reported in rice seeds hydroprimed for 48 hours as hydropriming enhanced speed of germination of treated seeds upto 66.81 %over untreated Prasad et al.(2012) \cite{10}.

### Mean germination time

All seed invigoration techniques significantly reduced mean germination time in comparison to untreated seeds except EMR 100mT for one hour. Plasma treated seeds (2.47 days) showed significant reduction in mean germination time in comparison to all the evaluated seed invigoration technique. Ling et al. (2014) \cite{11} reported that plasma treatment significantly reduces time taken for mean germination by soybean seeds over untreated seeds. Whereas EMR 200 mT exposure to seeds (2.67 days) resulted in decline in mean germination time significantly over control but statistically at par with hydropriming (2.71 days). These results were found in association with Martinez et al. (2017) \cite{13} in case of maize and wheat seed. They treated the seeds of both crops with magnetic radiation of 125 mT for 24 hours and reported that mean germination time was reduced upto 24.47% and 13.20% in maize and wheat respectively over untreated seeds. Canak et al. (2016) \cite{3} reported that mean germination time of hydroprimed seeds (5.24 days) for 17 hours reduced significantly over untreated seeds (5.41 days). Similar results are also reported by Junhaeng et al. (2015) \cite{9, 10} in barley and maize. In the present investigation, untreated seeds (3.04days) took maximum time to germinate the seedlings which was at par with EMR 100 mT strength (2.94 days).

### Rate of water imbibition

Invigorated seeds imbied more water during both the duration of soaking over control but during the initial hours imbibition was considerably high than successive hours. Seeds exposed to plasma and EMR 200 mT magnetic radiation resulted in significantly higher water absorption over untreated seeds at 6 hour duration and maintained the same trend of higher water uptake even in the later stages of soaking. Plasma technique increased water uptake through seeds upto 42.77% and 23.90% at 6 and 12 hours of soaking respectively while EMR 200 mT resulted in 28.19% and 18.56% increment.

These results are found in the strong agreement with Bormashenko et al. (2012) \cite{2} in case of lentil, beans and wheat in which alteration in the seed surface observed after the air plasma treatment for the time span of 15 seconds to 2 minutes. Increased roughness in the seed coat was observed enhanced water uptake through seed in contrast to control.
seeds. High imbibition of water significantly increase germination percentage and germination rate significantly over untreated seeds. Plasma treated soybean seeds absorbed 14.03% water in comparison to control which lead to quick and higher germination percentage Ling et al. (2014) [11].

Fig 1: Impact of different seed invigoration techniques on water uptake through seed at 6 and 12 hours after soaking.

Conclusion
From the present investigation, it may summarize that physical methods of seed invigoration improves seed germination and seedling vigour parameters of wheat seed. Among all the seed invigoration parameters, air plasma treatment for 6 minutes improved seed germination, speed of germination and mean germination time. Rate of water imbibitions was also increased in plasma treated seeds. In addition to this treatment, EMR 200 mT for one hour also improved physiological seedling vigour parameters. The accurate mechanism and science behind enhanced seedling vigour parameters due to exposure of plasma and magnetic radiation are yet to be explored. Therefore, further studies are essential for optimum utilization of these physical seed invigoration techniques.

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