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REVIEW ON EFFICIENT APPROACHES TO SEED INVIGORATION FOR QUALITY ENHANCEMENT

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ABSTRACT

The main aim of this review paper is to synthesize all the knowledge and information that have been generated through years of research and organized study in the field of seed invigoration techniques by various authors. Tremendous efforts were made in order to derive the successful results for seed quality enhancement through seed invigoration techniques. This paper summarizes all the possible research outputs and information derived in seed invigoration of different crops. Thus seed invigoration is physiological and biochemical changes related to synchronous germination, velocity, and increased seed germination. Priming is an invigoration technique that enhances the physiological and biochemical events in seeds during suspension of germination by low osmotic potential and negligible matric potential of the imbibing medium. Seed priming has emerged as an effective approach for increasing seed vigor and germination synchronization, as well as, seedling growth and field establishment under adverse environmental conditions. Various seed priming techniques have been developed, including hydro-priming (soaking in water), halo-priming (soaking in inorganic salt solutions eg. NaCl, $CaCl_2$, KNO_3), osmo-priming (soaking in solutions of different organic osmotica), thermo-priming (treatment of seeds with low or high temperatures), solid matrix priming (treatment of seed with solid matrices placing seeds between saturated jute mat layers), hardening (alternate soaking of seeds in tap water and drying before sowing) and bio-priming (hydration with biological compounds). In addition to these, seed pelleting, seed coating, physical methods such as magneto-priming, magnetic resonance invigoration, ionizing radiations are vital techniques for seed quality enhancement. All of these techniques have been increasingly used in various crops in different countries. Most recently very efficient and eco-friendly and economical approaches of seed invigoration have been emerging in the field of seed science and technology.

Keywords: Hydropriming; Biopriming; Magneto-priming; Seed coating, Seed invigoration

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BACKGROUND

Different pre-sowing seed treatments have successfully been integrated for vigor enhancement. Seed invigoration treatments have been developed to improve seed performance during germination and seedling early growth. Invigoration is method widely used to improve the physiological quality of seeds, especially seed vigor, through physical or chemical treatment. Seed invigoration is physiological and biochemical changes related to synchronous germination, velocity, and increased seed germination (Sutariati et al., 2018).

The development of seed invigoration treatments really started with seed priming. Priming is an invigoration technique that enhances the physiological and biochemical events in seeds during suspension of germination by low osmotic potential and negligible matric potential of the imbibing medium (Singh et al., 2017). Priming aimed to control seed hydration so that all seeds reached the same stage of germination before sowing; subsequent germination was rapid and synchronous. This was achieved by allowing seeds to imbibe from a solution of polyethylene glycol (PEG) such that imbibitions ceased when the seed water potential equaled that of the PEG solution, and was achieved at a seed moisture content below that required for germination (radicle protrusion). Improvements in the rate and uniformity of germination have been achieved in a range of species (Heydecker and Coolbear, 1977). Seed priming has been successfully demonstrated to improve germination and emergence in seeds of many crops specially vegetables and small seeded grasses (Singh et al., 2017). Seed priming has proved promising, and valid result, for many seed including the legume seeds (Bradford, 1986).

In priming, seeds are soaked in different solutions with high osmotic potential. This prevents the seeds from absorbing in enough water for radicle protrusion, thus suspending the seeds in the lag phase (Singh et al., 2017). Seed priming has been commonly used to reduce the time between seed sowing and seedling emergence and to synchronize emergence. Halo-priming of seeds in pre-sowing treatments in an osmotic solution allows seeds to absorb water, but restricts radicle occurrence through testa until the primed seeds are sown for germination under salt stress conditions. Primed seeds usually show improved germination parameters (Hardegree and Van Vactor, 2000).

PRIMING AND IMPORTANCE IN SEED TECHNOLOGY

Priming refers to the mechanism controlling the hydration level within seeds so that the metabolic activity required for germination can occur. ‘Priming’ is a well-established treatment for enhancing seed quality throughout the transient activation of the pre-germinative metabolism that includes antioxidant functions and DNA repair processes (Paparella et al., 2015). Seed priming has emerged as an effective approach for increasing seed vigor and germination

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synchronization, as well as, seedling growth and field establishment under adverse environmental conditions. Seed priming is increasingly considered a better approach to enhancing rapid and uniform emergence and to achieving high seedling vigor and better yields in vegetables, floriculture, and some field crops (Toklu et al., 2015). Priming is pre-sowing, seed priming technique is a physiological method based on controlled hydration treatment to absorb water before radicle swelling and plant growth under stressed condition. Methods of seed priming have been described in detail by (Bradford, 1986). Various seed priming techniques have been developed, including hydro-priming (soaking in water), halo-priming (soaking in inorganic salt solutions eg. NaCl, $CaCl_2$, KNO_3), osmo-priming (soaking in solutions of different organic osmotica), thermo-priming (treatment of seeds with low or high temperatures), solid matrix priming (treatment of seed with solid matrices placing seeds between saturated jute mat layers), hardening (alternate soaking of seeds in tap water and drying before sowing) and bio-priming (hydration with biological compounds) (Golezani et al., 2016)

In the context of seed technology, physical methods have showed several advantages over conventional osmopriming protocols (Bilalis et al., 2012). Physical methods for seed invigoration offer several advantages over conventional treatments based on chemical substances. First, they reduce the use of fertilizers, thus decreasing pollution of on-farm produced raw materials. Another advantage is that physical methods may be also used for seed disinfection before sowing and during the storage. Possible approaches include the treatment with electromagnetic waves (EWs), magnetic fields (MFs), the ultrasounds (US), and ionizing radiations (IR). Food treatment with IR, which enhances its microbiological safety and storability (Araújo et al., 2016). One of the most investigated physical pre-sowing seed treatments in agriculture is based on the use of MFs which have been described as eco- friendly, cheap, and non-invasive technique. Additionally, the impact of other types of physical treatments, such as, gamma (g), and X ionizing, UV and microwave (MW) radiation will be addressed in this review (Araújo et al., 2016).

REVIEW ON APPROACHES OF SEED INVIGORATION

Hydropriming

Hydro priming generally enhances seed germination and seedling emergence. Hydropriming defined as soaking of seeds in water. Hydro–priming significantly improved seedling establishment and early vigor of mung bean, resulting in faster development, and higher yields. The favorable effects of hydro-priming on seed germination and establishment have widely been studied in different plant species (Golezani et al., 2016). Hydropriming is a simple, low cost, and environmentally friendly technique for improving seed germination and seedling vigor of lentils (GHASSEMI-GOLEZANI et al., 2008). The results show that hydropriming treatment was

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superior in seedling growth rate among all priming applications. Several researchers reported the positive effect of hydropriming on seedling emergence rate, seedling establishment, early vigor, and the faster development of the seedling. Priming with PEG-6000 resulted in the highest number of plants per square meter at late sowing in both wheat varieties. Thus, seed priming with PEG appears to have promoted stand establishment at late sowing under field conditions.

The results of studies performed with various priming in inbred maize indicated that hydropriming was more effective than osmopriming treatments (PEG and urea). Similar results also observed in sweet corn where seed germination and vigor decreased with osmoconditioning, although germination significantly enhanced by water soaking (Dezfuli et al., 2008). However, osmopriming has been shown to activate processes related to germination, by increasing superoxide dismotase (SOD) and peroxidase (POD).

Invigoration of milk thistle seeds by hydro-priming resulted in significantly higher seedling emergence percentage and lower emergence time in the field. It was also revealed that 16 hours hydro-priming was found effective in improvement in seed invigoration and seedling emergence. Thus, this hydro-priming duration could be used as a simple method for enhancing seed vigor and field emergence of milk thistle a medicinal plant species. The seed priming was carried out by soaking the seeds in distilled water for 8 and 16 hours. All priming treatments were performed in an incubator adjusted on 20±1°C under dark conditions. After hydro-priming, seed samples were dried at 20-25°C for 24 hours before seeding was actually started.

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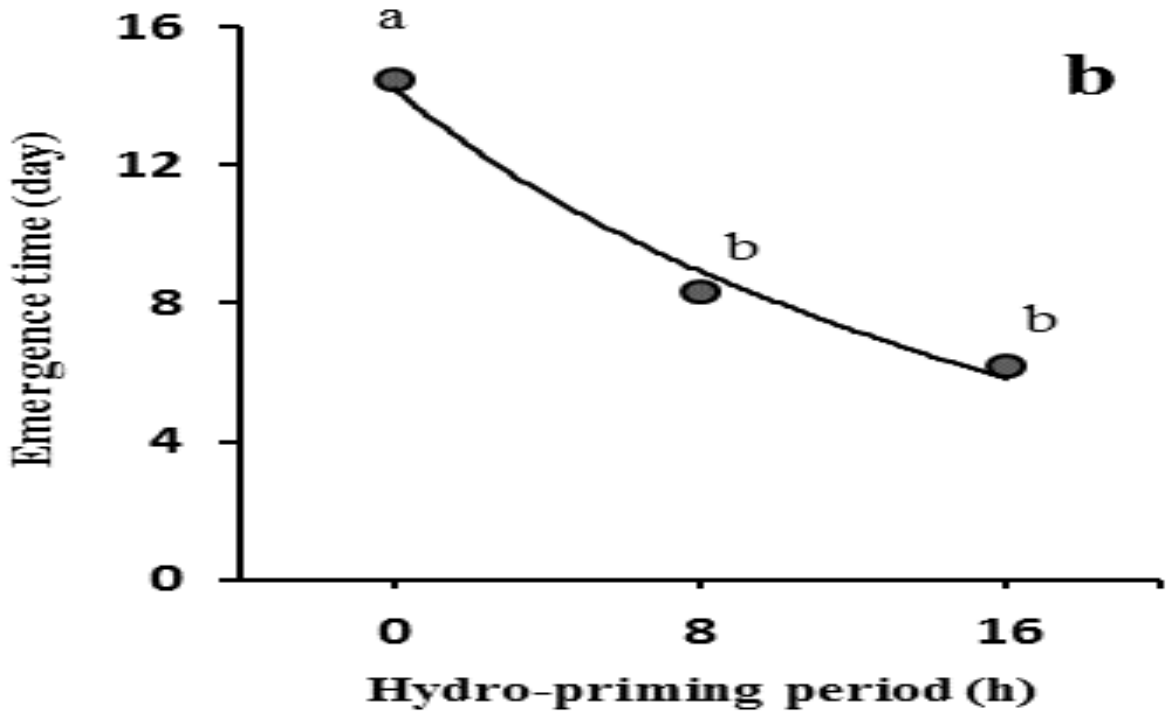

Figure 1: Field emergence percentage (a) and time (b) of milk thistle in response to hydro-priming duration. Different letters indicate significant difference at P≤0.05 (Golezani et al., 2016).

Biopriming

Biopriming is another vital tool in seed invigoration. This is performed by soaking seeds in the biological extracts from sea weeds and other plant products that are equally effective for seed treatment and seed enhancement. It is a process of biological seed treatment that refers to combination of seed hydration (physiological aspect of disease control) and inoculation (biological aspect of disease control) of seed with beneficial organism to protect seed with the help of beneficial fungi and bacteria. It is an ecological approach and an effective alternative to chemical control unique from chemical seed treatments by their utilization of living microorganisms. Different steps of biological seed treatment with antagonist have been presented in the Figure 2.

Importance of Biopriming

- One of the best alternate to chemical method.
- Uses various Bio-control agents.
- Provides protection to seed forming coat of antagonists

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- Safe for environment and human health

Fungal Antagonist for biopriming

- *Trichoderma viridae*
- *Trichoderma harzianum*
- *Trichoderma hamatum*
- *Gliocladium roseum*
- *Chaetomium bostrycoides*

Bacterial Antagonist for biopriming

- *Pseudomonas fluroscence*
- *Pseudomonas aureofaciens*
- *Pseudomonas aeruginosa*
- *Serratia polymuthica*
- *Pseudomonas chlororaphis*
- *Bacillus subtilis*

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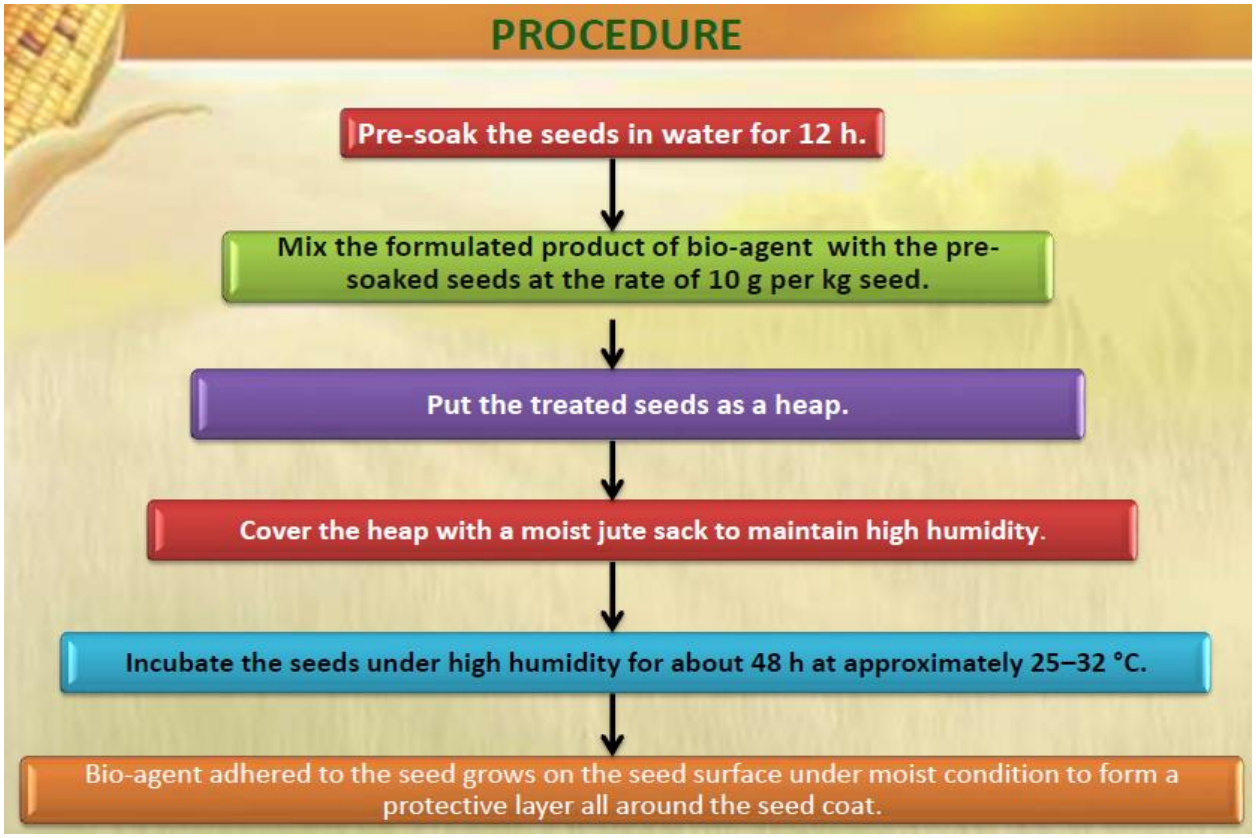

Figure 2: Procedure of biological seed treatment with antagonist

Influence of biopriming on seed germination and seedling growth behavior of the Brinjal and Tomato was illustrated with treatment of seeds with the extract of seaweeds *Gracillaria corticata* J. agri., *Kappaphycus alverazii* and mixture of both as biopriming agents. Fifty seeds each of brinjal and tomato was soaked with extracts for 48 hours at room temperature and dried to original weight at desired moisture content. It was observed that seed biopriming with 4% concentration of both extract mixture as bio-agent may be economical to use for enhancing germination parameters compared to other bio-agents. Thus this technique of biopriming is proved to be more economical, very cheap and easily applied by farmers and nursery workers than plant growth regulators and hormones. Effects of different bio-priming agents are distinctly visible in the germination percentage and mean germination time of tomato and brinjal (Patel et al., 2017).

Thermal Hardening

In hardening, seeds are exposed to alternate wetting and drying in distilled or tap water and such hydration-dehydration cycle may be repeated twice, thrice. Most recently a new technique for

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rice seed invigoration has been developed in which both seed hardening and osmoconditioning were successfully integrated. Hardening carried out in various salt solutions instead of tap or distilled water. It was concluded that osmohardening in CaCl2 solution (having an osmotic potential of –1.5 MPa) was best for vigor enhancement compared with other salts and simple hardening. It was then thought that alternate low and high temperature treatments may be effective for vigor enhancement in both types of rice (FAROOQ et al., 2005).

Thermal hardening of the rice seeds (both *indica* and *japonica*) was performed at different heating and chilling patterns showed significant role on germination and seedling vigor. The rice seed was exposed to five different patterns of heating-chilling as explained by (FAROOQ et al., 2005) in their study. The vigor enhancement as a result of alternating low and high temperatures may also be due to the hardening process, as observed in alternate wetting and drying. However, Heating followed by chilling followed by heating in *indica* and Chilling followed by heating followed by chilling in *japonica* rice were proven the best treatments for vigor enhancement (FAROOQ et al., 2005).

Hormonal Priming

Hormonal priming is soaking of seed in hormone solution is referred as hormonal priming. GA3, Salicylic acid, Ascorbic acid, Cytokinins etc can be used for this purpose (Kumari et al., 2017). The effect of hormonal priming with ABA, salicylic acid, or ascorbic acid on wheat germination and seedling growth under normal and saline conditions was evaluated (Afzal et al., 2006). The Gibberellic Acid and Salicylic Acid effects on seed germination and seedlings growth of wheat (Triticum aestivum L.) under salt stress conditions have been understood (Kumari et al., 2017). Priming with gibberellic acid (GA), salicylic acid (SA) and ascorbic acid (ASC) increased germination characteristics of aged seeds (Kumari et al., 2017). A hormonal priming treatment of Lentil with GA and kinetin at five different concentration of 0, 50, 100, 150 and 200 ppm were performed. The study revealed that seed priming with higher doses of GA and cytokinin may improve germination and vigorous performance of lentil. When lentil seeds treated with150-200ppm hormonal solution concentrations, FGP improved 43% and 52% respectively.

In another study conducted in maize seed treatment with different hormonal agents showed significant effect on seed germination and seed vigour parameters. Priming with GA3 (100ppm) increased the germination (%) and seed vigour in Maize. Soaking of seed with GA3 solution is advantageous to obtain healthy seedlings. Similarly, hormonal priming with Salicylic acid and halopriming with CaCl2 is alternative way to achieve good germination and seed vigor in crops (Kumari et al., 2017).

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Halopriming and Osmopriming

Halopriming is a pre-sowing soaking of seeds in inorganic salt solutions, which enhances germination and seedling emergence uniformly. NaCl, KCl, $KN0_3$, and $CaCl_2$ is used for this purpose. Osmopriming is the most widely used type of seed priming in which seeds are soaked in aerated low water potential solution. Priming of seeds in osmoticums has been reported to be an economical, simple and a safe technique for seedling establishment and crop production under stressed conditions (Guzmán and Olave, 2006). Among the various halopriming agents used for seed invigoration, $CaCl_2$ is found highly appropriate for seed treatment with enhanced germination and vigor characteristics.

Solid Matrix Priming and Chemo-priming

Solid matrix priming (SMP) is a technique developed by John Eastin (1990). In SMP, a solid matrix is used to regulate water imbibition by seeds. SMP alone or in combination with fungicides or biological agents has improved the rate and uniformity of emergence of vegetable seeds and reduced damping-off diseases. Combinations of seed priming with either fungicide or biological treatments to reduce damping-off disease in field-planted okra seed were evaluated. The term chemo-priming is used to indicate that fungicide-treated seeds rather than untreated seeds were used in the priming process. In bio-priming, untreated seeds were primed and 1 g of biological formulation was applied to the seeds using 1% carboxymethylcellulose (CMC) (Conway et al., 2001).

The efficacy of solid matrix priming techniques, alone or in combination with fungicide seed treatment on seedling emergence and reduction of damping-off of okra in field soil naturally infested with *Pythium ultimum* was performed. The following treatments were evaluated: thiram + carboxin (chemo-primed) (commercially applied), biological seed treatment (bio-primed) (*Trichoderma harzianum* isolate OK-110, 1 g suspended in 1% carboxymethylcellulose, untreated seed (control), and a 1% CMC control. Chemo-primed seeds had a more uniform and faster emergence compared with untreated seeds. (Conway et al., 2001).

SPM is a priming method wherein seeds are moistened for a given time at fixed temperature in an organic or inorganic solid matrix carrier to which water has been added (Harman and Taylor, 1988)). The study on shunken-2 sweet corn with disinfection and solid matrix priming suggested that the combination of SMP and disinfection with Sodium Hypochloride (SH) can be viable approach for seed treatment to fungicides to improve uniformity and stand establishment (Parera and Cantliffe, 1992).

Seed Coating

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Application of coating substance to the seed to enhance seed placement and performance without altering shape or placing chemicals on the seed coat which regulate and improve germination (Copeland and McDonald, 2012).

Advantage

- Enables accurate and even dose of chemicals and reduces chemical wastage.
- Improve the appearance and dust free handling.
- To apply fungicides, insecticides, micronutrients directly to seed.
- Allow easy flow of seed in automatic seeding.
- Act as a temperature switch and water intake regulator.

Disadvantage

- Coated seeds fetch high cost, than the bare seeds
- Improper coating and improper dilution of coating material may deteriorate the whole seed lot.

Types of seed coating

Seed Coatings

- It is the coating applied to the seed that does not obscure its shape. It may be fungicide, microbiological treatments and micronutrients
- Its major benefit is that the seed enhancement material is directly placed on the seed as compared to the broad casting.

Film Coatings

It's a sophisticated process of applying precise amount of active ingredients in form of thin film along with the liquid material directly on to the seed surface without obscuring its shape

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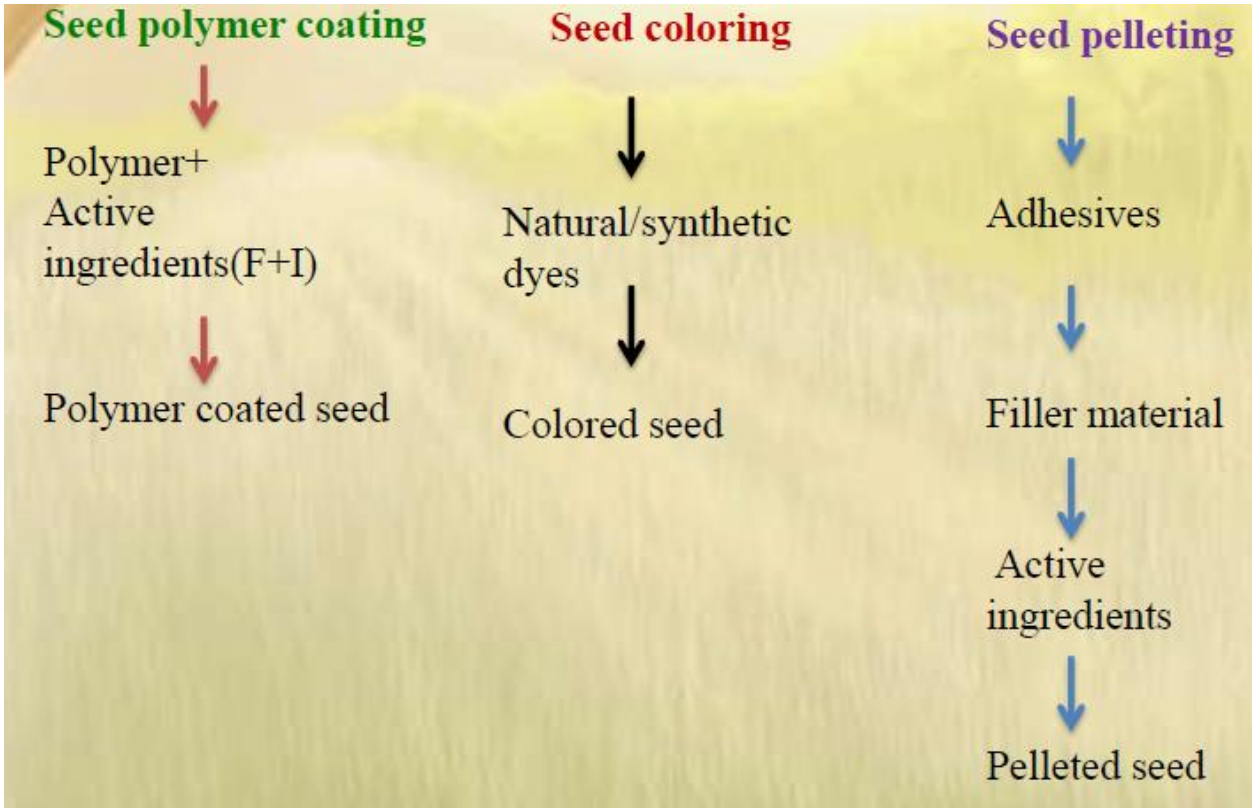

Figure 3: Steps in seed coating (Copeland and McDonald, 2012)

Seed Pelleting

It is the process of enclosing a seed with a small quantity of inert material just large enough to facilitate precision planting. Also, it is the mechanism of applying needed materials is such a way that they affect the seed or soil at the seed soil interference. Inert material creates natural water holding media and provide small amount of nutrients to younger seedlings (Halmer, 2006).

Seed in rotating drum is wetted, and blends of powered materials (e.g. chalk, clays, perlite, lime, peat, talc) plus water-attracting or hydrophobic materials are progressively added, along with more water, until desired pellet wt. or size increase is achieve. Wet-coated seed then dried with heated air, usually in separate equipment (Halmer, 2006).

Advantage of Pelleting

- Singling of seed by prevention of clogging
- Precision planting with increased seed size
- Attraction of moisture.

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- Supply of growth regulators, nutrients.
- Stimulation of germination.
- Influence of micro-environment.
- Saving of chemicals/fertilizers applied to soil.
- Supply of oxygen.
- Reduces seed rate.
- Uniform field establishment.
- Increase yield.
- Remedy for sowing at problematic soils.
- Protection from birds, animals and insects.

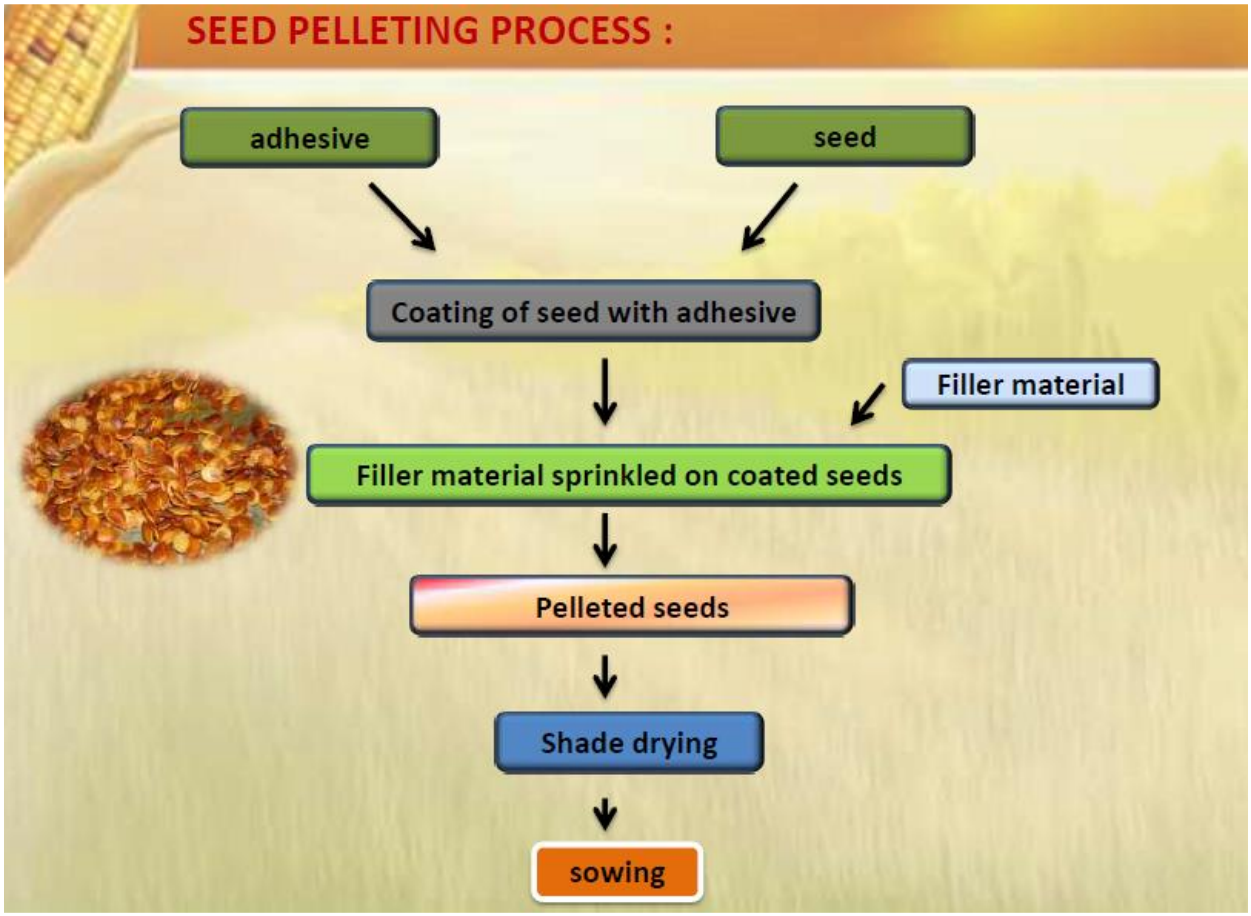

Figure 4: Seed pelleting process in vegetables (Halmer, 2006)

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Magneto-Priming

The impacts of magnetic field (MFs) on many biological processes in plants, such as growth, development, and metabolism have been well understood. Both static magnetic field (SMF) and electromagnetic field (EMF) are used in agriculture for seed priming (the so called 'magneto-priming') with proven beneficial effects on seed germination, vigor and crop yield. Beneficial effects of pre-sowing magnetic treatment on improving germination parameters and biomass accumulation has been described for several species. Magneto-primed seeds showed improved germination rate, vigor and seedling biomass. They also provide tolerance to biotic and abiotic stresses as a result of antioxidant response activation. Increased antioxidant enzyme activities of superoxide dismutase, catalase and glutathione reductase were explained in magneto-primed cucumber. Additionally, MFs treatments have been potentially used for minizing drought or disease induced adverse effects on crop productivity (Araújo et al., 2016).

Priming Seeds with Ultraviolet Radiation

Solar UV radiation classified into UV-C (200-280 nm), UV-**B** (280-320 nm), UV-A (320-400 nm). Numerous studies focused to investigate on global impact of UV exposure on plants from ecosystem to whole plants. However, very little work emphasized on UV impact in seed biology and germination. UV-C radiation is non-ionizing and it penetrates superficially into the plant tissues, which supports its potential as a germicidal agent. Seed treatments with low doses of UV- C (3.6kJm□2) were used to elicit host resistance to black rot in cabbage (*Brassica oleracea* L.). This UV-C seed treatment also improved the quality and growth response of cabbages under greenhouse conditions. In another study, investigated the impact of UV-C pre-sowing treatments in lettuce (*Lactuca sativa* L. 'Romaine'). Lettuce seeds were UV-C-treated by exposure to 0.82 and 3.42kJ m^2 doses and resulting seedlings were challenged with salt stress. The results showed that UV-C treated seedlings were able to mitigate the impact of excessive salinity, possibly as result of the enhanced free radical scavenging activity detected in their leaf tissues (Araújo et al., 2016).

Harmful effects of UV-B on plant physiology are DNA damage, protein and membrane injury that affecting photosynthesis and plant growth. Effects of UV-B irradiation on seed germination, seedling growth, and plant development were investigated on mash bean. Although germination rate and final germination percentage not affected by UV-B. Some deleterious effects were evident reduction in root and shoot growth. UV-B enhanced total soluble phenols (Araújo et al., 2016). UV radiation for seed invigoration is proved unfavorable. UV-A is less hazardous component of UV radiation. Although information on possible use of UV-A radiation as seed invigoration treatment is very limited. Pre-sowing UV-A treatment stimulated germination rate

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and seedling performance in mung bean. Positive effects of UV-A and UV-C radiation have been highlighted on germination seedling vigor as well as seed health (Araújo et al., 2016).

SUMMARY

Seed invigoration treatments have been developed to improve seed performance during germination and seedling early growth. Invigoration is method widely used to improve the physiological quality of seeds, especially seed vigor, through physical or chemical treatment. Seed invigoration is physiological and biochemical changes related to synchronous germination, velocity, and increased seed germination. Priming is an invigoration technique that enhances the physiological and biochemical events in seeds during suspension of germination by low osmotic potential and negligible matric potential of the imbibing medium. Various seed priming techniques have been developed, including hydro-priming (soaking in water), halo-priming (soaking in inorganic salt solutions e.g. NaCl, $CaCl_2$, KNO_3), osmo-priming (soaking in solutions of different organic osmotica), thermo-priming (treatment of seeds with low or high temperatures), solid matrix priming (treatment of seed with solid matrices placing seeds between saturated jute mat layers), hardening (alternate soaking of seeds in tap water and drying before sowing) and bio-priming (hydration with biological compounds), seed coating as well as seed pelleting are found most promising. One of the most investigated physical pre-sowing seed treatments in agriculture is based on the use of MFs which have been described as eco- friendly, cheap, and non-invasive technique. Various seed invigoration techniques that have been developed and in practice have been reviewed on this paper. Each of these priming methods has its merits over the other methods. Recently most innovative methods are being enforced for the seed priming to enhance the seed germination and increased vigor and synchronization of plant development.

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