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Physiological, Biochemical and Molecular Mechanisms of Seed Priming: A Review

A. Diya, R. Beena, V.G. Jayalekshmy 10.18805/LR-4638

ABSTRACT

Legumes being a major source of protein have a wide range of economic importance. But the major constraints in growing legumes are poor germination, seedling emergence, non uniform stand establishment and crop mortality leading to lower pulse productivity. Thus, pre-sowing seed treatments are critical parameters which attribute to efficient plant growth and high yield. Uniform seed germination, seedling growth and uniform establishment are the critical stages during the growth of crops. Seed priming is a very promising presowing treatment employed to improve seed germination, better crop establishment, enhance the seed quality and induces tolerance against biotic and abiotic stresses in plants thereby providing a balanced healthy diet to the citizens. Priming is a cost effective and feasible strategy involving controlled hydration of seeds followed by drying to stimulate the pre-germinative metabolic activities to occur promoting rapid germination of seedlings, break dormancy and enhance crop yield. Enzymatic, metabolic and biochemical processes of the primed seeds attributes to rapid and uniform seedling emergence. Seed priming methods comprises of conventional methods including hydro priming, osmopriming, biopriming, chemical priming and the advanced methods including nano priming and priming with physical agents. In this review paper, the underlying physiological, biochemical and molecular aspects of priming in pulses were discussed.

Key words: Biochemistry, Legume, Molecular mechanims, Physiology, Seed priming.

Department of Seed Science and Technology, College of Agriculture, Vellayani-695 522, Kerala, India.

Corresponding Author: R. Beena, Department of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University, Thiruvananthapuram-695 522, Kerala, India.
Email: beenaajithkumar@gmail.com

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Legumes are a major source of protein. They are mainly grown for human consumption, forage and silage for livestock and also as green manures. Seed is a primary input for legume production. It requires water, nutrients, oxygen, suitable climatic conditions for initiating germination process. Water uptake by the fertilized embryo encompasses 3 phases including initial rapid uptake of water termed as imbibition followed by a lag phase and an increased water uptake leading to crop growth (Bewley and Black, 1994; Bradford, 1995). Seeds become tolerant to desiccation during phase 1 and 2, whereas seeds become intolerant to desiccation during phase 3 (Taylor *et al*., 1998).

Employment of poor quality seeds results in low productivity and low crop yield. Seed enhancement refers to the post harvest seed treatment aiming at improved germination, seedling growth and facilitates seed delivery during sowing. Various seed enhancement techniques aims at improving the performance of seeds owing to better yield. It comprises of hydration of seeds, seed coating and seed conditioning technologies. Presowing hydration treatments comprises of uncontrolled and controlled water uptake systems. Controlled water uptake consists of controlled hydration with water, priming with solution, solid particulate matter. During priming, the water uptake is regulated, thus preventing the completion of phase 3 (Taylor *et al*., 1998). Thus priming is a commonly adopted pre sowing seed treatment which induce uniform seed germination and result in good stand establishment owing to improved crop yield. Effective germination will improve seedling growth owing to higher quality and yield even under unfavourable conditions (Nakaune *et al*., 2012; Manikanta *et al*., 2020).

Seed priming improves germination, seed vigour, root and shoot length, biomass production and produce tolerance against biotic and abiotic stresses.It also improve various biochemical processes including synthesis of various antioxidant enzymes, soluble sugar, regulates hormonal functions and thus improve plant growth. Priming plays an important role in enhancing crop growth under various abiotic stresses. The seedlings from primed seeds exhibit enhanced activities of catalase (CAT), peroxidase (POD), glutathione reductase (GR). They also regulate proteins like aquaporins, tonoplastic intrinsic proteins, dehydrins, lateembryogenic proteins. Priming also initiate various metabolic processes including protein synthesis, DNA repair (Bray *et al*., 1989; Jisha *et al*., 2013; Paparella *et al*., 2015).

Seed priming

Seed priming is the controlled hydration which allows the seeds to imbibe water but prevent radicle protrusion

(Heydecker and Gibbins, 1978). Germination process can be completed in three phases in case of both primed and unprimed seeds. In primed seeds, during phase I water enters in to the seed by imbibition. Phase II is hydration which allows controlled imbibition and induction of the pre-germinative metabolic activities and prevention of radicle emergence. Germination and post germination events occur during phase III (Rajjuo *et al.,* 2012).

In plant defense, priming is deûned as a physiological process by which a plant prepares to respond to imminent abiotic stress more quickly or aggressively. Moreover, plants raised from primed seeds show sturdy and quick cellular defense response against abiotic stresses. The various methods of priming include hydropriming, osmopriming, hormonal priming, biopriming *etc.* (Jisha *et al.,* 2013). Miladinov *et al*., 2020 reported that seed primimg can mitigate salinity stress in soybean,

Methods of seed priming

The various methods of seed priming comprises of conventional methods and advanced methods. The conventional method comprises of hydropriming, osmopriming, solid matrix priming, biopriming and the advance techniques include priming with nanoparticles and physical agents.

Conventional methods of seed priming

Hydropriming

Hydropriming is a feasible and economic technology involving soaking of seeds in water for a specific period of time and then drying back to the original moisture content before sowing. This enhances the seed hydration as well as water uptake efficiency of seeds during harsh environmental conditions like drought and heat stress (Maurya *et al*., 2020). Hydropriming resulted in earlier and uniform seed germination, improved vigour index I and vigour index II (Shukla *et al*., 2018). Water soaking improve the yield attributing characters like number of primary branches per plant, number of pods per plant, number of seeds per pod, test weight, seed yield, nitrogen content and seed protein content (Das and Jana, 2016).

Osmopriming

Osmopriming is the priming of seeds in aerated solutions like polyethylene glycol (PEG), NaCl, KNO_3, followed by rinsing in distilled water and shade drying (Venkatasubramanian and Umarani, 2007). It is a commercial technology where seeds are subjected to a definite level of imbibition as uncontrolled water uptake may lead to the accumulation of reactive oxygen species as well as oxidative damage to nucleic acid, proteins and lipid membranes (Maurya *et al*., 2020). The various agents utilized for osmopriming are PEG, sodium chloride, sodium nitrate, magnesium chloride, potassium nitrate *etc*.

Halopriming

Venkatasubramanian and Umarani (2007) suggested that halopriming is the soaking of seeds in solution of inorganic salts like NaCl, KNO_3 followed by rinsing in running water and shade drying. Seed performance of various crops can be improved by inclusion of plant growth hormones like salicylic acid, ascorbate, kinetin, *etc*. (Nawaz *et al*., 2013).

Biopriming

Biopriming is the inoculation of seeds with beneficial microorganism. Biopriming with a combination of microbes enhanced seed germination percentage, speed of germination, shootlength, root length, seedling fresh and dry weight, vigour index I and II (Vishwas *et al*., 2017). Bioprimimg contribute to longer-term plant health or plant growth promotion (Bennett and Whipps, 2008). Rhizobacteria are used as inoculants to enhance crop yield and for biological control of fungal pathogens. Certain strains of rhizosphere bacteria stimulate plant growth and are, therefore, called plant growth-promoting rhizobacteria (PGPR) (Tonelli *et al*., 2011). Biopriming was also found to alleviate the detrimental effects of drought stress and improved yield and yield components, nutrient uptake and regulated the activities of antioxidants (Nawaz *et al.,* 2020).

Hormonal priming

Hormonal priming is the pre-sowing seed treatment with the employment of various hormones like. GA_3, salicylic acid (SA), ascorbate, kinetin, *etc*. which promote better growth and seedling development (Sharma and Parikh, 2020). Rouhi and Sepehri (2020) reported that hormonal priming with GA_3 mitigated the negative effects of aging and drought stress.

Chemical priming

Chemical priming is the soaking of seeds in various types of chemicals which may be natural or synthetic in origin like choline, chitosan, ethanol, zinc sulphate, copper sulphate, potassium di hydrate phosphate, selenium which enhances crop growth and induce resistance to various abiotic stresses (Maurya *et al*., 2020). Primimg using boron enhanced the antioxidant activity in alfalfa seeds (Xia *et al*., 2020).

Nutrient priming

Nutrient priming is the soaking of seeds in a definite concentration of macro or micro nutrients for a specific time period before sowing of seeds (Shivay *et al*., 2016). Seed priming with micronutrient regulate water uptake by increasing osmosis during seed germination (Maurya *et al*., 2020). Nutrient priming with zinc resulted in highest germination parameters like germination percentage, mean daily germination, mean germination time, co-efficient of rate of germination, germination index, speed of germination, time to reach 50 per cent germination, vigour index, highest zinc and boron content (Raj *et al*., 2020) maximum pods per plant, higher grain yield and biological yield (Arif *et al*., 2007).

Physiological, biochemical and molecular aspects of priming in legumes

During priming, phase I include the activation of priming memory, repairing DNA and mitochondria, respiration and energy metabolism, ROS signalling and antioxidant defense system, cell cycle initiation and induction of stress response genes such as dehydrin (DHY), aquaporin (AQP) and hormone signalling. Phase II is the germination phase in which priming memory is recruited upon second rehydration and proteins are synthesised by using new mRNA. Phase III is the post- germination phase in which mobilization of stored reserve, elongation of radicle cell and finally radicle emerges out by rupturing the seed coat (Chen and Arora, 2013).

Activation of priming memory

Primed seeds can retain the memory of previous stress and enable protection against oxidative stress through earlier activation of the cellular defense mechanism, reduced imbibition time, upsurge of germination promoters and osmotic regulation (Marthandan *et al*., 2020).

Pre-germinative metabolism

At the physiological level, seed priming trigger the pre-germinative metabolism with the beginning of imbibition phase. During the onset of water uptake, various metabolic changes occur in the seed. Increased biosynthesis of protein, DNA and nucleotide were reported at the end of priming (Bray *et al*., 1989). During the rehydration process of seed priming, several biochemical activities like *denovo* synthesis of protein and nucleic acid, adenosine triphosphate production, accumulation of phospholipids and sterols, activation of antioxidants and also DNA repair mechanisms are promoted (Paparella *et al.,* 2015). Even though plants have evolved mechanisms to tolerate adverse conditions including temperature extremities, some seeds become vulnerable to these stresses during their initial developmental stages leading to lipid peroxidation, oxidative damage of protein and DNA (Kranner *et al*., 2010). Oxidative damage of DNA leads to necrotic cell death. So, it is necessary to repair the DNA which allows embryonic cells for proper DNA replication and cell development (Kranner *et al.,*2010; Waterworth *et al*., 2011).

α and β tubulin subunits of protein which are involved in the maintenance of cellular cytoskeleton and cell division are regulated during priming (Varier *et al.,* 2010). Black gram seeds primed with *Spirulina platentis* improved the structure of seeds owing to higher α amylase synthesis and also increased the activity of dehydrogenase and maintained it even during accelerated ageing (Thinh, 2021). Seed priming triggers the pre-germinative metabolism and seed repair response by the activation of DNA repair and antioxidant ezyme mechanism thus ensure proper seedling emergence and growth (Paparella *et al*., 2015).

ROS has a key role in regulating seed germination (Paparella *et al*., 2015). ROS produced during seed imbibition is related to enhanced seed vigour under extreme environ.mental conditions (Liu *et al*., 2007). The antioxidant system in seed can be understood by monitoring the expression profiles of genes encoding SOD (Paparella *et al*., 2015). Thus seed priming activates various physiological and biochemical processes during the pre-germinative phase owing to better seed germination and uniform seedling establishment.

Improved water uptake

Improvement of germination in primed seeds could be linked to mechanical and physical effects of pre-treatment. Primed seeds exhibit a faster imbibition in comparison with non-primed ones. Improvement of germination by priming is directly related to the modification of seed water relations. Primed seeds of *Trifolium repens* exhibited seed coat tears and circular depressions that favour seed imbibition. X-ray photographs also showed tissue detachment leading to free space between the cotyledons and radicle, which was never observed for control unprimed seeds. The voids created inside the seed as a result of priming make water flow easier, thus contributing to tissue hydration (Galhaut *et al*., 2014). Priming induced modification in aquaporin gene expression, which is involved in the faster imbibition of primed seeds (Chen and Arora, 2013). Osmo-priming improved plant water relations exhibited by maximum relative water content, water potential, osmotic potential under full turgor pressure in chickpea (Farooq *et al*., 2017).

DNA repair

DNA damage distorts the genomic integrity and may even cause death of the organism. Plant DNA is always prone to endogenous and environmental adversities and effective detection and repair of DNA damage are essential to ensure the stability of the genome. DNA repair mechanisms mainly including Base- and Nucleotide-Excision Repair (BER and NER) together with antioxidants are regarded as key pre-germinative metabolic processes (Wojtyla *et al.,* 2016). In primed seeds the repair of damaged DNA occur prior to replication (Varier *et al*., 2010).

The DNA repair is stimulated during rehydration of seed in order to overcome the growth inhibition during seedling growth. The exposure of seeds to various unfavourable conditions during maturation and storage owes to oxidation of DNA, loss of DNA integrity and finally trouble the cell cycle events (Bray and West, 2005). So, it is very essential to repair the DNA in order to facilitate proper germination and seed vigour. The pregerminative metabolic processes comprises of various antioxidant system along with Base Exersion Repair (BER) and Nucleotide Exertion Repair (NER) (Huang *et al*., 2007; Wojtyla *et al*., 2016). In barrel clover seeds, Tyrosyl-DNA Phosphodiesterase (MtTdp1α, MtTdp1β) genes which encode α- and β- tyrosyl-DNA phosphodiesterase and the DNA repair genes encoding topisomerase (MtTop1α and MtTop1β) were found to be upregulated during imbibition (Macovei *et al*., 2010; Wojtyla *et al.,* 2016). Tyrosyl-DNA phosphodiesterase 1 (TDP1) removes the stalled topoisomerase I-DNA by cleaving a 32 -phosphotyrosyl bond (Forti *et al.,* 2020).

The increased DNA damage during seed imbibition is marked by enhanced levels of 7,8-dihydro-8-oxoguanine (8-oxodG), as reported in *Medicago truncatula*.

(Balestrazzi *et al*., 2011). The PEG priming of *M. truncatula* seeds also showed the involvement of AtTFIIS and MtTFIIS in DNA repair mechanisms during seed imbibition (Macovei *et al.,* 2010; Wojtyla *et al.,* 2016).

Energy and carbohydrate metabolism

Seed priming is also found to improve the energy metabolism in plants. Seed germination involve degradation of storage substances like starch and generation of small molecular weight substances like sucrose and glucose. The starch metabolism during germination and seedling growth clearly depicts the seed vigour under stress condition. The ability of plants to convert starch in to soluble sugars shows their survival capacity under wide range of environmental conditions (Hussain *et al*., 2016; Stephen *et al*., 2021). Amylase is involved in the break down of starch in to transportable form of sugar (Pravallika *et al*., 2020). Priming helped to maintain the seed metabolism and synthesise α-amylase by improving the seed structure (Thinh, 2021). Even though chilling stress reduced α- amylase activity, total and reducing sugar content, trehalose content and sucrose content in chickpea, osmopriming resulted in increased activity of α- amylase and sugar contents under optimal as well as chilling stress condition in chickpea (Farooq *et al*., 2017). Seed primimg influenced the early stages of growth of cowpea under saline conditions (Nabi *et al*., 2020).

Better imbibition, seed vigour and plant growth

Water uptake by a dry seed is triphasic. Imbibition (Phase 1) is marked by the rapid uptake of water due to the lower water potential of the seed than the surroundings.This phase is similar in both primed and non-primed seeds. Phase II is the activation phase where pre-germinative metabolic activities including synthesis of starch, reorganization of cell membrane, degradation of food reserves occur to induce radicle protrusion. In Phase III (radicle protrusion phase) root and seedling growth occur (Ruttanaruangboworn *et al*., 2017). A change in the nutrient homeostasis, particularly calcium and nitrogen were noted in primed seeds. The primed seeds boost imbibition compared to unprimed seeds (Galhaut *et al*., 2014)

Priming improved germination percentage, (Thinh, 2021), speed of germination, shoot length, root length, dry matter production, maximum vigour index I and II (Gunasekar *et al*., 2017; Thinh, 2021). A similar result was found by seed priming with gibberellic acid in cowpea (Arun *et al*., 2016).The reproductive parameters like number of primary branches, number of pods per plant, number of seeds per pod, grain and biological yield also increasedas a result of priming (Gupta and Singh 2012; Das and Jana 2016). Seed priming alleviated chilling induced growth inhibition in chickpea marked by increased seedling dry weight, specific leaf area, coefficient of uniformity of emergence (CUE), emergence index (EI)(Farooq *et al.,* 2017). Zinc seed priming improved various growth parameters like early germination seedling establishment, improved shoot and root length, number of secondary roots per plant. seedling dry weight in chickpea (Ullah *et al*., 2019). Priming also enhanced chlorophyll content, total soluble protein, proline, flavanoid and phenolic content (Khan *et al*., 2019).

Reactive oxygen species

The reactive oxygen species like OH^-, O^{2-}, $H_2O_2^-$ are generated during seed germination, dessication and ageing leading to seed deterioration. They are involved in growth processes during seed development and involve in radicle protrusion during seed germination. The regulate gene expression during seed development, dormancy and germination. But excessive accumulation of ROS lead to oxidative stress, lipid peroxidation, cell injury, nuclear and organelle DNA damage, enzyme inactivation and disturbance in seed development and germination (Bailly, 2004). Priming with $CaCl_2$ reduced the generation of reactive oxygen species and improved photosynthesis in faba beans (Nouairi *et al*., 2019). Seed priming balances the production of ROS and thus moderate the chilling injury by alternate respiratory pathway with the level of antioxidants and active oxygen scavenging system (Pal *et al*., 2013).

Antioxidant defense system

Antioxidant system comprises of enzymatic (APX, CAT, SOD) and non enzymatic (GSH, AsA) systems. Each antioxidant exhibit a specific function, catalase coverts hydrogenperoxide into hydrogen and water, ascorbate peroxidase induce H_2O_2 catalysis. Ascorbic acid being an electron donor stimulate the degradation of H_2O_2 by APX. Glutathione and its enzymes involve in ascorbic acid regeneration (Hussain *et al*., 2019).

Glutathione is involved in many cellular processes under stress and is a substrate for glutathione S-transferase and glutathione peroxidase (Foyer and Noctor, 2005). Higher CAT, SOD and APX content in osmoprimed chickpea seedlings protect the plants against oxidative damage under chilling stress (Farooq *et al.,* 2017). The SOD plays an important role in catalyzing the dismutation of superoxide, while CAT and POD contribute in scavenging of H_2O_2 (Anjum *et al.,* 2015). Large amount of reactive oxygen species are produced during imbibition, ROS is responsible for seed vigour (Liu *et al*., 2007). Hydropriming for 6h significantly increase the activity of superoxide dismutase and catalase (Shukla *et al*., 2018).

Membrane integrity

Seed priming improves the cell membrane integrity by counteracting lipid peroxidation. The reduced leakage of electrolytes shows that seed priming may have contributed to limited oxidation of phospholipids, which are the active compounds of membranes, which indicate that during the hydration process cell membranes reorganize themselves to attain the original structure, disturbed while drying, or because of the rapid entering of water into cells during the initial period of seed soaking (Badek *et al.,* 2016). Hydropriming is reported to have improved the membrane integrity by reducing the electrolyte leakage in mung bean (Shukla *et al*., 2018).

A negative correlation exist between lipid peroxidation and crop growth. Lipid peroxidation is directly related to seed deterioration. Priming reduced lipid peroxidation in black gram (Thinh, 2021). Excessive generation of reactive oxygen species lead to oxidation and depolymerisation of nucleic acid, peptide bond disruption, oxidation of carbonyl and thiol groups, oxidation of polysaccharides and poly unsaturated fatty acids, lipid peroxidation in both cellular and organic membrane, thus adversely affect normal membrane functioning and cause oxidative stress, malfunctioning of cell, cell death and ultimately seed death (Kranner *et al.,* 2010). Priming was found to be effective in improving moisture stress tolerance by circumventing MDA content (Farooq *et al*., 2020) and reducing lipid peroxidation.

Cell cycle regulation

In primed seeds, cell cycle events occur earlier upon imbibition in water compared to unprimed seeds. The cell cycle is arrested at the G2 phase during priming allowing the synchronization of cells. The higher or lower cell cycle activity can be linked to higher or lower ratio of 4C DNA (G2 phase) and 2C DNA (G0/G1 phase) (Chen and Arora, 2013). Thus of the cell priming induces better germination performance through the activation of cell cycle.

Protein metabolism

Seed germination is an important process in the life cycle of a plant. During imbibition, the embryonic cells transforms from an inactive quiescent stage to a highly active metabolic stage. (Gallardo *et al*., 2001). Germination begins with the imbibition by a dry seed and terminates by the radicle protrusion (Bewley and Black, 1994). The proteins related to metabolic processes, protein synthesis, signalling and stress are identified during phase II of imbibition. Thus, phase II of seed imbibition is critical during seed priming (Cheng *et al*., 2016).

The enzymes involved in mobilization of storage proteins are either synthesized or activated during seed priming. α and β tubulin subunits, which are involved in the maintenance of the cellular cytoskeleton and are constituents of microtubules involved in cell division, are abundant during priming. Hydropriming improved seed protein content in lentil (Das and Jana, 2016). Black gram seeds primed with *Spirulina platensis* reported high protein content before and after ageing (Thinh, 2021). Accumulation of β-tubulins during priming has been observed in many species in relation with reactivation of cell cycle activity (Varier *et al.,* 2010). Proteins synthesised during priming are essential for radicle protrusion *eg*: plasma membrane intrinsic protein, tonoplast intrinsic protein which allow cell to cell water transport, expansion, organ development, germination (Chen *et al.,* 2012). A hydropriming specific protein catalase isoform accumulated during radicle emergence (Varier *et al*., 2010). Catalase is required to reduce the damage caused by ROS accumulated as a result of hydropriming induced oxidative stress. The abundance of low molecular weight heat shock proteins (LMW HSPs) increased in osmoprimed seeds. This confirms that water stress generated by high osmotic potential induces specific changes in protein synthesis (Gallardo *et al.,* 2001). The enzyme L-isoaspartyl protein methyltransferase, increase in response to priming (Varier *et al*., 2010).

Hormonal regulation

Seed maturation followed by germination passes through two phases: 1) transition from embryo development to maturation, 2) switch from quiescent dry state to germination (Kermode, 1990). These transitions are regulated by the interaction between growth promoters (GA) and retardants (ABA) (van der Geest, 2002; Nambara *et al*., 2010). The seed environment retains the embryo in the developmental stage until they are fully developed and accumulated reserve materials and arrest germination. ABA prevents germination and encourages embryo to continue in the developmental phase (Kermode, 1990).

GA is crucial for seed germination. Endogeneous GA promotes germination by weakening the endosperm cells surrounding the radicle tip (Groot and Karssen,1987). Gibberellic acid being and important growth promoter is involved in seed germination. Black gram seeds primed with *Spirullina platensis* recorded the highest gibberellic acid content (Thinh, 2021). Bewley (1997) explained about the antagonistic role of ABA and GA in improving germination. Prevention of radicle exertion can be achieved by incubating with ABA, whereas GA overcome the ABA mediated inhibition and promote germination (Bewley, 1997). The increased level of GA just before radicle protrusion implicates the importance of *denovo* biosynthesis of GA for controlling radicle protrusion. GA reduces the level of ABA by affecting ABA biosynthesis. Exogenous application of GA increase ethylene and auxin synthesis. (Ogawa *et al*., 2003).

Aquaporins

Plant growth mainly depends on water absorption from the soil and its movement from the roots to other plant parts and finally to the atmosphere. The radial component of the symplastic route is regulated largely by a family of water channel proteins called aquaporins (Amodeo *et al*., 1999). Plants possess various families of aquaporins, which reside in the plasma membrane, the tonoplast, or in other cellular membranes (Tyerman *et al*., 1999). Aquaporins (control water transport between cells and mediates cell expansion and organ growth (Chrispeels and Agre, 1994; Chrispeels and Maurel, 1994; Maurel *et al*., 2002). The primed seeds show better water uptake than unprimed seeds (Galhaut *et al*., 2014). Aquaporins helps in cell expansion finally leading to better germination (Chen and Arora, 2013).

Dehydrins

Dehydrin, group 2 late embryogenic abundant proteins are important in adaptation of plants to abiotic stresses. They accumulate during seed maturation during salt stress, heat stress, dehydration or freezing. Dehydrins can be employed as molecular markers for plant abiotic stress tolerance. (Hanin *et al*., 2011). Exogeneous application of γ- aminobutyric acid resulted in the accumulation of dehydrins and DHN coding genes (*SK2, Y2K, Y2SK* and *dehydrin b*) under salt stress in white clover (Cheng *et al*., 2018).

Photosynthetic ability

Chlorophyll is the most important parameter to measure the photosynthetic ability of plants. Priming with $CaCl_2$ alleviated cadmium induced stress and improved photosynthetic parameters like internal CO_2 concentration, transpiration rate, stomatal conductance, intrinsic water use efficiency, chlorophyll a, b in faba beans (Nouairi *et al.,* 2019). Seed priming with $FeSO_4$ enhanced chlorophyll a and b in mung bean (Khan *et al*., 2019). Osmopriming improved leaf net carbondioxide assimilation rate and photochemical efficiency of PSII under suboptimal and chilling stress conditions in chickpea (Farooq *et al*., 2017). Osmopriming improved chlorophyll content under well watered and water deficit conditions in lentils (Farooq *et al*., 2020). Priming with β amino butyric acid increased chlorophyll a, b, total chlorophyll and carotenoid content of seedlings (Jisha and Puthur, 2015).

Gene expression

In Barrel clover various genes involved in DNA repair and antioxidant response genes were identified during hydropriming and biopriming. *APX, SOD, OGG1, FPG* and *MT*2 genes were upregulated in response to hydropriming and biopriming which indicated that primed seedlings are better equipped with antioxidant system and efficient DNA damage system (Forti *et al.,* 2020).

Priming with PEG delayed the upregulation of genes *MtTdp1α* and *MtTdp1β* and their transcript level peaked during highest DNA damage . The increased DNA damage during seed imbibition is marked by enhanced levels of 7,8-dihydro-8-oxoguanine (8-oxodG), as reported in *Medicago truncatula* (Balestrazzi *et al.,* 2011).

CONCLUSION

Seed priming is an innovative technology for achieving faster and uniform emergence, vigorous stand establishment and higher productivity in crops under normal and stressful conditions. Vigorous growth and improved stress tolerance in plants raised from primed seeds is due to more effective energy metabolism, enhanced activation of different enzymes, increased DNA synthesis, hormonal regulation and quick cellular defense responses. So species specific priming technologies can be adapted to overcome the challenges of the environmental extremities.The exact molecular mechanisms behind seed priming are not fully explored. Therefore, future goals of agricultural scientists are to identify novel genes, proteins and transcription factors, which are regulated due to seed priming under various stresses.

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