Pulsed magnetic field improves seed quality of aged green pea seeds by homeostasis of free radical content

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Abstract To elucidate the mechanism responsible for magnetic field induced seed invigoration in aged seeds an experiment was conducted on six year old garden pea seeds stored under controlled (20 °C and 40% RH) condition. Aged seeds were magnetoprimered by exposing to pulsed magnetic field (PMF) of 100 mT for 1 h in three pulsed modes. The 6 min on and off PMF showed significant improvement in germination (7.6%) and vigor (84.8%) over aged seeds. Superoxide and hydrogen peroxide production increased in germinating primed seeds by 27 and 52%, respectively, over aged seeds. Nicotinamide adenine dinucleotide (reduced) (NADH) peroxidase and superoxide dismutase involved in generation of hydrogen peroxide showed increased activity in PMF primed seeds. Increase in catalase, ascorbate peroxidase and glutathione reductase activity after 36 h of imbibition in primed seeds demonstrated its involvement in seed recovery during magnetoprimering. An increase in total antioxidants also helped in maintaining the level of free radicals for promoting germination of magnetoprimered seeds. A 44% increase in level of protein carbonyls after 36 h indicated involvement of protein oxidation for counteracting and/or utilizing the production of ROS and faster mobilization of reserve proteins. Higher production of free radicals in primed seeds did not cause lipid peroxidation as malondialdehyde

content was low. Lipoxygenase was involved in the germination associated events as the magnitude of activity was higher in primed aged seeds compared to aged seeds. Our study elucidated that PMF mediated improvement in seed quality of aged pea seeds was facilitated by fine tuning of free radicals by the antioxidant defense system and protein oxidation.

Keywords Antioxidant enzymes · Green peas · Lipoxygenase · Magnetoprimering · Pulsed magnetic field · Vigour

Introduction

Pea is an important grain legume cultivated in many regions of the world. It forms an important source of dietary protein (20–30%) for the vegetarian population and also has sufficient amount of carbohydrate, dietary fibre, vitamins and minerals. Fresh green pods are harvested for vegetable purpose and can be consumed as fresh, frozen or canned form. In India, production of green peas in 2013 was 4 million tons, harvested from 0.4 million hectares (FAOSTAT 2013). Post harvest seed quality can be affected due to poor storage i.e. during the seed production chain, if the indented seeds are not lifted by the seed agencies or due to over production in a particular year, the seeds have to be stored and carried over to the next season. Storage under ambient conditions can result in loss of viability and vigour which can be further escalated under high temperature and high humidity. Even with controlled storage facilities, sometimes, seed germination and vigour falls resulting in rejection of expensive seeds. Priming treatment like osmopriming has been demonstrated to improve germination and help in reinvigoration of aged

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sunflower seeds (Bailly et al. 2000). Beneficial effects of static magnetic field (SMF) in terms of increased vigour of seedlings have been reported in many crops (Florez et al. 2007; Shine et al. 2011, Bhardwaj et al. 2012). The stimulatory response of pulsed magnetic field (PMF) seed treatment on plant growth and development has also been reported in soybean (Radhakrishnan and Ranjitha Kumari 2012). In our previous study, static magnetic field delayed ageing in soybean under accelerated ageing and maintained its quality (Kumar et al. 2014). We also reported reclamation of six year old aged pea seeds stored under controlled conditions by using static magnetic field (Nagarajan et al. 2012).

Antioxidant defense system has been related to germination in aged or primed seed (Bailly et al. 1998, 2000). Reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) are generated as a result of aerobic metabolism in cytosol, chloroplasts, mitochondria and the apoplastic space. Report on the positive role of ROS in germination and dormancy release show that superoxide and H_2O_2 act as secondary messengers in plant developmental processes (Mittler et al. 2004). Hydrogen peroxide acts as a priming factor that brings about changes at proteome, transcriptome and hormonal levels thereby promoting germination in pea (Barba-Espin et al. 2011). H_2O_2 production in the plants results from the reactions catalysed by cell wall associated NADH peroxidase, oxalate oxidases, Nicotinamide Adenine Dinucleotide Phosphate (reduced) (NADPH) oxidase and amine oxidases (Bolwell and Wojtaszek 1997). In our earlier studies, we found that magnetic field resulted in enhanced production of reactive oxygen species mediated by cell wall peroxidase in soybean (Shine et al. 2012). The primary scavenger of ROS is superoxide dismutase which converts O_2^- to H_2O_2 . Catalase (CAT) dismutates H_2O_2 into water and molecular oxygen, whereas ascorbate peroxidase (APOX) decomposes H_2O_2 by oxidation of co-substrates such as phenolic compounds and/or antioxidants. The involvement of catalase has been implicated in seed recovery of aged sunflower seeds (Kibinza et al. 2011). APOX mediated H_2O_2 catalysis is dependent on Ascorbic acid (AsA)—Reduced glutathione (GSH) cycle. Ascorbic acid serves as electron donor to activate H_2O_2 degradation by APOX and glutathione and enzymes such as glutathione reductase (GR), dehydroascorbate reductase, monohydroascorbate reductase are responsible for AsA regeneration.

At cellular level, ROS are also known to regulate the cellular redox status, to cause the carbonylation of proteins and to trigger specific gene expression (Bailly et al. 2008). This oxidation of reserve proteins helps in their mobilization during germination by increasing their susceptibility to proteolytic cleavage (Job et al. 2005). Lipoxxygenase

(LOX) catalyses the formation of hydroperoxides by the oxygenation of linoleic and linolenic acid (Siedow 1991). The hydroperoxides may lead to production of ROS like superoxide (Rosahl 1996). Lipoxxygenase can also play a role in post germination processes during seed germination by mobilizing the storage lipids that serve as a major carbon source for seedling during germination in dark (Rodriguez-Rosales et al. 1998).

In our previous study, we found dry seed pre-treatment with static magnetic field of 100 mT 1 h, being effective in increasing vigour (based on seedling dry weight) by 40.9% over the untreated controls in six year old naturally aged pea seeds (Nagarajan et al. 2012). To the best of our knowledge, in continuation with these studies, we found that very limited literature is available on the effect of pulsed magnetic field on improving seed quality traits like germination and vigor with no report on the effect of pulsed magnetic field in reclamation of aged seeds. The present study was therefore conducted to assess the (1) response of PMF in improving seed quality of aged green pea seed (2) role of reactive oxygen species metabolism in recovery of aged seed by magnetopriming.

Materials and methods

Seed material

Aged seeds of green pea cv. ‘Bonneville’ stored for six years under controlled storage facility (20 °C and 40% RH) were obtained from Regional Station, Indian Agricultural Research Institute, Karnal, India. The germination percentage and seedling vigour (based on dry weight) of aged seed at the beginning of the experiment were 82% and 33.6 respectively. An electromagnetic field generator EM-60 (Testron, New Delhi, India) with variable magnetic field strength (50–300 mT) and a gap of 10 cm between pole pieces was used for seed-treatment. All treatments in the experiments were run simultaneously along with control under similar condition.

Optimization of dose of pulsed magnetic field treatment

In our previous report, static magnetic field dose of 100 mT for 1 h increased vigour index I and II by 45.4 and 40.9% respectively in aged green pea seeds compared to unexposed controls (Nagarajan et al. 2012). The electromagnetic field of 100 mT was applied in pulses of 2 min ON followed by 2 min OFF i.e. 4 min periodic cycle for one hour interval which resulted in 15 cycles of exposure to the seeds in this treatment. Similarly, pulses of 3 or 6 min allowed the seeds to be exposed to 10 and 5 cycles

respectively in other treatments. SHAM exposure (exposure under exactly the same conditions and setup except for magnetic field) was carried out by placing the seeds for 1 h between the poles of electromagnet (in switched off mode). For germination and related parameters, both pulsed magnetic field treatments and sham (control) had 4 replicates of 25 seeds per replicate. In case of biochemical experiments, three replicates (5 g seeds) were considered for each treatment. Based on the observations (results discussed in next section), the treatment with 5 cycles of PMF i.e. 12 min pulse was selected for biochemical studies in aged green pea seeds.

Germination percentage

The germination of the seeds was determined using “between papers” method (ISTA 1985). One hundred seeds in four replications of 25 seeds each were placed between two layers of moist germination papers and incubated at 25 °C in an upright position. After 7 days the germinated seeds were evaluated for normal, abnormal seedling, un-germinated and dead seeds. Germination percentage was given on the basis of normal seedling only.

Rate of germination

Hundred seeds in four replication of 25 each were placed on moistened filter paper in Petri-dish and kept in an incubator at 25 °C. Daily germination count was taken until no more seed germinated. The rate of germination ($\Sigma n/t$) was calculated as:

$$\sum n/t = \frac{\text{Number of seeds germinated}}{\text{Day of the first count}} + \dots + \frac{\text{Number of seeds germinated}}{\text{Day of the final count}}$$

where, n = Number of seeds germinated and t = Time of count.

Seedling growth and seedling vigor

Root and shoot length of seedlings were recorded on linear scale and seedlings dried overnight in an oven at 90 °C. The weight of the seedlings was measured and Vigour Indices I and II calculated using the formula given by Abdul-Baki and Anderson (1973).

Determination of superoxide (O_2^-)

Superoxide (O_2^-) production was measured by the reduction of Na,3'-[1-[(phenylamino)-carbonyl]-3,4-tetrazolium] (4-methoxy-6-nitro) benzenesulfonic acid hydrate (XTT) (Schopfer 2001).

Determination of hydrogen peroxide (H_2O_2)

Hydrogen peroxide was estimated by forming titanium-hydro peroxide complex (Mukherjee and Choudhari 1983).

Determination of malondialdehyde (MDA)

The level of lipid peroxidation was determined by measuring malondialdehyde (MDA) according to the procedure given by Heath and Packer (1968). The MDA content was calculated using MDA extinction coefficient $\epsilon = 155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Estimation of total antioxidants

The total antioxidants were determined by measuring the absorbance of the reaction mixture containing supernatant, 7.5 mM neocuprine, ammonium acetate and 10 mM copper chloride at 450 nm (Apak et al. 2008).

Enzyme extraction and assays

Seeds were homogenized in potassium phosphate buffer (1:5 w/v) (pH 7.0) containing 1 mM EDTA and 1% polyvinyl pyrrolidone, with the addition of 0.2 mmol L^{-1} ascorbate in case of ascorbate peroxidase assay to protect APOX activity. The homogenate was centrifuged at $10,000g$ for 30 min at 0 °C and used for measuring various antioxidant enzyme activities. Total soluble protein was measured by Bradford method using Bovine Serum Albumin (BSA) as a standard for all treatments (Bradford 1976) and used for calculating the specific activity of enzymes.

Superoxide dismutase (SOD) activity

SOD activity was measured by monitoring the enzyme induced decrease in absorbance of formazone by nitroblue tetrazolium (NBT) with superoxide radicals (Dhindsa et al. 1981). The 3 ml reaction mixture contained sodium phosphate buffer (50 mM, pH 7.8), 13 mM methionine, 25 mM NBT, 0.1 mM EDTA, 2 μM riboflavin, 50 mM sodium carbonate and 0.1 ml enzyme extract. The tubes containing reaction mixture were illuminated for 15 min under two 15 W fluorescent lamps. One unit of SOD was defined as the amount of enzyme required for causing 50% inhibition of the reduction of NBT as monitored at 560 nm.

NADH peroxidase (POX) activity

Formation of H_2O_2 catalyzed by NADH peroxidase ($\epsilon = 4.23 \text{ mM}^{-1} \text{ cm}^{-1}$) was measured spectrophotometrically at 510 nm (Ishida et al. 1988). The seeds were incubated for 24 h in 2 ml reaction mixture containing

50 mM Na-acetate buffer (pH-6.5), 1 mM 4- aminoantipyrine, 1 mM 2,4-dichlorophenol, 50 mM MnCl_2 and 0.2 mM NADH.

Catalase (CAT) activity

CAT activity was assayed for 3 min by measuring the disappearance of H_2O_2 ($\epsilon = 39.4 \text{ mM}^{-1} \text{ cm}^{-1}$) at 240 nm absorbance (Aebi 1984). The 3 ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.0), 10 mM H_2O_2 for 0.1 ml enzyme extract.

Ascorbate peroxidase (APOX) activity

The ascorbate peroxidase activity (APOX) was estimated by recording the decrease in absorbance at 290 nm ($\epsilon = 2.8 \text{ mM}^{-1} \text{ cm}^{-1}$) for 1 min in 3 ml reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.5 mM H_2O_2 and 0.1 ml enzyme extract (Nakano and Asada 1981). The reaction was started by adding the enzyme extract. Correction was done for the low, non enzymatic oxidation of ascorbic acid by H_2O_2 .

Glutathione reductase (GR) activity

Glutathione reductase assay measured the amount of red coloured complex formed by reduced glutathione with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) (Smith et al. 1998). The 3 ml reaction mixture contained 66.67 mM potassium phosphate buffer, 0.33 mM EDTA, 0.5 mM DTNB in 0.01 M potassium phosphate buffer pH-7.5, 66.67 μM β -Nicotinamide adenine dinucleotide phosphate (β -NADPH), 666.67 μM glutathione oxidized (GSSG), 0.1 ml of enzyme extract. Reaction was started by adding 0.1 ml of 20 mM oxidized glutathione and increase in absorbance at 412 nm was recorded.

Estimation of protein bound carbonyls

Protein carbonyls were detected using a molar absorption coefficient for aliphatic hydrazones as $22 \text{ mM}^{-1} \text{ cm}^{-1}$ (Levine et al. 1994) by using Sigma-Aldrich protein carbonylation kit. An equal volume of freshly prepared dinitrophenylhydrazine (DNPH) solution was added to the homogenate followed by incubation at 25 °C for 10 min. The proteins were precipitated with pre-cooled TCA solution and separated after centrifugation at $13,000 \times g$ for 2 min. The pellet was washed with acetone to remove free DNPH and sonicated with 200 μl of 6 M guanidine. The sample was transferred to 96 well plate and absorbance measured at 375 nm.

Lipoxygenase (LOX) activity

LOX activity was determined by the method of Gallego et al. (1996). To the homogenate 35 μl of linoleic acid dissolved in 5 μl Tween-20 was added as a substrate and final volume made to 100 ml with 0.1 M phosphate buffer (pH 6.5). The reaction mixture was incubated at low temp (−20 °C) and absorbance read at 234 nm for 10 min.

Statistical analysis

Germination and related parameters were expressed as mean $\pm$ S.E. of 4 replicates of 25 seeds/replicate. The experimental design was completely randomized and data were analyzed by one way analysis of variance (ANOVA) using the software Windostat version 7.1.0.28, Indostat Services, Hyderabad, India. Least significant difference (LSD) was calculated for each trait at probability level of $p < 0.010$. For biochemical analysis, three replicates were taken for treatment and control and the data represented as $\pm$ S.E. of 3 replicates at different intervals of imbibition.

Results and discussion

Seed viability, rate of germination and vigour

Controlled storage facilities are not able to maintain seed quality in terms of germination and vigour when stored for prolonged period of time. The storage of green pea seeds at 20 °C and 40% relative humidity for 6 years resulted in decline of germination and vigour index (on seedling dry weight) to 82 and 33.6% respectively. Ageing of seeds under storage induced seed deterioration resulting in loss of seed vigour and viability. Seed priming of aged seeds by various techniques has been employed in many crops to reclaim the aged seeds of wheat (Dell'Aquila and Taranto 1986), cauliflower (Fujikura and Karssen 1992) and sunflower (Bailly et al. 2002). We treated aged green pea seeds with pulsed magnetic field and improved the germination and vigour by 6 and 84.8% respectively (Table 1). All the doses of PMF used for seed treatment were effective in enhancing seedling length and dry weight although maximum increase (35 and 24% in seedling length and dry weight respectively) was observed in 100 mT (1 h) magnetic treatment applied in pulse of 6 min on and off. Moreover, this treatment could also bring about increase in seedling vigor I and II compared to untreated aged seeds (Table 1).

Table 1 Effect of pulsed magnetic field on germination, rate of germination and seedling parameters in aged green pea seeds

	Germination (%)	Rate of germination (no. of germinated seeds/day)	Seedling length (cm)	Dry wt. (g)	Vigour-I ^a	Vigour-II ^b
Aged + unprimed	82.25 ± 1.436	6.54 ± 1.404	17.13 ± 0.402	0.366 ± 0.002	1854.95 ± 77.725	33.66 ± 1.292
Aged + 100 mT, 1 h (5 cycles*)	88.50 ± 1.258	8.16 ± 1.219	23.13 ± 0.201	0.452 ± 0.008	3620.50 ± 49.963	62.22 ± 1.138
Aged +100 mT, 1 h (10 cycles**)	80.50 ± 1.500	5.33 ± 1.409	18.75 ± 0.020	0.421 ± 0.004	1505.35 ± 52.332	32.40 ± 0.882
Aged +100 mT, 1 h (15 cycles***)	83.00 ± 1.000	9.29 ± 0.545	21.30 ± 0.580	0.439 ± 0.011	3238.60 ± 69.753	57.15 ± 1.261
LSD ≤0.010	3.247	4.012	1.700	0.024	275.994	5.158

Values in the table are mean ± standard error (number of replicates = 4)

^a Vigour index I = Germination % × seedling length (Root + Shoot)

^b Vigour index II = Germination % × seedling dry weight (Root + Shoot)

* 5 cycles = 6 min on/off at 100 mT for 1 h

** 10 cycles = 3 min on/off at 100 mT for 1 h

*** 15 cycles = 2 min on/off at 100 mT for 1 h

Fig. 1 Effect of pulsed magnetic field on (a) superoxide (O_2^-) and (b) hydrogen peroxide (H_2O_2) production from the aged green pea seeds treated with pulsed magnetic field. Values are means of 3 measurements. Error bars represent $\pm$ SE ($n = 3$)

Free radical production and scavenging by antioxidant system in germinating seeds

Seed invigoration of aged green pea seeds by PMF resulted in 27% higher production of superoxide anions (Fig. 1a). It was also accompanied by a concomitant increase in the level of hydrogen peroxide that remained over 1.6 fold in PMF treated seeds compared to untreated seeds at different stages of imbibition (Fig. 1b). The exogenous application of H_2O_2 has been observed to improve seed germination in various crops (Bailly et al. 2008). H_2O_2 has been designated as a “priming factor” that coordinates the initiation of seed germination in pea by altering proteome, transcriptome and hormone levels. It has been postulated to

induce a (Mitogen Activated Protein Kinase) MAPK-dependent decrease in abscisic acid level in the seed along with carbonylation of seed storage proteins (Barba-Espin et al. 2012).

The increase in the hydrogen peroxide levels in the initial stage of germination (30 h) in magnetically primed seeds could be explained by 66% increase in NADH peroxidase activity in the primed seeds (Fig. 2a). The production of hydrogen peroxide is mediated by several enzymes like peroxidases, oxalate oxidase, NADPH oxidase and amine oxidases (Bolwell and Wojtaszek 1997). NADH peroxidase mediated H_2O_2 production in magnetoprimed pea seeds may result in cell wall loosening and subsequent cell division leading to faster germination.

Fig. 2 Effect of pulsed magnetic field on (a) NADH Peroxidase (b) Superoxide Dismutase (c) Catalase (d) Ascorbate peroxidase and (e) Glutathione reductase activity at different stages of germination in

aged green pea seeds. Values are means of 3 measurements. Error bars represent $\pm$ SE ($n = 3$)

NADH peroxidase also helps in cell wall reconstruction of regenerating protoplast by the same mechanism (de Marco and Roubelakis-Angelakis 1996). However, in the later stages of germination the magnitude of increase in hydrogen peroxide in magnetoprimered seeds could not be explained by the same reaction as other enzymes involved in hydrogen peroxide production would have come into

play. SOD activity was high in PMF primed seeds, the magnitude of increase being more after 30 h of imbibition (Fig. 2b). H_2O_2 is generated by spontaneous dismutation of superoxide anions in reactions catalyzed by SOD. CAT activity did not show much variation in primed aged seeds up to 30 h of imbibition but increased to 1429.0 nmol/mg protein/min in primed seeds compared to 641.0 nmol/mg

Fig. 3 Effect of pulsed magnetic field on total antioxidant content at different stages of germination in aged green pea seeds treated with pulsed magnetic field. Error bars represent $\pm$ SE ($n = 3$)

protein/min unprimed seeds at 54 h of imbibition (Fig. 2c). Ascorbate peroxidase activity was also high in primed seeds with 43% more activity in primed seeds at 36 h of imbibition in comparison with unprimed aged seeds (Fig. 2d). GR activity like other antioxidant enzymes stayed higher in primed seeds compared to unprimed aged seeds (Fig. 2e) and may have led to increased glutathione production indicating its contribution in the scavenging process. Glutathione can remove H_2O_2 in cells through the activity of glutathione peroxidase (Schraufstatter et al. 1985). Our results corroborate the finding that showed enhancement in seedling vigour in osmoprimed aged sunflower mediated by increased activity of catalase and glutathione reductase enzymes (Bailly et al. 2002). The level of non enzymatic total antioxidant was also 13–52% higher in PMF primed seeds than for unprimed aged seeds (Fig. 3). The steep increase in CAT, APOX and GR activity, in addition to total antioxidant content after 36 h of imbibition coincided with the time of radical emergence in germinating seeds. These results indicated that H_2O_2 may act as a signaling molecule in metabolic processes leading to improved seedling vigour in primed seeds and its level for this function is tightly regulated by a fine tuning between production and scavenging. This homeostasis of ROS generates an ‘oxidative window’ for germination that allows the seed to perceive the environmental factors that help in completion of germination process. Above or below the “oxidative window for germination”, low or high amounts of ROS would not permit progress towards germination. The critical range of ROS in this window triggers protein oxidation, controls MAPK activation, inhibit phosphatases, activate Ca^{+2} channels and Ca^{+2} binding proteins, modulate redox potential and gene expression (Bailly et al. 2008).

Carbonylation of numerous proteins occurred during *Arabidopsis* seed germination, and the germinated seeds give rise to vigorous plantlets (Job et al. 2005). Protein carbonyls formed as a result of protein oxidation by free

Fig. 4 Effect of pulsed magnetic field on protein carbonyl content at different stages of germination in aged green pea seeds treated with pulsed magnetic field. Error bars represent $\pm$ SE ($n = 3$)

radicals were found to increase with time in both primed and unprimed germinating seeds (Fig. 4). It has been proposed that carbonylation of seed storage proteins would destabilize the compact seed storage protein complexes, thus increasing their susceptibility towards proteolytic cleavage (Job et al. 2005) followed by mobilization of proteins during germination.

MDA content and lipoxygenase activity

In general, ageing causes lipid peroxidation of membranes by lipoxygenase catalysed production of hydroperoxides. However, this did not find support from our data as malondialdehyde content was 9% lower in primed seeds at 42 h of germination against unprimed seeds (Fig. 5a). The decreased level of MDA indicated decreased lipid peroxidation that helps in maintaining the integrity of the membrane in primed seeds. PMF may have resulted in partial repair of the membrane damaged during ageing as has been observed in osmopriming of sunflower seeds where priming allows metabolic processes as DNA replication and ethylene biosynthesis (Bailly et al. 2002).

A several fold increase in lipoxygenase (LOX) activity was observed in primed seeds at 24 and 48 h of imbibition (Fig. 5b). This showed that lipoxygenase was probably involved in the pre and post germination processes in primed green pea seeds. The mobilization of storage lipids that are located in lipid bodies is essential for seedling growth and the oxidation of storage lipids during the process of germination is catalysed by lipoxygenase. The increase in LOX activity before the radicle protrusion could be due to the reaction that catalyses the oxygenation of polyunsaturated fatty acids in the phospholipid layer surrounding storage lipids. These lipids that were liberated by LOX become available to the cytosolic enzymes. The sudden increase in LOX activity after 36 h of imbibition in

Fig. 5 Effect of pulsed magnetic field on (a) malondialdehyde (MDA) content and (b) lipoxygenase activity at different stages of germination in aged green pea seeds. Values are means of 3 measurements. Error bars represent $\pm$ SE ($n = 3$)

PMF treated seeds could be the result of oxygenation of the linoleic residues of the storage lipids released from the lipid bodies by lipoxygenase. The oxidation of storage lipid after radicle emergence is triggered to supply carbon to the growing embryo.

Conclusion

Our study showed that pulsed magnetic field of 100 mT for 1 h (6 min on/off) can effectively reduce the damage caused by long term storage of green pea seeds under controlled conditions and thus reclaim the seed with reduced vigour. This improvement in vigour is mediated through ROS homeostasis maintained by antioxidative system in primed seeds. Also, oxidation of proteins by free radicals helped in mobilization of reserve proteins to the growing seedling of the primed seeds. The level of free radicals that promoted germination was not deleterious to the seed as malondialdehyde content was low and lipoxygenase was not involved in lipid peroxidation of the membranes.

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