ROLE OF REACTIVE OXYGEN SPECIES AND HORMONES IN SEED GERMINATION OF MAGNETOPRIMED CHERRY TOMATO SEEDS (*Lycopersicon esculentum* L.)

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चुम्बकीय-प्रवृत चेरी टमाटर बीजों के अंकुरण में प्रतिक्रियात्मक ऑक्सीजन प्रजाति और हारमोनों की भूमिका

ROLE OF REACTIVE OXYGEN SPECIES AND HORMONES IN SEED GERMINATION OF MAGNETOPRIMED CHERRY TOMATO SEEDS (*Lycopersicon esculentum* L.)

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CERTIFICATE

This is to certify that the thesis entitled **“Role of Reactive Oxygen Species and Hormones in Seed Germination of Magnetoprimed Cherry Tomato Seeds (*Lycopersicon esculentum* L.)”** submitted to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi in partial fulfillment of **Master of Science** in **PLANT PHYSIOLOGY**, embodies the results of *bonafide* research work carried out by **Mr. Mukesh Kumar Gupta Roll No. 20334** under my guidance and supervision, and that no part of this thesis has been submitted for any other degree or diploma.

This assistance and help availed during the course of investigation as well as source of information have been duly acknowledged by him.

Date: June 30, 2014
Place: New Delhi-12

(ANJALI ANAND)
Chairperson
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Dedicated to my parents….
I am blessed to be your son

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1. INTRODUCTION

Our country has achieved a good degree of stability in food production with estimates of 263 MT for the current year compared to 255.36 MT in 2012-13. This has generated interest in a need for providing health security to the citizens by supplying nutrition through balanced diet. The importance of consuming vegetables to ensure a balanced diet is being realized in all parts of the world due to health benefits of nutrients available from these crops. Vegetables play a major role in Indian agriculture by providing food, nutritional and economic security and more importantly, producing higher returns per unit area and time. India is the second largest producer of vegetables (ranks next to China) and accounts for about 15 % of the world's production of vegetables. The current production level is over 160 MT and the total area under vegetable cultivation is around 9.2 million hectares which is about 3 % of the total area under cultivation in the country (NHB, 2013**)**. In India, growing of vegetables is 4.8 times more remunerative than cereals and other field crops. Our demand of vegetables is expected to be 225 MT by 2020 and 350 MT by 2030 (www.iivr.org.in). Globalization has brought about changes in the living standards and living style of people, most noticeable being the change is the food habits among the new generation. As a consequence, demand-driven exotic vegetables production is increasing in India at the rate of 15 to 20 % per annum for self sufficiency as approx. 85 % is being imported from other countries.

India ranks second in the area as well as in production of tomato. Area under tomato cultivation is 0.865 mha, production 16.826 MT, productivity 20.07 tons/ha (APEDA /NHB, 2013). Cherry tomato is a small garden variety of tomato that has very high nutritive value, containing vitamin A, lycopene, beta-carotene, vitamin C and minerals like calcium, potassium etc. The cultivation of cherry tomato is a highly economical venture for the farmers as they have assured market through contract with national and international retail chains. However, under reducing land, declining natural resources and increasing biotic and abiotic stresses, the major challenge lies ahead to develop technologies that enhance quality and productivity of vegetables. The increasing cost of seeds of vegetables has made the researchers prioritize at obtaining a plant from each seed sown to make the crop economically viable for farmers. Thus, crop yields can be maximized by establishment of an adequate and uniform plant population for which good quality seed is a decisive factor. The benefits of agronomic inputs cannot be completely realized if the seed is of poor quality resulting in a poor stand. Rapid and uniform field emergence of seedlings is therefore a challenge to increase yield and quality as

a) Slow emergence results in smaller plants and seedlings which are more vulnerable to soil- borne diseases.

b) Extended emergence period of seedlings result in poor seedling stand establishment

Seed priming is a physiological seed enhancement method for overcoming poor and erratic seed germination in many vegetable and flower species. Seed priming techniques like hydropriming (soaking in water), osmopriming (soaking in solutions of different organic osmotica), solid matrix priming and halopriming (soaking in inorganic salt solutions) are being experimented extensively to increase the speed and synchrony of seed germination of vegetable and field crops (Nakaune *et al.* 2012). It reduces seed rate of production per hectare due to increase in the germination percentage finally reducing the cost of cultivation. Priming causes an effective invigouration in the seed which is an inception of metabolic processes that otherwise occur during imbibition that is subsequently fixed by drying the seed. These methods although effective in vigor improvement, have handling and storage problems. The pre-sowing seed treatment with magnetic field called “magnetopriming” is a physical priming technique that doesn’t hydrate the seed, allows storage of the seed at room temperature and is eco-friendly. Other priming treatments have a mandatory requirement of dehydrating the seed for storage before sowing but magnetopriming is carried out in dry seed and proves to be more useful. Several researchers have reported the beneficial effect of both static and oscillating magnetic fields in different plant species (Anna 2002; Fischer *et al.* 2004; Florez *et al.* 2007, Shine *et al.,* 2011, Bhardwaj *et al.,* 2012; Radhakrishnan and Ranjitha Kumari, 2012; Bilalis *et. al.*, 2013).

The stimulation in growth due to magnetopriming is mediated through the production of free radicals in the germinating seed (Shine *et al*., 2011, 2012). Reactive Oxygen Species (ROS) function as signaling molecules in plants thus regulating growth and development, programmed cell death and hormone signaling. For ROS to act as cellular messenger, seeds must be endowed with a ROS removing system that tightly regulates their concentration. Above or below the **"oxidative window for germination",** low or high amounts of ROS would not permit progress towards germination (Bailly *et al*., 2008). Antioxidant system is a multifactorial network composed of various enzymes such as catalase, superoxide dismutase, ascorbate peroxidase as well as non enzymatic compounds such as ascorbic acid and reduced glutathione. At cellular level, ROS are also known to regulate the cellular redox status, to cause the oxidation of proteins and to trigger specific gene expression (Bailly *et al*., 2008). ROS can react with all biological molecules including lipids, DNA and proteins. Because proteins have numerous biological functions, their oxidation may result in modification of their enzymatic and binding properties and lead to diverse functional changes. Oxidation of reserve proteins would help in their mobilization during germination by increasing their susceptibility to proteolytic cleavage (Job *et al*., 2005). Additional evidence on the putative role of ROS in germination comes from their interaction with plant hormones abscisic acid (ABA) and gibberellin (GA) (Finkelstein *et al*., 2008). Interaction of ROS with ABA and gibberellin signaling pathways has been shown in various plant physiological processes, which might be relevant to seed germination, since these hormones are known as major players in the regulation of seed dormancy (El-Maarouf-Bouteau and Bailly 2008, Finkelstein *et al*., 2008). GA has been described as an internal regulator involved in the induction of germination, whereas ABA is involved in establishment and maintenance of seed dormancy (Koorneef *et al*., 2002).

With the early production of ROS within the threshold levels so as to increase the rate of germination in magnetoprimed seed (Shine *et al*., 2011), it becomes pertinent to study the interplay of ROS and hormones in germinating magnetoprimed seeds. Keeping this in mind, the present investigation was conducted with the following objectives:

i. Optimization of static and pulsed magnetic field dose for seed invigouration and its effect on yield in cherry tomato
ii. To study ROS homeostasis (by antioxidant system and protein carbonylation) and hormone levels in germinating seeds

2. BACKGROUND

Horticulture is the fastest growing sector within Indian agriculture. Increasing per capita income, health consciousness, urbanization, shifting of farmers to high value vegetables due to higher income, favourable income elasticity of demand and annual growth rate for vegetables are important ingredients for fuelling vegetable growth in the country. Consequently, vegetable production assumes a greater role and importance within the agriculture sector and eventually in the national economy. India is the world's second largest producer of vegetables next only to China. It is estimated that all the horticulture crops put together cover nearly 23.96 million hectares area with an annual production of 262 million tonnes (NHB,2013). Though these crops occupy hardly 7 % of the cropped area yet they contribute over 18 % to the gross agricultural output in the country. For improved crop production, a main agricultural goal is to obtain rapid and uniform germination and seedling emergence once seeds are sown. Differences at these stages are generally not recovered, which directly impacts crop yield. Many physiological barriers in seeds may delay the onset, reduce the rate and increase the dispersion of germination events, leading to reduction in plant growth and final crop yield. The research on vegetable crops is oriented towards enhancing seed production and germinability of vegetable crop seeds. To increase the performance of commercial seed lots, the seed industry practices invigoration treatments known as priming, which consist of controlled imbibition of the seeds followed by dehydration back to their initial water content so as to permit their storage. Among seed priming techniques, hydropriming (soaking in water), halopriming (soaking in inorganic salt solutions), osmopriming (soaking in solutions of different organic osmotica), thermopriming (treatment of seed with low or high temperatures), solid matrix priming (treatment of seed with solid matrices) and biopriming (hydration using biological compounds) have been effectively used to improve seed performance in field (Ashraf and Foolad, 2005). The advantages and disadvantages depend upon plant species, stage of plant development, concentration / dose of priming agent, and incubation period. These treatments are thought to somehow reproduce early stages of germination, suggesting that the primed seeds advance germination time compared with untreated seeds. Although these methods are very effective in vigor improvement they are not eco-friendly and have handling problems. Thus, when the rational use of pre-sowing seed treatment is emphasized, greater importance is attributed to some physical methods for pre-sowing treatment of seeds, which are commonly regarded as being friendlier to the environment. Use of electro-magnetic energy (**magnetopriming**) for seed pretreatment is worthy of attention since its influence on the seeds can change the processes taking place in the seed and can stimulate plant development. The beneficial effects have numerous practical applications in modern agriculture like enhanced germination and seedling growth, reduce seed rate of production per hectare due to increase in the germination percentage finally reducing the cost of cultivation. Seed pre-treatment with magnetic field gains more relevance and significance when explored in vegetable crops as the seeds are of low volume and of high value. The feasibility of this technique is justified because of lesser-seed requirement compared to food crops.

2. 1 Magnetopriming- Seed priming with magnetic energy

Magnetopriming is a non invasive physical stimulation of dry seeds with magnetic field used for improving vigour and field emergence in seeds. The stimulating effects of magnetopriming on plant growth and yield under non-stressed condition have been observed in many crops (Phirke *et al*., 1996; Harichand *et al.*, 2002; Radhakrishnan and Ranjitha Kumari, 2012; Bhardwaj *et al.*, 2012; Bilalis, *et al.*,2013). Gubbels *et al.* (1982) observed that seed lots of flax (*Linum usitatissimum L.*), buckwheat (*Fagopyrum esculentum Moench*.), sunflower (*Helianthus annuus L.)* and field pea (*Pisum sativum L.)* exposed to a magnetic field produced earlier and more vigorous seedlings growth in some seed lots and increased the yield of sunflower. Suitable MF-pretreatment could speed up seedling development and increase total leaf numbers, in cauliflower and tomato (Amaya *et al.*, 1996; Samy, 1998).

It is believed that magnetic field influences the structure of cell membrane and increases their permeability and ion transport in the ion channels, which then affects some metabolic activity (Garcia and Arza, 2001). It has a long lasting stimulatory effect on plants leading to increased performance index (indicates photosynthetic efficiency) that consequently increases biomass in soybean plants (Shine *et al*., 2011).

2. 2 Effect of magnetopriming on germination and seedling growth

Magnetopriming is reported to affect seed germination and helps in crop establishment. The first report on the effect of magnetic field on growth of seedlings dates back to the work by Savostin (1930) where increase in the rate of wheat seedling elongation was observed under magnetic conditions. Later, Pittman and Omrod (1963), Bhatnagar and Dev (1977) and Pietruszewski (1999) recorded an increase in rate of germination of cereal seeds exposed to magnetic field. Alexander and Doijode (1995) noted that the application of an external magnetic field as a pre-germination treatment improved the germination and seedling vigour of low viability rice and onion seeds. Stationary magnetic field treatment of lettuce seeds led to faster germination than untreated seeds possibly due to an increase in water uptake rate (Garcia *et al.,* 2001). Celestino *et al*. (2000) reported enhanced germination and growth of *Quercus suber* seedlings exposed to electromagnetic field. Germination increased by 1.1-2.8 times in tomato seeds exposed to suitable magnetic field (Moon and Chung, 2000). Wheat and barley seeds treated with magnetic field showed early sprouting (Martinez *et al.*, 2000, 2002). Studies conducted by Rochalska (2002) on the germination and growth of wheat, triticale, maize and soybean, also indicated that magnetic field can be used as a method of seed vigour improvement. Exposure of seeds to magnetic field was found to accelerate sprouting and growth of the seedlings of cucumber (Yinan *et al.,* 2005). Podlesny *et al.* (2004, 2005) confirmed the positive effect of the magnetic treatment on the germination and emergence of both broad bean and pea cultivars. Soltani *et al*. (2006) have reported the positive effect of magnetic field on *Asparagus officinalis* and *Ocimum basilicum* seed germination and seedling growth. Vashisth and Nagarajan (2007, 2008) reported significant increases in germination, seedling vigor and shoot/root growth of one month old maize plants and chickpea seeds exposed to static magnetic fields. Alternating magnetic field treatments at low frequency (60 Hz) with combinations of three magnetic flux densities (20, 60 and 100 mT) and three exposure times (7.5, 15 and 30 minutes) were used as pre-sowing seed treatments in three maize (*Zea mays* L.) genotypes to increase seedling emergence rate, seedling dry weight and emergence in soil (Aguilar *et al.,* 2009). Similar results were also observed in maize by Zepeda-Bautista *et al.* (2010). In tomato, treating seed with stationary magnetic field improved germination percentage and rate of germination (Martínez *et al.,* 2009). Mean germination time (MGT) increased when the time of seed exposed to magnetic field was increased in wheat (Gholami and Sharafi, 2010). Irradiating maize seeds at magnetic induction levels of 160 and 560 mT also produced an increment in seed emergence rate and seedling emergence (Dominguez *et al.,* 2010).

2.3 Seed water uptake

Magnetically treated seeds absorb water faster and in larger quantities than untreated ones, with a change of the magnetic field from 0 to 10 mT; a behaviour that is explained in the framework of a model that assumes an interaction of the magnetic field with electrophysiological mechanism of osmo regulation (García-Reina *et al*., 2001). In wheat, Pittman and Ormrod (1970) reported that the seedlings grown from magnetically treated seed (180 mT) absorbed more moisture, respired more slowly, released less heat energy and grew faster than the untreated controls. In soybean, Kavi (1977) observed that soybean seeds exposed to magnetic field of 300 mT had increased capacity to absorb moisture. Francisco (2001) observed significant increase in the rate with which the lettuce seeds absorbed water in the interval 0-10 mT of magnetic treatment. An increment in the total mass of absorbed water in this interval was also observed. These results were consistent with the reports on the increase of germination rate of the seeds, and the theoretical calculation of the variations induced by magnetic fields in the ionic currents across the cellular membrane. The fields originate in changes in the ionic concentration and thus in the osmotic pressure which regulates the entrance of water to the seeds. The good correlation between the theoretical approach and experimental results provides strong evidence that the magnetic field alters the water relations in seeds, and this effect may be the explanation of the reported alterations in germination rate of seeds by the magnetic field. In cucumber, seed water status determined by transverse relaxation time (T_2) was significantly higher in magnetoprimed seeds (Bhardwaj *et al.*, 2012).

2.4 Production of Reactive Oxygen Species (ROS) during germination

Reactive Oxygen Species (ROS) are continuously produced during seed development, from embryogenesis to germination and seed storage (Fig. 2.1) (Slater *et al.*, 2008).ROS play a dual role in seed physiology behaving on one hand, as actors of cellular signaling pathways and on the other, as toxic products that accumulate under stress conditions. ROS, provided that their amount is tightly regulated by the balance between production and scavenging, appear beneficial for germination and to act as a positive signal for seed dormancy release. Such an effect might result from the interplay between ROS and hormone signaling pathways thus leading to changes in gene expression or in cellular redox status. The changes in ROS homeostasis would play a role in perception of environmental factors by seeds during their germination, and thus act as a signal controlling the completion of germination. However, uncontrolled accumulation of ROS is likely to occur during seed aging or seed desiccation thus leading to oxidative damage toward a wide range of biomolecules and ultimately to necrosis and cell death. The concept of the **"oxidative window"** for germination restricts the occurrence of the cellular events associated with germination to a critical range of ROS level, enclosed by lower and higher limits. Above or below

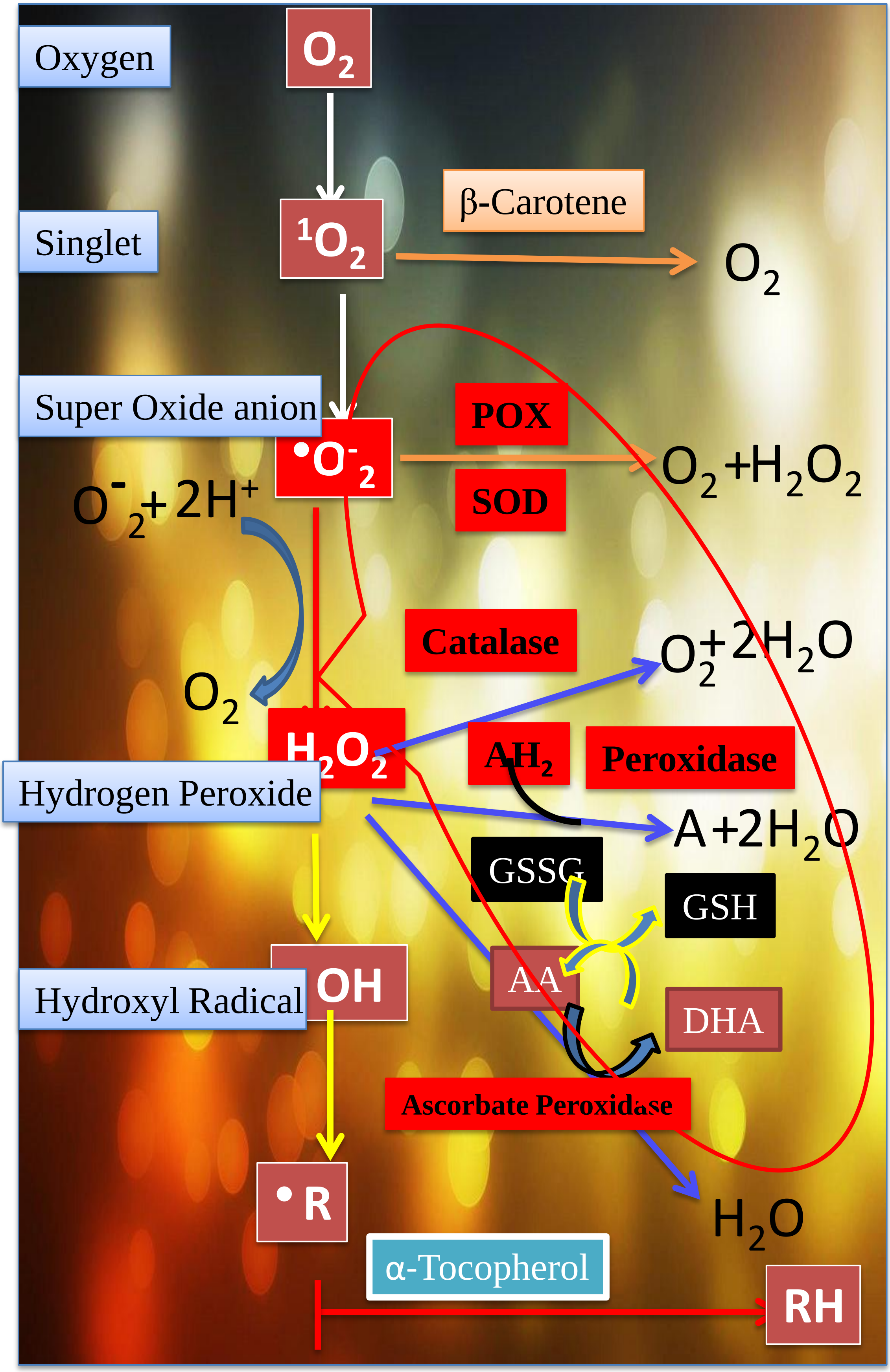

Fig. 2.1 Pathway of the formation of ROS and enzymes that scavenge ROS. (Slater *et al*., 2008)

the “oxidative window for germination”, weak or high amounts of ROS, respectively, would not permit progress toward germination (Bailly *et al., 2008).*

Germination is accompanied by extensive change in the redox state of seed proteins. The oxidative modification of proteins results from protein carbonylation by ROS, a process known to contribute to various diseases in humans, including aging, Alzheimer’s Disease, Parkinson’s Disease, cancer, and heart diseases. Various carbonylated proteins accumulate during imbibition of *Arabidopsis* seeds. This process targets specific metabolic enzymes, translation factors, and several molecular chaperones. Although accumulation of carbonylated proteins is usually considered in the context of aging in a variety of model systems, this is clearly not the case in seeds, because *Arabidopsis* seeds—despite containing large quantities of carbonylated proteins—can germinate at a high rate and yield vigorous plantlets (Job *et al*., 2005). It appears that the observed specific changes in protein carbonylation patterns are probably required for counteracting and/or utilizing the production of ROS caused by recovery of metabolic activity in the germinating seeds (Kranner *et al.*, 2010). Notably, the observed carbonylation of highly abundant seed storage proteins could reflect their role in scavenging the ROS produced during seed germination (Job *et al*., 2005). Moreover, H_2O_2 and superoxide anion accumulation during after-ripening are associated with changes in protein carbonylation, which has been proposed as a mechanism of dormancy alleviation in sunflower (Oracz *et al*. 2007) and *Arabidopsis* (Müller *et al*. 2009). It has been proposed that carbonylation can facilitate the mobilization of the seed storage proteins during seedling establishment, presumably by destabilizing their structure and promoting proteolytic attack (Job *et al*., 2005).

2.5 ROS scavenging by antioxidant enzymes

A homeostatis of ROS is maintained through increase in activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathione reductase (GR). Increase in SOD activity results in the scavenging of $O_2^{\cdot -}$ whereas CAT and GR detoxify H_2O_2 in germinating seeds. The activity of these enzymes increased in treated seeds that help in protecting the germinating seed from damaging effects of ROS. Osmopriming of aged sunflower seeds increased the activity of CAT and GR enzymes that was related to enhanced seedling vigor (Bailly, 2000) in primed seeds compared to aged seeds. Free radical scavenging enzymes such as CAT and SOD are synthesized during hydropriming of seeds to protect the cell from damage due to lipid peroxidation, which occurs due to the oxidative stress induced by hydropriming. These enzymes could also be effective in quenching the free radicals generated by lipid peroxidation occurring naturally and these effects are retained even after drying the primed seed (Varier *et al*, 2010).

2.6 Role of hormone in seed germination.

The phytohormone abscisic acid (ABA), a sesquiterpene compound resulting from the cleavage of carotenoids, controls storage reserve accumulation and desiccation tolerance of orthodox seeds (Nambara *et al*., 2010) This hormone induces the expression of late embryogenesis abundant (LEA) proteins, which become abundant during late maturation and is thought to act as chaperone to protect macromolecular structures against desiccation injury (Tunnacliffe *et at*., 2007). During seed development, ABA is produced in maternal tissues and in the embryo, but only embryonic ABA is necessary to impose a lasting dormancy (Nambara and Marion-Poll, 2003) and *de novo* ABA synthesis in the embryo during imbibition allows maintenance of dormancy (Kucera *et al.*, 2005). In barley, the embryonic ABA content decreased quickly after imbibition in non-dormant grains or in grains imbibed at 20 °C (where germination occurred) but remained high in dormant grains imbibed at 30 °C (where germination was prevented) (Benech-Arnold *et al*. 2006). Importantly, during seed maturation, ABA also exerts an inhibitory effect on mechanisms triggering precocious and deleterious germination of developing seeds on the mother plant (preharvest sprouting), thereby allowing the maturation process to be sustained and seeds to be formed that are endowed with appropriate reserves required for establishment of vigorous plantlets following germination. Therefore, to successfully germinate following imbibition and to counteract the inhibitory effect of ABA on germination, a nondormant seed must (*a*) establish a specific catabolism, decrease in sensitivity, and biosynthesis inhibition to reduce the active level of this hormone (4), and (*b*) synthesize another class of hormones represented by a large family of tetracyclic diterpenes, the gibberellins (GAs), which are essential germination activators (Groot& Karssen,1987; Koornneef & van der Veen, 1980; Sun & Gubler, 2004). GAs negatively regulate proteins behaving as repressors of germination, such as the GRAS transcriptional regulators involved in cellular differentiation (Peng & Harberd , 2002; Zhang *et al*., 2011). Besides these phytohormones, a number of diverse germination stimulants are known, some of which are presumably important as environmental sensors. Among them, several reactive oxygen species (ROS) exert control over germination, most likely in concert with NO, to regulate ABA catabolism and GA biosynthesis during seed imbibition (Liu *et al*., 2010). The balance of ABA/GA levels and sensitivity is a major regulator of dormancy status, where GAs promote progression from release through germination (Finkelstein *et al*. 2008). In the aleurone layer of cereals, the role of GAs is crucial after dormancy breakage, where it is required for reserve mobilization and germination (Jones & Jacobsen 1991; Gubler *et al*. 2002).

2.7 Interaction of ROS signaling with hormone signaling pathway

Hormonal signaling interacts with other signaling pathways; recent data have highlighted the importance of reactive oxygen species (ROS) signalling with respect to plant growth and development (Fujita *et al*., 2006). ROS are implicated in stress-induced damage and ageing, but evidence is increasing that they are also key components of signal transduction networks in plants (Møller *et. al*., 2007; El-Maarouf-Bouteau and Bailly 2008). The interaction between ROS and ABA has mainly been studied at the cellular level in guard cells, where ROS are considered second messengers in the ABA transduction pathway (Neill *et al*. 2008; Wang and Song 2008). H_2O_2 and ABA both induce stomatal closure, in seeds; these molecules have antagonistic effects on germination. ABA inhibits germination, whereas H_2O_2 breaks seed dormancy in several species including barley (Fontaine *et al*. 1994; Wang *et al*. 1998), rice (Naredo *et al*. 1998), wild oat (Hsiao and Quick, 1984), *Cinnamomum* *camphora* (Chien and Lin, 1994), *Zinnia elegans* (Ogawa and Iwabuchi, 2001), sunflower (Oracz *et al*. 2009), several warm-season grasses (Sarath *et al*. 2007) and *Arabidopsis* (Liu *et al*. 2010). H_2O_2 also accelerates the germination of non-dormant seeds of barley (Ishibashi *et al*. 2010) and pea, and stimulates the early growth of pea seedlings (Barba-Espin *et al*. 2010). ROS could play a key role in the transduction of the hormone signal in seeds since the interaction between hormones and ROS in other developmentally controlled processes in plants is known (Kwak *et al.,* 2008). The relationship between H_2O_2 and ABA is the most credible and documented. H_2O_2 is known to inactivate ABI1 and ABI2 type 2C protein phosphatases, two enzymes involved in ABA signaling (Meinhard *et al*., 2001). Furthermore, treatment of dormant barley seeds with hydrogen peroxide resulted in a decrease in endogenous ABA level (Wang *et al*., 1995) and alleviation of apple embryo dormancy by cyanide induces an increase in H_2O_2 level and a decrease in ABA content (Bogatek *et al.,* 2003). H_2O_2 would induce a MAPK-dependent decrease in abcisic acid (ABA) contents in the seed as well as the carbonylation of seed storage proteins (Bassel *et al*., 2008) favoring their mobilization, and some glycolytic enzymes that could stimulate the phosphate pentose pathway (Barba-Espín *et al.,* 2012). Interaction of ROS with ethylene and GAs, two hormones that are involved in dormancy alleviation of seeds of various species, has also been documented. It appears that ethylene and ROS transduction pathways share common components, GA_3 modifying the redox status of aleurone proteins during germination and ROS stimulating the expression of genes involved in GA signaling (El-Maarouf-Bouteau *et al.,* 2008). ROS play a key role in the hormone-regulated programmed cell death in barley aleurone cells, which is stimulated by GAs, which induce ROS accumulation, whereas ABA maintains low ROS concentrations through the activation of the alternative oxidase pathway and ROS-scavenging systems (Fath *et al*. 2002). An interaction between GA and ROS related to growth regulation has also been demonstrated by Achard *et al*. (2008), who showed that DELLA proteins, which are major negative regulators of GA signalling, activate ROS scavenging enzymes. Altogether these data suggest that ROS act directly, or as messengers of hormonal networks, as signaling molecules involved in the transition from a dormant to a non-dormant state. However, the putative mechanisms of action of ROS at the cellular level in the regulation of dormancy are far from being resolved. Till date, ROS have been shown to trigger protein oxidation during dormancy alleviation (Angelovici *et al* ., 2011) but they can also control MAP kinase cascade activation, inhibit phosphatases, activate Ca^{2+} channels and Ca^{2+} -binding proteins, modulate redox potential and gene expression.

2.8 Effect of magnetopriming on root and shoot parameters

Magnetically treated seeds of sunflower planted in soil resulted in significantly higher seedling dry weight, root length, root surface area and root volume in 1-month-old seedlings (Vashisht *et al.,* 2010). Phirke *et al*. (1996) found an increase in root, stem and fresh weight of tomato plants significantly exposed to magnetic field. Audus (1960) reported a strong magnetic effect on root development in magnetically treated seeds. Under water stress condition the increase in root growth of maize seedlings could explain their tolerance to stress (Anand *et al.,* 2012).

2.9 Effect of Magnetopriming on yield

Magnetic fields have been reported to exert a positive effect on plant growth and development (De Souza *et al.,* 1999; Martínez *et al.,* 2000), on growth of trees (Ruzic *et al.,* 1998), on the ripening of fruits and vegetables (Boe and Salunke, 1963) and on crop yield (Pietruszewski, 1993). The effects of pre-sowing magnetic treatments on the growth and yield of tomatoes were studied, and seeds exposed to 120 mT dynamic magnetic field enhanced the growth and yield in the late season (De Souza, *et al*., 2005). The improvement induced by the magnetic treatment was consistent with the results of other studies (Amaya *et al.,* 1999) where growth of tomato plant was enhanced. Also, Kavi (1983) observed that by subjecting the ragi (*Eleusine coracana Gaertn*) seed to a magnetic field of 100 mT gave higher yield. In wheat, Harichand *et al*., (2002) also observed that seed treatment with a magnetic field increased plant height, seed weight per spike and subsequent yield of crops.

Pittman (1977) reported that pre-seeding magnetic field treatment of barley (*Hordeum vulgare* L.) seeds resulted in seed yield increase in 13 out of 19 field tests. Freyman (1980) reported slight stimulus of net assimilation rate in two barley cultivars grown from magnetically treated seeds under controlled environment conditions, between 29 and 57 days after planting. Exposure of maize for 2-10 min to magnetic field ranging from 0.06 to 0.2 T stimulated germination and increased harvest by 29.5% (Antonow *et al.,* 1982). Pre-sowing exposure of safflower seeds to 72 mT of magnetic field for 10 min increased the number of secondary branches and yield (Faquenabi *et al.,* 2009). Seed magnetic treatment of 72 mT to 128 mT with exposure times between 13 and 27 minutes enhanced yield in soybean, cotton, and wheat (Phirke *et al.,* 1996). Yield of okra increased by 97.7% after exposure to static magnetic field as a result of increased flowers and fruits per plant (Naz *et al.,* 2012).

3. MATERIALS AND METHODS

The present investigation on the effect of electromagnetic field and duration of exposure on germination characteristics and yield of cherry tomato and the role of reactive oxygen species and hormones associated with germination process was conducted at the laboratory of Division of Plant Physiology and green house of Centre for Protected Cultivation Technology (CPCT), Indian Agricultural Research Institute, New Delhi during 2013-2014. Analytical facilities to conduct the biochemical work were availed at the Division of Plant Physiology, IARI. The experimental details and methodology used for execution of these studies are presented in this chapter.

3.1 SEED MATERIAL

Seed of cherry tomato (Selection 1) were obtained from CPCT, Indian Agricultural Research Institute, New Delhi and used for the study.

3.2 CHEMICALS

All the chemicals used in the study were of analytical grade and procured from SRL, Sigma, Hi Media, Merck and Fisher Scientific.

3.3 EXPERIMENTS & METHODOLOGY

3.3.1 Electromagnetic field generator

An electromagnetic field generator “Testron EM-60” with variable magnetic field strength (50 to 300 mT) with a gap of 10 cm between pole pieces was used for seed treatment (Fig. 3.1). The pole pieces were 15 cm in diameter and 35 cm in length. The number of turns per coil was 1600 and the resistance of the coil was 16 ohm. A DC power supply (80V/ 10A) with continuously variable output current was used for the electromagnet. A digital gauss meter model DGM-30 operating on the principle of Hall Effect, monitored the field strength produced in the pole gap. The probe was made of Indium Arsenide crystal and was encapsulated to a non- magnetic sheet of 5 mm × 4 mm × 1 mm and could measure 0-2 Tesla with full-scale range in increments of 5 mT. At low field strength (50 mT), width wise variation of magnetic field strength from centre to periphery was 0.6 and height wise 1.6% of the applied field. At high field (300 mT), they

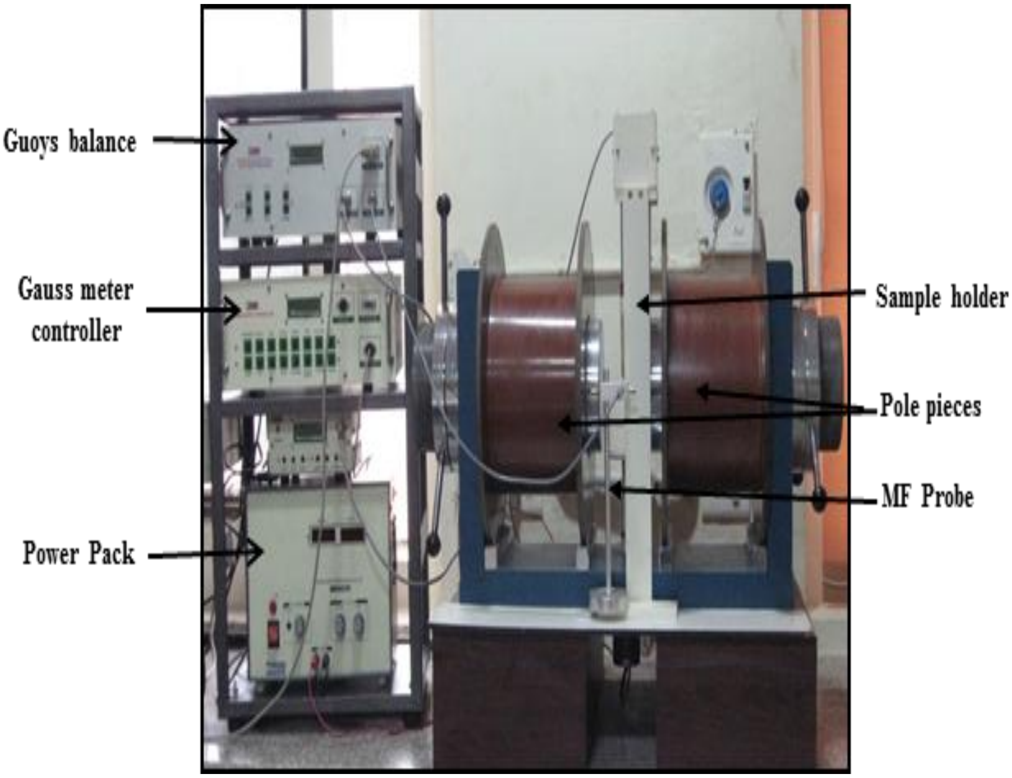

Fig.3.1 Electromagnetic field generator with variable magnetic field strength (50 – 250 mT) and a pole gap of 10 cm for seed treatment.

were 0.4 and 1.2% of the applied field, respectively. The local geomagnetic field was less than 6 mT and the direction of local geomagnetic field was north to south.

3.3.2 Optimization of static and pulsed magnetopriming dose for seed invigouration.

3.3.2.1 Static magnetic field

Cherry tomato seeds were exposed to static magnetic field of 50-150 mT in increments of 50 mT for varying durations of 30 min and 1 h, by placing in a cylindrical-shaped sample holder of 42 cm^3 capacity, made of a non-magnetic thin transparent plastic sheet. Three hundred visibly sound, mature, healthy seeds held in the plastic container were placed between the poles of the electromagnet having a uniform magnetic field. The required strength of the magnetic field was obtained by regulating the current in the coils of the electromagnet. A gauss meter was used to measure the strength of the magnetic field between the poles. The variation in temperature during the course of seed exposure was 25 ± 0.5^oC. For parallel control, seeds from the same lot used for magnetic field exposure were kept under conditions which had no influence of the induced magnetic field, as magnetic field generator was housed in a separate chamber. The field strength and duration was standardized for maximum enhancement of germination and vigour under laboratory conditions and best combination was selected for standardization in the pulsed mode.

3.3.2.2 Pulsed magnetic field

Among all the treatments in the static mode, 100 mT for 30 min was most effective in enhancing the germination parameters in magnetoprimed seeds and was further employed for treating seeds in pulsed mode. Four pulsed doses (the number of times the sample was exposed to magnetic field in 30 min) were designed for PMF treatment with 100 mT (30 min) applied in the cycle of 2, 3, 5 and 6 min on and off.

3.3.3 Germination- percentage

The germination of the seeds was determined by using “between papers” method given by ISTA (1985). One hundred seeds in four replications of 25 seeds each were placed between two layers of moist germination papers. The germination papers were rolled carefully ensuring that no excess pressure was placed on the seeds. These were wrapped in a sheet of wax paper to reduce surface evaporation of moisture and placed in the germination incubator at 25°C in an upright position. After 14 days, seeds were evaluated for normal, abnormal seedling, un-germinated and dead seeds. Germination - percentage was worked out on the basis of normal seedlings only and was transformed into arcsine values for statistical analysis.

3.3.4 Seedling growth and Vigour Indices

Ten normal seedlings from each replicate kept for germination, were randomly selected to measure shoot and root length in cm. They were subsequently dried at 80°C in an oven and weighed together till a constant weight was obtained.

Seedling vigour was calculated following Abdul-Baki and Anderson [1973] as

$$\text{Vigor index I} = \text{Germination\%} \times \text{Seedling length (Root +Shoot)}$$

$$\text{Vigor index II} = \text{Germination\%} \times \text{Seedling dry weight (Root +Shoot) (g)}$$

3.3.5 Rate of germination

Seeds were placed in Petri dish on a double layer of moistened filter paper and incubated in darkness in a germinator at 25°C. A daily germination count of the incubated seeds was taken until no more seeds germinated, and the speed of germination was calculated following Maguire (1962) as

$$\text{Speed of germination} = \sum (n/t)$$

Where n = number of seeds germinated and t = time of count

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

From the results of the optimization magnetic dose, it was observed that germination and related characteristics showed enhanced performance in the static magnetic field of **100 mT for 30 min** and **3 min on/off pulsed dose** of 100 m T for 30 min.

3.3.6 Seed water uptake

The water uptake by seeds during imbibition in double distilled water was determined in triplicate, each replicate having 80 mg seeds. Seeds were removed from the Petri plates at different time intervals, blotted dry and instantly weighed. The changes in weight due to imbibition were expressed as the amount of water absorbed by the seeds per dry weight which was calculated by the following formula.

$$\text{WU \%} = \frac{(\text{Fresh weight of seed} - \text{Dry weight of seed}) \times 100}{\text{Dry weight of seed}}$$

Where, WU stands for water uptake

3.4 Determination of superoxide ($O_2^{\cdot -}$)

Superoxide free radical was quantified by its capacity to reduce nitroblue tetrazolium chloride (NBT) in primed and unprimed seeds following the method of Chaitanya and Naithani (1994). Seed (40 mg) was homogenized in 2 ml of pre cooled phosphate buffer (0.2 M, pH 7.2). The homogenate was centrifuged at 10, 000 x g for 10 min at 4°C. Supernatant was used for estimation of superoxide anion. A 3 ml reaction mixture contained 100 µl supernatant in 0.75 mM NBT, 25 mM sodium carbonate, 0.1 mM EDTA and 13.3 mM L- methionine. Reaction mixture was incubated at 30 °C in a water bath for 10 min and the absorbance was recorded at 540 nm in a spectrophotometer. Superoxide anion content was calculated using a molar absorption coefficient of 12.8 mM^{-1} cm^{-1} and expressed as µmol g^{-1} fresh weight.

3.5 Determination of hydrogen peroxide (H_2O_2)

Hydrogen peroxide was estimated by formation of titanium-hydro peroxide complex (Mukherjee and Choudhari, 1983). Seeds (40 mg) were ground in 3 ml cooled acetone in a chilled mortar and pestle kept in ice bucket. The homogenate was filtered through Whatman No. 1 filter paper followed by addition of 2 ml of titanium reagent and 2.5 ml of ammonium hydroxide solution to precipitate the titanium–hydro peroxide complex. The reaction mixture was centrifuged at 10,000 x g for 10 min. The precipitate was dissolved in 5 ml of 2 M concentrated sulphuric acid and recentrifuged. The supernatant was read at 415 nm against blank and H_2O_2 expressed as µmol H_2O_2 g^{-1}f.w.

3.6 Assay of antioxidant enzymes

3.6.1 Enzyme extraction

Samples (40 mg) were crushed into a fine powder in a mortar and pestle with liquid N_2. Soluble protein was extracted by homogenizing the powder in 2 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% (w/v) polyvinyl pyrrolidone, with the addition of 0.2 mM ascorbate, in case of ascorbate peroxidase assay to protect APX activity. The homogenate was centrifuged at 10, 000 × g for 30 min at 4 °C and the supernatant was used for the following assays.

3.6.1.1 Superoxide dismutase (SOD) activity

Superoxide dismutase activity (SOD) (EC 1.15.1.1) was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT) (Dhindsa *et al*, 1981). The 3 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 25 mM NBT, 2 µM riboflavin, 0.1 mM EDTA, 50 mM sodium carbonate and 0.1 mL enzyme extract. The reaction mixture was illuminated for 15 min at a light intensity of 3600 lux. 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.

3.6.1.2 Peroxidase (POX) activity

Peroxidase activity (POX) (EC 1.11.1.7) was measured by monitoring the formation of tetraguaiacol (extinction coefficient 26.6 $mM^{-1}cm^{-1}$) from guaiacol (Rao *et al*, 1996). The POX reaction solution (3 ml) contained 0.5 mM phosphate buffer (pH 6.1), 16 mM guaiacol, 2 mM H_2O_2 and 20 µl enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 30 s using UV visible spectrophotometer.

3.6.1.3 Catalase (CAT) activity

Catalase Activity (CAT) (E.C.1.11.1.6) was determined by following the consumption of H_2O_2 ($\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$) at 240 nm absorbance for 3 min (Aebi, 1984). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 ml of enzyme extract in a volume of 3 ml.

3.6.1.4 Ascorbate peroxidase (APOX) activity

The ascorbate peroxidase activity (APOX) (EC. 1.11.1.11) was estimated by recording the decrease in absorbance at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) 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. 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 (Nakano and Asada, 1981).

3.6.1.5 Glutathione reductase (GR) activity

Glutathione reductase assay is based on the formation of red coloured complex by reduced glutathione with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) (Smith *et al*, 1988). The reaction mixture contains 10 mM potassium phosphate buffer, 0.33 mM EDTA, 0.5 mM DTNB, 2.0 mM NADPH, 20 mM GSSG (Oxidized Glutathione) , 0.1 ml of enzyme extract and double distilled water to make up the final volume to 3.0 ml. Reaction was started by adding 0.1 ml of 20 mM GSSG (oxidized glutathione). Increase in absorbance at 412 nm was recorded in UV-Visible spectrophotometer (Systronics, India).

3.7 Estimation of protein content

Protein content was assayed by Bradford's protein assay (1976). The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to proteins occurs. Standard protein was prepared in the range 5-100 µg/ml and 5 ml Bradford reagent (100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol and 100 ml of 85 % (w/v) phosphoric acid. The mixture was diluted to 1 L and the dye was filtered through Whatman no. 1 paper just before use) was added to standard and sample (100 µl). The mixture was incubated for 5 min and absorbance of the mixture read at 595 nm.

3.8 Determination of protein bound carbonyls

Protein carbonyls were detected spectrophotometrically following the method of (Levine *et al.* 1994) by using Sigma-Aldrich protein carbonylation kit. Seed samples (100 mg) were ground in 1 ml 100 mM phosphate buffer (pH 7.0) and centrifuged at 10,000 x g for 20 min at 4 ^{o}C and the supernatant used for further assay. Supernatants were lyophilized and redissolved in 100 µl of phosphate buffer (100 mM, pH 7.0) and used for the assay. Supernatants in 100 µl volume were mixed with 100 µl of freshly prepared dinitrophenylhydrazine (DNPH) solution, vortexed and incubated for 10 min at 25 ^{o}C. After incubation, proteins were precipitated with 30 µl of pre- cooled 100 % TCA solution, vortexed and incubated on ice for 5 min. The pellet was collected after centrifugation at 13000 × g for 2 min. Pellet was sonicated for 5 min after addition of 500 µl of ice-cold acetone and then incubated at -20 °C for 5 min followed by centrifugation at 13000 × g for 2 min. The pellet obtained after centrifugation was washed two times with acetone to remove free DNPH and then briefly sonicated with 200 µl of 6 M guanidine. The sample (100 µl) was transferred to 96 well plate and absorbance measured at 375 nm. Background control was taken by addition of 100 µl of water in one well. Carbonyl content was calculated using a molar absorption coefficient for aliphatic hydrazones as 22 mM^{-1} cm^{-1} and expressed as nmole carbonyl mg $protein^{-1}$.

3.9 Estimation of hormones by HPLC

3.9.1 Abscisic acid (ABA)

The amount of ABA was determined by HPLC in germinating cherry tomato seeds by following the method of Zeevart, 1980.

3.9.1.1 Preparation of sample

Cherry tomato seeds (40 mg) were collected at different times of imbibition (4, 8, 12, 24 and 36 h), frozen in liquid nitrogen and stored at -80 ^{o}C until further use. Frozen samples were homogenised three times in a total volume of 5 ml of 80 % v/v acetone (80 ml acetone, 1 ml glacial acetic acid and 100 mg of 2, 6 di-tert-butyl 4-methyl phenol in a total volume of 100 ml) and collected in a 10 ml volumetric flask. The homogenate was filtered through Whatman No. 1 filter. The filtrate was transferred to a beaker and flash evaporated in a flash vacuum evaporator for removing the acetone. After the evaporation of acetone from the extract, the lipid soluble material was deposited on the walls of the beaker. The deposit was dissolved in 2 ml of 1% acetic acid solution and the amber coloured aqueous solution collected in HPLC vials. The samples were filtered with 0.22 mm Millipore filter using 2.5 ml plastic syringe before injecting into the HPLC.

3.9.1.2 Estimation of ABA through HPLC

The calibration standard of ABA was prepared in three different concentrations 10, 50 75 and 100 ng/ml and 1,5,10 µg/ml. The standards were included with each group of samples loaded to the HPLC (Agilent 1100 series) at the same time as a control on detector response. The separation was achieved on a μBondapak TM/C18 P/N 841/6 S/N column with a stationary phase consisting of a Lichrosorb C-18 (Agilent, USA). A variable wavelength UV-visible detector was used at wavelength of 270 nm. The elution solvent was methanol and 1 % acetic acid (60:40) with a flow rate of 1 ml/min. A 20 µl sample of the extract was injected into the column and the retention time for ABA and standard was detected on the chromatograph.

Peak area was calculated to determine the concentration of ABA in the samples using ESTD (external standard) quantification procedure. Both calibration, which is the external standard, and extracted samples were analyzed under the same conditions as described previously. The results from the unknown samples were then compared with those of the calibration sample to calculate the amount in the extracted sample. ESTD procedure is practically easy to use, but needs excellent reproducibility in order to get reliable quantification from each run. To compensate for any instrumental drift, check samples were run every 20 sample and calibration curve was repeated for each group run.

3.9.2 Gibberellic acid (GA_3)

Gibberellic acid was estimated by HPLC in germinating cherry tomato seed at different time of imbibition by following the method of Wurst *et al*., 1984

3.9.2.1 Preparation of sample

Cherry tomato seeds (320 mg) were collected at different times of imbibition (4,8,12, 24 and 36 h),frozen in liquid nitrogen and stored at -80 ^{o}C until further use. The frozen samples were homogenized in 10 ml sodium phosphate buffer (50 mM, pH 7.5) containing 0.02% sodium diethyldithiocarbamate. The extract was kept for overnight at 4 ^{o}C in a shaker at 150 rpm and centrifuged the next day at 10,000 x g at 4^{o}C for 10 min. The supernatant was taken and volume made up to 5 ml with sodium phosphate buffer (50 mM, pH 7.5). The solution was partitioned with 2.5 ml diethyl ether in separating funnel and aqueous phase collected. The pH of the aqueous phase was adjusted to 2.5 using 1N HCL and again partitioned twice with 5 ml petroleum ether. The aqueous phase was recollected after partitioning and repartitioned thrice with diethyl ether. After collecting the aqueous phase, the extract was again partitioned twice with 2.5 ml ethyl acetate and ether phase collected. This ether phase was again partitioned twice with 2.5

ml of 0.2 M K_2HPO_4 and aqueous phase collected whose pH was adjusted to 2.5 by using concentrated H_3PO_4. The aqueous phase was later partitioned twice with 5 ml ethyl acetate and ether phase collected which was dried after filtering through a funnel containing sodium sulphate salt on the filter paper. The ethyl acetate extract was dissolved in 2 ml methanol and used for estimation of gibberellins. The samples were filtered with 0.22 mm Millipore filter using 2.5 ml plastic syringe before injecting into the HPLC.

5.6.2.2 Estimation of GA_3 through HPLC

The calibration standard of GA was prepared in eight different concentrations 50, 70, 100, 200, 400, 600, 800, 1000 µg/ml. The standards were included with each group of samples loaded to the HPLC (Agilent 1100 series) at the same time as a control on detector response. The separation was achieved on a µBondapak TM/C18 P/N 841/6 S/N column with a stationary phase consisting of Lichrosorb C-18 (Agilent, USA). A variable wavelength UV-visible detector was used at wavelength of 206 nm. The elution solvent was methanol and HPLC water (35:65) at pH 3 adjusted with phosphoric acid with a flow rate of 1 ml/min. A 20 µl sample was injected into the column and the retention time for GA and standard was detected on the chromatograph. Peak area was calculated to determine the concentration of GA in the samples using ESTD (external standard) quantification procedure. Both calibration, which is the external standard, and extracted samples were analyzed under the same conditions as described previously. The results from the unknown samples were then compared with those of the calibration sample to calculate the amount in the extracted sample. ESTD procedure is practically easy to use, but needs excellent reproducibility in order to get reliable quantification from each run. To compensate for any instrumental drift, check samples were run every 20 sample and calibration curve was repeated for each group run.

3.10 Green house experiment

3.10.1 Nursery establishment

Nursery of cherry tomato was raised in the trays on 5th Sept, 2013 at CPCT, IARI, New Delhi. The seedling tray (pro tray/plug tray) was filled with the growing medium (coco peat, vermiculite and Perlite in a ratio of 3:1:1). A small depression (0.5 cm) was made with fingertip in the center of the cell of the plug tray for sowing. One seed per cell was sown and covered with medium. The trays were filled with slightly moist media. A pinch of vermiculite was added in the individual cells after sowing and the trays shifted to polyhouse. The trays were irrigated lightly every day depending upon the prevailing weather conditions. Drenching with fungicides was done as a precautionary measure to prevent seedling mortality.

3.10.2 Transplanting of seedlings in greenhouse

Seedlings were ready for transplanting after 25 days of sowing in trays (Fig. 3.2). Seedlings of all treatments were transplanted with spacing of 50 x 50 cm (plant x row) in three replicates of 10 plants in each replicate. The plants were drip irrigated at regular intervals throughout the season. The average mean temperature during the growing season was 4 ^{o}C above the ambient and RH 60-70 percent in the polyhouse. The average PAR in the polyhouse was 75% of that observed under the ambient conditions during the crop season.

3.10.3 Harvest and yield components

Cherry tomatoes were harvested at light red/ red stage from different replicates of the treatments. In all, tomatoes were harvested eight times and data on number of fruits per plant and weight of fruits recorded. Average weight of the fruit was calculated from this data and the results have been presented.

3.11 Statistical Analysis

The experimental design was completely randomized design therefore, the data were analyzed by two way analysis of variance (ANOVA) using the software OPSTAT (http://hau.ernet.in/opstat.html). Data was expressed as mean values of the replicates $\pm$ SEM (n= no. of replicates). Least significant difference (LSD) was calculated for each trait at probability level of $P< 0.050$. Values for all enzyme activities are means of 3 replicates. For analysis of yield data, one way analysis of variance (ANOVA) using the software OPSTAT was carried out on three replicates per treatment consisting of eight plants per replicate.

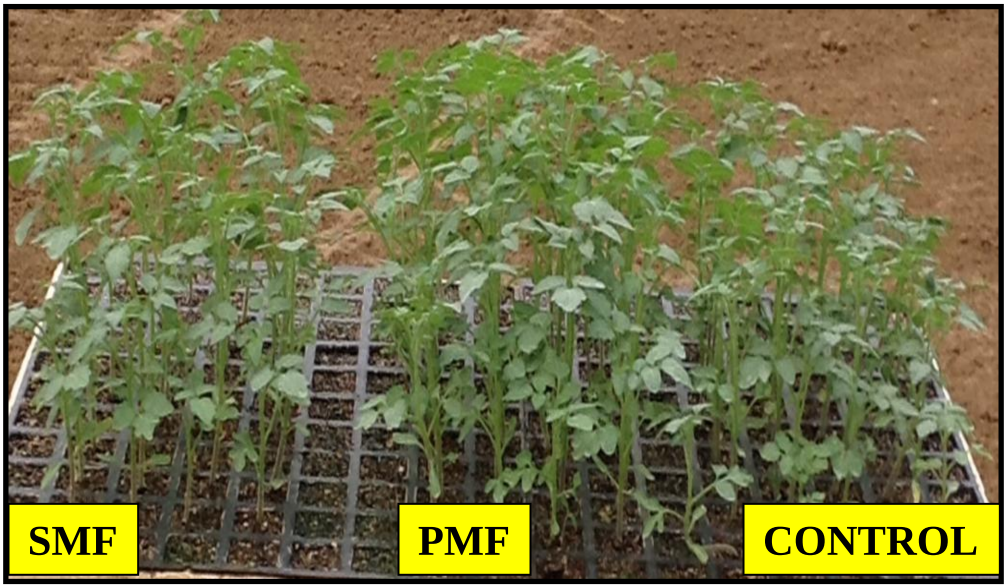

Fig.3.2. Twenty five day old nursery of primed and unprimed seeds of cherry tomato

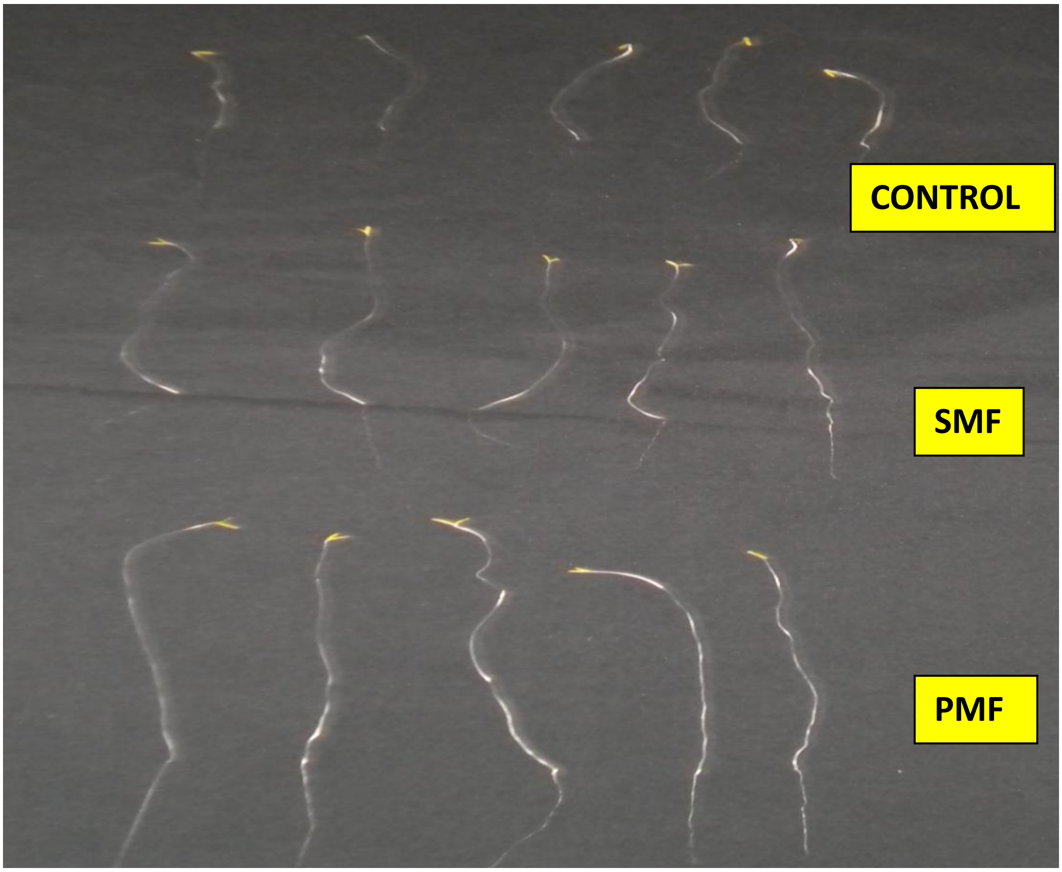

Fig. 4.1. Seedling growth (at 14 days after germination) in primed and unprimed cherry tomato seeds

4. RESEARCH PAPER – I

Static and Pulsed magnetic field exposure improves vigour and yield of cherry tomato

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Abstract

Cherry tomato seeds were magnetoprimed with various doses of static magnetic field (SMF) in the range of 50 – 150 mT for duration of 30 min and 1 h. SMF dose of 100 mT for 30 min gave maximum increase in germination characteristics and was selected and applied as pulsed dose. The seeds were exposed to pulsed magnetic field dose in the cycles of 2, 3, 5 and 6 min on and off and PMF dose of 3 min on and off cycle showed significant enhancement of 23 % in seedling vigour compared to other treatments. These two doses were evaluated for their field performance. Yield increased by 17 % in SMF and 27 % in plants from PMF primed seeds. There was no significant change in the number of fruits and the increase in yield was due to a significant increase in weight per fruit which was 6.4 g in control compared to 7.76 and 8.55 g in SMF and PMF respectively. Our results indicate that pulsed magnetopriming is more effective dry seed priming treatment that can be used for increasing productivity of cherry tomato.

Key words: Cherry tomato, Germination, Vigour index, Yield, Magnetopriming, Rate of germination

4.1 Introduction

Globalization has brought about changes in the living standards and living style of people, most noticeable being the change is the food habits among the new generation. As a consequence, demand-driven exotic vegetables production is increasing in India at the rate of 15 to 20% per annum for self sufficiency as approx. 85% is being imported from other countries. Tomato fruit is the most important vegetable world over, for table purpose as well as for processing. It is available throughout the year and is a rich source of energy, carotenoids, flavonoids, phenolics, mineral nutrients, vitamin C and dietary fibres which are beneficial and serve as protective ingredients for human health (Wold *et al.* 2004). Cherry tomato is a small garden variety of tomato that has very high nutritive value, containing vitamin A, lycopene, beta-carotene, vitamin C and minerals like calcium, potassium etc. The cultivation of cherry tomato is a highly economical venture for the farmers as they have assured market through contract with national and international retail chains. However, under reducing resources and increasing cost of vegetable, the major challenge lies ahead to develop technologies that enhance quality and productivity of vegetables. Crop yields can be maximized by establishment of an adequate and uniform plant stand. Rapid and uniform field emergence of seedlings is therefore a challenge to as slow emergence results in smaller plants and seedlings which are more vulnerable to soil- borne diseases thus leading to decline in productivity.

Seed priming is a physiological seed enhancement method for overcoming poor and erratic seed germination in many vegetable and flower species. Seed priming techniques like hydropriming (soaking in water), osmopriming (soaking in solutions of different organic osmotica), solid matrix priming and halopriming (soaking in inorganic salt solutions) are being experimented extensively to increase the speed and synchrony of seed germination of vegetable and field crops (Nakaune *et al.* 2012). The pre-sowing seed treatment with magnetic field called “magnetopriming” is a dry seed priming technique that doesn’t hydrate the seed, allows storage of the seed at room temperature and is eco-friendly. Other priming treatments have a mandatory requirement of dehydrating the seed for storage before sowing but magnetopriming is carried out in dry seed and proves to be more useful. Several researchers have reported the beneficial effect of both static and pulsed magnetic fields in different plant species (Anna 2002; Fischer *et al.* 2004; Florez *et al.* 2007, Shine *et al.,* 2011, Bhardwaj *et al.,* 2012; Radhakrishnan and Ranjitha Kumari, 2012, Bilalis *et. al.*, 2012; 2013). The aim of the study was to optimize the static and pulsed magnetic field dose for seed invigouration in cherry tomato and evaluate its field performance.

4.2 Materials and Methods

Seed of cherry tomato (Selection 1) were obtained from CPCT, Indian Agricultural Research Institute, New Delhi and used for the study.

4.2.1 Electromagnetic field generator

An electromagnetic field generator "Testron EM-60" with variable magnetic field strength (50 to 300 mT) with a gap of 10 cm between pole pieces was used for seed treatment. The pole pieces were 15 cm in diameter and 35 cm in length. The number of turns per coil was 1600 and the resistance of the coil was 16 ohm. A DC power supply (80V/ 10A) with continuously variable output current was used for the electromagnet. A digital gauss meter model DGM-30 operating on the principle of Hall Effect, monitored the field strength produced in the pole gap. The probe was made of Indium Arsenide crystal and was encapsulated to a non- magnetic sheet of 5 mm × 4 mm × 1 mm and could measure 0-2 Tesla with full-scale range in increments of 5 mT. At low field strength (50 mT), width wise variation of magnetic field strength from centre to periphery was 0.6 and height wise 1.6% of the applied field. At high field (300 mT), they were 0.4 and 1.2% of the applied field, respectively. The local geomagnetic field was less than 6 mT and the direction of local geomagnetic field was north to south.

4.2.2 Optimization of static and pulsed magnetopriming dose for seed invigouration.

4.2.2.1 Static magnetic field

Cherry tomato seeds were exposed to static magnetic field of 50-150 mT in increments of 50 mT for varying durations of 30 min and 1 h, by placing in a cylindrical-shaped sample holder of 42 cm^3 capacity, made of a non-magnetic thin transparent plastic sheet. Three hundred visibly sound, mature, healthy seeds held in the plastic container were placed between the poles of the electromagnet having a uniform magnetic field. The required strength of the magnetic field was obtained by regulating the current in the coils of the electromagnet. A gauss meter was used to measure the strength of the magnetic field between the poles. The variation in temperature during the course of seed exposure was 25 ± 0.5^{o}C. For parallel control, seeds from the same lot used for magnetic field exposure were kept under conditions which had no influence of the induced magnetic field, as magnetic field generator was housed in a separate chamber. The field strength and duration was standardized for maximum enhancement of germination and vigour under laboratory conditions and best combination was selected for standardization in the pulsed mode.

4.2.2.2 Pulsed magnetic field

Among all the treatments in the static mode, 100 mT for 30 min was most effective in enhancing the germination parameters in magnetoprimed seeds and was further employed for treating seeds in pulsed mode. Four pulsed doses (the number of times the sample was exposed to magnetic field in 30 min) were designed for PMF treatment with 100 mT (30 min) applied in the cycle of 2, 3, 5 and 6 min on and off.

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The germination of the seeds was determined by using “between papers” method given by ISTA (1985). One hundred seeds in four replications of 25 seeds each were placed between two layers of moist germination papers. The germination papers were rolled carefully ensuring that no excess pressure was placed on the seeds. These were wrapped in a sheet of wax paper to reduce surface evaporation of moisture and placed in the germination incubator at 25°C in an upright position. After 14 days, seeds were evaluated for normal, abnormal seedling, un-germinated and dead seeds.

4.2.4 Rate of germination

Seeds were placed in Petri dish on a double layer of moistened filter paper and incubated in darkness in a germinator at 25°C. A daily germination count of the incubated seeds was taken until no more seeds germinated, and the speed of germination was calculated following Maguire (1962) as

$$\text{Speed of germination} = \sum \left({}^{\mathrm{n}}/_{\mathrm{t}} \right)$$

Where n = number of seeds germinated and t = time of count

$$\sum\left({}^{n}/_{t}\right) = \frac{\text{Number of seeds germinated}}{\text{Day of the first count}} + \cdots + \frac{\text{Number of seeds germinated}}{\text{Day of the final count}}$$

4.2.5 Seedling growth and Vigour Indices

Ten normal seedlings from each replicate kept for germination, were randomly selected to measure shoot and root length in cm. They were subsequently dried at 80°C in an oven and weighed together till a constant weight was obtained.

Seedling vigour was calculated following Abdul-Baki and Anderson [1973] as

$$\text{Vigour index I} = \text{Germination\%} \times \text{Seedling length (Root + Shoot)(cm)}$$

$$\text{Vigour index II} = \text{Germination \%} \times \text{Seedling dry weight(Root + Shoot)(g)}$$

From the results of the optimization magnetic dose, it was observed that germination and related characteristics showed enhanced performance in the static magnetic field of **100 mT for 30 min** and **3 min on and off pulsed dose**.

4.3 Results

The results of optimization studies for improving seed vigour with static and pulsed magnetic field exposure are presented in **Table 1a and 1b** respectively. Graphs are drawn for individual parameters for depiction of data individually. The photograph showing seedling growth is presented in Fig. 4.1

4.3.1. Germination percentage

Static magnetic field exposure did not result in any significant change in the germination percentage of cherry tomatoes although SMF dose of 100 mT (30 min) resulted in 5 % increase compared to unprimed control (Fig 4.2 a).

On the basis of marginal increase observed under SMF exposure, 100 mT (30 min) was given as pulsed dose for 2, 3, 5, 6 min to cherry tomato seeds. Germination

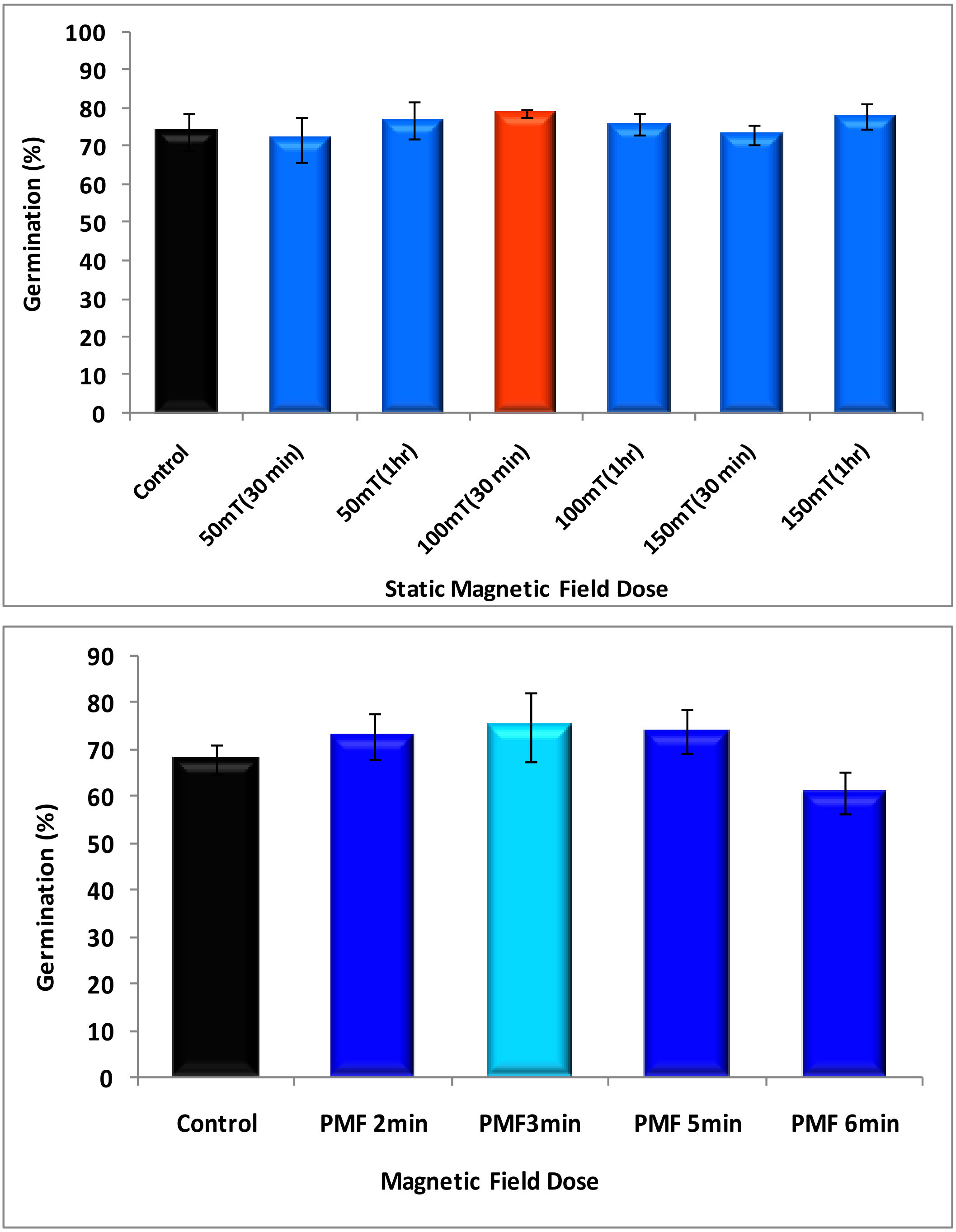


Fig 4.2. Effect of (a) Static and (b) Pulsed magnetic field on germination of cherry tomato seeds. Vertical line above the bar indicates ± S.E.M (n=4). LSD ($P < 0.05$) SMF= NS; PMF=NS

percentage was not significantly affected although there was 6% increase at PMF (3 min on/off) dose (Fig 4.2 b).

4.3.2. Rate of germination

Rate of germination showed insignificant increase by 8 - 16% at lower static magnetic field dose of 50- 100 mT for varying duration. The maximum rate of germination was recorded at SMF dose of 100 mT for 30 min duration (Fig 4.3 a). Rate of germination was adversely affected in MF dose between 100 mT (1h) – 150 mT (1h) with rate of germination falling below the rate observed in untreated control.

The 100 mT (30 min) dose applied as pulsed dose for 2, 3, 5, 6 min showed the maximum increase (21 %) in rate of germination at 3 min on and off pulse in comparison to untreated control (Fig 4.3 b).

4.3.3. Root and shoot length

SMF exposed seeds did not show a significant change in root length but shoot length was significantly higher. The shoot length increased by 9-20 % at varying doses of SMF compared to untreated control (Table 1a).

On the other hand, pulsed magnetic field treatment showed a significant increase in root length. Root and shoot length increased by 21 and 18 % respectively at PMF (3 min on and off (Table 1b).

Dry weight of ten randomly selected seedlings increased by 13 % in SMF dose of 100 mT (30 min) compared to unprimed control. Similarly, 19 % increase in seedling dry weight was observed in pulsed magnetic field dose applied in 3 min pulses.

4.3.5. Vigour indices

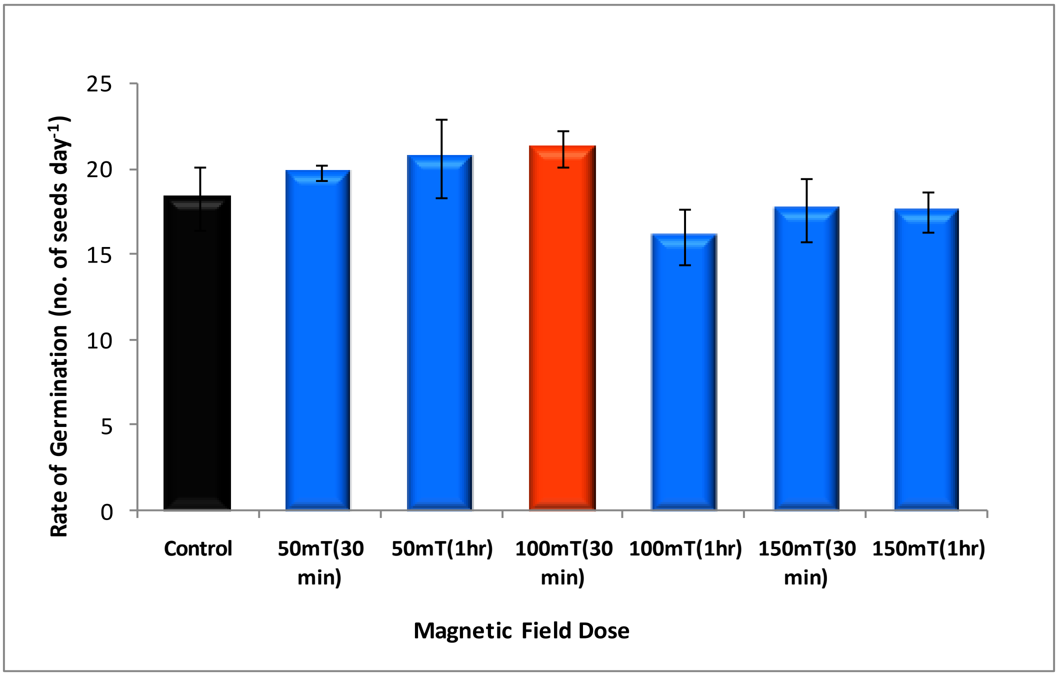
The static magnetic field strength and its duration had positive effect on vigor indices. Vigor index I increased in the range of 10-24 % and vigor index II in 9- 37% among the various treatments. Seeds treated with 100 mT (30 min) recorded the highest increase of 24 % in vigor index I (Fig.4.4 a) and 37 % in vigor index II (Fig.4.5 a). Increase in vigour was less when SMF dose was increased to 150 mT for 30 min and 1h.

Table 1a: Effect of static magnetic field on germination characteristics of cherry tomato

Magnetic field dose	Germination %	Root length (cm)	Shoot length (cm)	Seedling Dry wt. (mg)	Vigour Index I	Vigour Index II	Rate of germination (no. day^{-1})
50 mT (30 min)	72 .0 ± 5.89	4.20 ± 0.21	12.24 ± 0.22	5.40 ± 0.92	1,184.20 ± 97.18	0.402 ± 0.087	19.70 ± 0.32
50 mT (1h)	77.0 ± 5.00	4.00± 0.24	11.58± 0.36	6.25 ± 0.96	1,202.93 ± 80.43	0.484 ± 0.085	20.50 ± 0.08
100 mT (30 min)	79.0± 1.00	4.23 ± 0.34	11.30 ± 0.33	6.12 ± 0.36	1,222.99 ± 48.59	0.485 ± 0.033	21.20 ± 0.75
100 mT (1h)	76.0 ± 2.83	3.90 ± 0.28	11.37 ± 0.17	5.80 ± 0.32	1,160.88 ± 67.21	0.442 ± 0.037	16.03 ± 1.14
150 mT (30 min)	73.0 ± 2.49	3.69 ± 0.19	11.09 ± 0.37	5.82 ± 0.35	1,085.13 ± 96.56	0.415± 0.025	17.64 ± 1.30
150 mT (1h)	78.0 ± 3.46	4.20 ± 0.09	11.13 ± 0.34	4.95 ± 0.35	1,202.12 ± 64.47	0.388 ± 0.040	21.00 ± 0.82
Control	74.0 ± 4.46	3.42 ± 0.11	10.20 ± 0.36	4.87 ± 0.34	984.61 ± 47.64	0.380 ± 0.023	18.35 ± 1.20
C.D (P<0.05)	NS	NS	0.946	NS	NS	NS	3.114

Table 1b: Effect of pulsed magnetic field on germination characteristics of cherry tomato

Magnetic field dose	Germination %	Root length (cm)	Shoot length (cm)	Seedling Dry wt. (mg)	Vigour Index I	Vigour Index II	Rate of germination (no. day^{-1})
PMF (2 min)	73.0 ± 5.00	3.39 ± 0.15	10.39 ± 0.65	7.60 ± 0.59	1,126.5 ± 53.2	0.549 ± 0.03	21.15 ± 0.84
PMF (3 min)	75.0 ± 7.19	4.12 ± 0.14	10.31 ± 0.29	9.07 ± 1.06	1,169.73 ± 14.85	0.660 ± 0.04	24.35 ± 0.43
PMF (5 min)	74.0 ± 4.76	3.65 ± 0.09	11.26 ± 0.11	8.85 ± 0.44	978.40 ± 35.15	0.650 ± 0.05	22.45 ± 0.22
PMF (6 min)	61.0 ± 4.43	3.19 ± 0.19	8.83 ± 0.96	8.65 ± 0.56	836.29 ± 113.37	0.520 ± 0.01	22.43 ± 1.25
Control	68.0 ± 3.26	3.00 ± 0.09	9.51 ± 0.51	7.84 ± 0.63	849.54 ± 6.44	0.530 ± 0.04	22.02 ± 0.34
C.D (P<0.05)	NS	0.508	NS	NS	NS	0.105	NS



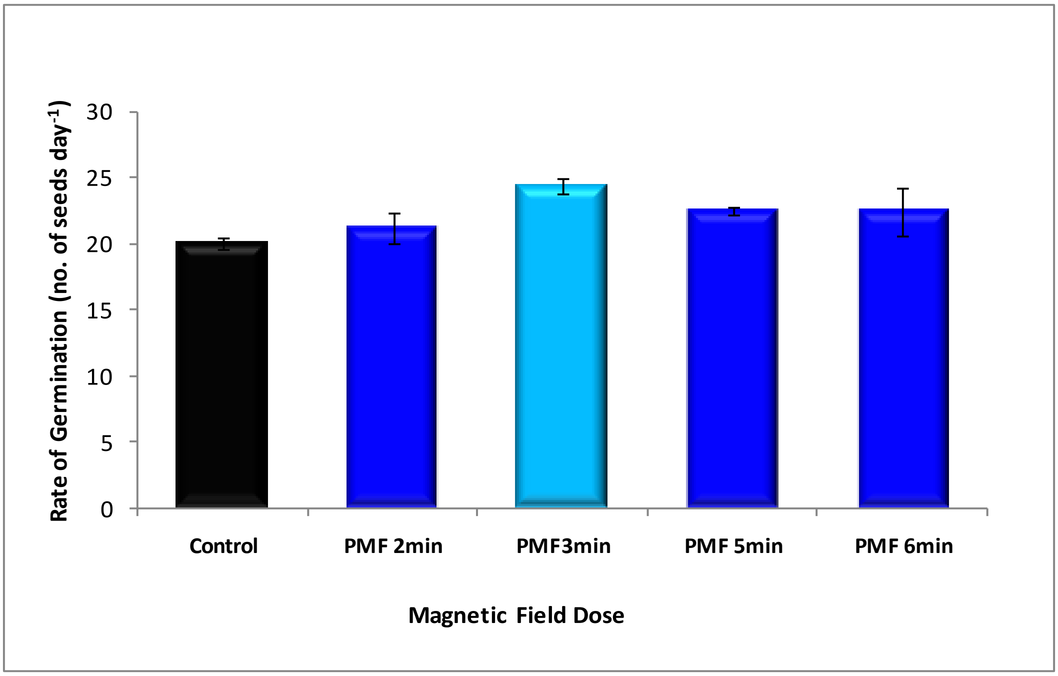


Fig 4.3. Effect of (a) Static and (b) Pulsed magnetic field on rate of germination in cherry tomato seeds. Vertical line above the bar indicates ± S.E.M (n=4). LSD ($P < 0.05$) SMF= 3.114; PMF=NS

Analysis of vigour indices in PMF treated seeds showed that PMF (3 min on and off) resulted in 20 and 23 % increase in Vigour Index I and II respectively over the untreated control (Fig. 4.4 b and 4.5b). PMF (6 min on and off) dose adversely affected the vigour indices as both the values became less than unprimed control.

Based on the results of all the germination related characteristics tested in **Static** and **Pulsed** magnetoprimed and unprimed seeds, SMF dose of **100 mT (30 min)** and PMF dose of **100 mT (30 min) applied as 3 min on and off cycle** was selected for evaluating the field performance and yield of the crop. The selected SMF and PMF dose was also used for ROS homoeostasis and its interaction with hormone signalling presented in **Chapter 5**

4.3.6 Yield components

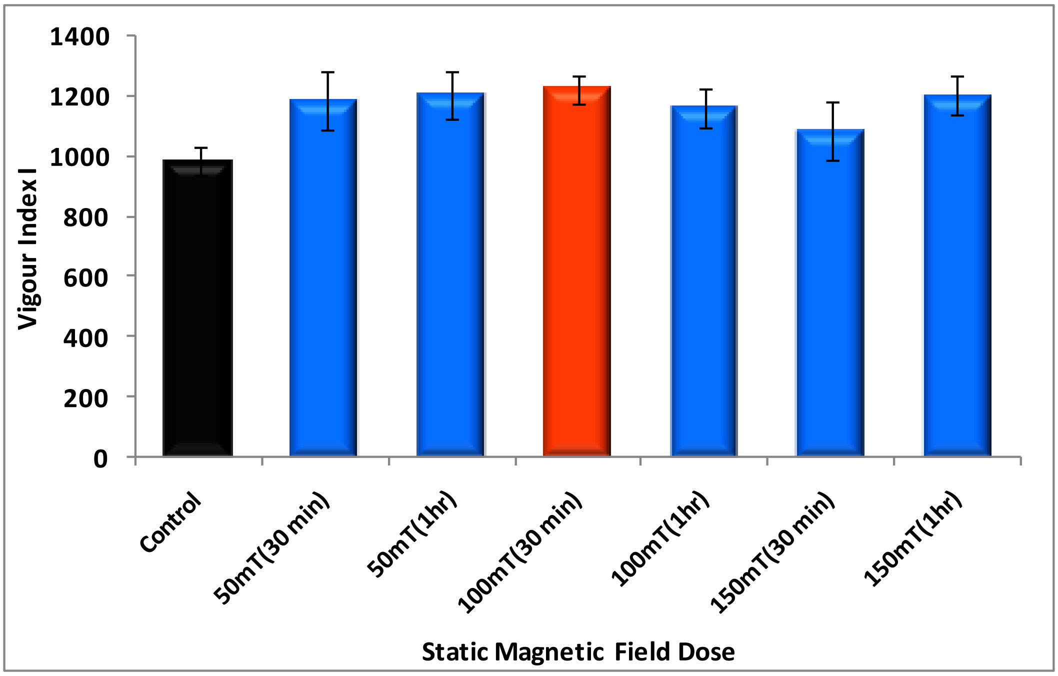
Flowering started at 22-26 days after transplanting in plants from pulsed and static magnetic field treatment which was 7-9 earlier than plants from unprimed control (Fig.4.6). Initiation of fruiting was at 41-43 and 42-45 days after transplanting in plants from pulsed and static magnetic field treatment which was 6 - 9 earlier than plants from unprimed control (Fig.4.7). The fruits were harvested at the light red/red stage (Fig. 4.8) starting from 97 days (1st week of January, 2014) after transplanting. Nine harvests were taken in all that continued uptil 16th April 2014. No significant difference was found between fruit number amongst the various treatments although there was 8-10 % reduction in number of fruits per square meter in fruits from SMF and PMF treated seeds compared to control. The average yield per square meter showed a non significant increase of 17 % in fruits from SMF treated seeds. However a significant increase of 27 % was observed in plants from PMF treated seeds (Table 2). Though the number of fruits was higher in control plants but the average fruit size was less than fruits from magnetoprimed plants. It was observed that average fruit weight was 6.4 g in control compared to 7.76 and 8.55 g in SMF and PMF respectively (Table 2).

Table 2. : Average number and yield of cherry tomato from plants magnetoprimed with static and pulsed magnetic field. Data are means of 3 replicate ± SE. Each replicate consists of eight plants

Treatment	Average no.of fruits plant^{-1}	Average yield of fruits plant^{-1} (kg)	Average no. of fruits m^{-2}	Average yield (kg m^{-2})	Average weight fruit^{-1} (g)
Control	197.2 ± 31.9	1.183 ± 0.076	788.8 ± 127.8	4.730 ±0.305	6.40± 0.40
SMF (100mT 30 min)	180.4 ± 10.3	1.391 ±0.059	721.7 ± 41.3	5.562 ± 0.237	7.76±0.53
PMF (3 min)	176.38 ± 10.2	1.503 ± 0.124	705.5 ± 41.0	6.012 ± 0 .495	8.55±0.51
CD (P< 0.05)	NA	0.217	NA	0.869	1.7

Table 3. Economic return in cherry tomato obtained due to higher yield from pulsed magnetopriming

Crop	Additional cost for seed treatment (Rs. 40/kg)	Yield (kg/100 m^2)		Additional yield (kg/100 m^2)	Additional return (Rs/100 m^2)	Economic return (Rs/Re invested for priming)
		Control	**PMF**			
Cherry tomato	**40**	**473.0**	**601.0**	**128**	**6400**	**160**



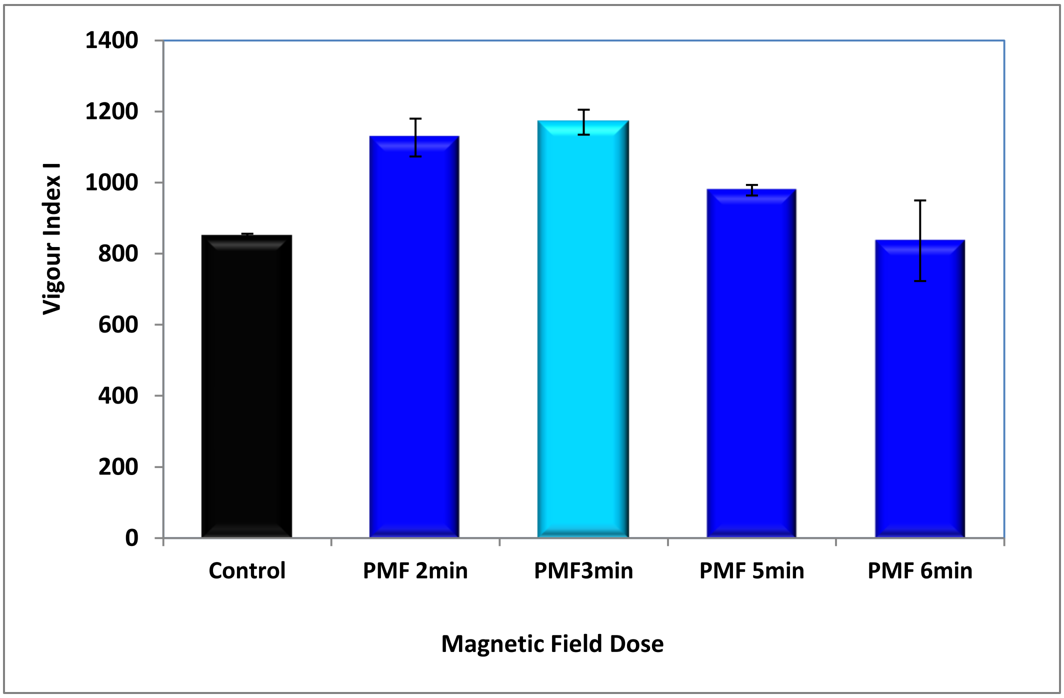


Fig 4.4 Effect of (a) Static and (b) Pulsed magnetic field on Vigour Index I of cherry tomato seeds. Vertical line above the bar indicates ± S.E.M (n=4). LSD ($P < 0.05$) SMF= NS; PMF=NS.

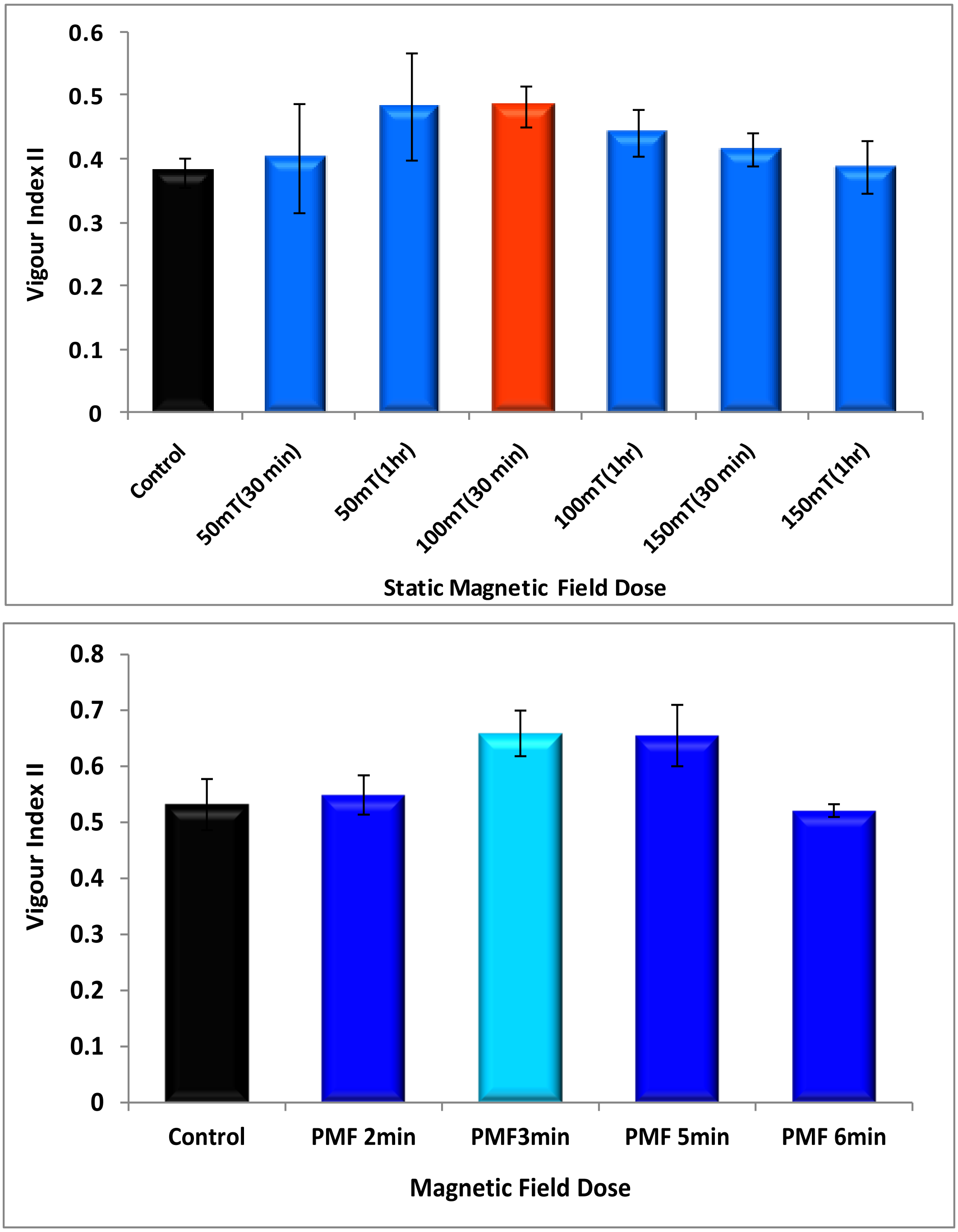


Fig 4.5 Effect of (a) Static and (b) Pulsed magnetic field on Vigour Index II of cherry tomato seeds. Vertical line above the bar indicates ± S.E.M (n=4). LSD ($P < 0.05$) SMF= NS; PMF=0.105

Fig. 4.6 Plants from Pulsed Magnetic Field treatment (left row) showing early flowering compared to control (right row)

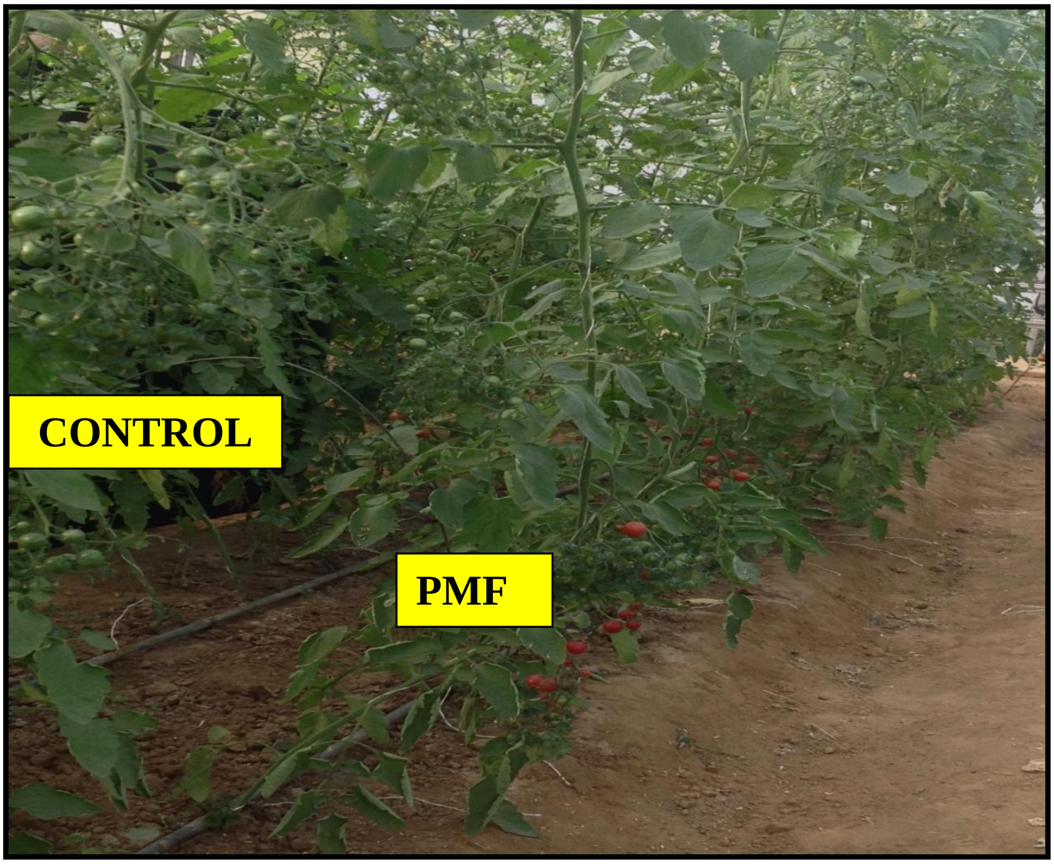


Fig. 4.7 Plants from Pulsed Magnetic Field treatment (right row) showing early fruit set compared to control (left row)

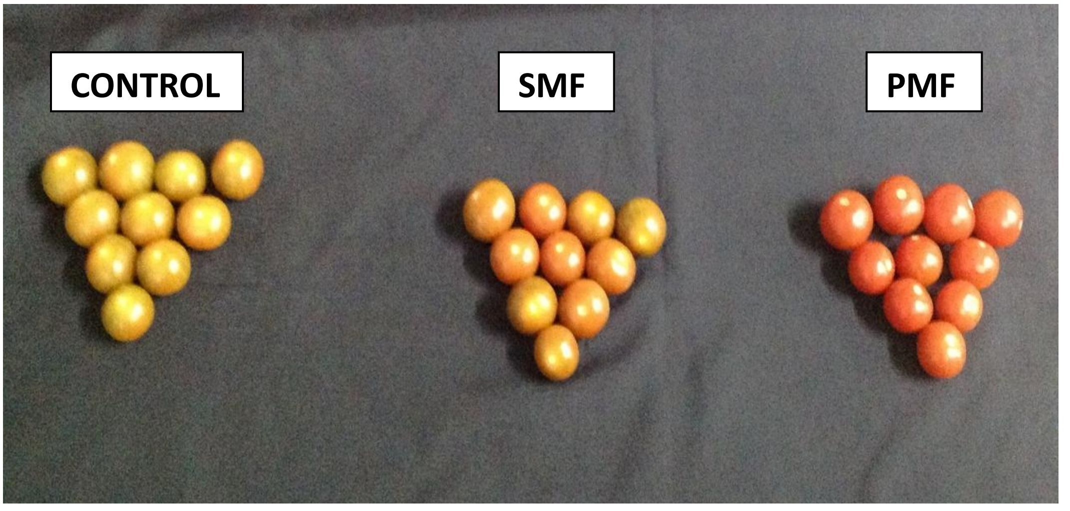


Fig.4.8 Different ripening stage in fruits of plants from magnetoprimed and unprimed seeds harvested at the same time.

4.4. Discussion

Seed forms a critical input for improving productivity in horticultural crops and with increasing cost of hybrid seeds, a plant from each seed sown is desirable. Rapid germination and emergence is an important factor for successful establishment of a plant. Pre-sowing physical and chemical treatments for seed priming are known to improve performance of seeds in the field. These treatments are successfully applied either to poor germinating seed lots or to seeds which are sown under different stress conditions. Magnetic treatment of seeds (magnetopriming) is one of the non invasive treatments that enhances the performance of many crop plants. It enables the 'poor' seed in the seed lot to catch up with development as expected in a 'good' seed. Several researchers have reported the beneficial effect of priming with magnetic fields (Martínez *et al.,* 2009; Dominguez *et al.,* 2010; Shine *et al.,* 2011; Bhardwaj *et al.,* 2012; Bilalis *et al.,* 2013). Our results demonstrated that magnetopriming of the seed with 100 mT (30 min) static and pulsed dose of **100 mT (30 min) applied as 3 min on and off cycle** enhanced all germination associated characteristics in cherry tomato. This observation suggested that there may be a resonance like phenomena which increases the internal energy of the seed that occurs at an appropriate combination of magnetic field and exposure time. Many models have been proposed to provide a theoretical understanding of the mechanism of magnetoreception in plants. Studies have revealed that phytoferritin occur in plant cells as crystalline magnetite (Fe_3O_4), ε-Fe_2O_3 and hematite (α- Fe_2O_3) (McClean *et al.*, 2001) and may interact strongly with the magnetic fields than do diamagnetic or paramagnetic materials. These particles can also affect the superoxide generated free radicals (Scaiano *et al.,* 1997). Enzyme catalysed reactions that involve intermediates with radical pairs (Grissom, 1995) and some porphyrins that form free radicals during mitochondrial respiratory chain may also be influenced by external magnetic field as it affects singlet-triplet conversions The radical pair mechanism (RPM) is currently the only physically plausible mechanism indicating the role of cryptochrome as a candidate for magnetoreception that results in generation of flavin-trytophan radical pairs (Occhipinti *et al.,* 2014).

Seed invigoration brought about by SMF and PMF exposure of the seeds can lead to enhancement of growth of plants during the vegetative phase. This may lead to early commencement of reproductive phase in vegetable crops as also reported in cucumber (Bhardwaj, 2012). Dayal and Singh (1986) found increase in height and number of primary branches when tomato seeds were exposed to MF varying from 15 to 155 mT. There were increased number of secondary branches and yield in safflower (Faquenabi *et al*, 2009) and broad bean (Podlensy *et al*, 2005) after treatment with MF. The effect of a permanent MF persisted in the Hill reaction of seedlings and in the mature plants of pea (Tarakanova, 1978). Magnetic field treatment of seeds have a long lasting stimulatory effect on plants as higher performance index for photosynthesis contributed to higher efficiency of light harvesting that consequently increased biomass in plants from treated seeds (Shine *et al*, 2012).

Similar to previous reports on crop yields from MF treated seeds, our study also showed an increase of 17 and 27 % in plants from SMF and PMF treated seeds respectively. Increased yield in tomato exposed to full-wave rectified sinusoidal non-uniform MFs induced by an electromagnet at 100 mT (rms) for 10 min and at 170 mT (rms) for 3 min was observed by De Souza *et al*. (2006). In the vegetative stage, the treatment led to a significant increase in the leaf area, leaf dry weight, and specific leaf area per plant consequently resulting in higher biomass. The earliness in flowering and fruit setting observed in magnetoprimed seeds can be exploited to the benefit of farmers as it can fetch a good market value for the seasonal horticultural crops by supplying fruits early. The economic return after treating the cherry tomato seeds with pulsed magnetic field so as to achieve an increased yield of 27% shows it to be very renumerative for the farmers (Table 3).

4.5 Conclusion

Static and pulsed magnetic field treatment can be used for improving the productivity of cherry tomato under controlled environment. Pulsed magnetic field treatment yields a better response and can be very renumerative and ecofriendly seed enhancement technology for yield improvement of cherry tomatoes.

RESEARCH PAPER – II

Oxidative signaling and hormone interaction during germination in magnetoprimed cherry tomato seeds

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Abstract

Cherry tomato seeds were exposed to static magnetic field dose of 100 mT (30 min) and pulsed magnetic field dose of 100 mT (30 min) applied in a cycle of 3 min on and off. Dynamics of seed water uptake showed an increase in uptake in pulsed primed seeds compared to static and untreated control. A maximum two fold increase was recorded in superoxide ion and hydrogen peroxide production in magnetoprimed seeds in both the doses over the control. Antioxidant enzyme system revealed that increased superoxide dismutase activity and oxidative reaction of peroxidase resulted in production of hydrogen peroxide during germination of magnetoprimed seeds. Increased ascorbate peroxidase activity during initial stages and catalase in later stages of germination helped in scavenging of hydrogen peroxide in germinating magnetoprimed seeds. Protein carbonyl content also increased in primed seeds to fine tune the levels of ROS and help in mobilization of seed reserve protein. The interplay of ROS with hormones suggested that GA signaling may lead to GA biosynthesis thus helping completion of germination. Pulsed magnetoprimed seeds showed better response in terms of invigouration and ROS related parameters than static magnetoprimed seeds.

Key words: Antioxidant enzymes, Abscisic acid, Cherry tomato, Gibberellic acid, Magnetopriming, Protein carbonylation, Seed water uptake

5.1 Introduction

Strategies for improving the growth and development of crop species have been investigated for many years. Seed priming, the controlled hydration and dehydration of seeds, is used to increase the rate and uniformity of seedling establishment of commercial vegetable and flower seeds (Bradford, 1986; Khan, 1992). Brocklehurst and Dearman (1983) found that seed priming improved the mean germination times in carrot (*Daucus carota* L.), celery (*Apium graveolens* L.), and onion (*Allium cepa* L.) seeds, but there were differences in response among species and seed lots with the slowest-germinating seed lots showing the largest improvements.

Priming treatments widely used in horticultural crops are hydropriming, osmopriming, halopriming and solid matrix priming. Magnetopriming (magnetic field priming) is a non invasive technique to improve the germination, vigour and yield in many field crops (Florez *et al.*, 2007; Bhardwaj *et al.*, 2012; Bilalis *et al.*, 2012; Bilalis *et al.*,2013). The stimulation in seedling growth due to magnetopriming is mediated through the production of reactive oxygen species (ROS) in the germinating soybean seed (Shine *et al.*, 2012). Increased level of free radicals in seeds after treatment of seeds with laser light and magnetic field accompanied by enhanced dynamics of seed germination have also been reported in faba bean and pea seeds (Podlesny *et al.*, 2001; 2005). Thus, ROS play a key signaling role in the achievement of major events of seed life, such as germination or dormancy release. ROS such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) are generated as a result of aerobic metabolism in mitochondria, peroxisomes and the apoplastic space in the germinating seeds. For ROS to act as cellular messengers, seeds have evolved specific ROS removing mechanisms to protect against the over production of ROS. There is a fine balance between the generation and scavenging of ROS by the up regulation of antioxidant defense system to prevent the oxidative damage. Protein carbonylation by ROS results in oxidative modification of proteins. Furthermore, the interaction of ROS with the ABA and gibberellin signaling pathways has been shown in various plant physiological processes, which might be very relevant to seed germination, since these hormones are known as major players in the regulation of seed germination (El-Maarouf-Bouteau and Bailly, 2008; Finkelstein *et al.* 2008). The present study was conducted to deduce a relationship between the ROS homeostasis maintained by antioxidative metabolism and protein carbonylation during the germination in static and pulse magnetoprimed seeds of cherry tomato. We also analyzed the hormone profile (ABA and GA) in germinating magnetoprimed seeds to study the interplay of hormones with ROS during germination.

5.2 Materials and Methods

Seed of cherry tomato (Selection 1) were obtained from CPCT, Indian Agricultural Research Institute, New Delhi and used for the study.

Cherry tomato seeds were exposed to static magnetic field of **100 mT for 30 min** and **pulsed dose** of 100 m T applied as **3 min on and off** for 30 min, by placing in a cylindrical-shaped sample holder of 42 cm^3 capacity, made of a non-magnetic thin transparent plastic sheet. Three hundred visibly sound, mature, healthy seeds held in the plastic container were placed between the poles of the electromagnet having a uniform magnetic field. The required strength of the magnetic field was obtained by regulating the current in the coils of the electromagnet. A gauss meter was used to measure the strength of the magnetic field between the poles. The variation in temperature during the course of seed exposure was $25 \pm 0.5^{o}C$. For parallel control, seeds from the same lot used for magnetic field exposure were kept under conditions which had no influence of the induced magnetic field, as magnetic field generator was housed in a separate chamber.

5.2 .1 Seed water uptake

The water uptake by seeds during imbibition in double distilled water was determined in triplicate, each replicate having 80 mg seeds. Seeds were removed from the Petri plates at different time intervals, blotted dry and instantly weighed. The changes in weight due to imbibition were expressed as the amount of water absorbed by the seeds per dry weight which was calculated by the following formula.

$$\text{WU\%} = \frac{(\text{Fresh weight of seed} - \text{Dry weight of seed})}{\text{Dry weight of seed}} \times 100$$

Where, WU stands for water uptake

5.2.2 Determination of superoxide ($O_2^{\cdot -}$)

Superoxide free radical was quantified by its capacity to reduce nitroblue tetrazolium chloride (NBT) in primed and unprimed seeds following the method of Chaitanya and Naithani (1994). Seed (40 mg) was homogenized in 2 ml of pre cooled phosphate buffer (0.2 M, pH 7.2). The homogenate was centrifuged at 10, 000 x g for 10 min at 4^oC. Supernatant was used for estimation of superoxide anion. A 3 ml reaction mixture contained 100 µl supernatant in 0.75 mM NBT, 25 mM sodium carbonate, 0.1 mM EDTA and 13.3 mM L- methionine. Reaction mixture was incubated at 30 ^oC in a water bath for 10 min and the absorbance was recorded at 540 nm in a spectrophotometer. Superoxide anion content was calculated using a molar absorption coefficient of 12.8 mM^{-1} cm^{-1} and expressed as µmole g $^{-1}$ fresh weight.

5.2.3 Determination of hydrogen peroxide (H_2O_2)

Hydrogen peroxide was estimated by formation of titanium-hydro peroxide complex (Mukherjee and Choudhari, 1983). Seeds (40 mg) were ground in 3 ml cooled acetone in a chilled mortar and pestle kept in ice bucket. The homogenate was filtered through Whatman No. 1 filter paper followed by addition of 2 ml of titanium reagent and 2.5 ml of ammonium hydroxide solution to precipitate the titanium–hydro peroxide complex. The reaction mixture was centrifuged at 10,000 x g for 10 min. The precipitate was dissolved in 5 ml of 2 M concentrated sulphuric acid and recentrifuged. The supernatant was read at 415 nm against blank and H_2O_2 expressed as µmol H_2O_2 g^{-1}f.w.

5.3 Assay of antioxidant enzymes

5.3.1 Enzyme extraction

Samples (40 mg) were crushed into a fine powder in a mortar and pestle with liquid N_2. Soluble protein was extracted by homogenizing the powder in 2 ml of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% (w/v) polyvinyl pyrrolidone, with the addition of 0.2 mM ascorbate, in case of ascorbate peroxidase assay to protect APOX activity. The homogenate was centrifuged at 10, 000 × g for 30 min at 4 °C and the supernatant was used for the following assays.

5.3.1.1 Superoxide dismutase (SOD) activity

Superoxide dismutase activity (SOD) (EC 1.15.1.1) was assayed by monitoring the inhibition of photochemical reduction of nitrobluetetrazolium (NBT) (Dhindsa *et al*, 1981). The 3 ml reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 25 mM NBT, 2 µM riboflavin, 0.1 mM EDTA, 50 mM sodium carbonate and 0.1 mL enzyme extract. The reaction mixture was illuminated for 15 min at a light intensity of 3600 lux. 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.

5.3.1.2 Peroxidase (POX) activity

Peroxidase activity (POX) (EC 1.11.1.7) was measured by monitoring the formation of tetraguaiacol (extinction coefficient 26.6 $mM^{-1}cm^{-1}$) from guaiacol (Rao *et al*, 1996). The POX reaction solution (3 ml) contained 0.5 mM phosphate buffer (pH 6.1), 16 mM guaiacol, 2 mM H_2O_2 and 20 µl enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 30 s using UV visible spectrophotometer.

5.3.1.3 Catalase (CAT) activity

Catalase Activity (CAT) (E.C.1.11.1.6) was determined by following the consumption of H_2O_2 ($\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$) at 240 nm absorbance for 3 min (Aebi *et al.*, 1984). The reaction mixture contained 50 mM phosphate buffer (pH 7.0), 10 mM H_2O_2 and 0.1 ml of enzyme extract in a volume of 3ml.

5.3.1.4 Ascorbate peroxidase (APOX) activity

The ascorbate peroxidase activity (APOX) (EC. 1.11.1.11) was estimated by recording the decrease in absorbance at 290 nm ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$) 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. 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 (Nakano and Asada, 1981).

5.3.1.5 Glutathione reductase (GR) activity

Glutathione reductase assay is based on the formation of red coloured complex by reduced glutathione with 5,5-dithiobis-2-nitrobenzoic acid (DTNB) (Smith *et al*, 1988). The reaction mixture contains 10 mM potassium phosphate buffer, 0.33 mM EDTA, 0.5 mM DTNB, 2.0 mM NADPH, 20 mM GSSG (oxidized glutathione) , 0.1 ml of enzyme extract and double distilled water to make up the final volume to 3.0 ml. Reaction was started by adding 0.1 ml of 20 mM GSSG (oxidized glutathione). Increase in absorbance at 412 nm was recorded in UV-Visible spectrophotometer (Systronics, India).

5.4 Estimation of protein content

Protein content was assayed by Bradford's protein assay (1976). The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to proteins occurs. Standard protein was prepared in the range 5-100 µg/ml and 5 ml Bradford reagent (100 mg Coomassie Brilliant Blue G-250 in 50 ml 95% ethanol and 100 ml of 85 % (w/v) phosphoric acid. The mixture was diluted to 1 L and the dye was filtered through Whatman no. 1 paper just before use) was added to standard and sample (100 µl). The mixture was incubated for 5 min and absorbance of the mixture read at 595 nm.

5.5 Determination of protein bound carbonyls

Protein carbonyls were detected spectrophotometrically following the method of (Levine *et al*. 1994) by using Sigma-Aldrich protein carbonylation kit. Seed samples (100 mg) were ground in 1 ml 100 mM phosphate buffer (pH 7.0) and centrifuged at 10,000 x g for 20 min at 4 ^{o}C and the supernatant used for further assay. Supernatants were lyophilized and redissolved in 100 µl of phosphate buffer (100 mM, pH 7.0) buffer and used for the assay. Supernatants in 100 µl volume were mixed with 100 µl of freshly prepared dinitrophenylhydrazine (DNPH) solution, vortexed and incubated for 10 min at 25 ^{o}C. After incubation, proteins were precipitated with 30 µl of pre- cooled 100 % TCA solution, vortexed and incubated on ice for 5 min. The pellet was collected after centrifugation at 13000 × g for 2 min. Pellet was sonicated for 5 min after addition of 500 µl of ice-cold acetone and then incubated at -20 °C for 5 min followed by centrifugation at 13000 × g for 2 min. The pellet obtained after centrifugation was washed two times with acetone to remove free DNPH and then briefly sonicated with 200 µl of 6 M guanidine. The sample (100 µl) was transferred to 96 well plate and absorbance measured at 375 nm. Background control was taken by addition of 100 µl of water in one well. Carbonyl content was calculated using a molar absorption coefficient for aliphatic hydrazones as 22 mM^{-1} cm^{-1} and expressed as nmole carbonyl mg $protein^{-1}$.

5.6 Estimation of hormones by HPLC

5.6.1 Abscisic acid (ABA)

The amount of ABA was determined by HPLC in germinating cherry tomato seeds by following the method of Zeevart, 1980.

5.6.1.1 Preparation of sample

Cherry tomato seeds (40 mg) were collected at different times of imbibition (4,8,12, 24 and 36 h), frozen in liquid nitrogen and stored at -80 ^{o}C until further use. Frozen samples were homogenised three times in a total volume of 5 ml of 80 % v/v acetone (80 ml acetone, 1 ml glacial acetic acid and 100 mg of 2, 6 di-tert-butyl 4-methyl phenol in a total volume of 100 ml) and collected in a 10 ml volumetric flask. The homogenate was filtered through Whatman No. 1 filter. The filtrate was transferred to a beaker and flash evaporated in a flash vacuum evaporator for removing the acetone. After the evaporation of acetone from the extract, the lipid soluble material was deposited on the walls of the beaker. The deposit was dissolved in 2 ml of 1% acetic acid solution and the amber coloured aqueous solution collected in HPLC vials. The samples were filtered with 0.22 mm Millipore filter using 2.5 ml plastic syringe before injecting into the HPLC.

5.6.1.2 Estimation of ABA through HPLC

The calibration standard of ABA was prepared in three different concentrations 10, 50 75 and 100 ng/ml and 1,5,10 µg/ml. The standards were included with each group of samples loaded to the HPLC (Agilent 1100 series) at the same time as a control on detector response. The separation was achieved on a µBondapak TM/C18 P/N 841/6 S/N column with a stationary phase consisting of a Lichrosorb C-18 (Agilent, USA). A variable wavelength UV-visible detector was used at wavelength of 270 nm. The elution solvent was methanol and 1 % acetic acid (60:40) with a flow rate of 1 ml/min. A 20 µl sample of the extract was injected into the column and the retention time for ABA and standard was detected on the chromatograph. Peak area was calculated to determine the concentration of ABA in the samples using ESTD (external standard) quantification procedure.

5.6.2 Gibberellic acid (GA_3)

Gibberellic acid was estimated by HPLC in germinating cherry tomato seeds at different time of imbibition by following the method of Wurst *et al*., 1984.

5.6.2.1 Preparation of sample

Cherry tomato seeds (320 mg) were collected at different times of imbibition (4,8,12, 24 and 36 h),frozen in liquid nitrogen and stored at -80 ^{o}C until further use. The frozen samples were homogenized in 10 ml sodium phosphate buffer (50 mM, pH 7.5) containing 0.02% sodium diethyldithio carbamate. The extract was kept for overnight at 4 ^{o}C in a shaker at 150 rpm and centrifuged the next day at 10,000 x g at 4^{o}C for 10 min. The supernatant was taken and volume made up to 5 ml with sodium phosphate buffer (50 mM, pH 7.5). The solution was partitioned with 2.5 ml diethyl ether in separating funnel and aqueous phase collected. The pH of the aqueous phase was adjusted to 2.5 using 1N HCL and again partitioned twice with 5 ml petroleum ether. The aqueous phase was recollected after partitioning and repartitioned thrice with diethyl ether. After collecting the aqueous phase, the extract was again partitioned twice with 2.5 ml ethyl acetate and ether phase collected. This ether phase was again partitioned twice with 2.5 ml of 0.2 M K_2HPO_4 and aqueous phase collected whose pH was adjusted to 2.5 by using concentrated H_3PO_4. The aqueous phase was later partitioned twice with 5 ml ethyl acetate and ether phase collected which was dried after filtering through a funnel containing sodium sulphate salt on the filter paper. The ethyl acetate extract was dissolved in 2 ml methanol and used for estimation of gibberellins. The samples were filtered with 0.22 mm Millipore filter using 2.5 ml plastic syringe before injecting into the HPLC.

5.6.2.2 Estimation of GA_3 through HPLC

The calibration standard of GA was prepared in eight different concentrations 50, 70, 100, 200, 400, 600, 800, 1000 µg/ml. The standards were included with each group of samples loaded to the HPLC (Agilent 1100 series) at the same time as a control on detector response. The separation was achieved on a µBondapak TM/C18 P/N 841/6 S/N column with a stationary phase consisting of Lichrosorb C-18 (Agilent, USA). A variable wavelength UV-visible detector was used at wavelength of 206 nm. The elution solvent was methanol and HPLC water (35:65) at pH 3 adjusted with phosphoric acid with a flow rate of 1 ml/min. A 20 µl sample was injected into the column and the retention time for GA and standard was detected on the chromatograph. Peak area was calculated to determine the concentration of GA in the samples using ESTD (external standard) quantification procedure.

5.7 Results

5.7.1 Seed water uptake

The temporal change in seed water uptake of primed and control seeds are depicted in Fig.5.1 Seed moisture increased by 8-11 % during the imbibition process in seeds treated with pulsed magnetic field compared to untreated seeds. Static magnetic field exposed seeds showed a marginal increase in seed water uptake against untreated control (Fig.5.1).

5.7.2 Dynamics of reactive oxygen species in germinating magnetoprimed seeds

5.7.2.1 Superoxide ion concentration

Superoxide ion production was high in both the magnetic treatments compared to control (Fig. 5.2). An approximately two fold increase was evident in both the treatments at the initial stage of imbibition. At later stages, no distinction was observed amongst the treatments as superoxide levels fell sharply at 12 and 24 h respectively in magnetically magnetoprimed and unprimed seeds respectively.

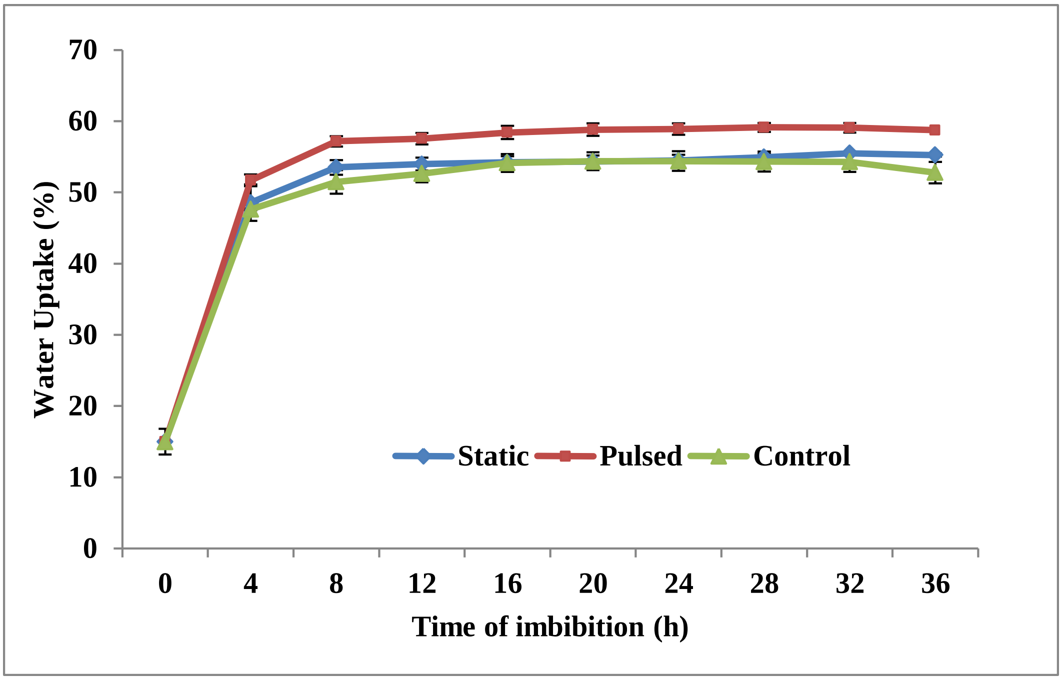

Fig.5.1 Change in water uptake at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Values are means of 3 measurement. Error bars represent ± S.E.(n=3)

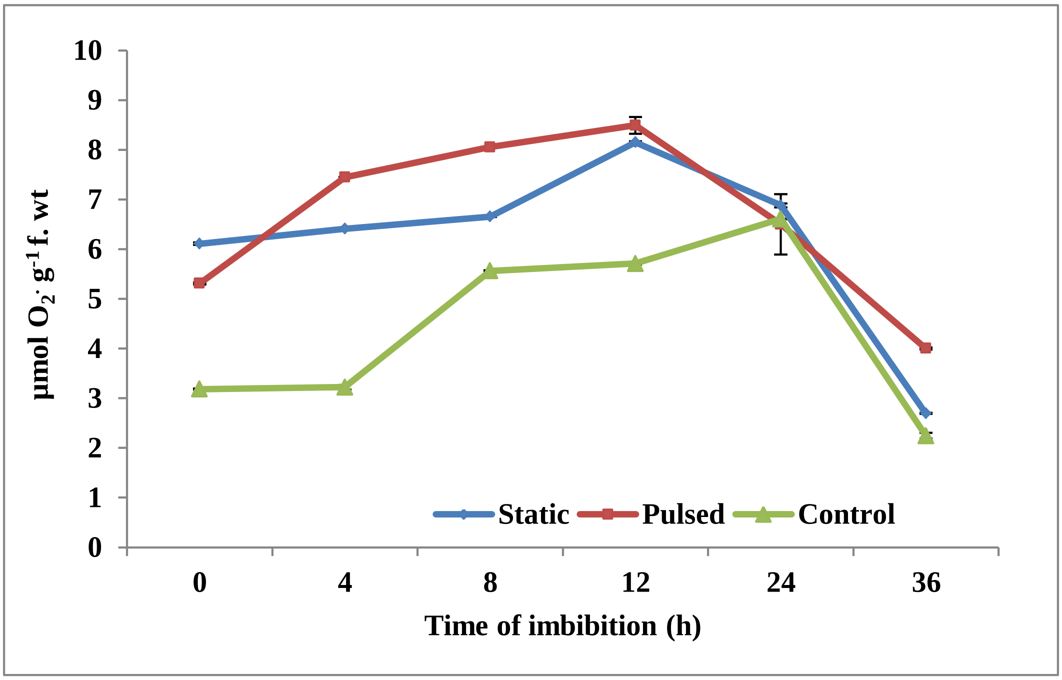

Fig. 5.2 Change in superoxide concentration at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Values are means of 3 measurement. Error bar represent ± S.E. (n=3).

5.7.2.2 Hydrogen peroxide content

The amount of hydrogen peroxide was more in magnetoprimed seeds compared to the control. A more than two fold increase was observed in PMF treated seeds after 12 h of imbibition compared to unprimed control. SMF treated seeds also recorded higher levels than control albeit to a lesser extent in the range of 29- 41 % between 12- 36 h of imbibition (Fig.5.3)

5.7.3 Antioxidant enzyme activity in germinating seeds

5.7.3.1 Superoxide dismutase (SOD) activity

SOD activity increased linearly with time in all the treatments although the increment was higher in PMF magnetoprimed seeds followed by SMF treated seeds (Fig.5.4). SMF primed seeds showed sudden increase in SOD activity after 12 h compared to unprimed control. The activity levelled off in unprimed control after 12 h of imbibition.

5.7.3.2 Catalase (CAT) activity

Catalase activity decreased with time in SMF and PMF magnetoprimed seeds and the decline was very steep in PMF treated seeds in the first 8 h but the activity doubled at 12 h of imbibitions (Fig.5.5). In SMF treated and untreated seeds the activity declined uptil 12 h followed by a gradual increase at 24 and 36 h of imbibition.

5.7.3.3 Guaiacol peroxidase (POX) activity

Magnetoprimed seeds showed higher peroxidase activity during germination than the unprimed control (Fig.5.6). SMF treated seeds showed intermediate activity amongst the three treatments throughout the germination process. PMF treated seeds showed 40 % increase in the first 8 h of imbibition followed by 75 % at 12 h compared to unprimed control. In static MF treatment the increase in activity was in the range of 4- 34%.

5.7.3.4 Ascorbate peroxidase (APOX) activity

Static and Pulsed magnetoprimed seeds had higher ascorbate peroxidase activity than unprimed seeds uptil 12 h of imbibition (Fig.5.7). SMF treated seeds had 60-70 %

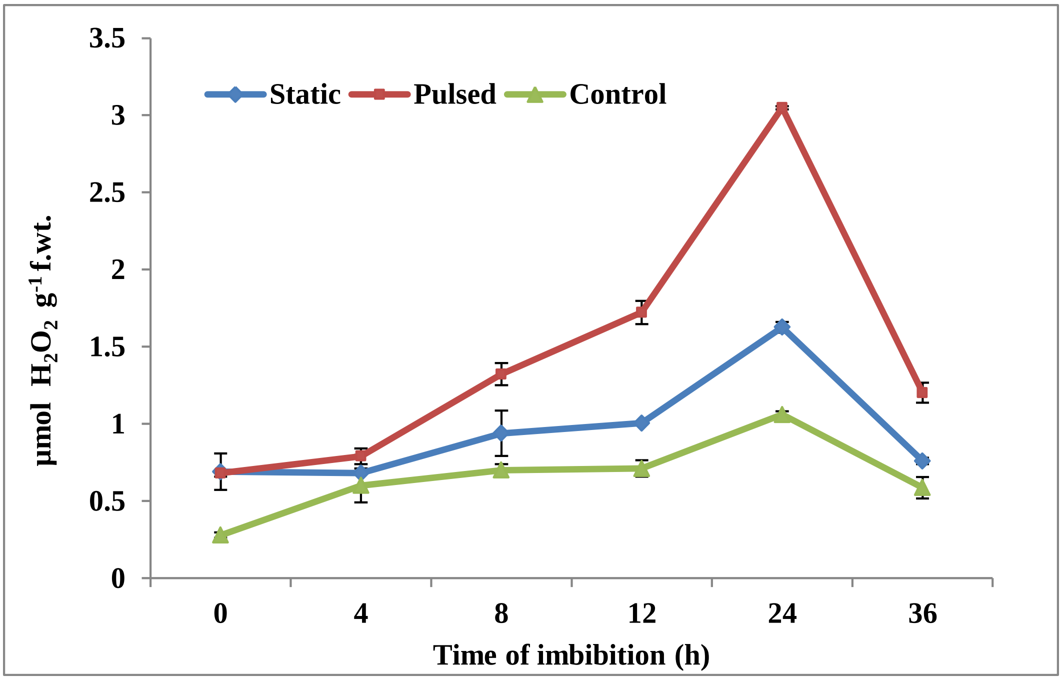

Fig. 5.3 Change in hydrogen peroxide concentration at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Value are means of 3 measurement. Error bar represent ± S.E. (n=3).

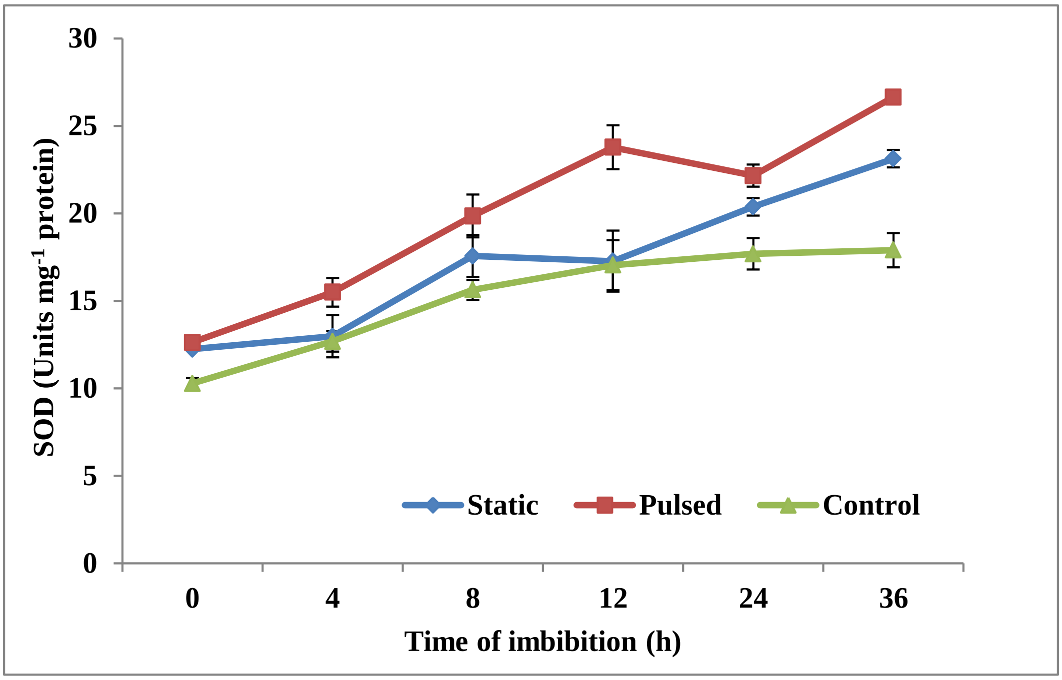

Fig 5.4 Change in superoxide dismutase activity at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Value are means of 3 measurement. Error bars represent ± S.E.(n=3)

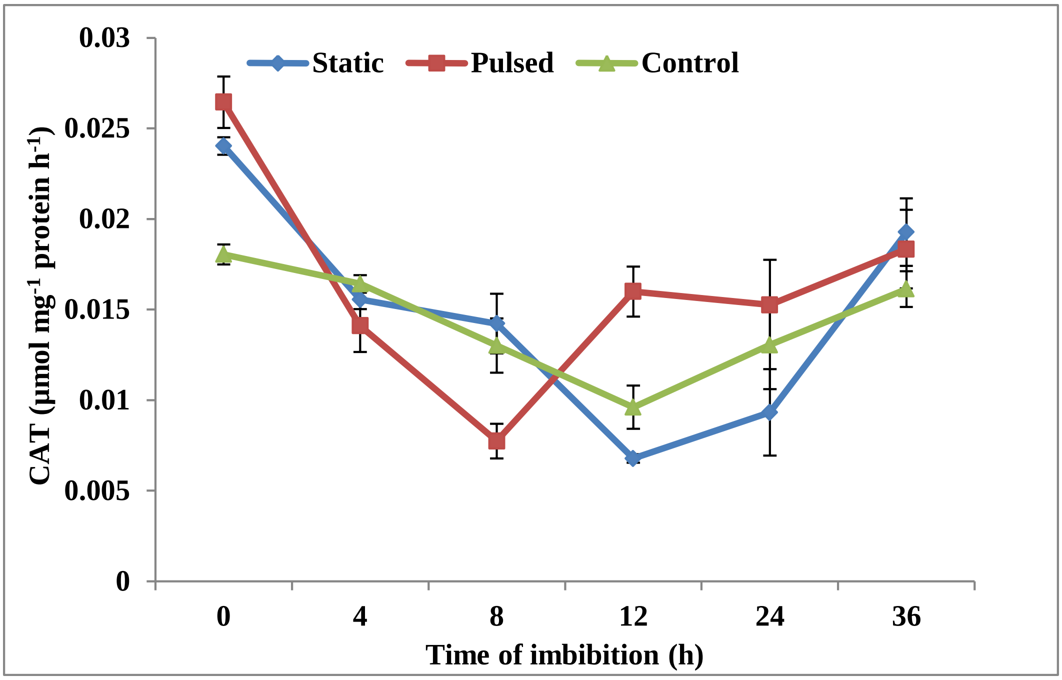

Fig.5.5 Change in catalase activity at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Values are means of 3 measurement. Error bars represent ± S.E.(n=3).

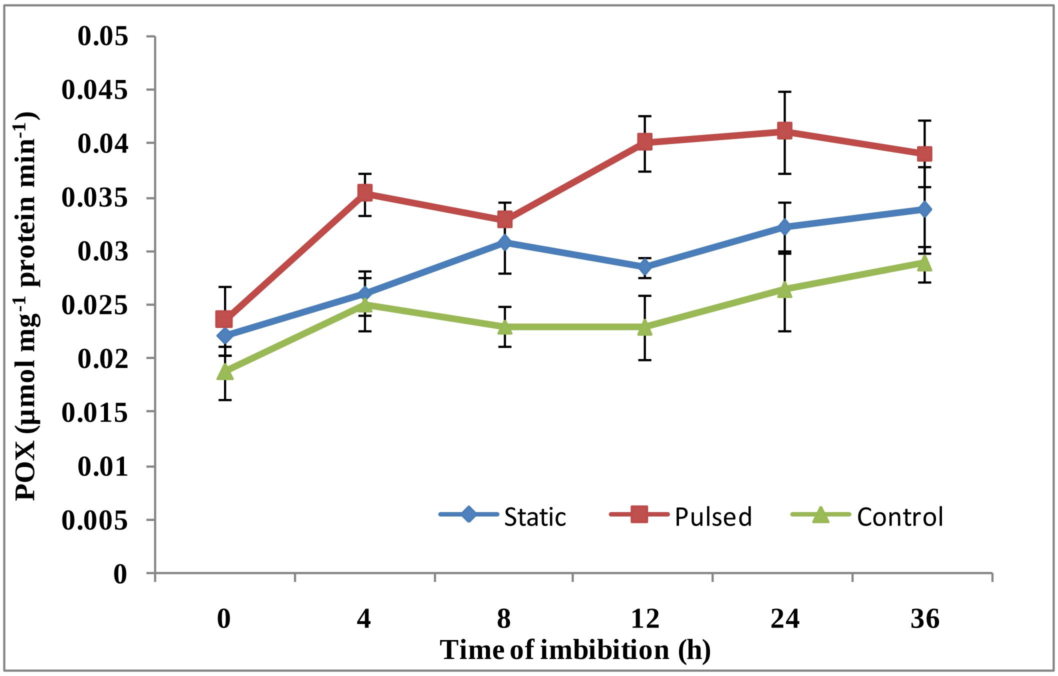

Fig.5.6 Change in guaiacol peroxidase activity at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Values are means of 3 measurement. Error bars represent ± S.E.(n=3)

more activity than control seeds during the germination process. Temporal changes in APOX activity showed that SMF treated seeds had 40-50 % more activity than PMF treated seeds.

5.7.3.5 Glutathione reductase (GR) activity

Glutathione reductase activity also declined till 8 and 12 h in SMF and PMF seeds respectively (Fig.5.8). It was observed to be less in magnetoprimed seeds than unprimed control from 12-24 hrs of imbibition. Maximum decline in GR activity was observed at 12 h in PMF (38 %) and 24 h in SMF (36 %) primed seeds compared to unprimed control.

5.7.4 Protein carbonyls in the germinating seeds

Protein carbonyls formed as a result of protein oxidation by free radicals were found to increase with time in all the treatments. Magnetoprimed seeds showed higher levels of oxidized protein than untreated seeds. PMF treatment resulted in 54 % increase in carbonyls levels compared to untreated control at 12 h of imbibition (Fig.5.9)

5.7.5 Total soluble protein content

Soluble protein content decreased in magnetoprimed seeds rapidly in comparison to unprimed control. It was found to be lowest in PMF primed seeds that showed faster rate of germination amongst all the treatments (Fig.5.10). All the treatments showed similar protein levels at 36 h of imbibition.

5.7.6 Abscisic acid and gibberellic acid content

Detectable levels of abscisic acid could be observed uptil 8 h in unprimed control and 4 h in magnetoprimed seeds. ABA level was approx. 2.5 fold higher in unprimed control at 4 hrs of imbibition. A time dependent decrease in ABA levels was evident in all treatments with ABA being detected uptil 8 h in control seeds compared to 4 h in magnetoprimed seeds (Fig.5.11). On the contrary, GA_3 could be detected (3.7 ng g^{-1} f wt.) in primed seeds after 8 h of imbibition. These levels fell to 0.9-1.3 ng g^{-1} f wt. at 12 h followed by a further decline at 24 and 36 h of imbibition. In case of pulsed MF treatment

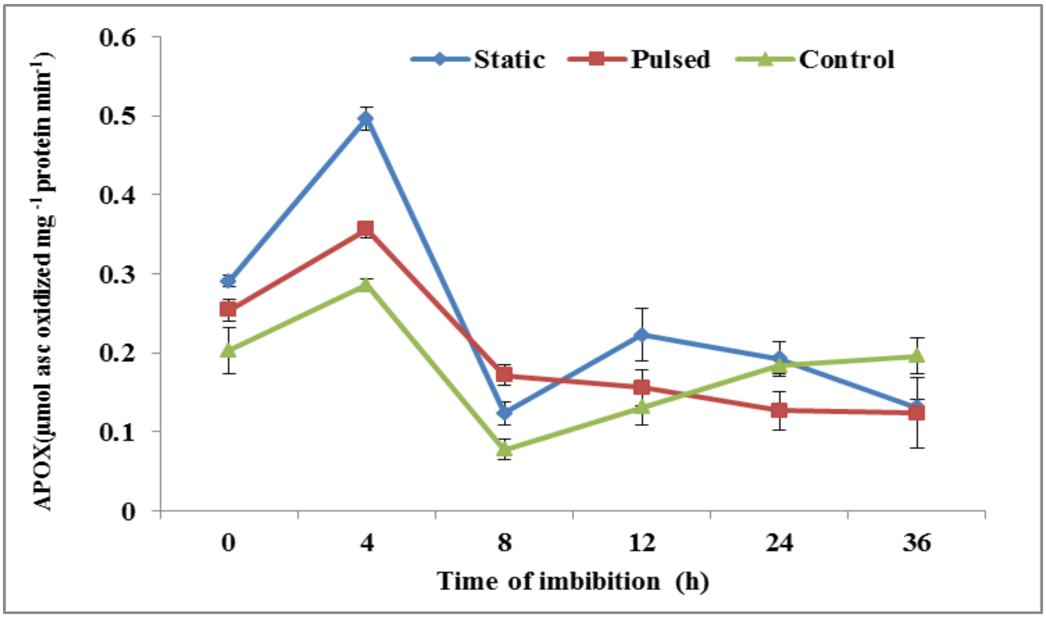

Fig.5.7 Change in ascorbate peroxidase activity at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Values are means of 3 measurement. Error bars represent ± S.E.(n=3)

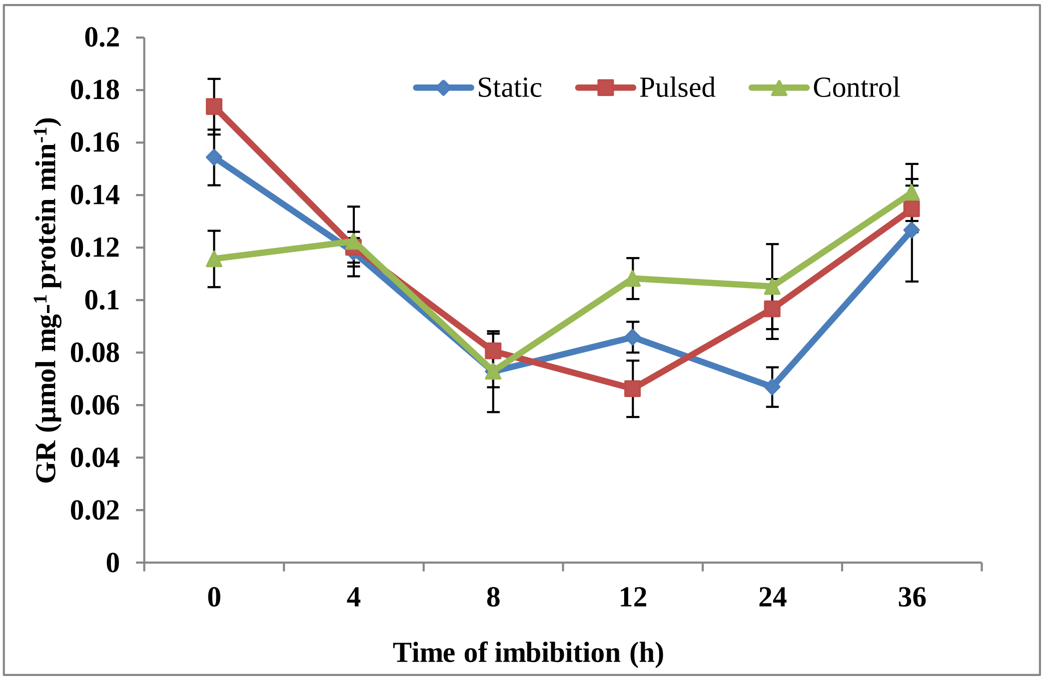

Fig.5.8 Change in glutathione reductase activity at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Values are means of 3 measurement. Error bars represent ± S.E.(n=3)

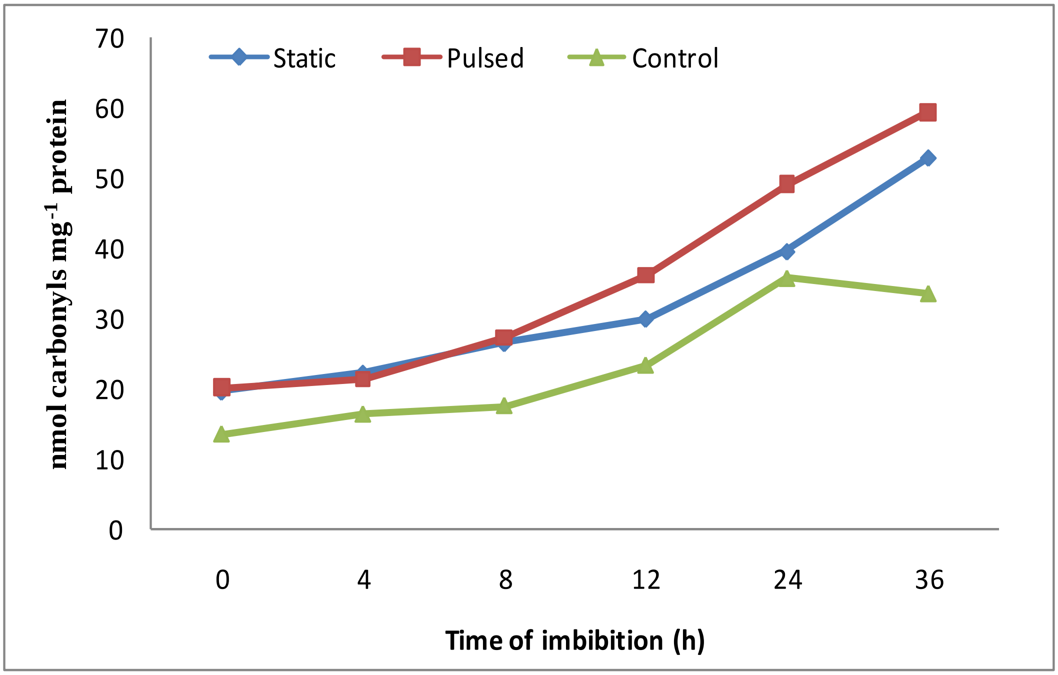

Fig.5.9 Change in protein carbonyl content at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds.

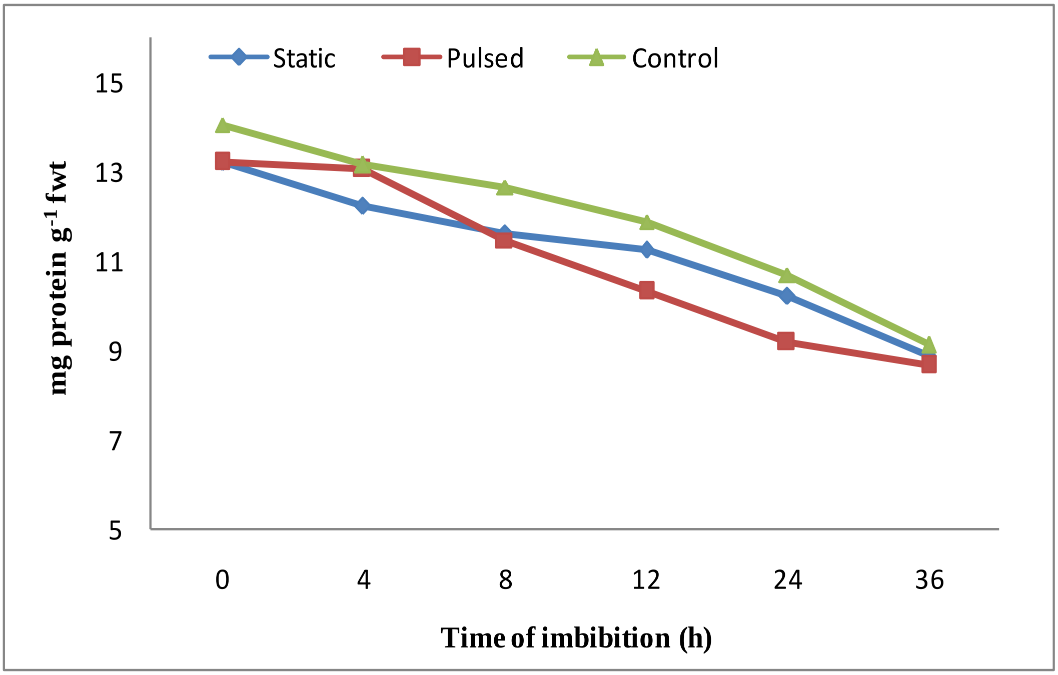

Fig.5.10 Change in soluble protein content at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds. Values are means of 3 measurement. Error bars represent ± S.E.(n=3)

GA_3 decreased by 75 % after 8 h but a 2 fold increase was observed at 36 h of imbibition. An increase in GA_3 content of unprimed control could be seen from 12 – 36 h of imbibition. (Fig.5.12)

Discussion

Seed priming is a pre-sowing technique to influence seed germination and seedling development by modulating pre-germination metabolic activity prior to emergence of the radicle and enhances germination rate and plant performance (Bradford, 1986). ROS production in seeds, earlier regarded as a symptom of oxidative stress as it was analyzed during seed dessication and aging has a dual role in seed physiology. ROS do not always act as harmful compounds and their fine tuning plays a key role in adjustment of gene expression during germination and dormancy release (Bailly *et al.,* 2008). Hydrogen peroxide (H_2O_2) (Morohashi 2002), nitric oxide (NO) (Caro & Puntarulo 1999; Sarath *et al.*, 2007), hydroxyl radicals (Schopfer 2001; Müller *et al.*, 2009) and superoxide radicals (Schopfer 2001; Kranner *et al.*, 2010) have been shown to accumulate during seed germination in various species. In our study, the two fold increase in the level of superoxide anion and hydrogen peroxide in magnetoprimed cherry tomato seeds corroborated with similar findings in faba bean (Podlesny *et al.,* 2005), soybean (Shine *et al.*, 2012) and cucumber (Bhardwaj *et al.,* 2012) to explain the enhanced germination characteristics leading to improved vigour. Magnetopriming may have resulted in production of the free radicals in dry state through non-enzymatic reactions such as lipid peroxidation (McDonald, 1999; Priestley, 1986; Wilson and McDonald, 1986) or the Amadori and Maillard reactions associated with free radical production and oxidation processes (Murthy and Sun, 2000; Murthy *et al.*, 2003;). However, the intracellular sources of ROS production are poorly documented. Presumably, most of the ROS produced should originate from mitochondria, since resumption of respiration in imbibed seeds might lead to electron leakage and increased production of ROS. Cellular signaling associated to the mechanism of seed germination is not triggered if the amount of ROS is too low or high. ROS regulates the germinating process by facilitating radicle protrusion or by protecting the embryo against pathogen attack (Bailly, 2004).

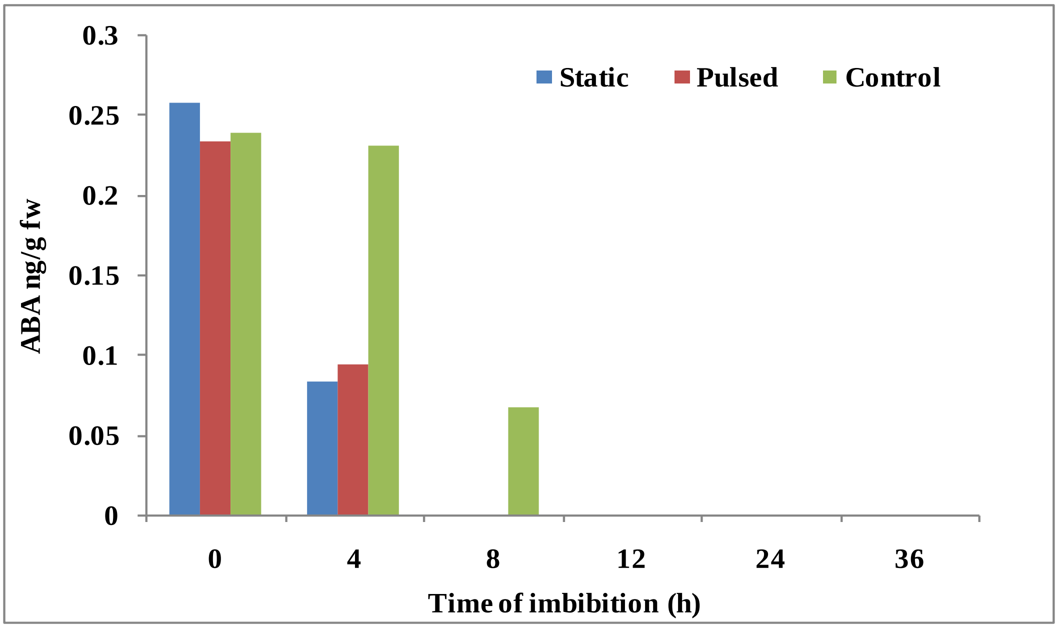

Fig.5.11 ABA content at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds.

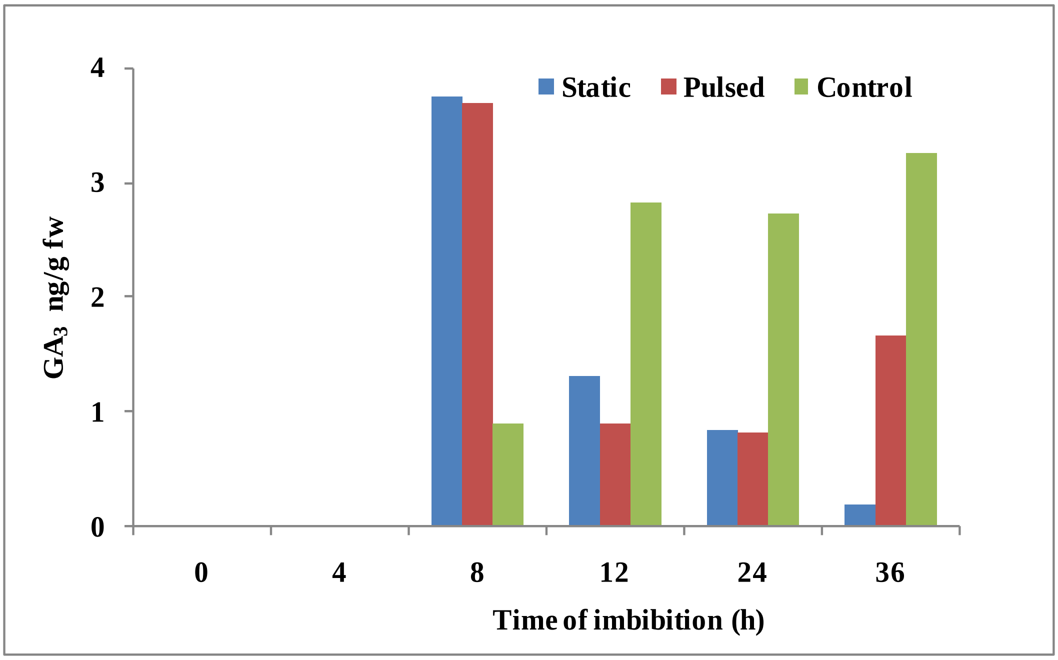

Fig.5.12 GA_3 content at different hours of imbibition at 25 °C in magnetoprimed and unprimed cherry tomato seeds.

Many models have been proposed to provide a theoretical understanding of the mechanism of magnetoreception in plants. Studies have revealed that phytoferritin occur in plant cells as crystalline magnetite (Fe_3O_4), ε-Fe_2O_3 and hematite (α- Fe_2O_3) (McClean *et al.*, 2001) and may interact strongly with the magnetic fields than do diamagnetic or paramagnetic materials. These particles can also affect the superoxide generated free radicals (Scaiano *et al*., 1997). Enzyme catalysed reactions that involve intermediates with radical pairs (Grissom, 1995) and some porphyrins that form free radicals during mitochondrial respiratory chain may also be influenced by external magnetic field as it affects singlet-triplet conversions. The radical pair mechanism (RPM) is currently the only physically plausible mechanism indicating the role of cryptochrome as a candidate for magnetoreception that results in generation of flavin-trytophan radical pairs (Occhipinti *et al.*, 2014).

The water uptake in pulsed magnetoprimed seeds was found to be more than static and control seeds. Pulsed magnetic field may influence the structure of the cell membrane and increases their permeability and ion transport in the ion-channels which affects the metabolic pathway activity (Labes, 1993; Chung *et al.*, 2008). Magnetic field exposure can also cause changes in the intracellular level of Ca^{2+} and ionic current density across the cellular membrane altering the osmotic pressure and the capacity of cellular tissue to absorb water (García- Reina *et al*., 2001). Specifically, the diamagnetic anisotropic properties of membrane phospholipids in magnetic field treatment reorient the phospholipids causing deformation of membrane channels (Rosen, 2003). Radhakrishnan and Kumari (2012) correlated decreased uptake of minerals in PMF treated seeds to increased membrane integrity of PMF treated seeds. MF treatment may have a direct effect on the calcium channels and pumps and excess Ca^{+2} is removed from cytosol to extracellular medium by Ca^{+2}-ATPases and/or Ca^{+2}/H^{+} antiporters in MF treated plants (Hajnorouzia et al., 2011). The increased physiological activity due to greater absorption of moisture by magnetoprimed seeds may be responsible for the increase in germination and seedling vigour. The presence of free water would allow ROS especially hydrogen peroxide to travel far within the cell from the production site (El-Maarouf-Bouteau and

Bailly, 2008). It has been demonstrated that hydration of seeds causes a release of free radicals from trapped state (Priestley *et al.*, 1985).

The ability of seeds to germinate has been linked to the accumulation of a critical level of H_2O_2, as this molecule is produced at a higher level in non-dormant imbibed seeds than in dormant imbibed seeds (Bailly *et al*. 2008). Report on the positive role of ROS in seeds show that superoxide and H_2O_2 act as secondary messengers in seed germination process (Barba-Espin *et al.,* 2011). An "oxidative window" for ROS ensures that germination proceeds at a faster rate helping in establishment of more vigourous seedling in magnetoprimed seeds (Fig. 5.13). In our study the levels of free radicals in magnetoprimed seeds that have a promotive effect in germination with their thresholds in that oxidative window were maintained through the antioxidative enzyme system. The increase in SOD activity in static and pulsed primed seeds is important for conversion of $O_2^{-\cdot}$ to H_2O_2. The enzyme peroxidase reduces H_2O_2 to water using various substrates as electron donors. This was clear from increased activity of ascorbate peroxidase activity in the early stage of imbibition in seeds primed with static field indicating that ascorbate is used as substrate during early stages of free radical scavenging in SMF treated seeds. APX 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 APX and GSH and enzymes such as glutathione reductase (GR), dehydroascorbate reductase, monohydroascorbate reductase are responsible for AsA regeneration. A declining GR activity in primed seeds would have impaired the production of AsA resulting in reduced APX activity in later stages of germination. Catalase dismutates H_2O_2 to water and oxygen but was observed to decrease in all treatments uptil 8 h of imbibition. The dynamics of CAT activity suggests that CAT did not play a role in the early breakdown of H_2O_2 although primed seeds had higher activity than unprimed control. There are several other enzymes that produce H_2O_2 (or active intermediates) in plants like peroxidases, oxalate oxidase, xanthine oxidase, membrane linked Nicotinamide Adenine Dinucleotide Phosphate oxidase (NADPH oxidase) and amine oxidase (Bolwell and Wojtaszek, 1997), among which cell wall peroxidase plays a major role in the generation of H_2O_2. Peroxidase is one of the key enzyme during the life cycle

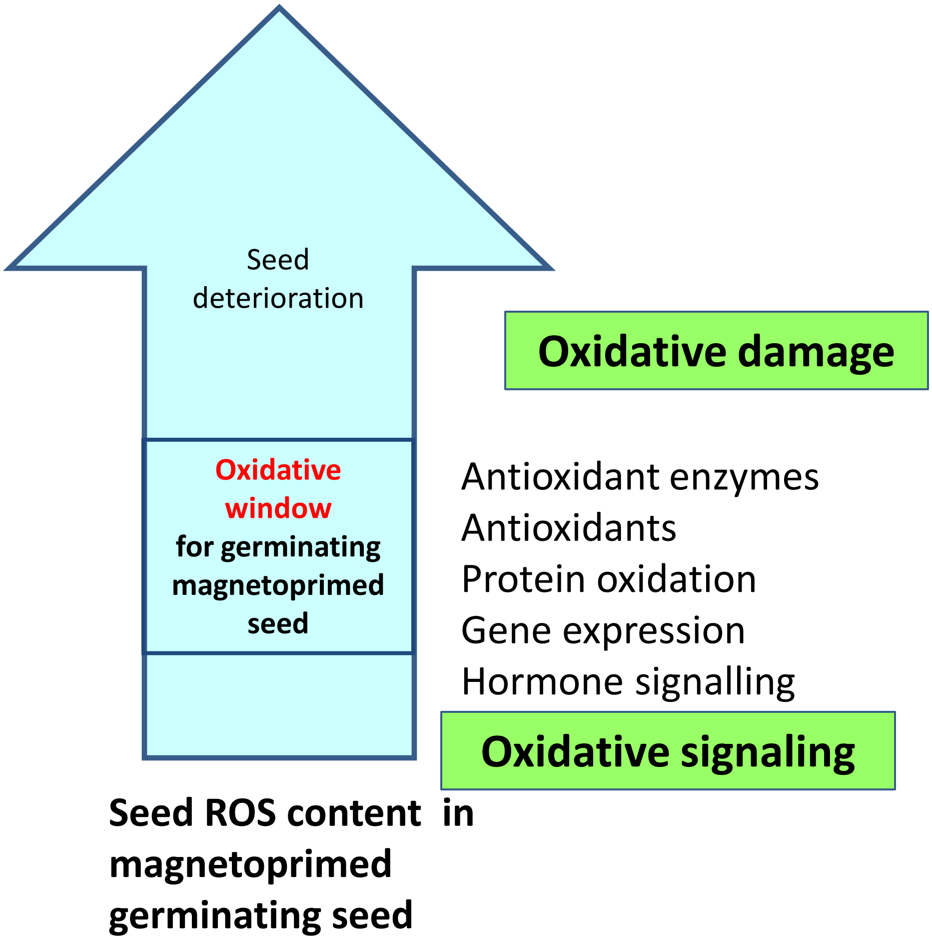

Fig. 5.13 ROS oxidative window for promotion of seed germination in magnetoprimed seeds

of plant, and its role can be antagonostic depending on the site of production and developmental stage of plant. Cytosolic peroxidase (compound I) is involved in the consumption of H_2O_2, whereas peroxidase (compound III) secreted in to the cell wall and surrounding medium is involved in the release of ROS (Passardi *et al*., 2004). Our study showed a very significant increase in non specific peroxidase activity in PMF and SMF magnetoprimed seeds. During the oxidative cycle of peroxidase, $O_2^{\cdot-}$ is converted to H_2O_2 by extracting electron from an electron donor (Liszkay *et al*., 2003)] i.e. $NAD^{\cdot}$ reduces O_2 to $O_2^{\cdot-}$ that can be converted to H_2O_2 by oxidizing extra NADH (Nicotinamide adenine dinucleotide) to $NAD^{\cdot}$. In addition, H_2O_2 can also be formed by spontaneous dismutation of $O_2^{\cdot-}$ as well as ascorbic acid mediated non enzymatic reduction of $O_2^{\cdot-}$ to H_2O_2, which can be further accelerated manifold by the activity of SOD (Scandalios, 1997).

Another attempt for explaining the possible role of ROS accumulation during germination was to study protein carbonylation, an irreversible oxidation process leading to a loss of function of the modified proteins. Carbonylation of numerous proteins occurs during *Arabidopsis* seed germination, although the germinated seeds gave rise to vigorous plantlets (Job *et al*., 2005). In our study, the amount of protein carbonyls was more in magnetoprimed seeds compared to untreated control with pulsed treated seeds showing a greater increase after 12 h of imbibition when observed with respect to static magnetoprimed seeds. The increase in carbonyls may result from increased levels of H_2O_2 or other radicals as levels of superoxide were less after 12 h of imbibition. Several reports have documented that specific change in protein carbonylation patterns might be required for counteracting and/or utilizing the production of ROS caused by the recovery of metabolic activity in the germinating seeds (Oracz *et al*., 2007). Cellular ROS may show some selectivity with respect to their targets. For example, H_2O_2, which is an oxidant, can react with specific molecules at specific sites (Halliwell and Gutteridge, 1999). During germination there is a marked change in the oxidized proteome, disclosing new carbonylated proteins of glycolysis/gluconeogenesis pathways (Thomas and ap Rees, 1972; Zhang *et al*., 2004). It has been proposed that carbonylation of seed storage proteins would help trigger their mobilization during germination by destabilizing the compact seed storage protein complexes, thus increasing their susceptibility towards proteolytic cleavage (Job *et al*. 2005). Our results explain the enhanced germination characters in pulsed magnetoprimed seeds over static MF treatment and provide the first evidence that protein oxidation could also play a key and necessary role faster mobilisation of reserve protein and increased seed vigour in magnetoprimed seeds.

Hydrogen peroxide is known to act as a priming factor that brings about changes at proteome, transcriptome and hormonal levels thereby promoting germination in pea (Barba-Espin *et al.*, 2011). The interplay between ROS and hormone signaling pathways lead to changes in gene expression or in cellular redox status that would play a role in the perception of environmental factors by seeds during their germination (Bailly *et al.,* 2008). H_2O_2 could act, directly or indirectly impairing the ABA transport from the cotyledon to the embryo inducing a decrease in ABA, stimulating the germination process (Barba-Espín *et al.,* 2011). Bahin *et al*., (2011) showed that the cross-talk between ROS and ABA or gibberellins metabolism and signaling might control barley seed dormancy. ABA levels decreased in the magnetoprimed cherry tomato seeds early during imbibition along with increasing ROS and H_2O_2. Germination in the presence of H_2O_2 in conjunction with high ABA levels during this period can be related to the inhibition of ABA signalling, or to GA signalling or biosynthesis activation, because the ABA/GA balance, rather than absolute hormone amounts, controls germination . If ABA signalling and sensitivity are not altered by ROS treatment while ABA content is high, it is reasonable to assume that the GA signalling was affected by the H_2O_2 treatment. GA levels could be detected at 8 h in magnetoprimed seeds followed by a decline. In untreated control these levels increased after 12 h of imbibitions. A proteomic study of seeds from the GA deficient *Arabidopsis ga1* mutant indicated a rather late implication of GAs in germination, occurring at a stage coinciding with or very close to radicle emergence (Gallardo *et al*., 2002). It appears that GAs, although required for the completion of germination, are not directly involved in many processes taking place during germination, such as the initial mobilization of seed storage proteins and lipids.

Our results showed the role of antioxidative enzyme and protein oxidation by ROS in maintaining the levels of free radicals within the oxidative window. The oxidized proteins that are produced as a result of ROS action might help in metabolizing seed reserves and improving vigour in magnetoprimed seeds. The interplay of ROS with hormones suggested GA signaling that may lead to GA biosynthesis and help in completion of germination. Based on our results we have proposed a model to explain the interplay between ROS and hormone signaling that can lead to improved vigour in magnetoprimed cherry tomato seeds (Fig.5.14).

Pulsed magnetoprimed seeds showed better response in terms of invigouration and all the biochemical changes discussed above were more during germination of pulsed rather than static magnetoprimed seeds.

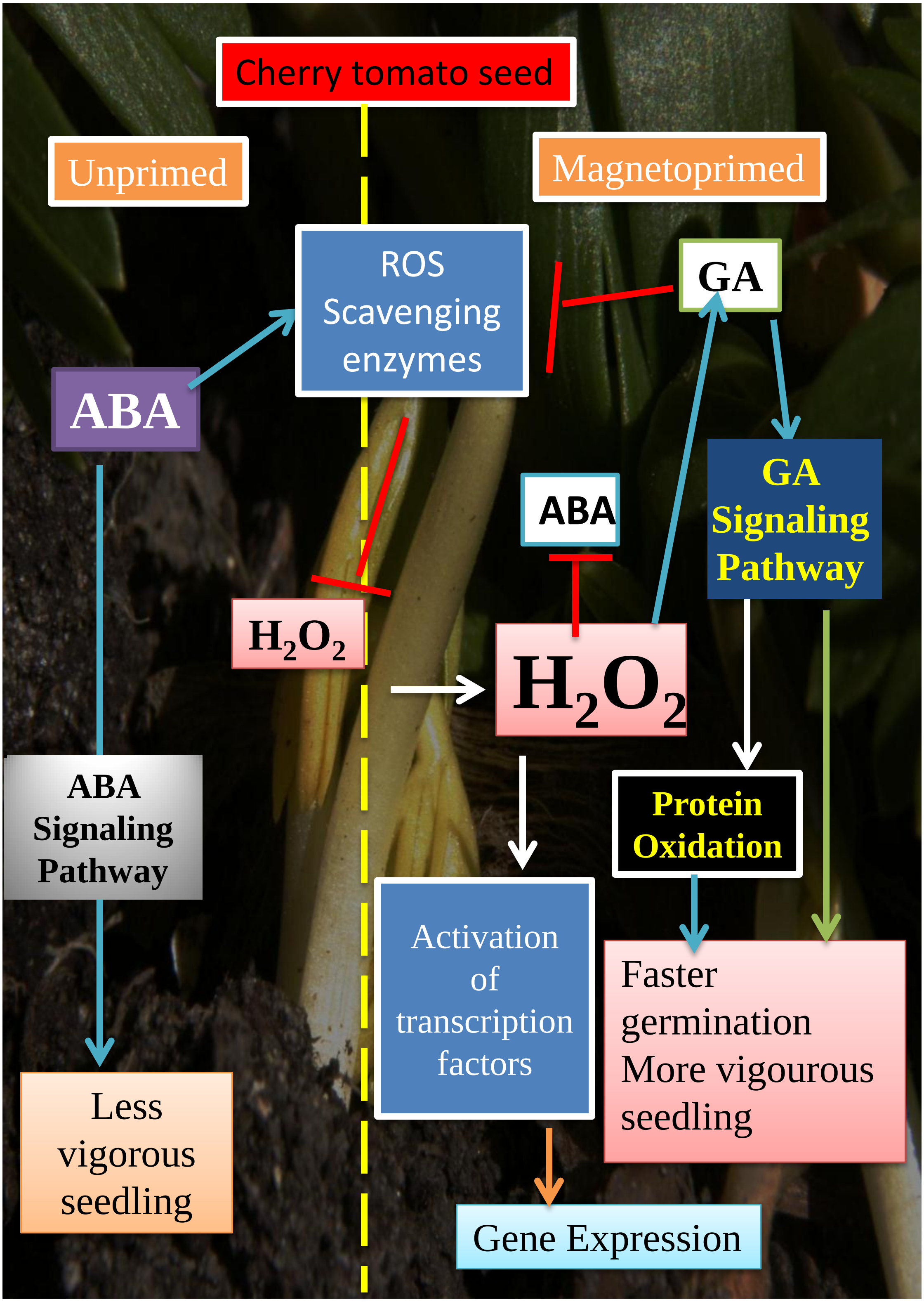

Fig. 5.14 Hypothetical model proposing a central role of ROS in increased seedling vigour of magneto primed seeds. Lower ABA concentration in magetoprimed seeds would be associated with higher level of ROS (H_2O_2) which could interfere with ABA and GA signaling pathway and induce protein oxidation. The size of the letters represent proportion of relative amount of compounds (ABA, GA_3 and H_2O_2)

6. DISCUSSION

6.1. Germination and related characteristics of magnetoprimed seeds of cherry tomato

Cherry tomato is a small garden variety of tomato that has very high nutritive value, containing vitamin A, lycopene, beta-carotene, vitamin C and minerals like calcium, potassium etc. The cultivation of cherry tomato is a highly economical venture for the farmers due to assured demand of this exotic vegetable in the national and international retail market. Various technologies are being explored for improving the productivity of exotic vegetables as the huge cost of hybrid seed demands that every seed should yield to its maximum potential. Rapid and uniform field emergence of seedlings is therefore a challenge as slow emergence results in smaller plants and seedlings which are poor yielders and susceptible to soil- borne diseases.

Various seed priming techniques like hydropriming, osmopriming, solid matrix priming and halopriming are used for overcoming poor and erratic seed germination in many vegetable crops and increasing the speed and synchrony of germination. Magnetopriming (exposure to magnetic field) is a non invasive physical stimulant used for improving vigour and emergence in seeds (Anna 2002; Fischer *et al.* 2004; Florez *et al.* 2007, Shine *et al.,* 2011, Bhardwaj *et al.*, 2012; Radhakrishnan and Ranjitha Kumari, 2012, Bilalis *et al.*, 2013). Our results demonstrated that magnetopriming of the seed with static dose of 100 mT (30 min) and pulsed dose of 100 mT (30 min) applied in the cycle of 3 min on and off enhanced all germination associated characteristics in cherry tomato. This observation suggested that there may be a resonance like phenomena which increases the internal energy of the seed that occurs at an appropriate combination of magnetic field and exposure time. Many models have been proposed to provide a theoretical understanding of the mechanism of magnetoreception in plants. Studies have revealed that phytoferritin occur in plant cells as crystalline magnetite (Fe_3O_4), ε-Fe_2O_3 and hematite (α- Fe_2O_3) (Mc Clean *et al.*, 2001) and may interact strongly with the magnetic fields than do diamagnetic or paramagnetic materials. These particles can also affect the superoxide generated free radicals (Scaiano *et al.*, 1997). Enzyme catalysed reactions that involve intermediates with radical pairs (Grissom, 1995) and some porphyrins that form free radicals during mitochondrial respiratory chain may also be influenced by external magnetic field as it affects singlet-triplet conversions. The radical pair mechanism (RPM) is currently the only physically plausible mechanism indicating the role of cryptochrome as a candidate for magnetoreception that results in generation of flavin-trytophan radical pairs (Occhipinti *et al.*, 2014).

6.2 Seed water uptake in germinating magnetoprimed seeds

The water uptake in pulsed magnetoprimed seeds was found to be more than static and control seeds. Pulsed magnetic field may influence the structure of the cell membrane and increase its permeability and ion transport in the ion-channels which affects the metabolic pathway activity (Labes, 1993; Chung *et al.*, 2008). Magnetic field exposure can also cause changes in the intracellular level of Ca^{2+} and ionic current density across the cellular membrane altering the osmotic pressure and the capacity of cellular tissue to absorb water (García- Reina *et al*., 2001). Specifically, the diamagnetic anisotropic properties of membrane phospholipids in magnetic field treatment reorient the phospholipids causing deformation of membrane channels (Rosen, 2003). Radhakrishnan and Kumari (2012) correlated decreased uptake of minerals in PMF treated seeds to increased membrane integrity of PMF treated seeds. MF treatment may have a direct effect on the calcium channels and pumps and excess Ca^{+2} is removed from cytosol to extracellular medium by Ca^{+2}-ATPases and/or Ca^{+2}/H^{+} antiporters in MF treated plants (Hajnorouzia *et al*., 2011). The increased physiological activity due to greater absorption of moisture by magnetoprimed seeds is responsible for the increase in germination and seedling vigour.

6.3 Production of reactive oxygen species in germinating magnetoprimed seeds

A maximum of two fold increase in level of superoxide anion and hydrogen peroxide was observed in SMF and PMF magnetoprimed seeds of cherry tomato. The ability of seeds to germinate has been linked to the accumulation of a critical level of H_2O_2, as this molecule is produced at a higher level in non-dormant imbibed seeds than in dormant imbibed seeds (Bailly *et al*. 2008). ROS thus play a key signaling role in the germination or dormancy release. ROS such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) are generated as a result of aerobic metabolism in mitochondria, peroxisomes and the apoplastic space in the germinating seeds. Approximately 2-3% of the oxygen used by mitochondria results into superoxide and hydrogen peroxide production. Report on the positive role of ROS in seeds show that superoxide and H_2O_2 act as secondary messengers in seed germination process (Barba-Espin *et al.,* 2011). In our study the level of free radicals that have a promotive effect in germination of magnetoprimed seeds was maintained through the antioxidative enzyme system and the presence of free water on imbibition allows ROS especially hydrogen peroxide to travel far within the cell from the production site (El-Maarouf-Bouteau and Bailly, 2008). It has been demonstrated that hydration of seeds causes a release of free radicals from trapped state (Priestley *et al.*, 1985).

6.4 Antioxidant enzyme system for ROS homeostasis

The increase in superoxide dismutase activity observed in static and pulsed primed seeds is important for conversion of $O_2^{-\cdot}$ to H_2O_2. There are several other enzymes that produce H_2O_2 (or active intermediates) in plants like peroxidases, oxalate oxidase, xanthine oxidase, membrane linked Nicotinamide Adenine Dinucleotide Phosphate oxidase (NADPH oxidase) and amine oxidase (Bolwell and Wojtaszek, 1997), among which cell wall peroxidase plays a major role in the generation of H_2O_2. Peroxidase is one of the key enzyme during the life cycle of plant, and its role can be antagonostic depending on the site of production and developmental stage of plant. Cytosolic peroxidase (compound I) is involved in the consumption of H_2O_2, whereas peroxidase (compound III) secreted in to the cell wall and surrounding medium is involved in the release of ROS (Passardi *et al*., 2004). Our study showed a very significant increase in non specific peroxidase activity in PMF and SMF magnetoprimed seeds. During the oxidative cycle of peroxidase, $O_2^{\cdot -}$ is converted to H_2O_2 by extracting electron from an electron donor (Liszkay *et al*., 2003) i.e. $NAD^{\cdot}$ reduces O_2 to $O_2^{\cdot -}$ that can be converted to H_2O_2 by oxidizing extra NADH (reduced Nicotinamide Adenine Dinucleotide) to $NAD^{\cdot}$. In addition, H_2O_2 can also be formed by spontaneous dismutation of $O_2^{\cdot-}$ as well as ascorbic acid mediated non enzymatic reduction of $O_2^{\cdot-}$ to H_2O_2, which can be further accelerated manifold by the activity of SOD (Scandalios, 1997).

The enzyme peroxidase reduces H_2O_2 to water using various substrates as electron donors. This was clear from increased activity of ascorbate peroxidase activity in the early stage of imbibition in seeds primed with static field indicating that ascorbate is used as substrate during early stages for free radical scavenging in SMF treated seeds. APX 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 APX and GSH and enzymes such as glutathione reductase (GR), dehydroascorbate reductase, monohydroascorbate reductase are responsible for AsA regeneration. A declining GR activity in primed seeds would have impaired the production of AsA resulting in reduced APX activity in later stages of germination. Catalase dismutates H_2O_2 to water and oxygen but was observed to decrease in all treatments uptil 8 h of imbibition. The dynamics of CAT activity suggested that CAT did not play a role in the early breakdown of H_2O_2 although primed seeds had higher activity than unprimed control. In the later stages the increase in CAT activity was in accordance with earlier results reported in MF treated tomato seeds (Xiao-ju and Guo; 1999).

6.5 ROS scavenging by protein carbonylation

Protein carbonylation is an irreversible oxidation process leading to a loss of function of the modified proteins. Carbonylation of numerous proteins occurs during *Arabidopsis* seed germination, although the germinated seeds gave rise to vigorous plantlets (Job *et al*., 2005). In our study, the amount of protein carbonyls was more in magnetoprimed seeds compared to untreated control with pulsed treated seeds showing a greater increase after 12 h of imbibition when observed in comparison with static magnetoprimed seeds. The increase in carbonyls may result from increased levels of H_2O_2 as levels of superoxide were less after 12 h of imbibition. Several reports have documented that specific change in protein carbonylation patterns might be required for counteracting and/or utilizing the production of ROS caused by the recovery of metabolic activity in the germinating seeds (Oracz *et al*. 2007). Cellular ROS may show some selectivity with respect to their targets. For example, H_2O_2, which is an oxidant, can react with specific molecules at specific sites (Halliwell and Gutteridge, 1999). During germination there is a marked change in the oxidized proteome, disclosing new carbonylated proteins of glycolysis/gluconeogenesis pathways (Thomas and ap Rees, 1972; Zhang *et al*., 2004). It has been proposed that carbonylation of seed storage proteins would help trigger their mobilization during germination by destabilizing the compact seed storage protein complexes, thus increasing their susceptibility towards proteolytic cleavage (Job *et al*. 2005). Our results explain the enhanced germination characters in pulsed magnetoprimed seeds over static MF treatment and provide the first evidence that protein oxidation could also play a key and necessary role for faster mobilisation of reserve protein and increased seed vigour in magnetoprimed seeds. Pulsed magnetoprimed seeds showed better response in terms of invigouration and all the biochemical changes discussed above were more during germination of pulsed rather than static magnetoprimed seeds.

6.6 Interaction of ROS with hormones (ABA and GA) in the germinating magnetoprimed seeds

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). The interplay between ROS and hormone signaling pathways lead to changes in gene expression or in cellular redox status that would play a role in the perception of environmental factors by seeds during their germination (Bailly *et al.* 2008). H_2O_2 could act, directly or indirectly impairing the ABA transport from the cotyledon to the embryo inducing a decrease in ABA, stimulating the germination process (Barba-Espín *et al.*, 2011). Bahin *et al*. (2011) showed that the cross-talk between ROS and ABA or gibberellins metabolism and signaling might control barley seed dormancy. ABA levels decreased in the magnetoprimed cherry tomato seeds early during imbibition along with increasing ROS and H_2O_2. Germination in the presence of H_2O_2 in conjunction with high ABA levels during this period can be related to the inhibition of ABA signalling, or to GA signalling or biosynthesis activation, because the ABA/GA balance, rather than absolute hormone amounts, controls germination (White *et al*. 2000; Cadman *et al*. 2006; Finch-Savage & Leubner-Metzger 2006). If ABA signalling and sensitivity are not altered by ROS treatment while ABA content is high, it is reasonable to assume that the GA signalling was affected by the H_2O_2 treatment. GA levels could be detected at 8 h in magnetoprimed seeds followed by a decline. In untreated control these levels increased after 12 h of imbibition. A proteomic study of seeds from the GA deficient *Arabidopsis ga1* mutant indicated a rather late implication of GAs in germination, occurring at a stage coinciding with or very close to radicle emergence (Gallardo *et al*., 2002). It appears that GAs, although required for the completion of germination, are not directly involved in many processes taking place during germination, such as the initial mobilization of seed storage proteins and lipids.

Based on our results, we have made an attempt to integrate the enhanced vigour in magnetoprimed seeds with ROS homeostasis through antioxidative metabolism and protein carbonylation. The role of hormones is envisaged in ROS mediated germination of magnetoprimed cherry tomato seeds.

Seed invigoration brought about by SMF and PMF exposure to the seeds led to enhanced growth of plants during the vegetative phase and earliness to flowering in cherry tomato as also reported in cucumber (Bhardwaj., 2012). Dayal and Singh (1986) found increase in height and number of primary branches when tomato seeds were exposed to MF varying from 15 to 155 mT. There were increased number of secondary branches and yield in safflower (Faquenabi *et al.*, 2009) and broad bean (Podlensy *et al.*, 2005) after treatment with MF. Data on yield showed that there was an increase of 17 and 27 % in plants from SMF and PMF treated seeds respectively. This was mainly attributed to increase in average weight of the fruit. Increased yield in tomato exposed to full-wave rectified sinusoidal non-uniform MFs induced by an electromagnet at 100 mT (rms) for 10 min and at 170 mT (rms) for 3 min was observed by De Souza *et al*. (2006). The earliness in flowering and fruit setting observed in magnetoprimed seeds can be exploited for benefit of farmers as it can fetch a good market value for the seasonal horticultural crops by supplying fruits early in the season.

7. SUMMARY AND CONCLUSION

The present investigation entitled **“Role of reactive oxygen species and hormones in seed germination of magnetoprimed cherry tomato seeds”** was conducted to understand the effect of magnetopriming on ROS homeostasis maintained by antioxidative metabolism and protein carbonylation during the germination in static and pulse magnetoprimed seeds of cherry tomato. The hormone profile (ABA and GA) in germinating magnetoprimed seeds was also evaluated to study the interplay of hormones with ROS during germination.

Cherry tomato (selection 1) seeds were magnetoprimed with different doses of static magnetic field in the range of 50 -150 mT for duration of 30 min and 1 hour. The best static magnetic field dose (100 mT for 30 min) was then applied as pulse in the cycle of 2, 3, 5 and 6 min on and off. Out of the various pulsed magnetic field doses 100 mT pulsed in 3 min on and off cycle showed enhancement of germination characters and significant improvement in seedling vigour.

Summary of the results is as under:

1. A significant increase was observed in root length and Vigour Index II of pulsed magnetic field dose where 100 mT was applied as pulse in the cycle of 3 min on and off for 30 min.
2. Germinating seeds of Pulsed magnetic field (3 min) resulted in faster uptake of water whereas marginal increase was observed in SMF treated seeds compared to control
3. A maximum two fold increase was observed in levels of free radicals at different stages of imbibition in germinating magnetoprimed seeds suggesting their role in oxidative signaling during vigourous seedling establishment in primed seeds.
4. The production of H_2O_2 was mediated through the increased activity of superoxide dismutase and oxidative reaction of cell wall peroxidase.

5. Increase in ascorbate peroxidase and catalse activity in the early and late stages of imbibition respectively aided in maintaining the levels of ROS within the oxidative window required for promotion of germination.
6. Increased protein carbonylation also explained its role in ROS scavenging and faster mobilization of reserve protein for more vigourous seedling establishment under pulsed mode.
7. Abscisic acid content decreased in all treatments during imbibition but gibberellic acid was observed during the later stages. This indicates that GAs are required for completion of germination and ROS may affect GA signalling for GA biosynthesis during seed germination.
8. Yield increased in magnetoprimed seeds, with a significant 27% improvement in case of PMF treated seeds. This increase was mainly due to significant increase in fruit size. The earliness to flower and set fruit in magnetoprimed seeds can be exploited as an economic advantage for higher market return.

Static and pulsed magnetic field treatment can be used for improving seedling vigour and subsequently the productivity of cherry tomato under controlled environment. Enhanced seedling vigour in magnetoprimed seeds was due to higher production of reactive oxygen species in the magnetoprimed seeds. Antioxidative enzymes and protein carbonyls helped in maintaining the levels of ROS within the oxidative window. The interplay of ROS with hormones suggested that GA signaling may lead to GA biosynthesis and help in completion of germination. Pulsed magnetic field treatment yielded a better response and can be very renumerative and ecofriendly seed enhancement technology for yield improvement of cherry tomatoes.

8. ABSTRACT (ENGLISH)

Role of reactive oxygen species and hormones in seed germination of magnetoprimed cherry tomato seeds

Cherry tomato seeds (Selection 1) were optimized for seed invigouration by magnetopriming of seeds. Static magnetic field dose of 100 mT for 30 min and pulsed dose of 100 mT applied in the cycle of 3 min on and off pulse for 30 min duration were found to be most effective in enhancing the germination parameters. A maximum of two fold increase over the control was recorded in superoxide anion and hydrogen peroxide production during the germination of magnetoprimed seeds in both the above treatments. Antioxidant enzyme system revealed that increased superoxide dismutase activity and oxidative reaction of peroxidase resulted in production of increased levels of hydrogen peroxide during germination of magnetoprimed seeds. Hydrogen peroxide was scavenged by coordinated action of ascorbate peroxidase and catalase. Reactive oxygen species (ROS) are involved in oxidative signaling and their levels are fine tuned to facilitate the improvement in germination in cherry tomato. Protein carbonyl content also increased in primed seeds for ROS homeostasis and mobilization of seed reserve protein towards faster seedling establishment in magnetoprimed seeds. The interplay of ROS with hormones showed that ABA levels decreased in the magnetoprimed cherry tomato seeds early during imbibition along with increasing levels of ROS and H_2O_2. GA_3 level was detected after 8 h in the magnetoprimed seeds followed by a decline suggesting that GA signaling may lead to GA biosynthesis thus helping in completion of germination. Pulsed magnetoprimed seeds showed better response in terms of invigouration and field performance. A significant increase of 27% was observed in yield of fruits under controlled condition. This was primarily due to the increase in average weight per fruit. Flower initiation and fruit set were also early in plants from the magnetoprimed seeds. This earliness can be advantageous in obtaining good market value of the crop.

9. ABSTRACT (HINDI)

सार

चुम्बकीय-प्रवृत चेरी टमाटर बीजों के अंकुरण में प्रतिक्रियात्मक ऑक्सीजन प्रजाति और हारमोनों की भूमिका

टमाटर बीजों में चुम्बकीय-प्रवृतता द्वारा बीज ओजीकरण के लिए चेरी टमाटर बीजों(सिलेक्शन 1) को तैयार किया गया।प्रयोग में लाई गई स्थिर चुम्बकीय क्षेत्र मात्रा 100 mT 30 मिनट के लिए और तरंगित मात्रा 3 मिनट तरंगित और अतरंगित चक्र के साथ 30 मिनट अन्तराल के लिए, अंकुरण मापदंडों को बढाने में सबसे ज्यादा प्रभावकारी सिद्ध हुई।दोनों उपरांकित उपचरणों में चुंबकीय-प्रवृत बीजों के अंकुरण के दौरान सुपरऑक्साइड एनायन और हाइड्रोजन पेरोक्साइड के उत्पादन में नियंत्रण के ऊपर अधिकतम दोहरी वृद्धि दर्ज की गई। एंटीऑक्सीडेंट एंजाइम तंत्र ने यह प्रदर्शित किया कि बढ़ी हुई सुपरऑक्साइड डिसम्यूटेज गतिविधि और पेरोक्साइड की ऑक्सीकरण अभिक्रिया चुंबकीय-प्रवृत बीजों के अंकुरण के दौरान हाइड्रोजन पेरोक्साइड के बढे हुए स्तर में फलित हुई। असकोरबेट पेरोक्साइड और कैटालेज की समन्वित क्रिया से हाइड्रोजन पेरोक्साइड अपमार्जित हुआ। प्रतिक्रियाई ऑक्सीजन प्रजाति (आरओएस) ऑक्सीकरणित संकेतन में शामिल थे और उनके स्तर चेरी टमाटर के अंकुरण को सुगम बनाने के लिए पूर्णतः विवरणित थे। प्रोटीन कार्बोनिल मात्रा भी प्रवृत बीजों में आरओएस स्थिरीकरण के लिए बढ़ी और चुंबकीय-प्रवृत बीजों में बीज संचित प्रोटीन तीव्रतर अंकुर बनने की ओर संघटित हुए। आरओएस के हार्मोनों के साथ अंतर्द्वंद ने यह प्रदर्शित किया कि आरम्भिक अंतःशोषण के दौरान आरओएस और हाइड्रोजन पेरोक्साइड के वर्धित स्तर के साथ साथ, चुंबकीय-प्रवृत चेरी टमाटर बीजों में एबीए स्तर भी घट गए। चुंबकीय-प्रवृत बीजों में जीए स्तर 8 घंटे के बाद सूचित हुआ जो कि एक गिरावट के साथ अनुगमित हुआ, जो यह संकेत करता है कि जीए संकेतन जीए जैवसंश्लेषण की ओर अग्रसर कर सकता है और इसलिए अंकुरण को पूर्ण होने में सहयोग करता है। तरंगित चुंबकीय-प्रवृत बीजों ने ओजीकरण और क्षेत्र-प्रदर्शन के मापदंड पर बेहतर प्रतिक्रिया दी। नियंत्रित परिस्थिति में फलों की उपज में 27% की महत्वपूर्ण वृद्धि देखी गई। यह मुख्यतः औसत भार प्रति फल में बढ़ोत्तरी के कारण हुआ। चुंबकीय-प्रवृत बीजों से पुष्प-उपक्रमण और फल तैयार होना भी पौधों में शीघ्र हो गए। यह आरम्भ में तैयार होने का गुण फसल के लिए अच्छा बाजार मूल्य प्राप्त करने में लाभदायक हो सकता है।

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