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Quantum Biology and Plants – New insights for Plant Science and Crop Improvement.

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Quantum Biology and Plants – New insights for Plant Science and Crop Improvement.

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Abstract:

Quantum Biology is an emerging discipline involving the study of quantum physical phenomena in biology and medicine. This applies to plant biology through the effects of static and Radiofrequency electromagnetic fields on plant growth and development; many of these effects being of potential economic interest. Quantum theory predicts that weak magnetic fields can alter the reaction rates (product formation) of biochemical reactions such as those catalyzed by metabolic enzymes or implicated in the biological activity of flavoprotein receptors such as cryptochromes. A further predicted consequence of electromagnetic fields is their effect on the synthesis of cellular ROS (reactive oxygen species), which are also central regulators of many agronomically important plant processes. The main goal of this communication is to briefly summarize the known effects of magnetic fields in biological systems with the view to identifying practical applications for plants. Current understanding of quantum physical mechanisms for modulating biological reactions will be discussed in terms of magnetic field effects on the cryptochrome blue-light receptor and on the formation of ROS (reactive oxygen speces). In conclusion, we will suggest workable methods to achieve low cost, environmentally friendly, and broadly applicable crop improvement strategies using tools from Quantum Biology.

Keywords:

Cryptochrome, RadioFrequency, Magnetic Field, Quantum Biology, Reactive Oxygen Species, Radcial Pair Mechanism, Plant Biotechnology.

Abbreviations:

mT; milliTessla
ROS; reactive oxygen species
RF; radiofrequency
PEMF; pulsed electromagnetic field
EMF; electromagnetic field
Hz; Hertz

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1. Introduction:

All living things are exposed to the Earth's magnetic field, which is generated by movement of molten metal at the Earth's core. This so-called geomagnetic field is a directional force that curves upwards from the magnetic South Pole, extends above the upper atmosphere, and then curves downwards towards the North Pole (Wiltschko and Wiltschko 1995). The most crucial role of the Earth's magnetic field is to deflect the ionizing particles brought to the upper atmosphere by solar wind and cosmic rays. These would otherwise destroy the ozone layer and render all life on Earth impossible (European Space Agency, 2020). However, the geomagnetic field also has a much more direct impact on biological organisms, and can trigger a biological response under appropriate conditions. Among the most widely accepted and best understood of these effects is the ability of living organisms to respond to direction of the magnetic field, for instance for directional orientation in migratory birds (Wiltschko and Wiltschko 1995; Wiltschko and Wiltschko 2019) and in many other organisms (Nyqvist et al 2020; Johnson and Lohmann 2005; Lefèvre and Bazylinski 2013; Monteil and Lefèvre 2020). There are also countless cellular and behavioural effects that have been documented as responsive to electromagnetic fields, which are found in organisms ranging from microbes to man (see eg. Hong 1995; Binhi 2002). In this article we shall focus primarily on effects of the very weak magnetic fields (0 – 1 milliTessla), within around an order of magnitude of the Earth's geomagnetic field (0.04 – 0.05 milliTessla). These forces are too weak to trigger classical biochemical or thermal reactions but can in principle interact with biological organisms by Quantum Biological processes.

1.1 Magnetic Fields modulate agronomically important traits in plants.

In the case of plants, magnetic fields impact on a vast array of growth and developmental phenomena, many of which have been recently comprehensively reviewed (see eg. Galland and Pazur 2002; Maffei M, 2014; da Silva and Dobranszki 2016; Radhakrishnan 2019). These include both directional and intensity – dependent effects of applied magnetic fields. Examples of directional effects include reported alignment of root growth with the Earth's magnetic field in certain cultivars of wheat and maize (Bogatina etal 1986, Zhadin 2001) as well as radish and beets (Schreiber 1958; Novitskaya et al. 2004), Roots were found to adopt either a North/South or East/West plane under field conditions. In addition, rotation of plants within the geomagnetic field induced non-symetrical effects on plant growth depending on the direction of rotation. For example slow rotation of plant seedlings (at 1 - 6 RPM) in a clockwise direction to the Earth's magnetic field caused differential growth in comparison to rotation in a counterclockwise direction in *Cyclamen*, *Phaseolus,* and *Avena* seedlings. The resulting puzzling observation was that rotating plants in one direction as compared to rotation in the other direction, changed root and shoot elongation growth, water uptake, and onset of senescence (reviewed in Galland and Pazur 2002).

In addition to these directional effects, a multitude of physiological changes could be induced in plants by changing the intensity of the magnetic field. For example, simply decreasing the intensity of the Earth's geomagnetic field to near Zero induced alterations in the root meristems; could increase or decrease fresh weight; alter mitochondrial morphology; alter water uptake; shorten or lengthen time to flowering; reduce or enhance seedling growth rates; lead to formation of binuclear or tetranuclear cells; cause ultrastructural changes in organelles; and cause alterations in gene expression and enzymatic function in different plant models. Such low level magnetic field-induced changes were documented in cultivars of plants including *Hordeum*, *Triticum*, *Vicia*, *Pisum*, *Linum*, *Zea*, *Lepidum*, *Daucus*, *Belladonna, Allium, Arabidopsis* and *Quercus* (reviewed in Galland and Pazur 2002; Maffei, 2014; da Silva and Dobranszki 2016).

Increasing the magnetic field strength above that of the Earth's field likewise caused both positive and negative effects on plant growth and metabolism. The most well-studied of these effects have been in improving the efficiency of seed germination, including breaking of seed dormancy, which was observed in seeds from barley, corn, chickpea, beans, wheat and some tree species (reviewed in Maffei, 2014). It should be noted however that effects on seed germination generally required exposing them to vastly increased magnetic field strengths as compared to the Earth's magnetic field (at least 100 X the geomagnetic field and higher) (see eg. Mahajan and Pandy, 2014; Iqbal et al. 2012; Cakmak et al. 2010). Because such high magnetic field strengths affect physical properties of solutes and growth media such as ion hydration and formation of calcium carbonate, the effects of such high magnetic fields on seed germination may be related to changes in solute composition rather than to interaction of the magnetic fields with biomolecules (Li et al 1997). Effects on plants of increasing the magnetic field strength in a more moderate range (.05 – 1 mT) show effects on plant elongation growth, enzymatic and metabolic activity, tolerance against environmental stresses and development (Maffei 2014, Galland and Pazur 2005, Radhakrishnan 2019), including in relation to cryptochrome-dependent response to the magnetic field in *Arabidopsis* (see below). Changes in plant growth and development have also been noted in response to pulsed magnetic fields. These so-called ELF – MFs (extremely low frequency magnetic fields) can be generated by electrical currents that are pulsed at intervals, generally of 10 to 300 Hz, and thereby trigger corresponding magnetic field pulses of equal frequency and relatively low intensity. Natural sources of ELF-MFs could be electric installations and power lines, for instance. ELF-MFs appear to affect many of the same plant growth and developmental processes as do changes in the static magnetic field strength, possibly by triggering the same underlying mechanisms (reviewed in Maffei 2014).

Finally, in the context of Quantum Biology and plants, it should not be ignored that a vast literature exists on the physiological effects of Radiofrequency (RF) oscillating magnetic fields on growth and development (reviewed in Vian et al 2016). Radiofrequency fields are non-ionizing electromagnetic fields in the MHz to GHz frequency range that can be generated, among other sources, by telecommunications and wireless technology. The effects on plants of radiofrequency fields at non-thermal intensities (ie at low energy levels that do not elevate their temperature) have been studied for literally decades, and many of the same growth and developmental processes in plants appear to be affected that have shown response to static magnetic fields. For example effects on plant elongation growth, metabolism, and gene expression have been documented at various RF frequencies on cultivars including *Vigna*, *Phaseolus*, *Zea*, *Solanum*, *Lemna*, *Triticum*, *Nicotiana*, etc (Vian et al 2016). Certain RF effects on plants can, in principle, be explained through mechanisms from Quantum biology (see below)

1.2 Difficulty of Interpreting Electromagnetic Field Effects in Plants

Despite these literally hundreds of individual case studies showing promising, potentially economically significant magnetic effects in different plant species, this field has remained highly controversial. First and foremost, problems have been methodological. Effects of magnetic fields are often subtle, producing only modest change in any measured physiological response to exposure. As a consequence, magnetic field conditions and protocols need to be precisely defined and all additional experimental factors that might affect the outcome of a study need to be strictly controlled. Therefore, among the biggest problems has been that the magnetic field exposure conditions in many trials, whether of static, pulsed, or Radiofrequency signals, are not standardized, and have in some cases been poorly controlled and insufficiently defined (summarized in Maffei et al 2014; Pazur and Galland 2005). This was not only due to insufficiently accurate measuring protocols, but also failure to exclude possible external inhomogeneities in the setup which may also affect the outcome (eg. contaminating sources of electromagnetic fields, surrounding metal, or fluctuations in light, temperature or humidity). Insufficient signal definition and control were particularly serious considerations in assessing the accuracy of data from Radiofrequency field experiments (Vian et al 2016). As a consequence, very few studies have been independently replicated and many such attempts have led to contradictory results.

In addition to these methodological difficulties with the exposure systems, a further problem for advancing the field arises from the many different biological protocols used by different studies. These span the range from investigating changes in fresh weight of crop species under field conditions to changes in enzymatic activity in proteins extracts in the laboratory; all the while using vastly differing magnetic field exposure times, intensities, and signal types. This methodological heterogeneity has completely obscured possible common features, underlying mechanisms or cause-effect relationships. For instance, it is impossible to know whether changes in calcium homeostasis reported in *Pisum* in response to a Low Level magnetic field in one study (Belyavskaya 2001) is the cause of changes in gravitropic growth observed in roots by another study (Kato, 1990), or whether these two Low Level field effects are completely unrelated. Understanding of underlying phenomena is furthermore compromised by the many different plant species, developmental stages, tissue types and environmental conditions used in these studies.

However, perhaps the most serious problem in the field has been an insufficient understanding of the underlying mechanisms involved. The identity of the biological magnetoreceptor(s) and the primary event(s) in triggering a biological response in plants have remained completely unknown. A testable hypothesis on the mechanism by which magnetic fields interact with biological systems is essential for design of rational experiments and meaningful new technological applications in plants.

2. The Radical Pair Mechanism of Biological Magnetosensing.

There are currently only a few models providing a mechanism for how very weak magnetic fields interact with biological systems. The most well-characterized of these involves ferrimagnetic minerals such as magnetite (Fe_30_4), which can form chains that align with the geomagnetic field (Johnsen and Lohmann 2005). They have been proven to provide the basis for orientation by magnetotactive bacteria and other microbes (Lefèvre and Bazylinski 2013). Magnetite crystals are also found in plants, animals and humans, and have been implicated in orientation responses in birds and some mammals. Magnetite is sensitive to the polarity of the magnetic field (ie points in the direction of the North Pole) just as is an iron-based man-made magnetic compass, and is in this way thought to convey directional information. However, magnetite-based mechanisms have not been shown to underlie any plant magnetic field effects (Galland and Pazur 2002; Maffei M, 2014; da Silva and Dobranszki 2016; Radhakrishnan 2019) and it is not obvious how such a system could trigger intensity-dependent physiological effects on plants that occur independently of the magnetic field direction.

An alternative to a ferromagnetite-based magnetosensing mechanism was derived from the application of quantum physical principles to biochemical reactions via the so-called Radical Pair mechanism (reviewed in Hore and Mouritsen 2016; Kominis 2015; Rodgers 2009). Magnetic fields near to the strength of the Earth's geomagnetic field are far too weak to trigger cellular biochemical reactions as they are many orders of magnitude below thermal energy minima for breaking of chemical bonds. However, once a chemical reaction is initiated in a molecule by some other energy source (eg. light or metabolic forces), then weak magnetic fields can interact with short-lived reaction intermediates that are formed in the course of these reactions to affect the outcome. An example would be a photoreceptor-induced reaction, where light triggers the biochemical reaction in a receptor, but whose amplitude can subsequently be modified (accelerated or slowed down) by a magnetic field. In this way, the biological response (extent of chemical activation) of the photoreceptor to a given light intensity could be either increased or decreased by an applied magnetic field. Another example where magnetic fields could modulate reaction rates would be in redox reactions, which can be initiatied by metabolic factors, and whose rate of product formation (eg metabolites such as ROS) could be altered. As a consequence, the radical pair mechanism provides a theoretical framework whereby magnetic fields could influence the efficiency of numerous biochemical reactions within the cell.

The targets for such a mechanism are biochemical reactions that form specific spin correlated radical pair intermediates. In this mechanism, a biochemical reaction is triggered by light or some other energy source in the cell. Atoms with unpaired electrons (a so-called 'radical pair') are generated by this initial step. When they are first formed, quantum theory dictates that the electron spins of the correlated radical pair are initially in opposite directions (the so-called singlet state). The singlet state then converts to parallell electron spins (the so-called triplet state). *The effect of the magnetic field is to intervene in this biochemical reaction by altering the rate of singlet – triplet interconversion in the intermediate state radical pair.* If the rate of product formation from the singlet intermediate state is different from that of the triplet state, the magnetic field can in this way influence the speed of a suitable biochemical reaction in the plant cell. The radical pair mechanism has been supported experimentally by demonstrations in model organic compounds and isolated proteins (Hore and Mouritsen 2016, Rodgers 2009).

It should be noted that many alternative suggestions have also been made concerning how electromagnetic forces may impact on biological systems, and that have also been extensively reviewed (see eg. Binhi 2002, Binhi 2017). We do not consider them here because no experimental evidence in their support has yet been provided. By contrast, the Radical Pair mechanism offers possible explanations for many of the known effects discussed below, and also suggests protocols for practical applications that may lead to crop improvement.

2.1 Biological Consequences of the Radical Pair Mechanism.

There are actually a vast number of cellular processes that form free radical reaction intermediates and whose rates could therefore, in principle, be either speeded up or slowed down in the presence of altered magnetic fields. In particular, metabolic and redox enzymes involved in oxidative respiration, ATP synthesis and photosynthesis are prime candidates for radical pair-based magnetosensitive reactions. In addition, the radical pair mechanism could mediate directional effects, since effect on electron spin would also depend on radical pair orientation with respect to the magnetic field inclination. Thus, if enzymes could somehow be ordered or aligned within a biological system, then their reaction rates could be altered only by suitably oriented incoming magnetic fields. In sum, the radical pair mechanism could in principle account for both directional and intensity-dependent magnetic field effects on biological systems, particularly at low magnetic field strength.

In practice, however, the theoretical effects of a magnetic field on biochemical reaction rates would be relatively modest, with maximal predicted change in singlet/triplet interconversion rates on the order of only a few percent (see Kominis 2015 and references therein). Furthermore, quantum effects of weak magnetic fields require distinct spatial and energetic relationships between the radical pairs and the surrounding molecules and media. It is therefore not currently possible to predict, even theoretically, whether a given cellular biochemical reaction would form magnetically sensitive radical pair intermediates *in vivo*, and what their optimal response characteristics might be. Finally, to see an effect of magnetic fields in whole organisms, the magnetically sensitive reaction step must be in some way rate limiting for a given physiological or growth response – otherwise, small changes in their efficiency would not lead to any measureable changes in the organism. This leads to one of the principal problems with defining magnetic field effects in biological systems, namely that primary effects on cellular biochemical reaction rates are likely to be subtle, dependent on environmental context, and may not lead to observable physiological consequences (Kominis 2015). Indeed, studies with promising candidate flavoprotein enzymes *in vitro* did not yield any measureable magnetic field effects on redox reaction rates, despite the formation of Radical Pair intermediates (Messiha et al 2015). Even in instances when measureable effects of the Radical Pair mechanism are observed *in vitro*, such as changes in electron transfer and triplet yields in bacterial (Hoff et al. 1977) or plant photosynthesis (Sonneveld et al. 1981), these do not necessarily occur at magnetic field intensities or time scales relevant to modulating photosynthetic rates *in vivo* (reviewed in Cao et al. 2020). Thus magnetic sensitivity of a reaction *in vitro* is no guarantee that there will be a magnetosensitive response to this same reaction occurring *in vivo*.

As a result of these limitations, the only identifiable effects of the Radical Pair mechanism that can ever be visualized *in vivo* will be those regulating receptors or signaling pathways where *even small changes in reaction rates* could lead to measureable change in biological response. In keeping with this prediction, the most well-characterized Radical-Pair based biological response known to date, which occurs in migratory orientation in birds, is thought to require a photoreceptor-based mechanism (Wiltschko and Wiltschko 2019). A second proven instance of the Radical Pair mechanism *in vivo* occurs in mammalian cell cultures, where a magnetic field can alter rate in synthesis of ROS (reactive oxygen species), a critical cellular signaling molecule to which cells are uniquely sensitive to small concentration differences (Usselman et al 2014; 2016). Nonetheless, and with this caveat in mind, Radical Pair based magentosensing mechanisms are predicted to be fairly universal and have indeed recently also shown to likely occur in plants. These experiments will be described below.

3. Cryptochrome, a putative biological magnetoreceptor.

The possibility that cryptochrome photoreceptors might serve as biological magnetoreceptors was first suggested by Ritz and colleagues with respect to migratory orientation in birds (Ritz, 2000). This was because birds require blue/UV light for navigation to the Earth's magnetic field direction, and cryptochromes were the only known photoreceptors at the time that could theoretically form radical pairs upon activation by light. In fact, cryptochromes are evolutionarily ancient flavoprotein receptors mostly studied for their roles in blue light perception, and are found throughout the biological Kingdom, including in plants and animals (Chaves et al 2011, Foley et al 2020, Wang and Lin 2020, Vechtomova et al 2020). Cryptochromes have many physiological functions in the different organisms including mediating de-etiolation, growth and development in plants, and regulating the circadian clock in insects and mammals. Photochemically, cryptochromes can be activated through flavin reduction by illumination with blue light, generating reduced flavin redox states. This process is accompanied by the formation of radical pair intermediates, and subsequently triggers an activated conformational state that mediates biological activity (Ahmad, 2016; see also Hammad et al 2019 for more detailed discussion).

Because all cryptochromes can undergo comparable redox chemistry, they would be expected to generate similar Radical Pair intermediates, and thereby could all, at least in principle, be sensitive to electromagnetic fields.

3.1 Cryptochromes and the Avian Compass

In the case of migratory birds, the idea that cryptochromes may function as magnetosensors gained particular traction when it was determined that cryptochromes were located in the retina in cell types suitable for magnetosensing. One variant, avian Cry1a, was found to be located in structured arrays in the UV cones consistent with a directional sensing role. Further experiments showed that the wavelengths of light that activated avian Cry1a in the living retina match the wavelengths of light that are required for bird directional sensing (reviewed in Wiltschko and Wiltschko 2019). It was suggested that, in order for magnetosensing to occur, the biological activity of the avian cryptochrome should change depending on its orientation with respect to the geomagnetic field. In this way the bird could detect the magnetic field inclination by simply turning its head and comparing cryptochrome signal intensity when looking in different directions (Wiltschko and Wilstchko, 2019). Additional circumstantial evidence of a role in magnetoreception was obtained from studies of isolated cryptochrome proteins of different species (insects, plants, and some vertebrates), including of photolyases, which are evolutionarily related ancestral proteins to cryptochromes (Vechtomova et al 2020). These studies showed that all cryptochrome-like proteins tested, regardless of biological origin, formed radical pair intermediates upon illumination and that their rates of photoreduction (leading to activation) could be manipulated by applied magnetic fields *in vitro* (see eg. Mouritsen and Hore 2016). However, *in vivo* studies of light sensitivity in living birds showed that these *in vitro* reactions could not be involved in the avian magnetic compass *in vivo* since magnetic orientation does not rely on light-triggered reactions (reviewed in Hammad et al 2019; Wiltschko et al. 2019). As a result, although it is fairly conclusively established that birds have a magnetic compass based on the Radical Pair mechanism (Mouritsen and Hore 2016), conclusive proof is still lacking that cryptochromes are the magnetic field receptors in birds. There is furthermore an ongoing discussion concerning which cryptochrome(s) may be involved and on the possible identity of the magnetosensitive radical pairs. These questions are likely to be resolved only by better methods for purifying and detecting magnetic field effects on avian cryptochromes, and from applying insights on the mechanism of cryptochrome magnetosensing derived from other experimental systems (below).

3.2 Cryptochrome-dependent magnetic field effects in Drosophila and Mouse.

As a more direct test for possible magnetosensitive properties of cryptochrome, other biological systems have been examined on the assumption that the basic chemistry involved should not change between cryptochromes in different organisms. Species that were examined included the *Arabidopsis*, *Drosophila*, and *Mouse* model systems. What all of these systems have in commmon is that endogenous cryptochrome function has been well characterized, and they are amenable to genetic approaches including mutation and knocking out of the cryptochrome genes themselves (Chaves et al 2011, Foley et al 2020). Because the cryptochrome receptor in these systems is soluble and therefore not oriented, it was not expected to obtain directional effects of the magnetic field as observed in birds. However, intensity dependent effects on cryptochrome function should still occur via the radical pair mechanism (see above Section 2). The consequence should be to either enhance or reduce cryptochrome function in the presence of an altered magnetic fields.

Accordingly, the following simple experimental approach was used to determine whether magnetic field effects were actually caused by cryptochrome. Living organisms were exposed to a magnetic field in the 0 – 1mT range, which is in reasonable proximity to the intensity of the Earth's magnetic field of 0.05mT and therefore suitable for a Radical Pair based mechanism. Specific physiological traits that were known to involve cryptochrome function were measured in the presence or absence of applied magnetic fields in these organisms. If a changed response to magnetic field exposure was detected, then the same traits were examined in a mutant organism that lacked cryptochrome. In this way, a role for cryptochrome in magnetic field sensing could be established.

A large proportion of animal studies have been performed in the *Drosophila* model system. These involved detection of behavioural responses, where exposure to magnetic fields could induce fruitflies to move towards magnetic fields, or else display a natural avoidance response. The extent of negative geotaxis, whereby flies preferentially move away from the gravity vector, were also affected. In addition, cellular functions were affected such as the period length of the *Drosophila* circadian clock, inducing seizures in *Drosophila* larvae, and promoting neuronal activity in motor neurons (reviewed in Foley et al 2020). What was particularly significant about these studies is that each and every one of these magnetic field effects required the presence of cryptochrome, and involved phenotypic traits that had been shown, at least partially, to be under cryptochrome control. These magnetic field effects (MFEs), although statistically significant were fairly minor (generally no more than 10 – 20% change caused by the magnetic field), and many also required precise calibration of illumination conditions (blue light intensity) to visualize. This is in principle consistent with the Radical Pair mechanism. However, in at least one instance, it appeared that full length cryptochrome (containing the flavin) was not required for an MFE (Fedele et al. 2014b), which excludes a radical pair-based mechanism if Cry is indeed the magnetoreceptor for this response. In sum these experiments proved that cryptochrome is involved in response to the magnetic field in fruitflies, but leaves open the question whether cryptochrome is an actual magnetoreceptor and, if so, it obeys the Radical Pair mechanism.

In the mouse (*Mus musculus*) system cryptochrome is best known for its role in the regulation of the circadian clock. This does not appear to require either light or flavin redox chemistry and thereby could not be regulated by magnetic fields via the Radical Pair mechanism (reviewed in Chaves et al. 2011, Michael et al 2017). However, there are two recent reports of magnetic field effects in mouse that involve cryptochrome. The first involves stimulation of ROS (reactive oxygen species) synthesis in cell cultures exposed to low frequency pulsed electromagnetic fields (Sherrard et al 2018); the second involves stimulation of repair to damaged neuronal networks in mouse brain by pulsed electromagnetic fields (Dufor et al. 2019). These effects were entirely absent in cryptochrome - deficient knockout mouse mutants. These results are conclusive in implicating cryptochrome in the responses to electromagnetic fields in mammals. However, as in the case of the fruit fly studies, they do not prove that cryptochrome is the actual magnetosensor, nor do they prove the underlying magnetosensing mechanism involved.

3.3 Cryptochrome - dependent magnetic field effects in *Arabidopsis.*

Arabidopsis cryptochromes (cry1 and cry2) regulate many aspects of growth and development, which have been extensively documented (see eg. Chaves et al 2011, Wang and Lin 2020). Among the most well-studied are the seedling de-etiolation and the inhibition of hypocotyl elongation response, in which stem elongation of germinating seedlings (hypocotyl growth) is inversely proportional to the blue light intensity. Exposure of *Arabidopsis* seedlings to a 0.5 mT magnetic field (10X the geomagnetic field) under precisely controlled illumination conditions resulted in a 10 – 20% decrease of hypocotyl growth inhibition, which was interpreted as increase in biological activity of cryptochrome caused by the magnetic field (Ahmad et al 2007) – see Fig.1. Although initially challenged (Harris et al 2009), effects on hypocotyl growth inhibition by exposure to magnetic fields were replicated under optimised conditions in several subsequent studies (Xu et al 2012, Pooam et al 2019, Hammad et al 2020, Albaqami et al 2020). Other experiments showed that seedlings exposed to a magnetic field that was less than that of the Earth's geomagnetic field (Low Level Field) also showed an effect on hypocotyl growth, in this case increased growth (decreased in cryptochrome activity) (Xu et al 2012, Albaqami et al. 2020). Importantly, all of these effects on seedling hypocotyl growth required blue light and intact cryptochrome.

FIGURE 1. Example of Magnetic Field effect on Arabidopsis seedling growth. To detect the effect of exposure to a 0.5mT magnetic field (10X the strength of the geomagnetic field of .05mT) Arabidopsis seeds were plated in stacks (left panel). The control stack was placed at earth strength (.05mT) magnetic field and the test stack was placed inside a triaxial Helmholz coil which generated a homogeneous static magnetic field in the vertical axis for 3 – 5 days. Seedling growth was measured after this time in both exposed and control plates; exposure to the magnetic field resulted in decreased hypocotyl length (right panel) indicative of enhanced cryptochrome biological activity (Pooam et al 2019).

The photoperiodic initiation of flowering is another well-studied effect mediated by *Arabidopsis* cryptochrome, in particular by *cry2*. Mutants in *cry2* showed significantly delayed flowering time as compared to wild type plants, both in long and short day growth conditions (reviewed eg. in Wang and Lin 2020). The effect of exposure to magnetic fields on the number of days to flowering of *Arabidopsis* plants was tested in low intensity white (Xu et al 2012) or blue (Xu et al 2015) light at reduced magnetic field intensity (Low Level Field). There was indeed a measureable delay (around 15%) in days to flowering, consistent with decrease in cryptochrome function under Low Level field exposure condtions. Mutants of cry2 did not show a delay in flowering at Low Level Field as compared to the control condition. Plants grown only in red light where cryptochrome is not active, also did not show a delay in flowering in a Low Level magnetic field as compared to the Earth's field control condition, indicating cryptochrome was involved in this response. In support of these results, exposure to Low Level magnetic fields were also observed to delay *Arabidopsis* flowering by 4 days by an independent laboratory. Such exposure furthermore delayed the expression of multiple genes involved in floral initiation including AP1, AGL24, FT and FLC, although in this study plant growth was performed at much higher white light intensity and had not been linked to cryptochrome function in this case (Agliassa et al. 2018).

In keeping with their effect on flowering time, exposure to Low Level magnetic fields were also observed to alter plant hormone signaling pathways known to be involved in floral initiation. These were assayed under limiting blue light growth conditions where the role of cryptochrome on the magnetic field response had been established (Xu et al 2012, 2015). Levels of several GA (giberellin) variants were reduced in plants exposed to Low Level Fields, as well as decrease in expression of multiple GA oxidase and floral genes LFY and SOC1, consistent with the delay in flowering (Xu et al 2016). Auxin signaling pathways were also altered, in particular expression of auxin transporter genes PIN1, PIN3 and PIN7 was upregulated as well as that of auxin transcriptional repressor genes (Xu et al 2017). The ensuing change in auxin distribution and regulation was hypothesized to contribute to the delay in floral initiation. Importantly, all of these many changes induced by LLF exposure observed in these studies were completely lacking in cryptochrome mutants.

3.5 is plant cryptochrome an actual magnetosensor? – Evidence from cryptochrome phosphorylation and protein degradation experiments in *Arabidopsis.*

From these abundant previous examples it could be definitely concluded that cryptochrome receptors are implicated in numerous magnetically sensitive responses in *Arabidopsis*. However, these studies have not proved that cryptochromes function as actual magnetoreceptors, nor do they provide the underlying mechanism involved.

In fact, there is increasing evidence that cryptochromes may not, in fact, be the actual magnetosensor for all of the magnetically sensitive phenotypes in which they have been implicated. For example, in fruitflies it has been shown that effects of cryptochrome on the circadian clock do not require that the flavin be present for a magnetosensitive response to occur, making it doubtful that cryptochrome could be the actual magnetosensor in this particular response (Fedele et al 2014b). In the case of plants, it has been reported that cryptochrome regulation of some genes occurs in continuous darkness (eg. of HYH, PKS1) but is nonetheless modulated by Low Level magnetic fields. Since there is already no known mechanism whereby cryptochrome receptors might regulate gene expression in complete darkness, this finding evidently also excludes a role for cryptochrome as magnetosensor by any known mechanisms (Agliassa et al 2018). In addition, there are reports of *Arabidopsis* genes whose expression is altered by exposure to magnetic fields coming from different directions, which is therefore unlikely to be mediated by cryptochrome (which is not oriented in plants). Finally, numerous changes in gene expression in response to magnetic field exposure reportedly occur in red light and in cry1cry2 double mutant seedlings, thereby excluding any magnetosensing role in these responses for cryptochrome (Agliassa et al 2018; Dhimana and Galland 2018).

It is therefore evident that cryptochromes are not the only magnetosensing receptors in plants. The possibility that alternate magnetosensing mechanisms to the Radical Pair mechanism exist in plants can also not be excluded.

In spite of this added complexity, there is increasing evidence that cryptochromes indeed have the capacity to function as primary magnetosensors in plants. This follows from studies using a characteristic of *Arabidopsis* cryptochromes to undergo conformational change upon activation by light. One consequence is that light-activated cryptochromes have an exposed of C-terminal domain (CCT), which makes it accessible to cellular kinases and subsequent phosphorylation. Thus, a change in the activation state of the receptor in vivo can be followed by simple Western blot analysis assessing phosphorylation (upper-shifted band) in response to illumination. A further direct assay for conformational change *in vivo* is available in *Arabidopsis* cry2 , where changes in the protein surface after illumination leads to exposure of ubiquitination sites which followed by cry2 protein degradation (reviewed in Chaves et al 2011, Wang and Lin 2020). Therefore, the extent of biological activation of cry2 in response to blue light can be followed by Western blot analysis of cry2 protein degradation in cellular extracts after blue light exposure (the greater the decrease in cry2 protein, the greater the initial cry2 biological activation). The critical difference between these experiments and the many physiological or behavioural assays described above is that the effect of the magnetic field on the *in vivo* activiation state of cryptochrome receptor itself is being monitored.

To detect the effect of a magnetic field on the formation of the biologically active state of Cry2, the rate of *Arabidopsis* cry2 degradation was measured in seedlings that had been exposed to altered magnetic fields and compared to control plants. Indeed, cry2 protein concentration was shown to be measurely reduced in seedlings exposed to Low Level magnetic fields, consistent with a decrease in biological activity (Xu et al 2012, Agliassa et al 2018). Converelsy, cry2 degradation rates was enhanced at increased magnetic field strength consistent with increase in biological activity (Ahmad et al, 2007). Furthermore, the extent of phosphorylation of cryptochrome was reduced by exposure to Low Level magnetic fields (Xu et al 2014, Agliassa et al 2018, Albaqani et al 2020) whereas it was increase at higher magnetic field strength (Pooam et al 2019, Hammad et al 2020) consistent with enhanced biological activity. In support of these findings, a comparable experiment conducted in *Drosophila* showed a comparable differences on the extent of degradation of cryptochrome after exposure to a magnetic field (Fedele et al 2014), consistent with a direct effect of the magnetic field on receptor activation. It should be emphasized that these changes are subtle and incremental, and can only be visualized under highly controlled conditions and at sub-saturating concentrations of blue light where cryptochrome is not at maximum activation.

In summary, these data from multiple different experimental systems and laboratories have now provided conclusive proof that cryptochromes are 1) involved in mediating physiological responses to external magnetic fields *in vivo* and 2) can function directly as a biological magnetoreceptor. These results still do not, however, provide evidence that the Radical Pair mechanism is involved.

4. Cryptochrome and the Radical Pair Mechanism

A remaining question concerns the underlying mechanism for cryptochrome magnetosensing, for example whether there is indeed a quantum sensing (radical pair dependent) mechanism as has been proposed to occur during avian orientation. In biological terms, what this means is that some step in the course of the cryptochrome photocycle must form radical pairs, and that these can be modulated by exposure to electromagnetic fields to either accelerate or slow down the rate of cryptochrome activation.

4.1 How could the Radical Pair mechanism act on Arabidopsis cryptochromes.

The plant cryptochrome photocycle has been extensively reviewed (see eg. Chaves et al 2011, Ahmad, 2016; see also Hammad et al. 2020 for more detailed discussion regarding confusion in the literature). In the case of *Arabidopsis* cry1, the bound FAD flavin occurs in the oxidised (FADox) redox state in the dark. The receptor is in a closed conformational state and is biologically inert. Upon illumination with blue light, the flavin undergoes a chemical reaction leading to formation of the neutral radical (FADH°) and reduced (FADH-) redox states. This is the primary photochemistry that all light-sensitive cryptochromes undergo. Flavin reduction in turn triggers a conformational change in the protein allowing interaction of cryptochromes with signaling partners such as transcription factors, kinases, phosphatases, etc. Flavin photoreduction is very rapid, occurring over a few milliseconds, and induces many of the phenotypic consequences described above. It is accompanied by the formation of radical pairs intermediates (Trp° /FadH°).

Once reduced, the flavin undergoes a spontaneous reoxidation reaction which restores the dark-adapted resting state (FADox). This is a slow reaction, on the order of several minutes, and inactivates the cryptochrome receptor. This reoxidation reaction does not require light and also is accompanied by the formation of radical pair intermediates, possibly including superoxide or other reactive oxygen derivatives as well as FADH°. Importantly, the flavin reoxidation reaction also forms hydrogen peroxide (H_20_2) as an end product. This molecule is in and of itself an important signaling intermediate in many plant defense and adaptation responses (see below). The Arabidopsis cryptochrome photocycle is summarized in Fig.2.

In sum, there are at least two reaction steps in the course of cryptochrome photocycle which could in principle be altered by the magnetic fields; the step of flavin photoreduction or that of flavin reoxidation. In either case, the effect of the magnetic field would be to alter cryptochrome biological activity by changing the rate of formation of the active state (forward reaction) or the rate of disappearance of the active state (reoxidation reaction).

In fact, initial attention has been focussed on magnetic field sensitivity of the forward photoreduction reaction, which has many attractive theoretical points. It has also been experimentally demonstrated *in vitro* that forward electron transfer rates can be altered by static magnetic fields in cryptochromes from various sources, including *Arabidopsis* (see eg. Hore and Mouritsen, 2016). Nonetheless, *in vivo* physiological experiments using alternating light/dark pulses showed that *Arabidopsis* cryptochrome is not sensitive to the magnetic field during light-triggered forward electron transfer, but only during the flavin reoxidation reaction (Pooam et al 2019, Hammad et al 2020). Similar physiological effects were observed in the case of avian magnetoreception (Wiltschko et al 2016). Therefore the extensive literature exploring the Radical Pair mechanism in cryptochromes through studies of forward electron transfer in isolated proteins is not relevant to biological function. Further progress will instead require identification and study of the radical pair formed during flavin reoxidation (see Hammad et al 2020 for a more complete analysis)

In sum, this section provides the most detailed case study to date of how a magnetic field can interact with a biological system to give a physiological response. The *Arabidopsis* cryptochrome photoreceptor responds to a magnetic field in the course of the flavin reoxidation reaction (transition from active to inactive resting state) by altering its rate. This results in a change in the equilibrium concentration of the activated state of cryptochrome. In other words, either accelerating or decreasing the flavin reoxidation rate will alter the concentration of the active (FADH°) redox form over time. Magnetic field effects can therefore be visualized as small changes in cryptochrome-dependent biological activity (Fig. 2), which is in fact observed (see Section 3).

4.2 Cryptochromes and Radiofrequency Fields

In the absence of direct experimental evidence, there are currently two main lines of evidence that a radical pair mechanism indeed underlies cryptochrome magnetosensitivity. Firstly, there is indirect evidence coming from migratory birds, where one of the signature predictions of the Radical Pair mechanism is that certain frequencies of oscillating (Radiofrequency) fields in the 1 – 10MHz range should interfere with the bird's directional sensing capability. This prediction is related to the calculated spin chemical properties of the radical pair intermediates formed in the presence of an Earth strength magnetic field (Ritz et al. 2004, Hore and Mouritsen 2016). This prediction has indeed been experimentally demonstrated in many elegant studies where birds were exposed to extremely weak Radiofrequency fields and became unable to sense the direction of the Earth's magnetic field (Niessner C and Winklhofer M, 2017). In fact, no other known explanation fits such a finding other than the Radical Pair mechanism, which is currently hypothesised to involve cryptochrome in avian orientation.

A second line of evidence consistent with the Radical Pair mechanism modulating cryptochromes comes from *Arabidopsis*, where activation of the cryptochrome receptor *in vivo* can be directly monitored by cryptochrome phosphorylation experiments (see section 3.5 above). A prediction of the Radical Pair hypothesis is that the response of plant cryptochrome to the Earth's magnetic field should be also disrupted in the presence of 1- 10MHz Radiofrequency osciallting fields. A recent study has indeed shown that exposure to a 7MHz magnetic field reduces the phosphorylation of *Arabidopsis* Cry1, indicating reduced biological activity. This same effect is observed when removing the Earth's magnetic field (as simulated by placing seedlings in a Low Level magnetic field) (Albaqami et al 2020). This is the first instance where sub-thermal (weak) radio frequency waves have been shown to modulate a biological receptor.

Nonetheless, in spite of these promising indications, a conclusive demonstration of the Radical Pair mechanism in relation to cryptochrome photochemistry still awaits identification of the relevant radical pair, however, and direct demonstration of its sensitivity to both static and osciallting magnetic field exposure in the isolated protein consistent with the physiological response.

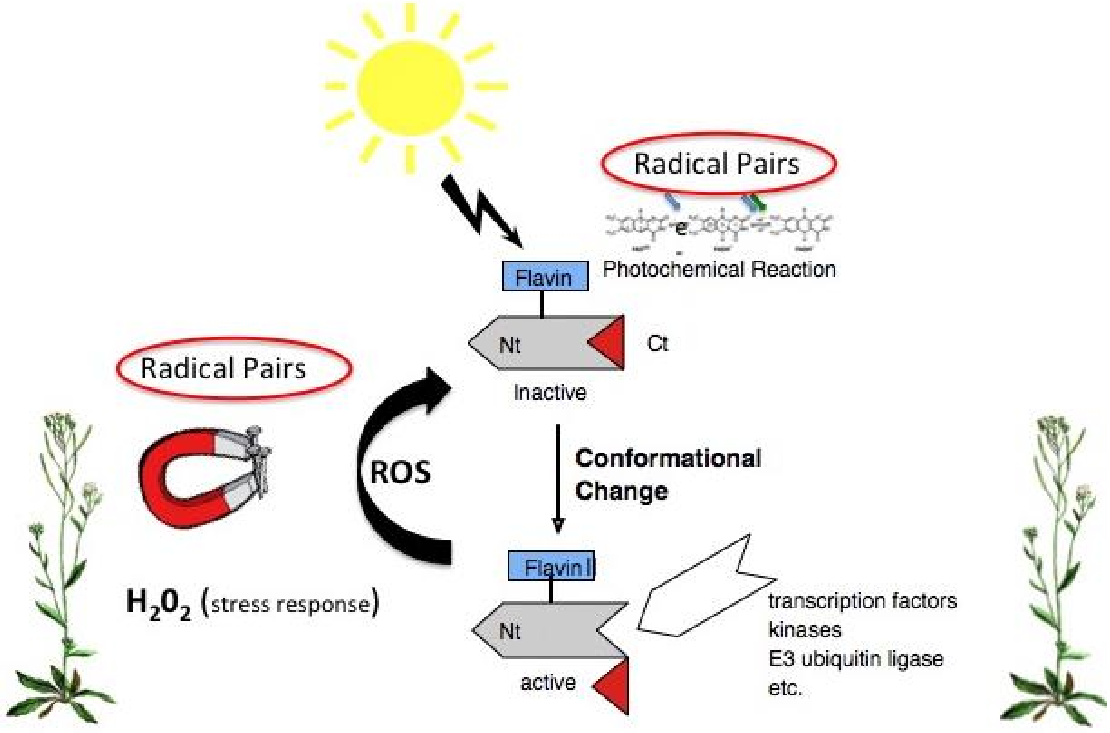

Fig. 2. *Arabidopsis* cryptochrome and the Radical Pair mechanism. In the dark, cryptochrome adopts and inactive conformational state with the flavin in the oxidised redox state (FADox). Upon illumination by light, cryptochrome undergoes a photochemical reaction whereby flavin is converted to reduced redox states. This process if accompanied by the formation of radical pairs. The redox changes trigger a conformational change in the receptor such that the C-terminal domain becomes exposed. The receptor is now activated and can interact with transcription factors, ubiquitin ligases, kinases and other signaling intermediates. The reduced (activated) flavin state is converted to the oxidized (inactive) receptor form by molecular oxygen in a reaction that produeces reactive oxygen species (ROS), including hydrogen peroxide (H202) which has many known physiological effects on plants including response to biotic and abiotic stress . This step also forms radical pairs. The magnetic field modulates the reoxidation step. Changing the rate constant of flavin reoxidation alters the concentration of the biologically active (reduced flavin) conformational state and also slows or accelerates the production of ROS and hydrogen peroxide. These are the possible effects of the magnetic field on cryptochrome via the Radical Pair mechanism (summarized in Hammad et al 2020).

5. Practical Considerations for use of Electromagnetic Fields in Agriculture.

From the above synopsis, there are a number of important issues to consider before any application of quantum biology to crop improvement or agriculture is attempted. First and foremost, effects of electromagnetic fields mediated by the Radical Pair mechanism are predicted to be relatively minor, as they can only slightly modulate the rates of ongoing physiological reactions. Such minor changes in metabolic enzyme activity or photoreeptor activation rate might not lead to much consequence to the organism, for example under optimised growth conditions or in full sunlight. To the contrary, magnetic field effects require carefully controlled, subsaturating reaction conditions to reliably detect. Indeed this is the case for plant cryptochrome responses to magnetic fields, which require that blue light be precisely regulated at relatively limiting intensities – too much or too little light and these effects will be completely undetectable (see eg. Xu et al 2012, Xu et al 2015, Pooam et al. 2019). Humidity, temperature, and soil quality could also significantly affect the ability to manipulate a given desired phenotypic trait by magnetic field exposure. It is therefore necessary from the start to target only those plant traits where a magnetic field effect is robust enough to occur under natural conditions, and which would make some real, reliably obtained difference to a desirable plant trait.

Another important consideration for applicaton of magnetic fields is cost. There is simply no way to apply a static magnetic field over large land areas in a cost efficient way. Currently static magnetic fields are produced by Helmholz coils, industrial magnets, or electronic devices driven by electrical power supplies. Therefore, the only agronomically feasible application of magnetic fields to crop improvement has for now been in promoting seed germination and the breaking of dormancy. Seed treatment prior to planting can indeed be done over a short time interval in a limited volume. Even so, this effect requres very high magnetic field strengths and likely does not involve a quantum biological mechanism (reviewed in Gallland and Pazur, 2005, Maffei, 2014).

In summary, electromagnetic fields do in principle modulate many plant traits of agronomic importance. However, simply exposing a large field, under natural conditions of full sunlight, to a continuous application of an artificial magnetic field is highly unlikely to have any measureable or reproducible consequences.

6. Case study: Successful Use of Pulsed Electromagnetic Fields in Medicine

An important clue as to how electromagnetic fields may nonetheless be efficiently used in manipulating crop plants in the field comes from intriguing results obtained by the medical community in the treatment of human disease. For more than 50 years, doctors and veterinarians have been using so-called ‘Pulsed Electromagnetic Fields’ or PEMFs to promote the healing of wounds or bone injuries as well as for other medical conditions (Markov, 2015). These pulsed fields are generated by simple electrical coils through which a current is passed at 10 – 300Hz, to induce pulsed magnetic fields at intensities with maximum magnetic field stength in the 2 – 10mT range. Significantly, such stimulation is only applied for short time intervals (10 to 15 minutes), and only once or twice a day. However the treatments are provided repetively over several days or weeks. Because the effects are additive, even weak PEMF stimulation over time result in the significant, measureable, and reproducible benefits for healing and cellular repair processes observed in a wide range of medical conditions (Markov, 2015).

Until recently, the underlying mechanism for the efficacy of these treatments was unknown, and indeed there has been much speculation on possible cellular ‘induced electrical currents’ or other physical phenomena (see eg. Binhi 2002). However, it has now been reported that, in mammalian cell cultures, PEMF effects are in fact comparable to those caused solely by a static magnetic field, for example Low Level field exposure (see eg. Martino et al 2010, Usselmann et al 2014). Specifically, a weak magnetic field (either generated by the PEMF devices or else by manipulating the static magnetic field strength using a Helmholz coil) can both cause a rapid, transient increase in the concentration of Reactive Oxygen Species (ROS) in exposed mammalian cell cultures (Sherrard et al 2018). Furthermore, the effect on production of ROS in cell cultures requires functional mammalian cryptochrome, consistent with a possible underlying Radical Pair mechanism.

6.1 Transient rise in ROS stimulates cellular defense and repair mechanisms.

ROS (Reactive Oxygen Species) are highly reactive free radicals derived from molecular oxygen. which are formed in the course of metabolic and redox reactions. Many of the enzymes that can generate ROS also generate radical pair intermediates, hence providing a potential means whereby they can be modulated by a magnetic field. Significantly, cryptochromes from a variety of species are also shown to generate ROS as byproducts of the flavin redox cycle, during reoxidation from their reduced (active) to oxidized (inactive) flavin state (Chaves et al 2011, Hammad et al 2020) – see also Fig.2. Thus, modulation of cellular ROS by quantum biology forces is a likelyconserved feature of many biological systems.

Cellular ROS are toxic to all life on earth at high concentrations, causing a condition known as oxidative stress (Sies, 2015). However, weak PEMF and static magnetic fields induce only a relatively low and transient rise in the concentrations of ROS, which does not lead to any known cellular damage (Markov,2015). Instead, at moderate doses, ROS is known to function as a signaling intermediate that actually induces beneficial outcomes in many organisms. These beneficial effects occur as a result of inducing ROS signaling pathways that promote resistance to biotic or abiotic stress, protect against acute and chronic inflammation, and regulate senescence (ageing). Moreoever these effects of ROS are highly conserved in organisms throughout the biological Kingdom including plants (Schieber and Chandel 2014). In mammalian cell cultures, it was proposed that the transient, mild increases in cellular ROS induced by PEMF should be sufficient to modestly stimulate cellular defense mechanisms (Sherrard et al 2018). Since these pulses of ROS are then repeated at frequent intervals over a period of days, cellular resistance should increase at each progressive iteration and thereby ultimately amplify the cellular repair mechanisms of exposed cells. This would provide a potential explanation for virtually all of the therapeutic effects observed with PEMF treatments (see Sherrard et al. and references therein).

6.2. Magnetic field stimulates cellular synthesis of ROS by the Radical Pair mechanism.

Finally, there is now direct experimental evidence that therapeutic effects of magnetic field exposure in mammalian cell cultures can be explained in terms of the magnetic field acting through the Radical Pair Mechanism. It was shown that a 7 MHz oscillating field (Radiofrequency) resulted in stimulation of ROS in cell cultures similarly as did exposure to altered magnetic fields and PEMF (Castello et al 2014,Usselmann et al 2014). Even more significnatly, it was shown that the effect of the Radiofrequency field was dependent on its angle of inclination with respect to the geomagnetic field (Usselmann et al. 2016). These are signature predictions of the Radical Pair mechanism as had been previously demonstrated to hold for bird orientation, and these data can not be explained by any alternative magnetosensing mechanism (Mouritsen and Hore 2016).

In sum, the transient stimulation of ROS in mammalian cell culture has multiple beneficial effects, and can be induced by short periods of either static, pulsed, and/or Radiofrequency electromagnetic field stimulation.

7. Applying Electromagnetic Fields to Crop Improvement.

We have seen that electromagnetic field effects on biochemical reactions in living cells are likely to be subtle and difficult to visualize or accurately control, especially under natural conditions. Building a strategy for crop improvement based upon modulation of individual identified cellular receptors or signaling pathways by magnetic field exposure is therefore problematic.

However, a relatively more robust consequence of Quantum forces interacting with biological systems appears to be their modulation of cellular levels of ROS. These are likely to be byproducts of multiple metabolic enzymes or redox reactions susceptible to the Radical Pair Mechanism and therefore provide a more reliable output for magnetic field exposure. Because (1) ROS is such a powerful and universal regulator of cellular defensive and repair mechanisms; because (2) ROS acts at extremely low intracellular concentrations meaning that even weak stimulation by a magnetic field could cause an physiological effect ; because (3) effects of multiple ROS stimulation events can be cumulative over time; and (4) most importantly because ROS stimulation can be induced by oscillating

(Radiofrequency) fields able to inexpensively reach a large surface area, we conclude that manipulation of cellular ROS may serve as the most promising avenue for applications of the magnetic field to agriculture.

In the next sections we provide a rationale and blueprint for how to design crop improvement strategies based on exposure to transient, repetitive Radiofrequency Field stimulation.

7.1 Role of ROS (reactive oxygen species) signaling pathways in plant stress response.

Many beneficial and agronomically useful plant traits can be controlled both directly and indirectly through cellular ROS signaling pathways (see eg. Amir et al 2019, Choudhury et al 2013, Khedia et al 2019). These can mediate plant resistance to biotic and abiotic stress conditions, including temperature, salinity, heavy metals, heat, cold, and pathogens. ROS signaling occurs as a result of evolutionarily conserved signaling pathways that induce multiple genes and cellular mechanisms contributing to protection against stress. Indeed, a brief transient increase in reactive oxygen concentration (ROS) is an early feature of plant responses to many stressors including pathogens, temperature, wounding, and salinity; these oxidative bursts are thought to trigger the ensuing defensive mechanisms for the many different cellular pathways.

7.2 'Induced Systemic Tolerance' – stress resistance induced by pre- exposure to ROS.

Of particular relevance to this review, a phenomenon known as 'induced systemic tolerance' or 'priming' has been shown to render crop plants of many varieties resistant to both biotic and abiotic stress (Hossain et al 2015, 2018). This occurs when plants in the field are pre-exposed briefly to very mild concentrations of ROS, for example by spraying or immersing them in H202 (one of the more stable and easily diffusible Reactive Oxygen Species), or else by exposure to brief temperature shock, which also transiently induces ROS (Hossain et al. 2018). Pre-exposure to ROS alleviated effects of subsequently applied drought stress, heat stress, cold stress, and heavy metal stress in a large variety of economically important monocot and dicot plant species including wheat and maize seedlings. It was further demonstrated that such pre-exposure to mild concentrations of ROS modulated gene expression, plant growth, metabolic processes, photosynthesis, proline accumulation, antioxidants, and other detoxification mechanisms which all help provide resistance to subsequent salt stress (Hossain et al 2015). All of these effects are consistent with a transient increase in cellular ROS, due to its wide-spread and far-reaching effect on induction of these cellular defense mechanisms.

ROS signaling is a highly complex process involving multiple distinct signaling intermediates, cellular pathways, and mechanisms. It is furthermore subject to self-regulation and homeostasis, such that effects of small changes in ROS concentrations can not be easily predicted or assayed. It is therefore highly significant that a measureable protection from almost all known abiotic stressors can be reproducibly achieved by even brief exposure of plants to such poorly defined, mild changes in cellular ROS as can be achieved by externally applying H202. It is moreover noteworthy that pre-exposure to ROS appears to work for both monocot and dicot plant species, and therefore provides a viable mechanism for manipulating almost all useful crop plants.

Finally, these beneficial effects on stress resistance were observed after only one pre-exposure session. What if there were repetitive bursts of priming provided throughout the entire growth season? The expected outcome would be greatly improved stress tolerance, resistance to disease, and crop yield.

Most significantly of all, stimulation of ROS by magnetic fields obeys the Radical Pair mechanism in mammalian systems and has also been widely observed to occur in plants. Therefore it should be possible to use calibrated Radiofrequency fields to obtain a comparable response (see eg. Hore and Mouritsen 2016).

7.3 PROTOCOL: Repetitive RF (radiofrequency) Exposure for use in Crop Improvement

From the above discussion, it is evident that targeted exposure to RF fields is almost ideally suited as a tool for crop improvement via a mechanism that transiently stimulates ROS. In support of this method, there is already considerable experimental evidence that stimulation of ROS and ROS signaling pathways occurs in a variety of plants in response to RF field exposure (reviewed in Vian et al 2016).

We therefore propose a methodology which borrows from the PEMF stimulation protocols used in medicine (Section 6). Instead of a continuous exposure, RF stimulation should be conducted only over brief intervals (10 – 30 minutes a day) to obtain a 'priming' effects without inducing long-term adaptation of plants to the signals and thereby loss of response. The protocol is as follows:

Firstly, the exact exposure conditions for Radiofrequency firlds will need to be determined in the lab, ie

- optimal frequency and intensity
- optimal exposure time and numper of repetitions per day
- optimal effect on stress responsive pathway of interest (ie monitor induced tolerance to drought, salt, heavy metal, and other stresses) to give maximal benefit for a given crop species

Secondly, suitable antennae must be installed in the field. This will require:

- building the antennae to specifications
- devising a power supply for use under field conditions
- proper positioning of the antennae so as to cover the correct surface area and deliver the signal at the correct angle to the Earth's magnetic field for maximum effect (see section 6.2)

Thirdly, once the antenna has been calibrated and installled in the field:

- Switch on antennae 10 – 30 minutes per day, once or twice a day
- Use for all subsequent growth seasons without needing further modification.

The outcome of this PROTOCOL in the immediate term should be to significantly improve yield for crops grown under marginal growth conditions of limited water, high salinity, temperature extreme, exposure to pathogens. In direct support of this methodology, there have been isolated reports that even a single application of a poorly defined RF signal reportedly increased subsequent tolerance to abiotic (heavy metal and UV/B) stress in wheat and glastum (Qiu Z et al 2011, Chen 2006).

In the longer term, further research may allow us to fine tune the plant cellular processes to be addressed by given signal types, and better adapt them to individual species and growth conditions. In direct support of this methodology, even a single applications of a poorly defined RF signal reportedly caused tolerance to abiotic (heavy metal and UV/B) stress in wheat and glastum.

8. Summary and Conclusion.

In this review, we have briefly summarized the current literatue on electromagnetic field effects on plants in the light of a single possible underlying mechanism for magnetosensing, namely the Radical Pair mechanism of chemical magnetosensing. We summarize the evidence that the Radical Pair Mechanism provides a means by which very weak electromagnetic fields can modify the rates of cellular reactions to achieve physiological changes *in vivo*. In particular, we summarize evidence that the activation of Cryptochrome, a putative magnetoreceptor in plants and animals, can be achieved through the Radical Pair mechanism. For an applied perspective, we first review the literature on successful applications of weak magnetic fields in medicine, in particular on the transient induction of cellular ROS to increase cellular resistance to disease. Then, we apply insights from this biomedical approach to suggest inducing resistance to biotic and abiotic stress by stimulating transient ROS in crop plants by targeted use of Radiofrequency exposure.

The unique feature of this review is that we seek to provide clarity in what is traditionally a large and confusing field by focussing only on a known, reasonable magnetosensing mechanism. This allows us to explain many contradictions and areas of confusion arising in the literature. In particular we emphasise that magnetic field effects on chemical reactions via the Radical Pair mechanism can only be observed if they occur at a rate limiting physiological step. This means that absence of a magnetic field effect does not necessarily mean the system cannot respond under optimised conditions. In other words 'absence of evidence does not mean evidence of absence', and many contradictory studies in the literature arise from even minor changes in methodology. Most importantly, our approach has enabled us to suggest realistic biological targets (ie ROS) likely to be directly modulated by magnetic fields in a large number of systems, and how to use RadioFrequency fields to achieve economically useful effects in plants.

It should be noted, however, that not all magnetic field effects can be explained by the function of cryptochrome, or even by the Radical Pair mechanism acting on other biological reactions. For example, effects of HF (high frequency) fields in the GHz range cannot be explained through currently known Quantum Physics principles. It is therefore likely that additional mechanisms, whether magnetite-based or otherwise, remain to be discovered, including additional means of applying electromagnetic fields to biotechnology and agriculture.

9. References

Agliassa C, Narayana R, Christie JM, Maffei ME (2018)a Geomagnetic field impacts on cryptochrome and phytochrome signaling. J Photochem and Photobiol B 185: 32-40.

Agliassa C, Narayana R, Bertea CM, Rodgers CT, Maffei ME (2018)b Reduction of the geomagnetic field delays Arabidopsis thaliana flowering time through downregulation of flowering‐related genes. Bioelectromagnetics 39: 361-374.

Ahmad, M. Photocycle and signaling mechanisms of plant cryptochromes (2016) Curr Opin Plant Biol 33: 108-115.

Ahmad M, Galland P, Ritz T,Wiltschko R, WiltschkoW (2007) Magnetic Intensity affects cryptochrome-dependent responses in Arabidopsis thaliana. Planta 225, 615–624.doi:10.1007/s00425-006-0383-0

Albaqami M, Hammad M, Pooam M, Procopio M, Sameti M, Ritz T, Ahmad M, Martino CF (2020) Arabidopsis cryptochrome is responsive to Radiofrequency (RF) electromagnetic fields. Sci Rep. 9;10(1):11260. doi: 10.1038/s41598-020-67165-5.

Amir R, Hussain S, Noor-ul-Ain H, Hussain A, Yun BW (2019) ROS Mediated Plant Defense Against Abiotic Stresses. In Plant Biotechnology: Progress in Genomic Era (pp. 481-515). Springer, Singapore.

Belyavskaya NA (2001) Ultrastructure and calcium balance in meristem cells of pea roots exposed to extremely low magnetic fields. Adv Space Res 28:645–650

Binhi V (2002) Magnetobiology 1st Edition. Underlying Physical Problems ISBN: 9780121000714, Imprint: Academic Press.

Binhi VN, Prato FS (2017) Biological effects of the hypomagnetic field: An analytical review of experiments and theories. PLoS One 12(6):e0179340. doi: 10.1371/journal.pone.0179340.

Cakmak T, Cakmak ZE, Dumlupinar R,TekinayT (2012) Analysis of apoplastic and symplastic antioxidant system in shallot leaves:impacts of weak static electric and magnetic field. J. Plant Physiol. 169, 1066–1073.doi:10.1016/j.jplph.2012.03.011

Cao J , Cogdell RJ , Coker DF , Duan H-G , Hauer J et al. 2020. Quantum Biology Revisited. Sci. Adv 6 : eaaz4888.

Castello PR, Hill I, Sivo F, Portelli L, Barnes F, Usselman R, Martino CF (2014) Inhibition of cellular proliferation and enhancement of hydrogen peroxide production in fibrosarcoma cell line by weak radio frequency magnetic fields. Bioelectromagnetics 35: 598-602.

Chaves I, Pokorny R, Byrdin M, Hoang N, Ritz T, Brettel K, et al. (2011) The cryptochromes: blue light photoreceptors in plants and animals. Ann Rev Plant Biol 62: 335–364.

Chen YP (2006) Microwave treatment of eight seconds protects cells of Isatis indigotica from enhanced UV-B radiation lesions.Photochemistry and Photobiology, vol. 82, no. 2, pp. 503–507.

Choudhury S, Panda P, Sahoo L, et al. (2013). Reactive oxygen species signaling in plants under abiotic stress. Plant Signaling & Behavior 8(4), e23681.

da Silva JAT, Dobranszki J (2016) Magnetic fields: how is plant growth and development impacted? Protoplasma 253 : 231–248

Dhimana SK, Galland P. Effects of weak static magnetic fields on the gene expression of seedlings of *Arabidopsis thaliana. J Plant Physiol* **231**: 9-18.

Dufor T, Grehl S, Tang AD, Doulazmi M, Traoré M, Debray N, Dubacq C, Deng Z-D, Mariani J, Lohof AM, Sherrard RM (2019) Neural circuit repair by low-intensity magnetic stimulation requires cellular magneto-receptors and specific stimulation patterns. Sci Adv 5:eaav9847.

European Space Agency (2020) https://www.esa.int/Science_Exploration/Human_and_Robotic_Exploration/Lessons_online/The_effects_of_magnetic_fields

Fedele G, Green EW, Rosato E, Kyriacou CP (2014a). An electromagnetic field disrupts negative geotaxis in Drosophila via a CRY-dependent pathway. Nat Commun 5: 4391.

Fedele G, Edwards MD, Bhutani S, Hares JM, Murbach M, Green EW, Dissel S, Hastings MH, Rosato E, Kyriakou CP (2014b). Genetic Analysis of Circadian Responses to Low Frequency Electromagnetic Fields in *Drosophila melanogaster*. Plos Genetics https://doi.org/10.1371/journal.pgen.1004804

Foley LE and Emery P (2020) ***Drosophila*** **Cryptochrome: Variations in Blue.** J Biol Rhythms 35(1):16-27. doi: 10.1177/0748730419878290. Epub 2019 Oct 10.

Galland P, Pazur A. Magnetoreception in Plants. (2005) J. Plant Research 118: 371 – 389.

Hammad M, Albaqami M, Pooam M, Kernevez E, Witczak J et al. (2020) Cryptochrome mediated magnetic sensitivity in Arabidopsis occurs independently of light-induced electron transfer to the flavin. Photochem & Photobiol Sci. 10.1039/c9pp00469f.

Harris SR, Henbest KB, Maeda K, Pannell JR,Timmel CR,Hore PJ et al.(2009).Effect of magnetic fields on cryptochrome-dependent responses in Arabidopsis thaliana. J. R. Soc. Interface 6: 1193–1205. doi:10.1098/rsif.2008.0519

Hoff AJ, Rademaker H, van Grondelle R, Duysens LNM (1977) On the magnetic field dependence of the yield of the triplet state in reaction centers of photosynthetic bacteria. Biochim Biophys Acta 460:547–554

Hong FT (1995). Magnetic field effects on biomolecules, cells, and living organisms. BioSystems 36 : 187-229.

Hore PJ, Mouritsen H (2016) The radical pair mechanism of magnetoreception. Ann. Rev. Biophys.45: 299–344.

Hossain MA, Bhattacharjee S, Armin SM, Qian P, Xin W, Li HY, Burritt DJ, Fujita M, Tran L (2015) Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. Front Plant Sci. 16;6:420. doi: 10.3389/fpls.2015.00420

Hossain MA, Li ZG, Hoque TS, Buritt D, Fujita M, Munné-Bosch S (2018). Heat or cold priming-induced cross-tolerance to abiotic stresses in plants: key regulators and possible mechanisms. Protoplasma 255: 399–412

Iqbal M, Muhammad D, Hag ZU, JamilY, Ahmad M (2012) Effect of pre-sowing magnetic field treatment to garden pea (Pisum sativum L.) seed on germination and seedling growth. Pak J Bot 44: 1851–1856.

Johnsen S, Lohmann K (2005) The Physics and Neurobiology of Magnetoreception. Nat Rev Neurosci 6 : 703-12. doi: 10.1038/nrn1745.

Kato R (1990) Effects of very low magnetic field on the gravitropic curvature of Zea roots. Plant Cell Physiol 31:565–568

Khedia J, Agarwal P, Agarwal P (2019) Deciphering hydrogen peroxide-induced signalling towards stress tolerance in plants. 3 Biotech 9(11):395. doi: 10.1007/s13205-019-1924-0.

Kominis I (2015) The radical-pair mechanism as a paradigm for the emerging science of quantum biology. Mod. Phys. Lett. B 29: 1530013

Lefèvre CT, Bazylinski DA (2013) Ecology, Diversity, and Evolution of Magnetotactic Bacteria. Microbiology and Molecular Biology Reviews 77 : 497 – 526.

Li Z, Qu A, Zhu W, Yang Y, Shu F (1997) Genetics effect of water treated by magnetic field (4200 GS) on root tip cells of Vicia faba. Chin Environ Sci 17:437–439

Maffei M E. Magnetic Field Effects on Plant Growth, Development and Evolution. Frontiers in Plant Science 2014, 5: 00445.

Mahajan TS, Pandey OP (2014).Magnetic-time model at off-season germination. Int. Agrophys. 28: 57–62.doi:10.2478/intag-2013-0027

Markov M (2015) XXIst century magnetotherapy. Electromagn Biol Med 34:190 - 196

Martino CF, Perea H, Hopfner U, Ferguson VL, Wintermantel E (2010) Effects of weak static magnetic fields on endothelial cells. Bioelectromagnetics 31: 296-301

Michael AK , Jennifer L. Fribourgh 1, Russell N. Van Gelder , Partch CL (2017) Animal Cryptochromes: Divergent Roles in Light Perception, Circadian Timekeeping and Beyond. Photochem Photobiol 93: 128–140.

Monteil CL, Lefevre CT (2019) **Magnetoreception** in Microorganisms. Trends Microbiol. 28(4):266-275. doi: 10.1016/j.tim.2019.10.012.

Nießner C, Winklhofer M (2017) Radical-pair-based magnetoreception in birds: radio-frequency experiments and the role of cryptochrome Journal of Comparative Physiology A **:** 203**:** 499–507.

Novitskaya GV, Kocheshkova TK, Feofilaktova TV, Novitskii YN (2004) Effect of choline chloride on the lipid content and composition in the leaves of principal magnetically-oriented radish types. Russ J Plant Physiol 51:361–371

Nyqvist D, Durif C, Johnsen MG, De Jong K, Forland TN, Sivle LD (2020) Electric and **magnetic** senses in marine animals, and potential behavioral effects of electromagnetic surveys. Mar Environ Res. 155:104888. doi: 10.1016/j.marenvres.2020.104888.

Pooam M, Arthaut LD , Burdick D , Link J , Martino CF, Ahmad M (2019). Magnetic sensitivity mediated by the Arabidopsis blue-light receptor cryptochrome occurs during flavin reoxidation in the dark. Planta 249: 319 —332

Qiu Z, Li J, Zhang Y, Bi Z, Wei H (2011) Microwave pretreatment can enhance tolerance ofwheat seedlings toCdCl2 stress. Ecotoxicology and Environmental Safety 74: 820–825, 2011.

Radhakrishnan R (2019) Magnetic field regulates plant functions, growth and enhances tolerance against environmental stresses *Physiology and Molecular Biology of Plants* 25: 1107–1119

Ritz T, Adem S, Schulten K (2000) A model for photoreceptor-based magnetoreception in birds. Biophys J 78:707–718.

Ritz T, Thalau P, Phillips J, Wiltschko R, Wiltschko W (2004) Resonance effects indicate radical pair mechanism for avian magnetic compass. Nature 429: 177-180

Rodgers CT (2009). Magnetic field effects in chemical systems. Pure Appl Chem 81: 19–43.

Schieber, Chandel (2014) Current Biol 24: R453 – 62
https://www.cell.com/current- biology/fulltext/S0960-9822(14)00326-1

Schreiber K (1958) An unusual tropism of feeder roots in sugar beets and its possible effect on fertilizer response. Can J Plant Sci 38:124

Sherrard RM, Morellini N, Jourdan N, El-Esawi M, Arthaut L-D *et al* (2018) Low-intensity electromagnetic fields induce human cryptochrome to modulate intracellular reactive oxygen species. PloS Biol 16*,* e2006229.

Sies H (2015) Oxidative stress: a concept in redox biology and medicine. Redox Biol. 4: 180–183.

Sonneveld A, Duysens LNM, Moerdijk A (1981) Sub-microsecond chlorophyll a delayed fluorescence from photosystem I. Magnetic field-induced increase of the emission field. Biochim Biophys Acta 636:39–49

Usselman RJ, Hill I, Singel DJ, Martino CF (2014) Spin biochemistry modulates reactive oxygen species (ROS) production by radio frequency magnetic fields. Plos One. 9: e93065.

Usselman RJ, Chavarriaga C, Castello PR, Procopio M, Ritz T et al (2016) The Quantum Biology of Reactive Oxygen Species Partitioning Impacts Cellular Bioenergetics. Sci Rep. 6: 38543.

Vechtomova YL, Telegina TA, Kritsky MS (2020) Evolution of Proteins of the DNA Photolyase/**Cryptochrome** Family. Biochemistry (Mosc). 85(Suppl 1) : S131-S153.
doi: 10.1134/S0006297920140072.

Vian A, Davies E, Gendraud M, Bonnet P. (2016). Plant Responses to High Frequency Electromagnetic Fields. BioMed Research International, 2016: 1830262.

Wang Q, Lin C (2020) Mechanisms of Cryptochrome-Mediated Photoresponses in Plants. Annual Review of Plant Biology 71:103-129.

Wiltschko R, Wiltschko W (1995) Magnetic Orientation in Animals. Zoophysiology 33: 1 – 297.

Wiltschko R, Ahmad M, Nießner C, Gehring D, Wiltschko W (2016) Light-dependent magnetoreception in birds: the crucial step occurs in the dark. J R Soc Interface. 13: 118 .

Wiltschko R, Wiltschko WJ (2019) Magnetoreception in birds. R Soc Interface. 16(158):20190295. doi: 10.1098/rsif.2019.0295.

Xu C, Yin X, Lv Y, Wu C, Zhang Y , Song T (2012) A near-null magnetic field affects cryptochrome-related hypocotyl growth and flowering in Arabidopsis. Adv Space Res 49: 834 —840.

Xu C , Lv Y , Chen C, Zhang Y, Wei S (2014) Blue light-dependent phosphorylations of cryptochromes are affected by magnetic fields in Arabidopsis, Adv. Space Res 53: 1118 —1124.

Xu C, Li Y , Yu Y, Zhang Y, Wei S (2015) Suppression of Arabidopsis flowering by near-null magnetic field is affected by light, Bioelectromagnetics 36: 476 —479

Xu C, Zhang Y, Yu Y, Li Y, Wei S (2017) Suppression of Arabidopsis flowering by near-null magnetic field is mediated by auxin, Bioelectromagnetics 39: 15 – 24. 10.1002/bem.22086

Xu C, Yu Y, Zhang Y, Li Y, Wei S (2016) Gibberellins are involved in effect of near-null magnetic field on Arabidopsis flowering, Bioelectromagnetics 38: 1 – 10. 10.1002/bem.22004

Zhadin MN (2001) Review of Russian literature on biological action of DC and low-frequency AC magnetic fields. Bioelectromagnetics 22:27–45.