African Journal of Biotechnology Vol. 12(3), pp. 275-283, 16 January, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2905
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Effects of moderate static magnetic field presowing treatment on seedling growth and oxidative status in two *Raphanus sativus* L. varieties

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Accepted 31 December, 2012

Improvement of seed performance by static magnetic field (SMF) constitutes a safe ecological way to substitute chemicals use. In laboratory conditions, we studied the effects of presowing seeds of two varieties of *Raphanus sativus* (Red: R.R, Red and White: R+W) by moderate SMF on seedlings' growth and oxidative status and this by using different intensities (100 and 200 mT) and exposure times (2 and 3 h). For radish, the SMF pretreatment of 100 mT/3 h induced significant enhancement of 8 day-old seedling biomass production and this by about 26 and 29% for respectively the RR and the R+W varieties. However, R.R seems to be more sensitive to SMF than R+W based on biomass production. Moreover, for the beneficial pretreatment of 100 mT/3 h, SMF-induced effects were also observed when white light was substituted by blue one using light filters. Besides, catalase (CAT) activity was significantly stimulated in radish plantlets (about 36% in cotyledons). Nevertheless, assimilatory pigments decreased ($p<0.05$) significantly (by about an average of 25%) but malondialdehyde (MDA) content remained unvaried. To conclude, SMF-presowing -at the treatment of 100 mT/3 h- allowed, in our experimental conditions, the improvement of *R. sativus* initial growth while keeping a good oxidative status.

Key words: Performance, presowing, radish, static magnetic field.

INTRODUCTION

In natural conditions, restoration of seed metabolism during early seed germination (phases I and II), is accompanied by initiation of metabolic processes (Weitbrecht et al., 2011) mainly resumption of energy metabolism, cellular repairs and protective mechanisms (Nonogaki et al., 2010). The early germination phases are reversible allowing seedlings survival to sudden water stress; this property is used in seed biostimulation to substitute the use of chemicals in agriculture and its associate environmental adverse effects. Seeds priming advances seed metabolism leading to an improvement of germination, enhancement of seedling performance and tolerance of plants to stress (McDonald, 2000). However, priming decreases longevity of high vigor seeds so post priming treatments are needed (Varier et al., 2010) but physical methods (laser, magnetic field, ultrasound, microwave) allow avoiding this disadvantage. In fact, these methods introduce energy in cells, inducing molecular transformations and improvement in yield for suitable treatments. Comparison of different physical methods in different seeds species concluded that beneficial effects were more pronounced after magnetic field (MF) treatments (Aladjadjiyan, 2007).

Particularly, static magnetic field (SMF) presowing has beneficial effects on different developmental stages and

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Abbreviations: SMF, Static magnetic field; **MF,** magnetic field; **R+Hyp**, radicle + hypocotyl; **Hyp+C,** hypocotyl + cotyledons; **R.R,** red radish; **R+W,** red and white radish; **ROS,** reactive oxygen species; **Chl,** Chlorophyll; **CAT,** catalase; **MDA,** malondialdehyde.

at the suitable treatments (Souza et al., 2005) especially on the first ones that determine plant establishment. In most studied species, SMF presowing treatment induces positive effects on germination (Shine et al., 2011; Aksyonov et al., 2007) through improving its rate and speed. Seedlings growth responses to MF presowing include stimulation or inhibition depending on treatment. In rice, an increased biomass of seedlings is observed when seeds are exposed to a weak electromagnetic field (EMF) (1 nT for 12 h, Alexander and Doijode, 1995) or moderate SMF (125 and 250 mT during 10 min, 20 min, 1 h and chronic exposure, Florez et al., 2004). Pulsed MF (24 h exposure) has an inhibitory effect on mung bean growth (Huang and Wang, 2007). MF presowing effect would be organ dependent; roots seem to be much more susceptible than shoots (Kato et al., 1989). Under continuous exposure, weak EMF induces an inhibitory effect on flax, pea and lentils roots for 0.5 and 2 nT intensities whereas no effect is observed for 1nT (Belyavskaya, 2004). The beneficial effects of SMF presowing on seedling growth are not only the result of stimulation of reserve mobilization and respiration initiated in germination but also of metabolic changes as protein biosynthesis from mRNAs, gene transcription and cellular repairs (Racuciu et al., 2008; Shabrangi and Majd, 2009).

On the other hand, in dry seed and- in spite of low metabolism-, there is a production of free radicals as reactive oxygen species (ROS) whose concentrations increase following seed resumption metabolism and whose levels, concentration and life time are modified by MF (Hunt et al., 2009). At low level, ROS might be involved in MF perception (Okano, 2008) particularly the superoxide ion $O_2^{\cdot -}$ (Solov'yov and Schulten, 2009) in the radical-pair proposed model (Solov'yov et al., 2007). Among the proposed MF perception models, the radical-pair one is thought to be the most relevant as it induces responses measurable for strengths weaker than geomagnetic field (Timmel et al., 1998). According to this model, responses to MF are the result of photo-induced radical-pair reactions in cryptochromes (Harris et al., 2009); blue light photoreceptors (380 < ʎ < 450 nm) ubiquitous among living taxa and involved in plants development and photomorphogenesis. Using *Arabidopsis thaliana* mutants continuously submitted to SMF (500 µT), Ahmed et al. (2006) showed the involvement of cryptochromes in hypocotyl reduction and anthocyanin accumulation. Also, MF activates the antioxidative defence improving plant tolerance to biotic and abiotic stresses (Rochalska and Grabowska, 2007). This might be the result of stimulation of enzymes activities implicated in the detoxification of $O_2^{\bullet -}$ (SODs) and H_2O_2 (catalases, peroxidases) (Atak et al., 2007; Celik et al., 2009).

In this context, we studied the influence of seed pretreatment by moderate SMF in two varieties of *Raphanus sativus*, "Red" R.R and "red and white" R+W, on seedling growth stages using different intensities (100 and 200 mT) and exposure times (2 and 3 h). Besides, we compared the effects of natural and blue lights during SMF presowing exposition, on biomass production. Finally, the oxidative status was evaluated by oxidative damage (malondihaldehyde, MDA and assimilatory pigments contents) and detoxification capacity (CAT activity). These results would contribute to find markers related to seed performance after SMF presowing that could improve, in field conditions, growth of this edible cruciferous known for its antioxidant properties.

MATERIALS AND METHODS

Plant material and seedlings' growth

Seeds of two varieties of crop specie: the radish *Raphanus sativus*, "R.R " and "R+W" known for its pharmacological and therapeutic properties (Beevi, 2010) were used in the present work. All were provided by the Seeds Laboratory of the ministry of Agriculture, Tunis, Tunisia.

Radish seeds (RR and R+W) were sterilized with 1% sodium hypochlorite for 2 min, washed with distilled water then placed in 9 cm Petri dishes. Three replicates with 13 seeds in each Petri dish was used in the experimental design, thus groups of 39 seeds were subjected to each magnetic treatment and analogous groups non-exposed to MF were used as the control.

The study was carried out under laboratory conditions, with natural light and an average room temperature of 25°C. Magnetic exposure was carried out directly after putting seeds to germinate. All biochemical parameters were measured on eight-day-old seedlings, in photosynthetic organs (cotyledons) and non-photosynthetic organs (Hyp + R).

Plant aerial (Hyp+C) and underground organs (Radicles) weights were measured with 10-5 g accuracy. Plant drying was carried out at 100°C in a vacuum oven during 48 h.

Magnetic treatment

The pre-sowing magnetic treatments were administered using an electromagnet. Petri dishes are placed between the two 10 cm apart interferes of the electromagnet having a cooling and electric current intensity regulation systems. This device generated a SMF .The required magnetic inductions were obtained by the regulation of the intensity of the direct current provided by a generator: Lake Shore Magnet Power Supply Model 647 in the insulation of coils feeding the electromagnet (Figure 1).

No magnetic field other than that of the geomagnetic field was detected within the experimental electromagnet when switched off and applied magnetic fields were far higher than 0.042 mT corresponding to the local geomagnetic field in the laboratory measured by an Earth Magnetometer Model EM 2 by Alpha Lab, Inc.

Seed treatments were as follows:

* Exposure to a dynamic magnetic field of 100 mT for 2 h.
*Exposure to a dynamic magnetic field of 100 mT for 3 h.
*Exposure to a dynamic magnetic field of 200 mT for 2 h.
* Exposure to a dynamic magnetic field of 200 mT for 3 h.
*Control: exposure to the local geomagnetic field only.

The choice of the above magnetic treatments was made from bibliography (Racuciu, 2008; Vashisth and and Nagarajan, 2010).

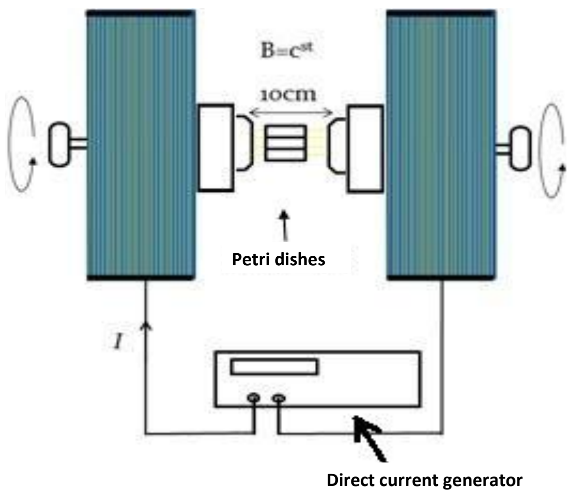

Figure 1. The experimental electromagnet apparatus. Three Petri dishes are placed in the air gap between the two iron bars to expose the seeds to the static magnetic field.

Oxidative status

It was evaluated in eight day old seedlings exposed or not to SMF at 100 mT during 3 h since this treatment corresponded to the pick of performance for radish in our experimental conditions.

Oxidative damage was evaluated by measuring the contents of photosynthetic pigments (Chl a, b and carotenoids) and MDA. Detoxification was estimated by measuring CAT activity.

All spectrophotometric analyses were conducted at 25°C in a Shimadzu UV/Visible Light spectrophotometer.

Assimilatory pigments' content

Eight-day-old seedlings' leaves were extracted in 80% cold acetone and the absorbance of the extract was determined at 663, 645 and 480 nm. Chlorophyll a, b and carotenoids quantities were calculated in accordance with Arnon method (Arnon, 1949).

Lipid peroxidation

Lipid peroxidation was determined using the 2-thiobarbituric acid (TBA) reaction followed by measurement of MDA content (Heath and Packer, 1968). Fresh tissue was ground in 0.25% TBA prepared in 10% TCA solution (10 ml/1 g fresh weight). The mixture was incubated at 95°C for 30 min, quickly cooled in an ice bath and centrifuged at 10 000 x *g* for 10 min. The absorbance of the supernatant at 532 nm was read and corrected for unspecific turbidity by substracting the value at 600 nm. The blank was 0.25% TBA in 10% TCA. The MDA concentration was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}.

Catalase activity was determined by monitoring the disappearance of H_2O_2 by measuring the decrease in absorbance at 240 nm (extinction coefficient of 0.036 mM^{-1} cm^{-1}) of a reaction mixture containing 50 mM potassium phosphate buffer (pH = 7.0), 10 mM H_2O_2 and enzyme extract (Aebi, 1984) for 4 min.

Enzyme activity was expressed per gram fresh weight. One unit of enzyme was defined as the amount necessary to decompose 1 μmol of substrate/min at 25°C.

Magnetic treatment under different illuminations

This experiment was carried out in a dark room for best control of luminosity. All radish seeds (RR and R+W varieties) are magnetically treated by the beneficial treatment (100 mT/3h). Nevertheless; controls correspond to seeds exposed to white light and seeds protected from light during magnetic treatment (obscurity).

Another group of three Petri dishes formed the treated group by blue light. Lid of each Petri dish was covered by blue colour filter (λ = 446 to 520 nm) (U 19530 by 3 B Scientific), inferior facet was protected by huge black paper to prevent the passage of each light but blue. Then, Petri dish was exposed to magnetic field. This was repeated until obtaining a group of three treated Petri dishes; the fragmentation was done for providing better quantity of blue light.

In eight day-old plantlets, we measured fresh and dry biomass after desiccation (100°C during 48 h).

Statistical analyses

The data represent the mean values ± SE of five replicates for biochemical parameters (CAT, MDA, Chla, Chlb and Carotenoids). For growth parameters, it represents the mean value of at least 30 replications. The data were analyzed using STATISTICA (version 8) by the application of one-way ANOVA followed by Tukey Post Hoc Test. The tests were performed separately for cotyledons and R + Hyp samples for biochemical parameters and for Hyp+C and R for growth ones. The differences were considered significant if p was at least $\leq$ 0.05. Means followed by the same letter(s) were not significantly different.

RESULTS

Seedling growth biomass production

In *R. sativus*, SMF effects on biomass production would vary with species but are neither intensity nor time SMF presowing treatment dependent. For R.R, SMF presowing treatment increased significantly ($p<0.05$) plantlet fresh weight except for the higher intensity 200 *mT* applied during 3 h which was insignificant (Figure 2D). The treatment 100 mT/3h gave the best seedling growth with an increase of 26% in fresh biomass as a result of radicle growth's stimulation (58%) (Figure 2B). Moreover, the treatments 100 mT/2h and 200mT/2h led to a significant increase ($p<0.05$) of 16 and 20% respectively in fresh biomass production.

For R+W variety, SMF did not induced any significant effect in seedling growth for 100 mT/2h (Figure 2E) and 200 mT/3h (Figures 2G and H). However, an increase of 29 and 16% in plantlet fresh biomass production had been observed respectively for 100 mT/3h and 200 mT/2h when compared to the controls (Figures 2F and G).

Similarly, these treatments had a remarkable effect ($p < 0.05$) on plantlets dry weights. For R.R, and concordant with fresh weight, SMF gave a significant rise ($p<0.05$) to dry biomass production except for the treatment of 200 mT/3h (Figure 4D). A stimulation in seedlings 'biomass production has been observed for all other treatments (100 mT/2h by 23%, 100 mT/3h by 22% and 200 mT/2h by 19%) as a result of radicle stimulation (Figure 3B).

For R+W variety, dry weight was significantly enhanced ($p<0.05$) for 100 mT/3h (Figure 3F) and 200 mT/2h (Figure 3G) by about 18 and 17%, respectively.

Based on these observations, we can consider that the treatment 100 mT/3h corresponded to the peak of performance for studied radish varieties. In fact, this treatment was chosen to effectuate biochemical dosages and magnetic field treatment under different illuminations.

Effects of blue light exposure during SMF presowing treatment

The SMF presowing treatment 100 mT/3h had beneficial effects on biomass production in radish studied varieties (Figures 3B, 3F; 4B, 4F). Magneto-perception might involve light since biomass production of plantlets was reduced when exposure of seeds of the two radish varieties to SMF occurred in the absence of light at the suitable treatment of 100 mT/3h. For R.R, a significant fall ($p <0.05$) of fresh and dry biomass production by 33% (Figure 4A) and 21% (Figure 4B), respectively was observed. These effects are also observed for the R+W variety whose fresh and dry biomass production were significantly downgraded ($p <0.05$) respectively by 29 (Figure 4C) and 20% (Figure 4D) through radicle biomass reduction. However, presowing by SMF under blue light (λ = 446 to 520 nm) allowed the correction of the growth reduction noticed when SMF treatment occurred in absence of light. For R.R, the observed upgrade was by 39 and 23% for fresh (Figure 4A) and dry plantlets biomass production, respectively (Figure 4B), whereas, it was by 34 and 21% for the R+W one.

Oxidative seedlings status

Presowing treatment by SMF (100 mT, 3h) induced an oxidative stress as shown by the increase of the activity of the H_2O_2 detoxifying enzyme: catalase in studied radish varieties.

Its activity had improved in cotyledons by about 37 and 36% for both studied varieties (Figure 5B) and by about 23 and 30% for respectively RR and R+W in non-photosynthetic organs (Figure 5E). SMF presowing induced oxidative damages since a significant decrease ($p<0.05$) in cotyledons chlorophylls a and b and carotenoids in R.R (Figure 5A) and R+W variety (Figure 5D) had been shown. This decrease was by about 25, 23 and 29%, respectively for Chl a, Chl b and carotenoids in RR variety. Moreover, this same effect was by about 27, 28 and 22% in R+W one. However or probably as a result of efficient detoxification, SMF presowing treatment did not exhibit any effect on MDA content neither in radish cotyledons (Figure 5C) nor in non photosynthetic organs (Figure 5F).

DISCUSSION

There are great difficulties to compare MF effects in plants because of difference in species (diamagnetic composition) and experimental conditions. In the used experimental treatment, nature (MF, EMF and SMF), intensity (from nT to T ranges), duration (second to hours or continuous) and frequency (acute or chronic) constitute other challenges. In addition, most studies concluded that magnetic effects are not correlated with intensity or duration of exposition, beneficial treatments and peak of performance rather correspond to a misunderstood combination of these two parameters.

SMF-presowing effects on seedlings growth stage and oxidative responses

Seedling growth is supported by reserves breakdown accumulated in cotyledons during seed maturation but also via photosynthesis in these organs whose contribution would vary with species (Zheng et al., 2011). In SMF pre-sowed radish, the observed increased biomass production is rather related to an enhanced reserves mobilization than assimilates production, photosynthetic pigments contents (chlorophylls and carotenoids) being decreased for the beneficial treatment (100 mT/3h) in cotyledons of both radish varieties. Thus might be the result of their oxidation by SMF induced ROS accumulation. In another hand, during SMF presowing and for the suitable treatment (100 mT/3h that improved biomass production in the two studied radish varieties), blue light can be substituted by white one during SMF presowing. This result is in agreement with

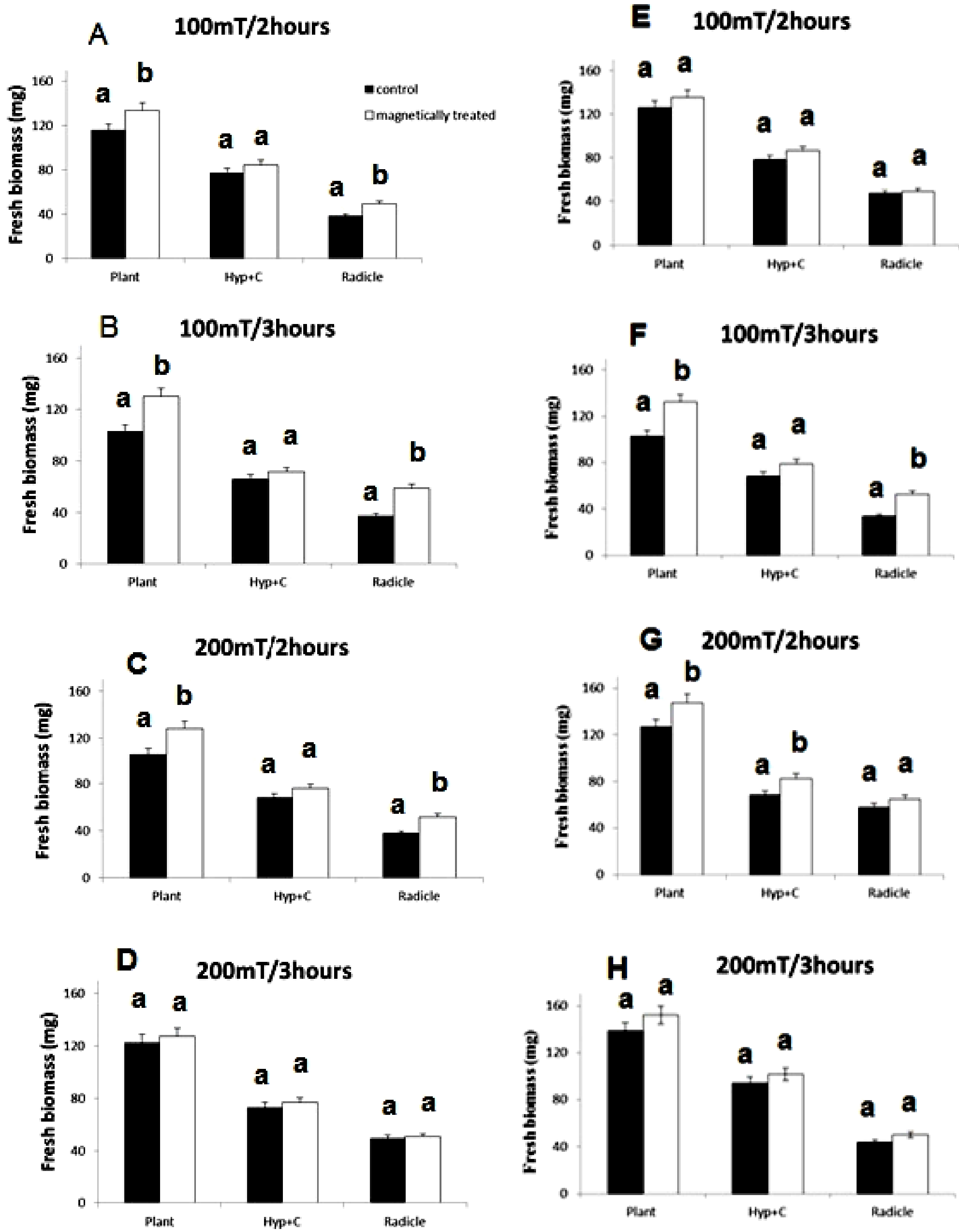

Figure 2. Variations of fresh biomass production (mg) of 8 old plantlets of two varieties, "Red" (A, B, C, D) and "Red and White" (E, F, G, H) of radish pre-sowed by static magnetic field at different intensities (100 and 200 mT) and exposure times (2 and 3 h) ± SE error bars, n at least=33. Means followed by the same letter(s) were not significantly different at p <0.05 according to Tukey test.

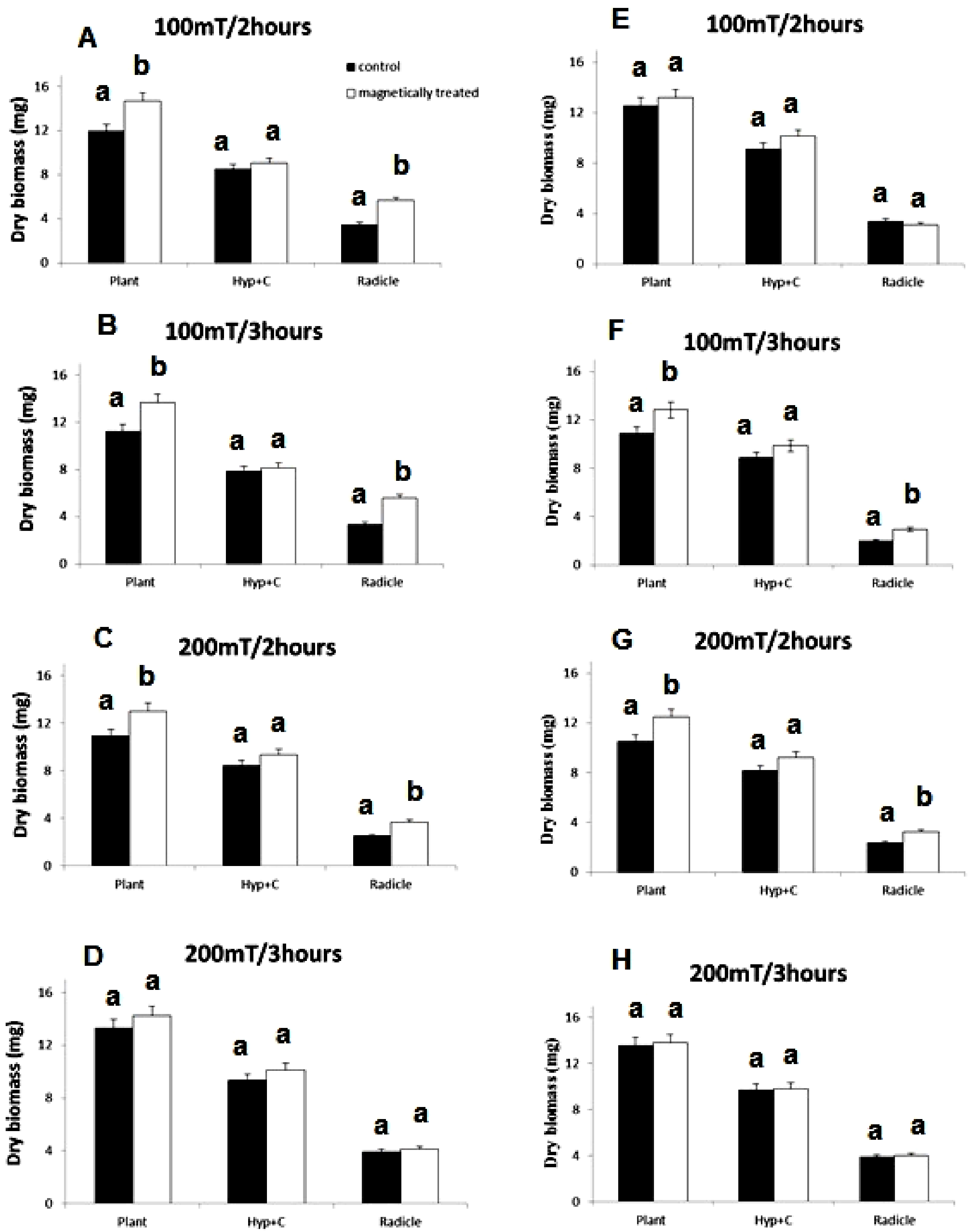

Figure 3. Variations of dry biomass production (mg) of 8 old plantlets of two varieties, "Red" (A, B, C, D) and "Red and White" (E, F, G, H) of radish pre-sowed by static magnetic field at different intensities (100 and 200 mT) and exposure times (2 and 3 h) ± SE error bars, n at least=33. Means followed by the same letter(s) were not significantly different at $p<0.05$ according to Tukey test.

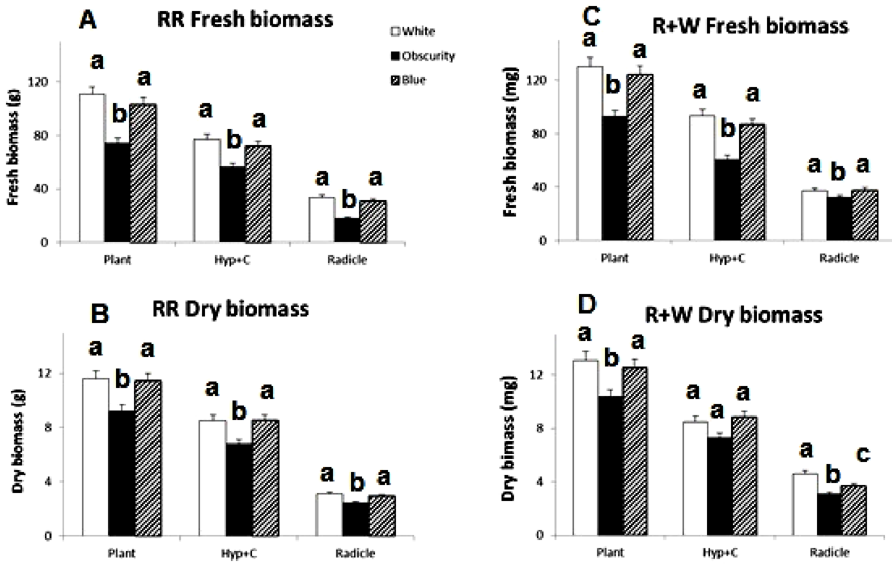

Figure 4. Effects of different illuminations (natural and blue lights, obscurity) during static magnetic field (100 mT during 3 h) presowing exposure on fresh and dry biomass production of 8 days old seedlings of Raphanus sativus varieties, “Red” and “Red and White”± SE error bars, n at least = 33. Means followed by the same letter(s) were not significantly different at p <0.05 according to Tukey test.

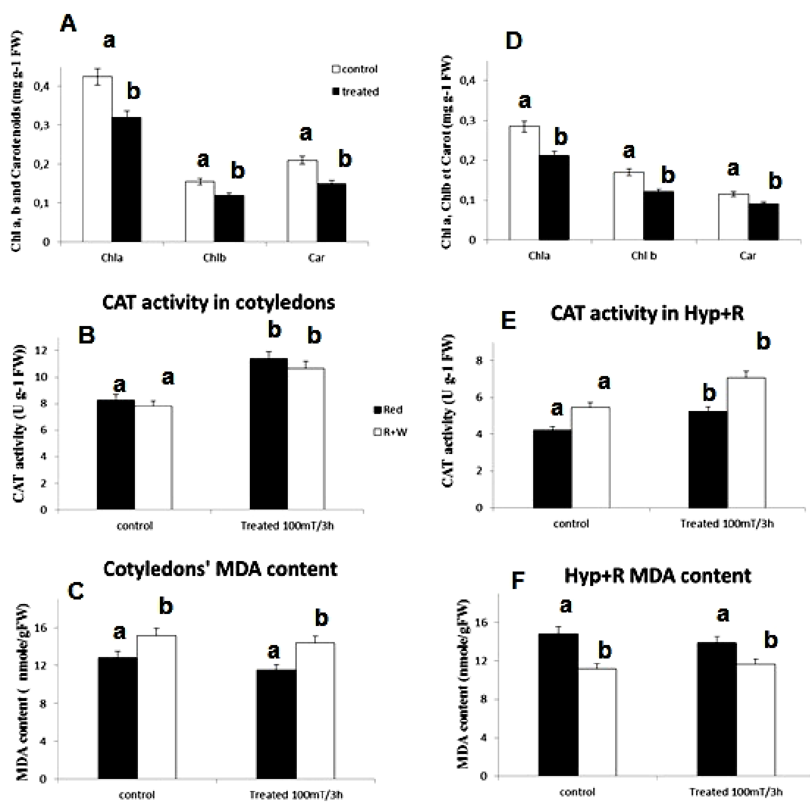

Figure 5. Variations of assimilatory pigments (chla and b, carotenoids) contents (mg g-1 FW), catalase activity (U g-1 fresh weight) and MDA content (nmol g-1 fresh weight) in plantlets (8 days old) of two radish varieties (“Red” and “Red and White”) submitted to static magnetic field presowing treatment (100 mT during 3 h) ± SE error bars, n = 4. Means followed by the same letter(s) were not significantly different at p <0.05 according to Tukey test.

the proposed involvement of the blue photoreceptors, cryptochromes, in magnetic field perception (Ahmad et al., 2006). This later involves ROS (Okano, 2008) particularly the superoxide ion $O_2^{\cdot -}$ acting at low level (Solov'yov and Schulten, 2009).

The SMF growth responses also depend on radish capacities to counteract ROS production increased by the germinative metabolism resumption in normal conditions (and following magnetic field treatment. In radish, the beneficial SMF treatment (100 mT/3h) for both germination and seedling growth, allowed stimulation of catalase activity counteracting the enhanced H_2O_2 production resulting probably of an increased SODs activity in plantlets organs. In *Glycine max*, increased of both SOD and catalase activities are observed allowing an efficient detoxification. Thus, it is thought to be the result of the influence of MF on the metal SOD cofactors and in particular Zn^{2+} and Cu^{2+} (Ḉelik et al., 2009). The enhanced ROS detoxification leads, at beneficial SMF treatments, to oxidative damages reduction as observed in sunflower for which membrane integrity was ameliorated (Vashisth and Nagarajan, 2010) probably by lowering iron absorption **(**Hajnorouzi et al., 2011) that prevents $OH^{\bullet -}$ formation by Fenton's reaction. In radish whose germination medium was water, the suitable presowing SMF treatment (100mT/3h) did not increased lipid peroxidation as MDA content was unvaried in the two studied varieties. Thus might be the result of an increased activity of glutathione S-transferase (GTS) induced by MF treatment (Rochalska and Grabowska, 2007). These enzymes detoxify peroxidised lipids and reduce lipid peroxidation improving seedlings growth under stressful conditions (Roxas et al., 2000) as SMF treatment. Moreover, in cruciferous vegetables as radish, GTS also conjugates isothiocyanates (Lin et al., 1998) implicated in their antioxidative defence and responsible for their antioxidant and therapeutic (Beevi et al., 2010) properties.

Radish presowing responses to SMF is variety dependent

The differential growth responses to SMF presowing observed in the two radish varieties might be related to difference in paramagnetic and diametric components (Penuelas et al., 2004) conferring a lower diametric susceptibility to the R+ W variety by comparison to the Red one. However, on the basis of the studied oxidative parameters and for the beneficial treatment (100 mT/3h), responses of the studied varieties were approximately similar. Radish varieties' detoxifying capacities might be related to other non studied enzymatic and non enzymatic detoxifying processes. MF has been shown to stimulate peroxidase activities (Shabrangi and Majd, 2009) but also accumulation of antioxidant secondary metabolites as anthocyanins (Ahmad et al., 2006) that would contribute to a better oxidative status supporting the better seedling growth of the R.R by comparison to the R+W one.

Conclusion

This study constitutes a contribution to determine suitable SMF presowing treatment (s) in radish on the basis of seedling biomass production and oxidative responses. In our experimental conditions, the treatment 100 mT/3h was beneficial for the studied radish varieties; it corresponded to the peak of performance. Nevertheless, R.R exhibited more sensibility to SMF than R+W variety. For this SMF presowing treatment, blue light might be substituted to white one and might be used in seed biotechnology to improve initial developmental stages.

ACKNOWLEDGEMENTS

The authors want to thank Dr Pierrette Fleurat Lessard for the helpful comments and suggestions on the manuscript.

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