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# Introducing the "NewMed Effect":A New Phenomenon which Mimic Radiation Induced Bystander Effect and Amplifies the Biopositive Effects of Very Low Doses of Gamma Radiation

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**In this paper we present our findings regarding a phenomenon which mimic bystander effect and significantly amplifies the stimulatory effects of low dose radiation. Thirty *Triticum aestivum* grains served as the "control group" while in the 2nd group 30 grains were exposed to gamma radiation emitted by Tc-99m. In the 3rd group, irradiated seeds were transferred to a new culture medium. The seeds in the 4th group were discarded after irradiation andt new un-irradiated cells were transferred to the irradiated medium. Exposure of *Triticum aestivum* to very low levels of ionizing radiation in the range of a few mSv enhanced root length, stem length, germination capacity, germination speed, fresh weight and the chlorophyll content. It was interestingly discovered that transferring irradiated seeds to a new medium, enhances root length, stem length, dry weight and the chlorophyll content. To the best of our knowledge this is the first report on the phenomenon we called it "NewMed effect". As far as we know, this is the 1st report on the stimulatory bio effects of exposure to very low doses of gamma radiation in *Triticum aestivum*. Further studies are needed to shed light on different aspects as well as potential applications of this effect.**

**Key words:** Hormetic Effects, Low Dose Radiation,
Bystander Effect, Growth, Wheat, *Triticum aestivum.*

The bystander effect that refers to the effects in normal non-exposed cells adjacent to the irradiated or targeted cells (Hall 2003) has significantly challenged the concept that genetic and biochemical alterations are restricted only to the directly irradiated cells (Baskar 2010). Both direct effects and bystander effects are dependent on factors such as the type of radiation and cell type (Baskar *et al*. 2007). Bystander effect that may either increase or decrease the radiation induced cancer risk has led to a major paradigm shift about the effects of ionizing radiation. The mechanisms of bystander effects are not yet clearly understood but it seems that this effect is induced by direct cell contact communications or release of specific materials from irradiated

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cells. It has been shown that bystander effect may cause stimulated cell growth or genotoxicity (intense double strand breaks (DSB), micronuclei (MN), mutation and decreased cell viability) in the non-irradiated bystander cells (Han *et al*. 2010). Some evidence indicate that in cultured cells, soluble factors released from the irradiated cells are responsible for bystander effects. For example, increased clonogenic stimulation in the bystander cells can be diminished by dilution of the culture medium of irradiated cells (Baskar *et al*. 2007; Ryan *et al*. 2008). When non-irradiated cells were co-cultured with cells exposed to low dose alpha particles with absorbed doses ranging 1-10 cGy, stimulated cell growth and increased MN and DSB were observed in the bystander cells (Han *et al*. 2010). Nitric oxide (NO) and transforming growth factor-1 (TGF-1) seem to play a role in increased cell proliferation in the non-irradiated bystander cells. On the other hand, increased proliferation (shortened cell cycle) in bystander cells does not let them have enough time to repair DSBs. Therefore, increased probability of mutation from the misrepaired or un-repaired DSBs can increase the risk of carcinogenesis in bystander cells (Han *et al*. 2010).

Over the past several years our laboratory has been focused on the health effects of exposure to elevated levels of natural ionizing radiation in high background natural radiation areas (HBNRAs) of Ramsar (Mortazavi *et al*. 2005a; Mortazavi *et al*. 2005b; Mortazavi and Karam 2005; Mortazavi and Mozdarani 2012; Mortazavi and Mozdarani 2013; Mortazavi *et al*. 2012b; Mortazavi *et al*. 2005c) and we have previously published the first report on the induction of adaptive response in the residents of these areas (Ghiassi-Nejad *et al*. 2002). A small part of Ramsar city with 1000-2000 population has high levels of natural radiation. The mean dose for the residents of HBNRAs of Ramsar is 10 mSv y_1 but some of the residents receive doses as large as 260 mSv y_1 (Mortazavi and Mozdarani 2012). Altogether our findings showed no apparent harmful health effects and we suggested that global research on the residents of HBNRAs help scientists better justify if the old linear no-threshold model (LNT) of radiation risk is appropriate as the basis for public health measures in these areas (Mortazavi *et al*. 2005c).During our experiments, we realized that plants grown in the soil samples from HBNRAs of Ramsar showed increased germination, growth rate, wet weight and root length compared to those grown in ordinary soil. We also worked on phenomena which mimicked bystander effect by either transferring un-irradiated seeds to the irradiated culture medium, or transferring irradiated seeds to un-irradiated culture medium. In this paper our new findings regarding this phenomenon which mimic radiation induced bystander effect and significantly amplifies the stimulatory effects of low doses of ionizing radiation are discussed.

## MATERIALS AND METHODS

Wheat seeds were soaked in tap water for 12 hours at 25 C in the dark. Then, their external surfaces were disinfected by sodium hypochlorite 5%) and rinsed 3 times with double distilled water. A hatch of 30 grains served as the "control group" was placed on a sterile moist filter paper in a Petri dish and only sham exposed. In the 2nd group "Technetium group", 30 grains, were exposed to gamma radiation emitted by Tc-99m at a distance of 20 cm from the radiation source. The exposure terminated after 24 hours. In the 3rd group, seeds received the exposure treatment of the 2nd group but after irradiation, the seeds were transferred to a new culture medium (distilled water). Finally, the seeds in the 4th group again received the exposure treatment of the 2nd group but irradiated seeds were discarded and new un-irradiated cells were transferred to irradiated medium. The average dose received by the seeds during 24 hours of exposure was calculated by Monte Carlo (MC) simulation. The 3D geometry of MC simulation is shown in Figure 1. Figure 2 presents the 2D geometry of MC simulation showing the location of the cells in dose calculations. All Petri dishes were incubated at 22±1° C for 8 days.

To measure the seed germination potential, the daily count of the germinated seeds were carried out for eight days at specified time intervals. Seeds with root lengths of over two mm were considered as germinated (Melki 2010). Germination Capacity percentage (GC%) was calculated according to the following equation (Melki 2008):

GC (%) = " Number of germinated seeds after 8 days" /"Total number of seeds" ×100

All tests were repeated three times, and the results of root length stem length, germination capacity, germination speed, fresh weight, dry weight and the chlorophyll content were statistically analyzed by ANOVA test using SPSS software.

## RESULTS

The mean (±SD) dose received by the seeds during 24 hours of exposure as calculated by Monte Carlo (MC) simulation was 1105.4 ± 378.8 ìSv (ranged 592.1 ± 1939.3 ìSv).

### Root Length

The mean root lengths in the control and exposure groups are shown in Figure 3. As it was expected, exposure of the seeds to low levels of gamma radiation has led to increased root length (P<0.001). Interestingly, when irradiated seeds were transferred to a new culture medium, the root length increased significantly again (P<0.05). On the other hand, when irradiated seeds were discarded and new seeds were transferred to the irradiated culture medium, the mean root length in this group was not significantly different from that of the control group (P=0.98).

### Stem Length

Figure 4 shows the mean stem lengths in the control and exposure groups. Again as it was expected, exposure to low levels of gamma radiation significantly increased the stem length (P<0.001). In contrast with root length findings, when irradiated seeds were transferred to a new culture medium, the stem length did not increased significantly (P=0.78). Showing a similar pattern of results, when irradiated seeds were discarded and new seeds were transferred to the irradiated culture medium, the mean stem length in this group was not significantly different from that of the control group (P=0.82).

### Fresh Weight

The mean fresh weights in the control and exposure groups are shown in Figure 5. Again showing a similar pattern of results, exposure to low levels of gamma radiation has led to increased fresh weight (P<0.001). Again, in contrast with root length findings, when irradiated seeds were transferred to a new culture medium, the fresh weight did not increased significantly (P=0.31). Again, when irradiated seeds were discarded and new seeds were transferred to the irradiated culture medium, the mean fresh weight in this group was not significantly different from that of the control group (P=0.22).

### Dry Weight

The mean dry weights in the control and exposure groups are shown in Figure 6. In contrast with our previous findings, exposure to low levels of gamma radiation did not significantly increase the dry weight (P=0.40). Interestingly, in spite of this, when irradiated seeds were transferred to a new culture medium, the dry weight increased significantly (P<0.001). In contrast with our previous findings, when irradiated seeds were discarded and new seeds were transferred to the irradiated culture medium, the mean dry weight in this group was significantly lower than that of the control group (P<0.001).

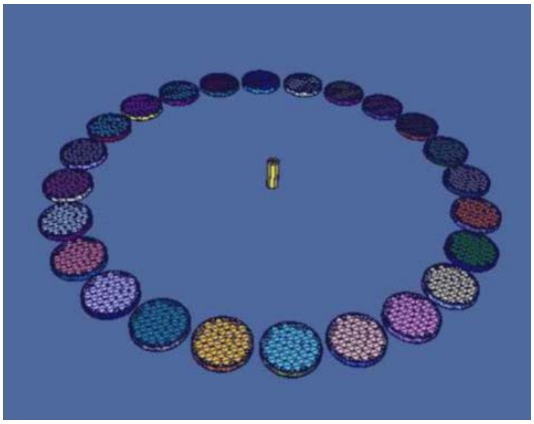


**Fig. 1.** The 3D geometry of MC simulation

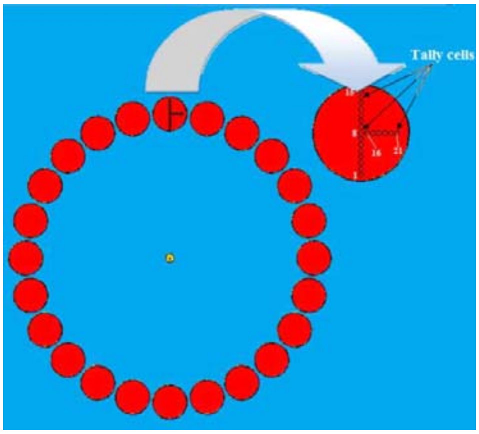


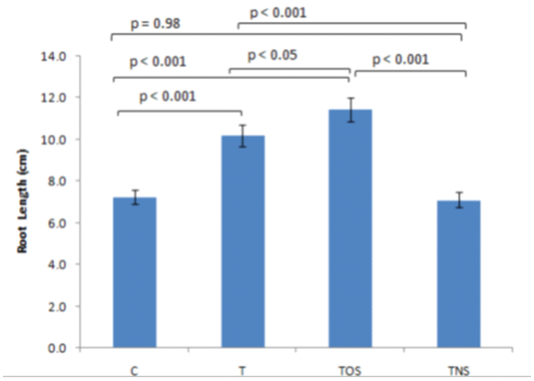
**Fig. 2.** The 2D geometry of MC simulation which shows the location of the cells in dose calculation

### Germination Capacity

Figure 7 shows the mean germination capacity in the control and exposure groups. Again as it was expected, exposure to low levels of gamma radiation significantly increased the germination capacity (P<0.001). In contrast with root length findings, when irradiated seeds were transferred to a new culture medium, the germination capacity did not increased significantly (P=0.99). Showing a similar pattern of results, when irradiated seeds were discarded and new seeds were transferred to the irradiated culture medium, the mean germination capacity in this group was significantly lower than that of the control group (P<0.05).

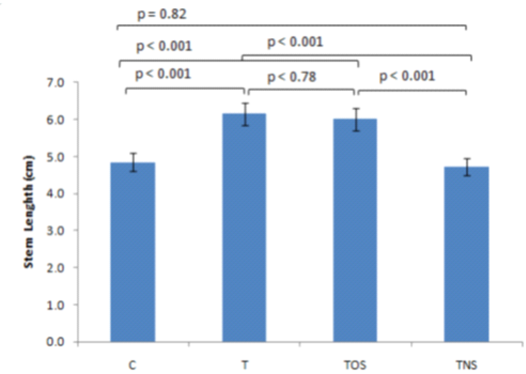
### Germination Speed

The mean germination speeds in the control and exposure groups are shown in Figure 8. Again, exposure to low levels of gamma radiation



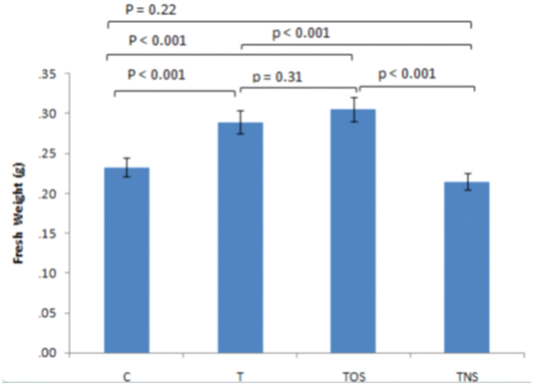
T: Seeds exposed to gamma rays from a Tc-99m source
TOS: The same exposure condition but irradiated seeds were transferred to a new culture medium.
TNS: The same exposure condition but new un-irradiated seeds were transferred to the irradiated culture medium
Error Bars indicate standard deviation (SD).

**Fig. 3.** The mean root length in the control and exposure groups



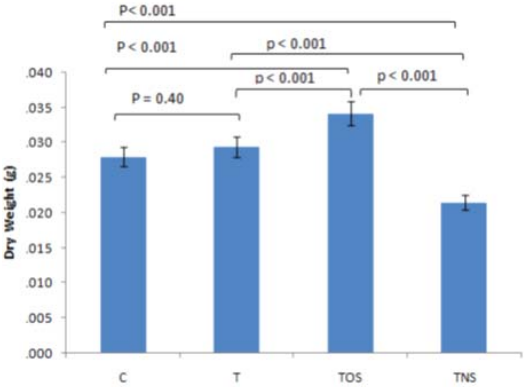
T: Seeds exposed to gamma rays from a Tc-99m source
TOS: The same exposure condition but irradiated seeds were transferred to a new culture medium.
TNS: The same exposure condition but new un-irradiated seeds were transferred to the irradiated culture medium
Error Bars indicate standard deviation (SD).

**Fig. 4.** The mean stem length in the control and exposure groups



T: Seeds exposed to gamma rays from a Tc-99m source
TOS: The same exposure condition but irradiated seeds were transferred to a new culture medium.
TNS: The same exposure condition but new un-irradiated seeds were transferred to the irradiated culture medium
Error Bars indicate standard deviation (SD).

**Fig. 5.** The mean fresh weight in the control and exposure groups



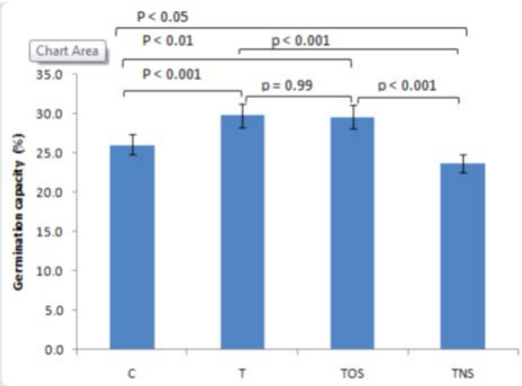
T: Seeds exposed to gamma rays from a Tc-99m source
TOS: The same exposure condition but irradiated seeds were transferred to a new culture medium.
TNS: The same exposure condition but new un-irradiated seeds were transferred to the irradiated culture medium
Error Bars indicate standard deviation (SD).

**Fig. 6.** The mean dry weight in the control and exposure groups

significantly increased fresh weight (P<0.001). Again, in contrast with root length findings, when irradiated seeds were transferred to a new culture medium, the fresh weight did not increased significantly (P=0.99). Again, when irradiated seeds were discarded and new seeds were transferred to the irradiated culture medium, the mean fresh weight in this group was not significantly different from that of the control group (P=0.99).

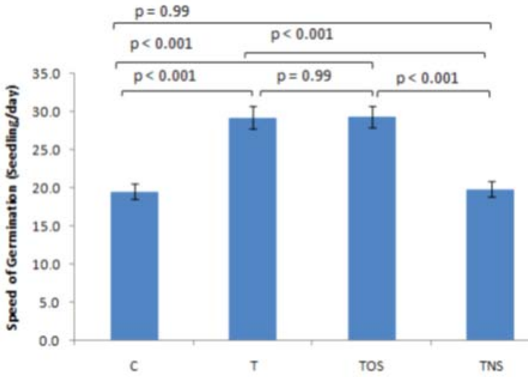
## Chlorophyll Content

Figure 9 shows the mean chlorophyll contents in the control and exposure groups. Again as it was expected, exposure to low levels of gamma radiation significantly increased the germination capacity (P<0.05). In a similar pattern with our previous results about root length, when irradiated seeds were transferred to a new culture medium, the germination capacity significantly increased (P<0.05). On the other hand, when irradiated seeds were discarded and new seeds were transferred to the irradiated culture medium, the mean germination capacity in this group was significantly higher than that of the control group (P<0.05).



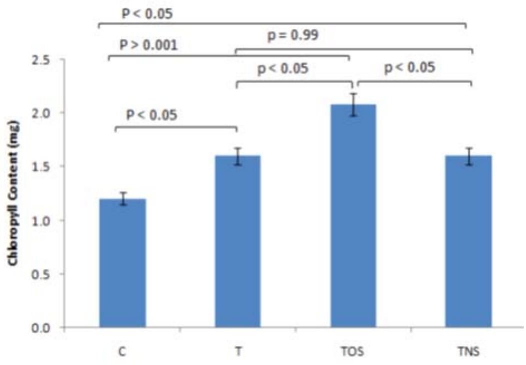
T: Seeds exposed to gamma rays from a Tc-99m source
TOS: The same exposure condition but irradiated seeds were transferred to a new culture medium.
TNS: The same exposure condition but new un-irradiated seeds were transferred to the irradiated culture medium
Error Bars indicate standard deviation (SD).

**Fig. 7.** The mean germination capacity in the control and exposure groups



T: Seeds exposed to gamma rays from a Tc-99m source
TOS: The same exposure condition but irradiated seeds were transferred to a new culture medium.
TNS: The same exposure condition but new un-irradiated seeds were transferred to the irradiated culture medium
Error Bars indicate standard deviation (SD).

**Fig. 8.** The mean germination speed in the control and exposure groups



T: Seeds exposed to gamma rays from a Tc-99m source
TOS: The same exposure condition but irradiated seeds were transferred to a new culture medium.
TNS: The same exposure condition but new un-irradiated seeds were transferred to the irradiated culture medium
Error Bars indicate standard deviation (SD).

**Fig. 9.** The mean chlorophyll content in the control and exposure groups

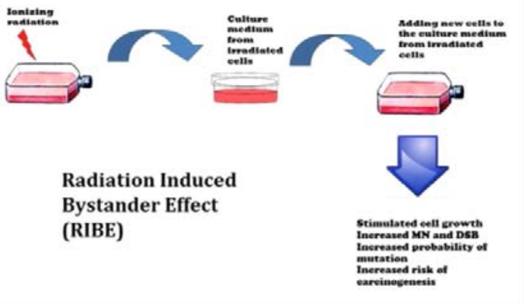


**Fig. 10.** Graphical representation of the radiation induced bystander effect

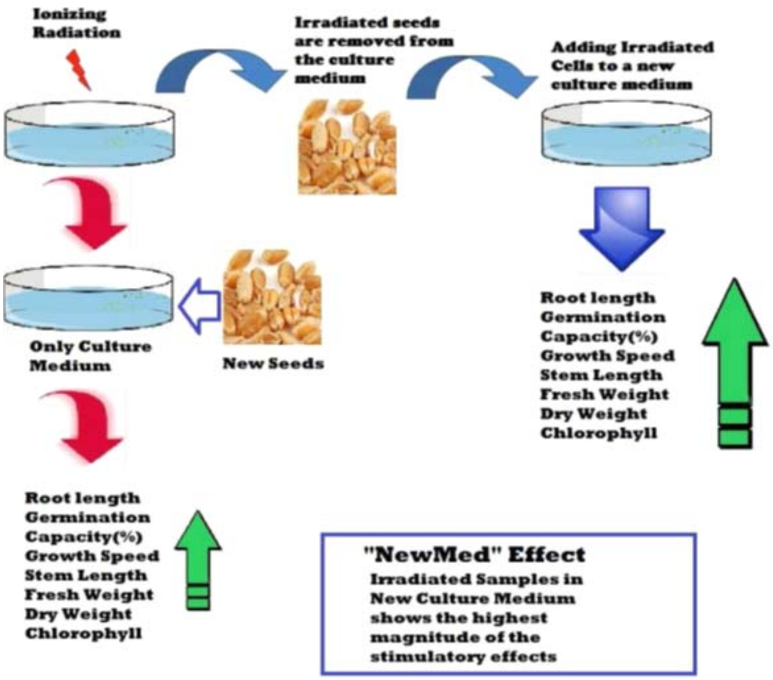


**Fig. 11.** The Graphical representation of the NewMed effect. When irradiated seeds were transferred to a new un-irradiated culture medium, the biopositive effects of low doses of ionizing radiation were significantly amplified compared to those of irradiated seeds which were not transferred to a new un-irradiated culture medium

## DISCUSSION

To the best of our knowledge this the first report on a phenomenon we called it “NewMed” effect. Furthermore, as far as we know, this is the 1st report on the stimulatory bioeffects of exposure to very low doses of gamma radiation in *Triticum aestivum*. The findings of this study clearly showed that exposure of *Triticum aestivum* to very low levels of ionizing radiation in the range of a few mSv enhances important factors such as root length, stem length, germination capacity, germination speed, fresh weight and the chlorophyll content. Since a great proportion of the plant water uptake is taking place by root systems and one of the first responses of plants to water stress is the root elongation (Taiz L 2002), enhancement of this response through various factors, including irradiation can improve their resistance to drought. Further research in this field can open new horizons in global food production and potential applications of this phenomenon overcoming drought and food shortage. It is worth mentioning that a dangerous hunger crisis has been warned by the United Nations because the world grain reserves are so risky low that severe climate in the food-exporting countries could trigger such a global catastrophe. Although wheat (*Triticum aestivum*) is an important food source in the world, it fails to grow properly in many areas due to unfavorable climate and soil conditions. Our findings in this section of the study are generally in line with reports which indicated a broad spectrum of hormetic effects due to stressors such as ionizing and non-ionizing radiation, heat, caloric intake, and even exercise, on plants, fungi, bacteria, protozoa, and animals, including humans (Barceló and Poschenrieder 2002; Calabrese and Baldwin 2000; Feinendegen 2005; Garzon and Flores 2013; Hayes 2007; Hayes 2009; Ji *et al*. 2006; Mortazavi *et al*. 2014a; Mortazavi *et al*. 2012a; Mortazavi 2013a; Mortazavi 2013b; Mortazavi 2013c; Mortazavi 2014; Mortazavi *et al*. 2011; Mortazavi *et al*. 2013; Mortazavi *et al*. 2014b; Rattan 2008; Tlili *et al*. 2011). Feinendegen has previously reported that low doses in the mGy range cause a dual effect on cellular DNA; a low risk of DNA damage and the induction of an adaptive protection against DNA damages from many, mainly endogenous, sources. He has reported that exposure to doses in the range of background radiaton, the damage to DNA is orders of magnitude lower than that from endogenous sources (i.g. reactive oxygen species) (Feinendegen 2005).

As the most important finding in these studies, we discovered that the biopositive effects of low doses of ionizing radiation were significantly amplified compared to those of irradiated seeds which were not transferred to a new un-irradiated culture medium. We found that in *Triticum aestivum* transferring irradiated seeds to a new un-irradiated culture medium, significantly enhances root length, stem length, dry weight and the chlorophyll content. It should be noted that as an abiotic stress, electromagnetic radiation can induce oxidative processes in plant cells. Moreover, exposure to radiation in the presence of O2, induces the production of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radical (Fan X. 2004). As plants are sessile and cannot escape the biotic and abiotic stresses, they depend on neatly developed signaling networks, including ROS signaling pathways, to regulate various developmental processes and responses to environmental stimuli (Heidarvand L 2010; Petrov VD 2012). It has been shown that the amount of ROS produced is related to the severity of stress; if the cells are exposed to heavy stress, large quantities of ROS will be produced as toxic byproducts of metabolism (Mittler R 2011; Potters G 2010; Thomas 2008). ROS have the ability to react with essential molecules, such as nucleic acids, proteins, lipids and carbohydrates, and alter their normal properties. In contrast, a weak stress induces low levels of ROS and these level of ROS can regulate various processes of plant growth and development through different signaling pathways, Such as calcium and protein kinase (MAPK) (Rathinasabapathi 2013). In the light of these events, we believe that in spite of this fact that exposure to low levels of ionizing radiation leads to stimulatory effects in irradiated seeds which leads to enhanced growth, agents released in the culture medium by irradiated seeds can limit the growth of these seeds. This concept can entirely explain the necessity of removing the irradiated medium and transferring the irradiated seeds to a new medium for obtaining better growth characteristics. As it has been shown that oxidative stress can be an important mediator of radiation induced bystander effect (Harada *et al*. 2008; Widel 2012), it can be hypothesized that oxidative stress induced by low doses of ionizing radiation has triggered the “NewMed effect” in irradiated seeds. Further studies are needed to investigate if this effect can be observed at cellular level. On the other hand, different aspects of this phenomenon and its potential applications should be further studied.

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