International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 8 Issue 11 ǁ 2020 ǁ PP. 69-72

Influence of Nano-Magnetism on Tomato Fruit Ripening

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Abstract: *Nanoparticle induced plant developmental process has been in the lime light in recent years. Magnetic field intensity towards fruit ripening has been a subject of interest. In the present study, magnetic nanoparticles along with a strong magnetic field influence the fruit ripening process artificially. The results of the present study will be useful for future research scientists working in this field.*
Keywords: Nanoparticle, magnetic field, ripening, ethylene, tomato.

Date of Submission: 12-11-2020 Date of acceptance: 28-11-2020

I. INTRODUCTION

All living creatures experience the force of earth's magnetic field (geomagnetic, GMF) for growth and development, including plants. Although weak (nearly 35uT near equator and 70uT near poles) various plant physiological process including seed germination, flowering, root growth and even fruit ripening are influenced by earths geomagnetic field [1]. This natural geomagnetic effect on biological systems has attracted the attention of biologists more than a decade. Exogenous influence of magnetic fields in the form of magnetized water to magnetic nanoparticles have long been used to improve various plant developmental processes in plants [2, 3]. Among all, fruit ripening a genetically driven programme in nature is also said to be an event influenced by earth's magnetic field (auxin like effect) [4]. Widely consumed tropical edible fruits including tomato, banana, mango and papaya ripe at a faster rate, due to their short shelf life [5,6].

Farmers and commercial vendors face huge losses due to the short shelf life of the edible fruits in addition to the untimely rainfall during ripening season. Due to the untimely rain and hails, fully ripened fruits drop from the plants in large number causing an inevitable loss to farmers and traders. Despite these setbacks, time taken to transport from field to markets also infers. As a precautionary step to these downfalls, commercial vendors and farmers started using the application of artificial chemical ripener's in mature pre-ripened fruits to setup controlled fruit ripening artificially. However, most of the chemicals used in this process are scientifically proven to be hazardous (ethereal, herbicides, calcium carbide, etc.) affecting human health on consumption [7,8,9,10], whereas spraying some ripening influencer gases like acetylene and ethylene are time consuming and expensive for large scale setup. Application of nanomaterials in improving agricultural processes has been the current trend in agroindustry [11, 12, 13, 14]. Metal based nanoparticle usage have resulted in successful outcomes on plant development over a decade [15, 16, 17]. Magneto nanoparticle reception in particular have resulted in improved seed germination, pollen transformation and faster growth in plants [14, 18, 19].

Compared to the impactful studies in mammals, minimal information is known about magneto-reception in plants, especially in controlled fruit ripening [20, 21, 22]. A previous attempt in using a low intensity static magnetic field has failed to trigger fruit ripening in tomato [23]. Considering the advantages of magnetic nanoparticles in agriculture applications and an earlier failure in using low-static magnetic field, the present study is designed combing both magnetic nanoparticles as well as a strong magnetic field in influencing the ripening in climacteric model fruit tomato (*micro-tom*). In the present study, we hypothesized that an electrical impulse could be generated through a strong magnetic field which can cause the magnetic force to influence the adhesion of magnetic nanoparticles inside fruit tissues and enforce a genome wide molecular regulation.

II. METHODS

Mature seeds of tomato (*Micro-tom*) were germinated in separate pots under normal greenhouse conditions with 16/8h day/light conditions. Flowers from mature plants were hand pollinated using a vibrator and uniformly labelled. Uniformly grown mature green fruits at 22DAP were used in all the experiments. Test control solution were prepared without adding magnetic nanoparticles. Plant control fruits were obtained from greenhouse only during the experimental period and used. Experimental solution contained polyethyleneimine-modified magnetic nanoparticles (Fe_3O_4, Nanoeast, China) [24] mixed with sterilized water (1:100). Two strong permanent magnets (Chemicell, Germany) of 0.3T magnetic intensity were used throughout the experiment. For

molecular experiments, total RNA was extracted using the commercial plant RNA kit (Qiagen, China) following manufacturer's protocol, after which cDNA was synthesized using a commercial kit (TAKARA, Japan), following manufacturer's protocol. For gene expression analysis, transcript levels as determined by qPCR (SYBR-qPCR mix, Transgene Biotech, China) were normalized to Actin expression. Primers used in this study are listed in Table.1. The mean values of data were measured from three replicates and standard error of the means was calculated. Data were analyzed by Origin 8.0 plotting software, and student's *t* test was used for assessing significant differences among the means.

III. RESULTS AND DISCUSSION

To test this, we initially used a low intensity magnet (single) along with application of magnetic nanoparticles. In addition, we also experimented the application of magnetic nanoparticles alone as well as a strong magnet alone. However, all these techniques did not cause any rapid change in the fruit ripening process (data not shown). We then used two strong intensity magnets of around 0.3T magnetic strength in combination of magnetic nanoparticles. The two magnets were placed at a close distance around 10cm from each other (Figure.1).

Briefly, we applied magnetic nanoparticle (MNPs-Fe_3O_4) solution in a ratio of 1:100 (dissolved 10µl nanoparticles in 100ml sterilized water solution). We note here that a higher concentration beyond this level also fetched the same results. So, we fixed 1:100 concentration throughout the experiment. We then selected the tomato fruits of uniform developmental stage (22 days after pollination; DAP). We filled the bisected petri plate with control solution (sterilized water alone) and test solution (nanoparticle dissolved in sterilized water) and placed the plate in between the two strong magnets of 0.3T strength (Figure.1). We initially optimized the time period for the effect of magnetic field. Interestingly, we observed that time periods of both 4 hours and 12 hours resulted in similar kind of effect on fruit ripening, whereas time limit below 4 hours caused a reduction in fruit ripening effect, thus we optimized a 4 hour treatment, enough to cause a trigger in ripening related molecular responses. After a 4 hour treatment, we air dried the experimental control as well as magnetic nanoparticle treated fruits for up to 20 minutes. Upon which, we placed the fruits in an air tight plastic transparent containers and allowed to ripen in light and temperature controlled growth chambers (25°C and16/8h photoperiod). Interestingly, magnetic nanoparticle treated fruits started to show ripening (blotchy) symptoms within 3 days after storage, whereas the plant control and the experimental control fruits did not show any signs of ripening as early as 3 days. It took 8 days for the plant control as well as experimental control fruits to show early ripening symptoms (Figure.1). From these results, we came to a conclusion that magnetic nanoparticle only in combination with a strong magnetic field fastens fruit ripening.

Ripening is a process controlled by several molecular events at genome level, so we further need to find out whether this nanoparticle influence has caused any change at the molecular level [25]. To test this, we extracted RNA from the fruit pericarp tissue and using real time qPCR assay, we investigated the expression of two critical ripening related ethylene biosynthesis marker genes *ACS2* and *ACS4* [26, 27]. Concurrent to the ripening phenotype, expression of both *ACS2* and *ACS4* genes were highly upregulated in magnetic nanoparticle treated fruits compared to both plant control and experimental control fruits (Figure.1). High upregulation of ethylene marker genes indicate higher ethylene biosynthesis in magnetic nanoparticle treated fruits. Collectively, these data suggest that a magnetic field alone nor a magnetic nanoparticle alone cannot cause ripening changes, whereas in combination they can trigger a genome wide alteration especially in triggering ethylene biosynthesis mediating the complex fruit ripening process. Although a complete genome wide study on underlying molecular mechanism remains to be identified, these findings highlight the value of magnetic nanoparticles on controlled fruit ripening influenced by a strong magnetic field, providing a new insight into the plant reception to an external stimuli. Recently, Samanta et al., reported that magnetic spicules generated around magnetic flux cause solar heating in the solar atmosphere, this finding concurs with our observation suggesting that magnetic spicules generated from strong magnetic fields might be the possible reason to trigger magnetic nanoparticles in causing thermal heat to influence fruit ripening [28]. In support, previously published reports highlight that, magnetic nanoparticles also serve as nano-heaters in certain self-healing inonomer matrix [29].

Current systems in achieving controlled fruit ripening largely depend on the usage of several hazardous chemicals, fruits artificially ripened using these chemicals face a great threat to consumers, as such there exist a lack of an appropriate approach to execute controlled fruit ripening. In the present study, we used nanomagnetism tool that applies a strong magnetic field enforcing the activation of magnetic nanoparticle in creating a thermal energy causing changes at molecular level (ethylene biosynthesis) in fruit tissues. For instance, previous investigations led on this field have applied only a low intensity static magnetic field without magnetic nanoparticles which were proven to be unsuccessful. Furthermore, since nanomagnetic effect can cause changes at molecular level, future researchers can carry out genome wide analysis to dissect the entire regulatory pathway mediated by this effect.

IV. CONCLUSION

Overall, our technique of fastening fruit ripening artificially using nanomagnetism will serve as a useful tool to farmers (controlled ripening), commercial vendors (remedy to hazardous chemical based ripening) and much importantly consumers (free from consuming chemically ripened fruits). Although our technique has been currently performed in small scale, chances for establishing large scale setup based on this technique is very high, as similar kind of large scale setups for ripening fruits using ethylene gas have been previously established (http://www.rinac.com/banana-ripening-chambers-manufacturers.html). Furthermore, this method holds a valuable asset of promise in providing novel insight of how energy (magnetic) from one form is able to transform energy (thermal) to cause molecular regulation of fruit ripening. We propose that when established in large scale, this nanomagnetic technique will posit a great deal for large set of targets (fruits and vegetables) in establishing controlled ripening.

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Table 1. Primers used in this study

Gene Name	Forward (3'-5')	Reverse (3'-5')
SlACS2 (Solyc01g095080)	GAAAGAGTTGTTATGGCTGGTG	GCTGGGTAGTATGGTGAAGGT
SlACS4 (Solyc05g050010)	GCTCGGAGGTAGGATGGTTTC	GTTCCTCTTCCATTGTGCTTGT
SlACTIN (Solyc11g005330)	TGTCCCTATTTACGAGGGTTATGC	AGTTAAATCACGACCAGCAAGAT

Figure.1

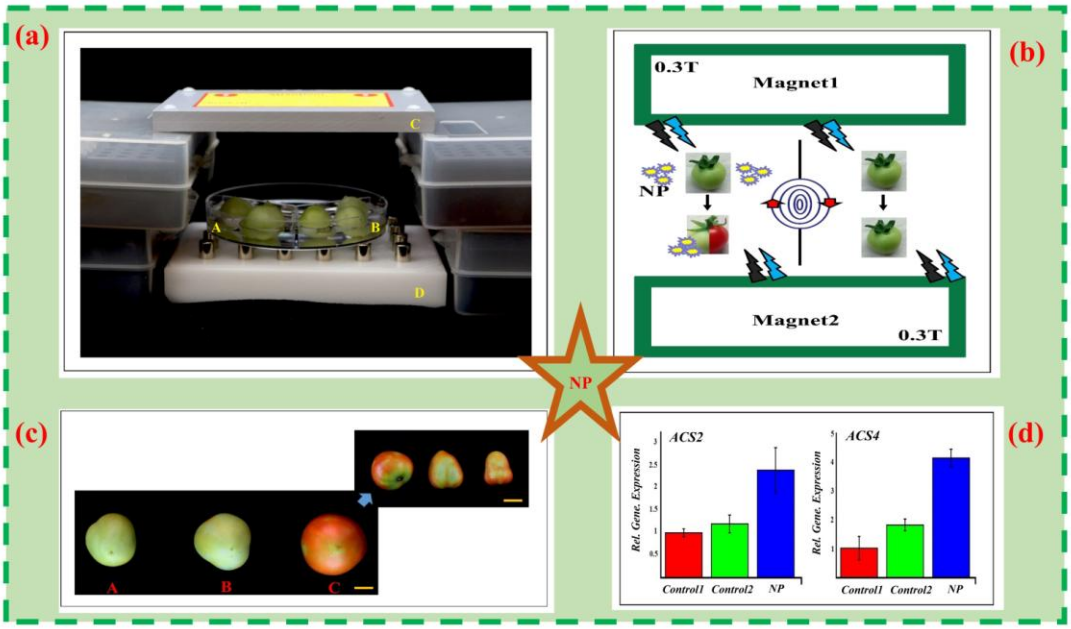

Figure.1 (a) Experimental setup used in the study (A) represents mature green fruits dipped in sterilized water mixed with magnetic nanoparticles (B) Represents mature green fruits dipped in sterilized water alone (C) & (D) two permanent magnets used in the study. (b) Graphical representation of the setup and possible influence of magnetic intensity and nanoparticles on fruit ripening. (c) Phenotype of fruits stored in temperature controlled growth chamber 8 days after storage (A) plant control (B) Test control (C) magnetic nanoparticle treated (upper row shows the blotchy ripening phenotype observed in magnetic nanoparticle treated fruits). (d) Expression analysis of ethylene biosynthesis marker genes *ACS2* and *ACS4* in 8 days stored fruits after nanomagnetic treatment. *Actin* was used as the internal control. All the experimental data are the result of three biological replicates. NP; Nanoparticle.