Plant Physiology and Biochemistry 145 (2019) 195–204

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Gold nanoparticles-based biosensor can detect drought stress in tomato by ultrasensitive and specific determination of miRNAs

Keyvan Asefpour Vakilian[a,b,*]

[a] Department of Agrotechnology, College of Abouraihan, University of Tehran, Tehran, Iran
[b] Private Laboratory of Biosensor Applications, Hamadan, Iran

ARTICLE INFO

Keywords:
miRNA-1886
tas14 dehydrin gene
Optical biosensor
Aggregation
Linear range

ABSTRACT

Drought stress can significantly affect the yield and quality of tomato production. However, the development of a sensitive and specific method for the determination of drought stress is somehow challenging since plant common morpho-physiological and biochemical characteristic are not generally specific to biotic and abiotic stresses. As a solution, the concentration of miRNAs in plant tissues can be a selective and specific indicator of plant stress. In this study, an optical biosensor based on gold nanoparticles is introduced to determine miRNA-1886 in tomato plant roots. Results showed that irrigation levels from 100% to 60% of field capacity increased the concentration of miRNA-1886 in a range from ca. 100 to 6800 fM (fM) causing a linear change in the biosensor response ($R^2 = 0.97$). Results also revealed that in contrast with plant conventional morpho-physiological and biochemical characteristic, miRNA-1886 concentration was not significantly affected ($P < 0.01$) by other stresses, i.e., salinity and temperature during the growth period. The biosensor introduced in this study is a reliable method to study stress-related functions of miRNAs in plants and their application in specific plant stress determination.

1. Introduction

microRNAs (miRNAs, miRs) are endogenous, single-stranded non-coding RNAs which are found in animals and plants (Bej and Basak, 2014). The mature miRNAs are usually approximately 21 nucleotides in length of which more than 10,000 miRNAs have been identified and characterized so far for plant species (Liang et al., 2018; Yang et al., 2019). miRNAs function as negative post-transcription regulators by specific binding and cleavage of their target mRNAs, or by repression of the target mRNA translation (Wu et al., 2009; Shriram et al., 2016).

miRNAs have crucial roles in plant physiology: they involve abiotic and biotic stress responses (Nejat and Mantri, 2018), plant hormonal regulation (Li et al., 2016; Singh et al., 2016), and developmental processes (Noman and Aqeel, 2017). They regulate gene expression through targeted cleavage of messenger RNA (mRNA) or translation repression (Guleria et al., 2011; Zeng et al., 2014). Light, carbon dioxide, temperature, nutrients and water availability, salinity, and heavy metal pollution are major abiotic stresses plants frequently encounter in nature (Pessarakli, 2010). Upon exposure to stress, plant miRNA expression is modulated in a manner which is generally spatial (plant tissue) and temporal (developmental/growth stage) specific (Shriram et al., 2016; Sunkar, 2010). To reduce the damage caused by the stress, plants exert gene expression reprogramming, resulting in osmolyte accumulation for osmotic adjustment and up-regulation of antioxidant pathways for reactive oxygen species (ROS) homeostasis (Sunkar, 2010). With the identification of stress-responsive miRNAs, which act at the post-transcriptional level, useful information on their role in improving the stress tolerance mechanism of plants can be obtained.

During the last two decades, hundreds of research studies have been dedicated to the changes in plant miRNA expression in response to biotic and abiotic stresses. *Arabidopsis thaliana*, *Oryza sativa*, and *Panicum virgatum* are among the plants whose stress-related miRNA functions are studied comprehensively (Noman and Aqeel, 2017). Studies have shown that some miRNAs function in several stresses exerting an up (down)-regulation process whilst there are many miRNAs that involve only one stress, or at least, only one of their functionalities has been identified. miRNA-166, miRNA-169, miRNA-172, miRNA-319, miRNA-393, miRNA-394, and miRNA-396 are some examples of miRNAs that function in many plant abiotic stress-related processes (Wang et al., 2014; Xie et al., 2014; Gao et al., 2016; Ghani et al., 2018; Patel et al., 2019). For better understanding of plant physiology at molecular level in stress-related studies, it is desirable to investigate the behavior of miRNAs that tend specific to the study stress. This specificity has two main advantages: first, miRNA regulation is responsible for

---

[*] Department of Agrotechnology, College of Abouraihan, University of Tehran, Tehran, Iran.
E-mail address: keyvan.asefpour@ut.ac.ir.

https://doi.org/10.1016/j.plaphy.2019.10.042
Received 3 September 2019; Received in revised form 29 October 2019; Accepted 31 October 2019

the study stress and other possible environmental factors do not affect the experimental results; and second, the development of an analytical system to measure the miRNA expression (or in some cases, concentration) will be easier.

Drought stress is one of the most important environmental stresses which can undesirably affect the successful production of agricultural crops (McWilliam, 1986). In drought conditions, physical and/or environmental factors lead to a shortage of water in plants resulting in their growth and yield reduction (Anjum et al., 2011; Abdul Jaleel et al., 2009). Severe drought stress significantly affects the physiological and biochemical processes of a majority of plants (Massonnet et al., 2007). Plant responses to drought stress are usually studied based on physiological parameters such as relative water content, stomatal reactions, photosynthesis, osmotic adjustment, active oxygen species and antioxidative enzymatic responses (Huang et al., 2016; Martinez et al., 2018; Jafari et al., 2019). One of the most important crop plants that is sensitive to drought is tomato (*Solanum lycopersicum* L.) (Goñi et al., 2018). In general, plant response to drought stress in tomato depends on duration and severity of the drought period as well as its inherent tolerance mechanisms (Iovieno et al., 2016; Patanè et al., 2016). In a pioneering study, Din and Barozai (2014) investigated the miRNAs available in tomato and their targets. They showed that a total of 109 miRNAs belonging to 106 families can be characterized from the tomato expressed sequence tags. miRNA-1886 is one of these miRNAs that functions when the plant is exposed to drought stress. Therefore, monitoring of plant miRNA-1886 during the exposure to drought stress can provide us with valuable information about tomato plant physiology.

To study the effects of plant stress on miRNA functions, several methods including northern blot and polymerase chain reaction (PCR) have been widely used during the last two decades. However, these methods suffer from weak analytical characteristics, e.g., limit of detection, response linear range, and precision (Petralia et al., 2017). Furthermore, these techniques are time-consuming and require expensive equipment (Mourya et al., 2012). However, biosensors, having a living unit as their receptor (Asefpour Vakilian and Massah, 2018) have been introduced as a promising analytical method for fast and sensitive determination of miRNAs in biological samples (Rafiee-Pour et al., 2016). Therefore, the design and development of a low-cost colorimetric biosensor for specific and ultrasensitive determination of miRNA-1886 seems to be useful. Furthermore, using metallic nanoparticles, especially gold nanoparticles (AuNPs) can improve the performance of the sensor because of their high extinction coefficient and strong distance- and size-dependent optical property (Hakimian and Ghourchian, 2019; Caporali et al., 2019). In these biosensors, the color of solution containing the AuNPs and target analyte changes upon AuNPs aggregation due to the coupling of AuNP surface plasmons (Kaur et al., 2015; Hakimian et al., 2018).

The objectives of this study are: (a) the design and development of an AuNPs-based optical biosensor for ultrasensitive and specific determination of miRNA-1886, (b) to monitor the effects of drought stress on plant miRNA-1886 concentration along with other plant characteristics, and (c) to investigate the functionality of miRNA-1886 as an specific indicator of drought stress in tomato plants.

2. Material and methods

2.1. Biosensor preparation

Chemicals. The HPLC purified oligonucleotide sequences including sly-miRNA-1886 (5′-UGAGAGAAGAGAGAUGGAAAA-3′, validated by miRBase), two three-base mismatched sly-miRNA-1886, i.e., 5′-UGAG UGAAGACAGAUGGAUAA-3′) and (5′-UCAGAGUAGAGAGAUGGU AAA-3′) and thiolated probe (5′- AAAAAAAAATTTTCCATCTCTCTTCT CTCATTTTTTTTTT-HS-3′) were prepared at Private Laboratory of Biosensor Applications, Hamadan, Iran. $HAuCl_4$, polyethylenimine (PEI) (50% solution, M_n ~1200, M_w ~1300), trisodium citrate dihydrate ($Na_3C_6H_5O_7.2H_2O$), HCl, and Tween-20 were purchased from Sigma-Aldrich (USA). 3.5 kDa molecular weight cut-off (MWCO) membrane with a thickness of 200 μm was prepared in Membrane Research Laboratory, Sharif University of Technology, Tehran, Iran.

Preparation of citrate-capped AuNPs (C–AuNP) and C–AuNP/probe. C-AuNPs were synthesized by citrate reduction of $HAuCl_4$ according to Ji et al. (2007). In brief, 1.5 mL of sodium citrate 1% was added to 21 mL of boiling $HAuCl_4$ solution (0.8 mM), whilst vigorously stirring until its color changed from pale yellow to deep red. The solution, was then stirred for an additional 15 min and gradually cooled down to room temperature. To prepare C–AuNP/probe, 400 μL of C-AuNPs was mixed with 2 μL of Tween-20 and 400 μL of thiolated probe (1 μM). The product was left for 48 h in room temperature and then, centrifuged for 23 min at 10,000 rpm. Finally, the supernatants were removed and the oily red precipitate was re-dispersed in 200 μL of deionized water.

Preparation of PEI capped AuNPs (P–AuNP) and P–AuNP/miRNA-1886. P-AuNPs were synthesized by thermal reduction according to Hakimian et al. (2018). Briefly, 100 μL of PEI (42 mM) was added to 3 mL of $HAuCl_4$ (1.5 M) under vigorous stirring at constant pH of 7.4 which was adjusted by adding HCl to the solution. Afterwards, the temperature of the solution was increased so its color changed from yellow to red which is an indication of the reduction process. P-AuNPs were then dialyzed against deionized water using the membrane. The resulting red solution was stored at 4 °C before use. For the preparation of P–AuNP/miRNA-1886, 5 μL of the miRNA-1886 solution with specific concentration was incubated with 40 μL of synthesized P–AuNP for 30 min at room temperature.

Optical sensing of miRNA-1886. By mixing 5 μL of C–AuNP/probe and P-AuNPs/miRNA-1886, probe-target hybridization results in the reduction of distance between nanoparticles and an interparticle cross-linking aggregation happens. As an indicator of reaction, the color of mixture changes from red-pink to pink and the absorption intensity decreases at 530 nm (Hakimian et al., 2018). Since the UV–Vis absorptions at 530 and 750 nm indicate the quantity of dispersed and aggregated AuNPs, respectively (Chen et al., 2008), the absorbance ratio of 750/530 nm can be considered as an indicator of probe-target hybridization, and consequently, the concentration of target (miRNA-1886). In this study, UV–Vis absorption spectra of the aggregated particles were recorded after 15 min of reaction using a multi-mode microplate reader (SpectraMax iD5, Molecular Devices, USA).

2.2. Plant material and experimental design

Tomato seeds (*Solanum lycopersicum*, cv. Artemis F1) were purchased from Harris Seeds (New York, USA). Seeds were surface sterilized with NaClO for 1 min and rinsed thoroughly with distilled water. Seeds were firstly grown in plug trays using growth medium of vermiculite: peat: perlite (3: 2: 1). On day 21, seedlings were transferred to 5-L pots containing the same growth medium of plug trays. Macro and micro nutrient fertilization management of the plants was according to Duffy and Dé;fago (1999) and Heeb et al. (2005). The relative humidity of growth room was adjusted at 70 ± 5% under a light intensity of $120\,\mu mol m^{-2} s^{-1}$.

2.3. Drought stress conditions

Complete and equally irrigation of all pots was continued until the eighth true leaf stage (~35 days after planting) with 100% of field capacity. After that, for the control plants, tap water was supplied daily to maintain soil water potential close to the field capacity. Soil moisture was measured daily by a time-domain reflectometry (TDR) device (PMS-714, LUTRON, Taiwan). Drought treated plants were stressed by withholding water until soil water potential became different with field capacity to study mild, moderate and severe drought stresses (Beltrano and Ronco, 2008; Jafari et al., 2019). In this study, control (W_0) with 100% of field capacity along with four water treatments, i.e., 90% (W_1), 80% (W_2), 70% (W_3) and 60% (W_4) of field capacity were studied with four replications.

Plant characteristics for drought investigations were measured three times: 45 (T_1), 55 (T_2), and 65 (T_3) days after planting. Since some of the measurements were destructive, $5 \times 4 \times 3 = 60$ pots were dedicated to the drought stress study.

2.4. Other stress conditions

To investigate the specificity of the study miRNA towards drought stress, the effects of three levels of salinity (control $1\ \mathrm{dS\ m^{-1}}$ [S_0], $2\ \mathrm{dS\ m^{-1}}$ [S_1], and $3\ \mathrm{dS\ m^{-1}}$ [S_2]), three levels of cold (control 18 °C [C_0], 15 °C [C_1], and 12 °C [C_2]), and three levels of heat (control 18 °C [H_0], 21 °C [H_1], and 24 °C [H_2]) were studied on plant characteristics. The treatments began after reaching the eighth true leaf stage. To obtain the desirable levels of salinity, different amounts of NaCl were added to the irrigation water whilst the temperature of the growth room was controlled using a wall mounted air conditioning system (24 K, LG, Korea) for temperature treatments. The night temperature of the growth room was 5 °C lower than the day temperature during the growth period. Measurements of salinity and temperature were conducted with $0.05\ \mathrm{dS\ m^{-1}}$ and 0.1 °C accuracy. Since the salinity, cold, and heat investigations were carried out with four replications and plant characteristics were measured one time on the last day of the experiment (day 65), $3 \times 4 = 12$ pots were dedicated for each of these stresses.

2.5. Morpho-physiological and biochemical measurements

In this study, several plant characteristics including morphological, physiological, and biochemical traits were measured for stress investigations. Tomato fruits were harvested and removed from the plants before the measurements. Spectral adsorption of the samples was recorded using a spectrophotometer (UV-1700, Shimadzu, Japan).

Morphological parameters including plant height (PH), root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW) and shoot dry weight (SDW) were measured. All the weights were measured using a scale with an accuracy of 0.1 g. In order to determine the dry weight of the plants, roots and shoots of the samples were placed in an oven at 80 °C for 48 h, and weighed afterwards (Sheikh et al., 2005). The plant height was measured using a line with an accuracy of 0.1 cm.

Relative water content (RWC) was measured according to Goñi et al. (2018). Fresh weight (FW) of the selected leaves was immediately measured after cutting. In order to obtain the turgid weight (TW), the leaves were immersed in distilled water in a closed Petri dish and incubated under normal room temperature and dim light for 18 h. After the imbibition, leaves were properly wiped to remove the water from the surface and then weighed. Afterwards, the leaves were put in a convection oven for 24 h at 80 °C to obtain dry weight (DW). RWC was calculated using Eq. (1).

$$\mathrm{RWC}\ (\%) = \frac{(\mathrm{FW} - \mathrm{DW})}{(\mathrm{TW} - \mathrm{DW})} \times 100 \tag{1}$$

Chlorophyll index (CI) was measured in the leaves using a Field Scout CM1000 chlorophyll meter (Spectrum Technologies, IL, USA). This chlorophyll meter estimates chlorophyll content based on ratios of the amount of ambient and reflected light at 700 and 840 nm.

Catalase (EC. 1.11.1.6) activity (CA) was measured using the method described by Havir and McHale (1987). One-gram sample of leaves was weighed and ground with an ice-cold pestle and mortar with 10 mL of phosphate buffer (50 mM, pH 7.0). The homogenates were then centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant filtered through two layers of cheesecloth was used for the determination of enzymatic activity. The reaction mixture consisted of 0.1 mL of enzyme extract and 2.8 mL of phosphate buffer (pH 7.4, 0.1 M) containing Na_2EDTA (4 mM). The biochemical reaction was started by adding 0.1 mL of H_2O_2 (0.01 M) to the reaction system. Samples without H_2O_2 were used as blank. CA was calculated by the differences in spectral adsorptions in 240 nm at 30 s intervals for 2 min after the initial biochemical reaction. A change of 0.01 units per minute in absorbance was considered to be equal to one-unit CA.

Anthocyanin contents (AC) of leaves were determined according to the method explained by Mancinelli (1990). One-gram leaf sample was firstly extracted in 3 mL of methanol-HCl (1% HCl, v/v); the leaf samples were then kept at 4 °C for 48 h. Finally, the extract was filtered through two layers of cheesecloth and the AC was measured by reading spectral adsorptions at 530 and 657 nm.

Phenol content (PhC) of leaves was determined according to Singleton and Rossi (1965) using Folin-Ciocalteu solution with slight modifications. 0.5 g of the fresh leaf tissues were homogenized in liquid nitrogen, then extracted with 5 mL of 80% methanol, and centrifuged at 10,000 rpm for 10 min. 100 μL of the supernatant was mixed with 1.8 mL of Folin-Ciocalteu solution and then kept 5 min at room temperature. Then, 1.2 mL of 20% Na_2CO_3 was added and the volume of the solution was adjusted to 6 mL by adding distilled water. The mixture was incubated for 1 h at 80 °C and the adsorption was measured at 765 nm against methanol.

Proline content (PC) of the leaves was measured according to Bates et al. (1973). Proline was extracted from 0.5 g of the leaf samples by grinding in 10 mL of 3% sulpho-salicylic acid and the mixture was then centrifuged at 10,000 rpm for 10 min. 2 mL of the supernatant was then added into test tubes containing 2 mL of freshly prepared acid-ninhydrin and 2 mL of glacial acetic acid. The tubes were placed in water bath for 1 h at 90 °C and the reaction was terminated in ice-bath. The mixture was then extracted with 5 mL of toluene and vortexed for 15 s. After keeping the mixture in darkness for at least 20 min at room temperature to separate the toluene and aqueous phases, the toluene phase was carefully collected into test tubes and the absorbance of the fraction was measured at 520 nm.

tas14 gene expression (tGE) was measured according to Goñi et al. (2018). In brief, total RNA was isolated from 50 mg of the ground leaf material using Total RNA Purification Kit (Norgen Biotek, ON, Canada) according to the manufacturer's instructions. Expression analysis of tas14 dehydrin gene (*Solyc02g084850.2*) was performed by real time-PCR using LightCycler 96 System (Roche, UK). Quantitative PCR was performed using about 300 ng of total purified RNA and the LightCycler RNA Master SYBR Green I one-step kit (Roche, UK) according to the manufacturer's instructions. The expression level of the tomato actin gene (*Solyc01g104770.2*) was used as the reference. The primers' sequences used were as follows: tas14, forward 5′-TCATCACCATGAGGG GCAAC-3′ and reverse 5′-ACCTTCATGTTGTCCAGGCA-3'; Actin, forward 5′-TCTTGAAGCGTTTTAAAAGATG GC-3′ and reverse 5′- TCACC AGCAAATCCAGCCTT-3'. The quality and concentration of the sequences were analyzed using HPLC before the experiments.

miRNA-1886 concentration (miRNA) was measured using the biosensor described at section 2.1. Total RNA was isolated from 50 mg of ground leaf material using Total RNA Purification Kit (Norgen Biotek, ON, Canada) according to the manufacturer's instructions. 5 µL of obtained sample was used to synthesis P-AuNPs/miRNA-1886 which was later mixed with C–AuNP/probe for spectroscopic determination of miRNA-1886.

2.6. Statistical analysis

The data obtained from each stress source investigation were individually subjected to analysis of variance (ANOVA) using LSD test at the significance level of 0.01 in SAS 9.0 programming environment.

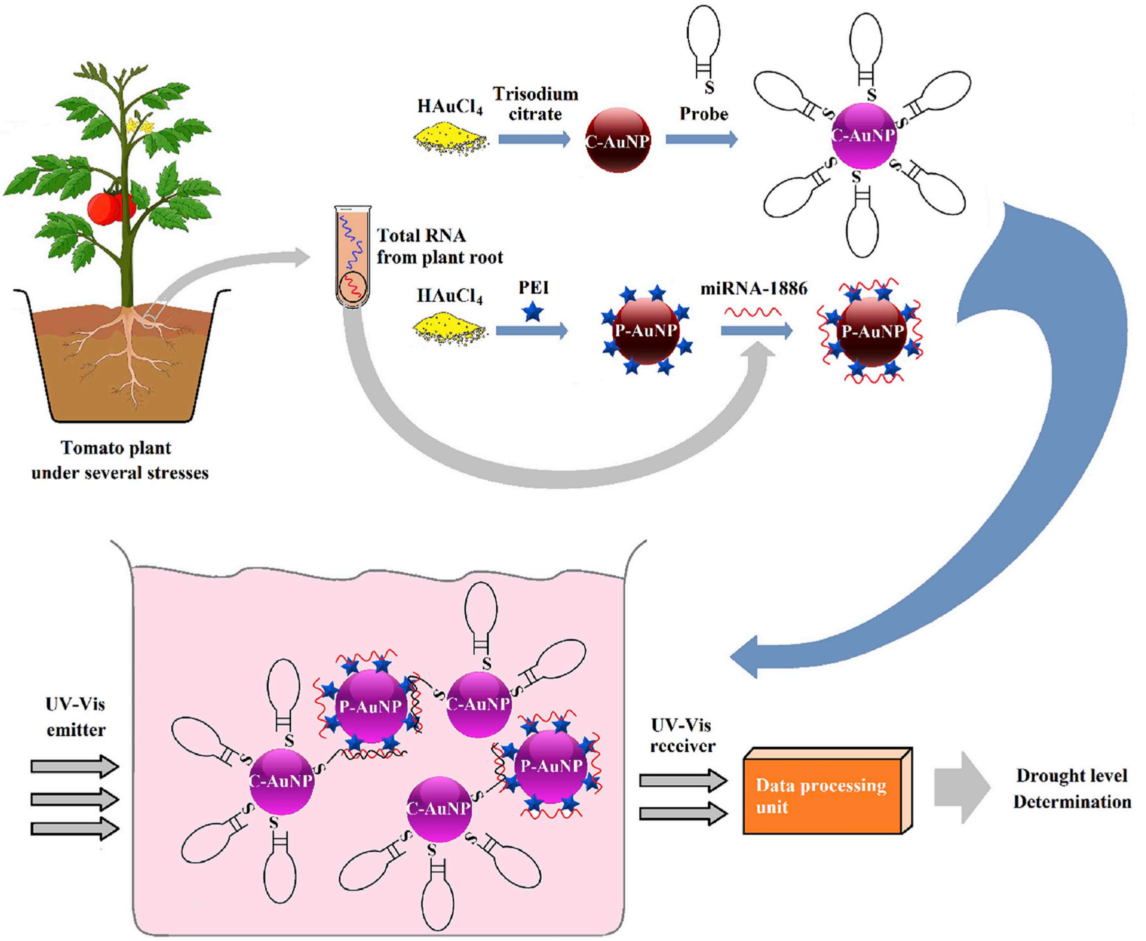

Fig. 1. Schematics of drought level determination using an optical biosensor to detect miRNA-1886 concentration in tomato plant root.

3. Results and discussion

3.1. Sensing mechanism for miRNA concentration determination

Sly-miRNA-1886 is one of the known miRNAs in tomato plants that involves the plant response towards drought conditions (Din and Barozai, 2014). It consists of 21 nucleotides in its length having a similar sequence with ath-miRNA-1886 in *Arabidopsis thaliana* except four mismatches (German et al., 2008). In this study, the concentration of sly-miRNA-1886 in plant root has been measured as a specific indicator of plant drought stress. To do this, an optical biosensor which its scheme is brought in Fig. 1 was used for miRNA concentration determination. The detailed procedure of biosensor preparation can be found in Section 2.1. Probe-target (sly-miRNA-1886) hybridization begins by the addition of C–AuNP/probe to P–AuNP/miRNA-1886. This hybridization results in a reduction of distance between nanoparticles and, finally, the nanoparticles aggregation happens. The aggregation changes the color of mixture and consequently, its UV–Vis absorbance. Since the performance of the biosensor depends on successful hybridization of probe-miRNA target, the thiolated probe was designed carefully. The thiolated probe molecules contained a nine-nucleotide thymidine spacer at their 3’ end which provides higher optimal immobilization and hybridization efficiency (Pö;hlmann and Sprinzl, 2010).

3.2. Analytical performance of the biosensor

Before the determination of miRNA concentration in plant root samples using the optical biosensor, it was necessary to investigate the analytical performance of the developed biosensor. In order to achieve the calibration curve of the biosensor, aliquots of miRNA solution were added by pipette to the inert electrolyte to obtain miRNA-1886 standards with concentrations in the range of 0-10^6 fM. Since the UV–Vis absorptions at 530 and 750 nm indicate the quantity of dispersed and aggregated AuNPs, respectively, samples were analyzed by the biosensor and their absorbance in 530 and 750 was recorded. To remove background signal from the results and to normalize the experimental data in a manner that the results of the experiments can be comparable with each other, $\delta A_{(530/750)}$ was used which is defined as $\delta A_{(530/750)} = |[A_{(530/750)} - A_{(530/750)P}]/A_{(530/750)}|$ where $A_{(530/750)}$ is the absorption ratio of C–AuNP/probe + P–AuNP/miRNA-1886 at 530 nm to that at 750 nm and $A_{(530/750)P}$ is the absorption of C–AuNP/probe + P–AuNP at 530 nm to that at 750 nm (Hakimian et al., 2018).

Fig. 2 depicts $\delta A_{(530/750)}$ as a function of log [miRNA-1886]. As shown in this figure, $\delta A_{(530/750)}$ increased by increasing the concentration of miRNA-1886. The linear range of the biosensor is shown with green color indicating the linear response of the biosensor ($R^2 = 0.97$) at concentrations between 0 and 10^4 fM. The consideration of $\delta A_{(530/750)}$ as the biosensor response provided us with a calibration curve in which the biosensor response is zero when the target miRNA is absent. According to Fig. 2, The sensitivity of the biosensor was 0.011 $\delta A_{(530/750)}$/log [miRNA-1886].

By investigating the expression of identified miRNAs in plant organs, e.g., seedlings, leaves, flowers, and roots, and its comparison with molar mass of plant miRNAs, the linear range obtained in this study is suitable for *in vivo* measurement of a large number of identified plant miRNAs (Jones-Rhoades and Bartel, 2004). The term precision is used to describe the degree of freedom of a measurement system from

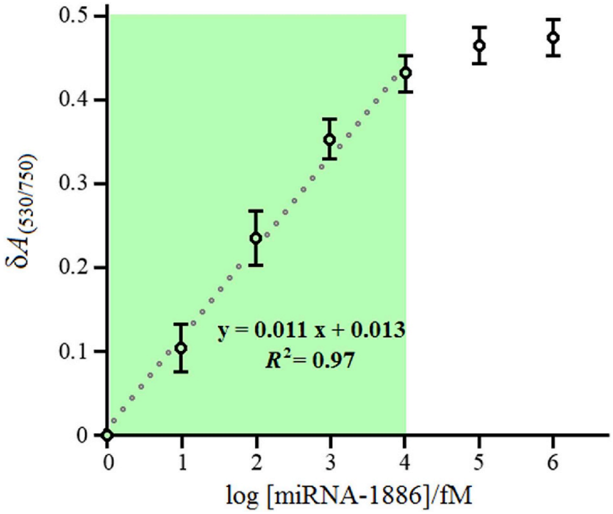

Fig. 2. $\delta A_{(530/750)}$ as a function of log [miRNA-1886]. Each data stands for mean and standard deviation of five independent measurements. The green area shows the linear range of the calibration curve.

random errors (Bendat and Piersol, 2011). Thus, a high precision measurement instrument will give only a small spread of readings if repeated readings are taken of the same quantity. The standard deviation of the measurements ($n = 5$) in Fig. 2 reveals the acceptable precision of the biosensor.

The limit of detection (LOD) was defined based on signal-to-noise approach (Shrivastava and Gupta, 2011). A signal-to-noise ratio of 3:1 was considered in this study and LOD was obtained equal to 2 fM. To study the specificity of the biosensor, 10^4 fM of the target miRNA along with the same concentration of two three-base mismatched miRNA-1886 were analyzed using the biosensor individually with five replications. The biosensor response for each of the miRNAs is brought in Fig. 3. As shown in this figure, the biosensor response for target miRNA is approximately four times higher than that for three-base mismatched miRNAs which indicates specificity of the developed biosensor towards the study sly-miRNA-1886. This is because of the stable hybridization of probe and target miRNA which makes the biosensor a reliable method to measure sly-miRNA-1886 in liquid biological samples.

Similar to the findings of this study, several studies have shown that optical biosensors which work based on nanoparticle aggregation can exert specific behavior towards target miRNA when the probe is designed correctly (Joshi et al., 2015; Qin et al., 2017; Peng et al., 2018; Li et al., 2019). Although northern blot (Winter and Diederichs, 2011), qRT-PCR (Zhi et al., 2010), and microarrays (Konishi et al., 2012) as conventional methods have been widely used for miRNA measurement by analysts, their general limitations are low sensitivity, low specificity, being time-consuming, and their label-requirements (Kilic et al., 2018). In this situation, using a biosensor can be a solution for reliable determination of miRNA. Although an optical biosensor is developed in this study, amperometric (Cheng et al., 2014), impedimetric (Congur et al., 2015), and fluorescence-based (Almlie et al., 2015) biosensors are also introduced for miRNA measurements. Electrode pre-treatments along with costly equipment can be considered as the most important limitations of electrochemical miRNA determination whilst fluorescence-based methods are not sensitive (Kilic et al., 2018). These are some of the reasons why researchers are becoming more and more attracted to optical miRNA biosensors.

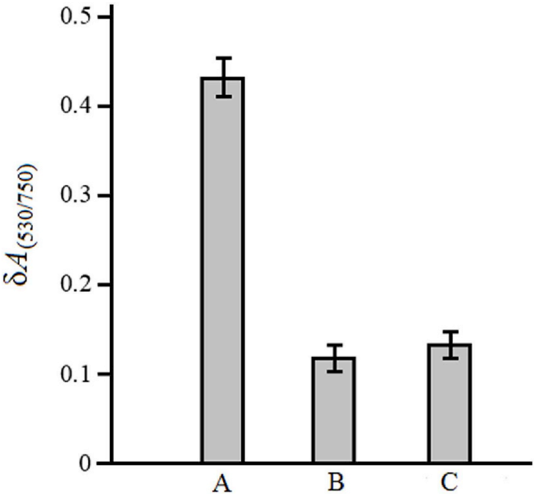

Fig. 3. Biosensor response for 10^4 fM of (A) sly-miRNA-1886 (5′-UGAGAGAAGAGAGAUGGAAAA-3′), (B) the first three-base mismatched sly-miRNA-1886 (5′-UGAGUGAAGACAGAUGGAUAA-3′), and (C) the second three-base mismatched sly-miRNA-1886 (5′-UCAGAGUAGAGAGAUGGUAAA-3′).

3.3. Effects of drought stress on tomato plant characteristics

So far, according to the results obtained by the biosensor performance evaluation, it has been shown that the introduced optical biosensor is a promising technique in target miRNA concentration determination. The main hypothesis of this study is that sly-miRNA-1886 concentration (which is determined in total RNA samples from tomato plant root using the developed biosensor) can be a specific indicator of plant drought stress. In this subsection, effects of drought stress on plant root sly-miRNA-1886 concentration along with other plant characteristics are brought and the next subsection is dedicated to the specificity of miRNA-1886 concentration towards tomato plant drought stress.

Table 1 shows tomato plant characteristics including morphological, physiological, and biochemical traits in various drought levels, measured at 45 (T_1), 55 (T_2), and 65 (T_3) days after planting. W_0 to W_4 indicate 100%–60% of field capacity, respectively. According to the table, all plant characteristics were significantly affected by severe drought stress ($P < 0.01$). However, mild and moderate drought levels did not significantly affect the plant characteristics except tas14 gene expression (tGE) and sly-miRNA-1886 concentration.

Morphological characteristics including PH, RFW, RDW, SFW, and SDW generally decreased by increasing the severity of drought stress in all sampling times. This decrease was significant at irrigations lower than 70% of field capacity. Similar results have been reported indicating that severe drought stress can undesirably affect the morphological characteristics of tomato plants (Subramanian et al., 2006). However, Nahar and Ullah (2011) reported that drought stress up to 70% of field capacity can improve tomato plant's morphological characteristics. The possible reason for this contradiction might be due to the differences in plant cultivar, soil texture, and growing conditions.

RWC is considered as a measure of plant water status and is used as one of the most meaningful indices for dehydration tolerance (Pirzad et al., 2011; Anjum et al., 2011; Jafari et al., 2019). As shown in Table 1, RWC significantly decreased by increasing the levels of water stress in all sampling times. Similarly, Subramanian et al. (2006) found out RWC decreases by exposing tomato plants to severe drought conditions. It is reported that the negative effects of water stress on leaf RWC reduces depending on the plant cultivar (Sharma and Sharma, 2008; Wang et al., 2012). Significant decrease in leaf RWC can be due to the unavailability of water in soil, or root system, which are not able to compensate water deficiency (Gadallah, 2000). A comparison of RWC of leaves in different sampling times reveals that RWC is higher in the initial stages of the leaf development and decreases as the dry matter accumulates and leaf matures (Anjum et al., 2011). CI, as another important physiological plant trait, also decreased in severe drought conditions (Table 1). Relative chlorophyll content has a positive correlation with photosynthetic rate (Buttery and Buzzell, 1977; Li

Table 1

Tomato plant characteristics in various drought stress levels. W_0 to W_4 indicate 100%–60% of field capacity, respectively.

Sampling time	Water treatment	PH (cm)	RFW (g)	RDW (g)	SFW (g)	SDW (g)	RWC (%)
T_1	W_0	53.2 ± 2.6^{e}	94.7 ± 11.3^{d}	7.5 ± 0.5^{d}	686 ± 31^{cd}	34.0 ± 1.7^{c}	94.8 ± 2.5^{a}
	W_1	52.9 ± 2.7^{e}	95.2 ± 12.8^{d}	7.6 ± 0.8^{d}	729 ± 30^{c}	32.7 ± 2.1^{c}	95.0 ± 2.1^{a}
	W_2	50.2 ± 2.6^{e}	97.9 ± 9.4^{d}	7.6 ± 0.9^{d}	704 ± 42^{c}	32.9 ± 1.9^{c}	94.3 ± 1.9^{a}
	W_3	45.3 ± 1.9^{f}	92.8 ± 8.9^{d}	7.4 ± 1.1^{d}	647 ± 28^{d}	27.7 ± 2.2^{d}	91.8 ± 2.7^{b}
	W_4	41.8 ± 1.5^{g}	73.0 ± 12.1^{e}	6.0 ± 1.3^{e}	593 ± 36^{e}	30.4 ± 1.8^{d}	91.2 ± 2.2^{b}
T_2	W_0	62.2 ± 2.7^{cd}	135.2 ± 9.1^{bc}	11.0 ± 1.6^{b}	817 ± 43^{b}	41.5 ± 2.0^{b}	92.1 ± 1.0^{b}
	W_1	61.1 ± 2.1^{d}	121.9 ± 9.7^{bc}	11.9 ± 1.7^{b}	798 ± 30^{b}	40.3 ± 1.7^{b}	92.3 ± 1.8^{b}
	W_2	59.0 ± 2.4^{d}	136.1 ± 8.1^{bc}	11.1 ± 1.4^{b}	802 ± 33^{b}	36.9 ± 1.8^{b}	91.7 ± 1.5^{b}
	W_3	53.4 ± 1.9^{e}	113.4 ± 7.7^{c}	9.6 ± 1.1^{c}	731 ± 31^{c}	33.2 ± 2.1^{c}	86.9 ± 1.9^{c}
	W_4	51.3 ± 1.8^{e}	112.3 ± 11.8^{c}	9.6 ± 1.3^{c}	727 ± 49^{c}	31.1 ± 2.0^{c}	87.1 ± 1.5^{c}
T_3	W_0	81.9 ± 1.9^{a}	172.0 ± 11.0^{a}	15.1 ± 2.6^{a}	951 ± 53^{a}	51.7 ± 2.5^{a}	91.0 ± 2.2^{b}
	W_1	75.8 ± 2.9^{b}	163.7 ± 13.2^{a}	15.7 ± 1.7^{a}	925 ± 47^{a}	53.0 ± 2.2^{a}	91.7 ± 1.7^{b}
	W_2	74.1 ± 1.8^{b}	177.1 ± 9.8^{a}	14.1 ± 2.2^{a}	939 ± 40^{a}	49.9 ± 2.8^{a}	90.9 ± 1.8^{b}
	W_3	66.0 ± 1.9^{c}	144.3 ± 8.7^{b}	12.5 ± 1.5^{b}	835 ± 44^{b}	39.3 ± 2.0^{b}	86.8 ± 2.0^{c}
	W_4	60.2 ± 2.6^{d}	149.1 ± 13.1^{b}	11.9 ± 2.6^{b}	827 ± 49^{b}	38.9 ± 2.7^{b}	85.9 ± 1.6^{c}

Sampling time	CI (%/%)	CA (units mg^{-1})	AC (A530 g^{-1} Fwt)	PhC (mg GAE g^{-1} Fwt)	PC (mg g^{-1} Fwt)	tGE (%)	miRNA (fM)
T_1	240.4 ± 8.6^{c}	0.22 ± 0.06^{bc}	0.88 ± 0.07^{f}	0.20 ± 0.03^{d}	4.22 ± 0.26^{e}	14 ± 3^{f}	134 ± 23^{h}
	212.5 ± 9.0^{e}	0.22 ± 0.06^{bc}	0.90 ± 0.05^{f}	0.19 ± 0.04^{d}	4.38 ± 0.27^{e}	87 ± 5^{e}	255 ± 54^{g}
	195.1 ± 8.9^{f}	0.24 ± 0.04^{b}	1.07 ± 0.08^{e}	0.21 ± 0.03^{d}	5.43 ± 0.21^{d}	214 ± 35^{d}	743 ± 47^{f}
	144.4 ± 9.9^{h}	0.24 ± 0.04^{b}	1.16 ± 0.09^{e}	0.38 ± 0.03^{c}	5.47 ± 0.33^{d}	459 ± 19^{c}	1023 ± 94^{e}
	107.8 ± 9.2^{i}	0.25 ± 0.05^{b}	1.53 ± 0.08^{c}	0.49 ± 0.05^{b}	6.62 ± 0.25^{b}	523 ± 43^{b}	2321 ± 136^{c}
T_2	271.8 ± 10.2^{b}	0.20 ± 0.06^{c}	0.69 ± 0.05^{g}	0.18 ± 0.03^{d}	4.04 ± 0.39^{e}	17 ± 6^{f}	150 ± 19^{h}
	250.7 ± 8.9^{c}	0.21 ± 0.04^{c}	0.85 ± 0.08^{f}	0.18 ± 0.03^{d}	4.16 ± 0.40^{e}	107 ± 9^{e}	269 ± 33^{g}
	215.2 ± 7.5^{e}	0.21 ± 0.05^{c}	1.11 ± 0.07^{e}	0.19 ± 0.06^{d}	5.22 ± 0.34^{d}	221 ± 40^{d}	821 ± 56^{f}
	189.0 ± 8.1^{f}	0.26 ± 0.04^{b}	1.26 ± 0.12^{d}	0.35 ± 0.06^{c}	5.89 ± 0.28^{c}	473 ± 36^{c}	2109 ± 201^{d}
	153.2 ± 7.7^{h}	0.30 ± 0.03^{a}	1.61 ± 0.11^{b}	0.51 ± 0.04^{b}	6.90 ± 0.30^{a}	550 ± 44^{b}	5622 ± 322^{b}
T_3	293.3 ± 10.1^{a}	0.19 ± 0.05^{c}	0.60 ± 0.07^{g}	0.15 ± 0.03^{e}	3.76 ± 0.29^{f}	15 ± 4^{f}	162 ± 27^{h}
	268.2 ± 9.2^{b}	0.20 ± 0.05^{c}	0.68 ± 0.07^{g}	0.16 ± 0.03^{e}	4.07 ± 0.28^{e}	93 ± 8^{e}	304 ± 47^{g}
	232.1 ± 8.4^{d}	0.22 ± 0.06^{bc}	1.28 ± 0.09^{d}	0.20 ± 0.04^{d}	5.65 ± 0.33^{d}	264 ± 42^{d}	1005 ± 66^{e}
	206.2 ± 8.5^{ef}	0.31 ± 0.04^{a}	1.30 ± 0.15^{d}	0.39 ± 0.05^{c}	5.99 ± 0.34^{c}	462 ± 53^{c}	2418 ± 194^{c}
	174.5 ± 7.9^{g}	0.31 ± 0.06^{a}	1.75 ± 0.16^{a}	0.57 ± 0.05^{a}	6.87 ± 0.46^{a}	622 ± 69^{a}	6833 ± 354^{a}

PH: plant height, RFW: root fresh weight, RDW: root dry weight, SFW: shoot fresh weight, SDW: shoot dry weight, RWC: relative water content, CI: chlorophyll index, CA: catalase activity, AC: anthocyanin content, PhC: phenolic compounds, PC: proline content, tGE: tas14 gene expression, miRNA: sly-miRNA-1886 concentration.

Data are the means ± SD ($n = 4$).

Different small letter within the same column indicate significant differences between treatments based on LSD test ($P < 0.01$).

et al., 2006). The decrease in total chlorophyll content under drought stress has been considered as a typical symptom of oxidative stress (Anjum et al., 2011; Ghobadi et al., 2013). Table 1 also shows that CI increased as the control plants became darker green in color over time. Similarly, as the treatment plants started to exhibit signs of water deficiency, the yellowish appearance in the leaves was visible.

CA of treatment plants was significantly higher than that of control plants (Table 1). A similar phenomenon was observed by Yang et al. (2009), Gholami et al. (2012), Bolat et al. (2014), and Jafari et al. (2019) who found that enzyme levels were higher in drought-affected plants than in control plants. To reduce the effects of oxygen species production due to the drought stress, plants have complex enzymatic antioxidant system, such as catalase scavenging systems (Apel and Hirt, 2004). Similarly, anthocyanin, phenol, and proline contents in water-stressed plants were higher than those in the control.

Table 1 shows that tas14 dehydrin gene (*Solyc02g084850.2*) expression (tGE) and sly-miRNA-1886 concentration increased significantly by even mild and moderate drought stress levels. tGE was measured with respect to a reference gene expression and was found at low constitutive levels in control plants. Similar to other late embryogenesis abundant proteins, high amounts of dehydrins accumulate in plant embryos in later stages of embryo maturation and desiccation (Hanin et al., 2011). Dehydrins are generally present in young plant organs and those exhibiting rapid cell division or cell elongation (Rorat et al., 2004). In this study, tGE was measured in plant leaves using PCR. TAS14 mRNA is induced in tomato seedlings and mature plants upon treatment with salinity, abscisic acid and/or mannitol (AbuQamar et al., 2009; Roychoudhury et al., 2013). Investigations have revealed the positive correlation between dehydrin gene expression or dehydrin protein accumulation, and plant stress tolerance (Muñoz-Mayor et al., 2012; Kosová et al., 2014). As a result, researchers have used dehydrins as a plant molecular marker for stress tolerance (Hanin et al., 2016; Binott et al., 2017; Wei et al., 2019; Jia et al., 2019).

Finally, the optical biosensor developed in this study was used to measure sly-miRNA-1886 concentration in control and treatment plants in different sampling times results of which are brought in Table 1. As shown in the table, miRNA concentration increased by increasing the severity of the drought stress. Moderate and even mild stress conditions significantly increased the miRNA concentration in total RNA of the plant root. According to Table 1, miRNA concentration in control and treatment plants varied in the range of 134 and 6833 fM. Due to fact that the linear range of the developed biosensor was between 0 and 10^4 fM, the biosensor was appropriate to measure the plant miRNA concentration even in severe drought stress conditions.

3.4. *Specificity of miRNA-1886 towards tomato drought stress*

Table 2 shows the characteristics of tomato plants exposed to various salinity and temperature stresses, individually. According to the table, all the characteristics except sly-miRNA-1886 concentration were significantly affected by the applied stress. Some of the plant traits were significantly affected only by the severe stress levels ($P < 0.01$) whilst other traits were affected by both moderate and severe stresses. As can be seen in Table 2, tomato plants were more sensitive to salinity compared to temperature stress. Similar results have been reported by Rivero et al. (2014). In along with Cheng et al. (2009) and Yu et al. (2009), Table 2 shows that tomato plants can tolerate heat than cold.

According to Tables 1 and 2, significant changes in all the studied plant characteristics except sly-miRNA-1886 concentration reveal that they were not specific to drought stress since they were affected by salinity and temperature stresses. Similar results have been reported by Mäkelä et al. (2000), Latef and Chaoxing (2011), and Goñi et al. (2018). Even tas14 dehydrin gene expression was affected ($P < 0.01$) by salinity and temperature stresses. When plants are exposed to various stresses related to cellular dehydration, e.g., drought, salinity, and temperature, dehydrins accumulate to high amounts in all vegetative

Table 2
Tomato plant characteristics in various salinity and temperature stress levels. The subscripts used for the treatments are declared in the text.

Treatment	PH (cm)	RFW (g)	RDW (g)	SFW (g)	SDW (g)	RWC (%)	CI (%/%)	CA (units mg^{-1})	AC (A530 g^{-1} Fwt)	PhC (mg GAE g^{-1} Fwt)	PC (mg g^{-1} Fwt)	tGE (%)	miRNA (fM)
S_0	81.9 ± 1.9^{a}	172.0 ± 11.0^{a}	15.1 ± 2.6^{a}	951 ± 53^{a}	51.7 ± 2.5^{a}	96.0 ± 2.2^{a}	240.4 ± 8.6^{a}	0.19 ± 0.05^{c}	0.60 ± 0.07^{c}	0.15 ± 0.03^{c}	3.76 ± 0.29^{c}	338 ± 40^{b}	162 ± 27^{a}
S_1	72.9 ± 2.2^{b}	132.4 ± 11.2^{b}	14.8 ± 1.7^{a}	819 ± 36^{b}	41.3 ± 1.5^{b}	93.1 ± 1.2^{b}	184.1 ± 6.6^{b}	0.32 ± 0.07^{b}	1.84 ± 0.11^{b}	0.32 ± 0.07^{b}	6.24 ± 0.36^{b}	347 ± 36^{b}	177 ± 19^{a}
S_2	56.1 ± 4.4^{c}	124.2 ± 9.3^{c}	9.9 ± 0.3^{b}	748 ± 57^{c}	32.2 ± 3.1^{c}	91.4 ± 1.9^{c}	94.1 ± 5.9^{c}	0.57 ± 0.09^{a}	2.25 ± 0.15^{a}	0.84 ± 0.08^{a}	8.82 ± 0.32^{a}	404 ± 59^{a}	165 ± 22^{a}
C_0	81.9 ± 1.9^{a}	172.0 ± 11.0^{a}	15.1 ± 2.6^{a}	951 ± 53^{a}	51.7 ± 2.5^{a}	96.0 ± 2.2^{a}	240.4 ± 8.6^{a}	0.19 ± 0.05^{c}	0.60 ± 0.07^{c}	0.15 ± 0.03^{c}	3.76 ± 0.29^{c}	338 ± 40^{a}	162 ± 27^{a}
C_1	74.1 ± 1.6^{b}	149 ± 9.6^{b}	14.3 ± 1.3^{a}	911 ± 39^{b}	43.4 ± 1.5^{b}	94.8 ± 1.0^{a}	209.5 ± 8.2^{b}	0.27 ± 0.05^{b}	1.53 ± 0.09^{b}	0.29 ± 0.05^{b}	4.83 ± 0.53^{b}	319 ± 27^{a}	154 ± 21^{a}
C_2	65.6 ± 2.7^{c}	131 ± 12.2^{b}	11.0 ± 0.9^{b}	843 ± 31^{c}	36.8 ± 1.7^{c}	92.3 ± 1.3^{b}	148.7 ± 7.7^{c}	0.41 ± 0.10^{a}	2.05 ± 0.11^{a}	0.64 ± 0.07^{a}	6.03 ± 0.47^{a}	280 ± 33^{b}	157 ± 18^{a}
H_0	81.9 ± 1.9^{a}	172.0 ± 11.0^{a}	15.1 ± 2.6^{a}	951 ± 53^{a}	51.7 ± 2.5^{a}	96.0 ± 2.2^{a}	240.4 ± 8.6^{a}	0.19 ± 0.05^{c}	0.60 ± 0.07^{c}	0.15 ± 0.03^{c}	3.76 ± 0.29^{c}	338 ± 40^{b}	162 ± 27^{a}
H_1	78.4 ± 1.9^{b}	165.5 ± 8.9^{a}	15.5 ± 1.4^{a}	939 ± 48^{a}	48.9 ± 2.1^{a}	95.6 ± 1.7^{a}	217.3 ± 9.0^{b}	0.24 ± 0.06^{b}	0.83 ± 0.10^{b}	0.26 ± 0.03^{b}	4.31 ± 0.42^{b}	341 ± 27^{b}	165 ± 19^{a}
H_2	72.3 ± 1.5^{c}	143.2 ± 11.3^{b}	12.4 ± 0.5^{b}	872 ± 36^{b}	42.3 ± 2.0^{b}	91.6 ± 2.1^{b}	176.8 ± 7.9^{c}	0.31 ± 0.05^{a}	1.23 ± 0.12^{a}	0.47 ± 0.06^{a}	5.14 ± 0.39^{a}	383 ± 41^{a}	170 ± 22^{a}

PH: plant height, RFW: root fresh weight, RDW: root dry weight, SFW: shoot fresh weight, SDW: shoot dry weight, RWC: relative water content, CI: chlorophyll index, CA: catalase activity, AC: anthocyanin content, PhC: phenolic compounds, PC: proline content, tGE: tas14 gene expression, miRNA: sly-miRNA-1886 concentration.
Data are the means ± SD ($n = 4$).
Different small letter within the same column for each treatment indicate significant differences between treatments based on LSD test ($P < 0.01$).

tissues (Skriver and Mundy, 1990; Parihar et al., 2015). Stress-inducible dehydrin encoding genes contain abscisic acid, drought and cold responsive elements, and myeloblastosis and myelocytomatosis regulatory elements in their promoter regions (Hanin et al., 2011; Skubacz et al., 2016). Their expression is regulated by abscisic acid-dependent and abscisic acid-independent signaling pathways.

Table 2 shows that sly-miRNA-1886 concentration was not significantly affected by salinity and temperature stresses. It seems that the miRNA concentration (and its up-regulation) is specific to drought stress and can be considered as a reliable marker of drought conditions. This finding can be useful in precise drought stress studies when other plant morpho-physiological and biochemical traits can be easily influenced by other sources of stress. Compared to TAS14 mRNA, miRNA-1886 is a small 21-nucleotide non-coding RNA which is found in plants. Although up-regulation of miRNA-1886 can be measured with conventional methods, e.g., PCR and northern blot (Din and Barozai, 2014), the optical biosensor based on Au nanoparticle aggregation which is developed in this study is a reliable method for specific determination of sly-miRNA-1886 in tomato plant root.

According to Table 1, when concentrations more than ca. 200 fM of sly-miRNA-1886 is detected by the biosensor, it is expected that the plant is exposed to the drought stress and due to the fact that the behavior of sly-miRNA-1886 is specific towards drought stress, it can be concluded that this change in sly-miRNA-1886 concentration belongs to drought conditions. Similar to this funding, other miRNAs have been considered as specific markers of metabolic activities in living organisms (Chen et al., 2012; Sun and Lai, 2013). Although a search in bibliographic databases shows that a large part of investigations on the role of miRNA in metabolic activities belong to studies on human (Tilghman et al., 2012; Rager et al., 2013; Clump et al., 2019), studies on identification of plant miRNAs and their target genes responsive to plant stress have been growing rapidly since 2000s. For example, the role of miRNA-172 in salinity stress of Arabidopsis (Li et al., 2016), miRNVL5 in drought stress of wheat (Gao et al., 2016), miRNA-1450 in temperature stress of soybean (Zeng et al., 2010), miRNA-901 in drought stress of rice (Zhou et al., 2010) are already investigated. Based on the results of this study and similar reports, miRNAs are reliable candidates for investigating the effects of physical and environmental stresses on plants since they exert specific response compared to the other plant characteristics.

4. Conclusions

In this study, an optical biosensor was developed based on gold nanoparticles aggregation which was capable of detecting mild, moderate, and severe drought stress in tomato plants by ultrasensitive determination of sly-miRNA-1886. Results showed that the analytical characteristics of the biosensor including linear range, LOD, precision, sensitivity, and specificity were acceptable for reliable determination of small length miRNAs. Results also showed that in contrast with plant conventional morpho-physiological and biochemical characteristic, i.e., PH, RFW, RDW, SFW, SDW, RWC, CI, CA, AC, PhC, PC, and tGE, miRNA-1886 concentration was not significantly affected ($P < 0.01$) by other stresses, i.e., salinity, cold, and heat during the growth period. This specificity of miRNA-1886 concentration towards drought stress can be a new insight in investigating the functions of miRNAs in plants and their applications in specific plant stress determination. Further studies are required to gather valuable information on the dependence of plant miRNA behavior on plant cultivar and stress severity towards biotic and abiotic stresses using optical biosensors.

Contribution

The experiments and paper preparation were carried out by the sole author.

Declaration of competing interest

The author declares that there are no conflicts of interest.

References

Pöhlmann, C., Sprinzl, M., 2010. Electrochemical detection of microRNAs via gap hybridization assay. Anal. Chem. 82 (11), 4434–4440.

Abdul Jaleel, C., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H.J., Somasundaram, R.A., Panneerselvam, R., 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. Int. J. Agric. Biol. 11 (1), 100–105.

AbuQamar, S., Luo, H., Laluk, K., Mickelbart, M.V., Mengiste, T., 2009. Crosstalk between biotic and abiotic stress responses in tomato is mediated by the AIM1 transcription factor. Plant J. 58 (2), 347–360.

Almlie, C.K., Larkey, N.E., Burrows, S.M., 2015. Fluorescent microRNA biosensors: a comparison of signal generation to quenching. Anal. Methods 7 (17), 7296–7310.

Anjum, S.A., Xie, X.Y., Wang, L.C., Saleem, M.F., Man, C., Lei, W., 2011. Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res. 6 (9), 2026–2032.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399.

Asefpour Vakilian, K., Massah, J., 2018. A portable nitrate biosensing device using electrochemistry and spectroscopy. IEEE Sens. J. 18 (8), 3080–3089.

Bates, L.S., Waldren, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39 (1), 205–207.

Bej, S., Basak, J., 2014. MicroRNAs: the potential biomarkers in plant stress response. Am. J. Plant Sci. 5 (05), 748.

Beltrano, J., Ronco, M.G., 2008. Improved tolerance of wheat plants (*Triticum aestivum* L.) to drought stress and rewatering by the arbuscular mycorrhizal fungus Glomus claroideum. Effect on growth and cell membrane stability. Braz. J. Plant Physiol. 21 (1), 29–37.

Bendat, J.S., Piersol, A.G., 2011. Random Data: Analysis and Measurement Procedures, vol. 729 John Wiley & Sons, USA.

Binott, J.J., Owuoche, J.O., Bartels, D., 2017. Physiological and molecular characterization of Kenyan barley (*Hordeum vulgare* L.) seedlings for salinity and drought tolerance. Euphytica 213 (7), 139.

Bolat, I., Dikilitas, M., Ercisli, S., Ikinci, A., Tonkaz, T., 2014. The effect of water stress on some morphological, physiological, and biochemical characteristics and bud success on apple and quince rootstocks. Sci. World J. 769732, 1–8.

Buttery, B.R., Buzzell, R.I., 1977. The relationship between chlorophyll content and rate of photosynthesis in soybeans. Can. J. Plant Sci. 57 (1), 1–5.

Caporali, S., Muniz-Miranda, F., Pedone, A., Muniz-Miranda, M., 2019. SERS, XPS and DFT study of xanthine adsorbed on citrate-stabilized gold nanoparticles. Sensors 19 (12), 2700.

Chen, S.J., Huang, Y.F., Huang, C.C., Lee, K.H., Lin, Z.H., Chang, H.T., 2008. Colorimetric determination of urinary adenosine using aptamer-modified gold nanoparticles. Biosens. Bioelectron. 23 (11), 1749–1753.

Chen, B., Li, H., Zeng, X., Yang, P., Liu, X., Zhao, X., Liang, S., 2012. Roles of microRNA on cancer cell metabolism. J. Transl. Med. 10 (1), 228.

Cheng, L., Zou, Y., Ding, S., Zhang, J., Yu, X., Cao, J., Lu, G., 2009. Polyamine accumulation in transgenic tomato enhances the tolerance to high temperature stress. J. Integr. Plant Biol. 51 (5), 489–499.

Cheng, F.F., Zhang, J.J., He, T.T., Shi, J.J., Abdel-Halim, E.S., Zhu, J.J., 2014. Bimetallic Pd–Pt supported graphene promoted enzymatic redox cycling for ultrasensitive electrochemical quantification of microRNA from cell lysates. Analyst 139 (16), 3860–3865.

Clump, D.A., Pickering, C.R., Skinner, H.D., 2019. Predicting outcome in head and neck cancer: miRNAs with potentially big effects. Clin. Cancer Res. 25 (5), 1441–1442.

Congur, G., Eksin, E., Erdem, A., 2015. Impedimetric detection of microRNA at graphene oxide modified sensors. Electrochim. Acta 172, 20–27.

Din, M., Barozai, M.Y.K., 2014. Profiling microRNAs and their targets in an important fleshy fruit: tomato (*Solanum lycopersicum*). Gene 535 (2), 198–203.

Duffy, B.K., Défago, G., 1999. Macro-and microelement fertilizers influence the severity of Fusarium crown and root rot of tomato in a soilless production system. Hortscience 34 (2), 287–291.

Gadallah, M.A.A., 2000. Effects of indole-3-acetic acid and zinc on the growth, osmotic potential and soluble carbon and nitrogen components of soybean plants growing under water deficit. J. Arid Environ. 44 (4), 451–467.

Gao, S., Yang, L., Zeng, H.Q., Zhou, Z.S., Yang, Z.M., Li, H., Sun, D., Xie, F., Zhang, B., 2016. A cotton miRNA is involved in regulation of plant response to salt stress. Sci. Rep. 6, 19736.

German, M.A., Pillay, M., Jeong, D.H., Hetawal, A., Luo, S., Janardhanan, P., Kannan, V., Rymarquis, L.A., Nobuta, K., German, R., De Paoli, E., Lu, C., Schroth, G., Meyers, B.C., Green, P.J., 2008. Global identification of microRNA–target RNA pairs by parallel analysis of RNA ends. Nat. Biotechnol. 26 (8), 941.

Ghani, A., Din, M., Barozai, M.Y.K., 2018. Convergence and divergence studies of plant precursor microRNAs. Pak. J. Bot. 50 (3), 1085–1091.

Ghobadi, M., Taherabadi, S., Ghobadi, M.E., Mohammadi, G.R., Jalali-Honarmand, S., 2013. Antioxidant capacity, photosynthetic characteristics and water relations of sunflower (*Helianthus annuus* L.) cultivars in response to drought stress. Ind. Crops Prod. 50, 29–38.

Gholami, M., Rahemi, M., Kholdebarin, B., Rastegar, S., 2012. Biochemical responses in leaves of four fig cultivars subjected to water stress and recovery. Sci. Hortic. 148, 109–117.

Goñi, O., Quille, P., O'Connell, S., 2018. *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. Plant Physiol. Biochem. 126, 63–73.

Guleria, P., Mahajan, M., Bhardwaj, J., Yadav, S.K., 2011. Plant small RNAs: biogenesis, mode of action and their roles in abiotic stresses. Genom. Proteom. Bioinform. 9 (6), 183–199.

Hakimian, F., Ghourchian, H., 2019. Simple and rapid method for synthesis of porous gold nanoparticles and its application in improving DNA loading capacity. Mater. Sci. Eng. C 103, 109795.

Hakimian, F., Ghourchian, H., Sadat Hashemi, A., Arastoo, M.R., Rad, M.B., 2018. Ultrasensitive optical biosensor for detection of miRNA-155 using positively charged Au nanoparticles. Sci. Rep. 8 (1), 2943.

Hanin, M., Brini, F., Ebel, C., Toda, Y., Takeda, S., Masmoudi, K., 2011. Plant dehydrins and stress tolerance: versatile proteins for complex mechanisms. Plant Signal. Behav. 6 (10), 1503–1509.

Hanin, M., Ebel, C., Ngom, M., Laplaze, L., Masmoudi, K., 2016. New insights on plant salt tolerance mechanisms and their potential use for breeding. Front. Plant Sci. 7, 1787.

Havir, E.A., McHale, N.A., 1987. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. Plant Physiol. 84 (2), 450–455.

Heeb, A., Lundegårdh, B., Ericsson, T., Savage, G.P., 2005. Effects of nitrate-, ammonium-, and organic-nitrogen-based fertilizers on growth and yield of tomatoes. J. Plant Nutr. Soil Sci. 168 (1), 123–129.

Huang, S., Van Aken, O., Schwarzländer, M., Belt, K., Millar, A.H., 2016. The roles of mitochondrial reactive oxygen species in cellular signaling and stress response in plants. Plant Physiol. 171 (3), 1551–1559.

Iovieno, P., Punzo, P., Guida, G., Mistretta, C., Van Oosten, M.J., Nurcato, R., Bostan, H., Colantuono, C., Costa, A., Bagnaresi, P., Chiusano, M.L., Albrizio, R., Giorio, P., Batelli, G., Grillo, S., 2016. Transcriptomic changes drive physiological responses to progressive drought stress and rehydration in tomato. Front. Plant Sci. 7, 371.

Jafari, S., Garmdareh, S.E.H., Azadegan, B., 2019. Effects of drought stress on morphological, physiological, and biochemical characteristics of stock plant (*Matthiola incana* L.). Sci. Hortic. 253, 128–133.

Ji, X., Song, X., Li, J., Bai, Y., Yang, W., Peng, X., 2007. Size control of gold nanocrystals in citrate reduction: the third role of citrate. J. Am. Chem. Soc. 129 (45), 13939–13948.

Jia, Y., Bai, J.Q., Liu, M.L., Jiang, Z.F., Wu, Y., Fang, M.F., Li, Z.H., 2019. Transcriptome analysis of the endangered *Notopterygium incisum*: cold-tolerance gene discovery and identification of EST-SSR and SNP markers. Plant Divers. 41 (1), 1–6.

Jones-Rhoades, M.W., Bartel, D.P., 2004. Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. Mol. Cell 14 (6), 787–799.

Joshi, G.K., Deitz-McElyea, S., Liyanage, T., Lawrence, K., Mali, S., Sardar, R., Korc, M., 2015. Label-free nanoplasmonic-based short noncoding RNA sensing at attomolar concentrations allows for quantitative and highly specific assay of microRNA-10b in biological fluids and circulating exosomes. ACS Nano 9 (11), 11075–11089.

Kaur, H., Kumar, R., Babu, J.N., Mittal, S., 2015. Advances in arsenic biosensor development–a comprehensive review. Biosens. Bioelectron. 63, 533–545.

Kilic, T., Erdem, A., Ozsoz, M., Carrara, S., 2018. microRNA biosensors: opportunities and challenges among conventional and commercially available techniques. Biosens. Bioelectron. 99, 525–546.

Konishi, H., Ichikawa, D., Komatsu, S., Shiozaki, A., Tsujiura, M., Takeshita, H., Morimura, R., Nagata, H., Arita, T., Kawaguchi, S., Hirashima, S., Fujiwara, H., Okamoto, K., Otsuji, E., 2012. Detection of gastric cancer-associated microRNAs on microRNA microarray comparing pre-and post-operative plasma. Br. J. Canc. 106 (4), 740.

Kosová, K., Vítámvás, P., Prášil, I.T., 2014. Wheat and barley dehydrins under cold, drought, and salinity–what can LEA-II proteins tell us about plant stress response? Front. Plant Sci. 5, 343.

Latef, A.A.H.A., Chaoxing, H., 2011. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. Sci. Hortic. 127 (3), 228–233.

Li, R.H., Guo, P.G., Michael, B., Stefania, G., Salvatore, C., 2006. Evaluation of chlorophyll content and fluorescence parameters as indicators of drought tolerance in barley. Agric. Sci. China 5 (10), 751–757.

Li, W., Wang, T., Zhang, Y., Li, Y., 2016. Overexpression of soybean miR172c confers water deficit and salt tolerance but ABA sensitivity in transgenic Arabidopsis thaliana. J. Exp. Bot. 67, 175–194.

Li, Y., Li, X., Meng, Y., Hun, X., 2019. Photoelectrochemical platform for MicroRNA let-7a detection based on graphdiyne loaded with AuNPs modified electrode coupled with alkaline phosphatase. Biosens. Bioelectron. 130, 269–275.

Liang, Y., Guan, Y., Wang, S., Li, Y., Zhang, Z., Li, H., 2018. Identification and characterization of known and novel microRNAs in strawberry fruits induced by Botrytis cinerea. Sci. Rep. 8 (1), 10921.

Mäkelä, P., Kärkkäinen, J., Somersalo, S., 2000. Effect of glycinebetaine on chloroplast ultrastructure, chlorophyll and protein content, and RuBPCO activities in tomato grown under drought or salinity. Biol. Plant. 43 (3), 471–475.

Mancinelli, A.L., 1990. Interaction between light quality and light quantity in the photoregulation of anthocyanin production. Plant Physiol. 92 (4), 1191–1195.

Martinez, V., Nieves-Cordones, M., Lopez-Delacalle, M., Rodenas, R., Mestre, T., Garcia-Sanchez, F., Rubio, F., Nortes, P.A., Mittler, R., Rivero, R., 2018. Tolerance to stress combination in tomato plants: new insights in the protective role of melatonin. Molecules 23 (3), 535.

Massonnet, C., Costes, E., Rambal, S., Dreyer, E., Regnard, J.L., 2007. Stomatal regulation of photosynthesis in apple leaves: evidence for different water-use strategies between two cultivars. Ann. Bot. 100 (6), 1347–1356.

McWilliam, J.R., 1986. The national and international importance of drought and salinity effects on agricultural production. Funct. Plant Biol. 13 (1), 1–13.

Mourya, D.T., Yadav, P.D., Mehla, R., Barde, P.V., Yergolkar, P.N., Kumar, S.R., Thakare, J.P., Mishra, A.C., 2012. Diagnosis of Kyasanur forest disease by nested RT-PCR, real-time RT-PCR and IgM capture ELISA. J. Virol Methods 186 (1–2), 49–54.

Muñoz-Mayor, A., Pineda, B., Garcia-Abellán, J.O., Antón, T., Garcia-Sogo, B., Sanchez-Bel, P., Flores, F.B., Atares, A., Pintor-Toro, J.A., Moreno, V., Bolarin, M.C., 2012. Overexpression of dehydrin tas14 gene improves the osmotic stress imposed by drought and salinity in tomato. J. Plant Physiol. 169 (5), 459–468.

Nahar, K., Ullah, S.M., 2011. Effect of water stress on moisture content distribution in soil and morphological characters of two tomato (*Lycopersicon esculentum* Mill) cultivars. J. Sci. Res. 3 (3), 677–682.

Nejat, N., Mantri, N., 2018. Emerging roles of long non-coding RNAs in plant response to biotic and abiotic stresses. Crit. Rev. Biotechnol. 38 (1), 93–105.

Noman, A., Aqeel, M., 2017. miRNA-based heavy metal homeostasis and plant growth. Environ. Sci. Pollut. Control Ser. 24 (11), 10068–10082.

Parihar, P., Singh, S., Singh, R., Singh, V.P., Prasad, S.M., 2015. Effect of salinity stress on plants and its tolerance strategies: a review. Environ. Sci. Pollut. Control Ser. 22 (6), 4056–4075.

Patanè, C., Scordia, D., Testa, G., Cosentino, S.L., 2016. Physiological screening for drought tolerance in Mediterranean long-storage tomato. Plant Sci. 249, 25–34.

Patel, P., Yadav, K., Ganapathi, T.R., Penna, S., 2019. Plant miRNAome: cross talk in abiotic stressful times. In: Genetic Enhancement of Crops for Tolerance to Abiotic Stress: Mechanisms and Approaches, vol. I. Springer, Cham, pp. 25–52.

Peng, Y., Li, Y., Li, L., Zhu, J.J., 2018. A label-free aptasensor for ultrasensitive Pb2+ detection based on electrochemiluminescence resonance energy transfer between carbon nitride nanofibers and Ru (phen) 32+. J. Hazard Mater. 359, 121–128.

Pessarakli, M., 2010. Handbook of Plant and Crop Stress, third ed. CRC Press, USA.

Petralia, S., Sciuto, E.L., Di Pietro, M.L., Zimbone, M., Grimaldi, M.G., Conoci, S., 2017. An innovative chemical strategy for PCR-free genetic detection of pathogens by an integrated electrochemical biosensor. Analyst 142 (12), 2090–2093.

Pirzad, A., Shakiba, M.R., Zehtab-Salmasi, S., Mohammadi, S.A., Darvishzadeh, R., Samadi, A., 2011. Effect of water stress on leaf relative water content, chlorophyll, proline and soluble carbohydrates in *Matricaria chamomilla* L. J. Med. Plants Res. 5 (12), 2483–2488.

Qin, X., Engwer, C., Desai, S., Vila-Sanjurjo, C., Goycoolea, F.M., 2017. An investigation of the interactions between an E. coli bacterial quorum sensing biosensor and chitosan-based nanocapsules. Colloids and Surfaces B Biointerfaces 149, 358–368.

Rafiee-Pour, H.A., Behpour, M., Keshavarz, M., 2016. A novel label-free electrochemical miRNA biosensor using methylene blue as redox indicator: application to breast cancer biomarker miRNA-21. Biosens. Bioelectron. 77, 202–207.

Rager, J.E., Moeller, B.C., Doyle-Eisele, M., Kracko, D., Swenberg, J.A., Fry, R.C., 2013. Formaldehyde and epigenetic alterations: microRNA changes in the nasal epithelium of nonhuman primates. Environ. Health Perspect. 121 (3), 339–344.

Rivero, R.M., Mestre, T.C., Mittler, R.O.N., Rubio, F., Garcia-Sanchez, F., Martinez, V., 2014. The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. Plant Cell Environ. 37 (5), 1059–1073.

Rorat, T., Grygorowicz, W.J., Irzykowski, W., Rey, P., 2004. Expression of KS-type dehydrins is primarily regulated by factors related to organ type and leaf developmental stage during vegetative growth. Planta 218 (5), 878–885.

Roychoudhury, A., Paul, S., Basu, S., 2013. Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress. Plant Cell Rep. 32 (7), 985–1006.

Sharma, S., Sharma, N., 2008. Effect of rootstocks on leaf water potential, water relations, antioxidant activities and drought tolerant in flemish beauty pear under water stress conditions. Indian J. Plant Physiol. 13 (3), 266–271.

Sheikh, F., Torchy, M., Valizadeh, M., Shakiba, M., Pasban Islam, B., 2005. Drought resistance evaluation in spring rapeseed cultivars. J. Agric. Sci. 15 (1), 163–174 (In Persian).

Shriram, V., Kumar, V., Devarumath, R.M., Khare, T.S., Wani, S.H., 2016. MicroRNAs as potential targets for abiotic stress tolerance in plants. Front. Plant Sci. 7, 817.

Shrivastava, A., Gupta, V.B., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. Chronicles Young Sci. 2 (1), 21–25.

Singh, S., Parihar, P., Singh, R., Singh, V.P., Prasad, S.M., 2016. Heavy metal tolerance in plants: role of transcriptomics, proteomics, metabolomics, and ionomics. Front. Plant Sci. 6, 1143.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. Am. J. Enol. Vitic. 16 (3), 144–158.

Skriver, K., Mundy, J., 1990. Gene expression in response to abscisic acid and osmotic stress. The Plant Cell 2 (6), 503.

Skubacz, A., Daszkowska-Golec, A., Szarejko, I., 2016. The role and regulation of ABI5 (ABA-Insensitive 5) in plant development, abiotic stress responses and phytohormone crosstalk. Front. Plant Sci. 7, 1884.

Subramanian, K.S., Santhanakrishnan, P., Balasubramanian, P., 2006. Responses of field grown tomato plants to arbuscular mycorrhizal fungal colonization under varying intensities of drought stress. Sci. Hortic. 107 (3), 245–253.

Sun, K., Lai, E.C., 2013. Adult-specific functions of animal microRNAs. Nat. Rev. Genet. 14 (8), 535.

Sunkar, R., 2010. MicroRNAs with macro-effects on plant stress responses. In: Seminars in Cell & Developmental Biology, vol. 21. Academic Press, pp. 805–811 8.

Tilghman, S.L., Bratton, M.R., Segar, H.C., Martin, E.C., Rhodes, L.V., Li, M., McLachlan, J.A., Wiese, T.E., Nephew, K.P., Burow, M.E., 2012. Endocrine disruptor regulation of microRNA expression in breast carcinoma cells. PLoS One 7 (3), e32754.

Wang, S., Liang, D., Li, C., Hao, Y., Ma, F., Shu, H., 2012. Influence of drought stress on the cellular ultrastructure and antioxidant system in leaves of drought-tolerant and drought-sensitive apple rootstocks. Plant Physiol. Biochem. 51, 81–89.

Wang, B., Sun, Y.F., Song, N., Wei, J.P., Wang, X.J., Feng, H., Yin, Z.Y., Kang, Z.S., 2014. MicroRNAs involving in cold, wounding and salt stresses in *Triticum aestivum* L. Plant Physiol. Biochem. 80, 90–96.

Wei, H., Yang, Y., Himmel, M.E., Tucker, M.P., Ding, S.Y., Yang, S., Arora, R., 2019. Identification and characterization of five cold stress-related rhododendron dehydrin genes: spotlight on a FSK-type dehydrin with multiple F-segments. Front. Bioeng. Biotechnol. 7, 30.

Winter, J., Diederichs, S., 2011. MicroRNA Northern blotting, precursor cloning, and Ago2-improved RNA interference. In: MicroRNA and Cancer. Humana Press, Totowa, NJ, pp. 85–100.

Wu, L., Zhang, Q., Zhou, H., Ni, F., Wu, X., Qi, Y., 2009. Rice microRNA effector complexes and targets. The Plant Cell 21 (11), 3421–3435.

Xie, F., Wang, Q., Sun, R., Zhang, B., 2014. Deep sequencing reveals important roles of microRNAs in response to drought and salinity stress in cotton. J. Exp. Bot. 66 (3), 789–804.

Yang, F., Xu, X., Xiao, X., Li, C., 2009. Responses to drought stress in two poplar species originating from different altitudes. Biol. Plant. 53 (3), 511.

Yang, M., Lu, H., Xue, F., Ma, L., 2019. Identifying high confidence microRNAs in the developing seeds of Jatropha curcas. Sci. Rep. 9 (1), 4510.

Yu, C., Wang, H.S., Yang, S., Tang, X.F., Duan, M., Meng, Q.W., 2009. Overexpression of endoplasmic reticulum omega-3 fatty acid desaturase gene improves chilling tolerance in tomato. Plant Physiol. Biochem. 47 (11–12), 1102–1112.

Zeng, H.Q., Zhu, Y.Y., Huang, S.Q., Yang, Z.M., 2010. Analysis of phosphorus-deficient responsive miRNAs and cis-elements from soybean (*Glycine max* L.). J. Plant Physiol. 167 (15), 1289–1297.

Zeng, H., Wang, G., Hu, X., Wang, H., Du, L., Zhu, Y., 2014. Role of microRNAs in plant responses to nutrient stress. Plant Soil 374 (1–2), 1005–1021.

Zhi, F., Chen, X., Wang, S., Xia, X., Shi, Y., Guan, W., Shao, N., Qu, H., Yang, C., Zhang, Y., Wang, Q., Wang, R., Zen, K., Zhang, C.Y., Zhang, J., Yang, Y., 2010. The use of hsa-miR-21, hsa-miR-181b and hsa-miR-106a as prognostic indicators of astrocytoma. Eur. J. Cancer 46 (9), 1640–1649.

Zhou, L., Liu, Y., Liu, Z., Kong, D., Duan, M., Luo, L., 2010. Genome-wide identification and analysis of drought-responsive microRNAs in *Oryza sativa*. J. Exp. Bot. 61 (15), 4157–4168.