

Chapter 5

Improvement and Effective Growth of Plants' Environmental Stress Tolerance on Exposure to Microwave Electromagnetic Wave Effects



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Abstract The usage of microwaves in cooking, in radar, and in communications is well known to most people who cook, and who use mobile phones. We have discovered that microwave energy can be used in agriculture, albeit the outcome does not only appear to be a thermal energy source but also a medium to carry information. Consequently, this chapter introduces the reader to the influence that microwaves have in plant growth. A peculiar feature of the microwave stimulus is that it suffices to irradiate the plant seeds, or else the first plant leaf for a very short time, after which plant growth follows its natural course. In addition to the discussion of the enhancement of plant growth by microwaves, pest repellents, and heat resistance, among others, the chapter also describes a novel method to irradiate plants with the use of drones to deliver the microwave radiation.

Keywords Microwaves · Electromagnetic wave effects · Growth enhancement · Environmental stress tolerance

5.1 Brief Review of Research on Plants with Microwave Irradiation

Most reports that examined the effects of irradiating plants with microwaves using WiFi and mobile phones have noted a negative growth of plants when subjected to the microwaves emitted from these sources. In this regard, Soran and coworkers [1]

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studied the influence of microwave radiation at bands corresponding to a wireless router (WLAN) and mobile devices (GSM) on leaf anatomy, on the essential oil content, and on the volatile emissions from such plants as *Petroselinum crispum* (Parsley), *Apium graveolens* (Celery), and *Anethum graveolens* (Dill). It appears that microwave irradiation resulted in thinner cell walls, and smaller chloroplasts and mitochondria as well as enhanced emissions of volatile compounds, particularly the monoterpenes and green leaf volatiles. Seemingly, the effects were stronger for the WLAN-frequency microwaves. Additionally, they found a direct relationship between microwave-induced structural and chemical modifications of the three plant species investigated, and concluded that their collective data demonstrated that pollution from generated microwaves may constitute a stress to the plants. On the other hand, only a few studies [1–3] have reported positive effects of microwaves with regard (i) to the enhancement of essential oil content by the GSM-frequency microwaves, albeit the effect of WLAN-frequency microwaves was inhibitory [1], and (ii) to the continuous microwave irradiation during the entire period from germination to the growth of plants such as, for example, the enhanced growth of roots in spinach and green beans [2, 3].

In a related study, Verma et al. [4] exposed the tomato fruit with a double dose of 9.3-GHz microwave radiation that yielded high lycopene content, total protein content, together with phenolic and flavonoid content in unripe, ripe, and overripe stages of two tomato varieties (NS-585 and NS-2535). Moreover, the activity of cell-wall degrading enzymes, such as polygalacturonase, pectinmethylesterase, and β -galactosidase, decreased in the double-dosed microwave irradiated fruit of both varieties. As well, postharvest exposure to microwaves could be applied to increase the shelf-life of the tomato fruit [4].

A recent study by Miler and Kulus [5] examined the ambiguous impact of microwaves on the DNA of plant cells, as they recognized that the usage of this electromagnetic radiation (e.g., 2.45 GHz, 800 W cm^{-2}) as a source of variation in mutation breeding could be very advantageous. Accordingly, their goal was to examine the influence of microwave radiation on the in vitro regeneration and acclimatization efficiencies as well as on the genetic and phenotypical variation of chrysanthemum *Alchemist*. Thus, they subjected leaf explants, with or without callus, to a microwave treatment for various periods and in different environments. It appears that microwave irradiation affected negatively shoot formation if it were applied for long periods, although it did not affect the rooting and acclimatization steps that were fully successful. Chrysanthemums produced from microwave-treated explants had longer shoots with inflorescences of greater diameter and altered shapes [5]. They further noted that the microwave treatment affected the generative phase by prolonging the bud coloration period. Approximately 22% of the plants regenerated from the microwave-treated explants, which demonstrated that band profiles were different from the reference control. The authors concluded that microwaves are an efficient and easy-to-access tool in mutation breeding of chrysanthemum *Alchemist* [5].

Several studies have considered that microwave effects are mainly due to a temperature increase on microwaving potting soils and plants. Nonetheless, there

is also a report by Saitou et al. [2] and Iguchi et al. [3] that considered the temperature rise to be extremely small, and that the effect was a peculiarity of the microwaves. In other words, any effects by the microwaves were nonthermal in nature. However, neither of these studies noted that there was no evidence for a nonthermal microwave effect.

5.2 The Obvious Question Is Then: Can Microwaves Affect Plant Growth?

First of all, it is relevant to recall that the term *Microwave* is a general term that describes electromagnetic waves at wavelengths between 1 m and 1 mm – i.e., electromagnetic waves with frequencies from 300 MHz to 300 GHz. These microwaves are used, for example, in communications such as mobile phones, satellites, and television broadcasting, and for heating and cooking as done in microwave ovens [6]. Our recent research has focused toward the application of the microwaves' electromagnetic wave energy to chemical reactions, to the synthesis and processing of (nano)materials, and to biological fields that involve physics and chemistry. In particular, to maximize the advantages of the microwaves' electromagnetic wave energy, we carried out several investigations from which we discovered and/or proposed applications of microwave-specific phenomena that could not be imitated by conventional heat sources, nor by any other type of energy sources.

The series of researches in the use of microwave electromagnetic wave effects led us to consider a new avenue of investigation as to how plants might be affected and how they might perform if they were exposed to weak microwaves at output microwave power of several μ -Watts. The objective here was that if plants felt microwaves as a stress, they may not relax that stress as humans normally would. Accordingly, the question relates as to how plants change to relieve that stress. Will the plants die/wither if irradiated with microwaves or will they show no change? Our first approach to answer this query then was to use the *Arabidopsis thaliana* (Columbia-0) plant that we had available 14 days after sowing. Using our 2.45-GHz microwave synthesis device, we proceeded to irradiate this plant with these microwaves initially for 1 h using the lowest possible output power so as to minimize/suppress any temperature rise that might otherwise affect the plant as thought by others [7]. For comparison, a control experiment was also carried out on the *Arabidopsis thaliana* plant that was sown and allowed to grow for a 14-day period but was not exposed to microwave radiation. The growth of the two plants was then observed in a growth chamber (artificial meteorological device) in which we could control the temperature, the humidity, the illuminance, and adjust the light/dark cycles to optimal conditions for growth. While no changes were expected, it was not what we observed. In fact, the *Arabidopsis thaliana* plant that had been microwave-irradiated revealed an enhanced growth rather than the expected *no change*. Nonetheless, we continued to observe the extent to which the difference

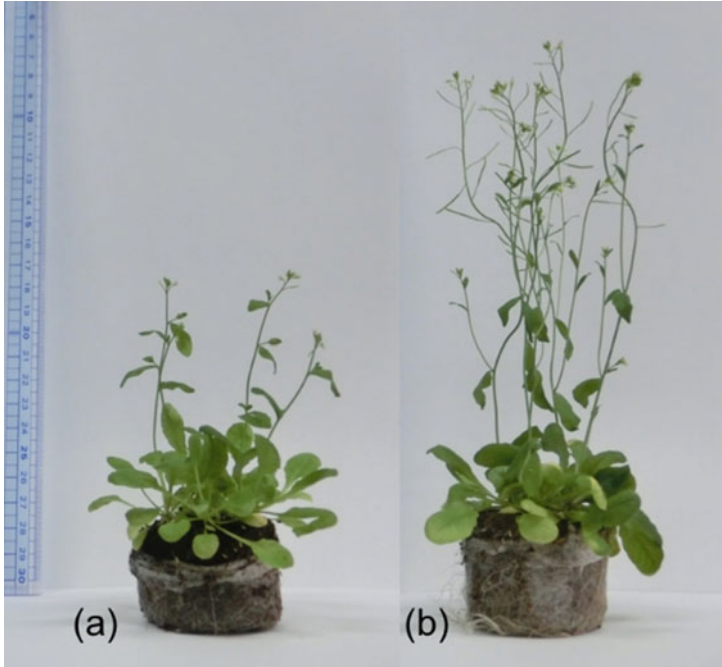


Fig. 5.1 Growth comparison of *Arabidopsis thaliana* after 1-h irradiation of microwave (photograph taken 38 days after sowing) [7]: (a) nonirradiated with microwaves (control), (b) irradiated with microwaves

in growth might be between the two plants. The results displayed in Fig. 5.1 show a photograph of the two *Arabidopsis thaliana* plants 38 days after sowing [7]. Despite being grown under the same environmental conditions, irradiating one of the plants with microwaves for just 1 h and subsequently allowed the plant growth to continue its normal course showed that the microwaves promoted its growth by nearly a factor of two (Fig. 5.1b) compared to the control *Arabidopsis thaliana* plant that had not been exposed to microwave radiation (Fig. 5.1a). The average inflorescence length of the microwaves was ca. 16 cm, while for the control plant it was on average about 8 cm.

We also discovered that irradiation with microwaves promoted the transition of *Arabidopsis thaliana* to the reproductive growth phase. In other words, microwaves had a positive effect on plant growth and should thus be considered as a kind of **microwave-induced stimulation**. Subsequent to these early experimental observations, and as a result of conducting additional screening experiments under various conditions of microwave power, irradiation timing, and irradiation times, among others, we found recently that a 1-s irradiation period is in fact sufficient to influence plant growth. Thus, contrary to our initial approach of irradiating for a 1-h period, it appears that the plants need not be exposed to microwave radiation for such a long period.

The significance of social and academic contributions of our research efforts is not inconsequential. As a social significance, it is important to note that the change in the growth rate by irradiating for just 1-s period with weak microwaves is indeed remarkable. That is, if seedling companies were to irradiate the seeds with microwaves in advance, even for just a very short time, the farmers need not then irradiate plant constituents with microwaves to enhance plant growth. On the other hand, the academic significance is that irradiation with microwaves for only 1 s, and then only once, can have a lasting and significant effect on plant growth in the future. As well, a comprehensive analysis of the plant genes [8] revealed that *Arabidopsis thaliana* irradiated with microwaves at μ -Watts output power suffered no genetic modification. In this regard, it is worth noting that the quantum energy of microwaves is 10^{-5} eV, so that chemical bonds cannot be broken by the microwaves.

Examination and use of electromagnetic wave effects have been a long-standing goal of our research efforts. Two decades ago we discovered that when a photocatalyst, whose extensive use has been in environmental remediation, is irradiated with microwaves together with UV-light, its photocatalytic activity was enhanced several times [9]. The photocatalyst referred to is titanium dioxide (TiO_2), an *n*-type semiconductor that whenever the nanoparticles are irradiated with less than 387 nm UV-light (band gap energy: 3.2 eV [10]) in aqueous media leads to the oxidative decomposition of water (H_2O) and generates reactive oxygen active species such as the $\bullet\text{OH}$ radicals, which can oxidatively decompose organic pollutants in aqueous media using only photon energy. In general, it is possible to decompose not only pollutants in aqueous ecosystems but also atmospheric pollutants. In spite of the thousands of academic studies and patents, however, water purification by the photocatalytic method has hardly been put into practical use because this treatment method is slow compared to other chemical purification methods that use, for example, ozone or hypochlorous acid. In other words, our efforts succeeded in enhancing the catalytic activity of the photocatalyst on exposure to both microwaves and UV/Visible light energy. The significance of that study [9] was that the photocatalytic activity was not a response to heat. Stated differently, the reaction efficiency of photocatalysts cannot be enhanced without utilizing the electromagnetic wave effects of the microwaves.

As an example, the degradation of the rhodamine-B (RhB) dye in aqueous media with dispersed TiO_2 semiconductor nanoparticles under both UV and microwave irradiation [11, 12] is reported in Fig. 5.2 [13]. Some decolorization was observed in the decomposition of RhB by TiO_2 irradiated only with UV light (TiO_2/UV). On the other hand, the photodegradation of RhB is clearly accelerated on exposing the TiO_2 simultaneously to both UV light and microwave radiation ($\text{TiO}_2/\text{UV}/\text{MW}$). Heating the RhB aqueous solution in which the TiO_2 dispersion was heated using a conventional heater while also irradiating with UV-light showed no enhancement of the activity of the photocatalyst ($\text{TiO}_2/\text{UV}/\text{CH}$) [11], even though the temperature of the water matched the heating rate from a solution exposed to microwave radiation. Moreover, no accelerated reaction was observed when the temperature was greater than the temperature reached on microwave heating. Accordingly, it appears that the role(s) of the microwaves was to streamline electron transfer inside the photocatalyst

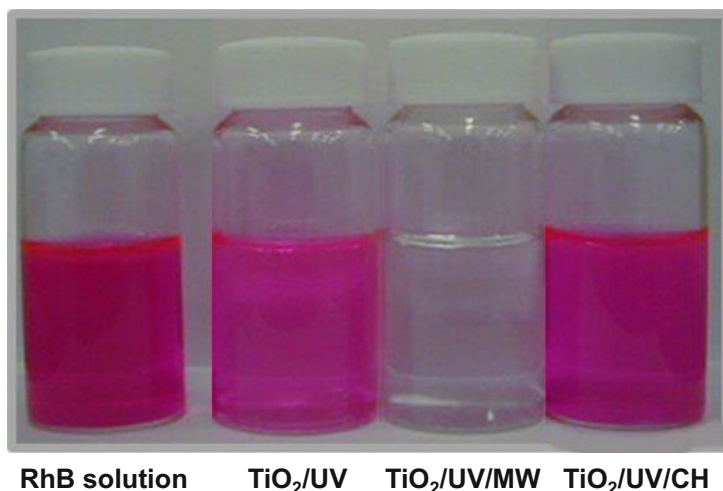


Fig. 5.2 Visual comparison of color fading in the degradation of RhB solutions (0.05 mM) subsequent to being subjected to various degradation methods for 150 min. From left to right: initial RhB solution; RhB subjected to photo-assisted TiO₂ degradation (TiO₂/UV); RhB subjected to integrated microwave–/ photo-assisted TiO₂ degradation (TiO₂/UV/MW); RhB subjected to photo- and thermal-assisted TiO₂ degradation (TiO₂/UV/CH). Reproduced from [13]. Copyright 2009 by Elsevier B.V

TiO₂ particles and suppress the recombination of the photogenerated electrons with the photogenerated holes [14]. Seemingly, microwave compatibility is good for reactions that require light energy, because they both consist of electromagnetic waves. The series of photocatalytic studies led us to envisage the existence of phenomena other than thermal effects from the microwaves as electromagnetic waves. In addition, we imagine next that microwaves are likely to affect what is originally driven by the electromagnetic waves from UV-Visible light photon energy.

We should not forget that microwaves are commonly used daily as a heat source (microwave ovens). However, we may hypothesize intuitively as to what the actual role of microwaves is in plant growth – thus the question: how does microwave electromagnetic energy directly affect plants?

5.3 Are Microwaves Used as Electromagnetic Energy?

Is the role of microwaves on plants simply as an electromagnetic wave source, or is it simply a heat source? We have recently been conducting multifaceted research on this question. For example, while the *Arabidopsis thaliana* on the 14th day after sowing was irradiated with microwaves for 1 h, we patiently monitored any changes in temperature using a plurality of optical fiber thermometers, and not least by

thermography. We noted that the maximum temperature change under microwave irradiation for 1 h was about 1.4 °C, from which we infer there was no thermal effect by the microwave irradiation. The expressions of heat shock protein (HSP), which is a heat response gene of *Arabidopsis thaliana*, and its regulator, HSF, were analyzed after microwave irradiation. There were no changes in these even when irradiated with microwaves. That is, under the conditions of the experiment, the temperature of the *Arabidopsis thaliana* plant increased neither macroscopically nor microscopically even when they were exposed to microwave radiation.

5.4 What Then Is the Role of Microwaves?

Since the growth of inflorescence stems of plants was enhanced by the microwaves, we proceeded to analyze the expression of genes and proteins involved in plant flowering induction and formation of reproductive organs. Results showed that the expression of the FT (FLOWERING LOCUS T) gene, which controls flowering induction by microwave stimulation, increased at 18 days after sowing. Similarly, we found that the expression of the FT protein also increased. In addition, since the expressions of the MYB30 gene and the FT gene (which are involved in flowering induction) increased, the stimulation of plants by microwaves promoted the flowering induction.

Next, the autofluorescence of chlorophyll was examined immediately after microwave irradiation on *Arabidopsis thaliana* 14 days after sowing (Fig. 5.3) in order to investigate the effect of microwaves on photosynthesis. Chlorophyll fluorescence is

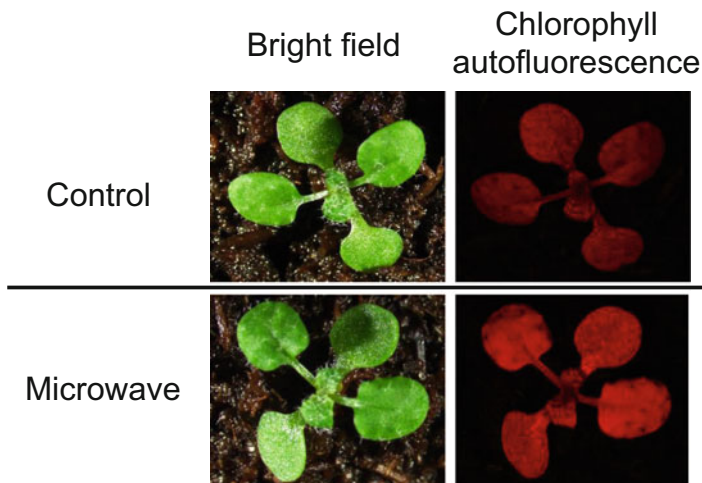


Fig. 5.3 Observation of chlorophyll autofluorescence from the *Arabidopsis thaliana* plant using a fluorescence microscope: Upper panel: control (nonirradiated microwave plant) and Lower panel: Microwave (plant irradiated with microwaves)

light that is re-emitted by the chlorophyll molecules during their decay from an excited state to their ground state. It is used as an indicator of photosynthetic energy conversion in higher plants, algae, and bacteria. In our case, microwave-treated plants revealed greater chlorophyll autofluorescence intensity compared to the nonmicrowave treated plant control. Chlorophyll autofluorescence is also known as a way for chlorophyll to dissipate excess energy not used for photosynthesis, but the plant appears to dissipate light energy even more when it is irradiated with microwaves. Even with its condensing antennae, chlorophyll is unable to capture microwave energy as the wavelength of the 2.45-GHz microwaves used is 12.24 cm, and thus chlorophyll cannot initiate its normal function of storing and using light energy to convert carbon dioxide (absorbed from the air) and water into glucose. In fact, however, we do find that microwaves do have some effect on the photosynthetic process.

5.5 Can Microwaves Improve Environmental Stress Tolerance?

5.5.1 General Situation of Plant Growth when Exposed to Environmental Stresses

It is well-known that the actual crop yields are within about 65 to 87% of the maximum value, assuming that the crop yields have reached their maximal value when the plants grow under ideal environmental conditions. However, the crop yields are reduced when plants are subjected to environmental stresses. Within this context, Table 5.1 shows the loss yields resulting from environmental biotic stresses

Table 5.1 Worldwide highest harvest yields, average harvest yields, and average loss yields of crops caused by environmental stresses on eight types of grains. Average loss yields from environmental stresses increased with both biological loss (disease, insect damage, and weeds) and nonbiological loss (drought, salt damage, flooding, and low temperatures) [15]

Grain	Highest harvest yields (kg/ha)	Average harvest yields (kg/ha)	Average loss yields from environmental stresses (kg/ha)	
			Biological losses	Nonbiological losses
Corn	19,300	4600	1952	12,700
Wheat	14,500	1880	726	11,900
Soybean	7390	1610	666	5120
Sorghum	20,100	2830	1051	16,200
Oats	10,600	1720	924	7960
Barley	11,400	2050	765	8590
Potato	94,100	28,300	17,775	50,900
Sugar beet	121,000	42,600	17,100	61,300

(biological stresses such as disease, insect damage, weeds) and abiotic stresses (nonbiological stresses such as drought, salt damage, flooding, and low temperatures) for eight types of grains [15]. The results show that environmental stresses do play a noninsignificant role that reduces crop yields. In particular, the yield loss due to abiotic (nonbiological) factors exceeds 50%. Accordingly, new technologies are required for increasing plant production under environmental stresses. Microwaves are thought and are expected to elicit results that are more resistant to these environmental stresses.

5.5.2 Heat Stress

Global temperature increases year by year as a result of global warming, and thus both the natural environment and the ecosystems are likely to undergo some associated changes. Because of this warming effect, some crops are being replaced by varieties that are resistant to temperature increases. Accordingly, can microwaves help to overcome this challenge? As a consequence, the following experiment was conducted in response to this challenge. To do so, *Arabidopsis* plants with and without microwave irradiation were subjected to high temperature (44 °C for 7 days) and scored in survival rate. We found that irradiation with microwaves improved the survival rate by $\geq 30\%$, even in a high temperature environment (see Fig. 5.4).

Next, it is relevant to describe possible molecular biological changes resulting from the application of microwaves through an analysis using a DNA microarray of the *Arabidopsis thaliana* plant subjected to microwave irradiation for 1 h at 14-day period after sowing. We confirmed an increased expression of the abscisic acid signaling genes *Nced3*, *Abi2*, and *Abi3* that play an important role in the regulation of heat tolerance, and of the *bZip28* gene that is involved in response of plants to various stress conditions including heat. Accordingly, we inferred that the heat response mechanism of plants was not activated by the microwave treatment,

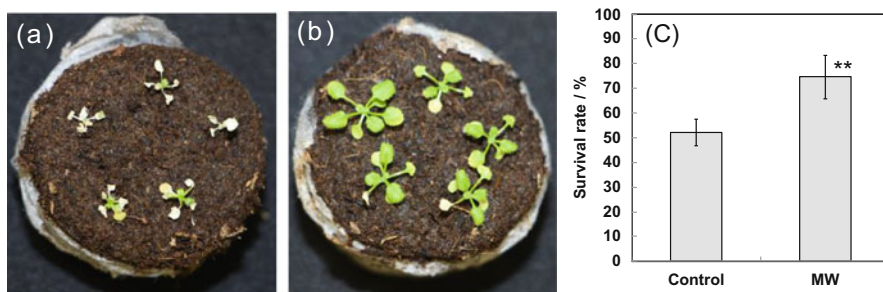


Fig. 5.4 Photographs that confirm the survival rate of *Arabidopsis thaliana* (a) unirradiated and (b) microwave irradiated in a high temperature environment (44 °C, for 7 days); (c) comparison of the survival rate of *Arabidopsis thaliana* after 7 days ($n = 20$; **: Significant at 1% level of t-test; bars show standard error) [8]



Fig. 5.5 Photograph of strawberries after a daytime temperature exceeding 30 °C. (a) Fruits from nonirradiated microwave strains, (b) fruits from microwave-irradiated strains [8]

although the mechanism of protecting plant cells from various stresses, including heat, was indeed activated.

As a further experiment, we examined the heat tolerance using strawberries as the commercial plant. Strawberry plants in the reproductive growth period were cultivated outdoors after irradiating them with microwaves for a 1-h period. Strawberries are known to shift from reproductive growth to vegetative growth at temperatures above 25 °C. Therefore, strawberries are not suitable for fruiting in a high temperature environment. Consequently, it was relevant and instructive to examine the presence or absence of any improvement in plant growth that might have been caused by microwaves. We found that the duration of fruiting was significantly longer, and the size of fruits was larger for the Strawberries that had been subjected to microwave irradiation than for the nonirradiated Strawberries. In addition, 51 days after planting, the temperature continued to exceed 30 °C; this caused the control strawberries to preclude harvesting the fruits after this period. By contrast, however, the strawberries that had been irradiated with microwaves continued fruiting after this period and were of excellent quality (see, for example, Fig. 5.5).

Currently, only 12% of the land worldwide is cultivated, as other lands are unsuitable for cultivation owing to present temperature conditions. In addition, global warming may well reduce arable land in the future. Even in such land and harsher conditions, however, we predict that agriculture could benefit if seeds and seedlings were preirradiated with microwaves.

5.5.3 *Stresses from Pests*

Agricultural chemical products have brought about prosperity to humanity because of their many effects on plant growth and increased crop yields to satisfy the never ending demands to feed the ever increasing world population. However, agricultural chemicals that previously consisted of natural products (such as manure or compost) now consist of chemically synthesized pesticides, especially since World War II, and over the years their effects on agriculture have improved significantly, albeit some beneficial and others deleterious. In this context, the negative societal image of

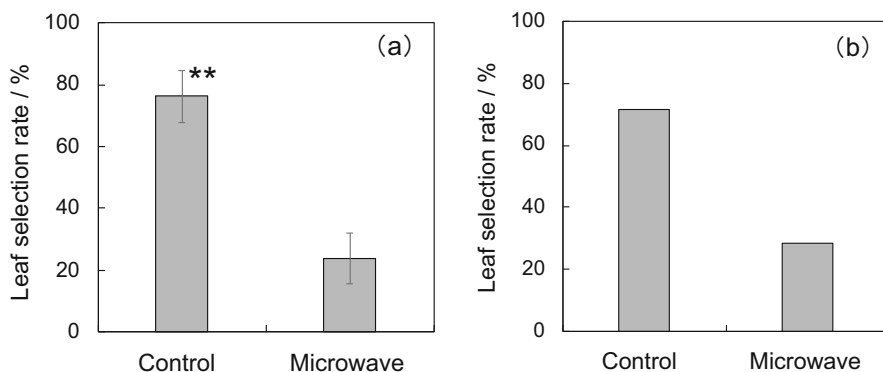


Fig. 5.6 Leaf selection rate by cabbage white butterfly larva ($n = 21$; **: Significant at 1% level of t-test; vertical bars refer to standard error); (a) *Arabidopsis thaliana*, (b) Arugula [8]

pesticides on commercial plants has grown, so much so that vegetables are now being marketed as being “pesticide-free” and are considered as higher value-added products (note the label “biological” and more costly products in your supermarkets). Accordingly, it was relevant for us to begin examining the pest repellent effect of microwave-irradiated plants following our many years of research on Microwave-assisted Chemistry and microwave effects on materials syntheses and processing. The principal motivation for starting this research is that plants grown by irradiating with microwaves might show an increase in external stress resistance. Pests also cause external stresses, which may thus increase the amount of various chemical repellent substances that plants produce. Indeed, when plants are injured by insects, the plants have a natural tendency to protect themselves (i) by releasing the insect damage/injury-resistant plant hormone known as Jasmonic Acid, (ii) by inducing the herbivore-induced plant volatile (HIPV), and (iii) by producing well-known reactive oxygen species [16]. At this juncture, our recent studies are focused on investigating whether irradiation with microwaves for a given time period of plant growth would affect these three defense responses. Accordingly, we conducted leaf preference tests using *Arabidopsis thaliana* and Arugula as our model plants and cabbage white butterfly larvae (*Pieris rapae*) as our model feeding pests.

In our experiments, the cabbage white butterfly larva was placed at the center between the *Arabidopsis thaliana* that had been irradiated with microwaves for 1 h and the nonirradiated *Arabidopsis thaliana*. Then, we assessed which plant the larva selects between these two cases (Fig. 5.6a). Figure 5.6b shows the results of a preference test using Arugula grown for 1 month after sowing the seeds that has been irradiated with microwaves. Results showed that microwave irradiation reduced the feeding damage caused by the cabbage white larvae. Similar results were observed from the data of the pest repellent rate of strawberries and Komatsuna (Japanese mustard spinach) in actual alley cultivation. An egg-laying preference test using the cabbage white butterfly was also conducted, results from which the proportion of the cabbage white butterfly, selected as the spawning plant, was

reduced by 1/3 or less by irradiating with microwaves. Furthermore, irradiation with microwaves significantly increased the synthesis of the amount of pest repellent produced. A sensory evaluation of vegetable foods subjected to microwaves and without exposure to microwave radiation revealed no difference in the five human sensorial responses, including taste. Since the repellent effect can be improved by electrical power used to generate microwave radiation, it would thus be possible to secure a safe and plentiful crop production without worrying about residual pesticides.

5.6 Microwave Irradiation Methodology

The advantages of our technique are that irradiation is achieved using only weak microwave power for a short time period at the beginning of plant growth, or else at some stage on the seeds, such that no further treatment would be required. Therefore, it is not necessary to arrange for microwave irradiation for the whole life of plant growth. In addition, it is not necessary to install a microwave device in an open cultivation field, in a greenhouse cultivation field, or in a plant factory, or in similar venues. Two types of microwave irradiation methods can be considered at the production site, one of which is a method of fixing the microwave irradiation port and performing the irradiation treatment while moving the plant. For example, a production process could be constructed by placing the buds (or seeds) in their early growth stage on a conveyor belt and irradiating them with a microwave irradiation device. Our second method would involve irradiating a fixed plant while moving the microwave irradiation port. In the latter case, the production process could be constructed by continuously applying microwave stimulation by irradiating with microwaves using a moving object such as a drone (Fig. 5.7).

Currently, attempts are being actively made to convert agriculture to IoT, a platform where embedded devices are connected to the internet, so they can collect and exchange data with each other. In addition, this platform enables the devices to interact, collaborate, and learn from each other's experiences as humans do daily. To the extent that this method is also entirely powered by electricity, it would be easy to incorporate it into the IoT agriculture. As well, to the extent that various phenomena can be controlled and expressed by changing the microwave irradiation pattern, it is possible to carry out detailed production according to the number of plants and the order for each habitat.

5.7 Concluding Remarks

There is a saying in Japan that states "Stepping on wheat seeds makes the sprouts grow stronger". This saying likens the phenomenon of deepening the roots and thickening the stems by stepping on the sprout of wheat. It is also a saying that can be applied to the difficulties and hardships faced by the young generation that should be



Fig. 5.7 Photograph illustrating a drone connected to a compact GaN semiconductor microwave generator with an antenna used to continuously apply a microwave irradiation pattern to the plants [8]

taken as an investment toward their future growth. The microwaves stress technology is similar to this. It is a technology that induces and activates the original power of plants by microwave irradiating their seeds or sprouts. In other words, the significance of microwave irradiation is that microwaves act as a trigger, that is, as a kind of catalyst that raises the potential of plants and their growth.

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