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Determination of moisture content in vegetative cultivated plants by the method of millimeter wave spectroscopy for the tasks of increasing plant productivity

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Investigation of the genetic and breeding orientation have shown that it is inefficient to study the genotype-environment (GTE) interaction in plants at the molecular level, since the GTE effect disappears without a trace on it, being an emergent property that occurs when gene products interact with labile ones during the day, weeks, months by limiting environmental factors. Such studies should be carried out for higher system levels, namely, at the next stages of life organization — organismal, population, ecological, phytocenotic. Since the most powerful „lever"for increasing plant productivity and yield, GTE, cannot be traced at the molecular level, knowledge of the molecular structures of the genome without knowledge of the dynamics of limiting environmental factors and the interaction of gene products with them does not contribute to the creation of high technologies for ecological genetic increase in productivity and yield plants. The accumulated world experience shows that rapid methods for measuring traits in plants, which give a holistic characterization of a complex dynamic system (this is what GTE is), can be created by interdisciplinary approaches, primarily using physical measurement methods. Within this approach, the article proposes to use the method of millimeter (MM) wave spectroscopy, which is very sensitive to changes in the water supply of plant tissues for the selection of drought-resistant plants.

Keywords: plant productivity, principle of background traits, moisture content, millimeter wave spectroscopy.

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Introduction

Science is facing an acute problem of increasing cultivated plant productivity and yield and one of them is hereditary drought resistance increase [1]. Therefore, it is important to understand epigenetic processes in genotype−environment interaction (GEI) which occur only at rather high organism organization levels (ontogenesis, population, agrocenosis, biogeocenosis). These life arrangement levels provide food and clothing of plant origin. Theory of ecological-genetic organization of quantitative traits was proposed by Russian researchers after discovery of a new epigenetic phenomenon on non-perennial culture — gene product set shifts determining the same quantitative attribute with change in limiting environmental factor [2]. Search for plant genotypes resistant to unfavorable environment conditions to ensure efficient breeding includes knowledge of genetic and physiological systems, including adaptivity, using of optimization systematic approaches rather than reduction-mechanized ones. As breeders?' vast accumulated experience shows, plant breeding may be useful if breeder has identification methods even without knowledge of quantitative production trait genetics [3].

Breeding traditionally identifies genotype of an individual genotype by its phenotype by means of long-term progeny testing. In this case, unique genotype incidence in real populations is so low and populations are so vast that up to 80−90% specimens in splitting population are rejected by phenotypes at the first breeding stages. When there is a lack of reliable genotype identification methods by phenotypes, major part of valuable forms is permanently lost and wont be supplemented by more accurate assessment of descendant groups at subsequent breeding stages. For express identification without alternation of unique genotype generations by their phenotypes by quantitative traits during selections from splitting hybrid and wild plant populations, Dragavtsev has proposed a background trait principle [4].

The background trait principle means that if a quantitative trait with zero (or near-zero) genetic dispersion can be found in any plant population, then variability of this trait will be only ecological. Traits with zero or very low genotypic variability are referred to as background traits. In limiting environmental factor conditions, e.g. drought, leaf water content trait has this property. When a phenotype having a positive deviation by this trait from the average population value is a modification located in the best microecological niche, and if the selection trait is shifted in this specimen towards positive direction against the background trait, this will be a modification and this specimen need not be selected. If the background trait in another specimen is defined at the average population value level and the selection trait is shifted towards the positive direction, this is a recombination (or mutation) required for effective breeding. For this purpose a term „phenomics" [5] has been introduced.

In order to detect the plants with the best genotype in drought conditions within the shortest time, it is necessary to measure moisture content in plants using a noninvasive method. Such approach may be referred to as express measurement which significantly saves breeder's time and labor and allows to study a larger number of plants than other existing contact labor-consuming methods for moisture content measurement in plants.

On the drought background as an unfavorable limiting environmental factor, genetic variability of moisture content in plants is reduced to zero. Stress mechanisms are activated, ecological and phenotypical variability takes place [6]. Using physical measurement of leaf water content which is a background trait in drought conditions, the best genotypes for hereditary drought resistance increase of the cultivated plants may be identified by selection traits.

A method of millimeter (MM) wave spectroscopy, which is very sensitive to the change in water supply of plant tissues, is proposed herein. electromagnetic MM band waves are featured by high absorption of electromagnetic waves of this band by water. absorption in free water is more than 20 dB/mm. This is higher than in all fluids on Earth. So using MM waves, small changes in water content in various media may be recorded. The change is water content will cause change in complex permittivity of ε-medium which can be measured by MM spectrometry methods. Change of ε-medium, in turn, changes absorption in it and the complex reflectance r of its surface. Measurement of r does not require penetration into the medium, i. e. is noninvasive. I.e. the proposed methods are, on the one hand, non-destructive and may be used for intra vitam measurements, on the other hand, real-time methods may be developed using MM spectroscopy.

For recent 50 years, study of plants using their dielectric properties has become one of traditional agrophysical research methods with a lot of publications devoted to it worldwide [7–41]. Study of dielectric properties of plants is widely used in a broad agricultural application range. Results of the first investigations in this field are presented in [7–10]. Thus, in [8], vegetation cover was studied using microwave radiometry. Since that time, study of vegetation covers using radiometric methods has been one of the important themes in terms of plant study by dielectric methods [11,32,39]. Using remote microwave sensing of vegetation covers, water content in field plants, water potential of vegetation cover, water stress in agriculture may be determined [39]. In order to carry out these studies, dielectric properties of plants (branches, leaves, roots, fruit) shall be known. A lot of publications [8–25,28–34] are devoted to this problems and define dielectric properties of many agricultural culture, including fresh fruit and vegetables [17,18,27,35]. Thus, in [8], dielectric properties of corn and wheat leaves and stems were studied in 1 to 8.5 GHz bands depending on water content. Dielectric properties play an important role in study of fruit maturity and ripeness [14–19,21–23,29–31,34], which is important both for prompt crops picking and provision of necessary storage conditions.

Humidity is one of the most important plant life variables. One of the first publications focused on the study of water motion in soil–plant–atmosphere chain was [6]. It describes an approximate solution of differential equation describing soil moisture flow to the plant root which absorbs water with regularly varying rate. This makes it possible to simulate hydraulic behavior of a culture plant. Using this water exchange model in plants, models were built for plants as water media in order to determine water content in plants by various methods [9,10,13,19,23,27,29,30,33,37–40]. It [32] was clarified which information may be found from microwave observations, their sensitivity to plant water content measurement, and relation between microwave water content indicators and usually used drought condition indicators.

In various frequency ranges, various models of water content effect on dielectric properties were developed [8,9]. In [8], water content in plants was simulated using model liquids taking into account salinity (presence of NaCl in plant's water). It was found that frequencies below 5 GHz with salinity about 10% and higher cause water content errors. Another publication focused on the influence of water stress on plants and their dielectric properties is [20].

In [9,10], microwave dielectric behavior of plant material is assessed depending on water content, microwave frequency and temperature. [9] shows experimental measurements of dielectric spectrum in 0.2 to 20 GHz band for different types of plant material, including leaves, stems and trunks in different water content conditions. Measurements were carried out by a coaxial probe method which can be used to determine permittivity of thick materials such as tree trunks and of thin materials such as leaves. According the obtained experimental data [10], a plant was simulated as a complex dielectric with Debye–Cole double dispersion, including water both in „free"and „bound" form.

many applications, including remote sensing of vegetation cover and water content measurements require simulated model for leaves which ensures precise complex permittivity corresponding to geometric structure and nature of vegetation itself. Many such models were described in [11] and references therein.

Various techniques were used for study, including radiometry, wideband and narrowband measurements in different frequency bands and different techniques, including waveguide, resonator, coaxial methods for various applications described above.

This paper is devoted to the development of physical methods of plant breeding based on the genotype–environment model [1–4]. For this purpose, [5] introduces a new term „Phenomics". Phenomics of plants offers a set of new technologies to accelerate the progress of understanding of gene function and environment. This will enable breeders to develop new agricultural germplasms to support future agricultural production. This review [5] describes plant physiology with regard to „omics“, some of new high-capacity phenotyping tools were addressed, including high resolution physical methods, and their applications in plant biology, functional genomics and plant breeding are discussed.

1. Materials and methods

This paper is devoted to the development of physical methods of intra vitam study of water content for plant breeding based on the genotype–environment model [1–4]. This model requires fast simultaneous intra vitam water content determination in large amount of plants at a certain height from ground. For this purpose, the MM wave water content measurement method is offered herein. The frequency band selection is defined by the following features — plants are water media with high water content. Water as one of the most polar substances on Earth has dielectric relaxation dispersion in microwave and MM band region (approx. 17–24 GHz depending on temperature). Therefore, the investigations will be performed in these bands. This method is based on two phenomena. First, complex permittivity dispersion for materials with complex composition is approximately equal to the additive sum of complex permittivity dispersions of individual polar components of this mixture. The second phenomenon is that dielectric relaxation is manifested, in particular, in very high attenuation of electromagnetic waves of this band in water and water media. And in frequency band near the relaxation frequency of any polar component, complex permittivity has the strongest dependence on the percentage composition of this very polar component in a composite material. This is due to the fact that the change of dielectric properties of polar materials is the highest in this very relaxation region. Based on this, microwave and MM water content measurement methods were developed for non-destructive and noninvasive study methods of water composites [41]. MM water content measurements have a set of important benefits compared with microwave water content measurements and other water content study methods described above. These benefits ensure the following physical phenomena:

1) MM wave absorption in water $\alpha > 15$ dB/mm, which is much higher than that of virtually all known materials;

2) with the wavelength reduction, absorption in water grows faster than in other materials.

Therefore,

• MM waves ensure higher sensitivity to water content than microwaves;

• MM waves ensure better spatial resolution than microwaves which is important for detention of localized water media in a non-polar matrix;

• MM waves are less sensitive than microwaves to the presence of conductive impurities in water which is important for plants, because conductive impurities, e.g. salts, occur in them;

• permittivity values of non-polar substances at MM frequencies are beyond their limit values which are predominantly constants which do not depend on frequency;

• all this ensures maximum accuracy for determining water content and bio-objects, which are water media, using MM spectroscopy in nonpolar matrices.

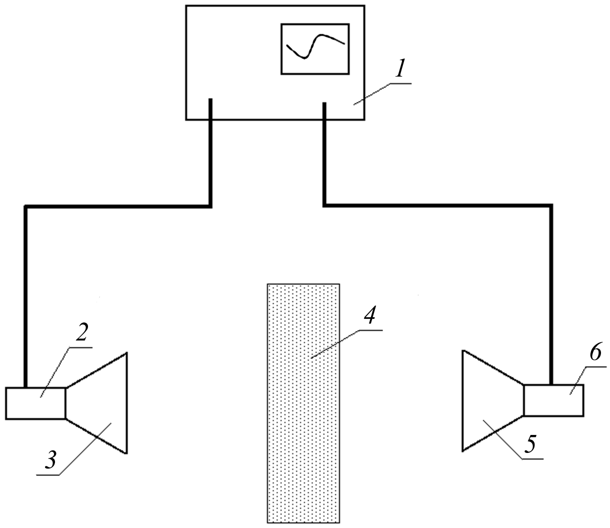

Figure 1. Measurement setup flow-chart: *1* — panoramic meter, *2, 6* — coaxial waveguide transitions, *3* — transmitting horn antenna, *4* — sample, *5* — receiving horn antenna.

Therefore, the plant is simulated as a bio-organic composite, solid part of plants is treated as a non-polar matrix, and water, without salt impurities effect, is a high-polar filler of this matrix.

The purpose of the experiment was to determine reflectance, transmittance and attenuation ratio in Peperomia obtusifoli leaves within 26.5–40 Ghz. The flow chart is shown in Fig. 1. Signal from the transmitting horn antenna *3* partially passed through the sample *4* and was received by the receiving horn antenna *5*. The part of signal was reflected from the sample and arrived into the transmitting antenna *3*. Panoramic meter *1* recorded dependences of transmittance (T, dB) and attenuation ratio (R, dB) on frequency. To avoid undesired measurement errors caused by mutual movement of the setup components, all structural components were rigidly attached to the guide rail. General view of the setup is shown in Figure 2. The test sample was tightly pressed against the horn *3*. The distance between the horn was about 55 mm.

After the measurement of dependences T and R of a sample on frequency, curves were approximated by second-order polynomial by least square method and average values were calculated by frequency band. Then, using T and R, attenuation ratio α of electromagnetic wave of a sample with thickness L are used, by equation (1):

$$\alpha = \frac{1 - T - R}{L}, \tag{1}$$

where T and R were substituted for times and L — for mm.

Visual inspection of Peperomia obtusifoli leaves (Figure 3) shows that the leaf face has glossy appearance and is lighter and leaf rear side is more opaque and dark. Leaf thickness was measured by electron micrometer. Each leaf was measured 3 times (without index, index a and index b). Without index and index a — leaf was pressed with its inner light side to the horn. Index b — leaf was pressed to the horn with its outer dark side. Measurements were carried out directly on the plant, i.e. without removal of leaves. After the measurements, leaves were removed from the plant, weighed and then dried. Drying time was 10 h. All measurements are summarized in the table. Diagrams were plotted using the obtained data — Figure 4–6.

The obtained results are analyzed below. It should be noted that leaf surfaces are very different. On one side there

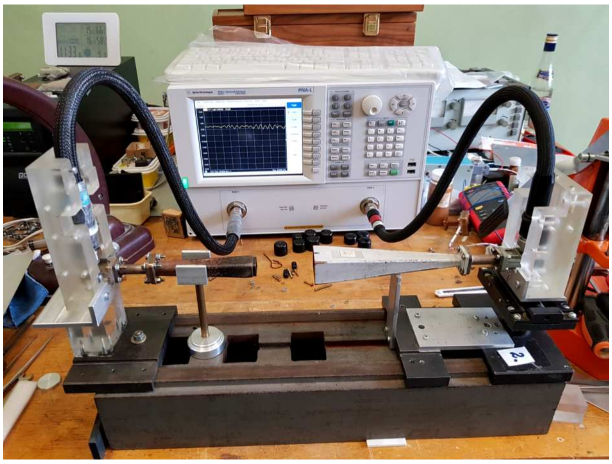

Figure 2. General view of the setup.

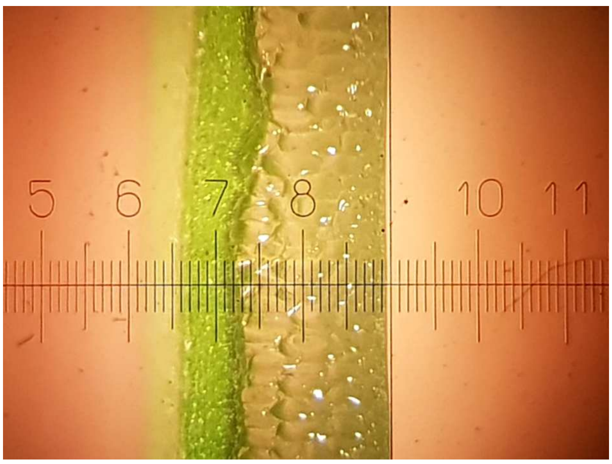

Figure 3. Peperomia obtusifoli leaf cross-section Division value 0.05 mm.

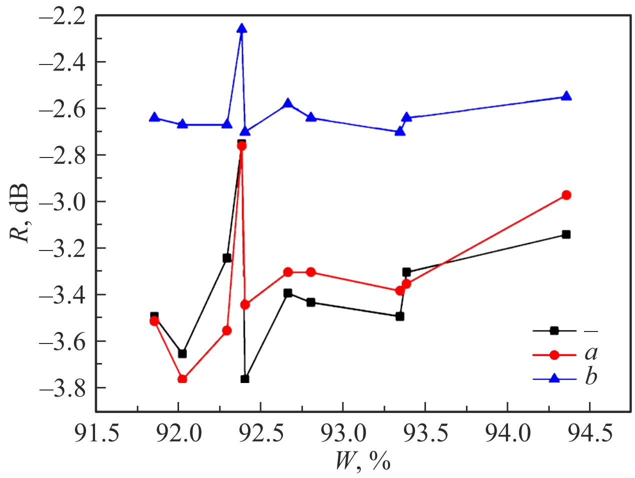

Figure 4. Dependence of R on water content W.

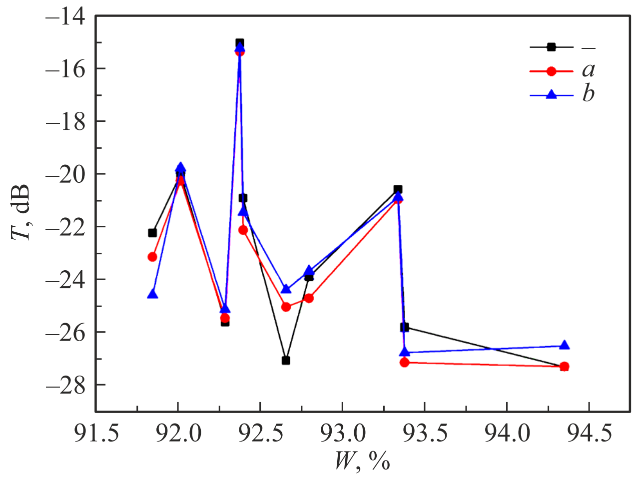

Figure 5. Dependence of T on water content W.

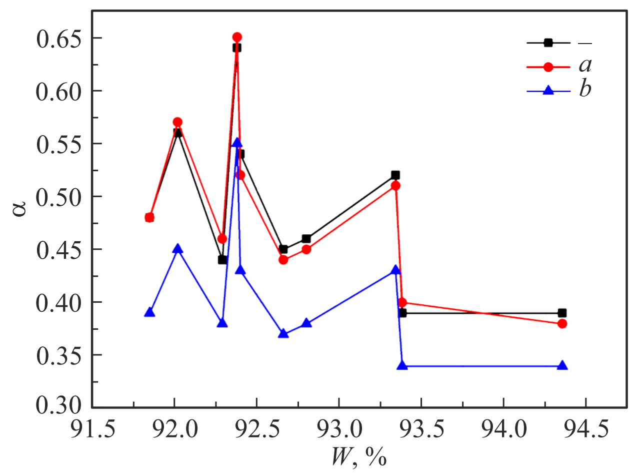

Figure 6. Dependence of α on water content W.

Note	, mm	m_1, g	m_2, g	W, %	№	R, dB	T, dB	Thickness α
Leaf 1	0.68	1.706	0.130	92.38	1	−2.75	−15.07	0.64
					1*a*	−2.76	−15.4	0.65
					1*b*	−2.26	−15.27	0.55
Leaf 2	1.05	2.972	0.198	93.34	2	−3.49	−20.61	0.52
					2*a*	−3.38	−20.99	0.51
					2*b*	−2.70	−20.91	0.43
Leaf 3	1.20	4.343	0.319	92.66	3	−3.39	−27.09	0.45
					3*a*	−3.30	−25.06	0.44
					3*b*	−2.58	−24.41	0.37
Leaf 4	1.30	5.082	0.287	94.35	4	−3.14	−27.32	0.39
					4*a*	−2.97	−27.31	0.38
					4*b*	−2.55	−26.53	0.34
Leaf 5	1.35	3.594	0.238	93.38	5	−3.30	−25.82	0.39
					5*a*	−3.35	−27.16	0.40
					5*b*	−2.64	−26.79	0.34
Leaf 6	1.0	5.011	0.400	92.02	6	−3.65	−20.04	0.56
					6*a*	−3.76	−20.28	0.57
					6*b*	−2.67	−19.80	0.45
Leaf 7	1.18	7.425	0.535	92.80	7	−3.43	−23.92	0.46
					7*a*	−3.30	−24.73	0.45
					7*b*	−2.64	−23.70	0.38
Leaf 8	1.20	6.342	0.489	92.29	8	−3.24	−25.63	0.44
					8*a*	−3.55	−25.48	0.46
					8*b*	−2.67	−25.15	0.38
Leaf 9	1.15	4.725	0.385	91.85	9	−3.49	−22.26	0.48
					9*a*	−3.51	−23.17	0.48
					9*b*	−2.64	−24.61	0.39
Leaf 10	1.05	4.090	0.311	92.40	10	−3.76	−20.94	0.54
					10*a*	−3.44	−22.15	0.52
					10*b*	−2.70	−21.48	0.43

is thick skin, and on the other side water is visible inside the leaf. Therefore, reflectance and transmittance for different sides are different. First, reflectance and transmittance of Peperomia obtusifoli leaves depend on the type leaf surface condition. Second, reflectance and transmittance depend on the leaf height on the plant stem. And finally, dependence of reflectance and transmittance on water content measured by drying is questionable. Significant dispersion between water content and reflectance/transmittance is observed.

Conclusions

The proposed MM water content measurement method provides a fundamental opportunity to measure water content in plants by a noninvasive method by electromagnetic wave reflection or transmission, almost immediately, because the measurement rate depends to a great extent on data processing rate. For different plants , different sensors are required depending on the leaf thickness and sizes, surface condition and water content. Plant leaf — is a heterogeneous medium, therefore, additional investigations are required to obtain more clear dependence of reflectance/transmittance on water content.

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Conflict of interest

The authors declare that they have no conflict of interest.

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