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The Hygroscopic Property of *Laurus Nobilis* (Daphne) Leaves (Hydrosynthesis)

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Abstract

It has been observed that Laves Nobles (Daphne) whose root system was isolated together with dry soil has a high capacity of receiving water from air having moisture content above 80%. Some fractions called as PI, P2, P3, P4, P5, P6, pigments have been chemically isolated and these were observed to be hydrosynthesized in vitro. All the pigments show maximum absorbance in the range of 325-360 nm. On the other hand it was realized that they are very hygroscopic substances to the degree of approximately three times greater than $CaCl_2$ (Calcium chloride).The mechanism by which hygroscopic pigments absorb water from air has been proved to be more efficient than that of root systems.

Key words: Laurus Nobilis (Daphne), Leaves, Hydrosynthesis hydrophile, hygroscopic, pigments.

INTRODUCTION

The photosynthesise which occurs in green leaf, takes form at the end of photosysthem activities or photochemical center which is found in leaf cell (1-6). the different pigments which have physical, chemical characteristics such as chlorophyll a,b, caratenoids, and phycobilin forms the main structure of this photosysthems found in choloroplast (1,2).

This study has been done by depending on the logic of chemical energy cycle phenomenon mat photon energy absorb from the sun accumulating by the help of photosynthesize or leaf. However, it is aimed to investigate collecting the water molecule in the air by the leaves of vegetables that can be in form of green continuously, absorbing less water by the root systems but can develop easily and grow on the hard rock.

MATERIAL AND METHOD

MATERIAL

In the investigation; as a subject of an experiment a daphne (Laurus Nobilis) one year old sapling and a green daphne leaf older than one year was used. For this purpose the sapling of daphne root system has mainly cleared from soil and installed into a glass container or a nylon bag by dry soil. So, root system and a soil with it was isolated from water and damp enviroment. In this case, the sapling of daphne was put into a decicator that has 80%-100% relative humidity and an airing. It is maintained that the relative humidity in the decicator is 80%-100% during the experiment.

The Extraction of Special Hygroscopic Compounds from Daphne Leaf

The extraction of the matters includes daphne leaf was obtained not demolishing the cell structure of the leaf. The mix that was obtained from daphne leaf was brought a concentrate solvent after evaporated by the help of rotator. The concentrate solvent was waited at the glass container at 4°C until 48-50 hour. At the end of this time, it was seen that the general mix formed two layers, dark red or dark brown upper layer (F1),and in the jell form of yellow -red lower layer (F2). F1 and F2 fractions are examined by separating from each other.

The thin layer chromatography, the difference of pH equivalence gradient and without ethylalcohol christalization of compounds methods were used in chemical puring of hygroscopic compounds the Fl fraction included.

Fl, F2 Fractions and the Hygroscopic of Other Matters

For this purpose heat controlled oven and sensitive scales was used. The F1, F2 fractions that was taken for experiment and other matters, were firstly dried (100°C) than weighed and they were weighted after being hold on 80%-100% relative humidity environment along 24 hours to 850 hours after the water that was absorbed from air was calculated according to time.

Microscopic Testing of Daphne Leaf

For this purples a basic microscope was used as different from normal daphne leaf the existence of colored points that covered all the leaf and seen on microscope on daphne leaf taken for extraction (6 hours later) appears. Unless the extraction time is increased (until 6-20 hours), the colored points that leaf included melts degree to degree and disappears by being reduced. By losing these colored points, the extraction colored matters that occurred from leaf is completed and hard daphne leaf becomes soft like usual plant leaf.

The Methods of Structure Analyze

The testing of structural characteristics of P1, P2, P3, P4, P5 and P6 pigments of Fl fraction which are obtained from daphne leaf was done at TUBITAK-Gebze Research Center. By this reason, usual spectroscopy, U.V spectroscopy, F.R. spectroscopy, H^1, NMR, atomic adsorption and DSC analyze methods of suggested pigments were used.

Discoveries and Discussion

Root system, isolated from air and water and situated in a glass container or nylon case with dry soil, shows normal growing of the daphne sapling whose leaves are on air in which moisture ratio %80-%100 at long time (10 months) obtains the water which it needs from the air moisture by its leaves.

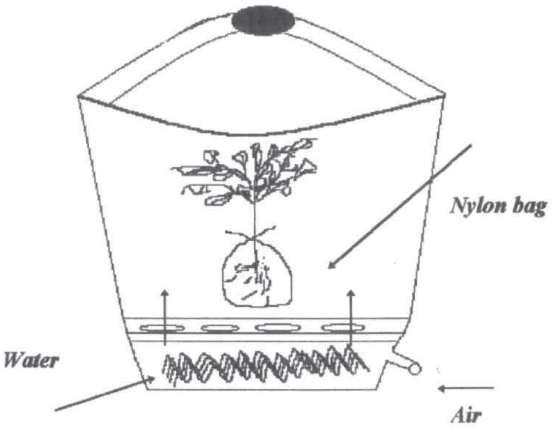

Figure l. The daphne sapling that is isolated from water and moisture soil of root system.

As shown in figure 1, according to the prepared conditions, it is becoming important that, the possibility of the daphne which is impossible to take water by root system can provide it from the moisture on the air by it leaves in order to grow not to dry.

According to the investigation and observations done, it is thought that as in the photosynthese, on other function is being found to make this duty on leaf. So, there can be advantage to accept that there are some centers which provide water necessity on leaf. The mechanism which catches water molecules from air and transfer it into the plant cells is called 'hydrosynthese' from different photosynthese on leaf.

The characteristics of special matters that are created these centers on the leaf. In order to prove the existence these hydrosynthese centers which is suggested.

In theoretically, the matters are taken form of 'hydrosynthese' centers must be more sensitive then all known organic and inorganic compounds as hygroscopic sensitivity. In this case, these matters can catch water molecules, in the air easily and quickly (as the Ferro molecules accumulate in two surroundings). Other wise the matters which create the hygroscopic antennas on upper surface of leaf or on leaf mouths can easily and quickly catch the water molecules and transfer then into the inner structure.

As ifs known, the hydroscopic characteristic matters on leaf cell such as carbohydrates, proteins, amino acids, different hydropicomlex compounds.(4..6) are enough quantity. The issue, to determine which one or which ones have possibility to realize these compounds that was told.

For this reason, all the compounds which can be soluble in water and found in it not to demolish the daphne leaf cell must be extracted and required to differ the special hygroscopic compounds from extracted matters.

So, it is observed that the matters include the concentrate extract taken can easily be separated into two fraction (pH 6.5) of layer by itself having been saved at +4°C for 48-50 hours in special glass back to make base the concentrated extract pH (pH 8-10) has been provided to differ into these layers of solvents more quickly.

For this purpose, it is necessary to be extracted the compounds which exist in the water and can ideally dissolve with out destroying the cell-structure of the leaf and to be separated hygroscopic compounds amongst the matters in the extracts taken.

The matters that have less hygroscopic characteristic but can be soluble in water can form layers differing from the matter. Which have high hygroscopic degree by forming jell because of unwatering characteristic.

We can say as colloid and ideal solvent rules the fraction (Fl) which forms upper layer of concentrate extract includes high hygroscopic tolerant matters, however the fraction which

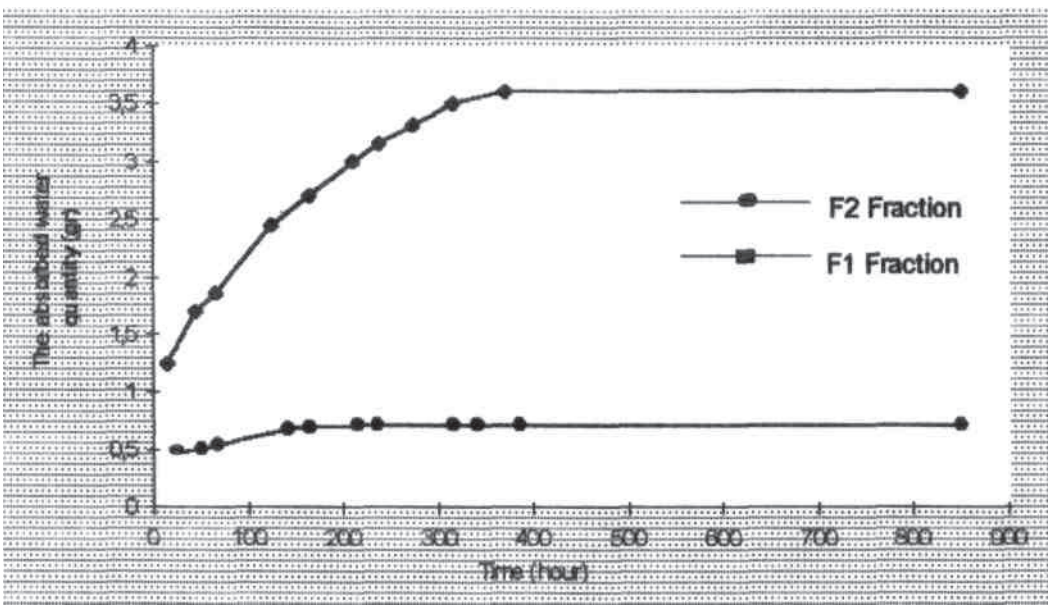

Figure 2. The curve that shows the water quantity which is absorbed from air on 80-100% moisture ratio of Fl and F2 fractions.

has forming of precipitation (F2) includes low-hygroscopic tolerant matters. After Fl and F2 fractions differed from each other, the results shown in Figure-2 have ascertained, the hygroscopic characteristics tested one by one.

As shown in graphics after Fl fraction is dried 2-4 hours in oven (100 °C) material is formed liquid by absorbing the water in air again if it is left on 80% -100% moisture condition The absorbing water from air is at long period (850 hour), it continues until the solvent becomes saturated by water.

At the end, it is observed that the Fl fraction that is taken for experiment absorbs three times higher water than its own mass. By the same method,at the end of the testing of the water absorbation characteristic from air of F2 fraction, it is understood that the hygroscopic degree of this fraction is very low and absorbs moisture in 0.5 ratio from its own mass if it is compared to Fl fraction.

By venturing the high hygroscopic character of fl fraction,it is understood which chemical compounds it includes and this mix includes colored compounds (pigments) called as Pl, P2, P3, P4, P5, and P6 at studies according to investigate the hygroscopic characteristic of compounds.

In normal water (pH 7) P1 and P2 pigments that forms precipitate are unsolved compounds if Fl fractions pH(6.5) is lowered until 1 by 0.1 HC1 (or diluted HC1); by this way they are matters that have dark brown colors, and can easily soluble on basic condition (pH 10-12).

In colored matters of P3 and P4 that form precipitate when Fl fractions (pH 6.5) are increased to 10-12 value by the help of 0. IN NaOH (or diluted NaOH) are easily solved compounds at normal water (pH 7) different from PI and P2 pigments.

P5 and P6 pigment precipitates after from crystal by the effect of ethyl alcohol from extract different from P1, P2, P3, P4 pigments.

Moreover, being differed of P1, P2, P3, P4 colored compounds that is taken from P1 fraction, has crystallized in unwatered alcohol by the aim of chemical purring. The hygroscopic characteristics and structure analyze of the pigments obtained by the way were tested.

The hygroscopic characteristics of the pigments P1, P2 (precipitate by HC1) and P3, P4 (precipitate by NaOH) that includes Fl fraction seems more interesting.

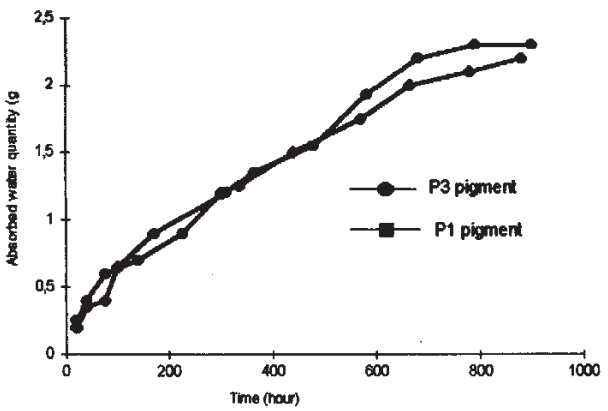

Figure 3. Figure 2 the cure that shows the quantity of absorbed water of the pigments PI and P3 have 80-100 moisture.

While these pigments are separated, they can absorb water 20 times more than their own mass, being different from total Fl fraction.

The other pigments (P5 and P6) that includes Fl fraction are hygroscopic compounds, their hygroscopic degrees are lower than the hygroscopic degrees of P1, P2, P3, P4 pigments.

Naturally it is the subject mouth investigation why the hygroscopic degree of Fl fraction this lower than the pigments that form this fraction.

The reason of this for us, P1, P2, P3, P4, P5 and P6 pigments that are found in Fl fraction is the forming of the hydrogen or salt types chemical bonds by the help of hydrophile groups by them selves. So, the bonds, that form between pigments degree is lower thanthe hygroscopic degree of fraction.

The compounds are separated from each other because of spoiling of temporary bonds that forms between pigments with the variation of the environment pH. The increase of hydrophile groups that are coming into being at their structures can cause increasing of the pigments hygroscopy.

So, the hygroscopic degree of free pigments must be under the hygroscopic degree of F1 fraction. The table one was prepared by comparing of the hydroscopic degree of hygroscopic compounds as $CaCl_2$ saccarose with the hygroscopic degree of free pigments (P1 and P3).

Table 1. The hygroscopic characteristic of different compounds.

No	The tested water	The water quantity that this absorbed by the mass or 1gr	The hydroscopic degree according to $CaCl_2$
1	$CaCl_2$	7	1
2	Saccarose	1	0.14
3	F1 fraction	38	0.54
4	F2 fraction	0.5	0.07
5	P2 pigment	20.5	3
6	P3 pigment	20.8	3
7	Soil	0.67	0.09

As shown in Table-1 the $CaCl_2$ that has high hydroscopic characteristic by taking the moisture from air for 7 times of its mass loss the capability of much more absorbance of water after becoming saturated. After saccarose 1 time of its mass, Fl fraction three times of its mass, Pl and P3 pigments 20 times of their masses absorb water from air at the same condition loss their hydroscopies. However when the hydroscopy degrees of the soil and F2 fractions are compared with other examples we observe that its hydroscopic character is so little that it is unimportant

From the otherside, as shown in table-l, the hydroscopic degree of PI and P2 pigments are 3 times bigger than $CaCl_2$ hydroscopic degree. According to these results, in daphne leaf, the existence of Pl, P2, P3 and P4 pigments that 3 times more than the hydroscopic degree of $CaCl_2$ strengthen the thesis of these pigments have important functions by taking the water and transporting by the help of plant leaf.

As being experimental to prove P1, P2, P3, P4, P5 and P6 pigments are related with water absorbing and transporting or not,artificial leaf model has been used in vitro condition. For that reason, after giving a leaf shape to filter paper, it is dried in the oven (100°C) by its lower and top layers are absorbed the prepared solvent from Fl fraction or P1, P2, P3 and P4 pigments.

The preparation in this type paper leaf model is settled down in the specific conditions which have 80-100% moisture by putting down perpendicular to do mouth of measure container (Figure 4).

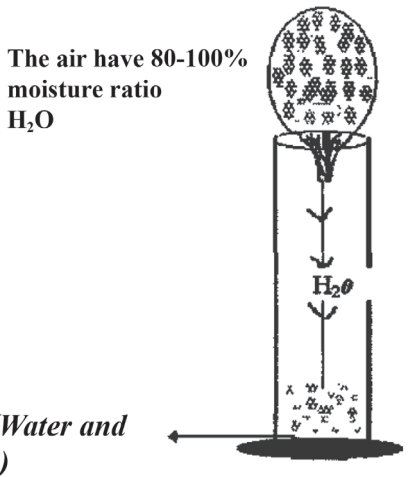

Figure 4. The artificial leaf model and "hydrosynthese"

The leaf model in this position absorbs the moisture found in the air 24-48 hours. (In vitro hydrosynthese) and cause the pigments which were absorbed by paper changing them into liquid form to flow down.

The flowing of water continues until the port formed by pigments that are absorbed on the paper is completely washed. After the paper is cleared from pigments, the absorbing water flowing into the bottom completely ends.

It is observed that the clear water without pigments moves into the handle of the paper leaf at in first 8-10 hours time of the experiment. In this time, as the observed water quantity increases, the hydroscopic pigments that are absorbed on the paper are becoming liquid and stars to flow through the handle of the leaf with water. If the pigments are brought in connecting chemically position on the paper (like in plant leaf) to form liquid by solving in water that was absorbed and flow of the pigments can be prevented at that time. So, to flow of the absorbed water through the handle of leaf can be provided purely.

At these conditions, the possibility of studying at long time is being obtained by the "paper leaf" model that is formed from pigments.

The below results are obtained from the spectrum analyze that is done by separating Fl fraction and it contains P1, P2, P3, P4, P5 and P6 pigments. The Fl fraction shows maximum adsorption (Fl=325-360 nm.) at 325-360 nm. wave length in visual light spectrum. Although P1 and P2 pigments taken purely from Fl fraction have maximum adsorption at 325-332 nm. wave length in P3 and P4 pigments this valve is between 325-340 nm. So, these pigments which are not so different from each other and have dark red, orange red or orange yellow colors according to visual light spectrum can be thought that they are the varies derivatives of similar compounds according to their chemical structure.

The mass spectrum analyze shows that P1, P2, P3 and P4 pigments are also different derivatives of similar compounds. According to mass spectrum, pigments molecular weights are close to each other and differ from M+ 460 to - M+480. It is observed that the melting point of these pigments are between 475°C to 560°C.

The elemental analyze of pigments shows that chemical structure contains. C, H, N and O. It is especially useful to point out the oxygen quantity from these elements is high (%30-50).

To compare with infrared spectrums of pigments shows the functional group region of spectrums are the same (4000-15000 cm-l) or very similar, but finger mark region(1500-400 cm-1) is different from each other.

The existence of adsorption pigs at infrared spectrums 3500(OH),1730(CO),1620, 1585, 1455 cm^{-1} (aromatic cycle, COOH) supports that PI and P2 pigments are possible from antracinon derivatives, P3 and P4 pigments from triphenylmetane derivatives and even if P5,P6 compounds from 1,2 benzedicarboxylic acid derivatives.

On the other hand the possibility has increased that the physical properties of P1, P2, P3 and P4 pigments purred chemically (indicator, the color of crystal color, pH<7 which is yellow color, pH >8 gives dark red color etc.) have similarities with the pigments of triphenylmetane and antracinon, these compounds are antracinon and triphenylmetane derivatives (7).

Further more, since the structure evaluating of P1, P2, P3, P4, P5 and P6 pigments have tested by us, it is abstained to define the sensitive chemical structure of these compounds definitely.

In this study the commentaries will be continued biologically mostly be cause the subject of plant physiology is important.

According to the researches the existence of the hygroscopic degree of pigments more than 3 times from $CaCl_2$'s hygroscopitidy in daphne leaf,shows that compounds have important role in taking and transporting water in the plant themselves.

The event that the leaf model prepared in the basement of these pigments catch the water from air, and push it through the bottom makes the idea possible that the similar functions can occur in living daphne leaf. If the pigments in daphne leaf didn't have the functions catching the water from air and transferring it to the plants cells,it wouldn't be possible to grow without taking water with its root system for the daphne sapling isolated in a nylon bag in dry soil for along time (10 times),and would have dry. In one opinion, daphne sapling root system of which was isolated obtains water to the plant with its leaves by taking not only CO_2 and O from the air but water molecules as well, and transmitting it to the plant cells (3,4).

To provide the important function such as transferring into the plant cells and the water absorbance of leaf it needs to form special "hydrosysthems" that is similar to photosynthese which forms from P1, P2, P3, P4, P5 and P6 pigments so the hydrosysthems which form from hydroscopic pigments

in daphne leaf must have the capability of catching the water molecules on air and transferring into the plant cells. Although it is proofed that free pigments can do this kind of function in vitro condition (the dried pigments taking water from moisture of air more than 20 times of its mass, the study of leaf model being done on paper from pigments)it is useful to think these pigments are working more effectively by creating locations with special proteins in vitro condition. So, the private structural occurrence occurred by in lively leaf or hydrosynthese centers will be work as it occurs in photosynthesis.

Here, apact from photosynthesis, catching water molecules in the air and transferring them to the plant cells get importance. Along term studies are needed with modern equipment's and methods to prove this research which is commended theoretically on the studies and observations being done.

However,according to the studies and our claim, we can explain the character of lively daphne leaf to take water from air and transfer it into the cells as shown below schematically (fig. 5).

The moisture that enter the cell from leaf mouth caught by the water molecules found in the air (CO_2, O_2, water etc.) epydermeces or the hygroscopic P1,P2,P3,P4 P5 and P6 pigments at inner cell or structure units by proteins of these pigments. So, let the water molecules caught be flown into the water tanks found in cells by the help of "Hydrosynthese "centers after they are stored. By the help of ksilem capillaries when the water which first flows through body then root system reaches they roots sucking it creates micro moistured region by pushing some of the water into the soil.

In this position, since the soil is moistured around the soil of roots, the movement through inner cells of plants of cations and anions will continue as in the meaning of plant physiology (8,12). At the end of different, anions and cations found in soil will diffuse all the plant cells by arriving firstly sucking roots, then ksilem capillaries after that leaf cells.

One of the points which must be cleared on this study which is related with the water transferred to the sucking roots then the soil from the leaf is that the water can be given to the soil in a big amount and as a result of this plant can have a water problem. According to us,the cell which forms the system of sucking roots ;makes a selecting between the different ions that enters plant (10,12),and forms system that prevents the water found in the plant cells from going out.

This system which controls the excessive water that flows from leaf to root system into the soil is useful to be thought that it is one of the factors that hinder the unwatering of plant cells.

If the subject is criticized from the point of evoluation,we think that this study is not contrast to the explanation of classic physiology on water taking of the plants and transmitting it.

Existence of hydrophilic pigments which have higher hydrophilics in leaf cells and tissues with protein, carbohydrates and hydrophile matters can cause osmatic pressure which occurs in cells, increase more and more so that water can be sucked easily by the root system is provided. In other word, the existence of hydroscopic pigments in the leaves of the plants which provide their own water only with their roots make them strong against the drought and provide them use the water possibilities found in the soil better.

On the other hand,being different from taking water plants from soil by root systems in some green plants (daphne. pine etc). In all season by developing of root system in evaluation, fhe hydroscopic pigments found on leaf the taking water system from air (Hydrosyntese) is developed. By this, these type of plants have gained twice more safety quarantine against the struggle of drought for the sake of life.

Daphne which is one of the plants that we think its syntheses system developed has chosen an appropriate life condition to the system. In the condition, moisture ratio changes between %80-100. Such a moisture contain can provide the need of water completely when the hydrosynthese required to work.

Another appraising of the subject related with evaluation according to us is that the event of taking water at plants occurs before differentiation of "hydrosynthese" event. "Hydrosynthese" event has been continuing at simple plants (land mosses etc). Although the root systems are not

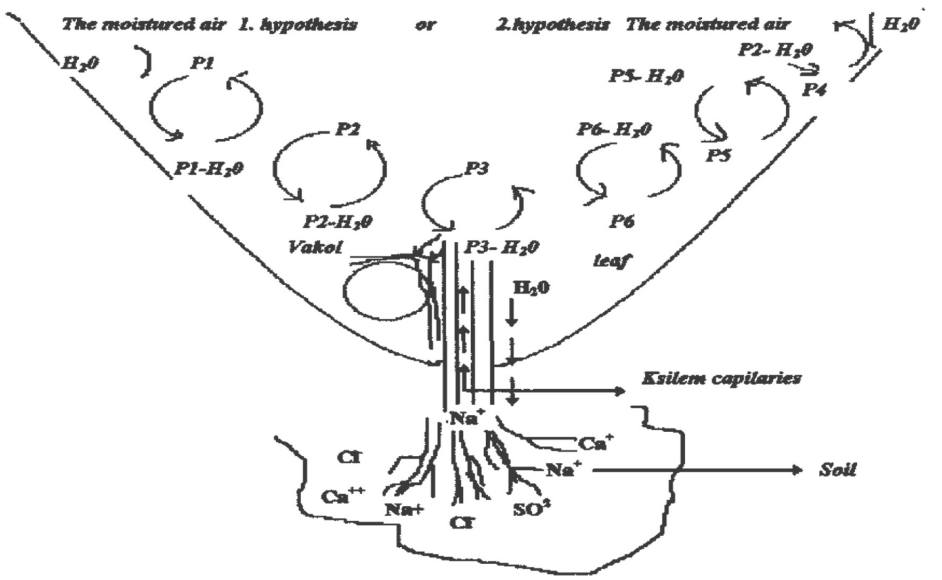

Figure 5. The conducting of water through leaf to root.

developing, they are able to assure their own water needs. According to us, this type of simple structural plants take the probability of the absorbing water from air by the help of hydroscopic compounds found in their cells In other word the hydrosynthese event has transferred from the plants that have high structure organization to the plants with simple organization and developing it be came perfect. By changing the life conditions, some plants that are exposed to differantion have lost their "hydrosynthese system" partically not completely be cause they do not need it.

However some of them (daphne etc.) have developed by developing "hydrosynthese" system in its leaves with reverse root system. These plants that have two side taking water mechanism (daphne etc.) have great advantage in struggling for the sake of life.

RESULT

At the end of the study;

1. In vitro condition, the daphne sapling of which root mechanism has been isolated with the soil has a mechanism to adsorb the water from the moisture in the air with its leaves and this new observed mechanism of the green leaf is called "hydrosynthese".
2. It is explained that the special hydroscopic pigments (P1,P2,P3,P4,P5 and P6) made "hydrosynthese" at daphne leaf have separated from leaf chemically and it is prooofed that these pigments are making "hydrosynthese" from air at in vitro condition.
3. It is proofed that the hydroscopic pigments that are separated from daphne leaf had maximum adsorption 325-360 ran. In visual spectroscopy and these pigments hydroscopic degree more than three times from CaC12's hydroscopic degree.
4. Showing that the daphne plant supplying its water need with its leaf and root system'it is emphasizes that be cause of this "hydrosynthese" event done in the leaves has more advantage.

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