

### **(3) Summary of Open Seminar**

#### **1) Studies on Salinity Tolerance in Crops (9:30-12:00, Jul. 17, 1997)**

##### **Variation and Physiological Mechanism of Salinity Tolerance in Rice**

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Although it is well known that rice is a relatively sensitive crop to soil salinity, there is a broad variation in the range of salinity tolerance among rice varieties. According to Yeo et al. (1990), tall varieties, normally low-yielding, are generally more salinity-tolerant than agronomically favorable semi-dwarf varieties. Despite of many and long-time efforts by rice breeders to introduce the salinity-tolerant trait into high-yielding varieties, so far very few reports on a successful case. It has to be still necessary to clarify the mechanisms of salinity tolerance, which would be a cause of the extensive intraspecific variation. In this seminar, the proposed physiological mechanisms of salinity tolerance are summarized, briefly along with up-dated results on the effects of salt stress on photosynthetic and oxidative/antioxidative reactions in rice plants. The most Characteristic, which tolerant varieties show under salt stress during vegetative growth, is the ability to restrict the flow of sodium ions into the shoot part and keep the better K/Na or Ca/Na ratios (Yamanouchi, 1993). However, it seems that its physiological and biochemical mechanism(s) are not yet well explained even after a series of excellent studies by Tsuchiya (reviewed in 1996). The salt-tolerant mechanism in the seed-germinating or reproductive-growth stage is necessarily different from that in the vegetative-growth stage. New approaches of JIRCAS to breed salt-tolerant high-yielding rice varieties are also introduced in this seminar. These are 1): Utilization of artificial mutant populations; 2): Utilization of somaclonal variants; 3): Studies on the physiology of salinity tolerance in wild rice species; and 4): Evaluation of alien genes which are responsible for salinity tolerance and their introduction to rice plants.

##### **Possibilities of Molecular Breeding of Salt-tolerant Crops**

*Mariko SHONO\**

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The accumulation of salts in agricultural soils is a problem that has been with man since he started cultivation. The majority of crops are relatively sensitive to salts, and their growth is limited by the saline soil which includes high concentrations of Na<sup>+</sup> ions. Na<sup>+</sup> ions has two possible toxic effects on cells. One is the specific toxicity of the ions on defined cellular systems and the other is the nonspecific osmotic effect. Regulation of ion concentration in cytoplasm is crucial for plant growth under saline conditions because it leads to a disturbance of ion homeostasis. The existence of a Na<sup>+</sup>-activated ATPase was demonstrated in the plasma membrane of *Heterosigma akashiwo*, a wall-less unicellular marine alga. The ATPase activity and the ATP-dependent transport of Na<sup>+</sup> ions had close resemblance to those of animal Na<sup>+</sup>/K<sup>+</sup>-ATPases (Na<sup>+</sup> pump). In a further investigation, the Na<sup>+</sup>-ATPase was solubilized and partially purified. The purified Na<sup>+</sup>-ATPase was a 140-kDa single polypeptide. If the cDNA of this Na<sup>+</sup>-ATPase can

be isolated, it may become a good candidate for recombinant DNA technology to improve salt tolerant crops. Some higher plants accumulate special organic solutes, such as polyols (sorbitol, mannitol), sugars (trehalose, sucrose), and some amino acids and derivatives (proline, betaine), which serve as osmoprotectants following exposure to NaCl stress. The *betA* gene codes for choline dehydrogenase (CDH) which catalyzes the first step of betaine biosynthesis pathway in *E. coli*. We introduced the *betA* gene into rice plant and confirmed the accumulation of betaine in the transgenic rice. The transgenic rice showed salt and drought tolerance more than non-transgenic rice.

Recombinant DNA technology for crop improvement can be used in new breeding programs with increased salt resistance when some particular genes from any organism, like *betA* gene, are identified as salt resistance related genes.

### **QTL Mapping of Salinity Tolerance in Rice by Using Molecular Marker**

*Seiji YANAGIHARA\**

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Salt in irrigation water or salt accumulation in soil leads a growth deficiency and serious yield reduction of rice. To solve the problem, we can approach from improving irrigation system, new varieties or something else. We have started QTL (Quantitative trait loci) mapping of salinity tolerance to aim tagging genes concerns on salinity tolerance either positive or negative. The RI (Recombinant inbred) population of 142 lines have been used for QTL mapping which was established in China by single seed descent procedure from Tesanai 2/CB cross. RI lines were evaluated for seedling survival date in culture solution with electrical conductivity, EC=12 dS/m by adding NaCl. A linkage map consisted of 46 RFLP (Restriction fragment length polymorphism) markers was constructed by using 53 markers. It could show 11 linkage groups. Only one marker locus RG13 on chromosome 5 was detected as significant marker for salt tolerance and it explained 11.6% of the observed phenotypic variance for SD (Survival day). The allele from Tesanai 2 increased salt tolerance at the locus. Linkage map is under expansion and further significant loci for salt tolerance are expected. To obtain genes for salt tolerance, cDNA libraries were constructed from salt stressed Teasnai 2 seedlings and its control. Differential screening is carrying on to detect salt stress specific genes.

## **2) Current topics of soil physics and their application to the arid land studies (13:00-16:00, Dec. 8, 1997)**

### **Studies on Characteristics of Heat and Water Transfer in Soils**

*Toshitugu MOROIZUMI\**

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The characteristics of heat and water transfer in soils were investigated from both the laboratory and field experiments and the numerical analysis. Firstly, experiments and simulations to determine the characteristics of heat transfer were conducted. It became clear that most of heat transfer can be

explained only by the heat-conduction and heat-loss terms, and the influence of natural convection on heat transfer can be neglected. Secondly, the heat and water transfer experiments were conducted using an insulated soil column with simulated ground water at the bottom. Experimental data were simulated by using the one-dimensional model of coupled flow, considering the heat-loss. The influences of hydraulic conductivities on the simulation were also investigated, using two estimated hydraulic functions. Finally, the heat and water transfer in the unsaturated soil zone under field conditions were investigated by applying the coupled model to the soil temperatures and pressure heads which were measured under natural conditions. And the influences of a correction coefficient and surface boundary condition on the changes of temperature and pressure head in the soils, and the estimation of evaporation by a water balance method were also discussed.

### **Simple Methods for Measuring the Water Retentivity and Permeability of Soils**

*Naomasa NISHIMURA\**

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For the measurement of the unsaturated hydraulic conductivity, a simple method is proposed using the soil under the condition of the steady evaporation without troublesome problems such as the clogging of the filter in the apparatus and its incomplete contact with the soil sample. The experiment using the apparatus made for this purpose is not only quite easy in practice, but also the water retentivity can be measured using the same apparatus constructively as used for this experiment. But the suction gradient can not be controlled in this method. So the nonlinear distribution of the suction through the soil sample must be considered for the precise calculation of the unsaturated hydraulic conductivity. For this purpose, two techniques of calculation are proposed, called 'the dual method' and 'the series method'. The calculated values of the unsaturated hydraulic conductivity by these two methods using the experimental data during the steady evaporation almost agree with the results of measurements by the suction method.

On the other hand, for the measurement of water retention, a simple method is proposed using a soil slab in the constant-rate stage of drying. For this purpose, we made an instrument by which the suctions can be measured at the upper and lower locations in the soil slab in which one-dimensional vertical unsaturated flow occurs. The structure and the manufacturing method of the instrument are explained in this paper. Also, another instrument was used for measuring the total weight of a soil slab equivalent to that for the suction measurements. By using these instruments, experiments were done to measure the drying process caused by the evaporation from the upper surface of the slabs of three typical soils which differ remarkably from each other in void ratio. On the other hand, two approximations were introduced with respect to the suction profile and the suction-water content relation of the soil slab at the constant drying stage in order to calculate the water retention using the measured data. The accuracy of these approximations was also examined. The water retentions of the soil slabs were calculated using these approximations. The results showed that the water retention can be obtained continuously in low suction range with accuracy by this method in case of sand, sandy loam, and well-structured soils which have high saturated conductivity.

In addition, another method is proposed for the estimation of unsaturated hydraulic conductivity using the same measured data at the end of constant-rate stage of drying of the soil slab. This method needs the experimental data of saturated hydraulic conductivity and water retention in high suction range of the soil sample in addition to the water retention in low suction range obtained from the measurements during the

constant-rate stage. An empirical function of unsaturated hydraulic conductivity versus water content was also introduced in order to calculate numerically the suction change in soil slab under evaporation by using finite element analysis. By means of these informations, unknown parameter in the function of unsaturated hydraulic conductivity could be determined so as to agree the analytical result of suction change with the experimental one at the end of constant-rate stage of drying.

### **The Use of Frequency Domain Reflectometry for the Measurement of Liquid Water Content with Change of Soil Water Phases**

*Beiping, Ji \**

\* Arid Land Research Center, Tottori University

Using FDR (Frequency Domain Reflectometry), That freezing soil dielectric constant ( $\epsilon_f$ ) is temperature dependent has been researched during the soil freezing and thawing procedures. What is measured directly by TDR (Time Domain Reflectometry) or FDR (Frequency Domain Reflectometry) is not soil water content or unfrozen soil water content ( $w_u$ ) but frozen soil dielectric constant ( $\epsilon_f$ ).

In this paper, using the two-phase frozen soil model, which is originally advanced by the author. The equation describing the relation between  $\epsilon_f$  and  $w_u$  has been deduced. The results are as follows:

1. That  $\epsilon_f$  has temperature dependence in frozen soil as well as in unfrozen soil has been researched with FDR method.
2. The same soil samples are used in freezing and thawing procedures, the measurement of dielectric constant with FDR shows that  $\epsilon_f$  in freezing procedure is higher than  $\epsilon_f$  in thawing procedure. In both procedures,  $\epsilon_f$  varies rapidly at around 0 . Furthermore, the increasing gradient of  $\epsilon_f$  in thawing procedure is greater in this temperature range.
3.  $\epsilon_f$ , which is dependent of initial soil water content, increases with increasing initial soil water content.
4. At around 0 ,  $\epsilon_f$  increases a little at first, then decreases rapidly. This phenomenon is also observed in water overcooling procedure. And the reason is not found yet. The author is considering that because water dielectric constant is temperature dependent, before water molecule changes to ice, accumulative charge catcher takes place.
5. According to the electric-magnetic wave theory and soil physical characteristic, the equation describing the relation between  $w_u$  and  $\epsilon_f$  and the analytical solution of this equation have been deduced. The quantitative analysis of every physical parameter has been done with this analytical solution. Absolutely frozen soil dielectric constant (no liquid water)  $\epsilon_s$  is dependent of soil water content, porosity and frost heave.
6. With generally analysis of every variable, the value of  $\epsilon_s$  varies from 3.2 to 5.4. And all the variables appearing in the equation are not artificial but have their own physical meaning.
7. According to the calculation of  $w_u$  in the frozen soil, that  $w_u$  is initial water content dependent and the hysteresis behavior are cleared. Furthermore  $w_u$  and  $\epsilon_f$  have the similar temperature dependence.
8. This analytical solution is not only used for FDR, but also for other measurement method with  $w_u$  can

be obtained by using f.

### 3) Studies on Plant-Plant Interactions by Allelopathic Compounds (10:00-17:00, Dec. 19, 1997)

#### Biology and Regulation of Parasitic Weeds

Yasutomo TAKEUCHI

Weed Science Center, Utsunomiya University

Parasites are quite distinct from other weeds in forming intimate attachments to their host crops, whether to roots as in *Striga*, *Orobanche* and *Aeginetia* species, or to shoots as in *Cuscuta* and mistletoe species.

Seeds of root parasites are extremely small, between 200 and 400  $\mu\text{m}$ , and they can survive more than 10-20 years in the fields. In the life history of hemi-parasite, *Striga* and holo-parasite, *Orobanche*, the first stage is called "after-ripening" or "post-harvest ripening". During conditioning stage, a moist environment is required for several days together with suitable temperature in order to render the ripe seed responsive to germination stimulants. Germination is followed by "haustrial initiation" induced by a second host-derived chemical, and followed by development, "attachment" to the host root and, lately, "penetration of the vascular tissue". Shoot apices normally develop only after contact has been established with a host root. *Striga* and *Orobanche* may need hormones from a host for shoot development. Hemi-parasite, *Striga* shoots will emerge from the underground 4-5 weeks after the attachment to host roots. On the other hand, more than half of the life cycle of holo-parasite, *Orobanche* and *Aeginetia*, is subterranean before flowering.

As water and foodstuffs are transported from hosts to parasites, stunting and yield reduction occur in hosts. This is achieved by high rate of osmotic pressure and transpiration of ever-open stomata in parasites. Stimulative and inhibitory chemical-signals are exchanged in both directions between parasites and their hosts. Members of gibberellins of both the early-13- and early-non-hydroxylation pathways were identified from both *Orobanche minor* and its host, *Trifolium repens*. And the parasite may also have the ability to produce other gibberellins, such as GA<sub>38</sub> and GA<sub>47</sub>.

*Orobanche minor* and *A. indica* will flower following flowering of their host plants in Japan. However, little information is available on the induction of flowering in root parasites.

Control methods include herbicides, mechanical, cultural and biological means. Biological control includes germination stimulants, insects, fungi, trap crops, catch crops and breeding for resistance. Ethylene, a germination stimulant, gives excellent control of *Striga* by inducing "suicidal germination". Selective translocated-herbicides can kill the weeds. With the availability of glyphosate-resistant lines of host crops, the use of glyphosate for selective control of parasites may also become a common practice. Breeding for resistance in *Striga* has been undertaken as a very promising control measure. Strong dependence of parasites on their host plants for developmental signals may eventually lead to more effective control methods by disruption of the signals they require.

### ***Striga* on sorghum: Host-parasite relationship**

A. G. T. BABIKER

Arid Land Research Center, Tottori University

Witchweeds (*Striga* spp.) are economically important root parasitic weeds on several poaceous and leguminous crops. *S. hermonthica*, the most damaging and widely distributed species, has become the major biotic factor threatening sorghum production in Sub Saharan Africa. Losses in grain yield are often significant (>70% ) leading to abandonment of land and migration of villages. The prevailing drought conditions coupled with poor soil fertility and intensified monocropping of sorghum have increased infestation and spread of the parasite. The life cycle of the parasite is strongly cued to that of its host. *Striga* has evolved very specific recognition systems to host-derived molecular signals, that allow close monitoring of the environment and precise timing and regulation of developmental events. The parasite produces a large number of seeds of minute size and limited reserves. A newly ripened seed is effectively dormant. To germinate a seed requires an after ripening period, a pretreatment in warm moist conditions followed by exposure to an exogenous germination stimulant exuded by the host roots. The labile nature of the host-derived stimulants, sorgoleones and strigolactones, ensures germination in close proximity of the host root. Following germination the radical elongates, chemotropically, towards the host root. Close to or on contact with the host root a haustorium, the organ for attachment and penetration, is induced in response to a second host-derived signal. The haustorium inducer, 2,6-dimethoxy-*p*-benzoquinone (2,6 DMBQ) is synthesized by enzymatic degradation of the host root surface components. Guided by lytic enzymes and probably by signals provided by the complex internal chemistry of the host, the haustorium attaches to, penetrates the host root and establishes connections with the xylem elements. *Striga* shoot, presumably under the influence of unidentified host-derived signals, develops and remains subterranean for 4 to 6 weeks. During the subterranean phase of development the parasite inflicts considerable damage to its host. A *Striga* infected plant shows chlorotic leaves, water deficit symptoms and stunted shoot growth. Root growth, on the other hand, may be stimulated. The pathological effect of the parasite is associated with inhibition of photosynthesis and disruption of host hormonal balance. *Striga*, drastically, reduces cytokinins and gibberellins and increases abscisic acid and farnesol in the host xylem sap. Host defense mechanisms include avoidance, localized necrosis, suberization, callose deposition and wall lignification. The significance of the host-parasite relationship with regard to crop field performance and efficacy of control measures will be discussed.

### ***Orobanche minor* Germination Stimulants Produced by Red Clover**

Takao YOKOTA

Department of Biosciences, Teikyo University,

The broomrapes are root parasites belonging to the genus *Orobanche* which is the most important in the family Orobanchaceae. Among the various species of the genus *Orobanche*, *O. ramosa*, *O. aegyptiaca*, *O. minor*, *O. cernua* and *O. crenata* are major crop parasites which are distributed mainly in Mediterranean countries. It has been demonstrated that *Orobanche* seeds germinate when exposed to stimulants excreted by the roots of the host plants. We have investigated components produced by red

clover roots which promote the germination of seeds of *O. minor* Smith.

Red clover was grown hydroponically using tap water under continuous fluorescent light. Germination stimulants were extracted from ca. 1700 liters of the medium by using XAD-4. The extract was purified by Sephadex LH-20 chromatography and the active fraction was purified further by HPLC using a C18 column resulting in the separation of three stimulants I, II and III in the order of elution. Although stimulant I is not further pursued because of its very low activity, stimulants II and II were further purified to homogeneity by HPLC with an Aquasil column and Phenyl column.

Stimulant II was a new compound and named orobanchol. It had very similar retention times to those of strigol on HPLC with C18 and silica supports although it was eluted slightly earlier than strigol on Phenyl-HPLC. Stimulant II had an UV maximum at 242 nm. GC-MS analysis of its TMSi ether suggested that compound II is a double-bond isomer of strigol. Stimulant III was identified with aletrrol on the basis of the HPLC behaviors, <sup>1</sup>H-NMR and GC-MS.

### **Synthesis of All Eight Stereoisomers of the Germination Stimulant Sorgolactone and Their Biological Activities**

*Yukihiro SUGIMOTO*

Arid Land Research Center, Tottori University

The naturally occurring sesquiterpene sorgolactone belongs to the class of “strigolactones”, which are highly potent germination stimulants for seeds of the parasitic weeds *Striga* and *Orobanche*. Because of the lack of spectroscopic detail due to the small amount of sorgolactone obtained (5 µg from 300,000 sorghum seedlings) and the extremely laborious isolation procedure, the proposed structure could not be ascertained. Therefore the total synthesis of the stimulant was undertaken to verify this tentative structure and to unambiguously establish the absolute stereochemical configuration. Evaluation of respective bioactivities of sorgolactone stereoisomers was also conducted to provide more insight in the structure activity relationships of these germination stimulants.

Two racemic diastereomers of the ABC-part of sorgolactone were prepared and coupled with homochiral latent D-ring synthons. In this manner, 4 mixtures of 2 separable, protected sorgolactone diastereomers were obtained. Deprotection gave all eight target compounds as single isomers. Combination of the relative *trans*-configuration of the natural sorgolactone ABC-part (determined by NMR) with the absolute stereochemistry between the C-ring stereogenic centers C8bC3a and C2' of naturally occurring sorgolactone (determined by CD-spectrometry) leads to the conclusion that the proposed absolute structure of natural sorgolactone is correct.

The germination stimulatory activity of all eight stereoisomers was assayed using seeds of *S. hermonthica* and *O. crenata*. The results clearly indicate that the sorgolactones are considerably less active in germinating *Orobanche* seed. However, as far as the absolute stereochemistry is concerned, the trend is the same as for *Striga* seeds. Only those isomers containing the same absolute stereochemistry as “natural” sorgolactone at 2 adjacent stereocenters exhibit significant germination stimulatory activity at critical concentrations.

### **Novel Germination Stimulants for *Orobanche minor* and *Striga hermonthica* Seeds**

Koichi YONEYAMA

Weed Science Center, Utsunomiya University

Among fungal metabolites examined for their effects on seed germination of important root parasites, witchweed [*Striga hermonthica* (Del.) Benth., *Striga*] and clover broomrape (*Orobanche minor* Smith, *Orobanche*), cotylenins (CNs) and fusicoccins (FCs), metabolites of fungi *Cladosporium* sp. 501-7W and *Fusicoccum amygdali* Del., respectively, induced high seed germination (>50%) of both parasites at a concentration of  $10^{-5}$  M. It should be noted that there has been no report on potent stimulants for *O. minor* seed germination, except for strigol-related compounds (strigolactones).

Inhibitors of ethylene biosynthesis [2-(2-aminoethoxyvinyl) glycine (AVG)] and its action [silver thiosulfate (STS)] reduced *Striga* but not *Orobanche* germination induced by CNs and FCs. This suggests that induction of *Striga* germination by CNs and FCs, as in the case with the true natural stimulant “strigol”, requires both ethylene biosynthesis and action, while that of *Orobanche* does not.

FCs and CNs display various physiological effects in plants, promoting seed germination and stimulating stomatal pore opening both in antagonism with abscisic acid (ABA). Under our experimental conditions, ABA was found to inhibit seed germination elicited by CNs and partial recovery of the ABA inhibition by CNs was observed. However, it is not clear whether CNs and FCs antagonize ABA in seed germination of *Striga* and *Orobanche*.

FCs are suggested to bind to the receptors which are involved in the regulation of key enzymes including plasma membrane H<sup>+</sup>-ATPase and soluble nitrate reductase. Recently, receptors of FCs have been identified as 14-3-3 proteins which are known to play a pivotal role in signal transduction of ethylene and phytochrome. Therefore, promotive effect of CNs on seed germination of *Monochoria vaginalis*, which requires light and flooded conditions for germination, may be through their effects on phytochrome and ethylene signaling pathways. Although receptors of germination stimulants in *Striga* and *Orobanche* seeds have not been identified, our results suggest that 14-3-3 proteins may be possible candidates for the receptors. The involvement of ethylene in *S. hermonthica* and *S. asiatica* germination induced by strigolactones and other chemicals may support this supposition.

### **Structures and Biosyntheses of Phytotoxins in *Cochliobolus spicifer* and *Bipolaris sorokiniana*:**

#### **C<sub>3</sub> Unit Addition Reaction in Phytotoxin Biosyntheses**

Hiromitsu NAKAJIMA

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Two fungal metabolites, cochliosporin A from a strain (D-5) of *Cochliobolus spicifer*, a pathogen of leaf spot disease in Gramineae, and sorokinianin from a strain (OB-25-1) of *Bipolaris sorokiniana*, a pathogen of spot blotch or foot and root rot diseases of barley and other grasses, were isolated and characterized. The deduced chemical structure of cochliosporin A indicates that it is composed of two portions; polyketide origin portion structurally related to spiciferinone, a phytotoxin of *C. spicifer* and C<sub>3</sub> unit portion. Sorokinianin is assumed to be composed of helminthosporol-type sesquiterpene portion and C<sub>3</sub> portion. Although they have β-hydroxy-γ-lactone as C<sub>3</sub> unit portion in common, sorokinianin shows phytotoxicity, whereas cochliosporin A does not.

To clarify the biosynthetic origin of sorokinianin we conducted the following experiments: stable



isotope labeled precursor feeding experiments; a replacement culture experiment with the candidates for the C<sub>3</sub> unit precursor, together with prehelminthosporol, the major metabolite of strain OB-25-1. In the [1,2-<sup>13</sup>C<sub>2</sub>] acetate feeding experiment the <sup>13</sup>C NMR data indicates that 7 intact units of acetate are incorporated into sorokinianin and its labeling pattern, except for the  $\gamma$ -lactone portion, coincides with that of the helminthosporol-type metabolite. The labeling pattern of the  $\gamma$ -lactone portion suggests that it is derived from the acetyl CoA-related metabolic pathway. In the replacement culture experiment addition of prehelminthosporol increased the production of sorokinianin to 4-6 times that without prehelminthosporol. The simultaneous addition of prehelminthosporol and fumaric acid markedly stimulated the production of sorokinianin to ca. 20 times that of the control. The same effect was observed for succinic acid, whereas alanine, serine, propionic acid and glycerol had no effect. Administration of [2,3-<sup>13</sup>C<sub>2</sub>] succinic acid to the culture medium resulted in an efficient incorporation of <sup>13</sup>C into the C<sub>3</sub> portion of sorokinianin.

The results of these experiments suggest that sorokinianin is biosynthesized from prehelminthosporol by the reactions that include the addition of a TCA cycle intermediate to prehelminthosporol, decarboxylation and lactonization.

### **Potent Allelochemicals in Wild Plants, *Polygonum sachalinense* and *Heracleum dulce***

*Hiroyuki NISHIMURA*

Department of Bioscience and Technology, School of Engineering, Hokkaido Tokai University

Chemical interactions among plants have long been recognized. Many scientists have shown that certain plants produce secondary metabolites which have the ability to inhibit growth and germination of other plants. The genus *Polygonum* has about 300 species and is distributed all over the world. *P.sachalinense* is a perennial grass found in Honshu and Hokkaido. The wild plant forms colonies along roadsides and river banks in gravelly soils. Judging from its ability to rapidly colonize, we hypothesize that allelopathy contributes to its aggressiveness.

The root exudates from *P.sachalinense* in a recirculating system significantly inhibited lettuce seedling growth. The rhizomes and roots of *P.sachalinense* were extracted with 70% acetone. Bioassay of the neutral-acidic fraction on the TLC agar plate showed the inhibitory activity corresponded to the two yellow pigment bands. Two orange needles were isolated and identified as anthraquinone compounds: emodin and physcion. Both compounds exhibited inhibitory activities against the seedling growth of several testing plant species. Glucosides were isolated from *P.sachalinense* and were identified as emodin-1-*O*- $\beta$ -D-glucoside and physcion-1-*O*- $\beta$ -D-glucoside, which showed no phytotoxic activity against lettuce seedlings. Emodin and physcion occurred in relatively high concentrations in the rhizome with roots, fallen leaves and the soil beneath the plant. The results indicate that these anthraquinones are responsible for the observed interference and are potent allelochemicals.

In the course of our research on roles of secondary metabolites in an ecosystem, we also have investigated allelochemicals in *Heracleum dulce* (Umbelliferae). The weed vigorously grows in a wild place and forms colonies along roadsides and river banks. The root exudates from *H. dulce* showed an inhibition on lettuce seedling growth using the recirculating system. From the results of the TLC agar plate bioassay using lettuce, timothy grass and green amaranth, several growth inhibitory bands were observed on the chromatogram of the neutral-acidic fraction. At least, eleven inhibitors were isolated and identified. Among those inhibitors, significant compounds which may play a role in allelopathy are

furanocoumarins; bergapten, pimpinellin and isopimpinellin etc.

#### **4) Mechanism of Water Erosion in Marine Saline Soil (13:00-16:00, Jan. 7, 1998)**

##### **Measures of Improving the Engineering Properties of Soft Clay Ground in Ariake Bay Area**

*Masateru TAKAYAMA*

Faculty of Agriculture, Kyushu University

Marine clay sediment, with its surface now above sea level, covers several hundred kilometers around Ariake Bay. Low strength and high compressibility of Ariake clay have led to widespread geotechnical problems such as subsidence by overpumping of ground water, ground settlement by the construction of structures such as dikes and agricultural facilities, and the rupture of canals due to excavation of the ground. To cope with those problems several methods to improve the soft clay ground, such as the installation of piles, preloading by earth embankment, and chemical stabilization of soil with cement and lime, have been performed in Ariake Bay area. In the present report, the geotechnical properties of Ariake Bay clay sediment are reviewed, and the methods to improve the soft clay ground employed in this area are presented.

##### **The Role of Salinity in the Behavior of Marine Clays**

*Masami OHTSUBO*

Faculty of Agriculture, Kyushu University

The present report describes mineralogy, chemistry and geotechnical properties of quick and nonquick clay profiles in Ariake Bay, and the correlation of the mineralogy and chemistry of the clays with their geotechnical properties. The chemistry was discussed in terms of saline environment at the time of deposition, and postdepositional weathering processes. The major factors which increase the activity of the clay were both smectite and salinity in pore water for the nonquick clays. There was a negative correlation between the sensitivity and salinity in pore water, and a positive correlation between the sensitivity and liquidity index for the combined data on quick and nonquick clays. The compression index of undisturbed clays was found to be represented as a function of the liquid limit and sensitivity.

#### **5) Present status of afforestation in semi-arid areas (14:00-17:00, Jan. 27, 1998)**

##### **Afforestation techniques in semi-arid areas of Chile**

*Tadayoshi KOMIYA*

Forest Agency, Tokyo

The project on Erosion Control and Afforestation in the Catchment Basins of Semi-arid areas, Chile, which has been conducted by the Japan International Cooperation Agency (JICA) and the Corporacion Nacional Forestal (CONAF), was commenced in 1993. The main purpose of this project is to improve the quality of life of the peasants by the rehabilitation of soils.

*Eucalyptus* (*E. globulus* and *E. camaldulensis*), *Acacia*, and several native species such as quillay (*Quillaja saponaria*) and maiten (*Maytenus boaria*) were produced in the nursery.

In afforestation, techniques such as shelterbelts, agriculture terraces and mulch for maintaining humidity, are applied in this project. Another remarkable one is the Japanese "cluster" planting technique which consists of three specimens planted together.

Erosion control is also very important for the success of afforestation, and techniques applied in this project are retention ponds, infiltration ditches, sandbag parapets, and dams.

### **Present status of afforestation in semi-arid areas of Kenya**

*Hiroshi MASUKO*

Japan International Cooperation Agency (JICA)

The Kenya/Japan Social Forestry Training Project in Kitui has been conducted by the Kenya government and the Japan International Cooperation Agency (JICA) since November, 1986. It is composed of two major schemes, namely, Social Forestry Training and Pilot Forest. Under Social Forestry Training scheme, 3100 people were trained at Muguga and Kitui training centers.

The Pilot forest scheme is composed of Field, Nursery and Extension section. The purpose of Pilot forest is to identify, develop and elaborate basic and appropriate afforestation techniques for ASAL (Arid and Semi-arid Land) conditions through various biological and technological researches. Disseminating these findings is also conducted through the extension and training components to the targeted groups.

This Project established more than 300ha of trial plantations supporting over 72 indigenous and exotic species.

## **6) Salt Accumulation Mechanism in Root Zone**

**- Movement of Water, Salt, and Heat Transport - (9:30-12:00, Jan 27, 1998)**

**On the Four Electrodes System to Measure Soil-Water Content in the Field**

**- Theoretical and Practical Development –**

*Masaki TOMINAGA*

National Research Institute for Earth Science and Disaster Prevention

Four-electrode-method, commonly known as Wenner method, to measure specific resistance of the earth is developed not only theoretically but also practically. The method has such strong points mainly from hardware that measurement can be done in short period, electrodes do not interfere with the change of soil-water content, and sensors are dependable mechanically when they are set under the ground.

Considering these strong points and through theoretical analysis, the “Normalized apparent specific resistance” is proposed to be measured in order to observe the dynamical change of soil-water content responding to the time-varying rain input at the surface of the ground. To establish fast measurement system, electronic circuit is developed and used successfully in the experiment of rain infiltration.

**Monitoring System for Water Flow and Solute Transport  
- Simultaneous Measurement of Water and Salt -**

*Mitsuhiro INOUE*

Arid Land Research Center, Tottori University

At the Arid Land Research Center of Tottori University, a national joint-use research institute, a monitoring system for water flow and solute transport has been set up inside a glasshouse of the Arid Land Dome which is 15 meters high and 36 meters in diameter. This new experimental facility allows experiments in which the movement of water and salt can be monitored in large soil columns with 798 mm in diameter. Soil matric potential head, soil temperature, bulk soil electric conductivity and volumetric water content in the large soil columns can be determined using underground suction gauges (UNSUC), thermocouple sensors, four-electrode salinity sensors, and amplitude domain refractometry sensors (ADR), respectively. In this seminar, the characteristic of the measuring system was described. The measuring accuracy of various sensors and electric balance was discussed. The validity of a monitoring system for water flow and solute transport was also discussed.

**7) Salt Natural Environment and Research Theme in Arid Land (13:30-15:00, Feb. 12, 1998)**

**Meteorological Environment and Agriculture in Arid Land**

*Taichi MAKI*

Division of Agrometeorology, National Institute of Agro-Environmental Science

Revegetation has been promoted in arid and semi-arid lands of China. However, desertification has also increased because of the over-development, the over-grazing and the over-deforestation that were caused by the increase of human population.

The crops which should be produced in arid land of China require drought resistance, wind tolerance, heat tolerance, cold hardiness and salt tolerance. Furthermore, the belt of the windbreak forest is the essential equipment in order to manage farming under the severe environment and the systematic improvement of environment is also needed. On this improvement, it is necessary to evaluate the amount of useful water and water consumption not to develop excessively. On those understandings, the meteorological improvement by the windbreak forest and net, and their effects on promoting the crop growth were reviewed showing the results of research which was made in Tulpan, Northwestern China to evaluate the availability of the windbreak forest in the boundary region between the desert and the oasis.

### **Challenge to Sustainable Social Growth –Environment• Food• Energy-**

*Yoshitaka NITTA*

Division of Research and Development Management, Central Research Institute of Electric Power Industry

The coal consumption which occupies about 80 percent of primary energy in China causes the air pollution and the acidic rain. On the other hand, the 340 thousand km<sup>2</sup> of alkaline soil is distributed in China, and the soil improvement by the gypsum is required. Taking an attention to such situation in China, the current research for a integrated measure of environmental protection was reviewed. The desulfarization device of coal was conducted in electric power industries and the factories to reduce the air pollution. In the process of desulfarization the gypsum is produced secondary and this gypsum is useful as a soil improvement material to convert the vast dead land to the agricultural land. It is necessary to search the new research like this, for the problems related to the human survival in 21<sup>st</sup> century, which include the increasing population, the lack of energy and food resource and air and water pollution.

## **8) Plant biology in temperate dry areas of Asia (10:00-16:00, Feb. 20, 1998)**

**Environmental and vegetation change since the Tertiary to Quaternary in Central Asia**

*Arata MOMOHARA*

Faculty of Horticulture, Chiba University

In the Paleo-Eocene (c.a. 65 - 35 myBP), the Tethys Sea was distributed widely in the area including the recent Mediterranean Sea, Aral Sea, and Western Siberia Lowland. During the Oligocene (c.a. 35 - 23 myBP) the Tethys Sea emerged into land areas and flood plains were distributed in Central Asia. Deciduous broad-leaved forests with Fagaceae and *Acer* developed in and around Kazakhstan between the Oligocene and Middle Miocene. This area was an important corridor for plant migration between Asian and European Continents that were separated before the Eocene. In the Middle Miocene (c.a. 15 myBP), the uplift of the Tibetan Plateau caused the dry climate and the appearance of steppe vegetation including *Ephedra*, *Cenopodiaceae*, *Compositae*, and *Gramineae* in central Asia. The dendroflora characterizing the Oligo-Miocene flora in central Asia became extinct and survived in Caucasia in part. Since the Pliocene (c.a. 5 - 1.7 myBP) to Pleistocene (since 1.7 myBP), the dry climate in northern China developed stepwise with the cooling of the earth. Loess sedimentation began in 2.4 myBP in the Loess Plateau and the desertification became strong in the late Early Pleistocene in and around the Tarim Basin. The palynological and pedological data in the inner Mongolia between 8 and 5 thousand years BP indicate vegetation covered more widely than in the recent.

**A Phytogeographical Sketch in Central Asia, with Special Reference to the Desert Flora of Xinjiang Province of China**

*Tatemi SHIMIZU*

Honorary professor, Kanazawa University

The outline of phytogeography in Central Asia has been mentioned by various authors such as Good (1974), Liu et al. (1983) and Takhatajan (1986). According to them, the arid areas of Xinjiang Province are divided into two floristic provinces, though the detailed floristics have not been presented yet. At this seminar, I tried to make floristic comparison between North and South Xinjiang based on distributional data of some representative plant taxa characteristic of desert. Only 40 (29%) of 148 species of the *Chenopodiaceae* and 9 (40%) of 21 species of the *Zygophyllaceae* were found to be common. Only one species of 9 native *Calligonum* (*Polygonaceae*) and only one species of 20 native *Ferula* (*Apiaceae*) occur in the south.

The desert flora of Xinjiang is quite different between north and south. The flora of South Xinjiang is much poorer than the north.

**Arid land vegetation and soil in Central Asia**

*Takeo TANIMOTO*

Faculty of Agriculture, Utunomiya University

The relationships between natural vegetation and many environmental factors, such as water condition, soil types, topography and human impact, were investigated around Taklimakan desert, Xinjian Uygur Autonomous, western China.

*Phragmites communis* community, *Populus euphratica* community, and *Tamarix hispida-Phragmites communis* community are distributed on low lands, river sides and near oasis. The development of these communities depends on high underground water level.

*Ephedra przewalskii* community, *Reaumuria songarica* community, and *Calligonum roborovskii* community are found in wadis and gubi type deserts. These communities depend on flood water in spring.

*Halostachys caspica* community, *Kalidium foliatum* community, and *Salicornia europaea* community are distribute in saline sites, low lands and sometimes in and near salt marsh.

The vegetation changes are observed everywhere around Taklimakan desert at present. Their main causal forces are over-grazing, shortage of water supply and salt accumulation.

### **Drought resistance of desert plants in Central and East Asia and its ecological significance**

*Tatsuaki KOBAYASHI*

Faculty of Horticulture, Chiba University

There are varieties of mechanism in drought resistance as physiological, morphological and phenotypic responses. The flora of North-American cold desert, which is climatically equivalent to Central-Asian desert, is characterized by long-lived shrubs and perennial grasses. The flora of Kazakhstan desert is characterized by long-lived shrubs and ephemeral annuals. The physiological tolerance of Kazakhstan shrubs is higher than that of North-American cold desert shrubs. On the other hand, the ephemerals do not have physiological tolerance to drought. It means that the dry condition is so severe in Central Asia, therefore, the ecosystem is not saturated to energetic capacity. The flora of Chinese sandy land is characterized by short-lived shrubs and perennial grasses. It means that the dry condition is more mild in semi-arid East Asia. It also suggests that temporal climatic variance and anthropogenic disturbance affect East-Asian sandy ecosystems.

### **Responses of Antioxidative Systems to Draught and Salt Stress in Higher Plants**

*Kiyoshi TANAKA*

Faculty of Agriculture, Tottori Univ.

Leaves close their stomata under water stress. This is a major temporary adaptive change that prevents water loss from the plants, but CO<sub>2</sub> concentration is lowered at the same time within cells resulting in the decrease of net photosynthesis. Photosynthetic electron transport is, however, maintained at a higher rate in the stressed leaves as compared to the large decrease in the rate of CO<sub>2</sub> fixation. This imbalance causes the overreduction of photosystem and facilitate the transfer of electrons to O<sub>2</sub> followed

by the formation of  $O_2^-$  and  $H_2O_2$ , and then other reactive oxygen species such as  $OH \cdot$  and  $^1O_2$  which can damage cell structure and function. High salt causes the reduction of water potential within cells resulting the decrease in  $CO_2$  concentration in the cells in the same manner. High salt is also known to inhibit RuBISCO and phosphoenolpyruvate carboxylase, which may cause the overreduction in photosynthetic electron transport chain. Salt brings about the increase in active oxygen in cells by decreasing the activity of superoxide dismutase which is a representative antioxidative enzyme.

When plants were subjected to water stress, plants increased several antioxidative enzymes and substances such as superoxide dismutase, glutathione reductase, tocopherol and so on. Plant accumulated hydrogen peroxide and malondialdehyde under draught stress. Transgenic alfalfa with elevated activities of mitochondrial superoxide dismutase showed higher tolerance to draught in terms of photosynthesis, membrane integrity and growth than control one.

### **9) Energy Balance over the Ground Surface of the Semi-Arid Areas (13:00-17:00, Mar. 4, 1998)**

#### **Researches on Energy and Water Balances over the Ground Surface Stared from Sand Clock**

*Junsei KONDO*

Emeritus Professor of Tohoku University

We can see phenomena commonly occurred in arid lands in the ordinary life. In the sand clock, the velocity of falling sand and the movement and distribution of the moisture are determined by the interrelationship among air, sand particle and moisture. We can understand the complex nature of the earth from the observation of the simple sand clock. for example, if the sand in the clock is heated by sun light for 24 hours, moisture contained in the sand is evaporated and cloud is formed in the upper part of the clock. The cloud disappear and the moisture return in to the sand after sunset.

The more the wind speed grow, the faster the trunks of trees and laundry of thick texture dry up. However, evaporation ratio does not depend on wind speed if it become moderately dry. This is the feature of evaporation occurred in arid lands. In arid lands, evaporation ratio is determined by three factors ; soil type, soil moisture and relative humidity of the atmosphere. Diffusion of molecules is dominant in case of water movement in moderately dry soil, thus evaporation ratio depends on the structure of soil particles. Evaporation can be restrained using this mechanism. for example, mulches of gravel, sand, straw or withered grass, which farmers traditionally use, reduce evaporation. Farmers have studied the nature, and know it well.

Snow and glacier are precious water resources in arid lands where precipitation is scarce. White snow well reflects sunlight, and limit heat release from the ground surface because of its low heat conductivity. Since snow changes the energy balance over the ground surface as above mentioned, it store the water above the ground for a long period.

Recently, many researches on the hydrological volumes (precipitation, evapotranspiration, runoff = water resource, underground water storage) in every corner of the world. Although observations of these volumes are not so difficult in the experimental sites, they are very hard to conduct for the large area composed of various surfaces. There are methods to evaluate evapotranspiration from various surfaces using the climatological features of hydrological factors in order to overcome the difficulty. "New potential evaporation", which can be calculated using only the routine meteorological data, is derived and the



climatic features of hydrological factors are revealed.

No matter how much precipitation is, water resources is scarce if potential evaporation exceeds precipitation, and vice versa. From this point of view, climatic humidity index WI (= precipitation / potential evaporation) is determined. Then hydro-climatic division is conducted depending on WI ; arid ( $WI \leq 0.1$ ), semi-arid ( $0.1 < WI < 0.3$ ), semi-humid ( $0.3 < WI \leq 1$ ) and humid ( $1 < WI$ ). The results show that annual variations of WI are substantial, which leads repetition of flood and drought years, in semi-arid and semi-humid areas.

### **Seasonal Variations of Energy and Water Balances over the Bare Ground Surfaces in Several Locations of China.**

*JianQing XU*

Faculty of Science, Tohoku University

Objective of this study is to investigate energy and water balances over bare ground surfaces in various locations in China. In this study, four representative soils (Soil-1 : Kuroboku, Soil-2 : Ranzhou Rome, Soil-3 : Narita Sandy soil, Soil-4 : Sand) are examined. Transportational processes of water of both vapor and liquid phases in the soils are parameterized. Then, the method to calculate energy and water balances using routine meteorological data (daily average air temperature, difference of maximum and minimum air temperature, duration of sunshine, daily precipitation, daily average vapor pressure and atmospheric pressure), which is applicable from arid to humid areas, is made. Daily and seasonal variations of energy and water balances of thirty representative locations in China is calculated by means of this method.

Principle results are as follows :

- (1) In semi-arid areas, the deeper the soil, the more the annual average soil moisture. The incremental ratio increases with the annual precipitation. Sensible heat flux is larger in dry season, and latent heat flux is larger in rainy season.
- (2) In arid areas, most of the net radiation is consumed for sensible heat flux. Annual average soil moisture does not vary with the depth. Surface soil moisture depends on the atmospheric relative humidity.
- (3) Snow in winter increase water resources.
- (4) Annual evaporation is closely related with annual precipitation. In arid areas, annual evaporation is nearly equal to annual precipitation. However, in humid areas where precipitation is abundant, there are maximum values of annual evaporation, which depend on the soil type. In humid areas, annual evaporation is more in loamy soils and less in sand.
- (5) Average soil moisture from 20cm to 60cm depths is almost same as the field capacity in humid areas but equilibrated with atmospheric relative humidity in arid areas.
- (6) The areas from Tibet to Altai, where sensible flux is evident, heat up the atmosphere.
- (7) Distribution maps of climatic humidity index WI (=precipitation / potential evaporation), which is the comprehensive index of energy and water balances, is presented.

### **10) Natural Environment and Research Theme in Arid Land (13:30-15:00, Mar. 27, 1998)**

#### **Desiccation and Soil Moisture Change Related to Evapotranspiration**

*Masatoshi AOKI*

Faculty of Agriculture, Tokyo University of Agriculture and Technology

The observation of meteorological profiles in a tropical rainforest located in the northern part of Thailand was introduced, and based on the observational results the change in meteorology and the desiccation process of soil in accordance with the deforestation was discussed. In addition, it is available for the oversea research to use the remote measurement by the telephone circuit because of the difficulty to arrive at the research point and to stay there for a while. An example of this remote measurement system which was applied in Hokkaido was introduced. It was reviewed that recently, we could get the observational data from oversea through the communication satellite at anytime even if we were in Japan.