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## **Pharmaco-Chemical Studies on the Aqueous Methanolic Extract of *Diospyros lotus* Leaves**

A. Said, Usama W. Hawas, Salwa M. Nofal, K. Rashed and A. Huefner

This study evaluated phytochemical and some biological activities of the aqueous methanolic extract of the leaves of *Diospyros lotus* L. Eight phenolic compounds were isolated and identified as gallic acid, methylgallate, ellagic acid, kaempferol, quercetin, myricetin, myricetin 3-O- $\beta$ -glucuronide and myricetin 3-O- $\alpha$ -rhamnoside. The structures were established on the basis of chemical and spectral analysis. The extract was tested for antioxidant, analgesic, anti-inflammatory and hepatoprotective activities. The 70% methanolic extract of *D. lotus* up to 5 g kg<sup>-1</sup> b. wt. revealed no obvious toxic effects in mice and its antioxidant activity showed a marked significant scavenging activity. On the other hand, the extract indicates a central and peripheral analgesic effect. The acute paw oedema response was significantly reduced. The protective effect of the extract against acetaminophen induced liver damage was indicated by the reduction of the elevation of the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) that caused by acetaminophen. The phenolic compounds isolated from *Diospyros lotus* in this study were found to be isolated for the first time from the genus *Diospyros*. (*Research Journal of Phytochemistry* 3 (1): 1-12, 2009; **doi**: 10.3923/rjphyto.2009.1.12)

## **Phytotoxic and Chemical Investigations of a Nigerian Medicinal Plant**

A. Falodun, A.M.A. Chaudhry and I.M. Choudhary

*Pyrenacantha staudtii* is a medicinal plant endemic in Nigeria and used ethnomedicinally for the treatment of various diseases by the populace. In continuation of the studies on chemical constituents of *Pyrenacantha staudtii*, the study was aimed at validating the phytotoxic activity of the leaf extract and through a systematic chemical procedure, isolation and identification of the chemical principles of *Pyrenacantha staudtii* leaf was established. The investigation of the chemical constituents of *Pyrenacantha staudtii* leaves has led to the isolation and characterization of two known compounds oleanilic acid and  $\beta$ -amyrin. The compounds were isolated first time from *P. staudtii*. The structures of the compounds were established by spectral (MS and NMR) and chemical methods. The methanolic extract of *P. staudtii* exhibited significant phytotoxic activity

against *Lemna minor* L. at  $10 \mu\text{g mL}^{-1}$ . The result of the study has justified the phytotoxic potential of the plant. (*Research Journal of Phytochemistry* 3 (1): 13-17, 2009; doi: 10.3923/rjphyto.2009.13.17)

### **Antihyperglycaemic Activity of Cycloart-23-ene-3 $\beta$ , 25-diol Isolated from Stem Bark of *Pongamia pinnata* in Alloxan Induced Diabetic Mice**

Sachin L. Badole and Subhash L. Bodhankar

*Pongamia pinnata* (L.) Pierre (Fabaceae) has been used in traditional medicine for treatment of diabetes. The aim of the research was to study the antihyperglycaemic activity of cycloart-23-ene-3 $\beta$ , 25-diol (code name compound B2) isolated by column chromatography method from stem bark of *Pongamia pinnata* in alloxan induced diabetic mice. The structure of compound B2 was elucidated by spectroscopical data. Diabetes was induced in mice by alloxan ( $80 \text{ mg kg}^{-1}$ , i.v.). Compound B2 was administered orally. Serum glucose level was determined at 0, 2, 4, 6 and 24 h. The onset was at 2nd h; peak effect at 6th h and the antihyperglycaemic effect was sustained until 24th h. Results obtained in the present study indicated antihyperglycaemic activity of cycloart-23-ene-3 $\beta$ , 25-diol (B2). (*Research Journal of Phytochemistry*, 3 (1): 18-24, 2009; doi: 10.3923/rjphyto.2009.18.24)

### **Suppression of Colon Cancer Development in an Azoxymethane-Fisher 344 Rat Model by Cranberry**

R. Sunkara, M. Verghese, L.T. Walker and L. Shackelford

The present study investigated the effect of cranberries on development of colon tumors induced by azoxymethane in Fisher 344 male rats. Fifty five rats were divided into five groups and fed with control (AIN 93) or treatment diets: cranberry meal (5, 10%) cranberry juice (2.5, 5%). Two AOM ( $16 \text{ mg kg}^{-1}$  b.wt.) injections were given weekly for 2 weeks for induction of colon tumors. At 45 weeks of age, all rats were killed and colons were evaluated for tumor incidence, size of tumor and tumor multiplicity. Selected hepatic phase 1 (CYP2E1), phase 11 (GST) and antioxidative enzyme (catalase and SOD) activities were determined. Tumor size and tumors/tumor bearing rat were higher ( $p \leq 0.05$ ) in the control group. Number of tumors was lower in cranberry fed rats compared to control. Administration of cranberry to rats increased ( $p < 0.05$ )

hepatic enzyme activities by 1.2-3.7 fold compared to control fed rats. These results indicate that feeding cranberry (meal and juice) inhibited colon tumors induced by AOM and enhanced the activity of hepatic enzymes. (*Research Journal of Phytochemistry* 3 (2): 25-34, 2009; doi: 10.3923/rjphyto.2009.25.34)

### **Bioactive Chemical Constituents of *Stereospermum kunthianum* (Bignoniaceae)**

A. Falodun, I.M. Qadir, C.F. Poh, K.I. Omogbai Eric and M.I. Choudhary

One iridoid and 2 phenylpropanoid glycosides were isolated from the stem bark of *S. kunthianum* together with mixtures of  $\beta$ -sitosterol and  $\beta$ -sitosterol glucoside. Their structures were determined by IR, HRESIMS and 1D and 2D NMR experiments and their enzyme inhibitory effect evaluated using xanthine oxidase. The inhibitory activities of 6-O-trans-p-coumaroyl-decinnamoyl-globularimin-Stereospermiside, (3, 4-dihydroxyphenyl)-ethyl-O- $\alpha$ -rhamnopyranosyl (1 $\rightarrow$ 3)-4-O-cinnamoyl- $\beta$ -D-glucopyranoside and 1,6-di-O-cinnamoyl- $\beta$ -D-glucopyranoside 1-3 were evaluated and compared to the standard positive control. (*Research Journal of Phytochemistry* 3 (2): 35-43, 2009; doi: 10.3923/rjphyto.2009.35.43)

### **Analysis of Essential Oil Constituents in Hydro-Distillates of *Calotropis procera* (Ait.) R.Br**

W. Okiei, M. Ogunlesi, E. Ofor and E.A.S. Osibote

The essential oil from the dried leaves of *Calotropis procera* was analyzed by GC-MS. The three major components in the oil are phytol and its isomers 3, 7, 11, 15-tetramethyl-2-hexadecene-1-ol (37.59%) and 6,10,14-trimethyl-2-pentadecanone (15.31%). The essential oil was collected in two modes: one mode is a continuous distillation for 4 h and another mode involves hourly collection of fractions over a period of 4 h, thus providing fractionated samples. This novel procedure makes it possible to identify other components which might not have been detected in the unfractionated sample. Such other components include tetradecanal, isophytol and 1-docosanol. The usefulness of phytol in the management of inflammatory diseases suggests that the plant may be useful in the management of arthritis. 6, 10, 14-trimethyl-2-pentadecanone, a mosquito repellent may be useful for malaria control. (*Research Journal of Phytochemistry* 3 (3): 44-53, 2009; doi: 10.3923/rjphyto.2009.44.53)

## **Estimation of Protocatechuic Acid in Greater Cardamom Fruit Extracts by HPTLC Method**

R. Manek, N.M. Patel, A. Bhargava, J. Vaghasiya, N. Jivani and S. Koradia

The aim of present study was development of simple, rapid and accurate HPTLC method for estimation of Protocatechuic acid in various extracts of *Amumum subulatum* Roxb. fruit constituents (Family Zingiberacea), commonly known as Badi Elaichi or Greater Cardamom. The powdered drug was subjected to extraction by soxhlet apparatus using methanol and acetone separately as well as petroleum ether (40-60), chloroform, methanol and water successively. The extracts were screened for presence of various phytoconstituents using preliminary chemical tests. Protocatechuic acid was estimated in methanol and acetone extract by HPTLC method. Detection and quantification was performed at wavelength 254 nm. The acetone and methanol extracts were found to contain 1.04846 and 0.8634% w/w protocatechuic acid, respectively by using validated method. Since, this method resolves and quantifies protocatechuic acid accurately and precisely, it can be useful for quantification of the compound in herbal formulation. (*Research Journal of Phytochemistry* 3 (3): 54-62, 2009; doi: 10.3923/rjphyto.2009.54.62)

## **Comparison of *in vitro* Antioxidant Activity of *Trigonella foenum-graecum* and *T. corniculata* Seeds**

M. Semalty, A. Semalty, G.P. Joshi and M.S.M. Rawat

Successive methanolic and direct ethanolic extracts of *Trigonella foenum-graecum* and *T. corniculata* seeds were prepared and were investigated for their potential antioxidant activity against DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radicals. Seed extracts of *T. corniculata* showed better antioxidant activity than that of *T. foenum-graecum*. Ethanolic extract of *T. corniculata* was the most effective antioxidant among the extracts with 90.24% DPPH radical scavenging activity at 500  $\mu\text{g mL}^{-1}$ . The antioxidant activity of the extracts increased with the increasing amount of the concentration. It was concluded that the seeds of *T. corniculata* had better antioxidant than *T. foenum-graecum*. Moreover the ethanolic extracts showed significantly better activity than the successive methanolic extracts. (*Research Journal of Phytochemistry* 3 (3): 63-67, 2009; doi: 10.3923/rjphyto.2009.63.67)

## ***In vitro* Investigation of Antioxidant Phenolic Compounds in Extracts of *Senna alata***

J. Okpuzor, H. Ogbunugafor, G.K. Kareem and M.N. Igwo-Ezike

Extracts of *Senna alata* were investigated for antioxidant phenolic compounds using High Performance Liquid Chromatography (HPLC). The dried aerial plant parts were macerated into powder and extracted in different organic solvent systems consisting of methanol, hexane, chloroform, ethyl acetate, butanol and water. Each extract was dried under reduced pressure using a rotary evaporator, freeze-dried and stored at a temperature of 4°C. The extracts were then subjected to high performance liquid chromatography studies. Two major phenolic compounds Naringin and Apigenin, were identified in some of the fractions of *Senna alata*. The presence of these flavonoids in *Senna alata* may explain its wide use in ethnomedicine practice for the treatment of hypertension, sickle cell anemia and diabetes in Southwestern Nigeria. (*Research Journal of Phytochemistry* 3 (4): 68-76, 2009; doi: 10.3923/rjphyto.2009.68.76)

## **Antimicrobial Activity of the Essential Oil and the Fractional Samples Obtained from the Leaves and Seeds of *Phyllanthus amarus* (Euphorbiaceae)**

M. Ogunlesi, W. Okiei, E.A.S. Osibote and C. Muotoh

The aim of this study is to investigate the alleged antimicrobial activity of *P. amarus*. Fresh leaves and seeds of the plant were air-dried, pulverized and the essential oil extracted into hexane by hydrodistillation over a period of 4 h. In addition, hourly fractions were collected and sensitivity tests were carried out on twelve microorganisms including yeast, Gram-positive and Gram-negative bacteria. All the samples of essential oil and fractions demonstrated activity against the microorganisms except *Pseudomonas aeruginosa*. The activity of the essential oil collected over 4 h exceeded that of the control 0.05% ciprofloxacin, for *Staphylococcus aureus* (isolate) and *Bacillus subtilis*. The results indicate the use of the plant as an antimicrobial. Thus, there is scientific basis for the use of the plant in the treatment of bacterial and fungal diseases. (*Research Journal of Phytochemistry* 3 (4): 77-84, 2009; doi: 10.3923/rjphyto.2009.77.84)

## **Variation of Lipopolysaccharide among the Three Major *Agrobacterium* Species and the Effect of Environmental Stress on the Lipopolysaccharide Profile**

H.H. Arafat, K. Tanaka, H. Sawada and K. Suzuki

Lipopolysaccharide (LPS) is a variable component among the bacterial species as well as strains of a single species and this characteristic is helpful for discrimination between strains. However, we have only limited information about LPS variation and influence by environment in *Agrobacterium* strains. In this study, we analyzed variation of lipopolysaccharide (LPS) among 34 *Agrobacterium* strains; 9 strains of *A. tumefaciens*, 15 strains of *A. rhizogenes*, 9 strains of *A. vitis* and one *A. rubi* strain. Most of the *A. tumefaciens* strains and every *A. rhizogenes* strains had high and low molecular weight LPS molecules (LPS I and LPS II, respectively). On the contrary, every *A. vitis* strains and two exceptional *A. tumefaciens* strains lacked LPS I but had a single LPS II band. The LPS profiles were stable phenotype in the *Agrobacterium* strains. Abiotic stresses such as high salinity, high and low pH and high and low temperature were given to representative strains in each species. Only a little alternation in the LPS profiles was observed under the stress conditions except the high temperature to LPS I. Cultivation at 35°C or higher resulted in a significant size reduction of LPS I in *A. tumefaciens* C58 strain down to the size similar to that of LPS II which attenuated the tumor formation. On the contrary, cultivation at the high temperature induced the exceptional *A. tumefaciens* strain MAFF 03-01001 to synthesize LPS I, which was absent at lower temperature in the strain. This phenomenon has never been observed so far at least in the family *Rhizobiaceae*. (*Plant Pathology Journal* 8 (1): 1-8, 2009; doi: 10.3923/ppj.2009.1.8)

## **Effect of Combined Use of *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01 on Biological Control of *Rhizoctonia solani* on *Solanum melongena* and *Capsicum annuum***

S. Abeysinghe

A combination of two compatible biological control agents, *Bacillus subtilis* CA32 and *Trichoderma harzianum* RU01, both antagonistic to the pathogen *Rhizoctonia solani*, was used to control damping-off in *Solanum melongena* and *Capsicum annuum*. Radial growth of the mycelium of *R. solani* was inhibited by *T. harzianum* RU01 in dual Petri plate assay. *T. harzianum* RU01 was capable to invading the whole surface of the pathogen colony, sporulating on it and

suppress the production of sclerotia of *R. solani*. Microscopic studies showed the hyphae of *R. solani* surrounded by the *T. harzianum* RU01 and subsequent disintegration. *B. subtilis* CA32 produced a zone of inhibition only with the pathogen and no signs of antagonism between the bacteria and *T. harzianum* RU01 on dual Petri plate assay. Significant plant protection was achieved when either *B. subtilis* added to the seeds or *T. harzianum* added to soil. However, when combine application of biocontrol agents, seed bacterization and *T. harzianum* application to soil, significantly enhanced the plant protection from *R. solani*. Soil application of *B. subtilis* and seed application of *T. harzianum* either singly or in combination did not protect from *R. solani* infection indicating that the importance of mode of application of biocontrol agents. (*Plant Pathology Journal* 8 (1): 9-16, 2009; doi: 10.3923/ppj.2009.9.16)

### ***In vitro* Antifungal Activity of Essential Oils and Their Compounds on Mycelial Growth of *Fusarium oxysporum* f. sp. *gladioli* (Massey) Snyder and Hansen**

L.L. Barrera-Necha, C. Garduño-Pizaña and L.J. García-Barrera

The increasing recognition and importance of phytopathogenic fungi, the difficulties encountered in their control and the increase in resistance to antifungal have stimulated the search for natural alternatives. The antifungal effects of essential oils and their compounds were investigated on mycelial growth inhibition bioassays of *Fusarium oxysporum* f. sp. *gladioli*. The essential oils have been used empirically. In general, a significant antifungal effect was observed with *Cinnamomum zeylanicum*, *Thymus vulgaris* and *Syzygium aromaticum* oils which had total inhibition at 100, 150, 200, 250 and 300 ppm. *Teloxys ambrosioides*, *Mentha piperita* and *Citrus aurantifolia* oils exhibited a dose dependent inhibition on mycelial growth to increase the dose of 100 at 300 ppm. While *Allium sativum*, *Capsicum* sp., *Ruta chalepensis* and *Eucalyptus globulus* oils had no antifungal activity at different concentration tested. All compounds with the exception of cineole had a fungicide or fungistatic effect. (*Plant Pathology Journal* 8 (1): 17-21, 2009; doi: 10.3923/ppj.2009.17.21)

### **Alkaline Seed-Bed: An Innovative Technique for Manifesting *Verticillium dahliae* on Fennel Seeds**

Khalid M. Ghoneem, Wesam I.A. Saber and Mohamed A. Elwakil

*Verticillium dahliae* attacks a wide range of plants including fennel causing a wilt disease. The fungus grows slowly on seeds when tested at the seed health laboratories. This habit character allows saprophytes to impair the fungal growth

and interfere the identification on both Moist Blotters (MB) and the Deep-Freezing Blotters (DFB). Since, these two techniques are not efficient enough to detect this fungus, the researchers planned to search for an alternative technique for detecting this fungus. Soaking three layers of blotters used as seed-beds in water solutions alkalined with KOH or NaOH at pH 10 presents the optimum seed-bed condition for manifesting the fungus on seed. This seed-bed condition also suppress the growth of saprophytes, so as the fungus was transparently shown on seeds. The *in vitro* study presents pH 9.5 as the optimum condition for the growth, sporulation and maximum glucose coefficient of the fungus. So far, it is recommended to use the alkalined seed-bed when searching for *V. dahliae* on fennel seed. (*Plant Pathology Journal* 8 (1): 22-26, 2009; **doi:** 10.3923/ppj.2009.22.26)

## **Epidemiology of Potato Blackleg in Warm Climate**

Nabil S. Farag, Ahmad A. Gomah and Naglaa A.M. Balabel

Potato (*Solanum tuberosum* L.) planted in warm climate in September, in Egypt, showed no above ground symptoms of blackleg. The harvested crop showed severe tuber collapse similar to that being produced by the soft rot disease. Tentative identification of the isolated bacteria revealed no affiliation to the soft rot bacterium *Erwinia carotovora* ssp. *carotovora*. Verification of identity was made by PCR that showed its close similarity, to *E. carotovora* ssp. *atroseptica* at 119 bp, compared to an authentic Dutch isolate PD 4202. The present study suggested that the quality of water phase in the field soil may play an important role in rot progress at the time of harvest. Tonicity, cation(s) content and pH of the water phase of soil, suspending the bacterial inoculum, were found important in this regard. The rot increased under hypertonic stress ( $10\text{ g}^{-1}\text{ NaCl}$ ) was attributed to a greater proliferation of bacteria on the expense of nutrients withdrawn from tuber cells. Further increase ( $20\text{ g}^{-1}\text{ NaCl}$ ) in tonicity, however, decreased tuber rot, indicating negative correlation between the aggressiveness of strain to potato tubers and their osmotic tolerance. Pathogen suspended in hypotonic solution produced greater rot possibly due to greater invasion by bacteria and/or maximization of the intercellular spaces following cell turgidity. The monovalent and divalent cations at approximately similar osmotic strength decreased the rot symptoms. This effect may be attributed to their effect on a group of depolymerases with different optimal conditions and/or their effect on changing optimal pH for pectinolysis. The tuber rot was recorded over a wide range of pH. Further investigations are needed to study in depth other edaphic factors related to epidemiology of blackleg disease in warm climate. (*Plant Pathology Journal* 8 (1): 27-31, 2009; **doi:** 10.3923/ppj.2009.27.31)

## Identification of *Puccinia pimpinellae* on Anise Plant in Egypt and Its Control

Wesam I.A. Saber, Khalid M. Ghoneem, Mohammed M. El-Metwally and Mohamed A. Elwakil

An emerging problem for the wider adoption of anise plantation in Egypt is the damage caused by the rust fungus. The detailed description and taxonomic studies (using light and scanning electron microscopy) show that such an obligate parasite fungus (*Puccinia pimpinellae*) is autoecious microcyclic (uredinial-telial stage only). Among tested Apiaceae plants, the host range test proved the specificity of the rust fungus to anise. To the researcher's knowledge, this is the first investigated record of a rust fungus on *Pimpinella anisum* plants in Egypt. The effectiveness of some plant resistance elicitors and two active chitinase producers; *Bacillus subtilis* Bio4 and isolated *Trichoderma harizianum* CH<sub>4</sub> (both of them recorded the highest clear zone/colony size ratio on chitin agar plates) in controlling anise rust disease and on growth and yield of anise were evaluated in two successive growing seasons. Spraying chitosan at 1000 ppm was the most potent in reducing Disease Severity (DS) and Incidence (DI) as well as improving plant height, chlorophyll content, inflorescence No. plant<sup>-1</sup> (74.2 and 76), 1000-fruit weight (2.94 and 2.83 g) and anise yield (646.8 and 670.0 kg fed<sup>-1</sup>), during both seasons. *B. subtilis* Bio4 and *T. harizianum* CH<sub>4</sub> showed moderate effect on the tested parameters. (*Plant Pathology Journal* 8 (2): 32-41, 2009; doi: 10.3923/ppj.2009.32.41)

## Screening of Transgenic Tobacco for Resistance Against *Cucumber mosaic virus*

K. Nadarajah, N.M. Hanafi and S.L. Tan

Coat protein (*CP*), Movement Protein (*MP*) and Overlapping (*OVG*) genes were isolated from a Malaysian *Cucumber mosaic virus* (*CMV*) isolate via RT-PCR and transformed into *Nicotiana tabacum* through *Agrobacterium tumefaciens*-mediated transformation. Out of the thirty six independently transformed lines developed from the three different genes and the mutants of *MP* and *OVG*, five lines were tested for resistance against *CMV* by challenge inoculations using three different concentrations (1:10, 3:10 and 5:10) of *CMV* macerated in 0.1 M sodium phosphate buffer (pH 7.0). The transgenic lines exhibiting complete resistance remained symptomless even when re-inoculated with 1:10 concentration of virus. The level of viral RNA accumulation in inoculated leaves was significantly (at least 2-3 times) lower compared to the control

untransformed plants. The upper leaves which were analysed for systemic spread of the infection had much lower levels of viral RNA accumulation compared to the inoculated leaves. Amongst the three genes and two mutant lines that were generated in this study, we found that the *CP* and *MP* genes were able to provide a better level of resistance to the plants compared to the overlapping gene. (*Plant Pathology Journal* 8 (2): 42-52, 2009; doi: 10.3923/ppj.2009.42.52)

### **Plant Resistance to TSWV and Seed Accumulation of Resveratrol within Peanut Germplasm and its Wild Relatives in the US Collection**

M.L. Wang, D.L. Pinnow, N.A. Barkley and R.N. Pittman

Biotic and abiotic stress may induce peanut plants to produce a high amount of resveratrol. The relationship between an individual plant's response to biotic stress caused by *Tomato spotted wilt virus* (TSWV) and the accumulation of resveratrol in the seed was investigated. Twenty peanut accessions and six wild relatives were selected from the US peanut germplasm collection and planted with two replicates. Individual plant response to natural-TSWV infection was observed and recorded in the field. Leaf tissues from each individual plant were collected and tested by an Enzyme-Linked Immunosorbent Assay (ELISA) using specific antiserum for TSWV. Seeds harvested from individual plants were used for quantification of resveratrol by High Performance Liquid Chromatography (HPLC) analysis. Extensive resveratrol variation in the seeds was detected among TSWV negative and positive plants. Among the accessions evaluated in this study, the specific genotype of each individual definitely played a major role on the capability for synthesis and accumulation of resveratrol. However, the synthesis and accumulation of resveratrol within an accession may not only be affected by a plant's response to TSWV, but also by other biotic and abiotic stress that an individual plant encounters in its environment. (*Plant Pathology Journal* 8 (2): 53-61, 2009; doi: 10.3923/ppj.2009.53.61)

### **Characterization of a Novel Far-Eastern Potato Virus Y Isolates**

Yury G. Volkov, Nadezhda N. Kakareka, Larissa A. Balabanova, Zinaida N. Kozlovskaya and Mikhail V. Sapotsky

Potato Virus Y (PVY) isolates differed in pathogenicity and molecular properties were found in potato and wild plants in the Far East of Russia. The results of linking research of nucleotide sequence similarity and polymorphism of P1 gene region and serological and biological assays suggested that the viruses

originated from a recombination and/or host adaptation events involving the ordinary type virus (PVY<sup>0</sup>) that led to development of necrotic type virus (PVY<sup>N/NTN</sup>) properties in the Far-Eastern PVY isolates. (*Plant Pathology Journal* 8 (2): 62-67, 2009; doi: 10.3923/ppj.2009.62.67)

### **Physiological Race of *Fusarium oxysporum* F. sp. *Lycopersici* in Kurdistan Province of Iran and Reaction of Some Tomato Cultivars to Race 1 of Pathogen**

J. Amini

In this research, eleven isolates of *Fusarium oxysporum* were collected from tomato plants displaying wilt symptoms in fields in Kurdistan province. Race 1 of pathogen was obtained from Moscow Timiryazev Agricultural Academy in Russia. Pathogenicity of the collected isolates and race 1 of the pathogen were evaluated in glasshouse conditions. Pathogenicity tests and race determination were conducted using root-dip inoculation with different tomato cultivars, Bely naliv-241 (not resistant), Blagovest (resistant to race 1) and Benito (resistant to both races of 1 and 2). The experimental design was a completely randomized type with six replications (pots) containing two seedlings per pot. Disease severity was measured five weeks following inoculation by using a scale of 0 to 4. The criteria used to assess the response of different cultivars were; leaf disease index, plant height and vascular discoloration index. Results showed that Bely naliv-241 lacking any resistance gene wilted four weeks after inoculation, but cultivars Blagovest and Benito did not develop symptoms to any of the isolates tested. The reaction of race 1 and the Iranian isolates were similar in pathogenicity suggesting that all of the isolates belong to *Fusarium* f. sp. *lycopersici* race 1. Also, Reaction 23 tomato cultivars against to *F. o. f. sp. lycopersici* indicated that 6 of the them were resistant, 5 were intermediately resistant, 6 were tolerant, 3 were susceptible and the rest 3 were found to be very susceptible. (*Plant Pathology Journal* 8 (2): 68-73, 2009; doi: 10.3923/ppj.2009.68.73)

### **Using Arbuscular Mycorrhizal Fungi and *Rhizobium leguminosarum* Biovar *phaseoli* Against *Sclerotinia sclerotiorum* (Lib.) de Bary in the Common Bean (*Phaseolus vulgaris* L.)**

E. Aysan and S. Demir

In this study, the effects of Arbuscular Mycorrhizal Fungi (AMF) *Glomus mosseae* (Gm), *Glomus fasciculatum* (Gf) and *Rhizobium leguminosarum* biovar *phaseoli* (Rlp), which are the important members of rhizosphere and

biological control agents, were examined on the patho-system of *Sclerotinia sclerotiorum* (Lib.) de Bary (Ss) and common bean. The colonization and nodulation of two biological control agents exhibited differences as a result of reciprocal interactions of these items as well as the effect of the Ss. Nodulation of Rlp particularly decreased in triple inoculation. In addition, colonization of AMF significantly decreased in treatment of Ss+AMF than control AMF. Treatments of single inoculations of AMF and Rlp isolates reduced disease severity by 10.3-24.1%. It was determined that single biological control agents inoculations were more effective than dual inoculations (AMF+Rlp). When the morphological parameters of common bean were considered, all of the morphological parameters values were decreased in treatments which present pathogen isolate. Besides this, all biological control agents increased total contents of P and N in treated plants compared to the controls. (*Plant Pathology Journal* 8 (2): 74-78, 2009; doi: 10.3923/ppj.2009.74.78)

### **The Use of Antioxidants and Microelements for Controlling Damping-Off Caused by *Rhizoctonia solani* and Charcoal Rot Caused by *Macrophomina phaseoliana* on Sunflower**

K.M. Abd El-Hai, M.A. El-Metwally, S.M. El-Baz and A.M. Zeid

Seed soaking method or foliar spray of antioxidants (citric acid and salicylic acid at 10 mM) and microelements (manganese and zinc at 2 g L<sup>-1</sup>) were tested to control of the damping-off and charcoal rot diseases of sunflower (varieties Sakha 53 and Giza 102). Field treatments in two different localities i.e., Tag El-Ezz, Dakahlia province and El-Serow, Damietta province were carried out. The high frequency isolated fungi (*M. phaseolina* and *R. solani*) presented in Tag El-Ezz location. On the other hand, *M. phaseolina* was isolated at a high frequency compared with *R. solani* in both locations. Sakha 53 was highly susceptible compared with Giza 102 when artificially infected with both *M. phaseolina* and *R. solani*. Laboratory results showed that salicylic acid alone or in combination with citric acid completely inhibited the linear growth of both pathogens i.e., *M. phaseoliana* and *R. solani* *in vitro*. A positive correlation between the concentrations of Rizolex-T 50 and its effect on the fungal growth were recorded. The dose of 3 g L<sup>-1</sup> prevented the growth of *R. solani* linear growth *in vitro*. The greenhouse results revealed that Giza 102 variety was highly susceptible to the infection by *R. solani*. *M. phaseoliana* showed severe symptoms in both sunflower varieties. On contrary, Giza 102 variety was tolerant to damping-off and charcoal rot diseases than Sakha 53 under field conditions. The application of Rizolex-T 50 followed by citric acid showed a highest

percentage of healthy plants followed by the combination of citric acid and salicylic acid. The application of manganese combined with zinc was more effective than the microelements alone. All treatments of antioxidants and microelements significantly reduced the incidence of charcoal rot disease. On the other hand, no significant differences between Rizolex-T 50 and salicylic acid treatments was shown. Sakha 53 variety gave the highest values of plant height and number of leaves plant<sup>-1</sup> while Giza 102 recorded the highest values of stem diameter and flower head diameter. The application of citric acid combined with salicylic acid maximized the plant height followed by the mixture of manganese and zinc. Manganese treatment followed by the mixture of citric acid and salicylic acid then zinc were the most effective in increasing the number of leaves plant<sup>-1</sup>. While, Rizolex-T 50 had no significant effect on plant height and number of leaves plant<sup>-1</sup>. Microelements were more effective than antioxidants on enhancing the stem and flower head diameters. The combination between manganese and zinc followed by Rizolex-T 50 recorded the maximum values of the stem and flower head diameters. Giza 102 variety recorded the highest values of 100 seeds weight, total phenols, photosynthetic pigments and the percentage of seed oil when the above applications were carried out. Sakha 53 variety showed the highest plant yield under the above treatment. The mixtures of citric and salicylic acids were highly effective in increasing plant yield. The highest values of photosynthetic pigments were shown in salicylic acid treatment followed by Rizolex-T 50. Total phenols content was highest due to Rizolex-T 50 application followed by salicylic acid. Except zinc, seed oil concentration increased significantly in both antioxidants and microelements treatments on oil concentration. (*Plant Pathology Journal* 8 (3): 79-89, 2009; *doi*: 10.3923/ppj.2009.79.89)

### **Seed-Borne Pathogens of Faba Bean in Egypt: Detection and Pathogenicity**

M.A. Elwakil, I.M. El-Refai, O.A. Awadallah, M.A. El-Metwally and M.S. Mohammed

This study was undertaken to study the seed-borne fungi of faba bean that attack the plants and reduce their yield in Egypt. The results provide a database for further study to control the pathogens. Twenty-six seed samples representing six faba bean cultivars collected from different parts of Egypt were used in this investigation. The blotter and deep-freezing methods were used. Surface- and non-surface-sterilized faba bean seeds were tested to detect and isolate the associated seed-borne fungi. The following 20 fungal species belonging to 13 genera were observed and identified: *Aspergillus flavus* (Link ex. Gray), *Aspergillus niger* (Van Tieghem), *Aspergillus ochraceus* (Wilhelm), *Penicillium*

*digitatum* (Pers.:Fr.) Sacc., *Penicillium italicum* (Wehmer), *Alternaria alternata* (Fr.) Keissler, *Botrytis faba* (Sardina), *Cephalosporium* sp., *Cladosporium cladosporioides* (Frensen. de Vries), *Epicoccum nigrum* (Link), *Fusarium oxysporum* (Schlechtendahl), *Fusarium semitectum* (Berkeley and Ravenel), *Fusarium solani* (Mart.) Sacc., *Fusarium verticillioides* (*moniliforme*) (Sheld), *Rhizoctonia solani* (Kühn), *Rhizopus stolonifer* (Ehr. ex Fr.), *Stemphylium globuliferum* (Vestergr.) E.G. Simmons, *Trichothecium roseum* (Pers.) Link, *Verticillium dahliae* (Ehrenp) Vuill. The blotter method yielded a greater number of fungi than the deep-freezing method on both surface and non-surface sterilized seeds, but the deep-freezing method was better for slow-growing fungi. The pathogenicity test revealed that the most commonly isolated fungi from pre- and post-emergence damping-off and stunted seedlings were *F. verticillioides*, *R. solani*, *Cephalosporium* sp. and *V. dahliae*. These fungi significantly reduced the photosynthetic pigments in faba bean leaves. *Fusarium verticillioides* caused the greatest reduction in chlorophyll content (A, B and total chlorophyll). *Fusarium oxysporum* and *V. dahliae* significantly reduced carotenoid content. *R. solani* significantly reduced total phenols content when compared with the other tested fungi. (*Plant Pathology Journal* 8 (3): 90-97, 2009; doi: 10.3923/ppj.2009.90.97)

### **Isolation and Evaluation of Indigenous Fungal and Bacterial Isolates as Potential Bioagents Against Broomrape (*Orobanche cernua*) in Jordan**

S.J. Goussous, K.M. Hameed and I. Saadoun

Isolation of microorganisms antagonistic to *Orobanche* was attempted using *Orobanche* plants and rhizosphere soil of *Orobanche*-infected crops in Jordan. Six fungi (*Cephalosporium* sp., *Cylindrocladium* sp., *Epicoccum* sp., *Fusarium* sp., *F. oxysporum* and *F. solani*) were isolated. Of these, isolates that belonged to the *Fusarium* genus were the most common (more than 80% of isolates). Pathogenicity tests of these isolates on *O. cernua* stems indicated that *Cylindrocladium* sp., *Fusarium* sp., *F. oxysporum* and *F. solani* were most effective. These organisms caused total necrosis of inoculated stem tissues. *Epicoccum* sp. caused moderate damage (60% severity); while *Cephalosporium* sp. was least effective causing only localized necrosis. Only *Cylindrocladium* and *Fusarium* sp. isolates infected *Orobanche* inflorescence, causing maceration of these tissues as well as total destruction of seeds. Several bacterial isolates, including *Pseudomonas* and *Actinomyces* sp., were also recovered from soil sampled from different agricultural fields. These bacteria were found to be pathogenic in varying degrees to *Orobanche* stems and inflorescence. A

previously isolated *Streptomyces* sp., R9, was also assessed in this study for its ability to inhibit *O. cernua* seed germination. Results showed a germination rate of 1-1.4% and 2.9-3.8% for R9 crude and lyophilized culture filtrates, respectively. In comparison, seed germination rates were 33.8-42.2% and 6.2-9.1% for water and culture medium controls, respectively. This study indicates that a wide range of microorganisms could be employed as antagonists to *Orobancha*. Their use as potential bioherbicides to control *Orobancha* appears promising. (*Plant Pathology Journal* 8 (3): 98-105, 2009; doi: 10.3923/ppj.2009.98.105)

### **Effect of Crop Rotation on the Soil Pathogen Population Dynamics and Canola Seedling Establishment**

S.F. Hwang, H.U. Ahmed, B.D. Gossen, H.R. Kutcher, S.A. Brandt, S.E. Strelkov, K.F. Chang and G.D. Turnbull

Impact of long-term crop rotations on populations of soilborne pathogens in the genera *Fusarium*, *Pythium* and *Rhizoctonia* and on canola seedling establishment and development was evaluated under controlled conditions. Soil samples were collected from two crop rotation experiments conducted at two sites in Saskatchewan, Canada. A part of the 2006-soil sample of each rotation was sterilized to compare canola seedling growth with and without soilborne pathogens. With 2007-soil, the sterilization treatment was replaced with a fungicide seed treatment (Apron Maxx) to assess the potential to reduce seedling diseases. Populations of *Fusarium*, *Pythium* and *Rhizoctonia* sp. were estimated in the soil of each rotation using dilution plating onto selective media for each fungus. Higher seedling emergence and increased growth of canola were obtained in the sterilized soil or due to seed treatment. *Fusarium* was the predominant genus followed by *Pythium* and *Rhizoctonia* sp. in the soil of both sites. This study suggests that diverse crop in the rotation may reduce the populations of *Fusarium*, *Pythium* and *Rhizoctonia* in the soil and may contribute to improve the overall growth of canola. (*Plant Pathology Journal* 8 (3): 106-112, 2009; doi: 10.3923/ppj.2009.106.112)

### **The Occurrence of Aflatoxins in Maize and Distribution of Mycotoxin-Producing Fungi in Eastern Kenya**

J.W. Muthomi, L.N. Njenga, J.K. Gathumbi and G.N. Chemining'wa

Aflatoxin poisoning resulting from consumption of contaminated maize has continued to recur in a yearly pattern in Eastern Kenya. The largest mycotoxin-

poisoning epidemic in the last decade was reported in Kenya in 2004. Therefore, this study was carried out to determine the occurrence and levels of mycotoxin-producing fungi and aflatoxin B<sub>1</sub> in maize and soils from Eastern Kenya. Maize, soils and mill dust samples were collected from farmers and traders in Machakos to determine the incidence of mycotoxin-producing fungi and aflatoxins during the 2007 harvest season. Fungal isolation was done by plating on agar medium, while aflatoxin B<sub>1</sub> was determined by ELISA. The most frequently isolated fungi were *Fusarium* and *Aspergillus* species and the *Aspergillus* species identified were *A. flavus*, *A. niger*, *A. terreus* and *A. versicolor*. *Aspergillus flavus* was frequently isolated from mill dust and soils from under the stores. Aflatoxin levels of up to 160 µg kg<sup>-1</sup> were detected in samples from areas with high *A. flavus* isolation and in whole maize than in semi-processed grain. Most mill dust samples were contaminated with aflatoxin up to 80 µg kg<sup>-1</sup>. The results indicate that *A. flavus* is the main producer of aflatoxins in maize Machakos and high inoculum levels of the fungus are present in soils, near stores and maize mills. Therefore, management of aflatoxin poisoning should include reduction of *A. flavus* inoculum in farms and storage environment. (*Plant Pathology Journal* 8 (3): 113-119, 2009; doi: 10.3923/ppj.2009.113.119)

### **Causal Agents of Root Rot and the Effect of Vesicular-Arbuscular Mycorrhizal Fungi in Seedlings of *Rhodiola rosea* in Alberta, Canada**

S.F. Hwang, H.U. Ahmed, K. Ampong-Nyarko, S.E. Strelkov, R.J. Howard and G.D. Turnbull

*Rhodiola* (*Rhodiola rosea*) is a plant with adaptogenic properties and is suitable for cultivation in Alberta, Canada. Disease surveys indicated the occurrence of root rots in rhodiola plantations in the Province. A total of 74 fungal isolates were associated with discoloration and rotting in the crown and root regions of the plants. Among these, 15 isolates were identified as *Fusarium* sp., three as *Pythium* sp. and eight as *Rhizoctonia* sp. This is the first report of root rot in rhodiola in Alberta, Canada. These soil-borne pathogens are the potential threat to the quality and quantity of rhodiola production. Experiments were conducted to determine the effect of vesicular-arbuscular mycorrhizal fungi and these soil pathogens on rhodiola growth and development under greenhouse conditions. Overall results indicated that *Fusarium*, *Pythium* and *Rhizoctonia* sp. are all capable of reducing rhodiola biomass. However, biomass was significantly higher when vesicular-arbuscular mycorrhizal fungi were applied in conjunction with these pathogens or in non-inoculated controls. This suggests that vesicular-arbuscular

mycorrhizal fungi could be used as a management tool for the control of seedling root rot diseases of rhodiola. (*Plant Pathology Journal* 8 (3): 120-126, 2009; doi: 10.3923/ppj.2009.120.126)

### **Expression of Phenylpropanoid Pathway Genes in Chickpea Defense Against Race 3 of *Ascochyta rabiei***

H.R. Kavousi, H. Marashi, J. Mozafari and A.R. Bagheri

The fungal disease, ascochyta blight, caused by *Ascochyta rabiei* is a major yield limiting factor of chickpea (*Cicer arietinum* L.) around the world. Expression analysis of genes induced in general defense response can provide clues to elucidate major defense mechanisms against pathogen infection in chickpea plants. The role of key phenylpropanoid pathway enzymes response to *Ascochyta rabiei* in chickpea was studied under greenhouse conditions using a reverse transcription and semi-quantitative polymerase chain reaction (SQ-PCR). Transcript accumulation of four genes encoding phenylalanine ammonia-lyase (PAL), chalcon synthase (CHS), isoflavone reductase (IFR) and Flavanone 3-Hydroxylase (F3H) induced in response to race 3 of *A. rabiei* was compared in resistant and susceptible genotypes. Results obtained in this study showed that in resistant genotype all 4 phenylpropanoid pathway genes: PAL, CHS, IFR and F3H were rapidly up regulated 6 h after inoculation with race 3 of *A. rabiei*. However, transcripts of PAL and IFR genes were rapidly accumulated in both resistant and susceptible cultivars. Therefore, induction of key enzymes of phenylpropanoid pathway appeared to be an important defense mechanism of chickpea plants against *A. rabiei*. (*Plant Pathology Journal* 8 (3): 127-132, 2009; doi: 10.3923/ppj.2009.127.132)

### **The Use of Bread Yeast as a Biocontrol Agent for Controlling Seed-Borne Fungi of Faba Bean**

M.A. Elwakil, O.A. Awadallah, I.M. El-Refai, M.A. El-Metwally and M.S. Mohammed

Present objective was to study this phenomenon on the common seed-borne fungi of faba bean in soil amended with composted organic wastes and infested with the most commonly isolated fungi from *Vicia faba* seed. *In vitro* studies showed that the yeast was effective in reducing the linear growth of *Cephalosporium* sp., *F. verticillioides*, *F. oxysporum*, *F. solani*, *R. solani* and *V. dahliae*. Pre- and post-emergence damping-off caused by *Cephalosporium* sp., *F. verticillioides*,

*F. oxysporum*, *F. solani*, *R. solani* and *V. dahliae* was reduced significantly when seeds of faba bean were coated with a water suspension ( $10^9$  cfu mL<sup>-1</sup>) of the yeast before sowing in soil supplemented with compost type (1) (prepared by Mansoura manufacturer with organic waste from city garbage) or type (2) (consisted of 1 ton of horticultural waste and 100 kg sheep manure). Soil was artificially infested with the fungi isolated from faba bean seeds. The above treatment significantly increased plant growth parameters including height, shoot and root length, number of branches/plant, number of pods/plant, pod weight/plant, fresh weight and dry weight. Photosynthetic pigments (chlorophyll A, chlorophyll B and carotenoids) were also increased by the treatments. Total phenols content in the treated plant leaves was higher than in the control plants. (*Plant Pathology Journal* 8 (4): 133-143, 2009 doi: 10.3923/ppj.2009.133.143)

### ***In vitro* Assay of Factors Affecting the Growth of Pathogens Associated with Diseases on Dragon Fruit (*Hylocereus* spp.) in Peninsular Malaysia**

M. Masyahit, K. Sijam, Y. Awang and M.G.M. Satar

Knowing the unfavorable environment for the growth of a pathogen can be utilized as the basic information in developing appropriate strategies to prevent disease occurrence on dragon fruit. Several environmental factors including temperature, pH and salinity, as well as biotic factor including three antagonistic bacteria species, namely *Bukholderia cepacia*, *B. multivorans* and *Pseudomonas aeruginosa* against *Bipolaris* sp., *Colletotrichum gloeosporioides*, *Botryosphaeria* sp. and *Monilinia* sp., were investigated. Mycelial growth of all tested fungi was constantly inhibited by a temperature of 35°C, while a temperature of 25°C was quite suitable for their growth. A temperature of 30°C was favorable for the growth of *Colletotrichum gloeosporioides*. Under different pH condition, the growth of tested fungi was mostly inhibited by extreme pH of 4 and 10. The salinity assay showed that *Monilinia* sp. was not affected by all treatments among tested fungi. Only concentration 100 ppm could reduce the growth of *Bipolaris* sp., though its inhibition statistically affected on 4 and 6 Days after Incubation (DAI). Meanwhile, the *in vitro* examination of antagonistic bacteria resulted in *Bukholderia multivorans* which was highly effective in inhibiting the growth of examined fungi, except *Monilinia* sp., which was more significantly influenced by *B. multivorans* and *B. cepacia*. The proper combination of environmental modification may be useful for the growth of crop in the field as well as the storage life of the fruit at postharvest preservation. (*Plant Pathology Journal* 8 (4): 144-151, 2009; doi: 10.3923/ppj.2009.144.151)

## **Sensitivity of *Penicillium digitatum* and *P. italicum* to Imazalil and Thiabendazole in Morocco**

H. Boubaker, B. Saadi, E.H. Boudyach and A.A. Benaoumar

Green and blue molds (caused by *Penicillium digitatum* and *P. italicum*, respectively) are the main postharvest diseases of citrus fruits in Morocco. Following packing houses reports of reduced efficacy of fungicides used to control these diseases, a survey was conducted during 2005-2006 packing season to characterize, both qualitatively and quantitatively, the resistance of *P. digitatum* and *P. italicum* to imazalil (IMZ) and thiabendazole (TBZ). Isolates of *P. digitatum* and *P. italicum* were obtained from decayed citrus fruits collected from commercial citrus packing houses located in the Souss-Massa-Draa (SMD), South of Morocco and were evaluated *in-vitro* for their sensitivity to IMZ and TBZ. Of the 290 *P. digitatum* isolates, 19% (55/290) were resistant to IMZ and 37% (107/290) were resistant to TBZ tested at discriminatory concentrations of 0.1 or 20  $\mu\text{g mL}^{-1}$ , respectively. In contrast, only 2.5 (5/204) and 21% (44/204) of *P. italicum* isolates collected from packing houses were resistant to IMZ and TBZ, respectively. No resistance to TBZ and IMZ were detected in *Penicillium* sp., isolates collected from a citrus orchard which has no known history of fungicide use. The proportion of collected isolates that were resistant to both fungicides was 1.5% for *P. italicum* and 10% for *P. digitatum*. The mean  $\text{EC}_{50}$  values for *in vitro* inhibition of mycelial growth of the *P. digitatum* resistant-isolates were between 0.81 and 0.98  $\mu\text{g mL}^{-1}$  for IMZ, whereas those of TBZ were between 39.23 and 50.84  $\mu\text{g mL}^{-1}$ . The mean  $\text{EC}_{50}$  values for *P. italicum* resistant-isolates ranged from 0.53 to 0.61  $\mu\text{g mL}^{-1}$  for IMZ and from 52.97 to 59.92  $\mu\text{g mL}^{-1}$  for TBZ, whereas the mean  $\text{EC}_{50}$  values for orchard collected isolates were 0.04  $\mu\text{g mL}^{-1}$  for IMZ and 0.16  $\mu\text{g mL}^{-1}$  for TBZ. The data will provide a baseline for monitoring resistance to IMZ and TBZ in populations of *P. digitatum* and *P. italicum* in the SMD commercial citrus packing houses in the future. (*Plant Pathology Journal* 8 (4): 152-158, 2009; doi: 10.3923/ppj.2009.152.158)

## **Integrated Management of *Meloidogyne incognita* Infecting Soybean by Certain Organic Amendments, *Bacillus thuringiensis*, *Trichoderma harzianum* and Oxamyl with Reference to NPK and Total Chlorophyll Statuus**

A.G. El-Sherif and Amona F.A. Ismail

The integrated control of *M. incognita* infecting soybean cv. Giza 21 using camel manure, dried leaf powder of marigold, *Trichoderma harzianum* 100% filtrate,

*Bacillus thuringiensis* singly or in combination with oxamyl under greenhouse condition (22±3°C) indicated that the concomitant treatments obviously gave better results than single ones did. Moreover, *B. thuringiensis* plus oxamyl at half dose each surpassed all other tested materials in percentage increase of total plant fresh and shoot dry weights (99 and 88 %), respectively, followed by *T. harzianum* filtrate plus oxamyl (95 and 61%) in this respect. On the other hand, pots received *T. harzianum* filtrate plus oxamyl ranked first in suppressing final nematode population (90%), root galling (65%) and egg mass numbers (62.79%), followed by B.t. plus oxamyl, marigold powder +oxamyl and then camel manure + oxamyl, where their reduction percentage values amounted to 88.7, 62.5 and 61%; 88.5, 59.87 and 58.9% and 67, 50.5 and 52.97%, respectively. Of the tested singly applications, *T. harzianum* filtrate showed the best results in improving plant growth and suppressing nematode development criteria, followed by B.t., whereas, marigold powder and camel manure achieved the lowest values in this respect comparing to nematode alone. Regarding the N, P and K concentrations in leaves of soybean plants inoculated with *M. incognita* treated with certain organic matters or fungal filtrate or bacterium alone or mixed with oxamyl, they were obviously enhanced by all tested materials whereas the opposite results was recorded for total chlorophyll content comparing to nematode alone. (*Plant Pathology Journal* 8 (4): 159-164, 2009; doi: 10.3923/ppj.2009.159.164)

### ***Puccinia pimpinellae*, a New Pathogen on Anise Seed in Egypt**

K.M. Ghoneem, M.A. Elwakil and A. El-Sadek Ismail

Routine seed health inspection of anise seeds showed *Puccinia pimpinellae* to be a commonly observed fungus on seed samples collected from different locations and the commercial markets of Egypt. Symptoms were shown as black discolorations on seeds. Masses of uredio- and teliospores of the fungus were visually seen. In some samples, a seed washing technique was essential to inspect for the presence of the fungal spores. This is the first report of *Puccinia pimpinellae* as a seed-borne pathogen of anise in Egypt. (*Plant Pathology Journal* 8 (4): 165-169, 2009; doi: 10.3923/ppj.2009.165.169)

### **A Revised Check-List of the Bryophytes of A4 Square of Turkey**

Turan Özdemir

The checklist includes a list of genera, species, subspecies and varieties of A4 square (including Trabzon, Giresun, Rize, Gümüşhane, Bayburt and Artvin

regions of Turkey) adopted by Henderson. It consist of 469 taxa of Bryophyta, 2 of them belong to Anthocerotopsida, 95 to the Hepaticopsida and 382 to Bryopsida. The result of the studies done in the study areas 112 taxa (10 Hepaticopsida and 102 Bryopsida) have been added as new record to A4 square from 2000 up to now (September 2008). Of these, 12 taxa (6 Hepaticopsida and 6 Bryopsida) are new to Turkey for A4 square in last ten years. (*International Journal of Botany* 5 (1): 1-35, 2009; *doi*: 10.3923/ijb.2009.1.35)

### **Distribution of Flowering Plants and Cyanobacteria in Relation to Soil Characters in Bahariya Oasis, Egypt**

I.B.M. Ibraheem and E.A. Al-Sherif

Baharia Oasis in one of the famous Oases in western desert of Egypt. This study dealt with the distribution of flowering plants and cyanobacteria in the Oasis in relation to each other and to physicochemical characters of soil. Fifty six species of flowering plants and 29 cyanobacterial species were identified in seven different habitats. The data revealed that the flowering plants and algal taxa were controlled by the edaphic factors and physico-chemical characters of the soil. In the present study, both positive and negative correlations between flowering plants and cyanobacterial taxa were obtained confirming the controversial effect of cyanobacterial crust on vascular plants. (*International Journal of Botany* 5 (1): 36-46, 2009; *doi*: 10.3923/ijb.2009.36-46)

### **The Distribution of C<sub>3</sub> and C<sub>4</sub> Photosynthetic Species of the Centrospermeae Along an Altitudinal Gradient in Western Kenya**

S. Sikolia, J.C. Onyango, E. Beck and J.I. Kinyamario

Two hundred and seventy eight species of the Centrospermeae were collected at different sites in Western Kenya representing gradients of altitude and aridity. Climate data were obtained from meteorological research stations. Species were examined for C<sub>3</sub> or C<sub>4</sub> photosynthesis using the anatomical Kranz syndrome,  $\delta^{13}\text{C}$  values and carbon dioxide compensation points. C<sub>4</sub> photosynthesis is a feature of modern members of dicotyledoneae is of multiple evolutionary origins. It evolved independently in members of the same family and was found in one to several genera and then often only with two to three species. C<sub>4</sub> species are concentrated in lowland habitats subjected to high temperature, low

precipitation and high evaporation. High  $\delta^{13}\text{C}$  values is associated with low water availability which is a physiological syndrome and also a feature of saline habitats. The  $\text{C}_3$  representatives of the Centrospermeae dominate in more moist and colder habitats, especially at higher altitudes. Only a few  $\text{C}_4$  species occur at high altitudes (3000-4000 m) namely *Sagina gallica*, *Silene abyssinica* and *Melandrium nordiflorum*. The transition zone between  $\text{C}_3$  and  $\text{C}_4$ -dicot is rather narrow between 1500 to 1700 m and thus much lower than that recorded for the monocots (2000-2200 m). The general pattern of  $\delta^{13}\text{C}$  values distribution along the altitudinal gradient show that the values of -10.60 to -16.55, -17.75 to -18.87 and -18.89 to -32.42‰ that corresponds to altitudinal ranges, 0-1500, 1550-1700 and 1800-4200 m, respectively. The low altitudes are associated with drought and high temperatures.  $\text{C}_4$  and  $\text{C}_3$  dicot species can be intercropped to increase bioproductivity for the betterment of the flora and fauna in the semi-arid and arid ecosystem.  $\text{C}_4$ -species are potential candidates for exploitation in the agroforestry systems especially for long-term management programmes. The present study may also be relevant for better understanding of global change with respect to the diversity of photosynthetic pathways, herbivory and vegetation dynamics. (*International Journal of Botany* 5 (1): 47-57, 2009; doi: 10.3923/ijb.2009.47-57)

### **The Effects of Aluminum on Fiber and Protein Bound Condensed Tannin, Polyphenols and Some Growth Index in Two *Sorghum* Cultivars**

H.A. Malmir, A. Mostajeran, A. Almodares, G. Asghari, A. Afkhami and H. Nassiry-Hachellu

The effect of Al on fiber and protein bound condensed tannin, polyphenols, tannin and some growth index in two cultivars of *Sorghum* (cult. 132 and 552) has been investigated. The medium culture was river sand and peat in 3:1 ratio, respectively. All pots received Hoagland's nutrient solution with and without  $\text{AlCl}_3$ . The first plant samples were obtained 30 day after sowing and the others were obtained each two weeks interval (days, 30, 44, 58, 72, 86, 90, 114 and 128) up to the end of plant's vegetative growth. Plant dry weight, growth rate and leaf area were measured and then total tannin, fiber and protein bound condensed tannin, total polyphenols in eight successive harvests were compared. The results indicated that the mean growth rate for cultivars 132 and 552 in control plants were 255 and 196 mg/plant/day, respectively and when Al contributed in the growth medium, the mean growth rates were reduced to 224 and 170 mg/plant/day in cultivars 132 and 552, respectively (almost 36% reduction). In average, leaf expansions were

6.4 and 4.5 cm<sup>2</sup> day<sup>-1</sup> in cultivar 132 and 552, respectively and Al significantly decreased (p<0.001) the leaf area by 11.3 and 7.1% in cultivar 552 and 132, respectively (p<0.001). Al causes to increase the concentration of Protein Bound Condensed Tannin (PBCT) in leaves of two cultivars with different patterns. In cultivar 132, PBCT was accumulated almost in a rate of 0.075 mg g<sup>-1</sup> DW day<sup>-1</sup> in plant during growing season. In contrast, in cultivar 552 the amount of PBCT was the same at early stage of growth in plants treated with Al and then increased slightly afterward. Although the root's PBCT were lower in cultivar 552, their amounts were decreased up to the end of growing season. This behavior was completely different in cultivar 132. Adding Al into nutrient media would change the pattern of PBCT in root. The amounts of PBCT in control plants were higher than fiber bound condensed tannin. However the trend was different in different cultivars respect to Al toxicity. Amount of total polyphenols in control plants were higher in cultivar 132 (90.9 mg g<sup>-1</sup> DW) than cultivar 552 (52.6 mg g<sup>-1</sup> DW) during growing season however Al has no significant effect on the amount of total polyphenols except at late stage of growth in which Al increased total polyphenols in both cultivars. Total tannin in cultivar 132 was peaked at middle stage of growth and was lower at younger and elder leaves. Al causes to increase the total tannin at elder leaves. (*International Journal of Botany* 5 (1): 58-66, 2009; doi: 10.3923/ijb.2009.58-66)

### **Carbon Dioxide Compensation Points of Some Dicots of the Centrospermeae Species and Their Ecological Implications for Agroforestry**

S. Sikolia, E. Beck and J.C. Onyango

The present studies on carbon dioxide compensation point (l) considered species from tropical semi-arid, snowline and saline ecosystems. The aim of the study was to establish the ecological range of the CO<sub>2</sub> compensation point of species in the semi-arid/arid, snowline/or saline conditions. Secondly, to determine the effect of biomass on the rate of carbon dioxide assimilation in relation to the ecological efficiency of the C<sub>3</sub> and C<sub>4</sub> photosynthetic pathways. Four and six-seven week old plants were used to assimilate carbon dioxide in the gas chamber until a constant reading was attained by Infra Red Gas Analyzer (IRGA). The carbon dioxide uptake concentrations (assimilations) were continuously measured by pumping a stream of the air through a closed gas tight, circuit with IRGA while temperature and light intensity and previous growth conditions were maintained constant. The dry weight of the plant was measured using a digital balance after the experiment. The CO<sub>2</sub> compensation points of the C<sub>4</sub> plants vary between 8-20 ppm. The CO<sub>2</sub>

compensation points of the C<sub>3</sub> plants vary between 40-60 ppm. The age and biomass of the plant influenced the rate of carbon dioxide assimilation in the C<sub>4</sub> species and C<sub>3</sub> species. The C<sub>4</sub> plant attained the CO<sub>2</sub> compensation point faster than C<sub>3</sub> plant under the same physiological conditions. The C<sub>4</sub> plant photosynthesized below 40 ppm of carbon dioxide concentration. The C<sub>3</sub> plant ceased carbon dioxide assimilation below 40 ppm of carbon dioxide concentration. Thus, an ideal ecological canopy set-up should consist of a C<sub>4</sub> overstorey and a C<sub>3</sub> understorey for efficient photosynthetic performance and yield. Potential C<sub>4</sub> overstorey species including *Amaranthus* species and *Kochia scoparia*, should be intercropped with potential C<sub>3</sub> understorey species like *Chenopodium album* and *Phytolaca dioica* by farmers and horticulturalists in Agriculture. The intercropping practice is economical, viable and apt in agroforestry systems, especially in the semi-arid and saline conditions socialized by nomadic tribes in Kenya. Perkerra irrigation project can act as satellite agroforestry research station, including Kerio valley and Turkana regions. (*International Journal of Botany* 5 (1): 67-75, 2009; doi: 10.3923/ijb.2009.67-75)

### **Sequence and Expression Analysis of EgSAPK, a Putative Member of the Serine/Threonine Protein Kinases in Oil Palm (*Elaeis guineensis* Jacq.)**

Huynh Ky, Le Vinh Thuc, Siew-Eng Ooi, Z. Ishak, P. Namasivayam and S. Napis

In present study, EgSAPK (EU805512), an oil palm transcript coding for a putative SAPK protein kinase, have been molecular characterized. The cDNA for EgSAPK isolated from an oil palm cell suspension culture is 1470 bp in length with a longest Open Reading Frame (ORF) of 963 bp. No translation start codon could be identified so EgSAPK cDNA sequence is lacking the 5'-end. The deduced protein sequence shares 89% identity with the serine/threonine protein kinase SAPK9 from rice (AB125310.1). Real time PCR results showed that the expression levels of EgSAPK varied in different oil palm tissues and the EgSAPK gene shares a similar expression pattern with the SAPK gene of rice. Furthermore, the transcription of the EgSAPK gene in green embryo, white embryo and embryogenic calli tissues were higher than in non-embryogenic calli tissues. Southern blot analysis showed that the EgSAPK gene might be present as a single copy gene in the oil palm genome. These results suggest that EgSAPK may have a similar function as the SAPK gene of rice and thus can be a candidate marker for oil palm somatic embryogenesis. (*International Journal of Botany* 5 (1): 76-84, 2009; doi: 10.3923/ijb.2009.76-84)

## **Bryophyte Diversity Within Urban Areas: Case Study of the City of Belgrade (Serbia)**

M. Sabovljević and S. Grdović

The bryophyte diversity and urban bryophyte flora of the city of Belgrade was studied. In total 210 taxa were recorded, with 23 hepatics and 187 mosses. Comparing to some other urban areas of Europe, bryophyte diversity of the Belgrade metropolitan belongs among the richest in Europe, however bryophytes are not abundant. Among bryophytes of Belgrade city, there are 14 nationally red-listed species and two internationally threatened. According to the frequency of records, the Belgrade bryophytes are classified to rare, common and spread. Rare species within the urban area are 94, 69 are common to find and only 24 are spread and easy to record in Belgrade wide area. Urban metropolitan areas are different from native but gives various condition in small shelters for rich diversity of small organisms like bryophytes. (*International Journal of Botany* 5 (1): 85-92, 2009; doi: 10.3923/ijb.2009.85-92)

## **Hybridization and Polyploidy: Cytogenetic Indications for *Hoffmannseggella* (Orchidaceae) Species Evolution**

Júlia Yamagishi-Costa and Eliana Regina Forni-Martins

In the present research through chromosome counts utilizing root meristems and immature ovaries and/or observation of meiotic behavior from floral buds, we analysed seven *Hoffmannseggella* species and confirmed the basic number of  $x = 20$ . From the seven species analyzed, one presented polyploidy (*H. briegei*) (Blumensch. ex Pabst) V.P. Castro and Chiron,  $2n = 80$ ), one presented both diploid ( $2n = 40$ ) and polyploid ( $2n = 80$ ) cytotypes (*H. rupestris* (Lindley) V.P. Castro and Chiron) and the five remaining species presented  $n = 20/2n = 40$  chromosomes. Polyploid species/cytotypes presented aneusomatic root tissues. Meiotic abnormalities, like monovalents, early disjunction of bivalents and putative tetravalents were observed in several species. We suggest that hybridization and polyploidy are, if not the major, at least very important mechanisms for the evolution of species and that these events are probably occurring in the present, possibly being responsible for many taxonomic divergences within the group. (*International Journal of Botany* 5 (1): 93-99, 2009; doi: 10.3923/ijb.2009.93-99)

## Compatibility, Growth and Production Potentials of Mustard/Lentil Intercrops

M.M. Rahman, M.A. Awal, A. Amin and M.R. Parvej

An experiment was conducted to analyse the interspecies compatibility and production potentials of mustard and lentil in intercrop association. The experiment comprised four planting systems viz., sole mustard, sole lentil, single row (1:1 i.e., one row of mustard followed by one row of lentil) and double row (1:2 i.e., one row of mustard followed by two rows of lentil) intercropping. The stands height and number of branches (primary and secondary) per plant were maximum and minimum in sole and single row intercropped plants, respectively. Higher leaf area index and total dry matter production was observed in sole cropped mustard or lentil while those were lower in 1:2 intercropped mustard or 1:1 intercropped lentil plants. Maximum seed yield, 1.26 t ha<sup>-1</sup> (or 1.30 t ha<sup>-1</sup>) was harvested from sole crop of mustard (or lentil) which was about 40 and 48% (or 34 and 12%) higher than that of the mustard (or lentil) yield obtained from single and double row intercrop mixtures, respectively. Combined seed yield from double row mixture was the maximum (1.8 t ha<sup>-1</sup>) and was respectively 11, 30 or 28% higher than that obtained from single row intercropped stands, sole mustard or lentil. Single and double row intercropping systems respectively resulted 25 or 41% increase in land equivalent ratios. Area time equivalency ratio was also increased by about 14 and 31%, respectively for single and double row intercropping systems. The competitive ratio of each population is approached to be unity in both intercropping systems reflecting the proper balance of the natural resources between associated species resulted better yield. The results suggest that mustard and lentil populations are well compatible in intercrop association and 1:2 row ratio mixture would be better for their profitable production. (*International Journal of Botany* 5 (1): 100-106, 2009; doi: 10.3923/ijb.2009.100-106)

## Post-Fire Regeneration of a *Pinus brutia* (Pinaceae) Forest in Marmaris National Park, Turkey

Ç. Tavşanoğlu and B. Gürkan

Post-fire recovery of a *Pinus brutia* Ten. forest on ophiolitic rocks was studied in Marmaris National Park, southwestern Turkey. Three study sites burned in different years (1999, 1995 and 1979) and a study site not burned for at least 45 years were studied from September 2000-2001. Although some opportunistic species had established in one-year-old site, species composition had been recovered again in older sites. Change in the plant species cover during

early post-fire succession was basically different between seeders and resprouters; cover of the seeders increased, but that of resprouters did not change. Post-fire growing line of *P. brutia* individuals during 22 year period was fitted to a linear regression model ( $r^2 = 0.9995$ ,  $p < 0.001$ ). In conclusion, post-fire regeneration of *P. brutia* forests on ophiolitic rocks in Marmaris National Park fits to the general autosuccessional model in Mediterranean Basin and fire is a complementary element for these forest ecosystems. (*International Journal of Botany* 5 (1): 107-111, 2009; doi: 10.3923/ijb.2009.107-111)

### **Ratooning Potential of Interspecific NERICA Rice Varieties (*Oryza glaberrima* × *Oryza sativa*)**

K.A. Sanni, D.K. Ojo, M.A. Adebisi, E.A. Somado, O.J. Ariyo, M. Sie, I. Akintayo, D.D. Tia, S.A. Ogunbayo, B. Cissé, M. Sikirou and M.A. Adekoya

Field experiment was conducted in Deve (6°48'N, 1°47'E, 72 masl) in the Savannah zone of Benin Republic, to examine the increase in grain yield due to ratooning. Eighteen upland interspecific varieties (NERICA1-18) and their *Oryza glaberrima* (CG 14) and one of the *O. sativa* (WAB 56-104) parents were used in a Randomized complete block design with three replications. The first (main) crop was harvested at mass maturity, after which the tillers were hand mowed to stubbles of about 10 cm tall. These were then left without any further input, until the ratooned plants were ready for harvest. The result showed a large variation in the ratoon performance among NERICA, with ratoon ability ranging from 13% (NERICA 2) to 39% (NERICA 14 and 17). Total grain yield (main plus ratoon) was significantly different ( $p < 0.001$ ) from that of the main harvest. The maximum total grain yield was 6.14 t ha<sup>-1</sup> for NERICA 2 followed by NERICA 15 and 11 (6.02 and 6.01 t ha<sup>-1</sup>, respectively). The yield increase of more than 1.5 t ha<sup>-1</sup> (the average yield of upland rice in Sub-saharan Africa) recorded in NERICA, with no additional input was very encouraging. This will presumably increase with additional input during ratoon. Therefore, NERICA rice is able to fructify twice, hence farmers can harvest more rice and make more profit. (*International Journal of Botany* 5 (1): 112-115, 2009; doi: 10.3923/ijb.2009.112-115)

### **Floristic Composition of Lake Al-Asfar, Alahsa, Saudi Arabia**

Ashraf M. Youssef, Mohamed A. Al-Fredan and Adel A. Fathi

The vegetation communities of the shores of Lake Al-Asfar; a large salt lake in Al-Hofouf, Al-Hassa, Saudi Arabia; were studied. The aim of the research was to study the relationship between the distribution of vegetation along salt lake

shores in relation to soil and climatic conditions. Four distinct lake shore habitats were examined. A total of 72 stands along the study area of the lake were investigated. It was concluded that soil texture, pH, soil moisture content, mineralization as well as the climatic factors were likely to be key factor in determining the distribution of vegetation communities along the shores and habitats of the lake. The study included: list of species and their families, growth forms, frequencies, densities, abundances, recurrence, diversity richness, heterogeneity and evenness in each of the four habitats along the lake. A total of 39 plant species belonging to 20 families were identified from the four studied habitats. More than 61% of the species recorded were perennial shrubs (PSH). Diversity richness indices were 2.02, 2.22, 3.05 and 4.91 in the inundated wet zone (Site I), moist zone (Site II), semi-dry zone (Site III) and arid zone (Site IV), respectively. Heterogeneity was from 2.01-3.10 (Shannon-H') and evenness was 0.89 to 0.98. The heterogeneity in species composition among the sites was moderate indicating that each site has its own unique flora. Those dominant communities occurring on highly and moderate saline soils of the four habitats (I, II, III and IV) along the lake included *Phragmites australis*, *Halocnemum strobilaceum*, *Zygodium mandavillei* and *Haloxylon salicornicum*, respectively. (*International Journal of Botany* 5 (2): 116-125, 2009; doi: 10.3923/ijb.2009.116-125)

## **A Checklist of Lekki Lagoon Diatoms**

T.A. Adesalu and D.I. Nwankwo

The diatoms of Lekki lagoon for the first time were studied at monthly intervals for two years (June 2003-May 2005). Two hundred and thirty seven (203 pennate and 34 centric forms) diatom species belonging to 50 genera were recorded. Among the pennate diatoms, the most frequent species were *Achnanthes conspicua*, *Bachysira follis*, *Bacillaria paradoxa*, *Craticula cuspidata*, *Cymbella ventricosa*, *Decussata placentula*, *Eunotia incisa*, *Frustulia rhomboides*, *Gyrosigma balticum*, *Luticola mutica*, *Nitzschia radiosa*, *Pinnularia biceps*, *P. gibba*, *Placoneis exigua*, *Plagiotropis* sp., *Sellophora pupila* and *Synedra ulna* var. *longissima*. The holoplanktonic forms included *Synedra ulna* (Nitz.) Ehr., *Synedra acus* Kutzing and *Tabellaria fenestrata* (Lyng.) Kutzing. The centrals were ably represented by *Aulacoseira* and its varieties, *Cyclotella* and *Terpsinoe musica*. In this study, fifty new diatoms species were recorded for Lagos lagoon complex while *Aulacoseira herzogii* is new record for Nigeria. Community structure analysis shows a highly diverse environment. (*International Journal of Botany* 5 (2): 126-134, 2009; doi: 10.3923/ijb.2009.126-134)

## **Effects of Salinity Stress on Growth, Ions Partitioning and Yield of Some Cowpea (*Vigna unguiculata* L. Walp.) Cultivars**

Victor Désiré Taffouo, Joseph Kemdem Kouamou, Louis Marie Tchiengue Ngalangue, Bop Alain Nandjou Ndjeudji and Amougou Akoa

In this study, twenty one cowpea cultivars (*Vigna unguiculata* L. Walp.) were tested for their salt tolerance at different degrees of salinity; 0, 50, 100 and 200 mM of NaCl, in both the laboratory and field conditions. In the laboratory, Na<sup>+</sup>, K<sup>+</sup>, K/Na ratio, plant height, roots dry weights, stems and leaves were investigated. In the field conditions, yield components (weight of 1000 seeds, number of pods per plant, total chlorophyll and grains yield) were determined in harvesting phase. Results showed that K<sup>+</sup> concentration, K/Na ratio, seedlings height and total chlorophyll were significantly decreased by salt solutions, especially by 200 mM and the magnitude of reduction varied according to cultivars. Na<sup>+</sup> was significantly increased with increasing NaCl concentrations in all plant organs. Roots dry weights as well as stems and leaves decreased significantly in all cultivars with increasing salinity except in organs of Bambey 21 (V11), IT97K-556-4 (V3) and IT04K-332-1 (V10) cultivars. Under field conditions, the weight of 1000 seeds, the number of pods per plant and grains yield were affected by soil salinity at 50 mM of all cultivars except in Bambey 21, IT97K-556-4 and IT04K-332-1. The results obtained during vegetative growth and harvesting phase suggested that Bambey 21, IT97K-556-4 and IT04K-332-1 cultivars were relatively tolerant to salinity than others. Bambey 21, IT97K-556-4 and IT04K-332-1 cultivars could be grown in environments with varying salinity. (*International Journal of Botany* 5 (2): 135-143, 2009; *doi*: 10.3923/ijb.2009.135-143)

## **A Multifunctional Acetyl-CoA Carboxylase Gene Confers Freezing Tolerance in *Arabidopsis thaliana***

Azura Amid and Gareth J. Warren

The *sfr3-1* mutation causes freezing-sensitivity in *Arabidopsis thaliana*. Through mapping, sequencing and transgenic complementation, *sfr3-1* was revealed as a missense mutation in ACC1, which is an essential gene encoding multifunctional acetyl-CoA carboxylase. Suppression of ACC1 expression by RNA interference produced a freezing-sensitive phenotype with some similarity to that of *sfr3-1*. The dCAPS primers and PCR confirmed that *sfr3* gene encodes multifunctional acetyl-CoA carboxylase. Microarray and real-time PCR experiments demonstrated that the expression of ACC1 increase only 1.48 fold in wild-type and 1.35 fold in mutant in response to cold treatment. Studies also suggested that

the *sfr3-1* mutation is more likely to be a temperature-sensitive mutation as the *sfr3* mutant cuticle becomes leaky only at low temperature and this was confirmed by cuticular defects analysis. (*International Journal of Botany* 5 (2): 144-152, 2009; doi: 10.3923/ijb.2009.144-152)

### **Variation of Some Nutritional Constituents and Fatty Acid Profiles of *Chlorella vulgaris* Beijerinck Grown under Auto and Heterotrophic Conditions**

Mostafa M. El-Sheekh and Alaa A. Fathy

This study is an attempt to evaluate the nutritional value of *Chlorella vulgaris* Beijerinck grown under autotrophic and heterotrophic conditions concerning their content of carotenoids, protein, proline, total free amino acids and fatty acids. Chlorophyll a (Chl.a) content of autotrophic cells of *C. vulgaris* was double that estimated in heterotrophic cells, while chlorophyll b (Chl.b) content of autotrophic cells was nearly half the value recorded for heterotrophic cells. Carotenoids (Car.) content of heterotrophic cultures decreased by 30.82% compared to that value of autotrophic cells. There was a slight decrease in the protein content of *C. vulgaris* under heterotrophic conditions. When the composition of total free amino acids and proline of *C. vulgaris* grown under autotrophic conditions is compared to that grown heterotrophically, it was observed that a significant increase in total free amino acids and proline in heterotrophic cultures. The percentage of most fatty acids of heterotrophic cells was relatively higher than autotrophic ones. There was no qualitative difference between autotrophic and heterotrophic cultures, except for the fatty acid 16:02 which was absent under autotrophic conditions. Present results showed that *C. vulgaris* has quite a simple qualitative fatty acids composition compared to other chlorophycean species, considering production of natural food supplements and/or natural pharmaceutical products, it is strongly recommended using autotrophic cells of *Chlorella* rather than using those of heterotrophic cells for such purpose. (*International Journal of Botany* 5 (2): 153-159, 2009; doi: 10.3923/ijb.2009.153-159)

### **Identification of Peach Genotypes (*Prunus persica* (L.) Batsch) in the North-Central Region, Mexico**

C.M. Melgoza Villagómez, H. Silos Espino, A. Nava Cedillo, L.L. Valera Montero, C. Perales Segovia, C.A. Martínez Guerra, G. Martínez Guerra, A. Cruz Hernández and O. Paredes López

Twenty four peach genotypes from the Central North Region of Mexico, were characterized based on morpho-physiological traits. Fruit weights from the

genotypes Roxana (135 g), San Gabriel C-167 (141.9 g) and Zacatecas landrace (162.3 g) were the higher, each in its group, since all genotypes were grouped according to their readiness to harvest as early-, middle- and late-harvest, respectively. RAPDs analysis yielded 52 monomorphic and 93 polymorphic fragments that were related to desirable characteristics from the *Prunus* genotypes. This information provide us tools for early individual identification of high-performance trees when still growing in the nursery. Therefore, growers may use this technique for assisted breeding program on their *Prunus* genotypes. (*International Journal of Botany* 5 (2): 160-165, 2009; **doi:** 10.3923/ijb.2009.160-165)

### **Development of Elephant Apple Fruit Quality as Affected by Postharvest Ethanol Application and Temperature**

A.B.M. Sharif Hossain, A. Nasrulhaq Boyce and Haji Mohamed

Experiments were conducted to study the effects of temperature and ethanol application on the development and quality of the elephant apple fruit. Various treatments were carried out, viz., at room temperature (RT, 28°C), in exposed sunlight (ES, 35°C), in 70% ethanol (ET, 28°C), under plastic covering (PC, 28°C), under plastic covering plus 70% ethanol (PCET, 28°C), at low temperatures (LT, 12°C) and at freezing temperature (FT, -1°C). The longest preservation time was observed in fruits preserved in FT (-1°C) and shortest in fruits preserved in ES (35°C) whilst the maturity index was highest in ES (35°C) treatments and lowest in FT (-1°C) compared to the other treatments at the end of the experiments. However, fruits kept at FT (-1°C) exhibited chilling injury symptoms. Total Soluble Solids (TSS) was highest in ES (35°C) and PCET (28°C) from the 1st to the 7th harvest compared to other treatments. A similar increasing trend in TSS was observed in all the treatments. On the contrary, Titratable Acidity (TA) was highest in FT (-1°C) and LT (12°C) from the 1st till the 7th harvest. Similarly a declining trend of TA was found in all the other treatments. TSS was found to be higher in pulp than in peel in the ethanol treatment at 6, 12, 24, 48 and 72 h. However, TA was higher in peel than pulp. The results showed that low temperatures (LT and FT) and plastic covering with 70% ethanol (PCET) delayed ripening in elephant apple fruits and were the best preservation techniques. (*International Journal of Botany* 5 (2): 166-170, 2009; **doi:** 10.3923/ijb.2009.166-170)

## **Pharmacognostical Comparison of Three Species of *Himatanthus***

José Luiz Pinto Ferreira, Ana Cláudia Fernandes Amaral, Renata Bastos de Araújo, Júnior Ribeiro Carvalho, Carolyn Elinore Barnes Proença, Sandra Aparecida Padilha Magalhães Fraga and Jefferson Rocha de Andrade Silva

The species *Himatanthus sucuuba* (Spruce ex Müll. Arg.) Woodson shows a wide range of use in folk medicine and other Amazonian species of this genus: *H. bracteatus* (A.DC.) Woodson and *H. stenophyllus* Plumel are also used by the great similarity between them. This study describes the macroscopic and microscopic morphological variation of leaves and stem bark of these species collected in the Amazonas state (Brazil). The contour of the leaf lamina, apex and the petiole aspects and the venation pattern were important features. The barks of *H. bracteatus*, differently from the other two species, did not present prismatic calcium oxalate crystals. Additionally, the extracts of the leaves, barks and latex of these species and the iridoids, plumieride (major in aqueous extracts of the leaves and latex from *H. bracteatus* and *H. sucuuba*) and isoplumieride (minor in all samples), were analyzed by HPTLC. The chromatographic profiles and the morphological analyses provided data for differentiation among the species. (*International Journal of Botany* 5 (2): 171-175, 2009; doi: 10.3923/ijb.2009.171-175)

## **A Preliminary Study on the Antibacterial Activity of *Quercus brantii* Against Bacterial Pathogens, Particularly Enteric Pathogens**

A. Safary, H. Motamedi, S. Maleki and S.M. Seyyednejad

The antibacterial activity of *Q. brantii* fruits ethanolic and methanolic extracts were examined using agar disc diffusion method against eight bacteria (*Salmonella typhi*, *Proteus mirabilis*, *Shigella dysenteriae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Brucella melitensis*, *Bordetella bronchiseptica*, *Pseudomonas aeruginosa*). These extracts had inhibitory effect at various concentrations (0.5, 0.1, 0.2, 0.3 and 0.4 g mL<sup>-1</sup>) against tested bacteria. The ethanolic extract had the highest activity (30 mm) against *Br. melitensis* and *B. bronchiseptica* while the lowest activity (7 mm) was demonstrated by the methanolic extract on *E. coli*. Studies on the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the

methanolic extract on tested microorganisms showed that the highest MIC (20 mg mL<sup>-1</sup>) and MBC (32 mg mL<sup>-1</sup>) were demonstrated against *Sh. dysenteriae*, *B. bronchiseptica* and *P. mirabilis* had the highest MIC and MBC values (32 mg mL<sup>-1</sup>) for the ethanolic extract. (*International Journal of Botany* 5 (2): 176-180, 2009; doi: 10.3923/ijb.2009.176-180)

### **The Effect of Stratification on Seed Germination of *Jasminus fruticans* L. (Oleaceae): A Contribution to a Better Insight on the Species Germination Ecology**

E. Pipinis, E. Milios, M. Aslanidou, O. Mavrokordopoulou and P. Smiris

A germination experiment was carried out in the laboratory to evaluate the effect of stratification on seed germination of *Jasminus fruticans*. Nine different treatments were used to break the embryo imposed dormancy. Seeds were subjected to warm stratification (20/25°C) for 1 or 2 months, cold stratification (2-4°C) for 1, 2 or 3 months and to the following warm plus cold stratification combinations: 1 month warm plus 1 month cold stratification (1W+1C), 1 month warm plus 2 months cold stratification (1W+2C), 2 months warm plus 1 month cold stratification (2W+1C) or 2 months warm plus 2 months cold stratification (2W+2C). Maximum germination (86.00%) and minimum mean germination time (11.26 days) were attained after 3 months of cold stratification without warm stratification. Seeds that were subjected to 2 months cold stratification exhibited 70.50% germination, whereas those stratified for 1W+2C or 2W+2C exhibited 69.00 or 67.50%, respectively. One month of cold stratification resulted in a germination percentage equal to 21%, whereas seeds that were subjected to warm stratification for 1 or 2 months prior to 1 month cold stratification gave germination percentages equal to 18.50 and 20.00%, respectively. None of the control seeds or those that were warm stratified for 1 or 2 months germinated. Results revealed that several months of cold stratification (3 months) were required to overcome physiological dormancy and to enhance *Jasminus fruticans* seed germination. (*International Journal of Botany* 5 (2): 181-185, 2009; doi: 10.3923/ijb.2009.181-185)

### **Management of Iron Deficiency Stress in Citrus through Soil Application of Vivianite to a Calcareous Soil**

Tarek G. Ammari, Alaeddin B. Tahboub and Taleb R. Abu-Zahra

Iron deficiency is a common abiotic stress in citrus trees grown on calcareous soils, where considerable reduction in yield is expected if not treated. In this

study the effectiveness of synthetic vivianite  $[(\text{Fe}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O})]$  to prevent Fe chlorosis in citrumelo Swingle (*Citrus paradisi* Macf. x *Poncirus trifoliata*) a susceptible rootstock to Fe deficiency stress, was investigated. One-year old citrumelo plants were grown on calcareous soil-sand mixture under greenhouse conditions and treated with: (1) no Fe (as control); (2) 1.6 g FeEDDHA plant<sup>-1</sup> (3) 5.4 g vivianite plant<sup>-1</sup>. Chlorophyll measurements were performed on the youngest fully expanded leaves in terms of SPAD index and at the end of the experiment leaf chlorophyll and Fe concentrations and growth vigor (young shoot dry weight) were determined. Vivianite was as effective as the FeEDDHA. Vivianite significantly prevented the development of Fe chlorosis. Chlorophyll concentration of plants treated with vivianite was significantly higher than those of control plants although vivianite-treated plants had almost equal leaf Fe concentration as control plants, vivianite significantly improved the vigor of citrus plants similar to the FeEDDHA compared to the control treatment. These results suggest that vivianite is an effective alternative to the environmental-unfriendly and expensive Fe-chelates for preventing Fe deficiency in citrus orchards. (*International Journal of Botany* 5 (2): 186-189, 2009; doi: 10.3923/ijb.2009.186-189)

### **Effect of Waste Water on Heavy Metal Accumulation in Hamedan Province Vegetables**

M. Cheraghi, B. Lorestani and N. Yousefi

The objective of this research was to elucidate the effect of waste water on heavy metal concentration in vegetables. To this purpose a region fertilized with waste water (Najafi Boolvar, Hamedan, Iran) was chosen as a polluted area and a region without any waste water pollution (Heydareh, Hamedan, Iran) as a control area. Eight kinds of vegetables were collected from both areas, separately and after preparing, Pb, Zn, Cu and Mn concentrations of them, were measured by using the atomic absorption spectrophotometer. Present results showed that concentration of some heavy metals in vegetables grown in Najafi Boolvar was several times as high as that in Heydareh. According to the results of present study, waste water have special problems for vegetables and thus for human health, because of ability of accumulating heavy metals in soil and biological accumulation of these elements in food chain. (*International Journal of Botany* 5 (2): 190-193, 2009; doi: 10.3923/ijb.2009.190-193)

## **A Floristic List and Phenology of Plant Species of Lawat Area District Neelum, Azad Jammu and Kashmir, Pakistan**

M.E.U.I. Dar and Z.H. Malik

The objective of this study was to collect the detailed information of plant biodiversity and phenological pattern of plant species in the area. For this purpose, detailed surveys were done during the months of March to November in 2005 and 2006. One hundred and eighty plant species were recorded from Lawat hills belonging to 66 families and 144 genera. The families Asteraceae, Balsaminaceae, Gentianaceae, Lamiaceae, Poaceae, Polygonaceae, Primulaceae, Ranunculaceae, Rosaceae, Scrophulariaceae and Umbellifereae were recorded with major contribution to the flora of the investigated area. Fifty one plant species (29%) flowered from March to May, 83 plant species (45%) flowered from the month of June and July, while 46 plant species (26%) flowered from August to September. Eleven plant species in the investigated area were reported as evergreen. (*International Journal of Botany* 5 (2): 194-199, 2009; *doi*: 10.3923/ijb.2009.194-199)

## **Epigeal Cryptocotyly in *Madhuca indica* J. F. Gmel. (Sapotaceae)**

A. Mundhra and N.D. Paria

An unusual case of epigeal cryptocotyly found in *Madhuca indica* J. F. Gmel. (Syn. *Bassia latifolia* Roxb.) of Sapotaceae is described and discussed in this study. The morphological characters shown by *M. indica* seedlings such as thickened hypocotyl, thick woody seed coat and non photosynthetic haustorial cotyledons are closely related to its epigeal cryptocotyly. The incidence of epigeal cryptocotyly germination in angiosperms seems to be scarce in available literatures. Such knowledge of germination and seedling morphology can throw some light in the silvicultural practices of this tree. (*International Journal of Botany* 5 (2): 200-202, 2009; *doi*: 10.3923/ijb.2009.200-202)

## **A Late Tertiary Pollen Record from Niger Delta, Nigeria**

O.E. Ige

This study presents the results of palynological investigations carried out on Atala-1 well, Niger Delta, Nigeria. The samples yielded pollen and spores and the identification of the pollen and spores, their relative diversity and abundance

provide data on which the palaeoenvironmental interpretations were based. Nine informal lithological units were delineated for the well, characterized by silty clay and very fine to very coarse sand grains which are typical of Benin and Agbada formations of the Niger Delta. Four pollen zones (PZ I-IV) were recognized from the pollen diagram and attempt was made at the reconstruction of the vegetation for the zones. The early part of the zone (I) was characterized by unstable wet and dry climatic conditions characterized by contrasting fluctuations between the percentage occurrence of *Rhizophora* sp. and Poaceae. The vegetation was gradually dominated by mangrove swamp vegetation towards the later part of the zones (II-IV), indicating a wet and moist climates for the period and a rise in sea level. (*International Journal of Botany* 5 (3): 203-215, 2009; *doi*: 10.3923/ijb.2009.203-215)

### **The Bryophyte Flora in the Urban Area of Aydin (Turkey)**

M. Kirmaci and E. Ağcagil

In this study, the bryophyte diversity and urban bryophyte flora of the city of Aydin was investigated. Research area was divided into three zones and nearly 500 bryophyte specimens were collected in 13 representative stands. One hundred and twenty three moss species belong to 22 families and 78 genera, 22 liverwort species belong to 14 families and 19 genera and one hornwort species were found in the area. *Fossombronia echinata* and *Crossidium crassinerve* which were recently recorded from Turkey were collected from the area as a second distributional locality. *Tortula muralis*, *Didymodon vinealis*, *Grimmia pulvinata*, *Bryum argenteum* and *Orthotrichum diaphanum* are the most common species found in the city center where high pollution exists. The protected areas in the city centre such as gardens, cemeteries, school yards etc. are necessary in order to protect of bryophytes. These areas are important to provide various habitats to small organism like bryophytes. (*International Journal of Botany* 5 (3): 216-225, 2009; *doi*: 10.3923/ijb.2009.216-225)

### **The Bryophyte Flora of Honaz Mountain (Denizli/Turkey)**

Mesut Kirmaci and Adnan Erdağ

The bryophyte flora of Honaz Mountain of Aegean Region (Western Turkey) has been investigated. One hundred and seventy five moss species belonging to 24 families and 64 genera, 20 liverwort species belonging to 14 families and

16 genera and one hornwort species were found on the area. *Orthotrichum rivulare* Turner and *Weissia breutelii* Müll. Hal. which was recently recorded from Turkey are collected from the area as a second distributional locality. And also some interesting taxa which are *Didymodon validus*, *Orthotrichum cupulatum* var. *bistratosum* and *Phascum cuspidatum* var. *schreberianum* collected from study area and discussed in the text. (*International Journal of Botany* 5 (3): 226-235, 2009; doi: 10.3923/ijb.2009.226-235)

### **Evaluation of Mycorrhizae Symbiosis Efficiency with Barley (*Hordeum vulgare* L.) through <sup>32</sup>P Uptake under Soils Contaminated with Heavy Metals**

M.R. Ardakani, S. Teimuri, M. Rezvani, H. Fathollahi, A. Khorasani, F. Rejali, A. Raza and F. Zafarian

This study designed to investigate more precise of mycorrhizal symbiosis in order to increasing mineral absorption by plant root system. Three pot experiments, radioactive with <sup>32</sup>P, non-radioactive and a trial with selected strain (from first and second trials) with heavy metals (Cd, Co and Pb) contaminated soil were set up for evaluation the efficacy of four mycorrhizae strains including *Glomus mosseae*, *G. etanicatum*, *G. intraradices*, mixed strains (combination of *G. mosseae*, *Gigarpora hartiga* and *G. fasciculatum*) in order to investigate the uptake, translocation and distribution of <sup>32</sup>P, P and also dry matter in barely in a glass house. Radioactive phosphorus (<sup>32</sup>P) was used in this study. Results revealed that *G. mosseae* had the highest amount of P uptake in comparison with other strains. It indicates that differences exist among mycorrhizae strains towards <sup>32</sup>P uptake and its transportation to shoot. Increased strain count of *G. mosseae* was found in contaminated pots in trial with contaminated soil along with higher P concentration in root and shoot than non-inoculated plant roots. (*International Journal of Botany* 5 (3): 236-243, 2009; doi: 10.3923/ijb.2009.236-243)

### ***In vitro* Growth of Wheat (*Triticum aestivum* L.) Seedlings, Inoculated with *Azospirillum* sp., Under Drought Stress**

M.H. Arzanesh, H.A. Alikhani, K. Khavazi, H.A. Rahimian and M. Miransari

This research was conducted to determine: (1) the effects of drought on wheat seedlings growth under *in vitro* and dark conditions and (2) if inoculation of wheat seedlings with *Azospirillum* sp. can alleviate the unfavorable effects of drought on

the growth of wheat seedlings. *In vitro* planted seedlings were subjected to different drought intensities using poly ethylene glycol and were inoculated with 25 *Azospirillum* strains including the isolated ones and the standard strains of *A. halopreaferanse*, *A. brasilense*, *A. irakense* and *A. lipoferum*. Different strains of *Azospirillum* sp. enhanced seedlings growth and adjusted their water behavior under drought. Such results in combination with the previously related results indicate that *Azospirillum* sp. are able to enhance plant growth and production under different physiological and ecological conditions. (*International Journal of Botany* 5 (3): 244-249, 2009; doi: 10.3923/ijb.2009.244.249)

### **Rapid Isolation of Genome DNA Suitable for PCR from Tropical Almond (*T. catappa*) Plant Populations**

B.O. Oboh, L.A. Ogunkanmi and N. Agwu

This study was conducted to develop a rapid and efficient method for the isolation of genomic DNA from the tropical woody tree, *Terminalia catappa* L. Fresh young leaves from 35 trees were sampled for the extraction of genomic DNA. The methodology employed excluded the use of liquid nitrogen and an ultracentrifuge with various modifications in the quantities and reagents used. The result of the extraction showed that genomic DNA of good quality and quantity with a spectrophotometric ratio of between 1.7-2.0 for the trees sampled. Result further showed that the extracted DNA on 1% agarose gel had high molecular weight bands following electrophoresis. Thus, we concluded that the modified protocol used for the extraction of genomic DNA in *T. catappa* which can be easily adapted to other crops produced DNA of good quality and quantity which can be used for PCR based studies. (*International Journal of Botany* 5 (3): 250-254, 2009; doi: 10.3923/ijb.2009.250.254)

### **Evolutionary History of the Genus *Pistacia* (Anacardiaceae)**

M.G. Al-Saghir

*Pistacia* L. belongs to the family Anacardiaceae (cashew family), order Sapindales. *Pistacia vera* L. (cultivated pistachio) is by far the most economically important species in the genus. It has edible seeds and considerable commercial importance. The evolutionary history and the phylogenetic relationships between species within the genus are not well understood. A better understanding of these relationships is needed to make the species more useful for plant improvement or

genetic studies. The objective of this perspective is to provide additional insight into understanding the evolutionary history of *Pistacia*. In conclusion, *Pistacia* is a monophyletic genus and it contains two sections (*Lentiscella* and *Pistacia*) and it is originated in the Paleocene epoch. This is based on Anacardiaceae being pantropical in distribution with North and South America representing major diversification centers of the family including the geographical distribution of *Pistacia*. This perspective provides additional insight into understanding the evolutionary history of the genus *Pistacia* to make the species more useful for plant improvement or genetic studies. (*International Journal of Botany* 5 (3): 255-257, 2009; doi: 10.3923/ijb.2009.255.257)

### **First Record of *Cheimonophyllum* Singer from Turkey**

A. Kaya

This study deals with the first record of pleurotooid fungus *Cheimonophyllum candidissimum* (Berk. and M.A. Curtis) Singer (*Cyphellaceae*) growing on *Alnus* sp. wood from Kahramanmaraş, Turkey. (*International Journal of Botany* 5 (3): 258-260, 2009; doi: 10.3923/ijb.2009.258.260)

### **Phytosociological Attributes of Wadi Gaza Area, Gaza Strip, Palestine**

M.M. Abou Auda, K.F. El-Sahhar and N.Y. Deeb

Wadi Gaza area, Gaza Strip, Palestine was subjected to a phytosociological study through 24 trips in the period from March to September 2007. This area has a characteristic semi-arid climate and locates in a transitional zone between Mediterranean, Negev and Sinai regions. Nine quadrats (10×10 m) at buttom, bank and open field wadi in six locations; namely, Al-Brikat, Al-Nabaheen, Al-Saoud, Al-Bahr, Al-Rabowa and Abu-Malaa representing the entire area of Wadi Gaza, Gaza Strip were chosen to study the vegetation, including species cover-abundance, species frequency, relative frequency, community similarity, in addition to soil analysis. Some quadrats were pure stand of one species; e.g., *Tamarix nilotica* in Al-Rabowa and *Arundo donax* in Abu-Malaa. Some species like *Cynodon dactylon* were restricted only to one place (wadi bank) of the location (Al-Brikat) due to the lower degree of animal grazing and the moisture availability. *Alhagi graecorum* and *Silybum marianum* recorded the highest species frequency in the studied area. Unique occurrence of some salinity tolerant

species were observed in Al-Bahr (a coastal location). The highest similarity was found between Al-Brikat wadi bank and Al-Nabaheen wadi open field. In contrast to the dissimilarity between both of Abu-Malaa and Al-Rabowa wadi bottom and other locations as they were pure stands and more or less unique locations. Physical and chemical structure of soil varied according to soil texture, pH values, salinity, moisture, sewage water, urban effect and bordering agricultural fields. (*International Journal of Botany* 5 (4): 261-269, 2009; doi: 10.3923/ijb.2009.261.269)

### **Pollen Grains of Lagos Lagoon Swamp and Hinterland Vegetation-I**

O.H. Adekanmbi and O.T. Ogundipe

In order to aid pollen identification, which is the bedrock of palynological studies, 14 plant species belonging to 4 families were subjected to standard palynological sample preparation. Taxa in the collection belong to the families Acanthaceae, Amaranthaceae, Apocynaceae and Aracaceae. Pollen grains belonging to the family Acanthaceae are mostly prolate in equatorial view and trigonal to circular in polar view. Family Amaranthaceae pollens are eurypalynous comprising of different morphological types of pollen, ranging from inaperturate to polyporate. Genera in the family Apocynaceae exhibit palynological extremes indicated by variety in the shape of the pollen grains, aperture, size and ornamentation of the studied species. Pollen grains in Aracaceae also exhibit variations ranging from monocolpate to trichotomosulcate nature of aperture. Light micrographs, detailed descriptions of the species and where possible Scanning Electron Micrographs are provided. It is established from this study that identification of palynomorphs should not pose a problem to the application of palynology even in fields other than biostratigraphy such as forensic studies, mellisopalynology, and medicine (e.g., alleviation of pollinosis). (*International Journal of Botany* 5 (4): 270-278, 2009; doi: 10.3923/ijb.2009.270.278)

### **Xylem Conductivity and Anatomical Traits in Diverse Lianas and Small Tree Species from a Tropical Forest of Southwest Mexico**

Mario Gutiérrez, Rubén San Miguel-Chávez and Teresa Terrazas

Seven lianas and four small trees collected from a tropical rainforest of Southwest Mexico were studied to relate vessel diameter and vessel frequency to the relative

hydraulic conductivity (RC), vulnerability to cavitation and anatomical traits on the secondary xylem. The seven liana species and four small tree species represented ten different families. Two liana species (*Passiflora ligularis* A. Juss. and *Vitis tiliifolia* Humb. and Bonpl.) showed the highest vessel diameters, RC and vulnerability to cavitation. A small tree (*Petrea volubilis* L.) presented the lowest values for vessel diameter, RC and cavitation. Narrow vessels determined the vessel frequency per mm<sup>2</sup> (-0.58) while wider vessels showed low influence (-0.24). Wider and narrow vessels determined RC and vulnerability to cavitation ( $r = 0.59$  to  $0.76$ ). Generally, wider vessels presented solitary distribution on the secondary xylem in liana and small tree species and narrow vessels were grouped in clusters. Liana species presented parenchyma in diverse forms, while parenchyma was scanty in the small tree species. The eleven species showed a broad range in RC and vulnerability to cavitation and showed diversity in anatomical traits on secondary xylem indicating that they have different anatomical adaptations with similar growth habit. (*International Journal of Botany* 5 (4): 279-286, 2009; doi: 10.3923/ijb.2009.279.286)

### **Effect of Topography and Soil on the Distribution of under Canopy Trees of *Garcinia* (Guttiferae) in Lowland Forest of Peninsular Malaysia**

M. Nazre, A. Latiff and M.K. Mohamad-Roslan

The distribution and aggregation of species in tropical forests is known to have certain preferences based on the edaphic factors such as topography types and soil series and this study was carried out to see the distribution of 16 *Garcinia* species in lowland dipterocarp forest in Pasoh Forest Reserve with those edaphic factors. Even though there have been many studies in large scale areas with a larger group of species to see the relationship of edaphic factors with species distribution, very few studies have been carried out on individual species especially for under-canopy or lesser known species. *Garcinia* is an under-canopy tree species in tropical South East Asian forests with no commercial values but mostly known because of their edible fruits. Based on published topography and soil series maps in 50 ha plot, the preferences of *Garcinia* trees were analysed and calculated. Results showed that *Garcinia* trees could be found on all types of topography and soil but most trees are distributed very well on the flatland and well-drained alluvium soils rather than other areas. The least number of stems found are in the top (highest) topography and poorly-drained (prone to flood) alluvium soil. However, at species level, there are significant preferences of each

*Garcinia* species on the different type of topography and soil. This shows that different type of topography and soil play significant effect on the distribution of *Garcinia* trees in tropical lowland forest. (*International Journal of Botany* 5 (4): 287-294, 2009; doi: 10.3923/ijb.2009.287.294)

## **Pollen Grains of Asteraceae and Analogous Echininate Grains**

O.H. Adekanmbi

Seven plant species belonging to 3 families were subjected to standard palynological sample preparation in order to provide additional tool in pollen identification. Taxa in this study include the families Asteraceae, Convolvulaceae and Malvaceae. Pollen grains belonging to the family Asteraceae are unique in being characteristically echinate. They can be differentiated from similar pollen in other families by the relatively small size of both the pollen and the spines and the irregular arrangement of the spines. Pollen of *Ipomoea aquatica* belonging to the family Convolvulaceae has spines like found in Asteraceae but can be distinguished in that those of Convolvulaceae are remarkably bigger in size. *Hibiscus rosasinensis* a species in the family Malvaceae share the same echinate characteristics but peculiar in possessing isolated spines, which stand out conspicuously. The pollen grain is differentiated from Convolvulaceae pollen in being larger. Light micrographs, detailed descriptions of the species and where possible Scanning Electron Micrographs are provided. This study is expected to be useful in palaeoecology research, petroleum exploration, honey industry and in forensic investigations. (*International Journal of Botany* 5 (4): 295-300, 2009; doi: 10.3923/ijb.2009.295.300)

## **Macromycetes of Genç District (Bingöl-Turkey)**

Y. Uzun, A. Kaya, A. Keleş, M.E. Akçay and İ. Acar

The study was conducted on the macrofungi specimens collected from Genç district (Bingöl-Turkey) between 2006-2009 to determine the macromycota of the region. Seventy eight taxa belonging to 23 families in *Ascomycetes* and *Basidiomycetes* classes were identified. Agaricaceae, Tricholomataceae and Strophariaceae are the most crowded families in the region. All of the taxa are new for the district. *Omphalotus olivascens* var. *olivascens* (*Marasmiaceae*) is determined as new record for Turkish macromycota. (*International Journal of Botany* 5 (4): 301-306, 2009; doi: 10.3923/ijb.2009.301.306)

## ***Wolffia columbiana* Can Switch Between Two Anatomically and Physiologically Separate States: Buoyant for Invasion and Starch Rich for Colonization**

Michael Witty

Turion formation is poorly studied in all of the *Wolffia* species and only narrow studies have been done. In this study details of *Wolffia columbiana* ecology and physiology are related to anatomy and histochemistry. We used a combination of histochemistry and a new method of pressing tissue into one focal plane to reveal anatomical features that are not visible using conventional methods. Previously unknown organs, wax coated substomatal cavities, are described which play a crucial role in *Wolffia* ecology. Tank experiments were used to determine the behavior of *Wolffia columbiana* plants in response to light and dark conditions. A physiological mechanism for transition between an invasive floating population and dormant benthic population using oxygen floatation is described, which involves *Wolffia columbiana* behaving like a small bathyscaphe. This mechanism is combined with accumulation of large stores of starch and is an adaptation to colonization and overwintering. (*International Journal of Botany* 5 (4): 307-313, 2009; doi: 10.3923/ijb.2009.307.313)

## **Characteristics of Cogon Grass Rhizomes and its Perforation of a Maiden Cane Rhizome**

J.J. Muchovej, O.U. Onokpise and S.K. Bambo

Cogon grass (*Imperata cylindrica*) is one of the most aggressive grasses world wide and spreads by an extensive rhizome system. This study adds observational detail to growth of cogon grass rhizomes and provides for a mechanism by which cogon grass is able to perforate other species of plants. During a competition study between *I. cylindrica* and native grasses, where the plants were grown in 30 cm pots, under greenhouse conditions, the underground systems were harvested by removing the root ball from the pot, then removing the potting mixture in order to un-potted for determination of length and weight of rhizomes and roots. In one pot, a unique situation was observed where a rhizome of cogon grass had perforated and traversed a rhizome of maiden cane. Aside from the physical damage, the rhizome of the maiden cane did not appear to be diseased. This study describes the rhizomes of the two plants and documents penetration. While cogon grass can penetrate other below ground parts of other species of plants, it does not appear to provide for major damage. (*International Journal of Botany* 5 (4): 314-316, 2009; doi: 10.3923/ijb.2009.314.316)

## **Ethnobotanical Utilization and Conservation of Chewing Sticks Plants Species in Ekiti State, Nigeria**

Joshua Kayode and Michael A. Omotoyinbo

Surveys and direct field observation were carried out to determine the endangered chewing stick species in Ekiti State, Nigeria. The survey involved the use of semi-structured interviews which were conducted with a fairly open framework that allowed for focused, conversational and two-way communication. Also group interviews were conducted in order to determine group consensus on the chewing sticks plant species. The relative abundance of the identified species was determined by the time it would take to physically come across the plant specimen in the study area. Results obtained revealed that a total of 49 plant species belonging to 28 different families were observed to be in use as chewing sticks. Most of these species were indigenous species; previous studies had revealed that these species were essentially rich in various natural products. The relative abundance test revealed that, 33, 51 and 16%, respectively, of the chewing sticks species were presently common, frequent and occasional on the abundance scale used in this study. Most of the species were uncultivated species whose wildlings were usually preserved in the study area and were in high demand in the study area. Other products widely derived from these species in the study area were identified. Thus the endangered species required urgent conservation efforts. Features that could enhance their conservation in the study area were defined. (*Research Journal of Botany 4 (1): 1-9, 2009; doi: 10.3923/rjb.2009.1.9*)

## **Induction of Seed Germination in *Cistus heterophyllus* (Cistaceae): A Rock Rose Critically Endangered in Spain**

José A. Navarro-Cano, Diego Rivera and Gonzalo G. Barberá

Seed germination from the only European population of the Iberian-North African endemism *Cistus heterophyllus* Desf. was studied by using germination inducing pretreatments. A seeding-cultivation method with flowerpots in a greenhouse was also tested and compared with the usual germination method using Petri dishes in a growth chamber. Seeds were collected in three different years. Germination percentages ca. 43% were obtained without pretreatment. This can be considered a high percentage in the genus *Cistus* and abnormally large for an isolated population composed by only nine individuals. A large variability in seed germination was found among the samples from the same population collected in different years. The dry-heat pretreatment ( $87\pm 3^{\circ}\text{C}$  for 12 min and soaking in

water at 20°C for 48 h) significantly increased the germination percentage, reaching 81.5%. Germination in greenhouse was as effective as in growth chamber. The seeds maintained their viability until at least 6 years after collection and storage in a dry atmosphere at laboratory temperature. These results mean the first contribution to the germination ecophysiology of this species and they are fundamental to the recovery plan of the last remnant population of *C. heterophyllus* in Spain. (*Research Journal of Botany* 4 (1): 10-16, 2009; doi: 10.3923/rjb.2009.10.16)

### **Taxonomic Significance of Foliar Epidermis in Some Members of Euphorbiaceae Family in Nigeria**

D.O. Aworinde, D.U. Nwoye, A.A. Jayeola, A.O. Olagoke and A.A. Ogundele

A detailed morphological study of the leaf epidermis of the tropical genera of some species *Acalypha*, *Bridelia*, *Euphorbia*, *Hura*, *Jatropha*, *Manihot* and *Ricinus* in Nigeria is presented. The study revealed several interesting epidermal features some of which have not previously been reported in the genera. Leaf epidermal characters such as pattern of epidermal cells, types of stomata and presence of trichomes are constant in some species and variable in others and thus of great significance in understanding the relationships between and within species. Leaves are amphistomatic in all species except in *Bridelia ferruginea*, *Euphorbia heterophylla*, *Euphorbia pulcherrima* and *Jatropha gossypifolia* which are hypostomatic. The stomata length, width, density and index also vary in different species. (*Research Journal of Botany* 4 (1): 17-28, 2009; doi: 10.3923/rjb.2009.17.28)

### **Female Gametophyte in Two Kenyan Species of *Inversodicraea*- (Podostemaceae)**

S. Sikolia and J.C. Onyango

This study aims to elucidate the ontogeny and organization of the female gametophyte. Further, provide evidences for the strike phenomenon in the Podostemaceae. The female gametophyte ontogeny in *Inversodicraea bifurcata* Engl. *I. keniensis* sp. nov. Nagendran et Sikolia conforms to the Apinagia type of the monosporic category. The primary chalazal nucleus degenerates at the two-nucleate stage. The strike phenomenon met in the family is discussed. The organized female gametophyte is four-nucleate, four-celled and consists of two pear shaped synergids, a large central egg cell and a polar cell. Filiform apparatus

are present in the synergids. Based on the female gametophyte ontogeny, there are no antipodal cell(s). Earlier reports of double fertilization are not confirmed, but only single fertilization take place in the family. The nucellar plasmodium is rationalized on its organization and ontogeny and is formed before fertilization in *Inversodicraea*. Effects of tension force and lytic enzymes during nucellar plasmodium organization and its ultimate significance are discussed. (*Research Journal of Botany* 4 (1): 29-39, 2009; doi: 10.3923/rjb.2009.29.39)

### **Accumulation of Raphides Crystals in *Euterpe oleracea* Mart. Embryo**

M.A.M. Neto, A.C. Conceição, A.S. Mendes, R.C.L. Costa and A.K.S. Lobato

The aim of this study was to determine if the crystals of the calcium oxalate present in the *Euterpe oleracea* embryo has function of calcium reserve during the germination process and if the accumulation is dependent of fruit production. In the experiment 1 the design was entirely randomized, with 3 evaluation periods (September, November and December). In the experiment 2 the design was entirely randomized, with 3 treatments (without cultivation, MS-CaCl<sub>2</sub> after 30 cultivation days and MS + CaCl<sub>2</sub> after 30 cultivation days). The results not confirm the function of calcium reserve of the raphides in *Euterpe oleracea* embryos, however was determined that the calcium oxalate crystals of the embryo are monohydrated and that the production and accumulation is dependent of the season, in which the accumulation is higher in the period of smaller fruit production. (*Research Journal of Botany* 4 (1): 40-47, 2009; doi: 10.3923/rjb.2009.40.47)

### **An Illustrated Description of *Selaginella imbricata* and *Selaginella yemensis* from Saudi Arabia**

Abdulrahman M. Al-Shehri and Gamal M.A. Lashin

*Selaginella imbricata* (Forssk) Spring and *Selaginella yemensis* (Swart) Spring, are described in detail in light of numerous recent collections from Saudi Arabia. The two species are recorded for the first time in El-Baha region and Shammaran and Muhayl valley of Asir region. The two species are investigated and compared with those of other countries. A key to the two species is providing. The presence of *Selaginella* species in five different localities in Saudi Arabia indicates that the species are native and not invasive plants. (*Research Journal of Botany* 4 (1): 48-54, 2009; doi: 10.3923/rjb.2009.48.54)

## **Phytosociological Investigation and Life Form Pattern of Grazinglands under Pine Canopy in Temperate Zone, Northwest Himalaya, India**

S. Kukshal, B.P. Nautiyal, A. Anthwal, A. Sharma and A.B. Bhatt

In temperate region of Northwest Himalaya, drier slopes are dominated by *Pinus roxburghii* and are known for rich ground herbaceous flora predominated by grasses. These regions serve as grazingland for livestock and cattle. Present study deals with vegetation analysis, phytosociology and life form pattern of such grazingland between 1100-1400 m a.s.l. across the altitudinal gradient and varying slopes. *Capillipedium parviflorum* is identified as dominant species based on Importance value index, although the area is exhibited by large number of herbs in comparison to grasses and sedges. Vegetation of the area is contagiously distributed and predominantly represented by therophytes and geophytes indicating the degree of anthropogenic activities. The native vegetation is disturbed by overgrazing and life forms of the flora of each of the association are maintained by the intensity of grazing. In the sites under observations, besides grazing, fire was main detrimental factor for dominating the flora by therophytes. Codominance of geophytes may be assigned to its propagation through underground perennating organs as the fire type in these ecosystems is crown fire type. The study describe all these features. (*Research Journal of Botany* 4 (2): 55-69, 2009; doi: 10.3923/rjb.2009.55.69)

## **Rapid and Efficient Method of Genomic DNA Extraction from Pistachio Trees (*Pistacia vera* L.)**

M.G. Al-Saghir

This study was conducted to develop a rapid and efficient protocol for extracting high quality DNA from Pistachio trees suitable for PCR and molecular studies. Genomic DNA was extracted from 12 Pistachio trees using modified QIAGEN DNeasy Plant Mini Kit. The results showed that the modified protocol successfully produced a sufficient amount of DNA with high quality, which was highly confirmed by the purity index values of DNA samples (1.45 to 2.01). In conclusion, the modified protocol can produce high quality DNA from Pistachio trees suitable for PCR studies such as RAPD and AFLP and it can be easily adjusted for other *Pistacia* species. (*Research Journal of Botany* 4 (2): 70-73, 2009; doi: 10.3923/rjb.2009.70.73)

## **Cryopreservation of *Brassia rex* Orchid Shoots Using PVS2 Technique**

N. Johari, C.L. Keng, X. Rathinam, U.R. Sinniah and S. Subramaniam

*In vitro* grown shoots of *Brassia rex* orchid hybrid was cryopreserved by means of plant vitrification solution 2 (PVS2) technique. For the preculture treatment, the shoots were excised into two standard sizes of 0.5-1.0 and 1.0-1.5 cm and were precultured on half-strength Murashige and Skoog (MS) semi solid medium supplemented with different concentrations of sucrose (control (0.06 M), 0.1, 0.25, 0.5 and 0.75 M) for 24 and 48 h. For the PVS2 dehydration treatment, the 0.1 M precultured (48 h and 1.0-1.5 cm) shoots were chosen for further experiment where the shoots were dehydrated in PVS2 solution at various durations (5, 10, 15, 20, 25 and 30 min) at 0 and 24°C for positive and negative storage in Liquid Nitrogen (LN). The viability of the cryopreserved cells were determined by 2, 3, 5-triphenyltetrazolium chloride (TTC) assay and chlorophyll extraction techniques. The best condition of PVS2 treatment was at 20 min of PVS2 treatment at 0°C prior to storage in liquid nitrogen. In chlorophyll determination based on chlorophyll assay, the highest concentration of total chlorophyll concentration (56.250 µg g<sup>-1</sup>) was obtained from shoots that were dehydrated for 25 min in PVS2 solution at 0°C without storage in liquid nitrogen. (*Research Journal of Botany* 4 (3): 74-88, 2009; doi: 10.3923/rjb.2009.74.88)

## **Genetic Diversity of Indian Liverwort *Plagiochasma appendiculatum* Revealed by RAPD Marker**

A. Soni, A. Kumar, V. Nath and A. Niveden

Genetic diversity of the Monoceious thalloid liverwort *Plagiochasma appendiculatum* was investigated by Rapid Amplified Polymorphic DNA (RAPD) analysis. The species is explained and demonstrated with its genetic diversity on the basis of morphological variations. Samples were collected from different parts of India growing on different habitat at variable altitude. After the study of its morphology, it has been observed that the population of this taxon shows significant variation in plant size, shape, colour, ventral scales, appendages of scales, rhizoids, position of male and female receptacles etc. Based on such morphological variations, we have used the RAPD marker to estimate the genetic diversity within and between the populations. Approximately 75% of the variations have been observed within and between genotypes of *P. appendiculatum* as

revealed with both phenotypic and genotypic data. The RAPD markers are being used increasingly to analyze the phylogenetic relationship among the liverworts to give the exact framework of taxonomic identification of naturally occurring liverwort *P. appendiculatum*. (*Research Journal of Botany* 4 (3): 89-100, 2009; **doi**: 10.3923/rjb.2009.89.100)